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Performance and Histological Responses of Internal Organs of Broilers Fed Ammonium Hydroxide Treated *Moringa oleifera* Seed Cake Diets.

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ABSTRACT

A 56- day feeding trial was conducted to investigate the effects of dietary raw and Ammonium hydroxide treated Moringa oleifera seed cake (AHMOSC) on performance, serum and haematological characteristics and histology of broiler chickens. One hundred and fifty day- old Arbor acre chickens were used in this experiment. They were fed five different diets as follows; Diet 1-the control devoid of Moringa oleifera seed cake (MOSC), diet 2-raw MOSC, diets 3, 4, and 5 – MOSC soaked in 10, 20, and 30% Ammonium hydroxide solution. Broilers fed graded level of AHMOSC had similar (p<0.05) DWG, FI and FCR with the control (61.73 to 61.99 vs. 59.99; 103.75 to 104.74 vs. 102.51 g/bird/day and 1.67 to 1.69 vs. 1.71, respectively, and were significantly (p>0.05) better than value for raw MOSC. The haematological indices and serum chemistry of chickens fed treated MOSC were poorer than the control but significantly (p<0.05) better than those of raw MOSC. Histology examination of the liver sections of the birds fed with raw MOSC in diet of the birds showed abnormal liver architecture due to the presence of anti-nutritional factors in the feed. With the inclusion of the graded level of ammonium hydroxide treated MOSC in diet of the birds, the liver architecture appears normal with characteristic delineation. In conclusion, treatment of MOSC with Ammonium hydroxide was effective in reducing its anti-nutritional contents and can be used as protein supplement in poultry diet. This study recommends the use of 20% ammonium hydroxide in treatment of MOSC for improvement of its nutritive value.

INTRODUCTION

The biggest constraint to poultry production in Nigeria is cost of feed, which accounts for about 60 to 80% of the recurrent expenditure in intensive poultry production (Oluyemi and Roberts, 2000). This is because feedstuffs used in formulating and compounding diets for poultry are also in high demand for human consumption and industrial uses (Anike and Okeke, 2003) thus, alternative feeding stuff must be sourced to prevent competition between man, industry and animal. Plant proteins are cheaper and therefore their use in large quantity ensures less expenditure as compared to animal Proteins. Some nutrients chemicals however, possess well known toxic properties if consumed in excessive amounts or without adequate preparation. The toxic chemicals in feeds when consumed by animals can cause a variety of actions in the body or can react in some adverse ways between the nutrients and toxins in vivo. For instance, some feed chemical toxins exert their action by interfering with nutrients retention, availability and some are known to adversely affect the utilization of nutrients while others have been reported to influence negatively the metabolism of nutrients, all these chemicals which influence the metabolism negatively are called anti-nutritional factors.

The anti-nutritional factors present in these feeds include trypsin, chymotrypsin and amylase inhibitors, aflatoxins and polyphenolic compounds which tend to inhibit and obstruct the activity of digestive enzymes thereby causing digestive losses (Singh et al, 2007). To find ways of reducing feed cost there have been numerous efforts by researchers, nutritionists and producers to find alternatives by considering novel feedstuffs which are safer, cheaper, locally available and nutritionally adequate and majorly that contain lesser toxin in which case their method of detoxification will be cheaper as feed ingredients.

Overwhelmed by the numerous economic benefits of *Moringa oleifera* tree in the tropical world where the plant is considered a miracle tree due to its industrial, medicinal and nutritional attributes, the area of its anti-nutritional or toxic factors remains to be fully dealt with. However, there is no information on the use of ammonium hydroxide for the processing of *Moringa oleifera* seedcake in nutrition of poultry. The constrain in the use of this present novel feed resources attributed to the ANFs present in MOSC could be ameliorated using aqueous ammonium hydroxide for the removal of condensed tannin (Griffiths,1991).

MATERIALS AND METHODS

Study Area

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria.

Experimental Animals and their Management

20kg of *Moringa oleifera* seed cake used for this work was obtained from Moringa processing plant at Afe Babalola University, Ado-Ekiti, Nigeria. Raw (unprocessed) *Moringa oleifera* seed cake was divided into four equal portions of 3Kg each. A portion was left untreated, while each of the other three portions was treated by soaking in 5Litres of 10, 20 and 30% ammonium hydroxide solution respectively for 24hours. The seed cake was later removed from solution and properly dried in the sun to a constant weight and milled for replacing soyabean in the diets. Five experimental diets were formulated to meet NRC (1994) requirement for day old broiler chicks. A corn-soybean reference diet (diet 1) is devoid of *Moringa oleifera* seed cake (MOSC), diet 2 contained raw MOSC, while the other three diets contained processed *Moringa oleifera* seed cake using ammonium hydroxide before inclusion in the diet at the same 5% level of inclusion corresponding to diets 1, 2, 3, 4 and 5 respectively (Table 1). One hundred and fifty day-old Arbor acre breed of broiler birds used for this experiment were obtained from Novic Farms, Ibadan, Oyo State Nigeria. The experiment was designed as a complete randomized design. The dietary treatments contained three replicates with 10 chicks per replicate. All the birds were raised under similar management practices throughout the eight week experimental period. The birds were given feed and water *ad-libitum* and weighed weekly. The composition of the experimental diets for the broilers is presented on Table 1.

Table 1: Composition (%) of the Experimental Diets

	Inclusion doses of treated MOSC (%)				
Ingredients (%)	**0 (control)**	**0 (Raw)**	**10**	**20**	**30**
Maize	52.00	52.00	52.00	52.00	52.00
AHMOSC	0.00	3.10	3.10	3.10	3.10
Wheat offal	10.00	10.00	10.00	10.00	10.00
Soya bean meal	31.00	27.90	27.90	27.90	27.90
Palm oil	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
Bone meal	1.15	1.15	1.15	1.15	1.15
Oystershell	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
*Vit/Min premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**

AHMOSC=Ammonium hydroxide *Moringa oleifera* seed cake. 0.50 premix supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,000 IU; vitamin E, 50 IU; vitamin B1, 1 mg; vitamin B2, 3 mg; vitamin B6, 1 mg; vitamin B12, 10 µg; vitamin K, 2 mg; copper (cupric sulphate), 75 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40mg; zinc, 90 mg, iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; sodium, 10 g

Data Collection

At the end of the experiment, one broiler per replicate in all the treatments was taken at random for blood collection by decapitation. Blood for biochemical indices was collected in bottles without EDTA for the analysis of the biochemical parameters such as total protein, albumin, globulin, urea, and transaminase activities (serum aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP)). The blood samples for haematological parameters were pretreated with ethylene diamine tetra-acetic acid (EDTA). Haematological indices examined include White Blood Cell (WBC), Red Blood Cell (RBC), Packed Cell Volume (PCV), Haemoglobin (Hb). Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were calculated from the values of the RBC, PCV and Hb respectively, according to the methods of Jain (1986). Histological examination of liver was carried out by taken its representative tissue samples during slaughtering and immediately fixed in 10% formalin-saline solution for 24 h before preparing the histological sections by using Paraffin method technique. All the excised sections were stained with hematoxylin and eosin stain, examined under light microscopes (X40) and then photographed by using Canon digital camera.

Data Analysis

Data collected on the response criteria were subjected to analysis of variance in a completely randomized design model and differences between treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS

Performance characteristics of broiler chickens fed ammonium hydroxide treated Moringa oleifera seed cake based diets

The performance characteristic of broilers fed ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) diets is presented on Table 2. Except for birds on raw diet (treatment 2), the average daily weight gains, the feed intake values and the feed conversion ratio of the birds were not significantly ($p>0.05$) affected by the dietary inclusion of the graded level of ammonium hydroxide treated MOSC for the replacement of soyabean. The feed conversion ratio favoured birds on the 20% ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) diet having the least mean (1.67) compared with the control. There was no mortality recorded during the course of this trial.

Table 2: Performance Characteristics of Broiler Chickens Fed *Ammonium Hydroxide* Treated *Moringa Oleifera* Seed Cake based Diets

Parameters	Inclusion doses of treated MOSC (%) 0 (control)	0 (Raw)	10	20	30	SEM
Daily weight gains (g/bird/day)	59.993[a]	42.340[b]	61.763[a]	61.873[a]	61.993[a]	1.366
Feed intake (g/bird/day)	102.507[a]	92.307[b]	104.477[a]	103.747[a]	104.737[a]	3.972
FCR	1.707[b]	2.180[a]	1.693[b]	1.677[b]	1.687[b]	0.045

FCR = feed conversion ratio, SEM= Standard error of mean*=P<0.05Means with different superscript along the same rows are significantly (P<0.05), MOSC=*Moringa oleifera* seed cake

Nutrient digestibility of broiler chickens fed ammonium hydroxide treated seed cake based diets

The nutrient digestibility of broilers fed with ammonium hydroxide MOSC diet is presented on Table 3. The nutrient digestibility of crude protein, fat and fibre of birds on control diet and the ammonium hydroxide treated groups were not significantly (p>0.05) different with the inclusion of graded level of ammonium hydroxide treated MOSC in the diet of the birds. The trend in the crude protein, fat and fibre digestibility revealed that the birds on 30% ammonium hydroxide treatment (diet 5) had the best nutrient digestibility compared with its counterparts, the raw and the control diet.

Table 3: Nutrient Digestibility of Broiler Chickens Fed *Ammonium Hydroxide* Treated Seed Cake based diets

Parameters (%)	Inclusion doses of treated MOSC (%) 1 0(control)	2 0(Raw)	3 10	4 20	5 30	SEM
Crude protein	76.870[a]	55.300[b]	76.107[a]	75.440[a]	76.007[a]	1.432
Crude fibre	68.993[a]	48.690[b]	71.023[a]	71.153[a]	71.293[a]	1.571
Fats	65.997[a]	46.570[b]	67.937[a]	68.057[a]	68.197[a]	1.503

SEM= Standard error of mean; [ab] Means with different superscript along the same rows are significant (P<0.05), .MOSC=*Moringa oleifera* seed cake.

Haematological indices of broiler chickens fed ammonium hydroxide treated Moringa oleifera seed cake based diets.

Haematological indices of the birds fed with ammonium hydroxide treated *Moringa oleifera* seed cake based diets is presented in Table 4. The red blood cells, PCV, MCH and MCHC were significantly (p<0.05) affected with an irregular pattern in the values with the inclusion of graded level of ammonium hydroxide treated MOSC in the diet of the birds. The mean cell volume, haemoglobin and white blood cell of the birds were not statistically (p≥0.05) different by the inclusion of the graded level of ammonium hydroxide treated *Moringa oleifera* seed cake in the diet of the birds.

Table 4: Haematological Indices of Broiler Chickens Fed *Ammonium Hydroxide* Treated *Moringa Oleifera* Seed Cake based diets.

	Inclusion doses of treated MOSC (%)					
Parameters	**1 0(control)**	**2 0(Raw)**	**3 10**	**4 20**	**5 30**	**SEM**
RBC (x 10^{12}/l)	2.36^{ab}	2.520^{a}	2.215^{b}	2.355^{ab}	2.180^{b}	0.148
Haemoglobin (g/dl)	8.75^{a}	9.300^{a}	9.000^{a}	8.950^{a}	8.650^{a}	0.397
PCV (%)	31.20^{a}	32.600^{a}	30.250^{ab}	31.400^{ab}	29.800^{b}	1.412
MCV (fl)	132.25^{a}	129.600^{b}	136.550^{a}	133.350^{a}	137.500^{a}	4.048
MCH (pg)	37.15^{c}	36.950^{c}	40.600^{a}	38.050^{bc}	39.950^{ab}	1.264
MCHC (g/dl)	28.05^{c}	28.550^{bc}	29.750^{a}	28.500^{bc}	29.050^{ab}	0.4147
WBC(x 10^{9}/l)	231.00^{a}	233.850^{a}	234.750^{a}	238.900^{a}	232.500^{a}	4.482

SEM= Standard error of mean=P<0.05; Means with different superscript along the same rows are significantly (P<0.05); RBC = Red Blood Cell PCV = WBC White Blood Cell, Packed Cell Volume, MCV = Mean Cell volume, MCH = Mean Cell Haemaglobin, MCHC= Mean Cell Haemoglobin Concentration, .MOSC=*Moringa oleifera* seed cake.

Serum biochemical indices of broiler chickens fed ammonium hydroxide treated Moringa oleifera seed cake based diets

The biochemical indices of the broiler chickens fed dietary ammonium hydroxide treated MOSC based diet is presented on Table 5. There was a significant (p<0.05) increase in the total protein levels with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diet of the birds. The albumin levels revealed a decrease in the trend with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diet of the birds. The serum alanine amino transaminase (ALT) and alkaline phosphatase (ALP) levels of the birds significantly (p<0.05) decreased linearly except raw diet (treatment 2) with the inclusion of ammonium hydroxide treated *Moringa oleifera* seed cake (MOSC) in the diet of the birds.

Table 5: Serum Biochemical Indices of Broiler Chickens Fed *Ammonium Hydroxide* Treated *Moringa Oleifera* Seed Cake based Diets

	Inclusion doses of treated MOSC (%)					
Parameters	**0(control)**	**0(Raw)**	**10**	**20**	**30**	**SEM**
Urea (mmol/l)	4.657^{b}	8.070^{a}	4.707^{b}	4.520^{b}	4.657^{b}	0.127
Total protein (g/l)	13.927^{c}	24.230^{a}	5.817^{b}	15.620^{b}	14.060^{c}	0.230
Albumin (g/l)	2.727^{b}	6.163^{a}	2.307^{bc}	2.020^{c}	2.157^{c}	0.261
AST(U/L)	70.000^{b}	90.030^{a}	64.210^{bc}	54.737^{c}	61.053^{bc}	6.894
ALT(U/L)	19.480^{c}	30.443^{a}	20.827^{c}	24.480^{b}	10.830^{d}	1.136
ALP (U/L)	35.680^{a}	36.287^{a}	25.020^{b}	23.580^{b}	22.960^{b}	3.948

SEM= Standard error of mean*=P<0.05Means with different superscript along the same rows are significantly (P<0.05);NS=Non Significant (P>0.05);. AST = Aspartate Amino transaminase, ALT = Alanine Amino transaminase ALP = Alkaline phosphatase MOSC=Moringa oleifera seed cake.

Histology of the liver of broiler birds fed ammonium hydroxide treated Moringa oleifera seed cake diets

The photomicrographs showing the liver of the birds fed the ammonium hydroxide treated *Moringa oleifera* seed cake (AHMOSC) diets is given in Figure 1 (a-e). The sections of the liver revealed degeneration of the hepatocytes and coagulative necrosis with the inclusion of raw MOSC in the diets of the birds, and were less marked in birds fed treated diets, whereas no changes were noticed in birds fed control and 30% ammonium hydroxide treated MOSC diet. . With the inclusion of graded level of AHMOSC in the diets of the birds, the density of the hepatocyte appears normal with characteristic delineation.

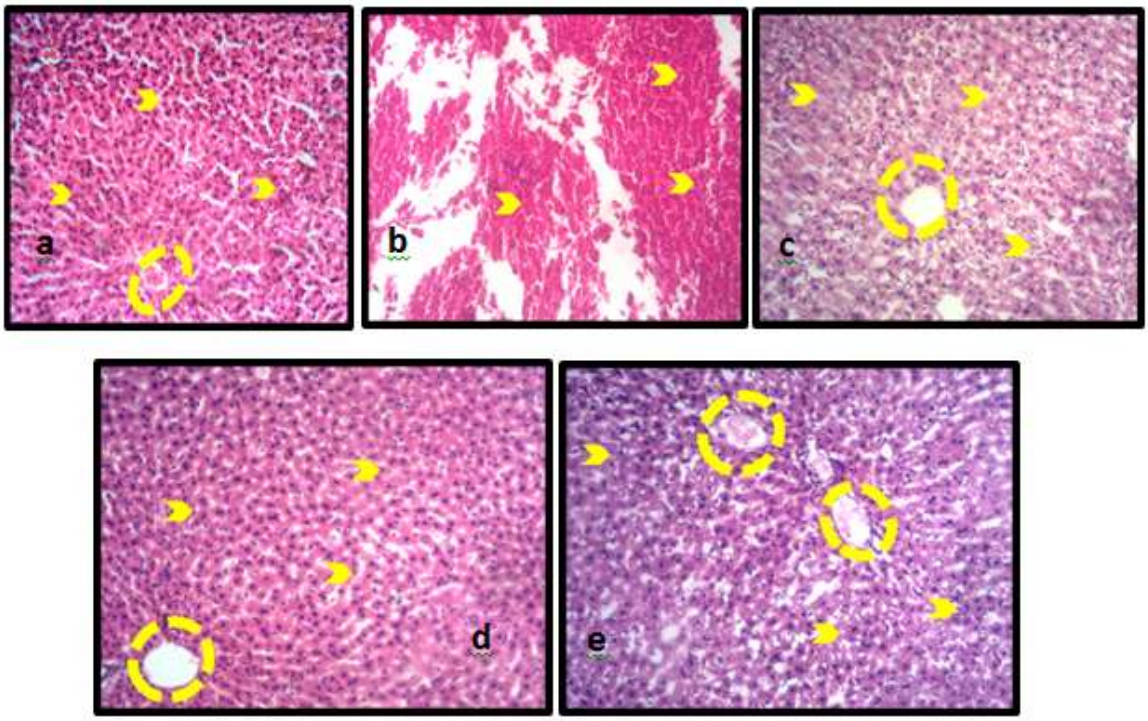

Figure 1 (a-e): Photomicrographs showing the liver of broilers fed the experimental diets, control (a) with normal hepatocyte, Raw MOSC (b) with severe haemorrhage of hepatic blood vessel, 10%AHMOSC (c) with mild hemorrhage of hepatic blood vessel, 20%AH MOSC (d) with mild hemorrhage of hepatic blood vessel and 30%AHMOSC (e) with normal hepatocyte.. H/E Stain Mag. X400

DISCUSSION

Growth performance characteristics: The observed improved growth performance of birds with the inclusion of graded level of ammonium hydroxide in the diets of the birds indicate the efficiency of the processing method to reduce the various anti-nutrients inherent in MOSC thereby enhancing its better utilization by the birds. This present study agrees with the findings of Barlett (1998) that found reduction in toxicity in mustard meal after alkali heating in broilers.

The significant increase in the feed intake of the animals fed ammonium hydroxide treated MOSC diet compared to those on the raw MOSC may be an indication of reduction in the level of tannins in the treated feed sample. Tannins are known to cause reduction in feed intake (probably by irritating the gut) due to their astringency or bitter taste, thereby reducing the palatability of the feed (Reed, 1995). Ferreira et al., (2008) reported that higher levels of 7.5 and 100 g/kg moringa seed meal supplementation depressed intake, growth rate and live weights of the chickens. Also, the significant improvement observed in the feed conversion ratio of the birds placed on ammonium hydroxide treated MOSC based diet, when compared with those on the raw MOSC diet, is indicative of a better utilization of the feed. It showed that the animals reared on the feed, gained more weight with little amount of the feed consumed. There was an indication that birds fed the control diet and the treated groups had better feed conversion ratios. The best FCR observed in birds on the control diet and the treated groups was due to the absence of anti-nutritional factors (ANFs) in the control diet and subsequent reduction in the ANFs present in MOSC by the adopted processed method.

This result confirms earlier findings of Emiola et al., (2003) that reduced ANFs as a result of improved

processing techniques enhanced birds' performance. Absence of mortality in this study is an indication that most of the anti- nutrients in the treated MOSC diets have been eliminated or significantly reduced. Some of these anti-nutrients are known to cause death in animals.

In this study, the nutrient digestibility of the birds fed the treated MOSC was better than those fed with raw MOSC diet and the better nutrient digestibility of the birds fed with the doses of ammonium hydroxide treated MOSC implies the effectiveness of the processing of raw MOSC for broilers which may be due to reduction in anti-nutritional factors present in MOSC that enhanced better feed intake and efficient utilization of the diet by the birds resulting in increased nutrient digestibility. This work is in line with the findings of Aletor (1993) that ANFs like tannin in the diets of livestock causes decreased feed consumption in animals especially monogastrics, binds dietary proteins including digestive enzymes by forming complexes that are not readily digestible hence tannins in the raw diet of the birds (treatment 2) may have formed complexes with the digestive enzymes in the birds fed untreated MOSC compared with their counterparts in the control diet and those fed with the treated group thus limiting the digestibility of protein and other nutrients and the undigested feed voided out without the nutrients utilized and retained by the animals.

Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb) and Total Protein (TP) were observed to be better for the control diet than for the MOSC diets although the differences were not significant ($p>0.05$). Apata (2004) reported a similar finding when processed *Prosopis africana* seeds were fed in the diet of laying hens. The author argued that with proper processing, grain legume seeds can be incorporated in poultry diets without any adverse effects.

The observed non-significant ($p>0.05$) difference in serum urea between the control and the treated groups is in agreement with the findings of Eggum (1970); Esonu *et al*; (2001); Iyayi and Taiwo (2003) who reported that serum urea and total protein contents depend on both the quantity and quality of the protein supplied in the diet. The significant ($p<0.05$) increase in serum urea observed in raw diet (treatment 2) is an indication that, the more the test feed was included in the diet of broilers, the more the birds survive at the expense of their body response. The observed significant ($p<0.05$) increase in total protein, and albumin with the inclusion of graded level of ammonium hydroxide in the diets of the birds attest to the nutritional adequacy of treated MOSC in meeting the protein needs of the birds. Onifade and Tewe (1993) had earlier reported that serum protein, albumins and globulins are generally influenced by total protein intake. ALP, AST and ALT decreased with the inclusion of graded level of ammonium hydroxide in the diets of the birds. The increase in ALT values in birds in treatments 2, compared with the control and treated group suggest that there may be a likelihood of liver damage by the raw MOSC.

Histological observations: The histological structure of livers from different experimental groups fed ammonium hydroxide treated MOSC diets is illustrated in Figure 1 (a-e). It is clear from Fig. 1a that the hepatocytes of the control chicks are normal with some dark-stained eosinophilic cells surrounding the central vein. This structural appearance of hepatocytes was also observed in hepatic sections of birds fed 10,30 and to a little extent 50% ammonium hydroxide treated diets Fig. 1(c-e) respectively. However, some congested areas, moderate hypertrophy of hepatocytes, necrotic areas and infilterable fluids could be seen in all sections. The sections of the liver revealed degeneration of the hepatocytes and coagulative necrosis with the inclusion of raw MOSC in the diets of the birds, and were less marked in birds fed treated diets, whereas no changes were noticed in birds fed control and 30% ammonium hydroxide treated MOSC.

Progressive changes in the liver architecture could be seen in Fig. 1b, where chicks were fed with the raw MOSC diets. There are many focal sinusoids in/between many compressed hepatic cords accompanied with necrotic areas, infilterable fluids and dilated central vein engorged with blood. These observations are supported by the microscopic examination of livers, where they were greatly enlarged in birds fed with the raw MOSC diets.

CONCLUSION

From this study, broilers fed treated MOSC diet performed better than those fed raw MOSC diet while those fed the control diet performed significantly better than those fed raw and treated MOSC diet respectively. The adopted treatment method in this study was not sufficient to totally detoxify the anti-nutrients present in MOSC. The best result obtained from replacing soyabean with treated *Moringa oleifera* seed cake diet was 30% Ammonium hydroxide treatment at 5% inclusion level.

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