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THE EFFECT OF PROBIOTIC AND ENZYME MIXTURE CONTAINING PHYTASE ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, MEAT QUALITY AND BIOCHEMICAL CONSTITUENTS OF PLASMA OF BROILER CHICKS FED DIFFERENT PROTEIN LEVELS

Aggoor, F. A. M.; F. S. A. Ismail and E. A. Shakmak
Poultry Production Dept., Faculty of Agric., Mansouria University,Egypt.

ABSTRACT

The influence of dietary protein levels and two types of pro-nutrients e.g. enzyme mixture containing phytase or probiotics on growth performance, carcass characteristics, meat quality and plasma biochemical constituents of broiler chicks was studied herein as means of improving protein utilization and decreasing feed cost. A complete randomized factorial design was conducted including two levels of crude protein 22 and 20% CP in the starting and 20 and 18% CP in the growing-finishing periods. Each CP level was fed either without or with enzyme mixture containing phytase or probiotics. Thus, there were 6 dietary experimental treatments, each one was fed to 24 one d-old unsexed broiler chicks divided equally among four replicates of 6 chicks each housed in a battery (30×35×40 cm). At the end of the experiment, 3 chicks of each treatment were slaughtered to determine carcass characteristics and meat quality traits. Furthermore, four plasma blood samples of each treatment were collected to determine some biochemical constituents. Also, nutrients digestibility and economic efficiency of treatments were also calculated.

Increasing protein level significantly increased growth, decreased feed intake thus improved feed conversion ratio (FCR), protein conversion ratio (PCR) and economic efficiency. Pro-nutrients supplementation significantly increased growth, decreased feed intake and improved FCR, PCR and economic efficiency, with probiotics being more efficiently than enzyme mixture containing phytase, and this was clear in each protein level. Enzyme mixture containing phytase or probiotics significantly improved digestibility of dry matter and crude fibre. Increasing protein level significantly increased front part and decreased hind part, meanwhile protein level and /or pro-nutrients did not significantly affect chemical composition and physical characteristics of meat. Enzyme mixture containing phytase increased plasma Ca and inorganic phosphorus (iP), the later was significantly increased by probiotics supplementation, too. Plasma ALT was significantly decreased due to probiotics supplementation, showing the improvement in liver functions.

In conclusion, low crude protein diet e.g. 20 and 18% in the starter and grower-finisher diets, respectively could be fed to broiler chicks when supplemented with probiotics cocktail containing *Bacillus subtillis* fermentation extract, Brewers yeast extract, lactic acid, citric acid, calcium propionate, sodium aluminosilicate and DL-methionine without adverse effects on growth performance and carcass quality, moreover it improved economic efficiency.

Keywords: Broilers, protein level, enzymes, probiotics, growth, meat quality.

INTRODUCTION

Protein nutrition represented a major challenge to poultry production especially in the region where protein rich feedstuffs are limited. One of the possible approaches to reduce the feed cost for poultry is the use of the low crude protein (CP) corn-soybean meal diet supplemented with amino acids and/or enzymes. Improving corn-soybean low-CP diet by amino acid supplementation/and or enzymes has received great interest, especially after the restriction set on the use of render feeds, environmental pollution and the need to least cost diet formulation, increasing availability of amino acids, and /or pronutrinets (Aletor *et al*., 2000; Sohail *et al*., 2003; Jiang *et al*., 2005; Corzo *et al*., 2005; Waldroup *et al*., 2005; Yamazaki *et al*., 2006; Ghazalah *et al*., 2006). However, methionine, lysine and /or pro-nutrients may allow low-CP feed to be formulated (Sohail *et al*., 2003; Corzo *et al*., 2005) and such approach is valuable for pollution control and decrease heat generated from metabolism of excess amino acids, however remains need further effort (Waldroup *et al*., 2005). The use of low-CP diet was linked with decreasing growth and increasing fat deposition, and impaired FCR and this depends on chick's age and magnitude of protein restriction.

The use of prebiotics and probiotics in poultry nutrition are widely emphasis as a mean of improving animal health, control pathogens and increased nutrient utilization through keeping healthy gut ecology (Makeld, 1991; Patterson and Burkholder, 2003; FAO, 2006, Ghazalah *et al*., 2007; Piray *et al*., 2007;). Multienzymes containing β-glucanase, α-amylase, cellulase, pectinase, xylanase, hemicellulase without or with protease and phytase could improve feed utilization and overcame the antinutritional factors of feedstuffs, improve gut health and immune response (Makeld, 1991; Jeroch *et al*., 1995; Attia *et al*., 2001; Kocher *et al*., 2002; Saleh *et al*., 2003; Yonemochi *et al*., 2003; Choct, 2006). However, studies addressed the impact of enzymes on dietary protein/amino acid utilization is rare (Zanella *et al*., 1999; Attia *et al*., 2003; Yonemochi *et al*., 2003; Selle *et al*., 2006). Thus, the effect of probiotic and enzyme mixture containing phytase on growth performance, carcass characteristics, meat quality and plasma biochemical constituents of broiler chicks fed different protein levels was investigated herein as means of improving protein utilization and reducing feeding costs of broiler chicks.

MATERIALS AND METHODS

Experimental design, birds and diets

The experimental design was complete randomized factorial design including two levels of crude protein being 22 and 20% CP in the starter and 20 and 18% CP in the growing- finishing diets, respectively (Table 1) fed without or with enzyme mixture containing phytase (Natuzyme[1]) and probiotics (Nutri-Bio Plus[2]).

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[1] Natuzyme®, (www. Alboraqmisr.com, 33511 Mansoura-Egypt, E-mail info@alboraqmisr.com)) was added at 1 g/kg. It is multifunctional feed enzyme mixture containing cellusase, xylanase,β-glucanase, α-amylase, protease, pectinase, and phytase. It also contains hemicellulases, amyloglycosidases and pentosanases activities.

[2]Nutri-Bio Plus®, is a grower promoter of AMECO-BIOS& CO, 339 W. Lemon Ave, Arcadia, CA 91007, USA, Email:amecobies@gmail.com). It's recommended dose of use is 200-500 g/ton feed. It is composed of *Bacillus subtillis* fermentation extract 130g, Brewers yeast extract 170g, lactic acid 20g, citric acid 10g, calcium propionate 100g, sodium aluminosilicate 550g and DL-methionine 20g.

Thus, there were 6 dietary experimental treatments, each diet was fed to 24 one d-old unsexed broiler chicks divided equally among four replicates of 6 chicks each housed in a battery (45×35×40 cm). The chicks (n=144 of Ross strain) were wing banded and distributed randomly among the experimental diets at day of hatch with keeping similar initial live body weight among replicates and treatments. The diets were based mainly on corn, soybean meal and corn gluten meal and formulated based on NRC (1994) Tables of feedstuffs, and met nutrient requirements of broiler chicks (NRC, 1994) except for protein in the low protein diet. Feed and water were offered *ad libitum* throughout the experiment. Chicks were kept under similar managerial and hygienic conditions and illuminated with 23 h light/d up to 49 d of age.

Criteria of response

Birds were weighed (g) individually at 14, 28 and 49 d of age, and feed intake was recorded by replicate at the same time and FCR ratio was calculated on a replicate basis. Protein conversion ratio was calculated by dividing protein intake by body weight gain for the whole experimental period. Coefficient of apparent digestibility of nutrients of the total gut was calculated according to Attia *et al*. (2007) using three replicates of one male each/ treatment. At 7 wk of age, 3 chicks were taken randomly from each treatment, and slaughtered; the remaining carcass after bleed, plucked and eviscerated was weighed (dressed weight) and divided into front and hind parts and weighed. Liver, gizzard, heart and spleen were separated and individually weighed. The carcass parts were expressed as relative to live body weight. A sample of breast meat and thigh meat (1:1; Wt:Wt) and the experimental diets were chemically analyzed for dry matter (DM, crude protein (CP), ether extract (EE) and crude ash (CA) according to AOAC (1990). Meat tenderness and water holding capacity (WHC) were measured according to the method of Volvoinskaia and Kelman (1962). Colour intensity of meat and drip were determined according to the method of Husani *et al*. (1950), whereas pH value was measured by a pH meter as described by Aitken *et al*. (1962) At 49 d of age, four blood samples of each treatment were collected in heparinzed tubes. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20^{o}C until analysis. Concentrations of total protein (Henry *et al*., 1974), albumin (Doumas *et al*., 1977), total lipids (Chabrol and Charonnat, 1973), total cholesterol (Watson, 1960), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Retiman and Frankel, 1957), Ca (Sendroy, 1944), inorganic P (Gomorri, 1942) were determined of each treatment. Globulin was calculated by difference between total protein and albumin, and albumin to globulin ratio was calculated. Furthermore, economic evaluation for all experimental diets was calculated as described by Zeweil. (1996).

Statistical Analysis

Data were analyzed using the GLM procedure of Statistical Analysis Software (SAS) version 6.11 (SAS® Institute, 1990, Cary, NC, USA) using two-way factorial design. Mean difference at $P \leq 0.05$ was tested according to Duncan New multiple range test (Duncan, 1955). When a significant interaction P value was obtained (<0.05), mean differences were compared using LSD, and the values were presented.

Table (1): Composition and calculated analyses of the experimental diets

Ingredients,%	Starter diets		Grower-finisher diets	
	22%	**20%**	**20% CP**	**18%CP**
Yellow corn	60.00	63.90	63.89	67.90
Soybean meal (44% CP)	19.00	18.80	18.99	18.99
Corn Gluten meal (60%)	13.78	10.03	10.03	6.25
Commercial oil blend	2.63	2.73	2.72	2.79
Dicalcium phosphate	1.77	1.80	1.32	1.36
Lime stone	1.45	1.50	1.43	1.40
Vit+Min mixture[1]	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
DL-methionine	0.02	0.12	0.00	0.07
L-lysine Hcl	0.29	0.32	0.22	0.28
Wash building sand	0.46	0.20	0.80	0.36
Total	**100.0**	**100.0**	**100.0**	**100.0**
Calculated values				
ME-**kc**al/kg diet	3192	3192	3186	3191
Crude protein, %	22.1	20.2	20.1	18.3
Methionine, %	0.45	0.50	0.38	0.40
Methionine plus cystine, %	0.84	0.85	0.73	0.72
Lysine, %	1.03	1.02	0.95	0.96
Ca , %	1.00	1.00	0.90	0.90
Available P, %	0.45	0.45	0.36	0.37
Determined values				
Dry matter,%	89.51	89.46	89.71	89.47
Crude protein	21.73	19.72	19.83	17.89
Ether extract, %	5.21	5.34	5.25	5.36
Crude fibre, %	2.43	2.39	2.41	2.38
Crude ash, %	9.81	9.84	9.71	9.78

[1] Vitamins and minerals mixture provide per kilogram of diet vitamin A (as all-trans-retinyl acetate); 12000 IU; vitamin E (all rac-α-tocopheryl acetate); 10 IU; k_3 3mg; Vit.D_3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; choline chloride, 500 mg; vitamin B_{12}, 10µg; vitamin B_6, 1.5 mg; thiamine (as thiamine mononitrate); 2.2 mg; folic acid, 1 mg; D-biotin, 50µg. Trace mineral (milligrams per kilogram of diet) Mn, 55; Zn, 50; Fe, 30;Cu, 10; Se, 0.1 and Ethoxyquin 3mg.

RESULTS AND DISCUSSION

Growth performance:

Results presented in Table (2) indicated that decreased protein level by 2% either in starting or in the growing-finishing diets significantly decreased growth of broiler chicks by2.7, 5.1, 2.1 and 0.7% during the period from 1-14, 15-28, 29-49 and 1-49 d of age.

This result indicates that decreasing protein level from 22 to 20 % in the starting and from 20 to 18% in the growing-finishing periods significantly impaired growth of broiler chicks. Whereas, the most adverse effects was observed during 15-28 of age period. This may be due to the shortage of available protein/amino acids for muscle protein synthesis during this period. Similar results were reported by Attia *et al*. (1998 and 2001) who found that decreased protein level in broiler diets significantly impaired growth.

However, Ferguson *et al*. (1998) pointed that if essential amino acid requirements are met, dietary CP can be decreased by nearly two percentages before production is adversely affected. In this concern, Gous (1998) reported that amino acid and energy requirements of broilers vary widely with newer genotype, and it is unclear whether these requirements are truly known.

Table 2. Effect of dietary crude protein level and/or feed additives on growth of broiler chicks (g\bird\period) from 1-49 d of age

Dietary treatments	BWG (g\bird\period) during				Number of dead chicks
	1-14 d of age	15-28 d of age	29-49 d of age	1-49 d of age	
Effect of dietary crude protein level,%					
22-20	275.7[a]	556.2[a]	1399.9[a]	2231.9[a]	0
20.18	268.3[b]	528.0[b]	1370.7[b]	2216.5[a]	0
P-Value	0.01	0.0001	0.01	0.0001	---
Pooled SEM	2.05	3.09	8.17	7.95	---
Effect of feed additives					
Without	243.7[b]	530.1[b]	1369.3	2140.9[c]	0
Natuzyme	287.8[a]	532.8[b]	1388.2	2208.8[b]	0
Probiotics	284.5[a]	563.5[a]	1398.5	2246.5[a]	0
P-Value	0.0001	0.0001	NS	0.0001	---
Pooled SEM	2.51	4.67	10.01	9.74	---
Interaction effect between dietary crude protein and feed additives					
High- (-)	250.7[b]	539.4[ab]	1382.5	2172.5	0
High- Natuzyme	282.6[a]	556.8[ab]	1404.6	2243.9	0
High- Probiotics	294.0[a]	572.5[a]	1412.9	2279.4	0
Low- (-)	236.8[c]	520.8[bc]	1356.2	2109.2	0
Low- Natuzyme	293.1[a]	508.8[c]	1371.7	2173.6	0
Low- Probiotics	275.0[ab]	554.5[ab]	1384.1	2213.6	0
P-Value	0.0001	0.04	NS	NS	---
Pooled SEM	3.55	6.60	NS	NS	---
LSD	24.3	45.2	--	--	--

a, b and c means within the same column within the same treatment not having similar superscripts are significantly different ($P<0.05$). NS $P\geq0.05$.

There were significant positive effects of pro-nutrients on growth of broiler chicks during 1-14, 15-28 and 1-49 d of age. The results indicated that Natuzyme and probiotics similarly improved growth of broiler chicks during 1-14 d of age. Meanwhile, only probiotics improved growth of broilers during 15-28 d of age compared to the unsupplemented control (6.3%) and Natuzyme supplemented-group (5.8%) during 15-28 d of age. During 29-49 d of age, there were lacks of significant effect of pro-nutrients on growth of broiler chicks. This may be due to maturation of digestive tract in terms of enzyme secretions, capacity and ecology (Attia *et al*., 2001; El-Deek *et al*., 2003).

For the whole experimental period, Nautzyme improved growth by 3.2%, while probiotics improved growth by 4.9% compared to the unsupplemented control. The probiotics supplemented group was also efficiently (p≥0.05) better than the enzyme cocktail containing phytase. The improved performance of enzymes supplemented group could be attributed to better digestibility of nutrients, limit antinutritional factors, better gut health and immune response (Jeroch *et al*., 1995; Zanella *et al*., 1999; Kocher *et al*., 2002; Saleh *et al*., 2003; Yonemochi *et al*., 2003; Choct, 2006; Selle *et al*., 2006; Attia *et al*., 2007; 2008). On the other hand, the effect of probiotics could be explained by the effect of probiotics on gut health and elimination of the harmful substances including the indetectable level of mycotoxicns due to its contents of *Bacillus subtillis* fermentation extract, Brewers yeast extract, lactic acid, citric acid, calcium propionate, sodium aluminosilicate and DL-methionine. Similar results were reported by Makled (1991), Patterson and Burkholder (2003), Sun *et al*. (2005), FAO (2006), Piray *et al.* (2007) and Ghazalah *et al* (2007).

The results indicate that, there was significant interaction between protein level and pro-nutrient supplementations during the early periods of age until 28, and lack of significance thereafter and for the whole period, too. It was found that both Natuzyme and probiotics similarly improved growth of broiler chicks during 1-14 d of age period, when comparison was made in each protein level. On the other hand, during 15-28 d of age period, enzymes had no significant effect within each protein level, while within the low protein diet probiotic was more efficient than Natuzyme, once again this may be due to the multi-nutritional effects of probiotics due to its contents of *Bacillus subtillis* fermentation extract, Brewers yeast extract, lactic acid, citric acid, calcium propionate, sodium aluminosilicate and DL-methionine. There was no significant interaction due to pro-nutrients on growth of chicks during 29-49 and 1-49 d of age, however, it is clear that pro-nutrients improved growth and the effect being constant with each protein level with probiotics was more efficient than Natuzyme. This may indicate that enzyme secretion was stabilized after 28 d of age, while the experimental hygienic condition and quality of the diet continue to contribute to the positive response to probiotics. There were no dead chicks during the experimental period (Table 2), meaning that decreasing protein level and/or pro-nutrients supplementations had no harmful effect on livability. These results are in agreement with those reported by Attia *et al.* (1998, 2001 and 2003).

Results presented in Tables (3) indicate that decreased protein level by 2% in the starting and growing-finishing diets significantly increased feed intake of broiler chicks during the period from 1-14, 15-28, 29-49 and 1-49 d of age. The increased feed intake for the whole experimental period amounted to 1%. This indicates that, however, of low magnitude, birds ate in an attempt to compensate for the decrease in protein and/or amino acid needs. These results are in agreement with those reported by Lipstein *et al*. (1975), Baker (1986) and Attia *et al*. (2001). There were significant negative effects of pro-nutrients on feed intake during all the experimental period.

It was found that probiotics had strong negative effect on feed intake compared to Natuzyme. The decrease for the whole experimental period amounted to 3.7 and 1% respectively compared to the unsupplemented control and Natuzyme supplemented group, respectively. Meanwhile, only Natuzyme decreased feed intake by 2.8% compared to the unsupplemented control. The results indicate that, there were significant interaction between protein level and pro-nutrients during only 1-14 d of age and lack of significance thereafter and for the whole period, too. Natuzyme and probiotics decreased feed intake in the control and low protein diet by different magnitude, and probiotics had stronger effects within each protein level. Natuzyme and probiotics had also different responses when comparison was made over protein levels. For the whole experimental period, the highest and the lowest feed consumed was for groups fed low protein unsupplemented diets and probiotic-supplemented high protein diet, respectively, with difference (4.8%) was insignificant.

Table 3. Effect of dietary crude protein level and/or feed additives on feed intake of broiler chicks (g\bird\period) from 1-49 d of age.

Dietary treatments	Feed conversion ratio (feed \ gain) during				Economic efficiency
	1-14 d of age	15-28 d of age	29-49 d of age	1-49 d of age	
Effect of dietary crude protein level,%					
22-20	1.458[b]	1.902[b]	2.316[b]	2.108[b]	18.32[a]
20-18	1.535[a]	2.034[a]	2.385[a]	2.175[a]	16.00[b]
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001
Pooled SEM	0.009	0.009	0.002	0.0012	0.071
Effect of feed additives					
Without	1.687[a]	2.103[a]	2.412[a]	2.256[a]	13.1[c]
Natuzyme	1.399[b]	1.967[b]	2.337[b]	2.113[b]	17.8[b]
Probiotics	1.404[b]	1.834[c]	2.302[c]	2.056[c]	20.5[a]
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001
Pooled SEM	0.012	0.012	0.003	0.0015	0.087
Interaction effect between dietary crude protein and feed additives					
High- (-)	1.625[b]	2.054[b]	2.379	2.211[b]	14.7[d]
High- Natuzyme	1.407[d]	1.859[c]	2.298	2.079[d]	19.0[b]
High- Probiotics	1.344[e]	1.792[d]	2.272	2.032[e]	21.3[a]
Low- (-)	1.749[a]	2.153[a]	2.445	2.301[a]	11.5[d]
Low- Natuzyme	1.392[d]	2.073[b]	2.376	2.146[c]	16.7[c]
Low- Probiotics	1.464[c]	1.877[c]	2.333	2.079[d]	19.8[b]
P-Value	0.0004	0.002	NS	0.0001	0.0001
Pooled SEM	0.017	0.017	0.004	0.002	0.123
LSD	0.047	0.049	-	0.006	**1.46**

a, b and c means within the same column within the same treatment not having similar superscripts are significantly different (P<0.05). NS P≥0.05.

Results presented in Table (4) indicated that increased protein level by 2% in the starting and growing-finishing diets significantly improved FCR of broiler chicks by 5.0, 6.5, 2.9 and 3.1% during the period from 1-14, 15-28, 29-49 and 1-49 d of age, respectively. This result indicates increasing protein level from 20 to 22 % in the starter diet and from 18 to 20% in the growing-finishing diets significantly improved FCR of broiler chicks. Whereas, the best improvement was observed during 15-28 d of age period. Similar results were reported by Attia *et al*. (1998 and 2001) who found that increasing protein level in broiler diets significantly improved FCR.

There were significant positive effects of pro-nutrients on FCR of broiler chicks during 1-14, 15-28 and 1-49 d of age. The results indicate that Natuzyme and probiotics similarly (~17%) improved FCR of broiler chicks during 1-14 d of age. Meanwhile, probiotics and Natuzyme improved FCR of broilers during 15-28, 29-49 and 1-49 d of age by 6.5 and 12.8%, 3.11 and 4.6% and 6.3 and 8.9% of the same order, respectively compared to the unsupplemented control. The results demonstrate that probiotics have stronger effect (P≥0.05) than Natuzyme, and the effect of pro-nutrients decreased with advanced age of chicks. Similarly, Makled (1991), Saleh *et al*. (2003), Yonemochi *et al*. (2003), Meng *et al*. (2005), Sun *et al*. (2005), Choct (2006), Selle *et al.* (2006), Piray *et al*. (2007), Ghazalah *et al*. (2007) and Attia *et al*. (2007; 2008) reported that enzymes and probiotics improved feed utilization, and the effect depends on dietary composition, age and/or strain or type of chicks.

The results indicate that, there were significant interaction between protein level and pro-nutrient supplementations on FCR during the early periods of age until 28 day, and lack of significance thereafter. It was found that both Natuzyme and probiotics improved FCR of broiler chicks during 1-14, 15-28 and 1-49 d of age, however, probiotics had stronger effect within each protein level when comparison was made within or over protein levels. For the whole experimental period, Nautzyme improved feed conversion by 6.0 and 6.7% for the high- and low-protein diet, while the corresponding effect for probiotics was 8.1 and 9.6%, respectively confirming the former conclusion. The present results are in agreement with those reported by Cowan *et al*. (1996) and Zanella *et al*. (1999) who indicated that enzyme supplementation should allow a reduction in CP formulation as well as ME, revealing the improvements in protein and ME utilization. This means that the effect of pro-nutrients was slightly better in the low protein diet than in the high-protein diet. It is interesting to report that feed conversion of any pro-nutrients supplemented group was better than the positive control, meanwhile the feeding unsupplemented low protein diet impaired FCR, however, Natuzyme and probiotics overcame this negative effect and probiotics was more efficient.

Results in Table (4) indicate that economic efficiency was improved with increasing protein level and/or pro-nutrient supplementation, with the effect of probiotics was stronger than enzyme mixture containing phytase within each protein level. The highest economic efficiency was recorded by probiotic supplemented-high protein diet and probiotic supplemented-low protein diet. Whereas, the lowest economic efficiency was recorded by low protein unsupplemented-diet**.** These results are in agreement with those reported by Attia *et al*. (2001; 2003) and Ghazalah *et al*. (2006).

Table 4. Effect of dietary crude protein level and/or feed additives on feed conversion of broiler chicks (g\bird\period) and economic efficiency from 1-49 d of age

Dietary treatments	Feed conversion ratio (feed \ gain) during				Economic efficiency
	1-14 d of age	15-28 d of age	29-49 d of age	1-49 d of age	
Effect of dietary crude protein level,%					
22-20	1.458^{b}	1.902^{b}	2.316^{b}	2.108^{b}	18.32^{a}
20-18	1.535^{a}	2.034^{a}	2.385^{a}	2.175^{a}	16.00^{b}
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001
Pooled SEM	0.009	0.009	0.002	0.0012	0.071
Effect of feed additives					
Without	1.687^{a}	2.103^{a}	2.412^{a}	2.256^{a}	13.1^{c}
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Probiotics	1.404^{b}	1.834^{c}	2.302^{c}	2.056^{c}	20.5^{a}
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001
Pooled SEM	0.012	0.012	0.003	0.0015	0.087
Interaction effect between dietary crude protein and feed additives					
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Low- (-)	1.749^{a}	2.153^{a}	2.445	2.301^{a}	11.5^{d}
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LSD	0.047	0.049	-	0.006	**1.46**

a, b and c means within the same column within the same treatment not having similar superscripts are significantly different ($P<0.05$). NS $P\geq0.05$.

Protein conversion ratio and apparent nutrient digestibility:

Data for protein conversion ratio and apparent nutrient digestibility are presented in Table (5). It was found that protein conversion ratio was significantly improved by decreasing protein level, and pro-nutrient supplementation, and the effect of pro-nutrient was clear in each protein level. However, the effect of probiotic was stronger than enzymes mixture containing phytase. The best protein conversion ratio was from probiotic supplemented-low protein diet and the poorest was from the unsupplemented high-protein diet. These results are in line with those reported by Kies *et al*. (2001), Attia *et al*. (2001) and Choct (2006).

Digestibility of nutrients was not significantly affected by dietary protein level, nor there a significant interaction between protein level and pro-nutrients supplementation. On the other hand, Natuzyme and probiotics had a significant positive similar effect on only digestibility of DM and CF, however, apparent digestibility of CP and EE and CA were not significantly affected by pro-nutrients supplementations. Similar results were reported by Cowan *et al*. (1996) Choct (2006), Selle *et al*. (2006), Piray *et al.* (2007) and Attia *et al.* (2001; 2007; 2008).

Table 5. Effect of dietary crude protein level and/or feed additives on apparent digestibility of nutrients (%) and protein conversion ratio (g protein intake\ g gain) of broiler chicks

Dietary treatments	Protein converse-on ratio	Digestibility of nutrients (%)				
		Crude protein	Ether extract	Crude fibre	Ash retention	Dry matter
Effect of dietary crude protein level, %						
22-20	0.464^{a}	78.46	78.23	31.86	30.03	78.97
20.18	0.435^{b}	79.08	78.59	32.70	30.14	78.96
P-Value	0.0001	NS	NS	NS	NS	NS
Pooled SEM	0.00028	0.630	0.233	0.811	0.678	0.385
Effect of feed additives						
Without	0.473^{a}	76.93	77.08	29.25^{b}	28.84	76.64^{b}
Natuzyme	0.443^{b}	79.56	79.13	33.75^{a}	30.48	80.02^{a}
Probiotics	0.431^{c}	79.81	79.01	33.85^{a}	30.94	80.26^{a}
P-Value	0.0001	0.04	NS	0.01	NS	0.0003
Pooled SEM	0.00034	0.772	0.855	0.993	0.831	0.472
Interaction effect between dietary crude protein and feed additives						
High- (-)	0.486^{a}	76.89	77.13	29.38	29.63	76.75
High- Natuzyme	0.457^{c}	78.89	78.81	32.91	29.98	79.71
High- Probiotics	0.447^{d}	79.61	78.73	33.29	30.50	80.47
Low- (-)	0.460^{b}	76.98	77.04	29.13	28.05	76.53
Low- Natuzyme	0.429^{e}	80.23	79.45	34.59	30.98	80.32
Low- Probiotics	0.416^{f}	80.02	79.28	34.39	31.38	80.04
P-Value	0.0001	NS	NS	NS	NS	NS
Pooled SEM	0.0005	1.091	1.208	1.405	1.174	0.667
LSD	**0.004**	---	---	---	---	---

[a, b and c] means within the same column within the same treatment not having similar superscripts are significantly different ($P<0.05$). NS $P\geq0.05$.

Carcass characteristics and meat quality:

Data for carcass characteristics and body organs are presented in Tables (6 and 7). Results indicate that percentage dressing, inedible parts, liver, gizzard, heart, giblets and spleen were not significantly influenced by dietary protein level, nor there was a significant interaction between protein level and pro-nutrients supplementation in these parameters in addition to front and hind parts of carcasses. Furthermore, pro-nutrients supplementation did not significantly affect these parameters, too. On the other hand, the control diet increased the front part and decreased the hind part significantly. These results are similar to those reported by Piray *et al.* (2007) and Attia *et al.* (2001; 1998; 2007; 2008). They reported that enzymes and probiotics improved availability of nutrients and overcame the anti-nutritional substances.

Data for chemical composition of meat e.g. percentage DM, CP, EE and ash are presented in Table (8), while physical characteristics e.g. pH, color, tenderness and WHC are displayed in Table (9). Results indicate that protein level and/or pro-nutrients supplementations had no significant effect on chemical composition and consequently on physical characteristics of meat. These results are similar to those reported by Attia *et al.* (2001; 2003; 2008). These authors indicated that protein level and enzyme supplementation had no effect on chemical composition and physical characteristics of meat.

Table 6: Effect of dietary crude protein level and/or feed additives on carcass characteristics (%) of broiler chicks of 49 d old.

Dietary treatments	Carcass characteristics, %			
	Dressing	Edible parts	Front	Hind parts
Effect of dietary crude protein level				
22-20	63.02	32.61	33.16	28.34
20-18	62.71	32.74	34.35	29.88
P-Value	NS	NS	0.06	0.05
Pooled SEM	0.023	0.023	0.408	0.481
Effect of feed additives				
Without	62.65	32.61	33.62	29.03
Natuzyme	62.96	32.71	33.76	29.19
Probiotics	62.98	32.70	33.89	29.11
P-Value	NS	NS	NS	NS
Pooled SEM	0.692	0.692	0.500	0.589
Interaction effect between dietary crude protein and feed additives				
High- (-)	62.80	32.60	32.83	29.99
High- Natuzyme	63.27	32.44	33.68	29.59
High- Probiotics	63.00	32.78	32.96	30.06
Low- (-)	62.50	32.62	34.42	28.07
Low- Natuzyme	62.67	32.98	33.83	28.81
Low- Probiotics	62.97	32.61	34.82	28.16
P-Value	NS	NS	NS	NS
Pooled SEM	0.979	0.979	0.707	0.833

NS P≥0.05.

Table 7. Effect of dietary crude protein level and/or feed additives on body organs (%) of broiler chicks of 49 d old

Dietary treatments	Body organs, %				
	Liver	Gizzard	Heart	Giblets	Spleen
Effect of dietary crude protein level					
22-20	2.282	1.405	0.682	4.37	0.211
20-18	2.342	1.489	0.718	4.55	0.212
P-Value	NS	NS	NS	NS	NS
Pooled SEM	0.053	0.030	0.032	0.082	0.010
Effect of feed additives					
Without	2.538[a]	1.473	0.729	4.74[a]	0.209
Natuzyme	2.196[b]	1.435	0.688	4.32[b]	0.224
Probiotics	2.203[b]	1.433	0.683	4.32[b]	0.210
P-Value	0.005	NS	NS	0.01	NS
Pooled SEM	0.065	0.037	0.039	0.100	0.012
Interaction effect between dietary crude protein and feed additives					
High- (-)	2.509	1.405	0.688	4.60	0.200
High- Natuzyme	2.236	1.371	0.679	4.29	0.235
High- Probiotics	2.098	1.439	0.681	4.22	0.198
Low- (-)	2.567	1.541	0.771	4.88	0.219
Low- Natuzyme	2.156	1.500	0.698	4.35	0.213
Low- Probiotics	2.306	1.425	0.686	4.42	0.203
P-Value	NS	NS	NS	NS	NS
Pooled SEM	0.092	0.052	0.055	0.141	0.017

a, b means within the same column within the same treatment not having similar superscripts are significantly different (P<0.05). NS P≥0.05.

Table 8. Effect of dietary crude protein level and/or feed additives on chemical composition of fresh muscle of broiler chicks of 49 d old

Dietary treatments	Chemical composition of fresh muscle (%)			
	Moisture	Protein	Ether extract	Ash
Effect of dietary crude protein level				
22-20	74.74	19.69	4.157	1.399
20-18	74.54	19.79	4.287	1.330
P-Value	NS	NS	NS	NS
Pooled SEM	0.120	0.235	0.184	0.038
Effect of feed additives				
Without	74.83	19.57	4.210	1.397
Natuzyme	74.61	19.75	4.240	1.365
Probiotics	74.49	19.91	4.220	1.332
P-Value	NS	NS	NS	NS
Pooled SEM	0.147	0.287	0.225	0.046
Interaction effect between dietary crude protein and feed additives				
High- (-)	75.08	19.38	4.113	1.497
High- Natuzyme	74.61	19.77	4.217	1.350
High- Probiotics	74.53	19.93	4.140	1.350
Low- (-)	74.57	19.77	4.303	1.297
Low- Natuzyme	74.61	19.73	4.260	1.380
Low- Probiotics	74.46	19.89	4.290	1.313
P-Value	NS	NS	NS	NS
Pooled SEM	0.208	0.406	0.319	0.065

Dietary treatments	Biochemical constituents									
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/glb ratio	Total lipids (mg/dl)	Cholesterol (mg/dl)	Ca (mg/dl)	iP (mg/dl	AST (U)	ALT (U)
Effect of dietary crude protein level,%										
22-20	4.79	2.31	2.48	0.931	686	176.6	12.26	6.340	11.99	6.444
20-18	4.73	2.28	2.45	0.931	680	173.8	12.17	6.516	11.97	6.606
P-Value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM	0.053	0.027	0.027	0.0046	6.8	3.749	0.125	0.109	0.230	0.128
Effect of feed additives										
Without	4.79	2.32	2.47	0.939	676	172.6	11.81[b]	6.123[b]	11.85	6.856[a]
Natuzyme	4.76	2.29	2.47	0.927	689	178.1	12.75[a]	6.608[a]	12.11	6.556[ab]
Probiotics	4.74	2.27	2.47	0.919	682	174.9	12.09[b]	6.555[a]	11.99	6.163[b]
P-Value	NS	NS	NS	NS	NS	NS	0.001	0.03	NS	0.02
Pooled SEM	0.065	0.033	0.034	0.057	8.4	4.590	0.153	0.133	0.282	0.157
Interaction effect between dietary crude protein and feed additives										
High- (-)	4.87	2.37	2.50	0.948	686	171.8	11.69	6.078	11.97	6.893
High- Natuzyme	4.72	2.29	2.43	0.942	694	177.3	12.91	6.563	11.64	6.453
High- Probiotics	4.78	2.27	2.51	0.904	678	180.7	12.19	6.380	12.38	5.987
Low- (-)	4.70	2.27	2.43	0.934	666	173.3	11.93	6.168	11.73	6.820
Low- Natuzyme	4.81	2.30	2.51	0.916	683	178.9	12.59	6.653	12.57	6.660
Low- Probiotics	4.69	2.28	2.41	0.946	687	169.1	11.98	6.730	11.61	6.338
P-Value	NS	NS	NS	0.0001	NS	NS	NS	NS	NS	NS

NS P≥0.05.

Table 9. Effect of dietary crude protein level and/or feed additives on physical characteristics of meat of broiler chicks of 49 d old

Pooled SEM	0.092	0.047	0.048	0.008	11.8	6.492	0.216	0.189	0.399	0.222

NS P≥0.05.

Biochemical constituents of blood plasma

Results for biochemical composition of blood plasma are shown in Table (10). Results indicate that protein level and the interaction between protein level and pro-nutrients had no significant effect on plasma total protein, albumin, globulin, total lipids, cholesterol, liver functions as assayed by AST and ALT enzymes as well as plasma Ca and P. Furthermore, pro-nutrients had only significant effects on plasma Ca and inorganic phosphorus and ALT enzyme. Results indicate that Natuzyme increased plasma Ca and iP and had no significant effect on plasma ALT compared to the control group. Plasma albumin/globulin ratio was only significantly affected by the interaction between protein and pro-nutrient supplementation. It was found that probiotic and enzyme supplementation to the high and low protein-diet significantly increased plasma Glb/Alb ratio compared to the other treatment groups. The increase in plasma Ca and iP due to enzyme supplementations could be due to the presence of phytase in enzyme cocktail which is well known for improving mineral availability (Kies *et al*., 2001; Choct, 2006; Panda *et al*., 2007 and Selle *et al*., 2006). These results are in agreement with those reported by Attia *et al*. (2001 and 2003). On the other hand, probiotics significantly increased plasma iP and decreased plasma ALT compared to the unsupplemented control. The increase in plasma iP due to probiotic supplementation may be due to the presence of citric acid in the cocktail (Brenes *et al*., 2003; Ebrahimnezhad *et al*., 2008). They reported that citric acid improved phytate phosphorus utilization by complex with Ca and reduces the formation of more stable Ca-phytate complexes, and/or citric acid may change the intestinal pH for better phytase activity.

Results indicate that decreasing protein level did not affect liver functions and plasma mineral contents, furthermore, supplementation of enzymes mixture increased plasma iP, showing the positive effect on minerals availability.

In conclusion, low crude protein diet being 20 and 18% in the starting and growing-finishing diets respectively could be fed to broiler chicks when supplemented with probiotics cocktail containing *Bacillus subtillis* fermentation extract, Brewers yeast extract, lactic acid, citric acid, calcium propionate, sodium aluminosilicate and DL-methionine without adverse effect on growth performance and carcass quality, while improved economic efficiency.

Table (10). Effect of dietary crude protein level and/or feed additives on biochemical constituents of blood plasma of broiler chicks

Dietary treatments	Physical characteristics of meat			
	pH	**Colour**	**Tenderness**	**WHC**
Effect of dietary crude protein level,%				
22-20	6.494	0.210	2.780	5.551
20-18	6.491	0.184	2.832	5.673
P-Value	NS	NS	NS	NS
Pooled SEM	0.077	0.014	0.041	0.066
Effect of feed additives				
Without	6.361	0.202	2.800	5.677
Natuzyme	6.528	0.189	2.800	5.535
Probiotics	6.588	0.200	2.818	5.625
P-Value	NS	NS	NS	NS
Pooled SEM	0.095	0.017	0.051	0.081
Interaction effect between dietary crude protein and feed additives				
High- (-)	6.387	0.210	2.743	5.643
High- Natuzyme	6.473	0.211	2.797	5.463
High- Probiotics	6.623	0.210	2.800	5.547
Low- (-)	6.337	0.194	2.857	5.710
Low- Natuzyme	6.583	0.169	2.803	5.606
Low- Probiotics	6.553	0.191	2.837	5.703
P-Value	NS	NS	NS	NS
Pooled SEM	0.134	0.025	0.072	0.114

[a,b,] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$). NS = not significantly.

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تأثير إضافة البروبيوتك و مخلوط الإنزيمات المحتوي علي الفيتيز علي الصفات الإنتاجية و جودة اللحم و بعض المكونات البيوكيميائية في بلازما الدم لدجاج اللحم المغذي علي مستويات مختلفة من البروتين

فؤاد عبد الواحد عجور، فوزي صديق إسماعيل و السيد أحمد شقمق
قسم إنتاج الدواجن-كلية الزراعة-جامعة المنصورة

أجريت هذه الدراسة بهدف تحسين الاستفادة من مستويات البروتين المختلفة (٢٢أو ٢٠% في مرحلة البادي) و (٢٠ و ١٨% في مرحلة النامي- الناهي) في علائق دجاج اللحم عند إضافة مخلوط الإنزيمات التجاري المحتوي علي الفيتيز و البروبيوتك و ذلك في علائق نباتية مكونه طبقا لتوصيات المجلس القومي لبحوث بأمريكا ١٩٩٤، و بهذا تكونت ٦ معاملات تجريبية في تصميم عاملي يتكون من مستوين من البروتين الخام و ثلاث معاملات تحت كل مستوي و غذيت العلائق في الفترة من ١-٤٩ يوم من العمر و عند نهاية التجربة ذبحت ثلاث طيور من كل معاملة و أجريت تجربة لدراسة كل من صفات النمو و معاملات الهضم و خصائص الذبيحة و جودة اللحم بعض مكونات بلازما الدم البيوكيميائية

وأظهرت النتائج آلاتي:-

١- زاد معدل النمو معنويا نتيجة التغذية علي مستوي البروتين الموصي به، بينما نقص استهلاك العلف و تحسنت الكفاءة التحويلية للغذاء و معدل تحويل البروتين و الكفاءة الاقتصادية، زادت منشطات النمو من معدلات النمو و خفضت من استهلاك العلف و حسنت من الكفاءة التحويلية للغذاء و معدل تحويل البروتين و الكفاءة الاقتصادية و كانت كفاءة البروبيوتك أفضل من مخلوط الإنزيمات المحتوي علي الفيتيز، كما أدي إضافة مخلوط الإنزيمات أو البروبيوتك الي العلائق المنخفضة في البروتين الخام إلي تحسين معدلات النمو و الكفاءة التحويلية للغذاء و معدل تحويل البروتين و الكفاءة الاقتصادية بحيث أصبحت أفضل من مجموعة الكنترول الايجابي و كانت كفاءة البروبيوتك أفضل من مخلوط الإنزيمات في كلا مستوي البروتين.

٢-أدت إضافة البروبيوتك و مخلوط الإنزيمات إلي تحسنا معنويا في معامل هضم المادة الجافة و الألياف الخام بالمقارنة بمجموعة الكنترول.

٣-أدي زيادة مستوي البروتين إلي زيادة معنوية في النسبة المئوية للجزء الأمامي للذبيحة و نقص في نسبة الجزء الخلفي و الصدري، ولم تتأثر جودة اللحم من حيث التحليل الكيماوي و الصفات الطبيعية للحوم بالمعاملات تحت الدارسة.

٥- أدي مخلوط الإنزيمات إلي زيادة معنوية في محتوي بلازما الدم من الكالسيوم و الفسفور، بينما أدي إضافة البروبيتك إلي زيادة معنوية في محتوي بلازما الدم من الفسفور و نقص في محتوي بلازما الدم من أنزيم ALT مما يظهر التحسن في وظائف الكبد.

ومن هذا يتضح إمكانية استخدام العلف النباتي المحتوي علي ٢٠% بروتين خام في علائق البادي و ١٨% بروتين خام في علائق النامي و الناهي عند تدعيمه بالبرويتويك دون نتائج سالبية علي معدلات النمو والكفاءة التحويلية للغذاء و الكفاءة الاقتصادية مع تحسن الكفاءة الاقتصادية مما يستدعي إجراء مزيد من البحوث لخفض تكلفة العلف.