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Optimizing the utilization of soybean and benniseed (in poultry feeds) through dietary supplementation with microbial phytase

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ABSTRACT

The effects of microbial phytase (Natuphos® 5000) supplementation of corn-soybean and benniseed based diets were studied with two weeks old broiler birds on the parameters of growth performance, nutrient retention and amino acid digestibility. One hundred and twenty chickens within a weight range of 190 to 210g were allotted in a completely randomised 2 x 3 factorial arrangement to six dietary treatments. Body weight gain (g) 1074.17 ± 12.26, feed intake (g) 1913.17 ± 15.60 and protein efficiency ratio 2.54 ± 0.16 were significantly ($p < 0.05$) increased by phytase supplementation of soybean meal and benniseed diets. Significant ($p < 0.05$) increases were also obtained for apparent retentions of dry matter (DM) 68.68 ± 3.72, nitrogen (N) 56.21 ± 2.83, phosphorus (P) 39.00 ± 2.14 and calcium (Ca) 58.78 ± 3.16 and apparent faecal digestibilities of protein (%) 8.11 ± 56 and amino acids. There were however significant ($P < 0.05$) differences in the magnitude of response by soybean meal and benniseed to phytase supplementation. For soybean meal diets, apparent retentions of N 55.74 ± 2.83 and p 37.88 ± 2.14 and protein digestibility 80.36 ± 1.20 increased rapidly ($p < 0.05$) with 400 FTU/kg phytase but marginally with 800 FTU/kg phytase. On the contrary for benniseed diets, supplementation with 400 FTU/kg phytase increased amino acid digestibility marginally while 800 FTU/kg phytase significantly ($p \leq 0.05$) increased the digestibility of all amino acids.

Keywords: Soybean meal, benniseed, microbial phytase, broiler chickens, nutritional evaluation.

Introduction

Phosphorus (P) and nitrogen (N) are essential nutrients in several metabolic processes, but the excretion of high amounts of either element produces environmental problems in the watercourses.

About two-third of the P contained in the grains of cereals, legumes and oleaginous seeds is present as phytic acid (commonly termed phytate). Phytate-P (pp) is poorly available in non-ruminant animals because they lack the enzyme phytase, which hydrolyses phytate into *myo*-inositol and inorganic phosphate.

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Phytate is also able to bind with proteins at low and neutral pHs [1-2] and inhibits α-amylases, trypsin, tyrosinase and pepsin in the intestinal tract [3-4]. These complexes decrease the activity of digestive enzymes with a subsequent decrease in the digestibility of dietary proteins. Phytic acid, being a strong acid, can also form salts with important minerals such as calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe) and potassium (K), reducing their solubility [5].

The addition of phytase enzyme to feeds has been used to reduce the pollutant residues, through improving the utilization of phytate-bound minerals in pig and poultry diets and decreasing the use of inorganic sources. The amount of phytate degraded by dietary phytase inclusion reduces the need for inorganic P addition by 1 to 1.2 g/kg in practical diets for pigs and poultry [6]. Data indicated that adding phytase increased the apparent digestibility of Ca and P in broilers and reduced the amount of P excreted [7], improved the apparent utilization of dry matter (DM), N and P in turkey poults and increased the retention of P and N in pigs [8].

The purpose of this study is to examine the response of broilers to microbial phytase added to corn-soybean meal and corn-benniseed diets. The efficacy of supplemental phytase was evaluated at three concentrations in terms of its effect on performance, apparent retentions of DM, N, P, PP and Ca and apparent faecal digestibilities of protein and amino acids.

MATERIALS AND METHODS

The study was carried out at the Department of Farm Animal Ethology and Poultry Production, Institute of Animal Husbandry and Animal Genetics, University of Hohenheim, Stuttgart, Germany, in accordance with the Guidelines issued by the German Regulations for Care and Treatment of Animals for Scientific Purposes [9].

Experimental Design

The microbial phytase (E.C.3.1.3.8) used in this experiment was Natuphos® 5000, a commercial enzyme preparation (BASF Corporation, Ludwigshafen, Germany) with a phytase activity of 5000 units/g. One phytase unit (FTU) is defined as that amount of phytase activity, which liberates inorganic phosphorus from 0.5 mM sodium phytate solution, at a rate of 1 µmol/minute at pH 5.5 and 37°C. The experimental design was a completely randomized 2 x 3 factorial arrangement of treatments. The variables were feed ingredients of two plant proteins (soybean meal and benniseed) and phytase additions of 0, 400, and 800 phytase units (FTU) per kg of feed. The basal unsupplemented soybean meal and benniseed diets contained 0 FTU/kg. Diets were formulated to be isocaloric and isonitrogenous based on nutrient values for the soybean meal and benniseed used (Table 1). Starter diets provided 0.70% total P while Ca:total P ratio was maintained at 1.4 : 1 to meet NRC [10] requirements. All feed was in mash form.

Chicks and Management

One day-old broiler chickens (n =150) obtained from a commercial hatchery, were placed in electrically heated battery brooders and offered a commercial starter diet (230 g crude protein/kg) *ad libitum*. At the end of two weeks, 120 of these chicks within a weight range of 190 to 210g were allotted in a completely randomized arrangement to six dietary treatments. Each treatment was sub-divided in four replicates of 5 birds each and housed in separate pens. Room temperature was maintained at 32 ± 1°C during the first

week and gradually decreased to 18°C by the end of the fifth week.

The birds received constant fluorescent illumination and were allowed free access to food in mash form and water. Individual body weight and pen feed intake were recorded. A total collection of excreta was made during the 5th week for three days for the derivation of apparent retentions of DM, N, P, PP and Ca and digestibilities of protein and amino acids. During this period, feed intake was measured and all excreta voided were collected and stored at –20°C. Frozen excreta samples were freeze-dried, weighed, finely ground and pooled for chemical analyses.

Chemical Analysis

The feed ingredients, formulated diets and excreta samples were analyzed to determine DM, crude protein, crude fibre, total fat, ash, sugar, starch, Ca, P and titanium oxide using methods recommended by VDLUFA [11]. Phytate phosphorus was analyzed using the anion exchange chromatographic method [12]. Feed samples were analyzed for phytase by incubation at pH 5.5 and 37°C [13] and phytase activity was calculated as follows: phytase units/kg = (P x 1000)/(W x 60), where P was micromoles of P liberated by phytase in 60 minutes, W was sample weight (g) and 60 was the incubation time (minutes). Amino acid analyses were conducted with a Beckman Multicrom B 4255 amino acid analyzer, after 24 h hydrolysis with 6N hydrochloric acid. All samples were analyzed in duplicate.

Calculations

The apparent faecal digestibility coefficients of crude protein and amino acids and retention values of DM, N, P, PP, and Ca were estimated by using 0.5% titanium oxide at the expense of corn as an indigestible marker. The following equation was used to calcu the digestibility and reta coefficients:

$$\text{Apparent nutrient digestibility} = \frac{(NT/TO)_d - (NT/TO)_e}{(NT/TO)_d}$$

Where $(NT / TO)_d$ = ratio of nutrient titanium oxide in diet, and

$(NT / TO)_e$ = ratio of nutrient a titanium oxide in excreta.(22).

Statistical Analysis

Multivariate Analysis of Varian (MANOVA) was used to analyze th data using the General Linear Modelin Procedure [14]. The sources of err that were analyzed included effects protein source, phytase level and the interactions. Significance level was s at $P \leq 0.05$.

RESULTS

The chemical compositions of soybea meal and benniseed and of th corresponding diets are shown i Tables 2 and 3. The values for crud protein and starch were higher in soybean meal while total fat, ash, non starch polysaccharides and apparen metabolisable energy were higher in benniseed. Soybean meal contained lower concentrations of methionine, methionine + cystine and total amino acids but values for histidine and threonine were higher in soybean meal than benniseed.

The performances of broiler chicks fed the various dietary treatments are shown in Table 4. Body weight gains were significantly ($P \leq 0.05$) increased by soybean meal diets supplemented with 400 and 800 FTU/kg phytase and benniseed diet supplemented with 800 FTU/kg. Significant main effects of protein source ($P = 0.0833$) and phytase

level (P = 0.0563) were observed for soybean meal but protein source x phytase level interaction was not significant. Feed intake followed a similar trend to weight gain except that birds on benniseed diet supplemented with 800 FTU/kg phytase, consumed more feed (P ≤ 0.05) than those on soybean meal diet supplemented with the same units of phytase. The efficiency of feed utilization measured as feed: weight gain ratio, was not influenced by phytase supplementation but soybean meal diets were better utilized as birds on this diet consumed less feed for increased body weight. There were significant (P ≤ 0.05) increases in PER values of soybean meal diet supplemented with 400 FTU/kg phytase and of benniseed diet supplemented with 800 FTU/kg phytase. The differences between 400 FTU/kg and 800 FTU/kg phytase levels were significant (P < 0.05) for benniseed diet but not significant for soybean meal diet.

The results of apparent retentions of DM, N, P, and Ca are shown in Table 5. In the unsupplemented soybean meal and benniseed diets, apparent DM retentions were 67.77 and 65.37% respectively. The addition of phytase to soybean meal and benniseed diets significantly (P ≤ 0.05) increased nutrient retention in both diets but the magnitude of increase was more for benniseed diet supplemented with 800 FTU/kg than soybean meal diet similarly supplemented. Apparent N retention was significantly (P ≤ 0.05) increased by 400 FTU/kg phytase for both diets and there was a significant phytase level effect (P = 0.027) and significant protein source x phytase level interaction (P = 0.061).

A significant phytase effect (P ≤ 0.05) was also observed for P retention with both feed ingredients. The increases obtained for soybean meal diets supplemented with 400 and 800 FTU/kg phytase were 11.58% and 14.87% respectively while for benniseed diets they were 13.47 and 13.53% respectively. The apparent retention of phytate-P was significantly (P ≤ 0.05) lower for unsupplemented soybean meal diets (11.51%) than unsupplemented benniseed diets (16.46%). Supplementation with phytase followed the same trend with P retention except that significant effects of protein source (P = 0.0296) and phytase level (P = 0.0639) were observed while interaction between protein source x phytase level was not significant (Table 5).

The effects of phytase on Ca retention were similar across all treatment levels. For this parameter, supplementation with 400 and 800 FTU/kg phytase significantly (P ≤ 0.05) increased Ca retention in both diets but the magnitude of response was greater for soybean meal than benniseed diets.

The apparent digestibilities of protein shown in Figure 1 and Table 6, were similar for unsupplemented soybean meal and benniseed diets (0 FTU/kg phytase) but improved significantly (P≤ 0.05) with 400 FTU/kg phytase supplementation. Further supplementation with 800 FTU/kg phytase gave additional but nonsignificant increases for both diets (P > 0.05).

Apparent amino acid digestibilities (Table 6) were also significantly (P ≤ 0.05) increased by microbial phytase supplementation but the response for benniseed was poorer and different from soybean meal. Whereas supplementation of benniseed diet with 400 FTU/kg phytase significantly (P≤

0.05) increased the digestibilities of only arginine and leucine, soybean meal diet supplemented with the same units of phytase significantly ($P < 0.05$) increased the digestibilities of threonine (7.7%), valine (7.4%), leucine (6.3%) and basic amino acids; arginine (3.3%) and lysine (3.1%). Supplementation of both diets with 800 FTU/kg phytase significantly ($P < 0.05$) increased the digestibilities of amino acids in both diets, the values ranging from 4.5 to 7.3% for soybean meal and 5.4 to 7.3% for benniseed diets. Branch-chained isoleucine (5.4%) and basic amino acid, lysine (4.5%) gave the least increases in apparent digestibility values for benniseed diets.

DISCUSSION

The chemical composition data in Table 1 underscore the potential value of benniseed as a source of nutrients for farm animals. The crude protein content, though lower than that of soybean meal, compares favorably with full fat soybean [15] and surpasses those reported for *Canavalia, Vicia and Phaseolus* species [16, 17]. The contents of total P, Ca, starch and metabolisable energy were higher in benniseed than soybean meal, a confirmation that benniseed is a feedstuff of high nutritional value. The differences in the chemical compositions reflect differences in the inherent characteristics of the two oilseeds. A striking feature is the difference in the methionine and methionine + cystine contents, which were higher in benniseed than soybean meal. Although soybean is known to have the most complete amino acid profile among plant proteins, it is deficient in methionine [18] and borderline in cystine [19], which makes it necessary to fortify soybean–based diets with synthetic methionine or methionine + cystine to ensure that these amino acids are not limiting. Studies have shown that benniseed, *Sesamum indicum*, has a high potential as a protein replacer, but that the toxicity conferred by heat-stable allelochemicals severely restrict its use in monogastric animal nutrition [20]. The two plant proteins used in this study were processed by heat treatment in order to remove the heat-labile toxic factors before inclusion in the basal diets at rates designed to supply the recommended amounts of N, P and amino acids for starter broiler chickens [10].

The inability of poultry birds to utilize dietary phytate was clearly demonstrated by the significant increases in feed intake and body weight gain of broiler chicks fed corn-soybean and corn-benniseed diets supplemented with microbial phytase. These results, which agreed with some previous reports [7, 8, 21], demonstrate that supplemental phytase is effective for optimum growth in poultry. The improved body weight gains attributable to the supplemental phytase were associated with increased feed intake and also better protein utilisation. The PER values for soybean meal and benniseed diets were significantly increased by phytase supplementation, suggesting an improvement in protein digestibility of feeds and increased availability of amino acids for the birds.

Phytase supplementation resulted in significant improvements in apparent retention of DM, N, P, PP and Ca, which are consistent with the observed increases in the faecal digestibilities of protein (fig. 1) and amino acids (Table 6). Similar improvements have been reported in other studies with broilers for apparent nitrogen retention [22], phosphorus retention [23], phytate phosphorus retention [24], calcium retention [25] and faecal amino acid

digestibility [21]. *In vitro* studies have shown that phytate-protein complexes are insoluble and less subject to attack by proteolytic enzymes than the uncomplexed protein [26]. Owing to its non-specific action, phytate also binds with the major proteolytic enzymes, pepsin [27] and trypsin [4], released into the gut lumen during digestion, eventually leading to lowered digestibilities of N and amino acids. It is therefore likely that when phytase hydrolyses the ester bonds to release P from phytate-P, it will also release the phytate-bound proteins and remove the negative effects of phytate on proteolytic enzymes, thereby increasing the digestion and absorption of protein and amino acids. *In vivo* data generated in lactating sows, lend support to the proposition that phytate inhibits trypsin activity and that this inhibition is partially reversed by supplemental phytase [28]. The improvement in Ca retention was expected because phytase liberates Ca from the Ca – phytate complex and as the availability of P increases, the availability of Ca also increases because both are part of the same complex [6].

The reasons for the significant differences in response to phytase supplementation by soybean meal and benniseed are not clear but two possible modes of action may be proposed to explain the differences between the two plant proteins. First, many plant seeds contain endogenous phytase whose activities may vary for different plants [29]. The digestibility coefficients of plant feed ingredients with higher endogenous enzyme activities tend to be higher than those with lower endogenous activities and addition of phytase to such feedstuffs would therefore be expected to yield a lower absolute response.

Secondly, plants differ generally in their storage sites of phytate and variation in response to supplemental phytase may relate to the respective storage sites of the compound [29]. In maize, phytate is concentrated in the germ [30], in wheat it is in the aleurone layer [29], in peanuts it is concentrated in the crystalloids [31] and in soybean, phytate is associated with the protein bodies [30]. The storage site of phytate in benniseed is not known but may be different from that of soybean. The 'locational' differences may influence the susceptibility of the native phytate to hydrolysis by exogenous phytase, and thus explain some of the variability in the results described in this study.

Overall, the findings of this study emphasize the relevance of phytate–P and phytate – protein complexes in practical poultry nutrition and demonstrate the usefulness of microbial phytase as a tool to lower the excretion of P and N in farm animals. The results show that phytic acid is a potent anti-nutritional factor which can impair the availability of P, Ca, protein and amino acids and eventually depress growth in broilers. It was demonstrated that the adverse effects of dietary phytate could be overcome by supplemental microbial phytase. The beneficial effects of phytase, for the most part, was due to an improvement in the digestibility of phytate – bound nutrients which provided the birds with additional nutrients that were limiting in the unsupplemented diets.

However, phytase effects were inconsistent and varied, although, the level of response was generally more for soybean meal than benniseed diets. Consequently, the benefits of phytase supplementation of diets containing feed ingredients of varying plant origin, may not be entirely predictable but may depend on various factors such as the

type of feed ingredient, the dietary content of phytate, storage sites of phytate and endogenous phytase activities in the ingredients.

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Table 1: Composition of Experimental Diets, g/kg (as fed basis)[1]

	Soybean			Benniseed		
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn (Hohenheim)	538.00	538.00	538.00	515.00	515.00	515.00
Soybean meal (48% protein)	346.00	346.00	346.00	278.70	278.70	278.00
Benniseed	-	-	-	161.00	161.00	161.00
Soyaoil	69.00	69.00	69.00	4.00	4.00	4.00
Limestone	10.00	10.00	10.00	8.80	8.80	8.80
Dicalcium phosphate	16.00	16.00	16.00	12.00	12.00	12.00
Choline Chloride	2.00	2.00	2.00	2.00	2.00	2.00
Sodium bicarbonate	5.20	5.20	5.20	5.20	5.20	5.20
L-Lysine-HCl	5.00	5.00	5.00	5.50	5.50	5.50
Methionine	1.00	1.00	1.00	0.30	0.30	0.30
Vitamin-Premix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Trace element premix[3]	0.80	0.80	0.80	0.80	0.80	0.80
Phytase (PU)[4]	-	400	800	-	400	800
Titanium oxide (TiO_2)	5.00	5.00	5.00	5.00	5.00	5.00
Calculated Composition[5]						
ME, (MJ/kg)	13.53	13.50	13.52	13.57	13.57	13.59
Crude Protein	234.00	230.00	235.00	238.00	240.00	236.00
Calcium	10.00	12.00	10.00	11.00	10.00	10.00
Total Phosphorus (P)	07.10	07.10	07.30	07.70	07.50	07.50
Calcium: Total P ratio	1.42	1.69	1.36	1.42	13.30	1.33
Starch	367.90	367.00	367.20	347.60	348.00	347.00
Sugar	47.10	47.00	47.20	39.20	39.00	39.00
Lysine	15.70	15.70	15.60	14.70	14.50	14.50
Methionine	04.00	04.20	04.10	04.30	04.20	04.30
Analysed Composition						
Dry Matter	902.70	900.00	898.50	911.20	895.60	902.50
Crude Protein	237.10	236.20	237.20	240.90	235.90	239.30
Crude Fibre	35.10	34.20	35.90	34.60	39.40	38.00
Phytic acid	106.60	106.00	106.50	117.00	115.20	121.00
Phytate P.	02.70	02.70	02.70	03.20	03.10	03.40
Total P.	07.20	07.40	07.00	07.50	07.20	07.10
Nonphytate	04.50	04.70	04.30	04.30	04.10	03.70
Calcium	11.20	12.00	12.00	11.20	12.00	12.10
Calcium: Total P ratio	1.56	1.62	1.71	1.76	1.66	1.70
Gross Energy (MJ/kg)	17.50	17.70	17.55	18.58	18.57	18.57

[1] Calculated to meet or exceed the requirements of broiler starter diet (NRC, 1994)

[2] Vitamin mixture provided the following per kilogram of diet: vitamin A(retiny1 acetate), 8,800 IU; cholecalciferol, 2,200 IU; DL-α-tocophery1 acetate, 11 IU; meanadione sodium bisulfite, 2.2mg; riboflavin, 4.4 mg; D-calcium pantothenate, 8.8 mg; nicotinic acid, 44 mg; pyridoxine hydrochloride, 2.2 mg; folic acid, 0.55 mg; d-biotin, 0.11 mg; thiamine hydrochloride, 2.5 mg; vitamin B_{12}, 6.6 µg; chlorine, 220 mg; and ethoxyquin, 125 mg.

[3] Mineral mixture provided the following per kilogram of diet: Mn, 60 mg; Zn, 50 mg; Fe, 30 mg; Cu, 5 mg; I, 1.06 mg; and Se, 0.1 mg.

[4] One unit of phytase (PU) is defined as the quantity of enzyme that releases 1µmol of inorganic P/min from 0.00015 mol/L sodium phytate at PH 5:5 at 37°C.

[5] Calculated composition based on values determined for individual ingredients.

Table 2: Composition of Soybean and Benniseed (g/kg)[1]

Ingredient	Soybean meal	Benniseed
Dry matter	915.0	960.0
Crude protein (N x 6.25)	483.0	380.0
Total non-starch polysaccharides	43.8	49.0
Ether extract	38.6	212.9
Crude ash	48.9	56.7
Total Phosphorus (P)	7.4	8.0
Phytate P.	4.2	5.4
Nonphytate P.	3.7	3.0
Calcium	8.6	10.8
Sugar	6.4	4.5
Starch	10.3	9.7
Apparent metabolizable energy (MJ/kg)	14.5	17.8
Lysine	27.8	27.4
Methionine	6.5	7.9
Methionine + Cystine	11.7	13.4
Histidine	13.8	12.6
Threonine	19.1	11.5
Total amino acids	143.4	152.8

[1]All except apparent metabolisable energy are determined values.

Table 3: Crude Protein and Amino Acid Concentrations in Experimental Diets, g/kg (air dry basis)

	Soybean			Benniseed		
Ingredients	0	400	800	0	400	800
Dry matter	902.70	900.00	898.52	911.20	895.60	902.56
Crude protein (N x 6.25)	237.12	236.25	237.20	240.90	235.92	239.36
Essential amino acids	8.50	8.74	8.51	8.28	8.42	8.51
	9.90	10.47	10.26	9.66	10.12	10.35
Threonine	4.00	4.03	4.14	4.00	3.91	3.80
Valine	9.00	9.55	9.43	8.74	9.00	9.00
Methionine	19.80	20.35	20.15	19.23	19.32	19.55
Isoleucine	11.60	11.96	11.50	11.39	11.50	11.50
Leucine	5.80	5.98	5.75	5.75	5.75	5.75
Phenylalanine	15.20	15.53	15.40	14.26	14.72	14.84
Histidine	14.50	15.00	14.49	16.56	16.91	16.79
Lysine	3.70	3.82	3.68	4.00	3.91	3.91
Arginine						
Cystine						
Non-essential amino acids						
Aspartic acid	3.22	3.28	3.30	5.20	5.10	5.20
Serine	2.68	2.66	2.70	2.88	2.90	2.90
Glutamic acid	28.44	28.40	28.42	35.60	34.70	35.00
Alanine	2.82	2.85	2.81	2.77	2.80	2.81
Tyrosine	4.20	4.22	4.19	5.16	5.12	5.11

Table 4: The Effect of Microbial Phytase Supplementation on Performance Parameters of Broiler Chicks Fed Corn-Soybean/Benniseed Diets[1]

TREATMENT		MEASUREMENTS[3]				
Ingredient	Microbial phytase (PU/kg diet)[2]	Total body weight gain (g)	Total feed intake (g)	Feed/gain (g/g)	Gain/feed (g/g)	PER[4]
Soybean	0	1004.67	1903.83	1.90	0.53	2.54[b]
	400	1050.83	1876.17	1.79	0.56	2.77[a]
	800	1074.17	1913.17	1.79	0.56	2.81[a]
	SEM[5]	12.26	15.60	0.05	0.004	0.16
Benniseed	0	1078.67	1995.00[b]	1.85	0.54	2.63
	400	1092.33	2029.83[b]	1.86	0.54	2.67
	800	1103.00	2137.44[a]	1.93	0.52	2.77
	SEM[5]	16.00	22.10	0.08	0.04	0.09
		Probabilities				
Source of variation						
Protein source effect		0.1833	0.1155	0.1336	0.1674	0.0171[*]
Phytase level effect		0.5563	0.5607	0.4699	0.5092	0.5118
Protein source x phytase level		0.8648	0.6951	0.1202	0.622	0.3122

[1]Data represent means of six dietary treatments of 20 broiler chicks during the period 14 – 35d posthatching

[2]PU, Phytase Unit/kg diet

[3]Values within a classification in the same column followed by different letters are significantly different ($P \leq 0.05$)

[4]Protein efficiency ratio: g weight gain/g crude protein intake

[5]Standard error of mean

[*] $P \leq 0.05$

Table 5: Apparent retentions of Dry Matter (DM), Nitrogen (N), Total Phosphorus (P), Phytate Phosphorus (PP) and Calcium (Ca) in Broilers Fed Soybean/ Benniseed-Corn Based Diets with Phytase Supplementation[1]

TREATMENT		MEASUREMENTS				
Protein source	Microbial phytase (PU/kg diet)[2]	DM retention	N retention	P retention	PP retention	Ca retention
Soybean	0	67.77	52.33[c]	33.95[c]	11.51[c]	43.54[c]
	400	68.69	55.74[a]	37.88[a]	13.61[c]	50.26[b]
	800	68.68	56.21[a]	39.00[a]	18.72[b]	58.78[a]
	SEM	3.72	2.83	2.14	2.25	3.16
Benniseed	0	65.37	51.88[c]	34.14[c]	16.46[b]	47.70[c]
	400	66.92	56.81[a]	38.74[a]	21.52[b]	50.13[b]
	800	67.34	57.88[a]	38.76[a]	34.54[a]	53.71[a]
	SEM	3.60	3.11	2.00	3.81	4.45
Main effects						
Soybean		68.38	54.76[a]	37.06[b]	14.06[c]	50.86
Benniseed		66.70	55.54[a]	37.54[a]	22.97[b]	50.87
Phytase effect (Units/kg)						
	0	67.07	53.11[c]	34.35[c]	13.22[c]	48.33[b]
	400	67.31	54.27[b]	38.06[a]	17.57[c]	49.55[b]
	800	68.34	57.10[a]	39.42[a]	22.75[b]	54.48[a]
		Probabilities				
Source of variation						
Protein source effect		0.2119[ns]	0.7593	0.4862	0.0296[*]	0.0774[+]
Phytase level effect		0.2149	0.0276[*]	0.5205	0.0639[+]	0.0180[*]
Protein source x Phytase level		0.1217	0.0614	0.0205[*]	0.7951	0.0133[*]

[1]Data represent means of six dietary treatments of 20 chicks each during the period 14 – 35d post hatching

[2]PU, Phytase Unit/kg diet

[abc]Means within columns with no superscripts differ significantly ($P < 0.05$)

Table 6: Apparent Total Tract Digestibility Coefficients (%) of Protein and Amino acids in Soybean and Benniseed Diets for Broiler Chickens Supplemented with Microbial Phytase

MAIN EFFECT[1]									
Protein Source	SOYBEAN[3]			BENNISEED[3]			Probabilities		Phytase
Amino Acids / Level (PU)[2]	0	400	800	0	400	800	Protein source effect	Phytase level effect	Protein source x phytase interaction
Protein	75.85±1.60[c]	80.36±1.20[b]	82.11±0.56[a]	72.16±1.44[d]	79.63±0.92[bc]	82.80±1.12[a]	0.2716	0.0134	0.2716
Isoleucine	82.85±1.78[b]	84.65±1.55[a]	87.20±1.25[a]	78.96±1.50[c]	84.22±1.25[a]	86.54±1.36[a]	0.1045	0.0110	0.4945
Luecine	82.00±1.15[b]	84.56±1.49[a]	87.09±1.25[a]	77.77±1.16[c]	84.49±0.98[a]	88.95±0.86[a]	0.0065	0.0188	0.0021
Phenylanine	84.17±1.32[b]	86.50±0.46[a]	89.05±0.39[a]	79.01±1.31[c]	85.90±1.32[a]	87.86±0.68[a]	0.0029	0.0210	0.1897
Histidine	83.22±1.20[b]	85.63±1.28[a]	88.69±0.89[a]	79.55±2.46[c]	86.00±1.53[a]	87.37±1.26[a]	0.4161	0.0112	0.1962
Tyrosine	83.55±1.11[b]	85.67±1.51[a]	88.72±1.27[a]	80.05±1.87[c]	86.64±2.61[a]	87.71±1.00[a]	0.2707	0.0214	0.3080
Arginine	82.74±1.35[b]	85.76±1.28[a]	88.18±0.75[a]	79.83±2.35[c]	85.07±1.37[a]	87.71±1.21[a]	0.4972	0.0021	0.4280
Threonine	82.52±0.58[b]	84.61±0.72[a]	88.29±0.62[a]	79.99±2.10[b]	82.05±1.36[b]	87.58±0.89[a]	0.4743	0.0133	0.4140
Valine	82.26±0.83[b]	84.40±1.05[a]	88.24±0.77[a]	80.81±2.43[c]	84.82±1.97[ab]	88.16±1.15[a]	0.5697	0.0220	0.8994

[1]Treatment means ± standard error
[2]PU. Phytase Unit/kg diet
[3]Means within row for each main effect separately lacking a common superscript letter differ (P ≤ 0.05)

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