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Effect of microbial 6-phytase on amino acid-digestibility of caecectomised laying hens fed low lysine-based diet

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Introduction

Earlier report by McNab (1994) showed that all dietary components including amino acids (AA), and minerals, are important when formulating diets for poultry, but critical attention will have to be given to the dietary AA in form of protein, as approximately 25% of the cost of practical poultry diets is attributed to AA. One of the most important AA in poultry nutrition is lysine, which are mostly inadequate in most plant foods and thus often added into diets in practical formulations. The cost of such addition is enormous on finished feeds. In recent past years, the importance of phytase in enhancing phytate-P utilization and its potential to improve the utilization of phytate-bound protein or AA have received considerable research attention in poultry nutrition despite the fact that the application of phytase as a tool to improve AA digestibility largely remain speculative in laying hens (Agbede *et al*., 2009a&b). Ravindra *et al.*(2001) reported improvement in nitrogen and lysine digestibility by 5.0% and 5.6% respectively when broilers were fed lysine deficient diet supplemented with phytase at 1000 FTU/kg while phytase supplementation at 500FTU/kg in lysine-adequate diet though led to the improvement in the digestibility of most AA, the increase was less pronounced.
In an *in vitro* study, lysine mono hydrochloride was incubated with bran, as a source of phytate, at pH 4.5 (Rutherford *et al.,* 1997). While incubation without phytase reduced the recovery of free lysine by 22% the addition of phytase led to 9% loss, suggesting phytate-free lysine interaction, which led to the conclusion that phytate may reduce the *in vivo* utilization of supplementary lysine. This was corroborated by the report of Biehl and Baker (1997). Reports abound on the positive effect of phytase on P and Ca utilization in poultry but information on such effect on AA, especially lysine digestibility in laying hens when fed lysine deficient diet is rare. Thus a significant and/or marginal improvement on lysine digestibility in laying hens through the addition of a microbial phytase is envisaged to reducing the cost of finished feeds which may be of economic importance to farmers in developing countries that depends largely on lysine importation. This paper thus examines the effect of phytase on lysine digestibility in caecectomised laying hens when fed diets containing adequate or marginally deficient dietary lysine. Also, evaluated are the effects of phytase on other AAs digestibility and P, Ca and nitrogen utilization.

Material and Methods

The pullets used for the trial were obtained when they were 16 weeks old from Avian Specialist at Ibadan, Nigeria and kept individually in balance crates. The hens were caecectomised when

they were 18 and 22 weeks old. The caecectomised procedure followed Angkanaporn et al. (1997) and Green et al. (1987) description. The experiment followed a 2x2-factorial arrangement in a completely randomized design with 2 basal diets. The first diet contained adequate lysine with (ALys Phy+) or without (ALys Phy-) phytase supplementation while the second diet contained marginally low lysine with (LLys Phy+) or without (LLys Phy-) phytase supplementation. Fourteen hens were used in two periods. The Ronozyme P 5000 (CT) used was obtained from DSM Nutritional Products, Basel. The hens were randomly allocated to the diets. The hens were fed for 5 days acclimation period and another 5 days of excreta collection period. Each hen was offered an amount of 120g/d of its diet in two equal meal per day at 08:00 and 15:00 h. Feed residues were gathered daily and weighed before and during the excreta collection period. Feed intake was recorded 5 days before and during excreta collection period. Hens were weighed before and at the end of the excreta collection period. To minimize votalization of the excreta, collection were done 3 times per day (7:00, 14:00 and 20:00 h) into separate and pre-weighed plastic buckets. Excreta were freeze dried and analyzed.

Results and Discussion

Table 1 showed that reduction of lysine in the LLys diets resulted to a depressed dry matter intake (DMI) with numerical improvement in N-utilization but phytase addition was not significant (P>0.05). This is contrary to the earlier report by Novak *et al.,* (2004) that low dietary lysine did not significantly lead to any reduction in feed consumption. Feeding varying levels of lysine and total sulphur amino acids had no effect on the feed consumption in Dekalb delta laying hens (Novak *et al.*, 2004). Also, in this study, the effect of phytase has no significant effect on the DMI, which is also contrary to the report by Panda *et al.,* (2005) in laying hens. The difference in Mean Digestibility (MD) of DMI was 1.4% -units in ALys and 3.8% -units in LLys.

Table 1: Dry matter intake and N-utilization of caecectomised laying hens

Lysine:	Adequate		Low		Pool SEM	*P-value (ANOVA)*		
						Lys	Phy	Lys X Phy
Phytase:	-	+	-	+				
Initial weight, kg	1.65	1.49	1.56	1.59	0.072			
Final weight, kg	1.67	1.47	1.60	1.59	0.080			
Dry matter Intake, g/d	104.6	106.1	97.1	100.9	0.548	0.048	0.390	0.709
Nitrogen Utilization, mg/d	0.65	0.68	0.66	0.70	0.047	0.499	0.224	0.787

Numerical improvement (Table 2) in the digestibility of 17 amino acids (AA) with phytase supplementation were observed without significant Lys x Phy interaction except in lysine (P<0.001). Addition of phytase to a lysine deficient diet significantly improved digestibility of lysine, while the digestibility of all other AAs were numerically enhanced in both ALys and LLys diets (except Alanine). This was in agreement with the report of Ravindran *et al.,* (2000) that addition of phytase to a lysine deficient diet significantly improved not only the ileal digestibility of lysine but also of other amino acids in broilers. This also suggests an enhanced efficiency of phytase activity at a reduced lysine level. From this study, at 1000 U/kg diet, phytase increased average amino acid digestibility by 8% in low lysine diet which almost restored the diet to the level of AA digestibility of ALys diet, which was 9%. Adding phytase to LLys diet improved the AA digestibility by 3.4 to 8.8%-units except that of lysine which was 32%-unit. This is in agreement with the reports of Ravindran *et al.,* (2000b) and Cowieson *et al.* (2006) which stated that phytase could enhance the digestibility of AA by 1.0 to 15% in broilers while Liu *et al,* (2007) and Agbede *et al.* (2009a) reported an improvement of 2 to 8% for laying hens. Hence, AA digestibility responses to phytase supplementation are variable in terms of digestibility effect

in all amino acids (AA). This may be as result of differential interactions between (AA) and phytase or it may be associated with the ability of phytase to increase the loss of endogenous compounds (e.g mucins) that are rich in certain amino acids (Cowieson *et al.,* 2006). In addition, higher AA digestibility unit of lysine (32%) in LLys, diet as improved by phytase supplementation may be linked to reduction in pH and hydrolysis of binary protein-phytate complexes of soybean which are formed below the isoelectric point of protein (pH < 5-6). At low pH, phytic acid interacts with α-NH_2 groups and with the side groups of basic amino acids which include arginine (isoelectric point 10.8), histidine (isoelectric point 7.6) and lysine (isoelectric point 9.7). This thus suggest that phytate could interact with free lysine and/ may be as result of quick broken of the resistance barrier in soybean meal by phyatse supplementation and thus made available the free lysine for the metabolic need which is in agreement with Han, (1988) that phytate from cottonseed is more resistance to phytase activity than phytate from soybean meal when measured *in vitro*.

Table 2: Amino acid digestibility of caecectomised laying hens

Lysine	Adequate		Low			*P-value (ANOVA)*		
Phytase	-	+	-	+	Pool SEM	Lys	Phy	Lys X Phy
Alanine	0.78	0.89	0.83	0.82	0.296	0.843	0.285	0.158
Arginine	0.79	0.89	0.82	0.87	0.080	0.897	0.189	0.608
Aspartic acid	0.80	0.87	0.78	0.82	0.077	0.455	0.284	0.814
Cystine	0.83	0.88	0.86	0.90	0.066	0.421	0.204	0.848
Glutamic acid	0.72	0.83	0.72	0.80	0.093	0.895	0.220	0.837
Glycine	0.82	0.88	0.83	0.88	0.069	0.867	0.198	0.896
Isoleucine	0.79	0.85	0.79	0.85	0.076	0.986	0.260	0.997
Leucine	0.81	0.90	0.83	0.91	0.080	0.768	0.122	0.799
Lysine	0.75	0.77	0.55	0.81	0.084	0.088	0.000	0.001
Histidine	0.73	0.79	0.75	0.81	0.084	0.739	0.308	0.960
Methionine	0.89	0.92	0.87	0.92	0.060	0.902	0.212	0.718
Phenylalanine	0.85	0.91	0.86	0.91	0.069	0.995	0.149	0.834
Proline	0.87	0.93	0.90	0.93	0.067	0.718	0.244	0.689
Serine	0.87	0.94	0.86	0.93	0.075	0.889	0.200	0.972
Threonine	0.81	0.91	0.85	0.89	0.075	0.841	0.188	0.527
Tryosine	0.81	0.86	0.80	0.87	0.081	0.977	0.294	0.806
Valine	0.83	0.91	0.86	0.89	0.220	0.840	0.180	0.527

Table 3 also showed that the improvement in P-retention of hens fed LLys diet and ALys diet are 7.5% and 4.2%, respectively due to phytase supplementation while the improvement in utilization are 3.2 % and 5.7%, respectively for ALys Phy+ diet and LLys Phy+ diet which is in agreement with the report of Nelson *et al.,* (1971). Also the P-intake, Ca-intake and P-excreted were significantly improved by dietary lysine level, although phytase supplementation marginally influenced P and Ca-intake and P and Ca-excreted in ALys Phy+ and LLys Phy+ but were not significant. This suggests a possible efficient metabolic activity of lysine. While the percentage reduction of marginal improvement of P excreted as affected by phytase supplementation in ALys and LLys diets are 1.4% and 3.8%, Ca excreted are 1.4% and 3.8%, respectively.

Conclusions and Outlook

The present study indicates that numerical improvement due to phytase and non significant interaction between phytase and lysine in laying hens could be observed in AA when laying hens are fed with diet containing low level of lysine. Phytase supplementation in low lysine diet could lead to significant improvement in lysine digestibility in laying hens. Also, low dietary lysine in

the diets of laying hens could depress feed intake, P-utilization and phosphorus retention. In general, the inclusion level of lysine in laying hen diet must be met for phytase to be effective with respect to AA digestibility and mineral utilization.

Table 3: Mineral utilization of caecectomised laying hens

Lysine	Adequate		Low		Pool SEM	*P-value (ANOVA)*		
Phytase	-	+	-	+		Lys	Phy	Lys X Phy
Phosphorus								
Intake, mg/d	321.7	326.4	210.6	218.9	1.447	0.000	0.379	0.804
Excreted, mg/d	163.7	161.5	132.7	128.3	0.866	0.000	0.536	0.842
Retention, mg/d	158.0	164.9	77.9	84.2	1.262	0.000	0.049	0.730
Utilized, %	48.9	50.5	36.6	38.8	0.549	0.000	0.044	0.582
Calcium								
Intake, mg/d	5074.7	5148.4	4698.4	4883.7	3.825	0.040	0.390	0.709
Excreted, mg/d	1697.6	1554.2	1443.4	1151.7	4.038	0.056	0.197	0.655
Retention, mg/d	3377.1	3594.2	3255.7	3732.0	4.504	0.971	0.119	0.551
Utilized, %	66.5	69.7	69.1	76.4	0.573	0.181	0.133	0.542

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