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The growth Promoting Effect of Beta-glucan in Comparison with Sodium Butyrate on Broiler Chicks

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Key words

Broiler, growth, sodium butyrate, beta-glucan, molecular studies.

ABSTRACT:

The present study was designed to evaluate the effects of yeast beta-glucan (YBG) in comparison with sodium butyrate (SB) on the performance of broiler chickens, with special attention to their molecular, hematological, biochemical and histophathological alterations. Therefore 240 one day-old Cobb broiler chicks were divided into 4 groups. The first group(n=60): chicks received basal ration and water without any treatment (and considered as a control group), the second group (n=60): chicks treated with 25µg YBG/ml. drinking water/day, the third group (n=60): chicks treated with 0.49 mg SB/ml. drinking water/day and the fourth group (n=60): chicks treated with 0.98 mg SB/ml. drinking water/day. The chicks received the treatment daily for 42 days. The obtained results showed that administration of YBG or SB showed a significant improved average weight gain (AWG). The growth promoting effects of these agents is a result of the improvement in the expression of *Growth Hormone Secratogauge Receptor* (GHSR) and *Insulin-Like Growth Factor 1 Receptor (IGF1R)*. Both *GHSR* and *IGF1R* were significantly improved by 3.5 and 4 folds respectively in chicks treated with YBG(25 µg/ml. drinking water/day) , 2 and 2.25 folds respectively in group treated with SB (0.49 mg/ml. drinking water/day) and 3 and 2.75 folds respectively in group treated with SB (0.98 mg/ ml. drinking water/day). YBG and SB (0.49 mg or 0.98 mg/ ml. drinking water/day each alone) significantly increased packed cell volume (PCV)% and red blood corpuscles (RBCs) count in comparison with the control. YBG and SB (0.98 mg/ ml. drinking water/day)significantly increased hemoglobin concentration(HB)% and white blood corpuscles (WBCs) counts. Chicks treated with SB (0.49 mg or 0.98 mg/ ml. drinking water/day each alone) showed a significant increase in serum total protein, albumin and cholesterol in comparison with the control group. Moreover, in chicks treated with SB (0.98 mg/ ml. drinking water/day) AST was significantly increased in comparison with the control group. All treated groups showed significant reduction in total bilirubin level compared with control group. Other tested parameters in all treated groups were not significantly changed compared to control group. Intestines of all treated groups showed significant improvement in length of intestinal villi in comparison with the control group. From the obtained results, we can conclude that YBG and SB can be successively used as new alternatives of antibiotic growth promoting agents in broiler chicks. YBG is considered the most important alternative followed by SB in the high concentration level then SB in the low concentration level.

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1. INTRODUCTION

Recently, antibiotic growth promoters in poultry industry has been banned because the hazard effects on human health by the development of microbial resistance to these products (Roe and Pillai, 2003). Several alternatives to antibiotic growth promoters have been proposed, as yeast beta-glucan and organic acids.

Yeast beta-Glucan is prebiotic glucose polymers that naturally occur in yeasts, molds, algae, mushrooms, bacteria, oats and barley. Immunostimulation is one of the most important properties of beta-glucans. They are classified as biological response modifiers. It can be used in human and veterinary medicine and pharmacy due to their biological activities (Vlatka et al., 2010). Beta-glucan is a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon and thus improving host health (Yeo et al.,2009).

Sodium butyrate is a recently used organic acid in poultry diet to enhance the performance and the

immune response of birds (Dibner and Buttin, 2002). It is rapidly absorbed to provide energy to the epithelial cells (Jozefiak et al., 2004) and promote sodium and water absorption (Friedel and Levine, 1992). It increases the epithelial cell growth and the proliferation index in the intestinal crypts (Leeson et al., 2005), also it has trophic effect on the gut mucosa. Supplementation of these additives in the diet of broilers enhanced nutrient utilization, growth and feed efficiency.

The previous studies on YBG and SB in broiler chicks have mainly focused on their phenotypic effects. However, the mechanisms of their growth promoting effect in broiler chicks have not been clearly determined. So the aim of the present work was to study the effect of oral administration of YBG or SB on the growth performance in broiler chicks by estimation of both phenotypic changes in body weight, body weight gain and feed conversion ratio and the changes in the expression of the growth related gene, IGF1R and the digestibility and food conversion ratio (FCR) related gene, GHSR, as one of the possible mechanisms of action of these agents. Our work extended also to evaluation of some hematological, biochemical parameters and histopathological alteration caused by YBG or SB administration in broiler chicks.

1. MATERIALS AND METHODS

2.1. Drugs:

Yeast beta-glucan (Tri-Glumix®), Produced by Phytogenix Co., New York, USA. Sodium butyrate (Ding su®), Produced by Xiamen Fujian Co., China. All the diagnostic kits used for assaying the hepatic and renal functions were obtained from Bio-diagnostic Company, Egypt. Other chemicals used during the experiment were obtained from EL-Gomhoria Company, Egypt.

2.2. Birds and Experimental design:

240 one day-old Cobb broiler chicks were obtained from El-Jazera Company for parents, Alexandria, and then were randomly divided into four groups as previously mentioned; The experiment lasts for 42days. All groups were fed on the same basal starter and grower rations based on corn and soybean according to *NRC, (1994).*

2.3. Performance parameters

They include ABW, AWG, feed intake, feed conversion ratio (FCR) were evaluated according to the method described by Oliveira et al., (2008) as follow:

Average Body weight (ABW): The chicks were weighted individually at the beginning of the experiment, afterward chicks were weekly weighted and the live body weight change was taken.

Average Body weight gain: was calculated as differences between two successive weights.

$$\text{Body weight gain} = W2 - W1$$

Feed intake: was calculated by difference between the weight of the offered feed per week and the remained part, and then divided by the number of birds in each group to measure the weekly feed intake per bird.

Feed conversion ratio (FCR): was calculated by dividing the amount of feed consumed (g) during the week by the gain in weight (g) during the same week.

Relative growth rate (RGR) was estimated (*Crampton and Lioyd, 1959)* as;

$$RGR = \frac{100\,(W2-W1)}{\frac{1}{2}(W2+W1)}$$

After necropsy, liver, two kidneys, spleen, stomach and bursa were dissected out, grossly examined and weighted. The organ weight index of each organ was calculated (Matousek, 1969) as; Organ weight index = Organ weight / live body weight x 100

2.4. Blood analysis:

At the end of experiment (day 42) ten birds of each group were slaughtered. Two blood samples were collected from each bird. The first blood sample was collected on heparin for hematological studies. The second blood sample from each slaughtered bird was collected without anticoagulant, centrifuged at 3000 r.p.m for 15 minutes for separation of the serum which kept frozen at -20 ºC for biochemical analysis. PCV%, erythrocytic and total leucocytic counts was determined (Dacei and Lewis, 1984). Hb concentration was determinedusing the colorimetric method (Wintrobe, 1965). Serum ALT, AST, ALP, total proteins, choloestrol, triglycerides, albumin, total bilirubin, urea and creatinine activities were measured colorimetrically according to Reitman and Frankel, (1957), Kind and King, (1954),Friedman and Young, (1997), Johnson et al., (1999),Walter and Gerade, (1970),Coulomb and Farreau, (1963) and Husdan and Rapoport, (1968) respectively. Serum globulin value was measured according to the method described by Coles, (1974).

2.5. Histopathological techniques:

Parts of the liver, kidney, spleen, bursa, stomach and intestines from five slaughtered birds from each group

were collected for histopathological examination (***Harries, 1989).***

Also, the middle-length of jejunum of five birds of each group, a 2-cm long segment were transected for histopathological examination for measurement of the length and width of the intestinal villi by using micrometer eye piece (***Rezaian et al., 2007).***

2.6. Molecular studies:

Five birds of each group were weighted and slaughtered then dissected. Samples of breast muscles were stored at -80 ºC, for molecular studies including Semi-quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) method was used to detect the changes in transcription levels of the two genes in muscles of chickens treated with YBG or SB (0.49 or 0.98 mg/ml. drinking water/day) in comparison with control chicks.

2.7. Methods used for molecular studies:

Methods used for molecular studies include the following:

1. RNA extraction according to the***(Esghaei et al., 2012)***.

2.Quantification of DNA and RNA using Nanodrop (Uv-Vis spectrophotometer Q5000/USA)(Boesenberg-Smith et al., 2012).

3.Reverse Transcription of RNA into cDNA (two steps):(El-Magd et al., 2013)

4. Polymerase chain reaction (PCR) (Daliri et al., 1999)

5.Agarose Gel Electrophoresis(Buitkamp et al., 1991)

.6.Image Analysis

7. Molecular Data Analysis.

2.8. Statistical analysis

The obtained data were statistically analyzed for variation among groups using GLM procedure of SAS computer program ***(SAS, 1987).*** Data were presented as means plus or minus the standard error. The minimum level of significance was set at P< 0.05.

3. RESULTS.

3.1. Performance Parameters:

3.1.1. Effect on average body weight of broiler chicks:

Chicks treated with YBG or SB (0.49 or 0.98 mg /ml.D.W./day) showed a significant increase (P<0.05) on ABW` in comparison with the control chicks. Within the treated groups, chicks treated with SB (0.98 mg/ml.D.W./day) showed the highest body weight followed by chicks treated with YBG then chicks treated with SB (0.49 mg /ml.D.W./day)

3.1.2. Effect on average body weight gain of broiler chicks:

Chicks treated with YBG or SB (0.49 or 0.98 mg /ml.D.W./day) showed a significant increase (P<0.05) in the AWG in comparison with the control chicks. Within the treated chicks, chicks treated with SB (0.98 mg/ml.D.W./day) showed the highest AWG followed by chicks treated with YBG then chicks treated with SB (0.49 mg /ml.D.W./day).

3.1.3. Effects on relative growth rate (RGR):

YBG treated chicks showed no significant differences (P<0.05) in RGR in comparison with the control chicks. However, it has been found that chicks treated with SB (0.49 mg or 0.98 mg/ml. D.W. /day) showed a significant improvement (P<0.05) in RGR in comparison with the control chicks. Within the treated groups, there were no significant differences between all treated groups in RGR.

3.1.4. Effects on organ weight index:

All treated chicks showed no significant differences (P<0.05) in organ weight index in comparison with the control chicks.

3.1.5 Effect on feed conversion ratio (FCR) of broiler chicks:

FCR was significantly decreased (P<0.05) in chicks treated with SB (0.98 mg/ml.D.W./day) in comparison with the control chicks. However, there was no significant difference in FCR of the other two treated groups in comparison with the control chicks.

3.2. Hematological findings:

There was a significant increase (P< 0.05) in the PCV% and RBCs count of all treated chicks in comparison with the control chicks. Within the treated groups, the chicks treated with SB (0.98 mg/ml.D.W./day) showed a significant increase (P< 0.05) in the PCV% and RBCs count in comparison with the other two treated groups. There was no significant difference between chicks treated with YBG and chicks treated with SB (0.49 mg /ml.D.W./day) in PCV% and RBCs count.

The Hb% and WBCs of chicks treated with YBG and chicks treated with SB (0.98 mg/ml.D.W./day) were significantly increased (P< 0.05) in comparison with the chicks treated with SB (0.49 mg /ml.D.W./day) and control chicks. The WBCs count of chicks treated with YBG was also significantly increased (P< 0.05) in comparison with chicks treated with SB (0.98 mg /ml.D.W./day) without significant differences on

Hb%. Also, there was no difference between Hb% and WBCs of chicks treated with SB (0.49 mg/ml.D.W./day) in comparison with control group.

3.3. Biochemical findings:

There was a significant increase (P< 0.05) in serum protein, albumin and globulin of chicks treated with SB (0.49 or 0.98 mg /ml.D.W./day) in comparison with the control chicks. While there were no significant differences in serum protein, albumin and globulin of chicks treated with YBG in comparison with control chicks. Within the treated groups of chicks there were no significant differences in serum protein, albumin and globulin of at 42 days from drug administration. There were no significant differences in albumin/globulin ratio between all treated groups of chicks and control chicks.

There was no significant difference (P< 0.05) in the ALT and ALP level between all treated groups of chicks and control chicks.

The AST level of chicks treated with SB (0.98 mg/ml.D.W./day) was significantly increased (P< 0.05) in comparison with the other three groups of chicks. While there was no difference between AST levels of the chicks treated with YBG, chicks treated with SB (0.49 mg/ml.D.W./day) and control chicks.

The serum urea and creatinine levels were not significantly different between all treated groups of chicks and control chicks.

There were no significant differences on serum triglycerides levels of all treated groups of chicks and control chicks.

The cholesterol levels of chicks treated with SB (0.49 or 0.98 mg/ml.D.W./day each alone) were significantly increased in comparison with chicks treated with YBG and control chicks. There was no significant difference between both groups of chicks treated with SB in cholesterol level. Also, there was no significant difference between chicks treated with YBG and control chicks in cholesterol levels.

The total bilirubin level of all treated groups of chicks was significantly decreased in comparison with control group. Also, the total bilirubin level of chicks treated with SB (0.49 mg/ml.D.W./day) was significantly decreased in comparison with chicks treated with SB (0.98 mg/ml.D.W./day).

3.4. Histopathological findings:

3. 4. a. Intestines:

Chicks treated with YBG showed a significant increase in intestinal villi length without significant differences in intestinal villi width in comparison with the control chicks(Figure 1). Chicks treated with SB (0.49 mg or 0.98 mg/ml. D.W./day) showed a significant increase in intestinal villi length and width in comparison with the control chicks (Figure 2,3).Within the treated groups, chicks treated with SB (0.98 mg/ml. D.W./day) showed a significant increase (P<0.05) in intestinal villi length and width than chicks treated with SB (0.49 mg/ml. D.W./day) than chicks treated with YBG (Figure 1)..

3. 4. b. Liver:

Chicks treated with YBG showed a normal histological structure of the hepatic cords, hepatocytes and central vein in comparison with the control chicks (Figure 4). Chicks treated with SB(0.49 mg/ml. D.W/day) showed a normal histological structure of the hepatic cords, hepatocytes and mild congestion of central vein in comparison with the control chicks (Figure 5).Chicks treated with SB(0.98 mg/ml. D.W/day) showed a normal histological appearance of hepatocytes and congestion of central vein in comparison with the control chicks.

3. 4. c. Kidney:

Chicks treated with YBG showed a congestion of renal blood vessels in comparison with the control chicks (Figure 7).Moreover, chicks treated with SB(0.49 mg or 0.98 mg/ml. D.W/day each alone) showed a congestion of intertubular blood capillaries in comparison with the control chicks.

3. 4. d. Spleen:

Chicks treated with YBG showed relatively normal red and white pulp in comparison with the control chicks (Figure 9). While, chicks treated with SB (0.49 mg or 0.98 mg/ml. D.W/day each alone) showed a hyperplasia of red pulp.

3. 4. e. Bursa of Fabricious:

Chicks treated with SB (0.49 mg or 0.98 mg/ml. D.W/day each alone) showed a normal histological structure with mild lymphoid depletion (Figure 11). Chicks treated with YBG showed a mild to moderate lymphoid depletion.

3.4. f. Stomach:

All treated chicks showed a normal histological structure of stomach in comparison with the control chicks.

3.5. Molecular results:

3.5.a. RNA extraction and cDNA production:

After mRNA extraction, the quality and integrity of total RNA were assessed by inspection of the ribosomal RNA bands (18S and 28S) in ethidium bromide stained 1% agarose gels which were visualized by ultraviolet (UV) light**(Figure 2).**

The concentration and purity of the extracted RNA were determined using Nanodrop and the results revealed that the isolated RNA is pure, also revealed presence of considerable higher concentrations of RNA (ranged from 350 to 650 ng/µl) **(Figure 3).**

3.5.b. Effects on IGF1R mRNA relative expression as compared to GAPDH using semi-quantitative RT-PCR:

Changes in transcription levels of *IGF1R* gene in muscles of treated chicks in comparison with the control chicks and to the housekeeping gene, *GAPDH*, were presented in **(Figure 4).**

mRNA expression of *IGF1R* was significantly increased in the treated groups of chicks in comparison with the control group ($P<0.05$). Among the three treated groups, chicks treated with YBG showed the highest expression level (about 4 fold more in comparison with the control) at 42 days from drug administration. While, chicks treated with SB (0.98 mg/ml. D.W. /day) showed a moderate increase in *IGF1R* gene expression(about 2.75 fold more in comparison with the control). Chicks treated with SB (0.49 mg /ml. D.W. /day) showed the lowest expression (about 2.25 fold more in comparison with the control).

3.5.c. Effects on GHSR mRNA relative expression as compared to GAPDH using semi-quantitative RT-PCR:

Changes in transcription levels of *GHSR* gene in muscles of treated groups of chicks in comparison with control chicks and to the housekeeping gene, *GAPDH*, were presented in **(Figure 5).**

mRNA expression of *GHSR* was significantly increased in the treated chicks in comparison with the control chicks at 42 days from drug administration ($P < 0.05$). Among the three treated groups, chicks treated with YBG showed the highest expression level (about 3.5 fold more in comparison with the control).While chicks treated with SB (0.98 mg/ml. D.W. /day) showed a moderate increase in *GHSR* gene expression(about 3 fold in comparison with the control). Chicks treated with SB (0.49 mg/ml. D.W. /day) showed the lowest expression (about 2 fold in comparison with the control) at 42 days from drug administration.

Table (1): The effect of the oral administration of yeast beta-glucan (YBG) (25 µg/ml.D.W.) given daily for 42 days on performance parameters of broiler chicks:

periods	parameters	Control	Yeast beta-glucan (25 µg/ml.D.W./day)
On 1st wk	ABW0	32.55±0.48^{a}	32.50±0.31^{a}
	AWG1	97.52±2.11^{a}	97.38±1.49^{a}
	FCR1	1.00±0.02^{a}	1.03±0.02^{a}
On 2nd wk	ABW1	128.96±2.74^{b}	131.73±1.78^{ab}
	AWG2	218.83±8.97^{b}	229.48±7.93^{a}
	FCR2	1.15±0.02^{a}	1.19±0.01^{a}
On 3rd wk	ABW2	346.20±6.40^{b}	358.22±4.06^{ab}
	AWG3	402.49±3.24^{a}	398.45±2.54^{a}
	FCR3	1.37±0.01^{ab}	1.40±0.01^{a}
On 4th wk	ABW3	749.40±9.09^{a}	759.50±6.24^{a}
	AWG4	485.99±10.49^{b}	593.35±5.61^{a}
	FCR4	1.74±0.03^{a}	1.72±0.02^{a}
On 5th wk	ABW4	1240.10±18.01^{b}	1356.20±12.26^{a}
	AWG5	656.53±17.01^{b}	696.56±8.16^{a}
	FCR5	1.71±0.04^{b}	1.75±0.02^{a}
On 6th wk	ABW5	1864.20±32.98^{b}	2063.10±20.85^{a}
	AWG6	586.73±8.46^{a}	601.50±11.12^{a}
	FCR6	2.15±0.03^{ab}	2.02±0.04^{b}
Total	ABW6	2460.20±36.62^{b}	2634.80±32.16^{a}
	AWG7`	2448.10±39.44^{b}	2616.70±29.34^{a}
	FCR7	1.69±0.02^{a}	1.67±0.02^{ab}

*Means carrying different letters with the same raw are significantly different ($P<0.05$).

ABW=Average Body Weight. **AWG=Average Weight Gain**

FCR=Feed Conversion Ratio *Values are expressed as mean ± S.E., *N= 60

Table (2): The effect of the oral administration of sodium butyrate (SB) (0.49 mg /ml.D.W.) given daily for 42 days on performance parameters of broiler chicks:

Periods	parameters	Control	Sodium butyrate (0.49 mg/ml.D.W./day)
On 1stwk	ABW0	32.55±0.48a	32.33±0.32a
	AWG1	97.52±2.11a	97.90±2.18a
	FCR1	1.00±0.02 a	1.03±0.03a
On 2ndwk	ABW1	128.96±2.74b	130.50±2.23ab
	AWG2	218.83±8.97b	239.10±5.45a
	FCR2	1.15±0.02 a	1.08±0.01b
On 3rdwk	ABW2	346.20±6.40 b	368.50±4.39a
	AWG3	402.49±3.24a	397.10±4.11a
	FCR3	1.37±0.01ab	1.36±0.01b
On 4thwk	ABW3	749.40±9.09a	771.42±6.42a
	AWG4	485.99±10.49b	562.30±5.18a
	FCR4	1.74±0.03 a	1.69±0.02a
On 5thwk	ABW4	1240.10±18.01b	1319.70±11.86a
	AWG5	656.53±17.01a	641.60±5.01a
	FCR5	1.71±0.04b	1.72±0.01ab
On 6thwk	ABW5	1864.20±32.98 b	1966.60±12.34a
	AWG6	586.73±8.46a	615.70±18.53a
	FCR6	2.15±0.03ab	2.20±0.06a
Total	ABW6	2460.20±36.62 b	2578.70±26.81a
	AWG7`	2448.10±39.44b	2553.70±29.26a
	FCR7	1.69±0.02a	1.67±0.02ab

*Means carrying different lette1rs with the same raw are significantly different (P<0.05).

ABW=Average Body Weight. **AWG=Average Weight Gain**

FCR=Feed Conversion Ratio *Values are expressed as mean ± S.E., *N= 60

Table (3): The effect of the oral administration of sodium butyrate (SB) (0.98 mg /ml.D.W.) given daily for 42 days on performance parameters of broiler chicks

periods	Parameters	Control	Sodium butyrate (0.98mg/ml.D.W./day)
On 1stwk	ABW0	32.55±0.48^{a}	32.75±0.32^{a}
	AWG1	97.52±2.11^{a}	102.20±2.18^{a}
	FCR1	1.00±0.02 ^{a}	1.03±0.02^{a}
On 2ndwk	ABW1	128.96±2.74^{b}	134.92±2.17^{a}
	AWG2	218.83±8.97^{b}	267.10±4.13^{a}
	FCR2	1.15±0.02 ^{a}	1.08±0.01^{b}
On 3rdwk	ABW2	346.20±6.40 ^{b}	397.00±4.58^{a}
	AWG3	402.49±3.24^{b}	438.40±4.96^{a}
	FCR3	1.37±0.01^{a}	1.32±0.01^{b}
On 4thwk	ABW3	749.40±9.09^{b}	848.67±8.24^{a}
	AWG4	485.99±10.49^{b}	580.40±3.07^{a}
	FCR4	1.74±0.03 ^{a}	1.57±0.01^{b}
On 5thwk	ABW4	1240.10±18.01^{b}	1411.30±12.72^{a}
	AWG5	656.53±17.01^{a}	655.30±4.06^{a}
	FCR5	1.71±0.04^{a}	1.66±0.01^{b}
On 6thwk	ABW5	1864.20±32.98 ^{b}	2071.30±12.46^{a}
	AWG6	586.73±8.46^{a}	610.80±16.39^{a}
	FCR6	2.15±0.03^{ab}	2.22±0.05^{a}
Total	ABW6	2460.20±36.62 ^{b}	2698.40±26.08^{a}
	AWG7`	2448.10±39.44^{b}	2654.20±26.95^{a}
	FCR7	1.69±0.02^{a}	1.62±0.02^{b}

*Means carrying different lette1rs with the same raw are significantly different (P<0.05).

ABW=Average Body Weight. AWG=Average Weight Gain

FCR=Feed Conversion Ratio *Values are expressed as mean ± S.E., *N= 60

Table (4): The effect of the oral administration of yeast beta-glucan (YBG) (25 µg/ml.D.W.) given daily for 42 days on hematological, biochemical parameters and histopathological alterations of broiler chicks:

Parameters		Control	Yeast beta-glucan (25 µg/ml.D.W./day)
Hematological parameters	PCV%	23.10± 0.28b	25.20± 0.53a
	Hb g/dl	12.66±0.23 b	13.50±0.17 a
	RBCs 10 6/cmm	1.65± 0.03 b	1.95 ± 0.02 a
	WBCs 103/cmm	25.25 ± 0.45 b	32.35 ± 0.63 a
Biochemical parameters	Total serum proteins (g/dl)	4.26± 0.05 b	4.41± 0.09ab
	serum albumin (g/dl)	2.37± 0.09 b	2.45± 0.02ab
	serum globulin (g/dl)	1.89± 0.10 a	1.98± 0.09 a
	albumin/globulin ratio	1.31 ± 0.10 a	1.26± 0.05 a
	ALT (U/L)	10.31 ± 1.02 a	12.40 ± 0.89 a
	AST (U/L)	161.10 ± 5.33 a	163.70 ± 5.70 a
	ALP (U/L)	175.36±1.63 a	175.62±1.30 a
	Urea (mg/dl)	12.77± 0.28 a	12.93± 0.52 a
	Creatinine (mg/dl)	0.36± 0.02 a	0.36± 0.03 a
	Triglycerides (mg/dl)	93.76± 5.05 a	88.42± 5.32 a
	Cholesterol (mg/dl)	95.60± 7.36 a	96.46± 3.99 a
	Bilirubin (mg/dl)	0.22±0.01 a	0.15±0.01b
Histopathological Alterations	Villus length (µm)	1194.58±28.98b	1458.02±36.14 a
	Villus width (µm)	113.81±4.28 a	113.04±4.32 a

*Means carrying different letters with the same raw are significantly different (P<0.05)
PCV=Packed Cell Volume Hb= Hemoglobin RBCs= Red Blood Corpuscles WBCs= White Blood Corpuscles ALT=Alanine Aminotransferase AST=Aspartate Aminotransferase ALP= Alkaline Phosphatase.
*Values are expressed as mean ± S.E., *N= 10

Table (5): The effect of the oral administration of sodium butyrate (SB) (0.49 mg /ml.D.W.) given daily for 42 days on hematological, biochemical parameters and histopathologicalalterationsof broiler chicks:

Parameters		Control	Sodium butyrate (0.49 mg/ml.D.W./day)
Hematological parameters	PCV%	23.10± 0.28b	25.80± 0.87a
	Hb g/dl	12.66±0.23 a	12.62±0.19 a
	RBCs 10 6/cmm	1.65± 0.03 b	2.11 ± 0.07 a
	WBCs 103/cmm	25.25 ± 0.45 a	25.60±0.39 a
Biochemical parameters	Total serum proteins (g/dl)	4.26± 0.05 b	4.55± 0.09 a
	serum albumin (g/dl)	2.37± 0.09 b	2.53±0.03 a
	serum globulin (g/dl)	1.89± 0.10 b	2.02± 0.10 a
	albumin/globulin ratio	1.31 ± 0.10 a	1.28±0.07 a
	ALT (U/L)	10.31 ± 1.02 a	11.51 ± 0.61 a
	AST (U/L)	161.10 ± 5.33 a	162.70 ± 3.90 a
	ALP (U/L)	175.36±1.63 a	175.24±1.45 a
	Urea (mg/dl)	12.77± 0.28 a	12.00± 0.18 a
	Creatinine (mg/dl)	0.36± 0.02 a	0.37± 0.01 a
	Triglycerides (mg/dl)	93.76± 5.05 a	96.79± 2.46 a
	Cholesterol (mg/dl)	95.60± 7.36b	122.52± 2.45 a
	Bilirubin (mg/dl)	0.22±0.01 a	0.14±0.01b
Histopathological Alterations	Villus length (µm)	1194.58±28.98b	1559.66±24.14 a
	Villus width (µm)	113.81±4.28b	127.70±3.67 a

*Means carrying different letters with the same raw are significantly different (P<0.05).
*Means carrying different letters with the same raw are significantly different (P<0.05)
PCV=Packed Cell Volume Hb= Hemoglobin RBCs= Red Blood Corpuscles WBCs= White Blood Corpuscles ALT=Alanine Aminotransferase AST=Aspartate Aminotransferase ALP= Alkaline Phosphatase.
*Values are expressed as mean ± S.E., *N= 10

Table (6): The effect of the oral administration of sodium butyrate (SB) (0.98 mg/ml.D.W.) given daily for 42 days on hematological, biochemical parameters and histopathologicalalterations of broiler chicks:

Parameters		Control	Sodium butyrate (0.98mg/ml.D.W./day)
Hematological parameters	PCV%	23.10± 0.28b	28.00±0.94a
	Hb g/dl	12.66±0.23 b	13.40±2.17 a
	RBCs 10 6/cmm	1.65± 0.03 b	2.41 ± 0.10 a
	WBCs 103/cmm	25.25 ± 0.45 b	29.20±0.36 a
Biochemical parameters	Total serum proteins (g/dl)	4.26± 0.05 b	4.55± 0.08 a
	serum albumin (g/dl)	2.37± 0.09 b	2.53±0.03 a
	serum globulin (g/dl)	1.89± 0.10 b	2.02± 0.08 a
	albumin/globulin ratio	1.31 ± 0.10 a	1.27±0.05 a
	ALT (U/L)	10.31 ± 1.02 a	12.85 ± 1.00 a
	AST (U/L)	161.10 ± 5.33 b	182.60 ± 7.03 a
	ALP (U/L)	175.36±1.63 a	175.77±0.99 a
	Urea (mg/dl)	12.77± 0.28 a	12.59± 0.23 a
	Creatinine (mg/dl)	0.36± 0.02 a	0.36± 0.01 a
	Triglycerides (mg/dl)	93.76± 5.05 a	95.80± 2.46 a
	Cholesterol (mg/dl)	95.60± 7.36b	116.70± 2.36 a
	Bilirubin (mg/dl)	0.22±0.01 a	0.17±0.01b
Histopathological Alterations	Villus length (µm)	1194.58±28.98b	1756.09±20.91 a
	Villus width (µm)	113.81±4.28b	123.11±4.06 a

*Means carrying different letters with the same raw are significantly different (P<0.05)
PCV=Packed Cell Volume Hb= Hemoglobin RBCs= Red Blood Corpuscles WBCs= White Blood Corpuscles ALT=Alanine Aminotransferase AST=Aspartate Aminotransferase ALP= Alkaline Phosphatase. *Values are expressed as mean ± S.E., *N= 10

Figure (1): The effect of oral administration of yeast beta-glucan (25µg/ml. drinking water) andsodium butyrate (0.49 mg or 0.98 mg/ml. drinking water each alone) given daily to broiler chicks for 42 day on intestinal villi length and width:

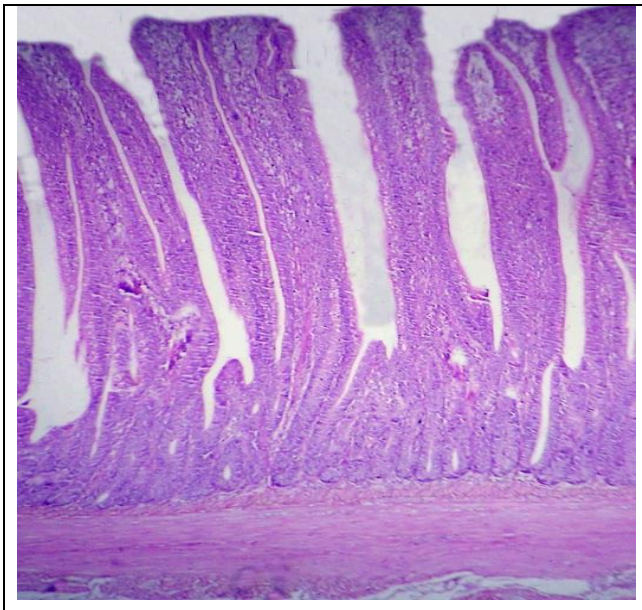

Fig. (1a): Intestinal villi of a chicken of control group showing a normal histological structure. H&E (X 160).

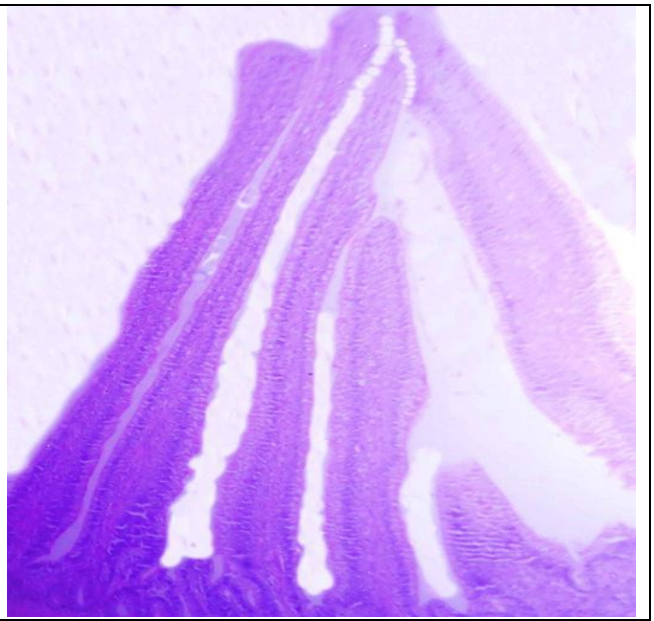

Fig. (1b):): Intestine of a chicken treated with YBG (25 µg/ml.drinking water/day) showing a significant increase in the length of the intestinal villi. H&E (X 160).

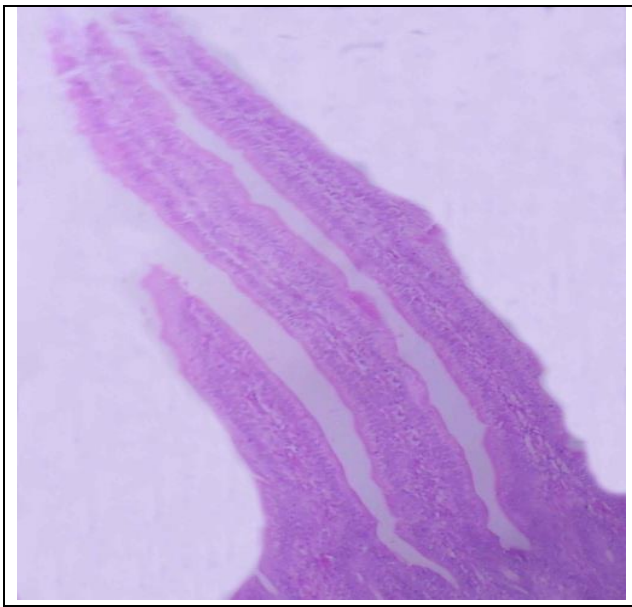

Fig. (1c): Intestine of a chicken treated with sodium butyrate (0.49 mg/ml.drinking water/day) showing a significant increase in the length and width of the intestinal villi. H&E (X 160).

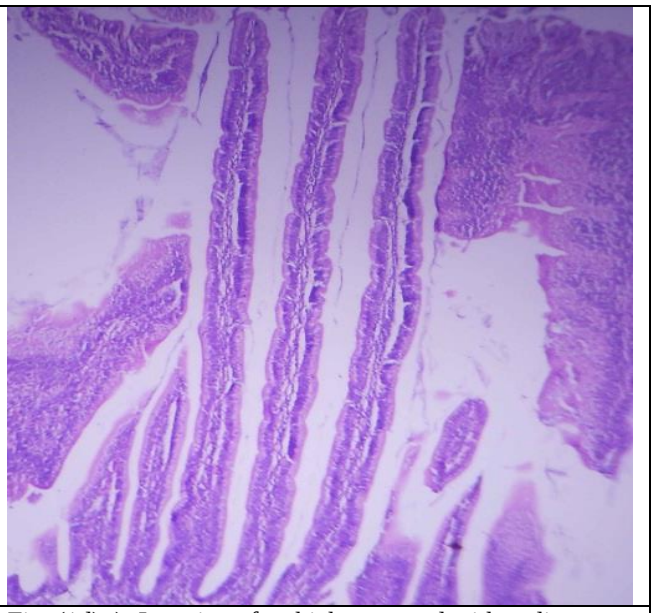

Fig. (1d):): Intestine of a chicken treated with sodium butyrate (0.98 mg/ml.drinking water/day) showing a significant increase in the length and width of the intestinal villi than other groups. H&E (X 160).

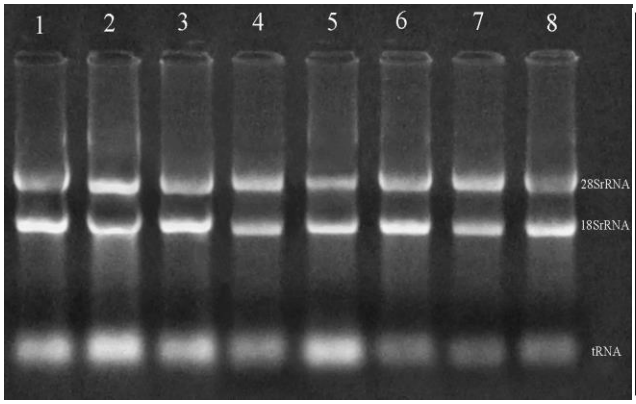

Figure (2): Ethidium bromide stained agarose gel showing extracted RNA from tissues samples of chicks showing intact 18s and 28s bands of rRNA:

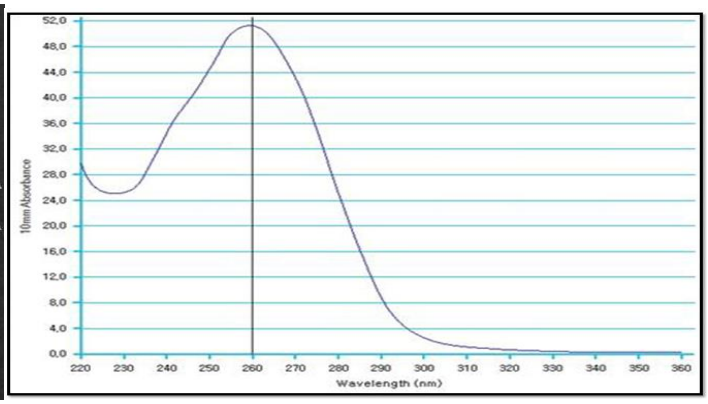

Figure (3):Nanodrop curve showing concentration and purity of extracted RNA from a representative sample which is 625ng/ul. In this curve the upper top presents at 260 and the bottom at 230 which indicates the presence of pure RNA:

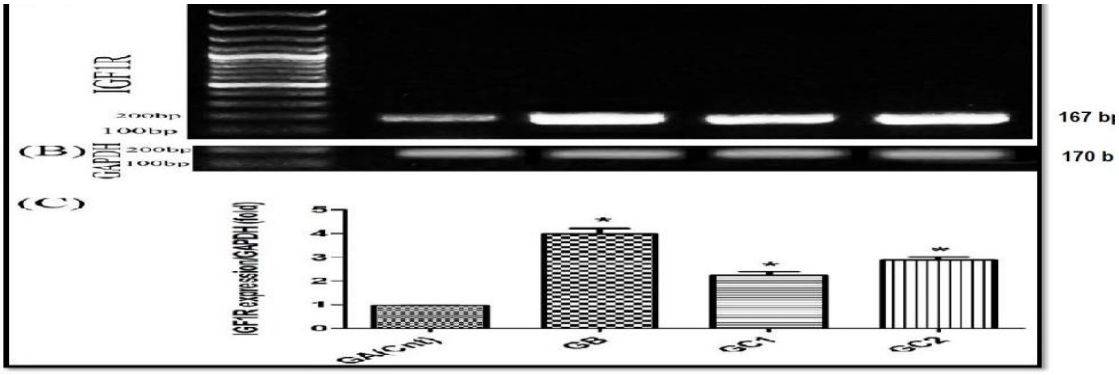

Figure (4): IGF1R gene expression levels in the skeletal muscles of control and treatment groups:
A) Ethiduim bromide stained agarose gel of RT-PCR products *IGF1R* gene with size of 167 bp (lane 1: **control group** (GA) and lane 2-4 treatment groups; lane 2: **YBG** (GB); lane 3: **SB (0.49 mg/ml.D.W./day)** (group C1), and lane 4: **SB (0.98 mg/ml.D.W./day)** (group C2) compared to B) the house keeping gene, *GAPDH*, with size of 170 bp. C) Band intensity was quantified using Image J software and the ratio of *IGF1R* to *GAPDH* was calculated. Mean ratios of five samples of each group performed on different samples and data are expressed as the mean ±S.E.M. are represented on this figure, relative to the mean ratio of the control group. *IGF1R* gene expression levels were significantly higher in treatment groups (GB, GC1 and GC2). * denotes significant difference from control group ($P < 0.05$).

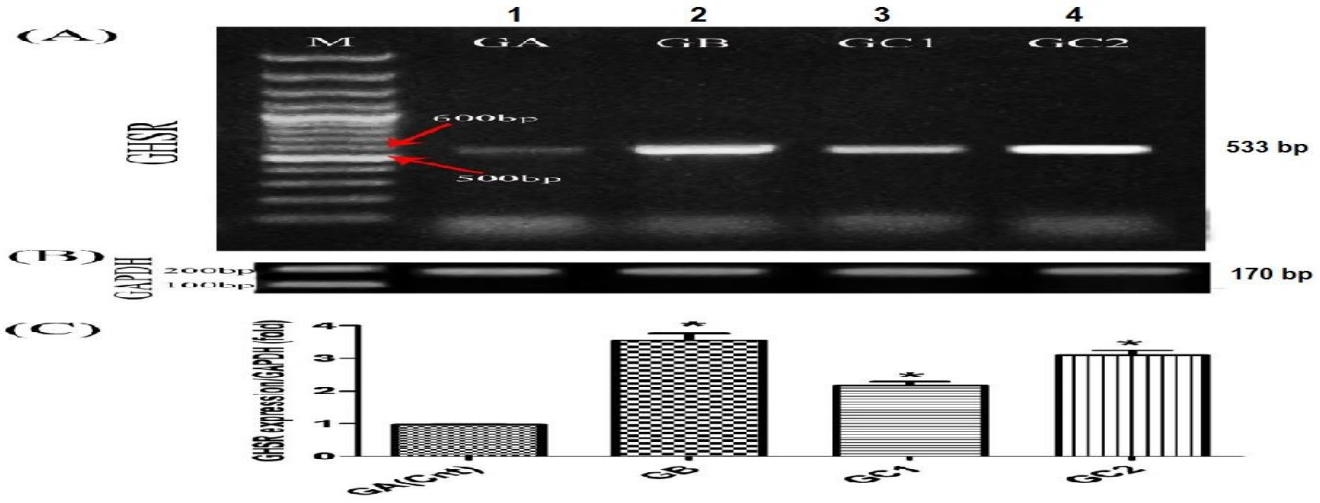

Figure (5): GHSR gene expression levels in the skeletal muscles of control and treatment groups:
A) Ethiduim bromide stained agarose gel of RT-PCR products *GHSR* gene with size of 533 bp (lane 1: **control group** (GA) and lane 2-4 treatment groups; lane 2: **YBG** (GB); lane 3: **SB (0.49 mg/ml.D.W./day)** (group GC1), and lane 4: **SB (0.98 mg/ml.D.W./day)** (group GC2) compared to B) the house keeping gene, *GAPDH*, with size of 170 bp. C) Band intensity was quantified using Image J software and the ratio of *GHSR* to *GAPDH* was calculated. Mean ratios of five samples of each group performed on different samples and data are expressed as the mean ±S.E.M. are represented on this figure, relative to the mean ratio of the control group. *GHSR* gene expression levels were significantly higher in treatment groups (GB, GC1 and GC2). * denotes significant difference from control group ($P < 0.05$).

4.

DISCUSSION:

The present work showed that YBGsignificantly improved AWG without significant improvement in FCR especially during grower/finisher periods of broiler chicks. This increase in the AWG may be due to that YBG prevent some digestion problems, like constipation and stomach troubles(Gardiner, 2000).Also, YBG stimulates defense mechanisms in the body, activating digestive enzymes, increasing the absorption of nutrients from feed owing to its ability to bind pathogenic bacteria and neutralizing toxins excreted by such bacteria (Zaghini et al., 2005).

Moreover, YBG improves the growth rate by its immunostimulant effect. By the use of YBG as feed additive, infections were lowered, improving growth and lowering needs for antibiotics addition (Donzis, 1993).

Also, the improvement on the expression of Ghrelin receptor gene by YBG may attribute the increasein the intestinal villi length, where Ghrelin promotes intestinal cell proliferation and inhibits apoptosis during inflammatory states and oxidative stress (Waseem et al., 2004).

The obtained results are compatible with those reported by Zhang et al. (2008) they concluded that the AWG varied quadratically with the dietary supplementation levels of YBG in the broiler chicks.

Our results are incompatible with the results obtained by Dritz et al., (1995) they concluded that the addition of 0.10% YBG decreased growth performance of weanling pigs during the first 7 days post-weaning.

Regarding to the hematological findings, in the present study, there was a significant increase in PCV%, Hb% and RBCs count of chicks treated with YBGcompared to the control chicks.

This increase may be attributed to the improved health condition caused by YBGand to the stimulated immune system. Also, increased hematopoietic activity was demonstrated with inclusion of YBG.

Those results are compatible with the results obtained by Al-kassie et al. (2008) they concluded an increase in PCV, Hb% and R.B.Cs count in YBG-treated chicks compared with the control chicks..

In the present study, there was a significant increase in total leukocyte count in YBG-treated chicks. This may be attributed to immunostimulatory and immunomodulatory effect of YBG. Where, YBG modulate both specific and non-specific immune responses in various animals (Chae et al., 2006) and release some kind of cytokines (Li et al., 2005).

These results are compatible with those reported bySalim et al. (2011)they reported that a relatively high levels of dietary YBG enhanced some cellular immune responses of chicks by modulate macrophage chemotaxis activity.

Concerning the biochemical parameters, the obtained results revealed that, there was no change in the AST, ALT and ALP activities in YBG-treated chicks. This normal level of hepatic enzymes due to that YBG is a safe biological modulator without noticeable side effects on the animal body, and that confirmed with our histopathological findings as the hepatic tissue showed normal histological structure in chicks treated with YBG.

The obtained results are compatible with those reported byBernard et al. (2000) they reported that the dietary treatments with YBG did not have significant effects on the activities of AST and ALT in broiler chicks.

Our results are incompatible with those reported by Hayhoe (2012) who concluded that a significant elevation of serum ALP in chicks treated with YBG in comparison with the control pigs.

Our results showed that there was no significant difference in concentration of serum triglycerides and cholesterol between YBG-treated chicks and the control chicks.

Our results are compatible with those obtained byAn et al. (2008) they concluded that the concentrations of various lipid fractions and components of serum were not significantly different in broiler chicks treated with YBG.

Our results are incompatible with those reported by Mensink (2006) who found that broiler which gave juice that contained YBG for 5 weeks showed a reduction of 5% in cholesterol level.

Our results revealed that there were no significant changes in serum urea and creatinine levels between chicks treated with YBG and the control chicks. This normal level attributed to that YBG is a safe biological modulator without noticeable side effects on the animal body, and that confirmed with our histopathologcal findings as the kidneys of chicks treated with YBG showed normal histological structure except a mild congestion of renal blood vessels.

Our results are compatible with those reported bySalim et al. (2011) they revealed that there were no

significant changes in uric acid and creatinine levels in broiler chicks treated with β-glucan compared with the control group.

Our molecular results showed an increase in transcription levels of IGF1R gene in muscles of YBG-treated chicks by 4 folds in comparison with the control chicks and to the housekeeping gene, GAPDH. The anabolic effect of these genes included improved immune state of the treated chickens. So, improving the food intake and more efficient in fighting diseases (Gardiner, 2000).

Our results are compatible with those reported by Guobin et al. (2011) they reported that IGFs are important positive modulators of body and muscle growth in mammals and chickens.

In the present study, it was found that an increase in transcription levels of *GHSR* gene in muscles of YBG-treated chicks by 3.5 folds in comparison with the control group and to the housekeeping gene, GAPDH. GHSR is a candidate gene for food intake and feed conversion rate and so its low transcription level decreases body weight gain (Zigman et al., 2005).

Our results are compatible with those reported by Zhang et al. (2008) they concluded that an in increase average daily gain and an improvement in the FCR were observed in YBG-treated chickens due to the improvement in the digestibility of the nutrients which is the main function of Ghrelin hormone.

Ourresults suggested that organic acid could replace antibiotics in broiler chicken's diet for realizing optimum performance. SB (0.49 mg/ml D.W./day) was not significantly sufficient to maintain the performance. SB (0.98 mg/ml D.W./day) in the diet was adequate for optimum AWG and FCR.

Butyrate has an important role in development of the intestinal epithelium. In this study, SB (0.49 mg or 0.98 mg/ml D.W./day each alone) improved the villus length and width in the jejunum. Such increase in the villi length and width improves nutrients absorption allowing an improvement in growth rate.

Our results are compatible with those reported byTaherpour et al. (2009) they concluded that higher levels of SB are required for optimum AWG and FCR.

The obtained results are incompatible with those reported byAntongiovanni et al. (2007) they suggested that a lower level (0.2% butyrate) is sufficient to maintain performance of broiler chickens.

Regarding to the hematological parameters, there aresignificant increase in the PCV%, Hb% and RBCs count in the SB-treated chicks. This increase may be due to the increase in the number of F-reticulocytes, an increase in the number of erythroid progenitors and the number of F-programmed progenitors induced by SB inclusion (Constantoulakis et al., 1988).

Those results are compatible with the results reported byBetty et al. (2011)they concluded that short-chain fatty acid induces fetal globin expression and erythropoiesis in vivo.

In the present study, there was significant increase in the number of WBCs in SB-treated chicks (0.98 mg/ml D.W./day). This may be attributed to the lowering effect of SB on the intestinal PH improving the local immune status of the intestinal tract. This acidic PH enhances growth and multiplication of beneficial bacteria which support the immune system increasing WBCs production (Van Immerseel et al., 2006).

These results are compatible with the results obtained byRicke (2003a) who concluded that short chain fatty acids used successively as antimicrobial agent improving the immune status

Concerning the biochemical parameters, there were significant increases in the total protein and albumin in SB-treated chicks. This may be attributed to increase of proteolytic enzymes activity/ improvement of pancreatic secretions, stimulating the activity of digestive enzymes, increase of nutrient digestibility, the increase in the absorption rate caused by increased intestinal villus length. Those effects provide more amino acids available for serum total protein and albumin synthesis Tung and Pettigrew (2006).

In the present study, there is numerical increase in the level of serum AST in SB-treated chicks (0.98 mg/ml D.W./day) as compared with the control. This increase confirmed by our histopathological findings in which there was mild to moderate congestive of the hepatic blood vessels and to the high effort performed by the liver following sodium butyrate administration as compared to the control chicks.

The normal levels of urea and creatinine in SB-treated chicks (0.49 mg or 0.98 mg/ml. D.W./day) in comparison with control chicks indicate normal kidney function. Where, most of butyrate absorbed from the gut lumen is metabolized before reaching portal vein blood, and 90 to 100% of butyrate appearing in the portal vein is subsequently extracted by the liver, such that arterial concentrations of butyrate are low (Reynolds et al., 1988).

These results were confirmed by our histolpathological findings where the kidneys of

chicks treated with sodium butyrate showed normal histological structure except a congestion of intertubular blood capillaries.

Those results are compatible with the results reported byMachado et al. (2012). They concluded that sodium butyrate decreases the activation of Nuclear Factor kappa B (NF-κB), an inducer of the inflammatory responses in the kidney, reducing inflammation and oxidative damage in the kidney of rats subjected to contrast-induced nephropathy.

In the present study there was a significant increase in the level of the serum cholesterol level of the SB-treated chicks in comparison with the control chicks.

This effect may be attributed to that SB decrease the expression of Lecithin-cholesterol acyltransferase (LCAT) gene in the hepatocytes. LCAT is an enzymethat converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of a lipoprotein particle.

These results are compatible with the results obtained by Skretting et al. (1997) who concluded that SB inhibits the expression of the human LCAT gene in the human hepatoma-derived cell line (HepG2) cells

In the present study, SB significantly decreases the level of total bilirubin in treated chicks in comparison with the control chicks.

This effect may be attributed to the improvement of hepatic cell functions induced by SB administration.

These results are compatible with the results obtained by Yang et al. (2014) who concluded that SB significantly decrease the level of total bilirubin in experimentally-induced acute liver failure in rats.

The obtained molecular results showed an increase in transcription levels of IGF1R gene in muscles of SB-treated chicks (0.49 mg or 0.98 mg/ml. D.W./day)by 2.25 and 2.75 folds respectively in comparison with the control group and to the housekeeping gene, GAPDH, were increased.

The anabolic effect of these genes on skeletal muscle tissues included improved absorption rate in the intestinal tract, stimulation of amino acids uptake and incorporation into protein, uridine and thymidine synthesis into nucleic acid, glucose uptake, cell proliferation and suppression of protein degradations (Florini et al. 1991).

Those results are compatible with the results reported byBeccavin et al. (1999)they concluded that IGF1 and its specific receptor IGF1R are two main growth factors that stimulate protein synthesis in muscle tissue.subsequenetly, the expression levels of IGFI and IGF1R genes were higher in the fast growing chicken than in the slow growing.

In the present study, the obtained results showed an increase in transcription levels of GHSR gene in muscles of SB-treated chicks (0.49 mg or 0.98 mg/ml D.W./day each alone) by 2 and 3 folds respectively as compared to control chicks and to the housekeeping gene, GAPDH. The increase be attributed to the rapid digestion and absorption of the nutrients in SB-treated chicks in comparison with the control chicks stimulating more release of ghrelin hormone and so more transcription of mRNA of GHSH gene.

Those results are compatible with the results reported byGalfi and Bokori (1990) they concluded that SB improves the AWG and FCR.

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