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EFFECTS OF NATIVE AND MICROBIAL PHYTASE ON LAYING PERFORMANCE, SHELL ASH AND PHOSPHORUS CONTENT OF HENS FED MASH AND PELLETED DIETS

S.G. Ademola[1*], T.E. Lawal[2], O. Odu[3], M.D. Shittu[4], H.O. Oladiran[1] and M.T. Adeshina[1]

[1]*Department of Animal Nutrition and Biotechnology, LAUTECH, Ogbomoso, Nigeria*
[2]*Department of Animal Science and Fisheries Management, Bowen University, Iwo, Nigeria*
[3]*Department of Animal Science Univ. of Ibadan, Ibadan, Nigeria*
[4]*Department of Animal Production and Health, LAUTECH Ogbomoso, Nigeria*

*E-mail: ademolasadiq@yahoo.com

ABSTRACT: *The study investigated effects of microbial phytase and native wheat bran phytase on laying performance, egg quality and shell phosphorus of hens fed two forms of diets. Five experimental diets were formulated for the study. Control and basal diets contained similar levels of nutrients. However, basal diet (T1) containing 15% wheat bran (WB) had lower available phosphorus (AVP). Diet forms (mash and pelleted) and microbial phytase supplementation (0 and 900 phytase unit (FYT) were arranged to examine their interaction effects. The 0 FYT microbial phytase represented the native wheat bran phytase activity in the mash diet only. T1 and T2 were mash and pelleted unsupplemented diets respectively. Diets in T3 and T4 were microbial phytase supplemented in mash and pelleted forms respectively. Laying hens fed unsupplemented mash basal diet (T1) had the highest hen day production (HDP) (P<0.024), and the best feed conversion (P<0.012). However, those fed mash supplemented diet (T3) had the lowest HDP and worst feed conversion. Microbial phytase supplementation to mash diet (T3) resulted in lowest egg mass of 45.35 gram daily (P<0.025). Pelleting the unsupplemented diet (T2) yielded poorer feed conversion than those fed unsuplemented mash diet (T1). Hens fed pelleted supplemented diet (T4) had slightly reduced HDP and significantly lower egg mass when compared to the control group. These hens had significantly highest yolk index (P<0.036) and egg shell with the most concentrated phosphorus content (P<0.002). It is concluded that native wheat bran phytase in mash diet containing 15% WB was effective for improved laying performance.*

Key words: *Hen Day Production, Microbial, Native Phytase Wheat Bran*

ORIGINAL ARTICLE

INTRODUCTION

Phytic acid is a major component of all plant seeds constituting 1-3 percent by weight of many cereals and oil seeds and typically accounting for 60 to 90 percent of the total phosphorus (Graf, 1983).The acid impedes the utilization of a number of important inorganic and organic compounds such as phosphorus, divalent cations and proteins as a result of insufficient endogenous phytase secretion by poultry. The poor digestive utilization of phytate phosphorus by laying hens and its consequences on diet cost, environment and, bioavailability of mineral and protein have led to efforts directed towards improving phytate digestion (Dilger et al., 2004). Strategies to overcome the poor utilization of phytate phosphorus by poultry include addition of expensive inorganic phosphorus, and microbial phytase preparation. Phytases have been identified in plants, micro-organisms and in some animal tissues (Konietzny and Greiner, 2002). Eeckhout and de Paepe (1994) reported that some feedstuffs contain 6-phytase activities (wheat, wheat bran, rye and barley) whereas others have little or no phytase activity (corn, oat, sorghum and oilseeds). Wheat bran has some phytase activity and it could be used as a viable source of phytase (Muhannad, 2010). Steiner et al. (2007) found that wheat bran contained 6-phytase activities ranging between 2349 and 9945 U/Kg.

Several authors have documented effects of heat treatment on pelleting native phytase from plant sources. Cavalcanti and Behnke (2004) reported that phytase activity may considerably reduce when wheat bran (WB) is processed into pellets because heat treatments destroy phytase. Also Hattingh (2002) asserted that some feed ingredients contained native phytase activity and that steam pelleting used in manufacture of many commercial poultry feeds resulted in significant losses of this intrinsic phytase activity. The limitation of most commercially available phytases was the inactivation of their activities when pelleted at a temperature greater than 70^{o}C

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(Jongbloed and Kemme, 1990). Moreover, variation in phytase activities among and within plant species with damaging effect of pelleting during feed manufacturing and the lack of availability of feed ingredients of high phytase activity, the presence of residual phytase activity often may not be considered in diet formulation when feed are pelleted (Kornegay, 1999). Varying responses have been reported on the effect of pelleting of feeds in relation to the availability of phosphorus from phytate. Pelleting of feed at 81^{0}C reduced the phytin content in a mixed rape seed, barley and pea diet by 7% (Skoglund et al., 1997). Improved utilization of the phytate phosphorus from a corn-soybean diet containing 25% wheat bran as a result of steam pelleting has been reported (Summers et al., 1967). However, reduction of phosphorus availability by pelleting of feeds containing phytase activity from wheat was observed by Jongbloed and Kemme (1990). Steam pelleting also failed to enhance phytate phosphorus availability from a corn-soybean diet when fed to laying hens (Pepper et al., 1969).The presence of high phytase activity in wheat bran and the inconsistency in responses of animals fed pelleted diets in relation to phosphorus availability stimulated the current investigation. Hence, the study assessed effects of microbial phytase preparation and native wheat bran phytase on laying performance, egg quality and egg phosphorus content of hens fed mash and pelleted corn-soybean meal-wheat bran-based diet.

MATERIALS AND METHODS

Site of the experiment

The study was carried out in Layer House of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. A period of 8 weeks was observed for the experiment.

Information on the commercial phytase product

The phytase enzyme used in this study was Ronozyme® NP (CT). The active agent of Ronozyme® NP is 6-phytase, produced by a strain of *Aspergillus oryzae*. One phytase (FYT) unit of Ronozyme® NP is defined as the amount of enzyme that liberates one µmol of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37^{0}C (von Holst, 2008). Wheat bran was the major source of native phytase activity in the basal diet and active only in mash diets. This is because heat treatment destroys native phytase of wheat bran in the pelleted basal diets.

Formulation of experimental diets

Five experimental diets were formulated for the study. Control diet (mash form) contained adequate levels of metabolizable energy, crude protein, calcium and available phosphorus. Control diet did not contain wheat bran. Basal diet contained15% wheat bran and the available phosphorus was however lowered by 0.16% when compared to control. A 2X2 factorial arrangement for form of basal diet (mash and pelleted) and microbial phytase supplementation (0, and 900 FYT/Kg) was used to examine interaction effects on measured parameters. Wheat bran was the major source of native phytase to mash basal diet which was represented by 0 FYT microbial phytase. Two basal diets in T1 and T3 were in mash form, and the other two diets (T2 and T4) were in pelleted form. A commercial microbial phytase preparation [Ronozyme® NP (M)] was supplemented in diets T3 and T4 while this phytase preparation was not added to diets in T1 and T2. Thus, two groups (T1 and T3) represented diets with native wheat bran phytase, but diet in T3 also contained supplemental microbial phytase preparation (Table 1). Pelleting of the diets (T2 and T4) was done at a temperature of 65^{0}C.

Table 1 - Ingredients composition of experimental diets

Parameters	Control	T1 (Mash)	T2 (Pellet)	T3 (Mash)	T4 (Pellet)
Corn	42.00	52.00	52.00	52.00	52.00
Soybean meal	20.90	20.90	20.90	20.90	20.90
Corn bran	24.65	-	-	-	-
Wheat bran	-	15.00	15.00	15.00	15.00
Fish meal (72%CP)	2.00	2.00	2.00	2.00	2.00
Bone meal	3.00	1.00	1.00	1.00	1.00
Oyster shell	6.85	8.50	8.50	8.50	8.50
Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25
Ronozyme NP (M)	-	-	-	+	+
Analysed composition					
Crude protein (%)	16.30	16.50	16.50	16.50	16.50
Crude fibre (%)	5.15	3.70	3.70	3.70	3.70
Calculated analysis					
Energy (Kcal ME/Kg)	2680.03	2687.68	2687.68	2687.68	2687.68
Methionine (%)	0.38	0.39	0.39	0.39	0.39
Lysine (%)	0.84	0.97	0.97	0.97	0.97
Available P (%)	0.58	0.42	0.42	0.42	0.42
Calcium (%)	3.68	3.54	3.54	3.54	3.54

*The following vitamins and trace elements were supplied per kg of the layer diet: 10,000IU Vit. A; 2,00IUVit. D_3; 23mg Vit K_3; 3mg Vit. B_1; 6mg Vit. B_2; 50mg Niacin; 10mg Calcium Pantothenate; 5mg Vit B_{12}; 1mg Folic Acid; 0.05mg Biotin; 400mg Choline Chloride; 120mg Mn;

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100mg Fe; 80mg Zn; 8.5mg Cu; 1.5 I; 0.3mg Co; 0.12mg Se and 120mg Antioxidant.
+ Ronozyme NP supplied 900 FYT phytase to the basal diet at 18mg/kg

Management of experimental laying hens

A total of 160 Nera Black laying hens (25 weeks old) were distributed to five experimental groups in a completely randomised design (CRD). The laying hens were kept in battery cage system. There were 4 replicates of 32 hens per treatment group. These birds were fed experimental diets *ad libitum* and clean water was also supplied to them. Vaccination and medication were carried out as at when necessary.

Data collection

Egg production was monitored on daily basis and number of eggs collected for each replicate was used in calculation for hen day production. Egg weight was estimated with the aid of a sensitive weighing scale. Feed intake and feed conversion were also determined on a replicate basis. A total of 240 eggs with 12 eggs per treatment selected each week for 4 weeks for egg quality assessment. Yolk index was expressed as a ratio of yolk length and yolk height.

Chemical analysis

Crude protein and crude fibre of the experimental diets were carried out using the methods of AOAC (2000). Broken egg shells were rinsed in water, air dried and ground with pestle and mortal. Eight shell samples per treatment were digested by dry ash-procedure at 600^{0}C for 6 hours to estimate eggshell ash. Furthermore, 8 egg shell samples per treatment were digested by wet-ash procedure using perchloric acid and nitric acid. Phosphorus concentration was determined colorimetrically (Genesys 5 Spectrophotmer; Thermo Electron Corporation, Madison, WI) at 410nm according to AOAC (1995) methods.

Statistical analysis

All data collected were analyzed by factorial analysis under CRD using SAS (1999). Significant means were separated using Duncan option of the same statistical software. A probability of 5 percent was considered significant ($P<0.05$).

RESULTS

Table 2 displayed laying performance and egg quality of hens fed either mash or pelleted basal diet containing 15% wheat bran with or without phytase supplementation. Dietary treatment significantly affected hen day production (HDP), egg mass, feed conversion and yolk index. Laying hens fed unsupplemented mash basal diet with native phytase activity from wheat bran (T1) had the best HDP ($P<0.024$) and significantly ($P<0.012$) improved feed conversion when compared to those fed other basal diets. Microbial phytase supplementation to mash basal diet (T3) caused significant reduction in HDP and pelleting basal diet with supplemental microbial phytase (T4) slightly reduced HDP. Laying hens fed unsupplemented pelleted diet (T2) had the heaviest egg mass which was comparable with those unsupplemented mash basal diet (T1) and control diet. Microbial phytase supplementation to mash and pelleted basal diets (T3 and T4) significantly reduced the egg mass when compared to others. Interaction effect of diet form and phytase supplementation significantly influenced the yolk weight ($P<0.047$) and albumen weight ($P<0.024$) with hens fed unsupplemented mash basal diet (T1) laid heavier egg yolks and lower albumen when compared to those fed other basal diets. Laying hens fed pelleted basal diet supplemented with microbial phytase (T4) had significantly higher yolk index and improved egg shell phosphorus content when compared to those fed other diets.

Table 2 - Laying performance and egg quality of laying hens fed mash and pelleted diets containing native wheat bran- and microbial- phytase

Parameters	Control	T1 (Mash)	T2 (Pellet)	T3 Phyt (Mash)	T4 Phyt (Pellet)	P-value	SEM	Diet Form	Phyt	Int.
HDP (%)	84.52^{a}	86.09^{a}	84.81^{a}	80.69^{b}	83.19^{ab}	0.024	1.05	0.518	0.002	0.062
Egg weight (g)	56.72	55.39	56.79	56.22	54.95	0.147	0.58	0.918	0.431	0.053
Egg mass (g/day)	47.92^{a}	47.68^{ab}	48.18^{a}	45.35^{c}	45.70^{bc}	0.025	0.68	0.565	0.006	0.924
Feed intake (g/bird/day)	108.41	104.61	108.63	108.48	110.57	0.233	1.73	0.115	0.132	0.600
Feed conversion ratio (feed/egg)	2.27^{b}	2.23^{b}	2.43^{a}	2.44^{a}	2.43^{a}	0.012	0.05	0.053	0.035	0.043
Yolk weight (%)	24.25	24.03	22.17	22.47	23.52	0.373	0.88	0.561	0.882	0.047
Albumen weight (%)	64.66	63.13	66.09	66.09	64.85	0.268	3.12	0.329	0.325	0.024
Shell weight (%)	11.09	12.93	11.65	11.44	11.63	0.115	0.60	0.321	0.173	0.159
Yolk index	0.43^{b}	0.46^{b}	0.43^{b}	0.43^{b}	0.62^{a}	0.036	0.05	0.131	0.138	0.059
Shell P (mg/100g)	0.41^{b}	0.45^{b}	0.50^{b}	0.47^{b}	0.73^{a}	0.002	0.06	0.010	0.030	0.069
Egg ash (%)	51.00	52.00	53.00	54.00	50.00	0.201	1.21	0.279	0.712	0.083

Means along the same row with different superscripts are significantly different ($P<0.05$). Phyt=phytase supplementation

DISCUSSION

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Monogastric organisms contained no or only negligible amount of endogenous phytase in the stomach and small intestine, these animals are therefore dependent on plant or microbial phytase (Pallauf and Rimbak, 1997).The positive response for laying hens fed unsupplemented mash basal diet containing 15% wheat bran revealed that native phytase from plant source could not be totally neglected if the diet was in mash form. It has been reported that hydrolysis of phytate within the digestive tract of poultry may be attributed to the action of phytase from one of three possible sources namely plant feed ingredients, animal-intrinsic phytase activities and microbial origin (commercial) phytase product (Ravindran et al., 1995). However, pelleting the basal diet adversely affected the potency of the native wheat bran phytase as the feed conversion of laying hens fed unsupplemented pelleted basal diet (T2) was poorer when compared to those fed unsupplemented mash basal diet (T1). Inactivation of the wheat bran phytase may be reason for the poor feed conversion. This finding is in consonance with the report of Cavalcanti and Behnke (2004) that heat treatment destroys phytase. Ullah and Mullaney (1996) also stated that losses in activity of phytase enzyme begin to occur when feeds are subjected to steam pelleting around 60^{0}C.

Phosphorus content in egg shell of control hens was not significantly different from those fed basal diets except those fed pelleted basal diet supplemented with microbial phytase (T4). This showed that lowering available phosphorus in layer diet from 0.58% to 0.42% did not compromise the availability of phosphorus for egg shell formation for hens fed basal diets containing wheat bran. Phosphorus was most concentrated in egg shell of hens fed microbial phytase pelleted diet. This observation corroborated with the finding of Summers et al. (1967) who observed improved utilization of the phytate phosphorus from a corn-soybean diet containing 25% wheat bran, as a result of steam pelleting.

The impact of phytase activity from wheat bran on utilization of phytate phosphorus and performance of poultry has been documented by several authors. Wheat bran's phytase may improve the absorption of phosphorus from cereals when given to simple stomach animals (Lesson and Summers, 2005). Yao et al. (2007) reported that ten percent of wheat bran replacing 0.05% inorganic phosphate did not influence either egg yield or nutrient utilization. These authors concluded that wheat bran phytase improved the performance and the utilization of dietary total phosphorus and crude protein of laying hens. The present study revealed that 15% wheat bran (with the intrinsic phytase activity) in unsupplemented mash layer diet achieved similar hen day production and egg mass with those of the control group, despite lowering available phosphorus by 0.16%. The improved laying performance of hens fed mash basal diet containing native wheat bran was in agreement with the conclusion of Cavalcanti and Behenke (2004) that wheat bran phytase improved plant phosphorus utilization and increased growth rate of broiler. Addition of microbial phytase preparation to mash basal diet (T3) did not further increase the laying performance of hens but it resulted in poorer feed conversion. Some authors have demonstrated that wheat bran or wheat bran (50%) supplemented with enzyme preparations have positive effect on the performance of broilers and laying hens (Abaza et al., 2004 and Ali et al., 2006). Higher dietary concentration of wheat bran may probably be the reason for the varying response observed in laying performance between the findings of these authors (Abaza et al., 2004 and Ali et al., 2006) and the present study.

In conclusion, native wheat bran phytase activity in the mash basal diet was effective to achieve maximum laying performance of hens but microbial phytase supplementation to the mash basal diet yielded poor feed conversion. Furthermore, pelleting of corn soybean meal diet containing 15% wheat bran adversely affected feed conversion of the hens. Phosphorus was more concentrated in the egg shell of hens fed pelleted diet supplemented with microbial phytase.

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