

Full Length Research Paper

The effects of *Saccharomyces cerevisiae* on performance and nutrients digestibility in broilers fed with diet containing different levels of phosphorous

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The effects of yeast culture (YC) with different levels of NPP (non phytate phosphorus) on performance, tibia and blood parameters and ileal digestibility of nutrients were investigated in broilers. A total of 720 one-day-old male broiler (Ross) chicks were divided into 48 groups and fed with 12 diets (4 groups per diet) for 42 days. The experimental design was a randomized complete block with a 3 × 4 factorial arrangement of treatments. The 12 treatments consisted of: 3 levels of NPP (normal, 80 and 60% normal level) and 4 levels of YC (0.0, 0.15, 0.3 and 0.45 % of diet). The results of this study showed that there were significant interactions ($p < 0.05$) between NPP level and the concentration of YC in the diet with body weight gain (BWG) and feed conversion ratio (FCR) during the 42 days of the experiment. In the lower levels of NPP, the improvement of FCR was dose dependent on YC. No NPP dietary × YC combination interactions were found in the other measured traits. The lower levels of NPP significantly ($p < 0.05$) decreased ileal digestibility of crude protein (CP) and calcium (Ca). The addition of YC significantly ($p < 0.05$) increased ileal digestibility of both P and Ca. The enhancement of NPP dietary significantly ($p < 0.05$) increased the concentration of P, Ca and ash in the tibia. Only birds fed with the diets containing 0.45% YC significantly had ($p < 0.05$) higher ash percentage than the other groups. Serum Ca and P were significantly ($p < 0.05$) affected by different levels of NPP in the diet. The effect of YC on concentration of serum P and total protein was significant ($p < 0.05$). In conclusion, the increased retention of P, CP and mineral utilization in deficient-NPP diets by YC resulted in increased availability of P and Ca to the broilers, which could have led to improved growth performance.

Key words: Yeast culture, phosphorus, broilers, performance, digestibility.

INTRODUCTION

Dietary requirements of P and its availability in feedstuffs of plant origin are important issues in poultry nutrition. About 60 to 70% of P in plant based ingredient commonly used for poultry diets occur as phytate P (myo-inositol hexaphosphate) which is poorly utilized by poultry (Perney et al., 1993, Sandberg, 2002). This is due to lack of adequate amounts of endogenous phytase in their gastro-intestinal tracts to fully hydrolyze the phytate

molecule (Ravindran et al., 1995; Punna and Roland, 1999). Phytate, in its native state, is also complexed with various cations, protein, lipids (Cosgrove, 1966), and starch (Thompson et al., 1984). Lan et al. (2010) showed that phytase supplementation significantly increased the dephosphorylation of phytate *in vitro* and *in vivo*. It has been shown that phytase supplementation improves growth, concentration of plasma P, tibia ash weight, tibia ash percentage, tibia-breaking strength, tibia phosphate, and retention of P in broilers (Han et al., 2009).

Yeast products are important natural growth promoters. Eckles and Williams (1925) first reported the use of *Saccharomyces cerevisiae* as a growth promoter for

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Table 1. Percentage inclusion and calculated composition of basal diet in starter, grower and finisher period.

Ingredient	0 – 10 days			11 – 28 days			29 – 42 days		
	(different levels of NPP)			(different levels of NPP)			(different levels of NPP)		
Corn	53.19	53.23	53.27	59.28	59.31	59.35	64.00	64.03	64.07
Soybean meal (44% CP)	31.55	32.06	32.60	27.58	28.06	28.53	22.96	23.43	23.90
Gluten meal (60% CP)	7.13	6.81	6.47	4.49	4.18	3.88	4.22	3.92	3.62
Soybean oil	3.00	3.00	3.00	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	2.04	1.45	0.85	1.80	1.27	0.73	1.85	1.32	0.78
Oyster shells	1.41	1.78	2.16	1.28	1.62	1.97	1.29	1.63	1.97
Commen salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
DL-Methionin	0.29	0.29	0.29	0.25	0.25	0.25	0.30	0.30	0.31
L-Lysin,HCl	0.53	0.51	0.50	0.46	0.45	0.43	0.52	0.51	0.49
Vitamin Premix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated composition									
ME (kcal/kg)	3010	3010	3010	3150	3150	3150	3200	3200	3200
CP (%)	23	23	23	20.10	20.10	20.10	18.50	18.50	18.50
Ca (%)	1.00	1.00	1.00	0.90	0.90	0.90	0.90	0.90	0.90
NPP (%)	0.5	0.4	0.3	0.45	0.36	0.27	0.45	0.36	0.27
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Lys (%)	1.44	1.44	1.44	1.20	1.20	1.20	1.00	1.00	1.00
Met + Cys (%)	1.09	1.09	1.09	0.94	0.94	0.94	0.80	0.80	0.80

¹Supplied per kilogram of diet: 600 IU vitamin A, 800 IU vitamin D, 83 mg vitamin E, 2.2 mg vitamin K3, 2 mg vitamin B6, 8 mg vitamin B12, 10 mg nicotine amid, 0.3 mg folic acid, 20 mg D- biotin and 160 mg choline chloride. ²Supplied per kilogram of diet: 32 mg Mn, 16 mg Fe, 24 mg Zn, 2 mg Cu, 800 µg I, 200 µg Co and 60 µg Se.

ruminants. Some studies have confirmed the effects of yeast culture (YC) in increasing concentrations of commensal microbes or suppressing pathogenic bacteria (Stanley et al., 2004) in monogastric animals. In addition, others have reported that yeast products affect nutrient digestibility (Thayer and Jackson, 1975; Thayer et al., 1978; Bradley and Savage, 1995).

The addition of live yeast to animal feed has been known to improve the nutritive quality of feed and performance of animals (Martin et al., 1989). *S. cerevisiae* (SC) yeast has been shown to contain biologically valuable proteins, vitamin B-complex, important trace minerals and several unique “plus” factors (Eckles and Williams, 1925). Some nutritional benefits identified are the ability to cause reduction in cases of infectious diseases (Line et al., 1997), to cause improvement of feed efficiency (Brake, 1991; Onifade and Babatunde, 1996) and availability of P (Moore et al., 1994; Day, 1977). To reduce the concentration of P in excreta, broilers can be fed to requirement, essentially by reducing overfeeding of P via decreasing the amount of P from inorganic sources (Levic et al., 2006). Angel et al. (2006) reported that under commercial conditions, broiler litter P was reduced by 30% when diet P was reduced by 10% which is of ecological importance.

The effect of yeast culture (YC) on bird's performance

with inadequate P in the poultry diet has not been fully investigated. Therefore, the purpose of this study was to evaluate the influence of non phytate phosphorus (NPP) levels and YC on growth performance, digestibility coefficients of nutrients and blood parameters in broilers.

MATERIALS AND METHODS

Experimental designs

One-day-old male broiler chicks (Ross 308, n = 720) were used to evaluate the effects of feeding YC on performance during a 42 days period. Birds were randomly assigned to 48 pens (15 birds/pen) and were fed with regular non medicated broiler starter (0 to10d), grower (11 to 28) and finisher (29 to 42) diets (Table 1). There were 12 dietary treatments: 3 levels of NPP (normal, 80% and 60% normal level) and 4 levels of YC (0.0, 0.15, 0.3 and 0.45 % of diet). YC used in this experiment was XP yeast culture prepared from *S. cerevisiae* containing approximately 4×10^7 cfu/g, a residue of the fermentation process used to produce the product (Diamond V Mills Inc., Cedar Rapids, IA).

Standard management practices of commercial broiler production were applied. All floor pens were measured as 1.3 × 1.5 m. Each pen contained one tube feeder and one bell-type waterer, and used litter top-dressed with about 5 cm of new shaving. All the chickens were maintained under uniform temperature and lighting control system during the entire period of the study. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were

recorded and analyzed at the end of each experimental period.

After being weighed on the 28th day of the experiment, three birds from each replicate were selected and transferred to metabolic cages (12 chicks per treatment). All these chickens were fed with experimental finisher diets containing Cr_2O_3 (0.3%) as the digestibility marker. On the morning of day 35, chickens were sacrificed by cervical dislocation. All of the ileal digesta between the meckels diverticulum and the terminal ileum (2 cm above the ileal-cecal junction) were obtained immediately and carefully. The digesta from each of the three birds per cage was pooled as one sample into a plastic bag and immediately stored at -20°C . Digesta were freeze-dried, ground through a 1.00 mm mesh screen, and mixed thoroughly before analyses. Dry matter, gross energy, CP ($\text{N} \times 6.25$, macro-Kjeldahl), calcium (Ca), and phosphorus (P) contents were determined according to AOAC (1990) methods. Samples also were analyzed for chromic oxide (Fenton and Fenton, 1979). The apparent digestibility values for dietary nutrients were calculated as follows:

$$\text{DD} = 1 - [(\text{ID} \times \text{AF}) / (\text{IF} \times \text{AD})]$$

Where, DD is the apparent digestibility of a nutrient in diet; ID is the concentration of an indigestible marker in diet; AF is the nutrient concentration in ileal digesta; IF is the indigestible-marker concentration in ileal digesta; and AD is the nutrient concentration in the diet.

To determine serum parameters at 35 days of age, three birds were randomly selected from each pen and 3 ml of blood was collected from the brachial vein from each bird. The blood samples were centrifuged for 15 min at $2500 \times g$, and the serum was separated and then stored at -20°C until the measurement of glucose, total protein, Ca and P in the serum using commercial diagnostic kits and according to the standard procedures (Ziestchem Diagnostic kits, Tehran, Iran) (AOAC, 1990).

After blood samples were collected, the birds (12 birds per treatment) were sacrificed by cervical dislocation, and the left tibias were immediately removed and cleaned. As described by Brenes et al. (2003), the tibias were subsequently dried at 105°C for 12 h, extracted with ether, dried again, and weighed. The dry, fat-free tibia bones were ashed at 550°C , and the ashes were weighed and used to determine concentrations of Ca and P. The P concentration of toe samples was determined by photometric method using the molybdo-vanadate procedure. The Ca concentration of toe samples was analyzed by dry ash method (AOAC, 1990).

Data were analyzed in a factorial experiment with randomized block design using the General Linear Model procedure of SAS® (SAS Institute, 1998). Means were compared for significant ($p \leq 0.05$) differences using the LSMEANS option of SAS.

RESULTS

The results of growth performance are summarized in Table 2. There were significant interactions ($p < 0.05$) between NPP levels and concentration of YC in the diet with some growth traits. Thus, the results presented are based on means of treatment and not mainly on effect. NPP \times YC combination interactions were found with BWG in all the experiment. In the whole experiment, the birds fed with diet containing normal level of NPP without YC had greater BWG than the rest groups. An interaction between NPP and YC was found with FI only at the finisher period. Interactions between NPP and YC were observed in finisher period and also in the whole experiment. In the lower levels of NPP, the improvement

of FCR was dose dependent on YC. The best FCR was found in birds fed with diets containing normal level of NPP and all used levels of YC.

No NPP \times YC combination interactions were found on digestibility of nutrients (Table 3). The effect of NPP concentration in the diet had significant effect ($p < 0.05$) on digestibility of CP and Ca, so that the use of lower levels of NPP made significant decrease ($p < 0.05$) on digestibility of these nutrients (Figure 1). Digestibility of Ca was dose dependent on YC (Figure 2). The addition of YC significantly ($p < 0.05$) influenced digestibility of P. The level of 0.30 % of YC made the highest digestibility of P.

The main effects of NPP and YC on blood and tibia parameters are shown in Table 4. There were no interactions between NPP and YC in all the parameters mentioned. Tibia parameters (tibia ash, P and Ca in tibia) were significantly ($p < 0.05$) affected by levels of NPP so that the enhancement of NPP dietary increased the concentration of P, Ca and ash in tibia. Only the highest level (0.45 % of diet) of YC made significant increase ($p < 0.05$) on tibia ash percentage. No significant effect of YC was observed in P and Ca concentration in the tibia.

Serum Ca and P were significantly ($p < 0.05$) affected by the level of NPP in the diet (Table 4). The birds fed with diets containing normal level of NPP significantly ($p < 0.05$) showed higher concentration of P and glucose and lower Ca in the serum when compared with birds fed with lower levels of NPP diet. The NPP diet did not affect the concentration of total protein and glucose in the serum. The effects of YC on the concentration of serum P and total protein were significant ($p < 0.05$). The addition of YC to diets linearly increased the concentration of P in the serum. The highest level of total protein in the serum was observed in birds fed with the diet containing 0.30% YC.

DISCUSSION

Because of the ability of phytase to improve nutrient utilization and performance, several phytase products derived mainly from *Aspergillus niger*, *Peniophora lycii* and *Escherichia coli* have been developed and are commercially available (Selle and Ravindran, 2007). Yeast, fungi, or bacteria can produce phytase that is needed to hydrolyze phytate into inorganic phosphate (Simons et al., 1990). Thayer et al. (1978) studied the effects of YC in turkey breeder hen diets and noted an improvement of phosphorus (P) utilization in hens fed with NPP deficient diets supplemented with YC.

The results of this study demonstrated that growth performance was significantly influenced by NPP dietary level. As expected, a decrease in the availability of phosphorus in the diet caused a negative effect on the overall performance of chicks. Birds fed with normal level of NPP had significantly higher body weight and better FCR compared to the birds fed with lower levels of NPP.

NPP deficiency was shown to result in reduced appetite, probably due to disturbance of the ratio

Table 2. The effect of added NPP and live yeast on growth performance in broilers during 42d of experiment.

Treatment	Level of NPP ¹	YC ²	Weight gain (g)				Feed intake (g)				Feed conversion ratio (g/g)			
			0-10 days	11-28 days	29-42 days	0-42 days	0-10 days	11-28 days	29-42 days	0-42 days	0-10 days	11-28 days	29-42 days	0-42 days
1	Recommend	0/00	180	1207	1225	2611 ^a	233	1746	2470 ^{abc}	4449	1.30	1.45	2.02 ^e	1.70 ^g
2	Recommend	0/15	180	1177	1209	2566 ^{ab}	227	1765	2491 ^a	4483	1.26	1.50	2.06 ^e	1.75 ^{efg}
3	Recommend	0/30	182	1178	1219	2580 ^{ab}	225	1734	2466 ^{abc}	4425	1.23	1.47	2/02 ^e	1.71 ^{fg}
4	Recommend	0/45	181	1163	1205	2550 ^b	226	1695	2434 ^{def}	4354	1.24	1.46	2/02 ^e	1.71 ^{fg}
5	80% recommend	0/00	168	1134	1145	2448 ^c	235	1752	2489 ^a	4476	1.40	1.55	2.17 ^d	1.83 ^{cd}
6	80% recommend	0/15	169	1121	1159	2449 ^c	237	1753	2454 ^{bcdde}	4445	1.40	1.56	2.12 ^{cd}	1.82 ^d
7	80% recommend	0/30	174	1134	1133	2442 ^c	235	1717	2421 ^f	4373	1.35	1.52	2.14 ^{cd}	1.79 ^{de}
8	80% recommend	0/45	174	1120	1174	2468 ^c	230	1720	2389 ^g	4338	1.31	1.54	2.03 ^e	1.76 ^{ef}
9	60% recommend	0/00	163	1039	1059	2263 ^e	227	1808	2462 ^{abcd}	4498	1.39	1.74	2.33 ^b	1.99 ^a
10	60% recommend	0/15	165	1061	1081	2307 ^{de}	230	1811	2481 ^{ab}	4522	1.39	1.71	2.30 ^b	1.96 ^{ab}
11	60% recommend	0/30	167	1048	1088	2303 ^{de}	236	1750	2441 ^{cdef}	4426	1.41	1.67	2.41 ^a	1.92 ^b
12	60% recommend	0/45	167	1076	1111	2354 ^d	234	1736	2430 ^{ef}	4400	1.40	1.61	2.19 ^c	1.87 ^c
SEM			1.7	17.3	12.6	20.5	3.3	10.6	10.9	16.6	0.02	0.03	0.02	0/02
P value														
Source of variation			<0.0001	<0.0001	<0.0001	<0.0001	0.022	<0.0001	0.005	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
Level of NPP											1	1	1	<0.0001
Level of YC			0.004	0.947	0.235	0.714	0.868	<0.0001	<0.0001	<0.0001	0.052	0.061	<0.0001	<0.0001
NPP × YC										1			1	
			0.836	0.413	0.083	0.052	0.148	0.152	0.036	0.284	0.171	0.173	0.030	0.035

¹ Non phytate phosphorus; ²Yeast culture; ^{a-g} means within columns with no common superscript differ significantly (P<0.05).

Table 3. Probability-values from the ANOVA for the effect of different level of NPP and YC on digestibility of nutrients in broilers.

Source of variation	DM	Energy	CP	Ca	P
Level of NPP ¹	0.478	0.353	<0.0001	0.299	0.0003
Level of YC ²	0.061	0.282	0.075	0.002	0.002
NPP × YC	0.802	0.729	0.907	0.922	0.814

¹ Non phytate phosphorus; ²yeast culture.

between Ca and NPP in diet (Olukosi et al., 2007). Therefore, in this study, the increase of NPP to the normal level in the diets resulted in

increased availability of P to the broilers, which could have led to improved appetite of the birds and hence improved feed intake and growth

performance.

Birds supplemented with YC showed better FCR Than non-supplemented birds especially in

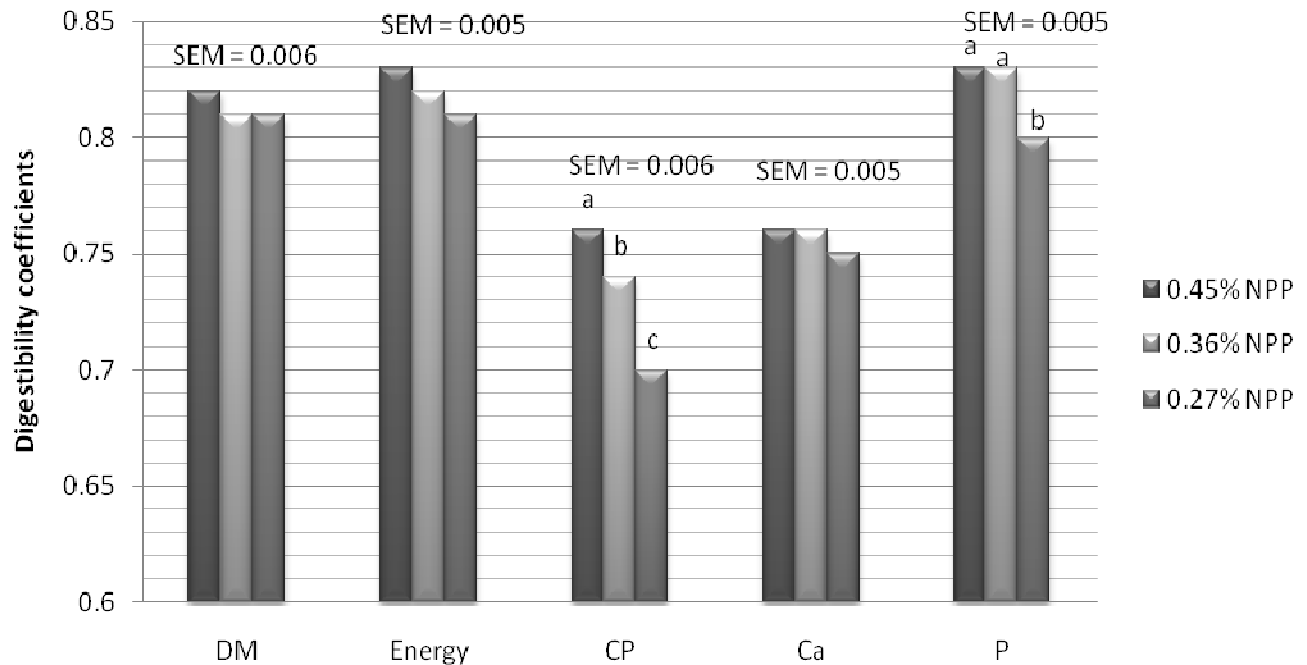


Figure 1. The effects of different levels of NPP on digestibility coefficient of dry matter (DM), energy, CP, Ca and P in broilers at 35 days old.

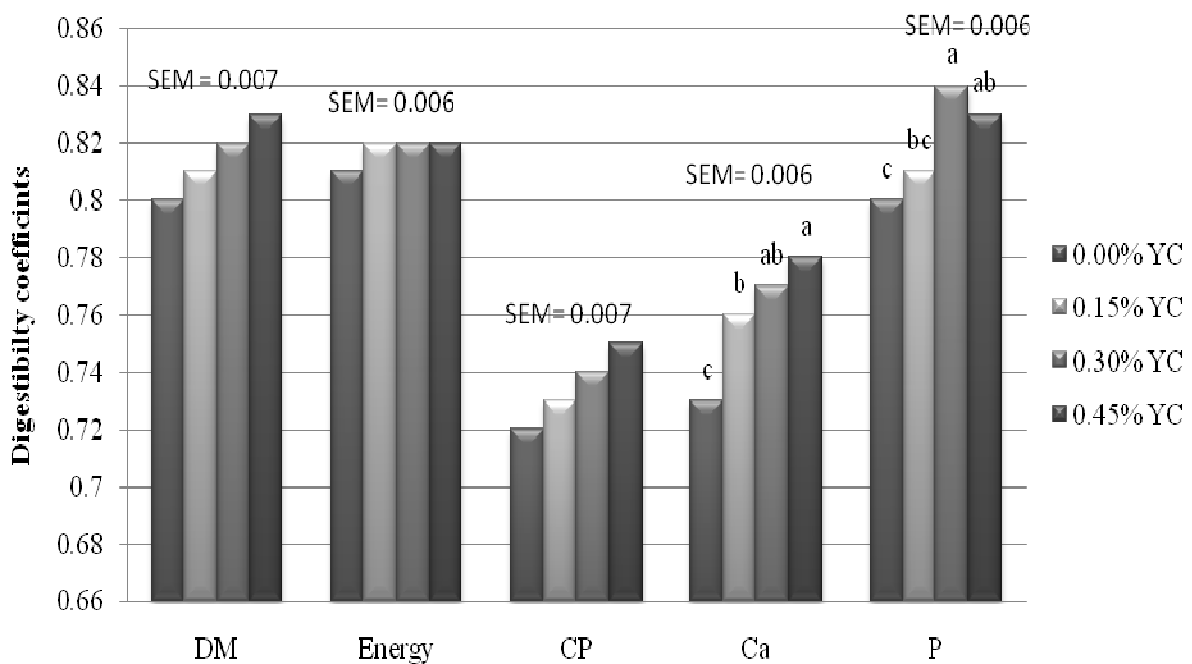


Figure 2. The effects of different levels of YC on digestibility coefficient of dry matter (DM), energy, CP, Ca and P in broilers at 35 days old.

lower level of NPP dietary. Studies with broilers (Thayer and Jackson, 1975), laying hens (Tonkinson et al., 1965), market turkeys (Savage et al., 1985), and turkey breeder hens (Thayer et al., 1978; Savage and Mirosh, 1990)

have shown that the supplementation of diets with YC resulted in enhanced growth performance. Moreover, incorporation of YC in feeds was reported to increase feed efficiency in growing chickens and breeder turkeys

Table 4. The effect of different level of NPP and YC on Tibia ash and blood parameters.

Treatment	Tibia Ash	P in Tibia	Ca in Tibia	Blood parameter			
				P	Ca	Protein	Glucose
Level of NPP¹							
Recommend	45.9 ^a	8.52 ^a	19.8 ^a	6.2 ^a	12.3 ^b	4.00	253.8
80% Recommend	43.9 ^b	8.17 ^b	18.9 ^b	6.1 ^b	13.0 ^a	4.10	246.9
60% Recommend	42.0 ^c	7.80 ^c	18.1 ^c	5.9 ^c	13.2 ^a	4.00	244.4
SEM	0.44	0.08	0.18	0.04	0.07	0.05	4.91
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.261	0.382
Level of YC							
0/00	43.2 ^b	8.06	18.16	5.9 ^b	12.8	3.8 ^c	248.0
0/15	43.3 ^b	8.10	18.7	6.0 ^b	12.8	4.0 ^b	242.5
0/30	44.0 ^{ab}	8.20	19.0	6.1 ^{ab}	12.9	4.2 ^a	251.2
0/45	45.3 ^a	8.29	19.3	6.2 ^a	12.8	4.1 ^{ab}	251.7
SEM	0.51	0.09	0.21	0.05	0.08	0.05	5.67
P value	<0.0001	0.290	0.113	0.010	0.997	0.0007	0.647

¹ Non phytate phosphorus; ² yeast culture.

^{a-9} Means within columns with no common superscript differ significantly (P<0.05).

(Thayer and Jackson, 1975; Day, 1977; Thayer et al., 1978). In contrast, Brewer (1983) reported a lack of effect on live weight and feed efficiency of market turkeys fed with diets supplemented with YC. In this experiment, birds fed with diets containing 80% of recommended NPP supplemented by YC were inclined towards the same FCR in birds fed with normal level of NPP. The results of this experiment showed that YC can improve performance in broilers fed with diet deficient in NPP. Selle et al. (2009) demonstrated that ileal P digestibility coefficients decreased when broiler chicks were fed with low-NPP diets which were similar to the apparent ileal P digestibilities obtained in this study. In this experiment, the digestibility of CP was affected by NPP dietary levels. Other studies (Yan et al., 2001, Shirley and Edwards, 2003) showed that reductions in NPP diet led to a decrease in nitrogen retention in the broilers.

Tibia ash and ash mineral contents are usually important parameter used in evaluating mineral reserves in animal bones. The results of this study showed that tibia ash percentage and tibia Ca and P of 35-day-old broilers were increased with enhancement of NPP dietary concentration. Similarly, Lou et al. (1998) showed that an enhancement of NPP in diet increased tibia ash percentage, ash, Ca and P contents. Also, it has been reported that feeding chickens with diets deficient in NPP resulted in a reduced percentage of tibia ash (Viveros et al., 2002).

The digestibility data showed that the addition of YC to a NPP deficient diet significantly improved the ileal digestibility of P and Ca. The increased digestibility of P and Ca in the broiler chicks by supplementing NPP deficient diets with YC, as compared with that of broilers fed with the NPP normal diet, was consistent with other reports (Thayer and Jackson, 1975; Thayer et al., 1978).

Nahashon et al. (1994) demonstrated an increased N and Ca retention in Single Comb White Leghorn laying hens fed with diets containing a direct-fed microbial product of bacterial origin. In this experiment, the addition of YC numerically increased CP ileal digestibility. Thayer et al. (1978) ascribed the increase of P utilization in turkey breeder hens to the presence of YC in the diet. Supplementation of YC at 0.45% of diet increased the amount of ash in the tibia. Increased mineral retention was also reported by Guevara et al. (1978) as increased tibia ash content in broilers fed with diets deficient in P and supplemented with 0.3 % YC diet. The ability of the YC to improve nutrient retention may be related partly to the phytase activity of the YC which facilitated a more efficient utilization of phytate P. Ohta et al. (1995) reported that dietary fructooligosaccharide also increased digestibility of Ca, Mg, Fe, Zn, and Cu. The role of oligosaccharide components in YC on digestibility of Ca and P or other minerals warrants further research. The beneficial effect of phytase has been related to the release of minerals and trace elements from complexes with phytic acid, and increase in digestibility and availability of macro- and microelements (Ravindran et al., 2008; Zhou et al., 2008), increased starch digestibility and increased utilization of protein (Woyengo et al., 2010). This is consistent with the growth performance data obtained in this study. From the results of this study, it could be concluded that the addition of a yeast or YC to poultry diets may increase the dietary utilization of the mineral present in the feed ingredients, and decrease waste disposal challenges currently facing the poultry industry by subsequently reducing the concentrations of nutrients especially P remaining in the excreta.

The enhancement of NPP dietary level increased serum P content, decreased Ca but had no effect on

serum total protein and glucose concentration in 35-day-old broilers. In this study, the same calcium levels were used in all the diets, and the balance between calcium and P was disrupted in the low-NPP diet. Similarly, Han et al. (2009) reported that the ratio of Ca to P in plasma of birds fed with diet having 0.35% NPP was more than this ratio in the plasma of birds fed with the diet of 0.13% NPP. This shift in calcium to phosphorus retention resulted in an improvement in broiler growth and tibia ash content. An increase in P retention in broilers fed with diets with 80% recommended NPP was related to a decrease in plasma P, indicating that birds had a greater ability to retain P from diets with lower NPP content (Keshavarz 2000; Ravindran et al., 2000). These results showed that broiler chicks fed with diet containing 0.30 and 0.45% YC had significantly higher serum total protein and glucose compared with the other groups.

Conclusion

This study demonstrated that the digestibility of P, CP and mineral utilization and serum P decreased with reduced NPP dietary levels. The results of this study indicated that increased digestibility of P and Ca in NPP deficient diets by YC resulted in increased availability of P and Ca to the broilers, which could have led to improved appetite of the birds and hence improved feed intake and growth performance. However, further studies are needed in order to clarify the influence of YC on the digestibility of mineral and other factors related to performance such as intestinal histomorphology.

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