Algal Crude Fucoidan Alone or with *Bacillus subtilis* DSM 17299 in Broiler Chickens Diet: Growth Performance, Carcass Characteristics, Blood Metabolites, and Morphology of Intestine

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Abstract

This study was conducted to evaluate the effects of algal fucoidan and probiotic *Bacillus subtilis* on growth performance, blood metabolites and intestinal morphology in broiler chickens. A total of 250 one-day-old Ross 360 male broiler chickens were randomly distributed into 5 treatments (6 replication pens/treatment) and reared for 42 d. The 5 dietary treatments were as follow: 1) a corn-soybean meal basal diet (control treatment); 2) a basal diet supplemented with antibiotic oxytetracycline; 3) a basal diet supplemented with the prebiotic fucoidan; 4) a basal diet supplemented with a probiotic product containing *Bacillus subtilis* spores; and 5) a basal diet supplemented with both the prebiotic and probiotic (served as a synbiotic). Birds received antibiotic and probiotic exhibited greater body weight gain (about 5.42% and 4.80%, respectively) than control treatment. The percentage of thigh and abdominal fat in birds fed probiotic diet were lower compared to the antibiotic treatment. The use of fucoidan and probiotic resulted lower ($P < 0.05$) serum concentration of triglyceride than those of antibiotic treatment. Supplementing of synbiotic increased villus height and villus height to crypt depth ratio in the jejunum ($P < 0.05$). Present study revealed that supplementing of probiotics with fucoidan could be advised as an effective synbiotic, instead of antibiotics, to improve the performance and health of broiler chickens.

Introduction

Antibiotics drugs are used in poultry industry to control, prevent and treat various bacterial infections and also to improve growth performance of birds. Today, the use of some antibiotics growth promoters (AGPs) in developing countries has simplified economic production of high quality poultry meat and eggs through the beneficial change of the gastrointestinal microbiota. However, unfortunately, these products may contain harmful concentrations of AGPs residues (Ashayerizadeh *et al.,* 2017; Mehdi *et al.,* 2018), that cause numerous health concerns in humans including allergic reactions, immunopathological diseases, carcinogenic effects, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity (Guetiya Wadoum *et al.,* 2016) and generation of resistant strains of pathogenic bacteria (Palanisamy *et al.,* 2017). Moreover, presence of AGPs in poultry manure threatens the health of the environment in terms of prevalence of antibiotic-resistance genes in manure-fertilized soil (Yang *et al.,* 2014). Since, many studies have been performed to find natural agents with the beneficial effects of AGPs.

Several alternatives for AGPs such as probiotics, prebiotics and phytobiotics have been more considered (Mehdi *et al.,* 2018). A probiotic defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Recently, prebiotic defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit (Gibson *et al.,* 2017). Fucoidans are a group of non-digestible and L-fucose-rich sulphated polysaccharides that present in the cell wall matrix of brown seaweeds and could potentially be used as prebiotic for human and animals (O'Sullivan *et al.,* 2010; Palanisamy *et al.,*

2017; Sweeney *et al.,* 2017). Fucoidans were reported to have various bioactivities including antibacterial, antiviral, anticoagulant, antithrombotic, antitumor, immunomodulatory, antioxidant, antiallergic, and anti-inflammatory (Flórez-Fernández *et al.,* 2018; Lim *et al.,* 2019; Palanisamy *et al.,* 2017). To the best of our knowledge, there is no report on the effect of fucoidan lonely or with probiotics on growth performance of broiler chickens.

Here, we hypothesized that fucoidan, as a prebiotic agent, could modify gut microbiota, alleviate intestinal mucosa inflammatory, and improve the growth performance of broiler chickens. Therefore, the present study was conducted to determine the effect of fucoidan, individually or in combination with probiotic as an alternative for antibiotic, on the changes of growth performance, and blood metabolites of broiler chickens.

Materials and Methods
Brown seaweed collection and Fucoidan extraction
A sample of *Sargassum tenerrimum*, collected from Gheshm Island, Iran, was washed with fresh water soon after collection in order to remove salt and sand, dried under hot-air drying at 50°C, and then kept in plastic bags at 4°C until use. Crude fucoidan were extracted according to the methods previously described by Wang *et al.,* (2015). Briefly, the dried alga sample was ground and mixed with distilled water (w/v = 1:10) and placed in a water bath maintained at 40°C for 15 min with shaking. The mixture was centrifuged at $3870 \times g$ for 10 min and the supernatant was collected. Ethanol was added to the supernatant to give a final ethanol concentration of 71.25% and shaken. The fucoidan were then recovered by centrifugation at $9170 \times g$ for 30 min, freeze-dried and milled.

Chickens and dietary treatments
The experimental protocols describing the management and care of animals were reviewed and approved by Ethical Committee at Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. A total of 250 one-day-old Ross 308 male broiler chickens were randomly allocated into 5 dietary treatments (i.e., including control) of 5 replicates as floor pens with 10 birds per each and reared for 42 days. A basal diet (Table 1) was formulated and considered as control treatment according to recommendation from Ross Broiler Nutrition Specification (Aviagen, 2014). Other four treatments were made by supplementing the basal diet with 1) 200 ppm antibiotic oxytetracycline, 2) 500 ppm prebiotic fucoidan, 3) 200 ppm probiotic GalliPro® (i.e., containing 4×10^9 CFU/g of *Bacillus subtilis* DSM 17299), and 4) combination of 500 ppm fucoidan with 200 ppm probiotic GalliPro® (served as synbiotic).

Table 1. Composition of the experimental diet (as - fed basis)

Item	1 to 10 d	11 to 24 d	25 to 42 d
Ingredient (%)			
Maize	54.62	57.19	61.84
Soybean meal	36.37	34.07	29.14
Corn gluten meal	2.78	1.79	1.64
Soybean oil	1.83	3.03	3.74
Limestone	1.18	1.08	1.00
Di-calcium phosphate	1.65	1.45	1.31
Salt	0.35	0.35	0.35
Vitamin mixture [a]	0.25	0.25	0.25
Mineral mixture [b]	0.25	0.25	0.25
DL-Met	0.31	0.26	0.24
L-Lys	0.31	0.20	0.20
L-Thr	0.10	0.06	0.04
Calculated content			
ME (Kcal/kg)	2,900	3,000	3,100
Crude protein (%)	22.23	20.80	18.89
Ca (%)	0.92	0.84	0.76
Available P (%)	0.46	0.42	0.38
Na (%)	0.15	0.15	0.15
Lys (%)	1.39	1.24	1.12
Met + Cys (%)	1.04	0.95	0.88
Arg (%)	1.40	1.32	1.18
Thr (%)	0.93	0.85	0.75

[a] Contained per kilogram of diet: vitamin A (trans - retinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 2,000 IU; vitamin E (DL - α - tocopherol acetate), 10 mg; vitamin K (bisulfate menadione complex), 1 mg; vitamin B_1 (thiamin mononitrate), 1 mg; vitamin B_2 (riboflavin), 5 mg; vitamin B_3 (Niacin), 30 mg; vitamin B_6 (pyridoxine - hydrochloride), 1.5 mg; vitamin B_8 (biotin), 0.05 mg; vitamin B_5 (D - calcium pantothenate), 10 mg; vitamin B_9 (folic acid), 1 mg; and antioxidant (butylated hydroxytoluene), 10 mg.
[b] Contained per kilogram of diet: Mn (manganese sulfate), 60 mg; Zn (zinc sulfate), 50 mg; Fe (ferrous sulfate), 30 mg; Cu (copper sulfate), 4 mg; I (potassium iodide), 3 mg; Se (sodium selenite), 0.1 mg; and Co (cobalt carbonate), 0.1 mg.

The broiler chickens received feed and fresh water *ad-libitum* throughout the experiment and temperature and other breeding management items were based on the strain guide. For each pen, body weight gain (BWG) and feed intake (FI) were obtained at 42 d of age. Mortality was recorded in a daily manner. The dead broiler chickens were weighed and feed conversion ratio (FCR) calculated using the following formula: total feed intake / (total final weight − total initial weight + total mortality weight). At the end of experiment, 2 broilers chicken per pen with body weights close to the pen average were selected for blood sampling, and then slaughtered and used for weighting of carcass, cuts, and internal organs (Huyghebaert and Pack, 1996). Also, total length of small intestine was removed and used for morphological study.

Blood parameters
Blood sample (5 mL) was drawn from wing vein and then centrifuged at $2000 \times g$ for 10 min at 4 °C. The collected serum was stored at -20 ℃ for further analysis. Serum glucose, cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c), albumin, total protein, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) enzymes activity were determined with an automatic biochemical analyzer (Abbott Alcyon 300, Abbot Laboratories, Abbott Park, IL, US) using commercial laboratory kits (Pars Azmoon Kits; Pars Azmoon, Tehran, Iran).

Tissue sampling
Segments of approximately 3 cm were taken from the midpoint of the duodenum, jejunum (between the bile duct entry and Meckel's diverticulum) and ileum (between the Meckel's diverticulum and cecum). Segments were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. All histological morphometric studies were performed on 5 µm sections, stained with haematoxylin and eosin, and examined by a light microscope (Zentek *et al.,* 2002). The slides were examined with an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera (Nikon Eclipse TS100, Japan). The images were analyzed using Image J analysis software V 1.32j (ImageJ,

National Institute of Mental Health, Bethesda, MD, USA) according to (Abramoff *et al.,* 2004).

Statistical Analysis
Data were subjected to ANOVA using the GLM procedure (SAS, 2009). Mortality data were converted to ARCSIN $\sqrt{(X /100)}$ prior to the analysis. Significant differences among treatments were identified by Tukey's HSD test. All statements of significance were based on a probability of $P < 0.05$.

Results
Growth performance
The effect of experimental treatments on BWG, FI, and FCR of broiler chickens are summarized in Table 2. Supplementing of antibiotics, probiotics, and synbiotics increased BWG and improved FCR of broiler chickens when compared to the control treatment ($P < 0.05$). However, BWG of broiler chickens received fucoidan was numerically more than control treatments. None of the treatments had significant effect on FI and mortality.

Carcass characteristics
The effect of experimental treatments on carcass yield and weight of internal organs in broiler chickens are shown in Table 3. The use of antibiotic in the diet of broiler chickens increased the percentage of thigh and abdominal fat pad than probiotic treatments ($P < 0.05$). Other comparisons were not significant between treatments for these items. Moreover, the percentage of other cuts and internal organs were not affected by treatments.

Blood profile
The effects of experimental treatments on selected blood profile of broiler chickens are shown in Table 4. Broilers fed with diet containing antibiotic had higher cholesterol serum concentration than synbiotic treatment. Supplementing of fucoidan and synbiotic significantly lowered the concentration of triglycerides and very low density lipoprotein-cholesterol (VLDL-c) compared to the antibiotic treatment. Furthermore, both fucoidan and synbiotic were effective in decrease of low density lipoprotein-cholesterol (LDL-c) concentration than control treatment ($P < 0.05$).

Table 2. Effect of dietary treatments on growth performance of broiler chicks from days 1 to 42 of experiment

Item [1]	Treatments					SEM [3]	P-value
	Control	Antibiotic	Fucoidan	Probiotic	Synbiotic [2]		
BWG (g)	2,416 [b]	2,547 [a]	2,491 [ab]	2,532 [a]	2,521 [ab]	14.52	0.021
FI (g)	5,068	4994	5,024	4,920	4,926	36.50	0.690
FCR (g/g)	2.09 [a]	1.96 [ab]	2.01 [ab]	1.94 [b]	1.95 [ab]	0.01	0.035
Mortality (%) [4]	0.22	0.25	0.22	0.19	0.22	0.03	0.990

[a,b] means in each raw with different superscripts are significantly different ($P <0.05$).
[1] BWG= Body weight gain; FI= Feed intake; FCR= Feed conversion ratio.
[2] Synbiotic = Fucoidan + probiotic.
[3] *SEM* = standard error of means.
[4] For statistical analysis, the original data were transformed via ARCSIN$\sqrt{(\times /100)}$.

Table 3. Effect of dietary treatments on carcass characteristics of 42-day-old broiler chickens

Item[1]	Treatments					SEM[2]	P-value
	Control	Antibiotic	Fucoidan	Probiotic	Synbiotic		
Carcass	64.64	65.11	64.52	64.30	64.42	0.50	0.991
Breast	23.78	24.58	23.87	24.87	22.62	0.48	0.671
Thigh	18.71 [ab]	19.65 [a]	18.55 [ab]	18.40 [b]	18.53 [ab]	0.14	0.035
Gizzard	1.60	1.74	1.76	1.70	1.86	0.04	0.544
Liver	2.24	2.06	2.21	2.01	2.43	0.04	0.184
bursa of Fabricius	0.16	0.18	0.21	0.20	0.18	0.05	0.704
Heart	0.67	0.64	0.60	0.65	0.66	0.01	0.654
Spleen	0.10	0.11	0.16	0.12	0.11	0.01	0.165
Abdominal fat	1.88 [ab]	1.95 [a]	1.80 [ab]	1.61 [b]	1.67 [ab]	0.008	0.033

[a-c] Means within a raw having different superscripts are significantly different ($P < 0.05$).
[1] Relative weight (g/100 g body weight) of the cuts and digestive organs of broiler chickens fed diets containing different rice bran contents.
[2] SEM = standard error of means.

Table 4. Effect of diet treatments on blood metabolites of 42-day-old broiler chickens

Item[1]	Treatments					SEM[2]	P-value
	Control	Antibiotic	Fucoidan	Probiotic	Synbiotic		
ALT (IU/L)	1.85	1.97	1.69	1.91	1.76	0.03	0.129
AST (IU/L)	222.33	229.33	225.33	225.00	226.00	1.82	0.869
ALP (IU/L)	2523.00	2551.00	2376.00	2662.30	2555.3	47.68	0.497
Creatine	0.25	0.27	0.24	0.31	0.27	0.009	0.216
TP	3.26	3.36	3.86	3.63	3.26	0.10	0.310
Albumin	1.43	1.46	1.96	1.73	1.43	0.09	0.364
Globulin	1.83	1.90	1.90	1.90	1.83	0.05	0.992
Cholesterol	128.66 [ab]	132 [a]	117.66 [ab]	116.00 [ab]	113 [b]	2.52	0.029
Triglyceride	85.33 [ab]	87.33 [a]	75 [b]	97.66 [ab]	74.66 [b]	1.70	0.019
HDL-c	69.33	75.00	82.33	76.00	78.00	2.60	0.682
VLDL-c	17.06 [ab]	17.46 [a]	15.00 [b]	15.93 [ab]	14.93 [b]	0.34	0.019
LDL-c	42.26 [a]	39.53 [ab]	20.33 [b]	24.06 [ab]	20.06 [b]	3.06	0.009

[a,b,c] means in each row with different superscripts are significantly different ($P < 0.05$).
[1] Wherever units are not listed, they are in g/dL; ALT= alanine transaminase; AST= aspartate transaminase; ALP= alkaline phosphatase; HDL-c= high density lipoprotein-cholesterol; VLDL-c= very low density lipoprotein-cholesterol; LDL-c= low-density lipoprotein-cholesterol; TP= total protein.
[2] SEM = standard error of means.

Table 5. Effect of dietary treatments on intestinal histology of 42-day-old broiler chickens.

Item[1]	Treatments					SEM	P-value
	Control	Antibiotic	Fucoidan	Probiotic	Synbiotic		
VH (μm)							
Duodenum	1453.03	1483.18	1467.10	1590.76	1585.36	21.36	0.063
Jejunum	1263.70 [b]	1440.05 [ab]	1332.65 [ab]	1456.09 [ab]	1464.60 [a]	26.80	0.028
Ileum	885.24 [b]	1203.18 [a]	1029.81 [ab]	1122.70 [ab]	1164.51 [ab]	38.56	0.032
VW (μm)							
Duodenum	169.29	163.78	179.74	174.80	164.83	3.31	0.562
Jejunum	162.20	145.66	179.20	144.38	166.41	8.61	0.738
Ileum	146.04	172.72	147.20	167.03	144.66	8.12	0.770
CD (μm)							
Duodenum	255.82	257.78	239.64	202.91	31.94	9.54	0261
Jejunum	188.24 [a]	175.34 [b]	180.82 [ab]	181.39 [ab]	185.15 [a]	1.56	0.001
Ileum	191.22	186.45	196.77	167.92	180.71	15.17	0.987
VSA (mm²)							
Duodenum	0.77	0.76	0.82	0.87	0.82	0.01	0.303
Jejunum	0.64	0.65	0.75	0.65	0.76	0.03	0.805
Ileum	0.40 [b]	0.65 [a]	0.47 [ab]	0.58 [ab]	0.51 [ab]	0.02	0.02
VH/CD							
Duodenum	5.78	5.89	6.25	7.90	7.42	0.32	0.102
Jejunum	6.73 [b]	8.34 [a]	7.36 [ab]	8.02 [a]	7.91 [a]	0.18	0.008
Ileum	5.057	7.51	5.49	7.86	6.63	0.63	0.627

[a,b,c] means in each row with different superscripts are significantly different ($P < 0.05$).
[1] VH= villus height; CD= crypt depth, VW= villus width, VSA= villus surface area; VH/CD= villus height to crypt depth ratio.
[2] SEM = standard error of means.

Intestinal morphology

The results of histomorphological measurements of the small intestine are shown in Table 5. The use of synbiotic and antibiotic significantly increased villus height in the jejunum and ileum, respectively. The use of basal diet alone or with synbiotic led to a deeper crypt in the jejunum compared to those of antibiotic treatment ($P < 0.05$). Moreover, broiler chickens received antibiotic had greater villus surface area in the ileum than both control and fucoidan treatment ($P < 0.05$). The ratio of villus height to crypt depth in the jejunum was improved by supplementing of antibiotic, probiotic, and synbiotic when compared to the control treatment ($P < 0.05$).

Discussion

Several studies previously confirmed the positive effect of antibiotics and probiotics and prebiotics on growth performance of broiler chickens (Al-Khalaifah, 2018; Tavaniello *et al.,* 2018; Wang *et al.,* 2017; Zaghari *et al.,* 2015). Recently, it is indicated that supplementing of diet whit a combination of fucoidan (80 *ppm*) and laminarin (250 *ppm*, as prebiotic) significantly increased BWG and FI, and also adversely affected FCR of broiler chickens during days 1 to 13 of experiment. However, in the study of Heim *et al.,* (2015) and Walsh *et al.,* (2013), the use of different levels of fucoidan had no significant effect on daily BWG and feed efficiency of other young monogastric animals. In the present study, supplementing of the synbiotic complex improved BWG and FCR, as well as antibiotic. It's reported that antibiotics successfully improve growth performance by modifying of gastrointestinal microbiota (i.e., especially killing pathogens) and so lowering of intestinal mucosal inflammation (Mehdi *et al.,* 2018), which have a significant effect on nutrient absorption. Also, anti-inflammatory effect of probiotics on the intestinal epithelial barrier of broiler chickens could be occurred by altering of gut microbiota and cooperating of beneficial microbes to enhance the intestinal integrity and immunity through competitive exclusion principle (Wang *et al.,* 2017). Hence, although FI was not affected by treatments, it seems that the better FCR in broiler received antibiotics and probiotic have been led to higher BWG after 42 days of experiment. However, supplementing of diets with different types and doses of prebiotics could led to variable and not always comparable results (Tavaniello *et al.,* 2018). As reported by (Sohail *et al.,* 2013), probiotic effectiveness could be dose- dependent and increase of fucoidan dosage in the diet may be significantly improves the growth performance of broiler chickens. Other factors including diets, stress and management have been showed enough potential to affect the effectiveness of feed additives (Tavaniello *et al.,* 2018).

In agreement to our results, it is reported that probiotics decrease the accumulation of abdominal fat of broiler chicken (Allahdo *et al.,* 2018). The decrease in abdominal fat pad by supplementing of probiotics may be explained by more production of bacterial short chain fatty acids regulating the balance between synthesis and oxidation of fatty acids (Allahdo *et al.,* 2018; Khatibjoo *et al.,* 2018). Thigh meat is composed of 8-10% lipid (Khatibjoo *et al.,* 2018), and a part of the difference of thigh yield between antibiotic and probiotic treatments could be related to their lipid content. Furthermore, the poultry industry uses antibiotics to improve meat production through the increase of intestinal nutrient absorption and so improvement of feed conversion ratio (Mehdi *et al.,* 2018).

It's previously reported that the use of probiotic and prebiotic had no adverse effect on total protein, albumin and glucose concentration in serum of broiler chickens (Manafi *et al.,* 2017; Park *et al.,* 2016). However, there is a little study on the effect of sulphated- prebiotic fucoidan on blood serum parameters in broiler chickens. The change of serum albumin and creatine concentration could be accruing by end-stage liver and/ or kidney disease, intestinal malabsorption syndromes, and protein-calorie malnutrition (Busher, 1990). So, according to the results of present study, it could be concluded that feeding fucoidan and the other additives has not led to these situations. Furthermore, it's reported that probiotic and prebiotic supplements can reduce the incidence of liver injury (i.e., causing by pathogense and/or their toxins) and maintain normal levels of hepatic enzymes (e.g., ALT, AST, and ALP) in serum by decreasing bacterial translocation and intestinal permeability in the intestine (Gratz *et al*., 2010). A part of significant reduction in serum cholesterol concentration in broilers received synbiotic could be due to reducing absorption and synthesis of cholesterol by lactic acid bacteria through de-conjugation of bile salts (Pereira and Gibson, 2002) or inhibit the activity of hydroxymethyl-glutaryl-coenzyme A (HMG-CoA) reductase; an enzyme associated in the cholesterol synthesis pathway thereby reducing cholesterol synthesis (Alkhalf *et al.,* 2010). Recently, Park *et al.,* (2016) found that fucoidan improves serum lipid levels by regulating the expression of key enzymes of cholesterol and triglyceride syntheses (e.g., HMG-CoA reductase, acetyl-CoA carboxylase and fatty acid synthase) in the liver through modulation of sterol regulatory element-binding proteins (SREBP)-2. VLDL-c is the precursor for LDL-c in the liver and contains about 50–60% of triglyceride (Liong *et al.,* 2007). So, it seems that the lower concentration of triglyceride in synbiotic treatment resulted in lower level of VLDL-c and LDL-c in serum.

Gut health and total surface area (i.e., including villus height and width) are important components involved in the improvement of nutrients absorption in the small intestine (Awad *et al.,* 2009). Intestinal pathogens are potential agent to adversely affect villus height and crypt depth (Ribeiro *et al.,* 2007). The decrease of villus height and increase of crypts depth can lead to inadequate nutrient absorption and lower performance (Wang *et al.,* 2017). On the other hand, from anterior to the posterior section of the small intestines, the major function of villi turns from digestion towards absorption (Wang *et al.,* 2018). So, it seems that a part of the better growth performance observed in broiler chickens under antibiotic and synbiotic treatment could be related to the improvement of their intestinal morphology, especially in the jejunum and ileum. A part of the increase of villus height in the jejunum in synbiotic treatment could be explained by the synergistic effects between fucoidan and probiotic bacteria (Tavaniello *et al.,* 2018), while antibiotics prevent pathogens from destroying villi (Wang *et al.,* 2018). Moreover, in the ileum, higher villus height provided more surface area for nutrients absorption and lead to higher body weight gain in broiler chickens received antibiotic treatment. Deeper crypt may be indicative of a faster turnover of the intestinal mucosa layer for villus renewal after injury which is energy consuming

process (Haldar *et al.,* 2011). However, the deeper crypt found in the jejunum of synbiotic treatment could be because of the need to renewing of villus cells after normal sloughing (Potten, 1998), not the action of pathogens and/ or their toxins. The determination of intestinal villus to crypt ratio is an appropriate indexes to evaluation the effects of various diets on gut microanatomy. The greater villus height to crypt depth ratio will lead to better growth performance in broiler chickens (Awad *et al.,* 2009). In the present study, a part of the high ratio in antibiotic treatment may be a result of broad-spectrum antibacterial activity of oxytetracycline.

Conclusion

In conclusion, fucoidan and probiotics could be included in the broiler chicken diet without interfering in the bird growth but improving the feed conversion ratio which will lead to the decrease of chickens manure and environmental pollution. Furthermore, these non-antibiotics feed additives improved the broiler chicken health in terms of serum lipid metabolites, intestine microanatomy, and absorption surface area. The decrease of thigh percentage an abdominal fat in broiler chickens under probiotic treatments could be thought-out as a "trade off" scenario between quantity and quality of broiler chicken carcass.

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