

SINGLE AND COMBINED EFFECTS OF MANNAN OLIGOSACCHARIDE (MOS) AND DIETARY PROTEIN ON THE PERFORMANCE AND IMMUNITY RESPONSE OF LAYING HENS

By

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Abstract: *This experiment was conducted to study the single and combined effects of mannan oligosaccharide and dietary protein on the performance, egg quality, immunity response, serum constituents and economical efficiency of laying hens which designed as factorial arrangement, three levels of crude protein (CP: 14.75, 13.25, and 11.75%) x two levels of mannan oligosaccharide (MOS: 0 and 0.1%). A total number of 72 Hy-Line W-36 laying hens 49 weeks old were distributed randomly into six equal groups each group containing 12 hens. **The results obtained could be summarized as follows:** 1- Level of CP% significantly ($P < 0.01$) affected each of egg production % (EP%), egg mass (EM), egg weight (EW), feed conversion (FC) and caloric conversion ratio (CCR). The level of 14.75% CP had numerically higher EP%, EM, EW than the level 13.25% CP and it had better ($P < 0.01$) FC or CCR than the other levels of CP%. The level of 13.25% CP caused insignificant increase in immune responses of SRBCs, CBH response of laying hens. 2- Adding MOS to layers diets significantly improved FC, crude protein conversion (CPC) and CCR. Also, it caused significant increases in shell %, yolk%, yolk index (YI), serum total protein and albumin while decreased egg albumen%. MOS supplementation caused insignificant increase in primary and secondary immune response of SRBCs and CBH response of hens compared with unsupplemented diet. 3- Hens fed the 14.75% CP unsupplemented diet with MOS had significantly higher ($P < 0.01$) EP% and EM than the groups fed 11.25% CP with or without 0.1% MOS diets, but, it insignificantly differed than other groups. The group of 14.75% CP + 0.1% MOS had the highest ($P < 0.01$) EW, the lowest FI ($P < 0.05$) and the best ($P < 0.01$) FC and CCR followed by group 13.25% CP + 0.1% MOS of EW, FC and CCR. 4- Eggs produced by layers fed diet contains 13.25% CP + 0.1% MOS had significantly higher shell% but*

lower albumen% than other groups. Eggs produced by group 11.75% CP+0.1% MOS had the higher YI than other groups. 5-The highest values of primary immune response against sheep red blood cells (SRBCs) were found in the groups fed diets contained 13.25% CP with or without MOS, whereas the highest value of secondary immune response was found in the group fed diet contains 11.75% CP+ 0.1% MOS followed by 13.25% CP+0.1% MOS group which had the highest value of cutaneous basophil hypersensitivity (CBH) response . The highest values ($P<0.01$) of serum total protein and albumin were of groups 14.75 % CP+0.1% MOS and 13.25% CP+0.1% MOS. These results favored the middle levels of CP (13.25%) + 0.1% MOS in diet of laying hens caused better effect in immunity responses of hens than others and the highest values of economical efficiency with the relative economical efficiencies were shown in the groups 14.75 % CP+0.1% MOS and 13.25% CP+0.1% MOS. It could be concluded that adding 0.1% MOS as natural feed additive in diets of laying hens which contain a level of CP that recommended by strain catalog or less with 1.5% were economically better in production and immunity without adverse effect on egg quality.

Key words: (Laying performance, crude protein, Mannan oligosaccharide, immune response, serum constituents).

INTRODUCTION

Using alternatives to antibiotic growth promotants in commercial chickens have become important mainly because of apprehensions about the possible development of resistant bacteria. At the same time, continuous use of antibiotic growth promotants in breeders may have one important ramification that could affect the poultry industry, reduction in the efficacy of antibiotics when used in progeny that are hatched to the same parents (**Shashidhara and Devegowda, 2003**). Also, antibiotic growth promotants resulted in the occurrence of resistant microorganisms which became one of the major problems in human medicine. One of many such classes of alternatives is the prebiotic mannan oligosaccharide (MOS). The MOS is derived from the outer cell wall of yeast. Mannose , the main component of MOS, is a unique sugar because many enteric bacteria have receptors that bind to it (**Griggs and Jacob, 2005**). The MOS supplementation is considered because it is not only shifts

gastrointestinal microflora balance toward beneficial organisms (**Spring *et al.*, 2000, Fairchild *et al.*, 2001**) but also resulted in significant improvement in antibody responses in broiler and layers (**MacDonald, 1995, Savage *et al.*, 1996, Cotter, 1997, Cotter *et al.*, 2000 Cotter *et al.*, 2002, Raju and Devegowda, 2002**). Supplementation of poultry diets with MOS results in improved production in terms of body weight gain and feed conversion (**Parks *et al.*, 2001**), which partly may be due to its hypothesized nutrient sparing effect and primarily to its influence on nutrient utilization in the gastrointestinal tract (**Kumprecht *et al.*, 1997, Savage *et al.*, 1997, Sonmez and Eren, 1999**).

Dietary protein content has a much consideration due to its high cost and its great effect on the production parameters of laying hens. Matching dietary protein and amino acids levels to the production requirement of laying hens is another important mean of reducing nitrogen emissions and excretion. Lowering the CP of the laying hens diets not only reduce nitrogen consumption but also means that less unutilized nitrogen is excreted. The response by the laying hens to dietary protein levels has been controversial for many years. **Fernandez, *et al.* (1973)** reported that increasing dietary protein level lead to an increase in egg production %. Also, average egg weight of layers increased as dietary protein level increased (**Summers, 1993**). Moreover, **Calderon and Jensen (1990)** observed an improvement in FC due to high dietary protein level. However, **Angelovicova (1994)** found that a low-protein diet containing 14.1 % CP reduced average daily FI and improved FC. **Glick, *et al.* (1983)** showed that diet deficient in protein (33% of requirement) could reduce numbers of lymphocytes in the thymus of chickens. However, the responses were varied by strain, dietary protein (**Cheema *et al.*, 2003**) environment, stress, production state and health status. Thus, protective immune responses require a supply of nutrients at the appropriate times and amounts (**Humphrey *et al.*, 2002**). The present experiment aimed to study single and combined effects of mannan oligosaccharide and dietary protein on the performance and immunity responses, serum constituents, and economical efficiency of laying hens

MATERIALS AND METHODS

The experimental work of the present study was carried out at the Poultry Research Station, Poultry Department, Faculty of Agriculture, Fayoum University from April to July 2003, to study single and combined effects of mannan oligosaccharide and dietary protein on the performance, egg quality, immunity response, serum constituents of laying hens. A total number of 72 Hy-Line W-36 laying hens at 49 weeks old were distributed randomly into six equal groups each group containing 12 hens in 12 replicates, one hen / replicate. The experiment was designed as factorial arrangement, three levels of CP (14.75, 13.25, and 11.75%) x two levels of mannan oligosaccharide (0 and 0.1%). The basal diets were formulated to satisfy nutrient requirements of laying hens according to the strain catalog recommendations (14.7 CP % and 2770 ME. K cal / kg). The composition and chemical analyses of the experimental diets are shown in Table 1. Artificial light was used beside the normal day light to provide 16-hour day photoperiod. Feed and water were provided *ad libitum*. Individual body weights were recorded at the beginning and the end (61 weeks of age) of the experiment to calculate live body weight changes (LBWC). Egg number (EN) and egg weight (EW) were recorded daily to calculate egg production % ($EP\% = EN \times 100 / 84 \text{ day}$) and egg mass ($EM = EN \times EW$). Feed intake (FI) was recorded weekly and it used to calculate feed conversion ($FC = FI / EM$), crude protein conversion ($CPC = FI \times CP\% / EM$) and caloric conversion ratio ($CCR = FI \times ME. K cal / EM$).

Egg quality measurements were determined monthly on eggs of the last three days. Twelve eggs / group were collected monthly throughout the experimental periods to determine egg shape index % (SI, **Carter, 1968**), shell thickness (ST) including shell membranes was measured using a micrometer at three locations on the egg (air cell, equator and sharp end), the percentage of shell, albumen and yolk were calculated. Yolk color (YC) was determined by matching the yolk with one of the 15 bands of the "1961- Roche improved yolk color fan. Yolk index % was calculated according to (**Well, 1968**), Haugh unit score (HU) was applied from a special chart using egg weight and albumen height which was measured by using a micrometer according to **Haugh (1937)**.

At 54 weeks of age four hens of each group were injected in wing vein with 0.2 ml of sheep red blood cells solution (SRBCs 9% suspension) and the blood samples were collected from the wing vein of these birds after one week to determine SRBCs primary immune response. The same birds were reinjected at 60 weeks of age and the blood samples were collected from these birds after 5 days to determine SRBCs secondary immune response in serum and determine the serum constituents. Antibody response against SRBCs were measured in serum using micro haemagglutination technique as described by **Yamamoto and Glick (1982)** and **Dix and Taylor (1996)**. The titers were expressed as the log 2 of the reciprocal of the highest dilution giving visible agglutination (**Atta et al., 1998**). Serum constituents were determined calorimetrically using kits according to **Weichselbaum (1946)** for total protein, **Dumas and Biggs (1972)** for albumin , **Allain, et al. (1974)** for total cholesterol and **Wahlefeld (1974)** for triglycerides.

To determine cutaneous basophil hypersensitivity (CBH) response, three hens from each group were randomly selected at 61 weeks of age and injected with 0.1 ml of phytohaemagglutinin –P (PHA-P, 100 µg/ml) subcutaneously in the right toe web, whereas. 0.1 ml saline was injected subcutaneously in the left toe web which served as the control. The thickness of both toe webs were measured in mm using a micrometer at 24 hr after injection. The CBH response was calculated as described by (**Atta et al., 1998**) as follows:

$$\text{CBH response} = \frac{\text{Thickness of right toe web (PHA-P) response}}{\text{Thickness of left toe web (saline response)}}$$

Economical efficiency of egg production was calculated from the input-output analysis which was calculated according to the price of the experimental diets and eggs produced. These values were calculated as the net revenue per unit of total cost. Analysis of variance was computed using the general linear model (GLM) procedure of statistical analysis system according to **SPSS (1999)**. Significant differences among means were evaluated using Duncan's multiple range test (**Duncan, 1955**).

RESULTS AND DISCUSSION

Productive performance of laying hens

Results presented in Table 2 showed that levels of CP% significantly ($P<0.01$) affected each of EP%, EM, EW, FC and CCR. The level of 14.75% CP had insignificantly ($P>0.05$) higher EP%, EM, EW than the level of 13.25% CP and, it had better FC or CCR than the other levels of CP% . However, the 11.75% CP had the lowest EP%, EM, EW (56.00%, 2413g and 51.42g respectively) associated with the worst FC and CCR being 3.13 g feed/ g EM and 8.68 calorie/ g EM. No significant effects of dietary protein on the FI, CPC and LBWC of laying hens were found. **Hammershoj and Kjaer (1999)** and **Bunchasak *et al.* (2005)** reported trend that increasing the egg weight with increasing dietary protein however, decreasing the level of protein improved CPC . Similar trends were found by **Bunchasak *et al.* (2005)** that high CP of 16 and 18 % tend to have better EP% and EM than the lower level of 14 % CP .However, **Hammershoj and Kjaer (1999)** reported that different levels of dietary protein did not affect EP%. On the other hand, **Harms and Russell (1995)** reported that the 10.95% CP satisfied the requirements needed for egg production , egg mass and egg content.

Adding MOS to diets of laying hens significantly improved FC, CPC and CCR than the unsupplemented diet (2.54 g feed/g EM, 0.342 g CP/g EM and 7.06 calorie/ g EM vs 2.86 g feed/g EM, 0.376 g CP/g EM and 7.94 calorie/ g EM respectively) , regardless of level CP level. Whereas, the MOS supplementation had no significant effects on EP%, EM, EW, FI and LBWC. Similarly, **Shashidhara and Devegowda (2003)** found that MOS supplementation had no influence on egg production of broiler breeders. On the contrary, **Guerrero ,1995, Berry and Lui , 2000 and Stanley *et al.*,2000** reported considerable improvement in egg production in the MOS-fed birds. Similar results of significant FC improvement were found in laying hens and Japanese quail at the same level of MOS supplementation were reported by **Chukwu and Stanley (1997)** and **Ghosh, *et al.* (2007)**. This is may be because MOS maintain gut health by adsorption of pathogenic bacteria containing type-1 fimbriae of different bacterial strains and remove the bacteria from gut (**Oyofe *et al.*, 1989 and Spring *et al.*, 2000**) and increase villus height, uniformity and

integrity also, increase in crypt depth is attributed to greater expenditure of energy to develop the absorptive surface (**Dawson and Tricarico, 2002, Loddi *et al.*, 2002 , Olivera, *et al.*, 2006 ,Mourao *et al.*, 2006 and Ghosh, *et al.*, 2007**)

Except for LBWC and CPC, there were significant treatment interaction effects on each of EP%, EM, EW, FI, FC, and CCR as shown in Table 2. The 14.75% CP unsupplemented diet with MOS had significantly higher EP% and EM (70.83% and 3118g) than either the 11.25% CP unsupplemented diet with MOS (54.17% and 2380g) or the 11.75% CP supplemented 0.1% MOS (57.84% and 2445g) whereas the former insignificantly differed than other groups for these traits. The level of 14.75% CP supplemented 0.1% MOS had significantly higher EW (55.46), however the level of 11.75% CP supplemented 0.1% MOS had lower EW (50.37g) than other groups. The level 14.75% CP unsupplemented MOS consumed more daily FI (94.12) than other groups whereas the 14.75% CP supplemented 0.1% MOS had the lowest FI of 85.24g. Therefore, the latter group had the best ($P<0.01$) FC of 2.17g feed/ g EM followed by 13.25% CP+0.1% MOS being 2.48 g feed/ g EM however poorer FC than other groups was shown by the 11.75% CP unsupplemented MOS being 3.28 g feed/ g EM. Similar trend were found for CCR.

Egg quality

Except YI, levels of CP% insignificantly affected all external and internal egg quality traits. The 11.75% CP had the highest YI of 56.82% as shown in Table 3. However, **Hammershoj and Kjaer (1999)** reported that with increasing dietary protein, albumen quality traits and egg shell % decreased. MOS supplementation resulted in significant increases in shell %, yolk% and YI of 10.73, 29.49 and 56.71%, respectively and a decrease in albumen% being 59.81% (Table 3). Similarly, **Berry and Lui (2000)** and **Shashidhara and Devegowda (2003)** reported that the MOS improved egg shell quality traits in older breeder females. There were significant differences in each of shell%, albumen% and YI ($P<0.05$) due to treatment interactions, may be due to improvement in calcium availability. Eggs produced by layers fed the 13.25% CP+0.1% MOS had significantly higher shell% but lower

albumen% than other groups (11.27% and 59.46%). Eggs produced by hens fed the 11.75% CP+0.1% MOS had the highest YI of 59.88% whereas those fed the 13.25% CP unsupplementation MOS produced significantly lower YI of 50.49% than other groups as shown in Table 3.

Immunity responses

Results presented in Table 4 indicated that the levels of CP% significantly affected the primary immunity. The level of 13.25% CP caused an increase in primary immune response ($P \leq 0.071$) of SRBCs of laying hens, however, insignificantly influenced either the secondary immune response of SRBCs or the value of CBH response of laying hens. MOS supplementation caused insignificant increase in primary ($P \leq 0.227$), secondary ($P \leq 0.055$) immune response of SRBCs and CBH response ($P \leq 0.126$) of hens, regardless of CP%. However, **Malzone *et al.* (2000)** and **Shashidhara and Devegowda (2003)** reported that hens fed diet supplemented with 0.05% MOS had higher SRBCs titers than controls at one week post-sensitization and significant increase in the values of antibody titers of broiler breeders. This effect on antibody titers may be due to the influence of the MOS on immune system and/or improvement of intestinal absorption of some related nutrients, such as Zn, Cu, Se. In addition to the reduction of the pathogenic bacteria load in the intestine and preventing the acute immune response against such bacteria (**Finucane, *et al.*, 1999** and **Spring *et al.*, 2000**). The interaction between levels of CP% and levels of MOS% showed no consistent trend in the values of primary and secondary immune response of SRBCs and CBH response. The highest values of primary immune response were found in the groups which fed diet contains 13.25% CP with or without MOS, whereas the highest value of secondary immune response was found in the group fed the 11.75% CP+0.1% MOS diet followed by the group fed diet contains 13.25% CP+0.1% MOS. The highest value of CBH response was found of the group fed diet contains 13.25% CP+0.1% MOS. The results indicate that the middle levels of CP (13.25%) + 0.1% MOS in diets of laying hens caused better effect in immunity responses of hens than others.

Serum constituents

The levels of CP % significantly affected triglycerides ($P \leq 0.05$), the lowest value was found in the group fed the 13.25% CP. However, there were no effect of the different levels of CP% used in this study on each of serum total protein, albumin, globulin, A/G ratio and cholesterol. Adding of 0.1% MOS in diets of laying hens caused significant increases ($P < 0.01$) in serum total protein and albumin compared with the hens fed the unsupplemented diet (10.09 vs 8.98g/dl and 6.36 vs 5.16g/dl), regardless of CP % however, insignificantly affected other serum constituents as shown in Table 4. Significant differences were found ($P < 0.01$) in serum total protein and albumin due to levels of CP% and MOS % interaction, the highest values were of groups 14.75 % CP+0.1% MOS and 13.25% CP+0.1% MOS being 10.34 and 10.24g/dl for total protein and 6.54 and 6.67 24g/dl for serum albumin, whereas, the lowest values were 8.68g/dl and 4.51g/dl of total protein and albumin for group 11.75 % CP+0.0MOS, respectively as shown in Table 4. it can be concluded that adding MOS to diets containing different levels of CP may be induced such improvement in synthesis of serum total protein and albumin.

Economical efficiency

Supplementing layer diets which differed in level of CP with 0.1% MOS improved economical efficiency and relative economical efficiency of laying hens compared with those fed the unsupplemented diet. The highest values of economical efficiency of 1.515 and 1.365 with the relative economical efficiencies of 116.00 and 104.49 were shown for the groups fed 14.75% CP+0.1% MOS and 13.25% CP+0.1% MOS. However, the worst values were found (0.967 and 74.01 of economical efficiency and its relative economical efficiency) for the group fed 11.75% CP+0.0 MOS as shown in Table 5. Similarly, **Namra (2006)** suggested that incorporation of 0.15 % baker's yeast in diet of quail layers apparently exhibited better amelioration in feed cost /egg than the control group. It could be concluded that adding 0.1% MOS as natural feed additive in diets of laying hens which contain a level of CP that recommended by strain catalog or less with 1.5% were economically better in production and immunity without adverse effect on egg quality.

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التأثيرات الفردية و المشتركة للمنان اوليجوسكريد و بروتين العليقة علي الأداء والإستجابة المناعية للدجاج البياض

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أجريت هذه التجربة لدراسة التأثيرات الفردية و المشتركة للمنان اوليجوسكريد و بروتين العليقة علي الأداء والإستجابة المناعية للدجاج البياض وصممت كتوزيع عاملي، ثلاث مستويات من البروتين الخام (١٤,٧٥، ١٣,٢٥، ١١,٧٥ %) x مستويين من المنان اوليجوسكريد (صفر، ٠,١%). ٧٢ دجاجة بياضة عمر ٤٩ أسبوع من سلالة الهاي لين دبلو ٣٦ وزعت عشوائياً إلى ٦ مجاميع متساوية كل مجموعة احتوت على ١٢ دجاجة. ويمكن تلخيص النتائج كما يلي

١-مستوى البروتين كان له تأثير معنوي جدا على كلا من نسبة إنتاج البيض ، كتلة البيض، وزن البيض ، معدل التحويل الغذائي ومعدل تحويل الطاقة. المستوى ١٤,٧٥ % بروتين هو الأعلى رقمياً في نسبة إنتاج البيض ، كتلة البيض، وزن البيض مقارنة بالمستوى ١٣,٢٥ % بروتين و المستوى ١٤,٧٥ % بروتين هو الأحسن (معنوياً جدا) في معدل التحويل الغذائي

ومعدل تحويل الطاقة ذلك مقارنة بالمستويات الأخرى من البروتين. والمستوى ١٣,٢٥ % بروتين سبب زيادة غير معنوية في الإستجابات المناعية ضد كرات الدم الحمراء للغنم و استجابة فرط الحساسية للدجاج البياض ٢- إضافة المنان اوليجوسكريد لعلائق الدجاج البياض حسنت معنويا كلا من معدل التحويل الغذائي ، معدل تحويل البروتين و معدل تحويل الطاقة ايضا سبب زيادة معنوية في نسبة قشرة البيض ، نسبة الصفار ، دليل الصفار ، البروتين الكلي بالسيرم والبيومين السيرم وسبب نقص في البيومين البيضة. إضافة المنان اوليجوسكريد سبب زيادة غير معنوية في الإستجابات المناعية الأولية والثانوية ضد كرات الدم الحمراء للغنم و استجابة فرط الحساسية للدجاجات البياض مقارنة بعدم الإضافة. ٣- الدجاجات المغذاة على عليقة بها ١٤,٧٥ % بروتين وغير مضاف لها المنان اوليجوسكريد كانت الأعلى معنويا جدا في نسبة إنتاج البيض ، كتلة البيض مقارنة بالمجموعات المغذاة على علائق بها ١١,٧٥ % بروتين ومضاف أو غير مضاف لها ٠,١ % منان اوليجوسكريد ولكن لم تختلف معنويا عن المجموعات الأخرى. مجموعة ١٤,٧٥ % بروتين المضاف لها ٠,١ % منان اوليجوسكريد هي الأعلى (معنويا جدا) في وزن البيضة و الأقل (معنويا) في كمية العلف المأخوذ و الأحسن (معنويا جدا) في معدل التحويل الغذائي ومعدل تحويل الطاقة يليها مجموعة ال ١٣,٢٥ % بروتين و ٠,١ % منان اوليجوسكريد في وزن البيضة، معدل التحويل الغذائي ومعدل تحويل الطاقة. ٤- البيض المنتج من دجاجات مغذاة على عليقة محتوية على ١٣,٢٥ % بروتين + ٠,١ % منان اوليجوسكريد كان أعلى معنويا في نسبة القشرة ولكن الأقل في نسبة الأليومين عن المجموعات الأخرى. والبيض المنتج من مجموعة ١٣,٢٥ % بروتين + ٠,١ % منان اوليجوسكريد كان أعلى معنويا في دليل الصفار. ٥- القيم الأعلى للإستجابة المناعية الأولية ضد كرات الدم الحمراء للغنم وجدت في المجموعات المغذاة على علائق بها ١٣,٢٥ % بروتين مضاف أو غير مضاف لها منان اوليجوسكريد في حين أن القيمة الأعلى للإستجابة المناعية الثانوية وجدت في المجموعة المغذاة على عليقة بها ١١,٧٥ % بروتين + ٠,١ % منان اوليجوسكريد يليها مجموعة ال ١٣,٢٥ % بروتين + ٠,١ % منان اوليجوسكريد والتي امتلكت أعلى قيمة لإختبار فرط الحساسية. القيم الأعلى (معنويا جدا) للبروتين الكلي بالسيرم والبيومين السيرم كانت للمجموعات ١٤,٧٥ % بروتين + ٠,١ % منان اوليجوسكريد و ١٣,٢٥ % بروتين + ٠,١ % منان اوليجوسكريد. هذه النتائج ايدت ان المستوى الأوسط من البروتين (١٣,٢٥ %) + ٠,١ % منان اوليجوسكريد في عليقة الدجاج البياض سبب أحسن تأثير في الإستجابات المناعية للدجاجات عن المجموعات الأخرى . والقيم الأعلى للكفاءة الإقتصادية ونسبة الكفاءة الإقتصادية كانت في المجموعتين ١٤,٧٥ % بروتين + ٠,١ % منان اوليجوسكريد و ١٣,٢٥ % بروتين + ٠,١ % منان اوليجوسكريد.

بذلك يمكن التوصية بإضافة ٠,١ % منان اوليجوسكريد كإضافة غذائية طبيعية في علائق الدجاج البياض والتي تحتوي على مستوى البروتين الموصى به تبعاً لكتالوج السلالة أو أقل مئة بمقدار ١,٥ % حيث كانوا الأحسن اقتصاديا في الإنتاج ومناخيا بدون تأثير سلبي على جودة البيضة.