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Effects of Dietary Supplementation of a Herbal Product (NBS Superfood) on Growth Performance, Intestinal Morphology, Immune Status and Blood Metabolites in Broiler Chickens

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Abstract

This study aimed to evaluate the effects of dietary Nutrition Bio-Shield Superfood® (NBS) supplement on the growth performance, intestinal morphology, immune response, and blood metabolites in broiler chicks. A total of 360 one-day-old Ross 308 broilers were used in a 42-d experiment. Based on a completely randomized design, the birds were allotted to 5 dietary treatments with 5 floor pen replicates of 12 birds each. Treatments included supplementation of 0, 0.5, 1, 1.5, and 2 g/kg of NBS to a basal diet. Feed conversion ratio (FCR) during 25-42 d and 1-42 d were linearly improved ($P \leq 0.05$) with increasing levels of dietary NBS. During these periods, FCR in groups that received 1 and 2 g/kg of NBS was significantly lower than the control group ($P < 0.05$). Dietary supplementation of NBS at 1 and 1.5 g/kg of the diet increased serum IgM level compared to all other treatments. Villus height (VH) and VH to crypt depth (CD) ratio showed a quadratic response to the treatments ($P = 0.05$ and $P = 0.04$, respectively), decreasing with the supplementation of 1.5 and 1 g/kg of NBS, respectively. The thickness of the muscle layer in the jejunum was increased by 2 g/kg of NBS compared to the control group. Feeding NBS had no significant effect on carcass traits and blood metabolites. In general, dietary NBS supplementation improves FCR, immune status, and jejunum histomorphology in broiler chickens.

Introduction

Antibiotics have been widely used in controlling infectious pathogens and increasing feed efficiency during the last decades (Engberg *et al.*, 2000). Despite having a strong therapeutic effect, they are now receiving much more attention and have become a significant cause for concern (Haque *et al.*, 2020). The presence of antibiotic residues in food and the environment endangers human and animal health (Haque *et al.*, 2020). Hence, there is a growing interest in finding effective options for controlling infectious diseases and limiting the spread of resistant bacteria.

One of the possible alternatives to antibiotics is plant-derived substances. Nutrition Bio-shield Superfood (NBS) is a healthy and suitable herbal supplement made from wheat grains (NBS Organic Company, Turkey). Wheat germ is rich in tocopherol, phytosterol, policosanol, thiamin, riboflavin, and niacin (Özcan and Ören, 2019). Arshad *et al*. (2013a, b) reported that dietary supplementation of wheat germ oil has beneficial effects on the stability and quality of the broilers' meat. In a recent study, Bayat *et al.* (2021) assess the impact of NBS on mice blood parameters. They added 50, 100, and 150 mg/kg BW of NBS to mice drinking water and found that the mean number of white blood cells and the neutrophil percentage increased, but lymphocytes counts percentage decreased. They concluded that the use of NBS improves the immune system of mice.

There is no published data about the effects of NBS powder in poultry. Thus, this experiment aimed to investigate the impact of NBS supplements on the growth performance, intestinal morphology, blood

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metabolites, carcass traits, and immune responses of broiler chickens.

Materials and Methods

Birds, diets, and housing

The present study was approved by the Animal Ethics Committee of the Ferdowsi University of Mashhad, Mashhad, Iran. A total of 300 one-day-old Ross 308 broiler chicks with an average BW of 45.12 ± 0.97 g were obtained from a local hatchery. The chicks were weighed using a digital balance (model PB 1501, Mettler, Toledo, OH) and distributed into 25-floor pens with 5 replicates of 12 chicks (6 males and 6 females), based on a completely randomized design. The pen size was 1.2 m × 1 m with wood shavings as a bedding material.

Table 1. Ingredients of NBS dietary supplement[1]

Composition	Amount, %	Minerals	Amount	Vitamins	Amount (mg)
Moisture	8.40	Total phosphorus **%**	0.44	B1	0.66
Total ash	1.80	Potassium **%**	2.31	B2	0.28
Fiber	11.26	Sulfur **%**	0.28	B3	2.70
Digestible nutrients	61.90	Magnesium **%**	0.32	B5	0.89
Carbohydrate g/100g	42.53	Calcium **%**	1.67	B6	0.89
Gross energy kcal/kg	4300	Boron **%**	0.62	C	52.40
ether extract	7.20	Iron (mg/kg)	241	A (IU)	530.0
Crude protein	20.60	Manganese (mg/kg)	49.80	D (IU)	483.0
Sugar	3.70	Zinc (mg/kg)	26.90	E (mg)	0.97
Cellulose	6.00	Copper (mg/kg)	13.6	K (µg)	63.60
Omega-3 fatty acids (mg/g)	48.42				
Omega-6 fatty acids (mg/g)	60.62				
Omega-9 fatty acids (mg/g)	22.16				

[1]Analyzed in Technology Development Center for Medicinal Plants. Department of Research and Development of Knowledge-Based Green Drug Researchers Company, Ardabil, Iran.

Table 2. Composition and calculated analysis of basal diets.

Ingredients (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (24-42 d)
Corn	41.94	35.99	31.86
Soybean meal (44% CP)	41.08	36.33	29.89
Wheat	10.00	20.00	30.00
Vegetable oil	2.51	3.74	4.75
Dicalcium phosphate	1.53	1.33	1.18
Limestone	1.42	1.31	1.21
Common Salt	0.21	0.21	0.21
DL- Methionine	0.42	0.37	0.34
L- Lysine HCl	0.21	0.16	0. 20
L-Threonine	0.08	0.06	0.06
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
$NaHCo_3$	0.10	0. 10	0. 10
Calculated composition, %			
Metabolizable energy (kcal/kg)	3000	3100	3200
CP	23.00	21.50	19.50
Linoleic acid	1.51	1.67	1.81
Ca	0.96	0.87	0.79
Available P	0.48	0.435	0.395
Sodium	0.16	0.16	0.16
Lysine	1.44	1.29	1.16
Methionine	0.76	0.68	0.62
Methionine + Cystine	1.08	0.99	0.91
Threonine	0.97	0.88	0.78
Arginine	1.50	1.36	1.18
Valine	1.10	1.04	0.95
Isoleucine	1.17	1.07	0.95

[1]Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 15,000 IU; vitamin D3, 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 mg; vitamin K, 5 mg; thiamin, 3 mg; riboflavin,10mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 70 mg; choline chloride, 1800 mg; folic acid, 2 mg; biotin, 0.4 mg; pantothenic acid, 20 mg.
[2]Mineral premix provided per kilogram of diet: Mn (manganese sulfate), 100 mg; Zn (zinc sulphate), 65 mg; Cu (copper sulfate), 5 mg; Se (Sodium Selenite), 0.22 mg; I (calcium iodate), 0.5 mg; and Co, 0.5 mg.

Each pen was equipped with a tray feeder for the first five days of the rearing period and thereafter a cylindrical feeder and two nipple drinkers. Each pen was considered as a replication, and the 25 pens were assigned to five dietary treatments as follows: 1) a basal diet without NBS supplementation (control), 2) basal diet + 0.5 g/kg of NBS, 3) basal diet + 1 g/kg of NBS, 4) basal diet + 1.5 g/kg of NBS and 5) basal diet + 2 g/kg of NBS. Nutrition Bio-Shield Superfood® is an herbal supplement produced from wheat grains by a green route (NBS Organic Company, Turkey). The ingredients of NBS powder were determined at Knowledge-Based Green Drug Researchers Company, Ardabil, Iran (Table 1).

The basal diet was formulated according to Ross 308 nutrient recommendations (Aviagen 2014b; Table 2). Rearing house temperature was set at 32°C on day one, and then decreased by 3°C per weak to reach 21°C at 28 d and remained constant until the end of the experiment. Relative humidity was kept between 60 to 65% throughout the experiment. During the first two days of age, 24 hours of light was provided for all birds, followed by 18 hours of light and 6 hours of darkness. Feed and water were provided *ad libitum* throughout the experiment. Other rearing operations were managed based on the Ross-308 management handbook (Aviagen 2014a).

Growth performance

The group weight of chicks in each pen was measured at one day of age and then at the end of starter (10 d), grower (24 d), and finisher (42 d) periods. The mean BW gain of chicks was obtained from the difference of their group weight at the beginning and end of each period divided by the number of birds at the end of the period. Feed intake (FI) was calculated by subtracting the residual feed from the feed given to each pen during the experiment. Mortality was recorded daily and the FCR was adjusted for it and presented as grams of feed consumed by the chicks in pen divided by grams of BW gain (Imari *et al.*, 2020).

Intestinal morphology

At 42 d, one male bird from each replicate was selected and killed by cervical dislocation. About 1 cm section from the midpoint of jejunum was separated, flushed with 0.9% saline, fixed in 10% buffered formalin, and kept for further processing. Tissue samples were taken out from the solution, dehydrated by a series of graded ethanol solutions, cleared in xylene, and infiltrated with melted paraffin. The infiltrated samples were embedded in paraffin blocks, and then tissue sections with a thickness of 5 to 6 μm were prepared using a rotating microtome. The sections were floated in 40°C distilled water, such that they were easily placed on the lamina after straightening the wrinkles. The slides were placed on a warm plate (45 °C), while drying, and the additional paraffin melted. The slides were stained with Hematoxylin and Eosin. All chemicals were purchased from Sigma-Aldrich Co. (Sigma-Aldrich Chemical Co, St. Louis, MO). Micrographs were taken using an Olympus BX41optical microscope (Olympus Corporation, Tokyo, Japan) equipped with a digital video camera. The images of tissue sections were analyzed by the stereological image software, Cast Image System, Version 2.3.1.3 (Visiopharm, Horsholm, Denmark). Histological measurements were performed on ten healthy villi from each tissue section. Morphometric indices included villus height (VH) from the tip of the villus to the crypt, villus width (VW; an average of VW at one-third and two-thirds of the villus), crypt depth (CD) from the base of the villus to the submucosa, and muscular thickness (MT) from the submucosa to the outer layer of the jejunum (Garcia *et al.*, 2007).

Blood collection and analysis

On 42 d, after 5 hours fasting, one male bird from each replicate was randomly selected, and 5 mL of blood sample was taken from the wing vein into a vacuum tube. Blood samples were kept at room temperature for 2 h and were centrifuged (3000 g; 10 min; 4°C). The collected sera were stored at –20°C until further analysis. Biochemical parameters were estimated using commercial laboratory kits according to the standard protocol (Pars Azmoon Co. Tehran, Iran). Measured parameters included glucose, uric acid, triglycerides, cholesterol (total high-density lipoprotein HDL-C and total low-density lipoprotein LDL-C). Serum glucose was measured using Glucose Hexokinase kit (catalog number 11876899 216, Roche Diagnostics, Indianapolis, IN), and uric acid concentration was measured using commercial reagent kits (catalog number P803-OU982-01, Pointe Scientific, Canton, MI). All samples were measured in duplicate.

Humoral immune response

To measure the immune response against sheep red blood cell (SRBC), a blood sample was taken from a ram and shed in a glass containing ethylenediaminetetraacetic acid (EDTA). The red cells were washed three times with Phosphate-buffered saline, and finally, a 5% solution of red blood cells was prepared in saline phosphate buffer. It should be noted that all the above steps were done in sterile conditions. At 35 days of age, one chick per replicate was injected intramuscularly with 0.5 mL of the aforementioned solution. To measure primary and secondary antibody response against SRBC, 7 days after each infusion, 2 mL of blood sample was taken from the wing vein. After blood clotting, sera are removed by centrifuge (3000 g; 10 min; 4°C). Collected sera were incubated for 30 min at 56°C to measure total anti-SRBC titers, immunoglobulin G

and immunoglobulin M. The titers of antibody were presented as the log2 of the highest dilution level of serum that agglutinated 0.05 mL of 2.5% suspension of SRBC in Phosphate-buffered saline (PBS; Eftekhari *et al*., 2018).

Carcass traits and internal organ measurements

At 42 days of age, one male chick with a BW close to the average live BW of the pen was selected, weighed, and slaughtered. After peeling, the carcass, breast, thighs, back and neck, wings, bursa of Fabricius, emptied gizzard, spleen, liver (with the gallbladder), pancreas, and abdominal fat pad were weighed and recorded separately. The results were presented as the percentage of live BW (Imari *et al.*, 2020).

Statistical analysis

The data were analyzed using the GLM procedure of SAS software (SAS, 2012) in a completely randomized design for variance analysis; the means were compared by Tukey's test ($P < 0.05$). A polynomial regression analysis was used to determine the effect of the dietary supplementation levels of NBS on the measured parameters (linear and quadratic). Orthogonal contrasts were performed to compare the means between treatments (control vs. other treatments).

Results

Growth performance

Table 3 shows the effect of NBS supplementation in the basal diet on BW parameters, daily weight gain (DWG), daily feed intake (DFI), and FCR of broilers at different ages. In the starter (1-10 d), grower (11-24), finisher (25-42), and whole experimental period (1-42 d), birds fed with diets containing NBS supplement had no significant difference compared to the control group ($P > 0.05$) in terms of BW and DWG. During the finisher period (25-42 days), birds fed with the diet supplemented with 1.5 g/kg NBS had significantly ($P < 0.05$) lower FI in comparison with the other groups but did not have a significant difference with the control group. However, the use of this supplement did not significantly affect FI during 1-10, 11-24, and 1-42 days of age compared to the control group. Dietary supplementation of NBS significantly affected the FCR of broiler chicks during 25-42 d ($P < 0.05$) and in the whole experimental period of 1-42 d ($P < 0.01$). Birds fed with diets containing 1 or 2 g/kg of NBS supplement had the lowest FCR in finisher (25-42 days) and in the whole rearing period compared to the control group ($P < 0.05$).

Table 3. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on growth performance in broiler chickens.

	Treatments[1]					*P*-Value				
	0	0.5	1	1.5	2	Linear	Quadratic	Treatments vs Control	Anova	*SEM*[3]
Body weight, g/bird										
10	223	221	224	219	218	0.15	0.62	0.32	0.49	2.71
24	771	744	750	777	741	0.66	0.95	0.41	0.60	19.54
42	1962	1934	2000	2046	2024	0.14	0.96	0.48	0.51	49.64
Daily weight gain, g/bird/d										
1-10	17.83	17.55	17.80	17.40	17.27	0.16	0.73	0.30	0.54	0.27
11-24	39.13	37.39	37.61	39.81	36.55	0.49	0.82	0.36	0.35	1.23
25-42	63.99	64.27	67.60	68.69	70.16	0.04	0.98	0.19	0.33	2.47
1-42	45.00	44.98	46.54	47.65	47.13	0.07	0.74	0.24	0.40	1.18
Feed intake, g/bird/d										
1-10	19.48	19.06	19.09	19.25	18.68	0.45	0.93	0.48	0.90	0.58
11-24	61.24	60.71	58.43	65.06	60.07	0.76	0.99	0.94	0.28	2.11
25-42	135.8^{ab}	131.6^{b}	126.2^{b}	146.3^{a}	131.6^{b}	0.61	0.77	0.67	0.02	3.92
1-42	83.89	81.18	80.12	86.78	80.52	0.85	0.93	0.41	0.13	2.04
Feed conversion ratio, g:g										
1-10	1.09	1.08	1.07	1.10	1.08	0.96	0.96	0.89	0.94	0.02
11-24	1.56	1.62	1.55	1.63	1.64	0.14	0.71	0.21	0.25	0.03
25-42	2.14^{a}	2.05^{ab}	1.87^{b}	2.13^{a}	1.88^{b}	0.05	0.62	0.06	0.02	0.06
1-42	1.83^{a}	1.80^{a}	1.67^{b}	1.86^{a}	1.68^{b}	0.04	0.99	0.07	0.002	0.03

[1]NBS Supplement, g/kg diet. $^{a–b}$Values in the same row with different letters are significantly different ($P < 0.05$).

[3]*SEM*: Standard error of the mean.

There were neither linear nor quadratic trends for BW, WG, DFI during different rearing periods and FCR during 1-10 and 11-24 days of age, except for FCR during 25-42 and 1-42 d, which was linearly affected by NBS supplement. As the NBS supplement level in the diet increased, the FCR of broilers

improved linearly. Orthogonal contrast between the control group and other treatments showed a tendency to a significant difference in FCR during 25-42 d and 1-42 d (P = 0.06 and P = 0.07, respectively). As shown in Table 3, birds receiving NBS supplement had better FCR in comparison with the control group during 25-42 d and 1-42 d (1.98 vs. 2.14 and 1.75 vs. 1.83, respectively).

Immune response

NBS supplementation had no significant effects on the total titer and IgG level on 42 days of age (Table 4). There was neither linear nor quadratic trend for total titer and IgG concentration in the blood serum of the broilers. Also, orthogonal comparison between control and NBS receiving birds did not significantly differ (P > 0.05) in total titer, IgM, and IgG concentrations. However, dietary supplementation of NBS at the levels of 1 and 1.5 g/kg of diet increased serum IgM level in comparison with all other treatments. In addition, IgM concentration showed linear and quadratic response (P = 0.02 and P = 0.0001, respectively) to the supplement. With increasing NBS to 1 g/kg of diet, the IGM concentration increased, and its concentration remained constant at 1.5 g/kg of diet and then decreased.

Table 4. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on immune response in broilers on 42 d[1].

	Treatments[1]					*P*-Value				
Items[2]	0	0.5	1	1.5	2	Linear	Quadratic	Treatments vs control	Anova	*SEM*[3]
Total titer	8.4	8.2	8.2	7.8	8.0	0.34	0.78	0.43	0.71	0.39
IgG	7.8	7.6	7.4	7.0	7.6	0.78	0.31	0.51	0.27	0.46
IgM	0.6[b]	0.6[b]	0.8[a]	0.8[a]	0.4[c]	0.02	0.0001	0.24	0.001	0.024

[1]NBS supplement, g/kg diet. Each mean represents five observations.
[2] Values are means of $\log_2$ of the reciprocal of the last dilution exhibiting agglutination.
[a–c] Values in the same row with different letters are significantly different (P < 0.05).
[3]*SEM*: Standard error of the mean.

Blood metabolites

Biochemical analysis of serum samples collected at 42 days of age is presented in Table 5. Dietary supplementation of NBS had no significant effect (P > 0.05) on glucose, triglycerides, cholesterol, LDL-C, and uric acid concentrations in the blood of broiler chickens. However, HDL-C level in birds fed with diets supplemented with 0.5 g/kg of NBS was marginally significant (P = 0.09). Moreover, blood metabolites did not show any linear or quadratic response to the supplement. Orthogonal contrast between the control group and NBS receiving birds did not significantly differ (P > 0.05) regarding blood metabolites.

Table 5. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on blood serum metabolites in broiler chickens at 42 d.

Items (mg/dL)	Treatments[1]					*P*-Value				
	0	0.5	1	1.5	2	Linear	Quadratic	Treatments vs control	Anova	*SEM*[2]
Glucose	256.0	266.4	278.4	238.8	262.2	0.23	0.46	0.63	0.12	10.13
Triglyceride	63.4	63.4	69.8	64.8	74.2	0.25	0.75	0.50	0.66	6.15
Cholesterol	126.2	143.4	139.2	127.8	131.4	0.85	0.32	0.37	0.62	9.16
HDL-C	47.0	53.2	50.4	44.8	45.6	0.14	0.13	0.57	0.09	2.33
LDL-C	57.75	66.0	65.0	61.20	60.2	0.99	0.09	0.16	0.39	3.30
Uric acid	7.16	7.16	7.64	8.16	7.34	0.84	0.79	0.92	0.83	0.63

[1]NBS Supplement, g/kg of diet
Each mean represents five observations.
[2]*SEM*: Standard error of the mean.

Morphological parameters of the jejunum

The effect of different NBS treatments on intestinal morphology characteristics on 42 days of age is presented in Table 6. Dietary supplementation of NBS significantly affected the thickness of the jejunal muscle layer (P < 0.05). Muscle layer thickness in the jejunum was increased by 2 g/kg of NBS compared to the control group. Villus height (VH) and VH to crypt depth (CD) ratio showed a quadratic trend in response to the dietary NBS levels (P = 0.05 and P = 0.04, respectively), decreasing with the supplementation of 1.5 and 1 g/kg of NBS, respectively. Orthogonal contrasts showed that VH on 42 days of age in NBS receiving birds are significantly (P < 0.05) longer than the control group (1463.4 vs. 1347.6 µm). In the case of the jejunum muscle layer thickness, the

orthogonal comparison showed that the thickness of the layer in birds receiving NBS was less than (P < 0.05) the control group (470.9 vs. 528.8 µm). Villus width and CD were not affected by the dietary treatments. They did not have a linear or quadratic trend, and there was no significant difference between the treatments and the control group in terms of the orthogonal comparison.

Table 6. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on jejunum histology in broiler chickens at 42 d.

Items (µm)	Treatments[1]					*P*-Value				*SEM*
	0	0.5	1	1.5	2	Linear	Quadratic	Treatments vs control	Anova	
Villus height	1347.6	1505.6	1496.0	1422.6	1429.4	0.60	0.05	0.04	0.17	48.13
Villus width	203.6	199.6	190.4	200.4	186.0	0.19	0.95	0.31	0.52	8.16
Crypt depth	157.2	157.0	142.0	159.0	169.4	0.39	0.15	0.98	0.42	9.84
Villus height :Crypt depth	8.68	9.64	10.86	9.28	8.49	0.76	0.04	0.33	0.27	0.84
Thickness of muscle layer	528.8^{a}	480.6^{ab}	472.2^{ab}	485.0^{ab}	445.8^{b}	0.009	0.56	0.008	0.05	17.84

[1]NBS Supplement, g/kg diet. Each mean represents five observations.
$^{a–b}$Values in the same row with different letters are significantly different (P < 0.05). *SEM*: Standard error of the mean.

Carcass traits

The mean percentage of carcass parts and internal organs at 42 days of age are shown in Table 7. Dietary supplementation of NBS had no significant effect on carcass parts and internal organs of broiler chicks on 42 d. Orthogonal contrast between the control group and NBS receiving birds did not show a significant difference (P > 0.05). Also, there were neither linear nor quadratic trends for carcass parts and internal organs in broilers on 42 d.

Table 7. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on carcass traits in broiler chicks at 42 d.

Items	Treatments[1]					*P*-Value				*SEM*
	0	0.5	1	1.5	2	Linear	Quadratic	Treatments vs control	Anova	
% of live body weight										
Carcass[2]	62.29	62.64	61.01	62.07	62.19	0.85	0.66	0.83	0.93	1.33
Breast	21.77	21.97	21.12	22.05	21.66	0.95	0.82	0.93	0.9	0.72
Thighs	18.62	19.04	19.01	19.01	19.45	0.36	0.97	0.41	0.88	0.54
back and neck	15.80	15.89	15.10	15.26	15.31	0.3	0.63	0.45	0.71	0.47
Wings	6.10	5.74	5.76	5.74	5.76	0.31	0.37	0.14	0.68	0.20
Bursa of Fabricius	0.14	0.12	0.16	0.16	0.17	0.19	0.9	0.62	0.59	0.02
Gizzard	1.76	1.75	1.93	1.85	1.86	0.48	0.64	0.55	0.85	0.13
Pancreas	0.27	0.25	0.28	0.28	0.25	0.92	0.75	0.8	0.89	0.02
Abdominal fat	1.39	1.27	1.28	1.16	1.53	0.64	0.05	0.53	0.21	0.11
Heart	0.51	0.51	0.50	0.54	0.48	0.62	0.48	0.84	0.58	0.02
Liver	2.17	2.25	2.23	2.24	2.16	0.94	0.56	0.74	0.97	0.12
Spleen	0.12	0.14	0.13	0.11	0.11	0.24	0.25	0.74	0.38	0.01

[1]NBS Supplement, g/kg diet. Each mean represents five observations.
[2]Carcases and their cuts were peeled before weighing.
SEM: Standard error of the mean.

Discussion

Growth performance

In this study, treatments containing NBS supplements were not significantly different from the control group in BW and DWG of broilers. NBS is a wheat grains-derived supplement that contains protein, essential vitamins, and minerals such as potassium, iron, zinc, D, E, and B vitamins, and essential fatty acids. Ellakany *et al.* (2017) reported that fermented wheat germ extract (FWGE) is a multi-compound supplement containing 2-methoxy benzoquinone and 2, 6-dimethoxy benzoquinone, that are likely responsible for some of its biological properties. They also observed that FWGE supplementation to broilers diet from day 1- 35 d at the doses of 0.5, 1.5, and 3 g/kg diet had a contrary effect with the results of the present study. All doses of FWGE in the study of Ellakany *et al.* (2017) increased BW, especially at 3 g/kg diet. There are many reports on the growth-promoting, immune-modulating, and antioxidant effects of FWGE which has a similar structure as NBS (Rafai *et al.*, 2011; Ellakany *et al.*, 2017; Liaqat *et al.*, 2020). Unlike this study, Salama *et al.* (2019) showed non-significant differences regarding BW,

DWG, carcass weight, and dressing percentages among rabbits in treatments fed with diets containing 0, 20, and 40% wheat germ meal.

There is no available data on the use of NBS supplement in broiler chickens' nutrition, but Arshad *et al.* (2013a, b) supplemented broilers' diet with wheat germ oil. They observed a significant improvement in broiler chicks' growth parameters, which is somewhat in agreement with the current study in terms of FCR. In the present study, FI was not significantly affected by dietary treatments. Moran and summers (1967) and Cave *et al.* (1968) studied the effects of WGM on broiler chickens and found an improvement in their growth performance and feed utilization.

NBS supplement improved FCR of the birds in comparison with the control group. Ellakany *et al.* (2017) reported that FWGE significantly decreased FCR in broilers ($P < 0.05$). In a study, Salama *et al.* (2019) showed that rabbits in the group receiving 40% autoclave-treated wheat germ meal achieved the lowest FCR.

The non-significant impact on growth performance with increasing wheat germ meal (WGM) may be due to an increase in anti-proteolytic factor (trypsin inhibitor) which inhibits enzymatic digestion of proteins or other hemagglutinin factors (Creek and Vasaitis, 1962). These data suggest that in future studies with NBS, nutrient digestibility needs to be measured in broilers. Also, Moran and Summers (1967) found that anti-trypsin activity was very high in raw WGM. Parrish and Bolt (1963) confirmed the impairment of growth rate, feed efficiency, and fat absorption in chicks due to feeding raw WGM and the presence of gluten. Also, Creek *et al.* (1961) reported that feeding raw WGM to chicks could lead to a reduction in growth rate and feed efficiency, accompanied by hypertrophy of the liver and impairment of fat and protein utilization.

The possible positive effects of wheat germ may be due to the nutrients present in it. In other words, when wheat germinates, the availability of its minerals, vitamins, and carbohydrates increases (Ellakany *et al.,* 2017). At the beginning of the germination process, the starch in the grain is broken down into simple sugars, glucose, and sucrose, which is done by the enzyme amylase. Germinated wheat contains vitamins A, B1, B6, B12, and E, folic acid, calcium, phosphorus, zinc, iodine, selenium, and magnesium. These nutrients are utilized when germ develops to mature wheat. (Banu *et al.,* 2020).

Immune response

NBS supplementation had no significant effects on total titer and IgG in response to SRBC administered to the birds in 42 d. While IgM antibody titer showed a significant increase in comparison with the control group. Increased immune responses have been previously reported with probiotics (Koenen *et al.,* 2004) and other similar supplements such as herbal extracts (Mathivanan and Kalaiarasi, 2007) in diets, which is consistent with the results of the present study. Bayat *et al.* (2021) added 50, 100, and 150 mg/kg BW of NBS to the drinking water of mice and reported that the NBS significantly improves cellular immune response. They observed the mean number of white blood cells, and the neutrophil percentage increased, but lymphocytes count decreased in the groups receiving NBS supplement. There is disagreement in the literature about the effect of different feed additives. Several authors have shown an increase in humoral immunity using additives (Kabir *et al.,* 2004; Khaksefidi and Ghoorchi, 2006), while some others observed no remarkable effect on antibody titers (Balevi *et al.,* 2001). Because their effectiveness depends on many factors such as type, level of application, method of administration (feed or water), frequency of use (single, intermittent or continuous), diet composition, age and species of birds, field hygiene level and environmental stressors (temperature, stocking density).

In this experiment, NBS Supplement did not significantly affect blood metabolites of glucose, triglycerides, cholesterol, LDL-C, HDL-C, and uric acid concentrations in broiler chicks on 42 days of age. Since the ban on antibiotics as a growth stimulant in poultry production, there has been a growing interest in exploring potential alternatives. NBS supplement can be one of the possible alternatives, like many other additives such as probiotics, prebiotics, organic acids, and enzymes. However, this supplement had no remarkable effect on blood metabolites in the current study.

Serum lipid profile is an important indicator for animal health and meat quality. Meat from broilers fed with diets supplemented with some feed additives are more appropriate for poultry markets due to its beneficial effect on human health (Fletcher, 2002). Thus, it is suggested that the effect of NBS supplement on meat quality be investigated in future studies.

Toghyani *et al.* (2010) did not observe the significant influence of experimental diets (flavophospholipol antibiotic, and 5 and 10 g/kg of thyme powder) on concentrations of total cholesterol, LDL-C, triglyceride, protein, and albumin in blood serum. Unlike this research, they observed that the feeding of the broilers with 10 g/kg of thyme as a natural growth promoter resulted in a marked increase in HDL-C concentration compared to other treatments.

Morphological parameters of the jejunum

Dietary NBS treatments did not affect villi characteristics and crypt depth in comparison with the control group. The authors could not find data about the effect of NBS supplements on intestinal histomorphology. But in a contrary to these results, de Souza *et al.* (2018) reported that birds fed diets with probiotics presented higher crypt depth than birds fed diets without probiotics. They observed that probiotics, as an additive and alternative to antibiotics, caused no effect on the villus height, villus width, and VH: CD ratio. Yakhkeshi *et al.* (2011) reported no significant differences among treatments (additives such as probiotics, prebiotics, organic acids, enzymes, and herbal extracts) regarding VH: CD ratio in the duodenum and ileum.

Garcia *et al.* (2007) demonstrated that dietary supplementation of medicinal plants increases VH in the broiler small intestine. They suggested that these plants reduce the number of harmful bacteria in the intestinal wall so that the production of toxic compounds decreases and the destruction of intestinal epithelial cells reduces. This function can lead to beneficial changes in intestinal morphology. In another investigation, plant extracts significantly increased short-chain fatty acids (SCFA) in the gut (Juskiewicz *et al*., 2011). These fatty acids can decrease the intestinal pH and improve the microbiome of the gut. The ratio of VH: CD is a good indicator of intestinal health and digestive tract maintenance (Pluske *et al.,* 1996). In general, the longer villi and higher VH: CD ratio are associated with increased epithelial cell turnover, thereby improving nutrient absorption (Xu *et al.,* 2003). This may explain, at least in part, the better FCR observed in the birds fed with diets supplemented with NBS supplement in the current study.

The broiler chickens fed with a diet supplemented with fermented wheat germ extract had a significantly higher average villus high in the small intestine than the control group (Ellakany *et al.,* 2017). The incidence of villus atrophy accompanied by a widening of the lamina propria, fusion of the villi, and leucocytic infiltration in the lamina propria was higher in the intestines of control chickens, indicating a less favorable microbial environment in the intestinal content (Ellakany *et al.,* 2017).

Carcass traits

The results showed that NBS supplementation had no remarkable effect on the carcass traits. The current results are consistent with Batista *et al.* (2007), who observed that dietary supplementation of antibiotics, prebiotics, probiotics, and synbiotics have no significant effect on carcass yield. Also, they did not observe differences in leg yield between control birds and those receiving feed additives. Abudabos *et al.* (2015) reported that prebiotics, probiotics, and a mixture of prebiotic and prebiotic (synbiotic) had no significant effect on carcass yield, breast muscle, drumstick, and abdominal fat pad in broilers. So that chicks received prebiotic had the highest performance. Al-Khalaifa *et al.* (2019) showed that there is no significant effect of the experimental diets (probiotics: 1 g/kg Lactobacillus and Bacillus coagulans, prebiotics: fructooligosaccharides (5 g/kg diet), and mannan-oligosaccharide on the abdominal fat pad, liver, heart, bursa of Fabricius, spleen, breast, and thighs.

Barroso *et al.* (2017) realized that carcass parameters and its cuts, including head, neck, feet, thighs, wings, liver, gizzard, breast are not significantly affected by the replacement of the growth promoters with probiotics. da Paz *et al.* (2010) showed that antibiotics, prebiotics, probiotics, and organic acids supplementation in birds' diet do not affect breast and thighs yield and liver, heart, and intestines' weight. de Faria *et al.* (2009) and de Souza *et al.* (2018) did not observe any differences in carcass traits by supplementation of antibiotic and probiotic as growth stimulators, consistent with the current research. These authors stated that many factors could interfere with additives. Therefore, the results are often inconsistent. Environment, health challenges, animal physiology, species are some of the factors that should be considered on evaluating NBS supplement in diets. The ineffectiveness of NBS supplementation on carcass characteristics and weight of digestive organs can be expressed as the nutrients required by the bird are provided through the diet and the addition of nutrient supplements including proteins, vitamins, minerals, and fatty acids will not improve the characteristics of the carcass. Therefore, NBS may need to be tested in diets that are marginally deficient in nutrients. Also, for this specific experiment, it is suggested that a microbiological challenge is needed so that this product may prove its function. Since NBS increased BW gain and improved FCR linearly, it is suggested that higher levels of this supplement be used in future studies.

Conclusion

In conclusion, this experiment revealed that supplementation diets with NBS improved FCR and the humoral immune response of broilers. However, it did not influence weight gain, blood metabolites, villi height and width, and carcass traits.

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