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Effect of toasting on phytic acid, protein and pH in a plant-based diet and its ingesta in fed catfish

C. KEMIGABO[1], J. KANG'OMBE,[2] W.L. JERE,[2] D. SIKAWA,[2] T.M. DHIKUSOOKA,[1] and C. MASEMBE[3*]
[1]National Agricultural Research Organization (NARO), Mbarara ZARDI, P.O Box 389, Mbarara, Uganda
[2]Department of Aquaculture and Fisheries Science, Lilongwe Universities of Agriculture and Natural Resources (LUANAR), P.O Box 219, Lilongwe, Malawi
[3]Department of Biology, Makerere University, P.O Box 7062, Kampala, Uganda
***Corresponding author:** (cmasembe@zoology.mak.ac.ug, cmasembe@gmail.com)

ABSTRACT
Feed accounts for up to 70% of production costs in aquaculture, hence reducing this cost is needed for rural development. Plant ingredients commonly used in feed contain phytic acid that forms indigestible complexes with nutrients thus reducing their use for fish growth. Toasting feed material is widely practiced to reduce antinutrients but its effect on phytic acid remains unclear. In the study, phytic acid, crude protein (CP) and pH in plant-based diets from untoasted and toasted ingredients and their ingesta along the gut were analysed using complex-metric titration, Kjeldahl method and micro pH meter, respectively. Phytic acid in the diet from untoasted and toasted ingredients differed ($p \leq 0.002$) while CP and pH did not ($p \geq 0.06$ and $p \leq 0.40$, respectively). The content of phytic acid in the diet doubled along the gut, CP halved and pH was 5.30 - 7.78. Diet from untoasted ingredients was not eaten thus no ingesta was analysed. Results showed that toasting plant ingredients had no effect on phytic, CP and pH implying that replacing animal protein with plant protein depends on proper pre-treatment of phytic acid.

Key words: catfish, pH, Phytic acid, plant diets, protein, toasting

RÉSUMÉ
L'alimentation représente jusqu'à 70% des coûts de production en aquaculture, d'où la nécessité de réduire ce coût pour le développement rural. Les ingrédients d'origine végétal couramment utilisés dans les aliments contiennent de l'acide phytique qui forme des complexes indigestes avec les nutriments, ce qui réduit leur utilisation pour la croissance du poisson. Le grillage des matières premières est largement pratiqué afin de réduire les anti-nutriments, mais son effet sur l'acide phytique demeure incertain. Dans la présente étude, l'acide phytique, la protéine brute (PB) et le pH des régimes alimentaires à base d'ingrédients végétaux grillés et non-grillés et leur ingesta le long de l'intestin ont été analysés en utilisant respectivement le titrage métrique complexe, la méthode Kjeldahl et le micro pH-mètre. La teneur en acide phytique diffère entre les régimes alimentaires à base d'ingrédients grillés et ceux à base d'ingrédients non grillés ($p \leq 0{,}002$), tandis qu'aucune différence n'a été observé au niveau de la teneur en protéine brute et du pH ($p \geq 0{,}06$ et $p \leq 0{,}40$, respectivement). La teneur en acide phytique du régime a doublé, la teneur en PB s'est réduite de moitié et le pH était de 5,30 à 7,78 le long de l'intestin. Les régimes alimentaires à base d'ingrédients non grillés n'ont pas été mangés, donc aucune ingesta n'a été analysée. Les résultats ont montré que le grillage des ingrédients végétaux n'a aucun effet sur le phytique, la PB et le pH, ce qui implique que le remplacement de la protéine animale par la protéine végétale dépend d'un prétraitement approprié de l'acide phytique.

Mots clés : poisson-chat, pH, acide phytique, régime alimentaire à base de plantes, protéines, grillage

INTRODUCTION

Fish is a rich source of essential nutrients which when consumed can counteract malnutrition among Ugandan children and women of the reproductive age concentrated in rural areas (Baruah *et al.,* 2004; FAO, 2014; Kikafunda *et al.,* 2014; Nyasimi *et al.,* 2014). Per capita fish consumption had however reduced to only 5.7 kg/year by the year 2012 which is below the 20 and 17 kg recommended by World Health Organization (WHO) and the Food and Agriculture Organization (FAO), respectively (MAAIF, 2011; Gordon *et al.,* 2013).This low fish consumption is attributed to high fish costs due to high demand that is projected to reach 930,000 t ha^{-1} by 2020 in Uganda (MAAIF, 2011). The natural fisheries which have been the main supply of fish have stagnated at 560,000

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t ha^{-1} and further reliance on this fish source has been considered an unsustainable option, triggering development of commercial fish farming to address the production gap (FISH, 2009; MAAIF, 2011). Among farmed fish species, catfish (*Clarias gariepinus*) comprises 60% of the total harvest and is used as both human food and as bait in the Nile perch line fishery around Lake Victoria basin (Isyagi, 2007; FAO, 2010; Chepkwemoi, 2013). It is farmed semi-intensively in earthen ponds and tanks with a good supply of quality feed. Feed costs constitute 60-70% of operating costs in catfish farming due increased use of conventional feed grade animal ingredients as human food and in making of livestock diets. Fish meal from small fish species such as the cyprinid silver fish (*Rastrineobola argentia*) has been a major component in quality fish feeds but its adoption as livestock and human food has increased its costs triggering use of plant materials in making fish feeds instead (FAO, 2012).

Plant materials have potential to supply essential nutrients (e.g. proteins, fats, carbohydrates and trace nutrients) for proper nourishment of farmed catfish fish but their inclusion in fish diets is limited by the antinutrients they contain such as phytic acid (Abdoulaye *et al.,* 2011; Kumar *et al.,* 2012). Phytic acid forms indigestible complexes with starch, proteins, fats and multivalent minerals, thereby denying their availability to the feeding fish (Reddy *et al.,* 1989; Mugendi *et al.,* 2010). Phytic acid also inhibits protease enzymes thereby reducing their ability to break down unbound proteins into absorbable amino acids for easy utilization (Von Der Haar *et al.,* 2014). When this partially digested feed is excreted by fish, it pollutes public water through nutrient enrichment (eutrophication). Therefore a combination of these two or more factors have a negative effect on the profitability and social credibility of catfish farming. Phytic acid can be reduced by conventional treatments such as autoclaving, soaking and boiling but this has an effect on the nutritional quality of the fish diets (Baruah *et al.,* 2004; Hussain *et al.,* 2011). Toasting is one of the traditional processing practices that deactivates trypsin inhibitors, reducing cyanoglucosides while preserving zinc and iron (Omoruyi, 2007). However, information on the effect of toasting on phytic acid in diets locally produced from a mixture of unrefined tropical plant materials is still lacking. Prior understanding of the phytic acid content in locally produced diets and how this relates to the bioavailability of nutrients such as proteins is necessary for development of alternative phytic acid pre-treatment strategies. This study analysed phytic acid, crude protein and pH in plant-based diets made from un-toasted and toasted ingredients and in their ingesta along the four major sections of the digestive tracts (gut) of catfish. It aimed at documenting the effects of toasting as a pre-treatment on the nutritional value of plant-based catfish diets with respect to the antinutrient phytic acid, protein content and pH.

MATERIALS AND METHODS

The study was conducted at Mbarara Zonal Agricultural Research and Development Institute (MBAZARDI), one of the public National Agricultural Research Organization's (NARO's) institutes with laboratory work on phytic acid and protein analysis conducted at the College of Veterinary Medicine, Makerere University (COVAB).

Figure 1. Map of Uganda showing the location of MbaZARDI (Adapted from lonelyplanet.com/Uganda)

Preparation of experimental diets. Secondary data on the proximate nutrient composition of commonly used feed grade materials was collected from the Department of Animal Science, School of Agriculture and Natural Sciences, Makerere University. The data were entered into Feed Win Computer feed formulation software of the Practical Training Center

(PTC+), Netherlands and used in the formulation of 35% crude protein plant based diets (one from toasted and the other from untoasted ingredients) while adopting the poultry window. Locally available ingredients were used (Tables 1 and 2) to make a diet that provides the minimum nutrient requirements for proper growth of the African catfish fry (NRC, 1993; Jobling, 1994; Hecht *et al.,* 1996; Robinson *et al.,* 2001; Kang'ombe *et al.,* 2007; Tacon, 2009; NRC, 2011). Feed ingredients were procured from primary proven processors to minimize adulterated ingredients (Nalwanga *et al.,* 2009) that compromise the formulae. Part of the ingredients was toasted at 80-100^0 C. The ingredients were ground to 0.02 mm and mixed by hand and pelleted into sinking pellets at 45-55^{0}C using a pelleting machine locally fabricated in Uganda. The pelleted fish diets were then kept at 4^{0}C until they were analysed for proximate nutrient composition following (AOAC, 2002). The amino acid profiles were determined using the High Performing Light Chromatography (HPLC) at Abingdon Health Laboratory Services, University of Birmingham, UK.

Dry matter of the two diet samples was determined using the oven method at 105^{0}C (AOAC, 2002). Phytic acid was determined using the complex-metric titration of residual iron (III) method (GarcõÂa-Estepa *et al.,* 1999) and crude protein percentage by the Kjeldahl method (AOAC, 2002). The pH of these diets was determined from the slurry of 10g samples soaked in water chilled to 4^{0}C using a Pen type digital Micro pH meter (Model ROHS).

Experimental fish and feed administration. Two sets of 55 fish each of 222-914g were obtained from on-station concrete tanks (3x2x 1.5) m with 400 litres where they had been raised on pelleted diets formulated from toasted ingredients. They were each stocked in 200 litre tanks on-station. One set was fed on a 35% crude protein diet formulated from untoasted ingredients while the other was fed on a diet with the same protein but formulated from toasted ingredients. Both sets of experimental fish were fed at a rate of 4% of body weight between 10:00 to 11:00 am daily guided by the fish's feeding response. Three fish samples were randomly seined out every one hour after feeding for five consecutive hours (up to 4:00 pm) into 20 Litre basins where they were killed by anethising them with excess clove oil (2.5ml/litre of water) according to guidelines to death as end point (Homeoffice, 2014).

Table 1. Ingredients used in formulation of the fish diets

Ingredient	Inclusion rate (%)
Cassava flour	4
Wheat pollard	8
Whole grain maize	16
Fish meal	27
Soybean	26
Bush beans	7
L-lysin Hcl	4
DL-methionine	2
Cotton seed cake	3
Fish oil	2
Fish mineral and Vitamin premix	0.002
Table salt	0.001
Sodium benzoate	1
Total	100.003

*The fish premix used was manufactured by NUTRINOVA International (USA).

Table 2. Nutrient composition of the formulated plant based diet (% dry matter and energy in kJ/g)

Parameter	Content
Dry matter	87.08
Ash	8.92
Crude protein	35.22
Crude Fibre	3.53
Fat	10.44
Gross energy (kJ/g)	30.00
Calcium	1.07
Potassium	1.27
pH	6.72- 6.79

The catfish samples in each batch were dissected and gut sections of stomach, anterior intestine, posterior intestine and rectum (30) removed using stainless steel scissors and surgical blades (No 22). (See Plate1).

[1]Vitamin A-900,000 IU ,Vitamin D3-200,000 IU, Vitamin E-30,000 IU, Vitamin K3-2.0 G, Thiamine(B1)- 15.0G, Riboflavin(B2)-10.0G,Niacin - 30.0G,Pantothenic acid - 30.0G,Pyridoxine (B6)- 10.0G,Folic acid - 2.5 G, Vitamin B12 - 0.01 G, Biotin-0.05G,Antioxidant - 125.0G,Manganese - 80.0G,Zinc - 50.0 G, Iron - 10.0 G, Copper - 10.0G, Iodine - 1.5G,Cobalt- 0.5G, Selenium - 0.3 G

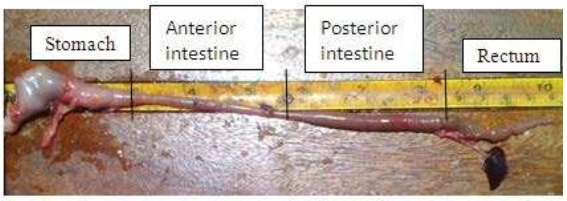

Plate 1. Catfish gut showing the different sections of the catfish digestive tract that were cut out.
(Picture taken by Kemigabo Chloe, 2015).

Determination of phytic, crude protein and pH in diets ingesta in fed fish. For determination of pH in ingesta, samples of freshly cut sections of the gut were crushed with their ingesta using a motor and pestle. Distilled water was added at a ratio of 1:10 w/v (Namulawa *et al.,* 2014). Using a digital Pen-type micro pH meter with a temperature reading (Model ROHS), pH values were recorded to the nearest 0.01. For phytic acid and protein analysis, ingesta contents from each section of the gut was placed into separate 10 ml plastic vials and chilled at 4^0C until analysed. Before use, the chilled ingesta samples were thawed and oven dried to a constant weight at 105^0C (AOAC, 2002). The dried ingesta samples were then analysed for phytic acid (GarcõÂa-Estepa *et al.,* 1999) and crude protein (AOAC, 2002).

Data handling and statistical analysis. Data were recorded and summarized in Ms Excel (Microsoft excel, USA) and exported into Paleontological statistics (PAST) software ver. 2.17 C developed by (Hammer *et al.,* 2001). Data on phytic acid, crude protein and pH in diets was analysed using the non-parametric Kruskal- Wallis ANOVA while data on the same parameters but in the ingesta was analysed by parametric one way ANOVA. In all cases significant differences were declared at 95% confidence interval ($p<0.05$) and multiple comparisons in the parametric ANOVA tests were conducted by Tukey's test.

RESULTS

The phytic acid percentage in diets formulated from untoasted ingredients (Table 4) differed from that in the diet made from toasted ingredients ($p \leq 0.002$) while their crude protein percentages did not differ significantly ($p \geq 0.894$). The pH values in the diets made from toasted and untoasted ingredients did not statistically differ ($p > 0.05$). The pH in the diet made from toasted ingredients ranged from 6.72 -7.08 with an average of 6.87 ± 0.16 while the pH of the diet made from untoasted ingredients ranged from 6.84 to 6.77 with an average of 6.83 ± 0.05.

Diet formulated from untoasted ingredients was not ingested by catfish. This made it impossible to get ingesta samples for use in the analysis for phytic acid and proteins along the digestive tract.

Table 3. Amino acid composition of the made diet as a percentage of crude protein

Major essential amino acids	Catfish requirement	Free amino acids	Hydrolysable amino acid
Argenine	4.3	0.004	4.488
Histidine	1.5	0	2.541
Iso Leusine	2.6	0	2.731
Leusine	3.5	0.004	4.846
Lysine	5.1	0.088	5.039
Threorenine	2.1	0.004	2.555
Tryptophan	0.5	0	-
Valine	3	0.004	3.029
Methionine + Cystine	2.3	0	1.234
Phenylamine + Tyrosine	5	0.004	3.029
Total	29.9	0.105	29.492

Table 5. Phytic acid and protein content in toasted and untoasted diets ingredients

Diet	Dry matter (%)	Crude protein (%)	Phytic acid (%)
Untoasted ingredients	88.59 ± 0.81	35.27 ± 2.30	5.89 ± 0.04
Toasted ingredients	87.07 ± 2.76	35.22 ± 0.51	5.67 ± 0.04

Phytic acid content (Fig. 2) in the ingesta along all sections of the gut (stomach, anterior intestine, posterior intestine and rectum) was significantly higher than that of the diet ($p<0.05$).

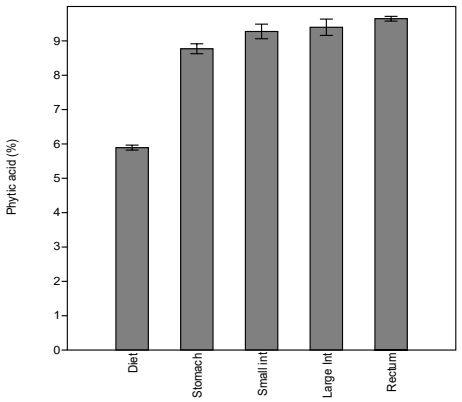

Figure 2. Variation of phytic acid between the diet and different sections of the gut.

Table 5. Phytic acid and crude protein (%) in the diet and ingesta of diets from toasted ingredients

Sample description	Composition of toasted diet and its ingesta		
	Dry matter (%)	Crude protein (%)	Phytate (%)
Diet (before ingestion)	87.07 ± 2.76	35.22 ± 0.51	5.67 ± 0.04
Ingesta in stomach	8.96 ± 1.11	29.25 ± 2.30	8.77 ± 0.07
Ingesta in anterior intestine	13.68 ± 6.03	21.19 ± 2.09	9.27 ± 0.11
Ingesta in posterior intestine	9.79 ± 4.38	20.79 ± 3.09	9.33 ± 0.12
Ingesta in the rectum	14.11 ± 1.54	18.44 ± 5.84	9.65 ± 0.04

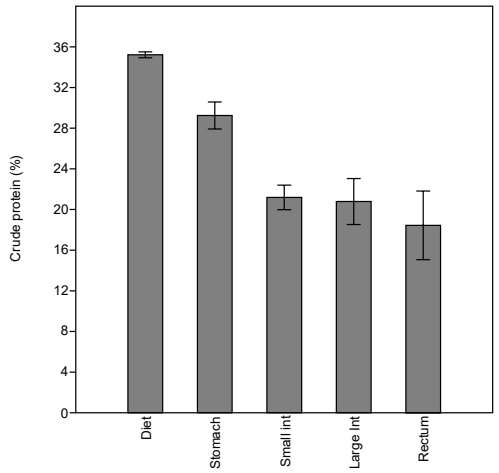

Figure 3. Variation of protein in the diet and ingesta between stomach and rectum

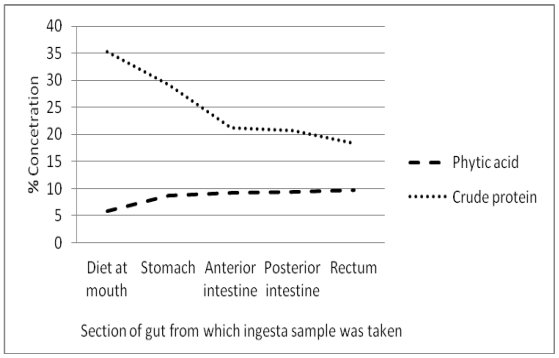

Figure 4. Phytic acid and crude protein variations in ingesta from toasted ingredients along the digestive tract.

Relationship between Crude protein and phytic acid content in ingesta. The phytic acid in the ingesta doubled between the stomach and rectum while the dietary crude protein decreased by half (50%) as it passed from stomach to the rectum (Fig. 3). There was negative linear correlation ($p< 0.0001$, R^2 adjusted =0.662) recorded between variations in protein and phytic acid percentage in the diet and ingesta from the toasted ingredients as previsiously reported (Hammer *et al.,* 2001) and as presented in Figure 4 and 5. On the other hand, the pH in the diet from toasted ingredients (6.72 - 7.08) did not differ from that of the diet from untoasted ingredients (6.77 - 6.89), $p> 0.05$. The pH variation in the ingesta was only recorded for the diet from toasted ingredients which was ingested (Figure 6).

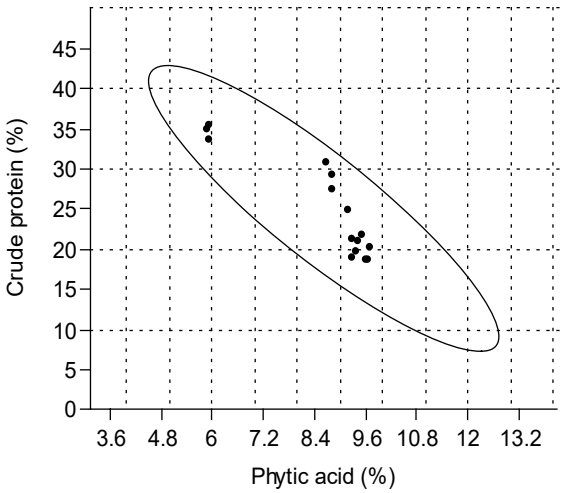

Figure 5. A correlation plot of crude protein and phytic acid percentage in the ingesta of the diet from toasted ingredients.

As shown in Figure 6 the pH values for ingesta in the small and large intestine differed from those in the stomach ($p< 0.05$) while those of ingesta from the rectum did not differ from those of the stomach

($p>0.05$).

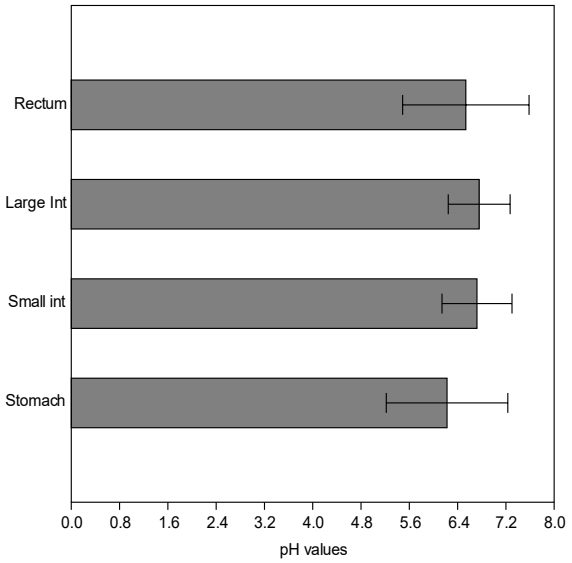

Figure 6. pH values in the different sections of the gut

DISCUSSION

The significantly lower phytic acid in the diet from toasted ingredients (5.65-5.76%) compared to the diet from untoasted ingredients (5.85-5.92%) could be attributed to activation of the innate phytase enzyme found in plant seeds that is responsible for breaking down phytic acid to release the bound nutrients during germination (Abdoulaye *et al.,* 2011). The phytic acid degradation process was likely to have occurred during toasting at low temperatures (60-70^{0}c) but as the heat increased, the phytase enzyme was inactivated thereby preventing further breakdown of phytic acid. This observation is in agreement with that of Omoruyi (2007) who reported that toasting destroyed small amounts of phytic acid in potatoes but conserved iron and zinc. Ndidi *et al.* (2014) observed similar changes after toasting Bambara nuts (*Vigna subterranean*). The recorded phytic acid percentages in both diets were however higher than 0.7-2.5% for most individual plant seeds e.g., 1-1.5% in soy,1% in maize, 2.5-5.8 in wheat bran, 0.75% in Indian whole bread made with mixed cereals (GarcõÂa-Estepa *et al.,* 1999) and comparable to the 5-7.5 % reported for rape seed (Serraino and Thompson, 1984; GarcõÂa-Estepa *et al.,* 1999; Abdoulaye *et al.,* 2011). This could have been attributed to the addiditive effects of using many ingredients to make the diet (wheat pollard, cassava, maize, soy bean, bush beans and cotton seed cake). Variation in the growing conditions of cereals and legumes used and the processing method applied could have also contributed to this variations as reported by Abdoulaye *et al.* (2011). It is also however expected that phytic acid would be higher in the unrefined tropical plant seed/grain materials compared to temperate ones. This is why toasting as compared to other pre-treatment methods is thought to increase nutrient availability in plant-based fish diets.

The increase in phytic acid percentage in the ingesta contents along the digestive tract of catfish fed on the diet from toasted ingredients suggests that phytic acid is not degraded by the catfish endogenous enzymes and thus accumulates along the gut. This is attributed to formation of indigestible complexes when phytic acid chelates the multivalent minerals and nutrients including proteins (Reddy *et al.,* 1989; Mugendi *et al.,* 2010).The elevation of phytic acid from 5.67 ± 0.04 to about 9.65 ± 0.04 in this diet would increase its chelating effect with minerals and nutrients in the diet and thus compromising nutrient bioavailability.

Lack of differences between crude protein in the diet from toasted ingredients (35.22 ± 0.51) and the diet from untoasted ingredients (35.27 ± 2.30) could be attributed to the fact that bound proteins are not readily destroyed by heat, but can rather be denatured. Just like any other heat treatment, toasting can alter the amino acid patterns within the protein molecules and cause changes in its biological function but may not change the protein molecule. This is in agreement with Masoero *et al.* (2005) and Ndidi *et al.* (2014) who did not observe any effects on protein breakdown after toasting peas and Bambara nuts, respectively. This however differed from observations of Balogun (2013) who reported a significant reduction in protein of Bauhinia seed that was toasted at 300^{0}C. Differences in the observations might have been due to differences in toasting temperatures and plant material involved.

The lower crude protein in ingesta of the diet from

toasted ingredients along the digestive system was attributed to digestion and absorption of protein along the gut. However, the overall absorption of only about 50% of the total protein was attributed to build up of phytic acid which might have bound protein through formation of insoluble complexes thus limiting its digestion (Abdoulaye *et al.,* 2011; Kumar *et al.,* 2012). This demonstrated that the potentially bound proteins in such diets could not be fully utilized by catfish to derive the minimum requirements, which could reduce its nutritional value and promote water pollution through nutrient enrichment. This observation was supported by the negative correlation between phytic acid and crude protein which showed that as phytic acid increased, protein bioavailability decreased.

The more or less neutral pH range in the diet from toasted ingredients (6.72-7.08) and untoasted ingredients (6.77 - 6.89) at a temperatures of 24^0 C was attributed to dominancy of plant proteins in the diet (Fig. 1). Plant protein is known to be deficient in essential amino acids particularly the sulphur-containing Cystine and Methionine as compared to animal proteins (Campbell and Campbell, 2006). This reduces the acid producing metabolites, maintaining a slightly close to a neutral pH even after the diet was supplemented with free methionine.

Lack of differences in pH in both diets could have been attributed to no changes in the amino acid profiles since there was also no significant difference in the protein content. However, the significantly higher pH in the small and large intestines as compared to the stomach and rectum was attributed to neutralization of stomach contents after the acid digestion phase common to most teleost fishes. Acid digestion is characterized by secretion of concentrated hydrochloric acid to activate zymogens and pepsinogen fluids in the endo-gastric cells to activate pepsin and trypsin-like enzymes for digestion of proteins ((Solovyev *et al.,* 2015). Accumulation of free amino acids as a result of protein hydrolysis in the stomach could also have influenced this pH. The stomach ingesta pH (5.30 – 6.75) observed in this study were however higher than pH 2-4 reported by Page *et al.* (1976) for channel catfish (*Ictalurus punctatus*), a species closely related to African catfish (*Clarias gariepinus*). It was also less than pH (2.5) reported for South American catfish by Hernandez *et al.* (2009), pH (1-2) for Nile tilapia reported by Evans *et al.,* (2005), pH (3.5–4.5) of Nile perch (*Perca fluviatili*s) and Zander (*Sander lucioperca*) reported by (Solovyev *et al.,* 2015). Gut pH vary between species based in diet composition, presence/absence of food, temperature, among others and was reported as a basis for diversity in fishes (Montgomery and Pollak, 1988). The increase in ingesta pH within the intestines (pH 6.3-7.7) was attributed to presence of sodium hydroxide, bicarbonate rich mucus cells in gut walls, alkaline bile salts and cholesterol produced by the pancreas, bile and liver to emulsify lipids as reported by De Silva and Anderson (1995). This trend of pH in the intestines is in agreement with the reported mild alkaline pH in the midgut of most teleost fishes (Smith, 1980).The intestinal ingesta pH of 6.3-7.7 was close to pH 6.5–7.2 reported by Solovyev *et al.* (2015) in the intestinal regions of perch and zander. The later reduction in ingesta pH within the rectum to more less the values in the stomach could have been influenced by a reduction in gastric enzyme production in most secretory cells and accumulation of feed fermentation products that include alkaline ammonia.

The fact that only the diet made from toasted ingredients was ingested and not the diet from untoasted ingredients was attributed to the sweet aroma imparted by toasting that increased acceptability. Similar results were reported by Jurkovic and Colic (1993) and Mridula *et al.* (2007) after toasting grains and cereals. The toast aroma was reported to be influenced by the toasting temperature with 80 - 110^0C for 30 minutes to one hour providing better aroma than lower and higher values (Sandhu *et al.,* 2015). This implied that toasting increased palatability which could have been through inactivation of heat labile toxic compounds such as tannins and some enzyme inhibitors as

previously reported (Serna-Saldivar, 2010; Medugu *et al.,* 2012). It should however be noted that toasting conducted under alkaline conditions can induce formation of unnatural anti-nutrients in form of toxic amino acid derivatives, e.g. oxidized forms of sulphur containing amino acids, D-amino acids, and lysine-alanine (LAL) thus reducing feed palatability/ nutritive value (Jurkovic and Colic, 1993).

CONCLUSIONS AND RECOMMENDATIONS

Toasting plant feed ingredients did not significantly reduce phytic acid content in plant based diets but conserved protein and pH thus combining it with more effective phytic acid degrading techniques such as fortification with digestive enzymes may enhance digestion and overall utilisation of plant protein as replacements for animal protein. Further investigations on the amount of protein bound by a unit of phytic acid might provide an insight on the effects of phytic acid contents on protein bioavailability (dose-response relationship) and guide development of more precise phytic acid reducing technologies. The current results provide a basis for development of effective pre-treatments for phytic acid reduction to enhance utilisation of plant based diets by fish.

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STATEMENT OF COMPETING INTERESTS

We the authors of this paper hereby declare that there are no competing interests in this publication.

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