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SUPPLEMENTATION OF DIET WITH MICROBIAL AND THERMO-STABLE 6-PHYTASE WITH RECOMMENDED AND DEFICIENT Ca AND P LEVEL ON BROILER PERFORMANCE

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ABSTRACT

An experiment was carried out to investigate the performances with 1080 male Cobb-500 broiler chicks using different levels of a microbial phytase in plant-based diet. Six dietary treatments were formulated as follows; the first 3 diets were balanced with Ca and Av. P according to the recommendation of Cobb-500 served as basal diet without phytase enzyme (T_1), basal diet with 100g phytase ton^{-1} feed (T_2) and basal diet with 200g phytase ton^{-1} feed (T_3), while the last 3 diets were deficient in Ca and Av. P and containing no phytase enzyme (T_4), diet with 100g phytase/ton feed (T_5) and diet with 200g phytase ton^{-1} feed (T_6). All 6 treatments were replicated 6 times. Body weight, feed consumption and mortality of broilers were recorded weekly. The highest body weight was found in T_3 with 200g phytase ton^{-1} feed with recommended Ca and Av. P level in the diet, followed by T_6 with 200g phytase/ton feed with deficient in Ca and Av. P level in the diet ($P<0.05$). The lowest weight was found in T_1 without phytase with recommended Ca and Av. P level in the diet. The feed consumption, feed conversion ratio and mortality of the birds during 35 days of experimental period did not differ significantly ($P<0.05$). The meat yield characteristics at 35 days of age were non-significant among different treatments ($P>0.05$). Significantly lower value of total ash (%), Ca (%) and total P (%) of tibia was found in control group (T_1) and T_4. The results of this experiment revealed that phytase supplementation at 200g ton^{-1} of feed improved broiler performances at either recommended or deficient level of Ca and Av. P.

Keywords: Broilers, Growth performance, Meat yield and Microbial Phytase

INTRODUCTION

The feed ingredients from plant origin used in poultry diet have some limitation that is the presence of anti-nutritional factor, phytic acid or phytate (Farrell, 1998), limits its use in poultry diet. Maize and soybean meal are the major feed sources in poultry

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diets. Again, rice polish, an important by-product of the rice milling industry, has the potential to be used as an alternative to grains in the poultry diet (Mujahid et al., 2003). More than 60% of P in maize and soybean meal (Reddy et al., 1982) and 90% in rice polish (Lamid et al., 2014) are in the form of phytate. Phytate not only does reduce P availability, but also decreases the absorption of elements such as zinc, iron, calcium and magnesium (Lamid et al., 2014). In addition, it decreases protein digestibility and energy use through inhibition of the enzymes such as pepsin, trypsin and α-amylase (Ravindran et al., 1995). Moreover, as the phytate P has low P availability (NRC, 1994), leads to use of inorganic P sources to meet the P requirement of poultry.

The inclusion of feed enzymes in poultry diets enhances nutrient utilization and performance by counteracting the negative influence of targeted substrates. A number of studies have shown that inclusion of microbial phytase in broiler diet releases the phytate bound P and to improve the utilization of P in plant derived ingredients including energy and amino acids (Selle et al., 2000). Phytase increased tibia ash in duck ration containing 20% rice bran (Farrell and Martin 1998). Leske and Coon (1999) reported that phytate P retention is dependent on feed ingredients and phytase efficacy is influenced by type of feed ingredient used in diets, phytase addition in defatted rice bran increased both phytate phosphorus hydrolysis and total P retention. Martin et al. (1998) reported improved amino acid digestibility when phytase was added to vegetable protein diets. Phytase are present in most cereals, but their activity varies widely amongst cereals (Bartnik and Szafranska, 1987). Farrell et al. (1993) reported significant beneficial responses when a microbial Phytase is added to chicken and duckling diets.

Recent advancement in enzyme technology has led to the development of new phytase formula, which is dust-free concentrated micro-granulated form developed for premix systems and thermo-stable exceptionally suitable to use in pellet system. Moreover, this type of microbial phytase greatly increases the amount of plant phosphorus available to the animal. It reduces both environmental impact and feed costs by dint of increasing live weight gain. In this experiment, a novel microbial 6-generation phytase will be evaluated by feeding diets containing either recommended or deficient level of dietary calcium and available phosphorus (available phosphorus; Av.P) in terms of growth performance and meat yield characteristics.

MATERIALS AND METHODS

Statement of the Experiment

The experiment was conducted at Nourish Research and Development Farm, Uzilapur, Gazipur to study the effect of microbial and thermo-stable 6-phytase (Ronozyme HiPhos) supplementation in broiler diet on performances and dressing parameters. A total of 1080 one day-old Cobb-500 commercial male broiler chicks were collected from the Nourish Poultry and Hatcheries Ltd. to carry out this research for a period of 35 days (from 10th April to 16th May, 2016).

Preparation of the experimental house

Broiler was reared in a gable type open sided house with a room of 2840 sq ft. The room was partitioned into 36 pens of equal size using wire net. Area of each pen was 49 sq ft. (7x7). Feeders, drinkers, buckets and all other necessary equipments were also properly washed and disinfected by 0.5% TH^{4+} solution. Rice husk was used as litter material.

Layout of the experiment

The experimental broiler chicks were equally and randomly divided and distributed into six dietary groups and each group was replicated into 6 sub-groups. Each dietary group consists of 180 chicks distributed into 6 replicated pens having 30 chicks in each replication.

Formulation of broiler diet

Broiler diets were formulated for two phases (starter and grower). Ingredient composition of broiler starter and grower diet were mainly corn, soybean meal, full fat soybean, meat and bone meal, soybean oil, mono-dicalcium phosphate, limestone etc. All diets were isocaloric and isonitrogenous. Feed was in pellet form and the nutrient requirements of Cobb-500 broiler strain were satisfied (according to the recommendation of Cobb-500 strain producing company). Ca and Available P (av. P) content of starter and grower diet as dietary treatment is shown in Table 1. Diets for each treatment was prepared separately and distributed into 6 treatments and kept in separate plastic containers for each replication.

Table 1. Ca and Available P content of starter and grower diet

Ingredients	Dietary Treatment					
	T1	T2	T3	T4	T5	T6
	Starter					
Ca	0.90	0.90	0.90	0.72	0.72	0.67
av. P	0.45	0.45	0.45	0.30	0.30	0.27
Phytase (gm ton^{-1})	0	100	200	0	100	200
	Grower					
Ca	0.80	0.80	0.80	0.62	0.62	0.57
av. P	0.36	0.36	0.36	0.21	0.21	0.18
Phytase (gm ton^{-1})	0	100	200	0	100	200

Management of experimental birds

The management practices were identical for all dietary groups. In all cases, *ad-libitum* feeds were offered to the broilers. Fresh and clean water was made available at all times.

Fresh and dried rice husk was used as litter material and spread over the floor at a depth of about 3cm. The chicks were provided with a temperature of 35°C at first week of age, decreasing gradually at the rate of 3°C per week continued up to 4 weeks of age. The birds were exposed to a continuous lighting period of 23 hours and a dark period of 1 hour in each 24 hours. Infectious bronchitis and New Castle disease vaccine were given at 3rd day and again booster dose was given at 25th day. Infectious Bursal disease vaccine was given at 14th day and again at 23rd day. A strict bio-security program was maintained and all birds were immunized regularly.

Processing of broilers

At the end of the trial, 3 birds were taken from each pen through random selection for recording dressing parameters and for total Ash, Ca and total P deposition in Tibia. Birds were slaughtered and allowed to bleed for 2 minutes and immersed in hot water (51-55°C) for 120 seconds in order to lose the feathers and this procedure was called semi-scalding, followed by removal of feathers by hand pinning. Then head, shank, viscera, giblet (heart, liver and gizzard) and abdominal fat were removed.

Data recording and statistical analysis

The following parameters were recorded during 35 days experimental period replication wise for each diet; weekly body weight, feed intake and feed conversion ratio, mortality when occurred, and meat yield parameters at the end of the experiment. All recorded and calculated data were statistically analyzed using analysis of variance technique by a computer using SAS statistical package program in accordance with the principles of Completely Randomized Design (SAS, 2009). DMRT was done to compare variations among treatments where ANOVA showed significant differences.

RESULTS AND DISCUSSION

Weekly body weight and weight gain

In respect to initial body weight, there was no significant difference (P>0.05) among the dietary groups. At the end of 35 days of age, the highest live weight (1798.79g/b) was found in broilers fed diet supplemented with phytase enzyme at the level of 200g ton^{-1} and fortified with Ca and Available P according to the recommendation of Cobb-500 (T_3). This was followed by broiler belonging to phytase supplementation at the level of 200g/ton (1760.52g/b) with deficient in Ca and Available P requirement (T_6), phytase supplementation at the level of 100g ton^{-1} (1718.72g/b) with recommended Ca and Available P level (T_3), phytase supplementation at the level of 100g/ton (1659.85g/b) with deficient in Ca and Available P level, and no phytase supplementation (1640.79g/b, T_4 and 1585.60g/bird, T_1). Significantly highest weight gain was found in T_3 and lowest in T_1 (Figure 1). The first 3 diets T_1, T_2 and T_3 were fortified with Ca and Available P according to the recommendation of Cobb-500, while the last 3 diets T_4, T_5 and T_6 were deficient in Ca and Available P level.

However, broilers receiving phytase supplemented feed had significantly higher weight than that of control ($P<0.05$) although their Ca and Available P level was fulfilled or not according to the requirement of Cobb-500.

Lelis et al. (2012) found diet supplemented with phytase increases growth performance of broiler. Singh et al. (2003) showed that phytase supplemented diets improved growth performance, relative retention of nutrients (N, Ca and P) and minerals (Ca and P) status in blood and bone in broiler chickens with a better efficacy in maize-based diets. Phytase is an enzyme that acts in the bonds of the phosphate group of phytate, releasing

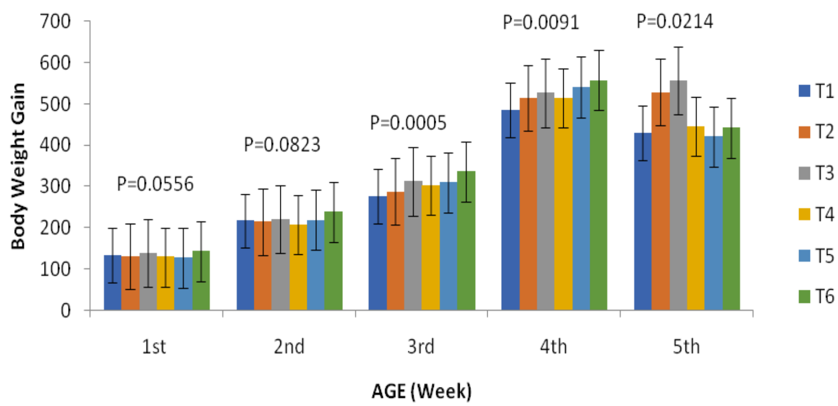

Figure 1. Pattern of weekly body weight gain of broilers fed diet supplemented with microbial and thermo-stable 6-phytase

P and other minerals that are part of this molecule (Cromwell and Coffey, 1991). This way, besides increasing P availability, the use of phytase also improves the availability of other minerals, such as magnesium, manganese and copper. Thus, its use in diets for broilers may provide positive responses on the digestibility of feeds and broiler performance, having a direct effect on productive efficiency. However, the worst result of control group (T_1) having Ca and Available P according to the need may be due to without supplementation of Phytase. A number of studies have shown that inclusion of microbial phytase in broiler diet releases the phytate bound P and to improve the utilization of P in plant derived ingredients including energy and amino acids (Selle et al., 2000).

Exogenous phytase is included in feed formulations not only to reduce phosphorus supplementation, but also to release minerals, particularly calcium, as well as amino acids and carbohydrates by the hydrolysis of phytate, improving nutrient utilization (Slominski, 2011). However, the calcium content of control may affect the growth performances. Dietary calcium content may negatively influence phosphorus utilization, particularly phytic phosphorus, due to the formation of insoluble complexes with calcium in the digestive tract, counteracting the effect of phytase, which is not able to hydrolyze such complexes (Leeson et al., 1996). Nelson et al. (1964) reported that dietary calcium levels higher than 0.70% at pH 6.0 promote the reaction of calcium with phytic acid, resulting in calcium phytate, which precipitates and cannot be broken down by phytase, consequently reducing phosphorus bioavailability. High dietary calcium levels change the pH of the upper sections of the digestive tract, inactivating phytase. Phytase activity is optimal at pH between 5.0 and 6.5, and it is reduced when pH is lower or equal to 3.0 (Casey and Walsh, 2004).

On the other hand, the better result of T_6 with phytase supplementation of 200g/ton and deficient in Ca and Available P level than control may be due to low inorganic calcium and phosphorus levels. Because, broilers fed diets with low inorganic calcium and phosphorus levels have higher capacity to hydrolyze phytate than those fed high levels of these minerals (Denbow et al., 1995). Therefore, minimal calcium and phosphorus levels need to be maintained in the diet; however, their interaction should not be overlooked, particularly considering phytase activity (Qian et al., 1997). Protein utilization and amino acid absorption may also be influenced by dietary phytic acid levels (Walk et al., 2012). These authors observed that phytic acid is a potent chelating agent. Its negative charges react with the positive charges of some amino acids (lysine, arginine, histidine), some proteins (including those involved in protein digestion, such as pepsin and trypsin), and carbohydrates (α-amylase), forming insoluble complexes, thereby reducing their availability and digestibility (Maenz, 2001) and consequently affecting metabolism and organ biometry. The better performance of broilers fed reduced Ca and P levels and phytase obtained in the present study suggest that phytase promoted better Ca and P utilization which is supported by the findings of Sousa et al. (2014). This is consistent with the results of Powell et al. (2011), who found that phytase is used more efficiently when dietary Ca levels are deficient.

Feed consumption and feed conversion efficiency

Total feed consumption did not significantly differ (P>0.05) among the treatments during 35 days experimental period, although higher value of feed consumption was found in the treatments where phytase supplemented feed at the level of 200g/ton was provided (Figure 2). Santos et al. (2013) found feed consumption did not differ significantly with phytase supplementation by the lower mineral diets during 21 days experimental period. Abeyrathna et al. (2014) stated that phytase supplementation did not affect feed consumption and FCR of Laying Japanese Quail. All these results agreed our results during 35 days experimental period in this experiment. Richtar et al. (1991) conducted an experiment on Ross broilers and reported that average feed intake across all P contents was 2447, 2531, and 2490 g for phytase 0, 500 and 100unit/kg respectively. However, improved feed intake was found by some researcher while using phytase enzyme in poultry diet (Naher, 2002). Naher (2002) carried out an experiment on utilization of rice polish-based diet with supplementation of phytase and carbohydrase in ducks. She showed that addition of mixed enzyme increased feed intake of ducks.

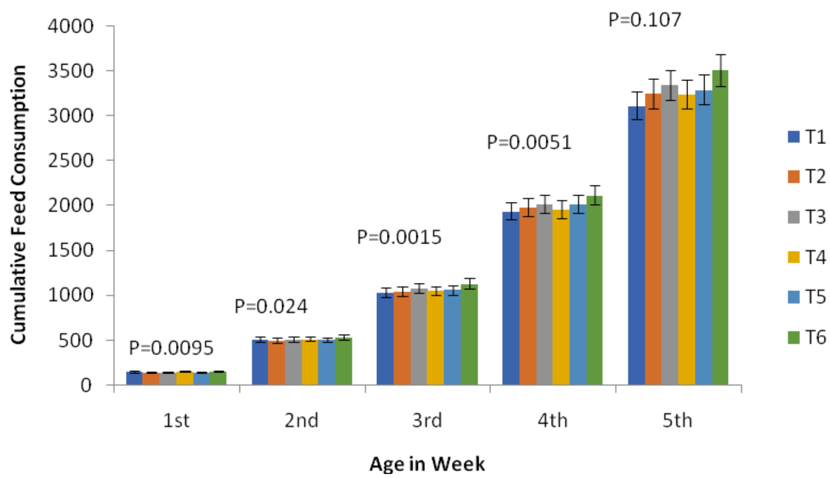

Figure 2. Pattern of cumulative feed consumption of broilers fed diet supplemented with microbial and thermo-stable 6-phytase

In respect to FCR at 1st week, there was significant difference ($P<0.05$) among the dietary groups (Table 2). FCR was better in T_3 and T_6 treatments (Phytase supplementation at 200g ton^{-1}) and worst was found in T_4 (No phytase supplementation with deficient in Ca and Available P). No significant differences were found at 2nd, 3rd, 4th and 5th week in FCR among the dietary treatments. During the whole experimental period, FCR did not show significant differences among the dietary treatment ($P>0.05$). Santos et al. (2013) stated that the use of high doses of phytase may allow significantly improved FCR in broilers. Abeyrathna et al. (2014) stated that phytase supplementation did not affect feed consumption and FCR of Laying Japanese Quail. However, many researchers reported that addition of phytase and carbohydrase on parboiled rice polish-based diet promoted feed conversion (Moshad et al., 2003; Naher, 2002; Rahman et al., 2009).

Table 2. Weekly feed conversion ratio of broilers fed diet supplemented with microbial and thermo-stable 6-phytase

Age (wks)	Dietary Treatment						Level of Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
1st Wk	1.13±0.01[abc]	1.09±0.03[bc]	1.05±0.02[c]	1.18±0.04[a]	1.16±0.03[ab]	1.08±0.01[c]	0.0077
2nd Wk	1.66±0.04	1.68±0.05	1.67±0.06	1.77±0.06	1.66±0.03	1.61±0.03	0.3474
3rd Wk	1.91±0.08	1.89±0.04	1.82±0.04	1.77±0.04	1.80±0.06	1.77±0.04	0.2581
4th Wk	1.88±0.06	1.83±0.05	1.79±0.02	1.77±0.04	1.77±0.03	1.77±0.03	0.3687
5th Wk	2.85±0.26	2.43±0.14	2.36±0.11	2.95±0.27	3.12±0.37	3.21±0.29	0.1135
FCR of total period	2.03±0.04	1.94±0.05	1.90±0.05	2.04±0.06	2.05±0.10	2.05±0.07	0.4432

Where T_1 = Phytase 0g/ton + Recommended Ca & Av. P, T_2 = Phytase 100g/ton + Recommended Ca & Av. P

T_3 = Phytase 200g/ton + Recommended Ca & Av. P, T_4 = Phytase 0g/ton + Deficient Ca & Av. P

T_5 = Phytase 100g/ton + Deficient Ca & Av. P, T_6 = Phytase 200g/ton + Deficient Ca & Av. P

Performance of broiler during whole experimental period

Table 3 represents the performance of broiler during whole experiment. In case of final body weight and body weight gain there was significant difference among the treatments (P<0.05). Higher body weight and body weight gain observed in a group where phytase supplementation was at highest level and lower in lowest level supplementation of phytase followed by control group. In case of initial body weight, total feed consumption, FCR for total period, mortality and survivability there were no significant differences among the dietary treatments (P>0.05).

Table 3. Performance of broilers during whole experimental period fed diet supplemented with microbial and thermo-stable 6-phytase

Age (wks)	Dietary Treatment						Level of Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Initial wt	43.9±1.08	43.0±0.29	42.6±0.68	42.7±0.55	43.4±0.47	42.4±0.70	0.62
Final weight	1585.6±48.6[c]	1718.7±46.7[abc]	1798.8±44.4[a]	1640.8±44.3[bc]	1659.9±42.1[bc]	1760.5±29.2[ab]	0.0143
Final wt gain	1541.7±48.9[c]	1675.7±46.6[abc]	1756.2±43.0[a]	1598.1±44.6[bc]	1616.4±42.3[bc]	1718.1±29.5[ab]	0.0138
Total feed Consumption	3114.4±52.83	3247.1±77.47	3344.2±145.8	3244.4±83.5	3290.9±74.79	3510.0±94.65	0.1070
FCR of total period	2.03±0.04	1.94±0.05	1.90±0.05	2.04±0.06	2.05±0.10	2.05±0.07	0.4432
Survivability	93.89±1.81	92.22±2.94	92.78±3.38	90.56±2.34	92.78±2.64	91.11±2.22	0.9520

Where T_1 = Phytase 0g/ton + Recommended Ca & Av. P, T_2 = Phytase 100g/ton + Recommended Ca & Av. P

T_3 = Phytase 200g/ton + Recommended Ca & Av. P, T_4 = Phytase 0g/ton + Deficient Ca & Av. P

T_5 = Phytase 100g/ton + Deficient Ca & Av. P, T_6 = Phytase 200g/ton + Deficient Ca & Av. P

Dressing parameters

Table 4 represents the dressing parameters of broiler among the dietary treatments during the experimental period. There was no significant difference in live weight, blood weight, feather and skin weight, carcass yield and visceral organ weight among the dietary treatments (P>0.05). Atapattu and Gamage (2007) stated that carcass parameters such as the weight of the pancreas, liver, digestive tract and gizzard of broiler were not affected by diet containing high levels of rice bran with microbial phytase. Moshad et al. (2003) found that total meat yield and breast meat significantly improved due to dietary supplementation of phytase and carbohydrase enzyme on parboiled rice polish-based diet. He also noted that there was no significant difference among giblet yield attributable to enzyme supplementation. Naher (2002) noted that dressing yield was significantly increased by enzyme supplementation in parboiled rice polish-based diet in meat type duck. Total meat and breast meat also significantly increased. Rahman et al. (2009) observed that addition of phytase and carbohydrase enzyme promoted meat yield characteristics at all parboiled rice polish levels.

Table 4. Dressing parameters of broilers fed diet supplemented with microbial and thermo-stable 6-phytase

Age (wks)	Dietary Treatment						Level of Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Blood weight (% of live weight)	4.03±0.43	4.31±0.47	3.74±0.22	3.84±0.63	4.22±0.25	5.09±0.37	0.2867
Feather & skin weight (% of live weight)	12.33±0.44	12.02±0.16	12.54±0.30	12.02±0.35	12.00±0.71	11.23±0.07	0.3203
Carcass yield (% of live weight)	66.30±1.02	66.54±0.92	67.29±0.82	67.71±1.05	67.08±2.25	69.28±0.64	0.5986
Visceral organ weight (% of live weight)	17.35±0.92	17.12±0.91	16.43±1.29	16.44±1.19	16.71±1.40	14.40±0.53	0.4750

Where T_1 = Phytase 0g/ton + Recommended Ca & Av. P, T_2 = Phytase 100g/ton + Recommended Ca & Av. P

T_3 = Phytase 200g/ton + Recommended Ca & Av. P, T_4 = Phytase 0g/ton + Deficient Ca & Av. P

T_5 = Phytase 100g/ton + Deficient Ca & Av. P, T_6 = Phytase 200g/ton + Deficient Ca & Av. P

Tibia content

It is shown from Table 5 that the significantly lower value of total ash (%), Ca (%) and total P (%) of tibia was found in control group (T_1, without phytase with recommended level of Ca and Av. P) and T_4 (Phytase 0 g ton^{-1} + Deficient Ca & Av. P). The results agreed with the results of Ceylan et al. (2012) and found that tibia P%

was significantly lower when diet was formulated with deficit level P without phytase.

Table 5. Total Ash (%), Ca (%) and total P (%) of tibia of broilers supplemented with microbial phytase

Treatment	Total Ash (%)	Ca (%)	Total P (%)
T_1	$46.54^b \pm 0.76$	$16.69^b \pm 0.21$	$7.75^b \pm 0.13$
T_2	$51.39^a \pm 0.47$	$18.35^a \pm 0.23$	$9.80^a \pm 0.12$
T_3	$52.48^a \pm 1.18$	$18.44^a \pm 0.32$	$9.49^a \pm 0.26$
T_4	$46.65^b \pm 0.56$	$16.84^b \pm 0.20$	$7.83^b \pm 0.53$
T_5	$50.62^a \pm 0.99$	$18.07^a \pm 0.51$	$9.32^a \pm 0.33$
T_6	$50.84^a \pm 1.15$	$18.32^a \pm 0.43$	$9.17^a \pm 0.55$
Level of Sig.	0.008	0.007	0.026

Where T_1 = Phytase 0g/ton + Recommended Ca & Av. P, T_2 = Phytase 100g/ton + Recommended Ca & Av. P

T_3 = Phytase 200g/ton + Recommended Ca & Av. P, T_4 = Phytase 0g/ton + Deficient Ca & Av. P

T_5 = Phytase 100g/ton + Deficient Ca & Av. P, T_6 = Phytase 200g/ton + Deficient Ca & Av. P

CONCLUSION

The study revealed that microbial and thermo-stable 6-phytase supplementation at the rate of 200g/ton of diet improved body weight gain of broilers at either recommended or deficient level of Ca and Av. P. On the other hand, microbial phytase supplementation did not affect total feed consumption, total FCR, meat yield characteristics, but significantly improve total Ash (%), Ca (%) and total P (%) of tibia. In conclusion, the present study reports that broiler growth performance may probably be enhanced through microbial and thermo-stable 6-phytase supplementation in their diet.

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