Egypt. Poult. Sci. Vol (31) (IV): (867-882)

MODULATION OF IMMUNITY AND SOME BIOLOGICAL FUNCTIONS OF JAPANESE QUAIL BY MANNAN OLIGOSACCHARIDE AND B-GLUCAN ADMINISTRATION

By

Zenat. A. Ibrahim

Poultry production Department, Faculty of Agriculture, Zagazig University, Egypt

.Received: 14/11/2011 Accepted: 28/11/2011

Abstract: *Three hundred sixty, 7 days old Japanese quail chicks were randomly divided into six groups of 60 chicks, with similar initial average body weight, in three replicates of 20 birds each. Different levels from natural product of yeast cell walls in form of (commercial suspension) containing 25 g/L MOS +30 g/L B-glucan were used in this experiment. The six treatment groups were used i.e negative and positive control (groups 1, 2) fed the basal diet. The other groups fed the basal diet and supplemented with different levels (0.5, 1, 1.5 and 2 ml/L respectively) of above suspension via drinking water from the second wk of age tell the end of experiment (six wk). All chicks from groups 2, 3, 4.5 and 6 were vaccinated against Newcastle Disease virus (Lasota strain vaccine) at day 21 and repeated at day 28, respectively to evaluate the primary and secondary antibody responses. Five birds from each group at day 35 were injected in the right wing- web with 0.1mL of phytohemagglutinin-L. The swelling of the wings were measured before injection, at 24, 48 and 72 hrs, post injection, to measure the cell mediated immunity response.*

The results indicated that, the highest (P< 0.001) body weight gain was recorded for the group fed on 0.5ml/L (12.5 mg/L MOS +15 mg/L B-glucan) (group 3). A similar trend was nearly obtained for the feed conversion ratio (FCR) in which, the low level of MOS+B-glucan significantly (P< 0.001) improved the feed efficiency as compared with the remaining treatments. Non Significant differences have been recorded in TP and Alb, but significant differences in Glb and A/G ratio were obtained compared with control chicks. Regarding to the total lipids, data clearly indicate significant decrease for chicks fed (25 mg/L MOS + 30 mg /L B-glucan) (group 4) compared with the control and other treatments. Triglycerides as well as cholesterol levels diminished significantly due to application of low doses. AST activity was non significantly reduced in treated quails. The high values of Lipase and amylase enzymes activities were found in (groups 3 and 4, respectively). The primary and secondary immune responses were improved for the chicks fed low levels of MOS and B-glucan compared with other supplemented groups. It is clearly observed that, the control treatments(group 1, 2) and the high level of MOS + B-glucan (group 6) had the lowest (P< 0.001) wing-web swelling at 24 h post PHA-L injection compared with the chicks received other levels of MOS+B-glucan (group 3,4 and 5). Histological observation support the performance data, where the growth performance of the treatment groups was significantly improved. It could be concluded that, treated Japanese quails with low levels of MOS and B-glucan improves growth performance, regulate lipid metabolism and enhance humoral and cellular immunity.

Key Words: Immunity, Biological Functions, Oligosaccharide, B-Glucan, Japanese Quail

INTRODUCTION

Prebiotics are short-chain carbohydrates consist of polymerization of two to sixty sugar's units. They are though to be non digestible by human or animal digestive enzymes (Cummings and Macfarlance, 2002).

Prebiotics are feed ingredients that stimulate selectivity to growth and activity of bifidobacteria and lactobacilli in the digestive tract of birds, and so, increase the natural resistance. Using of dietary additives such as Probiotics and Prebiotics has many beneficial effects on growth and feed efficiency (Dilworth and Day, 1978 and Crawford, 1979).

Mannan oligosaccharide (MOS) and B-glucan are present naturally in the cell wall of the yeast cell (*Saccharomyces cerevisiae*). They exerts a significant growth promoting effect by enhancing the animal's resistance to enteric pathogens. MOS is commercially available as a feed supplement and regarded as safe compound (Ferket, 2004). Based on the scientific literature, MOS enhances resistance to enteric disease and promotes growth by: (1) inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining (Oyofo *et al.*, 1989; Spring *et al.*, 2000; Duval-Iflah, 2001;Valancony *et al.*, 2001), (2) enhances immunity (Ferket,2002; Humphrey *et al.*, 2002), (3), qrush border mucin barrier (Iji *et al.*, 2001; Loddi *et al.*, 2002), (4) integrityof the gut lining (Sonmez and Eren, 1999; Ferket, 2002) (5) and reduces enterocyte turnover rate (Spring *et al.* 2000).

The benefits of MOS are based on specific properties that include modification of intestinal flora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system in the intestinal lumen. These properties have the potential to enhance growth rate, feed conversion efficiency and viability in commercial broilers and turkeys and to increase egg production. (Dilworth and Day, 1978, Crawford, 1979, and Hooge, 2004). In addition, prebiotics resulted in increase the activities of digestive enzymes in gastro-intestinal tract of broiler chicks (Dawlat *et al.*, 2006).

B-glucans are structural components of the cell wall of many bacteria, fungi, algae and yeast as well as cereal grains. They are belong to group of physiologically active compounds teamed biological response modifiers due to their ability to stimulate the immune system (Lowry *et al* 2005).

Therefore, the aim of the present study was to demonstrate the effect of different levels from natural product containing mixture of Mannan oligosaccharide and B-glucan (as prebiotics) on the growth performance, blood biochemical profile of Japanese quail. Also, the digestive enzymes activities and humoral and cellular immunity were studied.

MATERIALS AND METHODS

The present study was carried out at Poultry Production Farm, Faculty of Agriculture, Zagazig university, during summer season of year 2010.

Birds, Diets and Experimental Design

Three hundred sixty, 7 days old Japanese quail chicks were randomly divided into six groups of 60 chicks, with almost similar initial average body weight , in three replicates of 20 birds each. All birds were housed in battery cages with similar hygienic and managerial conditions. Feed and water were provided for *ad libitum* consumption throughout the experimental period. The grower diet was formulated to meet all requirements as recommended by NRC (1994). The composition of the basal diet is shown in Table (1).

Different levels from natural product of yeast cell walls in form of (commercial

suspension) containing 25 g/L MOS +30 g/L B-glucan were used in this experiment. The six treatment groups were used as negative and positive control (groups 1,2) fed the basal diet. The other groups fed the basal diet plus different levels of above suspension via drinking water from the second wk of age tell the end of experiment(six wk) as follows: (All groups were vaccinated, except only the first group).

Groups	Treatments
1	Negative control fed basal diet
2	Positive control fed basal diet
3	Fed basal diet+0.5 ml/L(12.5mg/L MOS+ 15 mg/L B-glucan)
4	Fed basal diet+ 1ml/L(25 mg/L MOS+30mg/L B-glucan)
5	Basal diet +1. 5 ml/L (37.5 mg/L MOS+ 45 mg/L B-glucan)
6	Basal diet + 2ml (50mg / L MOS +60 mg/L B-glucan)

Performance traits

The individual body weights (BW) were weekly recorded from the beginning of experiment till the sixth week. Then, the gain(BWG) was calculated. Feed consumption (FC) was also recorded and feed conversion ratio (FCR) was calculated as feed to gain (g feed/g gain) ratio.

Immunization and titration against Newcastle Disease virus (NDV)

All chicks from groups (2,3,4.5 and 6) were vaccinated against Newcastle Disease virus (Lasota strain vaccine) at day 21 and repeated at day28. Plasma samples were collected seven days after the first and second immunization respectively to evaluate the primary and secondary antibody responses (Hitchner *et al.* 1980). The antibody responses were measured using a micro– heamaggLutination technique as described by Dix and Taylor (1996). The antibody data were expressed as the log 2 of the reciprocal of the highest dilution given visible agglutination.

Cell mediated immunity response

Five birds from each treatment group at day35 were injected in the right wing- web with 0.1mL of phytohemagglutinin-L (Sigma Chemical Co., st. Louis, u 063178), (each 1m contains 100 ug PHA-L dissolved in sterile saline). The swelling of the wings were measured with micrometer before injection and at 24, 48 and 72 hrs, post injection. While the left wing was injected with 0.1 ml of the sterile saline and kept as control.

Blood biochemical analysis

At the end of the experimental period (6wk), six chicks from each group were randomly chosen and sacrificed in a horizontal position to reduce the antiperistalsis movement of intestinal segments and regurgitation of their contents. Blood samples were collected in heparinized tubes, centrifuged at 4000 rpm for 15 min. Plasma were decanted and stored at -20^{0}C for subsequent biochemical analysis.

Plasma total protein (TP,g/dl) according to Henry, (1974).Albumin (Alb,g/dl) by the method described by Doumas et al;(1971). Globulin(Glb) concentration was calculated by subtraction of plasma Alb from plasma TP. Total lipids (TL,g/L) was determined according to Knight *et al*, (1972) and cholesterol (TC mg/dl) according to Richmond, (1973). Triglycerides (TG,mg/dl) was also estimated by the method of Trinder, (1969). Activities (IU/L) of alanine transaminase (ALT) and aspartate transaminase (AST) were colorimetrically determind by using commercial kits according to Reitman and Frankel (1957).

Measurement of pancreatic enzymes activities

The contents and the adjacent epithelial lining of the duodenum and upper ileum were taken, mixed and about 1g of the

mixed content was immediately diluted with 10 ml of distilled water. All samples were centrifugated (3000 rpm/min) for 10 minutes, the supernatant fluid was taken then stored at -20 c^0 until the time of enzymatic analysis. Digestive enzymes activities were measured in the digestive fluid samples according to Pinchasov and Noy (1994) for amylase and Sklan *et al.* (1975) for lipase.

Histological studies

The same previous Six birds of each group were taken during slaughtering time for histological studies. Tissue samples from illum were taken, immediately fixed in 10% Formo- saline solution for ordinary histological technique. All sections (4-5 um thick) were stained with haemotoxylline and eosin (H& E) stains. Sections were examined under light microscope and then photographed using digital camera.

Statistical analysis

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 1994). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

Data listed in Table (2) clearly indicate significant increases in BW at 6 wk age and BWG of chicks fed on diets supplemented with all levels of MOS and B-glucan compared with control groups. It is of interest that, the highest ($P< 0.001$) BWG was recorded for the chicks fed on 12.5 mg/L MOS +15 mg/L B-glucan (group 3). A similar trend was nearly obtained for the FCR in which, the low level of MOS+B-glucan significantly ($P< 0.001$) improved the FCR as compared with the remaining treatments, while the higher levels of MOS+B-glucan did'nt improve the FCR as shown in Table (2). These results are in full agreement with those obtained by Benites *et al.* (2008) who found that addition of MOS at 1.0/0.5/0.5 (starter/grower/finisher) kg/ton diets had significantly greater BW at 42 d than birds fed control. No significant differences were found for FCR or mortality for any of the treatments. On the other hand, the results are in disagreement with those obtained by Waldroup *et al.* (2003), and Eren *et al.* (1999) who did not detect any improvement in BW when (1g/gk MOS diet) was fed to broilers.

MOS is reported to have at least three probable modes of action by which broiler performance may be improved: (1) adsorption of pathogenic bacteria containing type 1 fimbria with manose sensitive lectins, sometimes referred to as the ((receptor along)) mechanism (strongly binding to and decoying pathogens away from the sugar coated) intestinal lining , or stated another way, different bacterial strains can agglutinate MOS (oyofo *et al.*, 1989, spring *et al.*, 2000). (2) improved intestinal function or ((gut health)) for example, increases villi height, uniformity and integrity (Loddi *et al.*, 2002) and (3) immune modulation stimulates gut associated and systemic immunity by acting as a non pathogenic microbial antigen, given an adjuvant-like effect (Ferket *et al.*, 2002).

Chae *et al.* (2006) found that there was no significant effect of the feeding system or the β-glucan levels on the performance from 0 to 17 d but during 18–34 days birds housed on the open floor had significantly ($P< 0.001$) higher weight gain compared with those in cages.

Plasma protein profile

Data presented in Table (3) clarified the effect of feeding Japanese quail on different levels of MOS, and B-glucan on plasma protein profile. Non Significant differences have been recorded in TP and Alb, but significant differences in Glb and A/G ratio were obtained compared with control chicks. These findings in turn have influenced the A/G ratio as it is significantly declined in treated groups (3, 4 ,5 and 6)

than the control groups (1 , 2). The chicks fed 12.5 mg MOS+15mg B-glucan (group3) recorded the lowest value. These reduction may reflect an enhancement of Japanese quail immunity. The A/G ratio has been well known as an indicator for the metabolic activities and immune resistance. In birds, the low A/G ratio indicates more disease resistance and immune response (Griminger,1986). The improving effect of MOS and B-glucan on immunity of Japanese quail could be attributed to the influence of these feed additives on fed consumption, absorption, and utilization of nutrient as have been concluded by Zeweil (1997), Abdel-azeem (2002) and Tolba *et al.* (2004) on broilers.

Plasma lipids

The effect of MOS and B-glucan on plasma lipid profile is given in Table (4). Regarding to the total lipids (TL), data clearly indicate significantly decrease for chicks fed (25 mg/L MOS + 30 mg /L B-glucan), group (4) compared with the control and other treatments. Triglycerides (TG) as well as cholesterol (TC) levels diminished significantly due to application of low doses from MOS and B-glucan (group 3,4). Similar cholesterol depressing effect has been found in mule ducklings (Djouvinov *et al*, 2005), broilers (Mohan *et al*, 1996), and laying hens (De Roos and Katan, 1998 and Panda *et al*, 2003).

The dietary prebiotic concentration may be directly related to blood cholesterol content. The cholesterol-depressing effect could be attributed to the ability of probiotics and prebiotics to reduce cholesterol absorption and synthesis in gastrointestinal tract (Mohan *et al*. 1995and Kurtoglu *et al*. 2004).They showed that feeding on diets supplemented with mannan oligosaccharide reduced blood cholesterol. It may be due to MOS fermentation in gastrointestinal tract. The major products of MOS fermentation in the gastro-intestinal tract are short chain fatty acids which inhibit cholesterol synthesis (Cumming, 1991).

Liver enzymes

Regarding to liver function (expressed as plasma AST and ALT) data recorded in Table (5), clearly indicate non-significant variations between control and other treatments in the AST and ALT activities. In this respect, Abdel-Azeem, (2002) and Tolba et al. (2004) reported that prebiotic did not alter the activity of liver enzymes in plasma. These findings indicate no harmful effect of supplementation of broiler's diets with these biological materials on liver function. Consequently, it can conclude that MOS and B-glucan are safe in Japanese quail feeding.

Digestive enzymes

Digestive enzymes activities (lipase and amylase) in upper illume of gastrointestinal tract are represented in Table (6). The main features resulted by supplementation of Japanese quail diets with different level of MOS+B-glucan are increasing the activities of these digestive enzymes in significant manner. The high values of lipase and amylase enzymes activities were found in the level of 25 mg/L MOS+30 mg/L B-glucan and 12.5 mg/L MOS + 15 mg/L B-glucan (groups 4,3 respectively).

The observed high activities of these enzymes may be due to the pancreatic enzymes excreted in the gastrointestinal tract of treated Japanese quail . It is worthy to attribute the improvement of broilers growth performance to pronounced increases in digestive enzymes activities due to supplementation of diets with MOS (Dawlat *et al.* 2006) .On the other word, these additives may enhance nutrient utilization and improve FCR. In this connection, Osman (2003) reported that specific probiotic bacteria such as Bacillus and Lactobacillus produce digestive enzymes and interfere the physicochemical properties of gastrointestinal contents by which nutrients will be more accessible for digestive enzymes. In case of MOS supplemented, the elevation in

hydrolytic enzymes activities could be attributed to the ability of this material to activate the gastrointestinal micro-flora to produce more hydrolytic enzymes.

Humoral and cellular immunities

Antibody production

The effect of feeding Japanese quail on different levels of MOS+ Beta-glucan for a period of 6 wk on humoral immunity against NDV challenge are presented in Table (7).

It is clearly shown that chicks fed on (25 mg/L MOS+30 mg/L B-glucan) could enhanced the total antibody production estimated 7 d post the first immunization with NDV. While, the chicks of no supplementation (control) had the lowest primary antibody titers when compared with the other supplemented groups. Similar results with highly significance were completely achieved 7 d post second challenge with the same antigen.The results showed that the highest secondary antibody production was attained for chicks that fed on (12.5 mg/L MOS+15 mg/L B-glucan) followed by those given(25 mg/L MOS+30 mg/L B-glucan), group (3 and 4). It could be noticed that primary and secondary responses were improved for the chicks fed low levels of MOS and B-glucan compared with other supplemented groups and non supplemented one. Portions of cell wall structure of the yeast organism, saccharomyces contained (MOS) which elicit powerful antigenic properties. Ferket *et al.* (2002) suggested that an increase in antibody of the innate immune system to react to foreign antigenic material of microbial orgin. On the other hand, Asli *et al.* (2007) noticed that, the dietary supplemented with yeast during heat stress condition can improve the immune response of birds.

Cell-mediated immunity

From Table (8), it was noticed that injection of PHA-L significantly increased the wing-web swelling of chicks received 25 mg/L MOS+30 mg/L B-glucan (group 4). It is clearly observed that, the control treatments(group 1 ,2) and the high level of MOS + B-glucan (group 6) had the lowest ($P< 0.001$) wing-web swelling 24 h post PHA-L injection compared with the chicks received other levels of MOS+B-glucan (group 3,4 and 5).These results revealed that the cell mediated immunity of quail chicks may be enhanced by supplemental prepiotics.

It is well known that PHA-L is a lectin isolated from red kidney bean and stimulates T-cell proliferation with minimal effect on B-cells. It is considered a good in vivo measure of T-lymphocyte function. Cell-mediated immunity (CM) plays a major role in the response against intracellular bacteria and virus. (Qureshi et al. 1997). Several explanations were suggested for the obtained positive correlation between the immune response and the addition of MOS+B-glucan during the experimental period. Since MOS can enhance immunity system with beneficial actions (Ferket *et al*. (2002) and Humphary *et al* . (2002)). Such as prevention of intestinal colonization by diseas producing bacteria. Induces macrophages activation and toxin binding.

Lowery *et al.* (2005) found that β-glucan, as a feed additive (significantly ($P<0.05$) provided protection against *Salmonella* in young chickens. Huff *et al.* (2006) suggest that supplementation of broiler diets with beta-1,3/1,6-glucan may be valuable for decreasing production losses due to E. coli and respiratory disease.

Histological features

Histological examination of the intestinal sections from Japanese quail birds as influenced by different treatments are illustrated in Plates 1 to 6. It is clear that the villi diameters were increased in treatment groups (3-6) compared with the control ones (1 and 2). However, this increase was more obvious in groups that treated with 12.5mg/L MOS+15 mg/L B-glucan, 25 mg/L MOS + 30 mg/L B-glucan and 50 mg/L MOS+ 60 mg/L B-glucan (plates 3,4 and 6).This trend

was also observed at the level of 37.5 mg/L MOS+ 45mg/L B-glucan (group 5- plate 5) but the number of villi per unit area (microscopic field) was less than the other treatment groups.

Moreover, there were great variations in the crypts of Lieberkuhn size with supplemental additive especially in group 3, 5and6 (plates 3 , 5and 6). These crypts are known to secrete fluids containing different vital substances essential for the internal micro-environment of the small intestine segments. The crypts fluids are rabidly observed from the villi lumens to the epithelial cells of the villi, making a circulation from crypts to villi epithelium which results in a watery vehicle supply for improving absorption and utilization of nutrients, elaboration and production of antibodies and lymphocytes along with an increase in Goblet cells which secret substances responsible for reducing PH of the intestinal lumen.

A large intestinal surface area is a key for optimal digestive function; therefore the surface of the small intestine should be covered with long healthy villi. Yang *et al.* (2008) reported better energy digestion when including MOS in broilers. Several studies with MOS in poultry have looked at the intestinal structure and discovered longer villi and more shallow crypt (Baurhoo *et al.* 2009, Iji ., *et al.* 2001 Moraes and Ariki. 2002).A shallow crypt is a good indicator for an efficient small intestine, which requires fewer nutrients for renewal . With a low renewal rate the intestinal cells become more mature. followed by more efficient digestive enzyme production and nutrient absorption. To protect the villi and intestinal surface, the gut produces protecting mucus. This mucus is produced in specific Goblet cells. In general, the number of Goblet cells is an indicator of mucus production. Researchers found that goblet cell numbers were increased with MOS (Baurhoo *et al.* 2007).

It could be concluded that treated Japanese quails with low levels of MOS and B-glucan improves growth performance, regulate lipid metabolism and enhance humoral and cellular immunity. Generally, the administration of MOS and B-glucan were beneficial for Japanese quails.

Table (1): Composition and calculated analysis of Japanese quail grower diets.

Ingredient	Grower %
Yellow corn	54.56
Soybean meal (44%)	36.30
Protein Concentrate (52%)	6.80
Corn oil	0.95
Calcium Carbonate	0.70
Limestone	0.01
Lysine	0.03
Premix*	0.30
Salt (Nacl)	0.35
Total calculated analysis	100
Crude protein %	24
ME kcal/kg	2903.3
Lysine	1.3
Methionine	0.438
Calcium %	0.8
Available phosphorus (%)	0.315

Each kilogram of diet contains = VA, 4mg., D3, 2500 I.U., E, 10mg., B1, 2mg., B2, 5mg., B6, 4mg., B12, 10ug., Niacin, 25mg., Pantothenic acid, 10mg., Biotin, 50ug., Folic acid, 1000ug., and Coline chloride, 255mg. Selenium, 300ug., Copper, 10mg., Iodine, k, 2.0mg., Iron, 33mg., Manganese, 60mg.and , 60mg. Zinc.

Table (2): Growth performance of six wk old Japanese quail chicks fed on different levels of MOS+B-glucan.

Treatment/Traits	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.05 ml/L	Group 6 2ml/L	SEM*
Body weight (g) (1st wk)	20. 5	22.5	20.5	20.5	20.5	21.5	0.83
Body weight (g) (6wk)	180.32^{c}	182.5^{c}	217.75^{a}	198.39^{b}	206.52^{b}	206.73^{b}	1.11
Body weight gain (g)	159.81^{c}	160.58^{c}	196.97^{a}	177.62^{b}	185.69^{b}	184.43^{b}	1.13
Feed consumption(g)	446^{c}	431^{c}	408^{b}	418^{b}	503^{a}	438^{c}	2.44
Feed –conversion ratio	2.79^{c}	2.69^{c}	2.08^{a}	2.35^{b}	2.71^{c}	2.38^{b}	0.001

Means within a row with different superscripts are significantly different (P< 0.05)
*SEM= standard error of means.

Table (3): plasma protein profile of six wk old Japanese quail chicks fed on different levels of MOS+B-glucan.

Treatment/traits	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.05 ml/L	Group 6 2ml/L	SEM*
Total protein (g/dl)	4.33	4.48	5.28	4.41	4.73	4.73	1.24
Albumin (A) (g/dl)	2.30	2.19	2.09	2.11	2.29	2.27	1.72
Globulin (G) (g/dl)	2.03^{c}	2.28^{bc}	3.19^{a}	2.30^{bc}	2.46^{abc}	2.46^{abc}	0.81
A/G ratio	1.14^{ab}	0.96^{bc}	0.65^{c}	0.93^{bc}	0.94^{bc}	0.92^{bc}	0.30

Means within a row with different superscript are significantly different (P< 0.05)
*SEM= standard error of means.

Table (4): plasma lipids profile of six wk old Japanese quail chicks fed on different levels of MOS+B-glucan.

Treatment/traits	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.05 ml/L	Group 6 2ml/L	SEM*
Total Lipids (g/l)	11.97^{c}	12.03^{b}	12.66^{a}	11.81^{d}	11.99^{c}	11.88^{d}	2.392
Triglycerides (mg/dl)	312.50^{b}	320.25^{a}	297.29^{d}	300.75^{c}	318.25^{a}	319.33	2.142
Cholesterol (mg/dl)	146.1^{a}	168.9^{a}	92.6^{b}	106.8^{b}	120.1^{b}	109.68^{b}	1.117

Means within a row with different superscripts are significantly different (P<0.0001)
*SEM= standard error of means.

Table (5): Liver enzymes activities in plasma of Japanese quail fed on different levels of MOS +B-glucan.

Treatment/traits	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1. 5 ml/L	Group 6 2ml/L	SEM*
AST(IU/L)	39.25^{a}	37.00^{a}	35.00^{a}	34.00^{a}	37.00^{a}	35.25^{b}	0.597
Alt(IU/L)	4.07^{a}	4.22^{a}	4.15^{a}	4.17^{a}	4.17^{a}	4.02^{a}	1.93

Means within a row with different superscripts are significantly different (P< 0.05)
*SEM= standard error of means.

Table (6): Digestive enzymes activities in duodenum and upper ileum of Japanese quail fed on different levels of MOS+ B-glucan .

Treatment/traits	Group 1 (Negative Control	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.5 ml/L	Group 6 2ml/L
Amylase	1.87^{d} ±0.136	1.93^{c} ±0.125	2.20^{b} ±0.245	2.36^{a} ±0.118	2.21^{b} ±0.078	1.92^{c} ±0.085
Lipase	7.76^{d} ±0.301	8.28^{c} ±0.678	9.25^{a} ±0.518	9.40^{a} ±0.357	8.04^{c} ±0.201	8.95^{b} ±0.624

Means within a row with different superscripts are significantly different (P<0.05)

Table (7): Humoral immunity against NDV of 6wk old Japanese quail chicks fed on different levels of MOS+B-glucan.

Treatment/traits	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.5 ml/L	Group 6 2ml/L	SEM*
Primary	2.00^{d}	4.00^{b}	4.00^{b}	5.00^{a}	3.33^{c}	3.00^{c}	0.055
Secondary	2.00^{d}	4.00^{b}	6.00^{a}	5.00^{a}	4.66^{b}	5.00^{a}	0.277

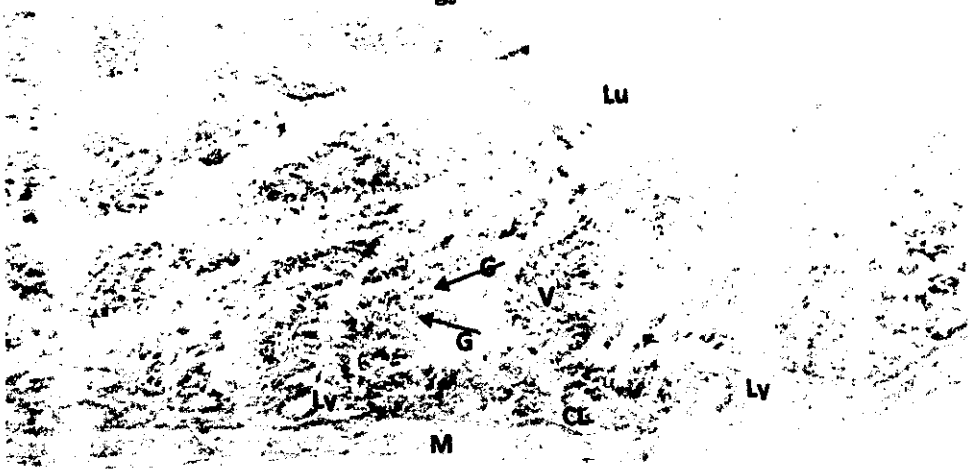
Means within a row with different superscripts are significantly different (P<0.0001)
*SEM= standard error of means.

Table (8): Cell-mediated Immunity of Japanese quail chick fed on different levels of MOS +B-glucan.

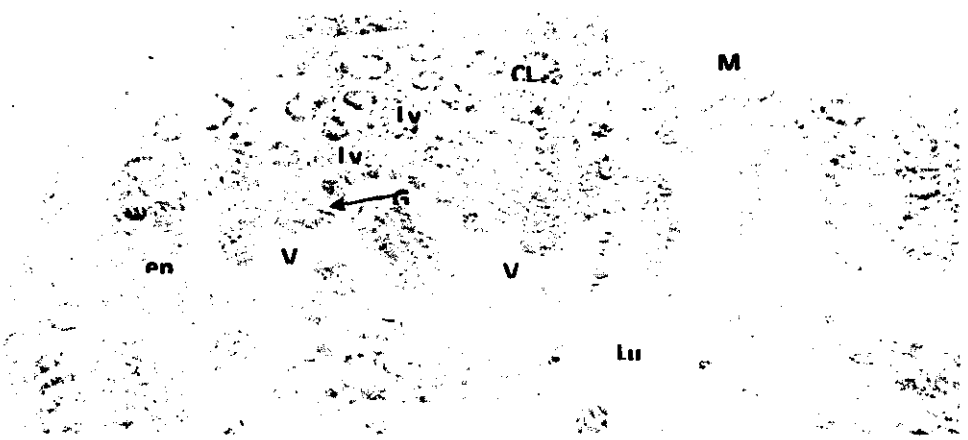
Time-post injection (hour)	Group 1 (Negative Control)	Group 2 (Positive Control)	Group 3 0.5 ml/L	Group 4 1ml/L	Group 5 1.5 ml/L	Group 6 2ml/L	SEM*
24	0.535^{bc}	0.505^{c}	0.607^{ab}	0.679^{a}	0.629^{ab}	0.524^{bc}	0.005
48	0.482^{a}	0.475^{a}	0.491^{a}	0.499^{a}	0.503^{a}	0.479^{a}	0.001
72	0.395^{a}	0.393^{a}	0.424^{a}	0.429^{a}	0.433^{a}	0.409^{a}	0.001

Means within a row with different superscripts are significantly different (P<0.0001)
*SEM= standard error of means.

Histology of ileum



Plate(1): T.s. of ileum from T1 quails (H&EX100)



Plate(2):T.S. of ileum from T2 quails (H&E X 100)

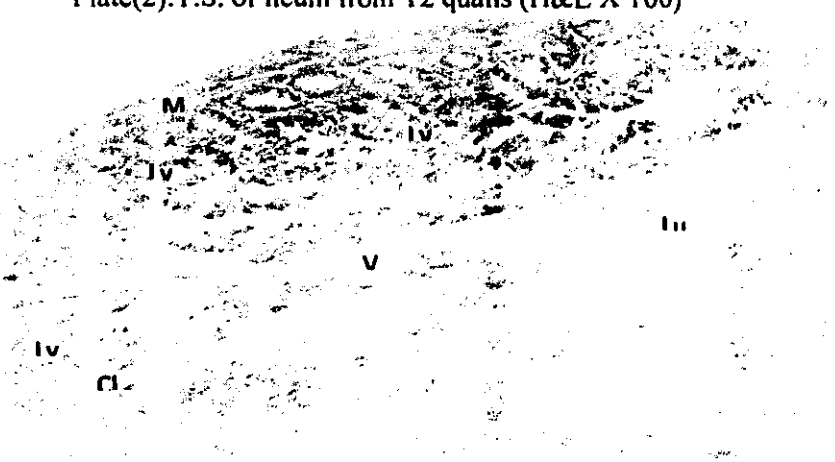


Plate (3) : T.s. of ileum from T3 quails (H&EX100)

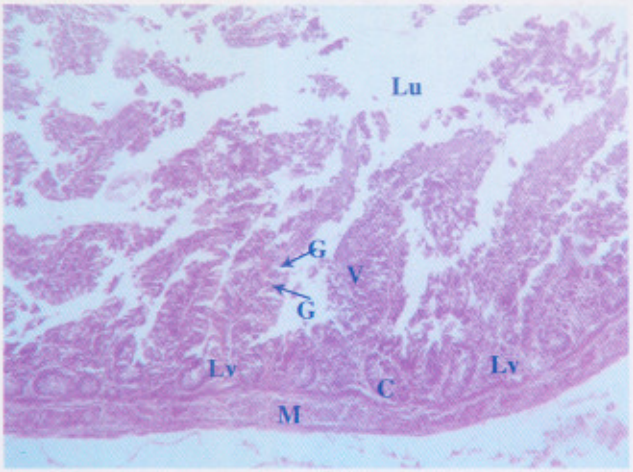


Plate (1) : T.s. of ileum from T1 quails (H&EX100)

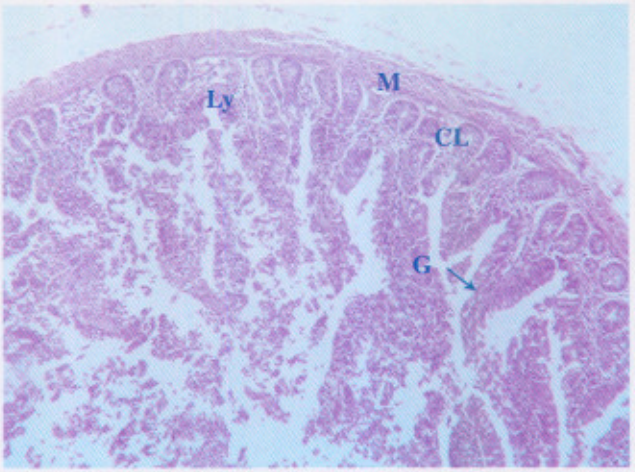


Plate (4) : T.s. of ileum from T4 quails (H&EX100)

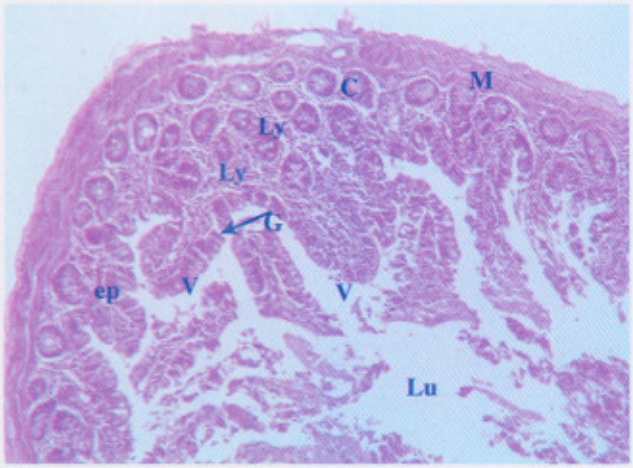


Plate (2) : T.s. of ileum from T2 quails (H&EX100)

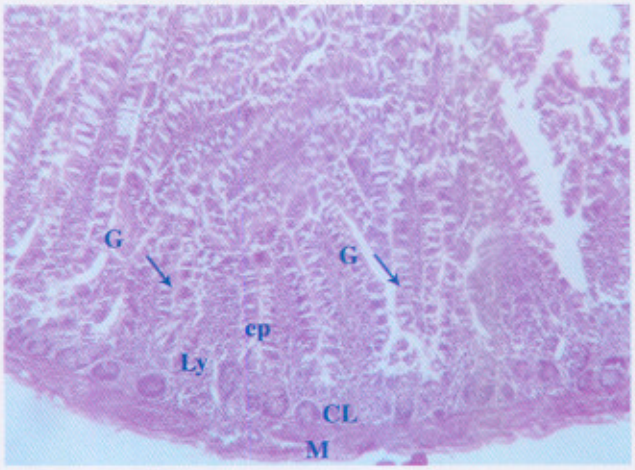


Plate (5) : T.s. of ileum from T5 quails (H&EX100)

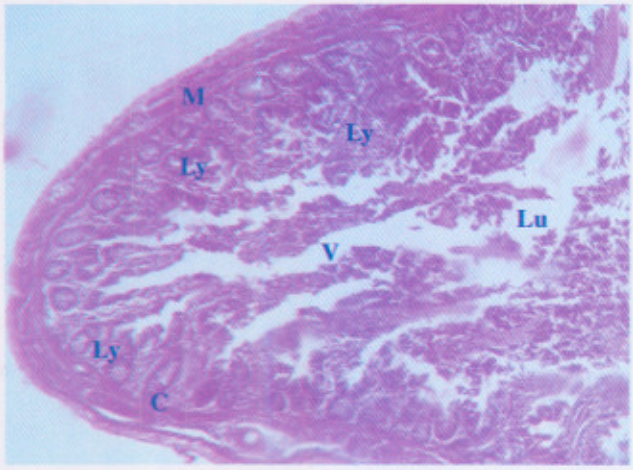


Plate (3) : T.s. of ileum from T3 quails (H&EX100)

Plate (6) : T.s. of ileum from T6 quails (H&EX100)

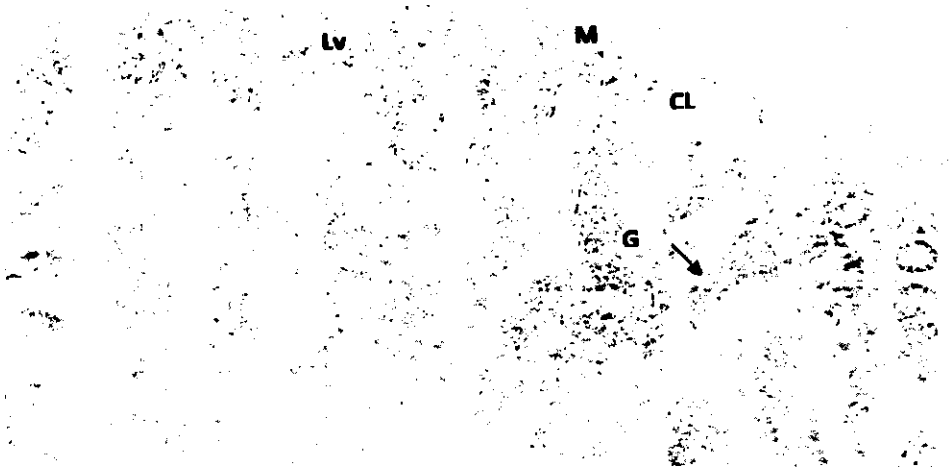
Abbreviation Key :

LY = Lymphocytes , V = Villi

CL = Crypts of Lieberkuhn , Lu = Intestinal Lumen

MM = Muscularies mucosa layer , ep = epithelial lining

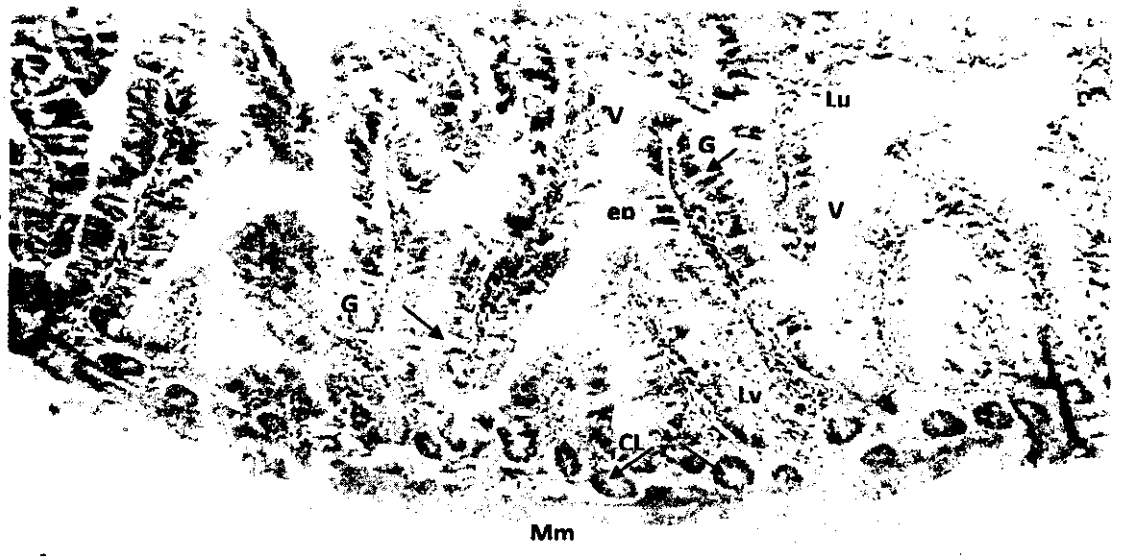
G = goblet cells



Plate(4): T.s. of ileum from T4 quails (H&EX100)

Plate (5) : T.s. of ileum from T5 quails (H&EX100)

Plate(5): T.s. of ileum from T5 quails (H&EX100)



Plate(6): T.s. of ileum from T6 quails (H&EX100

Abbreviation Key:
Ly=Lymphocytes , V=Villi
CL=Crypts of Lieberkuhn , Lu=Intestinal Lumen
MM=Muscularies mucosa layer , ep= epithelial lining
G=goblet cells

REFRENCES

Abdel-Azeem, F. (2002). *Digeston, Neomycin and Yeast supplementation in broiler diets under Egyptian summer conditions. Egypt. Poult. Sci. 22(1): 235-257.*

Asli,M.M, Hosseini, S. A, Lotfollahian,H and Shariatmadari,F (2007). *Effect of Probiotics, Yeast, Vitamin E and Vitamin C Supplements on Performance and Immune Response of Laying Hen During High Environmental Temperature. Int. J. of Poult. Sci. 6 (12): 895-900.*

Baurhoo, B; Phillip, L; Ruiz-Feria, CA (2007). *Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens". Poult. Sci. 86 (6): 1070–8.*

Baurhoo, B.; Ferket, P. R.; Zhao, X. (2009). *Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers". Poult. Sci. 88 (11): 2262–72.*

Benites.V, Gilharry. R, Gernat. A. G. and Murillo, J. G. (2008). *Effect of Dietary Mannan Oligosaccharide from Bio-Mos or SAF-Mannan on Live Performance of Broiler Chickens. j. APPL. POULT. RES. (17):471-475.*

Chae. B. J, Lohakare J.D., Moon. W.K., Lee. S.L., Parkand. Y.H. and Hahn. T.-W. (2006). *Effects of supplementation of β-glucan on the*

growth performance and immunity in broilers vet. Sci. vol. 80 (3): 291-298.

Crawford, J. S. (1979). *Probiotics in animals, Nutrition. Proceeding of Arkansas Nutrition Conference, PP.45:55.*

Cummings, J.H. (1991). *Production and metabolism of short-chain fatty acids in humans. Pages 11-17 in short-Chain Fatty Acids ; Metabolism and Clinical Importance. Rep .10th Rpss Conf. on Med. Res. A . F . Roche, ed. Ross Laboratories, Columbus, OH.*

Cummings, J. H. and G.T. MacFarlane (2002). *Gastro-intestinal effects of prebiotics. British J. of Nutrition (87): 145-151.*

Dawllat A. Salama; Mervat A. R. Ibrahim and sror.H A. (2006). *Fortification of broiler's diet with either probiotics or prebiotics have improved growth performance and some biological functions. J.Biol. Chem. Environ. Sci; Vol, 1 (4), 633-647.*

De Roos, N. M. and Katan, M. B. (1998). *Effects of probiotic bacteria on diarrhea, lipid metabolism and carcinogensis a review of papers published between 1988-1998. Poult. Sci . 77: 1259-1265.*

Dilworth, B. C. and Day, E. J. (1978). *Lactobacillus cultures in broiler Diets. Poult. Sci. 57:1101.*

Dix, M. C. and Taylor, Jr. R. L. (1996). *Differential antibody response in 6.B major histocompatibility (B) complex congenic chickens. Poult. Sci., 75:203-207.*

Djouvinov, D.; Biocheva, S.; Simeonova, T. and Vlaikova, T. (2005). *Effect of feeding lactina probiotic on performance, some blood parameters and caecal microflora of mule ducklings. Trakia J. of Sci . 3 (2) 22-28.*

Duncan, D. B. (1955). *Multiple ranges and multiple F-tests. Biometrics. 11:1-42.*

Duval-Iflah, Y.S., 2001. *Comparison of yogurt, heat treated yogurt, milk and lactose effects on plasmid dissemination in antibiotic mice. Ant. Van Leeu. Int. J. Gen. and Mol. Microbial., 79: 199.*

Doumas, B. T.; Watsom, W. A. and Bigges, H. G. (1971). *Albumin standard and the measurement of serum albumin with bromocresol green. Clin.Chem.Acta. 31:87-96.*

Eren, M., Deniz, G., Birickik, H., Gezen, S.S., Turkemen, I.I. and Yavuz, H.M. (1999). *Effects of supplementation of zinc bacitracin, mannan oligosaccharide and probiotic in broiler feeds on fattening performance. Vet. Fak. Derg. Ulu. University, 18: 73–84.*

Ferket, P.R., Parks C.W. and Grimes, J.L. (2002). *Benefits of dietary antibiotic and mannan oligosaccharide supplementation for poultry. In: Proc. Multi-State Poult. Feeding and Nutr. Conf., Indianapolis, Indiana USA. May 14-16, p: 22.*

Ferket, P. R. (2002). *"Use of oligosaccharides and gut modifiers as replacements for dietary antibiotics". Proc. 63rd Minnesota Nutrition Conference, September 17–18 (Eagan, MN): 169–82.*

Ferket, P.R., (2004). *Alternatives to antibiotics in poultry production: response, practical experience and recommendations. In: Lyons, T.P. and K.A. Jacques (eds.), Biotechnology in the Feed and Food Industry, Proceedings of Alltech's 20th Annual Symposium,* pp: *57–66. Nottingham University Press, UK.*

Griminger. P. (1986). *Lipid Metabolism in "AVIAN PHYSIOLOGY" edited by P.*

D Sturkie. 4th ed. Springer-Verlag, Inc., New Work, NY.

Henry, R. J. (1974). *Clinical Chemistry ,Harper & Row Publishers, New York.PP 181.*

Hitchner, S. B.; Domrmuth, C. H.; Purchase, H. G. and Williams, J. E. (1980). *Isolation and Identification of avian pathologists. Creative printing company Inc. Endwell. NY.*

Hooge, D.M., (2004). *Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993-2003. Int. J. Poult. Sci., (3): 163–74.*

Huff GR, Huff WE, Rath NC, Tellez G.(2006)*Limited treatment with beta-1,3/1,6-glucan improves production values of broiler chickens challenged with Escherichia coli.Poult Sci.85(4):613-8.*

Humphrey, B.D., Koutsos, E.A. and Klasing, K.C., (2002). *Requirements and priorities of the immune system for nutrients. In: Jacques, K.A. and T.P. Lyons (eds.), Biotechnology in the Feed and Food Industry, Proceedings of Alltech's 18th Annual Symposium, pp: 69–77. Nottingham University Press, UK.*

Iji, P.A., Saki, A.A. and Tivey, D.R. (2001). *Intestinal structure and function of broiler chickens fed on diets supplemented with a mannan oligosaccharide. J. Sci. Food Agric., 81: 1186–92.*

Knight, J. A.; Anderson, S. and Rawle, J. M. (1972). *Chemical base of the sulfo-phospho-vanilin reaction for estimation total serum lipids. Clin.Chem.18(3): 199.*

Kurtoglu, v.; Kurtoglu, F.; Seker, E.; Coskun, B.; Balevi, T. and Polat, E. S. (2004). *Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Additives and Contaminants, 21 (9): 817-823.*

Loddi. M.M., Nakaghi, L.S.O., Edens F., Tucci. F.M., Hannas. M.I., Moraes V.M.B. and Ariki. J., (2002). *Mannan oligosaccharide and organic acids on intestinal morphology integrity of broilers evaluated by scanning electron microscopy. Page 121. In: Proc. 11th European Poultry Sci. Conf., Bremen, Germany. Sept. 6-10.*

Lowry, v. k, Famell , M.B Ferto, P. J, swaggerty, C.L, Bahl, A. and Kougt , M.H (2005) *Purified B-glucan as an abiotic feed additive up-regulate the innate immune response in immature chickens against salmonella enteric serovar Enteritichs International Journal of Food Microbiology vol. 98 (3): 309-318.*

Mohan, B.; kadirvel. R.; Bhaskaran. M. and Natarajan. A. (1995) *Effect of probiotic supplementation on serum /yolk cholesterol and on egg shell thickness in layer. Br. Poult. Sci., 36:799-803.*

Mohan, B.; Kadirvel.R.; Natarajan. A. and Bhaskaran. M. (1996). *Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broiler. Br. Poult. Sci., 37: 395-401.*

Moraes and Ariki. J., (2002). *Mannan oligosaccharide and organic acids on intestinal morphology integrity of broilers evaluated by scanning electron microscopy. Page 121. In: Proc. 11th Europ. Poult. Sci. Conf., Bremen, Germany. Sept. 6-10.*

NRC (1994). *National Research Council. Nutrient Requirements of Poultry 9th ed. National Academy Press, Washington, D. C.*

Oyofo, B.A., DeLoach. J.R., Corrier. D.E., Norman. J.O., Ziprin, R.L. and Mollenhauer. H.H., (1989).

Prevention of Salmonella typhimurium colonization of broilers with D-mannose. Poult. Sci., 68: 1357–60.

Osman, M. (2003). *The influence of probiotic inclusion on the productive performance of commercial layers. Egyptian poult. Sci., 23:283-297.*

Panda, A. K; Reddy. M. R.; Rama. S.V.; Rao. S. V.; and Praharj. N.K. (2003) *Production performance, serum/yolk cholesterol and immune Competence of white leghorn layers as influenced by dietary Supplementation with probiotic. Trop. Anim. Health Prod. 35 (1): 85-94.*

Pinchasov,Y .and Noy. Y . (1994). *Early postnatal amylases in the gastrointestinal tract of turkey poults. Comp. Biochem. Phyosiol., 106:21-25.*

Qureshi, M. A.; Edens. F. W., and Harvenstein. G. B. (1997). *Immune system dysfunction during exposure to poult enteritis and mortality syndrome agents. Poult. Sci., 76: 564-569.*

Reitman,S. and Frankel. S. (1957). *A colorimetric methods for determining serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56-63.*

Richmond, N. (1973). *Preparation and Properties of cholesterol oxidase from Nacardia sp. And its application to enzymatic assay of total cholesterol in serum. Clin. Chem. 19 (12) :1350-6.*

SAS, (1994). *SAS Procedure Guide. Version 6.12 Edition. SAS Institute INC., Cary, NC, USA.*

Sklan, D.; Hurwitz. S.; Budowski. P .; and Ascarelli. I. (1975). *Fat digestion and absorption in chicks fed raw or heated soybean meal. Nutr., 105: 57-63.*

Sonmez, G. and Eren. M., (1999). *Effects of supplementation of zinc bacitracin, mannan oligosaccharide and probiotic into the broiler feeds on morphology of the small intestine. Vet. Fac. Dergisi Uludag University, 18: 125–38.*

Spring, P., Wenk. C., Dawson. K.A. and Newman. K.E., (2000). *The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella challenged broiler chicks. Poult. Sci. (79): 205–11.*

Tolba, A. A.; Sabry. M. M.; and Abuzead. S. M. M. (2004). *Effect of microbial Probiotics on Performance of broiler chicks under normal and Heat stress conditions 1-Lactobacillua or Pediococcus. Poult. Sci. 24(11): 351-367.*

Trinder ,P . (1969). *Ann. Clin. Biochem. 66:24-27.*

Valancony, H., Humbert. F., Rukelibuga. J., Bougon. M., Balaine. L. and Lalande. F., (2001). *Comparison of some substitutes for antibiotic additives in diets for turkey poults. Effects on production and on resistance to salmonella colonization. Sci. Tech. Avicoles, 35: 25–34.*

Waldroup, P.W., Oviedo-Rondon. E.O. and Fritts. C.A., (2003). *Comparison of Mos and antibiotic feeding programs in broiler diets containing copper sulfate. Int. J. Poult. Sci., 2: 28–31.*

Yang, Y.; Iji, P. A.; Kocher, A.; Thomson, E.; Mikkelsen, L. L.; Choct, M. (2008). *"Effects of mannan oligosaccharide in broiler chicken diets on growth performance, energy utilization, nutrient digestibility and intestinal microflora". British Poult. Sci. 49 (2): 186–94.*

Zeweil, H. S. (1997). *Evaluation of using some feed additives in growing Japanese quail diets. J.Agric. Mansoura Univ., 22(11) : 3611-3622.*

الملخص العربي

تعديل المناعه وبعض الوظائف البيولوجيه للسمان الياباني باضافة المنان اوليجو سكريدات والبيتاجلوكان

زينات عبد الجواد ابراهيم

قسم انتاج الدواجن- كلية الزراعه- جامعة الزقازيق

استخدم في هذه التجربه عدد ٣٦٠ كتكوت من السمان الياباني عمر ٧ يوم وقد تم تقسيم هذا العدد الى ٦ مجموعات في كل مجموعه ٦٠ كتكوت في ثلاثة مكررات في كل منها ٢٠ طائر. المجموعتين الاولى والثانيه استعملت كضابط سالب للاولى بدون معامله ولكن تم تحصينها والاخرى ضابط موجب بدون معامله ومحصنه. اما المجاميع من الثالثه الى السادسه فقد تم تجريعها في ماء الشرب يوميا من الاسبوع الثاني الى السادس بمستويات مختلفه (٠.٥ مل، ١ مل، ١.٥ مل و ٢ مل/لتر) من منتج طبيعي (على شكل معلق تجاري) يحتوي على جدر خلايا الخميره المحتويه على المنان اوليجو سكريدات والبيتا جلوكان بنسبة ٢٥جم/لتر و ٣٠ جم /لتر لكل منهما. تم تحصين الكنترول الموجب (مجموعه ٢) والاربعة مجاميع المعامله(٣و٤و٥و٦) بفيرس نيو كاسل (عترة لاسوتا) في عمر ٢١ و٢٨ يوم لتقدير الاستجابه المناعيه الاوليه والثانويه لهذه المحاميع وايضا تم حقن خمسة طيور من كل معامله لجميع المعاملات بمادة PHA-L في عمر ٣٥ يوم لتقدير المناعه الخلويه. وفي نهاية التجربه (٦ اسابيع) تم اخذ عينات من الدم لفصل البلازما وتقدير بعض التقديرات البيوكيميائيه مثل بروتينات البلازما، الدهون الكليه والثلاثيه والكوليستيرول، انزيمات الكبد، بعض الانزيمات الهضميه كما تم دراسة صفات النمو ومعاون التحويل الغذائي بالاضافه الى عمل قطاعات هستولوجيه في بعض الاجزاء من الامعاء.

اوضحت النتايج الاتي:

١- زيادة الاوزان الكليه ومعدلات النمو للطيور المعامله عن الكنترول وكان اعلى معدل نمو بعد المعامله (٠.٥ مل / لتر. مجموعه ٣) من المعلق التجاري كما اظهرت النتايج تحسين معنوي في معامل التحويل الغذائي لهذا المستوى ايضا.

٢- لايوجد اختلافات معنويه في مستوى البروتين الكلي والالبيومين في حين انخفضت نسبة الالبيومين/ الجلوبيولين للمجاميع المعامله بنسبه قليله من المعلق التجاري (٠.٥، ١مل/لتر. مجموعه ٣و٤) مما يدل على التحسين المناعي.

٣- بخصوص الدهون الكليه في بلازما الدم اظهرت النتائج انخفاضا معنويا عند معاملة (١مل/لتر. مجموعه ٤) واما الدهون الثلاثيه والكوليستيرول فقد انخفضت في جميع المستويات المعامله عن الكنترول.

٤- ادت المعاملات بالمنان اوليجو سكريدات والبيتا جلوكان الى انخفاض مستوى نشاط انزيمات الكبد في البلازما مما يعكس التاثير الواقي لهذه المواد.

٥- كما اظهرت النتائج زياده في بعض الانزيمات الهضميه (الاميليز والليبيز) والتي انعكست على الاداء الانتاجي للسمان وعضدتها نتائج الفحص الهستولوجي للامعاء.

٦- بخصوص كل من المناعه الخلطيه والخلويه اوضحت النتائج ان الطيور المعامله بمستويات قليله (٠.٥ و ١ مل/ لتر. مجموعه ٣ و٤) من المنان اوليجو سكريدات والبيتا جلوكان زياده في الاستجابه المناعيه الاوليه والثانويه عن المستويات الاعلى (٢ مل /لتر. مجموعه ٦) في حين لا توجد اي استجابه للكنترول السالب (مجموعه ١). اما المناعه الخلويه فقد اظهرت المجموعه المغذاه على (١مل/لتر) على اعلى استجابه للحقن بمادة PHA-L بعد ٢٤ ساعه من الحقن.

وتخلص هذه النتائج الى ان اضافة منان اوليجو سكريدات والبيتا جلوكان الموجودان طبيعيا في جدر خلايا الخميره الى ماء الشرب بنسب قليله يحسن من الاداء الانتاجي وينظم تمثيل الدهون ونشاط الكبد بالاضافه الى تحسين المناعه الخلطيه والخلويه لهذه الطيور.