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Effects of Mega Doses of Phytase on Growth Performance, Bone Status and Nutrient Excretion of Broilers Fed Diets Containing High Levels of Rice Bran

Research Article

K.G.S.C. Katukurunda[1*], N.S.B.M. Atapattu[2] and P.W.A. Perera[2]

[1] Department of Food Science and Technology, Faculty of Applied Science, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka
[2] Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

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*Correspondence E-mail: kgsckatukurunda@sci.sjp.ac.lk

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ABSTRACT

Phytate in poultry rations containing rice bran (RB) critically reduce poultry performance while increasing N and P excretion. The objective of this study was to determine whether the anti-nutritive problems associated with higher inclusions of rice bran in poultry rations (up to 40%) could be mitigated with mega doses of phytase. Twenty days old male broiler chicks (n=180) in 60 floor pens were fed on 10 dietary combinations of a completely randomized factorial design (2×5). Two dietary rice bran (RB) levels (20 or 40%) and five levels (0, 1000, 2000, 3000 and 4000 FTU/kg diet) of phytase (Natuphos 500) were main factors. Cage-wise daily feed/water intakes and body weights on day 28, 35 and 42 were determined. Weekly and total weight gain and feed conversion ratio were determined. Cr_2O_3 mixed diets were fed from day 35 to determine illeal digestibility of crude protein, phosphorus (P) and dry matter. On day 42, following a 12-hour fast, two birds from each pen were humanely slaughtered to determine visceral organ weights. Fat free tibia ash contents and latency-to-lie test done on day 28, 35 and 42 were used as bone parameters. Growth performance parameters were not enhanced significantly due to mega doses of phytase. Negative effects like body weight reductions affected latency to lie time increments and further confirmed by insignificancy of tibia ash increments. Phytase significantly improved the crude protein digestibility. The optimum levels of phytase for the best crude protein digestibility with 20% and 40% dietary rice bran were 3000 and 4000 FTU/kg, respectively. Digestibility values of P and dry matter also affected. Supplementation of mega doses of phytase improved illeal crude protein digestibilities but not growth performances and bone status. Mega doses of phytase did not mitigate the adverse effects of 40% rice bran included broiler diets.

KEY WORDS broilers, crude protein, mega doses, phytase, rice bran.

INTRODUCTION

Between 60% and 70% of the total production cost of poultry is associated with the cost of feedstuffs and thus cost of poultry feeds is of great economic importance (Attia *et al.* 2012). Cereals and their by products are the main energy source in commercial poultry diets. During recent years price of cereals, particularly maize increased sharply since its use for biofuel production. Consequently, attention should be paid to increase the use of cereal by products such as rice bran in poultry feeding to reduce the cost of feed production. Rice bran is widely used in poultry rations mainly in Asian region. It has been widely accepted that the inclusion of rice bran in excess of 20% in the diets results in significant reductions in growth performance while increasing the excretion of N and P (Arabi, 2013; Rutherfurd et al. 2002; Cowieson et al. 2004; Wu et al. 2004). Phytic acid present in rice bran complexes with minerals such as calcium, magnesium, iron and zinc and reduces the availability of P and other minerals which complex with it (Reddy et al. 1989). Phytic acid complexes with proteins, amino acids and proteolytic enzymes making the aminoacid less available (Selle et al. 2000). Higher levels of rice bran in diets reported to reduce protein and dry matter digestibility as well (Piyaratne et al. 2008; Samli et al. 2006; Gallinger et al. 2004).

Since both phytates and fiber acts as critical anti nutrients, supplementation of broiler finisher diets containing higher levels of rice bran with exogenous phytase is hypothesized to remove adverse effects of phytates. Since diets formulated to have higher levels of rice bran contain relatively a higher amount of phytate, higher levels of exogenous phytase than what is normally used in industry may be needed to hydrolyze all or much of the phytates. A number of studies (Sooncharernying, 1991; Wu et al. 2004; Edwards et al. 1988; Attia et al. 2012) have evaluated to impacts of mega doses of phytase in poultry, but none has used it in rice bran based diets. The objective of this study was to determine whether the anti-nutritive problems which associated with higher inclusions of rice bran in poultry rations up to 40% could be mitigated with mega doses of exogenous phytase.

MATERIALS AND METHODS

Birds and cage arrangement

A total of 200 broiler chicks (strain Cobb) were purchased from a local hatchery (Kekanadura, Sri Lanka) and brooded and managed according to the standard management practices. Chicks were fed on a commercial broiler starter diet (Prima Sri Lanka) until day 20. On day 20, 180 birds were weighed and allocated into 60 floor pens (75 cm×75 cm×75 cm) so that between cage weight variation was minimum.

Ration composition

Pens were randomly allocated into 10 dietary combinations of a completely randomized design, in 2 × 5 factorial arrangement. The main factors of the experiment were two dietary rice bran levels (20 or 40%) and five levels of phytase (0, 1000, 2000, 3000 and 4000 FTU/kg diet). Natuphos 500 (Baden Aniline and Soda Factory Corporation, Germany) used as the phytase source. The ingredient compositions of the diets are shown in Table 1. The maize meal level reduced with increasing dietary RB inclusion level. Fresh rice bran was collected from a local mill and immediately transported to the laboratory. Rice bran was heat stabilized (100 ˚C for 30 minutes). Rations were formulated using trial and error method on EXCEL and verified by using the software CUFA TOTALFEED.

Table 1 Ingredient compositions and calculated CP, CF, P and ME contents of two rations containing either 20 or 40% rice bran

Ingredient %	Level of dietary rice bran %	
	20	40
Yellow maize meal	44.4	23.905
Rice bran	20	40
Soya oil meal	23.8	24.45
Coconut oil	4.42	5.8
Fish meal	4.96	3.225
Dicalcium phosphate	0.94	0.95
Shell grit	0.94	1.14
DL-methionine	0.04	0.03
L-lysine HCl	0	0
Salt	0.25	0.25
Vitamin and mineral premix (local market)	0.25	0.25
Total diet composition	100.000	100.00
Crude protein (%)	20	20
Metabolizable energy (kcal/kg)	3139	3140
Non phytate P (%)	0.35	0.35
Ca (%)	0.9	0.9
Crude fibre (%)	4.9	6.8

Management of birds

Cages were provided with paddy husk litter. Each cage had a feeder and a drinker. Experimental diets and water was given *ad libitum* from day 20-42. Cage-wise daily feed and water intakes were calculated. Water intake was corrected for evaporation losses.

Growth performance parameters

Birds were weighed on day 28, 35 and finally on day 42. Mortality percentage, live weight on day 28, 35, 42, weekly and total weight gain and feed conversion ratio were determined as growth performance parameters. Cage-wise daily feed intakes were calculated as feed offered feed left over.

Determination of bone status

The fat free tibia ash contents and latency-to-lie test were used as bone status determination criteria. Right after killing left tibia of one bird from each cage was removed and frozen for analysis of fat free tibia ash. The latency to lie test was done on 28, 35 and 42 days on one randomly selected bird from each pen, on each day as described by Weeks et al. (2002).

Determination of nutrient digestibility values

Indigestible marker assisted illeal crude protein and dry matter digestibility and, the availability, dry matter and the availability of P were determined. All diets contained 2 g of Cr_2O_3/kg as an inert marker.

Chromic oxide mixed diets were fed from day 35-42. One randomly selected bird from each pen was killed on day 41 and illeal contents (extending from Mackel's diverticulum to a point of 40 mm proximal to the ileo-caecocolic junction) were collected immediately after slaughtering. Illeal contents were pooled according to ten dietary treatment combinations. Six randomly drawn samples from each composite were analyzed for Cr, CP and P. Illeal level digestibility/availability values were determined. Chromium, CP and P contents of the feed and illeal samples were determined according to the procedures given by AOAC (1995).

Apparent ileal nutrient digestibility %= $[\{(Nt/Cr)_d - (Nt/Cr)_i\} / (Nt/Cr)_d] \times 100$

Where:
$(Nt/Cr)_d$: ratio of nutrient to Cr in the feed.
$(Nt/Cr)_i$: ratio of nutrient to Cr in illeal digesta.

On day 42, following a 12-hour fast, two birds from each cage were humanely slaughtered. The carcasses were then manually eviscerated. Abdominal fat pad (including fat surrounding gizzard, bursa of F×abricius, cloaca and adjacent muscles) was weighed individually and calculated as a percentage of live weight of the bird.

Statistical analysis

The experiment was analyzed as a completely randomized design in 2 × 5 factorial arrangements. Minitab 17 (2013) was used for the analyses. Main effects of rice bran and phytase and interactions were determined. Effects were considered significant when (P<0.05) and regression equations (linear, cubic and sigmoid) were determined for phytase levels. In growth performance data analysis, cage means were used as replicates. In digestibility data analysis, samples taken from each composite (composite illeal samples from each treatment combination) were served as the replicates. In bone ash data analysis each bird was served as replicates. Latency to lie data analysis values of each bird selected for the test (minutes to lie) were served as replicate values.

RESULTS AND DISCUSSION

Growth performance, latency to lie and bone status

Effects of two dietary rice bran levels and five levels of phytase on growth performance, latency to lie and bone status of broiler chicken are shown in Table 2. Performance parameters such as live weight on day 23 and 42, weight gain, feed conversion ratio (FCR) were significantly infe rior for the birds fed 40% RB compared to those fed 20% RB. Feed intake not significantly influenced by dietary treatments.

In contrast, Deniz *et al.* (2007) concluded that feed intake was significantly reduced with the increasing level of rice bran in broiler diets. Feed conversion ratio was significantly increased with 40% dietary RB. None of the above mentioned growth parameters was influenced by phytase or RB phytase interaction.

Probably due to lower body weight, birds fed 40% RB showed significantly longer latency to lie time compared to those fed 20% RB on day 28 and 35. Phytase supplementation also resulted in longer latency to lie time. The latency to lie time on day 42 also increased significantly when phytase supplemented diets were fed.

However, RB level had no significant effect on the latency to lie on day 42. Tibia ash content was not significantly affected by RB. Gallinger *et al.* (2004) showed that the feed conversion ratio and tibia ash were more sensitive than weight gain to detect anti-nutritive factors in rice bran. They have further reported that high concentrations of rice bran (in excess of 20%) resulted in a significant reduction in body weight.

Gallinger *et al.* (2004) suggest that rice bran should be included in broiler diets at a level between 10 and 20% if strategies are not used to decrease the anti-nutritive activity. The short period of the trial possibly reduced the chances of significant reduction in tibia ash content in the present experiment.

Nutrient digestibility values

Effects of increasing levels of phytase supplementation on CP, P and dry matter digestibility of broiler chicken fed either 20 or 40% dietary rice bran are shown in Table 3. 20% RB fed chicks showed significantly (P<0.05) higher CP digestibility than those fed 40% RB. Meanwhile, phytase supplementation also increased the CP digestibility. Positive effects of supplemental phytase on protein digestibility and illeal % N digestibility have been reported elsewhere (Cowieson *et al.* 2004; Rutherfurd *et al.* 2002; Managi *et al.* 2009). There was a significant cubic relationship between incremental phytase and CP digestibility (Figure 1).

At both 20 and 40% RB levels incremental phytase increased the CP digestibility. However, always, CP digestibility of 40% RB diet was lower than that of 20% diet. At both RB levels, the maximum CP digestibility was reported with 4000 FTU/kg phytase. The relationship between phytase and CP digestibility at 20% RB could best be expressed as a quadratic relationship (Figurre 2), whereas the same for 40% RB was a cubic relationship (Figure 3).

Table 2 Effects of increasing levels of phytase supplementation on growth performance, latency to lie and bone status of broiler chicken fed either 20 or 40% dietary RB

Interaction effects		Live weight on day (g)		Weight gain (g)	Feed intake (g)	FCR	Latency to lie (min) on day			Tibia ash (%)
RB	Phytase (FTU/kg)	23	42				28	35	42	
20	0	907	2424	1517	2218	1.46	158	74.8	17.1	40.6
	1000	953	2398	1445	1915	1.35	161	79.0	19.0	44.1
	2000	947	2307	1360	2459	1.84	169	79.8	21.3	40.0
	3000	937	2157	1219	2152	1.82	168	78.8	17.1	41.8
	4000	885	2411	1525	2183	1.45	175	82.3	18.0	42.2
40	0	852	2147	1295	2281	1.77	120	53.8	12.1	45.6
	1000	936	2138	1201	2137	1.83	167	75.0	25.6	43.8
	2000	914	2008	1093	2242	2.06	150	65.0	18.6	43.5
	3000	877	2155	1278	2297	1.81	144	63.0	20.0	45.9
	4000	911	2205	1294	1944	1.57	144	59.5	25.3	44.3
Main effects										
RB	20	926	2339^{a}	1413^{a}	1801	1.6^{b}	167^{a}	79^{a}	19	42
	40	898	2131^{b}	1232^{b}	1711	1.81^{a}	144^{b}	63^{b}	20	45
Enzyme	0	879	2286	1406	2250^{b}	1.62^{bc}	142^{a}	65	15^{c}	43
	1000	945	2268	1323	2026^{c}	1.58^{bc}	153^{a}	76	23^{ab}	44
	2000	931	2158	1227	2351^{a}	1.98^{a}	159^{a}	73	20^{bc}	42
	3000	907	2156	1249	2225^{b}	1.82^{ab}	153^{a}	69	18^{bc}	44
	4000	895	2308	1409	2064^{c}	1.51^{c}	115^{b}	71	31^{a}	43
Significance level										
SEM		44.0	85	89	155	0.16	8.2	3.8	2.3	3.9
RB		NS	**	**	NS	*	***	***	NS	NS
Phytase		NS	NS	NS	NS	NS	*	*	*	NS
RB × phytase		NS	NS	NS	NS	NS	NS	NS	*	NS

RB: rice bran and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.
SEM: standard error of the means.

Accordingly, as far as CP digestibility is concerned, 3000 FTU/kg of diet can be recommended as the best phytase level for diets having 20% RB. Use of 4000 FTU is recommended for the diets having 40% RB.

Compared to control, 1000, 2000, 3000 and 4000 FTU phytase/kg increased the CP digestibility 16, 19, 21 and 24% (Figure 3). In contrast, Kornegay (1996), Kornegay (1999) standard response curves for phytase and protein digestibility, the linear increment of N digestibility at 750 and 450 FTU/kg phytase diets were 0.4 and 2.9%. Moreover Namkung and Leeson (1999) reported that phytase significantly increased total N digestibility by 2.5% when fed 1149 FTU/kg phytase included maize soybean diet. Ravindran *et al.* (2000a); Ravindran *et al.* (1999) and Ravindran *et al.* (2000b) reported a 2.7% of N digestibility increase in low phosphorus (P) diet and 1.8% of total N digestibility in adequate P diet with wheat and sorghum. Moreover Ravindran *et al.* (1999) reported 2.7, 2.8 and 4.7% of total N digestibilities due to of phytase (600 FTU/kg), xylanase (6600 EXU/kg) and both in combination (600 FTU/kg+6600 EXU/kg), respectively. However Zhang *et al.* (1999) reported that phytase did not increased the digestibilities of amino acids.

In this experiment the mega doses of phytase increased the CP digestibility in chicks by several magnitudes than above studies. The use of relatively poor quality diets having at least 20% RB may be a one reason for better response for phytase. Numerous studies (Arabi, 2013; Rutherfurd *et al.* 2002; Cowieson *et al.* 2004; Wu *et al.* 2004) have shown that phytase increased the amino acids availability and in-turn the performance. And reduce the excretion of N with faeces (Aggrey *et al.* 2002; Cowieson *et al.* 2004; Rutherfurd *et al.* 2002).

However, it needs to be noted that though phytase increased the digestibility values of CP, the growth performance values were not altered significantly. It is hypothesized that phytase mitigated the effects of high RB on protein digestibility but not the other adverse effects.

Rutherfurd *et al.* (2002) reported that inclusion of phytase in poultry diets with rice bran did not significantly alter the amino acid digestibility, except glycine. Furthermore they concluded that it is possible that the phytate in rice bran plays minor role in the amino acid-matrix interactions that occur within the feedstuff or that the phytate molecules associated with proteins were inaccessible to phytase because of static interference.

Table 3 Effects of increasing levels of phytase supplementation on CP, P and dry matter digestibility of broiler chicken fed either 20 or 40% dietary RB

Treatment factors		**Digestibility / availability (%) (%)**		
RB	Phytase (FTU/kg)	Crude protein	P	Dry matter
20	0	64.7	78.0	77.3
	1000	76.1	74.2	73.5
	2000	78.2	69.7	69.3
	3000	80.6	81.3	81.2
	4000	82.2	79.2	79.1
40	0	59.2	79.3	79.1
	1000	72.1	76.0	75.8
	2000	74.6	75.9	75.1
	3000	75.4	76.1	75.6
	4000	81.6	79.7	79.8
Mean effects				
RB	20	76	77	76
	40	73	77	77
Enzyme	0	62^{d}	79	78
	1000	74^{c}	75	75
	2000	76^{bc}	73	72
	3000	78^{b}	79	78
	4000	82^{a}	79	79
Significance level				
SEM		1.3	4.0	4.2
RB		***	NS	NS
Phytase		***	NS	NS
RB × phytase		NS	NS	NS

RB: rice bran.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.
SEM: standard error of the means.

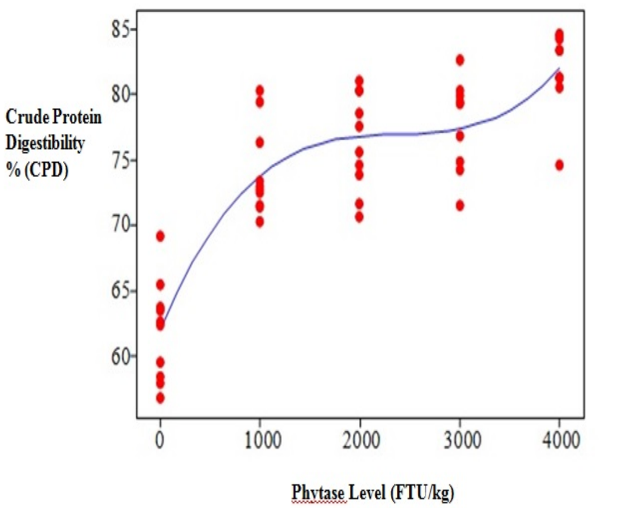

Figure 1 Relationship between incremental phyase level and CP digestibility (CPD, %), R-Sq: 80.4%, R-Sq (adj): 79.1%
CPD= 62.04 + 0.01834 Phy – $(0.000008 \text{ Phy})^2$ + $(0.000000 \text{ Phy})^3$

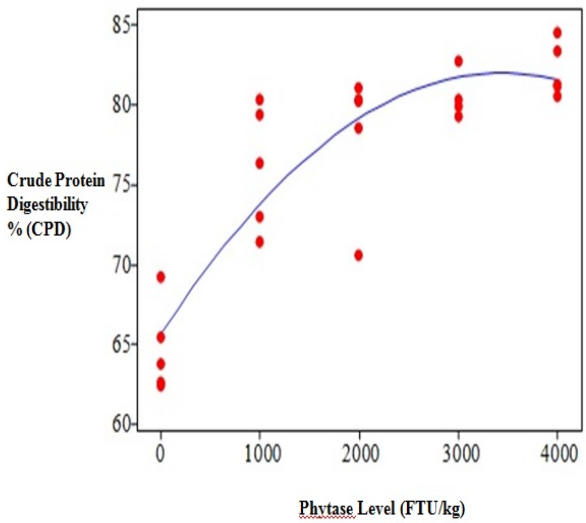

Figure 2 Crude protein digestibility (CPD, %) of 20% rice bran diets fed to broilers for phytase treatments, R-Sq: 79.7%, R-Sq (adj): 77.8%
CPD= 65.66 + 0.009546 Phy – $(0.000001 \text{ Phy})^2$

Contrary to above conclusions, the results of present experiment suggest that improvements in CP digestibility could be achieved when RB based diets are supplemented with phytase. Furthermore, for maximum CP digestibility higher level of phytase than what industry used is needed.

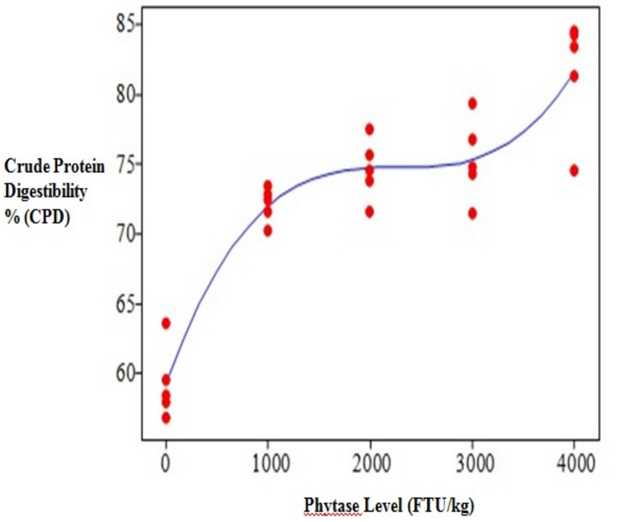

Figure 3 CP digestibility of 40% rice bran diets fed to broilers for phytase treatments, R-Sq: 89.8%, R-Sq (adj): 88.3%
CPD= 59.28 + 0.02043 Phy - $(0.000009\ \text{Phy})^2$ + $(0.000000\ \text{Phy})^3$

Diets having higher level of RB needs even higher levels of phytase. Numerous studies (Arabi, 2013; Rutherfurd *et al.* 2002; Cowieson *et al.* 2004; Wu *et al.* 2004) have shown that phytase increased the amino acids availability and in turn the performance and reduce the excretion of N with faeces (Aggrey *et al.* 2002; Cowieson *et al.* 2004; Rutherfurd *et al.* 2002). It is not possible to conclude whether excretion of N was reduced due to phytase since faecal level digestibility was not determined.

However, no response in growth performance suggests that utilization of amino acids might have been low (Cowieson *et al.* 2004). However, Attia *et al.* (2012) have concluded that high fiber diets increased the exogenous losses of nutrients. Therefore, suitability of illeal digestibility measurements is questionable under the present experimental conditions. Phosphorous and dry matter digestibility values were not significantly influenced by RB or phytase levels.

Table 4 Effects of increasing levels of phytase supplementation on relative weights of gizzard, liver, Pancreas and small intestines and relative lengths of small intestines of broiler chicken fed either 20 or 40% dietary RB

Treatment factors		**Relative weights (g)**				**Relative length of small intestine (cm)**
RB	Phytase (FTU/kg)	Gizzard	Liver	Pancreas	Small intestine	
20	0	53	55.2	5.8	164	131
	1000	55.2	57.8	5.2	165	122
	2000	55.2	57.6	5.8	167	120
	3000	51	54.4	6.4	160	128
	4000	54.8	51.8	5.6	163	116
40	0	50.6	58.2	6	164	122
	1000	51.4	57.8	5.4	151	122
	2000	54	55.8	6.2	142	111
	3000	57	56.2	5.8	151	118
	4000	54.8	54.4	5.4	169	126
Mean effects						
RB	20	54	55	6	164^{a}	123
	40	54	56	6	155^{b}	119
Enzyme	0	52	57	6^{ab}	164	126
	1000	53	58	5^{b}	158	120
	2000	55	57	6^{ab}	154	120
	3000	54	55	6^{a}	156	121
	4000	55	53	6^{ab}	166	119
Significance level						
SEM		1.05	0.8	0.12	1.88	1.22
RB		NS	NS	NS	NS	NS
Phytase		NS	NS	NS	NS	NS
RB × phytase		NS	NS	NS	NS	NS

RB: rice bran.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significant.
SEM: standard error of the means.

Our findings are contradictory to many studies (Arabi, 2013; Olukosi *et al*. 2010; Piragozliev *et al.* 2008; Munir and Maqsood, 2013) who reported increased P availability and bone ash contents in broilers, due to supplemental phytase. Extreme dietary P levels, inorganic undigested P, endogenous losses of P; inbuilt phytase action in the gut (Munir and Maqsood, 2013) may be reasons for the absence of response to phytase within the experimental limits. RB × phytase interaction also not significant in this experiment.

Determination of relative weights of digestive organs

Effects of increasing levels of phytase supplementation relative weights of gizzard, liver, pancreas and small intestines and relative lengths of small intestines of broiler chicken fed either 20 or 40% dietary rice bran are shown in Table 4. Digestive organs parameters did not show any significant influences due to RB or Phytase. However Deniz *et al*. (2007) concluded that there was an increase in relative size of the digestive organs when rice bran was included in the diet.

Gallinger *et al*. (2004) have also reported that the broilers fed a diet with 40% rice bran had higher pancreas and intestine weights than control and suggested the presence of other anti-nutritive factors other than phytate. In this experiment, the weights of pancreases and small intestines showed numerically increased by phytase levels and RB, respectively and the statistical significance (P>0.05) were absent. The other parameters such as weights of liver, gizzard and relative length of small intestine were not significant with RB inclusion, phytase and interaction between RB and phytase.

According to Gallinger *et al.* (2004) the concentrations of rice bran in excess of 20% in the diet produce significant reductions of body weight. Furthermore, feed conversion ratio and tibia ash contents were inferior with diets containing more than 10% of rice bran.

In this experiment, phytase did not show the expected performance of animals. Many studies have failed to achieve positive responses due to phytase and have reported a range of explanations including the differences in breeds and genetics (Edwards *et al*. 1988; Aggrey *et al*. 2002) slaughter age (Edwards *et al*. 1988), feeding operations such as alternative time feeding (Edwards, 2004), particle size of feed (Kasim and Edwards, 2000; Kilburn and Edwards, 2001; Kilburn and Edwards, 2004) and inappropriate mixing (Shirley and Edwards, 2003) unconsidered nutrient levels such as Ca, P and vitamin D (Edwards and Veltman, 1983; Edwards, 2004; Edwards, 1993), fiber (Ballam *et al.* 1984) and dietary Ca and P (Qian *et al*. 1996; Mitchell and Edwards, 1996).

CONCLUSION

Dietary phytate in poultry rations can seriously reduce the performance of birds because of the anti nutritive problems which associated with the utilization of nutrients. In this experiment the growth performance parameters were not enhanced significantly though the mega doses of phytase have increased the crude protein digestibility. The optimum levels of phytase for highest crude protein digestibility were different between two rice bran levels. The 20% rice bran included diet gave its highest crude protein digestibility at 3000 FTU/kg while 40% rice bran included diet gave its maximum crude protein digestibility at 4000 FTU/kg. Negative effects of 40% RB on growth performance, bone status and digestibility indices of P were not mitigated by phytase supplementation. The supplementation of mega doses of phytase found to increase illeal crude protein digestibilities but not the growth performances and bone parameters.

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