

## BLACK SEED EXTRACTED OIL FOR ENHANCING PRODUCTIVE AND PHYSIOLOGICAL STATUS OF BROILER.

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### SUMMARY

Effect of extracted black seed oil (BSE) on broiler performance was evaluated. Four groups of 60 chicks were divided in this study. The first group (control) fed basal diet, the other groups fed diet supplemented with 0.1, 0.2% extracted oil (BSE1 & BSE2) and 0.2% Flavophospholipol as antibiotic growth promoter (AGP). The results showed ( $P \leq 0.05$ ) higher body weight at 21 and 35 days of age for the group supplemented with BSE2 compared with other groups. Carcass and breast were greater ( $P \leq 0.01$ ) in birds fed diets supplemented with either the two supplementations of BSE or AGP than control group. Significant high concentrations have been recorded in total protein and globulin in BSE2 group followed by BSE1 and AGP. The A/G ratio was declined from 0.523 in control to range of 0.319 to 0.431 in the others. The activity of amylase enzyme recorded more than 5 folds in BSE2 and twice in BSE1 compared with both AGP and control groups. Protease activity in stomach recorded more than 4 folds in BSE2 and 2 folds comparing with AGP and control groups. This study showed encouraging improvement in immune response accompanied by live body weight for broilers fed diets supplemented with BSE compared with the other groups. It could be concluded that BSE addition to broiler diets may improve performance and immunity characteristics.

**Keywords:** *extracted black seed oil; Flavophospholipol; broiler performance; immunity.*

### INTRODUCTION

Antibiotic growth promoters have played an important role in animal growth by helping animal avoid bacterial diseases. Antibiotics appeared to act by reducing the pathogenic bacteria load and modifying the micro-flora in the gut. Supplementations of antibiotic additives to livestock diets by low-level can help prevent illness and improve performance (Hedde, 1984). However, their prolonged use has the potential to increase bacterial resistance and the level of drug residues in edible animal products. This will lead to the transfer of antibiotic resistance to human pathogens and will be harmful to human. (Hughes and Heritage, 2004; Kritas and Morrison, 2005).

Recently, the use of antibiotics as feed additives has been gradually restricted (Kocher, 2005; Cervantes, 2006; Plail, 2006) and prohibited in the European Union since 2006. Therefore, considerable efforts have been made to search for alternatives to antimicrobial feed additives over the past decade. One alternative to antimicrobial feed additives is essential oils derived from herbs and spices. The use of medical plants (herbs) has a long history throughout the world and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries. Herbal essential oils assist in colonization of the beneficial microbial population within the gastrointestinal tract to more balanced levels (Zaika, 1988; Jang *et al.*, 2007). Besides their antimicrobial properties (Ultee *et al.*, 2002), they also exhibit antioxidant (Basmacioglu *et al.*, 2004), antifungal (Bang *et al.*, 2000; Shin and Lim, 2004), digestion-stimulating, and enzymatic activities (Jamroz *et al.*, 2003, 2005; Hernandez *et al.*, 2004).

One of the herbs is the black seed. *Nigella* seeds (black seeds) are cultivated domestically in Morocco from the *Nigella sativa* plant and used as a spice, condiment and medicinal treatment. The very fact that black seed targets the vital workings of the immune system grants it power over a wide range of ailments, both as a treatment and preventative. Consequently, black seed has proved itself a forceful ally against many ailments, such as those caused by bacteria, viruses, and common allergies.

The goal of this present work was to study the productive performance, physiological status and immune response for replacing antibiotic growth promoter with the extract of black seed in broiler diets

## MATERIALS AND METHODS

### *Birds management:*

Two hundred and forty Hubbard broiler chicks one day old were used in this study. All chicks were vaccinated against the common viral diseases (NDV, IBDV) at the recommended periods. Two feeding phases were applied: starter, from 1-21 days and finisher from 21-35 days of age. Experimental diets were formulated to meet the nutrient requirements of the broiler chicks (NRC, 1994) which are presented in Table 1. The chemical composition of the basal diets was analyzed according to A.O.A.C. (1995). Feed and water were supplied *ad-libitum* and a constant (22L: 2D) light period was provided during the experimental periods.

**Table (1): Composition, calculated and chemical analysis of the basal diets**

Ingredients (%)	Starter (0-3 Weeks)	Finisher (3-5 Weeks)
Yellow corn	57.77	71.00
Soybean meal (44%CP)	25.5	13.60
Corn gluten meal	10.00	10.00
Vegetable oil	3.00	2.00
Bone meal	2.60	2.00
Limestone	0.30	0.60
Vit & Min Premix*	0.30	0.30
NaCl	0.25	0.25
L-Lysine	0.18	0.20
DL-Methionine	0.10	0.05
Total	100	100
A-Chemical analysis:-		
Crude protein %	22.16	18.00
Crude fiber %	3.53	3.13
Ether extract %	2.86	3.12
Ash %	6.77	6.58
B-Calculated analysis:		
ME (Kcal/Kg diet)	3139	3221
Calcium %	1.02	0.90
Available phosphorous %	0.45	0.35
Lysine %	1.15	0.90
Methionine %	0.51	0.40
Cystine %	0.38	0.33
Meth. + Cys. %	0.89	0.73

\*Each 3kg of vit-mineral mixture contain vit A 10m IU, vit D3 1mIU, vit E 10g, vit B1 1g, vit B2 4.0g, vit B6 1.5g, Nicotinic acid 20g, Pantothenic acid 10g, vit B12 0.01g, Biotin 0.05g, Folic acid 30g, Choline chloride 50g, Iron 30g, Manganese 40g, Copper 3g, Iodine 0.45g, Zinc 45g and Selenium 0.1g.

### *Supercritical carbon dioxide extraction of black seeds:*

The extraction was carried out in an automated and computerized laboratory-pilot extraction plant, SFF model as described by Rao *et al.* (2007). The extraction pressure was controlled by micrometering valves and the carbon dioxide used was Premier-X50S. In the present study, the highest extraction yield for black seed oils was 31.2% under the SFE conditions which the temperature was 50°C and the extraction pressure 400 bar.

### *Experimental design:*

The broiler chicks were divided randomly into four treatment groups of 60 chicks (3 replicates/20 chicks/each). The first group was served as control and fed basal diet, while, the other groups received the

basal diet supplemented with 0.1, 0.2% black seed extracted oil (BSE) and 0.2% Flavophospholipol (commercial pharastim 4%) as antibiotic growth promoter (AGP), respectively. The extracted oil was mixed with vegetable oil (as a carrier) which used in diet as one of the energy sources. Chicks weight was recorded at 7, 21 and 35 day of age. Body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR, g feed/g gain) were recorded during the same periods while the mortality rate was recorded daily.

***Blood parameters:***

At 35 d of age, ten birds from each experimental group were weighed and slaughtered by slitting the jugular vein, then scalded and defeathered. From each bird, 3 ml blood was collected in heparinised tubes. The blood samples were centrifuged at 3000 r.p.m / min for 15 minutes, clear plasma was separated then stored in a deep freezer at -20°C until biochemical analysis. Determination of plasma total protein and albumin were done colorimetrically by using available commercial kits (Diamond Diagnostics Company). The globulin values were calculated by subtracting the values of albumin from the corresponding values of total protein. Total lipids, triglycerides and total cholesterol were determined according to Zollner and Kirsch (1962) while low density lipoprotein (LDL) was determined according to Assmann *et al.* (1984). Liver enzymes activity (aspartate aminotransferase, AST and alanine aminotransferase, ALT) were determined according to Reitman and Frankel (1957). Calcium was determined according to Gindler and King (1972) while inorganic phosphorus was determined according to Amador and Urban (1972). Creatinine was determined colorimetrically by using available commercial kits (Diamond Diagnostics Company). Plasma concentration of thyroid hormones, triiodothyronine (T3) and thyroxine (T4) were determined using commercial enzyme immunoassay test kit (Taytec Incorporation, 7278 Aldercrest Dr., Mississauga, ON, L5N7N8, Canada).

***Immune Response against sheep red blood cells:***

Nine chicks per treatment (3/replicate) were injected at 7 days of age intravenously with 1 ml of 7 % suspension of sheep red blood cells (SRBC'S) in phosphate-buffer saline (PBS). At 14, 21, 28 and 35 days of age, blood samples were collected and centrifuged with SRBC'S to determine the primary and secondary antibody responses. The humeral immune response was detected according to Van der zijpp *et al.* (1983) and Bachman and Mashaly (1986). Antibody production was measured using a micro-titer technique (El-Kaiaty *et al.*, 2002). Immune organs (spleen, thymus (all lobes of both sides) and bursa of Fabricious) were removed and weighted from the slaughtered birds.

***Carcass traits:***

Carcasses were manually eviscerated; weighted and relative carcass parts (breast, thigh and drum) were calculated. Liver, heart, gizzard, and abdominal fat relative weights were recorded while the intestinal weight and length (cm) were measured.

***Digestive tract enzyme activity:***

Digestive tract (stomach and intestine) was emptied by gentle squeezing and their contents were taken, mixed and about 1g of the mixed content was immediately diluted with 10 ml of distilled water. All samples were centrifuged for 2500 r.p.m / min for 10 minutes, the supernatant fluid was taken then stored at -20°C until the time of enzymatic analysis. Amylase and protease activity of digestive content of stomach and intestine were determined according to Osman (1982) and Malik and Singh (1980), respectively.

***Histological technique for light microscope:***

Representative specimens of bursa of Fabricious (as a lymphoid organ), liver, and small intestine (juenium) for different groups were fixed in 10 % formalin-saline solution and prepared by the ordinary histological techniques. The sections were stained with haemotoxylline and eosin (H& E) stains according to the methods of Culling (1983). These sections were examined under X40 power using light microscope and photographed by using a suitable digital Camera.

***Statistical analysis:***

Data were statistically analyzed by using the General Linear models (GLM) procedures of SAS (SAS, 2001). Significant differences among treatment means were determined by Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

**Performance traits:**

The effects of dietary treatments on BW, FCR, and mortality are shown in Table 2. The two levels of BSE supplementation and AGP supplementation in the diets of broiler affect positively in the BW and the livability of the broilers at the growing period than those fed diets without supplementations.

**Table (2): Effect of feeding different substitutive levels of black seed on productive performance of growing chicks.**

Item	Control	BSE1	BSE2	AGP	Prob.
Live body weight (g) at:					
At 7 days	122.0 $\pm$ 4.49	127.0 $\pm$ 5.11	124.05 $\pm$ 3.99	126.0 $\pm$ 4.12	0.74
21days	658.37 <sup>c</sup> $\pm$ 10.13	700.35 <sup>b</sup> $\pm$ 11.25	765.58 <sup>a</sup> $\pm$ 15.16	702.39 <sup>b</sup> $\pm$ 13.22	0.032
35days	1614.96 <sup>c</sup> $\pm$ 15.3	1717.52 <sup>b</sup> $\pm$ 25.2	1877.72 <sup>a</sup> $\pm$ 30.3	1823.44 <sup>ab</sup> $\pm$ 20.2	0.006
Daily body weight gain (g) from:					
7-21 days	38.21 <sup>c</sup> $\pm$ 1.09	40.92 <sup>b</sup> $\pm$ 0.75	45.85 <sup>a</sup> $\pm$ 0.95	41.19 <sup>b</sup> $\pm$ 0.89	0.04
21-35days	68.62 <sup>c</sup> $\pm$ 1.99	72.75 <sup>b</sup> $\pm$ 2.02	79.31 <sup>a</sup> $\pm$ 3.01	72.91 <sup>b</sup> $\pm$ 2.99	0.01
7-35 days	53.09 <sup>c</sup> $\pm$ 1.15	56.77 <sup>b</sup> $\pm$ 1.39	62.47 <sup>a</sup> $\pm$ 2.58	59.08 <sup>ab</sup> $\pm$ 1.29	0.01
Daily feed consumption (g) from:					
7-21 days	58.92 $\pm$ 2.53	59.83 $\pm$ 3.46	61.93 $\pm$ 2.98	59.93 $\pm$ 2.78	0.58
21-35days	125.79 $\pm$ 4.58	126.5 $\pm$ 4.82	133.07 $\pm$ 4.15	130.79 $\pm$ 3.93	0.63
7-35 days	92.36 $\pm$ 6.23	93.21 $\pm$ 5.16	97.5 $\pm$ 7.12	95.36 $\pm$ 6.16	0.63
Feed conversion ratio (Feed/Gain) from:					
7-21 days	1.54 <sup>a</sup> $\pm$ 0.06	1.46 <sup>b</sup> $\pm$ 0.07	1.35 <sup>c</sup> $\pm$ 0.08	1.45 <sup>b</sup> $\pm$ 0.09	0.002
21-35days	1.84 <sup>a</sup> $\pm$ 0.08	1.74 <sup>b</sup> $\pm$ 0.09	1.68 <sup>c</sup> $\pm$ 0.05	1.79 <sup>b</sup> $\pm$ 0.06	0.01
7-35 days	1.74 <sup>a</sup> $\pm$ 0.02	1.64 <sup>b</sup> $\pm$ 0.05	1.56 <sup>c</sup> $\pm$ 0.06	1.67 <sup>b</sup> $\pm$ 0.07	0.008
Mortality %	5	3	2	4	-

<sup>a, b, c</sup> Means within the same row with different superscripts are significantly different.

The results showed significantly ( $P \leq 0.05$ ) higher body weight at 21 day of age for the group supplemented with BSE2 than other groups and the same trend was recorded at 35 day of age. The best LBW ( $P \leq 0.01$ ) was recorded for BSE2 group than control, BSE1 and AGP groups. Concerning daily weight gain, the groups supplemented with BSE1 & 2 and AGP showed higher daily weight gain than control group (Table 2). The highest values were recorded for BSE2 group followed by BSE1 & AGP groups which were equally similar at 7, 21 and at 35 day of age. However, no significant effects were observed on FC between treatments at all experiment periods. The results of performance traits were in agreement with Ahmed *et al.*, 2009. They reported the advantages for using natural additive supplementation (aucalyptus leaves) to compensate the positive effect of using antibiotic growth promoter (pharmastin 4%) on growth rate. The beneficial effect of growth promoter substances, such as antibiotics, on performance is related to a more efficient use of nutrients, which in turn results in an improved FCR (Devriese *et al.*, 1993). Recent scientific articles regarding dietary supplementation with the extracts of some plants indicated encouraging initial results (i.e., exhibited growth promotion, nutrient digestibility enhancement, and feed efficacy mechanisms) in broiler chickens without affecting bird mortality (Alçiçek *et al.*, 2003, 2004; Hernandez *et al.*, 2004; Jamroz *et al.*, 2005; Çabuk *et al.*, 2006; Garcia *et al.*, 2007).

**Carcass traits:**

The main results of carcass traits are set out in Table 3. Carcass, breast and thigh weights (as a percentage of carcass) were significantly greater ( $P \leq 0.01$ ) in birds fed supplements of either the BSE or AGP than the control group. No significant differences were observed for drum percentage for all groups. However, abdominal fat seemed to be lower in BSE groups than AGP and the control groups in respecting the role of black seed on reducing storing fats. These results agree with observations published from previous research on essential oils (Perdok *et al.*, 2003; Wald, 2003; Isabel and Santos, 2009). Moreover, the results of carcass traits in the present study suggest that dietary essential oils appear to have a beneficial effect on breast weight (Isabel and Santos, 2009).

**Blood parameters:**

Data presented in table 4 clarify the effect of feeding broilers on diets with different levels of BSE or AGP on plasma protein profile. Significant increases have been recorded in total protein and globulin in BSE2 group followed by BSE1 and AGP. No significant differences in albumin were observed compared with control group. These findings in turn have influenced the A/G ratio as it declined from 0.523 in the control to the range of 0.319 to 0.431 in the other treatments. These results are in agreement with Abd El-Motaal *et al.* (2008) who found that using 3 g eucalyptus leaves powder/ kg diet significantly increased the levels of plasma globulin and calcium compared with the control group. The reduction in A/G ratio may reflect an enhancement of broilers immunity. The improving effect of BSEs or AGP on the immunity of broilers could be attributed to the influence of these feed additives on feed consumption, absorption and utilization of nutrients as reported by Abd El-Azeem, 2002 and Tolba *et al.*, 2004. Regarding to total lipids, data clearly indicate significantly decreased in BSE2 group compared with the control. Plasma cholesterol and LDL levels were also significantly decreased in treated groups than control broilers. Similar results were reported by Ahmed *et al.* 2009 in growing Japanese quails. The reduction of cholesterol level may well be caused by inhibition of the culture within the intestine (Francis *et al.*, 1987). Sellars (1991) reported that the decrease in cholesterol level is attributed to deconjugating bile salts in the intestine, thus preventing them from acting as precursors in cholesterol synthesis while Ghazalah and Ibrahim (1998) referred that to the inhibition of their synthesis and not due to their redistribution among other body organs. Anderson and Gilliland (1999) reported that using natural additives like *Lactobacillus* reduces the blood cholesterol by direct breakdown of cholesterol and deconjugation of bile salt. Lutgens and Daemen (2004) reported that *Lactobacillus acidophilus* competitively inhibits HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway, thereby decreasing intra-cellular cholesterol synthesis. The decrease in hepatic intra-cellular cholesterol concentration results in compensatory increase in the expression of hepatic LDL receptors, which clear LDL from the circulation.

**Table (3): Effect of different levels of black seed extracted oil and antibiotic growth promoter supplementation on carcass traits.**

Item	control	BSE 1	BSE 2	AGP	Prob.
carcass%	59.13 <sup>c</sup> ±5.81	62.59 <sup>b</sup> ±4.71	62.76 <sup>a</sup> ±5.21	63.86 <sup>b</sup> ±7.32	0.009
gizzard%	2.017±0.33	2.201±0.51	2.493±0.26	2.109±0.42	0.076
liver%	2.683±0.44	3.466±0.25	2.691±0.34	3.075±0.31	0.081
heart%	0.421±0.04	0.526±0.05	0.540±0.04	0.473±0.05	0.059
spleen%	0.126±0.03	0.151±0.05	0.104±0.02	0.13±0.03	0.067
bursa%	0.115±0.06	0.148±0.05	0.131±0.06	0.131±0.06	0.064
abdominal fat%	1.491 <sup>b</sup> ±0.59	1.534 <sup>a</sup> ±0.57	0.711 <sup>c</sup> ±0.85	1.513 <sup>ab</sup> ±0.77	0.042
breast%	22.011 <sup>c</sup> ±0.19	22.876 <sup>ab</sup> ±0.26	23.769 <sup>a</sup> ±0.21	22.443 <sup>b</sup> ±0.19	0.038
thigh%	4.428 <sup>c</sup> ±0.96	4.706 <sup>b</sup> ±0.94	5.935 <sup>a</sup> ±0.99	4.567 <sup>b</sup> ±0.96	0.045
drum%	4.916±0.92	5.201±0.92	5.841±1.00	5.162±0.94	0.076
Intestine %	8.024 <sup>a</sup> ±0.95	7.856 <sup>b</sup> ±0.89	7.124 <sup>c</sup> ±0.92	7.940 <sup>b</sup> ±0.99	0.064

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

**Table (4): Effect of different levels of black seed extracted oil and antibiotic growth promoter supplementation on blood parameters of broiler.**

Item	control	BSE 1	BSE 2	AGP	Prob.
Total Protein g/dl	3.686 <sup>c</sup> ±0.35	4.423 <sup>b</sup> ±0.41	4.572 <sup>a</sup> ±0.27	4.425 <sup>ab</sup> ±0.32	0.04
Albumin g/dl	1.266±0.08	1.105±0.10	1.105±0.19	1.126±0.08	0.07
Globulin g/dl	2.420 <sup>c</sup> ±0.18	3.318 <sup>ab</sup> ±0.20	3.467 <sup>a</sup> ±0.16	3.299 <sup>ab</sup> ±0.21	0.05
A/G ratio	0.523	0.333	0.319	0.341	
Total lipids mg/dl	454.27 <sup>a</sup> ±12.5	445.16 <sup>b</sup> ±14.2	433.41 <sup>c</sup> ±19.4	449.72 <sup>ab</sup> ±11.3	0.008
cholesterol mg/dl	117.085 <sup>b</sup> ±13	123.065 <sup>a</sup> ±10	100.704 <sup>c</sup> ±12	120.075 <sup>ab</sup> ±11	0.01
LDL mg/dl	82.45 <sup>a</sup> ±1.23	68.24 <sup>b</sup> ±1.62	56.47 <sup>c</sup> ±5.62	59.11 <sup>c</sup> ±3.43	0.014
AST IU/L	30.20±3.12	29.40±1.10	30.40±2.29	29.80±1.19	0.75
ALT U/L	44.20±2.98	43.21±3.11	42.86±2.22	43.71±1.67	0.68
Ca (mg/dl)	9.86±0.16	10.21±0.76	10.15±0.27	10.04±0.33	0.68
P (mg/dl)	5.86±0.51	6.27±0.17	6.05±0.15	6.07±0.15	0.73
creatinine (mg/dl)	0.793±0.05	0.783±0.03	0.723±0.04	0.788±0.05	0.59
T3 ng/ml	1.70±0.16	1.81±0.01	1.73±0.19	1.77±0.09	0.06
T4 ng/ml	13.73±0.28	12.22±0.48	13.54±0.46	12.71±0.21	0.07

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

Regarding to liver function expressed as plasma AST and ALT, data recorded in Table 4 clearly indicate non significant variations between control and other treatments. Similarly, Abd El-Azeem, 2002 and Tolba *et al.*, 2004 reported that probiotics did not alter the activity of liver enzymes in plasma. The histological sections on liver confirmed these findings. The same trend was recorded for creatinine levels, as an indicator for kidney function, where no effects of BSEs or AGP were recorded. Concerning calcium and phosphorus levels, it was noticed that there were no significant differences among all groups. Concerning thyroid hormones, our results in table 4 indicate that plasma thyroid hormones concentrations were seems to be significantly differed among treatments. T3 levels raised in treated groups and free T4 levels declined compared with control group. These findings are in agreement with Abd El-Motaal *et al.* (2008) and Ahmed *et al.*, 2009. They found that using natural additives in diet (Eucalyptus) increased the levels of plasma thyroid hormone (T3) compared with the control group. These findings may reflect a good feed utilization, absorption and metabolism for birds when diets supplemented with BSE.

#### **Digestive enzymatic activity:**

Digestive enzymes activities (amylase, protease) in different segments of gastrointestinal tract are presented in Table 5. As a result of stomach pH, among groups, low amylase activity and no significant differences in samples taken from stomach. Although, high amylase activity was recorded in the small intestine contents. The activity of amylase enzyme was increased more than 5 folds in BSE2 and twice in BSE1 compared with either AGP or control group. On the other hand, protease activity was significantly different among groups when estimated in stomach and in small intestine. In stomach, protease activity was more than 4 folds in BSE2 and 2 folds comparing with AGP and control groups. Whereas, the same trend was found for its activity in small intestine. These data may explain our results for the superiority of supplemented broilers with BSEs in live body weights and blood profile. These findings may reflect a good feed utilization, absorption and metabolism for birds when diets supplemented with BSE.

**Table (5): Effect of different levels of black seed extracted oil and antibiotic growth promoter supplementation on enzymes activity of broiler**

Item	control	BSE 1	BSE 2	AGP	Prob.
In stomach content:-					
Amylase	0.448±0.199	0.717±0.184	0.820±0.172	0.524±0.189	0.077
Protease	15.216 <sup>c</sup> ±3.25	31.365 <sup>b</sup> ±5.75	65.952 <sup>a</sup> ±5.65	15.965 <sup>c</sup> ±4.89	0.010
In small intestine content:-					
Amylase	35.842 <sup>c</sup> ±5.17	80.150 <sup>b</sup> ±8.25	162.861 <sup>a</sup> ±15.02	38.126 <sup>c</sup> ±6.12	0.012
Protease	7.892 <sup>c</sup> ±2.45	14.659 <sup>b</sup> ±3.15	28.752 <sup>a</sup> ±4.23	8.268 <sup>c</sup> ±2.01	0.009

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

#### **Immune response traits:**

The same trend was observed also for immune response (Table, 6). Significantly high primary immune response (represented as the antibody titer against SRBC's) at 35 days of age were associated with either the BSE and AGP additive fed groups than the control. In general, the AGP group was higher than BSE1 and BSE2. Similar results were reported by Malzone *et al.* (2000) and Shashidhara and Devegowda (2003) who observed a significant increase in the antibody titers against SRBC's for birds fed 0.05 % of natural additives compared to the control. Similarly, Zulkifli *et al.* (2000) stated that the immune response of broilers increased when chicks were fed diet containing natural supplementations. The increase in antibody titers may be due to the influence of the microorganisms on immune system and/or the improvement in the intestinal absorption of micronutrients, such as: Zn, Cu and Se. In addition, it might be attributed to the reduction of the pathogenic bacteria load in the intestine which prevents the acute immune response against such bacteria (Finucane *et al.*, 1999 and Spring *et al.*, 2000).

**Table (6): Antibody titers against SRBC'S of broilers fed black seed extracted oil and antibiotic growth promoter supplementation (Mean ± SE).**

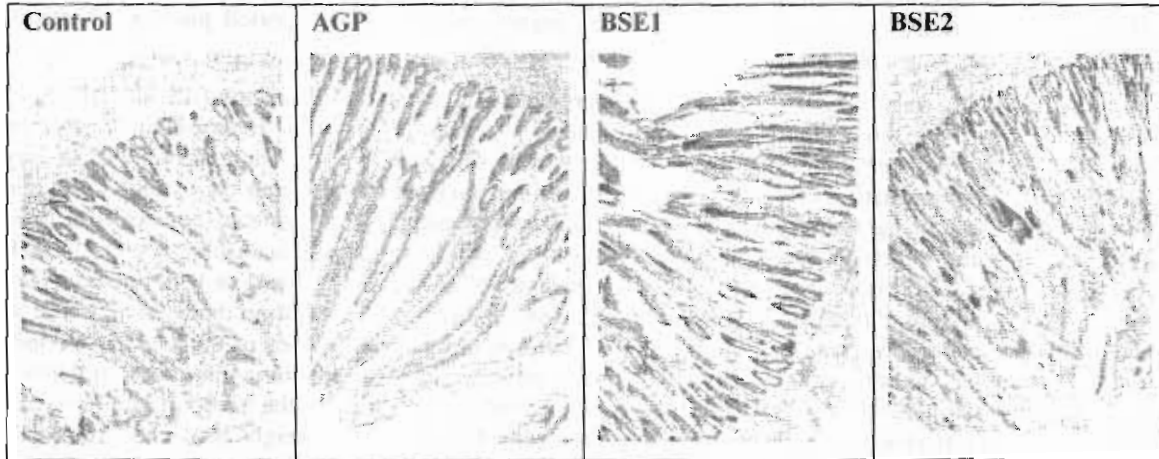
Item	Control	BSE 1	BSE 2	AGP	Prob.
7 days post primary SRBC's injection	4.23±0.36	4.43 <sup>b</sup> ±0.57	4.59 <sup>b</sup> ±1.35	4.72 <sup>a</sup> ±1.22	0.03
14 days post primary SRBC's injection	2.10±0.12	2.33±0.11	2.16±0.11	2.23±0.12	0.08
7 days post secondary SRBC's injection	4.74±0.78	4.54±1.32	4.63±0.99	4.71±1.21	0.13
14 days post secondary SRBC's injection	2.71±0.21	2.59±0.41	2.69±0.13	2.77±0.41	0.06

<sup>a, b, c</sup> Means within the same row with different letters are significantly differ.

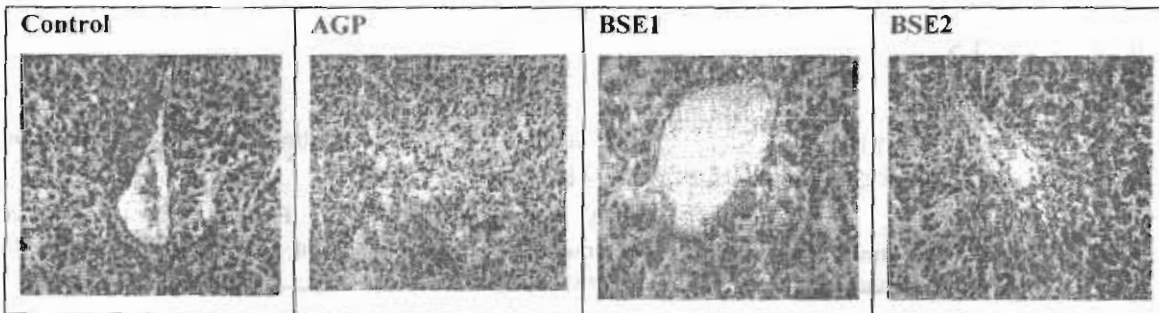
**Histological results:**

Histological examinations of the intestinal sections are illustrated in Figure (1). It is clear from the transverse sections (T.S) that the villi height increased in treated groups compared with the control one.

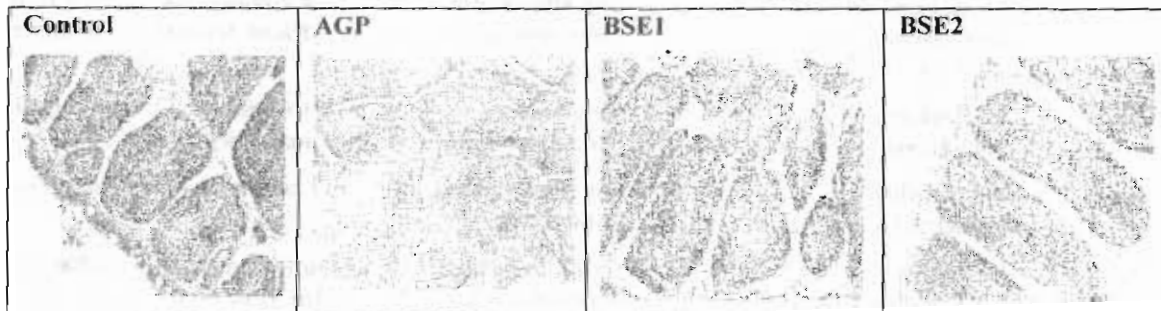
**Fig. (1): The histological structure (at 40X) of the small intestine from broilers fed different biological-additives and a control group.**



**Fig. (2): The histological structure (at 40X) of the liver from broilers fed different biological additives and a control group.**



**Fig. (3): The histological structure (at 40X) of the bursa of fabricious from broilers fed different biological additives and a control group.**



However, this increase was more obvious in groups that were fed basal diets supplemented with BSEs groups. Furthermore, there were great variations in the size and number of Crypts of Lieberkuhn associated with the supplemental additives. These Crypts are known to secrete fluids containing different vital substances essential for the internal micro-environment of the small intestine segments (Hodges, 1974). While the sections from the liver parenchyma of the control treatment Fig. (2) has normal hepatocytic structure with dilated central vein engorged with blood. Also, there were dark stained eosinophilic cells surrounding or near the central veins. There is moderate hypertrophy of liver cells especially in (AGP group) which may reveal hyperactivity of the liver cells or a compensatory effect due

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### الزيت المستخلص للحبة السوداء لتحسين الاداء الانتاجي و الحالة الفسيولوجية لدجاج التسمين.

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أجريت هذه الدراسة لتقييم تأثير مستخلص زيت الحبة السوداء (حبه البركة) علي أداء دجاج التسمين بدلا من منشطات النمو من المضادات الحيوية. تم تقسيم الكناكيت الي اربع مجموعات بكل مجموعة ٦٠ كتكوت. تم تغذية المجموعة الأولى علي العليقة الاساسية بدون إضافات بينما غذيت المجموعات الأخرى علي العليقة الاساسية مضافا اليها ٠,١% , ٠,٢% زيت مستخلص من الحبة السوداء و ٠,٢% فلافوسفوليبول كمضاد حيوي منشط للنمو علي التوالي. أظهرت نتائج هذه الدراسة زيادة معنوية (٠,٠٥) لوزن الجسم علي عمر ٢١ و ٣٥ يوم للدجاج المغذي علي زيت الحبة السوداء بمستوي ٠,٢% مقارنة بباقي المجموعات. كانت الأوزان النسبية للذبيحة لحم الصدر و الساق أعلى معنويا بمستوي ٠,٠١% للطيور المغذاة علي زيت الحبة السوداء بمستوياتها و المضاد الحيوي و ذلك بالمقارنة عن المجموعة الكنترول. تم تسجيل زيادات معنوية للبروتين الكلي و الجلوبيولين بالدم بالمجموعة المغذاة علي ٠,٢% زيت مستخلص ليها في المستوي ٠,١% زيت مستخلص و ٠,٢% مضاد حيوي. لم يلاحظ اختلافات معنوية بين الطيور بالمجموعات المختلفة بالنسبة لمستوي الالبيومين بالدم. أظهرت النتائج وجود تأثير علي نسبة الالبيومين للجلوبيولين بالدم حيث انخفضت من ٠,٥٢٣ الي ٠,٣١٩ و ٠,٤٣١ بالمجموعات المختلفة. أظهرت النتائج زيادة نشاط إنزيم الاميليز لأكثر من خمسة أضعاف للطيور المغذاة بزيت الحبة السوداء بنسبة ٠,٢% و بضعفين بتلك المغذاة بنسبة ٠,١% بالمقارنة بالمغذاة بالمضاد الحيوي و الكنترول. و من ناحية أخرى , كان النشاط لإنزيم البروتينيز مختلف معنويا بين المعاملات المختلفة عند تقديره بالقناة الهضمية : فقد سجل النشاط الإنزيمي للبروتينيز في المعدة أكثر من ٤ أضعاف في الطيور المغذاة علي الزيت المستخلص بنسبة ٠,٢% و ضعفين بالمقارنة بالمغذاة علي المضاد الحيوي و الكنترول. تشير النتائج بهذه الدراسة إلي تحسن مشجع للإستجابة المناعية للطيور مصحوبه بوزن الجسم لدجاج التسمين المغذي علي عليقة مضاف اليها مستخلص زيت الحبة السوداء بالمقارنة بالدجاج في المجموعات الأخرى. نستنتج من نتائج القطاعات الهستولوجية و الاداء الانتاجي لهذه الطيور في هذه الدراسة أن إضافة مستخلص الحبة السوداء لعلائق دجاج التسمين يمكن أن تحسن الاداء الانتاجي و مستوي المناعة لهذه الطيور.