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MINERAL COMPOSITION AND NUTRITIVE VALUE OF FRESH AND SUPPLEMENTED RICE STRAWS PRESERVED IN MANUAL STACKING, BALLING, AND WRAPPING STORAGE METHODS

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ABSTRACT

Rice straws are underutilized as feed due to cattle's preference for fresh straw. Rice straws have low nutritional value and are easily susceptible to microbial spoilage during traditional storage of loose stacking or manual open-air balling. Supplementing fresh rice straws with some minerals, energy, and protein could improve the nutritional value and storage stability under different preservation methods. The present study aimed to determine the effect of supplementing fresh rice straws with minerals, molasses, and urea, stored under three manual treatments: loose stacking, open-air balling, and airtight wrapping. Fresh rice straws were treated with 1% calcite-based mineral mixture, 0.6% molasses, and 0.05% urea and stored for 60 days. The supplemented rice straws were chopped and mixed with a basal diet composed of 55% elephant grass and 20% concentrate. Four experimental diets were then developed from the composite. That is the basal diet + fresh rice straw (FRS), basal diet+stacking supplemented rice straw (SSRS), basal diet+balling supplemented rice straw (BSSR), and basal diet+wrapping supplemented rice straw (WSRS). The experimental diets were fed to young Pesisir bulls using a 4x4 Latin Square design for eleven days. Fresh and supplemented rice straws were analyzed for dry matter, mineral content, blood hematology profile and protein content. The fresh rice straw had a relatively high potassium, iron, and manganese content but was low in calcium, sodium, phosphorus, copper, and selenium. Supplementation significantly increased the concentration of calcium, phosphorus, potassium, sodium, copper, and selenium in the preserved rice straw. Rice straw preserved in the wrapping way had the highest copper, cobalt, and selenium content. Feeding cattle with supplemented rice straw preserved in the wrapping method, decreased the blood monocyte value equivalent to that of the fresh straw. Supplementation of fresh rice straws with calcite-based minerals, molasses, and urea has the potential to increase some essential mineral content and maintained the nutritional value of rice straw preserved in an air-tied wrapping method.

Key words: Calcites, Hematology, Mineral, Pesisir cattle, Rice straw, Storage, Supplementation



INTRODUCTION

Rice is a staple food and widely cultivated in Indonesia. Rice straw as one of the byproducts of rice cultivation is a popular source of feed for ruminant animals. Rice straw is available almost free of cost since rice cropping is found almost in all villages. Rice straw is becoming increasingly important during the dry season due to the inadequate availability of green fodder. The use of rice straw in the cattle diet is however limited as a part replacement of the forage due to insufficient energy and nutrient supply, low voluntary intake, and digestibility. The daily intake of rice straw is limited to less than to less than 2% of animal body weight or 1.0-1.5 kg per 100 kg live weight [1,2]. Rice straw is characterized by low energy and essential nutrients (protein, mineral) and slowly degrades in the rumen due to high cell wall content of fiber, silica, and lignin [1,3]. The digestibility, nutritive value, and utilization of rice straw could be improved by physical and biological treatments [4,5]. However, the pre-feeding treatments are not economical and practically feasible for small-scale farmers because rice straw is usually fed to the animal in intact and fresh form [6].

Rice straw is normally available in large quantities during rice harvesting season, but most are burnt, or left in the rice field without protection and treatment, resulting in underutilization as feed due to its susceptible nature to physical and microbial damages. Rice straw is commonly collected during harvesting day and fed to cattle in intact and fresh form due to cattle's preference for fresh straw. Rice straw was also stocked in an intact and fresh form on the farm in open-air loose stacking or manual tied balling due to the seasonality of rice harvesting. However, the intact and fresh straw stored in traditional open-air conditions is susceptible to physical changes and microbial damage during storage due to high moisture content of about 65% [6,7,8]. Previous research has found that rice straw stored in open-air conditions significantly decreased the moisture content and palatable stem component and encountered undesirable microbial growth and physical changes of rot or toughness, resulting in poor organoleptic acceptance, lower fiber digestibility, and feed utilization efficiency [6, 8].

Rice straw needs to be utilized more as feed due to Indonesia's scarcity of green fodder and global concern about environmental pollution caused by burning the biomass in the rice field. The possible alternative for better utilization of intact and fresh rice straw for ruminant feeding is to improve voluntary intake, the availability of nutrients and energy, and storage stability by nutrient supplementation and preservation in a practical and effective long-term storage way. A previous study by Khalil *et al.* [8] on the effects of manual loose stacking, open-air tied balling, and



airtight wrapping to maintain the organoleptic value, palatable component, moisture, nutrient content, and nutritional value of stored rice straw supplemented with the calcite-based mineral mixture, molasses, and urea for feeding of the local cattle, found that wrapping was the most feasible method to preserve the moisture, nutrient content, and nutritional value of supplemented rice straw during storage. Wrapping of supplemented rice straw had desirable color, soft and brittle texture, pleasant flavor, minimized undesirable fungi growth, increased crude protein, and reduced crude fiber content.

The use molasses as readily available carbohydrate for cattle and enhance the development lactic acid based bacterial fermentation during the storage which might have positive effect of aroma, moisture content, digestibility of stored rice straw [9]. The addition of calcite-based minerals is intended to control the development of undesirable microorganisms and to make the stored rice straw more nutritionally complete [8,10]. Rice straw has a relatively high content of potassium (12.6 g/kg DM [11,12], but it is low in phosphorus, copper, zinc, calcium, and sodium content that does not meet animal requirements [13]. The present research aimed to evaluate the beneficial effect of calcite-base mineral, molasses, and urea supplementation on the mineral composition of rice straw stored for 60 days in manual loose stacking, open-air balling, and airtight wrapping and to document the changes in blood hematology, serum mineral and protein concentration of cattle fed on diets containing the above-preserved rice straws. The manual airtight storage might help retain the straw's original and supplemented mineral content.

MATERIALS AND METHODS

Preparation, Supplementation, and Storage of Rice Straw

About 1.8 tons of fresh rice straw were directly collected from rice fields by harvesting day in five different periods of 360 kg each. The straws were divided into three parts (120 kg per part). Each part was manually packed or arranged in three different preserved treatments: loss stacking (stacking), tie compacted balling (balling), and airtight compacted wrapping (wrapping). The stacking, balling, and wrapping of rice straw were done following the procedure described by Khalil *et al.* [8]. Stacking of rice straws was initiated by plugging a pole of 1.5 m length in the floor. The straws were gradually piled up and compacted around the pole. For balling, the rice straws were arranged and compacted gradually, stretched on three ropes, then rolled up, manually compacted, and tied to the two ends of the rope. Wrapping used black plastic sheet with a size of 3 x 1.5 meters. The rice straws



are first rolled up, compacted, and tied with rope, then airtightly wrapped up with the plastic sheet, and tied at the ends of the plastic.

During the stacking, balling, and wrapping, the straws were gradually sprayed with 0.6% (v/w) molasses and 0.05% urea solution and sprinkled with 1% (w/w) the calcite-based mineral mixture. Molasses of 2.160 g and feed-grade urea of 180 g were dissolved in 15 L of water before being sprayed on straws of 360 kg. The calcite-based mineral mixture composed of calcined limestone (20.9%), calcined oyster shell (18%), limestone (20%), dicalcium phosphate (20%), iodized kitchen salt (14%), cobalt ($\text{CoCO}_3 \cdot 6\text{H}_2\text{O}$) (0.1%), copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (1%), zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (1%) and premix (5%). The mineral concentrations were designed to complement the mineral deficiency of the local rice straw [11] and to meet the standard mineral requirement for cattle, according to National Research Council (NRC) [14]. The supplemented and packed straws were stored at room temperature for 60 days on a cattle farm.

Sample Preparation and Chemical Analysis

Samples of fresh rice straw were collected before straws were supplemented, stacked, rolled, and wrapped. Samples of stored rice straw were taken on the 60th day in three pilled positions (exterior, middle, and central). The straw samples were chopped and homogenized. One composite sample was formed per pile and dried for 72 hours in an oven. The air-dried samples were ground to pass through a 1-mm sieve in a hammer mill for moisture and mineral content. The moisture content was analyzed using proximate analysis by following the procedure described by AOAC [15]. The concentration of minerals was determined using the atomic absorption spectrophotometer [16]. All analysis results were reported on a dry matter basis.

Preparation Experimental Diets and Feeding Trial

The fresh and stored straws were chopped and mixed as much as 25% with a basal diet comprising 55% elephant grass and 20% concentrate. There were four experimental diets: T0: basal diet+fresh rice straw (FRS) (control), T1: basal diet+stacking supplemented rice straw (SSRS), T2: basal diet+balling supplemented rice straw (BSRS), and T3: basal diet+wrapping supplemented rice straw (WSRS). Concentrate composed of palm kern meal (44.3%), chopped cassava tuber peel (19.18%, soybean meal (17.28%), and rice bran (17.68%). The experimental diets were fed to four young local bulls of Pesisir cattle in a 4x4 Latin Square design for a seven-days adaptation period and subsequently four days for data collection for each period by following the feeding trial method described by Budiono *et al.* [17]. The animals were kept in individual pens, equipped with feed



troughs, and drinking water buckets by following the national guideline ethic for animal care based on the Republic of Indonesian law number 18 of 2009. There was no treatment or handling that harmed or impeded the freedom of the animals.

Collection and Analysis of Blood Samples

At the end of each data-collecting day, blood samples were collected from the jugular vein using 10-mL disposable syringes from all cattle to measure hematology and serum chemistry. About 3-4 ml of blood was immediately transferred to a test tube bottle treated with Ethylene Diamine Tetra Acetic Acid (EDTA) for the hematological test. Hematological parameters measured were: hemoglobin concentration (HGB), hematocrit (HCT), total red blood cell (RBC), total white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration (MCHC), platelets number count (PLT), percentage of lymphocytes (LYM) and monocytes (MONO). Determination of the hematological parameters was performed by using a Medonic Veterinary Hematology analyzer (Medonic CA 620; Medonic, Sweden).

The rest of the blood samples were allowed to clot overnight for serum separation. The serum was collected in a sterile vial to analyze total protein and minerals. The total protein, Ca, P, and Mg concentrations were analyzed by autoanalyzer (Mindray BC-2800). Blood sample preparation and analysis were carried out at the Chemical Laboratory of the National Veterinary Service Institute in Baso, Bukittinggi, West Sumatra, Indonesia.

Statistical Analysis

A one-way analysis of variance was conducted on the data using SPSS software program v. 20. Duncan's Multiple Range Test (DMRT) was applied to separate means. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The mineral composition of the fresh rice straws and the supplemented rice straws stored in different ways is presented in Table 1. The fresh rice straw (FSR) had a relatively high potassium, iron, and manganese content which are within the range of values previously reported from other areas of Indonesian [11,18]. It is well above the requirement for growing cattle of 6 g/kg, 50 mg/kg, and 20 mg/kg DM for potassium, iron, and manganese, respectively [14]. Plant normally contains high amounts of potassium [19]. Potassium is a significant cation in the intracellular fluid and plays an important role in regulating intracellular osmotic pressure [20]. The mean iron concentration in the fresh rice straw of 166 ppm was considerable high



due to the critical limit of iron of 50 ppm. Iron is essential for the formation of red blood cells and required for activity of various enzymes, while manganese plays an important role for activation of enzymes involved in amino acid metabolism, antioxidative reaction, bone development, and wound healing [20].

The mean calcium content of 3.5 g/ kg DM of the untreated fresh rice straw (FSR) was numerically adequate to meet the calcium requirement of growing cattle of 2-4 g/kg DM in the diet [14,21]. However, rice straw has a relatively high content of lignin and oxalate, which affects calcium digestibility [22,23]. Oxalate forms insoluble crystal compounds with serum calcium (Ca) or magnesium (Mg), resulting in low serum Ca or Mg levels [24]. The bioavailability of calcium and other macro minerals is also impeded because they bond with other substances in the rice straw forming an acid-insoluble ash [25]. Calcium is the most abundant mineral in the animal body. It plays a vital role in forming and maintaining bones and teeth and the normal function of nerves impulse and muscle contraction [20].

The sodium and phosphorus content in fresh straw (FRS) are critical essential macro minerals. The results obtained for sodium (0.36 g/kg DM) and phosphorus (0.52 g/kg DM) are lower than the recommended by NRC [14]. According to Wu [20], sodium regulates extracellular osmotic and blood pressure and feed intake. Sodium deficiency results in a decrease in osmotic pressure, dehydration, and hypertension. The recommended dietary sodium concentration in the ration for beef cattle is 0.6-0.8 g/kg DM [14]. Phosphorus level of 0.52 g/kg DM in the untreated fresh rice straw (FSR) was deficient for growing cattle. Growing cattle requires 2.0 to 3.4 g phosphorus in the diet DM [14,26,27]. Phosphorus deficiency affects feed intake, growth, and normal bone development due to its many biochemical functions as components of nucleotides, phosphocreatine phospholipid, and nucleic acid [25,27]. The ratio of phosphorus to calcium is also crucial since phosphorus works together with calcium in the formation of the body structure. The Ca:P ratio in the rice straw of about 6:1, far above the ideal ratio of 1:1 to 1:1.2 for optimal absorption and function [25].

Microminerals are essential for disease resistance, nutrient metabolism, and reproductive performance [20]. Copper of 0.08 mg/kg DM was the most critical micromineral in the fresh and untreated rice straw (FSR) since the standard requirement of growing cattle is 10 mg/kg DM [14]. Copper level in the cattle diet was suggested to be more significant than 20 mg/ kg [29]. Copper is an essential trace element, acts as a cofactor for various enzymes, and participates in redox and hydroxylation reactions with essential physiological functions [20]. A deficiency of copper in the body directly and indirectly affects iron metabolism and



biosynthesis of heme, melanin, and collagen [20]. Therefore, it implies that rice straw feeding needs to be supplemented with calcium, phosphorus, sodium, and copper sources.

Mineral mixture was added to rice straw primarily to make the stored rice straw more nutritionally complete and to control the development of undesirable microorganisms [10]. As shown in Table 2, except for magnesium, iron, and zinc, supplementation of rice straw with calcite-based minerals, molasses, and urea significantly increased macro and trace mineral content. The results obtained for the macro and trace minerals are above the standard requirement, according to NRC [14]. The Ca: P ratio narrowed from 1:7 in the fresh straw (FSR) to 1:3-4 in the supplemented rice straws. There was a significant effect of storage methods on trace minerals of copper, cobalt, and selenium. Rice straws preserved in airtight condition (wrapping up) had the highest copper, cobalt, and selenium (Table 1).

The airtight wrapping helps preserve the nutritional value of rice straw by creating anaerobic conditions and rapid decline in pH value by lactic acid bacteria, which helps to prevent spoilage by an aerobic microorganism [30]. The fermentation process reduced the water content in plant tissues, contributing to a relative increase in the concentration of nutrients [31]. Moreover, the decrease in pH potentially enhances the solubility and availability of certain minerals like calcium (Ca) and magnesium (Mg) [32]. On the other hand, some minerals could be lost during storage, primarily due to leaching. Water-soluble minerals like potassium could be lost through the effluent formed during fermentation. As mentioned above, fresh rice straw, fortunately, contained relatively high potassium (Table 1), which has no significant effect on the overall mineral content of the stored rice straw.

Table 2 shows the biochemical profile of cattle blood-fed on fresh and supplemented rice straws. Except for monocytes and mineral supplementation, the storage treatments had no significant effect on blood chemical values. A cattle-fed diet containing rice straw stored in the open-air stacking had the highest monocyte value of $0.97 \times 10^3/\mu\text{L}$ slightly higher than the standard value of $0.1-0.8 \times 10^3/\mu\text{L}$ reported by Roland *et al.* [33]. The monocyte values increased presumably due to the massive proliferation of unwanted aerobic microorganisms in the outer layer and decay in some parts of the inner pile, resulting moldy rice straw stored in the open-air storage conditions [8].

White blood cells (WBCs) or leukocytes play an essential role in immune defense [33]. The WBC recorded in this study is within the normal range (5–12 g/dl) [32] and close to the range values reported by Sarmin *et al.* [35] for female Peranakan



Ongole (PO) cattle in different reproductive stages but slightly lower than Simmental heifer reported by Yuherman *et al.* [11]. The similar WBC count obtained implies that the immune status of the cattle was at the same level across the treatment diets. Normal WBC in all treatments indicated that all cattle were healthy throughout the experimental period, indicating non-allergic conditions, free parasitism, and any foreign body in circulation [35]. The RBC counts reported in this study were close to the range 5.2 – 6.1 g/dl reported for Simmental heifer by Yuherman *et al.* [11] and typical standard values of 5.3-7.9 g/dl for cattle reported by McDowell [36]. The standard RBC value in this study implied that the feeding of fresh and preserved rice straws as component diets were able to supply an adequate amount of nutrients needed to prevent anemia.

The hemoglobin and hematocrit were positively correlated with the animal's nutritional status. The hemoglobin concentration (HGB) range in this study fell within the range of 9-15 g/dl reported by Roland *et al.* [33] but slightly higher than the values of 9.1-9.3g/dl obtained by Suharti *et al.* [37] for Bali calves fed with soybean oil calcium soap. The hematocrit was in the normal range of 21-30 % [34]. Erythrocyte parameters include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). MCV, MCHC, and MCH were within the normal range for cattle [33] and close to the range values reported by Suharti *et al.* [37] for Bali's calves.

The data in Table 2 shows no significant difference in the serum mineral and protein profile of cattle blood among the treatments. This study's average calcium, phosphorus, and magnesium concentrations were within the typical reference values reported by McDowell [36]. There was no significant effect of mineral supplementation and preserved ways on the blood mineral and protein. The serum concentrations of calcium, phosphorus, and magnesium are influenced by age, reproductive status, season, and soil type [35,38,39].

The serum protein concentration is a function of hormonal balance, nutritional status, water balance, and other health factors [34]. Healthy animals' total protein serum concentration typically varies between 6.5 – 8.5 g/dl [34]. The total serum protein concentration in this study was within the normal range, which implies that the test diets supplied adequate nutrients needed to maintain normal serum protein levels. However, the serum protein concentrations in this study tended to decrease in the cattle-fed diets containing rice straws stored in open-air stacking and rolling, presumably due to lower crude protein content and microbial contamination [8].

Storage of the supplemented rice straw in airtight conditions is in principle like the preservation process of ensiling, which is widely applied to grass other green fodder, and straw. Feeding wrapping supplemented rice straw had beneficial effects on cattle's growth performance and feed utilization efficiency. Cattle fed on FRS and WSRS had higher body weight gain and better FCR than the SSRS and RSRS. The live body weight gain (228.1 g/d) and FCR (17.2) of the WSRS are close to that of the FRS (LWG: 234.4 g/d; FCR: 14.7) [8]. Xu *et al.* [30] reported that the ensiling of rice straw in China effectively improved the feed's nutritional quality and daily LWG of lambs.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Fresh rice straw had a relatively high potassium, iron, and manganese content but was low in calcium, sodium, phosphorus, copper, and selenium. Supplementation significantly increased the concentration of calcium, phosphorus, potassium, sodium, manganese, copper, and selenium. The supplemented rice straw preserved in manual wrapping could maintain higher copper, cobalt, and selenium content than the stacking and balling. Feeding of the wrapped supplemented straw gave equivalent effects to that of the fresh straw on blood monocyte value. In conclusion, supplementation of the calcite-based mineral mixture, molasses, and urea positively affected mineral concentration, and the nutritional values of rice straw could be better maintained by storing it in manual wrapping.

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Authors' Contributions

Conceptualization, Khalil and Hermon, data collecting and analyzing: Dwi Ananta and Andri wrote the first draft: Khalil, review, and editing: Andri and Hermon. All authors have read, commented, and agreed on the final draft of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.



Table 1: Mineral composition of fresh and supplemented rice straws stored in manual stacking, balling, and wrapping methods

Parameter	Fresh straw (FRS)	Storage method:		
		Stacking (SSRS)	Balling (BSRS)	Wrapping (WSRS)
Macro minerals (g kg⁻¹DM)				
▪ Calcium	3.48 ^b ±1.01	5.67 ^a ±1.54	6.05 ^a ±1.15	6.61 ^a ±1.88
▪ Phosphorus	0.52 ^b ±0.09	1.12 ^a ±0.13	1.57 ^a ±0.30	1.55 ^a ±0.31
▪ Magnesium	1.21±0.47	1.47±0.32	1.89±0.54	1.66±0.34
▪ Potassium	16.56 ^b ±1.57	22.94 ^a ±2.94	24.86 ^a ±6.48	22.61 ^a ±4.36
▪ Sodium	0.36 ^b ±0.10	2.64 ^a ±0.50	2.15 ^a ±0.95	2.44 ^a ±1.34
Trace minerals (ppm)				
▪ Iron	166.14±21.61	168.28±14.75	179.37±29.04	170.79±33.82
▪ Manganese	295.37 ^b ±56.02	488.64 ^a ±37.51	506.06 ^a ±134.10	525.57 ^a ±72.30
▪ Copper	0.08 ^c ±0.02	16.29 ^b ±4.71	20.09 ^b ±4.10	55.99 ^a ±5.15
▪ Zinc	58.58±4.17	73.37±16.58	77.78±10.15	69.02±15.75
▪ Cobalt	6.33 ^d ±0.34	7.76 ^b ±0.23	6.79 ^c ±0.32	8.35 ^a ±0.32
▪ Selenium	0.21 ^d ±0.01	0.34 ^c ±0.06	0.37 ^c ±0.09	0.44 ^b ±0.03

a,b,c,d: Means within a raw with different superscripts differ significantly (P<0.05)



Table 2: Hematology value, mineral profile, and total protein concentration in blood serum of cattle fed diets containing fresh and supplemented rice straws stored in manual stacking, balling, and wrapping methods

Parameter	Fresh straw (FRS)	Storage method:		
		Stacking (SSRS)	BSRS (BSRS)	Stacking (WSRS)
Blood hematologic profile:				
– Total white blood count (WBC) ($\times 10^3 \mu\text{L}^{-1}$)	9.15 \pm 1.67	10.20 \pm 1.12	8.27 \pm 0.25	9.95 \pm 1.21
– Total red blood cell (RBC) ($\times 10^6 \mu\text{L}^{-1}$)	7.72 \pm 0.88	6.97 \pm 1.34	7.84 \pm 0.33	6.77 \pm 1.65
– Hemoglobin concentration (HGB) (g.dL $^{-1}$)	11.50 \pm 0.61	10.85 \pm 1.64	10.95 \pm 0.96	10.52 \pm 2.22
– Haematocrit (HCT) (%)	24.97 \pm 2.94	25.62 \pm 4.46	27.57 \pm 3.15	25.25 \pm 5.97
– Mean capsular volume (MCV) (fL)	36.17 \pm 1.65	36.50 \pm 1.62	36.75 \pm 2.59	36.27 \pm 1.60
– Mean capsular hemoglobin (MCH) (pg)	15.75 \pm 0.46	15.77 \pm 0.72	15.45 \pm 1.13	15.70 \pm 1.03
– Mean capsular Hb concentration (MCHC) (g.dL $^{-1}$)	41.47 \pm 4.01	40.45 \pm 3.86	41.80 \pm 0.98	42.12 \pm 1.32
– Platelets number (PLT) ($\times 10^3 \mu\text{L}^{-1}$)	124.50 \pm 40.95	167.00 \pm 53.16	140.50 \pm 70.50	148.50 \pm 30.88
– Lymphocyte count (LYM) ($\times 10^3 \mu\text{L}^{-1}$)	6.07 \pm 1.29	6.60 \pm 1.77	6.12 \pm 1.37	6.87 \pm 0.91
– Monocyte count (MONO) ($\times 10^3 \mu\text{L}^{-1}$)	0.67 ^b \pm 0.12	0.97 ^a \pm 0.50	0.87 ^a \pm 0.09	0.75 ^b \pm 0.05
Blood serum mineral and protein (mg.dL$^{-1}$):				
– Calcium	8.90 \pm 1.62	8.52 \pm 0.87	8.27 \pm 0.49	8.20 \pm 0.77
– Magnesium	2.27 \pm 0.27	2.25 \pm 0.17	2.30 \pm 0.24	2.42 \pm 0.33
– Phosphorus	8.62 \pm 1.64	7.25 \pm 2.12	6.90 \pm 1.02	6.45 \pm 1.69
Total protein (mg.dL $^{-1}$)	8.52 \pm 0.86	7.62 \pm 0.41	7.15 \pm 0.59	8.15 \pm 1.06

^{a,b}: Means within a raw with different superscripts differ significantly (P<0.05)



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