

PRODUCTIVE PERFORMANCE OF LAYING HENS FED DIETS CONTAINING DIFFERENT PLANT PROTEIN SOURCES WITH ENZYMES OR PROBIOTICS

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ABSTRACT

A factorial experiment (4×3) was conducted to evaluate the productive performance of laying hens fed diets containing four plant protein sources [soybean meal (SBM), sunflower meal (SFM), rocket seed meal (RSM) and their combination] either without or supplemented with probiotics (Nutri-Bio Plus, 0.5 g/kg) or enzyme preparation (Natuzyyme, 0.5g/kg). One hundred and eighty, 20-week-old Hy-Line W-36 hens were randomly assigned to 12 equal experimental groups, each with five replications. All birds were kept in community battery cages (3 birds per cage), set up in an open-sided laying house, and exposed to a daily photoperiod of 16 hr and managed similarly. Twelve pellets experimental diets were formulated to contain metabolizable energy of about 2800 kcal/kg and crude protein, of about 17%. Feed and water were provided *ad libitum* throughout the experimental period (20-44 weeks of age). The performance criteria included body weight, productivity (daily feed intake, egg production, egg weight, daily egg mass, feed conversion and economic efficiency), some egg quality traits, nutrients digestibility and certain blood parameters (total lipids, total protein, albumin, globulin, total calcium and inorganic P and activity of alanine aminotransferase and aspartate aminotransferase). The most important results can be summarized as follows: Apart from the effect of feed additive, feeding the SFM- and combined plant proteins produced positive effects on egg production rate, feed conversion, and economic efficiency from 20 to 44 weeks of age. Hens fed the SFM, RSM and combined plant protein-diets consumed significantly less feed but exhibited superior means of body weight gain, yolk index, yolk color score and shell thickness as compared to their control counterparts; other criteria were not affected. Also, significantly higher means of digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE) were observed for cockerels in response to feeding the SFM, RSM and combined plant protein-diets compared with those of the control group. Although hens fed the probiotics and enzyme-supplemented diets consumed significantly less feed, they achieved superior means of body weight gain, egg production rate, egg weight, feed conversion, economic efficiency, shell thickness, shell weight per unit surface area, yolk color score and Haught units as compared to the control group, regardless of the effect of dietary protein source, however; other parameters were not affected. Moreover, cockerels fed the probiotics and enzyme-supplemented diets achieved superior means of digestibility of DM, OM, CP, EE, CF and NFE as compared to their control counterparts. There were significant interactions between dietary protein source and feed additive only for body weight gain, shell thickness and shell weight per unit surface area, but not on other criteria of response. Based on these results, it could be concluded that sunflower meal and rocket seed meal can be used as safe feed ingredients in laying hens diets (at levels of 14-15% or their mixture at a weight ratio of 1:1), with or without enzyme or probiotics addition. Taking the economic aspect into account, the priority of choosing plant protein sources could be directed into SFM plus RSM, followed by SFM and then by RSM, in descending order.

Keywords: Sunflower seed meal, rocket seed meal, probiotic, enzyme, hen production, egg quality.

INTRODUCTION

Soybean meal (SBM) is the most widely used protein source in the formulation of poultry diets. However, when the price of soybean meal increases, poultry nutritionists look for sources of protein that are more economical to use in feed formulation. There are other competitively priced plant protein sources that their utilization in poultry diets is limited due to the presence of antinutritional factors or lack of appropriate processing technology. In Egypt, sunflower meal (SFM) and rocket seed meal (RSM) can be used, to a certain limit, as an alternative plant protein source for poultry, depending upon their nutritional quality.

Nowadays, large amounts of sunflower seeds are produced in Egypt, mainly for oil production. The locally produced SFM can be used as a feed ingredient for poultry and animal nutrition. Concerning the nutritive value of SFM, Villamide and San Juan (1998) reported that it has a variable CP content (29 to 45%), depending on the dehulling and oil extraction processes in an inverse relation to its crude fiber contents (14 to 32%). As compared to SBM, SFM is relatively richer in sulphur amino acids but markedly lower in lysine (Elkin, 2002) and available threonine (Leeson and Summers, 1997). In spite of its limited use for poultry because of its high fiber content and lysine deficiency (Villamide and San Juan, 1998), SFM was successfully used up to 20% in broiler and laying hens diets (Zatari and Sell, 1990; Vieira *et al.*, 1992; Sherif *et al.*, 1997a, b), and could reach 27% in laying hens diets, without compromising their productive performance (Sherif *et al.*, 2001). On the other hand, Swain *et al.* (1996) found an improvement in growth performance of broiler chicks in response to feeding diets containing high-crude fiber sunflower cake and supplemented with a multienzyme preparation. In addition, Kocher *et al.* (2000) observed superior nutrients digestibility for high SFM-diets by broilers due to enzyme addition.

Rocket (*Eruca sativa*) is one of the herbaceous plants of the Brassicaceae family. During the last decade, its cultivation in Egypt has obviously increased in harmony with the increasing demand for the volatile oils as pharmaceutical agents. The RSM, the by-product remaining after oil extraction, can be used as a cheaper source of plant protein for poultry as compared to SBM (El-Hindawy *et al.*, 1996; Craig, 1999; Abdo, 2003; El-Shafei *et al.*, 2007). With regard to the nutritive value of RSM as a feed ingredient for poultry, Abdo (2003) indicated that RSM contains 94.93% dry matter (DM), 16.11% ash, 24.53% crude protein (CP), 10.59% ether extract (EE), 19.72% crude fiber (CF), 29.05% nitrogen free extract (NFE), 1.16% lysine, 0.28% methionine, 0.43% cystine and 0.99% threonine. However, Osman *et al.* (2004) found that its content of DM, ash, CP, EE, CF and NFE are 92.73, 11.83, 36.03, 7.64, 7.69 and 36.81%, respectively. They also reported that RSM can be included up to 10% in broiler diets without adverse effects on growth performance. Recently, El-Shafei *et al.* (2007) illustrated the nutritional value of RSM as follows: 92.63% DM, 8.36% ash, 30.20% CP, 5.65% EE, 10.20% CF, 38.22% NFE, 0.45% Ca, 0.60% total P, 1.93% lysine, 0.35% methionine, 0.19% cystine and a metabolizable energy of 3265 kcal/kg. In the same study, they concluded that RSM can be used in

Japanese quails diets up to 14.92% without any adverse effects on their growth performance and carcass characteristics. On the other hand, rocket seeds, like other members of the Brassicaceae family, contain appreciable amounts of erucic acid, flavonoids and glucosinolates (Bennett *et al.*, 2006); the latter may depress appetite because of its bitter taste or through its indirect negative effect on the endocrine system of the bird (Tripathi and Mishra, 2007).

It is generally accepted that feed ingredients of plant origin contain a variety of components (*i.e.* antinutritional substances) that cannot be digested by monogastric animals because of the lack or insufficiency of endogenous enzyme secretions. In addition to being unavailable to the animal, these components also lower the utilization of other dietary nutrients, leading to depressed performance. Recently, the inclusion of commercial enzymes or probiotics in poultry diets has become a common practice, with different degrees of success depending upon the stress, health and nutritional status of the bird. The main targets for using feed enzymes are to increase digestibility (or availability) of nutrients, to break down the antinutritional factors, to achieve the least cost feed formulations and for environmental reasons (Bedford and Morgan, 1996; Bedford and Partridge, 2003). Beneficial effects of feeding probiotics-supplemented diets on the health status and productive performance of laying birds have been reported (Makled, 1991; Haddadin *et al.*, 1996; Ghazalah and Ibrahim, 1998; Siam *et al.*, 2004). Probiotics can also benefit the host animal by enhancing the synthesis of certain vitamins, providing digestive enzymes and increasing the production of volatile fatty acids that are finally metabolized in favor of the host (Fuller, 1989; Rolfe, 2000). Probiotics may also increase the uptake of nutrients in the gastrointestinal tract through their indirect effect on its permeability (Mulder *et al.*, 1997). Therefore, the present study was designed to evaluate the productive performance of laying hens fed diets containing different plant protein sources with or without probiotics or enzyme preparation.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Unit, Agricultural Research and Experimental Station, Faculty of Agriculture, Mansoura University. One hundred and eighty 20-wk-old Hy-Line W36 laying hens were used in this study. The birds were housed in community laying cages (3 pullets per cage); the width, length and height of each cage were 40, 50 and 40 cm, respectively. Pullets were randomly divided into 12 equal groups of 5 replications. The cages were placed in an open-sided laying house, supplied with artificial light to provide a suitable photoperiod of about 16 hours per day. Four isocaloric (2800 kcal ME/kg) and isonitrogenous (17 % CP) experimental diets were formulated based on soybean meal (SBM), sunflower meal (SFM) and rocket seed meal (RSM). Diet (1) contained SBM and corn gluten meal and served as a control diet. In diets 2, 3 and 4, SFM, RSM and their combination (1:1) provided 33 % of the crude protein content of the control diet, respectively. These four diets were either supplemented with probiotics (Nutri Bio Plus) or commercial enzyme preparation

(Natuzyne) or used without supplementation, thus twelve experimental diets were formulated and fed from 20 to 44 weeks of age. Nutri-Bio Plus is composed of *Bacillus subtilis* fermentation extract, brewers yeast extract, lactic acid and citric acid, calcium propionate, L-Methionine and sodium aluminosilicate. Natuzyne is a multienzyme preparation containing cellulase, xylanase, β -glucanase, α -amylase, protease, pectinase and phytase; it also contains hemicellulases, amyloglycosidases and pentosanases. Feed and water were supplied *ad libitum*. Composition and chemical analyses of the experimental diets are presented in Table 1.

Table (1): Composition and chemical analyses of the experimental diets¹ containing different plant protein sources

| Ingredient | SBM Diet 1 (Control) | SFM Diet 2 | RSM Diet 3 | Mix ² Diet 4 |
|-----------------------------------|----------------------------|---------------|---------------|----------------------------|
| Yellow corn | 59.09 | 58.16 | 57.34 | 56.34 |
| Soybean meal (44% CP) | 19.80 | 3.50 | 3.00 | 3.87 |
| Corn gluten meal (62% CP) | 4.90 | 7.00 | 7.20 | 6.90 |
| Sunflower meal (SFM) | | 15.00 | | 7.50 |
| Rocket seed meal (RSM) | | | 14.00 | 7.00 |
| Starch | 4.50 | 4.50 | 6.18 | 6.36 |
| Limestone | 9.00 | 9.00 | 9.00 | 9.00 |
| Dicalcium phosphate | 1.80 | 1.80 | 2.00 | 1.90 |
| Premix ³ | 0.30 | 0.30 | 0.30 | 0.30 |
| NaCl | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-Methionine | 0.16 | 0.09 | 0.25 | 0.16 |
| L-Lysine-HCl | 0.15 | 0.35 | 0.43 | 0.37 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis (NRC, 1994): | | | | |
| ME, kcal/kg | 2800 | 2802 | 2800 | 2800 |
| CP, % | 17.04 | 17.00 | 17.04 | 17.02 |
| EE, % | 2.53 | 2.71 | 3.76 | 3.18 |
| CF, % | 2.75 | 3.42 | 2.52 | 2.98 |
| Ca, % | 3.89 | 3.89 | 3.89 | 3.89 |
| Total P, % | 0.66 | 0.69 | 0.68 | 0.68 |
| Nonphytate P, % | 0.41 | 0.41 | 0.42 | 0.42 |
| Lysine, % | 0.89 | 0.89 | 0.89 | 0.89 |
| Methionine, % | 0.46 | 0.46 | 0.52 | 0.48 |
| Meth.+Cyst., % | 0.75 | 0.81 | 0.78 | 0.78 |
| Feed cost/kg diet (L.E.) | 1.18 | 1.18 | 1.22 | 1.14 |
| Determined analysis (AOAC, 1984): | | | | |
| Dry matter (DM); % | 89.45 | 89.66 | 89.53 | 89.84 |
| CP, % | 16.90 | 16.72 | 16.97 | 16.95 |
| EE, % | 2.49 | 3.07 | 3.90 | 3.11 |
| CF, % | 2.12 | 3.70 | 2.80 | 3.08 |
| Ash, % | 9.51 | 9.13 | 8.86 | 8.97 |
| NFE, % | 58.43 | 57.04 | 57.00 | 57.73 |

¹: Diets 1, 2, 3 and 4 were formulated after supplementation with zero, probiotics (0.5 g/kg) or enzyme preparation (0.5 g/kg).²: A mixture of SFM and RSM (1:1 wt/wt) was used to replace 33% of CP content of the control diet. ³: Each 3 kg of premix contained: vit A, 12,000,000 IU; vit D₃, 3,500,000 IU; vit E, 20 g; vit. K₃, 3 g; vit. B₁, 3 g; vit. B₂, 8 g; vit. B₆, 3 g; vit. B₁₂, 15 mg; Ca pantothenate, 12 g; Niacin, 40 g; Folic acid, 1.5 g; Biotin, 50 mg; Choline chloride, 600 g; Mn, 80 g; Zn, 75 g; Fe, 40 g; Cu, 10 g; I, 2 g; Se, 0.3 g; Co, 0.25 g and CaCo₃ as a carrier.

Data on hen-day egg production rate (EPR), daily feed intake (DFI), feed conversion (FC), egg weight (EW) and daily egg mass (DEM) were

periodically determined, on a 28-d period basis. Body weight gain (BWG) and economic efficiency of production (EEP) were also estimated for the whole experimental period. EEP was calculated as 100 times net revenue divided by total feed costs. While, net revenue was calculated as total revenue minus total feed costs.

Three egg quality tests (at 34, 35 and 36 weeks of hens' age) were performed and the average means were tabulated. A total of 360 eggs (30 eggs per treatment) were randomly chosen and used for egg quality tests. The measurements of egg quality included egg weight and its relative components (shell, yolk and albumen), egg shape index (ESI), yolk index (YI), shell thickness (ST), yolk color score (YCS) and Haugh units (HU: Haugh, 1937). Shell weight per unit surface area (SWUSA) was also calculated using the equation of Carter (1975).

At 25 weeks of birds' age, digestibility trials were conducted to evaluate the digestion coefficients of nutrients of the experimental diets using Hy-Line W36 cockerels. Each 3 cockerels were housed in a separate cage to serve as a metabolic age. Each group of cockerels was fed its respective experimental diet for a four-day pretest adaptation period, followed by a three days test period. Just after collection, the excreta were sprayed with 1% boric acid for the elimination of nitrogen loss due to a possible ammonia release. The excreta were dried in a forced-air oven at 70°C. Then, the excreta were allowed to equilibrate in moisture with atmospheric air before being weighed, finely ground, and stored in plastic bags until analysis. The chemical analysis of experimental diets and dried excreta samples were carried out according to the official methods of analysis (Association of Official Analytical Chemists: AOAC, 1984). The procedure described by Jakobsen *et al.* (1960) was used for separating the fecal protein fraction in excreta samples. Precipitated protein represents its undigested part in the excreta. Urinary organic matter was calculated according to Abou-Raya and Galal (1971).

Blood samples were collected from the wing veins of 44-wk-old birds in heparinized tubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Concentrations of plasma total protein (Henry, 1964), albumin (Doumas *et al.*, 1971), total lipids (Frings and Dunn, 1970), calcium (Tietz, 1987) and inorganic phosphorus (Goldenberg and Fernandez, 1966) as well as activity of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST: Reitman and Frankel, 1957) of laying hens were determined. Level of plasma globulin was calculated by subtracting level of plasma albumin from that of total protein. A completely randomized block design in a factorial arrangement of treatments (4×3), 4 dietary plant protein sources (SBM, SFM, RSM and a mixture of SFM and RSM at a weight ratio of 1:1) and 3 feed additives (Without addition, Probiotics and Enzyme) was used. The statistical processing of data was performed by using two-way analysis of variance of the GLM procedure of the Statistical Analysis System (SAS, 1990). When the main effects of dietary protein source and/or feed additives were significant ($P \leq 0.05$), means were separated by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance of laying hens:

Effect of dietary protein sources:

Regardless of the effect of feed additive, DFI, EPR, FC, EEP and BWG were significantly affected by dietary protein source during the experimental period (20-44 weeks of age), while EW and DEM were not affected (Table 2). The initial body weights of pullets were approximately similar at the beginning of the experiment. Birds fed the experimental diets containing SFM and RSM consumed significantly less feed ($P<0.01$) compared with their control counterparts (Table 2). The best EPR ($P<0.01$) was accomplished by hens fed SFM-containing diets, followed by that fed the diet containing the combined plant protein sources, and then birds fed either the RSM- or control-diets. Means of FC for hens fed the SFM- and combined plant protein-diets were significantly superior ($P<0.05$) compared with those of hens fed the control or RSM-containing diets. The best mean of EEP ($P<0.01$) was attained by hens fed the combined plant protein diet, followed by those fed SFM, the control and the RSM diets, respectively. It is interesting to note that feeding the experimental diets containing SFM, RSM and plant protein mixture resulted in significantly higher ($P<0.01$) BWG at 44 weeks of age compared with the control group fed the corn-soybean meal-based diet.

The better productive performance (EPR and FC) of hens fed diets containing SFM and plant protein mixture, in spite of their lower feed intake and consequently lower nutrients intake, indicates that these diets were utilized more efficiently than that occurred for the control diet. The depressed feed intake for hens fed SFM, RSM and plant protein mixture-containing diets might be related to certain dietary components which adversely affected the appetite of birds and/or palatability of diets. It is well known that notable reductions in voluntary feed intake of birds may result from certain nutritional imbalances, general or specific effects of antinutritional factors or presence of unpalatable compounds. The lower feed intake coincided with superior EPR and FC for hens fed SFM-containing diets in the present study are surprising and unexplainable, mainly because there is no available evidence that SFM can act as an appetite depressant or it has substantial amounts of antinutritional factors. But such an effect of SFM might have brought about as a consequence of a certain type of amino acid imbalance due to its low contents of digestible lysine (Villamide and San Juan, 1998) and/or available threonine (Leeson and Summers, 1997). On the other hand, RSM has been reported to contain antinutritional substances such as erucic acid, flavonoids and glucosinolates (Bennett *et al.*, 2006); the latter can depress appetite because of its bitter taste or through its indirect negative effect on the endocrine system of the bird (Tripathi and Mishra, 2007). However, relevance between feed palatability and feed intake is probably less important for poultry than for other classes of livestock because the senses of taste and smell in birds are not as well developed as in other animal species (Mawson *et al.*, 1993). According to Elwinger (1986), feed intake of laying hens was not

significantly affected by feeding diets containing a Swedish low glucosinolate rapeseed meal up to 13.5%, while egg production and feed conversion ratio were impaired linearly. But, Leeson *et al.* (1987) found that even complete replacement of soybean meal with rapeseed meal did not result in a significant reduction in feed intake of broilers and laying hens. Thus, the depressed intake of the RSM-containing diets, reported herein, could be resulted from indirect post-ingesting effects of such antinutritional factors (e.g. the toxic effect of its erucic acid) rather than from the reduced palatability *per se*.

Table (2): Productive performance of laying hens fed diets containing different protein sources with or without feed additives from 20 to 44 weeks of age

| Dietary treatments | DFI ¹ (g) | EPR ² (%) | EW ³ (g) | DEM ³ (g) | FC (g:g) ⁶ | EEP % ⁷ | IBW ⁸ (g) | BWG ⁹ (g) |
|----------------------------|-------------------------|-------------------------|------------------------|-------------------------|--------------------------|-----------------------|-------------------------|-------------------------|
| Main factors | | | | | | | | |
| Protein sources (A) | | | | | | | | |
| SBM | 98.21 ^a | 86.33 ^{bc} | 60.36 | 48.89 | 2.01 ^a | 112.49 ^c | 1345 | 178.0 ^b |
| SFM | 95.98 ^b | 89.76 ^a | 60.55 | 49.53 | 1.94 ^b | 123.99 ^b | 1339 | 202.1 ^a |
| RSM | 95.85 ^b | 85.81 ^c | 60.40 | 47.41 | 2.02 ^a | 109.27 ^c | 1350 | 197.7 ^a |
| Mix | 95.21 ^b | 87.71 ^b | 60.61 | 51.60 | 1.95 ^b | 129.98 ^a | 1351 | 200.3 ^a |
| SEM ¹ | 0.633 | 0.567 | 0.388 | 0.450 | 0.052 | 1.692 | 9.615 | 2.15 |
| Sign. level | ** | ** | NS | NS | * | ** | NS | ** |
| Feed additives (B) | | | | | | | | |
| 0.0 | 97.88 ^a | 82.55 ^b | 58.75 ^b | 47.41 | 2.16 ^a | 108.48 ^b | 1346 | 174.8 ^b |
| Probiotics | 96.05 ^b | 90.16 ^a | 61.49 ^a | 50.53 | 1.90 ^b | 123.77 ^a | 1344 | 204.6 ^a |
| Enzymes | 95.00 ^b | 89.50 ^a | 61.21 ^a | 50.14 | 1.89 ^b | 124.56 ^a | 1348 | 204.3 ^a |
| SEM ¹ | 0.548 | 0.491 | 0.336 | 0.184 | 0.045 | 1.465 | 7.925 | 1.92 |
| Sign. level | ** | ** | ** | NS | ** | ** | NS | ** |
| AB interaction | | | | | | | | |
| 1x1 | 98.94 | 81.40 | 58.98 | 48.38 | 2.05 | 103.58 | 1346 | 152.0 |
| 1x2 | 99.86 | 89.46 | 62.10 | 50.24 | 1.99 | 114.32 | 1343 | 191.3 |
| 1x3 | 95.82 | 88.14 | 60.00 | 51.10 | 1.98 | 119.58 | 1345 | 190.7 |
| 2x1 | 97.94 | 85.10 | 59.30 | 45.86 | 2.13 | 113.32 | 1336 | 179.0 |
| 2x2 | 94.96 | 92.36 | 61.16 | 51.48 | 1.84 | 129.90 | 1338 | 213.7 |
| 2x3 | 95.04 | 91.82 | 61.18 | 51.30 | 1.85 | 128.74 | 1343 | 213.7 |
| 3x1 | 97.94 | 81.02 | 58.40 | 49.26 | 2.26 | 98.04 | 1354 | 178.7 |
| 3x2 | 94.90 | 87.62 | 61.48 | 49.18 | 1.92 | 113.12 | 1346 | 205.7 |
| 3x3 | 94.72 | 88.78 | 61.38 | 49.78 | 1.90 | 116.66 | 1352 | 208.0 |
| 4x1 | 96.72 | 82.68 | 58.30 | 44.34 | 2.18 | 118.96 | 1349 | 188.7 |
| 4x2 | 94.48 | 81.18 | 61.22 | 51.24 | 1.85 | 137.74 | 1351 | 207.7 |
| 4x3 | 94.42 | 89.26 | 62.32 | 56.18 | 1.85 | 133.24 | 1353 | 204.7 |
| SEM ¹ | 1.09 | 0.98 | 0.67 | 0.56 | 0.09 | 2.93 | 11.85 | 5.84 |
| Sign. level | NS | NS | NS | NS | NS | NS | NS | * |

^{a-c}: For each of the main factors, means in the same column having different superscripts differ significantly at P<0.05.

¹⁻⁹: Refers to standard error of the means, daily feed intake, egg production rate, egg weight, daily egg mass, feed conversion, economic efficiency of production, initial body weight and body weight gain, respectively.

Effect of feed additives:

In agreement with the present results, Vieira *et al.* (1992) indicated that when SFM replaced up to 40.5 % of SBM protein, it had positive linear effects on feed conversion of laying hens. Similar results were also obtained

by Zatari and Sell (1990) who found that SFM can successfully be used in broiler diets up to 20%. Moreover, Sherif *et al.* (2001) reported that laying hens fed diets containing up to 27% SFM achieved slightly better means of productive performance and economic efficiency compared with their control counterparts. With waterfowls, Vetesi *et al.* (1998) indicated that SFM could completely replace dietary SBM with no adverse effects on their growth performance. Compared to the control, the insignificant slightly inferior productive performance (EPR, DEM and EEP) of laying hens fed the RSM-diets, reported herein, is in partial harmony with the findings of Osman *et al.* (2004), who reported poor growth performance in broiler chicks in response to feeding 15% RSM-containing diet.

Apart from the effect of dietary protein source, hens fed the diets supplemented with probiotics or enzymes consumed less feed ($P < 0.01$) and achieved significantly higher ($P < 0.01$) means of EPR, EW, FC, EEP and BWG during the experimental period (20-44 weeks of age) compared with the control group, while DEM was not affected (Table 2). The percent improvements in EPR, EW, FC, EEP, and BWG were 9.22, 4.66, 12.04, 14.09 and 17.07% in probiotic-supplemented groups and 8.42, 4.19, 12.50, 14.82 and 16.88% in enzyme-supplemented groups, respectively. The improvement in productive performance by laying hens fed probiotics or enzymes-supplemented diets may imply that the CP content (17%) of basal diet and/or its amino acid composition, used in this experiment, were marginally inadequate to meet the nutritional requirements of the experimental birds, and such inadequacy may have been met by these supplements.

In general, the improved productive performance of laying hens in the present study following feeding the probiotic-supplemented diets might be produced through its potential for secreting certain digestive enzymes and benefiting the host animal by enhancing the synthesis of certain vitamins, certain amino acids and increasing the production of short chain fatty acids; the latter contribute significantly to the energy supply of the animal (Fuller, 1989; Rolfe, 2000; Dibner and Richards, 2005). The positive effects of probiotics, reported herein, on the productive performance of hens are in accordance with those reported by Haddadin *et al.* (1996), Siam *et al.* (2004), Xu *et al.* (2006) and Gallazzi *et al.* (2008). With regard to the beneficial effects of probiotics, Haddadin *et al.* (1996) fed laying hens diets supplemented with liquid cultures of *Lactobacillus acidophilus* and found that egg production and feed conversion were significantly better than in the control group. In this respect, Siam *et al.* (2004) indicated that feeding diets supplemented with *Lactobacillus acidophilus*, *Bifidobacterium bifidum* or both had positive effects on egg production rate, egg weight and feed conversion of Bovans laying hens. In a later study, Xu *et al.* (2006) found that feeding probiotic (*Bacillus subtilis*)-supplemented diet produced significant improvements in egg production and feed conversion of Lohmann Brown layers. Recently, Gallazzi *et al.* (2008) demonstrated that egg production and feed conversion ratio of Hy-Line Brown pullets were improved in response to feeding probiotic (*Lactobacillus acidophilus* D2CSL)-supplemented diet. However, other researchers observed no beneficial effect of dietary probiotics

on the performance of laying hens (Mahdavi *et al.*, 2005; Mohiti Asli *et al.*, 2007). These inconsistent responses of laying hens to dietary supplementation with probiotics may be due to several interacting factors such as type of probiotic, dose of administration, viability of microorganisms, composition of the basal diet, plane of nutrition, age, strain, and physiological, environmental and stress status of the birds, level of husbandry, management and hygienic conditions and course of study (Patterson and Burkholder, 2003).

It is interesting to note that, although hens fed the enzyme-supplemented diets consumed less feed, they achieved superior EPR, EW, FC, BWG and EEP compared with hens fed the control diet (Table 2). So, such beneficial effects on productive performance are mainly associated with an improved digestibility of nutrients of diets induced by exogenous enzyme addition, as presented in Table 5. In the present study, the positive effects of dietary enzyme supplementation on laying hen performance are in partial agreement with the findings reported by (Van der Klis *et al.*, 1997; Jalal and Scheideler, 2001; Yakout *et al.*, 2003; Mandai *et al.*, 2005) in laying hens. However, other investigators observed no significant effect for enzyme addition in layer diets (Al Bustany and Elwinger, 1988; Senkoylu *et al.*, 2004). In this regard, Van der Klis *et al.* (1997) reported that production performance (except feed conversion ratio) was significantly improved by dietary supplementation of phytase. Similarly, Jalal and Scheideler (2001) indicated that supplementation of phytase in normal; corn-soybean meal diets improved feed intake, feed conversion and egg mass while egg production and egg weight were not affected. In addition, Yakout *et al.* (2003) demonstrated that productive performance of Mandarah laying hens were significantly improved in response to enzyme supplementation (Bio-Nutra[®]) to diets of different energy levels. Moreover, Mandai *et al.* (2005) found a significant increase in the nitrogen-corrected apparent metabolizable energy (AME_n) values for chickens, guinea fowl and quails due to enzyme supplementation, while AME_n value of rapeseed meal did not improve by enzyme addition. On the other hand, Attia *et al.* (2001) reported that phytase and/or a multienzyme mixture (Optizyme) could overcome the negative effects of feeding rice bran-based diet on productive and reproductive performance of Norfa laying hens. It is evident that effectiveness of feed enzymes for poultry depends on a variety of factors relating to enzyme (*i.e.* type of enzyme(s), its biological activity, level of addition, degree of specificity to substrate and its stability at high temperature), bird (*i.e.* age, nutritional, physiological and stress status) and diet (*i.e.* composition, form, physical features and method of processing); feeding programs and housing systems may also be involved. The effects of interaction between dietary protein source and feed additive in the present study were not significant for all criteria of productive performance of laying hens during the entire experimental period, with the exception of a significant dietary protein source by feed additive interaction on BWG of hens.

Egg weight and its components

Effect of dietary protein sources:

Irrespective of the effect of feed additive, egg weight and relative shell weight were significantly ($P < 0.05$) affected by dietary protein source during the period of egg quality examination (34-36 weeks of age), as shown in Table 3. Eggs sampled from hens fed SFM- and combined plant protein-diets were significantly heavier compared with those of hens fed the control or RSM-containing diets. This might imply that the samples were not quite representative since EW, as a criterion of productive performance of laying hens from 20 to 44 weeks of age, was not significantly affected by dietary protein source (Table 2). The slight differences observed in the relative weight of egg shell were inconsistent and may not be related to the effect of dietary protein source.

In the present study, the lack of significant differences in EW in response to feeding the SFM-containing diets (Table 2), is in line with the findings of Vieira *et al.* (1992) and Sherif *et al.* (1997a and 2001), who observed no significant effects of feeding SFM on the relative weights of yolk, albumen and shell. In addition, Mirza *et al.* (1993) and Senkoylu *et al.* (2004) found that feeding diets containing SFM did not significantly affect egg weight. The present results suggest that the inclusion of 14% RSM in laying hen diets had no negative effect on EW or its components. In line with the present results, Leslie *et al.* (1973) and Olomu *et al.* (1975) reported that EW was not affected by feeding rapeseed meal-containing diets to laying hens. In addition, Abd-El Motagally *et al.* (2000) observed no significant effects on EW and egg components when laying hens were fed diets containing up to 13% rapeseed meal. However, there is also some evidence in the scientific literature for a reduction in egg size in response to feeding the rapeseed meal-containing diets to laying hens.

Effect of feed additives:

Independently from the effect of dietary protein source, hens fed the diets supplemented with probiotics or enzymes laid significantly ($P < 0.01$) heavier eggs during the period of egg quality examination (34-36 weeks of age) compared with the control group (Table 3). The percent improvements in EW were 7.21 and 7.16% in probiotic- and enzyme-supplemented groups, respectively. However, egg components were not influenced by dietary supplementation with either enzyme or probiotics. Since higher EW for hens fed the supplemented diets (Table 3) coincided with superior EPR and FC (Table 2), and improved nutrient digestibility (Table 5), the increase in EW caused by feeding the supplement-diets might be produced through more efficient assimilation of nutrients in these diets. In disagreement with the present results, Haddadin *et al.* (1996), Mahdavi *et al.* (2005), Mohiti Aslj *et al.* (2007) and Gallazzi *et al.* (2008) found that probiotic supplementation to laying hen diets had no positive effect on egg weight. It has been suggested that the effectiveness of probiotics may be more obvious in stressed chickens (Jin *et al.*, 1997). On the other hand, Senkoylu *et al.* (2004) reported that enzyme supplementation to laying hen diets containing high-oil sunflower meal failed to affect egg weight. In addition, Yakout *et al.* (2003) reported that enzyme supplementation had no effect on egg components but significantly

improved egg weight. The effectiveness of feed enzymes for poultry depends mainly on their biological activity, composition, stability to temperature of feed processing, specificity to target substrate and other factors relating to the experimental diet and bird. The effect of interaction between dietary protein source and feed additive was not significant for egg weight and its components.

Table (3): Egg weight and its components¹ of laying hens fed diets containing different protein sources with or without feed additives

| Dietary treatments | EW ¹ (g) | YW ² % | AW ³ % | SW ⁴ % |
|----------------------------|---------------------|-------------------|-------------------|---------------------|
| Main factors | | | | |
| Protein sources (A) | | | | |
| SBM | 56.54 ^b | 26.13 | 62.03 | 11.84 ^a |
| SFM | 57.45 ^a | 26.39 | 62.31 | 11.31 ^b |
| RSM | 56.56 ^b | 26.01 | 62.24 | 11.74 ^a |
| Mix | 57.84 ^a | 26.00 | 62.42 | 11.57 ^{ab} |
| SEM ¹ | 0.338 | 0.138 | 0.166 | 0.116 |
| Sign. level | * | NS | NS | * |
| Feed additives (B) | | | | |
| 0.0 | 54.49 ^b | 25.98 | 62.55 | 11.46 |
| Probiotics | 58.42 ^a | 26.29 | 62.12 | 11.58 |
| Enzymes | 58.39 ^a | 26.13 | 62.17 | 11.70 |
| SEM ¹ | 0.293 | 0.119 | 0.144 | 0.100 |
| Sign. level | ** | NS | NS | NS |
| AB interaction | | | | |
| 1x1 | 53.86 | 26.10 | 62.85 | 11.05 |
| 1x2 | 57.58 | 26.54 | 61.12 | 12.34 |
| 1x3 | 58.19 | 25.75 | 62.12 | 12.13 |
| 2x1 | 54.40 | 25.70 | 62.80 | 11.49 |
| 2x2 | 60.09 | 26.96 | 62.22 | 10.81 |
| 2x3 | 57.87 | 26.49 | 61.90 | 11.61 |
| 3x1 | 54.20 | 25.98 | 62.26 | 11.75 |
| 3x2 | 57.32 | 25.98 | 62.32 | 11.70 |
| 3x3 | 58.16 | 26.06 | 62.17 | 11.76 |
| 4x1 | 55.50 | 26.12 | 62.31 | 11.56 |
| 4x2 | 58.69 | 25.69 | 62.84 | 11.48 |
| 4x3 | 59.33 | 26.20 | 62.12 | 11.69 |
| SEM ¹ | 0.586 | 0.238 | 0.288 | 0.200 |
| Sign. level | NS | NS | NS | NS |

¹: Means are average of three egg quality tests performed at the 34th, 35th and 36th weeks of age

^{a-b}: For each of the main factors, means in the same column having different superscripts differ significantly at P≤0.05.

¹⁻⁴: Refers to standard error of the means, egg weight, yolk weight, albumen weight and shell weight, respectively.

Eggshell and interior egg quality traits

Effect of dietary protein sources:

Apart from the effect of feed additive, both eggshell quality (as measured by ESI, ST and SWUSA) and interior egg quality (YCS, YI and HU)

traits were significantly affected by dietary protein source during the period of egg quality examination (34-36 weeks of age), as given in Table 4. The results indicated that eggs laid by hens fed the SFM-containing diets exhibited superior ($P<0.01$) means of ST, YCS, YI and ESI ($P<0.05$) while SWUSA was significantly ($P<0.01$) inferior to those of hens fed the control diet but HU was not affected. Feeding the RSM-containing diets produced similar positive effects on ST, YCS, YI, ESI and SWUSA but HU was not affected. Eggs produced by hens fed the combined plant protein-diets had significantly better means of YCS, YI, HU and ST but ESI and SWUSA were not affected.

Table (4): Eggshell and interior egg quality traits⁵ of laying hens fed diets containing different protein sources with or without feed additives

| Dietary treatments | ESI ² (%) | YCS ³ | YI ⁴ (%) | HU ⁵ | ST ⁶ (mm) | SWUSA ⁷ (mg/cm ²) |
|----------------------------|-------------------------|-------------------|------------------------|--------------------|-------------------------|---|
| Main factors | | | | | | |
| Protein sources (A) | | | | | | |
| SBM | 81.42 ^b | 7.59 ^b | 33.44 ^c | 73.49 ^b | 0.341 ^b | 97.37 ^a |
| SFM | 82.45 ^a | 7.89 ^a | 34.44 ^b | 73.92 ^b | 0.345 ^a | 93.40 ^b |
| RSM | 82.37 ^a | 7.92 ^a | 35.47 ^a | 72.89 ^b | 0.347 ^a | 96.71 ^a |
| Mix | 81.88 ^{ab} | 7.92 ^a | 35.68 ^a | 80.75 ^a | 0.348 ^a | 95.70 ^{ab} |
| SEM ¹ | 0.268 | 0.074 | 0.195 | 0.431 | 0.001 | 0.839 |
| Sign. level | * | ** | ** | ** | ** | ** |
| Feed additives (B) | | | | | | |
| 0.0 | 81.89 | 7.46 ^b | 34.52 | 73.69 ^b | 0.329 ^b | 93.27 ^c |
| Probiotics | 82.28 | 8.09 ^a | 35.03 | 75.63 ^a | 0.354 ^a | 96.10 ^b |
| Enzymes | 81.91 | 7.94 ^a | 34.72 | 76.46 ^a | 0.353 ^a | 98.02 ^a |
| SEM ¹ | 0.232 | 0.065 | 0.169 | 0.374 | 0.001 | 0.727 |
| Sign. level | NS | ** | NS | ** | ** | ** |
| AB interaction | | | | | | |
| 1x1 | 81.78 | 7.20 | 33.08 | 72.89 | 0.323 | 89.66 |
| 1x2 | 81.19 | 7.90 | 33.74 | 72.92 | 0.352 | 101.84 |
| 1x3 | 81.29 | 7.67 | 33.52 | 74.65 | 0.350 | 100.63 |
| 2x1 | 82.27 | 7.43 | 34.00 | 72.39 | 0.327 | 93.39 |
| 2x2 | 82.47 | 8.29 | 34.69 | 74.84 | 0.353 | 90.62 |
| 2x3 | 82.59 | 7.96 | 34.62 | 74.53 | 0.355 | 96.21 |
| 3x1 | 82.16 | 7.60 | 35.62 | 70.28 | 0.331 | 96.34 |
| 3x2 | 83.29 | 8.13 | 35.66 | 73.37 | 0.354 | 96.04 |
| 3x3 | 81.65 | 8.03 | 35.12 | 75.02 | 0.354 | 97.75 |
| 4x1 | 81.37 | 7.60 | 35.38 | 79.21 | 0.335 | 93.68 |
| 4x2 | 82.16 | 8.07 | 36.03 | 81.39 | 0.358 | 95.93 |
| 4x3 | 82.13 | 8.10 | 35.62 | 81.65 | 0.350 | 97.50 |
| SEM ¹ | 0.464 | 0.129 | 0.337 | 0.747 | 0.002 | 1.453 |
| Sign. level | NS | NS | NS | NS | ** | ** |

⁵: Means are average of three egg quality tests performed at the 34th, 35th and 36th weeks of age. ²⁻⁷: For each of the main factors means in the same column having different superscripts differ significantly at $P\leq 0.05$. ¹⁻⁴: Refer to standard error of the means, egg shape index, yolk color score, yolk index, Haugh unit, shell thickness and shell weight per unit surface area.

The inferior mean of SWUSA of eggs laid by hens fed the SFM-containing diets might be attributed, at least partly, to the concurrent decrease of shell weight of their eggs (Table 3).

The higher YCS of eggs produced by hens fed diets containing SFM, RSM or combined plant protein mixture is attributable to higher total xanthophylls contents in these diets (provided mainly by yellow corn and corn gluten meal) as compared to that of the control diet. Another contributing factor for the enhanced egg yolk pigmentation could be mediated as a result of the increased digestibility of EE in these diets (Table 5), and thus increasing the absorbability of fats and associated xanthophylls. The beneficial effects, reported herein, on egg quality measurements for feeding SFM-, RSM- or combined plant protein-diets could be due to more efficient utilization of nutrients, particularly, amino acids, fatty acids and minerals. However, the present results clearly indicate that eggshell and interior quality traits were not consistently affected by dietary protein source.

In partial agreement with the present results, Casartelli *et al.* (2006) found that hens fed SFM (up to 13%) in their diets produced eggs of superior shell quality (measured as egg specific gravity and percent shell weight), whereas HU was not affected. In addition, Abd-El Motagally *et al.* (2000) observed positive effects on YI, YCS and HU while ESI and ST were not affected when laying hens were fed diets containing up to 13% rapeseed meal. On the other hand, other researchers observed no adverse or positive effects on egg quality traits following to feeding the laying hens on SFM-containing diets (Vieira *et al.*, 1992; Sherif *et al.*, 1997, 2001).

Effect of feed additives:

Irrespective of the effect of dietary protein source, hens fed the diets supplemented with probiotics produced eggs with superior means ($P < 0.01$) of eggshell (as determined by ST and SWUSA) and interior quality (in terms of YCS and HU), during the period of egg quality examination (34-36 weeks of age), compared with their control counterparts whereas ESI and YI were not affected (Table 4). Also, feeding the enzyme-supplemented diets had positive effects ($P < 0.01$) on YCS, HU, ST and SWUSA as compared to those of the control group (Table 4), while ESI and YI were not affected.

In line with the present results, Nahashon *et al.* (1994) and Mohan *et al.* (1995) observed a slight improvement in eggshell thickness in hens supplemented with probiotics for 10 weeks during the period of peak production. Recently, working with Japanese quail hens, Zeweil and Ismail (1998) found that egg specific gravity, shell weight and shell thickness were significantly increased in response to feeding probiotic-supplemented diets. Moreover, Xu *et al.* (2006) reported positive effects of probiotics on eggshell thickness, yolk color and Haugh units. Recently, Gallazzi *et al.* (2008) found that dietary supplementation with probiotics had positive effects on HU and egg specific gravity but had no effect on shell thickness. Some of these authors attributed the beneficial effect of probiotics on eggshell quality to a favorable environment in the intestinal tract, which could help to assimilate more calcium. In this regard, Nahashon *et al.* (1994, 1996) reported positive correlations between the probiotic administration and retention rates of nitrogen, calcium and phosphorus in laying hens. However, no clear reason

could be offer at the present time for the positive effect of probiotics, reported herein, on the interior quality of eggs. Haddadin *et al.* (1996) and Nahashon *et al.* (1996) suggested that better eggshell quality of eggs produced by probiotics-fed hens is due to better gut health conditions and production of lactic acid which facilitate ionization and absorption of salts, particularly Ca and P. Probably, such acidification is also involved in the improvement of the interior quality of eggs. On the other hand, Roberts and Choct (2006) reported a positive effect for using the commercial enzyme products in laying hen diets on shell thickness, but Abdo (2003) and Wu *et al.* (2005) found that dietary enzyme supplementation exerted no significant effect on most of egg quality parameters. However, Yakout *et al.* (2003) reported that enzyme supplementation had no effect on egg quality indices but significantly improved egg yolk color. The interactions between dietary protein source and feed additive had no significant effects on all egg quality traits, except for ST and SWUSA which were significantly affected.

Nutrient digestibility of the experimental diets:

Effect of dietary protein sources:

It is important to point out that digestion trials, performed herein, were carried out using mature cockerels at 25 weeks of age. Regardless of the effect of feed additive, nutrient digestibility (DM, OM, CP, EE, CF and NFE) were positively affected by dietary protein source (Table 5).

Cockerels fed the experimental diets containing SFM, RSM and combined plant protein mixture had significantly higher digestibility for DM, OM, CF and NFE ($P<0.01$), and for CP and EE ($P<0.05$) compared with their control counterparts. The highest means of DM, OM, CF, NFE, CP and EE digestibility values were exhibited by cockerels fed the combined plant protein mixture-diets, respectively, while the corresponding lowest values were for the control diet. Digestibility of nutrients ranged between 67.67 to 69.20%, 73.67 to 75.20%, 29.52 to 31.23%, 64.41 to 68.60%, 79.67 to 81.20% and 79.47 to 80.68% for DM, OM, CF, NFE, CP and EE, respectively. The improved nutrient digestibility by cockerels in response to feeding the diets containing SFM, RSM and their combinations may be related to a better amino acid balance, enhanced uptake of nutrients and thus more efficient utilization. In partial accordance with the present findings, Abd-El Motagally *et al.* (2000) found that feeding Muscovy ducks on diets containing up to 30% SFM produced positive effects on CF digestibility, while those of NFE and OM were significantly decreased but had no significant influence on digestibility of CP and EE. However, Askbrant and Håkansson (1984) indicated that digestibility of nutrients (*i.e.* OM, CP, CF and total carbohydrates) and N retention were impaired when laying hens were fed diet containing 30% rapeseed meal. On the other hand, Abdo (2003), working with broiler chicks, reported that feeding RSM-containing diets resulted in significantly lower digestibility values for DM, OM, EE, CP, CF, NFE as well as N balance but improved Ca and P retention, while EE and CP digestibility were not affected.

Table (5): Nutrient digestibility of the experimental diets for Hy-line W36 cockerels as affected by feeding different protein sources with or without feed additives

| Dietary treatments | DM ^c (%) | OM ^d (%) | CP ^a (%) | EE ^b (%) | CF ^b (%) | NFE ^e (%) |
|----------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|
| Main factors | | | | | | |
| Protein sources (A) | | | | | | |
| SBM | 67.67 ^b | 73.67 ^b | 79.67 ^b | 79.47 ^b | 29.52 ^b | 64.41 ^b |
| SFM | 68.97 ^a | 74.97 ^a | 80.96 ^a | 80.45 ^a | 31.09 ^a | 68.39 ^a |
| RSM | 68.91 ^a | 74.94 ^a | 80.90 ^a | 80.45 ^a | 30.79 ^a | 68.34 ^a |
| Mix | 69.20 ^a | 75.20 ^a | 81.20 ^a | 80.68 ^a | 31.23 ^a | 68.60 ^a |
| SEM ^f | 0.187 | 0.190 | 0.187 | 0.191 | 0.262 | 0.178 |
| Sign. Level | ** | ** | * | * | ** | ** |
| Feed additive (B) | | | | | | |
| 0.0 | 67.21 ^b | 73.23 ^b | 79.20 ^b | 79.04 ^b | 29.21 ^b | 66.91 ^b |
| Probiotics | 69.53 ^a | 75.53 ^a | 81.52 ^a | 80.94 ^a | 31.43 ^a | 68.87 ^a |
| Enzymes | 69.33 ^a | 75.33 ^a | 81.32 ^a | 80.81 ^a | 31.33 ^a | 69.55 ^a |
| SEM ^f | 0.162 | 0.164 | 0.162 | 0.165 | 0.227 | 0.142 |
| Sign. level | ** | ** | ** | ** | ** | ** |
| AB interaction | | | | | | |
| 1x1 | 66.45 | 72.45 | 78.45 | 78.36 | 28.56 | 66.28 |
| 1x2 | 68.17 | 74.17 | 80.17 | 79.96 | 29.58 | 67.73 |
| 1x3 | 68.39 | 74.39 | 80.38 | 80.09 | 30.42 | 67.91 |
| 2x1 | 67.31 | 73.31 | 79.30 | 79.24 | 29.96 | 66.99 |
| 2x2 | 69.85 | 75.85 | 81.84 | 81.18 | 31.94 | 69.14 |
| 2x3 | 69.75 | 75.75 | 81.74 | 80.94 | 31.37 | 69.06 |
| 3x1 | 67.28 | 73.38 | 79.28 | 79.27 | 28.95 | 66.97 |
| 3x2 | 69.95 | 75.95 | 81.95 | 81.29 | 31.95 | 69.24 |
| 3x3 | 69.49 | 75.49 | 81.48 | 80.82 | 31.49 | 68.84 |
| 4x1 | 67.78 | 73.78 | 79.78 | 79.31 | 29.40 | 67.40 |
| 4x2 | 70.13 | 75.13 | 82.13 | 81.33 | 32.27 | 69.39 |
| 4x3 | 69.70 | 75.70 | 81.69 | 81.41 | 32.04 | 69.02 |
| SEM ^f | 0.325 | 0.328 | 0.324 | 0.330 | 0.455 | 0.312 |
| Sign. level | NS | NS | NS | NS | NS | NS |

^{a,b}: For each of the main factors means in the same column having different superscripts differ significantly at P<0.05. ^f: Refer to standard error of the means, dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen-free extract, respectively.

Effect of feed additives:

Apart from the effect of dietary protein source, feeding the probiotics or enzyme-supplemented diets significantly (P<0.01) improved the digestibility of nutrients (DM, OM, CP, EE, CF and NFE) as compared to those of the control cockerels. The improvement in nutrient digestibility by cockerels fed probiotics-supplemented diets might be due to an increased permeability of the gut enhanced by supplemental probiotics, and thus increasing uptake of nutrients, as suggested by Mulder *et al.* (1997). Probiotics has also been reported to have a positive impact on the intestinal balance and the competition between useful and pathogenic bacteria and thus enhancing the efficiency of feed utilization in favor of the bird (Fuller, 1989; Rolfe, 2000). On the other hand, exogenous enzymes have been

reported to improve digestion by removing antinutritional factors which interfere with the normal processes of digestion, by digesting the fiber components of the diet, or by creating an environment which encourages minimal bacterial fermentation in small intestine while encouraging beneficial bacterial fermentation in the caeca (Choct *et al.*, 1995; Bedford, 1996a, b; Bedford and Partridge, 2003).

In this regard, Bedford (1996a) stated that alteration of the fermentation profiles in the bird can significantly benefit the performance by more effective partitioning of ileal nutrients between the bird and resident flora, provision of nutrients in the caeca from fiber digestion, and by reduction in immunological challenge.

In harmony with the present findings, Kocher *et al.* (2000) observed superior nutrient digestibility for high SFM-diets by broilers due to enzyme addition. In addition, Mandai *et al.* (2005) found a significant increase in the nitrogen-corrected apparent metabolizable energy (AME_n) values of SFM for chickens, guinea fowl and quails due to enzyme supplementation, while AME_n value of rapeseed meal did not improve by enzyme addition. On the other hand, the improved nutrient digestibility of the present probiotic-supplemented diets is in line with the literature evidence that positive correlations exist between the probiotic administration and nitrogen, calcium and phosphorus retention in laying hens (Nahashon *et al.*, 1994 and 1996). Dietary protein sources and feed additives were not interrelated for digestibility of nutrients, measured in the present study.

Blood parameters of laying hens:

As shown in Table 6, neither dietary protein source nor the added supplements affected blood parameters of 44-wk-old laying hens, measured in the present study. The lack of significant differences among means of blood parameters of laying hens may be an indication that the current experimental diets had no adverse effects on productive performance and health status of birds.

The present results agree with those reported by Sherif *et al.* (2001), who fed laying hens graded levels of SFM-diets and found that plasma levels of glucose, total protein, total lipids and cholesterol as well as plasma activities of ASL and ALT were not affected. On the other hand, El-Shafei *et al.* (2007) fed Japanese quails diets in which RSM replaced 8.0 to 32% of soybean meal protein and found that blood plasma cholesterol and triglycerides were significantly decreased when its replacement value reached 32%, while total protein and activities of AST and ALT were not affected. Regarding the effect of probiotic supplementation on biochemical constituents of blood, Mohan *et al.* (1995) and Haddadin *et al.* (1996) observed a hypocholesterolaemic effect of probiotics in laying hens. Several mechanisms are present in the scientific literature for explaining such cholesterol lowering effect of probiotics. Of these mechanisms, Gilliland *et al.* (1985) suggested that some of the microorganisms present in the probiotics preparation could assimilate the cholesterol present in the gastrointestinal tract for their own cellular metabolism, thus reducing the amount absorbed. Another mechanism for the decrease of cholesterol in probiotics-fed animals, suggested by Fukushima and Nakano (1995), is that probiotics are able to

inhibit the activity of hydroxy-methyl-glutaryl-CoA, an enzyme involved in the intestine and responsible for the biosynthesis of cholesterol. In general, means of all blood parameters, measured herein, fell within the normal physiological range (Freeman, 1984), irrespective of the effect of dietary treatments.

Conclusion

Based on these results, it could be concluded that sunflower meal and rocket seed meal can be used as safe feed ingredients in laying hens diets (at levels of 14-15% or SFM plus RSM at a weight ratio of 1:1), with or without probiotics or enzyme addition. Taking the economic aspect into account, the priority of choosing plant protein sources could be directed to SFM plus RSM, followed by SFM and then by RSM in a descending order.

Table (6): Blood parameters of laying hens fed diets containing different protein sources with or without feed additives

| Dietary treatments | Total protein g/dL | Albumin g/dL | Globulin g/dL | Total Lipids g/L | AST U/L | ALT U/L | Ca mg/dL | P mg/dL |
|---------------------|--------------------|--------------|---------------|------------------|---------|---------|----------|---------|
| Main factors | | | | | | | | |
| Protein sources (A) | | | | | | | | |
| SBM | 4.96 | 2.44 | 2.52 | 20.56 | 115.67 | 6.61 | 22.81 | 5.71 |
| SFM | 4.92 | 2.40 | 2.52 | 20.74 | 115.44 | 6.56 | 22.73 | 5.63 |
| RSM | 4.68 | 2.32 | 2.36 | 20.61 | 115.56 | 6.44 | 22.90 | 5.88 |
| Mix | 4.73 | 2.35 | 2.38 | 20.66 | 116.00 | 6.35 | 22.89 | 5.82 |
| SEM ¹ | 0.129 | 0.056 | 0.075 | 0.449 | 4.124 | 0.236 | 0.426 | 0.186 |
| Sign. level | NS | NS | NS | NS | NS | NS | NS | NS |
| Feed additive (B) | | | | | | | | |
| 0.0 | 4.64 | 2.30 | 2.34 | 20.64 | 116.08 | 6.39 | 22.76 | 5.77 |
| Probiotics | 4.97 | 2.44 | 2.53 | 20.79 | 116.58 | 6.61 | 22.83 | 5.77 |
| Enzymes | 4.88 | 2.39 | 2.49 | 20.50 | 114.33 | 6.47 | 22.90 | 5.74 |
| SEM ¹ | 0.112 | 0.049 | 0.065 | 0.389 | 3.572 | 0.204 | 0.369 | 0.161 |
| Sign. level | NS | NS | NS | NS | NS | NS | NS | NS |
| AB interaction | | | | | | | | |
| 1x1 | 4.79 | 2.37 | 2.43 | 20.25 | 115.33 | 6.51 | 22.70 | 5.70 |
| 1x2 | 5.12 | 2.48 | 2.64 | 20.75 | 114.00 | 6.73 | 22.92 | 5.70 |
| 1x3 | 4.99 | 2.47 | 2.52 | 20.67 | 117.67 | 6.58 | 22.78 | 5.73 |
| 2x1 | 4.65 | 2.30 | 2.35 | 21.02 | 116.00 | 6.28 | 22.79 | 5.73 |
| 2x2 | 5.14 | 2.52 | 2.62 | 20.92 | 118.33 | 7.03 | 22.83 | 5.70 |
| 2x3 | 4.98 | 2.37 | 2.61 | 20.27 | 112.00 | 6.38 | 22.56 | 5.47 |
| 3x1 | 4.50 | 2.24 | 2.26 | 20.58 | 119.00 | 6.43 | 22.68 | 5.87 |
| 3x2 | 4.84 | 2.39 | 2.45 | 21.01 | 115.33 | 6.51 | 22.87 | 5.90 |
| 3x3 | 4.72 | 2.32 | 2.40 | 20.25 | 112.33 | 6.39 | 23.16 | 5.87 |
| 4x1 | 4.61 | 2.29 | 2.41 | 20.72 | 114.00 | 6.37 | 22.86 | 5.80 |
| 4x2 | 4.77 | 2.37 | 2.40 | 20.46 | 118.67 | 6.17 | 22.72 | 5.77 |
| 4x3 | 4.81 | 2.38 | 2.43 | 20.79 | 115.33 | 6.52 | 23.09 | 5.90 |
| SEM ¹ | 0.224 | 0.098 | 0.130 | 0.779 | 7.144 | 0.408 | 0.738 | 0.323 |
| Sign. level | NS | NS | NS | NS | NS | NS | NS | NS |

¹: SEM refers to standard error of the means.

NS: No significant differences were observed among dietary treatments for all blood parameters, measured herein.

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الأداء الإنتاجي لدجاج البيض المغذى على علائق تحتوي على مصادر مختلفة من البروتين النباتي والمدعمة بالمنشطات الحيوية أو الإنزيمات
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أجريت هذه الدراسة بهدف تقييم الكفاءة الإنتاجية لدجاج البيض المغذى على علائق تحتوي على أربعة مصادر من البروتين (كسب فول الصويا وكسب بذور عباد الشمس وكسب بذور الجرجير ومخلوط هذه الأكساب) بدون أو بعد إضافة منشط حيوي (نيوترن بيوبلاس بمعدل ٠.٥ جم/كجم) أو مستحضر إنزيمي (ناتوزايم بمعدل ٠.٥ جم/كجم). تم التوزيع العشوائي لعدد ١٨٠ دجاجة عمر ٢٠ أسبوعا إلى ١٢ مجموعة تجريبية متساوية بكل منها ٥ مكررات وتم تسكينها في بطاريات ذات أقفاص جماعية (بكل قفص ٣ دجاجات تمثل مكررة). تم تكوين ١٢ عليقة تجريبية متساوية في محتوياتها من الطاقة القابلة للتمثيل (٢٨٠٠ كيلو كالوري/كجم) والبروتين الخام (١٧%) وتم تقديم الغذاء والماء بحرية للمجموعات التجريبية المختلفة من الطيور حتى نهاية التجربة عند عمر ٤٤ أسبوعا. وتضمنت القياسات المأخوذة: وزن الجسم وإنتاجية الطيور (استهلاك الغذاء اليومي ومعدل إنتاج البيض ووزن البيضة وكتلة البيض اليومية ومعامل التحويل الغذائي) والكفاءة الاقتصادية وبعض صفات جودة البيض ومعاملات هضم المركبات الغذائية وبعض معايير بلازما الدم (مستوى الدهون الكلية والبروتين الكلي والألبومين والجلوبولين والكالسيوم الكلي والفسفور غير العضوي وكذلك نشاط إنزيمي الأستين أمينوترانسفيريز وأسبرتيت أمينوترانسفيريز في البلازما). وأمكن تلخيص أهم النتائج فيما يلي: بصرف النظر عن تأثير الإضافات العلفية، أحدثت التغذية على العلائق المحتوية على كسب بذور عباد الشمس ومخلوط الأكساب تأثيرات معنوية إيجابية على معدل إنتاج البيض، معامل التحويل الغذائي والكفاءة الاقتصادية خلال فترة التجربة (٢٠-٤٤ أسبوعا من العمر). أدت التغذية على العلائق المحتوية على كسب بذور عباد الشمس وكسب بذور الجرجير ومخلوط الأكساب إلى نقص معنوي في استهلاك الغذاء اليومي ومع ذلك حدث تحسن معنوي في زيادة وزن الجسم، صفات جودة البيض (معامل الصفار، درجة لون الصفار وسمك القشرة) ومعاملات هضم المركبات الغذائية (المادة الجافة، المادة العضوية، البروتين الخام، المستخلص الإثيري، الألياف الخام والمستخلص الخالي من الأزوت)، بينما لم تتأثر باقي القياسات. رغم الانخفاض المعنوي في استهلاك الغذاء اليومي للدجاج المغذى على العلائق المدعمة (بالمنشط الحيوي أو المستحضر الإنزيمي) فإن التغذية على تلك العلائق حققت تحسنا معنويا في زيادة وزن الجسم والمظاهر الإنتاجية (معدل إنتاج البيض، وزن البيضة، معامل التحويل الغذائي)، الكفاءة الاقتصادية، جودة القشرة (سمك القشرة ووزن وحدة المساحة من مسطحها) والجودة الداخلية للبيض (درجة لون الصفار ومقياس جودة البياض) ومعاملات هضم المركبات الغذائية بينما لم تتأثر باقي القياسات، وذلك بغض النظر عن تأثير مصدر بروتين الغذاء. كان للتفاعل بين مصدر بروتين الغذاء والإضافة العلفية تأثير معنوي على الزيادة في وزن الجسم وسمك القشرة ووزن وحدة المساحة من مسطح القشرة بينما لم يؤثر معنويا على باقي الصفات المدروسة. ونستخلص من نتائج هذه الدراسة أنه يمكن استخدام كسب بذور عباد الشمس وكسب بذور الجرجير كمكونات علفية آمنة للدجاج البياض (بمستويات تتراوح بين ١٤-١٥% أو مخلوطين بنسبة ١:١) في وجود أو عدم وجود الإضافات العلفية. كما أنه بأخذ الناحية الاقتصادية في الاعتبار فإن الأولوية في اختيار مصادر البروتين النباتي التي يمكن أن تحل محل جزء من بروتين العليقة ينصب على مخلوط كسب بذور عباد الشمس + كسب بذور الجرجير يليه كسب بذور عباد الشمس ثم كسب بذور الجرجير.