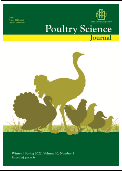

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Supplementing a Herbal Product (NBS Superfood) in Broiler Diets Varying in Energy and Protein Levels: Effects on Growth Performance, Intestinal Morphology, Immune Response and Blood Metabolites

Ali Almamury Ahmad Hassanabadi Saeed Zerehdaran & Hassan Nassiri-Moghaddam

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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Corresponding author
Ahmad Hassanabadi
hassanabadi@um.ac.ir

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Abstract

This study aimed to evaluate the effects of dietary supplementation of Nutrition Bio-Shield Superfood® (NBS) on the growth performance, intestinal morphology, immune response and blood metabolites in broiler chickens fed with low metabolizable energy (ME) and crude protein (CP) diets. A total of 360 one-day-old Ross 308 broilers were used in a 42-d experiment. The birds were allotted to 6 dietary treatments with 5 floor pen replicates of 12 birds each; based on a completely randomized design. Dietary treatments included a positive control diet (PC, standard diet), a negative control diet (NC, 3% reduction in ME and CP without NBS supplementation), and dietary supplementation of NBS at 0.5, 1, 1.5, and 2 g/kg to negative control diet (NBS0.5, NBS1, NBS1.5, NBS2, respectively). Body weight on d 10 and body weight gain during d 1-10 were lower ($P < 0.05$) in birds fed with NC and NBS diets compared to the positive control group. During later phases and d 1-42, no significant differences were observed in body weight, body weight gain, feed intake, and feed conversion ratio of broilers among the treatments. Supplementation of NBS or lowering the nutrients by 3% had no significant effect on immune response (total Ig, IgG and IgM concentrations) compared to positive control group. NBS supplementation decreased muscle layer thickness (MLT) in the jejunum ($P < 0.01$). Villus width (WV) and MLT showed a linear response to the treatments ($P = 0.02$ and $P = 0.02$, respectively), in this case, VW increased with the supplementation of NBS at 0.5 to 2 g/kg, and MLT decreased with these levels of dietary NBS. In general, dilution of nutrients by 3% of the standard diet and dietary supplementation of NBS had no significant effect on growth performance, immune response, blood metabolites and carcass traits in broiler chickens.

Introduction

Feed constitutes a major part of expenses in a poultry farm and thus the ever-rising feed cost become an important challenge faced by poultry industry. (MacDonald, 2010). Among all nutrients, protein and energy are two important items that make a lot of interest and challenges to the nutritionists since in fact, they are the primary and costly items affecting all products in poultry industry (Kamran *et al.*, 2008b). A diet reduced in crude protein (CP), metabolizable energy (ME) or other nutrients without any harmful effect on bird's health and productivity could be a viable option to consider by a nutritionist. Therefore, poultry researchers are trying to find modern procedures for an economical broiler production by increasing the utilization of energy and protein (EL-Sheikh, 2002). It has been shown that adding some photogenic plants to the diets improves nutrient utilization (Hafez and Attia, 2020). Interestingly, their effect is more pronounced when birds face with a challenge such as a nutritional one (Hafez and Attia, 2020).

Chickens are susceptible to pathogens (Broom and Kogut, 2019). Antibacterial additives like

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antibiotics used to be as a dietary supplement to reduce the amounts of pathogenic microorganisms in the birds' gastrointestinal tract and increase their growth and feed efficiency (Low *et al*., 2021). But, in the recent years, their routine use has been banned in many countries (Hafez and Attia, 2020). One of the possible alternatives to antibiotics is plant-derived substances such as essential oils, flavonoids, polyphenols, alkaloids and tannins. In poultry nutrition, a wide range of plant components have been investigated for their antibacterial, coccidiostatic, immunostimulatory and growth promoting properties (Low *et al*., 2021). A major by-product of wheat germ oil extraction is defatted wheat germ (DWG), which contains a variety of nutrients including relatively high protein (30%), carbohydrates, pigments, minerals and B vitamins (Zhu *et al*., 2006). Nutrition Bio-shield Superfood (NBS) is a healthy and suitable herbal supplement extracted from wheat grains by a green path (NBS Organic Company, Turkey). NBS is a feed additive similar to DWG, but its mechanism of action need to be clarified using reliable experiments. There have been very few studies on using NBS supplement as a feed additive in broilers feed (Bayat *et al*., 2021). Therefore, the aim of this study was to evaluate the effects of dietary supplementation of NBS powder on growth performance, intestinal morphology, immune response and blood metabolites in broiler chickens fed with varying ME and CP diets.

Materials and methods

Birds, diets and housing

A total of 360 one-day-old broiler chicks from Ross 308 strain were obtained from a commercial hatchery with an average body weight (BW) of 40 ± 0.17 g. The chicks were sexed and distributed into 30 floor pens with five replicates of 12 chicks each (6 males and 6 females), based on a completely randomized design. The pen size was 1.2 m×1 m with wood shavings as bedding material. Nutrition Bio-shield Superfood (NBS) used as a feed additive in preparing the treatments. It is a healthy and suitable herbal supplement extracted from wheat grains by a green path (NBS Organic Company, Turkey; Table 1).

Table 1. Chemical composition of NBS supplement powder[1] (DM basis)

Composition	Amount	Minerals	Amount	Vitamins	Amount
Moisture (%)	8.40	Total phosphorus (%)	0.44	B1 (mg/kg)	0.66
Total ash (%)	1.80	Potassium (%)	2.31	B2 (mg/kg)	0.28
Crude fiber (%)	11.26	Sulfur (%)	0.28	B3 (mg/kg)	2.70
Digestible nutrients (%)	61.90	Magnesium (%)	0.32	B5 (mg/kg)	0.89
Carbohydrate (%)	42.53	Calcium (%)	1.67	B6 (mg/kg)	0.89
Gross energy (kcal/kg)	4300	Boron (%)	0.62	C (mg/kg)	52.40
Ether extract (%)	7.20	Iron (mg/kg)	241	E (mg/kg)	0.97
Crude protein (%)	20.60	Manganese (mg/kg)	49.80	A (IU)	530.0
Sugar (%)	3.70	Zinc (mg/kg)	26.90	D (IU)	483.0
Cellulose (%)	6.00	Copper (mg/kg)	13.6	K (μg/kg)	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.
NBS: Nutrition Bio-Shield Superfood®.

Dietary treatments included: Positive control (PS), Negative control (NC, with a 3% reduction in ME and CP as basal diet), Negative control + 0.5 g/kg NBS (NBS0.5), Negative control + 1.0 g/kg NBS (NBS1), Negative control + 1.5 g/kg NBS (NBS1.5), Negative control + 2.0 g/kg NBS (NBS2). The Positive control 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 week 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. All birds were provided with 24 h light during the first two days of age, followed by 18 h light: 6 h dark thereafter. Feed and water were provided ad libitum throughout the experiment. Other rearing conditions were managed according to the Ross-308 management handbook (Aviagen, 2014a).

Growth performance

The group weight of chicks in each pen was measured at day one of age and then at the end of starter (d 10), grower (d 24) and finisher (d 42) periods. The mean body weight gain (BWG) was calculated from the weight gain of the birds per pen. Feed intake (FI) was calculated by subtracting the remaining feed from the feed provided in each pen during the experiment. The feed conversion ratio (FCR) was modified for mortality and was expressed as grams of feed consumed by birds in pen divided by grams of weight gain. Daily mortality was recorded in each treatment.

Table 2. Composition and calculated analysis of basal diets, as-fed basis.

Diet ingredients, %	Standard diets			Low-nutrient diets		
	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	41.94	35.89	31.66	46.35	40.20	35.83
Soybean meal (CP 44%)	41.08	36.33	29.89	38.46	33.84	27.59
Wheat	10.00	20.00	30.00	10.00	20.00	30.00
Soy oil	2.51	3.74	4.75	0.57	1.76	2.75
Dicalcium phosphate	1.53	1.33	1.18	1.54	1.35	1.19
Limestone	1.42	1.31	1.21	1.43	1.32	1.21
Common salt	0.21	0.21	0.21	0.21	0.21	0.21
DL- Methionine	0.42	0.37	0.34	0.44	0.39	0.36
L- Lysine HCl	0.21	0.16	0.20	0.29	0.24	0.28
L-Threonine	0.08	0.06	0.06	0.11	0.09	0.08
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
NaHCO3	0.10	0.10	0.10	0.10	0.10	0.10
Calculated composition %						
Metabolizable energy (kcal/kg)	3000	3100	3200	2910	3007	3104
Crude protein	23.0	21.5	19.5	22.31	20.86	18.92
Linoleic acid	1.51	1.67	1.81	1.19	1.35	1.48
Calcium	0.96	0.87	0.79	0.96	0.87	0.79
Available P	0.48	0.43	0.39	0.48	0.43	0.39
Sodium	0.16	0.16	0.16	0.16	0.16	0.16
Lysine	1.44	1.29	1.16	1.44	1.29	1.16
Methionine	0.77	0.69	0.63	0.77	0.69	0.63
Met + Cys	1.08	0.99	0.91	1.08	0.99	0.91
Threonine	0.97	0.88	0.78	0.97	0.88	0.78
Valine	1.10	1.00	0.90	1.06	1.00	0.90
Isoleucine	0.97	0.89	0.81	1.13	1.03	0.91

[1]Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 15,000 U; vitamin D3, 5000 U; vitamin E (DL-α-tocopheryl acetate), 80 mg; vita-min K, 5 mg; thiamine, 3 mg; riboflavin,10 mg; 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 sulphate), 100 mg; Zn (zinc sulphate), 65 mg; Cu (copper sulphate), 5 mg; Se (Sodium Selenite), 0.22 mg; I (calcium iodate), 0.5 mg; and Co, 0.5 mg.

Intestinal morphology

At 42 days of age, one male bird was selected from each replicate and killed by cervical dislocation. About 1 cm from the midpoint of the jejunum was removed, washed with 0.9% saline, fixed in 10% formalin buffer, and stored for further processing. The samples were taken out of solution and dehydrated by a series of graded ethanol solutions, cleared in xylene, and infiltrated with paraffin. Infiltrated tissue samples were embedded in paraffin blocks. The embedded samples were cut into a thickness of 5-6 μm using a rotating microtome. The sections were floated in 40 °C distilled water, so that they were easily placed on the lamina after smoothing. The slides were placed on a warm plate at 45 °C and the excess paraffin was melted while drying. Slides were stained with hematoxylin and eosin. All chemicals were sourced from Sigma Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO). The micrographs were taken with an Olympus BX41 optical microscope (Olympus, Tokyo, Japan) equipped with a digital video camera. Images were analyzed using ImageJ software (version 1.5). Morphometric measurements were performed on 10 healthy villi from each sample. Morphometric indices included villi height (VH) from the tip of the villi to the crypt, VW (average VW in one-third and two-thirds of the villi), and crypt depth (CD) from the base of the villi to the submucosa and muscle thickness (MT) from submucosa to outer layer of jejunum (Garcia *et al*., 2007).

Blood collection and analyses

On d 42, after 5 hours fasting, one male bird from each replicate was randomly selected and 5 mL blood sample was taken from the wing vein into a vacuum tube. Blood samples were kept at room temperature for 2 h and centrifuged (3000 g; 10 min; 4°C). The collected sera were stored at –20 °C until further analysis. Measured parameters included glucose (Glu), uric acid (UA), triglycerides (TG), total cholesterol (Chol), high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c). Serum glucose was measured using enzymatic kinetic methods (Glucose Hexok-inase kit, Roche Diagnostics, Indianapolis, IN), but other biochemicals were measured via commercial reagent kits (Pars Azmoon Co. Tehran, Iran).

Humoral immune response
To measure the immune response against sheep red blood cells (SRBC), a blood sample was taken from a ram and shed in a glass containing 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. 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 responses against SRBC, 7 days after injection, 2 mL 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 placed for half an hour at 56 °C for measurement of total anti-SRBC titers, IgG and IgM. The antibody titers data were shown as the log 2 of the highest dilution causing visible agglutination of 0.05 mL of 2.5% SRBC suspension in PBS (Eftekhari *et al.*, 2018).

Carcass traits and internal organ measurements
At 42 days of age, one male bird per pen was selected, weighed, and decapitated for measuring carcass yield. Weights of carcass yield, breast, thighs, back and neck, wings, gizzard, abdominal fat pad, liver, bursa of Fabricius, spleen, and pancreas were weighed separately and shown relative to the live body weight of the bird (Imari *et al.*, 2020).

Statistical analysis
The data were subjected to variance analysis using the GLM procedure of SAS software (2012) in a completely randomized design. Significant differences between means were compared by Tukey's test at a significance level of $P < 0.05$. The dose related effect of the NBS supplement was computed using orthogonal polynomial contrasts for the linear and quadratic effects. Orthogonal contrasts were used to determine the mean comparison among treatments.

Results
Growth performance
Growth performance parameters are summarized in Table 3. Birds fed with NC diet (3% reduction in ME and CP) either supplemented or not supplemented with varying levels of NBS powder showed a significant reduction in average BW at d 10 and daily BWG during d 1-10 of age compared to PC group. However, experimental treatments including NC diet and NC diet supplemented with different levels of NBS powder did not significantly affect BW and BWG in the post-starter period or in the whole rearing period compared to the PC group (Table 3). Orthogonal comparison between PC group and all NC groups showed a significant decrease ($P < 0.01$) in average BW in the starter and grower periods (210.6 vs 223.0 and 733.2 vs 771.0 g/bird, respectively) compared to the PC group. Also, daily BWG in NC birds, either supplemented or not supplemented with NBS powder, were significantly ($P < 0.01$) lower than PC group in the starter period (16.5 vs 17.8 g/bird/d). Orthogonal comparisons also showed that NBS supplementation to NC diet had no significant effect on BW and BWG in any rearing periods. There was a linear ($P < 0.01$) and a quadratic ($P < 0.01$) decrease in both average BW and daily BWG in the starter period in response to NBS levels (from 0 to 2 g/kg diet).

The FI and FCR were not significantly affected by diluting the diet (NC diet) or by dietary supplementation of NBS during the rearing periods. The independent comparisons also did not show significant differences between the groups receiving NBS with PC or NC in all of the experimental periods. Neither linear nor quadratic trends were observed in the FI and FCR of birds during the experiment. Except that, the FI of NBS receiving birds showed a quadratic trend ($P = 0.01$) during 1-10 days of age (Table 3).

Immune response
As shown in Table 4, anti-SRBC titers were not significantly affected by diluting the diet by 3% (NC diet) as well as the addition of NBS to the diluted diet compared to PC or NC during the rearing periods. The orthogonal comparisons also did not show significant differences between the groups receiving NBS compared to PC or NC in the experimental periods. Neither linear nor quadratic trends were observed in the total Ig, IgG and IgM concentrations in the birds' blood during the experiment (Table 4).

Morphological parameters of the jejunum
As shown in Table 5, the VH, VW, CD and VH:CD ratio in the jejunum of the birds was not significantly affected by diluting the diet by 3% (NC diet) and the addition of NBS to the diluted diet compared to PC or NC on 42 d. The orthogonal contrasts did not show significant differences in these histomorphological parameters between birds receiving NBS and PC or NC groups. Neither linear nor quadratic trends were observed in the VH, CD and VH: CD ratio of the birds at 42 d of age. However, the VW in the jejunum linearly increased in response to increasing dietary NBS levels but did not show quadratic trend (Table 5).

Blood metabolites
As shown in Table 6, Mean serum metabolite concentrations including Glu, TG, Chol, HDL-c, LDL-c and UA on d 42 of age were not significantly affected by NC diet (diluted ME and CP by 3%) compared to PC diet, as well as the supplementation of NBS to the diluted diet compared to PC or NC groups.

Table 3. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on the growth performance in broiler chickens[1] (1–42 d).

	Control diets [2]		NBS supplement [3]				*P*-value					
days	PC	NC	0.5	1	1.5	2	NBS vs PC	NBS vs NC	Linear	Quadratic	Anova	*SEM*
Body weight, g/bird												
10	223.0^{a}	214.0^{b}	212.0^{bc}	205.0^{c}	209.0^{bc}	213.0^{bc}	0.0002	0.15	0.002	0.001	0.002	2.66
24	771.0	739.0	732.0	724.0	740.0	731.0	0.01	0.64	0.08	0.11	0.25	13.68
42	1957.0	1988.0	1938.0	1977.0	2043.0	1975.0	0.70	0.94	0.58	0.95	0.89	63.02
Average daily weight gain, g/bird/d												
1-10	17.80^{a}	16.8^{b}	16.6^{bc}	16.0^{c}	16.4^{bc}	16.8^{b}	0.0001	0.15	0.002	0.0004	0.0009	0.24
11-24	39.13	37.15	37.17	37.10	37.95	37.02	0.06	0.85	0.25	0.25	0.43	0.82
25-42	74.79	78.09	76.24	78.85	81.59	78.34	0.27	0.84	0.26	0.63	0.75	3.22
1-42	45.13	46.75	45.06	46.00	47.33	45.97	0.54	0.68	0.58	0.83	0.87	1.48
Feed intake, g/bird/d												
1-10	19.48	18.22	18.19	17.13	18.85	18.80	0.08	0.96	0.56	0.01	0.07	0.52
11-24	52.31	50.64	51.84	47.29	54.45	49.40	0.55	0.96	0.66	0.72	0.26	2.10
25-42	139.7	145.3	139.2	136.1	147.8	142.2	0.77	0.52	0.70	0.76	0.65	5.36
1-42	81.95	83.69	81.30	78.21	85.99	81.91	0.97	0.58	0.87	0.64	0.49	2.72
Feed conversion ratio												
1-10	1.09	1.08	1.09	1.07	1.15	1.11	0.62	0.36	0.18	0.55	0.36	0.02
11-24	1.33	1.36	1.39	1.27	1.43	1.33	0.72	0.87	0.89	0.79	0.18	0.04
25-42	1.88	1.86	1.83	1.72	1.81	1.81	0.26	0.35	0.32	0.37	0.69	0.05
1-42	1.82	1.79	1.80	1.70	1.81	1.78	0.44	0.75	0.59	0.43	0.59	0.05

[a-b] Values in the same row with different letters are significantly different ($P < 0.05$).

[1]Each mean represents five observations.

[2] PC= Positive control (standard diet), NC=Negative control (with a 3% reduction in nutrients)

[3] NBS Supplement: NBS0.5=Negative control + 0.5 g/kg, NBS1= Negative control + 1.0 g/kg, NBS1.5= Negative control + 1.5 g/kg, NBS2= Negative control + 2.0 g/kg (Nutrition Bio-Shield Superfood® (NBS) powder is a herbal product derived from wheat grains).

SEM: Standard error of the means.

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

	Control Diets[2]		NBS Supplement[3]				P-value					
	PC	NC	0.5	1	1.5	2	NBS vs PC	NBS vs NC	Linear	Quadratic	Anova	*SEM*
Total Ig (Log_2)	8.4	8.0	8.6	7.6	7.6	7.4	0.37	0.82	0.15	0.74	0.57	0.52
IgG (Log_2)	7.6	6.0	7.8	6.8	7.4	6.6	0.52	0.28	0.80	0.92	0.67	0.86
IgM (Log_2)	0.8	0.8	0.8	0.8	0.4	0.8	0.72	0.75	0.60	0.87	0.87	0.27

[1]Each mean represents five observations.
[2] PC= Positive control (standard diet), NC=Negative control (with a 3% reduction in nutrients)
[3] NBS Supplement: NBS0.5=Negative control + 0.5 g/kg, NBS1= Negative control + 1.0 g/kg, NBS1.5= Negative control + 1.5 g/kg, NBS2= Negative control + 2.0 g/kg (Nutrition Bio-Shield Superfood® (NBS) powder is a herbal product derived from wheat grains).
SEM: Standard error of the means.

Table 5. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on jejunum histology in broiler chickens at d 42[1].

Items	Control diets[2]		NBS Supplement[3]				P-value					*SEM*
	PC	NC	0.5	1	1.5	2	NBS vs PC	NBS vs NC	Linear	Quadratic	Anova	
Villus height (μm)	1347	1379	1322	1395	1489	1447	0.31	0.61	0.06	0.64	0.31	55.4
Villus width (μm)	203.6	188.8	221.0	228.8	231.6	228.6	0.11	0.01	0.02	0.62	0.14	12.7
Crypt depth (μm)	157.2	155.4	175.0	159.9	173.2	156.4	0.29	0.19	0.53	0.12	0.20	6.7
Villus height:crypt depth	8.42	8.95	7.63	8.82	8.42	9.24	0.78	0.45	0.60	0.16	0.37	0.52
Thickness of muscle layer (μm)	528.8[a]	449.0[cd]	478.0[bc]	508.2[ab]	417.0[d]	483.8[abc]	0.02	0.34	0.03	0.09	0.0007	15.9

[a-b]Values in the same row with different letters are significantly different ($P < 0.05$).
[1]Each mean represents five observations.
[2] PC= Positive control (standard diet), NC=Negative control (with a 3% reduction in nutrients)
[3] NBS Supplement: NBS0.5=Negative control + 0.5 g/kg, NBS1= Negative control + 1.0 g/kg, NBS1.5= Negative control + 1.5 g/kg, NBS2= Negative control + 2.0 g/kg (Nutrition Bio-Shield Superfood® (NBS) powder is a herbal product derived from wheat grains).
SEM: Standard error of the means.

Also, the orthogonal contrasts did not show significant differences in these parameters between birds receiving NBS compared to PC or NC groups. Neither linear nor quadratic trends were observed in the blood parameters of the birds at d 42 of age. Except that, blood LDL-C concentration in NBS receiving birds showed a linear decrease ($P = 0.05$) in response to increasing levels of dietary NBS (Table 6).

Carcass traits

Carcass traits parameters are summarized in Table 7. Carcass cuts and internal organ weights of the broilers on d 42 were not significantly affected by lowering ME and CP by 3% compared to PC diet, as well as the supplementation of different NBS levels to the diluted diet compared to PC or NC groups. Also, the orthogonal contrasts did not show significant differences in these parameters between birds receiving NBS compared to PC or NC groups. Neither linear nor quadratic trends were observed in the carcass cuts and internal organs of the birds at 42 d of age. However, the orthogonal comparisons showed that relative weight of wings in NBS receiving birds was significantly lower than PC group. Other independent comparisons did not show any significant differences. Also, linear and quadratic trends were not observed in carcass parts and internal organs of the birds at 42 days of age, except for the relative weight of the thighs that showed a quadratic trend in response to the dietary NBS levels (Table 7).

Discussion

Growth performance

In this study, the level of protein and energy in the diet decreased by 3%, assuming that dietary supplementation of NBS can compensate for this challenge. But this effect was not observed. The major reduction in ME and other nutrients may compromise productivity. On the contrary, Ellakany *et al*. (2017) reported that 0.5, 1.5 and 3 g/kg diet of fermented wheat germ extract (FWGE), a product similar to NBS, significantly increased BW ($P < 0.05$), especially with the dose of 3 g/kg feed. Also, they observed that FCR values were significantly decreased ($P < 0.05$) in the FWGE treated groups at 35 days of age. In another study, Rafai *et al*. (2011) reported the incorporation of FWGE diet at 1 g/kg level improved the BWG of the pigs by 6% in average.

Saleh *et al*. (2014) reported that dietary supplementation of summer shield® (a herb mixture) significantly increased BWG, while FI was not affected at concentration of 1 g/kg or 2 g/kg, until 37 days of age, and the FCR decreased by supplementation of the herb mixture. They reported that this could be due to the growth-stimulating effects of some summer shield materials. Former studies showed that dietary supplementation of onion (Aji *et al*., 2011), and coriander (Hamodi *et al*., 2010) increase BW of broilers. It has also been reported that, plant materials such as spearmint increase FI and thus improve growth performance of broilers (Amasaib *et al*., 2013). But, feeding the NBS was not significantly effective on FI and FCR of broilers in the current study. It has been reported that medicinal plants modulate microflora of the gastrointestinal tract, consequently influence the growth performance of broiler chickens (Metzler *et al*., 2005). Kamel (2000), suggested that the improved growth performance and digestibility of broilers observed in most studies about herbal-supplemented diets may be due to the anti-fungal and antimicrobial effects of these substances.

In this study, a 3% reduction in dietary nutrients had no remarkable effect on broilers growth performance. Also, this reduction in nutrient levels has not been enough to significantly affect the performance of broiler chickens. Our results at the starter period were in agreement with findings of Hidalgo *et al*. (2004), Waldroup *et al*. (2005) and Kamran *et al*. (2008 a,b) who showed that BWG was decreased as dietary CP and ME were decreased. M Abdel-Hafeez *et al*. (2016) indicated that broiler chickens fed diets with normal ratio of ME to CP but with lower ME had lower feed efficiency and body weight than the broiler chicks fed with the control diet. However, broilers fed with normal CP and low ME diets and ratios close to the control group had approximately similar BW and FCR. Nkwocha *et al*. (2014) suggested that ME of broiler diets could be reduced to 2880 kcal/kg without affecting growth performance. The decrease in FI of the birds in low dietary nutrient level groups in this study was in agreement with the findings of Newcombe and summers (1985) who suggested that birds eat to almost full-gut capacity, so suggesting that appetite might be the main factor controlling FI of the broilers. M Abdel-Hafeez *et al*. (2016) showed that when the ME and CP decrease, the FCR was numerically increased, so decreasing the ME with normal ME to CP ratio should not be advised, while in this study, the reduction level of nutrients had no effect on FCR of chickens in different periods. Fetuga (1984) recommended a range of 23 - 24% CP and 2800 - 3000 kcal/kg ME in the starter and 19 - 21% CP with same ME level for the finisher periods of broilers.

Table 6. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on blood serum metabolites in broiler chickens at d 42[1].

Items (mg/dL)	Control Diets[2]		NBS Supplement[3]				*P*-value					*SEM*
	PC	NC	0.5	1	1.5	2	NBS vs PC	NBS vs NC	Linear	Quadratic	Anova	
Glucose	256.0	247.4	238.8	254.4	237.6	274.0	0.73	0.81	0.50	0.13	0.44	13.5
Triglyceride	63.4	61.0	65.6	59.2	54.2	61.4	0.56	0.88	0.41	0.80	0.69	5.0
Cholesterol	126.2	144.0	128.4	135.0	147.2	129.4	0.27	0.30	0.60	0.33	0.25	7.35
HDL-c	47.0	49.0	49.0	47.8	51.0	49.0	0.44	0.94	0.50	0.76	0.92	2.63
LDL-c	56.6	68.0	66.0	65.8	62.6	60.2	0.11	0.35	0.05	0.96	0.35	3.95
Uric acid	7.64	7.78	7.62	7.62	7.26	6.48	0.56	0.39	0.14	0.32	0.63	0.58

[1]Each mean represents five observations.
[2] PC= Positive control (standard diet), NC=Negative control (with a 3% reduction in nutrients)
[3] NBS Supplement: NBS0.5=Negative control + 0.5 g/kg, NBS1= Negative control + 1.0 g/kg, NBS1.5= Negative control + 1.5 g/kg, NBS2= Negative control + 2.0 g/kg (Nutrition Bio-Shield Superfood® (NBS) powder is a herbal product derived from wheat grains).
SEM: Standard error of the means.

Table 7. Effects of dietary supplementation of Nutrition Bio-Shield Superfood® on carcass parts and internal organs (% of live weight) in broiler chicks at d 42[1].

Carcass traits (% of live weight)	Control diets[2]		NBS Supplement[3]				*P*-value					*SEM*
	PC	NC	0.5	1	1.5	2	NBS vs PC	NBS vs NC	Linear	Quadratic	Anova	
Breast	21.77	22.38	21.86	21.89	21.05	22.80	0.98	0.40	0.84	0.52	0.64	0.75
Thighs	18.62	19.06	19.53	19.07	19.23	17.99	0.56	0.86	0.44	0.03	0.28	0.49
back and neck	15.79	14.41	15.89	15.90	15.02	16.04	0.85	0.01	0.41	0.54	0.10	0.46
Wings	6.09	5.53	5.62	5.64	5.54	5.29	0.009	0.97	0.01	0.62	0.08	0.18
Edible carcass	62.29	61.39	62.91	62.52	60.86	62.13	0.84	0.56	0.78	0.88	0.86	1.25
Bursa of Fabricius	0.14	0.16	0.16	0.17	0.22	0.14	0.29	0.57	0.28	0.18	0.20	0.02
Gizzard	1.75	1.74	1.96	1.69	1.87	1.70	0.55	0.49	0.78	0.14	0.08	0.07
Pancreas	0.27	0.27	0.26	0.26	0.25	0.27	0.78	0.81	0.81	0.69	0.98	0.02
Abdominal fat	1.39	1.52	1.18	1.48	1.33	1.60	0.65	0.14	0.50	0.29	0.43	0.13
Heart	0.51	0.53	0.54	0.49	0.50	0.52	0.94	0.66	0.67	0.84	0.86	0.02
Liver	2.17	2.52	2.21	2.36	2.37	2.35	0.22	0.14	0.53	0.56	0.31	0.11
Spleen	0.12	0.11	0.11	0.13	0.12	0.12	0.91	0.57	0.52	0.97	0.80	0.01

[1]Each mean represents five observations.
[2] PC= Positive control (standard diet), NC=Negative control (with a 3% reduction in nutrients)
[3] NBS Supplement: NBS0.5=Negative control + 0.5 g/kg, NBS1= Negative control + 1.0 g/kg, NBS1.5= Negative control + 1.5 g/kg, NBS2= Negative control + 2.0 g/kg (Nutrition Bio-Shield Superfood® (NBS) powder is a herbal product derived from wheat grains).
SEM: Standard error of means.

Immune response

In the current study, dietary supplementation of NBS had no significant effect on immune response in broilers. Ellakany *et al*. (2017) reported no significant differences between FWGE receiving birds and control regarding the RBCs and white blood cells (WBC) counts. In vitro and in vivo results have indicated that the cellular immunity improves in response to medicinal plants (Yakhkeshi *et al.*, 2001). Bayat *et al.* (2021) investigated the SRBC challenge on the WBCs count in mice. They reported that the mean number of WBCs in the groups receiving NBS powder at concentrations of 50, 100 and 150 mg/kg diet was significantly higher than the control group.

Saleh *et al.* (2014), indicated that feeding a herbal product enhanced the rates of the antioxidant enzyme glutathione peroxidase and immune-related parameters in broiler chickens. Dietary supplementation of FWGE (Stipkovits *et al*., 2004) or the use of NBS in our study may decrease the use of antibiotics. Also, wheat germ and NBS contain high vitamin E concentrations (Ellakany *et al.*, 2017; Bayat *et al.*, 2021). The antioxidative properties of vitamin E could protect body against free radicals produced in higher amount during stressful conditions. This factor prevents damage to body tissues and eliminates any infectious organism thus improving the body's defense mechanism. However, in this study, herbal compound (NBS) had no significant effect on immune response of broilers that may be due to many factors such as type, frequency (individual, intermittent or continuing) and level of usage, procedure of administration (water or feed), age, species, diet composition, field hygiene and surrounding stressors (stocking density, and ambient temperature).

Morphological parameters of the jejunum

Dietary supplementation of NBS had no significant effect on jejunum histology in broiler chickens. Among jejunum histomorphological traits, MLT was the only trait affected by the treatments in the current study. In agreement with current study results, Yakhkeshi *et al.* (2011) reported that herbal extract had no significant effect on the VH in ileum at 21 and 42 days of age. In addition, no significant differences were observed among treatments regarding to CD in duodenum, jejunum and ileum and VH: CD in duodenum and ileum at 21 and 42 days of age. Kósa *et al.* (2011) showed that the shortening of intestinal villus in the pigs fed with WGE supplemented diet was significantly milder regarding both duodenum and jejunum. Compared with the control group, the broiler chickens fed diet supplemented with FWGE had a significantly higher VH in the intestine on day 10, 21 and 42 of rearing period (Kósa *et al.*, 2011). They showed the incidence of villus atrophy accompanied by widening of the lamina propria, fusion of the villus, and leucocytic infiltration in the lamina propria were higher in the gut tract of control, indicating a lower favorable microbial environment in the intestinal contents. Wheat germ is rich in omega-3 fatty acids that can provide energy for intestinal villi growth, so its supplementation in diluted diets may help villi growth and development (Kósa *et al.*, 2011).

Blood metabolites

Dietary supplementation of NBS had no significant effect on glucose, triglycerides, cholesterol, LDL-C and uric acid concentrations in blood serum of broiler chickens compared to the control group. Ellakany *et al*. (2017) reported that with increasing the rate of supplement NBS in the broiler diet, the level of triglycerides decreased, but the level of glucose increased. In disagreement with these data, Saleh *et al*. (2014) indicated that summer shield supplementation improved plasma lipids profile by reducing total cholesterol, triglyceride, and LDL-C levels but increasing HDL-C concentration. M Abdel-Hafeez *et al.* (2016) showed that there were no significant differences between control and other groups with low ME diets in glucose, triglycerides, albumen, urea, and creatinine in broiler chicks. Also, Nkwocha *et al*. (2014) suggested that finisher broilers could withstand up to the level of 2880 kcal ME/kg decline with no deleterious effects on hematological characteristics. NBS contains phytosterols, substances that may decrease intestinal uptake of cholesterol via blocking its receptor (Trautwein *et al.*, 2003). This may reduce the harmful effects of cholesterol on the heart and arteries and the risk of heart attack.

Carcass traits

The NBS supplement did not influence the carcass traits in the current study. The present results agree with Lima *et al.* (2012) study in which, the yields of carcass and primal cuts of broilers were not significantly affected by additives such as whole corn germ (0 to 160 g/kg). It seems that sufficient amounts of amino acids and CP were obtained by feeding diluted diets and the treatments did not have a significant effect on carcass weight and characteristics. This indicates that half of the dietary protein in chickens is mainly deposited in breast meat (Garcia *et al*., 2007). Likewise, Lopes *et al*. (2019) observed that the yields of carcass, breast, and legs, abdominal fat deposition, and liver weight were not affected by whole corn germ.

Dalólio *et al.* (2015) reported that carcass yield and the cuts were not significantly different, when antibiotic alternatives were added to the birds' diet, as in the present study. On the contrary, in Albino *et al*. (2006) study, Probiotics increased breast yield, breast fillet and legs in broiler diet. Comparatively, da Rocha *et al*. (2010) reported higher breast yield in broilers received prebiotics, organic acids and probiotics in comparison to those received diets without additives. Also, Ellakany *et al*. (2017) reported that there was a significant effect on intestine, liver, gizzard, spleen, body fat, bursa of Fabricious weights in broiler chickens fed FWGE at 45 days of age. da Silva *et al*. (2011) did not find statistical differences in carcass traits of broilers, fed with diets supplemented with antibiotic and probiotic. Studies have shown that the use of antibiotic alternatives like probiotics as growth promoters can be used without adverse effects on animal performance (da Silva *et al*., 2011). It seems that in the present study, due to the hygienic conditions of rearing, low-stocking-density environment and proper management and diet, the effect of NBS supplementation is minimal and may be more effective in challenging conditions.

In the current study, reducing diet nutrients by 3%, did not significantly affect the carcass weight and internal organs of broilers. Dairo *et al.* (2010) observed a similar result by reducing ME and CP of diet on the carcass traits and internal organs. some researchers reported that carcass weight, percentage of carcass, breast, thigh and other organs such as heart, liver and abdominal fat were not affected by reducing ME and CP (Hai and Balha, 2000; Hidalgo *et al*., 2004). Also, M Abdel- Hafez et al. (2016) showed that different treatments had no significant effect on carcass traits either in normal ME:CP diets or in diets with a ratio close to the normal diets. It was reported that a balanced ME:CP ratio is important to achieve optimum carcass yield and meat quality in broilers. On the other hand, this might be due to a stable ME:CP ratio that was maintained across all the dietary treatments (Kidd *et al.,* 2004; Kamran *et al*., 2008a,b).

Conclusion

In conclusion, this experiment revealed that dietary supplementation of a herbal product, NBS, and lowering the ME and CP of diet by 3% had no significant effect on growth performance, humoral immune response, blood metabolites, jejunum histomorphology and carcass traits in broiler chickens.

References

Aji SB, Ignatius K, Ado AA, Nuhu JB, Abdulkarim A, Aliyu U, Gambo MB, Ibrahim MA, Abubakar H, Bukar MM, Imam AM & Numan PT. 2011. Effects of feeding onion (*Allium cepa*) and garlic (*Allium sativum*) on some performance characteristics of broiler chickens. Research Journal of Poultry Science, 4:22–27. DOI: 10.3923/rjpscience.2011.22.27

Albino LF, Feres FA, Dionizio MA, Rostagno HS, Vargas Júnior JG, Carvalho DC, Gomes PC & Costa CH. 2006. Uso de prebióticos à base de mananoligossacarídeo em rações para frangos de corte. Revista Brasileira de Zootecnia, 35:742-749. DOI: 10.1590/S1516-35982006000300015

Amasaib EO, Elrahman BH, Abdelhameed AA, Elmnan BA & Mahala AG. 2013. Effect of dietary levels of spearmint (Menthaspicata) on broiler chick's performance. Online Journal of Animal and Feed Research, 3:193–196.

Aviagen. 2014a. Ross broiler: management handbook. Huntsville (AL): Aviagen group.

Aviagen. 2014b. Ross broiler: nutrition specifications. Huntsville (AL): Aviagen group.

Bayat A, Khalkhali A & Mahjoub AR. 2021. Nutrition Bio-Shield Superfood: Healthy and live herbal supplement for immune system enhancement. International Journal of Food Science and Nutrition, 15: 6–9.

Broom LJ & Kogut MH. 2019. Deciphering desirable immune responses from disease models with resistant and susceptible chickens. Poultry Science, 98 (4): 1634 – 1642. DOI: 10.3382/ps/pey535

da Rocha AP, Abreu RD, Costa M, de Oliveira GJ, Albinati RC, da Paz AS, de Queiroz LG & Pedreira TM. 2010. Prebiotics, organics acids and probiotics in rations for broilers. Revista Brasileira de Saude e Producao Animal, 11:793–801.

da Silva WT, Nunes RV, Pozza PC, dos Santos Pozza MS, Appelt MD & Eyng C. 2011. Avaliação de inulina e probiótico para frangos de corte. Acta Scientiarum - Animal Sciences, 33: 19–24. DOI: 10.4025/actascianimsci.v33i1.9979

Dairo FA, Adesehinwa AO, Oluwasola TA & Oluyemi JA. 2010. High and low dietary energy and protein levels for broiler chickens. African Journal of Agricultural Research, 5: 2030–2038. DOI: 10.5897/AJAR10.254

Dalólio FS, Moreira J, Valadares LR, Nunes PB, Vaz DP, Pereira HJ, Pires AV & da Cruz PJ. 2015. Aditivos alternativos ao uso de antimicrobianos na alimentação de frangos de corte. Revista Brasileira de Agroecologia, 5. DOI: 10.21206/rbas.v5i1.281

Eftekhari SM, Hassanabadi A, Tahmasebi A, Golian A & Nassiri-Moghaddam H. 2018. The effect of hydroalcoholic extract of angelica (Heracleumpersicum) fruit on performance, immune responses, small intestine histology, haematological parameters and carcass characteristics of broiler chickens. Journal of applied animal research, 46:1336–1343. DOI: 10.1080/09712119.2018.1505621

Ellakany H, El-Sayed A, Soliman F & Elbestawy A. 2017. The effect of fermented wheat germ extract on biochemical, physiological and performance of broiler chickens. Alexandria Journal of Veterinary Sciences, 55: 91–97. DOI: 10.5455/ajvs.280407

El-Sheikh TM. 2002. Effect of strain, flock age and their interaction on hatched chick weight under different season. Egypt Poultry Science Journal. 22: 879–896.

Fetuga BL. 1984. Techniques in feed formulation. Paper presented at the feed mill management training workshop. Dept. of Agricultural Economics, University of Ibadan. April.

Garcia V, Catala-Gregori P, Hernandez F, Megias MD & Madrid J. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. Journal of Applied Animal Research, 16: 555–562. DOI: 10.3382/japr.2006-00116

Hafez HM & Attia YA. 2020. Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. Frontiers in Veterinary Science, 7: 516. DOI: 10.3389/fvets.2020.00516

Hai DT & Blaha J. 2000. Effect of low-protein diets adequate in levels of essential amino acids on broiler chicken performance. Czech Journal of Animal Science, 45: 429–436.

Hamodi SJ, Al-Mashhadani EH, Al-Jaff FK & Al-Mashhadani HE. 2010. Effect of coriander seed (*Coriandrum sativum* L.) as diet ingredient on broilers performance under high ambient temperature. International Journal of Poultry Science, 9: 968–971. DOI: 10.3923/ijps.2010.968.971

Hidalgo MA, Dozier WA, Davis AJ & Gordon RW. 2004. Live performance and meat yield responses to progressive concentrations of dietary energy at a constant metabolizable energy-to-crude protein ratio. Journal of Applied Poultry Research, 13:319–327. DOI: 10.1093/japr/13.2.319

Imari ZK, Hassanabadi A & Nassiri Moghaddam H. 2020. Response of broiler chickens to calcium and phosphorus restriction: effects on growth performance, carcass traits, tibia characteristics and total tract retention of nutrients. Italian Journal of Animal Science, 19:929–939. DOI: 10.1080/1828051X.2020.1808101

Kamel C. 2000. Natural plant extracts: Classical remedies bring modern animal production solutions. In: Proceedings of the III Conference of Feed Manufacturers of the Mediterranean: Feed Manufacturing in the Mediterranean Region Improving Safety: From Feed to Food. Brufau J (ed.), Institute Agronomique Mediterraneen de Zaragoza, Reus, Spain. 31–38.

Kamran Z, Sarwar M, Nisa M, Nadeem MA, Mahmood S, Babar ME & Ahmed S. 2008a. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poultry Science, 87: 468–474. DOI: 10.3382/ps.2007-00180

Kamran Z, Sarwara M, Nisa M, Nadeem MA, Mushtaq T, Ahmed T, Babar ME & Mushtaq MMH. 2008b. Effect of low levels of dietary protein on growth, protein utilization and body composition of broiler chicks from one to twenty-six days of age. Avian Biology Research, 1: 19–25. DOI: 10.3184/175815508X336639

Kidd MT, McDaniel CD, Branton SL, Miller ER, Boren BB & Fancher BI. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. Journal of Applied Poultry Research, 13: 593–604. DOI: 10.1093/japr/13.4.593

Kósa E, Glávits R, Ózsvári L, Jurkovich V & Brydl E. 2011. Effect of fermented wheat germ extract (FWGE) on the intestinal morphology. In Animal Hygiene and Sustainable Livestock Prod. pp. Poster Presentations, Vienna, Austria.

Lima MB, Rabello CB, Silva EP, Lima RB, Arruda EM & Albino LF. 2012. Effect of broiler chicken age on ileal digestibility of corn germ meal. Acta Scientific Veterinary Sciences, 34: 137-141. DOI: 10.4025/actascianimsci.v34i2.11812

Lopes EC, Rabello CB, Santos MJ, Lopes CD, Oliveira CR, Silva DA, Oliveira DP & Dutra WM. 2019. Performance and carcass characteristics of broilers fed whole corn germ. Brazilian Journal of Animal Science, 10: 48. DOI: 10.1590/rbz4820180247

Low CX, Tan LT, Ab Mutalib NS, Pusparajah P, Goh BH, Chan KG, Letchumanan V, Lee LH. 2021. Unveiling the impact of antibiotics and alternative methods for animal husbandry: A Review. Antibiotics (Basel). 10(5):578. DOI: 10.3390/antibiotics10050578

M Abdel-Hafeez H, SE Saleh E, S Tawfeek S, MI Youssef I & BM Hemida M. 2016. Effects of low dietary energy, with low and normal protein levels, on broiler performance and production characteristics. Journal of Veterinary Medicine and Research, 23: 259–274. DOI: 10.21608/JVMR.2016.43364

MacDonald JM. 2010. The economic organization of U.S. broiler production. Economic Information Bulletin No. 38. Economic Research Service, USDA. DOI: 10.22004/ag.econ.58627

Metzler B, Bauer E & Mosenthin R. 2005. Microflora management in the gastrointestinal tract of piglets. Asian-australas Journal of Animal Science, 9: 1353–1362. DOI: 10.5713/ajas.2005.1353

Newcombe M & Summers JD. 1985. Effect of increasing cellulose in diets fed as crumbles or mash on the food intake and WG of broiler and Leghorn Chicks. British Journal of Poultry Science, 26: 35–42. DOI: 10.1080/00071668508416784

Nkwocha GA, Agbabiaka LA, Anukam KU & Beketin TO. 2014. Growth response, carcass and blood characteristics of finisher broilers fed sorghum offal meal as dietary supplement. International Journal of Agricultural Science, 4: 392–398.

Rafai P, Papp Z, Jakab L, Tuboly T, Jurkovich V, Brydl E, Ózsvári L & Kósa E. 2011. The effect of fermented wheat germ extract on production parameters and immune status of growing pigs. Journal of Animal and Feed Sciences, 20: 36–46. DOI: 10.22358/jafs/66156/2011

Saleh AA, Ijiri D & Ohtsuka A. 2014. Effects of summer shield supplementation on growth performance, nutrient utilisation, and plasma lipid profiles in broiler chickens. Veterinary Medicine 59: 536–542. DOI: 10.17221/7818-VETMED

SAS (Statistical Analyses System). 2012. SAS/STAT Software, Version 9.4. Cary (NC): SAS Institute Inc.

Stipkovits L, Lapis K, Hidvegi M, Kosa E, Glavits R & Resetar A. 2004. Testing the efficacy of fermented wheat germ extract against mycoplasma gallisepticum infection of chickens. Poultry Science, 83: 1844–1848. DOI: 10.1093/ps/83.11.1844

Trautwein EA, Duchateau GS, Lin Y, Mel'nikov SM, Molhuizen HO & Ntanios FY. 2003. Proposed mechanisms of cholesterol-lowering action of plant sterols. European Journal of Lipid Science and Technology, 105: 171-185. DOI: 10.1002/ejlt.200390033

Waldroup PW, Jiang Q & Fritts CA. 2005. Effects of supplementing broiler diets low in crude protein with essential and nonessential amino acids. International Journal of Poultry Science, 4: 425–431. DOI: 10.3923/ijps.2005.425.431

Yakhkeshi S, Rahimi S & Gharib NK. 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. Journal of Medicinal Plants, 10: 37–53.

Zhu KX, Zhou HM & Qian HF. 2006. Proteins extracted from defatted wheat germ: nutritional and structural properties. Cereal Chemistry, 83: 69–75. DOI: 10.1094/CC-83-0069