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Buffalo and Goats in Asia: genetic diversity and its application

Proceedings of a seminar Kuala Lumpur, Malaysia, 10-14 February, 1991

Editor: N.M. Tulloh

Contents

Foreword

The buffalo is still a key component of village systems of agriculture in Asia and its well-being and productivity have a major influence on, and in some cases determine, the standard of living of the small farmers and their families in several countries in the region.

The two ACIAR projects reported in these proceedings (8515 and 8364) set out to establish whether crossbreeding among buffalo types and strains leads to an increase in productivity, measured as growth, fertility, milk production and draught capacity, and to investigate the extent of the genetic variation among buffalo populations and among goat populations that could be identified using existing biochemical and karyotyping technology.

One project (8515) consisted of a network of research institutions in Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and Australia. The other (8364) was centred on the University of Malaya and Universiti Pertanian Malaysia, drawing its experimental resources from the network and other institutions in the region, and included the genetic variation between goats as well as buffalo in the region in its mandate.

With the possible exception of crossbreeding, improving the productivity of animals via genetic options is slow relative to the possibilities presented by changes to the nutritional environment, or in the case of health, the possibilties available by using vaccines or chemicals. However, genetic changes are relatively inexpensive and long lasting and do not need continuous inputs to maintain their effectiveness.

These two projects have demonstrated two important principles in spite of their limited period of operation. The first is that crosses between swamp and river buffalo lead to productivity increases and the second is that there are substantial genetic differences among populations of swamp buffalo and among populations of goats from different regions in Southeast Asia.

Although much more research needs to be done on the theoretical and practical aspects of buffalo breeding, these projects have heightened the awareness of the potential benefits for the smallholder farmers that can be derived from a better utilisation of the buffalo genetic resources in the region.

G.H.L. Rothschild
Director, ACIAR

Welcome to Participants

THE objective of this coordination and planning meeting is to review the progress of ACIAR buffalo projects relating to the studies on gene karyotyping and on comparative studies between genotypes on growth, reproduction and feed utilisation. There is no doubt these studies are concerned with fundamentals of the science of buffalo production. From what we know, much has been accomplished since these two projects were implemented. Malaysia is fortunate to be part of the research team because its buffalo population is the smallest in the region — and that is fast declining.

The declining buffalo population in Malaysia is indeed ironical when we consider the importance given to the development of the livestock industry as a whole. There are many reasons for this. Perhaps the most significant is that the available technology is inadequate to propel buffalo production from subsistence to commercial farming. In a way, the failure to increase the buffalo population in Malaysia reflects, to a large degree, the inadequacies in research.

The buffalo population of the world is increasing at about 0.6% per annum and 90% of the population is in Asia. The rate of increase in the Indian sub-continent is four times that of the world, indicating the importance of buffalo in India. The role of buffalo as a major income generator in the Indian rural economy is well recognised. Buffalo constitute 30% of the total bovine population in India, and contribute about 58% of the milk produced. In order to sustain a high level of production efficiency, research is being undertaken in almost every discipline, e.g. genetics, nutrition and reproduction.

The scenario in Southeast Asia is somewhat different where buffalo are traditionally utilised for draught power in the rice paddy. With the rapid increase in farm mechanisation the use of buffalo for draught is in decline. Socio-economic changes in the region suggest that this decline in use for draught will continue.

A new perspective has to emerge for buffalo where Southeast Asia is concerned. It cannot be denied that the buffalo is well adapted to the environment but the slow growth rate, low milk yield and reproductive efficiency are constraints that have to be overcome. Our aim is to increase the production efficiency of the buffalo in order to yield milk, beef and draught power more economically. To achieve this, the level of research has to be enhanced. As an example, we all realise the importance of studies on the evaluation and conservation of genetic resources. But so far the research has been confined to some karyogenetic studies and I am happy to say that we have been carrying out such studies at Universiti Pertanian Malaysia since the late 1970s. However, there are other important areas, such as molecular, genetic and immunogenetic techniques and the study of biochemical polymorphism that need to be utilised in order to define the inter- and intra-breed genetic differences.

In nutrition also, more basic research needs to be undertaken to study rumen function since buffalo in Southeast Asia have to rely on low quality fibrous feed. Unfortunately, there are very few well-equipped laboratories available, nor is there sufficient expertise in this region to enable such research to be undertaken. Universiti Pertanian Malaysia has, however, initiated a research program on rumen microbiology with a view to understanding the role of rumen microbes in fibre digestion. In spite of the recent fire which destroyed the entire rumen microbiology laboratory, the research team has made significant contributions towards the understanding of microbial degradation of feed materials in the rumen.

Another area which requires more physiological studies is reproduction. There has been some good research done on this topic in the region, but we are still a long way from successfully controlling reproduction in buffalo.

The level and intensity of livestock research, especially on the buffalo, needs to be enhanced, but unfortunately the research capacity is limited. More collaborative studies have to be initiated between laboratories and scientists in the region and we are grateful to ACIAR for the leading role it has played in stimulating this activity. We in Malaysia are prepared to share our facilities and expertise and we know the same is true of other institutions in other countries. In the same spirit we all hope that ACIAR will continue to support research to improve buffalo production in the region.

Professor Syed Jalaludin Syed Salim
Department of Animal Science
Universiti Pertanian Malaysia
Serdang

Workshop Conclusions

Institutional

THERE is now substantially better coordination within the region between institutes involved in buffalo research; the exchange of information and the sharing of experimental material and results are now considerable.

The wide range of complementary skills required to adequately evaluate buffalo genotypes has been harnessed and the capability of research institutions has been increased by the provision of equipment. Opportunities for research training on topics of importance to the region have been enhanced.

The results from Project 8515 demonstrate that the productivity of the Australian swamp buffalo could be substantially increased by crossing to a suitable river genotype. This would favour domestication of feral populations, thus removing a serious threat to the ecology of northern Australia and the long-term health status of the Australian cattle industry, and encourage an expansion of trade in live buffalo and buffalo products.

The injection of a relatively small amount of funding to the several institutions involved in Project 8515 has produced results that could not have been generated within the foreseeable future by any single existing institution.

Scientific

Relative to the swamp types, F_1s (Murrah × swamp) have been shown to have higher yield and growth rate and similar draught capacity. Initial data indicate that intercalving intervals of F_1s are shorter and onset of puberty is earlier. The superior growth of the F_1s allows them to be used for work at an earlier age.

Differences in semen quality have been demonstrated between genotypes though the effect of these differences on fertility has not been investigated.

The proportion of animals showing post-partum oestrus and conceiving has been shown to depend on season. An interaction between genotype and nutrition has been demonstrated for the interval from calving to conception.

There are no apparent differences in digestive efficiency between the crossbred river × swamp and purebred swamp. Differences in water intake that may affect production efficiency have been recorded.

Methods for karyotyping of buffalo are now well established within the region. It has been established that only three karyotypes exist within crossbred populations derived from Murrah ($2n = 50$) and swamp types ($2n = 48$).

Techniques have been developed for typing biochemical markers in both goats and buffalo. Of the 70 markets targeted, 56 and 51 may now be applied to buffalo and goat populations, respectively.

Methods for studying genetic distances among populations have been established within the region. These studies have detected three major groups of swamp buffalo from among those sampled. The genetic distance between populations is not related to geographic distance and is similar in magnitude to values already established for European livestock breeds.

The genetic distance among goat populations is less than that among buffalo populations. In both species the proportion of heterozygotes is less than expected.

In an associated research project restriction length fragment polymorphism (RFLP) methods for studying mitochondrial DNA (mtDNA) of buffalo have been developed.

Socio-economic

Closer contact between scientists and farmers has been facilitated with a consequently greater appreciation of research needs and practicability of implementing new technologies in existing systems.

The generation of F_1 river × swamp genotypes in the villages has provided the focus for the introduction of additional technologies to village systems; e.g. deworming, vaccination, feed supplements.

Extension strategies that are appropriate to large-scale implementation of new technologies at the village level have been developed; e.g. methods for encouraging farmers to produce F_1 buffalo or cattle have been devised.

The ready acceptance of F_1 buffalo by the village farmers who participated in the project has been demonstrated, suggesting that acceptability of the F_1s will not be an impediment to their large-scale introduction.

Substantial increases in productivity have been achieved by crossing the swamp types to a river breed, suggesting that the economic return to smallholder farmers will be increased if such national programs are implemented.

The direct economic significance of the results to smallholder farmers and the potential for large national benefit to arise from these results should stimulate interest among national and international policy makers and funding agencies in the use of planned crossbreeding as a simple, cost-effective way of improving incomes in the region.

Future Research Directions

While Projects 8364 and 8515 have generated results that could be acted upon immediately, it should be recognised that areas of uncertainty remain, particularly in relation to the productivity of subsequent generations of crossbreds. These areas will need further investigation before it could be recommended confidently that present research results be adopted on a large scale. The deficiencies in knowledge that are considered to be most in need of attention and the areas of research in which cooperation could be strengthened or new initiatives adopted are included in the following.

Given that the continous production of F_1s is not sustainable, there is an urgent need to evaluate the growth, reproduction, draught capacity and milk yield of subsequent generations of crossbreds relative to the performance of the parental breeds over the range of environments and management systems that exist throughout the region. These comparisons should also be used to identify the breeding system that best retains the advantages of the F_1 and is appropriate for each set of circumstances.

Given that $2n = 49$ karyotypes will continue to segregate and could result in the formation of non-viable gametes regardless of the breeding system used, it is imperative that the practical significance of any possible effect of chromosomal imbalance on fertility, particularly of F_1 males and of both males and females of later generations, be determined as soon as sufficient animals of each generation and each karyotype become available. Effects on fertility should be examined for both AI and natural mating systems.

Large numbers of animals of known karyotype will be required to provide unequivocal answers to both of the above investigations and cooperation between regional centres will be necessary if the answers are to be obtained within a reasonable time.

Given that the number of exotic bulls that could be used for crossbreeding is likely to be very limited in all countries, efficient methods for the production of F_1s will have to be developed if crossbreeding is to have a significant impact at the national level. Investigations should include ways to improve, at the village level, the technology associated with the use of AI including oestrous detection and oestrous synchronisation.

Embryo transfer technology will have to be developed specifically for buffalo to overcome quarantine barriers to the free movement between countries of desirable exotic genotypes.

Crosses other than Murrah × swamp, including crosses among swamp populations, are likely to be more appropriate for some environments or management systems and the productivity of these crosses needs to be investigated. Since empirical testing of all possible crosses is impossible, potentially useful strains or types for use in these crosses could be identified on the basis of known characteristics, particularly mature size and milk yield potential, and estimates of genetic distance from the type or strain under improvement.

The widespread or indiscriminate use of crossbreeding is likely to result in the loss of potentially useful localised strains, in some instances before their characteristics can be documented, unless specific measures are implemented to conserve these strains. Since it is neither logistically nor economically feasible to conserve all populations, it is necessary to identify those that are considered to be most worthy of conservation. Identification of these strains could be on the basis of known characteristics and genetic distances.

Studies of genetic distances should be extended to other populations, other loci and other sources of variation including mitochondrial and genomic DNA to enable better identification of strains that could be potentially useful in genetic improvement programs.

The facilities, techniques and expertise developed for studying crossbreeding and genetic variation in buffalo populations could have application in the genetic improvement of other domestic species.

Genetic Identification of Strains and Genotypes of Swamp Buffalo and of Goats in Southeast Asia: rationale for their study

J.S.F. Barker,[1] T.K. Mukherjee[2] and M. Hilmi,[3] S.G. Tan[4] and O.S. Selvaraj[2]

GENETIC variation is the basic material for the animal breeder, and the effectiveness of the tools of selection and crossbreeding depends on the existing amounts of genetic variation.

In Asia and Oceania, review and documentation of genetic variation in different species of domestic animals, primarily between breeds and strains, was first undertaken by the Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO). In two workshops, one at Tsukuba in 1979 and one at Kuala Lumpur in 1981, the domestic animal genetic resources of the region were documented and the problem of their most efficient utilisation discussed. Subsequently FAO collected from some selected countries in Asia (Malaysia, Thailand and Sri Lanka) data on animal resources and their productivity measurements as a part of its overall program on the identification, evaluation and conservation of genetic resources. At all four World Congresses on Genetics Applied to Livestock Production (1974, 1982, 1986 and 1990), one of the important topics for discussion was conservation and management of indigenous and non-indigenous animal genetic resources. A data bank at the Institute of Animal Breeding, University of Hanover, has been established jointly by the Working Party on Animal Genetic Resources of the European Association of Animal Production (EAAP) and FAO. This covers information on the livestock breeds of Europe and other continents, although most of the current information pertaining to performance records is for European breeds.

For Asia and Oceania, as elsewhere in the developing world, the primary issue is not conservation (or preservation), but evaluation and appropriate utilisation of existing resources. Evaluation is the prerequisite to optimum utilisation — to provide the data on the comparative productivity and adaptability of the existing native strains, and of crosses, backcrosses, etc., both among them and with exotic breeds. Utilisation then involves the development of breeding programs (selection and/or crossbreeding) to increase productivity and efficiency of production.

Swamp Buffalo and Indigenous Goats

These two species are of primary importance throughout Southeast Asia and are utilised in a wide range of agricultural systems — from intensive agriculture with multiple cropping and plantation agriculture to extensive grazing systems. There is a multitude of local types, strains or geographically separated populations and presumably, they are generally well-adapted to the existing climatic-husbandry-economic conditions. But their productive performance and adaptability is generally poorly known, and they are under threat of genetic dilution or replacement by transfer of genetic material from exotic breeds; i.e. breeds of river buffalo from the Indian sub-continent and breeds of goat from Europe.

High production of breeds in developed countries relative to that of native strains in less-developed countries has led to unrealistic expectations of the potential for rapid improvement of productivity in the less-developed countries through importation. Whether this importation and crossbreeding with native strains be indiscriminate or planned, the nett result could be loss of the native genetic resources

[1] Department of Animal Science, University of New England, Armidale, NSW 2351, Australia
[2] Institute for Advanced Studies, University of Malaya, Malaysia, 59100 Kuala Lumpur, Malaysia
[3] Faculty of Veterinary and Animal Science, Universiti Pertanian, Malaysia 43400 UPM Serdang, Selangor, Malaysia
[4] Department of Biology, Universiti Pertanian, Malaysia 43400 UPM Serdang, Selangor, Malaysia

before their true value is known. Crossbreeding with exotic breeds may improve production over that of the native strains, some native strains may be better than others, crosses among some native strains may be better than the parent strains, but we cannot know whether they are or not without appropriate evaluation.

Optimum utilisation depends on knowledge gained from evaluation, but this poses the major problem — given the wealth of breeds, strains and geographical populations to be considered, it obviously will not be possible to evaluate all of them with the limited resources available.

At the first SABRAO Workshop, Barker argued that the solution to this dilemma is to determine the genetic relationships among the breeds, strains and populations of each species of livestock, so that they may be grouped into sets that are genetically similar, and then to include in evaluation experiments one representative from each set. The study of swamp buffalo and goats in Southeast Asia (ACIAR Project 8364) is the first to put this approach into practice.

The methodology derives from studies in evolutionary genetics. In brief, biochemical genetic methods are used to determine the genotypes of individual animals at loci specifying various proteins, usually enzymes, using the technique of electrophoresis. The loci analysed most likely have no (or little) effect on production traits of interest; the objective was to determine if populations are genetically different for what was hoped would represent a random sample of the genome. For the estimated relationships to be as reliable as possible, it was essential that a large number of loci be analysed. In this study, the aim was to assay at least 70 loci, but it must be noted that about two-thirds of these were expected to show no variation, and so would be uninformative. The final genetic relationship estimates therefore would be based on about 20–25 loci.

The results from this study should help to rationalise research programs, to minimise inefficient use of scarce resources in breed evaluation, and to facilitate choice of the most suitable and productive strains. In addition, the estimated relationships should be useful in predicting expected heterosis in crosses between populations.

Chromosome Analysis in Crossbreeding

As noted previously, crossbreeding of swamp buffalo with breeds of river buffalo has been promoted as one means of improving performance and productivity, by taking advantage of heterosis in the F_1 crossbreds. However, the river and swamp buffalo differ in chromosome numbers; $2n = 50$ and 48 respectively, as chromosomes 4 and 9 of the river buffalo karyotype are tandemly fused in the swamp buffalo. F_1 animals therefore have 49 chromosomes, and if the F_1 are mated inter se, all three chromosome configurations segregate in the F_2. What is not clear is whether $2n = 49$ animals suffer any decrease in reproductive capacity, or what the best breeding program should be from the F_1.

Histological and meiotic chromosome analysis done by T.A. Bongso and M. Hilmi on testicular biopsies taken from F_1 bulls ($2n = 49$) had revealed a large proportion of degenerating spermatocytes and abnormal spermatids, and an increased incidence of abnormal chromosome configurations (univalents and multivalents). Thus, detailed analysis of the karyotypes of F_1, F_2 and backcross animals was essential to characterise the segregation of different chromosomal types and to relate chromosomal configurations to reproductive performance.

Objectives of the Study

Given the background above, it is important to present here the objectives of the research program at the time it was initiated. As will become clear in the papers to follow, not all of these objectives have been met. Some were too ambitious at the time, but all need to be considered in discussing not only the results available to date but also the priorities for continuing and future studies of buffalo and goat populations, and their genetic improvement. The objectives of the study when the project started in 1986 were:

Genetic differentiation among goat and swamp buffalo strains

(a) To estimate gene frequencies at biochemical loci in breeds, strains and geographical populations of (i) swamp buffalo in Australia, Indonesia, Malaysia, Philippines, Thailand and Sri Lanka (note: the strains analysed will include all those in the Buffalo Genotypes Evaluation Project, No. 8515), and (ii) goats in Indonesia, Malaysia, Philippines, Thailand and Sri Lanka).

(b) To use these data to estimate genetic similarity (genetic distances) among the populations and thus define genetically similar sets.

(c) To provide the basis for and to stimulate appropriate comparative evaluation of representative populations from each set.

(d) To determine the effect of biochemically determined alleles and genotypes of each locus on

growth, reproductive performance, milk production, draught or components of feed utilisation. For the buffalo, data on these quantitative characteristics will be obtained for all animals in the Universiti Pertanian Malaysia (UPM) herd, and for all animals included in the subprojects of the ACIAR project 'Evaluation of Different Buffalo Genotypes for Draught, Meat and Milk Production' (Project No. 8515).

(e) To determine any relationship between biochemical loci and alleles with chromosome constitutions of the swamp and river buffalo (i.e. linkage and linkage disequilibrium). This analysis will use the data from the animals in Project 8515. If there are significant linkage disequilibria, particularly if different alleles at a biochemical locus are fixed in the swamp and river buffalo, electrophoretic assays will be valuable in assisting identification of putative F_1 or backcross animals in a crossbreeding population.

Analysis of swamp × river crossbreeding

(a) To undertake detailed cytogenetic studies (blood chromosome analysis, meiotic chromosome and synaptonemal complex analysis) on F_1, F_2 and backcross animals with 48, 49 and 50 chromosome constitutions to identify exact segregation patterns and to determine if different karyotypes exist within the 2n = 49 backcross and F_2 groups. This will be done on the UPM herd in Malaysia.

(b) To correlate this karyotypic data with the electrophoretic data collected from the same animals in the 'Genetic Differentiation among Strains' component of this proposal, to determine the pattern of inheritance in the various chromosomal types.

(c) To study semen characteristics of crossbred bulls with uneven karyotypes in search of reduced sperm counts (if any) related to unbalanced gamete formation as a result of abnormal meiotic segregation.

(d) To undertake a survey of the chromosome status of the four populations of Sri Lankan buffalo sampled for the genetic evaluation component of this project, as the current data showing a 2n = 50 rather than the normal 2n = 48 of swamp buffalo is based on a small sample from only one locality (Bongso, T.A., Kumaratileke, W.L.J.S. and Buvanendran, V. 1977. Ceylon Veterinary Journal 25, 9–11).

(e) To evaluate the chromosomal status of all animals being used in the ACIAR-funded project No. 8515.

Animal Production in Southeast Asia: present status and research directions

S. Jalaludin[1] and Y.W. Ho[2]

Abstract

Small and large ruminants in Southeast Asia are reared mostly by smallholder farmers with very limited resources. Although ruminant production, at present, contributes very little to farm income, there is potential to expand the production from ruminants and make better use of the resources available on-farm. Research will play a significant role in this expansion of ruminant production.

Present Status

THE efficiency of ruminant production in the tropics is lower than that in the temperate regions. Many reasons have been proposed to explain the relatively low productivity of tropical systems; among these are uneconomical land holdings, lack of capital, inferior genetic stock, primitive technology, unskilled farmers, poor quality feeds and parasites and other diseases. One of the constraints to ruminant production in some developing countries is the lack of feedstuffs although this may not be true for parts of Southeast Asia where there are large amounts of native grasses and forages in the countryside and also large quantities of by-products that could be utilised as ruminant feeds.

The governments in the region have made a concerted effort to increase ruminant production, not only to keep pace with the demand for products associated with rapid urbanisation and an improved standard of living, but also to create a more equitable wealth distribution between urban and rural populations. However, the lack of appropriate technology has hindered livestock development. The present available technology is inadequate to propel ruminant production from subsistence to commercial farming. As a consequence, the region is heavily dependent upon imports of dairy products and, to a lesser extent, meat. For example, Malaysia is only 37% self-sufficient for beef, 8% for mutton and 5% for total liquid milk equivalent.

[1] Department of Animal Science
[2] Department of Biology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selagnor, Malaysia

The slow growth in ruminant production in Southeast Asia, in spite of the availability of feed resources, may be attributed largely to over-dependence on foreign technology which is thought to be the key to progress. Transfer of technologies from advanced to less advanced countries has rarely been successful either technically or economically.

A case in point is the replacement of the local low-producing animals with 'superior' stock imported from developed countries. These exotic animals require a high level of management and are unable to adapt to the local environment. Many schemes in the past have failed as a result of over-reliance on imported stock and technologies. These schemes ignored the potential of optimising the use of indigenous breeds and locally-available feed resources.

Another major constraint to ruminant production in Southeast Asia is the farmers' view of the role of ruminants in the traditional farming systems. The ruminants in Southeast Asia, particularly cattle and buffalo, are kept by tradition for draught to work in the rice paddy but, with the rapid introduction of mechanisation to small farms, their role as work animals is being reduced. While there may be still some need for draught power this alone is unlikely to provide the incentive for farmers to increase animal numbers. Thus, for ruminant production to increase, it must be demonstrated to be a profitable venture.

The desired shift from subsistence to commercial farming can be affected when technologies such as improved genetic stocks, economical feeding systems, effective disease control methods and

techniques for increasing reproductive efficiency are available. Future technologies in developing countries must be based on local research which aims at modifying and improving the existing systems in ways that are acceptable to farmers.

It is imperative that research into ways to improve ruminant production be increased. In determining new research thrusts, it is necessary to take cognisance of the poor research support and weak infrastructure within the region and to develop research projects and co-operation to improve the efficiency of utilisation of the available human and physical research resources. The strength of ruminant research in the region, at present, is more in the applied fields than in the fundamental sciences. This is probably one of the main reasons for the slow technological progress.

An effective research program is one which is market-driven and user-oriented and takes into account both short-term needs as well as long-term objectives. The research program should be balanced for both applied and basic aspects of research and include a number of ruminant species. The following are research strategies for the enhancement of ruminant production in Southeast Asia.

Integrated animal production systems

In parallel with discipline-oriented research, studies must also be undertaken to produce integrated production technologies and systems. In Southeast Asia, the integration of crop with livestock has been practiced widely by traditional small farmers over a long period. However, the level of productivity from traditional systems is relatively low. This production concept nevertheless needs to be intensified and made commercially viable. It is particularly relevant to large plantations and estates but in these enterprises the technologies must be well developed. At present, there is a lack of expertise in integrated animal research. The following issues need to be addressed:

— Technologies in estate/plantation crop production are well known but there is very limited knowledge on forage inter-row cultivation and utilisation. Forage for these applications should withstand low light intensity and persist under grazing pressure.

— Since tropical forage is poorly utilised, strategic feeding of minerals and feed supplements (fermentable and by-pass) is likely to be beneficial.

— Characterisation of crop–animal systems to promote sustainability, including impact on soils and plants, nutrient dynamics, production and economics.

— Research on genetic improvement of ruminant species should continue in order to identify animals with maximum potential to benefit from the husbandry systems.

— Education, training and extension in crop–livestock systems is lacking. There is a need for teaching aids; e.g. text books and manuals on integrated farming systems. A regional network of researchers and research activities would strengthen the transfer of information. Co-operation between institutions within the region will encourage and facilitate rapid information flow.

Feeding strategies

Ruminant production in Southeast Asia has the potential to expand since in most regions there are adequate feed resources (both conventional and non-conventional) to support a larger population of ruminants than at present.

Conventional feeds The land mass under native grasses is extensive and needs to be developed, especially with the introduction of superior dairy breeds. The latter requires that pasture improvement programs at village level be accelerated to meet the demand for high quality feeds. To date, pasture development in Southeast Asia has largely failed because of the inappropriate technology used. The following constraints need to be overcome if pasture development is to succeed in the short term:

— lack of trained pasture specialists for extension work;

— limited choice of suitable pasture grass and legume species for use in specific situations; i.e. open ranches, smallholdings and under tree crops;

— inadequate information on agronomic techniques; e.g. establishment, fertilizer rates, grazing pressure effects;

— lack of pasture management know-how under different production systems;

— lack of strategies to utilise vast grazing resources in the region;

— inappropriate information on propagation and utilisation of tree legumes;

— poor soil types with low pH deficient in phosphorus and with excessive aluminium;

— lack of proper institutional support and infrastructure within the region.

To overcome these limitations, it is recommended that:

— forage propagation centres be established to grow promising species for seed and vegetative propagation, and

— existing grazing reserves be developed into cattle improvement centres as a means of accelerating ruminant production.

The centres, managed by government or farmers' cooperatives, would act as animal breeding stations, forage multiplication centres and technology transfer points. They would also be places for housing, propagation and distribution of imported animals and conserved fodder.

Non-conventional feeds Crop residues and by-products are available in large quantities in the Southeast Asian region. Research to improve the feeding quality of fibrous residues such as rice straw and palm press fibre is in progress. In spite of extensive information on the usefulness of rice straw and palm press fibre as animal feeds, little is fed to ruminants, at least in Malaysia. Reasons for this are:

— difficulty in collecting and transporting straw and fibres;
— lack of finance to treat straw or fibre;
— lack of skill to manage the technology effectively;
— uncertainty regarding the benefits to be derived;
— lack of labour on farms to apply the technology;
— competition with conventional feeds and forages.

In Malaysia, palm kernel cake (PKC) has been used extensively for fattening beef cattle and for supplementing dairy cattle on commercial farms. Very little is used on village farms. The major constraint in using PKC is its long-term effect on animal health due to its high mineral content, especially copper. More research is needed on this aspect.

While the feeding of agricultural by-products certainly has a place in any type of smallholder production system, there is also a role for permanent pasture since systems in Southeast Asia depend heavily upon free grazing to meet basic nutritional needs. Livestock production in Southeast Asia is largely subsistence but successful small- and large-scale commercial farms are emerging.

Genetic improvement

The indigenous stock of ruminant species (cattle, buffalo, sheep and goat) are poor producers. Most of them have slow growth rates, low milk yields and low reproductive rates. In the case of buffalo and cattle, research should be undertaken to determine:

— the extent of heterosis in various F_1 crossbreds with local breeds at different levels of stress, and its loss in subsequent generations;

— the efficiency of milk, meat and draught production, based on total lifetime performance.

To accomplish the above, it would be necessary to identify superior bulls and cows for the creation of nucleus herds.

Research should also be carried out to evaluate buffalo breeds and crosses with particular emphasis on the improvement of reproductive efficiency, draught power, growth, semen quality and comparative resistance to environmental stresses.

For sheep and goat, the following are recommended:

— evaluation of indigenous breeds of sheep and goats which are numerically more important and whose product is in keeping with the local needs, such as meat, milk and fibre;

— studies on reproductive behaviour of indigenous sheep to enable the use of hormones for synchronising of oestrus, super ovulation and induction of early maturity;

— development of two strains of Javanese Thin-Tailed sheep, one homozygous for the 'prolificacy' gene and the other without it;

— for meat production, selection on body weight gain should be encouraged;

— also for meat production crossbreeding of extremely coarse hairy wool breeds with exotic mutton breeds;

— selection within breeds of sheep and goats for genetic improvement of specific characteristics, including identification of outstanding individuals that can be widely used to augment normal selection and breeding methods.

The genetic resources in Southeast Asia are very diverse. Research on the evaluation of the biodiversity and conservation of genetic stock should be enhanced.

Molecular Studies and Biotechnology

New technologies such as gene manipulation and embryo transfer should be studied and applied where appropriate to promote rapid advances in the ruminant production in the region. However, before this can be accomplished, basic studies have to be undertaken on the following topics:

— comparative cytogenetics, molecular genetics, immunogenetics, and biochemical polymorphism.

So far, research undertaken under an ACIAR program in cytogenetics has succeeded in the identification of about 55 markers in goats and buffalo. Although there is a need to expand the program laterally to cover a wider area (China, India, Bangladesh, Burma and Vietnam), there is already sufficient information for the program to advance into gene technology application; e.g. gene mapping of buffalo and cattle, studies on the goat genome, DNA sequencing, RELP, gene transfer, etc. from cattle to buffalo.

In nutrition, more basic research needs to be undertaken on rumen function since ruminants in Southeast Asia will have to continue to rely on low quality fibrous feeds as their basal diet. Unfortunately there are very few laboratories (or expertise) in this region which are able to undertake such research. Universiti Pertanian Malaysia has initiated a research program on rumen microbiology to understand the role of rumen microbes in fibre digestion. The research team has made some contributions towards the understanding of microbial degradation of feed materials in the rumen. Further studies need to be carried out to:

- isolate, characterise and select rumen microorganisms with high cellulolytic activities from cattle, buffalo, sheep and goat;
- assess the role of these highly cellulolytic microorganisms in the digestion of various feed materials, particularly fibrous agro-byproducts;
- use information obtained from both the above to devise schemes by which the microorganisms can be manipulated and used to improve the nutrition of animals fed fibrous diets.

International, Regional and National Cooperation

As mentioned earlier, the regional and national research capabilities in many areas of animal production are weak. In order to expand research activities in the specified fields — ranging through genetics, nutrition, reproduction, diseases, molecular studies — it is proposed that research be undertaken on a concerted basis by and in the region.

The research program should be comprehensive to provide both short-term and long-term solutions. Research thrusts should encompass both basic and applied studies. We propose for consideration by the participants at this meeting an integrated regional animal research program headed by a coordinator and assisted by a sectional head for each discipline. This is to facilitate coordination and communication between scientists, reduce duplication and wastages, maximise the use of resources, allow a faster and smoother flow of information and, most importantly, attract more research fundings.

The lack of finance is a major research constraint. International agencies such as FAO, IAEA, ACIAR, IDRC and EEC could provide the financial support to ensure the successful implementation of the research programs.

Conclusion

Research findings need to be validated quickly to determine their value. As far as possible all validation trials should be conducted on farms which are in inherently capable of absorbing new technologies i.e. institutional and commercial farms. Once the application of technology is proven on farms, its use can be gradually extended to smallholder farmers. It will not be easy as the farmers in Southeast Asia are neither highly skilled nor knowledgable. However, the merits of on-farm research cannot be disputed.

On-farm trials should be conducted in the context of farming-systems research, involving the farmers. Moving to on-farm research will address technology mechanisms and diffusion models, markets and other sociological inputs as well as the publication of results. International donors should be encouraged to assist in developing and implementing on-farm research as a discipline in its own right.

In strengthening research capabilities in ruminant production within the region, special emphasis should be given to ensure development of expertise and centres of excellence; in this situation, the scarce resources will be well managed.

Biochemical Polymorphisms in River Buffalo

C.R. Balakrishnan* and S.L. Goswami*

Abstract

Biochemical polymorphism studies help not only in understanding the basic architecture of the species but are also useful when studying population dynamics, basic genetics, clinical genetics and gene mapping.

Buffalo seem to have a higher degree of conservation with regard to biochemical polymorphisms than other livestock. Of over 35 protein-enzyme studies, polymorphisms were found in less than half the loci. In most of them, the rarer alleles usually had a very low frequency (less than 0.1). In other cases wide variation in frequency of the different alleles occurred in buffalo from different countries. These data can be used for studying animal migration-gene flow patterns. The swamp buffalo seems to have distinctly different features from the river buffalo; thus the $tf^{D}(Tf^{B})$ fequency of the allele is much higher in the swamp type. Further, the swamp buffalo has a very high frequency of an albumin varient (Alb^{X}) which is absent in river buffalo.

DOMESTIC buffalo are farm animals of great economic importance. They provide milk, meat and draught power in many countries. The species has a distribution eastwards to the Far East and Southeast, and westwards to some countries in Africa and Europe. World population of this species is about 138 million and about half of them (74 million) are in India (FAO, 1987).

Biologically buffalo belong to the class *Mammalia*, subclass *Eutheria*, order *Ungulata* and the family *Bovidae*. *Bovinae* is a tribe of Bovidae and contains three genera, viz., *Bos*, *Bubalina*, and *Syncerina*. Domestic buffalo belong to the *Bubalina* while the African buffalo belong to *Syncerina*. There are two major types of domestic buffalo, viz: the river buffalo of the Indian subcontinent and the swamp buffalo of the South Asian region.

Biochemical polymorphisms are inherited variations of biomolecules. They are useful in studies of basic genetics, population dynamics, clinical diagnosis and in gene mapping. Although information on biochemical polymorphism in buffalo is scanty, an attempt has been made here to review the work on blood and milk proteins including enzymes in water buffalo. Haemoglobin, transferrin, albumin, amylase, ceruloplasmin, carbonic anhydrase, alkaline phosphatase, cell peptidase-beta and some other enzymes have been subjected to analysis in order to study polymorphisms in buffalo.

* National Dairy Research Institute, Karnal, 132001, India

Descriptions of Various Polymorphisms

Haemoglobin

Haemoglobin is one of the most thoroughly investigated protein molecules. In buffalo (*Bubalus bubalis*) two haemoglobin bands were reported in chromatographic studies by Giri and Pillai (1956). Vella (1958), while investigating haemolysates of water buffalo from Bali, also found two bands of haemoglobin in all the animals except one which had traces of a third band. Studies on Siamese buffalo by Loypetjra (1962) and on Indian buffalo by Sen et al. (1966) also indicated the existence of two haemoglobin bands. Naik and Sukumaran (1967) similarly found that, out of 350 buffalo typed, all had two bands except three individuals. In these three individuals only one band was observed. The proportion of haemoglobin was between 60 and 70% in the faster band, and 30–40% in the slower one. These workers did not observe any difference between foetal and adult haemoglobin, either by electrophoresis or by alkali denaturation techniques. Khanna and Braend (1968) and Khanna (1973) made similar observations. Of the 507 haemoglobin samples studied, 501 had two bands. Two rare phenotypic exceptions were found in six animals. One variant was observed in four animals which

exhibited three bands. In the other variant, observed in two animals, the relative proportions of haemoglobin in two band patterns were different from normal; the slower haemoglobin band was found to have a concentration of about half or less than the common type. Makaveev (1968) also observed two haemoglobin fractions in buffalo from Bulgaria. Similar results were also reported by Abe et al. (1969) in Formosan buffalo, Granciu et al. (1972) in Bulgarian buffalo, Tan et al. (1980) in Malaysian water buffalo, Vlaic et al. (1983) in Romanian buffalo, Prasad et al. (1983) in Indian buffalo and by Iozio and Annunzuata (1986) in Italian buffalo.

The above reports do not reveal any polymorphism of haemoglobin in buffalo although some exceptions in a small number of animals have been reported. The third band was proposed to have arisen by a mutation of the beta-chain. Several theories have been proposed for the universal presence of two bands and other minor variants but none have been tested experimentally. Variation and improvement in techniques such as isoelectric focusing, electrophoresis of separate chains, etc. may reveal more variation as has been the case with caprine haemoglobins (Braend et al. 1987).

Transferrin

Transferrin is a specific iron-binding protein. Its major function is transport of iron to the bone marrow and tissue storage organs. Transferrin plays a significant role in a cyclic process whereby iron derived from the catabolism of haemoglobin and other proteins is conserved by its return to the haemopoietic tissue. Transferrin also participates directly in the regulation and control of iron absorption and protects against iron intoxication (Putnam 1965). The occurrence of genetically controlled transferrin heterogeneity in human beings (Smithies 1957) stimulated intensive investigations in various species.

Loypetjra (1962) described polymorphism of transferrin in Siamese buffalo. He reported three phenotypes having mobilities equivalent to the transferrin AA, AB and BB of cattle (Makaveev 1968, 1970). Makaveev and Donev (1984) reported polymorphism in river buffalo from Bulgaria, imported Indian river buffalo and also in crosses between these two types. Three phenotypes controlled by two co-dominant alleles called Tf^B and Tf^C were observed. Three phenotypes with different nomenclatures have been reported in buffalo from various countries. Abe et al. (1969) designated the transferrin alleles as Tf^A and Tf^B in buffalo from Formosa; Zubareva et al. (1969) as Tf^A and Tf^B in buffalo from Russia; Naik et al. (1969) as Tf^A and Tf^B in buffalo from India; Masina et al. (1971) as Tf^D and Tf^E in buffalo from Italy; Granciu et al. (1972) as Tf^B and Tf^C in buffalo from Romania and Nasrat et al. (1976) as Tf^D and Tf^E in buffalo from Egypt. A third allele Tf^N was reported in Nili and Surti breeds of Indian buffalo by Khanna (1973) and Basavaiah et al. (1977). Tandon and Khanna (1983) reported six Tf phenotypes in some Indian breeds of buffalo. A fourth and fastest phenotype Tf^A has been found in swamp buffalo at a very low frequency by Amano et al. (1980). This particular allele of transferrin was also reported recently in buffalo from Bangladesh by Amano et al. (1987). The frequencies of these alleles differ in different breeds of buffalo at different locations (Table 1). There is a lack of standardisation for nomenclatue of transferrins. Standardisation would enable comparisons to be made between results from different laboratories on various breeds of buffalo.

Albumin

Albumin is a major serum protein and is of paramount importance because of its relative abundance, homogeneity, osmotic and transport function (Putnam 1965). The physiological consequences of the ion-binding behaviour of serum albumin and its affinity for dyes, drugs and other molecules have been emphasised by Bennhold (1962). Genetically governed polymorphism of albumin has been reported in various species (Lush 1966).

In Indian river buffalo, Khanna and Braend (1968) reported polymorphism in albumin. Three phenotypes controlled by two co-dominant alleles called Alb^F and Alb^S were demonstrated. Similar findings were reported by Makaveev (1968, 1970) in river buffalo of Bulgarian and Indian origin, by Masina et al. (1971) in Italian river buffalo, by Juneja and Chaudhary (1971) in Indian buffalo, Vlaic et al. (1983) in Romanian buffalo and by Kwan (1973) in Malaysian buffalo. Abe et al. (1969) in Formosan water buffalo and Osterhoff et al. (1970) in African buffalo found only one type of albumin when subjected to starch gel electrophoresis. Most of the breeds of river buffalo have a very high frequency (Table 2) of the slow type (Alb^S). Swamp buffalo lack this phenotype and instead have a variant (Alb^X) of lesser mobility (Amano et al. 1980, 1986 and 1987). A third phenotype slower than Alb^X was also reported in the Murrah by Tandon (1982). Whether this variant and Alb^X are the same or not is not known.

Amylase

Amylases are most important enzymes which catalyse the hydrolysis of the polysaccharides. Their substrates include starch and glycogen. Heterogeneity

Table 1. Gene frequencies of transferrin alleles in buffalo.

Genotype	Location	Number of samples	Tf^A	Tf^D or Tf^B	Tf^K or Tf^E, Tf^C	Tf^N	Reference
Egyptian		—	—	0.367	0.633	—	Nasrat et al. (1976)
Bulgarian		101	—	0.16	0.84	—	Makaveev (1968)
Italian		350	—	0.368	0.632	—	Masina et al. (1971)
Romanian		158	—	0.09	0.91	—	Granciu et al. (1972)
Italian		100	—	0.23	0.77	—	Rondolini et al. (1972)
Bhadawari		91	—	0.06	0.94	—	Khanna (1973)
Marathwada		37	—	0.05	0.95	—	"
Murrah:	Ambala	171	—	0.06	0.94	—	"
	Harianghata	149	—	0.18	0.82	—	"
	Hissar	129	—	0.10	0.90	—	"
	Izatnagar	210	—	0.16	0.84	—	"
	Jhansi	108	—	0.22	0.78	—	"
	Ludhiana	85	—	0.15	0.85	—	"
	Mathura	51	—	0.08	0.92	—	"
	Meerut	108	—	0.12	0.88	—	"
	Pantnagar	118	—	0.26	0.74	—	"
	Visakapatnam	109	—	0.12	0.88	—	"
Nagpuri		75	—	0.05	0.95	—	"
Nili		115	—	0.11	0.87	0.02	"
Pandarpuri		34	—	0.09	0.91	—	"
Surti		139	—	0.11	0.88	0.01	"
Murrah		434	—	0.107	0.889	0.003	Basavaiah et al. (1977)
Murrah-grade		333	—	0.108	0.881	0.001	"
Malaysian		?	—	0.16	0.78	—	Tan et al. (1980)
River		36	—	0.094–0.250	0.750–0.906	—	Amano et al. (1980)
Swamp		146	0.065–0.142	0.850–0.935	0–0.008	—	"
Murrah		542	—	0.146	0.864	—	Shankar & Bhatia (1982)
Murrah		—	—	0.170	0.830	—	Singh et al. (1987)
Lankan: Hambantota		26	—	0.269	0.731	—	Amano et al. (1986)
Minnelia		21	—	0.357	0.643	—	"
Murrah		10	—	0.200	0.800	—	"
Wild		5	—	0.100	0.900	—	"
Italian		303	—	0.358	0.642	—	Iozio & Annunzuata (1986)
Murrah		—	—	0.145	0.855	—	Naik et al. (1969)
Caucasian		261	—	0.280	0.720	—	Zubareva et al. (1969)

in the serum amylase has been reported in many species including livestock. In buffalo, three amylase phenotypes controlled by two co-dominant alleles Am^A and Am^B were reported by Makaveev (1968) and Zubareva et al. (1969). Khanna (1973, 1978) reported five amylase phenotypes governed by three alleles. Homozygous Am^C was not observed. Trehan and Nair (1972) observed two independent loci controlling amylase in buffalo. Bulgarian buffalo have a higher frequency of Am^B than Indian buffalo (Table 3) and it is very rare in Sri Lankan buffalo.

Ceruloplasmin

Ceruloplasmin is a glycoprotein and has oxidase activity. Each molecule binds eight atoms of copper and virtually all of the copper in plasma is so bound. This protein is of great biochemical and clinical interest, as there is a regular variation in the ceruloplasmin level in various diseases and during pregnancy. So far no variation in the electrophoretic band pattern has been revealed. Only a single serum ceruloplasmin band has been observed in various breeds of buffalo (Makaveev 1972, Khanna 1979, Tandon 1982, and Amano et al. 1986).

Carbonic anhydrase

Two co-dominant alleles Ca^F and Ca^S with three phenotypes have been reported at the carbonic anhydrase locus in buffalo, the Ca^F allele being more frequent (Soos et al. 1971, Trehan and Nair 1972, Makaveev 1975). Later Khanna and Tandon

Table 2. Gene frequencies of albumin alleles in buffalo.

Genotype	Location	Number of samples	Alb^F	Alb^S	Alb^X	Reference
Murrah		177	0.21	0.79	—	Juneja and Chaudhary (1971)
Italian		350	0.327	0.673	—	Masina et al. (1971)
Malaysian		39	0.205	0.795	—	Kwan (1973)
Italian		100	0.33	0.67	—	Rondolini et al. (1973)
Bhadawari		91	0.27	0.73	—	Khanna (1973)
Marathwada		37	0.11	0.89	—	"
Murrah:	Ambala	171	0.23	0.77	—	"
	Haringhata	138	0.07	0.93	—	"
	Hissar	129	0.08	0.92	—	"
	Izatnagar	206	0.10	0.90	—	"
	Jhansi	108	0.17	0.83	—	"
	Ludhiana	85	0.04	0.96	—	"
	Mathura	51	0.22	0.78	—	"
	Meerut	108	0.15	0.85	—	"
	Pantnagar	119	0.29	0.71	—	"
	Visakapatnam	109	0.21	0.79	—	"
Nagpuri		75	0.05	0.95	—	"
Nili		115	0.17	0.83	—	"
Pandarpuri		34	0.12	0.88	—	"
Surti		139	0.17	0.83	—	"
Egyptian		544	0.29	0.71	—	Nasrat et al. (1976)
River buffalo		36	0.062–0.125	0.875–0.938	—	Amano et al. (1980)
Swamp buffalo		146	0.035–0.267	—	0.733–0.965	"
Lankan: Hambantota		26	0.481	0.519	—	Amano et al. (1986)
Minnela		21	0.429	0.571	—	"
Murrah		10	0.100	0.900	—	"
Wild		5	0.700	0.300	—	"
?		300	0.35	0.65	—	Vlaic et al. (1983)
Italian		303	0.310	0.690	—	Iozio and Annunzuata (1986)
Murrah		66	0.280	0.720	—	Singh et al. (1987)
Romanian buffalo			0.320	0.680	—	Milovan and Granciu (1974)

(1980) revealed another allele Ca^C with a fast mobility and low frequency. Sri Lankan breeds of buffalo also have this polymorphism with the co-dominant alleles (Amano et al. 1986). However, Malaysian swamp buffalo lack carbonic anhydrase polymorphism (Kasim et al. 1981). Breed differences in distribution of various alleles are not marked (Table 4).

Alkaline phosphatase

The co-dominant alleles, namely Alp^A, Alp^B and Alp^C were described in Bulgarian and Indian × Bulgarian crossbred buffalo (Makaveev 1975). The allele Alp^A was the most frequent. Tandon (1982) reported two alleles in Indian breeds. Most of Sri Lankan and Southeast Asian breeds of buffalo have only one type of alkaline phosphatase isozyme. In these breeds it is the slower band in contrast to the study of Makaveev (1975) where the faster band was found to be prevalent. Iozio and Annunzuata (1986) reported three alleles with 0.505, 0.468 and 0.027 gene frequencies in Italian buffalo.

Cell peptidase B

Variation of cell peptidase B has been studied by Amano et al. (1986). They delineated three genes Pep-$B^{F'}$, Pep-B^F and Pep-B^S with decreasing mobility. Pep-B^F was most frequent in all the three breed groups, namely, Sri Lankan, Hambanlota and wild buffalo. Pep-$B^{F'}$ had a very low frequency. This locus has been found polymorphic in most of the buffalo populations from Southeast Asia, except those from North Sumatra and crossbred animals from Indonesia and the Philippines (Amano et al. 1986). No report on polymorphism in peptidase-B is available from other breeds of buffalo to compare or contrast with the above study.

Table 3. Gene frequencies of amylase alleles in buffalo.

Genotype	Location	Number of samples	Amy^C	Amy^A	Amy^B	Reference
Bulgarian		101	—	0.72	0.28	Makaveev (1968)
Murrah		22	—	0.90	0.10	"
Bhadawari		91	—	0.93	0.07	Khanna (1973)
Marathwada		37	—	1.00	—	"
Murrah:	Ambala	171	—	1.00	—	"
	Haringhata	126	0.03	0.85	0.12	"
	Hissar	129	0.01	0.98	0.01	"
	Izatnagar	134	—	0.93	0.07	"
	Jhansi	108	—	0.96	0.04	"
	Ludhiana	85	—	0.97	0.03	"
	Meerut	108	—	0.95	0.05	"
	Pantnagar	78	—	0.96	0.04	"
	Visakapatnam	109	—	0.97	0.03	"
Nagpuri		75	—	0.98	0.02	"
Nili		115	0.02	0.95	0.03	"
Pandarpuri		34	—	0.96	0.04	"
Surti		139	0.01	0.94	0.05	"
Malaysian swamp		?	0.09	0.82	0.09	Tan et al. (1980)
Lankan Hambantota		26	—	1.00	—	Amano et al. (1986)
Minnelia		21	—	0.952	0.048	"
Murrah		10	—	1.00	—	"
Wild		5	—	1.00	—	"

Table 4. Gene frequencies of carbonic anhydrase alleles in buffalo.

Genotype	Number of samples	Ca^C	Ca^F	Ca^S	Reference
Bulgarian	135	—	0.956	0.044	Makaveev (1975)
Murrah × Bulgarian	47	—	0.904	0.906	"
Murrah	90	—	0.844	0.156	Khanna & Tandon (1980)
Murrah	104	0.010	0.899	0.096	"
Malaysian swamp	77		No polymorphism		Kasim et al. (1981)
Lankan: Hambantota	26	—	0.904	0.906	Amano et al. (1986)
Minnelia	21	—	0.905	0.095	"
Murrah	10	—	0.850	0.150	"
Wild	5	—	0.900	0.100	"
Italian	303	—	0.900	0.100	Iozio & Annunzuata (1986)

Erythrocyte potassium

Potassium concentration in erythrocytes of buffalo is controlled by two alleles, K^{HK} and K^{LK} (Sengupta 1975 and George and Balakrishnan 1985). The range of means of K^+ in red cells was 37–41 mEq/L in low potassium (LK) and 87–91 mEq/L in high potassium (HK) type animals (Sengupta 1975). The K^{HK} allele appeared to be recessive to K^{LK}, however; it appeared that dominance of K^{LK} may not be complete.

Other blood polymorphisms

Serum acid phosphatase and lactic dehydrogenase polymorphisms were demonstrated by Makaveev (1972). Esterase D was reported to be controlled by two alleles by Tan et al. (1980). Malate dehydrogenase, acid phosphatase, glucose-6-phosphate dehydrogenase, 6-phospho-gluconate dehydrogenase, phosphohexose isomerase and phosphoglucomutase have been shown to be polymorphic with two alleles each in Indian buffalo by Tandon

(1982). He could not find any polymorphism in lactate dehydrogenase and esterases. Gazia and Agergaard (1982) described pre-albumin and adenosine deaminase polymorphisms. Glyoxalase in Malaysian buffalo is controlled by two alleles GLO^F and GLO^S with gene frequencies of 0.14 and 0.86, repectively (Kasim et al. 1981). A large number of swamp and river buffalo breeds from Sri Lanka and Southeast Asian countries lacked polymorphism at serveral loci including serum amylase-II, cell tetrazolium oxidase, serum-X protein, cell esterase-D, cell esterase-I, cell esterse-II, acid phosphatase, cell lactate dehydrogenase-A, cell lactate dehydrogenase-B, cell 6-phosphogluconate dehydrogenase, serum slow alpha-2 macroglobulin, cell phosphohexose isomerase and cell adenylate kinase (Amano et al. 1986).

Serologically detectable allotypes A1 and A2 at a multi-allelic locus were defined by Iannelli (1978) in buffalo. The third allele at this locus was responsible for the absence of both A1 and A2 specificities. Later two more allotype systems B and C characterised by one marker each were described in these buffalo (Iannelli and Capparelli 1981).

Polymorphism of vitamin D3 binding protein in Italian buffalo is controlled by three alleles namely A, B and C. The frequencies of these alleles were 0.414, 0.44 and 0.146, respectively (Masina et al. 1978).

Glucose-6-phosphate dehydrogenase has been found to be polymorphic in Italian buffalo. The respective allelic frequencies of faster and slower alleles were 0.52 and 0.48 (Iozio and Annunzuata 1986).

Milk protein polymorphisms

Milk protein polymorphisms in farm animals has been reported for lactoglobulin, beta-lactoglobulin and casein, but studies of buffalo have been very limited. No polymorphism has been found in beta-lactoglobulin of buffalo milk (Sen and Sinha 1961, Ganguli and Majunder 1968, Chiofalo et al. 1985). Bhattacharya et al. (1963) and Chiofalo et al. (1985) did not find genetic polymorphism in alpha-lactalbumin of buffalo.

Tandon (1982) observed polymorphism in alpha-casein controlled by two alleles, the slower one with a very low frequency. Two phenotypes of beta-casein have been found (Aschaffenberg et al. 1968, Tandon 1982). No polymorphism was detected in K-casein by various workers (Aschaffenberg 1968; Ganguli and Mazumder 1968) although both components of K-casein (AB) were found in buffalo.

Conclusion

One may wonder what is the purpose of studying biochemical polymorphisms. Is there any practical application for it? Dr R.W. Robertson (pers. comm.) warned that 'this activity [of recording variations in proteins] unless undertaken to answer specific questions in a precise and quantitative fashion, seems to have the same intellectual content as stamp collecting without asthetic appeal'. Those were the days (early 1970s) of peak quantitative approach to genetics. But with the advent of cytogenetics, clinical genetics, molecular techniques such as gene isolation, cloning and gene transfer, recombinant DNA methods and restriction enzyme usage, the same 'stamp collection' has resulted in a storehouse of basic material with which to work. Chromosome mapping, identification of markers linked with other important genes (those which have physiological, clinical, biochemical activity), prenatal diagnosis, etc. have become possible, at least in man where over 5000 such 'stamps' have been collected. In farm animals, particularly in buffalo, the progress in this area is abysmally slow. Work on buffalo is minimal because it is confined mostly to the Southeast Asian region of the Indian sub-continent where inputs for basic research are small. However, the importance of the buffalo as a major dairy-draught-meat animal is unquestionable, at least in this region. Serious efforts should be made to use most modern molecular technologies for studying genetic variation in buffalo.

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Electrophoretic Studies on Southeast Asian Buffalo and Goats: Methodology

S.G. Tan,[1] O.S. Selvaraj,[2] J. Roslin,[1] O. Aklima,[1] Y.Y. Gan,[1] T.K. Mukherjee[1] and J.S.F. Barker[3]

Abstract

In order to determine the genetic relationships among Southeast Asian populations of swamp buffalo (*Bubalus bubalis*) and Katjang goats (*Capra hircus*), we have attempted to develop methodologies for typing as many electrophoretic biochemical markers as possible from the blood of these two species. The main technique used was cellulose acetate electrophoresis (CAE), with polyacrylamide, starch and agarose gel electrophoresis (in that sequence) used as backup techniques where satisfactory bands or variants could not be detected for any particular marker on CAE.

Blood was separated into red cell, white cell and plasma, which were analysed independently. Following this strategy, we are now able to type for 56 electrophoretic biochemical markers from buffalo blood. Of these, 30 had not previously been reported as being electrophoretically typed from buffalo blood. Similarly, for goats we have now developed electrophoretic methodologies for typing 48 blood biochemical markers of which 25 had not been reported before.

THE Swamp water buffalo, *Bubalus bubalis* is indigenous to Southeast Asia, Southeast China and Assam in India (Mason 1974). Native Katjang goat (*Capra hircus*) populations of Southeast Asia are essentially unstudied and have been neglected from both the research as well as the development aspects. Little is known about the population genetics of Southeast Asian buffalo and goats. However, before effective crossbreeding and selection programs can be planned and launched for each species, knowledge about the genetic relationships among different populations of Swamp buffalo and of Katjang goats from various localities in Southeast Asia is desirable.

Electrophoresis provides a means of estimating gene frequencies for polymorphic markers that can be used to define different breeds and strains of organisms as well as their relationships to one another. However, little electrophoretic data are available on Southeast Asian buffalo and goats.

From the literature, a total of 27 blood biochemical markers of water buffalo (river and swamp) are demonstrable on starch gel electrophoresis (STAGE). Of these, 16 are polymorphic in one or more populations, namely albumin, transferrin, alkaline phosphatase, group-specific component (Gc), haemoglobin, esterase D, carbonic anhydrase, peptidase B, glyoxalase I, malate dehydrogenase, catalase, NADH diaphorase, prealbumin, adenosine deaminase and glutamate oxaloacetate transaminase (Tan et al. 1980, Amano et al. 1986). Of these, only variants for five systems (amylase, albumin, transferrin, Gc and haemoglobin) have been subjected to family studies and therefore are confirmed as being under Mendelian genetic control.

For goats, 26 biochemical markers have been reported (Tuñón et al. 1989, Shotake et al. 1986) on STAGE of which 13 are polymorphic; viz. albumin, amylase, nucleoside phosphorylase, alkaline phosphatase, transferrin, haemopexin, haemoglobin, esterase, leucine aminopeptidase, prealbumin, X-protein, diaphorase and esterase D. Family studies have been done only for transferrin, albumin, alkaline phosphatase, X-protein, diaphorase and haemoglobin.

Therefore, given the limited numbers of polymorphic biochemical markers reported for these two species and bearing in mind Nei's (1978) statement that the accuracy of any genetic distance estimate is

[1] Department of Biology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2] Institute for Advanced Studies, University of Malaya, 59100 Kuala Lumpur, Malaysia
[3] Department of Animal Science, University of New England, Armidale, NSW 2351, Australia

increased if the number of genetic markers used in its estimation is large, we have decided to develop methodologies for typing as many biochemical markers as possible. Blood is used as the source of biochemical markers because it is the most easily obtainable tissue for population studies of these two species. Cellulose acetate electrophoresis (CAE) is our main electrophoretic technique with polyacrylamide gel electrophoresis (PAGE), STAGE and agarose gel electrophoresis (AGE) as the back-up media for biochemical markers that cannot be satisfactorily resolved on CAE. Since buffalo and goat blood enzymes had hardly been typed on CAE, PAGE and AGE before, all the methods presented for these media have been developed by us. The STAGE procedures used were from the literature.

Materials and Methods

Tan et al. (1990) have described the procedures used in blood collection and processing and CAE analysis for 38 buffalo blood enzymes, while Selvaraj et al. (1990) have described the procedure for typing albumin. Similar procedures were used for goats except that 20ml of whole blood was drawn from each animal. We have now changed our recommended buffers for typing buffalo ME, MDH and NADH diaphorase to 0.02M citrate-phosphate pH 6.4 and the voltage used to 250V.

The horizontal PAGE technique used was as in Tan and Teng (1979). The gradient PAGE for typing goat post albumin and post transferrin was as in Gahne et al. (1977) except that the gel buffer was 0.1875M tris-borate pH 8.6, the electrode buffer was 0.065M tris-borate pH 8.6, the 14% gel layer was omitted and Whatman 17 was used as the sample insert. The STAGE used for buffalo amylase typing by affinity electrophoresis and maltase staining was as in Archibald (1981) using a 0.3M boric acid — 0.06M sodium hydroxide pH 8.0 (Khanna 1978) electrode buffer and 12% starch gel made with a gel buffer containing 0.014M tris-citrate pH 7.3 and 0.005M calcium chloride (Mazumder and Spooner 1970). The AGE used for typing goat amylase was as in Kompf et al. (1979) with Sigma A-6877 medium EEO agarose as medium and Whatman 17 as the sample insert. X-protein for goats was typed on STAGE following the procedure of Tucker et al. (1967).

The methodology used for typing other goat and buffalo biochemical markers on PAGE and CAE are as follows:

PAGE

Seven percent gel was used unless otherwise specified. Inserts were removed after running for 1hr and the run was resumed. Bromophenol-blue was used as the tracker dye.

1. Adenosine deaminase (Ada)
   Goat and buffalo plasma and RBC.
   Electrode buffer: 0.1M potassium phosphate pH 6.5.
   Gel buffer: 1 in 10 dilution of electrode buffer.
   Insert: Whatman 3 for goat red blood cells (RBC), Whatman 17 for buffalo plasma, goat plasma and buffalo RBC.
   Run: 200V, 4hr.
   Staining: As in Harris and Hopkinson (1976).
   1 band per sample. Since for both buffalo and goats, the RBC and plasma bands looked similar, we recommend that buffalo plasma and goat RBC be used for typing since they gave sharper bands and the staining time was shorter.

2. Catalase (Cat)
   Goat and buffalo RBC.
   Stock solution: 0.9M tris, 0.5M boric acid, 0.02M disodium EDTA pH 8.6.
   Electrode buffer: Dilute stock solution 1 in 7.
   Gel buffer: Dilute stock solution 1 in 10.
   Insert: Whatman 1
   Run: 300V, 7hr.
   Staining: As in Harris and Hopkinson (1976) using the staining procedure which did not require the incorporation of starch into the gel.
   1 band per sample.

3. Ceruloplasmin (Cp)
   Goat and buffalo plasma.
   Electrode buffer: 0.1M sodium hydroxide, 0.286M boric acid pH 7.6.
   Gel buffer: 0.01M tris, 0.0046M citric acid pH7.6.
   Insert: Whatman 17.
   Run 350V, 4hr.
   Staining: Stain gel in 50ml solution containing 0.625g sodium acetate trihydrate, 0.035ml glacial acetic acid pH 5.0 and 50mg O-dianisidine hydrochloride. Homozygotes showed 1 band and heterozygotes 2 bands per sample.

4. Erythrocyte Protein (EP)
   Goat and buffalo RBC.
   Electrode buffer: 0.05M tris-glycine pH 8.9.
   Gel buffer: 0.375M tris-HCl pH 8.9
   Gel: 7% containing 5% glycerol
   Insert: Whatman 3
   Run: 500V for 2hr. Remove inserts. Then run for another 5 hr at 800V.
   Staining: As for transferrin.
   Homozygotes showed 1 fast or slow band while heterozygotes showed 2 bands.

5. Esterase (Est)
Goat and buffalo plasma and RBC.
Electrode buffer.
Anode: 0.06M citric acid, 0.088M disodium hydrogen phosphate pH 4.5.
Cathode: 0.143M boric acid, 0.075M sodium hydroxide pH 9.0.
Gel buffer: 0.013M tris-citrate pH 4.8.
Gel: 7% containing 0.1% Kodak Photoflo-600.
Insert: Whatman 3
Run 300V, 3hr.
Staining: As in Steiner and Joslyn (1979).
For both buffalo and goats, the mobilities of the RBC and plasma esterases differ. Two zones of black alpha esterase and 1 zone of red beta esterase were observed in both plasma and RBC of both species. The migration rates of the plasma and RBC esterases differ from one another.

6. Glyoxalase I (Glo I)
(i) Buffalo RBC
Electrode buffer: 0.125M tris, 0.075M histidine hydrochloride pH 7.8.
Gel buffer: 1 in 20 dilution of electrode buffer.
Gel: 10% containing 0.3% soluble starch
Insert: Whatman 3
Run: 200V, 4hr
Staining: As in Taggart et al. (1978).
Homozygotes showed one fast or slow band and heterozygotes a broad band which overlapped the fast and slow bands.

(ii) Goat RBC
Electrode buffer: 0.026M barbitone sodium adjusted to pH 7.0 with trizma hydrochloride.
Gel buffer: 0.071M tris-HCl pH 7.5.
Gel: 10% containing 0.3% soluble starch.
Insert: Whatman 17.
Run: 150V, 4hr.
Staining: As for buffalo.
1 band per sample.

7. Glutathione peroxidase (Gpx)
Goat and buffalo RBC.
Electrode buffer: 0.2M disodium hydrogen phosphate adjusted to pH 7.5 with citric acid.
Gel buffer: 1 in 15 dilution of electrode buffer.
Gel: 5% containing 1% Kodak Photoflo-600 and 0.4% soluble starch.
Insert: Whatman 1
Run: 300 V, 5 hr, cathodal run.
Staining: The positive procedure of Agar and Board (1984) was used.
1 band per sample.

8. Inorganic pyrophosphatase (Pp)
Goat RBC
Electrode buffer: 0.1M tris, 0.1M maleic acid, 0.01M disodium EDTA, 0.01M magnesium chloride adjusted to pH 7.2 with 1M sodium hydroxide.
Gel buffer: 1 in 10 dilution of electrode buffer.
Gel: 10% PAGE containing 0.1% Kodak Photoflo-600.
Insert: Whatman 1.
Run: 200V, 5hr.
Staining: As in Harris and Hopkinson (1976)
1 band per sample.

9 Inosine triphosphatase (Itp)
Goat and buffalo RBC.
Electrode buffer: 0.44M boric acid 0.04M lithium hydroxide pH 7.2
Gel buffer: 1 in 10 dilution of electrode buffer.
Gel: 6%
Insert: Whatman 17
Staining: As in Harris and Hopkinson (1976)
1 band per sample.

10. Leucine aminopeptidase (Lap)
Goat and buffalo plasma.
Electrode and gel buffer: 0.1M tris-borate, 0.005M magnesium chloride pH 8.9.
Gel: 10%.
Insert: Whatman 17.
Run: 200V for 1 hr. Remove insert. Then 300V for 4 hr.
Staining: As in Randi et al. (1986).
1 band per sample for buffalo. 2 zones of activity in goat. The more anodal zone showed one band per sample while for the less anodal zone, homozygotes showed 1 fast or slow band and heterozygotes showed 2 bands.

11. Peptidase D (Pep D)
For goat and buffalo RBC and plasma.
Electrode buffer: 0.1M tris, 0.1M sodium dihydrogen phosphate pH 7.4.
Gel buffer: 1 in 20 dilution of electrode buffer.
Gel: 5% containing 0.1% Kodak Photoflo-600 for buffalo RBC and 7% for goat RBC and buffalo and goat plasma.
Insert: Whatman 3.
Run: 300V, 4hr.
Staining: As in Harris and Hopkinson (1976) except that we dissolved 0.1g 3-amino-9-ethyl carbazole in 2.5 ml N, N-dimethyformamide before adding it to the staining mixture.
1 band per sample. The plasma band migrated less anodally than the RBC band.

12. Phosphoglycolate phosphatase (Pgp)
Goat and buffalo RBC.
Electrode buffer: 0.1M tris, 0.1M maleic acid 0.01M disodium EDTA, 0.01M magnesium chloride.
Adjust to pH 7.2 with 1M sodium hydroxide (about 120ml needed).
Gel buffer: 1 in 10 dilution of electrode buffer.
Gel: 9% containing 0.1% Kodak Photoflo-600 for buffalo and 8% containing 0.1% Kodak Photoflo-600 for goat.
Insert: Whatman 17.
Run: 200V, 5hr.
1 band per sample.

13. Transferrin (Tf)
Buffalo plasma.
Electrode and gel buffers: As for Esterase
Gel: 10%.
Insert: Whatman 3.
Run: 300V, 3hr.
Staining: 0.1% Coomassie Brilliant Blue R250 in 33% methanol and 3% sulfosalicylic acid. After bands appear, destain in a mixture of 8% acetic acid, 25% methanol and 67% water (Hoste, 1979).
Homozygotes had three bands while heterozygotes showed four bands.

CAE

Buffalo. The methodologies for the following buffalo RBC enzymes were not presented in Tan et al. (1990).

1. Glycerol-3-phosphate dehydrogenase (alpha-Gpd).
0.1M tris-maleate-$MgCl_2$-EDTA pH 7.4.
6 applications. Run 1 hr at 200 V. 1 band per sample.

2. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh).
0.13M tris-EDTA-borate pH 8.9. 5 applications. 1.5 hr, 100 V. 1 band per sample.

Goat. The methodologies for typing the following 20 blood enzymes were similar to those for buffalo as presented in this paper and in Tan et al. (1990), viz. adenylate kinase, esterase D, glucose-6-phosphate dehydrogenase, alanine aminotransferase, glutamate oxaloacetate transaminase, hexokinase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, mannose phosphate isomerase, petidase A, B and C, phosphoglucomutase, 6-phosphogluconate dehydrogenase, nucleoside phosphorylase, pyruvate kinase, superoxide dismutase, triose phosphate isomerase and uridine monophosphate kinase. The buffers used for the following four enzymes were similar to those described for buffalo (Tan et al. 1990), except that Kodak Photoflo-600 was added to them for typing the goat enzymes, viz. red cell acid phosphatase, fructokinase, fumarase and glucose dehydrogenase. In goats although all samples showed 1 band per sample for nucleoside phosphorylase, some animals had bands with high activity and others with low activity as typed visually on the gel. This observation has been confirmed through spectrophotometric studies (Sekaran et al. 1989).

The methodology for the following goat enzymes differed from those for buffalo. All samples were RBC, run at 200V for 1hr and staining as in Richardson et al. (1986) unless otherwise stated.

1. Amylase (Amy)
Gelman's high resolution buffer (Product No. 51104), 0.06M tris-barbital-sodium barbital pH 8.8. 4 applications of plasma or RBC. 210V. Staining as in Archibald (1981). 2 zones per sample for both RBC and plasma. The less anodal zone was diffused. Score only the more anodal zone which showed 1 band per sample with high and low activity variation.

2. Alkaline phosphatase (Alp)
Gelman's high resolution buffer pH 8.8. 5 applications of plasma. 1 fast or slow or a broad band which overlapped the fast and slow bands or no band per sample.

3. Albumin (Alb)
Gelman's high resolution buffer pH 8.8. 1 application of plasma. 1 fast or slow or a broad band which overlapped the fast and slow bands per sample.

4. Carbonic anhydrase (Ca)
0.2M citrate-phosphate pH 6.4. 2 applications. Staining as in Tan et al. (1990). 1, 2 or no band per sample.

5. NADH Diaphorase (NADH-Dia) zones 1 and 2
0.02 citratephosphate pH 6.4 with Kodak Photoflo-600. 4 applications. 2 zones per sample, the less anodal zone 1 and the more anodal zone 2. For both zones homozygotes showed 1 fast or slow band and heterozygotes 2 bands.

6. Fructose-1, 6-diphosphatase (Fdp)
0.1M citrate-phosphate pH 6.4 with Kodak Photoflo-600. 6 applications. 90 minutes. 1 band per sample.

7. Glucose phosphate isomerase (Gpi)
0.02M citrate-phosphate pH 6.4 with Kodak Photoflo-600. 6 applications. 90 minutes. 3 bands per sample.

8. Haemoglobin (Hb)
   Gelman's haemoglobin buffer (Product No. 51126), 0.342M tris-glycine-EDTA pH 9.2. 1 application of diluted red cells (1 part of RBC to 4 parts of water). 210V. 1 fast or slow band for homozygotes and 2 bands for heterozygotes.
9. Lactate dehyrogenase (Ldh)
   0.02 citrate-phosphate pH 6.4 with Kodak Photoflo-600. 1 application. 70 minutes. 3 zones per sample of which only the most anodal is scorable.
10. Transferrin (Tf)
    Gelman's haemoglobin buffer pH 9.2. 2 applications of plasma. 210V. 1 fast or slow band for homozygotes and 2 bands for heterozygotes.

Results and Discussion

By using CAE, PAGE, STAGE and AGE we are now able to type for 56 electrophoretic blood markers in buffalo of which 30 have not previously been reported in the literature. Similarly, in goats we can now type for 48 markers of which 25 had not been reported before. In addition, we have also detected but have not yet found the optimal buffer systems for several other blood enzymes such as cytidine deaminase, mitochondrial-isocitrate dehydrogenase, mitochondrial-malate dehydrogenase, guanylate kinase and prealbumin in both species and post-albumin and post-transferrin in buffalo. Work will be continued to try and develop methodologies for typing these and other biochemical markers.

Our aim is to try and develop methodologies for as many blood electrophoretic biochemical markers as possible in these two species, because only about 30% of these (Harris 1980) are expected to be polymorphic and therefore of value in generating gene frequency data to be used in computing genetic distances. Using blood samples from animals with known pedigree, these methodologies are now being used to firmly establish the Mendelian genetic basis of those polymorphic markers for which family studies have not been done before. They are also being used to analyse population samples which we have collected from Australia (buffalo only), Malaysia, Indonesia, Philippines and Thailand to establish their genetic relationships. Collection of samples from additional localities is also planned.

In future we hope to use the more sensitive technique of polyacrylamide gel isoelectric focusing to supplement our current electrophoretic techniques, as well as to refine our analyses further by using molecular markers such as DNA restriction fragment length polymorphisms. The blood samples that we have collected and the methodologies that we have developed will make this the most extensive biochemical genetic study on Swamp buffalo and Katjang goats, both in terms of the numbers of animals analysed and in the numbers of biochemical markers typed. It would also allow a comparative study of the genetic variation present within and genetic distance between populations, based on both electrophoretic loci and molecular markers if this project were to be extended to include the use of DNA markers in future. More populations and genetic markers can be incorporated into our project data as these become available.

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Genetic Relationships among Populations of Swamp Buffalo in Southeast Asia

T.K. Mukherjee,[1] J.S.F. Baker,[2] S.G. Tan,[3] O.S. Selvaraj[1], J.M. Panandam,[1] Y. Yushayati[1] and Sreetharan[1]

Abstract

Biochemical polymorphisms of swamp buffalo are being studied, primarily to estimate genetic distances among various populations in Southeast Asia and the Northern Territory, Australia. This preliminary report presents data for genetic variation in and genetic distances among ten populations in four countries (Indonesia, Philippines, Thailand and Malaysia).

Of the 26 loci assayed to date using one of three electrophoretic techniques (cellulose acetate, starch gel or polyacrylamide), 10 were polymorphic and 16 monomorphic. Allele frequencies showed substantial differences among populations, particularly for carbonic anhydrase and malic enzyme, and these loci, together with malate dehydrogenase, had significant observed heterozygote deficiences in the majority of populations. Over all loci, the mean F_{ST} indicated significant genetic differentiation among the 10 populations.

Further analysis of population genetic differentiation, using the Nei genetic distance, showed that the distances among these populations are within the range exhibited for distances among breeds of European domestic livestock. There was essentially no relationship between genetic distances and geographic distances among populations, and we conclude that these swamp buffalo populations have been largely isolated from one another, and have differentiated genetically due to genetic drift.

As noted by Barker et al. (these Proceedings), our aim is to provide a comprehensive analysis of biochemical genetic variation, using at least 70 loci, in populations from different regions of four countries of Southeast Asia and from Australia. When the genotypes at a particular locus are determined for all individuals sampled from each population, the allele frequencies at that locus in each population can be estimated. The genetic relationship among any two populations is a function of differences between them in allele frequencies, with this relationship usually expressed in terms of a genetic distance. For example, if the two populations are homozygous for different alleles at a particular locus, the distance is the maximum possible, while if the allele frequencies in the two populations are identical, the distance is zero. The estimated genetic distances may vary among loci, and the most accurate measures of relationship will be obtained by averaging over many loci.

[1] Institute for Adanced Studies, University of Malaya, 59100 Kuala Lumpur, Malaysia
[2] Department of Animal Science, University of New England, Armidale, NSW 2351, Australia
[3] Department of Biology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Genetic relationships among breeds of a number of livestock species have been investigated using biochemical polymorphisms, where the main objective has been study of the origin and phylogeny of the various breeds. However, a variety of genetic distance (or genetic similarity) measures have been used in these studies, so direct comparisons are not possible. Even where the same measure is used, caution is necessary in comparing different studies where different loci are included. This is particularly important where only a few polymorphic loci are studied, as has been true for a number of investigations of breed and strain relationships.

The standard genetic distance of Nei (1972) has been used extensively in studies of evolutionary genetics of natural populations and in some livestock studies. The genetic distances among seven Spanish native cattle breeds ranged from 0.007 to 0.180 (Gonzalez et al. 1987). Van Zeveren et al. (1990) estimated distances of 0.0693–0.1030 among four pig breeds of Europe, and Zanotti Casati et al. (1990) of 0.0124–0.0599 among five Italian native sheep breeds. These values are generally within the range of 0.00–0.05 observed for local races of many species from insects to man (Nei 1987).

For swamp buffalo, few data are available. Amano and his colleagues have done a number of studies of morphological, blood group and biochemical variation in Asian livestock species, including buffalo (Amano et al. 1982, 1984, and references therein). However, their results for biochemical polymorphisms have been based generally on relatively small numbers of animals sampled from each locality (2 to about 25) and few polymorphic loci. Results for each country have been reported in separate papers, but Amano (1983) has summarised relationships among populations from different countries, based on eight polymorphic loci. Two separate samples are reported from the Philippines and Indonesia, but it is not specified whether each of these, and the single samples from other countries, represent animals from just one locality, or are pooled samples from a number of localities. The only other studies of biochemical polymorphism in swamp buffalo that we are aware of (Tan et al. 1980, Kasim et al. 1981) reported on enzyme and other protein loci in Malaysian swamp buffalo.

In this preliminary report, we present data for genetic variation at 26 loci and genetic distances among 10 populations, i.e. for those locus–population combinations for which assays have been completed. Assays of additional loci from these populations, and for all loci for samples from the Northern Territory (Australia), another locality in Thailand, and further samples from Malaysia are still in progress. Collections from Sri Lanka have not yet been done.

Materials and Methods

A total of 502 blood samples from swamp buffalo (Table 1) were collected from three geographically separated areas in each of Indonesia, Thailand and Malaysia and from one locality in the Philippines (Fig. 1). An outbreak of Foot and Mouth disease in two of the three originally selected regions in the Philippines prevented collection and importation of blood samples to the laboratory in Malaysia.

Methods of collection of whole blood, separation into plasma, red blood cells and white blood cells, and assay methods using starch, cellulose acetate and polyacrylamide electrophoresis for 56 genetic loci have been described by Tan et al. (1990 and these Proceedings). The 26 genetic loci reported here are listed in Table 2.

Table 1. Collection localities and numbers of animals sampled at each.

Country	Locality	No. sampled
Indonesia	Bogor	50
	Medan	50
	Ujung Pandang	54
Philippines	Musuan	57
Thailand	Cheng Mai	25
	Haadyai	30
	Surin	85
Malaysia	Sabah	50
	Sarawak	50
	Terengganu	51

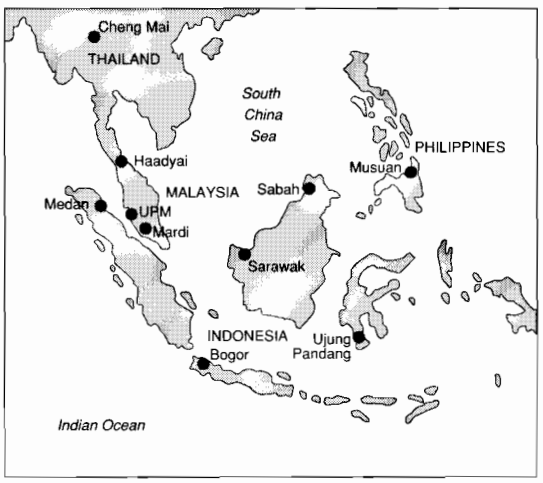

Fig. 1. Geographical localities at which populations were sampled.

Allele frequencies at all loci except for Ca and Est-D were estimated by the direct count method. For Ca and Est-D, null homozygotes were identified in a number of populations. As null heterozygotes would be scored as homozygotes for the other alleles present, an iterative search procedure was used to determine simultaneously the allele frequencies and the inbreeding coefficient that best fitted the observed phenotype frequencies. These allele frequencies and inbreeding coefficient then were used to estimate all genotype frequencies (including null heterozygotes) for the analyses of genetic variation in each population, differentiation among populations and genetic distances.

Genetic variation in each population was measured as the percentage of loci that were polymorphic and mean heterozygosity. Population structure and differentiation among populations were analysed using F-statistics. Genetic distances among populations were estimated using Nei's (1972) standard genetic distance and a dendrogram to depict relationships among the populations was produced using the unweighted pair group method with arithmetic

means (Sneath and Sokal 1973). Most analyses were done with the BIOSYS computer package (Swofford and Selander 1989), as recently modified (W.C. Black, pers. comm. 1990).

Table 2. Blood protein loci examined using electrophoretic techniques.

Locus symbol	Name of blood protein	Method of electrophoresis
Ak	Adenylate kinase	CAE
Alb	Albumin	CAE
Amy	Amylase	STAGE
Ca	Carbonic anhydrase	CAE
Dia_1-2	NADH Diaphorase Zone 2	CAE
Dia_1-3	NADH Diaphorase Zone 3	CAE
Est-D	Esterase D	CAE
G-Est	General Esterase	CAE
Fk	Fructokinase	CAE
Got	Glutamate oxaloacetate transaminase	CAE
G6pd	Glucose-6-phosphate dehydrogenase	CAE
Gpi	Glucose phosphate isomerase	CAE
Hk	Hexokinase	CAE
Idh	Isocitrate dehydrogenase	CAE
Ldh	Lactate dehydrogenase	CAE
Mdh	Malate dehydrogenase	CAE
Me	Malic enzyme	CAE
Mpi	Mannose phosphate isomerase	CAE
Np	Purine nucleoside phosphorylase	CAE
Pep-B	Peptidase-B	CAE
Pep-C	Peptidase-C	CAE
6Pgdh	6-Phosphogluconate dehydrogenase	CAE
Pgm	Phosphoglucomutase	CAE
Sod	Superoxide dismutase	CAE
Tf	Transferrin	PAGE
XP	X-Protein	STAGE

CAE — Cellulose acetate electrophoresis
STAGE — Starch gel electrophoresis
PAGE — Polyacrylamide gel electrophoresis

Results

Of the 26 loci studied, 10 (viz. Alb, Amy, Ca, Dia_1-3, Est-D, Mdh, Me, Pep-B, Pep-C and Tf) were polymorphic in at least seven of the 10 populations. The electrophoretic phenotypes for these polymorphic loci are shown in Figure 2, and further descriptions of relative band positions and staining intensity have been given by Selvaraj et al. and Tan et al. (these Proceedings). Loci were classed as monomorphic only after scoring at least 25 samples from each population.

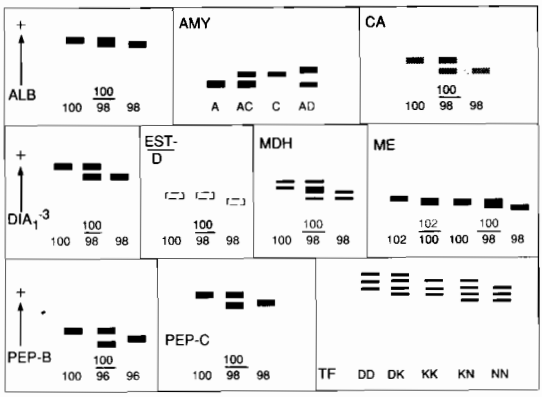

Fig. 2. Schematic representations of the electophoretic banding patterns for the polymorphic protein loci. For each locus, the x-axis shows alleles and the y-axis the distance run.

Genetic variability within populations

Allele frequencies for the polymorphic loci (Table 3) show substantial differences among populations, particularly for Ca and Me, and to a lesser extent for Dia_1-3 and Mdh. Only for Alb, Amy, Pep-B, Pep-C and Tf is the same allele the most common in all populations.

The percentage of loci that are polymorphic ranges from 23.1 in Sabah to 38.5 in a number of populations (Table 4). Observed heterozygosity (Table 4) is lowest for Sarawak (0.069) and highest for Haadyai (0.164), but apart from these extremes, is not significantly different among populations. For all populations except Haadyai, observed heterozygosity is less than expected. As more loci are assayed, these measures of genetic variability are likely to decrease, since loci known to be polymorphic from previous studies have been included here.

Hardy–Weinberg equilibria

The overall trend of an observed deficiency of heterozygotes (Table 4) may be analyzed in more detail by testing the significance of deviations of the observed genotype frequencies from those expected under Hardy–Weinberg equilibrium. These tests, for each locus in each population, are summarised in Table 5. Three loci (Ca, Mdh and Me), and to a lesser extent Est-D, show consistent observed deficiences of heterozygotes, while Pep-C shows a significant observed excess of heterozygotes in six populations, and an excess ($P < 0.10$) in three other populations. For Ca and Est-D, where null alleles were detected,

Table 3. Numbers of animals assayed (N) and allele frequencies for 10 polymorphic loci.

		Indonesia			Philippines	Thailand			Malaysia		
Locus	Allele*	Bogor	Medan	U.P.[1]	Musuan	C'Mai[2]	Haadyai	Surin	Sabah	Sarawak	T'ganu[3]
Alb											
(N)	—	50	50	53	47	25	29	50	50	50	51
	100	.980	.710	.698	.840	.620	.500	.600	.810	.860	.794
	98	.020	.290	.302	.160	.380	.500	.400	.190	.140	.206
Amy											
(N)	—	50	50	52	47	25	30	50	48	49	51
	A	.900	.980	.981	.979	1.000	.633	.900	1.000	1.000	.980
	C	.100	.020	.019	.021	.000	.367	.090	.000	.000	.020
	D	.000	.000	.000	.000	.000	.000	.010	.000	.000	.000
Ca											
(N)	—	49	50	53	47	24	29	50	50	50	51
	100	.092	.760	.670	.819	.083	.207	.500	.650	.660	.029
	98	.908	.240	.151	.181	.875	.793	.390	.120	.060	.941
	null	.000	.000	.179	.000	.042	.000	.110	.230	.280	.029
Dia_1-3											
(N)	—	50	50	51	47	25	30	50	50	50	51
	104	.470	.420	.520	.372	.360	.400	.330	.000	.000	.500
	100	.530	.580	.480	.628	.640	.600	.670	1.000	1.000	.500
Est-D											
(N)	—	45	50	49	47	21	30	50	50	50	51
	100	.678	.610	.765	.862	.857	.633	.600	.450	.470	.912
	98	.167	.090	.204	.138	.143	.117	.220	.090	.340	.000
	null	.156	.300	.031	.000	.000	.250	.180	.460	.190	.088
Mdh											
(N)	—	50	50	54	47	23	28	48	50	50	51
	100	.440	.390	.528	.681	.870	.857	.865	.790	.960	.716
	98	.560	.610	.472	.319	.130	.143	.135	.210	.040	.284
Me											
(N)	—	49	50	54	47	25	27	50	50	50	51
	102	.173	.210	.083	.223	.120	.074	.220	.000	.000	.451
	100	.755	.610	.731	.702	.860	.574	.640	.000	.960	.451
	98	.071	.180	.185	.074	.020	.352	.140	1.000	.040	.098
Pep-B											
(N)	—	48	50	54	47	24	29	50	50	50	51
	100	.615	.760	.741	.755	.813	.638	.770	.880	.750	.775
	96	.385	.240	.259	.245	.188	.362	.230	.120	.250	.225
Pep-C											
(N)	—	48	50	54	47	24	29	50	50	50	51
	100	.708	.760	.704	.883	.604	.759	.790	.780	.800	.627
	98	.292	.240	.296	.117	.396	.241	.210	.220	.200	.373
Tf											
(N)	—	48	50	51	53	25	30	54	50	49	49
	D	.271	.190	.284	.104	.300	.100	.213	.000	.000	.163
	K	.729	.750	.716	.868	.700	.900	.787	1.000	1.000	.837
	N	.000	.060	.000	.028	.000	.000	.000	.000	.000	.000

[1] Ujung Pandang, [2] Cheng Mai, [3] Terengganu

* For the terminology used to describe the alleles, see Tan et al. 1990, and these Proceedings.

the frequencies of null heterozygotes could not be estimated in those populations where no null homozygotes were scored. Thus the true heterozygote deficiencies may be greater than indicated by these tests for Ca at Bogor, Medan, Musuan and Haadyai, and for Est-D at Musuan and Cheng Mai.

Table 4. Measures of genetic variability at 26 loci in each population.

Population	Percentage of loci polymorphic	Mean heterozygosity	
		Observed	Expected
Bogar	38.5	.122 ± .038	.138 ± .040
Medan	38.5	.138 ± .041	.155 ± .043
Ujung Pandang	38.5	.125 ± .038	.154 ± .042
Musuan	38.5	.099 ± .029	.117 ± .034
Cheng Mai	34.6	.102 ± .042	.121 ± .036
Haadyai	38.5	.164 ± .050	.160 ± .043
Surin	38.5	.115 ± .036	.156 ± .043
Sabah	23.1	.085 ± .033	.089 ± .035
Sarawak	26.9	.069 ± .034	.085 ± .034
Trengganu	38.5	.102 ± .036	.125 ± .038

F-statistics

F-statistics (Table 6) estimated using the methods of Weir and Cockerham (1984) again show the substantial inbreeding within populations (F_{IS}) for the Ca, Mdh and Me loci. Ca and Me also make the highest contributions to among-population differentiation (F_{ST}), with substantial contributions also due to Alb, Amy, Dia_1-3, Est-D and Mdh. Over all loci, the mean F_{ST} (0.1748 ± 0.0496) indicates significant genetic differentiation among the populations.

Hierarchical F_{ST} estimates (Wright 1978) were computed to assess genetic differentiation among localities within countries, and among countries. The estimates of 0.170 for among localities within countries and -0.014 for among countries show that all of the differentiation is among localities, and that there is no structuring of localities among countries. Populations in the same country are just as likely to be genetically different as are populations from different countries.

Genetic distances

The matrix of genetic distance coefficients among each pair of populations is given in Table 7 and the dendrogram derived from these in Figure 3. The populations group into three clusters but, as expected from the hierarchical F_{ST} estimates, the two major clusters each include populations from Indonesia, Thailand and Malaysia. The population from Sabah shows the greatest differentiation from all other populations.

Table 5. Summary of chi-square tests for deviation from Hardy–Weinberg equilibrium.*

	Indonesia			Philippines	Thailand			Malaysia		
Locus	Bogor	Medan	U.P.[1]	Musuan	C'Mai[2]	Haadyai	Surin	Sabah	Sarawak	T'ganu[3]
Alb	ns	ns	ns	ns	ns	+	+	(+)	ns	(+)
Amy	ns	ns	ns	ns	Homo	ns	−	Homo	Homo	ns
Ca	ns	ns	ns	ns	−	−	−	−	−	−
Dia_1-3	ns	+	ns	ns	ns	+	−	Homo	Homo	ns
Est-D	−	ns	−	ns	ns	ns	−	ns	ns	ns
Mdh	−	−	−	−	−	ns	ns	ns	−	−
Me	−	−	−	−	−	−	−	Homo	−	−
Pep-B	ns	ns	ns	ns	ns	ns	ns	ns	+	(−)
Pep-C	+	+	+	ns	+	(+)	(+)	+	(+)	+
Tf	ns	ns	ns	ns	ns	ns	ns	Homo	Homo	ns

* ns = not significant
\+ = significant observed excess of heterozygotes, (+) = $0.05 < P < 0.10$
− = significant observed deficiency of heterozygotes, (−) = $0.05 < P < 0.10$
Homo = locus homozygous
[1] Ujung Pandang, [2] Cheng Mai, [3] Terengganu

Table 6. Summary of F-statistics at all loci.

Locus	F_{IS}	F_{ST}	F_{IT}
Alb	−.1215	.0901	−.0205
Amy	.1580	.1613	.2938
Ca	.4376	.3836	.6533
Dia_1-3	.1005	.1617	.2459
Est-D	.1962	.1044	.2802
Mdh	.3547	.1719	.4656
Me	.7025	.3141	.7959
Pep-B	−.0762	.0195	−.0552
Pep-C	−.3578	.0264	−.3220
Tf	−.0270	.0767	.0517
Weighted mean	.1465	.1716	.2930
Jackknife estimates over loci			
Mean	.1478	.1748	.3013
Standard deviation	.1113	.0496	.1296

*F_{IS} = fixation index of individuals relative to their locality population, or the correlation between genes within individuals within locality populations (inbreeding within each locality).

F_{ST} = correlation of genes between individuals within the same locality population, which is interpreted as a measure of the amount of differentiation among locality populations.

F_{IT} = fixation index of individuals relative to the total set of populations, or the correlation between genes within individuals.

Discussion

Although the geographically separate swamp buffalo populations of Southeast Asia are generally phenotypically very similar, there are nevertheless substantial genetic differences. The observed genetic distance values (0.006–0.082) are within the range of those observed among defined breeds of livestock.

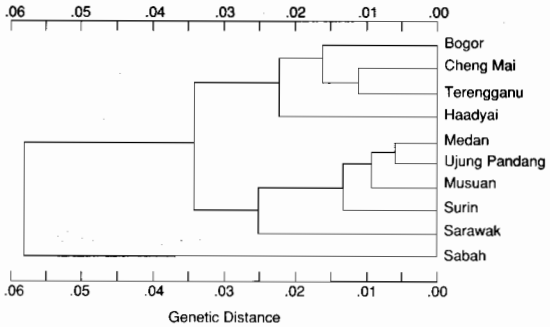

Fig. 3. Dendrogram of genetic relationships (genetic distances) among the ten populations.

Genetic variation among populations may result from differential selection (natural or artifical) or from genetic drift, while migration (gene flow) would tend to reduce variation. Swamp buffalo populations have not been subject to artifical selection programs and there is no a priori reason to expect markedly different natural selection pressures affecting the different populations. Thus the observed genetic differences most likely reflect genetic drift, possibly countered by migration. Migration of animals is most likely between neighbouring populations which should then be more similar genetically than more distant populations. However, this is not observed for these populations. The genetic distance matrix (Table 7) and the dendrogram (Figure 3) show that there is essentially no relationship between genetic distance and geographic distance. Even though there is likely to have been increasing migration between neighbouring populations in recent times, we conclude that these populations have been largely isolated from one another and have differentiated genetically due to genetic drift.

Table 7. Matrix of Nei (1972) genetic distances for each pair of populations.

Population	1	2	3	4	5	6	7	8	9	10
1. Bogor	—									
2. Medan	.027	—								
3. Ujung Pandang	.028	.006	—							
4. Musuan	.033	.010	.008	—						
5. Cheng Mai	.019	.038	.029	.032	—					
6. Haadyai	.027	.035	.033	.035	.018	—				
7. Surin	.028	.015	.012	.011	.015	.013	—			
8. Sabah	.082	.045	.054	.049	.081	.055	.041	—		
9. Sarawak	.055	.034	.030	.020	.043	.046	.018	.045	—	
10. Terengganu	.013	.035	.033	.034	.011	.021	.023	.074	.061	—

The generally observed deficiency of heterozygotes (Table 4) may also be indicative of genetic drift in populations of small effective size, rather than of deliberate inbreeding. In addition, observed heterozygote deficiencies in some populations may have resulted from the Wahlund effect, if all animals sampled were not from a single random mating population.

The average level of inbreeding within populations (F_{IS}, Table 6) and the measure of population differentiation (F_{ST}, Table 6) are heterogeneous among loci. As all loci have been subjected to the same breeding structure, this heterogeneity in inbreeding indicates some form of selection affecting gene and genotype frequencies at some of the loci.

To the extent that the genetic differences among populations remain as more populations are included, and as more loci are analysed, we predict that genetic drift will also have led to genetic differentiation at loci affecting performance, so that the populations would be expected to differ in their mean genetic merit for growth and draught ability, and for adaptive traits.

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Genetic Relationships among Populations of Southeast Asian Native Goats

O.S. Selvaraj,[1] T.K. Mukherjee,[1] S.G. Tan[2] and J.S.F. Barker[3]

Abstract

Biochemical polymorphisms of native goats in Southeast Asia are being investigated for some 50 loci, primarily to estimate genetic distances among various populations. This preliminary report presents data for genetic variation in, and genetic distances among, nine populations in four countries (Indonesia, Philippines, Thailand and Malaysia).

Of the 26 loci screened by either of two different electrophoretic techniques (cellulose acetate and starch gel), 11 were polymorphic and 15 monomorphic. Allele frequencies showed some differences among populations, but the same allele was the most common in all populations for all but three loci (amylase, purine nucleoside phosphorylase and transferrin). Consistent observed deficiencies of heterozygotes were found at six loci, and average inbreeding within localities was high ($F_{IS} = 0.2741 \pm 0.0911$). Over all loci, the mean F_{ST} was relatively small (0.040 ± 0.0146), but there was genetic differentiation among the nine populations. We suggest that gene flow among these populations has been sufficient to counter, to some extent, population differentiation due to genetic drift.

Two previous papers (Barker et al. and Mukherjee et al. these Proceedings) give the background and introduction to our studies of biochemical polymorphism in swamp buffalo and native goat populations. The primary aim is to understand the genetic structure of these populations and to estimate the genetic relationships among them.

Although there have been some substantial studies of biochemical polymorphism in goats (e.g. Tucker and Clarke 1980), very few studies have been done on the genetic relationships among breeds and populations of goats; perhaps surprisingly, past studies have been on Asian populations. However, these data are limited in that only a small number of local populations from the same region was considered, sample sizes often were small and the number of polymorphic loci per population was only 2–7. Caution is necessary therefore in comparing these studies with others. Nozawa et al. (1978) studied eight local populations of the Okinawa Islands and the Japanese Saanen breed. Measures of the standard genetic distance of Nei (1972) among the Okinawa populations ranged from 0.0003 to 0.0025 and the distances of these populations from the Japanese Saanen were 0.0046–0.0088. However, due to regular importations of the Japanese Saanen to Okinawa, there was an estimated gene flow of nearly 13% from the Saanen to the Okinawa goats, so that these latter distances would be less than that prior to this crossbreeding. Nei standard genetic distances among three populations of Indonesian native goats were in the range 0.0011–0.0054 (Katsumata et al. 1981). Equivalent ranges for six Korean native goat populations (Katsumata et al. 1982) and for five Sri Lankan populations (Shotake et al. 1986) were, respectively, 0.0001–0.0056 and about 0.0006–0.0032 (as interpreted from their Figure 4). For 14 native breeds of goat in Spain, Tuñón et al. (1989) reported Nei genetic distances in the range 0.003–0.097. These distances among Spanish breeds, which are substantially larger than the distances among local populations of native goats within countries in Asia, were based on large samples from each breed (36–115) and eight polymorphic loci.

In this preliminary report, we present data for genetic variation at 26 loci and genetic distances

[1] Institute for Advanced Studies, University of Malaya, 59100 Kuala Lumpur, Malaysia
[2] Department of Biology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3] Department of Animal Science, University of New England, Armidale, NSW 2351, Australia

among nine populations; i.e. for those locus-population combinations for which assays have been completed. Assays of additional loci, from these populations and for all loci for samples from an Australian feral goat population (an outgroup for the phylogenetic analysis) and further samples from Malaysia, are still in progress. Collections from Sri Lanka have not yet been done.

Materials and Methods

A total of 465 blood samples was collected from nine localities in Indonesia, Philippines, Thailand and Malaysia (Table 1, Fig.1). Methods of collection of whole blood, separation into plasma, red blood cells and white blood cells, and assay methods using starch and cellulose acetate electrophoresis for 51 genetic loci have been described by Tan et al. (1990 and these Proceedings).

Twenty-six blood genetic systems are reported here, comprising 23 erythrocyte systems: acid phosphatase (Acp), adenylate kinase (Ak), carbonic anhydrase (Ca), NADH-diaphorase 1 zone 1 (Dia_1-1), NADH-diaphorase 1 zone 2 (Dia_1-2), fructokinase (Fk), fructose-1,6-diphosphatase (Fdp), glucosephosphate isomerase (Gpi), glutamate oxaloacetate transaminase (Got), glutamate pyruvate transaminase (Gpt), glucose-6-phosphate dehydrogenase (G6pd), glucose dehydrogenase (Gdh), hexokinase (Hk), haemoglobin (Hb), isocitrate dehydrogenase (Idh), lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh), malic enzyme (Me), purine nucleoside phosphorylase (Np), phosphoglucomutase-2 (Pgm-2), 6-phosphogluconate dehydrogenase (6Pgd), superoxide dismutase (Sod), X-protein (XP); and three plasma systems: amylase (Amy), albumin (Alb) and transferrin (Tf).

All were analysed by cellulose acetate electrophoresis, except for X-protein where horizontal starch gel electrophoresis was done following the method of Tucker et al. (1967).

Table 1. Collection localities and numbers of animals sampled at each.

Country	Locality	No. sampled
Indonesia	Bogor	50
	Medan	50
	Ujung Pandang	48
Philippines	Musuan	51
Thailand	Cheng Mai	50
	Haadyai	39
Malaysia	MARDI/Univ. of Malaya	55
	Sabah	51
	Sarawak	71

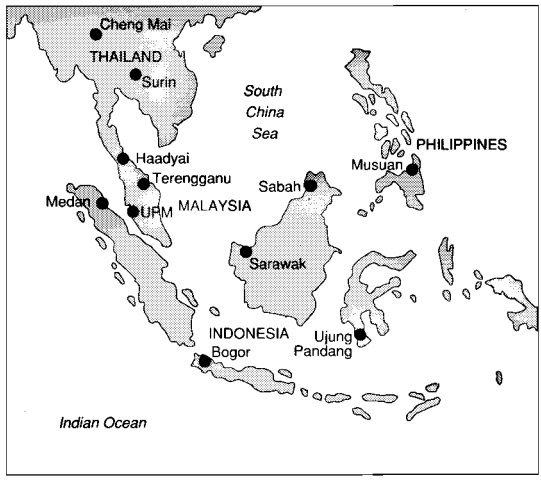

Fig. 1. Geographical localities from which populations were sampled.

Allele frequencies at the Alb, Hb, Mdh, Me and Tf loci were estimated by the direct count method. For Amy and Np, only two phenotypes (high (H) and low (L) activity) were observed on the gels, and family studies have shown high activity to be dominant at both loci. Assuming Hardy Weinberg equilibrium at each locus, the frequency of the low activity allele was estimated by the square root method, and then genotype frequencies were estimated. For the Ca, Dia_1-1, Dia_1-2 and XP loci, null homozygotes were identified in a number of populations. As null heterozygotes would be scored as homozygotes for the other allele present, an iterative search procedure was used to determine simultaneously the allele frequencies and inbreeding coefficient that best fitted the observed phenotype frequencies. These allele frequencies and the inbreeding coefficient then were used to estimate all genotype frequencies (including null heterozygotes) for the analyses of genetic variation in each population, differentiation among populations, and genetic distances.

Genetic variation in each population was measured by the percentage of loci that were polymorphic and the mean heterozygosity. Population structure and differentiation among populations were analysed using F-statistics. Genetic distances among populations were estimated using Nei's (1972) standard genetic distance, and a dendrogram to depict relationships among the populations was produced using the unweighted pair group method with arithmetic means (Sneath and Sokal 1973). Most analyses were done with the BIOSYS computer package (Swofford and Selander 1989) as recently modified (W.C.Black, pers. comm. 1990).

Table 2. Numbers of animals assayed (N) and allele frequencies for 11 polymorphic loci.

Locus	Allele	Indonesia			Philippines	Thailand		Malaysia		
		Bogor	Medan	U.P.[1]	Musuan	C'Mai[2]	Haadyai	MARDI/ UM[3]	Sabah	Sarawak
Alb										
(N)	—	50	50	48	51	50	39	55	51	71
	A	.760	.790	.719	.667	.750	.615	.927	.804	.782
	B	.240	.210	.281	.333	.250	.385	.073	.196	.218
Amy										
(N)	—	50	50	48	51	50	39	55	51	71
	H	.320	.380	.427	.333	.420	.397	.409	.520	.394
	L	.680	.620	.573	.667	.580	.603	.591	.480	.606
Ca										
(N)	—	50	50	48	51	50	39	55	51	71
	100	.470	.880	.542	.853	.640	.974	.855	.892	.690
	98	.410	.120	.458	.147	.070	.026	.109	.078	.021
	null	.120	.000	.000	.000	.290	.000	.036	.029	.289
Dia_1-1										
(N)	—	50	50	48	51	50	39	55	51	71
	102	.000	.000	.000	.069	.000	.000	.000	.000	.000
	100	1.000	1.000	1.000	.657	.870	.846	1.000	.941	.852
	98	.000	.000	.000	.235	.130	.154	.000	.059	.127
	null	.000	.000	.000	.039	.000	.000	.000	.000	.021
Dia_1-2										
(N)	—	50	50	48	51	50	39	55	51	71
	102	.280	.100	.135	.206	.170	.103	.018	.206	.239
	100	.720	.900	.865	.716	.830	.628	.973	.716	.556
	98	.000	.000	.000	.000	.000	.103	.009	.000	.000
	null	.000	.000	.000	.078	.000	.167	.000	.078	.204
Hb										
(N)	—	50	50	48	51	50	39	55	51	71
	A	.960	.900	.813	.824	.840	.897	.891	.873	.923
	B	.040	.100	.188	.176	.160	.103	.045	.059	.000
	X	.000	.000	.000	.000	.000	.000	.064	.069	.077
Mdh										
(N)	—	50	50	48	51	50	39	55	51	71
	100	.790	.770	.656	.735	.720	.526	.755	.647	.732
	98	.210	.230	.344	.265	.280	.474	.245	.353	.268
Me										
(N)	—	50	50	48	51	50	39	55	51	71
	102	.130	.080	.167	.186	.190	.141	.118	.186	.085
	100	.790	.750	.698	.725	.680	.744	.882	.745	.824
	98	.080	.170	.135	.088	.130	.115	.000	.069	.092
Np										
(N)	—	50	50	48	51	50	39	55	51	71
	H	.530	.550	.385	.333	.430	.500	.364	.480	.528
	L	.470	.450	.615	.667	.570	.500	.636	.520	.472
Tf										
(N)	—	50	50	48	51	50	39	55	51	71
	A	.680	.750	.604	.618	.610	.487	.445	.765	.556
	B	.290	.230	.323	.343	.370	.513	.509	.235	.423
	C	.030	.010	.042	.039	.020	.000	.018	.000	.014
	D	.000	.010	.031	.000	.000	.000	.027	.000	.007
XP										
(N)	—	50	50	48	51	50	39	55	51	64
	X1	.200	.120	.188	.196	.200	.346	.218	.206	.344
	X2	.710	.560	.594	.618	.470	.654	.527	.618	.563
	null	.090	.320	.219	.186	.330	.000	.255	.176	.094

[1] Ujung Pandang, [2] Cheng Mai, [3] MARDI/University of Malaya

Results

Of the 26 loci studied, 10 (viz. Alb, Amy, Ca, Dia_1-2, Hb, Mdh, Me, Np, Tf and XP) were polymorphic in all nine populations and one (Dia_1-1) was polymorphic in five populations. The electrophoretic phenotypes for these polymorphic loci are shown in Figure 2, and further descriptions of relative band positions and staining intensity have been given by Selvaraj et al. (1990) and Tan et al. (these Proceedings). Loci were classed as monomorphic only after scoring at least 25 samples from each population.

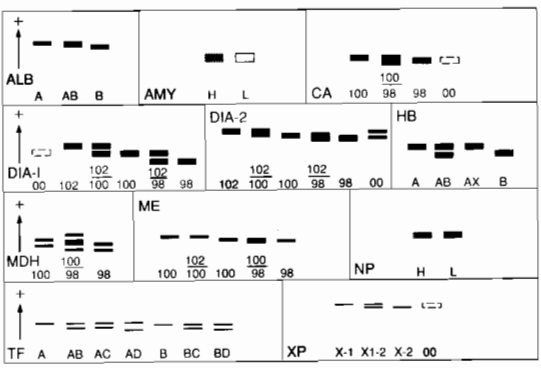

Fig. 2. Schematic representation of the electophoretic banding patterns for 11 polymorphic blood protein loci. For each locus, the x-axis shows alleles and the y-axis the distance run.

Genetic variability within populations

Allele frequencies for the polymorphic loci (Table 2) show differences among populations, but the same allele was the most common in all populations except for three loci (Amy, Np and Tf). Amy^L was most common in eight populations, but had a slightly lower frequency in Sabah (0.480). Differences among populations were greater for Np (Np^L range of 0.450 to 0.667), and for Tf (Tf^A range of 0.445 to 0.765, with Tf^C not present in two populations and Tf^D not present in five). The Tf^D allele is reported here for the first time in Southeast Asian goats. Although at low frequency in any one population, it is widespread throughout the region.

The percentage of loci that are polymorphic is 38.5 in Bogor, Medan, Ujung Pandang and MARDI/University of Malaya, where Dia_1-1 is homozygous, and 42.3 in the other five populations.

Observed heterozygosity (Table 3) is lowest for Ujung Pandang (0.102) and highest for Cheng Mai (0.138), but is not significantly different among populations. For all populations, observed heterozygosity is less than expected. As more loci are assayed, these measures of genetic variability are likely to decrease, since loci known to be polymorphic from previous studies have been included here.

Hardy–Weinberg equilibria

The overall trend of an observed deficiency of heterozygotes (Table 3) may be analyzed in more detail by testing the significance of deviations of the observed genotype frequencies from those expected under Hardy–Weinberg equilibrium. These tests, for each locus in each population, are summarised in Table 4. Two loci (Mdh and Me), and to a lesser extent Ca, Dia_1-2, Hb and XP, show consistent observed deficiences of heterozygotes. Only Tf shows no significant departures from Hardy Weinberg equilibrium in any population. For Ca, Dia_1-1, Dia_1-2 and XP, where null alleles were detected, the frequencies of null heterozygotes could not be estimated in those populations where no null homozygotes were scored. Thus the true heterozygote deficiencies may be greater than indicated by these tests for Ca at Medan, Ujung Pandang, Musuan and Haadyai, for Dia_1-1 at Cheng Mai, Haadyai and Sabah, for Dia_1-2 at Bogor, Medan, Ujung Pandang, Cheng Mai and MARDI/UM, and for XP at Haadyai.

Table 3. Measures of genetic variability at 26 loci in each population.

Population	Percentage of loci polymorphic	Mean heterozygosity	
		Observed	Expected
Bogor	38.5	.104 ± .034	.154 ± .042
Medan	38.5	.110 ± .037	.139 ± .039
Ujung Padang	38.5	.102 ± .032	.172 ± .045
Musuan	42.3	.134 ± .040	.182 ± .044
Cheng Mai	42.3	.138 ± .039	.180 ± .045
Haadyai	42.3	.133 ± .042	.170 ± .045
MARDI/Univ. of Malaya	38.5	.104 ± .037	.129 ± .040
Sabah	42.3	.114 ± .034	.158 ± .041
Sarawak	42.3	.120 ± .035	.175 ± .044

Table 4. Summary of results of chi-square tests for deviation from Hardy-Weinberg equilibrium.*

	Indonesia			Philippines	Thailand		Malaysia		
Locus	Bogor	Medan	U.P.[1]	Musuan	C'Mai[2]	Haadyai	MARDI/ UM[3]	Sabah	Sarawak
Alb	+	(+)	–	+	ns	(+)	ns	(+)	ns
Ca	–	–	–	–	ns	ns	–	–	ns
Dia_1-1	Homo	Homo	Homo	–	–	–	Homo	ns	–
Dia_1-2	–	–	–	–	–	ns	ns	–	–
Hb	ns	–	–	–	–	–	ns	ns	ns
Mdh	–	–	–	–	–	–	–	–	–
Me	–	–	–	–	–	–	–	–	–
Tf	ns	ns	ns	ns	ns	ns	ns	ns	ns
XP	–	ns	–	ns	ns	–	ns	–	–

*ns = not significant
\+ = significant observed excess of heterozygotes, (+) = $0.05 < P < 0.10$
– = significant observed deficiency of heterozygotes
Homo = locus homozygous
Amy and Np not included as genotype frequencies were estimated assuming Hardy-Weinberg equilibrium
[1] Ujung Padang, [2] Cheng Mai, [3] MARDI/University of Malaya

F-statistics

F-statistics (Table 5) estimated using the methods of Weir and Cockerham (1984) again show the substantial inbreeding within populations (F_{IS}) for the Ca, Dia_1-2, Mdh, Hb, Me and XP loci. However, the highest average within population inbreeding coefficient is shown by Dia_1-1, with large significant deficiencies of heterozygotes in four of the five populations that were polymorphic. Ca, Dia_1-1 and Dia_1-2 also make the highest contributions to among-population differentiation (F_{ST}). Over all loci, the mean F_{ST} (0.0406 ± 0.0146) indicates significant genetic differentiation among the populations.

Hierarchical F_{ST} estimates (Wright, 1978) were computed to assess genetic differentiation among localiues within countries, and among countries. The estimates of 0.042 for among localities within countries and -0.004 for among countries show that all of the differentiation is among localities, and that there is no structuring of localities among countries. Populations in the same country are just as likely to be genetically different as are populations from different countries.

Table 5. Summary of F-statistics at all loci.

Locus	F_{IS}*	F_{ST}	F_{IT}
Alb	–.1025	.0325	–.0667
Amy	.0120	.0039	.0158
Ca	.4993	.1579	.5784
Dia_1-1	.7378	.1070	.7658
Dia_1-2	.4534	.0750	.4944
Hb	.2577	.0272	.2779
Mdh	.5997	.0122	.6046
Me	.6484	.0062	.6506
Np	.0115	.0167	.0279
Tf	–.0123	.0359	.0241
XP	.3576	.0249	.3736
Weighted mean	.2769	.0410	.3066
Jackknife estimates over loci			
Mean	.2741	.0406	.3040
Standard deviation	.0911	.0146	.0909

*F_{IS} = fixation index of individuals relative to their locality population, or the correlation between genes within individuals within locality populations (inbreeding within each locality).
F_{ST} = correlation of genes between individuals within the same locality population, which is interpreted as a measure of the amount of differentiation among locality populations.
F_{IT} = fixation index of individuals relative to the total set of populations, or the correlation between genes within individuals.

Genetic distance

The matrix of genetic distance coefficients among each pair of populations is given in Table 6 and the dendrogram derived from these in Figure 3. The

populations group into one cluster of five populations and two each of two populations. As expected from the results of the hierarchical F_{ST} analysis, there is no obvious within-country clustering, except for Bogor and Ujung Pandang.

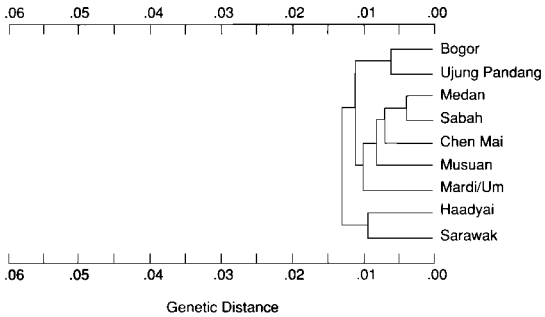

Fig. 3. Dendrogram of genetic relationships (genetic distances) among the nine populations.

Discussion

The native goats of southeast Asia show very extensive polymorphism for coat colour and therefore appear quite diverse phenotypically. However, this diversity seems to exist in all local populations, so there are no apparent phenotypic differences among populations, and no specific strains or breeds are recognised.

Nevertheless, as shown by the significant average F_{ST} (0.0406 ± 0.0146), there are genetic differences among these populations. Mukherjee et al. (these Proceedings) have discussed briefly factors affecting population differentiation in swamp buffalo. The conclusion drawn is that those populations have been largely isolated from one another, and that they have differentiated genetically due to genetic drift.

The overall results for the native goat populations are qualitatively similar — in the overall observed deficiency of heterozygotes (Table 3) and the significant population differentiation (F_{ST}). But the magnitude of population differentiation is much less for the goat populations than for the buffalo and the inbreeding within populations is greater.

The high level of within-population inbreeding (Table 5, $F_{IS} = 0.2741 \pm 0.0911$), whether due to deliberate inbreeding or small effective population size, might be expected to accentuate genetic drift and lead to increased population differentiation. But as the magnitude of F_{ST} is relatively small, it seems that there has been sufficient gene flow among these populations to counter this differentiation.

Further speculation should be left until data are available for more loci and for more populations. However, there are significant genetic differences among these native goat populations, so that they may be expected to differ in mean genetic merit for growth and other production traits.

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Table 6. Matrix of Nei (1972) genetic distances for each pair of populations.

Population	1	2	3	4	5	6	7	8	9
1. Bogor	—								
2. Medan	.010	—							
3. Ujung Pandang	.006	.009	—						
4. Musuan	.014	.010	.011	—					
5. Cheng Mai	.010	.007	.008	.007	—				
6. Haadyai	.020	.015	.017	.009	.014	—			
7. MARDI/UM	.016	.008	.011	.013	.009	.016	—		
8. Sabah	.011	.004	.010	.008	.007	.010	.010	—	
9. Sarawak	.010	.012	.016	.010	.007	.009	.013	.008	—

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Cytogenetic Aspects of Crossbreeding River and Swamp Buffalo

D.W. Cooper*

Abstract

There are two chromosomal races within the water buffalo (*Bubalus bubalis*), the 2n = 50 form of the river buffalo or Indian subcontinent type and the 2n = 48 form of the swamp buffalo or East Asian type. The 2n = 48 race is characterised by a 'tandem' fusion, which unites the short (p) arms of the number 4 and number 9 chromosome of the 2n = 50 race. It has been conjectured that the heterozygote 2n = 49 might be sub-fertile. The argument is advanced here that mutations which became fixed in a population are unlikely to be selectively disadvantageous. The evidence concerning the fertility of this fusion and the fertility of Robertsonian fusions in other mammals is briefly summarised. Few large-scale studies yielding decisive results have been carried out, and only one case of reduced fertility (the 1/29 fusion of domestic cattle) has been firmly established. The fertility of 2n = 49 buffalo inter-racial heterozygotes should be assessed in the long term. However, the possibility of reduced fertility does not seem so great as to inhibit the use of crosses between the races in order to upgrade the swamp buffalo.

WATER buffalo can be broadly divided into two types, river and swamp. River buffalo originate from the Indian subcontinent, have a chromosome number of 2n = 50, and are 'improved domesticated animals' with high milk and meat production. Swamp buffalo are mainly found in East Asia, have 2n = 48 and are much less highly selected for production purposes. A 2n = 50 form of the swamp buffalo exists in Ceylon, which suggests that the primary differentiation between the two chromosomal forms is geographic. The difference between the two forms is a translocation which has joined the 4 and 9 chromosomes of the 2n = 50 form to create the 2n = 48 form. A recent review of this and other aspects of buffalo crossbreeding can be found in Bongso and Mahadevan (1989).

The existence of the chromosomal difference is of concern for animal programs in which the river buffalo is being used to upgrade the swamp buffalo. An important question is whether the F1 and the 2n = 49 backcross animals heterozygous for the chromosomal rearrangement are likely to suffer reduced fertility. Emphasis has been placed upon the possibility that this could be so (Basrur 1989). The purpose of this paper is to examine the evidence and to make suggestions concerning the likely practical consequences of the chromosomal difference.

* School of Biological Sciences, Macquarie University, NSW 2109, Australia

Nature of Translocation and its Consequences

The translocation has been described by various authors as a 'tandem' fusion of chromosomes 4 and 9 (Wurster and Benirschke 1968, Di Berardino and Ianuzzi 1981, Bongso and Hilmi 1982). A tandem fusion is defined in cytogenetic terms as one which results from a break near the centromere of one chromosone and another break near the end in a second, followed by reciprocal rejoining (Rieger, Michaelis and Green 1968). This use of the word tandem is confusing to a non-cytogeneticist, since tandem usually implies that the components of the repeated elements run in the same direction. Chromosomes have just two main components, the p (small) and q (large) arms and so, with reference to the present problem, the fusions which run 4p4q9p9q and 9p9q4p4q would be tandem, whereas 9q9p4p9q is really 'anti-tandem', if the word tandem is used strictly. However, it is this last which differentiates the 2n = 50 and 2n = 48 forms of the water buffalo.

The most common type of translocation which occurs during evolution is the Robertsonian translocation. In these, two acrocentric chromosomes break near their centromeres (one in the p arm, the other in the q arm) and undergo a reciprocal exchange, creating a large bi-armed chromosome and a very small chromosome with two very short arms, which is usually lost. In centric fusions, the centromeres of two acrocentrics fuse, leading to a larger bi-armed chromosome, presumably without any loss of genetic material. When a rearrangement becomes fixed in evolution, there is a consequent reduction in diploid chromosome number of two, although the number of chromosome arms (nombre fondamentale = NF) remains unchanged. Robertsonian translocations account for the difference in chromosome number between domestic cattle ($2n = 60$) and the water buffalo with $2n = 50$ (Ianuzzi et al. 1987).

The 4/9 water buffalo translocation should have similar reproductive consequences to a Robertsonian translocation. In heterozygotes for a Robertsonian translocation unbalanced gametes arise only through mis-segregation of centromeres. This source of unbalanced gametes could occur in the 4/9 translocation. In addition, crossing over in the region between the two centromeres (the interstitial region) could do likewise. Since the intersitial region is quite short, the behaviour of the 4/9 translocation may approximate that of a Robertsonian translocation. Figure 1 and its caption give further details.

The reproductive consequences of a translocation depend not only on the proportion of unbalanced gametes produced, but also upon whether the resultant genotypes are selected against at the gametic or zygotic stage. If the former, i.e. unbalanced gametes do not take part in zygote formation, no reduction in fertility is likely. In the latter case, the zygotes with unbalanced genotypes are likely to abort early in development and, in domestic animals, will cause returns of service. The theoretical range of possible effects upon reproduction is from none to very considerable and we are not in a position to predict what might happen in a particular instance. Each translocation has to be evaluated empirically on its own merits.

It should be observed, however, that elementary population genetics suggest that those translocations which cause the least reduction in fertility are the ones which are most likely to become fixed in a population. Indeed, in the presumably unusual instances where the heterozygotes and homozygotes for the translocation have a selective advantage, the probability of fixation would be greatest. Translocations are a particular kind of genetic mutation. Most detectable mutations are deleterious, some are selectively neutral and a very small fraction are advantageous. It is the latter two classes which are likely to become fixed during the course of evolution. Thus, on general grounds, we would expect that translocations which have run the gamut of natural selection are unlikely to cause problems with fertility.

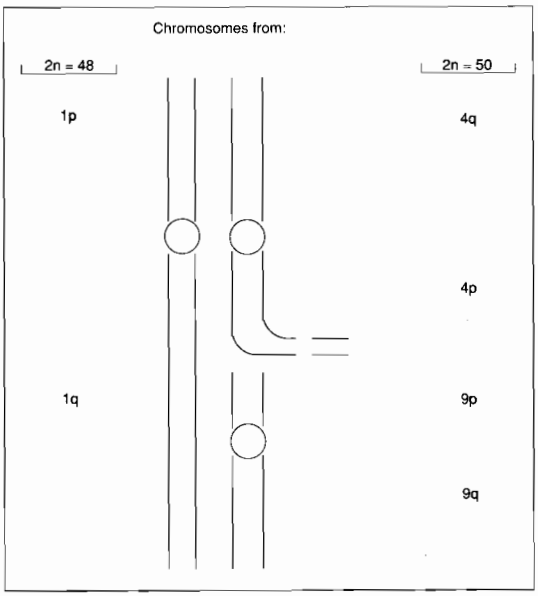

Fig. 1. Diagrammatic representation of a possible chromosomal composition and pairing behaviour in a heterozygote for the 4/9 'tandem' fusion and the original 4 and 9 chromosomes. The union of the the 4 and 9 of the $2n = 50$ form becomes the number 1 chromosome of the $2n = 48$ form (Di Berardino and Ianuzzi, 1981). Whether or not the 9 centromere and the nucleolar organiser region from the telomeric end of the 4p are included in the fusion is yet to be resolved; in this diagram it is assumed that the number 9 centromere of the $2n = 50$ form remains in the 1q arm of the $2n = 48$ form, but is inactive, while the 4p nucleolar region is lost. The gap in 4p represents the nucleolar organiser. The upper crossover would create chromosomes with unequal arm lengths at anaphase I, and consequently unbalanced gametes after meiosis II. It is difficult to say how likely such a crossover would be, since the translocation might be accompanied by some chiasmata suppression. The lower crossover would lead to dicentric and acentric fragments, although the likelihood of such a crossover is probably very small.

Data from Water Buffalo

Decisive data concerning the fertility of translocation are still being collected. Data summarised by Harisah et al. (1989) indicate that the segregation ratios in

F_2 and backcross animals are Mendelian (Table 1). Heterozygotes with 2n = 49 can produce six types of gametes with respect to their chromosomal constitutions, two balanced and four unbalanced. Harisah et al. (1989) point out that if all gametes from both sexes taking part in fertilisation are balanced, then there is a 1:2:1 expectation of 48:49:50 chromosomal forms in the F_2, whereas if all six possible gametes are produced in equal proportions, the expectations become 1:6:1. The data agree closely with the first expectation, but differ decisively from the second. The conclusion to be drawn from this is that a large proportion of unbalanced gametes cannot be produced by both parents. (However, it should be noted that if one sex of parent produced all balanced gametes, the other a large proportion of unbalanced ones, and if zygotic selection took place against unbalanced genotypes, then a 1:2:1 would also result.) There is thus at present no support for the notion of reduction in fertility through the production of unbalanced gametes by the 2n = 49 heterozygote. Histological and cytogenetic studies on spermatogenesis show that some selection against unbalanced gametes is probably taking place (Bongso and Hilmi 1982, Hilmi 1984 cited in Harisah et al. 1989).

A beginning has been made to assess other aspects of reproduction with the potential to affect the fertility of the heterozygous bulls as opposed to the homozygous 2n = 48 and 2n = 50 bulls (Bongso and Mahavedan 1989). These include measurements on testis size and dimensions, semen volume, sperm concentration and percentage of abnormal sperm. As indicated by Vidyadaran and Azmi (1989) much more data are needed to provide clear answers. It is significant that, in China, a large-scale crossbreeding program involving an F1 produced by crossing swamp buffalo × one breed of river buffalo followed by mating to a second breed of river buffalo has been very successful (Yongzuo 1989). This indicates that any depression of fertility which might have occurred was not of practical importance. It also needs to be stressed that separating the effects of chromosomal constitution from other genetic differences, which might have accumulated since the time of separation of the two chromosomal forms, will require a large and carefully designed study.

Evidence from Other Mammals

Much of the evidence on the behaviour of Robertsonian translocations in domestic animals has been conveniently summarised by Nicholas (1987). The most studied example is the 1/29 translocation which reaches quite high frequencies in certain cattle breeds. Very detailed work in Sweden has shown that heterozygotes have about a 5% reduction in fertility (Gustavsson 1979). By contrast three separate Robertsonian translocations have been described in sheep in New Zealand and they are without apparent effect upon fertility (Bruére 1975). These have been combined to reduce the diploid number from 54 to 48 in one group of sheep. Selection against aneuploid spermatocytes takes place in sheep (Stewart-Scott and Bruére 1987).

Robertsonian translocations can exist in polymorphic frequencies in wild populations. Two examples are the blue fox, *Alopex lagopus* (Christensen and Pedersen 1982) and the common shrew, *Sorex araneus* (Sharman 1956, Searle 1986). The latter is associated with hybrid zones. It is of interest that a hybrid zone for the chromosomal form of the water buffalo, as well as for other genetic markers, occurs in Bangladesh (Faruque 1989). A larger study of this area, to determine the extent of the hybrid zone, and to compare the fertility of the 2n = 48, 49 and 50 animals in it, is desirable.

Nicholas (1987) states:

> Apart from the 1/29 centric fusion in cattle, and the centric fusion in sheep discussed above, many other centric fusions involving various pairs of non-homologous chromosomes have been reported in domestic cattle, sheep, goats, pigs, and dogs, and in wild ungulates of various species. In all cases except the 1/29 in cattle, centric fusions have not been found to have any adverse effects on reproductive ability or on any

Table 1. Segregation ratios in F_2 animals; data from Harisah et al. (1989).

Chromosome No.	Malaysian data	Philippine data	Combined data	Expectations for: 1:2:1	1:6:1
48	8	1	9	11.5	5.75
49	6	16	22	23.0	34.50
50	7	8	15	11.5	5.75
Totals	21	25	46	46	46

χ^2_2 for a 1:2:1 = 1.65 ($P > 0.05$) and χ^2_2 for a 1:6:1 = 21.25 ($P < 0.001$). However note that χ^2_2 for heterogeneity = 9.78 ($P < .001$).

other characteristic. However, until large-scale population surveys are conducted as described above, our understanding of the effect of most of these centric fusions will remain incomplete.

Nicholas also draws attention to the reduced fertility associated with reciprocal translocations involving large interstitial regions in pigs, cattle and chickens.

Jacobs (1977) and Speed (1989) have summarised the considerable amount of data on structural rearrangements available in humans. Some Robertsonian translocations have normal fitness, some reduced fitness while reciprocal translocations generally exhibit reduced fitness. A feature of both is the association of the rearranged chromosomes with the XY bivalent during male meiosis. Most of these translocations come to be investigated for clinical reasons and so there is an inherent ascertainment bias in the data. A further complication is that members of the species *Homo sapiens* in Western societies do not seek to maximise their reproductive potential in the way which is demanded of domestic animals, and so a translocation leading to early zygotic elimination may merely slightly widen the interval between children, without affecting overall fitness.

Concluding Remarks

The expectation that translocations might lead to a reduction in fertility is based in part upon the predicted difficulties with meiosis and in part on the demonstrated difficulties in bearers of translocations, especially in humans, who have come to clinical attention. How often such reductions in fertility apply to a special class of single translocations which differentiates two interfertile populations within a species is a question which cannot at present be answered. If the chromosomal difference between the swamp and river buffalo were not known, one wonders whether questions would be being raised about the fertility of their hybrids. The advantage of interbreeding the two are so considerable (Bongso and Mahadevan 1989) that is necessary to ask how seriously to take the possibility of significantly reduced fertility. Both evidence from within the species and more generally from other mammals suggests that at this stage it is merely a matter for monitoring. Studies directed at deciding whether crossing over occurs in the 4p arm should have priority. If it does, the translocation may behave more like a reciprocal translocation; if it does not, its behaviour would be very similar to that of a Robertsonian translocation.

Acknowledgments

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Karyotypes of Water Buffalo Crosses (Swamp × River)

M. Hilmi*

Abstract

Two hundred and nine buffalo consisting of swamp, river and their various crosses were karyotyped. Karyotype analysis, based on the number of chromosomes, fundamental number and presence or absence of marker chromosomes (chromosomes 4, 9 and T or 4/9 tandemly fused chromosomes) revealed that in a population of crossbred buffalo there were only three karyotype categoroies present; i.e. those with chromosome complements of $2n = 48$, $2n = 49$ and $2n = 50$. The swamp buffalo, the river buffalo and their F_1 hybrid possess chromosome complements of $2n = 48$, $2n = 50$ and $2n = 49$ respectively, while the ¾ swamp types and the ¾ river types have two karyotypes each; i.e. $2n = 48$ and $2n = 49$, and $2n = 49$ and $2n = 50$, respectively. The F_2 hybrids (the offspring from inter se mating between breed types with $2n = 49$) have 3 karyotypes, i.e. $2n = 48$, $2n = 49$ and $2n = 50$. This study also reveals that the breed types of buffalo with similar chromosome numbers would have similar karyotypes.

WATER buffalo of Asia have been classified into swamp and river types based on their habitat and use under domestication (McGregor 1941). Phenotypically and cytogenetically the two types of buffalo can be easily distinguished. The swamp buffalo are slate-black in colour with white-grey chevrons on the brisket below the neck. They have long horns which grow horizontally from the head curving backwards to form a crescent. The river buffalo are larger in size and jet black in colour; these buffalo may have white markings on the forehead and tail-switch. They have relatively short horns which curl forward, downward and backward. Cytogenetically they are distinguished by their chromosome complements: the swamp buffalo has a diploid number of $2n = 48$ while the river buffalo has $2n = 50$. However, chromosome analysis based on the G-banded karyotypes has revealed that the two types of buffalo are related; although they possess different chromosome numbers they have almost the same amount of chromosome material (Hilmi 1984). The reduction in the number of chromosomes in the swamp buffalo karyotype is the result of a tandem fusion between the telomeric end of the short arm of the sub-metacentric chromosome number 4 with the centromeric end of the acrocentric chromosome number 9 in the river buffalo. The subsequent loss of the centromeric chromosome gives rise to a metacentric chromosome assigned as chromosome number 4 in the swamp buffalo karyotype. Henceforth, this 4/9 tandemly fused chromosome will be referred to as chromosome T. Because of this fusion, chromosome pair number 9 in the swamp buffalo karyotype is absent.

Crossbreeding between the two types of buffalo has been practiced in many Asian countries; this practice produces an F_1 hybrid with a diploid chromosome number of 49 which is a mixture of the haploid sets of each parent's karyotype (Bongso and Hilmi 1982). The hybrids are found to be fertile in inter se and backcross matings to produce second generation hybrids and backcrosses (Bongso and Hilmi 1982, Bongso et al. 1984).

Studies on meiotic chromosome preparations from testicular tissues of F_1 ($2n = 49$) hybrid males revealed that, due to the presence of the three unpaired autosomes, the synaptic possibilities at diakinesis included the formation of three univalents, a univalent with a bivalent (which may consist of either chromosomes 4 and 9, 4 and T, or 9 and T) or a single trivalent (Hilmi 1984). The possible pattern of segregation of these autosomes is presented in the Punnet Square (Fig.1). From the figure it can be seen that there are 8 or 6 possible types of gametes depending on whether the meiotic process is associated with non-disjunction or disjunction, respectively. It can also be seen that only the centre

* Department of Animal Sciences, Universiti Pertanian Malaysia, 43400 Serdang, Selangor, Malaysia

sets (shaded) of gametes are genetically balanced while the other gametes are unbalanced and those produced as a result of non-disjunction are severely unbalanced. Theoretically therefore, subsequent breeding involving animals with 2n = 49 chromosome complements could lead to the production of embryos and ultimately to offspring with many categories and sub-categories of karyotypes. Therefore the objective of this study was to investigate if there are karyotypes other than those with chromosome complements of 2n = 48, 2n = 49 and 2n = 50 in different buffalo breed types. The presence of different chromosome configurations or sub-karyotypes within each karyotype category was also investigated.

Gamete	23 0 27	24 4 29	24 9 28	24 T 29	25 4,9 30	25 4,T 29	25 9,T 30	26 4,9,T 32
23 0 27	46 0 54	47 4 56	47 9 55	47 T 56	48 4,9 57	48 4,T 58	48 9,T 57	49 4,9,T 59
24 4 29	47 4 56	48 44 58	48 4,9 57	48 4,T 58	49 44,9 59	49 44,T 60	49 4,9,T 59	50 44,9,T 61
24 9 28	47 9 55	48 4,9 57	48 99 56	48 9,T 57	49 4,99 58	49 4,9,T 59	49 99,T 58	50 4,99,T 60
24 T 29	47 T 56	48 4,T 58	48 9,T 57	48 TT 58	49 4,9,T 59	49 4,TT 60	49 9,TT 59	50 4,9,T 61
25 4,9 30	48 4,9 57	49 44,9 59	49 4,99 58	49 4,9,T 59	50 44,99 60	50 44,9,T 61	50 4,99,T 60	51 44,99,T 62
25 4,T 31	48 4,T 58	49 44,T 60	49 4,9,T 59	49 4,TT 60	50 44,9,T 61	50 44,TT 62	50 4,9,TT 61	51 44,9,TT 63
25 9,T 30	48 9,T 57	49 4,9,T 59	49 99,T 58	49 9,TT 59	50 4,99,T 60	50 4,9,TT 61	50 99,TT 60	51 4,9,TT 62
26 4,9,T 32	49 4,9,T 59	50 44,9,T 61	50 4,99,T 60	50 4,9,TT 61	51 44,99,T 62	51 44,99,TT 63	51 4,99,TT 62	52 44,99,TT 64

Fig. 1. Punnet Square showing the segregation of marker chromosomes 4, 9 and T in the gametes and the possible karyotypes of different buffalo crosses resulting from different combinations of the gametes.

Notes:
Numbers at left corner of each box indicate number of chromosomes.

Numbers and letters in centre row of each box indicate chromosome number (single or paired) where 4, 9 and T represent marker chromosomes used in this study.

Number at bottom right corner of each box indicates the fundamental number.

Shaded boxes indicate gametes or offspring possessing a chromosome complement in a balanced configuration.

Materials and Methods

A total of 209 buffalo was studied (Table 1).

Leucocyte cultures for chromosome preparations and constructions of karyotypes for the different genotypes of buffalo were carried out according to the methods of Hilmi (1984) modified from Basrur and Gilman (1964).

Karyotype analysis was based on the diploid chromosome number, the fundamental number and the types of marker chromosomes; i.e. the submetacentric chromosome number 4, the acrocentric chromosome number 9 and the metacentric 4/9 tandemly fused chromosome (designated as T).

Table 1. Genotypes and numbers of buffalo karyotyped.

	Countries			
Genotype	Malaysia	Thailand	Philippines	Total
Swamp	20	33	—	53
River	20	—	—	20
F_1 hybrid	21	8	53	82
¾ swamp	12	—	—	12
¾ river	21	—	—	21
F_2 ($F_1 \times F_1$)	21	—	—	21
Total	115	41	53	209

Results

Table 2 shows genotypes of buffalo classified according to karyotype, based on the diploid number of chromosomes. The chromosome complements of swamp, river and F_1 hybrid buffalo genotypes were 2n = 48, 2n = 50 and 2n = 49, respectively. The other genotypes (the ¾ swamp, the ¾ river and the F_2) are made up of more than one category. The first backcross animals possessed the two karyotype categories; the ¾ swamp type showed a chromosome complement of 2n = 48 and 2n = 49 while the ¾ river type exhibited animals with 2n = 49 and 2n = 50. The F_2 genotype consisted of three karyotype categories, namely, 2n = 48, 2n = 49 and 2n = 50.

Table 3 shows the chromosome analysis based on all criteria when genotypes with the same diploid chromosome number are listed separately. The table shows that karyotypes with the same chromosome complement also show the same fundamental number and the same set of marker chromosomes.

Discussion

The results of this study confirm that the chromosome complements of the swamp and river buffalo and their F_1 hybrids are 2n = 48, 2n = 50 and 2n = 49, respectively. They are consistent with those

Table 2. Chromosome complements of water buffalo genotypes.

Genotype	n	Chromosome complement		
		2n = 48	2n = 49	2n = 50
Swamp	53	53	—	—
River	20	—	—	20
F_1 hybrid	82	—	82	—
¾ swamp (F_1 × swamp)	12	7	5	—
¾ river (F_1 × river)	21	—	10	11
F_2 (F_1 × F_1)	21	8	6	7

reported by previous workers (Fischer and Ulbrich 1968, Songsri and Ramirez 1979, Bongso and Jainudeen 1979, Hilmi 1984, Bongso et al. 1984). The presence of three karyotype categories (2n = 48, 2n = 49 and 2n = 50) in F_2 hybrid buffalo and two karyotype categories each in the ¾ river buffalo (2n = 49 and 2n = 50) lends further support to earlier observations by Hilmi (1984), Bongso et al. (1984) and Bongso (1986). However, this result is in contrast to the expected result, as shown in the Punnet Square (Fig. 1) where six to eight types of gamete having haploid numbers ranging from n = 23 to n = 26 may be produced and where in the F_2 hybrid embryos or offspring, the possible karyotype categories that could be produced ranged from chromosome complements of 2n = 46 to 2n = 52. Therefore this suggests that the gametes with n = 23 and n = 26 which are severely unbalanced may be selected out during gametogenesis or are unable to fertilise, or if they are able to fertilise the embryos or the offspring die in utero. This assumption is supported by the histological and ultra-structual studies on hybrid testes reported by Hilmi (1984). He showed that, while the testicular tissues of the swamp and river buffalo (representing buffalo with 2n = 48 and 2n = 50 respectively) appeared normal, those of the F_1 hybrid buffalo (representing animals with 2n = 49) showed a large proportion of degenerating spermatocytes and abnormal spermatids during the process of spermatogenesis.

The results shown in Table 3 indicate that there is no sub-karyotype category. Thus, those buffalo genotypes with similar chromosome complements will have similar karyotypes. This could happen only when those gametes with balanced chromosome configuration are involved in a successful fertilisation. With reference to the Punnet Square, there are two such gametes; those gametes with n = 24 and n = 25 haploid chromosome complements belonging to that of the swamp type and river type respectively. Therefore, in the F_2 hybrids or offspring resulting from inter se mating of F_1 hybrids or in the offspring resulting from mating amongst buffalo having chromosome complements of 2n = 49, the distribution of karyotype categories 2n = 48, 2n = 49, 2n = 50 will be in the ratio 1:2:1. Otherwise, the expected ratio would be 1:6:1 (should the slightly unbalanced gametes be able to produce viable embryos or offspring). The report by Harisah (1988) that the karyotypes of the F_2 hybrid buffalo (2n = 48, 2n = 49 and 2n = 50) are distributed in the Mendelian ratio 1:2:1 lends support to the fact that, in the genotypes of buffalo with a karyotype having a chromosome complement of 2n = 49, only the balanced gametes are successful in producing viable offspring.

Table 3. Chromosome complements, fundamental numbers and marker chromosomes of buffalo genotypes.

Genotypes	Chromosome number (2n)	Fundamental number (NF)	Marker chromosome
Swamp	48	58	TT
¾ swamp	48	58	TT
F_2	48	58	TT
River	50	60	44, 99
¾ river	50	60	44, 99
F_2	50	60	44, 99
F_1	49	59	4T, 9–
¾ swamp	49	59	4T, 9–
¾ river	49	59	4T, 9–
F_2	49	59	4T, 9–

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Molecular Study of Mitochondrial DNA in Buffalo

Y.Y. Gan,[1] B. Norlia,[1] M. Mahyuddin,[1] T.I. Tengku Azmi[2], I. Latiff[2] and S.G. Tan[3]

Abstract

This project focused on the molecular study of mitochondrial DNA in buffalo. Mitochondrial DNA was extracted from blood platelets for the study of restriction fragment length polymorphisms (RFLP). In the preparation of mitochrondrial DNA probes, mitochondrial DNA extracted from liver tissues was used. Methods for the extraction of mitochrondrial DNA from liver tissues of buffalo have been developed. Results showed that 7.13 mg of mitochondrial DNA can be obtained from 200 gm of liver tissues of buffalo with the ratio of OD_{260} and OD_{280} equal to 1.821.

Mitochrondrial DNA was digested with restriction endonucleases and separated by agarose gel electrophoresis. It was then Southern-transferred onto nylon membrane. After hybridisation with the mitochondrial DNA probe labelled with ^{32}P, DNA patterns were obtained by autoradiography.

The molecular weight of buffalo mitochondrial DNA was determined to be approximately 16.5 kb. The patterns of mitochrondrial DNA in buffalo tissue digested with Bam HI were analysed. Four fragments of 7.4 kb, 5.2 kb, 2.9 kb and 1.0 kb were obtained, indicating that there were four Bam HI restriction sites in the mitochondrial DNA of buffalo.

RECENTLY, a lot of interest has arisen in cytogenetic studies of buffalo. Despite this, information for genetic polymorphisms among swamp and river buffalo is still lacking.

Our aim was to utilise the DNA restriction fragment length polymorphisms (RFLP) and the biochemical isoenzyme polymorphisms to study the genetic similarities and difference in buffalo. The study of biochemical polymorphisms has been reported elsewhere (Tan et al. 1980).

In this project, study of mitochondrial DNA (mtDNA) in buffalo was chosen instead of the nuclear genome for the following reasons: (1) the molecule of mtDNA is smaller (16 kb) than the nuclear genome; (2) the rate of mtDNA mutation is 7–10 times faster than that of the nuclear genome (Brown et al. 1982); (3) it is maternally inherited and it will not reshuffle during fertilisation. All of these advantages make it ideal for the study of phylogeny and evolution.

[1] Department of Biotechnology
[2] Department of Animal Science
[3] Department of Biology
Universiti Pertanian Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Human mitochondrial DNA was reported to be 15659 bp (Anderson et al. 1981). The mtDNA sequence of bovines was also published by Anderson et al. in 1982. Studies on mtDNA of *Drosophila* (De Salle et al. 1980, Shah et al. 1979), primates (Brown et al. 1982), yeast (Bonitz et al. 1980, Hudspeth et al. 1982) and mouse (Bibb et al. 1981) have also been reported. However, the study of mtDNA in buffalo is just beginning (Bhat et al. 1990).

In recent years several authors have used mitochondrial DNA for the study of evolution. Mitochondrial systems in animals appears to be much simpler than the single cell eukaryotes and the eukaryotic nuclear systems. The mammalian mitochondrial system with 22 tRNAs requires only half as many aminoacyl synthetase as the eukaryotic cytoplasmic system which requires 40 tRNAs (Anderson et al. 1981).

Animal mitochondrial DNA is 25000 times smaller than the nuclear genome of the smallest animals. Animal mitochondrial DNA arrangement appears to be more stable with no sequence rearrangement (inversion, transposition, deletion, addition) having been reported. The changes observed are reported to be due to mutation (Brown et al. 1982).

The other important characteristic of mitochondrial DNA is that it appears to be uniparentally (maternally) transmitted and free from genetic complexities as compared to the biparental mode of transmission. There is no genetic reshuffling during fertilisation, making it ideal for the study of phylogeny and evolution (Giles et al. 1980, Case et al. 1981, Schurr et al. 1990, Ballinger et al. 1991). However, multiple mitochondrial genotypes do exist in the maternal cytoplasmic genome (Hauswirth and Laipis 1982, Laipis et al. 1982, Olivo et al. 1983).

Availability of the nucleotide sequence from various mammals enables us to undertake comparative studies at the nucleotide level which provides knowledge on the process of molecular evolution of the mitochondrial genome (Brown et al. 1982, Saccone et al. 1983). The other method for the study of evolution is comparing changes in restriction endonuclease cleavage sites. Ferris et al. (1983) reported that, using this method, they were able to study mitochondrial DNA evolution in mice. The variation attributed to base substitution was encountered at about 200 of the 300 cleavage sites examined and a length mutation was located in this genome. Ferris et al. (1983) also reported that the average rate of point mutation divergence in mitochondrial DNA is 2–4 percent per million years. They have also provided genetic markers from new inbred strains that are useful in mouse developmental and cell biology.

Yonekawa et al. (1980) have studied the evolutionary relationships between laboratory mice and the subspecies *Mus musculus domesticus*. They concluded that the genetic background of laboratory mice was derived from *M. musculus domesticus*. They also suggested that intra-subspecific heterogeneity of *Mus musculus* is very small, although it can possibly be detected by increasing the sample size and using more restriction endonucleases.

In order to study variation in gene structure at the nucleotide sequence level, it is necessary to align the physical map with identifiable genes. Such maps are important in analysing sequence variation within an animal and between related individuals. Restriction maps of sheep and goat mitochondrial DNA and the analysis of evolutionary relationships between these DNAs, based on restriction endonucleases, have also been prepared. Upholt and David (1977) suggested that most differences between the mitochondrial DNA of sheep and goats were due to single nucleotide substitutions and that the most rapidly evolving segments in these two mammals were two regions close to the D-loop. Potter et al. (1975) have detected several intra-species mitochondrial differences but no difference has been found in different organs of a single individual.

Polymorphism of mitochondrial DNA in a maternal lineage of Holstein cows has been studied by Hauswirth and Laipas (1982). They found that there were two mitochondrial genoytpes existing within one Holstein cow maternal lineage. The process that gave rise to this phenomenon is clearly of fundamental importance in understanding the intra-specific mitochondrial polymorphisms and evolution in mammals.

Materials and Methods

The mtDNA used for the study of mtDNA restriction fragment length polymorphisms was extracted from the blood tissue. Blood was separated into different fractions. The red cells and the serum were used for isozyme analysis. White blood cells were stored at −50 °C for the analysis of nuclear DNA in the future. Mitochondrial DNA was extracted from the mitochondrial fraction separated from the serum. Hirt's extraction method (Hirt 1967) was used to extract the mtDNA, proteinase K, SDS, RNAase, salmon sperm DNA being used during the DNA extraction.

Agarose gel electrophoreses were performed using lambda DNA Hind III digests as standard molecular markers. After electrophoresis, the gels were stained with ethidium bromide solution and visualised under UV light. It was then Southern-transferred to a nylon membrane, baked at 80 °C for 3 hours and then stored in an envelope at room temperature.

Mitochondrial DNA extracted from liver tissues of buffalo was used as a probe. Random multiprime kit (Amersham) was used to label the DNA probe with ^{32}P. The reaction mix was incubated for 30 minutes at 37 °C. Nylon membrane was prehybridised in a prehybridisation solution (50% formamide, 6X SSC, 5X Denhardt's 0.5% SDS, denatured salmon sperm DNA and EDTA) at 60 °C in a belly dancer incubator for 2 hr. The prepared DNA radioactive probe was then added to the hybridisation solution and incubated at 65 °C for 48 hours. After hybridisation, the nylon membrane was rinsed carefully and thoroughly dried between Whatman 3 MM paper and autoradiographed.

Results and Discussion

The methods of extracting mtDNA from the liver tissues of buffalo have been developed. An amount of 7.13 mg of mitochondrial DNA was obtained from 200 g of liver tissue, which is equal to 0.035

mg/g tissue. The ratio of optical density readings of OD_{260} and OD_{289} was 1.821 indicating that good purity of DNA was obtained. This was considered as good yield compared to the work reported by Avise et al. (1978) who obtained only 0.002 mg/g liver tissue from a species of the rodent *Peromyscus*.

When lambda DNA digested with Hind III was used as a molecular marker, the molecular weight of the undigested mitochondrial genome in buffalo was estimated to be approximately 16.5 kb. Since the human mitochondrial genome was reported as 16569 bp by Anderson et al. 1981, bovine mitochondrial DNA has been documented as 16338 bp by Anderson et al. (1982) and the mouse mitochondrial genome as 16295 bp by Bibb et al. (1981). The results obtained for the buffalo mitochondrial genome reported here confirm that mitochondrial genome size in mammals is quite conservative and stable.

The size of mitochondrial DNA in invertebrates is much larger. Among invertebrates, *Drosophila* mitochondrial DNA has been well studied. The major differences in gene order between *Drosophila* and mammalian mitochondrial DNAs can be explained by a single translocation (Clary et al. 1982). The decrease of organelle DNA sizes from unicellular to multicellular organisms was further confirmed by comparing the genome sizes with yeast, filamentous fungi and vascular plants (Gan 1988). The mitochondrial DNA genome of yeasts was found to be five times larger than mammalian mitochondrial DNA, with the genome size as large as 108 kb. In filamentous fungi such as *Neurospora* and *Aspergillus*, the genome size was from 32 to 95.2 kb. The mitochondrial genome sizes for vascular plants range from 155 kb to 2400 kb, many times larger than the yeast mitochondrial genome. The genetic code of vascular plant mitochondrial DNA genome was similar to universal codon with only one exception; CGG codes for tryptophan and not for arginine (Gan 1988).

When the buffalo mitochondrial DNA was digested with Bam HI, four restriction fragments of 7.4 kb, 5.2 kb, 2.9 kb and 1.0 kb were obtained in the autoradiograph. These fragment sizes indicate that there are four Bam HI sites (ggatcc) in the buffalo mitochondrial genomes. There were some indications of polymorphisms between and within the river and the swamp buffalo but more samples are needed to confirm this DNA sequence difference. Laipis et al. (1979) have reported some restriction mapping of mitochondrial DNA of bovine based on restriction endonuclease cleavage sites. It will be interesting to compare the DNA sequence homology between the cattle and buffalo, which may shed some light on the phylogeny and the genetic distance of these two related groups.

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Future Studies of Genetic Differentiation among Swamp Buffalo and Native Goat Populations

J.S.F. Barker,[1] S.G. Tan[2] and T.K. Mukherjee[3]

IN the introductory paper to this first section of these Proceedings we have emphasised the rationale for the study of genetic differentiation among populations in terms of the evaluation and appropriate utilisation of animal genetic resources.

The first question then is — what are these resources? In the developed world, they are distinguished as separate breeds, where a breed may be defined (Turton 1974) as:

> A homogeneous, sub-specific group of domestic livestock with definable and identifiable external characters that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a homogeneous group where geographical separation from phenotypically similar groups has led to general acceptance of its separate identity.

But for most of the endemic (native) livestock of Southeast Asia, breeds are not recognised or defined. Thus for example, the swamp buffalo of Asia exist in many geographically separate populations, but all are phenotypically similar. Yet we should not assume that this phenotypic similarity implies genetic similarity. There may well be genetic differences at many loci, including those loci affecting productive, reproductive and adaptive traits.

Little is known of the history of the swamp buffalo and goats of Southeast Asia, but we can assume that they have been present throughout the region for some thousands of years, and that for much of this time, local populations would have been largely isolated from one another, i.e. little migration between populations. Given such isolation, inbreeding will accumulate within populations — not necessarily because of deliberate mating of relatives by human intervention, but simply because of the small effective size of the local populations. For alleles which are neutral to selection, this inbreeding is expected to lead to population differentiation — the process of genetic drift. Similar selection pressures, either natural or artificial, in different populations will oppose the drift and tend to keep them genetically similar, as will any migration between populations. Equally, different selection pressures in different populations will increase differentiation.

Of course, the histories of population size, selection pressures and migration are not known. Nevertheless, the present degree of genetic differentiation can be determined by a comparison of allele frequencies at polymorphic loci in the existing populations and by estimating genetic distances among each pair of populations. From these pair-wise genetic distances, a dendrogram can be constructed to show diagrammatically the relationships among the populations and their clustering into genetically similar groups.

In principle, this methodology appears straightforward, but there are several measures of genetic distances and several methods of constructing a dendrogram from any set of genetic distances. The genetic distance measures are mathematically rather diverse and different ones are often not related in any simple way. Thus different distance measures could lead to different interpretations of the relationships among some sets of populations, with no way of knowing which is closest to the true relationships. Although correlations among the various distance measures are generally high (Hedrick 1975, Chakraborty and Tateno 1976) particularly when applied to local populations within a species (e.g. livestock strains and breeds), there is need for further study of the application of different measures to our data for swamp buffalo and goats. For the present reports of our preliminary data (Mukherjee et al. and Selvaraj et al., these Proceedings) we have used the standard genetic distance of Nei (1972), simply

[1] Department of Animal Science, University of New England, Armidale, NSW 2351 Australia
[2] Department of Biology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3] Institute for Advanced Studies, University of Malaya, 59100 Kuala Lumpur, Malaysia

because it has been widely used in studies in evolutionary biology and of relationships among livestock breeds. For dendrogram construction, the unweighted pair group method with arithmetic means (UPGMA) that we have used seems generally superior; i.e. most likely to extract the true relationships (Rohlf and Wooten 1988).

These comments on methodology should not be taken to mean that the distances and dendrograms presented for swamp buffalo and native goat populations are in some way wrong. Given the assumptions inherent in deriving these methods, we are presenting one interpretation of the relationships among the populations. Other methods may give somewhat different results, but they are unlikely to be substantially different. As the work proceeds and more loci and more populations are assayed, we will be in a better position to compare different distance measures and methods. Even though our results are preliminary and based on a limited sample of loci, there are already two clear conclusions:

(1) there is little or no relationship between genetic distance and geographic distance, and

(2) genetic distances among Southeast Asian swamp buffalo populations are substantially greater than those among native goat populations.

Within this ACIAR project, more populations will be sampled from other countries and more loci assayed. In particular, samples of populations in Sri Lanka, Bangladesh and Myanmar need to be obtained. Sri Lankan populations are significant because of the reported phenotypically swamp-type animals with a chromosomal complement of $2n = 50$ (Bongso et al. 1977). Thus the Sri Lankan buffalo may be more closely related to the river buffalo, perhaps representing a less domesticated, unimproved river buffalo. Populations in Bangladesh and Myanmar are at the boundary between the river and swamp buffalo distributions. A barrier in this region may have allowed separate development of the two forms, although there may have been more recent introgression between them. Populations in this region, as in Sri Lanka, may therefore be less closely related to other Southeast Asian populations.

It must be emphasised that the study should not end with the samples of individuals collected from the particular set of populations in this project. New populations or data on additional loci can be added to the gene frequency data base at any time to update or expand the analyses. The first conclusion above indicates that additional populations should be studied to give a finer geographical scale of populations.

We particularly wish to encourage other workers to undertake study of additional populations — using the electrophoretic methods already developed in this project (Tan et al., these Proceedings) so that the results will be comparable and can be added to the data base.

Another aspect of the preliminary results that will need further analysis is the general observed deficiencies of heterozygotes at many loci in both species. Such heterozygote deficiencies may be due to inbreeding, unrecognised null alleles, selection or Wahlund effects (i.e. inclusion of two or more populations with different allele frequencies in the one sample). Analyses to attempt to distinguish these possible causes of heterozygote deficiencies will be done when data on more populations and more loci are available. However, it should be noted that the mean value of F_{IS} (within locality inbreeding) was much higher in the goat populations than in the swamp buffalo, namely 0.2769 and 0.1465, respectively. Clearly we need to know more about the breeding structure of both species in order to understand this difference. In addition, a more detailed analysis of genotype frequencies within each population is necessary.

To summarise then, the results obtained to date demonstrate the value of the work, most particularly in the substantial genetic differences among some of the swamp buffalo populations. We emphasise that these are preliminary results and that further data collection and more detailed analyses are necessary.

Finally, we should consider other related studies and future directions. Our primary interest is in the genetic structure of the buffalo and goat populations and the genetic differences among populations within each species. To study these, protein electrophoresis is being used. But protein electrophoresis underestimates the total level of genetic variation, because (1) protein differences are detected only if the amino acid substitution changes the net charge of the protein (and many do not), and (2) only a small fraction of the DNA actually codes for proteins. With regard to the extent of variation detected by electrophoresis, the resolution can be increased, often quite dramatically, by using sequential electrophoresis with varying pH or gel concentration (Coyne 1982, Keith et al. 1985). Barker (1985) has suggested that this procedure be applied to those loci that prove polymorphic on our standard assay procedure for the buffalo and goat populations.

This procedure would increase the accuracy of our estimation of genetic relationships. In addition, other methods should be considered. Advances in molecular biology in recent years allow the poten-

tial for finer analysis of genetic variation — ultimately by comparison of DNA sequences. Sequencing is now quite routine but still too expensive and slow for population analysis. However, restriction enzymes can be used to assay for single nucleotide changes in DNA, and to quickly survey particular regions of the DNA for polymorphism.

Restriction enzymes cleave DNA at specific sites — each enzyme recognising a particular short (4–8 base pairs) sequence in the DNA. After cutting the DNA with a restriction enzyme, the DNA is run on a gel and the cut fragments are separated according to their length. A base change in the recognition site leads to a restriction fragment length polymorphism (RFLP), where two short fragments are replaced by one long one.

RFLP variation is being actively studied in many domestic animals. By using a number of enzymes, each with different recognition sites, many polymorphisms can be assayed quite rapidly. Many of the analyses of RFLP variation to date have been of the mitochondrial DNA (mtDNA) — a small circular genome of 16–18 kilobases of DNA. The human, mouse and bovine mtDNA genomes have been completely sequenced, providing the basis for mapping restriction sites in other species; two recent reports give data for mtDNA polymorphism in Philippine native cattle (Watanabe et al. 1989) and in Indian river buffalo (Bhat et al. 1990). The methodology is well-established and at least one study has been initiated using mtDNA variation to analyse genetic differentiation among 14 cattle breeds — six *Bos taurus* from Europe, four *Bos indicus* from India, two breeds from West Africa and two from East Africa (E.P. Cunningham, pers. comm.).

The swamp buffalo and goat blood samples already collected for the electrophoretic assays could and should be used for mtDNA analysis. We will have extensive data on electrophoretic variation and mtDNA analysis of these same animals would provide a unique data set, allowing comparison of the genetic differentiation in the nuclear genome with that in the mitochondrial genome.

Our understanding of the recent evolution and genetic differentiation of current populations of these Southeast Asian species would thereby be increased dramatically.

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Comparative Evaluation of Reproductive Performance of Malaysian Swamp and River Buffalo and their Crosses

M. Mahyuddin*, W. Sharifuddin*, D. Ismail* and M. Hilmi*

Abstract

A study involving 48, 2–5 years old females, 16 from each genotype of swamp and river buffalo and their F_1 crosses was conducted at Universiti Pertanian Malaysia (UPM) Puchong farm to test the effects of plane of nutrition and genotype on productive and reproductive performance. Equal numbers of each genotype were placed in paddocks 1 and 2 respectively. Animals in paddock 1 received palm kernel cake (PKC) supplementation at the rate of 350 g/head/day provided once a week whereas animals in paddock 2 received none. All animals received a mineral mixture and had access to wallowing. An F_1 bull was placed in each paddock for breeding. Body weights, rectal temperatures, and plasma progesterone levels were monitored weekly in addition to examinations of the uterus which were made per rectum. Voluntary dry matter intakes were estimated by using the chromic oxide marker technique and the data were treated according to the physiological status of the female i.e. open, lactating or pregnant.

PKC supplementation under improved pasture in this study did not influence body condition nor did it improve reproductive performance. The body condition of the buffalo in both paddocks was similar although buffalo in the supplemented group had a slightly higher average daily weight gain and heifers in this group attained puberty at an earlier age. Voluntary feed intake in grazing animals was significantly affected by concentrate supplementation, physiological status and breed type. F_1 crossbred animals were more efficient in utilising feed than either the swamp or river buffalo.

RIVER buffalo were brought into Malaysia by Indian immigrants for milk. Their number has remained small (about 5 000) (Jainudeen 1983) and they are kept in the peri-urban areas in small herds. A crossbreeding program between river and swamp buffalo was initiated at Universiti Pertanian Malaysia (UPM) farm in 1977 to improve the genetic potential of the indigenous swamp buffalo.

Although reproduction in river buffalo has received greater attention than in swamp buffalo, there has been no attempt to compare the reproductive and productive performances of these two genotypes and their crosses in the tropics. The aim of this study was to compare the reproductive traits of the swamp and river buffalo and their crosses, when subjected to similar management. Since nutrient intake under range condition in buffalo is relatively unknown, the second objective of this study was to measure their grazing intake.

* Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia 43400 UPM Serdang, Selangor Malaysia

Materials and Methods

Forty-eight, 2–5 years old females, 16 from each genotype of swamp, river and their F_1 crosses were used in this study. Equal numbers of each genotype were allocated to paddocks 1 (group 1) and 2 (group 2). Animals in paddock 1 received palm kernel cake (solvent type) supplementation at the rate of 350 g/head/day provided once a week. All females except the river buffalo were obtained from the UPM farm. The water buffalo were purchased from outside farms. A three year old F_1 bull was introduced to each of the paddocks at the start of the experiment. These bulls were tested for libido before entering the experiment, although their semen quality was unassessed.

A pasture area of 23 ha of improved pasture at UPM Puchong farm was divided into 2 paddocks of equal size to accommodate the two feeding systems. The pasture was fertilised with urea at the rate of 250 kg N/ha/year in six equal applications. Animals in both paddocks had access to mineral salt licks and wallowing.

Blood samples were collected weekly in heparinised vacuum tubes for plasma progesterone radio immunoassays. In addition, the animals were weighed and their rectal temperature recorded at monthly intervals. Progesterone was assayed by radioimmunoassay in extracted aliquots of 0.5 ml plasma using 3H progesterone as a tracer (Jainudeen 1983). The limit of sensitivity, defined as twice the standard deviation of the blank value, was 0.1 ng/ml. All samples were assayed in duplicate. The precision of the intra- and inter-assay precision was 9 and 10%, respectively, with an average recovery of 96.5 ±12.6%. Results were corrected for percent recovery and plasma used.

Voluntary dry matter intake (DMI) was estimated using the chromic oxide marker in edible gel suspension technique as described by Dahlan et al. (1988). Chromic oxide marker (Cr_2O_3) in gel suspension was given orally to each animal in both groups twice daily (morning and evening) at the rate of 8 ml/head/day for a duration of 14 days. Pasture and concentrate samples were taken at the experimental sites. Fecal sample collections were made twice daily for a period of 9 days started from day 5 of the experiment. Concentration of chromium in the feces was determined using a spectrophotometer at an optical density of 372 nm. In order to determine differences in DMI animals were classified according to their physiological status i.e open (non-pregnant and non-lactating) lactating or pregnant.

Results and Discussion

Weight gain

The average weight gains of the buffalo are shown in Table 1. The animals in Paddock 1 (group 1) had a slightly better weight gain than those in Paddock 2 (group 2) for all genotypes. However, this difference was not statistically significant.

Table 1. Average daily weight gains (g ± SD) Swamp, river and crossbred buffalo.

Genotype	Group 1*	Group 2
Swamp	233 ± 6.4	207 ± 8.5
River	196 ± 9.8	187 ± 8.5
Crossbred	216 ± 9.5	205 ± 8.1

* Animal received palm kernel cake supplementation at the rate of 350 g/day, once a week.

Initiation of ovarian activity in heifers

The average ages at puberty for the heifers were 31 ±9.0 months and 41 ±8.7 months for groups 1 and 2 respectively. The difference between groups was not significantly different. The range of means for lengths of oestrus cycle for individual animals was 17–23 days. During the experiment six heifers became pregnant with an average of 3.5 cycles per conception. From the onset of puberty all non-pregnant heifers cycled regularly except for two heifers in group 1 and one in group 2 that showed short irregular cycles. Two heifers in group 1 had abnormally long cycles of up to nine weeks (Table 2). The average weight at onset of puberty for heifers in groups 1 and 2 were 355 ±18 kg and 338 ±27 kg, respectively.

Table 2. Oestrus cycle in heifers.*

	Group 1	Group 2
Number	5	9
Regular (2–3 weeks)	1	4
Long cycles (4–9 weeks)	2	—
Irregular	2	1
Pregnant	—	4

* After the collection of these data, two more heifers became pregnant.

Postpartum ovarian activity was monitored in 27 females. Since there were no differences in the performance of the two groups, the data were pooled and the performance of each genotype was compared. The reproductive parameters for each genotype are presented in Table 3. There were no statistical differences between genotypes for gestation length and calving interval. The resumption of ovarian activity was based on functional activity (plasma progesterone concentrations) rather than on morphological presence (rectal palpation) of corpus luteum. The average gestation lengths (d ± S.D.) for the three genotypes were 338 ±46, 319 ±30 and 320 ± 27 for swamp, river and their crosses, respectively.

The swamp buffalo had the shortest calving interval although the differences between genotypes were not statistically significant. The calving intervals obtained in this study are longer than the 532 days reported by Jainudeen (1983) for the same farm. The swamp buffalo exhibited a shorter period from calving to first postpartum oestrus than the river and crossbred buffalo. Plasma progesterone profiles indicated ovarian function in 33% of swamp and 57% of crossbred buffalo by 120 days postpartum while the river buffalo were in anoestrus beyond 120 days postpartum.

Table 3. Reproductive parameters (±SD) of buffalo genotypes.

Parameter	Swamp	River	Crossbred
Gestation length (d)	338 ± 46	319 ± 30	320 ± 27
Calving interval (d)	628 ± 203	790 ± 343	799 ± 384
Postpartum oestrus[1] (d)	128 ± 31	303[2]	145 ± 69

[1] Period from parturition to first postpartum oestrus
[2] Represented by 1 animal

Table 4. Feed intake as a percent bodyweight (PBW)*.

	Swamp	River	Crossbred
Bodyweight (kg)	456.6[a]	496.6[a]	568.1[b]
PBW	1.198[a]	1.193[a]	1.111[b]

* Within rows, different letters indicate statistical differences ($P < 0.05$).

Voluntary feed intake

Intake as a percentage of body weight (PBW) was significantly affected by palm kernel cake supplementation. The supplemented group had lower (1.12%) grazing intake than the non-supplemented group (1.22%). No difference in PBW was detected between the swamp and the river buffalo but the PBW of the crossbreds was significantly lower than that of the other two genotypes (Table 4) indicating that the F_1 crossbreds were more efficient in the utilisation of feed.

The PBW for animals of the different physiological status were 1.32, 1.15 and 1.13 for lactating, open and pregnant animals. Lactating animals had significantly higher PBW than either open or pregnant animals. The voluntary intake as a percent of body weight is generally low because of the limited grazing time the animals were subjected to during the study period.

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Reproductive Performance and Body Weight Changes of the Lankan and Murrah Buffalo in Sri Lanka

A.R. Mohamed[1] and M.G. Jayaruban[2]

Abstract

A comparative study of the reproductive performance and body weight changes was carried out on Lankan and Murrah buffalo reared together under dry zone farming conditions. Three comparisons were made, namely: (a) with supplementary feeding, (b) in a free-grazing system on natural vegetation and (c) response to treatment with Folligon (PMSG) and progesterone releasing intravaginal devices (PRID and EAZI-breed CIDR-B).

Supplementary feeding significantly reduced ($P < 0.05$) the calving to conception interval in the Lankan buffalo from 213.8 ± 115.7 to 76.1 ± 38.0 days compared with the Murrah from 312.8 to 213.6 ± 104.1 days. There were no significant effects of genotype or supplementary feeding on the interval from calving to involution of the uterus or on the onset of the first post-partum oestrous cycle. In addition, there was no significant difference between the genotypes in these two traits when they were reared and allowed to graze together on natural vegetation in the dry zone. Conception occurred mostly between the months of December and May with a peak incidence during January and February in both genotypes.

A combination of factors such as rainfall, decreased ambient temperature, duration of bright sunshine, day length and relative humidity, coupled with lower body temperature and availability of abundant natural herbage during the calving season appears to enhance conception rates in buffalo. Treatment with reproductive hormones did not significantly improve conception rates in the two genotypes but the Lankan buffalo appeared to show a better response than the Murrah.

IT is well known that buffalo have a low reproductive efficiency compared with cattle and it may be due to the occurrence of a seasonal pattern in calving and conception. Climatic changes are thought to affect both males and females in their physiological functions as well as the growth of the natural vegetation which supplies the required nutrients in free-grazing farming systems. A seasonal trend in calving and conception has also been observed in the indigenous (Lankan) and the imported river buffalo in Sri Lanka.

Over 90% of the buffalo population in the country are of the indigenous breed (Lankan buffalo) and the rest are of imported river types including Murrah, Surti, Nili-Ravi and their crosses. The Lankan buffalo (LB) are mainly reared for use as draught animals in rice cultivation (mainly tillage of rice fields and in threshing of the harvest) whereas the river buffalo are reared mainly for milk production. In phenotypical characters and behaviour, the LB appears to belong to the swamp type but cytogenetic studies have revealed that it is similar to the river type in having 50 pairs of chromosomes (Bongso et al. 1978).

The objectives of the present study were to: (1) compare the reproductive performance of Lankan buffalo with that of the Murrah breed, reared together under the dry zone farming conditions of Sri Lanka, using feeding practices similar for those adopted by the rural farmers in the region; (2) determine their reproductive potential under improved nutritional regimes; (3) determine the responses to hormonal therapy, to reduce the interval from calving to conception in the two genotypes.

The variations in the agro-climatic factors that are thought to affect reproduction and body weight gains were also monitored.

[1] Department of Animal Science, Faculty of Agriculture, University of Ruhana, Kamburupitiya, Sri Lanka
[2] Veterinary Research Institute, Peradeniya, Sri Lanka

Materials and Methods

The experiment was conducted at the buffalo farm, Polonnaruwa, situated in the dry zone low-country region of the North-Central Province of Sri Lanka between 1986 and 1990.

Study 1. A total of 32 pregnant heifers comprising 16 Lankan (LB) and 16 Murrah buffalo (MB) were initially selected for this study. Each genotype was divided into two groups, namely, a control group and a group given supplementary feeding with a concentrate ration. The allocation of animals to groups was made on a rotational basis soon after calving; i.e. the first calved animal of each genotype was allocated to the control group and the second calved animal to the supplementary feeding group, and so on, so as to have eight animals in each cell.

Each 1000 kg of the concentrate ration consisted of 200 kg maize meal, 614 kg rice polishings, 115 kg soya bean meal, 115 kg soya bean meal, 15 kg urea, 20 kg di-calcium phosphate, 20 kg sea shell powder, 15 kg common salt and 1 kg 'Zoo Dry VM 9006' (vitamin–mineral supplement). The concentrate ration was fed at the rate of 1.2 kg per 100 kg body weight per day until the confirmation of pregnancy by rectal palpation. Half of the daily ration was given in the morning and the other half in the evening. The MB were milked twice a day but the LB were not milked but their calves were allowed to suckle throughout the night for a period of one month, after which limited suckling was provided in the morning and in the evening. All animals were allowed to graze together freely during the day and were housed in the same shed at night and provided with rice straw ad libitum. Wallowing was provided for about one hour around midday after recording rectal temperatures. The rectal temperatures were measured at 3 to 4 day intervals (i.e. twice per week during the morning (0600–0730 hours), mid-day (1200–1330 hours) and evening (after 1700 hours), using a normal clinical thermometer. The daily ambient temperature and the relative humidity were recorded at 0600, 0800, 1000, 1200, 1300, 1400, 1600, and 1800 hours and also the maximum and minimum temperatures. The relative humidity was measured using an electronic digital humidity meter KM8001 (Astell Scientific Equipment, UK) and the ambient temperatures were measured using a laboratory-type maximum and minimum thermometer. The rainfall was recorded using a meteorological office pattern rain gauge (Snowdon) with a Camden measure. The data on the number of hours of bright sunshine (measured using a Cambell Stoke sunshine recorder), wind velocity in the region and the day length measurements were obtained from the data collected by the Department of Agriculture, Peradeniya, and the Meteorological Department, Colombo.

The normal calving to conception pattern and the other reproductive parameters of the animals were determined by measuring the progesterone concentrations in blood, by rectal palpation and by scrutinising the breeding records. Rectal examinations were performed once a week to determine the time of involution of the uterus, ovarian changes and confirmation of conception. Blood samples were collected twice a week and progesterone concentrations were measured by a radioimmunoassay technique using Amerlax M Progesterone RIA kits (Amersham (Australia) Ltd, Sydney). All animals were weighed weekly using a Ruddweigh Model KM 1 Microprocessor (Ruddwigh, Australia).

Study 2. In this study, the LB that had been used in the first experiment were allowed to graze together with a larger herd of MB where the village herds were also sent for grazing on natural vegetation comprising mixed foliage under shrub-jungles. The MB were given about 500 g of a commercially prepared concentrate ration or some rice bran at milking (to induce milk let-down). The climatological and other data were collected as in the previous study.

Study 3. Under the farming system adopted in Study 2, the two breeds were evaluated for the response to treatment with 'Folligon' (PMSG from Intervet, Holland), to a progesterone-releasing intravaginal device ('PRID', marketed by Bomac Laboratories) containing 1.55 g progesterone and 10 mg oestradiol benzoate and to EAZI-breed CIDR-B (Carter Holt Harvey Plastics Products, New Zealand) containing 1.9 g of progesterone in an inert silicone elastomer. The animals selected were those that were clinically normal but which had not shown any ovarian activity for at least four weeks prior to treatment during the normal breeding season.

A group of 11 LB and 12 MB were given 1000 I.U. of Folligon intramuscularly. A second group of 10 LB and 12 MB were selected for treatment with PRIDs which were removed after a period of 12 days. The third group of 15 LB and 23 MB were treated with the EAZI-breed CIDR-B intravaginal inserts which were removed after a period of 15 days.

Results

Factors affecting fertility

The analysis of progesterone profiles, breeding records and rectal examinations of LB and MB showed a similar pattern of seasonality in conception when reared together under normal dry zone farming conditions without additional supplementary feeding (Fig. 1). Most of the MB were found to conceive between the months of December and May whereas most of the LB conceived between

January and April. Peak conception rates occurred around January and February in both genotypes. Continuous low conception rates of below 10% occurred between June and November in the MB and from May to December in the Lankan breed.

The onset of the peak conception season was preceded by a rise in the monthly rainfall from September to January (Northeast monsoon) with a concomitant rise of the mean relative humidity between October and December (Fig. 2) followed by another slight increase in the rainfall in March and April (Southwest monsoon). The lowest rainfall of below 50 mm occurred between June and August. Around the month of peak conception, the mean ambient day-temperature was found to be the lowest (<29 °C), but remained above 32 °C between April and September (Fig. 2).

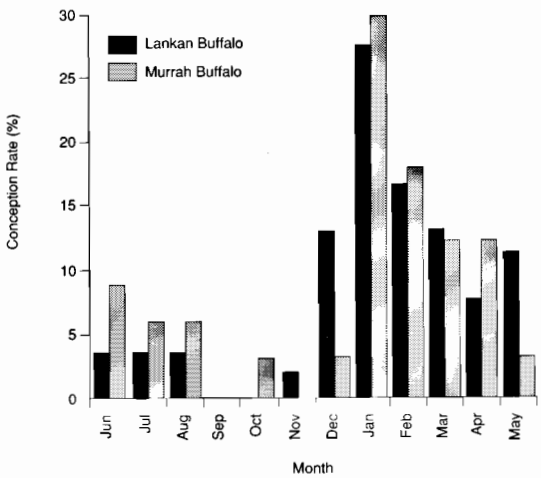

Fig. 1. Comparison of the conception pattern of the Lankan buffalo with that of the Murrah under dry zone conditions.

The monthly morning average rectal temperature (MRT) of the MB was found to vary between 37.4 °C and 37.9 °C, but the average midday rectal temperature (MDRT) ranged from 38.4 °C to 39.2 °C (Fig. 3). The midday and evening rectal temperatures remained elevated from June to October, and showed a diminishing trend to lower levels during the months from January to April to coincide with the months of high conception (Fig. 3). A similar pattern was also observed in the Lankan breed (Fig. 4). The number of hours of bright sunshine per day decreased to the lowest levels of 4.1 and 3.9 h during the months of January and February, respectively (Fig. 5). The month of June had the highest day length of 12.5 h which subsequently decreased to the lowest level of 11.7 h in December and began to rise gradually (Fig. 5.). Short day lengths of <12 h between November and February were also found to coincide with the period of peak conception (Fig. 5).

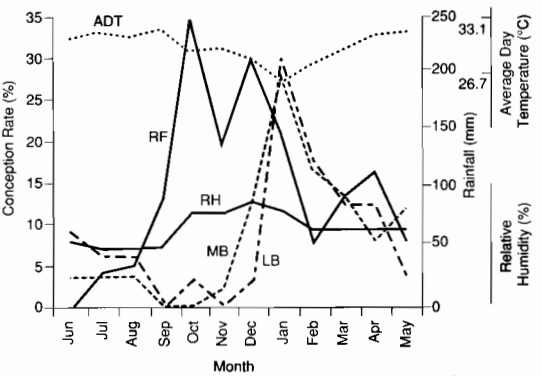

Fig. 2. Comparison of the conception rates of the Lankan (LB) and the Murrah buffalo (MB) with the changes in the monthly rainfall (RF), average day temperature (ADT) and average relative humidity (RH) under dry zone conditions.

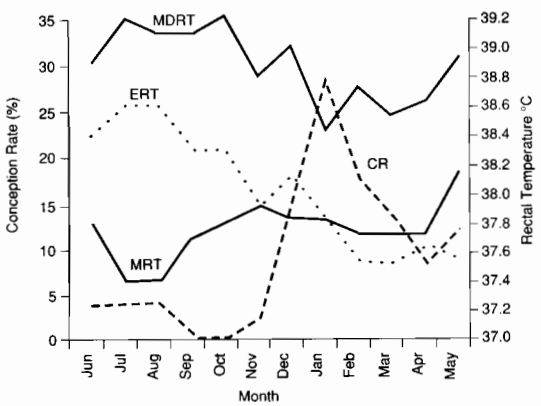

Fig. 3. Comparison of the conception rate (CR) of the Murrah buffalo with the changes in the morning (MRT), mid-day (MDRT) and evening (ERT) rectal temperatures.

Post-partum changes and conception under range conditions

The results of the post-partum measurements of the interval between calving and complete involution of the uterus, onset of the first oestrous cycle (as measured by the rise in the progesterone concentrations in blood of >0.5 ng/ml for 2 consecutive weeks) and the calving to conception interval in the two genotypes are shown in Table 1. No significant differences between the genotypes were observed in any of these measurements.

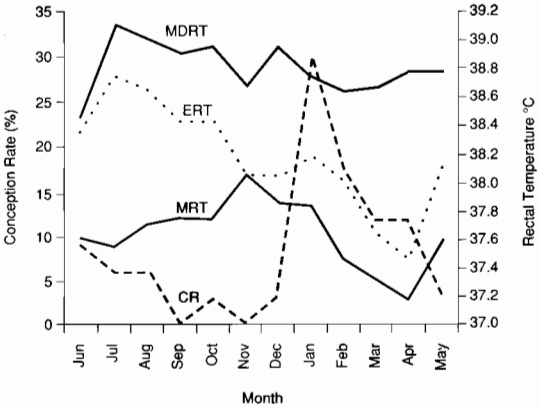

Fig. 4. Comparison of the conception rate (CR) of the Lankan buffalo with the changes in the morning (MRT), mid-day (MDRT) and evening (ERT) rectal temperatures.

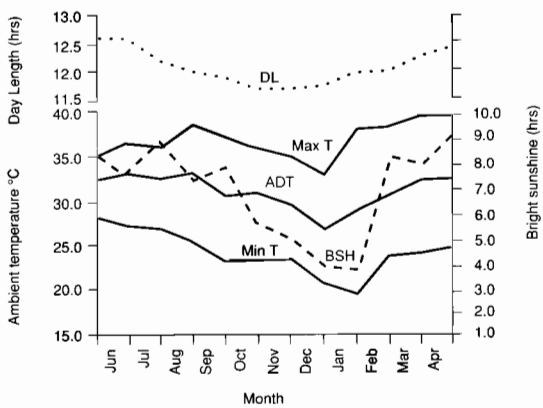

Fig. 5. Changes in the maximum (Max T), minimum (Min T) and average day temperature (ADT), day length (DL) and bright sunshine hours (BHS) in the dry zone.

Post-partum changes and conception under conditions of supplementary feeding

The proximate analysis of the supplementary feed was found to contain a dry matter content of 90.5%, ash 18.0%, crude fibre 12.2%, protein 19.5%, fat 6.3% and NFE 44.0%. The reproductive traits studied in the two genotypes fed with the supplementary ration and compared with their control groups are summarised in Table 2. Data were analysed using a 2 (buffalo genotypes) × 2 (with or without supplementary feeding) × 8 (replicates) factorial design (Steel and Torrie 1960). The mean interval from calving to complete involution of the uterus was found to be shorter (ns, $P<0.1$) for the LB than for the MB (Table 2); however there was no significant effect of supplementary feeding in the time required for this trait. The time interval from calving to the onset of the first oestrous cycle was lower in the LB than in the MB (Table 2) although the difference was not significant ($P<0.1$). The two groups of animals fed with the supplementary ration exhibited ovarian activity earlier than the respective control groups but again the differences were not statistically significant.

The calving to conception interval was significantly lower ($P<0.05$) within treatments, in the LB than in the MB (Table 2) and supplementary feeding significantly reduced ($P<0.05$) this trait in both genotypes.

Mean body weights at conception

The mean body weights at the time of conception for the two genotypes fed with and without the supplementary ration are given in Table 2. There was no significant effect of supplementary feeding for this trait. However, the MB were significantly heavier than the LB ($P<0.05$).

Supplementary feeding increased body weight gains in both breeds compared with the control groups, but all animals showed a loss in body weight around the months of September and October (Figs 6, 7, 8 and 9).

Table 1. Post-partum changes in Lankan and Murrah buffalo managed under range conditions.

	Involution of uterus (days) Mean ± SD (n)	Onset of first oestrous cycle (days) Mean ± SD (n)	Interval from calving to conception (days) Mean ± SD (n)
Lankan buffalo	26.4 ± 3.6 (28)	97 ± 73 (27)	175 ± 106 (30)
Murrah buffalo	30.0 ± 8.0 (21)	133 ± 91 (18)	250 ± 120 (22)

Table 2. Interval in days † from calving to involution of the uterus, to onset of first oestrus and to conception, and body weight (kg) at conception in the Lankan and Murrah buffalo fed the supplementary ration (SF) compared with their respective control groups (C).

	Lankan		Murrah	
	Control 8*	SF 8	Control 8	SF 8
Involution	22.8 ± 3.4	20.4 ± 5.0	25.3 ± 4.2	26.4 ± 3.9
First oestrus	69.6 ± 43.2	40.6 ± 10.6	92.4 ± 44.0	75.8 ± 67.7
Conception**	213.8 ± 115.7[a]	76.1 ± 38.0[b]	312.8 ± 115.8[c]	213.6 ± 104.1[a]
Weight	257.2 ± 27.8[a]	279.4 ± 24.7[a]	379.7 ± 29.3[b]	365.4 ± 25.5[b]

† Within rows, numbers with different superscripts are significantly different ($P<0.05$)

* Number of animals

** Interval from calving to conception

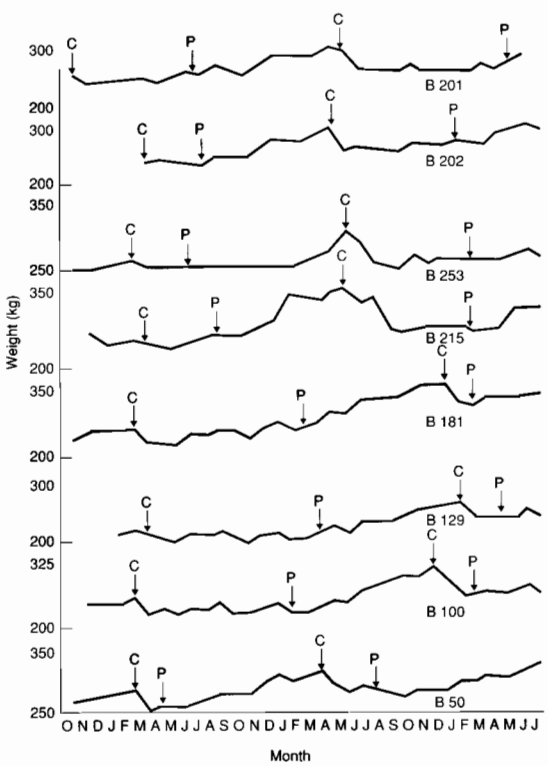

Fig. 6. Monthly body weight changes for each control Lankan buffalo (no supplementary feeding). C = calving date, P = date of conception. Individual identification numbers are shown.

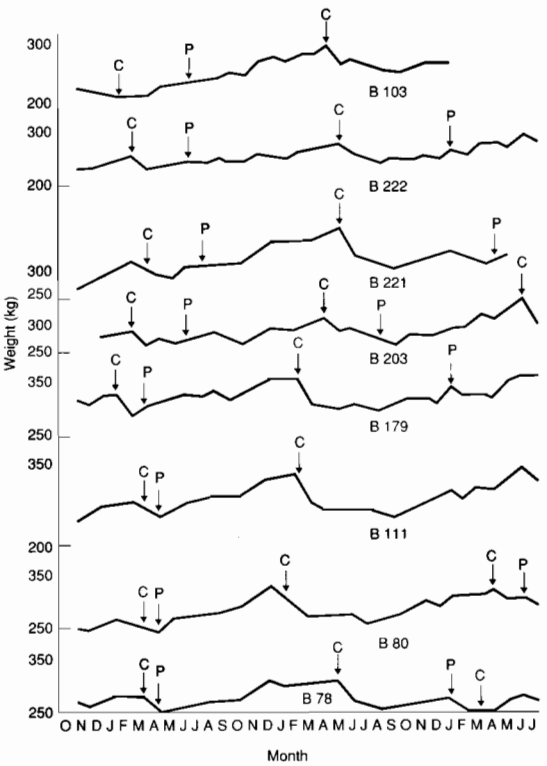

Fig. 7. Monthly body weight changes for each Lankan buffalo fed with supplementary ration. C = calving date, P = date of conception. Individual identification numbers are shown.

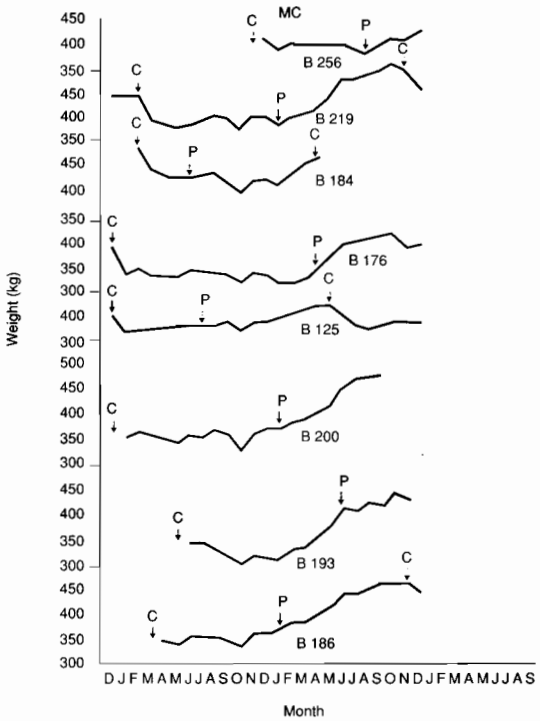

Fig. 8. Monthly body weight changes for each control Murrah buffalo (no supplementary feeding). C = calving date, P = date of conception. Individual identification numbers are shown.

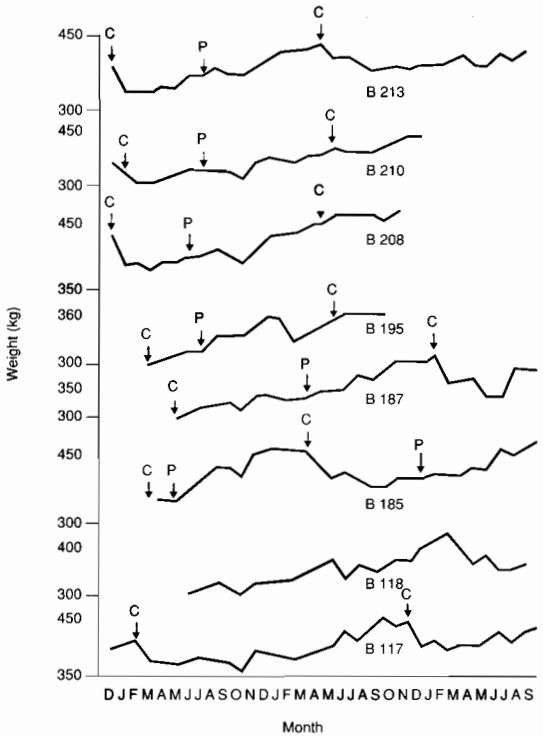

Fig. 9. Monthly body weight changes for each Murrah buffalo fed with supplementary ration. C = calving date, P = date of conception. Individual identification numbers are shown.

Response to treatment with reproductive hormones

The response to treatment with the three types of hormonal preparations used in the two genotypes namely, Folligon, PRID and EAZI-breed CIDR-B during the breeding season, are shown in Tables 3, 4 and 5, respectively. Generally, the LB were found to have a higher conception rate than the MB for all 3 treatments.

Table 3. Comparison of the conception rates of Lankan and Murrah buffalo treated with Folligon.

Animals	Lankan	Murrah
No. treated	11	12
No. conceived	5	4
% pregnant	45.0	33.3

Table 4. Comparison of the conception rates of Lankan and Murrah buffalo treated with PRID.

Animals	Lankan	Murrah
No. treated	10	12
No. retained the device	9	9
No. conceived	4	3
% Pregnant	40.0	25
% pregnant of animals which retained the device	44.4	33.3

Table 5. Comparison of the conception rates of Lankan and Murrah buffalo treated with EAZI-breed CIDR B.

Animals	Lankan	Murrah
No. treated	15	23
No. retained the device	10	17
No. conceived	6	6
% pregnant	40.0	26.1
% pregnant of animals which retained the device	60.0	35.3

Discussion

A marked seasonal trend in calving and conception has been observed in most countries where buffalo have been kept for draught, milk or meat production (Seyed Karam Shah 1988). Seasonal calving in Murrah buffalo (Mohamed 1989, Buvanendran et al. 1985, 1971) and in Lankan buffalo (Wijeratne 1962, de Silva et al. 1985 and Perera et al. 1987) has also been observed in the different regions of Sri Lanka. Calvings in both breeds were found to be high between the months of October and December associated with higher conception rates occurring between January and April in the LB; the patterns are similar to those reported by Kumaratileke, W.L.J.S. and Buvanendran, V. (1979). This has been mainly attributed to the climatic variations which are thought to have a direct effect on the physiological functions of the animal and indirectly affect the availability of required nutrients. In the dry zones of Sri Lanka, annual pasture production shows a bimodal pattern with excess forage being available from about the end of October to the beginning of March and another slight excess during May–June (Appadurai 1968). During the other months of the year, herbage availability is below the animals' requirement and needs to be supplemented for normal growth and production. The LB are mainly used for draught during the rice cultivation seasons ('Maha' and 'Yala'). Because the LB cow is a poor milk producer, farmers do not normally provide any supplementation and it is totally dependent on the natural vegetation for all its physiological requirements. As this dependence on natural vegetation under varying climatic conditions has continued for many centuries, the LB has become adapted for them and this is reflected in seasonal calvings and conceptions. The conception pattern of the MB in the region (bred there for over 3 decades) shows a similarity to that of the LB, indicating that the MB have become acclimatised to the dry zone conditions.

In the region studied, the two monsoon rains (Northeast and Southwest) produce two different peaks in annual herbage production. With the Northeast monsoon, herbage production increases and the animals show better body-weight gains; this period coincides with the main calving season (October–December) and is immediately followed by the period of peak conception. Figures 2 and 5 show that around the period of peak conception, a combination of climatic factors, including maximum, minimum and average day temperatures and the number of hours of bright sunshine and day length, remain at lower levels compared with those in other months of the year; in addition, these factors are coupled with a concomitant gradual fall in the relative humidity. In addition to the climatic factors, the measured morning, mid-day and evening rectal temperatures are at their lowest around the months of January to April (Figs 3 & 4). All these factors combine to minimise heat stress on the animals and, at the same time, provide abundant natural herbage for growth and production.

Post-partum changes

The interval from calving to involution of the uterus under field conditions in the LB was found to be 26.4 ± 3.6 days and for the MB it was 30.2 ± 8.0 days (Table 1). This is in close agreement with that observed for the LB by Perera et al., 1987 (32.9 ± 8.2 days) and for the MB Butchain et al., 1975 (37.2 days). However, shorter intervals (20.1 ± 2.9 days) have been observed by Usmani et al. (1987) for limited sucklers of Nili-Ravi buffalo in Pakistan. Post-partum supplementary feeding in the present experiment (Table 2) did not lead to any significant change in the time interval for involution of the uterus.

Under field conditions, no significant differences between the genotypes were observed in the intervals from calving to the onset of first oestrous cycle and conception. As shown in Figure 1, the two breeds showed a similar conception pattern. The MB had a calving to conception interval of 250 ± 119.7 days, and the LB an interval of 174.9 ± 105.7 days (Table 1) which was within the range of 5.1 to 7.5 months (approx. 153–225 days) reported by de Silva et al. (1985) in a survey carried out in 16 districts of Sri Lanka. In a group of 11 MB reared under more favourable environmental conditions in the mid-country wet zone of Sri Lanka (Peradeniya) Perera (1981) found the calving to conception interval to be 161.5 days. The experiment on supplementary feeding of LB and MB was carried out under favourable environmental conditions for conception but the animals selected were mostly from those that had calved just after the peak calving season (October–December). However, when supplementary feeding with a good quality concentrate ration at the rate of 1.2% of the body weight in addition to free-grazing during the day and ad lib rice-straw at night, the calving to conception intervals were 213.6 ± 104.1 days for MB and 76.1 ± 38.0 for LB, respectively (Table 2). This indicates that the LB had a superior reproductive potential to the MB. Moreover, a long calving to conception interval in the MB suggests that supplementary feeding alone cannot reduce the calving interval to an acceptable level in this genotype, although it can be done with the LB if fed during the favourable months of the year (Table 2). The observation that there was no significant difference in body weights at the time of conception between the supplemented group and its

respective control group of each genotype indicates that the weight of the animal also plays a significant role in successful conception. A similar observation was made by Patu et al. (1983) in a study on Indonesian swamp buffalo given a high and low level of nutrition; they suggested that a critical body weight must be reached for the commencement of ovarian activity. Moreover, a long calving to conception interval also suggests that nutrition and climate are not the only factors that are responsible for reduced reproductive efficiency, at least in the MB. The gene composition of the LB might be superior to that of the MB for reproduction. Seasonal differences in calving have been explained by Jalatge (1982) on the hypothesis that the buffalo population has a minimum 'refractory period' of 5 to 6 months following parturition before receiving a fertile service. Photoperiodism and seasonal variations as shown by a gradual decrease followed by a gradual rise in day length, bright sunshine hours, ambient temperature and a concomitant gradual decrease in humidity may also be playing a role in buffalo reproduction in the tropics. However, Kanai and Shimizu (1983), in a group of 8 cycling swamp buffalo examined for a period of one year under temperate conditions, reported that the animals were observed to be in oestrus throughout the year with no significant seasonal variations.

Response to hormonal therapy

Treatment of non-cycling animals of both genotypes with Folligon, PRID and EAZI-breed CIDR-B during the normal breeding season did not show any significant differences between the herd types. PRID rejection was observed in 10% of the LB and 25% in the MB, whereas rejection of EAZI-breed CIDR-B was 33.3% in LB and 26% in the MB reared together on natural vegetation among shrub-jungles. The cause for the rejection could not be identified. It is not known if they were expelled due to straining, to the thread becoming entangling while grazing around the bushes or while wallowing, or because the device in the vaginal canal was loosely placed or was pulled out by birds (especially crows) or by unknown persons. The low rejection rate of PRID observed in the LB may have been due to tight fitting of the device in the vaginal canal compared with that of the MB which has a comparatively larger lumen. Although no significant differences in the success rates were observed for the above treatments between the two genotypes, the LB showed a better response in conception rates than the MB (Tables 3,4). For the two types of intravaginal devices, the MB showed conception rates of 25% and 26% respectively, of the total animals treated. Jainudeen et al. (1981) observed that of 9 Malaysian buffalo treated with PRID all retained the device. However, ovulation occurred only in 4 animals (44%), whereas Rajamahendran et al. (1981) has reported a 26% conception rate in post-partum lactating buffalo cows treated with PRID and mated naturally. In the present experiment only 33.3% conceived among the MB which retained the PRID. Apparently, there are no published data with which to compare the conception rate obtained in the Lankan breed. Thus treatment with progesterone-releasing devices alone does not appear to be of much use under field conditions; however, usefulness may be increased if combined with pregnant mare serum gonadotropins (PMSG) as observed by Macmillan and Pickering (1988) in cows using a CIDR-B type intravaginal device (60% and 36% pregnant, in treated and control groups, respectively).

In conclusion, the comparative study has shown that the LB has a superior reproductive potential to the MB under conditions of better management. Under dry zone farming conditions as practiced by the villagers, where no supplementary feeding is provided, no significant differences were observed in the reproductive performance of the two genotypes studied. The LB also tended to show a better response to treatment with various reproductive hormones than the Murrah buffalo.

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A Comparative Study of Reproductive Performance and Growth of Female Swamp and Murrah × Swamp Buffalo under Village Conditions

M. Kamonpatana,* S. Sophon,* T. Jetana,* S. Sravasi,* R. Tongpan* and K. Thasipoo*

Abstract

This paper reports a study of the reproductive efficiency of swamp buffalo (SS) cows and a comparison of the growth and development of reproductive function in swamp buffalo and F_1 Murrah × swamp buffalo crossbreds (MS). The program was carried out on smallholder farms in two location, namely, Koksrisupan and Komalasai. At both locations oestrus was synchronised in first parity SS cows and mating was with either swamp or Murrah buffalo semen. Of the cows mated, 106 calved between the beginning of November 1986 and the end of February 1987, and their progeny were used to study growth and development.

Cows and their calves were weighed monthly from January 1987 to March 1990. All groups showed seasonal growth patterns. However, buffalo at Komalasai grew faster than those at Koksrisupan and the MS calves grew faster than the SS calves. In the SS cows, calving percentages averaged 40% or less. The percentage of these cows that resumed ovulating within 120 days of calving was higher at Komalasai than at Koksrisupan and this was reflected in a shorter calving interval, namely, 532 ± 152 compared with 682 ± 135 days. Within the age range of 24–38 months, a higher percentage of buffalo, both male and female, reached puberty in the MS than in the SS buffalo. The mean ages at the onset of puberty were lower at Komalasai than at Koksrisupan. Body weights at the onset of puberty were similar for both locations and genotypes.

IN Thailand, more information is needed about the reproductive performance and growth of swamp buffalo (SS) and of F_1 Murrah × swamp (MS) buffalo crossbreds on smallholder farms. The objectives of the work reported here were:

— to compare the reproductive performance of female swamp buffalo managed on smallholder farms at two locations, and
— to compare the growth and development of reproductive function of SS and MS buffalo of both sexes in the same locations.

The proposal was to breed both genotypes under village conditions using as dams, first parity swamp buffalo (i.e. after their first calf) which were mated by AI using swamp or Murrah semen. Reproductive performance of the cows was to be assessed in terms of conception rates, calving rates and calving intervals. The SS and MS calves were to be assessed in terms of growth rate, age at weaning, postweaning weight gain and age at puberty.

* Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Street, Bangkok 10330, Thailand

Materials and Methods

Locations

Koksrisupan and Komalasai were selected as locations for the work because they are typical of two important farming areas in Thailand. Koksrisupan is in a dry, foothills area where the soils are mainly sandy loams and there is poor feed availability for at least six months of the year. Komalasai is a mountainous area where good feed is available for about nine months of the year. There were 19 cooperating villages at Koksrisupan and 8 at Komalasai.

Oestrous synchronisation and animals available

At Koksrisupan, 166 SS cows were synchronised for oestrus by giving two injections of $PGF_{2\alpha}$ (Lutalyse® 15 mg/injection) 12 days apart. The cows

Table 1. Calving performance of cows* on village farms.

Year of calving†	Location	No. of cows mated	Conception rate (%)	Calving rate (%)
1986–1987	Koksrisupan	166	31.3	30.1
	Komalasai	139	41.7	40.3
1987–1988	Koksrisupan	35	37.1	37.1
	Komalasai	65	18.5	18.5
1988–1989	Koksrisupan	32	25.0	25.0
	Komalasai	30	30.0	30.0
1989–1990	Koksrisupan	32	††	††
	Komalasai	30	††	††

* Cows were mated by using AI at Koksrisupan after oestrous synchronisation and at Komalasai after observing oestrus.
† Calving was during the period from the beginning of November in one year to the end of February in the next.
†† Not available.

were inseminated twice — at 72h and 96h after the second injection. At Komalasai, 139 cows were inseminated at the time of oestrus as observed by the owners. Conception was detected by a progesterone assay of blood samples taken 24–26 days after the first insemination. The calving performance of the cows mated is shown in Table 1. The calving season was from the beginning of November in one year to the end of February in the next. The calving performance of the cows which calved in 1986–87 was also recorded in subsequent years and these details are also shown in Table 1. Records of mating and calving dates for these cows were used to calculate calving intervals.

Of the cows mated, 106 calved during the 1986–87 season and their progeny were used in the growth and development study. The numbers of calves available were: SS: 34 males, 39 females; MS: 10 males, 19 females. In 1988, additonal calves were added to the performance test group from 'spares' in the villages. Details of the number of animals available throughout the investigation are shown in Table 2. The fall in numbers in all groups between 1987 and 1990 reflects the unavailability of calves for various reasons.

Body weight determination. The 106 cows which calved in 1986–1987 and their surviving calves were weighed monthly from January 1987 to March 1990 using a Ruddweigh ® electronic scale. It was rarely possible to get birth weights and the birth weights shown were obtained by extrapolation of data from the regular monthly weighings.

Health control. All calves were subjected to a deworming program at ages of approximately 21, 90 and 180 days. The youngest group was dosed with Piperazine citrate (220 mg/kg BW) and the older age groups were given either Panacur® (176 mg/kg BW) or Rintal® (50 mg/kg BW).

Table 2. The numbers of buffalo calves of each genotype and sex available for performance testing in 1987 and changes in numbers during subsequent years.

		Swamp		Murrah × swamp	
Year	Location	Male	Female	Male	Female
1987	Koksrisupan	11	20	4	11
	Komalasai	23	19	6	8
1988	Koksrisupan	7	12	4	12
	Komalasai	24	25	6	10
1989	Koksrisupan	7	12	4	12
	Komalasai	7	7	3	2
1990	Koksrisupan	7	11	4	12
	Komalasai	7	7	3	2

Results

Reproductive efficiency of cows

Reproductive efficiency of buffalo cows in terms of conception rate and calving rate is shown in Table 1. The numbers of breeding cows available during 1988, 1989 and 1990 were the pregnant cows that calved in 1987; less than 2% of the cows aborted in the herds studied. In 1987, the animals from Koksrisupan were smaller than the animals from Komalasai and calving rates at Koksrisupan and Komalasai were 30.1% and 40.3% respectively. The higher calving rate at Komalasai may have been due to the fact that insemination was carried out during the peak of the breeding season when clear oestrous symptoms appeared in most of the fertile animals.

Effect of seasons on body weight of buffalo cows

From the middle of the dry season to the middle of the hot season (January–April) there was a substantial reduction of the body weights (BW) of both pregnant and nonpregnant buffalo cows (Fig.1). At the

end of the hot season (May–June) there is always a short period of intermittent rain before the rainy season (starting in July) which enables the animals to have green grass for grazing in the paddy field before ploughing. Most of the animals gain weight. It is obvious that fluctuation of BW was affected by the plane of nutrition. Shortage of feed supply was serious when the availability of a grazing area was not provided for the animals during the rice growing season.

Calving interval

More cows from Komalasai resumed ovulating (as assessed by progesterone assays) within 120 days of calving than from Koksrisupan (Table 3) and this resulted in a calving interval which was 150 days shorter at Komalasai. A different strain of swamp buffalo and the availability of green grass at Komalasai, as compared to Koksrisupan, may have contributed to the shorter calving interval.

Genotype evaluation

Body weights of all buffalo calves were adjusted to definite ages of 0, 8, 12, 18, 24 and 36 months and are shown in Table 4. Differences between means were not tested for statistical significance. Birth weights of MS males were heavier than the MS females, but in SS, birth weights of males and females were similar. The yearling BW of both MS males and females were higher than in the SS, the mean differences ranging about 32–36 kg in males and 25–33 kg in females. As age increased, the MS males and females grew faster than the SS so that, at the age of three years, the mean differences between MS and SS ranged from 78 to 116 in males and from 42 to 57 kg in females.

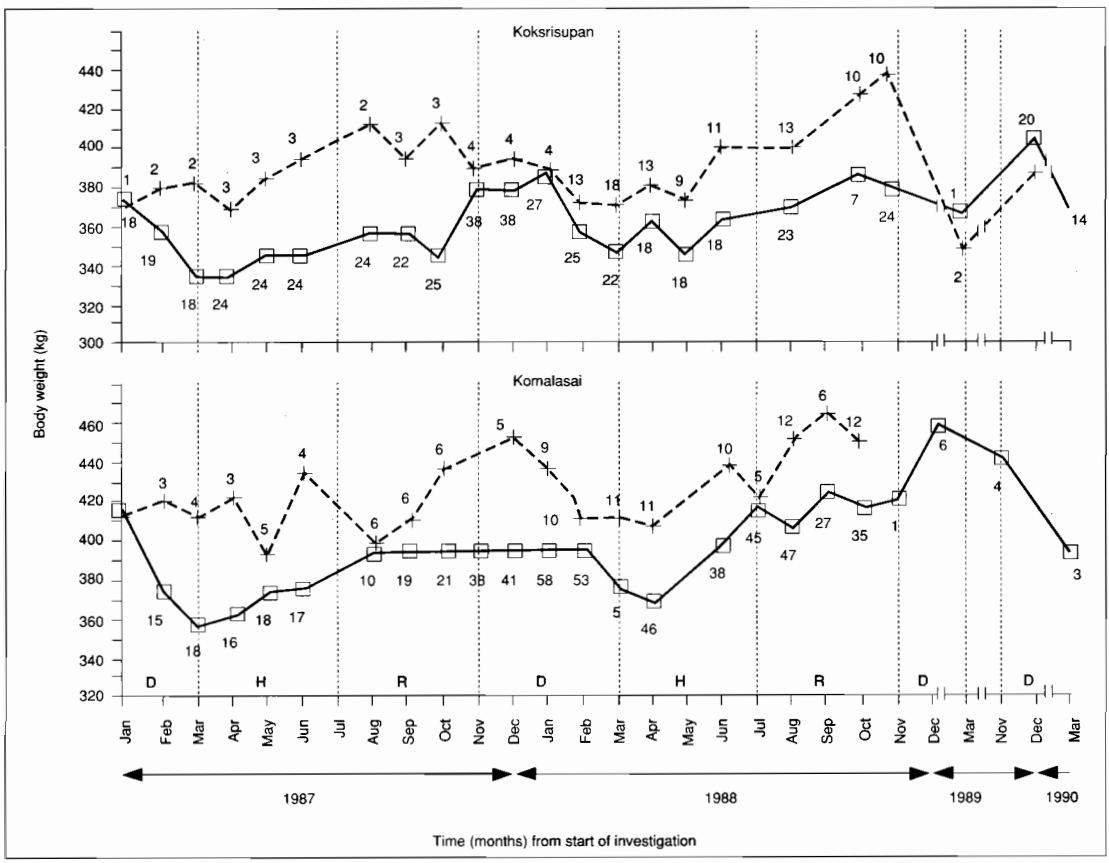

Fig. 1. Influence of seasons on body weight of pregnant (+) and non-pregnant (□) buffalo cows during 1987–1990 at Koksrisupan and Komalasai. The number of cows present on each weighing occasion is indicated on the graphs. D = dry season; H = hot season; R = rainy season. The calving season is from the beginning of November to the end of February.

Table 3. Effect of location on post-partum resumption of ovulation (as detected by progesterone assay) and subsequent calving interval. Ovulations are shown as percentages of total number of cows at each location.

Days post-partum	Location							
	Koksrisupan				Komalasai			
	SS*		SM**		SS		SM	
	n†	%	n	%	n	%	n	%
<60	2	9.5	1	7.1	1	3.8	2	25.0
60–90	3	14.3	0	0	5	19.2	1	12.5
90–120	3	14.3	4	28.6	10	38.5	2	25.0
>120	13	61.9	9	64.3	10	38.5	3	37.5
Total	21	100	14	100	26	100	8	100
Calving interval ± SD (days)	SS + SM in total of 19 animals 682 ± 135				SS + SM in total of 8 animals 532 ± 154			

* SS = Dams that delivered swamp calves
** SM = Swamp buffalo dams that delivered swamp × Murrah calves
† n = Number of cows

Table 4. Mean body weights (kg) ± SD of swamp and Murrah × swamp buffalo at various ages in the villages of Koksrisupan (Kok) and Komalasai (Kom).

Age (months)	Mean (×) ± SD n*	Swamp				Murrah × Swamp			
		Male		Female		Male		Female	
		Kok	Kom	Kok	Kom	Kok	Kom	Kok	Kom
0	X	29	30	29	29	33	32	29	30
	SD	5	6	5	6	5	7	6	7
	n	7	24	12	25	4	6	12	10
8	X	112	157	120	145	131	179	116	165
	SD	14	33	16	29	24	26	27	29
	n	7	24	12	25	4	6	12	10
12	X	156	196	148	161	192	228	173	194
	SD	16	38	47	32	5	20	46	47
	n	7	18	12	13	4	5	12	10
18	X	209	232	193	230	235	287	220	280
	SD	31	39	24	34	9	14	46	45
	n	7	17	12	8	4	5	12	9
24	X	252	268	236	258	316	346	277	353
	SD	32	48	37	44	37	9	56	53
	n	7	17	12	6	4	4	12	8
36	X	297	341	329	337	375	457	371	394
	SD	7	53	41	48	21	—	60	35
	n	3	5	5	2	2	1	6	3

* n = number of animals

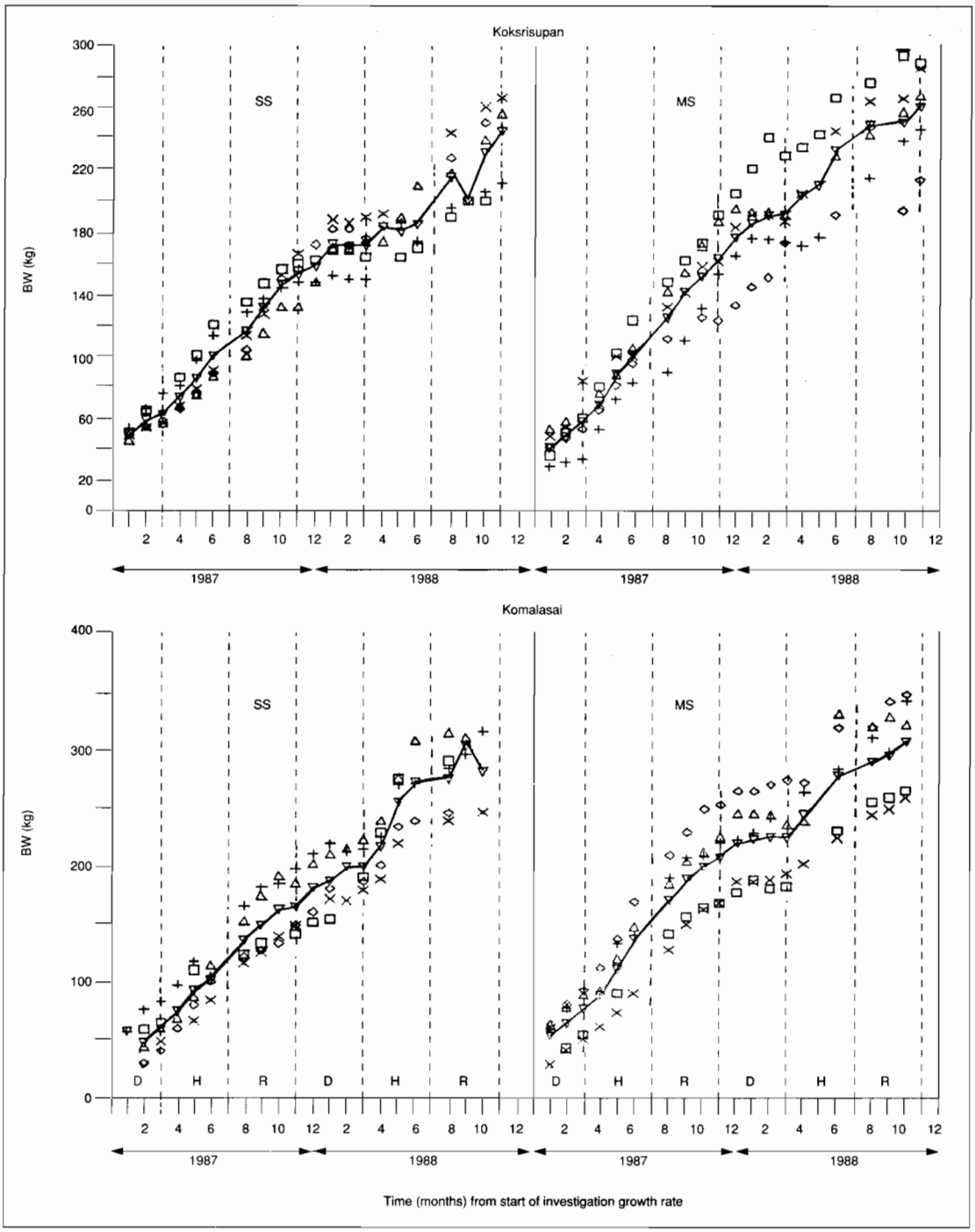

Fig. 2. Influence of season on growth rate of buffalo calves. D = dry season; H = hot season; R = rainy season. Within graphs, each symbol shows successive weights for an individual animal.

As shown in Figure 2, the growth rate of buffalo calves was reduced during the dry season when they were 12–15 months of age. Preweaning and post-weaning gain was difficult to determine in village buffalo because of weaning practices. In the villages, the calves were supposed to be naturally weaned at the age of about two months when the dams become pregnant. At this stage the dams did not always allow their calves to suck.

Growth rates in age groups ranging from 0–8, 8–12, 12–18, 18–24 and 24–36 months are shown in Table 5. Within sexes, MS buffalo, in most age groups, had higher growth rates than SS buffalo. There was no consistent difference between the growth rate of males and females.

Puberty

Puberty in males was assessed by the ages and weights when the animals were castrated, and in females by active ovarian activity. Age at castration was determined by the farmers after watching the sexual behaviour of young bulls. Ovarian activity was assessed by progesterone levels in two blood samples taken ten days apart, after the body weight of animals had reached 275 kg; progesterone levels were then checked every two months. Puberty of the animals was identified at the ages and body weights shown in Table 6. By the time the observations ended the mean percentages of animals reaching puberty at various ages (months ± SD), with data pooled from both locations were:

SS males: 33%, 29.5 ± 3.0; females: 28%, 35.2 ± 2.5.

MS males: 50%, 33.4 ± 5.6; females: 55%, 30.7 ± 4.0.

The mean body weights (kg ± SD) for these animals when reaching puberty were:

SS males: 355 ± 59, females: 342 ± 46
MS males: 392 ± 41, females: 377 ± 46

Conclusions

The program has shown that field work to assess the reproductive performance of buffalo cows and the growth and reproductive development of their progeny can be done effectively on smallholder farms. It was also apparent that the crossbreeding program and the management of the F_1 Murrah × swamp crossbreds were of interest to the farmers and did not cause them any serious inconvenience.

The reproductive efficiency of swamp buffalo cows was disappointing with calving percentages of 40% or less. There is great room for improvement here and the aim should be to achieve a calving percentage of at least 70. There were big differences between locations in the time taken for the postpartum resumption of ovulation and in calving interval. Further investigations are desirable to find out if these differences were due to genetic differences between swamp buffalo or to differences in feeding and management, or a combination of all these factors.

In terms of body weight, the MS crossbreds grew faster than the swamp buffalo and the MS are regarded as suitable for use in a fattening program for the meat market. In both locations, buffalo showed seasonal changes in growth rate but they grew faster at Komalasai than at Koksrisupan.

Table 5. Mean growth rates (g/day) ± SD of buffalo calves in villages at Koksrisupan (Kok) and Komalasai (Kom) during 1987–1990.

Range of ages (months)	Swamp				Murrah × swamp			
	Male		Female		Male		Female	
	Kok (n* = 7)	Kom (n = 24)	Kok (n = 12)	Kom (n = 25)	Kok (n = 4)	Kom (n = 6)	Kok (n = 12)	Kom (n = 10)
0–8	340 ± 63	530 ± 142	378 ± 72	466 ± 118	390 ± 140	575 ± 97	360 ± 96	625 ± 125
8–12	357 ± 160	321 ± 152	301 ± 122	347 ± 133	480 ± 180	350 ± 155	448 ± 227	449 ± 152
12–18	291 ± 97	231 ± 102	151 ± 72	186 ± 46	240 ± 50	330 ± 53	268 ± 116	329 ± 69
18–24	233 ± 149	176 ± 117	233 ± 138	187 ± 50	450 ± 200	334 ± 79	315 ± 126	359 ± 81
24–36	127 ± 21	193 ± 27	212 ± 85	225 ± 64	120 ± 90	330 (n = 1)	200 ± 84	220 ± 65

* n = number of animals

The onset of puberty in MS heifers was earlier than in the SS heifers and further work needs to be done to determine whether there are differences between these genotypes in calving interval.

Table 6. Puberty testing of 70 buffalo aged 24–38 months from Koksrisupan (Kok) and Komalasai (Kom).

	Swamp				Murrah × swamp			
	Male		Female		Male		Female	
	Kok	Kom	Kok	Kom	Kok	Kom	Kok	Kom
No. of animals								
Prepubertal testing	7	17	12	6	4	4	12	8
Reaching puberty	1	7	3	2	2	2	4	7
Percentage reaching puberty	14.3	41.2	25.0	33.3	50.0	50.0	33.3	87.5
Mean age ±SD (months) at puberty	30.1	29.4±3.3	37.0±0.8	32.6±0.7	37.3±1.1	29.5±5.8	33.8±3.5	28.9±3.2
Mean body weight ± SD (kg) at puberty	332	377±63	343±41	341±77	382±32	402±62	339±28	398±40

Comparative Growth Performance of Carabao and Carabao Crosses under Controlled (Institutional) and Smallholder Farms

B.A. Parker,* Z.M. Nava,* R.M. Lapitan,* A.N. del Barrio* and V.G. Momongan*

Abstract

The comparative performance of carabao, Nili-Ravi × carabao and Murrah × carabao crossbreds were determined under institutional and smallholder farmers' conditions. Data were obtained from body weight and measurements of 111 animals from birth up to three years of age and analysed using the method of least squares for disproportionate sub-class numbers. Analysis of main effects showed that location, breed and year were significant sources of variation affecting body weight, height, heart girth and body length. Sex was not a significant source of variation. Means showed that crossbreds raised in both institutional and farmers' herds were heavier than their carabao contemporaries. However, crossbreds raised in farmers' herds did better than those raised at the Institute. The Nili-Ravi crossbreds were heavier than the Murrah crossbreds in the farmers' herds at 30, 33 and 36 months of age. Differences between genotypes in height, heart girth and body length were generally parallel to differences in body weight. At the Institute, no significant differences in body measurements were observed between the crossbred groups.

THE swamp buffalo is an important integral part of the Filipino farmers' way of life. About 98 percent of the total swamp buffalo population are raised by smallholder farmers. On average, a typical farmer maintains 1 to 2 animals as a source of draught power, milk and meat. Mature carabaos weigh 390–550 kg, the male being heavier than the female, and some male carabaos can weigh as much as 650 kg. The carabao produces an average of 600 l of milk with a lactation period of 270–300 days. The Murrah and Nili-Ravi breeds, compared with the carabao, are bigger and have a higher milk yield than the swamp buffalo.

In the past, efforts have been made to improve the growth and milk yield performances of the carabao through breeding. Exotic buffalo genotypes such as Murrah and Nili-Ravi were used in the crossbreeding programs. The daily milk production of Carabao-Murrah crossbreds was higher than that of the Philippine carabaos. It was also reported that the crossbreds were significantly heavier than the carabaos at all ages up to 2 years. Since most of the studies were conducted in institutional herds, it was expected that there would be considerable variations between the performance of the carabaos in the institutional herds and the farmers' herds. It is not uncommon to find larger and heavier carabaos in the latter where management and feeding practices differ considerably. Thus, a comparative study would, therefore, be important.

The objective of this study was to evalute the growth performance of Carabao (PC), Carabao × Murrah (MX) and Carabao × Nili-Ravi (NRX) crosses under controlled (institutional) and smallholder farmers' condition.

Materials and Methods

Breeding groups

Matings were organised so that female carabaos were randomly inseminated with frozen semen from 2 breeds of sire, Nili-Ravi and Murrah. Frozen semen was obtained from a minimum of 6 bulls (per breed)

* Philippine Carabao Research and Development Centre, Institute of Animal Science, University of the Philippines at Los Baños, College, Laguna, 4031, Philippines

maintained at the AI stud farms of the donor countries. A carabao male × carabao female breeding group was kept as a control group for comparison. At least 10 animals per breeding group (5 males and 5 females) per test location were maintained. The test locations were the institutional herd at the University of the Philippines at Los Baños (UP) and the cooperating smallholder farmer herds in Lucban and Tayabas, Quezon. The institutional herd is about 84 to 95 km from Lucban and Tayabas, respectively. Environmental conditions for each location were recorded. The numbers of the animals in different locations during the years 1986 to 1990 are shown in Table 1.

Feeding and management

In the institutional herd, new-born calves were allowed to stay with their dams for 24 hours each day for one month and were gradually weaned upon reaching 10 to 12 months of age. Concentrates (18 to 20% CP) were given at the rate of 1 to 1.5% of the body weight up to 4 months of age and 15 to 16% CP concentrate up to 1 to 1½ years of age. Thereafter, 13 to 14% CP concentrates were given to the animals. Roughage which consisted mainly of legumes, grasses and farm by-products (rice straw, corn stover, etc.) were given ad libitum. Water was available at all times.

In the smallholder farmers' herds, new-born calves were allowed to stay with the dams until weaning (10 to 12 months of age). Feeding and management of the animals as practiced by the farmers were noted.

Calves in the institutional and farmers' herds were dewormed at one month of age and every 3 months thereafter.

Weighing and measurement of experimental animals

Weighing of the animals was done at birth (within 24 hours) and every 3 months thereafter until 3 years of age. Three consecutive weighings, with water and feed withdrawn overnight, were done in the institutional herd, while the animals raised by the farmers were weighed on two consecutive days. Body measurements (heart girth, height at withers and body length) were also taken during the weighing period. The average of the 2 or 3 weighings was used in the analysis of the data. The weights of the animals in the farmers' herds were adjusted to common ages (3-month intervals) taking into account the actual date of weighing and date of previous weighing. No adjustment was made to the data gathered from the institutional herd since the animals were weighed on specified dates at 3-month intervals.

Statistical analysis

The data were organised and coded in accordance with the data set format of the Statistical Analysis System (1982) program, general linear method procedure. Preliminary analyses were computed to evaluate the data set on the basis of several descriptive statistics such as frequencies, means, standard deviations and distribution of data among years, sexes and locations. Analyses indicated no significant difference between Lucban and Tayabas herds

Table 1. Number of animals within location, genotype, year and sex.

	UP				Lucban			
Breeds	Carabao	Nili-Ravi cross	Murrah cross	Total	Carabao	Nili-Ravi cross	Murrah cross	Total
Year								
1986	10	1	13	24	6	0	18	24
1987	0	2	3	5	3	0	7	10
1988	0	1	0	1	4	0	1	5
1989	0	2	0	2	0	9	0	9
1990	3	5	10	18	0	10	3	13
Total	13	11	26	50	13	19	29	61
Sexes								
Male	7	7	14	28	6	6	15	27
Female	6	4	12	22	7	13	14	34
Total	13	11	26	50	13	19	29	61

hence the data for these locations were pooled and, in the text from here onwards, these locations are referred to only as Lucban (LUC). Data for birthweight were complete only in the institutional herd with few data from the other locations; therefore, birthweight was not included as a dependent variable in the final analysis. In addition, in 1988 no comparisons within year and location were made due to the limited number of animals (Table 1). The final data set contained observations on 111 animals with disproportionate sub-class numbers, covering the years 1986 to 1990.

The method of least squares for disproportionate sub-class numbers was used for an analysis of variance and for determining least squares means. Two mathematical models were used. Model I, without interaction, with location, breed, year and sex as the main effects was used to assess the dependent variables based on any main effects. In Model II, with two-factor interaction, the main effects were the same as in Model I and its description is:

$$Y_{ijklm} = u + f_i + b_j + y_k + s_l + (fb)_{ij} + (fy)_{ik} + (fs)_{il} + e_{ijklm}$$

where:

$i = 1, 2$; $k = 1, 2, 3, 4$; $j = 1, 2, 3$; $l = 1, 2$;

Y_{ijklm} = observed value for the l-th sex in the k-th year by the j-th genotype in the i-th location;
u = overall population mean;
f_i = effect of the i-th location;
b_j = effect of the j-th breed group;
y_k = effect of the k-th year;
s_l = effect of the l-th sex;
$(fb)_{ij}$ = interaction effects for location and genotype;
$(fy)_{ik}$ = interaction effects for location and year;
$(fs)_{il}$ = interaction effects for location and sex;
e_{ijklm} = random error or effect peculiar to the ijklm-th observed value.

Tests of significance assumed that the effects of sires, dam and residual error (e) were random and uncorrelated with means of zero and variances of σ^2_s, σ^2_d and σ^2_e, respectively. Pairwise comparison of least squares means was done to determine which means differed significantly from one another. This was based on an SAS test for all possible probability values for the hypothesis that one least squares mean (if estimated) is equal to another least squares mean.

Graphs were prepared using the least squares means at three-month intervals to present variations of growth rates of the breeding groups within and among locations.

Results and Discussion

Body weight

Details of the least squares analysis are not presented but main effects showed that location, breed and year were significant sources of variation, affecting body weights from 3 months to 36 months of age. The effect of sex of the animals was not a significant source of variation. Significant ($P < 0.01$) genotype-environment interactions were also shown in the analysis of variance when the two-factor interactions were included in the model. These interactions may be ascribed to differences in the sensitivity of the different genotypes in different environmental conditions (Falconer 1989).

Carabao vs crossbreds. The variations in growth rate between crossbreds and carabao within and between locations are shown in Figure 1. In Lucban, the crossbred groups were significantly heavier than PC. At 12 months of age, NRX and MX were heavier than PC by 98.1 and 88.1 kg, respectively, while at UP, NRX and MX were heavier than PC by 54.2 kg and 43.9 kg, respectively. At 24 months of age, NRX and MX at Lucban were significantly heavier than PC by 150.1 and 130.2 kg, respectively. In China, Hau (1979) reported that the F_1 Murrah crossbreds were heavier and larger than the Chinese swamp buffalo. Cuong (1983) likewise reported that Murrah crossbreds at 12 months of age weighed from 160 to 200 kg compared with the Vietnamese swamp

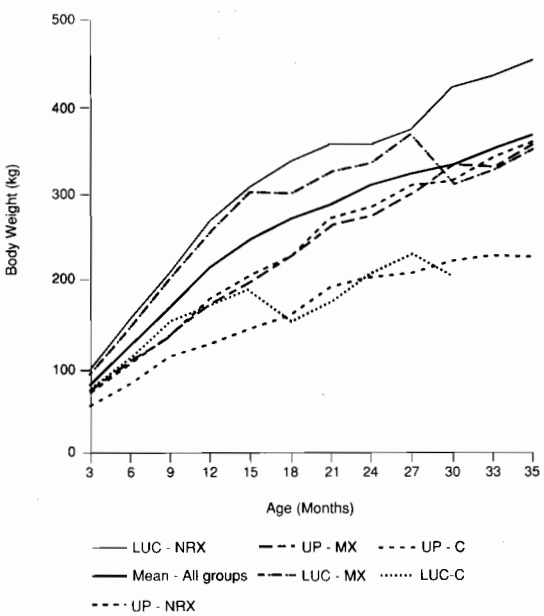

Fig. 1. Body weights of breed groups within locations.

bufflo weighing 130 to 150 kg. In Thailand, Bunyavejchewin et al. (1986) reported that F_1 Murrah-swamp crossbreds grew faster than the swamp buffalo. In contrast,however, our work showed that the weight of one-year-old Murrah-swamp crossbreds did not differ significantly from that of swamp buffalo. The average daily gain of swamp buffalo (0.43 kg/day) was higher than that of Murrah-swamp crossbreds (0.33 kg/day).

Variations in body weight between locations attributable to the effect of year are presented in Table 2. Within an age group, buffalo were generally heavier at Lucban than at UP. During 1986 and 1987, there were significant differences between locations in body weights of animals from 3 months up to 24 months of age. In 1986, significant differences between locations of 43.7 kg and 82.5 kg, respectively, were observed between the mean weights of

Table 2. Means* and standard errors, from the least squares analysis, for body weights (kg) at different ages within locations and years.

Age (months)	Location	Overall mean	Year			
			1986	1987	1989	1990
3		78.9				
	UP		59.3 ± 5.4a	78.7 ± 8.6b	68.7 ± 13.8abc	68.5 ± 4.8abcd
	Lucban		86.1 ± 6.5ce	88.5 ± 8.7cef	63.9 ± 12.4acdefg	80.6 ± 9.2cdefg
6		123.3				
	UP		100.4 ± 7.4 a	122.1 ± 12.3ab	110.1 ± 19.9abc	98.2 ± 6.9abcd
	Lucban		136.6 ± 8.1bce	146.6 ± 10.6bcef	106.6 ± 15.3abcdefg	112.3 ± 1.0abcdeg
9		170.1				
	UP		151.4 ± 8.2a	147.3 ± 15.7ab	155.1 ± 2.5abcefg	131.7 ± 8.7abcd
	Lucban		186.4 ± 10.1ce	195.6 ± 12.8cef	174.0 ± 19.1abdefg	157.2 ± 13.9abcdefg
12		244.9				
	UP		192.4 ± 8.8a	163.4 ± 16.9ab	192.2 ± 27.8abc	160.5 ± 9.3abcd
	Lucban		236.1 ± 11.2ce	288.3 ± 15.2cef	193.8 ± 21.1abcdefg	214.2 ± 15.3acefg
15		244.9				
	UP		219.2 ± 10.0a	185.8 ± 19.8ab	217.6 ± 32.7abc	191.1 ± 10.9abcd
	Lucban		288.7 ± 13.3e	279.2 ± 17.4cef	192.7 ± 25.9abcdg	237.3 ± 18.2abcfg
18		271.9				
	UP		243.3 ± 10.8a	206.9 ± 21.4ab	230.0 ± 35.4abc	221.9 ± 11.3abcd
	Lucban		317.6 ± 15.9e	317.4 ± 19.0e	180.8 ± 28.7bcd	246.0 ± 20.3abcd
21		288.6				
	UP		258.1 ± 8.8a	219.8 ± 17.4ab	238.4 ± 28.8abc	250.2 ± 9.2bcd
	Lucban		322.8 ± 14.8e	329.7 ± 19.3ef	207.7 ± 25.4abcd	287.7 ± 20.3abcdef
24		309.7				
	UP		275.0 ± 9.7a	241.3 ± 18.7abc	247.0 ± 30.8abc	281.6 ± 9.9acd
	Lucban		357.5 ± 16.1e	—	236.1 ± 30.8abdf	309.2 ± 22.1abcde
27		326.1				
	UP		227.5 ± 10.2a	244.3 ± 22.2ab	268.2 ± 38.2abc	303.3 ± 10.7acd
	Lucban		380.7 ± 17.5ae	—	246.5 ± 32.4abcd	340.5 ± 25.1cde
30		336.0				
	UP		288.5 ± 10.4a	253.7 ± 30.8b	300.4 ± 3.7c	325.0 ± 10.8cd
	Lucban		425.3 ± 20.8ab	—	196.4 ± 38.5a	323.2 ± 32.5cd
33		351.8				
	UP		296.5 ± 9.9a	234.5 ± 42.2ab	330.1 ± 31.9abc	340.1 ± 10.3cd
	Lucban		481.3 ± 26.3e	—	277.5 ± 32.4abcd	389.2 ± 21.8e
36		366.9				
	UP		317.3 ± 10.8a	259.3 ± 44.9ab	335.1 ± 34.0abc	351.5 ± 11.0cd
	Lucban		495.7 ± 28.3ae	—	302.0 ± 34.2abcd	411.7 ± 23.0ce

* Within age groups, means with no common letter are significantly different ($P<0.05$)

animals at 12 and 24 months of age. In 1987 at 12 months of age, the difference in mean weight between locations was very high (124.9 kg). In 1989 and 1990, the year effect was not a significant source of variation for differences between locations in body weight. It is possible that weighing procedures (fasting at UP but not on the farms) may have contributed to these differences between locations.

The differences in rainfall and atmospheric temperatures over years and locations are shown in Figure 2. The dry months in Lucban during the year 1986 were in February to May, while the dry months in UP were from January up to July. In 1987, both UP and Lucban had five months of dry season (January to May). The management system likewise differed between the two locations. In Lucban, the farmers fed their animals by tethering their animals under the coconut trees or in any vacant land where animals could graze. They also transferred the animals 2 or 3 times daily to another lot, thus the animals could select fresh roughage most of the time. On the other hand, animals at UP were confined and fed mainly with crop residues, particularly rice straws, and supplemented with concentrates throughout the whole experiment. Differences in the feeding management given to the animals had most effect on the growth performance of the carabaos (as opposed to the crossbreds) both in Lucban and UP. Rufener (1975) reported a decline in body weight of mature water buffalo in Northeast Thailand during the summer months from January to April because of feed scarcity.

Crossbred comparison. There were significantly heavier NRX and MX in Lucban than in UP (Table 3). At six months of age, the NRX and MX in Lucban were heavier than their corresponding crossbred groups at UP by 41.5 and 36.9 kg, respectively. Similarly, a significant difference was also observed at 24 months of age, where the NRX and MX at Lucban were heavier by 82.9 and 52.3 kg, respectively, than the corresponding NRX and MX at UP. At 36 months of age, NRX at Lucban was 91.6 kg heavier than the NRX raised at UP, but there was no significant difference between MX in the two locations. The NRX and MX crossbreds were only significantly different in weight at Lucban at 30, 33 and 36 months of age. The NRX were heavier than the MX crossbreds, the difference being 103.4 kg at 36 months of age. At UP, there was no significant difference observed between the two crossbred groups.

Body Measurements

The least-squares analysis of variance showed that location, breed and year were significant sources of variations for height, heart girth and body length measurements (details not presented). There was no significant effect of sex on heart girth and body length measurements during the early period of growth except for height from 24 months to 36 months of age. Increases in body measurements over a three-month interval were slight. A study by Famor (1986) showed increases in height, heart girth and body length, respectively of 2, 5 and 6.2 cm over a period of three months, for NRX and MX. In the present experiment, the average increase in height within a six month interval for NRX at UP was 6.6 cm compared with 5.6 cm in Lucban.

Height. Means for breed groups (Table 4) show that NRX were significantly bigger at Lucban than at UP at 12, 18, and 36 months of age. Furthermore, NRX were significantly greater in height than PC. There were no significant differences between NRX and MX at any age. In Thailand, Bunyavejchewin et al. (1988) reported that height of 12-month-old swamp buffalo was not significantly different from that of F_1 Murrah-swamp crossbreds.

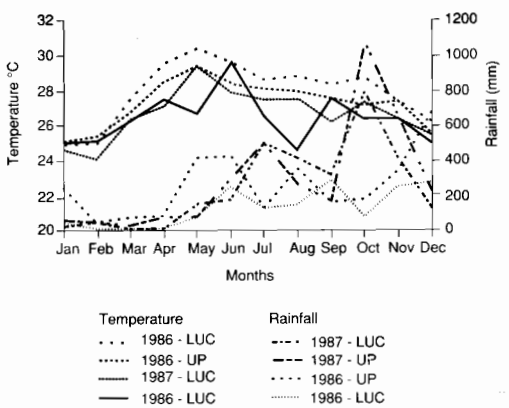

Fig. 2. Monthly temperature and rainfall during the year 1986-87 within locations.

Effect of year was evident (Table 5), for example, in 1986, buffalo aged 36 months were 14.5 cm taller at Lucban than at UP, whereas the difference in height at the same age in 1990 was only 3.5cm.

Heart girth. This is an important body measurement because it can be used to predict body weight. Regression equations were formulated using heart girth by Castillo (1981). In the present study, location, breed and year were significant factors affecting heart girth measurement. A location by year interaction was found significant which could have been due to differences in environmental conditions, particularly temperature and rainfall, between the two

locations. The other interactions tested were not significant, except at 18 months for a location × genotype interaction. Least-squares means showed significant differences between locations for heart girth of the Nili-Ravi crossbred group (Table 6). At 12, 18, 24 and 30 months of age, the heart girths of NRX in Lucban were 20.9, 33.1, 18.1 and 16.3 cm bigger than those in UP, respectively. The heart girth measurements between NRX and PC, within and between locations were significantly different. AT 12, 24 and 30 months of age, NRX in Lucban were bigger than PC by 13.9, 28.4 and 43.8 cm, respectively. Similarly, NRX at UP at the same ages, was bigger than its carabao contemporaries by 13.6, 19.6 and 25.9 cm, respectively. On the other hand, no significant differences in heart girth were observed between NRX and MX groups at ages 6 to 24 months.

Table 3. Means* and standard errors, from the least squares analysis, for body weights (kg) at 3-month intervals within locations and genotypes.

Age (months)	Location	Overall mean	Genotype		
			Carabao	Nili-Ravi cross	Murrah cross
3		78.9			
	UP		54.4 ± 10.4a	72.3 ± 5.7ab	75.1 ± 6.3b
	Lucban		68.9 ± 7.7abc	93.6 ± 11.6cde	91.6 ± 6.2cde
6		123.3			
	UP		82.2 ± 14.5a	111.5 ± 8.6b	105.9 ± 9.4abc
	Lucban		107.1 ± 40.5abc	153.0 ± 13.1d	142.8 ± 7.7ed
9		170.1			
	UP		112.0 ± 16.4a	150.0 ± 10.6ab	138.0 ± 11.7abc
	Lucban		151.4 ± 12.5ab	207.2 ± 16.3de	202.2 ± 9.6e
12		213.2			
	UP		126.5 ± 17.2a	180.7 ± 12.1b	170.4 ± 13.3b
	Lucban		170.8 ± 15.2bc	268.9 ± 17.4d	258.9 ± 10.9d
15		244.9			
	UP		145.2 ± 19.3a	205.5 ± 13.7b	196.3 ± 15.1bc
	Lucban		189.5 ± 19.5b	308.5 ± 21.1d	302.8 ± 1[illegible].2d
18		271.9			
	UP		160.3 ± 19.9a	229.7 ± 14.8b	225.7 ± 16.3b
	Lucban		153.2 ± 27.2a	338.2 ± 18.2c	304.8 ± 15.2c
21		288.6			
	UP		191.6 ± 14.8a	262.5 ± 12.3b	270.8 ± 11.3b
	Lucban		176.2 ± 11.3a	357.0 ± 17.2c	327.8 ± 51.1c
24		309.7			
	UP		203.5 ± 16.1a	274.7 ± 13.2b	285.4 ± 12.1b
	Lucban		207.5 ± 36.1a	357.6 ± 14.9c	337.7 ± 21.0c
27		326.1			
	UP		209.7 ± 16.6a	302.9 ± 14.8b	310.6 ± 12.7bc
	Lucban		229.8 ± 37.7ab	373.3 ± 15.6d	369.8 ± 21.9cd
30		336.0			
	UP		221.5 ± 18.1a	336.8 ± 15.3b	317.7 ± 14.8bc
	Lucban		206.7 ± 43.5a	420.3 ± 18.9d	311.8 ± 30.1bc
33		351.8			
	UP		227.3 ± 18.4a	330.6 ± 18.5b	340.7 ± 15.1bc
	Lucban		—	436.9 ± 18.7d	328.5 ± 29.5bc
36		367.0			
	UP		227.8 ± 19.5a	363.2 ± 19.6b	356.5 ± 15.9bc
	Lucban		—	454.8 ± 20.0d	351.4 ± 31.3bc

* Within age groups, means with no common letter are significantly different ($P<0.05$)

Table 4. Means* and standard errors for height (cm), from the least squares analysis, at 6-month intervals for locations and genotypes.

Age (months)	Location	Overall mean	Genotype		
			Caraboa	Nili-Ravi cross	Murrah cross
6		95.8			
	UP		87.1 ± 3.1a	95.1 ± 1.8b	93.9 ± 2.1bc
	Lucban		91.4 ± 2.3abcd	99.5 ± 2.6bcde	98.7 ± 1.6bce
12		109.1			
	UP		98.4 ± 2.5a	105.6 ± 1.8b	103.0 ± 1.95bc
	Lucban		103.8 ± 2.5bc	119.4 ± 2.5d	113.0 ± 1.6d
18		115.5			
	UP		104.0 ± 1.9a	110.8 ± 1.5b	110.9 ± 1.6bc
	Lucban		105.4 ± 2.7abc	119.7 ± 1.8d	120.2 ± 1.5d
24		119.4			
	UP		110.1 ± 1.6a	119.8 ± 1.3b	119.5 ± 1.2bc
	Lucban		107.2 ± 3.7d	121.6 ± 1.5bce	121.1 ± 2.1bce
30		122.1			
	UP		112.5 ± 1.7a	123.4 ± 1.5b	123.5 ± 1.4bc
	Lucban		110.0 ± 4.2a	126.2 ± 1.8bcd	119.7 ± 2.9bcd
36		125.2			
	UP		114.3 ± 2.0a	125.0 ± 2.0b	126.2 ± 1.7bc
	Lucban		—	130.9 ± 2.0cd	123.4 ± 3.2bcd

* Within age groups, means with no common letter are significantly different ($P < 0.05$)

Table 5. Means* and standard errors for height (cm), from the least squares analysis, at 6-month intervals for locations and years.

Age (months)	Location	Overall mean	Year			
			1986	1987	1989	1990
6		95.8				
	UP		93.1 ± 1.7a	96.4 ± 5.9ab	94.9 ± 4.3abc	91.7 ± 1.5acd
	Lucban		95.4 ± 1.7abcde	100.3 ± 2.5bcf	88.7 ± 3.2abcde	97.5 ± 2.4adef
12		109.1				
	UP		108.0 ± 1.3a	107.9 ± 2.5ab	109.2 ± 4.1abc	101.8 ± 1.4cd
	Lucban		111.5 ± 1.6abce	116.0 ± 2.4cef	103.9 ± 3.1abcdeg	108.1 ± 2.3abcdeg
18		115.5				
	UP		113.4 ± 1.1a	113.1 ± 2.1ab	113.2 ± 3.4abc	110.3 ± 1.1bcd
	Lucban		117.2 ± 1.6ce	122.7 ± 2.0f	105.8 ± 2.9d	114.6 ± 2.0abcde
24		119.4				
	UP		117.6 ± 0.1a	116.9 ± 1.9ab	115.6 ± 3.1abc	115.8 ± 1.0abcd
	Lucban		121.9 ± 1.6e	—	110.0 ± 3.1cd	118.0 ± 2.3abcde
30		122.1				
	UP		119.8 ± 0.1a	117.8 ± 2.9ab	121.5 ± 3.1abcd	120.1 ± 1.0abc
	Lucban		127.1 ± 2.0ce	—	108.9 ± 3.7b	119.9 ± 3.0abcde
36		125.2				
	UP		122.1 ± 1.1a	116.6 ± 4.6ab	125.3 ± 3.5abc	123.3 ± 1.1abcd
	Lucban		136.6 ± 3.0e	—	118.3 ± 3.5abcd	126.8 ± 2.4abcd

* Within age groups, means with no common letters are significantly different ($P < 0.05$)

Table 6. Means* and standard errors, from the least squares analysis, for heart girth (cm) at 6-month intervals for locations and genotypes.

Age (months)	Location	Overall mean	Genotype		
			Carabao	Nili-Ravi cross	Murrah cross
6		121.1			
	UP		110.1 ± 6.3a	115.1 ± 3.8ab	113.4 ± 4.3abc
	Lucban		117.6 ± 4.3abcd	130.3 ± 4.4de	122.6 ± 3.4abcde
12		145.3			
	UP		120.8 ± 4.5a	134.4 ± 3.2b	130.7 ± 3.5b
	Lucban		141.4 ± 3.8b	155.3 ± 3.7c	159.1 ± 2.9c
18		158.2			
	UP		135.5 ± 3.9a	149.6 ± 3.4b	166.1 ± 3.3bc
	Lucban		154.3 ± 7.6abc	182.7 ± 3.6d	175.4 ± 4.0d
24		166.9			
	UP		145.0 ± 3.3a	164.6 ± 2.7b	161.3 ± 2.5bc
	Lucban		154.3 ± 7.6abc	182.7 ± 3.6d	175.4 ± 4.3d
30		171.7			
	UP		147.6 ± 3.5a	173.5 ± 3.0b	165.8 ± 2.8bc
	Lucban		146.0 ± 8.9ac	189.8 ± 3.3d	166.9 ± 6.6bc
36		177.2			
	UP		150.8 ± 3.4a	178.5 ± 3.5b	174.1 ± 2.7bc
	Lucban		190.4 ± 3.4d	170.1 ± 5.2bc	—

* Within age groups, means with no common letter are significantly different ($P<0.05$)

Table 7. Means* and standard errors, from the least squares analysis, for heart girth (cm) at 6-month intervals for locations and years.

Age (months)	Location	Overall mean	Year			
			1986	1987	1989	1990
6		121.1				
	UP		117.2 ± 3.3a	121.4 ± 5.5ab	116.0 ± 0.0abc	110.8 ± 3.2abcd
	Lucban		127.4 ± 3.3bce	125.1 ± 5.3abcef	109.5 ± 5.9abcdef	126.4 ± 4.5abcef
12		145.3				
	UP		140.9 ± 2.4a	137.2 ± 4.5ab	134.3 ± 7.4bc	128.7 ± 5.2bc
	Lucban		147.6 ± 2.8acd	145.1 ± 4.1abcde	148.1 ± 5.0abcdeg	155.2 ± 3.7fdeg
18		158.2				
	UP		154.1 ± 2.0a	148.6 ± 4.1ab	148.9 ± 6.8bc	146.6 ± 2.2bcd
	Lucban		167.2 ± 3.1e	163.0 ± 3.9cef	139.7 ± 5.6bcd	156.4 ± 4.0abcef
24		166.9				
	UP		161.4 ± 2.0a	149.3 ± 3.8ab	160.2 ± 2.0bc	175.5 ± 3.4acd
	Lucban		175.5 ± 3.4de	183.4 ± 6.6def	153.6 ± 6.6abcfg	167.4 ± 4.6acdefg
30		171.7				
	UP		167.6 ± 2.2a	153.1 ± 5.7ab	160.8 ± 6.3abc	167.7 ± 2.0abcd
	Lucban		187.5 ± 3.9e	—	144.3 ± 7.2bc	164.7 ± 6.0abcd
36		177.2				
	UP		172.4 ± 2.1a	158.7 ± 7.6ab	165.8 ± 5.9abc	174.3 ± 1.9acd
	Lucban		201.0 ± 5.1e	—	157.4 ± 5.8c	182.0 ± 3.9d

* Within age groups, means with no common letter are significantly different ($P<0.05$)

Table 8. Means* and standard errors, from the least squares analysis, for body length (cm) at 6-month intervals for locations and genotypes.

Age (months)	Location	Overall mean	Genotype		
			Carabao	Nili-Ravi cross	Murrah cross
6		100.6			
	UP		86.3 ± 4.4a	98.8 ± 2.6b	98.5 ± 2.9bc
	Lucban		94.1 ± 3.3abcd	107.7 ± 3.6de	105.1 ± 2.3ce
12		118.8			
	UP		105.4 ± 3.5a	115.5 ± 2.4b	113.6 ± 2.7bc
	Lucban		107.0 ± 3.2ac	133.2 ± 3.4d	123.7 ± 2.2e
18		131.5			
	UP		111.5 ± 2.7a	125.5 ± 2.0b	124.5 ± 2.2b
	Lucban		115.8 ± 3.8a	138.0 ± 2.5c	137.1 ± 2.1c
24		139.6			
	UP		124.0 ± 2.7a	138.7 ± 2.2b	139.5 ± 2.0bc
	Lucban		131.8 ± 5.9acd	138.9 ± 2.5bcd	149.2 ± 3.5a
30		144.4			
	UP		127.7 ± 2.8a	146.9 ± 2.3b	144.9 ± 2.3bc
	Lucban		121.4 ± 6.6a	152.4 ± 2.9b	136.6 ± 4.6ac
36		146.8			
	UP		130.4 ± 2.6a	151.8 ± 2.6b	148.8 ± 2.1bc
	Lucban		—	153.4 ± 1.8bcd	155.3 ± 2.7bcd

* Within age groups, means with no common letters are significantly different ($P<0.05$)

Table 9. Means* and standard errors, from the least squares analysis, for body length (cm) at 6-month intervals within locations and years.

Age (months)	Location	Overall mean	Year			
			1986	1987	1989	1990
6		100.6				
	UP		92.9 ± 2.4a	103.9 ± 3.8b	96.9 ± 6.1abc	92.9 ± 2.1acd
	Lucban		102.1 ± 2.4bce	106.0 ± 3.3bcef	90.8 ± 4.6acde	98.8 ± 3.3abcdef
12		118.8				
	UP		116.0 ± 1.8a	119.1 ± 3.4ab	115.6 ± 5.6abc	108.2 ± 1.9cd
	Lucban		122.5 ± 2.3bc	129.4 ± 3.1e	109.3 ± 4.4abcdf	113.1 ± 3.1abcdf
18		131.5				
	UP		129.5 ± 1.5a	123.0 ± 2.8b	125.5 ± 4.7c	123.3 ± 1.5bcd
	Lucban		135.2 ± 2.1ce	137.3 ± 2.6e	120.5 ± 4.0bcd	128.2 ± 2.7abcde
24		139.6				
	UP		136.2 ± 1.6a	133.1 ± 3.1ab	132.3 ± 5.1abc	134.7 ± 1.7abcd
	Lucban		141.2 ± 2.7ace	—	135.6 ± 5.1abcde	143.3 ± 3.6ace
30		144.4				
	UP		140.5 ± 1.6a	139.9 ± 4.6ab	135.9 ± 5.0ab	143.0 ± 1.6abd
	Lucban		156.4 ± 3.6e	—	119.9 ± 5.8f	134.2 ± 4.7abd
36		146.8				
	UP		142.6 ± 1.9a	144.0 ± 9.3ab	151.0 ± 6.6abc	150.8 ± 2.3bd
	Lucban		157.2 ± 4.2bcd	—	142.3 ± 5.4abcde	155.7 ± 2.8abde

* Within age groups, means with no common letters are significantly different ($P<0.05$)

The effects of year on heart girth measurements taken at the two locations are shown in Table 7. For example, in 1986, with the exception of buffalo aged 12 months, all other age groups had significantly bigger heart girth measurements at Lucban than at UP. In contrast to this result, in 1990 heart girth measurements were significantly different between locations only for buffalo at the age of 12 months.

Body Length. Genotype means for body length at both locations are shown in Table 8. NRX and MX buffalo were generally longer, within age groups, at Lucban than UP. These differences were significant at the following ages: NRX — 6, 12 and 18 months and for MX — 12, 18 and 24 months.

Within and between locations, body length was generally greater in the NRX and MX buffalo than in the carabaos, although these differences were not all statistically significant.

The effect of year of measurement on body length was significant during 1986 (Table 9) when the mean body lengths of animal groups in the two locations were significantly different at all ages except 24 months. There were also significant differences in mean body length between locations in 1987 (at ages of 12 and 18 months) and in 1989 (age 30 months). There were no differences in 1990.

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Reproductive Performance and Milk Production of Philippine Carabaos and Phil-Murrah Crossbreds in a Simulated Smallholder Farmers' Environment

V.G. Momongan,* A.S. Sarabia,* A.R. Obsioma,* S.S. Capitan,* N.P. Roxas,* O.A. Palad* and E.C. dela Pena*

Abstract

There is a need to improve the productivity of the Philippine carabao, a swamp buffalo *(Bubalus bubalis)*, to meet the increasing demand for draught animal power, meat and milk through crossbreeding with the Murrah buffalo. However, there is also a need to evaluate the reproductive and productive performances of the crossbreds vis-a-vis the carabaos before adopting a large-scale crossbreeding program as a national policy. The results of this study showed that the Phil-Murrah crossbred reached puberty, conceived for the first time and gave birth to a calf one year earlier than the Philippine carabao. There was no difference in the number of services per conception and in the length of gestation period between the Philippine carabaos and the Phil-Murrah crossbreds. Postpartum reproductive performance showed no significant difference between the Philippine carabaos and the Phil-Murrah crossbreds in terms of the recurrence of first behavioural oestrus, service period and calving interval. However, the Phil-Murrah crossbreds produced more than twice as much milk per day as the Philippine carabaos. Moreover, the lactation period of the Phil-Murrah crossbred was about 49 days longer than that of the Philippine carabao.

THE Philippine carabao (*Bubalus bubalis*) is a swamp buffalo used by Filipino farmers mainly as a draught animal and, secondly, for meat and milk. On average, a carabao is used for 84 working days annually although, in a rice-based farming system, carabaos worked 98 days yearly (Alviar 1986). In some selected areas, caracows are milked for the production of soft cheese and milk-based confectioneries. Although there is a ban on the slaughter of carabaos of cerain ages in the Philippines, carabeef is sold in the local market as red meat.

In studies conducted at the village level, Momongan et al. (1984, 1985) revealed the low productivity of the Philippine carabaos which was attributed to a number of factors. In an effort to improve the size of Philippine carabaos, a breeding scheme was formulated to cross the carabaos with Murrah buffalo (Momongan et al. 1989). The carabao is expected to contribute to the draught ability and the Murrah buffalo to the size and milking ability of the crossbred. The crossbreeding program would also help in the multiplication of the dwindling carabao population. However, there is a need to compare the reproductive and productive performance of the crossbreds with the carabaos before adopting a large-scale crossbreeding program as a national policy. Moreover, the result of the evaluation would also provide a basis for the development of technological packages that include a genetic component for incorporation into existing systems for the development of buffalo productivity.

This study aims to evaluate the reproductive performance and milk production of the carabaos and the Phil-Murrah crossbreds in a simulated smallholder farmers' environment.

* Philippine Carabao Research and Development Centre, Institute of Animal Science, University of the Philippines at Los Baños College, Laguna 4031 Philippines

Materials and Methods

Experimental animals

Twenty Philippine carabaos and 20 Phil-Murrah crossbreds (Murrah × Philippine carabao) were

closely monitored to assess their reproductive performance (during pubertal, postpubertal, and postpartum stages) and milk production. All the animals were kept in community pens of eight animals per pen (four each of Philippine carabao and Phil-Murrah crossbred). They were allowed to graze by tethering from 0800–1100 hours and from 1400–1600 hours whenever feasible. Roughages consisting of legumes, grasses or farm by-products (rice straw, corn stover, etc.) depending on availability, were fed ad libitum every time the animals were in confinement. Concentrate mixtures containing 13–14% crude protein were given at a rate of 1.0–1.5% of body weight, depending on the quality of the roughage. In general, deworming was done every three months.

Determination of age at puberty, postpubertal and postpartum reproductive performance

To establish age of puberty, rectal palpation and a vasectomised bull were utilised to determine ovarian structures and occurrence of oestrus, respectively, in conjunction with measurement of plasma progesterone level. Twenty-ml blood samples were collected from each animal on the day of rectal palpation and plasma progresterone levels were analysed using the radioimmunoassay (RIA) technique. Ovaries were considered inactive when plasma progresterone level remained below 0.5 ng/ml. Heifers were allowed to cycle 3 to 4 times before they were bred but none was bred if it weighed less than 250 kg.

Services per conception were recorded and the gestation period was reckoned from the time of last service which resulted in a conception, as diagnosed by rectal palpation, until the calf was born. About two weeks before the expected parturition time, the animal was isolated from the rest of the herd in a maternity barn. Unless necessary, calving was allowed to progress without assistance. The calf was made to suckle the colostrum milk within 12 hours after calving.

Rectal palpation was utilised in the detection of pregnancy, postpartum uterine involution, ovarian structures and reproductive disorders. It was also used to estimate the anatomical dimensions of the reproductive tract.

Milk production

Milk production was recorded by getting the morning milk yield of the left and the right halves of the udder on alternate days starting one month after calving. The calf was separated at 1900 hours until after the morning milking. The calf was fed with the milk taken from the dam and a concentrate feed every time it was separated from the dam. The dam was also given a concentrate feed before milking time. Morning milk production from two quarters of the same side of the udder was recorded on alternate days. The calf was allowed to be with the dam after the morning milking at 0700 hours, and remained with the dam until 1900 hours after which the calf was again separated. Milk production was monitored throughout the lactaction period.

Progesterone assay

Plasma progesterone was determined using the radioimmunoassay technique as described by Kamonpatana et al. (1979). The lower limit of detection was 2.5 pg/ml; the recoveries for 500 pg/ml and 2 500 pg/ml were 98% and 95% (n = 30), respectively. The within and between assay coefficients of variations (CV) were 10.5% and 10.8%, respectively.

Statistical analysis

The data were analysed using ANOVA in a completely randomised design (CRD) comparing two treatments.

Results and Discussion

Prepubertal and pubertal performance

The Phil-Murrah crossbred heifers reached puberty 488 days (16 months) earlier than the Philippine carabao heifers with a weight advantage of about 74 kg in favor of the crossbreds (Table 1). In both genotypes, it appears that follicular development occurred much earlier than the commencement of first behavioural oestrus, as determined by a vasectomised bull together with other physiological manifestations of heat.

In terms of the development of the reproductive tract, the Phil-Murrah crossbred exhibited no significant difference in anatomical measurements compared with those of the Philippine carabao, indicating the size and weight of the animal at puberty had no bearing on the development of the reproductive tract in buffalo (Table 2). There were no significant differences between Philippine carabaos and Phil-Murrah crossbreds in diameter of the cervix, size of the body and horns of the uterus, as well as in the size of the ovaries.

The results of this study confirm the findings of Liu (1978) who claimed that Murrahs and Murrah-swamp crossbreds reached the age of puberty earlier than the local Chinese swamp buffalo. He reported the age of puberty for Murrah to be 431 days (range 314 to 643); for Murrah × Chinese swamp, 674 days (range 384 to 1203); and for Chinese swamp buffalo, 1405 days (range 659 to 1387).

Table 1. Pubertal performance (means† ± SD) of the Philippine carabaos and the Phil-Murrah crossbreds, with 20 animals/genotype.

Genotype[a]	Age (days) at development of first follicle	Age (days) at commencement of first behavioural oestrus[b]	Age (days) at first ovulation[c]	Weight at puberty (kg)	Body score[d]
PC	937 ± 145**	1282 ± 275**	1293 ± 286**	281 ± 31**	3.2 ± 0.4*
M × PC	681 ± 90	794 ± 107	817 ± 128	355 ± 43	3.5 ± 0.5

a: PC = Philippine carabao; M = Murrah buffalo
b: i.e. age at puberty
c: based on palpation of corpus luteum to assess development
d: based on a condition score of 1-5; 1 = emaciated, 5 = very fat
† Statistical difference within columns: *: $P < 0.05$, **: $P < 0.01$

Table 2. Anatomical measurements (means ± S.E., cm)[a] of the reproductive tract of the Philippine carabaos and the Phil-Murrah crossbreds at puberty, with 20 animals/genotype.

Genotype[b]	Diameter of the cervix	Diameter of the body of the uterus	Diameter of the mid-portion of the horns of the uterus	Dimensions of the ovaries (L × W)[c]
PC	2.2 ± 0.11	2.2 ± 0.10	1.7 ± 0.08	1.9 ± 0.76 × 1.6 ± 0.08
M × PC	2.1 ± 0.11	2.2 ± 0.10	1.8 ± 0.08	1.9 ± 0.08 × 1.5 ± 0.08

a: Measurements of left and right side for dimensions of the mid-portion of the uterine horns and for dimensions of ovaries, respectively, were not significantly different and have been pooled
b: PC = Philippine carabao; M = Murrah buffalo
c: L = length, W = width

Plasma progesterone profile

With approaching puberty, Day et al. (1987) postulated that in heifers, there is a decline in oestradiol receptors in the hypothalamus and/or pituitary accompanied by a decreasing negative feedback of oestradiol on luteinizing hormone (LH) secretion, thus, resulting in more frequent surges of LH secretion with approaching puberty. He further stated that, at puberty, oestradiol receptors in the hypothalamus and/or pituitary become variable and the increased oestradiol secreted by the Graafian follicles in the ovary would then have a positive feedback on the release of LH from the pituitary, thus causing ovulation and the eventual formation of a corpus luteum (CL). Thus, the presence of a CL in the ovary is a positive indication of ovulation, and the identification of progesterone in the plasma is an indication of a functional CL.

The trends of plasma progesterone secretion during prepubertal, pubertal and postpubertal stages in Philippine carabaos and Phil-Murrah crossbreds are shown in Figures 1 and 2, respectively. At the prepubertal stage, the plasma progesterone concentration was below 0.5 ng/ml. During this stage only developing follicles can be felt on the surface of the ovaries upon rectal palpation. However, as soon as sexual maturity was attained, as evidenced by ovulation and CL formation, there was a coincidental rise in progesterone level in the plasma. The mean pubertal plasma progesterone concentrations were observed to be 2.98 ± 1.45 ng/ml and 3.10 ± 1.36 ng/ml in Philippine carabaos and Phil-Murrah crossbreds, respectively. The length of the first oestrous cycle based on the plasma progesterone pattern ranged from 21 to 35 days for the Philippine carabaos and 21 to 28 days for the Phil-Murrah crossbreds.

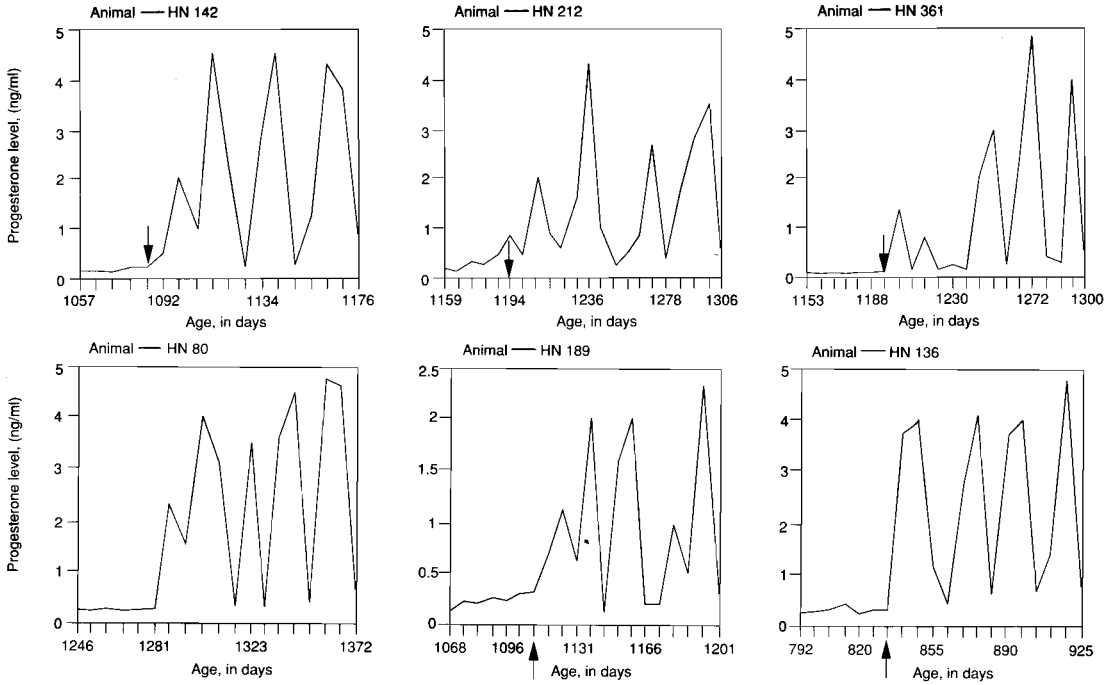

Fig. 1. Plasma progesterone levels (ng/ml) during pre- and post-pubertal stages in six PC heifers. The arrow in each diagram indicates the onset of puberty.

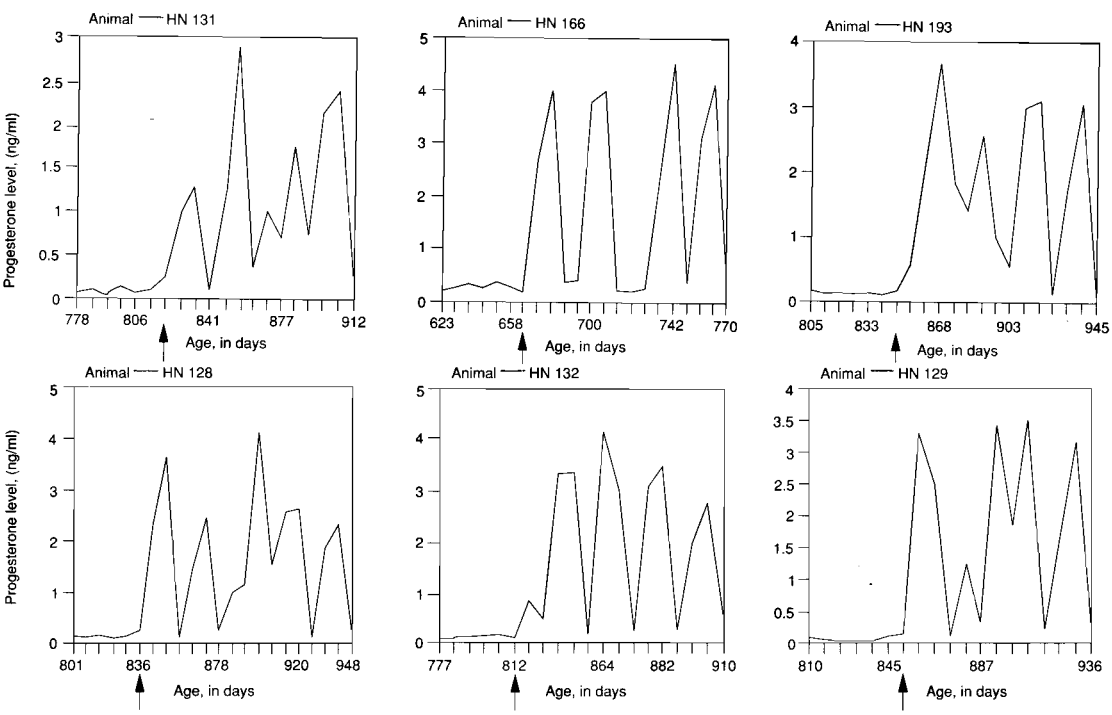

Fig. 2. Plasma progesterone levels (ng/ml) during pre- and post-pubertal stages in six M × PC heifers. The arrow in each diagram indicates the onset of puberty.

Postpubertal and postpartum reproductive performance

The Phil-Murrah crossbreds conceived and gave birth to calves one year earlier than the Philippine carabaos (Table 3). The ages at first calving in this study were greater than those reported from the literature by Momongan (1985) as 44.8 months (range: 32.4–61.7) for river buffalo and 54.6 months (range: 45.7–63.6) for swamp buffalo; however, the age at first calving reported for carabaos under the village conditions was 63.6 months (Momongan et al. 1984) and 66.0 months (Momongan 1985). There was no significant difference in the number of services per conception and in the length of gestation period between the Philippine carabaos and the Phil-Murrah crossbreds.

After calving, there was no signficant difference between genotypes in the length of time for the uterus to involute, for the recurrence of follicular development, for the animal to manifest first behavioural oestrus and for the CL to develop in the ovary, between the Philippine carabaos and the Phil-Murrah crossbreds (Table 4). However, it appears that more Phil-Murrah crossbreds exhibited silent heat because there were instances when CL could be palpated but the animals were not observed to show signs of heat.

The Phil-Murrah crossbreds had shorter service periods and calving intervals than the Philippine carabaos (Table 5) although these differences were not statistically significant. The calving interval reported in this study is longer than that reported by Momongan (1985) who recorded 482.4 days (range 430 to 551) for river buffalo and 567 days (range 448 to 726) for swamp buffalo. However, in a study conducted under village conditions, Momongan (1985) reported that the calculated calving interval for 82 multiparous cows was 26.3 ± 12.2 months (range 11.5–57.5); that is: 789 ± 366 days (range 345 to 1725).

Milk production on first lactation

The milk production of first lactation Philippine carabao and Phil-Murrah crossbreds is presented in Table 6. The improvement in average daily milk production in the Phil-Murrah crossbred over that of the Philippine carabao was 165%; and 235% improvement if based on total milk production. Likewise, the length of lactation of the Phil-Murrah crossbred was significantly longer ($P<0.05$) which contributed to the greater milk production of the Phil-Murrah crossbred compared with that of the Philippine carabao. Similar findings were reported

Table 3. Post-pubertal performance (means ± SD) of the Philippine carabaos and the Phil-Murrah crossbreds.

Genotype[a]	n[b]	Age at first conception (days)	n	No. of services/ conception	n	Age at first calving (days)	n	Gestation length (days)
PC	12	1585 ± 422**	12	2.5 ± 1.9	11	1924 ± 40**	11	322 ± 11
M × PC	20	1237 ± 388	20	2.4 ± 1.4	20	1556 ± 338	20	319 ± 6

a: PC = Philippine carabao; M = Murrah buffalo
b: n = number of animals
** Statistical differences within columns: $P < 0.01$

Table 4. Post-partum reproductive performance (means ± SD, days) of the Philippine carabaos and the Phil-Murrah crossbreds. Times are calculated from date of calving.

Genotype[a]	n[b]	Involution of uterus	n	First follicular development	n	First behavioral oestrus	n	First corpus luteum development
PC	11	50 ± 10.0	11	73 ± 22	11	146 ± 83	11	152 ± 84
M × PC	20	43 ± 12.0	20	55 ± 27	16	152 ± 52	16	143 ± 53

a: PC = Philippine carabao; M = Murrah buffalo
b: n = number of animals

by Le Xuan Cuong (1983) who claimed that the Vietnamese swamp buffalo produced only 2.0 kg of milk per day, while the crossbred produced 2.5 to 4.0 kg of milk per day. Similarly, Liu (1975) reported that ¾ Murrahs and ½ Murrahs in Taiwan produced 4.2 and 3.4 kg of milk per day, respectively. The significant improvement in milk production of the Phil-Murrah crossbred over that of the Philippine carabao is another justification for a crossbreeding program.

Table 5. Service period and calving interval of the Philippine carabaos and the Phil-Murrah crossbreds (means ± S.D.).

Genotype[a]	No. of animals	Service period (days)	Calving interval (days)
PC	7	423 ± 223	866 ± 256
M × PC	7	339 ± 174	675 ± 177

a: PC = Philippine carabao; M = Murrah buffalo

Table 6. Milk production of first lactation Philippine carabaos and Phil-Murrah crossbreds (means†).

Genotype[a]	No. of animals	Total milk production[b] (l)	No. of days actually milked (days)	Average daily milk production (l)
PC	7	194.8**	187.3*	1.04**
M × PC	7	652.5	236.4	2.76

† Statistical differences within columns: *: $P < 0.05$, **: $P < 0.01$

a: PC = Philippine carabao; M = Murrah buffalo

b: Milk production during the first month of lactation was not measured; the calf stayed with the dam during the first month post-calving. Milk production represents only morning milking.

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The authors are grateful to the University of the Philippines at Los Baños for providing facilities to conduct these studies, which form part of the research conducted under ACIAR Buffalo Project 8515 and GOP/FAO/UNDP Project PHI/86/005.

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Semen Characteristics of Different Buffalo Genotypes

M. Hilmi*

Abstract

Semen from water buffalo of different genotypes with varying chromosome complements was evaluated in order to observe the effect of unbalanced gamete formation in genotypes with chromosome complements of 2n = 49. This study showed that the ¾ swamp buffalo (2n = 48) is similar to its swamp ancestor in terms of sperm production and has a similar chromosome complement. Likewise the ¾ river buffalo (2n = 50) is related to the river buffalo. However, with regard to crossbred genotypes with 2n = 49, these relationships do not follow.

CROSSBREEDING by mating the river buffalo (2n = 50) and swamp buffalo (2n = 48) has been practiced in many Asian countries. This mating produces an F_1 hybrid with a chromosome complement of 2n = 49, the combination of the haploid sets of each parent (Bongso and Hilmi 1982). The karyotype of this hybrid consists of 4 pairs of sub-metacentric chromosomes, 19 pairs of acrocentric chromosomes and, in addition, 3 unpaired chromosomes: a large metacentric chromosome (a 4/9 tandemly fused chromosome), a sub-metacentric chromosome (chromosome number 4) and an acrocentric chromosome (chromosome number 9).

The F_1 hybrids are fertile: so also are the inter se matings of F_1 types to produce second generation hybrids (F_2) and the backcrosses to either parent type (¾ swamp and ¾ river buffalo). However, since the F_1 is heterozygous for a balanced tandem fusion, problems could occur during meiosis when homologous chromosomes pair during the first meiotic stage and segregate during the subsequent stages (Bongso et al. 1983). This has been demonstrated in meiotic chromosome analyses and in histological studies from semi-thin sections of testicular biopsies (Hilmi 1984). Both studies indicated that six to eight possible types of gametes could be produced, but only two out of these gametes have a genetically balanced configuration. The fate of the gametes with the unbalanced chromosome materials and their effect on semen values remain to be be investigated. Therefore, the objective of this study was to evaluate the semen of different genotypes of buffalo bulls, especially those with 2n = 49 chromosomes, to observe any effect of unbalanced gametes.

* Department of Animal Sciences, Universiti Pertanian, Malaysia 43400 Serdang, Selangor, Malaysia

Materials and Methods

Eleven buffalo bulls of different genotypes were used. These comprised two swamp (2n = 48), two ¾ swamp (2n = 48), three ¾ swamp (2n = 49), two ¾ river (2n = 49) and two ¾ river (2n = 50) buffalo. They were housed in a shed within individual pens when they reached two years of age, and were fed daily with grass ad libitum, supplemented with 2 kg of pelleted commercial feed. For the following six months, they were trained for semen collection using an artificial vagina. During training and semen collection various types of buffalo of both sexes were used, as dummies, as they showed certain preferences towards different genotypes or sexes. At the end of the six months, semen was collected and evaluated twice weekly, for a period of five weeks.

Results and Discussion

Vidyadaran and Azmi (1989) showed that swamp bulls responded to male swamp or ¾ swamp buffalo as dummies, whereas the ¾ swamp bulls exhibited individual preferences. However, the ¾ river bulls accepted the ¾ river or swamp buffalo of both sexes, but refused to mount the ¾ swamp buffalo. In this study, attempts were made to evaluate semen from two F_1 bulls that had been used for natural mating. However, these bulls had to be set aside since they refused to mount females of any

genotype. To enable semen collection from such bulls, female buffalo which are on heat may have to be used.

The results for semen characteristics of different genotypes (Table 1) correspond to those reported by Situmorang and Setipu (1989), and Ramakrishnan et al. (1989). Jainudeen et al. (1982) reported higher values for semen volume, sperm concentration and total sperm per ejaculate than those reported here. However, their higher values may have been due to the fact the bulls used were animals selected for collection of semen for artificial insemination. In the present experiment, there was no significant difference between the swamp buffalo (2n = 48) and the ¾ swamp buffalo (2n = 48), except for percent live sperm in the swamp buffalo. The semen characteristics for the ¾ river (2n = 50) were similar to those for Murrah buffalo (2n = 50), as reported by Bhattacharya et al. (1978). This result indicates that those animals of similar karyotype can be considered to possess a similar genotype irrespective of breed type.

There was no significant difference between the percentages of live sperm in the ¾ swamp (2n = 49) and the ¾ river (2n = 49) buffalo but values for these variables were lower than for genotypes with chromosome complements of 2n = 48 and 2n = 50. Buffalo bulls which are heterozygous for the balanced tandem fusion (2n = 49) produced a high percentage of abnormal sperm and a low percentage of live sperm.

It was also observed (Table 1) that the ¾ swamp genotype (2n = 49) had mean values for semen volume, sperm concentration and total sperm per ejaculate that were significantly higher than for animals with a chromosome complement of 2n = 48, but not significantly different from the ¾ river genotype with 50 chromosomes. This may indicate some expression of hybrid vigour. However, the ¾ river genotype with 2n = 49 has significantly lower values than the ¾ swamp genotype with 2n = 49. In fact one of the ¾ river genotype (2n = 49) used for this study produced aspermic semen. Situmorang and Setipu (1989) showed that their F1 bulls had low semen quality with low semen volume, low sperm concentration and low total number of sperm per ejaculate, but a high percentage of dead sperm and abnormal sperm. What mechanism causes the variation in the genotypes of buffalo with a chromosome complement of 2n = 49 remains to be investigated.

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Table 1. Semen characteristics (mean* ± SD) of water buffalo genotypes with chromosome complements of 2n = 48, 49 and 50.

Genotype	Number of bulls	2n	Semen volume ml	Sperm concentration × 10^9/ml	Total No. of sperm/ejaculate × 10^9	Live sperm %	Abnormal sperm %
Swamp	2	48	1.66 ± 0.60^a	0.992 ± 0.22^{ab}	1.695 ± 0.94^a	86.52 ± 5.64^a	8.95 ± 1.15^a
¾ Swamp	2	48	2.08 ± 0.50^a	0.923 ± 0.18^a	1.951 ± 0.71^a	84.50 ± 5.88^b	9.78 ± 1.59^a
¾ Swamp	3	49	2.85 ± 0.68^b	1.087 ± 0.20^b	3.072 ± 0.78^b	77.75 ± 5.57^c	30.07 ± 12.42^b
¾ River**	2	49	1.94 ± 0.81^a	0.165 ± 0.10^c	0.344 ± 0.12^c	67.60 ± 6.41^c	53.40 ± 5.73^c
¾ River	2	50	3.01 ± 0.97^b	1.075 ± 0.12^b	3.233 ± 1.11^b	80.50 ± 4.76^d	19.85 ± 3.45^d

* Means in the same column with different superscripts indicate the values were significantly different ($P < 0.05$). Significance testing is based on variations among replicates semen collections.

** Only semen volume was analysed from 2 animals. Other measurements were based on one animal as only one of the two animals produced semen.

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Comparative Growth Performance, Semen Quality and Draught Capacity of the Indonesian Swamp Buffalo and its Crosses

P. Situmorang* and P. Sitepu*

Abstract

The performance of Indonesian swamp, river and crossbred river × swamp buffalo was studied on an institutional farm and in villages. The body weight of crossbreds was significantly higher than that of their parents. Body weights (kg) of swamp and crossbred buffalo were (respectively) 22.4 and 24.9, 60.4 and 71.0, 102.0 and 126.3, 134.4 and 167.3 at 0, 3, 6, and 9 months of age. The superiority of the crossbred was also recorded for older buffalo. At 6 and 9 months of age, body weight was significantly higher in villages than on institutional farms. Male calves grew faster than females. Body size of crossbreds was significantly larger than that of swamp buffalo.

The gestation periods were 324 and 325 days for swamp and crossbred cows, respectively. Gestation periods were three days longer for male than for female calves.

Measurement of semen characteristics showed that all three genotypes produced about the same volume of semen per ejaculation but swamp buffalo had higher values for sperm concentration, total sperm, motility and percentage of live sperm than river and crossbred buffalo. Semen quality improved with the onset of the wet season and, in all genotypes, it was suitable for use in A.I. programs. The libido of river buffalo and crossbreds was significantly better than that of swamp buffalo and there was an indication that river and swamp buffalo bulls preferred to mate with cows of their own respective genotypes.

Both swamp and crossbred buffalo gave a similar response to a working load. Pulse rates, respiration rates and rectal temperatures were similar for swamp and crossbred buffalo 2 hours before work, during work and 2 hours after work.

DURING the last four years, the Indonesian Government has focused its effort to conserve and increase the population of buffalo. Although this species has proven its role in the farming system in Indonesia, it appears that more effort should be made to optimise its potential and to increase its population. In the early 1980s the buffalo population in Indonesia was about 2.5 million which was the seventh largest in the Asian and Pacific region (FAO 1983). According to the Directorate General of Livestock Services (DGLS), the population has increased during the five years to 1989 when it was 3.3 million.

Almost all of the Indonesian buffalo are of the swamp type with only a few hundred of the river type (Murrah) which are found mainly in North Sumatra. In some regions, the swamp buffalo has adapted naturally to the local environment; some distinguishing characteristics, such as distribution of colour (white especially), average size, behaviour and horn shape differ markedly between regions.

Both male and female buffalo are used primarily for draught purposes, not only in the wet area of Java but also in the drier areas of eastern Indonesia. In North Sumatra, a few of the river type, which were originally imported from India in the 19th century, are still maintaining their purpose as milk producers. Although the price of buffalo milk in this region is almost double that of milk from cattle, it appears that only people of Indian descent are interested in keeping these animals. The slow increase of the buffalo population in Indonesia is directly related to its low reproductive rate. Among all reproductive characteristics, a low conception rate

* Research Institute for Animal Production, PO Box 123, Bogor, Indonesia

is the most serious problem to be overcome. Moreover, an increasing demand for buffalo meat tends to result in over-slaughtering of these animals.

In order to increase the productivity, a cross-breeding program was launched by the Indonesian Government in 1950 (Toelihere et al. 1981).

The present study was conducted to compare Indonesian swamp buffalo and their crosses in terms of growth, semen characteristics and their physiological responses to a working load.

Materials and Methods

The studies were conducted under institutional conditions at the Research Institute for Animal Production (RIAP) and in West Java, Central Java and North Sumatra under village conditions to evaluate the performance of different buffalo genotypes for reproduction, growth and draught capacity.

The growth performance study

In the initial phase of this study, the growth performance of each genotype of buffalo (river and swamp) was evaluated by conducting surveys.

In order to produce the crossbreds required, female Indonesian swamp buffalo were randomly inseminated with frozen semen collected from river and swamp buffalo bulls. Semen was collected by artificial vagina (AV), diluted in a lactose-based solution to reduce the concentration of sperm to a level of not less than 100 million live sperm/ml; it was then frozen in vapour of liquid nitrogen for 8–10 minutes and stored. All females were inseminated with either swamp or Murrah semen, three and four days following synchronisation of oestrus, using $PGF_{2\alpha}$. Unless otherwise stated, the term 'crossbred' refers to the F_1 progeny of Murrah bulls mated to swamp buffalo cows.

Feeding and management On the institutional farm, new born calves were allowed to stay with their dams for 24 hours and weaned at four months of age. All calves were fed ad libitum with elephant grass and supplemented with 0.3 kg of rice-bran/day; water was available at all times.

In the villages, new born calves were allowed to stay with their dams all the time and no weaning was practiced. Feeding and managment of the animals, as practiced by the farmers, were noted.

Calves on the institutional farm were dewormed at one month of age while in the villages, deworming was not regularly practiced.

Body weight and surface measurements Body weight was recorded at birth (within 24 hrs) and every three months thereafter. In the surveys, body weight was recorded only during the visits. On the institutional farm, body weight was measured early in the morning, before feeding. The weights of the animals on the village farms were adjusted by interpolation between weighing dates, to provide estimated body weight at three-monthly intervals. Body measurements were made on calves at the institutional farm and during the survey. They included length of body (distance from the pin bone to the anterior edge of the shoulder), heart girth and height at withers.

Semen quality study

The study was designed to evaluate the semen quality collected from swamp, two river and three crossbred buffalo bulls which were evaluated during a period of six months. All bulls were fed ad libitum with elephant grass and no supplementation was practiced. Drinking water was available at all times. Bulls were housed individually in pens 2m × 2m and no exercise was allowed except when leading the bulls to the site of collection (25m from the stable). Both swamp and river bulls were used as teasers, to find out if there was a preference by swamp buffalo cows for a particular genotype of bull.

Semen collection Semen was collected once a week using an artificial vagina and the number of ejaculates obtained during a period of 15 minutes was recorded. The time spent from when a bull entered the site of semen collection until the first ejaculate was obtained was recorded as the libido test.

Each semen sample was examined for volume, motility (0–5 grades), sperm density, total sperm per ejaculate and percentage of live sperm.

Reproductive performance

The reproductive performance of Indonesian swamp buffalo, following synchronisation with $PGF_{2\alpha}$, was studied under both institutional and village conditions.

Synchonisation of oestrus This study was conducted under institutional conditions. Sixteen mature, non-pregnant cows were injected (intramuscularly) with 2ml Estrumate (equivalent to 500 μg cloprostenol). Blood samples were collected on the day of injection and after 2,3,4,5,6 and 7 days for plasma progesterone assays.

In a second trial, 20 cows were used and the procedure for synchronisation was the same as in the first trial. All cows were closely observed by joining them with a vasectomised bull. Oestrous behaviour (swelling of vulva, bellowing, raised tail and mucous discharges) was also recorded.

In another trial under village conditions, a total of 258 mature, non-pregnant cows were palpated per rectum to determine the presence of a corpus luteum (CL) and follicles. Cows with a palpable CL or follicle were assigned for synchronisation, while cows which possessed a small ovary and the absence of a CL or follicle were categorised as inactive ovaries and excluded from the experiment. The method of synchronisation and prostaglandin preparation used in the villages was similar to the procedures used under institutional conditions. All cows were inseminated by A.I. 2 and 3 days after synchronisation, depending on the oestrous status of each cow.

Fertility A pregnancy test was conducted by rectal palpation at day 60 following insemination. Conception rate was calculated as the number of animals conceived divided by the number of cows which were observed to be in oestrus and inseminated.

Effect of work on physiology and semen quality

A study was conducted to evaluate the effect of work on the physiology and semen quality of buffalo. Three swamp buffalo bulls and three crossbred bulls were individually housed in 2m × 5m pens and offered chopped rice straw ad libitum for three months. The animals were not allowed to wallow and no special management was given except for work in the morning, each day for three months. For one month of work each animal pulled a sledge with a load of 40–60 kg for four hours each day. At the same time, physiological measurements, including pulse rate, respiration rate and rectal temperature were taken two hours before, during, and two hours after the period of work. Body weight and semen quality were also recorded during the three months of this experiment.

Results and Discussion

Crossbred calves

On village farms, near Yogjakarta, 5 out of 12 crossbred calves were lost due to abortion in late pregnancy. Of three calves born on the institutional farm, one calf died during delivery. The incidence of dead calves is believed to have been due to poor management during the calving period. Characteristics of pure swamp buffalo, such as colour of the body and presence of chevrons, were observed in one third of all crossbred calves.

Body weight and size

The average body weights and measurements of size of the Indonesian swamp, river and crossbred buffalo, recorded in the survey, are shown in Tables 1 and 2. The average body weights at different ages, of calves born by using A.I., are shown in Tables 3, 4, and 5; the crossbreds are first cross Murrah × swamp buffalo.

The body weights of crossbreds were significantly higher ($P < 0.01$) than those of Indonesian swamp buffalo at all ages recorded (Tables 2 and 4). Similar results were observed also for heart girth, body length and height where the crossbreds were bigger

Table 1. Survey means (± SD) for body weight and size of River (Murrah) buffalo of both sexes at various ages.

Age (years)		Body weight (kg)		Body measurements (cm)					
				Girth		Height		Length	
		F*	M*	F	M	F	M	F	M
<1	n**	14	5	17	9	17	9	17	9
	x†	115	72	120	112	99	93	89	83
	SD	48	20	21	19	12	10	13	16
1–2	n	27	4	32	5	32	5	32	5
	x	283	211	165	135	120	104	116	97
	SD	41	24	23	30	11	15	13	15
2.5–4	n	19	3	32	3	32	3	32	3
	x	407	507	187	198	129	134	130	139
	SD	46	26	23	5	8	2	9	3
>4	n	63	7	63	7	66	7	64	7
	x	452	514	196	195	133	133	136	139
	SD	50	65	10	12	5	8	6	8

* M = male, F = Female
n** = number of animals observed
x† = mean

Table 2. Survey means (± SD)* for body weight and measurements of crossbred (Murrah × Swamp) and Swamp buffalo at various ages at Brebes and Ngawi.

Age (years)	Genotype	n†	Weight (kg)	Measurements (cm)		
				Girth	Height	Length
Brebes						
1	Crossbred	5	230 ± 40	158 ± 9	110 ± 2	103 ± 9
	Swamp	6	177 ± 28	147 ± 8	106 ± 5	101 ± 10
1.5	Crossbred	4	345 ± 26	172 ± 6	122 ± 9	126 ± 12
	¾ Swamp	4	285 ± 36	175 ± 11	120 ± 18	113 ± 6
	Swamp	3	230 ± 16	165 ± 6	107 ± 1	107 ± 4
2.5	Crossbred	3	434 ± 55	199 ± 10	129 ± 6	133 ± 15
	Swamp	2	290 ± 28	174 ± 2	120 ± 11	118 ± 3
3.5	Crossbred	5	445 ± 38	194 ± 7	129 ± 7	137 ± 10
	Swamp	2	354 ± 20	187 ± 4	118 ± 6	118 ± 2
5	Crossbred	8	450 ± 56	199 ± 11	131 ± 7	139 ± 9
	Swamp	8	383 ± 46	190 ± 11	122 ± 5	131 ± 9
Ngawi						
1.5	Crossbred	4	337 ± 42	174 ± 9	120 ± 6	118 ± 3
	Swamp	3	216 ± 17	162 ± 10	110 ± 5	106 ± 6
3	Crossbred	6	475 ± 83	200 ± 12	133 ± 5	129 ± 6
	Swamp	14	363 ± 14	197 ± 8	128 ± 2	134 ± 6
5	Crossbred**	—	—	—	—	—
	Swamp	4	435 ± 50	192 ± 11	122 ± 3	126 ± 5

† Number of animals

* Differences between genotypes, within age groups, were all statistically significant; for body weight: $P < 0.01$ and for size measurements: $P < 0.05$

** Data not available for this age group

Table 3. Mean body weights (kg) of Swamp buffalo and crossbreds under institutional and village conditions.

Location	Age (months)	Swamp		Crossbreds	
		Male	Female	Male	Female
Institutional farm					
Number of calves		2	2	1	1
	0	21.0	21.5	30.0	27.0
	3	55.5	59.5	76.0	74.0
	6	91.0	100.0	121.0	115.0
	9	131.0	132.0	162.0	157.0
	12	149.0	142.5	173.0	170.0
Villages					
Number of calves		3	2	4	3
	0	24.0	22.5	24.3	23.3
	3	64.0	61.0	70.5	69.0
	6	110.7	102.0	130.0	127.0
	9	140.7	131.0	168.5	160.0

than the Indonesian swamp buffalo (Tables 2 and 6). The results obtained in this study were similar to those reported by others in Malaysia, Philippines, Thailand, India and China (Yongzuo Xiao 1988).

At the age of one year, body weight of river (Murrah) buffalo (Table 1) was higher than that of swamp buffalo (Table 2) but was lower than that of the crossbreds (Tables 2 & 4). This comparison of results presented in different tables involves buffalo grown in different environments; however, the differences are thought to truly reflect the ranking in size of these three buffalo genotypes.

Table 4. Differences between genotypes in mean* body weight (kg). The table includes pooled data from both sexes.

Age (months)	Swamp (n = 9)	Crossbreds (n = 9)
0	22.4[a]	24.9[b]
3	60.4[a]	71.0[b]
6	102.0[a]	126.3[b]
9	134.4[a]	163.7[b]

* Values with different superscripts in the same row are significantly different ($P < 0.01$)

Table 3 shows a comparison of body weights of swamp and crossbred buffalo at various ages, grown under institutional and village conditions. Numbers of animals were small but indications were that, apart from the birth weights of calves born in the villages, crossbreds were generally heavier than swamp buffalo up to the age of nine months in both types of environment. Again, apart from birth weight, body weights of buffalo grown in the villages tended to be heavier than those of buffalo grown on the institutional farm.

Table 5. Mean* body weights (kg) of Swamp buffalo reared under different conditions. The table includes pooled data from both sexes.

Age (months)	Institutional farm		Villages	
	n	Weight	n	Weight
0	6	23.7	12	23.7
3	6	63.3	12	66.9
6	6	103.0[a]	12	119.8[b]
9	6	140.8[a]	12	153.2[b]

* Values with different superscripts in the same row are significantly different ($P < 0.05$)

The results reported here are in agreement with those reported by B. Parker in the Philippines where the carabaos and crossbreds raised by farmers' herd were generally heavier than those raised in an institutional herd.

The lower body weights of buffalo grown either under institutional or village conditions shown in Table 3 compared with those grown at Ngawi and Brebes (Table 2) were related to differences in age and in feed quality and management. All buffalo raised on the institutional farm were fed elephant grass ad libitum and no supplementation was given. In the villages, the roughage source was mainly field grasses which were sometimes mixed with tree legume. Additionally, the buffalo under institutional conditions were fenced in with access to animal houses at all times while buffalo under village condition were free to graze in the field during the day, although put into an animal house at night. There was also an indication that body weight of swamp buffalo was higher in Ngawi than in Brebes for both the 3.5 and 5 year olds (Table 2). This may have been

Table 6. Mean body measurements (cm) of Swamp and crossbred buffalo.

Age (months)	Sex*	Crossbred†			Swamp buffalo†		
		Girth	Height	Length	Girth	Height	Length
3	M	103	82	76	90	76	68
	F	101	80	74	90	78	73
6	M	123	92	90	105	87	79
	F	117	92	86	104	90	80
9	M	134	104	110	124	94	92
	F	130	100	107	125	93	91
12	M	135	106	114	128	98	97
	F	131	103	109	128	97	94

* M = male, F = female
† Number of animals: 2 of each genotype

due to better feeding practices of farmers in Ngawi than in Brebes. Feed analyses of samples collected from animal pens in Brebes and Ngawi showed that although protein levels were similar (12 ± 3% and 13.3 ± 4% for Brebes and Ngawi, respectively), the neutral detergent fibre (NDF) levels were lower in feeds from Ngawi than from Brebes (58 ± 8% and 67 ± 5%, respectively). This is an indication of a higher digestibility value for feeds in Ngawi than in Brebes. In addition, it was observed that in Ngawi feeds were always available in pens whereas in Brebes only a few farmers provided feed in pens. Therefore, the growth of buffalo in Brebes may have been limited by the feed quality and quantity.

When comparing body weights of males and females (Tables 1, 3 & 7), males tended to be heavier althought this did not reach statistical significance until the age of nine months.

The measurements of size (Tables 1, 2 & 6) were correlated with body weight and therefore reflected differences in body weight. However, relationships between measurements of size and body weight are not presented.

Table 7. Sex differences in mean* body weights (kg). The table includes pooled data from both genotypes.

Age (months)	Males		Females	
	n	Weight	n	Weight
0	10	24.1	8	23.1
3	10	66.1	8	65.3
6	10	115.5	8	112.5
9	10	152.0[a]	8	145.4[b]

* Values with different superscripts in the same row are significantly different ($P < 0.05$)

Gestation period

Gestation periods of Indonesian swamp buffalo raised in the villages or on the institutional farm are shown in Table 8. There was no significant effect of breed or sex of calf on length of gestation period although it tended to be longer for male calves than for female calves. These results are in agreement with the results reported by Brackel (1952) on cattle.

Sexual behaviour

There was an indication that swamp and river buffalo bulls showed a preference for mounting females of their own respective genotypes. Different results were found for crossbred bulls. Over a period of six months, three crossbred bulls were observed to make 65, 55 and 46 mountings, respectively; the frequency of mounting either swamp or river buffalo females by these bulls was 0 and 65, 11 and 44, and 32 and 14, respectively. This result shows that two crossbred bulls preferred to mount river type, and in contrast, the other bull which preferred to mount swamp type females.

Table 8. Effect of genotype, location and sex of calf on length of gestation period.

Genotype	Location	Sex of calf	n*	Mean length (days) of gestation period
Swamp	Institutional farm	Male	2	327
		Female	2	324
	Village	Male	3	325
		Female	2	320
Crossbred	Institutional farm	Male	1	328
		Female	1	326
	Village	Male	4	324
		Female	3	322

* n: number of buffalo cows

Semen characteristics

Semen characteristics of different genotypes, during a six-month evaluation period, are shown in Table 9. There was no significant difference in volume of semen obtained from the different genotypes but the swamp type produced significantly more concentrated semen ($P < 0.01$) than both the crossbred and the river buffalo. Among all bulls studied, the sperm collected from the swamp type showed the best motility and percentage of live sperm. The motility and percentage of live sperm collected from crossbred buffalo were still slightly better than those from river buffalo but they were not significantly different. The lower quality of semen collected from the crossbreds, compared with that from swamp buffalo, is in agreement with a recent report that crossbreds (F_1) have a higher rate of degeneration of spermatogenic cells than their parental genotypes (Bongso et al. 1983). Tayel, Moustaf and Jondet (pers. comm) have indicated that, although no desirable minimum number of live sperm per dose has yet been determined, they assume that at least 12 to 15 million live sperm are necessary. However, the concentration of 810 million obtained from the F_1 cross in this present study (Table 9) leaves no doubt that the semen is suitable for A.I. purposes. The highest total spermatozoa and concentration of ejaculate was recorded during the period of September to November (Table 10). The volume of

semen was also higher during October; however the difference was not statistically significant. Increasing quality and quantity of semen obtained during September and October is assumed to correlate with the quality of roughage given to the experimental animals. These results agree with the finding that improvement of semen quality is associated with the supply of highly nutritive green fodder (El Sawafi et al. 1974).

As with sperm concentration and total sperm per ejaculate, the motility and percentage of live sperm were also significantly higher ($P<0.01$) during the wet season (September to November) than during the dry season (June to August) (Table 10).

There were no significant interactions between breed and month of collecting for all semen characteristics evaluated.

Sexual desire was evaluated by recording the number of ejaculates produced during 15 minutes of collecting each week and the time required to obtained the first ejaculate (Table 11). The mean numbers of ejaculates obtained from river and crossbred buffalo were significantly greater ($P<0.01$) than the mean number obtained from swamp buffalo, but there was no significant difference in number of ejaculates between the river buffalo and the crossbreds. The time elapsed from introducing bulls to the collecting site until the first ejaculation was obtained shows (Table 11) that river buffalo gave the fastest response (3.3 min), followed by crossbreds (3.8 min) and then by swamp buffalo (5.5 min). This result supports those reported by Chenoweth (1982) who found that both libido and mating ability are known to be greatly influenced by genetic factors.

There was no significant effect of month of collection on the ejaculation time; however, the mean numbers of ejaculates obtained during October and November were significantly higher ($P < 0.05$) than in the other months of collection.

Oestrus and progesterone levels

The injection of 500 μg of cloprostenol caused a rapid reduction in the concentration of plasma progesterone. In one group of cows ($n=6$) it was 1.98 ± 0.86 μg/ml on the day of injection and fell to levels of 0.05 μg/ml or less 2 days after injection; it remained at this low level for 7 days. This result is in agreement with results reported by Kamonpatana et al. (1979) who found that injection with 25 mg $PGF_{2\alpha}$ (thromethamine salt) caused a rapid

Table 9. Means* for semen characteristics of different genotypes.

Genotype	n**	Sperm volume (ml)	Sperm concentration ($C \times 10^9$) C	Total sperm per ejaculate ($TS \times 10^9$) TS	Motility score	Live sperm %
River	2	1.4	0.65^A	0.91^A	2.5^A	72.5^a
Swamp	2	1.5	1.45^B	2.49^B	3.6^B	86.9^b
Crossbred	3	1.6	0.81^C	1.32^C	2.7^A	75.5^a

* Values with different superscripts in the same column are significantly different; lower case: $P < 0.05$, upper case: $P < 0.01$
** n = number of bulls

Table 10. Seasonal changes in means* for semen characteristics.

Month	Semen volume (ml)	Semen concentration ($C \times 10^9$) C	Total sperm per ejaculate ($TS \times 10^9$) TS	Motility score	Live sperm %
June	1.5	0.85^a	1.34^a	2.6^a	76.9^a
July	1.5	0.85^a	1.34^a	2.7^a	73.1^a
August	1.5	0.85^a	1.34^a	2.7^a	76.0^a
September	1.5	0.85^a	1.42^a	3.1^b	81.1^b
October	1.6	1.20^b	1.76^b	3.1^b	80.6^b
November	1.5	0.90^a	1.44^{ab}	3.2^b	79.8^b

* Values with different superscripts in the same column are significantly different ($P < 0.05$)

Table 11. Mean number of ejaculates (E) and mean time required to produce the first ejaculate (TE, min) for different buffalo genotypes, during a collection period of six months.

Months	River		Swamp		Crossbred		Mean*	
	E	TE	E	TE	E	TE	E	TE
June	2.1	3.1	1.5	5.4	2.0	3.4	1.9[a]	3.9
July	2.2	3.4	1.6	5.2	2.4	3.9	2.1[a]	4.1
August	2.2	3.3	1.5	5.5	2.3	3.9	2.0[a]	4.2
September	2.1	3.2	1.8	5.9	2.1	3.9	2.0[a]	4.3
October	2.6	3.3	1.7	5.4	2.6	3.9	2.3[b]	4.1
November	2.5	3.5	1.8	5.5	2.3	3.6	2.2[b]	4.1
Mean**	2.3[A]	3.3_a	1.6[B]	5.5_b	2.3[A]	3.8_c		

* Values with different superscripts in the same column are significantly different ($P < 0.05$)

** Values with different superscripts in the row of genotype means indicate significant differences in E ($P < 0.01$). In the same row, values with different subscripts indicate significant differences in ET ($P < 0.05$

regression of the CL and a decline in serum progesterone level within 24 hours of injection. Jainudeen (1976) reported that administration of a $PGF_{2\alpha}$ analogue did give effective induction of oestrus, followed by normal ovulation of buffalo. Although $PGF_{2\alpha}$ treatment has been effective for the induction of oestrus, the difficulty of detecting oestrus has been a major factor limiting the use of A.I. in buffalo.

The response of swamp buffalo to the $PGF_{2\alpha}$ injection is shown in Table 12. All cows treated had been previously palpated and showed normal ovaries. We detected a higher percentage of animals in oestrus under institutional conditions than in villages. This can be accounted for by differences in management and in levels of nutrition. Some of the animals treated in the villages were still rearing their calves and it is thought that this affected the condition of the ovaries. Anoestrus during the post partum period has been reported as a cause of low fertility, due to a delay in the resumption of ovarian activity; ovarian cyclicity is restored earlier in non-suckled buffalo (Jainudeen et al. 1983, Perera et al. 1987). Our result of detecting about 50 percent of the cows to be in oestrus on day 3 after $PGF_{2\alpha}$ injection (Table 12) is similar to the results reported in cattle by Lauderdale et al. (1975) and Hafs and Manns (1985).

After four days of observation following $PGF_{2\alpha}$ injection, the percentage of cows in oestrus was 76.1 and 80.6 for villages and institutional farm, respectively. In general most of the animals came into oestrus within three days of injection.

The study in three villages (Bantul, Kulon Progo and Sleman), involving 258 buffalo cows, showed that 17.3% of buffalo had inactive ovaries but no inactive ovaries were found at the institutional farms

Table 12. Response of Swamp buffalo to $PGF_{2\alpha}$ treatment.

Location	n*	Percentage of treated cows in oestrus Days after treatment			
		2	3	4	Total
Institutional farm					
Trial 1	20	20.0	50.0	10.0	80.0
Trial 2	16	18.6	53.9	9.9	80.6
Mean		19.3	53.9	9.9	80.6
Villages					
Bantul	33	24.2	42.4	9.2	75.8
Kulon Progo	62	17.7	45.2	12.9	75.8
Sleman	80	5.0	41.3	28.8	75.1
R.Bitung	65	7.7	53.8	15.4	76.9
Mean		13.7	45.8	16.6	76.1

* Number of cows treated

(Table 13). There was variation in the characteristics of the active ovaries between villages and between villages and the institutional farm (Table 13). The mean percentages of palpable CL, and follicles respectively, were: villages 25.7 and 57.0 and the institutional farm 40.0 and 60.0.

The oestrous behaviour of 20 cows following injection of $PGF_{2\alpha}$ is shown in Table 14. Mucous discharge was the dominant sign of oestrus. This sign, evident in all animals, first appeared 62.2 hours after $PGF_{2\alpha}$ injection and persisted for 58 hours. Homosexual behaviour was evident 58.6 hours after injection and persisted for 82.0 hours but there was considerable variation of intensity between animals. Heterosexual behaviour, although it was not as pronounced as homosexual behaviour, was more consistent in all animals and, therefore, is a more reliable

indicator of oestrus. The number of mounts was much higher during homosexual behaviour (17.3) than in heterosexual behaviour (8). Other signs of oestrus, such as an increasing frequency of urination, smelling vulva, restlessness, bellowing and raised tail, were infrequent and difficult to observe and record.

Table 13. Characteristics of ovaries of Indonesian Swamp buffalo.

Location	*n	Inactive ovaries (%)	Active ovaries (%)	
			Follicle	CL
Institutional farm	20	0	60.0	40.0
Villages				
Bantul	40	17.5	62.5	20.0
Kulon Progo	73	9.6	58.9	31.5
Sleman	145	24.8	48.7	25.5
Mean		17.3	57.7	25.7

* Number of cows examined

Table 14. Means (± SD) for behavioural signs of oestrus following $PGF_{2\alpha}$ injection of Swamp buffalo cows.

Signs	Time (hours)
Mucous discharge (n* = 20)	
Time after PG injection	62.0 ± 8.8
Duration of discharge	58.3 ± 8.6
Homosexual behaviour (n = 20)	
Time after PG injection	
Start of behaviour	58.6 ± 7.3
Completion of behaviour	82.0 ± 11.1
Number of mounts: 17.3 ± 10.5	
Heterosexual behaviour (n = 13)	
Time after PG injection	
Start of behaviour	51.2 ± 8.2
Completion of behaviour	78.0 ± 11.2
Number of mounts: 8.0 ± 3.1	

* n = number of cows

These results support the work of Jellinck and Avenell (pers. comm.) who found that the frequency of heterosexual behaviour was coincident with the surge in concentration of luteinizing hormone. They observed homosexual behaviour occasionally but found that it was too erratic to be useful. Jainudeen et al. (1983) also concluded that homosexuality was not a constant feature during oestrus in buffalo and they found that 50 to 70% of oestrous females detected by a teaser male did not exhibit any homosexual behaviour. Mucous discharge is a dominant sign of oestrus if close observation is used; however, we excluded this sign as a suitable indicator of oestrus because of the prolonged period over which it was present (58.3 ± 8.6h). Other behavioural studies have failed to demonstrate any satisfactory alternative to observing oestrus as a means of determining the appropriate time for mating or insemination (Avenell et al. 1982, Fletcher and Putu 1985).

Fertility

The data on conception rates are not tabulated. However, with the exception of Kulon Progo village, the conception rate obtained by using semen from river buffalo was very low (<10%). Although data were limited, a higher conception rate (67%) was obtained when swamp buffalo semen was used. The conception rate achieved with river bufffalo semen, by using A.I. in this study, was lower than that reported by De Guzman (1988) who found 30–35 % in most Asian countries. This difference is attributed mainly to the poor quality of river buffalo semen which we used.

The relatively high conception rate (67%) obtained when we used swamp bull semen is similar to the result reported by Avenell et al. (1982) who found that, under laboratory conditions, a conception rate of around 60% could be achieved from a single insemination 12–24 hours after the onset of oestrus. Therefore, we conclude that the low reproductive performance of Indonesian swamp buffalo is not a hereditary character but is an effect of the environment.

Some of the variation in conception rates within the locations studied was related to management including adequacy of the sheds provided, availability of water, health control, feeding, etc. It has been shown by Singh et al. (1984) that the provision of a shed and shower and/or wallowing facilities during summer, are very effective in improving fertility.

Effect of work on semen quality physiology

The effect of work on semen quality, with results pooled for different genotypes, is shown in Table 15. There were no significant changes in semen characteristics of both swamp and crossbred buffalo after working for four hours each day, for one month. There was a tendency for total volume, total concentration and total semen production to decrease during working periods and during the month after the commencement of the working period but these changes were not statistically significant.

Table 15. Effect of work on characteristics of buffalo semen.*

Time of semen collection	Volume (ml)	Sperm concentration ($C \times 10^9$) C	Total sperm per ejaculate ($TS \times 10^9$) TS	Motility score	Live sperm %
2 h before work	1.7	0.92	1.50	2.9	73.5
During work	1.4	1.02	1.45	2.9	76.5
2 h after work	1.4	0.96	1.36	2.9	75.5

* Mean values pooled for 3 swamp and 3 crossbred buffalo bulls

Table 16. Mean* pulse (P) rate, respiration (R) rate and rectal temperature (T) of swamp and crossbred buffaloes before, during and after work.

	P/min		R/min		T (C°)	
	Swamp	Crossbred	Swamp	Crossbred	Swamp	Crossbred
2 h before work	35.0[a]	35.3[a]	19.7[a]	15.7[a]	38.1[a]	38.0[a]
During work	79.7[b]	78.3[b]	68.7[b]	62.3[b]	40.1[b]	40.1[b]
2 h after work	40.3[a]	39.3[a]	25.7[a]	25.7[a]	38.3[a]	38.4[a]

* Values with different superscripts in the same column are significantly different ($P < 0.01$). Means are based on 3 swamp and 3 crossbred buffalo.

Physiological responses to work by the different genotypes are shown in Table 16. The pulse and respiration rates and rectal temperatures of swamp and crossbred buffalo were similar when compared two hours before, during work and two hours after work. However, work stress significantly increased all three measurements ($P < 0.01$). There is an indication that the respiration rate of crossbreds may be lower than that of swamp buffalo but the difference was not statistically significant.

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Performance and Physiological Characteristics of Different Buffalo Genotypes

P. Bunyavejchewin,[1] B. Tanta-ngai,[1] C. Chantalakhana,[1] S. Konanta,[2] O. Vechabussakorn[2] and P. Kalavibool[2]

Abstract

This study was conducted as a collaborative investigation between Kasetsart University and the Department of Livestock Development. Its purpose was to evaluate the production performance, some physiological traits and draught ability of swamp buffalo, pure Murrahs, ½ Murrahs and ¾ Murrah crossbreds.

The comparative study comprised three parts, namely: 1. A comparison of the growth and some physiological characteristics of the four different genotypes from birth to one year. 2. The evaluation of growth of swamp buffalo, ½ Murrahs and ¾ Murrah crossbreds after weaning for one year. 3. A comparison of the draught ability of swamp and ½ Murrah crossbreds.

The results showed that pure swamp buffalo and pure Murrahs grew slower than Murrah crossbreds. Differences between genotypes in heart rate and respiration rate were equivocal; however, swamp buffalo generally had higher body temperatures than the other genotypes. For the same amount of ploughing, the swamp buffalo generated less power than the half Murrahs; in this situation, the swamp buffalo were more efficient as draught animals; however, under village conditions, this difference in power output is not likely to be noticeable.

Research is needed to evaluate the carcass and meat quality and to estimate the production costs of swamp buffalo and Murrah crossbreds for the fattened prime meat market. In addition, more information of draught ability is needed to add the findings of this study.

THE swamp buffalo has been used as a draught animal in crop production, especially where rice is the main commodity in the integrated farming system in Thailand, since time immemorial. Buffalo breeding generally takes place through random natural mating, usually within breeds.

In 1978, 100 Murrah buffalo (90 heifers and 10 bulls) were introduced into Thailand from India by the Department of Livestock Development (DLD) for experimental production. They have been raised at two livestock stations of the DLD — Nhong Kwang and Surat-Thani. Crossbreeding between swamp buffalo and Murrahs has been conducted since introduction but not much information has been obtained.

[1] Buffalo and Beef Production Research and Development Centre, Kasetsart University, Bangkok 10903, Thailand
[2] Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok 10400, Thailand

This study was aimed at comparing swamp buffalo with Murrah crossbreds and pure Murrah for their growth performance, draught ability and some physiological characteristics.

Materials and Methods

The study was conducted in three parts.

Part I. Eleven swamp buffalo, 16 half Murrah crossbreds, 16 three-quarter Murrah crossbreds and 15 pure Murrahs, born in similar environments at DLD stations during August–November 1987, were used to compare their production performance and physiological characteristics. The calves (both sexes) with their dams were grazed on pasture in the morning and returned to a barn in the afternoon where straw and/or hay and water were provided, as well as mineral blocks. The calves were weaned at about eight months of age.

Calves were weighed at birth and then at two-monthly intervals. Thereafter and, at the time of weighing, height at withers, heart girth and length of body (distance from the pin bone to the anterior edge of the shoulder) were measured and physiological traits (body temperature (BT), heart rate (HR) and respiration rate (RR)) were recorded. The records of these traits were collected until animals reached one year of age.

The measurements used as criteria of growth were weaning weight, yearling weight and average daily gain. Similarly, comparisons of physiological traits were made at birth, weaning, and at one year of age. Least-squares methods were used to analyse the data. Thus, it was assumed that:

$$Y_{ijk} = u + a_i + b_j + e_{ijk}$$

where Y_{ijk} = measurement of k^{th} individual of i^{th} breed and j^{th} sex,

u = the grand mean
a_i = the effect of i^{th} breed,
b_j = the effect of j^{th} sex, and
e_{ijk} = the error of the experiment.

The least significant difference was used to compare the least-squares estimates of parameters within breeds.

Part II. Thirty weaned buffalo, half male and half female, involving three different genotypes (10 swamp buffalo, 10 half Murrah crossbreds and 10 three-quarter Murrah crossbreds) were used to compare growth performance in the same environment at Lumphya Klang Livestock Research and Breeding Center. All animals were put in a barn two weeks prior to the experiment and treated as if on the actual test to allow them to adjust to the testing procedure. At the beginning of the one-year test, water and feed were withheld for one night in order to weigh the animals the following morning.

For daily management, the calves were individually neck-chained. They were fed with a concentrate supplement (cassava chip and dry Leucaena leaf in the ratio of 3:1) early in the morning and then grazed all together in the pasture. In the evening, they were brought back to the barn and fed with hay and/or straw. Ample water and mineral blocks were also provided in the barn.

Data collected were monthly weights and heart girth measurements. In addition, associated measurements (body length and height) were recorded at the time of the initial and final weighings. Daily weight gains and final measurements were subjected to analysis of covariance due to the variation between animals at the start of the experiment. The treatment means were tested for differences by Duncan's New Multiple Range Test.

Part III. Fourteen observations of six pairs of two different buffalo genotypes, swamp buffalo and half-Murrah, were used to evaluate their draught ability by ploughing in rice fields. Within each pair, buffalo were about the same age; they belonged to the same owner and had the same management under village conditions in Surin province.

On each farm, one buffalo of each genotype worked separately. They ploughed for 90 minutes at the same time and on the same soil type (a sandy loam) for each set of observations. A spring dynamometer was attached between the yoke and plough to record the pull (force) produced while ploughing. A pair of ploughmen switched to handle each other's buffalo after half the working time.

Data recorded for draught ability were the force produced during working, ploughing speed and area ploughed. Physiological responses of buffalo while working were measured. Pulse rate (pulses per minute) was sensed at the base of the tail. Respirations per minute were recorded by observing flank movements. Rectal temperature was measured by inserting a thermister probe into the rectum.

A paired-comparison test was used to analyse different means.

Results

I. Growth and some physiological characteristics from birth to one year

Growth. Table 1 shows the production performances of the four buffalo genotypes in terms of birth weight, weaning weight, yearling weight and average daily gain, as well as body measurements in terms of heart girth, length and height. The growth of buffalo from birth to one year of age is also shown in Figure 1. It was found that, before weaning, growth in body weight of Murrah crossbreds was better than that of swamp buffalo and pure Murrahs; however, swamp buffalo were better than pure Murrahs. Differences in body measurements were the same direction. After weaning, contrary results were obtained, swamp buffalo having the highest daily weight gain followed by ½ Murrahs, pure Murrahs and ¾ Murrahs, respectively. Heart girth, length and height of the body were higher in swamp buffalo than in pure Murrahs, but were in between those of ¾ Murrahs and ½ Murrahs. The effects of genotype on length measured at birth, weaning weight, preweaning gain and heart girth measured at weaning, were statistically significant (Table 1).

Table 1. Least-squares estimates of means for various traits, for each genotype from birth to 1 year of age.

Parameters	Means	Genotype				Sex		C.V. (%)
		Murrah	¾ Murrah	½ Murrah	Swamp	Female	Male	
Production Traits								
At birth								
Weight (kg)	34.152	−2.083	+0.176	+1.970	−0.063	−0.974	+0.974	10.83
Body measurements (cm)								
— heart girth	77.058	−1.781	+2.643	+1.859	−2.722	−0.839	+0.839	5.39
— length	56.270	+0.737[a]	+1.043[a]	+2.030[a]	−3.810[b]	−0.900	+0.900	7.67
— height	71.441	−0.463	+0.290	−0.214	+0.387	−0.321	+0.321	5.68
Weaning								
Weight (kg)	195.261	−20.706[a]	+18.034[b]	+6.511[bc]	−3.839[c]	−5.772	+5.772	9.71
Body measurements (cm)								
— heart girth	141.534	−6.844[a]	+2.512[b]	+1.682[b]	+2.650[b]	−1.473	+1.473	4.22
— length	97.929	−1.296	+1.214	+0.664	−0.582	−0.688	+0.688	5.69
— height	108.538	−2.256	+0.796	−0.803	+2.263	−0.482	+0.482	3.82
One year								
Weight (kg)	234.878	−22.110	+5.769	+8.123	+8.123	−5.719	+5.719	10.50
Body measurements (cm)								
— heart girth	155.747	−4.630	+0.382	+2.943	+1.306	−1.503	+1.503	4.24
— length	110.028	−2.164	−0.194	+1.898	+0.461	−0.244	+0.244	4.33
— height	115.032	−1.506	+0.352	+0.082	+1.065	−0.878	+0.878	3.42
Average daily gain (kg/d)								
Pre-weaning	0.665	−0.071[a]	+0.060[b]	+0.025[b]	−0.014[ab]	−0.017	+0.017	13.92
Post-weaning	0.334	−0.017	−0.097	+0.012	+0.102	−0.017	+0.017	50.18
Physiological Traits								
At birth								
HR	116.486	−1.527	+11.572	−4.908	−5.137	−3.621	+3.621	14.13
RR	58.824	+4.947	−3.196	−3.014	+1.264	+1.534	−1.534	42.07
BT	39.414	+0.079	−0.084	−0.218	+0.223	−0.017	+0.017	1.95
Weaning								
HR	68.451	−2.812	+0.298	+3.100	−0.585	−1.414	+1.414	10.74
RR	29.081	−3.726[a]	−1.348[a]	−2.311[a]	+7.385[b]	+0.335	−0.335	23.25
BT	38.551	−0.464[a]	−0.173[a]	+0.222[b]	+0.415[b]	+0.008	−0.008	1.09
One year								
HR	65.832	+4.394	−0.376	+1.370	−5.387	+1.387	−1.387	11.76
RR	23.897	−0.351	−0.268	−0.608	+1.227	+0.189	−0.189	11.78
BT	38.288	−0.063	−0.087	+0.042	+0.108	+0.062	−0.062	1.36

Note: Least-squares constants in the same row with different superscripts are significantly different ($P < 0.05$)

The results for weight gain from weaning to one year show much variability. This period of growth was only four months. The calves may have been adjusting themselves to the environment without their dams. The expression of weight gain might have shown less variability within genotypes if the duration of this period had been prolonged.

The differences between genotypes in surface measurements were largely due to differences in body weight; however, at birth, swamp buffalo did appear to be shorter in body length but taller than the other genotypes.

Physiological traits. Average values are shown in Table 1. The highest values for heart rate at birth, weaning and one year of age were found in ¾ Murrahs, ½ Murrahs and Murrahs. For respiration rate, Murrahs had the highest rate at birth, while the rates at weaning and one year were highest in swamp buffalo. It is noticeable that, at every stage of measurement appearing in Table 1, body temperature of swamp buffalo was the highest. The results from least-squares analysis in Table 1 reveal that the effects of genotype on respiration rate and on body temperature at weaning were statistically significant.

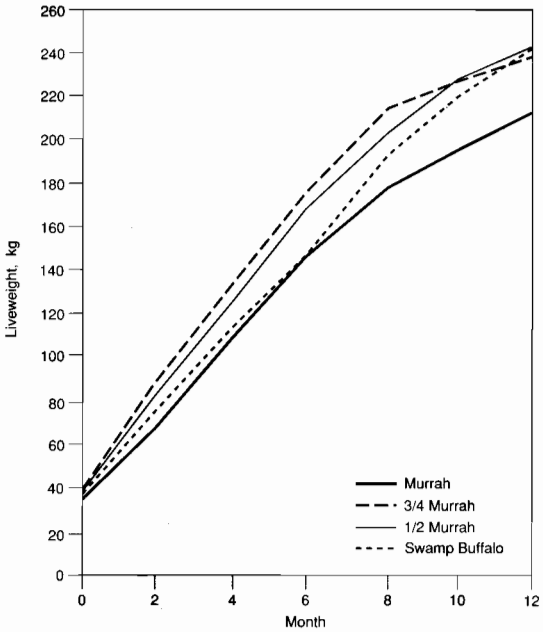

Fig. 1. Growth of buffalo from birth to one year of age. Weaning was at approximately eight months of age.

II. Growth after weaning

Buffalo live weights at the commencement of the experiment differed significantly between genotypes due to the limited availability of animals at that time. The average live weights and initial measurement at the start of the experiment are shown in Table 2. The results of the analysis of covariance shown in the table reveal that final weights after the one-year test differed significantly between genotypes. Swamp buffalo had a lower adjusted weight ($P < 0.05$) than ¾ Murrah crossbreds and halfbreds (352, 375 and 391 kg respectively), but no significant difference was found between the two crossbreds. For daily weight gain, which is the major criterion for the production performance of animals, halfbreds had the highest adjusted average daily gain (512 g/d) which was higher than that of swamp buffalo (405 g/d, $P < 0.05$) and ¾ Murrah crossbreds (468 g/d; $P > 0.05$n.s.). The actual and adjusted live weight changes of these genotypes are shown in Figures 2a and 2b, respectively. The associated parameters; heart girth, body height and length showed similar trends, but only body length was significantly different between genotypes.

Table 3 shows the effect of sex on growth performance of buffalo. Apart from an initial difference in live weight no significant difference was found

Table 2. The effect of genotypes on means* for growth performance of buffalo from weaning onwards for one year at Lumphya Klang Livestock Research and Breeding Center.

Item		Genotype		
		Swamp	½ Murrah	¾ Murrah
Number of animals		10	10	10
Initial measurements				
Weight (kg)		209.0[a]	217.6[a]	185.0[b]
Heart girth (cm)		145.6[a]	144.4[a]	136.6[b]
Height (cm)		109.5[a]	108.1[ab]	105.6[b]
Length (cm)		101.9	103.4	99.9
Final measurements				
Weight (kg)	— actual	356	402	359
	— adjusted	352[b]	391[a]	375[a]
Hearth girth (cm)	— actual	172.9	175.3	170.3
	— adjusted	171.2	174.2	173.1
Height (cm)	— actual	122.3	123.5	122.3
	— adjusted	121.7	123.4	123.0
Length (cm)	— actual	124.6	133.8	129.1
	— adjusted	124.5[b]	133.3[a]	129.6[a]
Daily weight gain (g/d)	— actual	403	505	477
	— adjusted	405[b]	512[a]	468[a]

* Means in the same row with different superscripts are significantly different ($P < 0.05$)

between sexes for any trait, in the analysis of covariance. In addition, there was no significant interaction between genotype and sex.

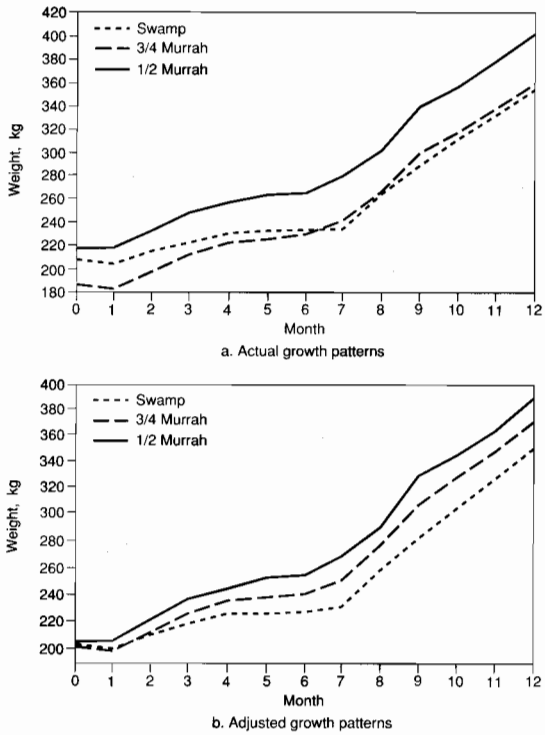

Fig. 2. Growth of buffalo from weaning onwards, for one year at Lumphya Klang Livestock Research and Breeding Center.

III. Draught Ability

Draught ability of swamp buffalo and ½ Murrahs was measured under village conditions. Table 4 shows that, when ploughing on the sandy loam soil, ½ Murrahs generated more draught force than swamp buffalo (33.36 vs 30.62 kg) and ploughed (highly) significantly faster (2.95 vs 2.60 km/hr). In terms of draught power, swamp buffalo generated less power than ½ Murrah ($P<0.01$). However, the work output (area ploughed) in 90 minutes was not significantly different between the two genotypes (544 and 552 m^2 for swamp and ½ Murrahs, respectively).

Comparing the physiological responses of the two genotypes after 90 minutes of work, the rise of temperature and of respiration rate seemed to be slightly higher ($P>0.05$n.s.) in swamp buffalo than in ½ Murrahs, while the latter had a greater increase ($P>0.05$n.s.) in pulse rate than the former.

From an engineering point of view, power is the major criterion to measure draught efficiency of animals. In that sense, the findings of this study show a trend for swamp buffalo to work slightly better than ½ Murrahs by producing less power while obtaining the similar work output. Since Thai farmers do not use buffalo longer than 2–3 hours at any one time in village conditions, the difference in work ability between the two genotypes is hardly noticeable. However, more observations are needed to confirm this finding.

Table 3. The effect of sex on means* for growth performance of buffalo from weaning onwards for one year at Lumphya Klang Livestock Research and Breeding Center.

Item		Sex	
		Male	Female
Number of animals		15	15
Initial measurements			
Weight (kg)		193.2[b]	214.5[a]
Heart girth (cm)		140.0	144.4
Height (cm)		108.2	107.3
Length (cm)		100.1	103.4
Final measurements			
Weight (kg)	— actual	367.5	377.3
	— adjusted	376.3	368.4
Heart girth (cm)	— actual	171.0	174.7
	— adjusted	172.1	173.6
Height (cm)	— actual	123.5	121.9
	— adjusted	123.4	122.0
Length (cm)	— actual	127.8	130.5
	— adjusted	128.3	130.1
Daily weight gain (g/d)	— actual	478	446
	— adjusted	473	451

* Mean in the same row with different superscripts are significantly different ($P < 0.05$)

Conclusion

The growth performances of the ¾ Murrahs and the ½ Murrahs were generally better than those of the swamp buffalo and Murrahs. So far as phyiological traits were concerned, the results were equivocal. Swamp buffalo tended to have lower heart rates (not significant) but higher respiration rates and rectal temperatures than crossbreds (not significant). In terms of draught ability, swamp buffalo were more efficient than the ½ Murrahs but, working under village conditions, this difference is not likely to be noticed.

The results presented here need to be confirmed by further investigations. It would also be appropriate to extend the work to include production costs, carcass evaluation and meat quality of these genotypes under conditions of management aimed at the meat market.

Table 4. Means (± SD) for draught ability and physiological responses of swamp buffalo and ½ Murrahs. Soil type: sandy loam.

Item	Swamp	½ Murrah	P
Environmental measurements			
Ambient temperature (°C)		25.9 ± 3.07	
Black Globe temperature (°C)		28.0 ± 4.11	
Relative humidity (%)		89.1 ± 5.53	
Draught ability			
Live weight (kg)	408 ± 17	414 ± 36	ns
Draught power[1] (kW)	0.21 ± 0.06	0.26 ± 0.04	**
Draught force (kg)	30.62 ± 10.21	33.36 ± 6.60	ns
Ploughing speed (km/hr)	2.60 ± 0.31	2.95 ± 0.29	**
Area ploughed in 90 mins (m^2)	544 ± 163	552 ± 160	ns
Physiological responses			
Δ Rectal temperature °C	1.49 ± 1.07	1.22 ± 1.08	ns
Δ Pulse rate per min	12.36 ± 11.90	15.54 ± 11.60	ns
Δ Respiration rate per min	22.36 ± 14.05	21.00 ± 15.88	ns

Notes: 1) $\text{Draught Power (kW)} = \frac{FV}{1000 \times 3.6}$

where F is draught force (kg), V is speed (km/hr) and kW (kilowatt)

Δ is the increase in physiological response after 90 minute work

Utilisation of Feedstuffs by Swamp, Murrah and Crossbred Buffalo

Z.A. Jelan[1] and Norhani Abdullah[2]

Abstract

Crossbred buffalo, which differ in chromosome make-up from swamp and Murrah buffalo, are believed to possess different production potentials which could be exploited for meat production and draught power. It is also possible that these genotypes may exhibit some differences in certain aspects of digestive physiology and efficiency in feed utilisation. This paper reports a series of studies on growth, nutrition, feed utilisation and some rumen fermentation characteristics of different buffalo genotypes. Studies on growth indicated that the ¼ and ¾ swamp buffalo were significantly superior in growth rate and had larger heart girth and greater height at the withers than the swamp type. Though not significant, the crossbreds showed greater feed intakes than the swamp buffalo. Generally, there were no marked differences among the genotypes in the rumen parameters measured nor in feed utilisation.

CROSSBREEDING the swamp with the larger river buffalo began in 1950 but, due to poor interest in buffalo farming and management, an organised crossbreeding program was not practiced. However, recently crossbreeding has been encouraged in order to improve meat and milk productivity and draught power. Several studies (Fisher and Ulbrich 1968, Bongso and Jainudeen 1979, Bongso et al. 1984) have shown that the F_1 hybrid with 49 chromosomes was found to be fertile. Inter se matings of the F_1 hybrids and backcrossing these hybrids with either of their parents have also produced hybrids of different chromosome make-up (Harisah 1988). Differences in genotype and chromosome make-up would be more common on farms where uncontrolled matings occur. Even though few experiments have been conducted to assess the effect of crossbreeding, these crossbred buffalo are believed to possess different production potentials.

This paper reports an investigation on the growth and feed utilisation of various buffalo genotypes, including crossbreds, in a controlled farming and management system. The main objective of the study was to determine the relationship between chromosome make-up, feed utilisation and growth in order to identify a suitable crossbred for a particular purpose; e.g. for meat production and draught.

[1] Department of Animal Sciences
[2] Department of Biochemistry
Universiti Pertanian Malaysia, Selangor, Malaysia

Material and Methods

Growth rate studies

Based on breeding records and karyotype analysis, three groups of 12 month old buffalo, each consisting of six swamp, six ¼ swamp (Murrah × [Murrah × swamp]) and six ¾ swamp (swamp × [Murrah × swamp]) types were used in a growth experiment lasting 30 weeks. The average body weights of these animals at the begining were: 200, 240 and 225 kg respectively. They were penned individually with individual feeding and drinking facilities. The buffalo were obtained from the University's buffalo farm. They were weaned at six months of age and were raised on improved pasture prior to the trial.

The buffalo which had an average body condition score of 3–4 (maximum score 6) were dewormed and vaccinated against foot and mouth disease and haemorrhagic septicaemia prior to the trial.

The animals were fed at 0900 hours and 1400 hours with a basal diet comprising (dry matter basis) 80% solvent extracted palm kernel cake (PKC) and 20% cut-and-carry grass (*Setaria kazangula* and *Brachiaria decumbens*). In addition, all animals were

offered 150 g/d of local fishmeal. Body weight (BW) was measured biweekly. Feed intake was measured on two occasions, each of three weeks duration. Measurements of height at withers, height at sacral crest and heart girth were made at intervals of four weeks.

Nutrient degradation and rumen fermentation.

Four swamp and four ¾ swamp buffalo of average age 14 months and 250kg body weight were each cannulated at the rumen (Jelan 1985) and fitted with a permanent rubber cannula (Macam Rubber Pty Ltd, Australia). They were individually penned, managed and fed rations similar to animals in the growth trial. After an adaption period of about 4 weeks the following studies were carried out:

Degradation of feed in nylon bags. Four bags containing standard PKC and four bags containing standard grass samples (*Setaria splendida*) were suspended in the rumen of each cannulated animal. At 24 and 48 hours two bags of each standard sample were removed, washed and the amount of dry matter (DM) and organic matter (OM) degraded were calculated (Mehrez and Orskov 1977).

Comparison of fermentation characteristics. Rumen samples were aspirated through a stainless steel tube permanently attached to the cannula at intervals of 3,6,9,12,15, and 22 hours after feeding. Rumen fluid from each animal was pooled and stored frozen in McCartney bottles pending analyses. Fluid was thawed, centrifuged and analysed for ammonia and total fatty acid concentrations.

Feed utilisation

Twelve buffalo bulls, aged 30–40 months, comprising four each of swamp, Murrah and F_1 crossbreds were individually penned in a trial of 16 weeks. These animals were obtained from the University's buffalo farm and had been raised on improved pastures. Prior to the experiment, all animals were dewormed and each was fitted with a rumen cannula. They were fed solvent extracted PKC ad lib. and a commercial mineral supplement.

Following an adjustment period of about eight weeks, feed and total water intakes were measured for eight weeks. Evaporative water loss from individual drinking bowls was deducted from the total daily water intake. Rumen fermentation was monitored at 3,6,12 and 24h after feeding. Samples of rumen fluid were stored, prepared and analysed as was done previously. All data were analysed with an analysis of variance program using a Statistical Analysis System (SAS) computer program (SAS 1979).

Results

Growth rate studies

The growth responses of buffalo to the improved diet are shown in Table 1. The growth rates of ¾ and ¼ swamp buffalo were significantly different ($P < 0.05$) from those of the pure swamp buffalo. Between the crossbreds, the ¼ swamp showed the fastest growth although not significantly different from that of the ¾ swamp buffalo.

The average changes in body measurements are also shown in Table 1. The changes in heart girth, height at withers and height at the sacral crest were parallel to changes in body weight and the levels of statistical difference are indicated in the table.

Table 1. Average growth rates (g/d) and changes in body measurements (mm/week) at specific parts of the body.*

Genotype	Growth rate	Heart girth	Withers	Sacral crest
Swamp	755.5^a	6.3^a	3.9^a	4.2^a
¾ Swamp	786.3^b	8.8^b	5.5^b	4.9^a
¼ Swamp	840.4^b	11.1^c	5.2^b	7.4^b

* Within columns numbers with different superscripts are significantly different ($P < 0.05$)

Table 2 shows the average daily feed intake by swamp buffalo and its crossbreds. There were no significant differences either in DM and or in OM intake among the genotypes although the 3/4 swamp buffalo seemed to utilise more DM and OM than the other genotypes.

Table 2. Average daily feed intake ($g/BW^{0.75}$) of buffalo fed a palm kernel cake and grass based-diet.

Buffalo genotype	Dry matter	Organic matter
Swamp	138.2	132.9
¾ Swamp	146.1	142.8
¼ Swamp	144.7	138.3

Degradation of nutrients and rumen fermentation

The degradation of standard samples of grass and PKC from nylon bags suspended in the rumen of swamp and ¾ swamp buffalo for 24 and 48 hours is shown in Table 3. The degradation of DM and OM from the nylon bags was not significantly different between genotypes.

Table 3. Degradation of nutrients (%) from nylon bags suspended in the rumen of swamp and ¾ swamp buffalo crossbreds.

Genotype	Grass		PKC	
	Dry matter		Organic matter	
	24h	48h	24h	48h
Swamp	35.8	44.5	58.8	68.5
¾ Swamp	34.3	46.8	57.9	65.8

Table 4 shows some characteristics of the fermentation of feed in two genotypes. There were no significant diferences between them with respect to the total volatile fatty acid production (TVFA), rumen pH and rumen ammonia concentrations on a diet based on PKC and grass. However, a generally higher level of TVFA was detected in the crossbreds.

Table 4. Characteristics of the rumen of buffalo fed a PKC and grass based-diet.

Genotype	TVFA (moles/L)	Rumen ammonia (mg/L)	Rumen pH
Swamp	148.8	122.2	6.2
¾ Swamp	153.5	118.7	6.2

Feed utilisation

Table 5 shows the feed and water intake of swamp, river and F_1 crossbred buffalo fed a PKC-based diet. There was no significant difference in DM intake among the three buffalo genotypes. However, greater DM intakes were observed in the Murrahs and hybrids than in the swamp buffalo. A significantly greater ($P < 0.05$) total water intake was observed in the swamp as compared to the crossbreds. However, greater total water intake was also observed in the Murrahs as compared with the F_1 buffalo even though this difference was not statistically different.

Table 5. The mean (± SD) for body weight, feed and total water intake (g/100 kg body wt) by swamp, Murrah and F_1 crossbred buffalo.

Attribute	Swamp	F_1	Murrah
Body wt (kg)	237.0 (25.4)	362.0 (90.6)	273.5 (6.4)
DM intake	1.7 (0.3)	1.8 (0.2)	1.8 (0.4)
Water intake*	5.9 (0.6)[a]	4.1 (0.9)[b]	4.9 (0.8)[ab]

* Within this row numbers with different superscripts are significantly different ($P < 0.05$)

Table 6 shows some rumen parameters in buffalo fed a PKC-based diet. The data were limited and tests for statistical differences were not made. The rumen ammonia concentrations and pH were higher in the crossbreds and Murrahs than in the swamp buffalo. The propionate concentration was greater in the swamp while the acetate and butyrate concentrations were similar among the genotypes.

Table 6. Average daily rumen pH, ammonia and molar proportions of volatile fatty acids in swamp, Murrah and F_1 crossbred buffalo.

	Swamp	F_1	Murrah
Rumen pH	5.4	6.0	6.3
Rumen ammonia (mg/L)	81.0	130.0	123.0
Acetate (%)	32.2	34.6	30.6
Propionate (%)	35.2	23.0	25.4
Butyrate (%)	24.2	26.6	27.2

Discussion

Information on the growth potential of various genotypes of crossbred buffalo of different chromosome number is limited. One of the most important constraints in studies involving buffalo genotypes has been the limited availability of crossbred animals of known breeding history and with similar body condition.

This study showed that crossbreds have significantly greater growth potential than the pure swamp buffalo, particularly on a high plane of nutrition. The larger size of the crossbred, as indicated by larger girth and greater height at both withers and sacral crest (Table 1) is an important trait. These animals may generate greater draught power than the swamp buffalo.

The faster growth rate of the crossbreds required a higher nutrient intake than that of the swamp buffalo of similar age (Tables 2 and 5). We are unable to conclude that there were any differences among the genotypes with respect to digestive physiology. It is unlikely that differences exist among them because there were no differences in DM and OM losses from feed samples in nylon bags suspended in the rumen; nor were there any differences between them in efficiency of rumen fermentation (Tables 3 and 4).

The lower total water intake seen in the F_1 crossbreds than in the swamp type and the Murrahs is not understood. It is possible that there are differences in their ability to regulate body temperature.

The fermentation patterns shown in Table 6 provide some evidence of possible differences between the genotypes when fed a PKC-based diet. Further study in this area, using a larger number of animals, is desirable.

It may be concluded from this study that the ¼ swamp buffalo (i.e. ¾ Murrah) is superior for growth to the swamp type and may be useful as a draught animal. However, the genetic potential of these animals can only be expressed when their nutritional requirements are met.

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Genetic Diversity and Sustainable Agriculture — Implications for Animal Production Systems

R.K. Munro* and D.B. Adams*

Abstract

Genetic selection of domestic animals can lead to a reduction in genetic diversity through the elimination of individuals or populations deemed to be undesirable. Genetic diversity is the basis of population change through selection or crossbreeding and any reduction in that diversity reduces the potential for future genetic change.

Genetic change may be required in the future because of changed demands for produce or because of environmental changes such as climatic change, or reduced availability or effectiveness of inputs to the agricultural system; e.g. fossil fuels, fertilizers and chemotherapeutics. The preservation of genetic diversity would increase the chances that those genotypes would exist or could be selected for.

Maintenance of genetic diversity in live animal populations carries penalties to productivity compared with a population of selected stock. This has implications for profitability of enterprises, generation of capital for national development and trading relationships. Cryopreservation of genetic material, where this is possible, also has costs related to the actual procedures and the research and development of techniques. Preserving genetic diversity, therefore, involves direct costs and the costs of opportunities foregone because of allocation of resources to this area. Allocation of resources to these competing demands raises many questions of equity at national and international levels. Programs to improve buffalo genotypes need to consider these concerns to ensure that the resulting changes to agricultural ecosystems are socially, economically and ecologically sustainable.

In their agricultural roles, animals have been subjected to many years of selection for increased productivity with progressive elimination of animals with the least desirable traits. In many instances the gains from this selection have been extremely profitable.

Along with other aspects of man's activities, agriculture is now being appraised in relation to its ecological sustainability. One aspect of the question of sustainability is the desire to preserve genetic diversity in populations of domestic animals, and the implications that genetic selection within agricultural environments holds for this concept. Appraisal of animal production systems, with a view to their ecological sustainability, is a new and pressing challenge and may require changes in approach from the traditional production-oriented ethos. Some departures from traditional approaches are presented in this paper with a view to stimulating constructive discussion of issues.

* Bureau of Rural Resources, PO Box E11, Queen Victoria Terrace, ACT 2601, Australia

The possibility of climatic change raises the possibility of environmental change on a dramatic scale. The changes that would be required in animal production systems to maintain previously existing levels of adaptation and productivity could be equally dramatic. The need for genetic diversity to accommodate those changes may be great.

Other changes to the environments of farm animals, though less dramatic, are already occurring as a result of changing agricultural practices. This paper describes some of the practices that modify the agricultural environment, such as the use of chemotherapeutics, and the interactions these may have with genetic selection in animal production systems. The role of selection in manipulating levels of genetic diversity and the relative suitability of governmental or other institutions for maintaining valuable genestocks are discussed. Various techniques can be used to preserve genetic diversity and the merits of some of these techniques and their relationships with the social and economic environment are explored.

In the context of the present ACIAR projects the important role that the buffalo occupies in the agricultural ecosystems of Asia is recognised. Increased productivity through genetic change of the buffalo population could contribute markedly to the overall productivity from agriculture. In the light of the possibilities alluded to above, it is important that the evaluation of buffalo genotypes, and an understanding of their interactions with the environment, precede or accompany attempts to alter the genetic composition of local genotypes or the management regimes of local production systems. In this way the possibility of increased productivity being achieved and sustained is enhanced.

Animal Production Systems, Selection, and Genetic Diversity

Selection has occurred within the context of specific agro-ecological environments. These environments have often been made more conducive to animal production by improvements to the quality and quantity of feeds, control of disease and reduction in bioclimatic stress. Many animal production systems now use relatively benign and static environments to allow high levels of production and efficiency. There is concern that livestock selected in these situations may lose the ability to adapt to new and probably less favourable environments and, therefore, be less efficient producers under those circumstances.

If production from animals is to be maintained at present levels, then both the suitability of genotype and environment need to be considered. The histories of introduction of 'improved' European livestock to harsh environments, including Australia, have graphically illustrated the consequences of mismatching genotype and environment.

Even where animals have been kept in the regions in which they evolved, their exposure to pathogens and other stressors has often been increased by changes to their environment. Examples are increased stocking density and associated social stress and unfavourable housing related to ventilation and humidity. In many instances the deficiencies of livestock in dealing with their environments have been overcome by vaccines, chemotherapeutics (e.g. antibiotics and anthelminthics) and by careful attention to nutrition and husbandry.

The opportunity to manipulate the environment of livestock may diminish in the future. Development of resistance to chemotherapeutics by parasites is now widespread in many production systems. The ability of future generations to use chemotherapeutics as an aid to production is already compromised and may have severe implications for maintaining high densities of animals in intensive housing systems or other systems, such as irrigated pastures, where exposure to parasite infection is high. Use of vaccines to control parasites is widely practiced and is often effective. However many serious diseases of livestock, such as those caused by blood-borne protozoa, may not be amenable to control by vaccines. In addition, new disease agents can emerge and effective vaccines may not be developed quickly or easily.

There is no guarantee that current standards of nutrition and husbandry will be sustainable in the future. Increasing competition between man and animals for high-quality feed ingredients and restricted availability of financial capital and other inputs to agriculture (e.g. fossil fuels, machinery and fertilizer) may lead to a return to simpler systems of animal production. Strains of animals adapted to high levels of nutritional and other inputs may no longer be the most appropriate. Concerns about real or perceived environmental issues may also create a demand for products from low-input systems.

A further problem that arises from these considerations of matching genotype to environment is how the match should be measured. Existing attitudes would support the use of production data, with high production being equated with suitability. However, the concept of sustainability raises questions about the time scale over which productivity is to be measured. This challenges conventional agricultural science to move from a 'mindset' of short-term gains to a fuller understanding of interactions in the agricultural ecosystem. This understanding should involve soil-plant-animal-atmosphere interactions and extend to the cultural and socioeconomic aspirations of the communities in which the practices occur.

Genetic Diversity and the Use of Chemotherapeutics

A major function of science is to predict the consequences of certain actions or events. In the case of agriculture, most researchers have restricted their predictions to increased output of a particular commodity over a relatively short period. Chemotherapeutics have provided several examples where the full consequences of their application over the long term require further investigation. Issues which require further consideration include:

— immediate gains to productivity, through use of chemotherapeutics, being offset by selection of resistant parasites and ultimate loss of production potential by reduced ability to control resistant parasite populations;

— effects of chemotherapeutics on the resistance of animals to parasites, especially when host resistance is depressed and no longer exerts continuous control on parasite populations which are then only exposed to intermittent control by chemotherapeutics;

— effects of chemotherapeutics on non-target species such as pasture plants and soil micro- and macro-fauna, and the long-term effects on nutrient cycles, soil fertility and predation of free-living stages of parasites.

The long-term productivity of agricultural systems, therefore, requires a sophisticated understanding of the production system, its inputs, outputs and processes. The vast range of ecological niches for animal production and the variety of adaptations of animals and husbandry systems needed to exploit them requires maintenance of genetic and human cultural diversity to support animal production systems.

Preservation of Diversity

There is a need to preserve genetic material from animals able to produce efficiently without the battery of supports currently employed in some production systems. In developed countries, large sections of particular animal industries can be controlled by one management group. In this situation, selection of animals in controlled environments may result in the loss of particular genotypes or particular genes from large parts of the population. The concern is that the loss is irreversible.

In 'developed' countries, the 'improvement' of domestic livestock may have already decreased genetic diversity, largely through reductions in the population sizes of minor breeds and strains and, to a lesser extent, through loss of alleles within breeds subjected to intensive selection. There may now be a need to establish indices and criteria for genetic diversity in domestic animals.

In Australia in the 1970s a government committee reported on the issue of genetic diversity in the Australian poultry industry (Standing Committee of Agriculture 1979a). It concluded that there was sufficient value in the concept to encourage characterisation and cataloguing of poultry breed resources in Australia and maintenance of much greater levels of genetic variety than formerly. Recommendations relating to the use and capacity of import quarantine stations were included to ensure that a flow of genetic material into the country was possible. Other reports have addressed similar issues in other industries in Australia (Standing Committee of Agriculture 1979a,b).

The Role of Institutions in Preservation of Diversity

Equity between generations should influence decisions that may allow the loss of unique genetic material. Loss of genetic material may hamper the ease or the extent to which animal breeders in future generations can adapt their animals to new environments or alter the nature of their products. The role of biodiversity is therefore essentially one of insurance to provide future generations with the greatest array of genetic material possible. As with all insurance, the questions of cost and chance can be raised. Would the cost of preserving genotypes exceed the possible benefits and, if so, would not commercial breeders see this opportunity and ensure they secure the benefits? Questions such as this are easier to answer with the benefit of hindsight. Commercial enterprises do not always survive to realise their long-term plans, nor would they necessarily have sufficient capital or expertise to respond to what could be major problems on a national or international scale. Selection of genotypes that will yield benefits in the future must also carry considerable risk because the nature of future environments is not known.

Responsibility for the maintenance of genetic diversity is communal and should not be assigned to commercial groups which are subject to short-term market forces. Governmental organisations, perhaps multi-national, may be appropriate for financing and managing schemes to maintain genetic diversity in domestic animals. Such schemes may never earn a profit according to contemporary commercial accounting systems. Nevertheless, they would safeguard the long-term wellbeing of animal production systems that contribute to the wellbeing of people and would act to preserve inter-generational equity.

Preservation of Genetic Diversity and Implications for Developing Countries

In many 'less developed' countries environmental manipulation has not occurred to the same extent as in 'developed' countries. Here genetic selection has led to development of regionally adapted strains (land-races) with greater variety and seemingly greater resistance to local environmental stresses than livestock from developed countries. It appears that the bulk of genetic diversity related to this resistance may reside in the animal populations of 'less developed countries'. As the value of these strains to animal industries in 'developed' countries becomes apparent, some interesting questions related to the strategies and equities of preservation will arise.

Preservation of biodiversity in animals is possible by two methods at present, cryopreservation or conservation of live animals.

Cryopreservation

Cryopreservation (Turner 1981) is the name given to a range of techniques that can be used to preserve living material for long periods at low temperatures. It has some features which make its use convenient and desirable for the preservation of genetic diversity.

1. Cryopreservation has the advantage of maintaining genetic material relatively cheaply, seemingly indefinitely and largely protected from environmental hazards and mutagens such as ionising radiation.
2. Techniques for cryopreservation of male and female germ cells and embryos already exist for a number of species and presumably these techniques could be extended to include others. It is important to note that cryopreservation has been difficult to achieve in some species (e.g. the pig) and substantial research and development may be needed to produce efficient cryopreservation systems for all target species.
3. Genetic material can be acquired from donor animals without the need to remove them from their environment or the breeding population. Local cultural associations with the human population need not be disturbed to any great extent.
4. Problems with equity between 'developed' and 'less developed' countries are not major. The technology can be transferred readily between countries. Collection of material from sources in 'less developed' countries imposes no great cost or disadvantage on those countries. There may be conflict over distribution of benefits that result from the use of genotypes after collection. This possibility should not delay plans for collection of endangered or valuable genotypes but should be resolved as quickly as possible. The prospect of a country having to buy back genes that originated within its indigenous populations would seem to be an undesirable outcome which, if not guarded against, may restrict access of collectors to gene pools.
5. Cryopreserved material is cheaper and easier to transport internationally than live animals. Disease and quarantine problems still exist with cryopreserved germ cells but embryos within their zona pellucida are largely resistant to infection by pathogens and so offer a means for allowing movement of genetic material with much reduced possibility of disease transmission (Shelton and Morris 1985, Bureau of Rural Resources 1989a,b,c).
5. Cryopreserved material can be re-introduced to existing populations of the same species as a source of genetic variation. It can also be used for comparisons with existing live animals to measure genetic changes since collection from that population occurred and it can be used as genetic material free of pathogens that may have infected live genestocks since cryopreservation.

Cryopreservation is, therefore, a valuable tool for preserving genetic material. Without live animal populations though, there seems little likelihood that preserved genetic material can contribute at all effectively to the genes of living organisms. Developments in gene insertion and chimaera technology are possible but seem to be many years in the future. The existence of live animals is therefore vital to the strategy of preservation of genetic diversity. In contrast to wildlife, there is little likelihood of extinction of domestic species. However, until a comprehensive program for cryopreservation of domestic species is a reality, preservation of genetic diversity in this area depends on conservation of live animals.

Conservation of live animals

Live animal preservation can be achieved in situations with minimal human intervention where populations rely largely on preservation of habitat to ensure survival ('lock up' preservation) or where they are maintained in their usual animal production systems and continue to contribute to meeting human needs ('in situ' preservation). In the former situation, yields of animal produce may not be harvested or may be harvested in diminished amounts because of reduced or absent management and husbandry inputs. Each of these arrangements has biological and socio-economic characteristics which may be advantageous under certain circumstances.

'Lock-up' preservation, with the low level of managerial intervention that this implies, brings with it the possibility of continuous adaptation of animal populations to changing environmental pressures. It has the advantage of low maintenance costs but also the disadvantages of high establishment costs and high opportunity costs due to committed and inflexible resource allocation which may not be maintained during periods of short-term crisis.

'In situ' preservation has the advantages of low establishment costs and continued contribution to the wellbeing of the human population. In this situa-

tion there is some motivation to maintain the preservation because of the immediate benefits. However, there is also motivation to introduce 'improvements' to increase these benefits. This is of course the beginning of the process perceived to reduce genetic diversity and generate the need for preservation. It also serves to demonstrate some of the problems of equity that may arise from policies of in situ genotype preservation, namely:

— there will be reduced options to increase productivity by changing husbandry and management procedures, by using other aids to productivity such as chemotherapeutics and selecting superior stock from the group for propagation;

— it will mean accepting lower levels of productivity in the hope of long-term gains because of

  (i) lower productivity which will contribute to lower standards of living, less opportunity for investment, development and social progress, perceptions of inefficiency and low social worth and a need to provide some form of compensation or recognition of the benefits foregone for the common good; and

  (ii) gains which may never eventuate, which may not be justified in light of the productivity foregone or which may not be of benefit to those who have borne the cost but may be of greater benefit to those who have created problems in other places.

Perceived benefits flow from the preservation of genetic diversity but many disincentives may apply to those engaged in preservation. These disincentives could be diminished by the purposeful creation of circumstances in which the existing custodians of genetic diversity could continue their stewardship of that diversity profitably and without disadvantage. One approach may be to retain and foster animal production over a wide range of environments as an effective method for promoting genetic diversity. Unhappily, this attractive option has potentially serious drawbacks. Without aids to production such as chemotherapeutics, exotic feedstuffs and exotic genotypes, or compensatory affirmative action, those regions of the world not capable of efficient animal production according to current commercial concepts would be disadvantaged. Other regions which are currently efficient in these terms would be able to compete to the detriment of local producers, local industry and local genotypes and their supporting cultural systems. Affirmative action to protect local markets is important in retaining local production and should be considered an integral part of maintenance of genetic diversity in farm animals.

There is a need to understand how an animal population is maintained and how husbandry is shaped by the socio-economic environment. Social and economic factors may be as important as the agricultural and genetic considerations which bear upon productivity and genetic diversity.

Conclusion

It appears from this appraisal that the issue of genetic diversity involves many aspects of the agricultural, social, economic and trading arrangements of nations. There is wide scope for thought in addressing the perceived and demonstrated need for stewardship of the genetic resources present in the world's domestic livestock and their near relatives. Attempts to improve the productivity of agricultural systems through introductions of new genetic material into local buffalo populations or genetic selection, need to be evaluated in terms of their long and short term effects on buffalo performance and the productivity of the ecosystem as a whole.

Present attitudes to genetic improvement may need to be reviewed in light of concerns over sustainability of animal production and the need to maintain a level of genetic diversity. The direct costs of maintaining a level of diversity, and the indirect costs of productivity foregone need to be assessed when determining what level of genetic diversity is desirable in animal production systems. Justifying the diversion of resources to genotype preservation from other pressing social and environmental problems, especially in less developed countries, also requires careful consideration. Many issues impinge on the challenge to maintain genetic diversity. Wise decision-making requires that all of them should be considered.

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International Assistance for Buffalo Research: has enough been done?

B.M.A.O. Perera*

Abstract

International support for buffalo research over the past two decades has resulted in substantial strengthening of national institutes in many Asian countries, both materially and intellectually. The agencies which have played leading roles include the Food and Agricultural Organisation, the International Atomic Energy Agency, the Australian Centre for International Agricultural Research, Deutsche Gesellschaft für Technische Zusammenarbeit, the Canadian International Development Research Centre and the Swedish Agency for Research Cooperation. The support has been of three main types: a) infrastructure development involving provision of equipment, training and expert services, b) funding of specific research projects to enhance knowledge on biology and productivity to solve problems which limit productivity, and c) promotion of information interchange. Although much of the knowledge needed to improve buffalo production through breeding, feeding, management and disease control now exists, there is a clear need to study applications at the village, small-farm level for improving productivity through practical, cost-effective and sustainable interventions. This requires a sound understanding of the target farming systems, a multidisciplinary approach, collaboration with livestock extension services and interaction with national development programs. Agencies which fund research, as well as institutes engaged in it, have an obligation to ensure that their efforts result in tangible benefits to the rural buffalo farmers.

SUBSTANTIAL support has been provided by international, bilateral and national funding agencies, particularly over the past two decades, towards improvement of buffalo production in Asia (Acharya and Lokeshwar 1989). The main objective has been to promote more efficient utilisation of the buffalo for agricultural production in village farming systems, thereby contributing to improvement of the socio-economic status of the small farmers. The basis of this support has been a firm conviction, within national governments as well as among international funding agencies, that the buffalo has an important role to play in the agricultural economies of most developing countries in Asia.

The water buffalo plays different roles in different countries, providing milk, meat, draught power and social security in different combinations and proportions. There are only two major types of buffalo (river and swamp) but they are reared in vastly differing environments and under a multitude of management systems. Therefore, in any attempt to improve buffalo production, the initial need is to identify the constraints to optimal productivity in each specific management system and to determine the areas where research is needed to overcome these constraints. The type of applied research needed to address these problems is certainly not easy to conduct. It calls for a sound understanding of the target farming system, requires an interdisciplinary approach and must judiciously combine empirical on-farm studies with advanced laboratory techniques. The ultimate aim should be to develop cost-effective interventions based largely on indigenous resources.

The objective of this paper is to summarise the type of support which has been given to water buffalo research and development in Asia through different international and bilateral funding agencies and to highlight some of the areas where useful research results have been generated. It also attempts to identify the reasons for the paucity of on-farm applied research which is required for translating the knowledge already available into meaningful field applications. The focus of the paper will be on the process and modalities of research support, rather than on specific areas or scientific disciplines in which future research is required.

* Animal Production and Health Section, Joint FAO/IAEA Division, International Atomic Energy Agency, PO Box 100, A-1400 Vienna, Austria

Past and Present Support

Food and Agricultural Organisation (FAO) Rome

FAO has been active in supporting livestock production in tropical Asian countries since the early 1950s. Specific attention to buffalo production, however, gained momentum only in the early 1970s, in response to recommendations emanating from several of its expert consultations. During this period there was much interest in establishing an International Buffalo Research and Development Centre and several Asian countries were in support of the idea. However, in the mid-1970s an FAO expert undertook a survey of buffalo production in seven Asian countries and, based on the finding that many of the countries had established national research and development programs of their own to improve buffalo production, recommended that the strategy should be to strengthen national centres and to link them through a regional network.

The main mechanisms through which FAO supports buffalo research are Technical Cooperation Projects (TCP) which are country-specific, and regional programs. One of the earliest TCPs was a cattle and buffalo development and extension project in Pakistan, initiated in 1974, for improving breeding techniques and extension services under rural conditions. The studies conducted under this project and those undertaken concurrently in India have enabled the development of semen freezing technology for the buffalo (Hultnaes 1972). The benefits have clearly transcended national boundaries and facilitated wider application of Artificial Insemination (AI) throughout the region in this species.

A second major undertaking was the strengthening of the Philippine Carabao Research and Development Centre, financed by UNDP. This project was initiated in 1981 and had several components, the main activities being aimed at improving the milk production of carabaos through crossbreeding with the river types, evaluating the suitability of different genotypes for use as milk, meat and draught animals under different management conditions, and studying various aspects of reproduction, management, nutrition and diseases (Ranjhan et al. 1987).

FAO has also assisted in the compilation and dissemination of information on the buffalo. An important early contibution was to assist Dr Ross Cockrill in the publication of his pioneering book on the buffalo (Cockrill 1974). With assistance from the Swedish International Development Agency (SIDA), FAO organised an international meeting on reproduction of buffalo in Karnal, India, in 1978 and published the proceedings (FAO 1979).

In 1983 a UNDP-funded regional project entitled 'Buffalo Development — Technical Cooperation among Developing Countries for Training Junior Scientists and Dissemination of Research Results and Technology' was initiated. Under the first phase of this project three training courses were conducted: (a) Technical and economic aspects of agro-industrial by-product utilisation (Sri Lanka and India) (b) smallholder buffalo production systems (Philippines) and (c) Clinical aspects of buffalo reproduction (Pakistan). Three compendia of research results and state-of-knowledge reports were compiled in each of the subject areas and were published by the International Buffalo Information Centre (IBIC) in Bangkok.

The concept of Technical Cooperation among Developing Countries (TCDC) was utilised to good advantage through this program, much of the local costs being met by the participating countries through the National Currency Fund (NCF) established under FAO's Animal Production and Health Commission for Asia and the Pacific (APHCA). Phase two of this project was aimed at strengthening the regional network of buffalo development centres in Asia and included three training workshops, study tours, extension demonstrations and the writing of monographs.

FAO organised a Roundtable on International Cooperation in Buffalo Research and Development at the Second World Buffalo Congress held in New Delhi, India, in December 1988 (Acharya and Lokeshwar 1989). Arising from the recommendations of this meeting, an International Network has been initiated and steps are being taken to secure core funding for supporting participating institutes to undertake specific research projects.

International Atomic Energy Agency (IAEA) Vienna

The Joint FAO/IAEA Division's Animal Production and Health Section has been supporting research on ruminant production in Asia since the early 1970s, and buffalo production specifically since 1978. The Section's research programs are aimed at solving problems which limit animal productivity in tropical and subtropical regions, with emphasis on improving nutrition, reproductive efficiency and health of livestock. Particular emphasis is placed on evaluating the productivity of indigenous and upgraded breeds, under traditional village-level or small-farm management systems, with the objective of devising simple, cheap and acceptable methods for overcoming constraints imposed by climate, management, nutrition or diseases (Dargie 1989).

In order to provide technical support for these activities, a laboratory was established at the FAO/IAEA Agricultural Laboratory in Seibersdorf (near Vienna), with facilities for development and adaptation of nuclear and related techniques which can be applied under conditions prevailing in developing countries. For example, standardised kits for progesterone measurement, based on a simple and robust RIA method, are currently being supplied to over 70 laboratories for studies on reproduction. Similarly, development work is being undertaken in collaboration with leading laboratories of the world for standardising ELISA kits for detecting antibodies and antigens associated with a variety of viral, bacterial and parasitic infections. Several of these (e.g. for rinderpest, brucellosis and trypanosomiasis) are now being distributed to institutes participating in FAO/IAEA projects. The laboratory also serves as an important training facility for counterparts in developing countries wishing to establish the above techniques.

The FAO/IAEA provides support to institutes in developing countries through Coordinated Research Programs (CRPs) and Technical Cooperation (TC) projects. The CRP scheme involves the award of research contracts to scientists to conduct specific research projects of relevance to the objectives of that particular program. The contract holders are provided with modest financial support for equipment, reagents and local costs. They also have access to standardised kits, technical advice and literature services from the Joint FAO/IAEA Division. The participants are brought together for Research Coordination Meetings (RCMs) which are held every 16–18 months to promote interchange of information. At these meetings the contract holders have the opportunity of discussing their work and future plans with resource persons (termed 'agreement holders') who have specific expertise in the area.

There have been two successive five-year CRPs dealing exclusively with the buffalo. The first phase of this program, entitled 'The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia' (1978–84) included 12 contracts from 7 countries (Bangladesh, India, Indonesia, Malaysia, Philippines, Thailand and Sri Lanka), and 4 agreements from 2 countries (Australia and Sweden). Four RCMs were held during this period and the papers presented at the final RCM, together with the conclusions and recommendations, were published as an IAEA Panel-Proceedings Series in 1984 (IAEA 1984). The second phase of the program (1984–89) had 14 contract holders from 8 countries (Bangladesh, Indonesia, Malaysia, Pakistan, Philippines, Thailand, Sri Lanka and Vietnam) together with 5 agreement holders from 4 countries (Australia, Japan, Malaysia and Sri Lanka). Three RCMs were held, and the proceedings of the final RCM were published in 1990 (IAEA 1990).

Within the framework of the IAEA's TC program, the Joint FAO/IAEA Division assists Member States to become more self-reliant by strengthening the capability of institutes within the country to conduct studies on problems facing livestock production. The assistance provided includes equipment, training of local staff and provision of expert services in specific areas of need.

Several TC projects in Asia have dealt exclusively with buffalo, or with large and small ruminants in general. In Sri Lanka two successive projects were implemented (1978–86) for strengthening facilities at the University of Peradeniya and at the Veterinary Research Institute for research on nutrition, reproduction and diseases. The research projects undertaken within this framework also received support from other donor agencies such as SAREC (Sweden) and ACIAR (Australia).

The studies on reproduction provided comprehensive information on effects of climate, management and nutrition on reproductive efficiency of buffalo under village conditions (de Silva et al. 1985, Perera 1987). A management strategy, involving restricted suckling of calves, was found to be effective in reducing the long period of postpartum anoestrus which is responsible for poor fertility in village buffalo. The nutritional studies provided information on mineral deficiencies occurring in different forages during different seasons of the year, and also resulted in the development of a practical method for improving the feeding value of rice straw through urea treatment (Jayasuriya and Karunaratne 1984). Studies on *Toxocara vitulorum*, a parasite causing heavy mortality in buffalo calves, provided the basic information needed for devising a practical method of controlling the parasite (Roberts 1990). It involves the use of a single dose of a cheap and readily available anthelmintic at the most appropriate stage; this is now being applied in many other Asian countries where the parasite is a major problem.

In Thailand, the University of Khon Kaen was provided with assistance to establish an RIA laboratory and to apply this technique in studies on village buffalo reproduction. The facilities for nutritional studies were also upgraded and several staff members were trained in these disciplines. Currently the Joint FAO/IAEA Division is operating a multidisciplinary TC project funded by the UNDP, involving studies on soils, plants and animals. The components in animal science have assisted several universities (Chulalongkorn, Kasetsart, Chiangmai and Khon Kaen) as well as the Department of Livestock

Development to strengthen their research capabilities in reproduction and disease diagnosis. Studies on reproductive endocrinology have yielded valuable information which is now being used for improving fertility under small-farm conditions (Kamonpatana et al. 1989).

The Joint FAO/IAEA Division is also implementing a UNDP country project in Indonesia, aimed at improving the capability for studies on interactions between nutrition and reproduction of ruminants and at establishing ELISA techniques for diagnosing the more important diseases of livestock. The recipient institutes are the National Atomic Energy Agency (BATAN), the Directorate General of Livestock Services (DGLS) and the Research Institute for Animal Diseases (BALITVET). Based on the studies undertaken at BATAN, a strategy has been developed for providing cheap supplementary feeding to cattle, buffalo and goats, in the form of multi-nutrient urea–molasses blocks (UMB). Field trials have shown that UMB supplementation increases production (growth, milk) and reproduction in animals fed low quality roughage diets in a cost-effective manner (Dargie 1989). The technology for making the blocks has been simplified so that it can be adapted to local conditions and, in collaboration with the DGLS and Provincial Directorates of Livestock Services, BATAN is now transferring the technology to village farmers.

In the animal disease component of this program a national training course was held on the use of ELISA for diagnosing brucellosis. The Regional Disease Investigation Centres of the DGLS have been provided with the necessary resources for using this technique for epidemiological studies aimed at formulating a national control policy for brucellosis.

In the Philippines, a project was supported to evaluate the productivity of village carabaos maintained under traditional conditions and to monitor the effects of changes in management, nutritional supplementation and improved health care. The participating institutes are the Philippine Nuclear Research Institute, the Bureau of Animal Industry, the University of the Philippines at Los Baños and the International Institute for Rural Reconstruction.

The Universiti Pertanian Malaysia was provided with assistance to establish RIA techniques for hormone measurement and to use nuclear and related techniques for studies on utilisation of agro-industrial by-products as animal feed. Studies conducted under this program have indicated methods for improving reproduction of buffalo by strategic weaning of the offspring (Jainudeen et al. 1983) and for using palm-press fibre (waste from the palm oil industry) for feeding ruminants. A further component of this project is a collaborative attempt between the University and the Department of Veterinary Services (DVS) to establish ELISA techniques for diagnosing important diseases of livestock. This will enable the UPM, DVS and the Veterinary Research Institute at Ipoh to undertake epidemiological studies on diseases such as brucellosis and to use this knowledge for formulating more effective control programs.

In Vietnam a program to strengthen capability for studies on reproduction of cattle and buffalo is underway at the Institute for Agricultural Technology in Ho Chi Minh City. Studies have been initiated to evaluate reproductive efficiency in different grades of crossbred buffalo (swamp × river) and cattle (indigenous × European), with the objective of determining the best genotypes for use under local conditions. Assistance is also being provided to establish ELISA techniques for diagnosis of livestock diseases (trypanosomiasis in cattle and buffalo, and pseudorabies in swine) at the National Institute for Veterinary Research in Hanoi and the Veterinary Research Institute in Ho Chi Minh City.

Australian Centre for International Agricultural Research (ACIAR) Canberra

ACIAR promotes research into improving agricultural production in developing countries, by mobilising Australian expertise to help developing countries to identify and solve problems through collaborative research programs. It contracts research groups in Australian institutes to work with partners in developing countries and provides supplementary support to complement the resources of the participating institutes.

Two successive projects were supported in India (1982–89) on increasing the efficiency of straw utilisation through the use of urea–molasses blocks (UMB). The response to supplementation has been spectacular in terms of improved growth and milk production and the technology is now being used on a wide scale by village farmers in several dairy cooperatives. A third project is currently underway to study the feasibility of incorporating anthelmintics in the UMB, thus providing a practical delivery system to control gastro-intestinal parasites in milking buffalo at the same time as providing supplementary nutrition. A project in Thailand (1984–88) examined chemical and physical characteristics of fibrous agricultural residues under different environments and looked at supplementation strategies for the different materials.

The two projects on genetics and breeding, which are being concluded at this workshop, have followed the structure of CRPs referred to above, with several participating countries tackling a common problem. The project on evaluation of different buffalo genotypes for draught, meat and milk production (1985–90) was aimed at encouraging research institutes in Southeast Asia to undertake comparative studies on different breeds and/or strains of buffalo in respect of nutrition, reproduction, growth and draught power. The program on genetic identification of strains and genotypes of buffalo and goats in Southeast Asia (1986–90) involves the analysis of patterns of inheritance of biochemical markers and chromosomal segregation in different river × swamp crosses.

A multidisciplinary project on studies of draught power systems is underway in Indonesia (Hoffmann et al. 1989). The three main components are farming systems research, nutrition-physiology and economics. On-site farm trials, ploughing contests, demonstrations and training of farmers in new technologies are part of the activities aimed at improving draught power output from swamp buffalo and its optimum utilisation by the village farmers.

In the field of animal diseases, a regional program is underway to establish improved methods for the diagnosis and control of livestock diseases in Southeast Asia using the ELISA technique. The buffalo diseases of importance for which ELISAs are being established are brucellosis and haemorrhagic septicaemia (HS). In the case of brucellosis the ELISA is being tested against conventional methods for use in sero-epidemiological surveys, while the objective of the HS work is to develop a typing ELISA for identifying HS-causing strains of *Pasteurella multocida* from field isolates (Dawkins et al. 1990).

A project on epidemiology of ephemeral fever in China has introduced new diagnostic techniques to study the epidemiology of this disease. In Sri Lanka, a project funded jointly by ACIAR, IAEA and SAREC to study the life cycle of the important helminth parasite *Toxocara vitulorum* yielded valuable information which was then utilised for developing an effective control strategy (see IAEA above). A second project in Sri Lanka looked at the feasibility of using vaccines for babesiosis and anaplasmosis in ruminants.

In Thailand support was provided for developing modern diagnostic methods for foot-and-mouth disease (FMD). The techniques established permit identification of the serotypes involved in an outbreak and therefore allow more rational vaccination strategies to be adopted.

Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) Bonn

GTZ is the organisation responsible for Technical Cooperation activities in the (former) Federal Republic of Germany. It has supported two major livestock development projects involving buffalo. The first, located in the Punjab District of Pakistan (1978–81) aimed at improving livestock production through initial surveys and subsequent studies on fodder production, AI, animal health services, milk marketing and use of male calves for meat production.

A follow-up project (1983–89) is located at Pattoki in the Punjab and is aimed at establishing economically independent farmers' organisations on a self-help basis, and providing them with improved extension services and marketing facilities. The research component of the project is integrated with the infrastructure development aspects and involves studies on management, nutrition, growth, health and reproduction. In 1987 GTZ sponsored an international symposium on buffalo reproduction in Islamabad and assisted in publication of the proceedings (PARC).

International Development Research Centre (IDRC) Ottawa

IDRC is a corporation funded by the Canadian Government, with an international Board of Governors. Its mandate is to support scientific and technical research by developing countries based on their own priorities. One of the seven Divisions within IDRC is Agriculture, Food and Nutrition Sciences (AFNS), with the objective 'access to food for the individual'.

IDRC is supporting a project, based in Malaysia and involving several Southeast Asian countries, to study the relationship between chromosome composition and production characteristics in different grades of swamp × river crossbred buffalo. In the Philippines, a project was undertaken to study various aspects of draught power, with the objective of providing information on constraints faced by small farmers and designing practical interventions. Two further projects on draught power are being supported in Thailand and India, with more specific emphasis on the role of nutrition, and aimed at evaluating the potential for improving draught power on small farms.

IDRC has supported the International Buffalo Information Centre (IBIC) at Kasetsart University in Thailand since its inception. This has resulted in

problems involving actual farming situations because such projects are difficult to execute and control and are of a long-term nature. There is also no guarantee that scientifically valid or statistically significant data which are publishable will be forthcoming from such investigations.

A further constraint is that scientists working in universities or research institutes sometimes have little contact with the field livestock services or national agencies involved in development and extension activities. While it is necessary that any livestock development program should have a research component aimed at monitoring the effects of any innovations that are applied, it is also clearly desirable for scientists to tailor their research around their national or local livestock development programs. The two sectors should ideally work in partnership, complementing the resources and expertise of one another. Donor agencies should promote and, where necessary, make their support conditional on such interaction.

Similarly, different funding agencies working in related fields within a country or a region should attempt to coordinate and link their activities. For example, one agency may be assisting a research institute in the development of techniques for disease diagnosis, while another is supporting the field veterinary services to undertake epidemiological studies. The two projects should obviously work together if the country is to derive the full benefits. Unfortunately, such collaboration is sometimes lacking.

Bridging the Gap

As stated in the introduction, the objective of most funding agencies is to generate research which would ultimately (but not in the too distant future!) help the small farmers of Asia to utilise the buffalo more efficiently for improving their economy. Developing the infrastructure for research in developing countries and providing scientists with the opportunity to play a dynamic role in national development is also an important consideration. However, the ultimate goal should not be clouded by the intermediary processes.

In my opinion, international funding agencies should strive for more coordination between one another for the type of support provided. They should also restrict institution building and limit infrastructure development to countries where a real need and justification exists. More support should be channeled to well-defined projects aimed at solving the problems faced by indigenous farmers. A greater degree of selectivity aimed at focusing support in priority areas and more vigorous technical direction as well as regular critical reviewing of the results is desirable. A proven method of achieving this is to provide for peer-reviews through research coordination meetings or workshops, which also promote interchange of information between scientists working on similar problems within the region.

Attempts should be made to foster interdisciplinary research aimed at tackling a specific problem from various angles and to ensure active interaction with related livestock development programs within the country. Collaboration with other field extension services and farmers' organisations should be encouraged where relevant.

The adoption of a structured approach along the following lines should be promoted in order to ensure that research goals stay relevant to local problems:

a) determine current status of production and the major constraints, through field surveys and longitudinal case studies;

b) study possible methods for overcoming the constraints, using local resources and simple interventions such as strategic supplementation of the diet, changes in management, control of diseases, etc.;

c) test these under local farming conditions and, if found to be effective and applicable, undertake wider dissemination through national livestock development and extension systems.

Admittedly, these are difficult and unpopular choices. However, the agencies which fund research, as well as the institutes engaged in it, have a responsibility to make the best use of available resources, and to ensure that some of the benefits reach the ultimate target, the rural farmers who form the backbone of agricultural production in most Asian countries.

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the development of a valuable repository of information on all aspects of buffalo production, which serves as a focus for formal as well as informal interchange of information. The 'Buffalo Bulletin' published by IBIC is a further contribution in this direction.

Swedish Agency for Research Cooperation with Developing Countries (SAREC) Stockholm

SAREC is an independent agency of the Swedish Government and has the objectives of strengthening research capacity in developing countries and facilitating their access to research results. It supports international, regional and national programs. In the field of buffalo production, SAREC's main support has been to a multidisciplinary program in Sri Lanka.

In 1980 SAREC funded a national workshop on buffalo research to document the present status and future priorities (SAREC 1980). Based on the recommendations, and subject to regular review by Swedish and Sri Lankan scientists, SAREC is supporting a broad-based program of research involving various aspects of management, nutrition, reproduction and diseases of buffalo, as well as studies on socio-economic aspects of buffalo production. Some of these studies have also received supplementary support from projects of the IAEA and ACIAR.

The results from these studies have provided valuable baseline information on buffalo production in Sri Lanka (see also IAEA above). With regard to nutrition, the information required for providing strategic supplementation with minerals, which are deficient in certain regions or during different seasons, now exists. Improvement of reproductive performance has been shown to be possible through improved nutrition and calf management practices such as limited suckling (Perera et al. 1988). Studies on the helminth parasite *Toxocara* (in collaboration with IAEA and ACIAR projects) have yielded the information needed for effective control through strategic use of anthelmintics. With regard to HS, studies on the carrier state and immunity after vaccination have provided more insights into the epidemiology of this disease.

Based on this work, an educational booklet containing information on the biology and husbandry of buffalo has been published (NARESA 1989).

Discussion

From the foregoing it is clear that much international and bilateral support has been channelled to buffalo research in Asia. The inputs from national sources have also been considerable, particularly in countries such as India, Pakistan and Thailand. These investments in infrastructure development have resulted in well equipped laboratories and adequately qualified staff in many Asian countries, with sufficient resources to handle the type of research needed to improve buffalo production under their own conditions.

Support has also been provided for specific research projects to study methods of improving buffalo production and for interchange of information between scientists within the region. The concept of coordinated research programs, woven around a common theme, with several participating countries forming a network has proved popular among donors as well as recipients.

In general, it can be concluded that the approach taken by many international agencies, that of strengthening national research capability and facilitating exchange of information as well as transfer of appropriate technology between developing countries, has served the needs of the region far better than the establishment of a centralised international centre.

Needs for the Future

In most countries of Asia, a considerable wealth of knowledge has been accumulated on the basic physiology and production characteristics of the buffalo. With regard to the dairy-type river buffalo, it can be stated that much of the information needed for improved breeding, feeding, management and disease control already exists. In the predominantly draught-type swamp buffalo, the diversity of environments and production systems precludes generalisations but the basic means to improvement exist.

The question is, how much of this has been tested and applied in real farming situations, and what has been the contribution towards improving production at the village level? Although some of the new knowledge and technologies have been translated into practical applications, my contention is that much more needs to be done in this direction. Even where practical, proven, applications exist mobilisation of appropriate national agencies responsible for dissemination of the technology among farmers has been slow.

There are several possible reasons for this trend. The first is that some research workers, by the very nature of their background and training, may not understand the problem from the farmer's viewpoint, or appreciate the importance of seeking a solution within the socio-economic framework of the farmer. The second is that they are cautious about tackling

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Results from the Buffalo Evaluation Project and Future Directions for Research

J.E. Frisch* and J.E. Vercoe*

Abstract

When this ACIAR project was initiated, we posed a number of questions relating to ways of improving the productivity of the swamp buffalo of Southeast Asia and offered suggestions that might lead to solutions to those questions (Frisch and Vercoe 1984). The solutions offered ranged from being solely genetic to solely environmental. However, the nature of the project was such that emphasis would be placed on genetic solutions while not forgetting most problems would be solved through some combination of both genetic and environmental changes. In the interval since the project was initiated, less than one buffalo generation has passed. It would therefore be extremely naive to think that complete investigation of possible genetic solutions could have been accomplished by project scientists. Nevertheless, it could be expected that the basis for improvement of productivity would have been established and knowledge relating to future directions for research effort would have been accumulated. This paper examines the degree of success achieved by the project in both areas of expectation with success being measured in terms of improvement in the major components of productivity in any country in the region. The desirability of increasing each of these major components has been presented previously (Frisch and Vercoe 1984).

Improvement of Growth Rate

THE relevance of increasing growth rate relates to concurrent increases in draught capacity and the amount of meat produced per unit of time, and to a likely reduction in the age at puberty. Studies within the project have demonstrated that F_1 (swamp × river) animals grow far more rapidly than the pure swamp types. In the Philippines, liveweights of F_1 (carabao × Nili-Ravi) and F_1 (carabao × Murrah) were about 50% higher at 12 months of age than those of straightbred carabao both in village and in institutional herds (Parker et al., these Proceedings). In Thailand, Bunyavejchewin et al. (these Proceedings) have reported that the F_1 (Thai swamp × Murrah) had higher weaning weight and post-weaning gains that were about 26% higher than those of the purebred Thai swamp. Kamonpatana et al. (these Proceedings) have reported consistent differences in growth to three years in favour of the F_1 ranging from 13 to 34% depending on age, sex and location. In Vietnam growth rate to 12 months of age was about 25% higher for the F_1 swamp × Murrah (Cuong and Trieu). In Indonesia (Situmorang and Sitepu, these Proceedings) the liveweight of the F_1 swamp × Murrah was significantly higher at all ages than that of the straightbred swamp both in village and institutional herds. By nine months the F_1 hybrids were about 22% heavier than the straightbreds. By three years of age the difference had increased up to 30%, depending on location, in favour of the F_1 hybrid. There has not been any report from project scientists from any country that contradict these results. Thus, improvements in growth rates of swamp buffalo can be expected over a wide range of environments and management systems simply by crossing to a suitable river breed. That this result has widespread applicability is supported by the absence of any reports of genotype × environment interactions for liveweights at any age. This effectively discounts the possibility that all of the improvements in gains have arisen from favoured treatment of the crossbreds. Thus a substantial genetic improvement in liveweight has been obtained in a single generation using relatively simple technology. To achieve gains of similar magnitude by selection within the swamp types, complex, large-scale recording schemes would have to be organised and then operate efficiently for probably 50 years

* CSIRO Division of Tropical Animal Production, Rockhampton, Queensland 4702, Australia

or more. Even if the lack of recording facilities and small herd sizes are ignored as major constraints to success of such a scheme, the time involved would alone make such a selection program impractical. The evidence is therefore very clear that at least initially, where rapid and substantial genetic improvement of growth rates of swamp buffalo is required, crossing to one of the large river breeds is at present the only feasible solution.

For these experimental results to have a significant impact at the national level, widespread acceptance of the crossbreds by farmers is essential; simple, effective methods for producing large numbers of F_1s must be developed and breeding programs that would ensure continuation of the gains achieved in the initial cross need to be tested. The question relating to the availability of feed for larger, faster-growing animals also needs to be addressed. Results from the project provide clear evidence that at least those farmers participating in the crossbreeding studies have been able to feed their crossbreds at such a level that they have been able to express their higher genetic potential for growth. In all countries the F_1 hybrids were substantially heavier than the swamp types reared under similar conditions. At a more general level, if it is assumed that the production of cereal straws and other agricultural and horticultural by-products is to retain proportionality with human numbers, it is likely that the maximum sustainable mass of buffalo will rise accordingly. Sociological reasons aside, it is likely that there is no biological penalty associated with this mass being composed of fewer, larger animals rather than a larger number of smaller animals, provided levels of feeding remain in proportion to size. In this respect, differences in digestive efficiencies of the swamp types and the crossbreds are likely to be small or non-existent (Jelan and Abdullah, these Proceedings) and results from cattle suggest that, at ad lib. levels of feeding, overall efficiency of feed utilisation by the larger crossbreds will be similar to that of the smaller swamp types (Frisch and Vercoe, 1984). Thus in those situations where an increase in growth rate and size are desirable or necessary, prime consideration must be given to crossing the swamp types to the appropriate river breed.

Improvement of Reproductive Rate

In those countries or regions where increases in numbers of buffalo or increased rates of turnoff are required, increases in reproductive rates of the existing breeds will be necessary. It may be that environmental solutions for increasing the characteristically low reproductive rates of swamp buffalo will be found. However, unless these solutions are very simple, inexpensive and readily available at the village level, they cannot be expected to have any significant impact at the national level on buffalo reproductive rates. The long-term solution, one that does not require continual inputs, is to use genotypes that have higher reproductive rates than the swamp types over a wide range of environmental conditions. The only possible way in which this could be achieved in the short term is by crossing the swamp types to other appropriate breeds or strains. Investigations of this possibility formed an integral part of the ACIAR-sponsored project. Comparative reproductive rates of straightbred swamp, river (either Murrah or Nili-Ravi) and their F_1 hybrids have been measured in several different countries.

To demonstrate statistically significant differences in reproductive rates of the different genotypes in all countries would require comparisons involving hundreds of animals at each location. Lack of resources precludes such a strict comparison. However, data have been obtained for several reproductive parameters that indicate that genetic differences do exist between different buffalo genotypes. In assessing these differences in reproductive performance, it is necessary to know whether genotype $\times$ environment interactions occur and if so under what conditions. It was with this aim that calving to conception intervals were measured in Sri Lanka on two groups of Lanka and Murrah buffalo, one group allowed to graze natural vegetation and the other allowed to graze but offered additional supplementary feed (Mohamed and Jayaruban, these Prodeedings). Both genotypes responded markedly to the supplementary feed with reduced calving to conception intervals but, more importantly in the context of long-term improvement of the reproductive rate, the calving to conception intervals of the Lanka buffalo that were grazed and not offered supplementary feed were the same as those of the Murrah group fed concentrate supplement (214 + 104 and 214 + 116 days, respectively). This indicates that a real genetic difference in reproductive capacity exists between the two genotypes.

In the Philippines, comparative reproductive performance of carabao (swamp) and F_1 carabao $\times$ river (Murrah and Nili-Ravi) was measured both in village and institutional herds (Momongan et al., these Proceedings). The F_1 hybrids reached puberty and produced their first calves about one year earlier than the straightbred carabao. Subsequent intercalving intervals of the F_1s were shorter (although not statistically significant) than those of their carabao contemporaries by about six months.

In Thailand, reproductive performance of the F_1 hybrids has been superior to that of the straightbred swamp type. Kamonpatana et al. (these Proceedings)

have reported that F_1 (swamp × Murrah) females reached puberty at a younger age than purebred swamp females at two locations that differed in the availability of feed. In Vietnam, mean intercalving intervals of Vietnamese swamp buffalo were reported to be about 100 days longer than those of F_1 swamp × Murrah hybrids (Cuong and Trieu pers comm.). The Malaysian comparison (Mahyuddin et al., these Proceedings) is anomalous in that intercalving intervals were excessively long when compared to either previous estimates from the same farm or relative to estimates from other countries. In the present report, although the proportion of F_1 swamp × Murrah females resuming ovarian activity by 120 days post-partum was higher than that of the purebred Malaysian swamp, intercalving intervals were reported to be higher for the F_1 (799 + 384 days) than for the swamp (628 + 203 days).

In all of the above examples, data have originated from small numbers of animals. However, the superiority of the F_1 hybrid over the straightbred swamp type is almost completely consistent. This is a strong indication that the superiority of the F_1 hybrids is real and is not due to chance alone.

Reproductive studies within the project have concentrated on females. However, a study in Indonesia (Situmorang and Sitepu, these Proceedings) has examined some variables associated with male fertility. This is important not only because of the contribution of the male to reproductive rates of females, but also because karyotype differences between swamp and river types may be associated with problems at spermatogenesis. In the Indonesian study, there were no significant differences in the volumes of ejaculates obtained from swamp, Murrah or their F_1 hybrid, but semen from swamp bulls had the highest sperm concentration. However, the F_1 and the Murrah produced a greater number of ejaculates during weekly 15 minute tests. Time to first ejaculation on presentation of a teaser animal was also shorter for the Murrah and F_1 genotypes. Numbers of animals involved were low but the data do indicate that libido and semen quality of the F_1s are satisfactory.

Thus, the evidence presented by project scientists indicates that the reproductive capacity of the F_1 hybrid female is superior to that of the various swamp strains. The improvement achieved by crossbreeding has been consistent and it is doubtful if any other method currently available could match the magnitude of the increases or the simplicity with which they were achieved. Crossbreeding must therefore be given serious consideration whenever there is a need to increase reproductive rates of the swamp types.

Improvement of Draught Capacity

One of the prime functions of buffalo throughout the region is to provide draught power, including power for land preparation. This, although the F_1 (swamp × river) may have superior growth and reproductive rates, it is unlikely to be acceptable to most smallholder farmers unless it also has superior or at least equivalent draught capacity to the existing swamp types. Investigations of comparative draught capacity were therefore an integral part of the project.

Comparisons in Thailand (Bunyavejchewin et al. these Proceedings) examined differences between F_1 (swamp × Murrah) and Thai swamp buffalo in physical measurements associated with draught capacity. The F_1 generated more power than the swamp but ploughed at a faster speed. The net result was that the actual areas ploughed in 90 minutes were very similar (552 and 544 m^2 for the F_1 and swamp types, respectively). To achieve this result, the width of cut must have been about 13% greater for the plough drawn by the swamp animals and this may account for their slower speed.

Differences between genotypes in physiological measurements in response to work were small. Increases in rectal temperature, pulse rate and respiration rate in response to work for 90 minutes were similar for both genotypes which supports the conclusion reached from the comparisons of physical performance that both genotypes had similar net draught capacity. Comparisons in Indonesia (Situmorang and Sitepu, these Proceedings) of changes in rectal temperature, pulse and respiration rates in response to work are in complete agreement with the results from Thailand.

Thus, although the comparisons have been of limited scope, what evidence there is suggests that the draught ability of the F_1 (swamp × river) is at least equivalent to that of the straightbred swamp types.

Improvement of Milk Yield

It has long been recognised that the potential milk yield of the swamp types can be increased by crossing to a dairy-type river breed. It may therefore be expected that further comparisons of milk production of F_1 and swamp type could add little, if any, additional information to what is already known. However, additional information that is required is whether the higher milk yield of the F_1 has a detrimental effect on reproductive rates, particularly when they are fed poor quality diets. In the Philippines (Momongan et al., these Proceedings), milk yields of F_1 (carabao × Murrah) and straightbred

carabao were compared when both genotypes were also rearing their calves. Comparative estimates of partial milk yields (overnight production only) were 653L and 195L for the F_1 and carabao respectively. Despite the higher yield of the F_1s their subsequent intercalving intervals were almost 200 days shorter than those of their carabao contemporaries. These results were obtained from animals fed adequate diets including concentrates but are further supportive evidence of the superiority of the F_1 as a multipurpose animal.

Additional Information Required

The comparisons conducted throughout the region as part of the project lead to the conclusion that considered overall, the performance of the F_1 (swamp $\times$ river) is superior to that of any of the swamp types to which the F_1 has been compared. No other method, genetic or environmental, can produce lasting improvements in overall productivity of the magnitude produced by crossbreeding in such a short time, Thus, wherever the need or the desire exists to increase overall productivity of the swamp types, crossbreeding must be seriously considered as the first step in the process. There are however several aspects associated with the production of crossbreds that need additional investigation before large-scale crossbreeding can be confidently recommended.

Male fertility

Crossing of the swamp ($2n = 48$) and river types ($2n = 50$) produces an F_1 that has 49 chromosomes. This unequal diploid number could be expected to lead to complications at meiosis with the possibility that reproductive rates of the F_1 could be reduced compared to those of the parental types. Comparisons by project scientists have produced strong indications that reproductive potentials of F_1 females are higher than those of the swamp types. However, the limited evidence from Indonesia (Situmorang and Sitepu, these Proceeedings) suggests that sperm concentration of the F_1s may be affected. This is not unexpected as previous studies using testicular tissue from F_1 bulls (see Barker et al., these Proceedings) have reported a high proportion of degenerating spermatocytes as well as other abnormalities. However, the practical significance of these conditions remains to be assessed both in AI and natural mating systems.

Since progeny of the F_1 must be either F_2 or backcross to either swamp or river types, their diploid numbers could be $2n = 48$, 49 or 50. Whether there are any differences in fertility of these different karyotypes has not been unequivocally demonstrated. The number of animals required to do so is large and regional cooperation will be required if a result is to be forthcoming within a reasonable time.

Identification of appropriate crosses

The Murrah and Nili-Ravi, which are quite closely related, were the only two river breeds used to generate the F_1s involved in the project. Within the swamp types, an unknown number of strains was used, unknown because there has been very limited differentiation of the swamp types from the different geographical regions. Mukherjee et al. (these Proceedings) have however begun investigations into genetic relationships between swamp types using biochemical polymorphisms and have reported significant genetic differentiation between ten populations sampled from Indonesia, Malaysia, Philippines and Thailand. While in all cases reported the F_1s produced from these different populations of swamp types have been shown to be more productive than the purebred swamp types, the possibility that other crosses would be more suited to particular regions or production systems remains to be investigated. F_1s of large mature size or high milk yield potential such as those obtained by crossing the swamp types to the Murrah or Nili-Ravi will not be the most appropriate animals in all circumstances. Other crosses utilising river breeds of lower milk yield potential or smaller mature size then the Murrah or Nili-Ravi, or different strains within the swamp types need to be investigated. Since the number of breeds and strains potentially available for crossbreeding is large, empirical testing of all possible combinations is neither logistically nor economically feasible. Initial testing must be confined to comparisons of genetically different groups that are known or thought to have the attributes desired in the crossbred. Methods for identification of these different groups have been outlined elsewhere (Barker et al., these Proceedings). Once the appropriate groups have been identified, effort could then be directed towards identifying the most desirable genotypes within those groups. This should be done as a matter of urgency before the implementation of any large-scale crossbreeding programs.

Development of appropriate breeding systems

Although the F_1 has been shown to have several productive advantages over the purebred swamp types it is only the first step towards the development of a breed that is more productive than the existing swamp types. Reproductive rates of the swamp types are too low to allow the continuous production of F_1s. Breeding policies must therefore be adopted that will maximise the productive advantages of the combination of swamp and river types. The options are

to interbreed, backcross or cross to a third unrelated breed. It is likely that whichever system is adopted, productivity will decline relative to that of the F_1. However, in some environments or production systems the decline may be of minor practical significance. At the current state of knowledge of buffalo genetics, the magnitude of any decline in productivity from the F_1 cannot be predicted. Even so, unless the comparative productivities of crossbreds with $2n = 48$, 49 or 50 were shown to be markedly different, it is doubtful if this knowledge would have any significant impact on short-term breeding policies. The production from the F_1 of some $2n = 49$ animals is unavoidable regardless of which breeding system is adopted. Low reproductive rates combined with the need to maintain or increase numbers dictate low culling rates and any decision to cull an animal is likely to be made for reasons other than its karyotype even if the karyotype was known. However, if it was shown that crossbreds with $2n = 49$ had a productive disadvantage, long-term breeding policy should aim at their elimination. This is likely to be an expensive and time-consuming exercise and reinforces the urgency to determine comparative productivities of $2n = 48$, 49 and 50 karyotypes before any large-scale crossbreeding schemes are implemented.

The question of karyotype aside, the main aim of any initial breeding policy should be to establish the relative proportions of swamp and river types required in the commercial animal. This is likely to vary depending on the environment and the production system used and will have to be determined for each particular set of circumstances. Since the opportunity for using within-breed selection to alter gene frequencies in village populations of draught buffalo is low, the required combination of breeds needs to be set in the first stages of crossbreeding. After that, interbreeding is likely to be the only feasible system for maintaining approximate combinations of the required genes.

Comparative draught capacity

The prime function of non-dairy buffalo is to produce draught power and additional evidence on the comparative draught capacity of crossbreds and swamp types would increase confidence in any decision to launch wide-scale crossbreeding programs. In this context, studies conducted under the Draught Animal Power project are of major significance.

Efficient production of F_1s

It is likely that the number of exotic bulls available for crossbreeding purposes will be very limited for at least several buffalo generations and AI will continue to be used as the main method for producing F_1s. However, project scientists have consistently reported low success rates where AI has been used in village herds. If crossbreeding is to make a significant impact at the national level, efficient, simple low-cost methods for improving the success of AI have to be developed. Areas for research might include methods for prolonging the life of sperm in the female reproductive tract or more efficient synchronisation and detection of oestrus.

Comparative resistance to environmental stresses

Most buffalo spend their entire life in relatively stressful environments and to function satisfactorily they must have a high level of resistance to the stresses of these environments. Differences in resistance are expressed through differences in mortality, particularly in calves, differences in growth or reproductive rates, or as differences in physiological responsiveness, particularly to work. Insufficient data on comparative mortalities of F_1s and pure swamp types have been generated within the project to decide whether biologically significant differences do exist. However, the limited evidence that is available suggests that there are no major differences in mortalities between the two genotypes. The comparative advantage of the F_1s over the swamp types for growth and reproduction and their similar physiological response to work also suggest that if there are any differences in resistance to environmental stresses between the two genotypes they are small. However, in all instances, it has been F_1 animals that have been compared to the swamp types. These F_1s are a unique generation with a unique combination of parental genes that is not found in any other generation. Because of this, resistance of the F_1 is likely to be close to that of the more resistant purebred parent. This may give a false impression of the level of resistance to be expected in subsequent generations of crosses. As segregation of the genes that control resistance occurs in the F_2 or if the F_1 is backcrossed to the less resistant parental breed, the mean level of resistance will decline. Lack of resistance could then become an important determinant of the comparative productivity of subsequent generations of crosses.

To be certain of the value of any particular cross to provide a sustained lift in productivity, the characteristics of the F_2 or backcross generations need to be known. Insufficient data are available from within the project from which to draw firm conclusions. However, in Thailand (Bunyavejchewin et al., these Proceedings) the lower post-weaning gains of both purebred and backcross Murrah com-

pared to pure swamp animals and the F_1 hybrid suggest that the Murrah has lower resistance to environmental stresses than the swamp type.

These results suggest that in those regions where buffalo are used mainly for purposes other than milk production, upgrading of the swamp type to Murrah will reduce productivity. Whether this reduction is associated with lower resistance to environmental stress of the Murrah (though not necessarily other river breeds) remains to be determined. Regardless of the reason, the limited data available do suggest that it will be crossbreds rather than purebreds that will be the most productive animals.

It would be very naive indeed to assume that all of the answers for solving the problems associated with low productivity of swamp buffalo are already known. The project has simply laid the foundations for finding solutions. Continuation and extension of these studies will be required if crossbreeding is to make an effective contribution to improvement of swamp buffalo populations at the national level. This will not happen without continued support for and interest in the buffalo project.

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Participants

Dr Tengku Azmi
Faculty of Veterinary Medicine and Animal Science,
Universiti Pertanian Malaysia,
43400 Serdang, Selangor
Malaysia

Dr Zainal Aznam Jelan
Department of Animal Sciences,
Universiti Pertanian Malaysia,
43400 Serdang, Selangor,
Malaysia

Dr C.R. Balakrishnan
National Dairy Research Institute,
Karnal-132001
(Haryana),
India

Prof. J.S.F. Barker
Department of Animal Science,
University of New England,
Armidale, NSW 2351.
Australia

Mrs Pakapun Bunyavejchewin
Buffalo & Beef Prod. Res. and Development Centre,
Kasetsart University,
Bangkhen, Bangkok 10900,
Thailand

Prof. R.H. Crozier
Department of Genetics and Human Variation,
La Trobe University,
Bundoora, Vic. 3083.
Australia

Mr Le Xuan Cuong
Agricultural Insitute of Vietnam,
Ministry of Agriculture (Cattle and Buffalo Research Department,
121 Nguyen Binh Khiem Street,
No. 1 District,
Ho Chi Minh City,
Vietnam

Dr G. Davis
CSIRO Division of Tropical Animal Production,
Tropical Cattle Research Centre,
Box 5545 Mail Centre,
Rockhampton, Qld. 4702.
Australia

Fongpaik Quai
59 Jalan 11/4,
46200 Petaling Jaya,
Kuala Lumpur,
Malaysia

Dr J.E. Frisch
CSIRO Division of Tropical Animal Production,
Tropical Cattle Research Centre,
Box 5545 Mail Centre,
Rockhampton, Qld. 4702.
Australia

Dr Y.Y. Gan
Department of Biotechnology,
Universiti Pertanian Malaysia,
43400 UPM, Serdang, Selangor
Malaysia

Ms Connie Halbeiten
Research Fellow,
Institute for Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

Dr Mohd Hilmi Abdullah
Faculty of Veterinary Medicine and Animal Sciences,
Universiti Pertanian Malaysia,
43400 Serdang, Selangor
Malaysia

Dr D. Hoffmann
Research Program Co-ordinator,
Animal Sciences,
ACIAR,
GPO Box 1571,
Canberra, ACT 2601,
Australia

Prof. Maneewan Kamonpatana
Faculty of Veterinary Science,
Chulalongkorn University,
Henri Dunant Street,
Bangkok 10330,
Thailand

Mr P. Lynch
Proceedings Editor,
ACIAR,
GPO Box 1571,
Canberra, ACT 2601,
Australia

Mrs B. Marler
Administrative Assistant,
CSIRO Division of Tropical Animal Production,
Tropical Cattle Research Centre,
Box 5545 Mail Centre,
Rockhampton, Qld 4702.
Australia

Prof. Mohamed Mahyuddin Dahan
Fakulti Sains & Teknologi Makanan,
Universiti Pertanian Malaysia,
43400 UPM,
Serdang, Selangor,
Malaysia

Dr A.R. Mohamed
Department of Animal Science,
Faculty of Agriculture,
University of Ruhuna,
Kamburupitiya,
Sri Lanka

Prof. Vincente G. Momongan
Institute of Animal Science,
University of the Philippines at Los Baños,
College, Laguna 4031,
Philippines

Prof. T.K. Mukherjee
Institute of Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

Dr R.K. Munro
Bureau of Rural Resources,
Department of Primary Industries and Energy,
GPO Box 858,
Canberra, ACT 2601.
Australia

Dr J.M. Panadam
Institute of Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

Dr Benedioto A. Parker
Institute of Animal Science,
University of the Philippines at Los Baños,
College, Laguna 4031,
Philippines

Dr B.M.A.O. Perera
Animal Production and
Health Section,
Joint FAO/IAEA Division,
PO Box 100,
A-1400 Vienna,
Austria

Mrs J. Sambhi
ACIAR Liaison Officer,
C/o Australian High Commission,
Kuala Lumpur,
Malaysia

Mr Selvaraj O.S.
Institute of Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

Dr F.H. Shah
Genetics Department,
Universiti Kebangsaan Malaysia,
Bangi, Selangor,
Malaysia

Dr P. Situmorang
Balai Penelitian Ternak (BPT),
PO Box 123,
Bogor 16001, West Java,
Indonesia

Dr P. Sitepu
Balai Penelitian Ternak (BPT),
PO Box 123,
Bogor 16001, West Java,
Indonesia

Mr Sreetharan
Institute of Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

Dr S.G. Tan
Department of Biology,
Universiti Pertanian Malaysia,
43400 UPM, Serdang, Selangor
Malaysia

Em. Prof. N.M. Tulloh
1 Wilgra Avenue,
Ashburton, Vic. 3147,
Australia

Dr J.E. Vercoe
CSIRO Division of Tropical
Animal Production,
Tropical Cattle Research Centre,
Box 5545 Mail Centre,
Rockhampton, Qld 4702.
Australia

Ms Yushayati Yusof
Institute of Advanced Studies,
University of Malaya,
59100 Kuala Lumpur,
Malaysia

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