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

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ABSTRACT

The influence of different dietary protein sources and two types of pro-nutrients e.g. enzyme mixture containing phytase and probiotics on growth performance, carcass characteristics, meat quality and plasma biochemical constituents of broiler chicks was studied herein as means of improving the utilization of diets containing different plant protein sources and decreasing feed cost. A complete randomized straight run experimental design including 10 dietary treatments was conducted. A basal control corn-soybean meal diet containing 22 and 20% CP in the starting and growing-finishing periods, respectively was formulated. Sunflower meal (SFM) and rocket (*Eruca sativa*) meal (RM) individually or as a mixture of (1:1; Wt::Wt) replaced 25% of soybean meal protein. Thus, there were four main experimental diets. Moreover, the diets containing SFM and/or RM was either supplemented or not with enzyme mixture containing phytase (Natuzyme) or probiotics (Nutri-Bio Plus). Thus, there were 10 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 (30×35×40 cm). At the end of the experiment (49 d of age), 3 chicks of each treatment were slaughtered to determine carcass characteristics and meat quality traits. Furthermore, four blood samples per treatment were collected to determine some biochemical constituents. Also, a digestibility trail and an economic efficiency study were carried out.

Although, substituting 25% of soybean meal protein by SMF and/or RM had no adverse effect on growth or feed conversion ratio (FCR) it improved protein conversion ratio (PCR) and economic efficiency of broiler chicks. Furthermore, Natuzyme and probiotics supplementation significantly improved growth, FCR, PCR and the economic efficiency, with the effect of probiotics was more efficient. Feed intake was significantly decreased due to inclusion of SFM and/or RM and further decreased by enzymes and probiotic supplementation, however, the effect of probiotics was stronger than enzymes. Enzyme supplementation improved significantly digestibility of dry matter of diet containing a mixture of SFM and RM. Sunflower meal and/or RM had no effect on dressing percentage, front and hind parts, meat quality and plasma total protein, globulin and albumin/globulin ratio, total lipids, cholesterol, inorganic phosphorous (iP), Ca and AST and ALT enzymes.

In conclusion, SFM and/or RM could be included in broiler diets to replace 25% of soybean meal protein without adverse effects on growth performance and economic efficiency. Furthermore, enzyme mixture and probiotics supplementation resulted in improved growth performance and economic efficiency. Whereas, probiotic supplemented-diet containing a mixture of SFM and RM resulted in the best productive performance and economic efficiency.

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

INTRODUCTION

Recently, protein nutrition represented a major challenge to poultry production especially in the region where protein rich feedstuffs are limited. The practical poultry feeding experienced to use soybean meal as a mean plant protein supplement since the discovery of cow mad disease (BSE) in the last decade (Attia *et al*., 2003; El-Deek *et al*., 2003). Formulating poultry diets based on corn-soybean meal has increased soybean meal demands and accordingly raised feeding cost. One of the possible approaches to reduce the feed cost for poultry is the use of alternative locally available protein sources. Diets containing soybean meal as a sole protein source may be deficient in methionine unless correctly formulated with other protein or synthetic amino acids (McNaughton and Deaton, 1981). Sunflower meal and rocket meal are by-products of sunflower oil and rocket oil extraction respectively, are available in the Egyptian market for use in animal feed and would be important when soybean meal is in shortage (Michel and Sunde 1985; Attia *et al*., 2003; El-Deek *et al*., 2003). Alib *et al*. (1988), Attia *et al*. (2003) and El-Deek *et al*. (2003) reported that sunflower meal containing 24.4-29% CP, 16.3-18.3% NFE, 14.6-15.8% CF and 2.9-3.3% ash. They added that the maximum amount of sunflower meal that could include in broiler diets appeared to be 15.0% in all mash diets and 30.0% in pelleted diets. Higher levels may adversely affect performance, however it could completely replace the supplemental protein if sufficient supplemental lysine is added (Deaton *et al*., 1979; McNaughton and Deaton 1981). They concluded in accordance with the results reported by Waldroup *et al*. (1970), Rad and Keshavarz (1976) and Zatari and Sell (1990a) that sunflower meal could replace 50.0% of soybean meal or included between 10.0-15.0% in the diet. Although, El-Sherif *et al*. (1995) reported that growth and feed-to-gain ratio of broilers fed diet containing 15.0% dehulled sunflower meal were poorer than those of the soybean meal-control ones. Rocket meal is expected to increase in the local market due to its use as a medical plant.

On the other hand, research work with rocket meal is limited. Osman *et al*. (2004), Fagbenro (2004) and El-Shafei *et al*. (2007) found that rocket meal contains 30-20-36.03% CP; 5.65-7.64% EE, 7.69-10.20% CF,8.36-11.83% ash and 36.81-38.22% NFE and could be used up to 10% in broiler diets, 20% of dietary protein in catfish and 15% in the diet for growing Japanese quail, respectively. Rocket meal had 0.34-0.35% methionine, methionine and cystine 0.42-.54%, 1.18-1.93% lysine, 0.98% threonine, 2.08% arginine and 1.27% valine (Osman *et al*., 2004; El-Shafei *et al*., 2007), and 0.45% Ca and 0.60% total phosphorus.

Fiber content of sunflower meal may restrict its use to 8.0% in broiler-growing diets and 16.0% in broiler-finishing diets, but not restrict its use in layer diets (McNaughton and Deaton, 1981). Sunflower meal NSP contains 11.5% uronic acid (pectin), 13.0% xylose and 7.7% arabinose (Carré and Brillouet, 1986). Sunflower meal NSP was higher than that of soybean meal, due to increased concentrations of cellulose and xylose in the insoluble NSP and uronic acids in soluble NSP (Irish and Balnave, 1993). It was concluded that formulating poultry diets required specific attention to the nutrient-diluting effects of sunflower meal fiber or NSP (Carré and Brillouet 1986; Francesh *et al*., 1995) and the limiting amino acids lysine, arginine and threonine (Cuca *et al*., 1973; Tsvetanov *et al*., 1984).

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, Piray *et al.*, 2007). Enzyme mixture or phytase may be a practical mean to improve performance and permit utilization of higher levels of agro-industrial by-product in monogastric animal nutrition (Attia *et al*., 2001). 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 (Jeroch *et al*., 1995; Saleh *et al*., 2003; Yonemochi *et al*., 2003; Gahzalah *et al.*, 2005; Meng *et al*., 2005; Choct, 2006). Thus, the effect of probiotic and enzyme mixture containing phytase on growth performance, carcass characteristics, meat quality and biochemical constituents of blood plasma of broiler chicks fed diets containing a mixture of different plant protein sources was investigated herein as means of improving nutrinet utilization and reducing feeding costs of broiler chicks.

MATERIALS AND METHODS

Experimental design, birds and diets

A complete randomized straight run design including 10 dietary treatments was conducted. A basal control corn-soybean meal and corn gluten meal diet containing 22 and 20% CP in the starting and growing-finishing periods, respectively was formulated. Sunflower meal and rocket (*Eruca sativa*) meal individually or as a mixture of (1:1) replaced 25% of soybean meal protein. Thus, there were four main experimental diets in the starting (Table 1), and growing–finishing periods (Table 2). Moreover, the diets containing sunflower meal and/or rocket meal was fed either without or with enzyme mixture containing phytase (Natuzyme[1]) and probiotics (Nutri-Bio Plus[2]). Thus, there were 10 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=240 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 formulated based on tables of feedstuffs, and met

[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.**

nutrient requirements of broiler chicks (NRC, 1994). 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.

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

Ingredients,%	**Starting diets**			
	Control	**25%SFM**	**25% RSM**	**25%SFM+RSM**
Yellow corn	60.00	55.50	60.00	57.50
Soybean meal (44% CP)	19.00	10.61	10.00	10.48
Corn Gluten meal (%)	13.78	11.48	11.30	11.29
Sunflower meal	0.00	14.48	0.00	7.24
Rocket meal	0.00	0.00	13.45	6.73
Commercial oil blend	2.63	3.82	2.39	3.16
Dicalcium phosphate	1.77	1.73	1.47	1.60
Lime stone	1.45	1.35	0.25	0.87
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	0.03	0.12	0.08
L-lysine	0.29	0.42	0.42	0.44
Wash building sand	0.46	0.00	0.00	0.00
Total	**100.0**	**100.0**	**100.0**	**100.0**
Calculated values				
ME-**kc**al/kg diet	3192	3213	3197	3203
Crude protein	22.1	22.2	21.7	22.0
Methionine %	0.45	0.44	0.50	0.48
Methionine plus cystine %	0.84	0.81	0.82	0.83
Lysine, %	1.03	1.01	1.02	1.03
Ca , %	1.00	0.96	0.95	0.98
Available P, %	0.45	0.43	0.43	0.43
Determined values				
Dry matter,%	89.51	89.38	89.74	89.18
Crude protein	21.73	21.81	21.77	21.86
Ether extract, %	5.21	6.18	5.08	6.01
Crude fibre, %	2.43	3.11	2.69	2.86
Crude ash, %	9.81	9.67	9.58	9.74

[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.**

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. 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 49 wk of age, 3 chicks were taken randomly from each treatment, and slaughtered; the remaining carcass after bleeding, was plucked and eviscerated then weighed (dressed weight) and divided into chest 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). Color 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)

Table (2).Composition and calculated analyses of the growing-finishing experimental diets

Ingredients,%	Growing-finishing diets			
	Control	25% SFM	25%RSM	25%SFM+RSM
Yellow corn	63.90	60.00	60.00	60.00
Soybean meal (44% CP)	18.99	9.50	10.00	9.83
Corn Gluten meal (%)	10.03	9.00	9.30	9.00
Sunflower meal	0.00	13.16	0.00	6.58
Rocket meal	0.00	0.00	12.23	6.11
Commercial oil blend	2.82	3.725	3.59	3.69
Dicalcium phosphate	1.80	1.76	1.53	1.65
Lime stone	1.20	1.215	0.24	0.72
Vit+Min mixture[1]	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
Dl-methionine	0.05	0.08	0.14	0.11
L-lysine	0.30	0.48	0.44	0.46
Wash building sand	0.31	0.48	1.93	1.25
Total	100.0	100.0	100.0	100.0
Calculated values				
ME-kcal/kg diet	3201	3212	3201	3206
Crude protein	20.21	20.25	20.11	20.12
Methionine %	0.43	0.44	0.48	0.46
Methionine plus cystine %	0.78	0.78	0.78	0.78
Lysine, %	1.00	1.00	1.00	1.00
Ca , %	0.91	0.91	0.91	0.95
Available P, %	0.43	0.44	0.48	0.46
Determined values				
Dry matter,%	89.71	89.50	89.39	89.65
Crude protein	19.83	19.76	19.71	19.84
Ether extract, %	5.25	6.31	5.10	6.18
Crude fibre, %	2.41	2.98	2.69	2.80
Crude ash, %	9.71	9.83	10.51	10.46

[1] As shown in Table 1.

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 -20ºC 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), and inorganic P (Gomorri, 1942) were determined by colorimetric method using commercial available kits. Globulin was calculated by differences between total protein and albumin. Furthermore, economic evaluation for all experimental diets was done 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 using Duncan New multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance, protein conversion ratio and economic efficiency

Growth of broiler chicks as affected by different plant protein sources without or with pro-nutrients is presented in Table (3). Data indicate that growth of chicks was significantly affected by dietary treatments at 14, 28 and 49 d of age and for the whole experimental period. The present results indicate that inclusion of 25% of SFM and/or RM had no harmful effect on growth of broiler chicks. However, a combination of SFM and RM significantly increased growth by ~2.5% of broilers at only 14 d of age compared to the control group when unsupplemented diets were compared. The superiority of diet containing combination of plant protein sources indicated a complementary effect which might have increased availability of amino acids and/or eliminate the anti-nutritional substance or amino acid imbalance (Irish and Balnave, 1993 and Attia *et al*., 1998; 2003). Attia *et al*. (1998; 2003), Senkoyla and Dale (1999) and Daghir (2008) concluded that SFM may be included in poultry diets up to 15% without adverse effect on growth when supplemented with adequate amino acids. On the other hand, results reported by Osman *et al.* (2004) reported that higher than 10% of RM in broiler diets adversely affect performance. Also, El-Shafei *et al*. (2007) indicated that RM could be used up to 15% in the diet for growing Japanese quail without adverse effect on growth performance. Whereas, Fagbenro (2004) found that RM at 20% of total dietary protein did not affect growth of African catfish.

Enzymes and probiotics supplementations to SFM and RM diets significantly increased growth compared to its negative control; however, probiotics was more efficient with each protein source. Difference between enzymes and probiotics was significant up to 28 d of age, and diminished in SFM and RM thereafter. However, probiotic had a superior significant positive effect on diet containing a combination of SFM and RM than enzyme supplemented-group. Results indicate that the effect of pro-nutrients depends on dietary composition and age of chicks, as probiotics had strong effect on SFM containing diets and up to 28 d of age. These results are in agreement with those reported by Soliman *et al*. (1996) and Attia *et al*. (1998; 2003) who reported that enzyme addition to duck and broiler diets containing different levels of sunflower meal had some beneficial effect on growth and feed utilization especially at the high sunflower meal level. However, Kocher *et al*. (2000) indicated that commercial enzyme products had no appreciated effect on sunflower meal containing-diet. On the other hand, the positive effect of probiotic cocktail 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. These results are in agreement with those reported by Makled (1991), Patterson and Burkholder, 2003; Sun *et al.* (2005), FAO (2006) and Piray *et al.* (2007).

For the whole experimental period, inclusion of SFM and/or RM in broiler diets to replace 25% of soybean meal did not significantly affect growth, indicating lack of negative effects. On the other hand, both Natuzyme and probiotics significantly improved growth compared to their negative control in each diet, however, difference was only significantly in favor of probiotics in the diet containing a combination of SFM and RM. It is clear that any diet supplemented with Natuzyme or probiotics had better growth than the positive control, showing the beneficial of pro-nutrients for improving nutrient utilization. It was found that the highest growth was from group fed a mixture of SFM and RM supplemented with probiotics, while the lowest growth was from unsupplemented group fed RM, with as significant difference between these groups.

There were no dead birds during the experimental period. Similarly, Attia *et al*. (1998; 2003), Suresh *et al*. (2000), Osman (2004), Fagbenro (2004) and El-Shafei *et al*. (2007) found that SFM and RM had no adverse effect on morality rate of birds.

Feed intake of broiler chicks as affected by different plant protein sources without or with pro-nutrients is presented in Table (3). Data indicate that feed intake of chicks was significantly affected by dietary treatments during 1-14, 15-28 and 29-49 as well as during 1-49 d of age. Results reveal that inclusion of 25% of SFM and/or RM significantly decreased feed intake of broilers during all experimental period compared to the control. The results indicate that group fed diet containing a mixture of SFM and RM consumed lower feed intake than those fed RM alone during 1-14, 15-28 and 1-49 d of age. Whereas, difference was insignificant between groups fed RM or SFM and those fed a mixture of SFM and RM during 29-49 d of age. These results indicate that a combination of SFM and RM had stronger negative effect on feed intake compared to any of them. For the whole experimental period, the decrease in feed intake due to inclusion of SFM and/or RM was 1.8, 1.2 and 2.2% respectively. These results are in agreement with those reported by Attia *et al*. (1998; 2003) with SFM fed to Peckin ducks and broiler chicks. No adverse effect of RM on feed intake of broiler chicks was also shown by Osman *et al*. (2004), also El-Shafei *et al*. (2007) found no effect of RM on feed intake of Japanese quail.

Enzymes and probiotics supplementation to different plant sources containing diets had further significant negative effect on feed intake compared to their negative control.

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Furthermore, probiotic had stronger negative effect than enzymes supplementation in each diet and this depends on dietary composition and age of chicks. For the whole experimental period, enzymes and probiotics decreased feed intake by 2.7 and 3.6% respectively, of SFM diet compared to their negative control. The corresponding value for RM diet was 2.2 and 3.6% respectively. Meanwhile, the corresponding value for diet containing a mixture of SFM and RM was 3.4 and 5.7% respectively. This indicates that enzymes and probiotics had stronger effect on diet containing a mixture of SFM and RM than diet containing SFM or RM only. The effect of enzymes and probiotics on feed intake is related to dietary composition and age of chicks (Jeroch *et al*., 1995; Attia *et al*., 2001). Kidd *et al*. (2001) reported that enzymes did not affect feed intake of broiler chicks. On the other hand, Piray *et al*. (2007) observed that prebiotic increased feed intake compared to the unsupplemented control group.

Feed conversion ratio, PCR and economic efficiency of broiler chicks as affected by different plant protein sources without or with pro-nutrients are presented in Table (4). Data indicate that FCR of chicks was significantly influenced by inclusion of SFM and/or RM during 1-14, 15-28 and 29-49 d of age and for the whole experimental period, too. The present results indicate that inclusion of 25% of SFM and/or RM had no adverse impact on FCR of broiler chicks. However, a combination of SFM and RM significantly improved FCR by ~6.2% of broilers during 1-14 d of age compared the control group when unsupplemented sources were compared. This may be due to the 2.5% increase in growth and 2.1% decrease in feed intake. Also, a combination of SFM and RM significantly improved FCR compared to the SFM or RM during the same period. During 15-28 and 29-49 d of age, there were no significant differences in FCR among the unsupplemented diets containing different plant protein sources. For the whole experimental period, FCR was numerically improved due to inclusion of SFM and RM compared to the control group. Meanwhile, inclusion of a mixture of SFM and RM resulted in significantly better (2.9%) FCR compared to the control group. Attia *et al*. (1998; 2003) Senkoyla and Dale (1999) and Daghir (2008) concluded that SFM may be included in poultry diets up to 15% without adverse effect on FCR when supplemented with adequate amino axids. On the other hand, results observed by Osman *et al.* (2004) with broiler chicks, El-Shafei *et al*. (2007) with Japanese quail and Fagbenro (2004) with African catfish indicated that up to 10-15% of RM in broiler and Japanese quail diets and 20% of total dietary protein did not adversely affect FCR. On the other hand, El-Sherif *et al*. (1995) reported that FCR of broilers fed diet containing 15.0% dehulled sunflower meal were poorer than those of the soybean meal-control ones.

Protein conversion ratio was significantly improved due to inclusion of SFM and /or RM in broiler diets, with the effect of RM was significantly better than SFM and the mixture was better than both. These results provided further evidences for the improvement in amino acids balance due to inclusion of different plant protein sources. Attia *et al*. (1998; 2003) reported similar results.

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Economic efficiency was significantly increased due to inclusion of SFM and /or RM in broiler diets, with the effect of RM was similar to that of RM and the mixture was better than both.

Enzymes and probiotics supplementations to SFM and/or RM containing diets significantly improved FCR compared to its negative control; however, probiotics was significantly more efficient within each protein source. Difference between enzymes and probiotics was significant up to 28 d of age, and diminished in SFM and RM thereafter, while remains significant in the diet containing the mixture of two tested plant protein sources with superiority of probiotics.

For the whole experimental period, both Natuzyme and probiotics significantly improved FCR and PCR compared to their negative control in each tested diet. Furthermore, the difference was significant in favor of probiotics in each tested ingredient. For example, enzymes and probiotics improved FCR of SFM diet by 6.4 and 9.3%, respectively. The corresponding values for RM diet were 5.0 and 8.0%, respectively. For diet containing the mixture, the improvements due to enzymes and probiotics were 7.0 and 11.6%, respectively. It was found that Natuzyme or probiotics supplemented-diet had better FCR and PCR than the positive control, showing the beneficial of pro-nutrients for improving nutrient utilization. It was found that the best FCR and PCR was from group fed a mixture of SFM and RM supplemented with probiotics, while the poorest FCR and PCR was from unsupplemented control group fed corn-SBM and corn gluten meal diet, with a significant difference (14.2 and 9.3%, respectively) between these groups. Results reveal that the effect of pro-nutrients depends on dietary composition and age of chicks, as enzymes and probiotics had strong effect on SFM containing diets. This indicates that sunflower meal was targeted by enzymes mixture and probiotic compared to RM diet. It should be mentioned, however, that the effect of enzymes or probiotics could not totally attributed to its effect on SFM and/or RM but also to the other components of the experimental diets such as corn, soybean meal and corn gluten meal (Attia *et al*., 2003; 2007; 2008; Saleh *et al*., 2003; Choct, 2006)

These results are in agreement with those reported by Soliman *et al*. (1996) and Attia *et al*. (1998; 2003) who reported that enzyme addition to duck and broiler diets containing different levels of sunflower meal had some beneficial effect on feed utilization especially at the high sunflower meal level. However, Kocher *et al*. (2000) indicated that commercial enzyme products had no appreciated effect on sunflower meal containing-diet. On the other hand, the positive effect of probiotic cocktail are in agreement with those reported by Makled (1991), Patterson and Burkholder, (2003), Sun *et al*. (2005), FAO (2006) and Piray *et al*. (2007).

Economic efficiency was significantly improved due to supplementation of enzymes mixture and probiotics, and the effect of probiotics was significantly stronger in each diet than the effect of enzyme mixture. The results indicate that the best economic efficiency was from probiotic supplemented-diet containing a mixture of SFM and RM, while the poorest was from the positive control.

Apparent digestibility of nutrients and ash retention:

Results for the effect of dietary composition and pro-nutrients supplementation on apparent digestibility of the experimental diets are shown in Table (4). Results demonstrate that SFM and/or RM and enzyme supplementation had no negative effects on apparent digestibility of nutrient and ash retention. The results indicate that enzyme supplementation to diet containing 25% of mixture of SFM and RM increased digestibility of dry matter by 4.7 and 4.2% compared to the positive and the negative control. This group also, had better digestibility of DM than the unsupplemented SFM or RM diet. There was no significant difference between enzymes supplemented diets or probiotics supplemented ones when comparison was made within or over different tested sources. The increase in dry matter digestibility could enhance ME value of the diet, and increase ME availability for growth (Jeroch *et al*., 1995; Cowan *et al*., 1996; Zanella *et al*.1999, Ghazalah *et al*., 2005 and Attia *et al*. 2007 and 2008). However, Rebolé *et al*. (1999) reported that enzyme addition to broiler diets containing 15% sunflower meal did not significantly improved digestibility of protein/amino acid, fat/fatty acids and ME.

Carcass characteristics and body organs:

Results for the effect of dietary composition and pro-nutrients supplementation on carcass characteristics and body organs are shown in Table (5). Results indicate that SFM and/or RM and enzyme supplementation had no significant effects on percentage dressing, inedible parts, front and hind parts and heart. Also Osman *et al.* (2004) and El-Shafei *et al*. (2007) reported that RM up to 15% in broilers and Japanese quail diets had no adverse effects on carcass characteristics and body organs.

SFM caused spleen hypertrophy compared to the other unsupplemented diets, while RM significantly decreased liver percentage compared to the other unsupplemented diets. The increase in spleen percentage due to inclusion of sunflower indicated the negative effect of sunflower NSP on gut ecology. Carré and Brillouet (1986) indicated that NSP of sunflower meal contain 11.50% uronic acid (pectin), 13.0% xylose and 7.7% arabinose. The fermentation of these substances may encourage the growth of pathogenic organisms (Attia *et al*., 1998; 2003) due to lack digestive matching enzymes. This was confirmed by the positive effect of probiotic on spleen, thus complete recovery was observed compared to the positive control.

It was found that probiotic supplementation decreased liver percentage of SFM diet compared to its negative control and the positive control, too. Supplementation with either enzymes or probiotic to 25% RM diet significantly decreased gizzard percentage compared to its negative control, and the effect of probiotic was more efficient, thus difference from the positive control was diminished. Difference in giblets percentage due to different unsupplemented protein sources was insignificant; however, proboitic supplementation to SFM and/or RM diets significantly decreased giblets percentage compared to its negative control. Enzyme supplementation exhibited similar effect on only the diet containing a mixture of SFM and RM. These results are similar to those reported by Piray *et al.* (2007) and Attia *et al.* (1998; 2001; 2007; 2008). These authors reported that pro-nutrients e.g. enzymes and probiotics improved availability of nutrients and overcame the anti-nutritional substances.

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Meat quality:

Data for chemical composition of meat e.g. percentage DM, CP, EE and ash and physical characteristics e.g. pH, color, tenderness and WHC are shown in Table (5). Results reveal that different protein sources 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.* (1998; 2003; 2008). These authors indicated that protein sources and enzyme supplementation had no effect on chemical composition and physical characteristics of meat.

Biochemical constituents of blood plasma

Results for biochemical composition of blood plasma and plasma Ca and iP are shown in Table (6). Results reveal that SFM and/or RM and pro-nutrient supplementation had no effect on plasma total protein, globulin and albumin/globulin ratio, total lipids, cholesterol, inorganic phosphorus, Ca and AST and ALT enzymes. These results are similar to those reported by El-Deek *et al*. (2003) with SFM, Osman *et al*. (2004) found no negative effect of RM up to 15% in broiler blood hemoglobin, plasma total protein, albumin, globulin and albumin to globulin ratio, while more than 5% significantly decreased plasma total lipids, cholesterol, GOT and GPT. Also, El-Shafei *et al*. (2007) found that RM had no adverse effects on blood contents of Japanese quail.

Results show that plasma albumin was significantly decreased due to inclusion of SFM and RM mixture compared to only the positive control. However, enzyme and probiotic supplementation restored plasma albumin to the level of the positive control. Also, enzyme supplementation to the diet containing a mixture of SFM and RM increased plasma Ca compared to the unsupplemented diet containing SFM and/or RM and the positive control, too. This increase in plasma Ca due to enzyme supplementation may be due to phytase containing of the enzyme mixture which is well known for improving mineral availability (Kies *et al*., 2001; Choct, 2006; Panda *et al*., 2007 and Selle *et al*., 2006). The results indicated that probiotics had no significant effect on plasma Ca level, however, differences from enzyme supplemented groups were not significant.

The present results indicate that inclusion of SFM and/or RM did not affect liver functions, furthermore, supplementation of enzymes mixture and probiotics had nor effect, too. These results are similar to those reported by Attia *et al*. (2001; 1998; 2003).

In conclusion, SFM and/or RM could be included in broiler diets to replace 25% of soybean meal protein without adverse effect on growth performance and economic efficiency; furthermore enzyme mixture and probiotics supplementation resulted in improved growth performance and economic efficiency. Whereas, probiotic supplemented-diet containing a mixture of SFM and RM resulted in the best productive performance and economic efficiency.

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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 علائق تحتوي علي مصادر مختلفة من البروتين النباتي

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أجريت هذه الدراسة بهدف تحسين الاستفادة من العلائق التي تحتوي علي مصادر مختلفة من البروتين (كسب الجيرجير، كسب عباد الشمس الغير منزوع القشرة أو مخلوطهما) التي حلت جزئيا محل (٢٥% من بروتين كسب فول الصويا) في علائق دجاج اللحم في مرحلتي البادي (٢٢% بروتين خام) والنامي- الناهي (٢٠% بروتين خام) عند إضافة مخلوط الإنزيمات التجاري المحتوي علي الفيتيز و البروبيوتك و ذلك في علائق نباتية متساوية في العناصر الغذائية مكونه طبقا لتوصيات المجلس القومي لبحوث بأمريكا ١٩٩٤، و بهذا تكونت ١٠ معاملات تجريبية يتكون من اربعة علائق اساسية و هي تلك الخالية من كسب عباد الشمس و/أو كسب الجيرجير و تكوين تحت العلائق المحتوية علي مصادر البروتين النباتي البديلة ٣ علائق غذيت في وجود أو عدم وجود مخلوط الإنزيمات التجاري المحتوي علي الفيتيز و البروبيتك و غذيت العلائق في الفترة من ١-٤٩ يوم من العمر و ذبحت ثلاث طيور عند نهاية التجربة و درست صفات النمو و معاملات الهضم وخصائص الذبيحة و جودة اللحم بعض مكونات بلازما الدم البيوكيميائية و الجدوى الاقتصادية وأظهرت النتائج آلاتي:-

١-لم يؤثر مصدر البروتين في الزيادة في وزن الجسم و الكفاءة التحويلية للغذاء، بينما حسن معنويا من معدل تحويل البروتين و الكفاءة الاقتصادية بينما قلل من معدل استهلاك الغذاء و أدي إضافة منشطات النمو الي زيادة وزن الجسم و تحسن الكفاءة التحويلية للغذاء، و معدل تحويل البروتين و الكفاءة الاقتصادية وقلل من معدل استهلاك العلف و كانت كفاءة البروبيوتك أفضل من الإنزيمات و كان أفضل معدل نمو و كفاءة تحويلية للغذاء و البروتين و كفاءة اقتصادية للمجموعة التي غذيت علي علف يحتوي علي ٢٥% من مخلوط كسب عباد الشمس و كسب الجيرجير المدعم بالبروبيوتك .

٢-أظهرت النتائج تحسين معامل هضم المادة الجافة نتيجة إضافة الإنزيمات للعلائق المحتوية علي مخلوط كسب عباد الشمس و الجيرجير فقط.

٣-لم تؤثر المعاملات التجريبية و إضافة منشطات النمو علي النسبة المئوية للتصافي و نسبة الجزء الأمامي أو الخلفي للذبيحة.

٤-لم تتأثر جودة اللحم من حيث التحليل الكيماوي أو الصفات الطبيعية للحوم باستخدام كسب عباد الشمس و/أو كسب الجيرجير حتي نسبة ٢٥% من بروتين كسب فول الصويا أو بإضافة منشطات النمو.

٥- لم تتأثر معظم مكونات الدم البيوكيمائية ووظائف الكبد أو الكالسيوم و الفسفور ببلازما الدم باستخدام كسب عباد الشمس و/أو كسب الجيرجير حتي نسبة ٢٥% من بروتين اكسب فول الصويا أو إضافة منشطات النمو.

ومن هذا يتضح إمكانية استخدام كسب عباد الشمس و /أو الجيرجير حتى نسبة ٢٥% من بروتين كسب فول الصويا دون تأثيرات سلبية علي صفات النمو بينما أدت إضافة البروبيوتك إلي العلائق المحتوية علي مخلوط من كسب عباد الشمس و الجيرجير إلي تفوق تلك العليقة معنويا في معدلات النمو و الكفاءة التحويلية للغذاء و معدل تحويل البروتين و الكفاءة الاقتصادية مقارنة بباقي المعاملات التجريبية الاخري مما يستدعي إجراء مزيد من الدراسات علي مثل هذه التركيبات من الأعلاف كمصادر علفية بديلة لدجاج اللحم.

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Table (3): Effect of different protein sources and supplementation with multienzymes containing phytase and probiotics on growth (g/bird/period) and feed intake (g/bird/period) of broiler chicks.

Criteria	Control	Sunflower meal			Rocket meal			Sunflower rocket mixture			SEM	P value
		–	Enzymes	Probiotics	–	Enzymes	Probiotics	–	Enzymes	Probiotics		
Growth of broilers during 1 to 49 d of age, g/chick												
Body weight, 1 d of age g	40.1	40.9	39.7	40.9	40.4	39.7	39.7	39.9	40.0	39.9	0.109	NS
BWG, 1-14 d of age	290.8^{f}	291.6^{f}	329.0^{cd}	341.9^{a}	290.3^{f}	324.6^{d}	336.4^{b}	298.1^{e}	330.9^{c}	345.8^{a}	1.824	0.001
BWG, 15-28 d of age	830.2^{e}	834.0^{e}	893.9^{cd}	928.3^{ab}	828.2^{e}	882.6^{d}	912.5^{bc}	837.5^{e}	897.5^{cd}	937.4^{a}	7.275	0.001
BWG, 29-49 d of age	2212.6^{d}	2214.6^{d}	2301.6^{bc}	2354.7^{ab}	2205.5^{d}	2288.2^{c}	2332.5^{abc}	2226.1^{d}	2311.1^{bc}	2375.7^{a}	17.81	0.001
BWG, 1-49 d of age	2172.5^{d}	2173.7^{d}	2262.0^{bc}	2313.9^{ab}	2165.1^{d}	2248.5^{c}	2292.9^{abc}	2186.2^{d}	2271.1^{bc}	2335.8^{a}	17.40	0.001
Feed intake of broilers during 1 to 49 d of age, g/chick/period												
Feed intake, 1-14 d of age	410.5^{a}	404.0^{bc}	399.5^{def}	392.3^{gh}	406.3^{b}	400.5^{cde}	396.5^{efg}	401.9^{cd}	395.6^{fg}	390.1^{h}	1.444	0.0001
Feed intake, 15-28 d of age	1114.8^{a}	1090.5^{b}	1016.6^{e}	999.0^{g}	1097.3^{b}	1034.0^{d}	1009.1^{ef}	1079.5^{c}	1003.6^{fg}	990.4^{h}	2.856	0.0001
Feed intake, 29- 49 d of age	3303.0^{a}	3248.4^{bc}	3199.6^{de}	3178.6^{ef}	3264.8^{b}	3227.1^{cd}	3192.5^{ef}	3241.5^{bc}	3163.3^{f}	3074.5^{g}	10.89	0.0001
Feed intake, 1-49 d of age	4828.1^{a}	4742.4^{bc}	4615.8^{e}	4569.9^{f}	4768.4^{b}	4661.3^{d}	4598.0^{ef}	4723.3^{c}	4562.4^{f}	4454.6^{g}	14.03	0.0001

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

Table (4): Effect of different protein sources and supplementation with multienzymes containing phytase and probiotics on feed conversion ratio (FCR; g feed/ g gain), protein conversion ratio (PCR; g protein/ g gain), economic efficiency and apparent digestibility of nutrients of broiler chicks

Criteria	Control	Sunflower meal			Rocket meal			Sunflower with Rocket meal			SEM	P Value
		−	Enzymes	Probiotics	−	Enzymes	Probiotics	−	Enzymes	Probiotics		
Feed conversion ratio (FCR; g feed/ g gain), protein conversion ratio (PCR; g protein/ g gain), economic efficiency												
FCR,1-14 d of age	1.687^{a}	1.661^{a}	1.427^{c}	1.347^{de}	1.671^{a}	1.425^{c}	1.377^{cde}	1.583^{b}	1.416^{cd}	1.310^{e}	0.024	0.0001
FCR,15-28 d of age	2.103^{a}	2.044^{a}	1.829^{c}	1.733^{de}	2.077^{a}	1.894^{b}	1.785^{cd}	2.044^{a}	1.788^{cd}	1.702^{e}	0.020	0.0001
FCR, 29-49 d of age	2.412^{a}	2.374^{ab}	2.295^{cd}	2.252^{d}	2.397^{a}	2.325^{bc}	2.274^{cd}	2.383^{a}	2.261^{d}	2.163^{e}	0.019	0.0001
FCR, 1-49 d of age	2.256^{a}	2.211^{ab}	2.069^{cd}	2.005^{f}	2.214^{ab}	2.104^{c}	2.037^{de}	2.190^{b}	2.036^{de}	1.936^{f}	0.017	0.0001
PCR, 1-49 d of age	0.486^{a}	0.478^{c}	0.446^{f}	0.432^{h}	0.482^{b}	0.454^{e}	0.439^{g}	0.474^{d}	0.440^{g}	0.441^{i}	0.00049	0.0001
Economic efficiency, 1-49 d of age	14.7^{i}	16.4^{h}	21.9^{e}	25.1^{b}	16.4^{h}	20.8^{f}	24.2^{c}	17.7^{g}	23.3^{d}	30.1^{a}	0.126	0.0001
Apparent digestibility of nutrients												
Dry matter	76.75^{c}	76.83^{c}	79.46^{ab}	78.84^{abc}	77.09^{bc}	79.52^{ab}	79.29^{ab}	77.16^{bc}	80.37^{a}	79.43^{ab}	0.548	0.0004
Crude protein	76.89	77.18	79.79	79.61	77.21	79.24	80.13	76.92	80.44	79.99	1.159	NS
Crude fibre	29.38	29.19	34.14	33.15	29.37	33.98	34.80	29.61	32.84	34.03	1.355	0.02
Crude ash	29.63	28.18	30.89	29.80	29.86	30.16	31.06	27.86	31.38	30.57	1.174	NS
Ether extract	77.13	77.15	79.55	79.73	78.42	80.40	79.17	77.39	79.21	79.52	1.125	NS

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

Table (5). Effect of different protein sources and supplementation with multienzymes containing phytase and probiotics on carcass characteristics (%), physical characteristics of meat and on chemical composition of fresh muscle of broiler chicks of broiler chicks

Criteria,%	Control	Sunflower meal			Rocket meal			Sunflower-rocket mixture			SEM	P value
		−	Enzymes	Probiotics	−	Enzymes	Probiotics	−	Enzymes	Probiotics		
Carcass characteristics (%)												
Dressing	62.8	63.7	63.9	63.9	62.5	63.0	63.7	63.9	64.2	64.5	0.990	NS
Inedible parts	32.6	31.52	31.66	31.81	32.81	32.68	31.96	31.51	31.59	31.30	0.990	NS
Front part	32.8	35.5	34.3	34.0	33.0	33.4	34.3	34.1	34.6	34.7	0.877	NS
Hind part	29.9	28.2	29.5	29.9	29.5	29.6	29.4	29.8	29.6	29.7	0.797	NS
Liver	2.51^{a}	2.44^{ab}	2.35^{abcd}	2.09^{e}	2.19^{cde}	2.23^{bcde}	2.18^{cde}	2.42^{abc}	2.22^{bcde}	2.16^{de}	0.085	0.009
Gizzard	1.41^{d}	1.52^{cd}	1.41^{d}	1.56^{c}	1.87^{a}	1.69^{b}	1.45^{cd}	1.47^{cd}	1.38^{d}	1.44^{cd}	0.046	0.0001
Heart	0.687	0.709	0.688	0.642	0.631	0.613	0.703	0.697	0.612	0.596	0.042	0.05
Giblets	4.60^{ab}	4.68^{a}	4.44^{abc}	4.29^{bc}	4.69^{a}	4.54^{ab}	4.34^{bc}	4.59^{ab}	4.21^{c}	4.20^{c}	0.096	0.007
Spleen	0.20^{bc}	0.25^{a}	0.23^{ab}	0.19^{c}	0.20^{bc}	0.19^{bc}	0.22^{bc}	0.21^{bc}	0.21^{bc}	0.19^{c}	0.013	0.009
Physical characteristics of meat												
Moisture,%	75.08	74.76	74.51	74.62	74.75	74.60	74.22	74.28	74.51	74.18	0.241	NS
Protein,%	19.38	19.44	19.64	20.08	19.67	19.80	20.12	20.04	20.02	20.14	0.480	NS
Ether extract,%	4.11	4.36	4.28	3.99	4.05	4.19	4.25	4.29	4.16	4.25	0.374	NS
Ash	1.49	1.40	1.46	1.45	1.43	1.34	1.35	1.31	1.25	1.38	0.062	NS
Chemical composition of fresh muscle												
Color	0.21	0.21	0.18	0.19	0.18	0.19	0.18	0.21	0.19	0.18	0.023	NS
pH	6.38	6.47	6.38	6.42	6.33	6.59	6.52	6.61	6.31	6.45	0.121	NS
Tenderness	2.74	2.79	2.81	2.81	2.77	2.77	2.85	2.85	2.87	2.84	0.087	NS
WHC	5.64	5.78	5.62	5.72	5.66	5.71	5.70	5.67	5.67	5.74	0.095	NS

NS = not significantly.

Table (6). Effect of different protein sources and supplementation with multienzymes containing phytase and probiotics on biochemical constituents of blood plasma of broiler chicks

Criteria	Control	Sunflower meal			Rocket meal			Sunflower-rocket mixture			SEM	P value
		−	Enzymes	Probiotics	−	Enzymes	Probiotics	−	Enzymes	Probiotics		
Total protein, g/dl	4.87	4.63	4.74	4.91	4.71	4.86	4.61	4.61	4.70	4.72	0.096	NS
Albumen, g/dl	2.37^{a}	2.24^{ab}	2.29^{ab}	2.32^{ab}	2.35^{ab}	2.34^{ab}	2.25^{ab}	2.22^{b}	2.30^{ab}	2.26^{ab}	0.040	0.02
Globulin, g/dl	2.50	2.39	2.45	2.59	2.36	2.52	2.36	2.39	2.40	2.46	0.065	NS
Alb/Glb ratio	0.948	0.937	0.935	0.896	0.996	0.929	0.953	0.929	0.958	0.919	0.023	NS
Total lipids, mg/dl	686	671	680	668	772	704	682	674	691	677	23.5	NS
Cholesterol, mg /dl	171.8	171.3	164.5	182.8	171.0	164.1	178.5	161.4	169.4	174.7	5.98	NS
AST	11.9	11.7	12.6	12.6	11.7	11.8	12.6	11.6	12.3	12.3	0.382	NS
ALT, U	6.89	6.73	6.21	7.04	6.51	6.24	6.63	6.27	6.71	6.42	0.223	NS
Ca, mg/dl	11.69^{c}	12.1^{bc}	12.7^{ab}	12.1^{bc}	12.0^{bc}	12.6^{ab}	12.3^{abc}	11.9^{bc}	13.1^{a}	12.2^{abc}	0.272	0.04
iP, mg/dl	6.08	6.39	6.57	6.59	6.29	6.79	6.43	6.17	6.75	6.70	0.209	NS

^{a,b,c} **Means in the same row followed by different letters are significantly different at ($p \leq 0.05$). NS = not significantly.**