Biotechnology in Animal Husbandry 37 (1), 45-64, 2021 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.087'65
https://doi.org/10.2298/BAH2101045S

EFFECT OF DIETARY INCLUSION OF *SILYBUM MARIANUM* OIL EXTRACTION BYPRODUCT ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND CECAL MICROBIAL POPULATION OF BROILER CHICKEN

Mehdi Shahsavan, Somayyeh Salari, Mohammadreza Ghorbani

Department of Animal Science, Food Science and Animal Science Faculty, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran. P.O. Box: 6341773637.
Corresponding author: Somayyeh Salari, somayehsallary@yahoo.com, S.Salari@asnrukh.ac.ir
Original scientific paper

Abstract: The aim of this study was to evaluate the effects of *Silybum marianum* oil extraction byproduct (SMOEB) supplementation on performance and some physiological parameters of broiler chickens. Treatments were consisted of different levels of SMOEB (0%, 3%, 6%, 9% and 12% of the diet). From day 8-21, dietary inclusion of 3%, 9% and 12% of SMOEB into the diet increased ($P<0.05$) feed intake and body weight gain of broilers. From day 22-42, increasing SMOEB level increased feed intake linearly. Increasing SMOEB level increased FI linearly and quadratically from day 8-42. On day 16, a higher value was recorded for wing-web thickness in birds that were received 9% of SMOEB in the diet only at 24h following the PHA-P injection ($P<0.05$). The higher value was observed for CBH response at 12 and 24h post-injection on day 21and day 35 in broilers were fed by 9% of SMOEB supplemented into the diet for toe web thickness. It can be concluded that SMOEB could be added to the diet of broilers without any adverse effect on performance parameters and also, it can improve immune parameters of birds at levels up to 12%.

Key words: Body weight, Broiler, By- product, Immunology, Microbiology

Introduction

Due to COVID-19 crisis and drastic changes in feed chain and feed supply, search for alternative feed supplies becomes urgently apparent and needs further research (*Hafez and Attia, 2020*). Also, poultry production has become more important for developing countries following increasing human population and demand for animal protein. According to *Longe (1986*), poultry production represents the fastest means for compensating the shortage of animal protein availability due to their fast rate of production and quick return on investment. Unfortunately, severe drought and shortage of water resources has increased the costs of conventional feedstuff production for animals in many developing countries including Iran. Therefore, low-cost by-product feeds are often used to decrease feed costs and improve farm profitability (*Rogers and Poore, 1994*). Chemical and physical properties of By-product can influence the production efficiency. For instance, the use of Agro-industrial by-products (AIBs) in animal feed can dramatically reduce the cost of feed (*Longe, 1986*). Considerable efforts have been made to improve the utilization of these AIBs in practical monogastric nutrition.

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs (*Fatehi et al., 2004*). The by-products provided through oil extraction process, which is a part of the medicinal plants production, can be used for animal nutrition.

Milk thistle (*Silybum marianum* L. Gaernt.), sometimes called wild artichoke, is a medicinal plant that has been used for thousands of years as a remedy for a variety of ailments (*Rainone, 2005*). Silybum marianum is used presently to treat alcoholic hepatitis, liver cirrhosis, liver poisoning, and viral hepatitis, and to protect the liver from side effects of medications (*Luper, 1998*). Standardized Silybum marianum extract is known as silymarin that is a combination of at least seven chemicals (*Muriel, 1992*) and it is a naturally occurring polyphenolic flavonoid (*Atanasoff et al., 2015*). Silymarin and its major constituent, silybin, have been reported to work as antioxidants scavenging free radicals and inhibiting lipid peroxidation. Some studies also suggest that they can protect against genomic injury, increase hepatocyte protein synthesis, decrease the activity of tumor promoters, stabilize mast cells, chelate iron, and slow down calcium metabolism (*Flora et al., 1998*). Flavonoids are a group of natural compounds known to have various pharmacological actions such as antioxydative, anti-inflammatory and diuretic (*Havesteen, 2002*). It has been reported that extracts of plants that are rich in flavonoids possess antimicrobial activity (*Tim and Andrew, 2005*). *Abed et al. (2015)* showed that the extracted flavonoids of Silybum marianum can act against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae*. In short, no study has been conducted in Iran to determine the optimal dietary inclusion level and the mode of action for these plant by-products in order to enhance the growth performance in poultry production. Therefore, the present study is aimed at investigating the efficacy of an oil extraction by-product of *Silybum marianum* on the performance, and some physiological parameters of broiler chickens.

Materials and Methods

All the procedures under taken in our study were approved by the Animal Ethics Committee at agricultural sciences and natural resources university of Khuzestan, Ahvaz, Iran.

Preparation and analysis of Silybum marianum oil extraction by-product

Silybum marianum oil extraction by-product (SMOEB) was prepared from the Barij Essence Pharmaceutical Company, Kashan, Iran. Four samples of SMOEB were prepared and were analyzed for dry matter (DM), crude protein (CP, crude fiber (CF), ether extract (EE), and ash according to AOAC (2002). Total phenolic and flavonoid content was analyzed in the study by *Senguttuvan et al., (2014).* The results are shown in Table 1.

Table 1. Chemical composition of *Silybum marianum* oil extraction byproduct (%)

DM	CP	EE	CF	Ash	Lignin	Total of Phenol*	Total of Flavonoid*	Tannin	ME[1]	NFE[2]
93	20	10	30	3.78	13.75	206.18	571.62	232.12**	1522.98	29.22

[1]Metabolizable energy value was calculated using an equation from NRC (cottonseed meal, expeller or solvent) (Janssen, 1989) (kcal/kg).
[2]Nitrogen free extract=100-(Moisture + CP + EE + CF + Ash)
*Values were analyzed according to micro/g DW
** Value was analysed according to mg/g DW

Birds, diets, and general procedures

One-day-old male chicks (Ross 308) were obtained from a local hatchery and were housed in floor pens, and were fed according to a standard broiler diet for week 1. Feed and water were provided *ad libitum* for their consumption. On day 8, totally 275 one-day-old male broiler chicks were weighed individually and were distributed randomly into 5 treatment groups, with 5 replicates (11 chicks) for each treatment group in a completely randomized design. Treatments were consisted of different levels of SMOEB (0%, 3%, 6%, 9% and 12% of diet). The feeding regimen was consisted of a starter (for day 8-21), and grower (for day 22-42) diet. The diets were formulated to meet the nutrient requirements of broilers according to *NRC (1994*). Mash feed and water were provided ad libitum throughout the experiment. The ingredients and chemical composition of the diets are shown in Tables 2 and 3. Feed was prepared weekly and was stored in airtight containers. Light intensity was kept on same level continuously for the first 3 days of post-hatching, after which a 23L:1D lighting schedule was maintained during the experiment. At day 1 of age, the temperature was set at 33°C and subsequently was reduced by 2°C/wk.

Table 2. Composition and nutrient contents of the basal diet and diets with increasing levels of *Silybum marianum* oil extraction byproduct (SMOEB) in the starter phase (d 8 to 21 posthatch)

	Levels of SMOEB (%)				
Ingredients (%)	0	3	6	9	12
Corn	58.69	56.84	54.85	51.63	48.61
Soybean meal (43% CP)	32.30	31.50	30.82	30.00	29.20
SMOEB	0.00	3.00	6.00	9.00	12.00
Wheat bran	1.76	0.87	0.00	0.00	0.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Soybean oil	0.70	1.20	1.70	2.70	3.52
Dicalcium phosphate	1.26	1.30	1.33	1.36	1.35
Limestone	1.24	1.23	1.23	1.23	1.23
Common salt	0.26	0.26	0.26	0.26	0.26
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15
Vitamin and mineral premix[3]	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.14	0.15	0.16	0.17	0.18
Calculated analysis (% unless stated otherwise)					
ME (kcal/kg)	2959	2959	2959	2959	2959
Crude protein	21.21	21.17	21.18	21.15	21.15
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	1.32	1.29	1.26	1.22	1.19
Calcium	1.00	1.00	1.00	1.00	1.00
Non-phytate P	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20

[1] Supplied per kilogram of diet: retinyl acetate, 1.55 mg; cholecalciferol, 0.025 mg; α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 10 mg; calcium pantothenate, 10 mg; choline chloride, 400 mg; nicotinamide, 50 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamin), 1.013 mg; Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg.

Table 3. Composition and nutrient contents of the basal diet and diets with increasing levels of *Silybum marianum* oil extraction byproduct (SMOEB) in the grower phase (d 22 to 42 posthatch)

	Levels of SMOEB (%)				
Ingredients (%)	0	3	6	9	12
Corn	65.41	62.39	59.33	56.23	53.38
Soybean meal (43% CP)	27.71	27.00	26.27	25.62	24.71
SMOEB	0.00	3.00	6.00	9.00	12.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Soybean oil	1.70	2.41	3.15	3.88	4.60
Dicalcium phosphate	0.90	0.90	0.95	0.95	0.95
Limestone	1.35	1.35	1.33	1.33	1.34
Common salt	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.17	0.17	0.17	0.17	0.17
Vitamin and mineral premix[3]	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.06	0.08	0.10	0.12	0.15
Calculated analysis (% unless stated otherwise)					
ME (kcal/kg)	3100	3100	3100	3100	3100
Crude protein	18.91	18.95	18.98	19.03	19.10
Methionine	0.38	0.38	0.38	0.38	0.38
Lysine	1.13	1.10	1.07	1.05	1.02
Calcium	0.90	0.90	0.90	0.90	0.90
Non-phytate P	0.35	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15	0.15

[1] Supplied per kilogram of diet: retinyl acetate, 1.55 mg; cholecalciferol, 0.025 mg; α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 10 mg; calcium pantothenate, 10 mg; choline chloride, 400 mg; nicotinamide, 50 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamin), 1.013 mg; Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg.

Performance

Weekly body weight gain (BWG) and feed intake (FI) of each pen were recorded. Feed conversion ratio was calculated by dividing FI with BWG.

Size of different organs

At the end of the experiment, 2 birds from each replicate (which were close to the mean BW of the replicate) were selected and slaughtered to evaluate the relative weights (based on BW) of the breast, thigh, pancreas, liver, gizzard, and abdominal fat. The length of intestinal segments including duodenum, from the pylorus to the distal portion of the duodenal loop; jejunum, the segment between the point of entry of the bile ducts and Meckel's diverticulum, ileum, from Meckel's diverticulum to the ileocecal junction and cecum (left and right) were also measured separately (*Sadeghi et al., 2015*).

Apparent ileal digestibility of nutrients

The chromic oxide (Cr_2O_3) marker method reported in the study by *Vries et al., (2014)* was used to measure ileal nutrient digestibility. Marker-containing diets (supplemented with 3 g/kg of Cr_2O_3) were given for 7 consecutive days, from day 36 to day 42 of the experimental period. At the end of study, 2 birds per replicate were killed by cervical dislocation and ileal contents, from Meckel's diverticulum to the ileocecal junction, and all were collected in sealed bags. The pooled digesta samples (on pen basis) were kept frozen at -20 ˚C until further analysis was performed. Feed samples and digesta were ground (using a 0.5 mm screen) prior to chemical analysis. The samples were analyzed for dry matter, organic matter, crude protein, and ether extract according to the standard procedures of *Association of Official Analytical Chemists (2002).* Chromium oxide content in the experimental diets and digesta was measured according to the study conducted by *Saha and Gilbreath (1991).* Digestibility coefficients were calculated using the following formula (*Hafeez et al., 2016*):

Eq. 1

$$\text{Apparent Digestibility}(\%) = 100 - [(\frac{\text{Cr2O3 diet}}{\text{Cr2O3 digesta}}) \times (\frac{\text{nutrient in digesta}}{\text{nutrient in feed}}) \times 100]$$

Gut microflora

On day 28, 2 birds per replicate and 10 birds per treatment were selected and cecal digesta (1 g) from each bird were transferred aseptically into 9 mL of sterile saline solution and were diluted serially. *Lactobacilli* was enumerated on De Man-Rogosa-Sharpe (MRS) agar, *E. coli* and *coli*- form were counted on Mac Conkey (MC) agar after incubation at 37˚C for 48 h in an anaerobic chamber, and for 24 h in an aerobic chamber, respectively (*Guban et al., 2006*). All samples were plated in duplicate.

Cellular immunology

In order to assess the *in vivo* cell-mediate immune response, we prepared a solution containing 1 mg/ml of phytohemagglutinin-P (PHA-P; Sigma Chemical Co., St. Louis, MO) by adding 1 ml of sterile PBS (0.15 M at pH = 7.4) to 1 mg of PHA using the septum. At 16 days of age, 8 birds per treatment (2 birds of each replicate) were selected. Then we injected 0.1 ml of PHA-P solution either intra- or sub-dermally into the right wing web of the elbow joint (or interdigitary skin or wattle) as well as 0.1 ml of sterile PBS (0.15 M at pH = 7.4) into the left wing web as a sham control. The thickness of the wing web was measured, using digital calipers (pressure-sensitive), to the nearest 0.05 mm immediately before and at 24 hour and 48 hours after injection. The mitogen stimulation index (SI) was calculated as follows: (*Grasman 2010*). Eq 2.

The mitogen stimulation index (SI) =
[(the increased thickness of right wing web) – (the increased thickness of left wing web)]

CBH response
The CBH response to phytohemagglutinin-P (PHA-P; Sigma Chemical Co., St. Louis, MO) was used to assess *in vivo* cell-mediated immune response. At 21, and 35 days of age, 8 birds per treatment received 100 μg of PHA-P in 0.1 mL of sterile PBS (0.15 M at pH = 7.4) which was injected intradermally in interdigital skin between the second and third digits of the right foot. 0.1 mL of PBS was injected into the left foot as a sham control. The thickness was measured, using digital calipers, to the nearest 0.05 mm immediately before and at 12, 24, and 48 hours post-injection, and the response was evaluated as follows: (*Akhlaghi et al., 2013*). Eq 3.
"Cutaneous basophilic hypersensitivity response = [(thickness of right toe web postinjection − thickness of right toe web preinjection) − (thickness of left toe web postinjection − thickness of left toe web preinjection)]

Lymphoid organs
At the end of the experiment, 2 birds from each replicate were slaughtered and the spleen and bursa of fabricius were weighed and calculated as a percentage of live BW.

Statistical analysis
Data were analyzed in a completely randomized design using General Linear Model procedures of SAS (*SAS Institute, 2001*). The following model was assumed in the analysis of all studied traits: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, (Eq.4)
Where Y_{ij} is the observed value for a particular character, μ is the overall mean, T_i is the effect of the i^{th} treatment, and ε_{ij} is the random error associated with ij^{th} recording. Data were analyzed considering the pen of birds as the experiment unit regarding performance parameters, and the individual chicken was measured as the experimental unit for the rest of the parameters. The mean values were compared using Duncan's multiple range test. Statistical significance was determined at $P<0.05$. Microbiological counts were subjected to base-10 logarithm transformation before analysis.

Results

Performance
Data representing the effect of dietary inclusion of SMOEB on performance of broilers are shown in Table 4. Mortality was lower than 3% with no differences

between the groups. From day 8 to day 21, dietary inclusion of 3%, 9% and 12% of SMOEB into the diet caused the increase (P<0.05) in feed intake (FI) and body weight gain (BWG) of broilers compared to those birds that were received 6% of SMOEB. From day 22 to day 42, FI was increased (P<0.05) by the inclusion of 12% of SMOEB into the diet compared to the other groups, while BWG was not influenced. The supplementation of SMOEB at the level of 12% into diet increased (P<0.05) FI compared to the birds receiving the control diet and the diets containing 3% and 6% of SMOEB from day 8 to day 42. Orthogonal contrast comparisons (Table 4) showed that FI quadratically decreased with increasing levels of SMOEB in d 22–42 (p = 0.022) and d 8–42 (p = 0.021). The FCR improved quadratically by SMOEB supplementation (p < 0.023) from d 8 to 42. However, there were no significant differences in feed conversion ratio (FCR) between treatments, during experimental growth phases, and over the whole period of experiment.

Table 4. Effect of treatments on growth performance traits of broilers at different phases

	Levels of SMOEB (%)							Contrast	
	0	3	6	9	12	SEM	P- Value	Linear	Quadratic
Starter phase (8 to 21 d)									
Feed intake (g/b)	853.8[ab]	880.3[a]	799.0[b]	878.6[a]	900.7[a]	21.10	0.034	0.187	0.073
Body weight gain (g/b)	430.6[ab]	445.8[a]	400.9[b]	440.4[a]	454.7[a]	11.70	0.046	0.266	0.078
Feed conversion ratio (g:g)	1.98	1.97	1.99	1.99	1.98	0.006	0.200	1.000	0.453
Grower phase (22 to 42 d)									
Feed intake (g/b)	2773.6[b]	2748.9[b]	2709.6[b]	2857.5[b]	3104.8[a]	76.677	0.016	0.006	0.022
Body weight gain (g/b)	1376.7	1450.1	1458.1	1471.0	1508.6	54.897	0.561	0.121	0.749
Feed conversion ratio (g:g)	2.01	1.89	1.85	1.94	2.05	0.085	0.424	0.736	0.067
Experiment overall (8 to 42 d)									
Feed intake (g/b)	3627.4[b]	3629.26[b]	3508.69[b]	3736.13[ab]	4005.57[a]	92.063	0.018	0.009	0.021
Body weight gain (g/b)	1807.4	1895.9	1859.0	1911.4	1963.3	63.944	0.524	0.126	0.947
Feed conversion ratio (g:g)	2.01	1.92	1.88	1.95	2.04	0.049	0.196	0.583	0.023

[a–b] Means with different superscripts within the same row differ significantly ($P \leq 0.05$)
SMOEB: *Silybum marianum* oil extraction byproduct

Size of different organs and length of intestine

The size of different organs is presented in Table 5. None of the dietary treatments led to remarkable changes in any of the organs (P>0.05). The length of the duodenum, jejunum, and cecum was increased in broilers fed by SMOEB at the levels of 6%, 9%, and 12% supplemented into the diet compared to control (P<0.05). The ileum length was enlarged significantly (P<0.05) at the levels of 9%

and 12% of SMOEB compared to the control group. Orthogonal contrast comparisons (Table 5) showed that length of duodenum, jejunum, ileum, and cecum linearly increased with increasing levels of SMOEB in the diet (p=0.0005, p=0.0006, p=0.001, p<0.0001, respectively).

Table 5. Effect of dietary treatments on relative organ weights (% of BW) and length of the intestine (cm) at 42 d of age in broilers

	Levels of SMOEB (%)							Contrast	
Size of different organs	0	3	6	9	12	SEM	P-value	Linear	Quadratic
Breast	21.85	21.68	23.36	21.88	22.34	0.542	0.256	0.484	0.362
Thighs	19.57	19.63	19.32	18.97	19.34	0.272	0.521	0.198	0.568
Pancreas	0.27	0.27	0.27	0.25	0.26	0.008	0.623	0.175	0.941
Liver	2.23	2.13	2.18	2.23	2.17	0.033	0.178	0.773	0.628
Gizzard	2.49	2.46	2.48	2.54	2.57	0.033	0.183	0.034	0.215
Abdominal Fat	1.60	1.50	1.501	1.82	1.92	0.147	0.184	0.052	0.197
Length of intestine									
Duodenum	27^{b}	29^{ab}	30^{a}	30^{a}	31^{a}	0.828	0.005	0.0005	0.155
Jejunum	72^{b}	79^{ab}	84^{a}	85^{a}	86^{a}	2.681	0.010	0.0006	0.110
Ileum	78^{c}	79^{bc}	89^{abc}	91^{ab}	94^{a}	4.076	0.022	0.001	0.795
Cecum	32^{b}	33^{b}	40^{a}	41^{a}	41^{a}	1.546	<0.001	<0.0001	0.145

$^{a–c}$ Means with different superscripts within the same row differ significantly ($P \leq 0.05$)
SMOEB: *Silybum marianum* oil extraction byproduct

Apparent ileal digestibility of nutrients

As shown in Table 6, apparent ileal digestibility of dry matter, organic matter, crude protein and ether extract were not influenced by the treatments. Orthogonal contrast comparisons (Table 6) showed that apparent ileal digestibility of organic matter increased quadratically by SMOEB supplementation (p=0.028).

Table 6. Effect of dietary treatments on apparent ileal digestibility of nutrients and cecal microbial population of broilers at d 42

	Levels of SMOEB (%)							Contrast	
Apparent ileal digestibility (%)	0	3	6	9	12	SEM	P-value	Linear	Quadratic
Dry Matter	77.1	77.3	77.4	77.1	76.9	0.240	0.601	0.422	0.184
Organic matter	79.5	79.9	80.0	79.8	79.5	0.159	0.231	0.724	0.028
Crude protein	72.8	72.0	72.5	72.4	72.6	0.239	0.337	0.965	0.189
Ether extract	63.1	62.6	62.9	62.8	63.0	0.144	0.216	0.960	0.068
Cecal microbial population (log CFU/g of digesta)									
Lactobacill	7.9	8.0	8.1	8.1	8.0	0.070	0.355	0.237	0.154
Coliform	7.8	7.6	7.8	7.9	7.9	0.153	0.635	0.267	0.445
E.Coli	7.8	6.9	6.8	7.1	7.1	0.357	0.348	0.331	0.153
Lactobacilli/E.coli	1.01	1.16	1.19	1.14	1.12	0.090	0.127	0.257	0.098
Total aerobes	7.7	8.0	7.9	8.1	8.1	0.130	0.176	0.078	0.459

SMOEB: *Silybum marianum* oil extraction byproduct

Intestinal bacterial colonization

The effects of SMOEB supplemented into diet on cecal microbial population of birds are shown in Table 6. Cecal population of *Lactobacillus*, *Coliform*, *E. coli*, Total aerobes and *Lactobacilli/E.coli* ratio were not influenced by the treatments (P>0.05).

Immunity

CBH response

Table 7 shows the CBH response in broiler chickens. On day 16, a higher value was recorded for wing web thickness in the birds that were received 9% of SMOEB in the diet only at 24hr following the PHA-P injection (P<0.05). Moreover, higher value of CBH response was observed in toe web thickness at 12 and 24hr post-injection on day 21 and day 35 in broilers that were fed by 9% of SMOEB supplemented into diet. The value of CBH response for wing web thickness quadratically increased (p=0.048) by increasing SMOEB levels in diet on day 16 only at 24hr following the PHA-P injection. On day 21, CBH response for toe web thickness at 12hr post-injection, linearly (p=0.016) and at 24hr (p=0.021) and 48hr (p=0.041) post-injection, quadratically, increased by supplementation of SMOEB in the diet. The CBH response quadratically affected by supplementation of SMOEB after 12hr (p=0.033) and 48hr (p=0.045) post-injection on day 35. The relative weight of bursa of fabricius was not influenced by the treatments (P>0.05; Table 7). However, higher relative weight of spleen was observed in broilers fed by 9% of SMOEB supplemented into diet compared to the other groups (P<0.05).

Table 7. Effects of dietary treatments on cutaneous basophilic hypersensitivity response to phytohemagglutinin-P (PHA-P) injection as well as the relative weight of lymphoid organs in broilers

	Levels of SMOEB[1] (%)							Contrast	
Immunity	0	3	6	9	12	SEM	P-value	Linear	Quadratic
Wing web thickness, mm (d 16)									
24h	0.20[b]	0.23[b]	0.23b	0.39[a]	0.17[b]	0.040	0.028	0.421	0.048
48h	0.19	0.21	0.21	0.32	0.16	0.052	0.343	0.840	0.225
Toe web thickness, mm (d 21)									
12h	0.27[b]	0.31[b]	0.37[ab]	0.45[a]	0.36[ab]	0.036	0.037	0.016	0.087
24h	0.23[c]	0.30[bc]	0.37[ab]	0.41[a]	0.33[abc]	0.031	0.032	0.014	0.021
48h	0.11	0.16	0.22	0.28	0.12	0.049	0.208	0.366	0.041
Toe web thickness, mm (d 35)									
12h	0.56[b]	0.57[b]	0.62[ab]	0.71[a]	0.55[b]	0.033	0.032	0.343	0.033
24h	0.45[b]	0.46[b]	0.49[b]	0.62[a]	0.36[b]	0.069	0.032	0.798	0.060
48h	0.21	0.37	0.37	0.41	0.28	0.063	0.254	0.365	0.045
Lymphoid organs [2] (d 42)									
Bursa of fabricius (%)	0.18	0.13	0.20	0.15	0.17	0.025	0.383	0.872	0.775
Spleen (%)	0.11[b]	0.11[b]	0.11[b]	0.15[a]	0.10[b]	0.012	0.045	0.866	0.178

[a–c] Means with different superscripts within the same row differ significantly ($P \leq 0.05$).
[1] SMOEB: *Silybum marianum* oil extraction byproduct
[2] Lymphoid organs are expressed as a percentage of live body weight

Discussion

Growth performance

To our knowledge, our study is the first study investigating the effect of various levels of SMOEB on broiler performance. The literature also lacks reliable experimental data regarding this topic. We showed that from day 8 to 21, the groups that were fed by 6%, 9% and 12% of SMOEB exhibit the highest FI and BWG and those that were fed by the diet containing 3% of SMOEB as well as the control diet had the lowest level regarding these parameters. Meanwhile, from day 22 to 42 and day 8 to 42, the birds that were fed by SMOEB at the level of 12% had highest FI and there was no significant difference in the BWG between treatments. The reason for this observation is presumably the high palatability caused in part by the high concentration of SMOEB (6%, 9% and 12% of the diet). Another reason for high values of FI could be related to the lignin content of SMOEB. As shown in Table 1, SMOEB contains 13.75% lignin, and thus any increase in the levels of SMOEB, can increase the lignin content of diets. This is also supported by the findings of *Ricke et al. (1982)* which they have observed the increase in FI in broilers caused by the dietary supplementation of lignin in the diet. Also, there are some reports indicated that low concentrations of tannins increased feed intake and thus increased performance of monogastric animals (*Huang et., 2018*). The high feed intake might have enhanced body weight gain of birds from day 8 to day 21. In our experiment, inclusion of SMOEB influenced feed consumption, but BWG and FCR were not influenced in whole period of experiment, suggesting that the primary effect of SMOEB can be on the feed intake. Similarly, Fani *Makki et al. (2013)* evaluated the ability of *Silybum marianum* seeds on performance of the broiler chickens contaminated with aflatoxin B1 (AFB_1) and they reported that *Silybum marianum* seeds increased FI and BWG, and improved FCR, however, AFB1 had a negative effect. *Gowda and Sastry (2000)* confirmed the improvement of BWG *caused by receiving Silybum marianum* seeds. They also attributed these effects to antioxidant activity that stimulated protein synthesis by the bird's enzymatic system. Perhaps the improvements in BWG of birds that were fed by high levels of SMOEB (6%, 9% and 12% of diet) from day 8 to day 21 is attributed to polyphenolic flavonoid compounds (*Atanasoff et al. 2015*) such as silymarin in SMOEB that has remained after processing by antioxidant activity. It can be pointed that flavonoid compounds like silymarin could have improved the body weight gain by influencing microbial population in digestive system (*Sťastnik et al. 2016*). Similarly, Tedesco *et al*. (2004) reported that the addition of *Silybum marianum* seeds to the broiler diets caused an improvement in body weight gain. In contrast to our findings, Suchy *et al*. (2008) reported that supplementation of *Silybum marianum* seed cakes at the levels of 0.2% and 1%, caused a decrease in BWG of broiler chickens. In the current study, both BWG and FCR were similar among birds were fed by the basal diet and the diet supplemented by different levels of SMOEB during the whole period of experiment. FCR is a measure representing how well a flock converts feed intake into weight gain. It is also the ability of the livestock to turn feed mass into body mass. Birds that have low feed conversion ratio are referred to as efficient food consumers. Presumably, due to the similar nature of SMOEB, corn, and soybean meal, broilers treated with these ingredients exhibited similar responses of BWG and FCR. As shown in Tables 2 and 3, SMOEB was replaced with corn and soybean meal in the control diet.

Size of different organs and length of intestine

Results shown in Table 5 indicate that relative weights of the breast, thigh, pancreas, liver, gizzard, and abdominal fat of broilers at 42 days were not influenced by dietary treatments. In contrast, Zahid & Durrani (2007) fed broilers by different levels of *Silybum marianum* seed, and found significantly higher breast weights at the level of 1.5 % of feed. *Fani makki et al. (2013)* investigated the interaction between different levels of *Silybum marianum* seeds (0.5% and 1%) and aflatoxin B_1 (0, 25 and 50 %) in broiler diet. They also reported that relative weight of thigh was not influenced by different levels of aflatoxin B_1 and *Silybum marianum* seeds. However, the highest weight of breast muscle was observed in birds fed by 1% of *Silybum marianum* seeds compared to the birds that were received different levels of aflatoxin B_1. *Schiavone et al. (2007)* reported that different levels of Silymarin (0, 40 and 80 mg/kg) influenced carcass and thigh yields of broilers negatively.

The reason for the increase in length of the duodenum, jejunum, ileum, and cecum on day 42 in chickens that were received high levels of SMOEB (9% and 12%) is presumably due to the high level of crude fiber (30%) in SMOEB as shown in Table 1. This result is supported by the findings of *Sadeghi et al. (2015),* who showed the length of the jejunum was increased in broilers fed by sugar beet pulp (3%) and sugar beet pulp/rice hull compared to those received rice hull (3%). Sugar beet pulp and sugar beet pulp/rice hull enlarged the ileum significantly compared to the other dietary treatments. The amount of crude fiber in sugar beet pulp and rice hull in that study was equal to 15.1% and 44.7%, respectively. In agreement with this, *Jiménez-Moreno et al. (2013)* reported longer intestines in chicks received sugar beet pulp compared to those fed by rice hull at6 days and 12 days of age. In contrast, *Saki et al. (2011)* demonstrated that supplementing a greater ratio of soluble fiber decreased ileum length at 14 days of age. The measurements in the present study confirm that intake of high fiber diets causes a significant increase in length of the caecum. This observation has been confirmed in previous studies on birds (*Savory & Gentle 1976; Savory 1992*), and also on other animal species such as rat (*Hansen et al. 1992; Zhao et al. 1995*) and pig (*Jorgensen et al. 1996*). These changes can influence the energy metabolism as visceral organs have a high rate of energy expenditure relative to their size (*Pekas and Wray 1991*).

Apparent ileal digestibility of nutrients

Depending on the dietary fiber levels, the diet passes the digestive tract at different speeds. This speed is a crucial digestion parameter (*Mateos et al. 2012*). The coefficients of total tract apparent digestibility of nutrients, such as the dietary metabolizable energy content, can be increased significantly if fiber is included in the diet *(Gonzalez-Alvarado et al. 2010)*. Also, several studies have reported that inclusion of moderate amounts of insoluble fiber sources in the diet can increase hydrochloric acid *(Jiménez-Moreno et al., 2010*), bile acids, and enzyme production (*Hetland et al., 2003*) and result in increased nutrient digestibility and growth performance of broilers fed by low-fiber diets (*Jiménez-Moreno et al., 2009 a, b; González-Alvarado et al., 2010*). In the current study, by increasing the level of SMOEB in the diet, crude fiber level of diet was increased, as shown in Table 1, SMOEB is contained of 30% of crude fiber. The difference in apparent ileal digestibility of nutrients was almost identical between diets that were supplemented by SMOEB and the control diet.

Intestinal bacterial colonization

It is important to consider that some feed additives originating from plant products have a profound effect on gut microflora either directly or indirectly *(Cowan 1999)*. In the current study, birds fed by the diets containing different levels of SMOEB showed a similar microbial population of cecum compared to those fed by control diet. Antimicrobial activity has been recognized as the major beneficial effect of phytogenics on animal production, although the exact antimicrobial mechanism has not been fully understood. Some *in vitro* studies demonstrated that flavonoids of *Silybum marianum* exhibited antimicrobial activity against intestinal microbes such as *Escherichia coli*, *Staphylococcus aureus, and Klebsiella pneumoniae* (*Abed et al., 2015*). Antimicrobial action of flavonoids is mediated by lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (*Helander et al., 1998*). But in the current study, although, SMOEB was contained of flavonoid (as shown in Table 1) but there was not significant alterations between treatments in terms of various species of microorganisms. On the other hand, in an *in vivo* study, it seems that the findings regarding the effect of flavonoids including *Silybum marianum* on gastrointestinal microflora are not consistent with the present study, even though *Silybum marianum* has been recognized generally as an antimicrobial agent. Therefore, it is speculated that the *in vivo* antimicrobial property of phytogenic in birds can be influenced by basal diet and environmental conditions.

Immunity

The PHA-P skin response test is an *in vivo* method for measuring T lymphocyte-mediated immunity. It measures the swelling caused by inflammatory leukocyte and fluid infiltration after an intradermal injection of PHA-P. Mitogens such as PHA-P are derived from lectins, which are considered as plant or bacterial proteins that bind to specific sugar components of glycoproteins attached to the surface of cells. PHA-P specifically binds to the surface of T lymphocytes. In the skin response test, PHA-P stimulates T lymphocytes to release lymphokines, resulting in an increase in vascular permeability and the influx in a variety of leukocytes. A large increase in skin thickness indicates a strong T cell-mediated immune response (*Grasman, 2010). Ezekowitz and Hoffman (1998*) demonstrated that spleen cells are consisted of T cells, B cells, and macrophages. When antigens intrude into the body, the spleen notifies T and B cells, thus stimulating cell-mediated immunity. In the current study, the levels of 6% and 9% of SMOEB increased the influx in a variety of leukocytes to injection site of PHA-P. In addition, it had been shown that certain non-genetic factors such as dietary nutrient concentrations can alter to some extent the expression of the genes responsible for immune responsiveness *(Rama Rao et al., 2003).* Also, cell-mediated immunity system of birds is consisted of cells with the ability of quick cleavage, which requires adequate nutrient material (*Vegad, 2002). Wang et al. (2008)* and *Brenes et al. (2008)* reported that plant extracts rich in polyphenols, as natural antioxidants are good candidates (with safety and toxicity effects), because they are obtained easily from natural sources and they can prevent lipid oxidation in food products efficiently. In the current study, the improvement in cellular immunity caused by supplementing high levels of SMOEB (especially 9% of the diet), can be partly due to polyphenolic flavonoid compounds such as silymarin in SMOEB which have remained after processing. As reported by *Basaga et al. (1997*) milk thistle can support the immune system through its powerful antioxidant, as well as by its direct effects on immune cells. *Chand et al. (2011)* showed that Silymarin, as antioxidant, has protective action against the oxidative damages on the immune organs (bursa of fabricius, spleen and thymus). Moreover, *Mitaru et al. (1984)* reported that the lignin content of the diets appears to have some adverse effects on protein and can bind to some of amino acids such as methionine. Also, *Rama Rao et al. (2003)* reported that methionine levels lower than 0.50 % in broiler diet would generate a poorer immune cell response compared to higher concentrations. In general, the level of requirement of methionine for increased immunity is higher rather than the one for growth. Therefore, the decline in cellular immunity in birds that were fed by 12% of SMOEB can be related to the lignin content of diets. This means the lignin content of diets, only in high levels of SMOEB (12%), could suppress the cellular immunity. Similarly, as shown in Table 1, in our experiment, SMOEB is contained of 13.75 % of lignin.

Al-Khalifa et al. (2012) reported that the thymus, spleen, and bursa of fabricius are the main immune organs in poultry. During an immune response, mature lymphocytes and other immune cells interact with antigens in these tissues. Consequently, in some cases immune tissue development can indicate immune system response. *Cazaban and Gardin (2012)* showed that in young birds the bursa of fabricius plays an important role in boosting immunity. In the current study, SMOEB did not influence the growth of the bursa of fabricius. But chickens fed by diets containing 9% of SMOEB had significantly greater spleen weights compared to chickens fed by 0, 3%, 6% and 12% of SMOEB. It is not clear that why the increased spleen weight was not maintained after feeding chickens by 9% of SMOEB supplemented in diet. *Fani Makki et al. (2013)* investigated the interaction between different levels of *Silybum marianum* seeds (0.5% and 1%) and aflatoxin B_1 (0, 250 and 500 mg/kg) in broilers diet, and found out that in diets containing AFB_1, bursa of fabricius weight was decreased significantly, whereas spleen weight did not change significantly by the treatments.

According to our results, SMOEB can be used in broiler chick diets at levels up to 12%. Also, not only the use of SMOEB as by-product in the diet had not negative effect in broiler performance, but also it improved immune parameters of birds.

Acknowledgement

Special thanks to the Agricultural Sciences and Natural Resources University of Khuzestan (Khuzestan, Iran).

Uticaj uključivanja u ishranu nusproizvoda ekstrakcije ulja *Silybum Marianum* na porast, imunološki odgovor i mikrobiološku populaciju develog creva brojlerskih pilića

Mehdi Shahsavan, Somayyeh Salari, Mohammadreza Ghorbani

Rezime

Cilj ovog istraživanja je bio da se procene efekti dodavanja u ishranu nusproizvoda ekstrakcije ulja *Silybum marianum* (SMOEB) na performanse i neke fiziološke parametre pilića brojlera. Tretmani su se sastojali od različitih nivoa SMOEB (0%, 3%, 6%, 9% i 12% obroka). Od 8. do 21. dana, uključivanje 3%, 9% i 12% SMOEB u ishranu povećalo je (P<0,05) unos hrane i prirast telesne mase brojlera. Od 22. do 42. dana, povećanjem nivoa SMOEB linearno se povećavao unos hrane. Povećavanjem nivoa SMOEB povećavao se FI linearno i kvadratno od 8. do 42. dana. Veća vrednost za debljinu mrežice krila zabeležena je 16. dana kod brojlera koji su primile 9% SMOEB u ishrani samo 24 sata nakon injekcije PHA-P (P <0,05). Viša vrednost zabeležena je za CBH odgovor 12 i 24 sata nakon injekcije, 21. i 35. dana kod brojlera koji su hranjeni obrokom sa 9% SMOEB-a za debljinu mrežice prstiju. Može se zaključiti da bi SMOEB mogao da se doda u ishranu brojlera bez ikakvog negativnog uticaja na parametre performansi, a takođe može da poboljša imunološke parametre brojlera na nivoima do 12%.

Ključne reči: telesna masa, brojler, nusproizvod, imunologija, mikrobiologija

Disclosure of interest
The authors report no conflict of interest.

References

ABED I. J., AL-MOULA R., GHUSOON A. A. (2015): Antibacterial effect of flavonoids extracted from seeds of *Silybum marianum* against common pathogenic bacteria. World Journal of Experimental Biosciences, 3, 36-39.

AKHLAGHI A., ZAMIRI M. J., JAFARI AHANGARI Y., ATASHI H., ANSARI PIRSARAEI Z., DELDAR H., EGHBALIAN A.N., AKHLAGHI A. A., NAVIDSHAD B., YUSSEFI KELARIKOLAEI K., HASHEMI S. R. (2013): Oral exposure of broiler breeder hens to extra thyroxine modulates early adaptive immune responses in progeny chicks. Poultry Science, 92, 1040–1049.

AL-KHALIFA H., GIVENS D. I., RYMER C., YAQOOB P. (2012): Effect of n-3 fatty acids on immune function in broiler chickens. Poultry Science, 91, 74–88.

AOAC (2002): Association of Official Analytical Chemists (AOAC), 18th ed. Official Methods of Analysis, Gaithersburg, MD.

ATANASOFF A., GIRGINOV D., ZAPRYANOVA D., NEDELKOV K., CAGILTAY F., SECER F. S. (2015): Effects of vitasil on proximate composition and some biochemical parameters of common carp (Cyprinus carpio L.). Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series 45, 7-12.

BASAGA H., POLI G., TEKKAYA C., ARAS I. (1997): Free radical scavenging and antioxidant properties of silibin complexes on microsomal lipid peroxidation. Cell Biochemistry and Function, 15, 27-33.
BRENES A., VIVEROS A., GON I., CENTENO C., SAYAGO-AYERDY S. G., ARIJA I., SAURA-CALIXTO F. (2008): Effect of grape pomace concentrate and Vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poultry Science, 87**,** 307-316.
CAZABAN C., GARDIN Y. (2012): Part1: Bursa of Fabricius is a visual indicator. World Poultry Magazine**,** 27**,** 30-31.
CHAND N., DIN MUHAMMAD F., DURRANI R., SUBHAN QURESHI M., SHAHIBZADA S. U. (2011): Protective effect of milk thistle (*Silybum marianum*) against aflatoxin B_1 in broiler chicks. Journal of Animal Science, 24, 1011-1018.
COWAN M, M. (1999): Plant products as antimicrobial products. Clinical Microbiology Reviews, 12, 564-582.
EZEKOWITZ R. A. B., HOFFMAN J. (1998): Innate immunity. Current Opinion Immunology, 10, 9-53.
FANI MAKKI O., AFZALI N., OMIDI A. (2013): Effect of different levels of Silymarin (*Silybum marianum*) on growth rate, carcass variables and liver morphology of broiler chickens contaminated with Aflatoxin B_1. Poultry Science, 1, 105-116.
FATEHI M., FARIFTEH F., FATEHI-HASSANABAD Z. (2004): Antispasmodic and hypotensive effects of Ferula assa foetida gum extract. Journal of Ethnopharmacology, 91, 321-324.
FLORA K., HAHN M., ROSEN H., BENNER K. (1998): Milk thistle (*Silybum marianum*) for the therapy of liver disease. The American Journal of Gastroenterology, 93,139-143.
GONZALEZ-ALVARADO J. M., JIMÉNEZ-MORENO E., GONZALEZ-SANCHEZ D., LAZARO R., MATEOS G. G. (2010): Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 d of age. Animal Feed Science and Technology 162, 37-46.
GOWDA S. K., SASTRY V. R. B. (2000): Neem (*Azadirachta indica*) seed cake in animal feeding-scope and limitation- Review. Asian-Australasian Journal of Animal Sciences 13, 720-728.
GRASMAN K. A. (2010): In vivo functional test for assessing immunotoxicity in birds (ED.), Immunotoxicity testing: Methods and Protocols, Methods in Molecular Biology (pp. 387-397) Humana Press, Product.
GUBAN J., KORVER D. R., ALLISON G. E., TANNOCK G.W. (2006): Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of Lactobacillus salivarius, and reduced bile salt deconjugation in the ileum of broiler chickens. Poultry Science 85, 2186–2194.
HAFEEZ A., MANNER K. M., SCHIEDER C., ZENTEK J. (2016): Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on

performance and nutrient digestibility in broiler chickens. Poultry Science 95, 622-629.

HAFEZ M. H., ATTIA Y. A. (2020): Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak, Frontiers in Veterinary Sciences.

HANSEN I., BACH KNUDSEN K. E., EGGUM B. O. (1992): Gastrointestinal implications in the rat of wheat bran, oat bran and pea fiber. British Journal of Nutrition 68, 451-462.

HAVSTEEN B. H. (2002): The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics 96**,** 67–202.

HELANDER I. M., ALAKOMI H. L., LATVA-KALA K., MATTILA-SANDHOLM T., POL I., SMID E. J., GORRIS L. G. M., Von Wright A. (1998): Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural and Food Chemistry 46, 3590-3595.

HETLAND H., SVIHUS B., KROGDAHL A. (2003): Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. British Poultry Science 44, 275–282.

HUANG Q., LIU X., ZHAO G., HU T., WANG Y. (2018): Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition 4, 137-150.

JIMÉNEZ-MORENO E., FRIKHA M., DE COCA-SINOVA A., L´AZARO R. P., MATEOS G. G. (2013): Oat hulls and sugar beet pulp in diets for broilers. 2. Effects on the development of the gastrointestinal tract and on the structure of the jejunal mucosa. Animal Feed Science and Technology 182, 44–52.

JIMÉNEZ-MORENO E., GONZALEZ-ALVARADO J. M., GONZALEZ-SANCHEZ D., LAZARO R., MATEOS G.G. (2010): Effects of type and particle size of dietary fibre on growth performance and digestive traits of broilers from 1 to 21 d of age. Poultry Science 89, 2197–2212.

JIMÉNEZ-MORENO E., GONZALEZ-ALVARADO J. M., GONZALEZ-SERRANO A., LAZARO R., MATEOS G.G. (2009a): Effect of dietary fibre and fat on performance and digestive traits of broilers from one to twenty-one d of age. Poultry Science 88, 2562–2574.

JIMÉNEZ-MORENO E., GONZALEZ-ALVARADO J. M., LAZARO R., MATEOS G. G. (2009b): Effects of type of cereal, heat processing of the cereal, and fibre inclusion in the diet on gizzard pH and nutrient utilization in broilers at different ages. Poultry Science 88, 1925–1933.

JORGENSEN H., ZHAO X. Q., KNUDSEN K. E. B., EGGUM B. (1996): The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. British Journal of Nutrition 75, 379-395.

LONGE O. (1986): Replacement value of biscuit waste for maize in broiler diets. Nigerian Journal of Animal Production 13, 70-78.

LUPER S. (1998): A review of plants used in the treatment of liver disease: Part 1. Alternative Medicine Review 3, 410-421.
MATEOS G. G., JIMÉNEZ-MORENO E., SERRANO M. P., LÁZARO R. P. (2012): Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. Journal of Applied Poultry Research 21, 156–174.
MITARU B. N., BLAIR R., REICHERT R. D., ROE W. E. (1984): Dark and yellow rapeseed hulls, soybean hulls and a purified fiber source: Their effects on dry matter, energy, protein and amino acid digestibilities in cannulated pigs. Journal of Animal Science 59, 1510-1518.
MURIEL P., GARCIAPINA T., PEREZ-ALVAREZ V., MOURELLE M. (1992): Silymarin protects against paracetamol induced lipid peroxidation and liver damage. Journal of Applied Toxicology 12, 439-442.
NRC (1994): Nutrient Requirement for Poultry, (9th ed.). Washington, DC: National Academy Press.
PEKAS J. C., WRAY J. E. (1991): Principal gastrointestinal variables associated with metabolic heat production in pigs: statistic cluster analyses. Journal of Nutrition 121, 231-239.
RAINONE F. (2005): Milk thistle. American Family Physician 72,1285–1288
RAMA RAO S. V., PRAHARAJ N. K., REDDY M. R. (2003): Interaction between genotype and dietary concentrations of methionine for immune function in commercial broilers. British Poultry Science 44, 102-112.
RICKE S. C., VAN DER. AAR P. J., FAHEY G. C. JR., BERGEN L. L. (1982): Influence of dietary fibers on performance and fermentation characteristics of gut contents from growing chicks. Poultry Science 61, 1335-1343.
ROGERS G. M., POORE M. H. (1994): Alternative feeds for reducing beef cow feed costs. Veterinary Medicine 89, 1073–1084.
SADEGHI A., TOGHYANI M., GHEISARI A. (2015): Effect of various fiber types and choice feeding of fiber on performance, gut development, humoral immunity, and fiber preference in broiler chicks. Poultry Science 94, 2734-2743.
SAHA D. C., GILBREATH R. L. (1991): Analytical recovery of chromium from diet and faeces determined by colorimetry and atomic absorption spectrophotometry. Journal of the Science of Food and Agriculture 55, 433–446.
SAKI A. A., MATIN H. R. H., ZAMANI P., TABATABAI M. M., VATANCHIAN M. (2011):Various ratios of pectin to cellulose affect intestinal morphology, DNA quantitation, and performance of broiler chickens. Livestock Science 139, 237–244.
SAS institute (2001): SAS User's Guide. Release 8.2 Ed. Cary, NC: SAS Institute Inc.
SAVORY C. J., GENTLE M. J. (1976): Changes in food intake and gut size in Japanese quail in response to manipulation of dietary fibre content. British Poultry Science 17, 571-580.
SAVORY C. J. (1992): Metabolic fates of U-^{14}C-labelled monosaccharides and an enzyme-treated cell-wall substrate in the fowl. British Journal of Nutrition 67, 103-114.

SCHIAVONE A., RIGHI F., QUARANTELLI A., BRUNI R., SERVENTI P., FUSARI A. (2007): Use of *Silybum marianum* fruit extract in broiler chicken nutrition: Influence on performance and meat quality. Animal Physiology and Animal Nutrition 91, 256-62.
SENGUTTUVAN J., PAULSAMY S., KARTHIKA K. (2014): Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pacific Journal of Tropical Biomedicine 4 (Suppl 1), 359-367.
ST'ASTNIK O., JUZL M., KARASEK F., STENCLOVA H., NEDOMOVA S., PAVLATA L., MRKVICOVA E., DOLEZAL P., JAROSOVA A. (2016): The effect of feeding Milk Thistle seed cakes on quality indicators of broiler chickens meat. Potravinarstvo® Scientific Journal for Food Industry. Potravinarstvo 10, 248-254.
SUCHY JR. P., STRAKOVA E., KUMMER V., HERZIG I., PISARIKOVA V., BLECHOVA R. (2008): Hepatoprotective effect of milk thistle (*Silybum marianum*) seed cakes during the chicken broiler fattening. Acta Veterinaria Brno 77, 31-38.
TEDESCO D., STEIDLER S., GALLETTI S., TAMENI M., SONZOGNI O., RAVAROTTO L. (2004): Efficacy of Silymarin-Phospholipid complex in reducing the toxicity of *aflatoxin* B_1 in broiler chicks. Poultry Science 83, 1839-1843.
TIM T. P., ANDREW J. L. (2005): Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26, 343–356.
VEGAD I. L. (2002): Role of nutrients in the avian immune response. Overcoming immunosuppression in poultry. Poultry Research Information and Scientist`s Meet.
VRIES S., KWAKKEL R. P., PUSTJENS A. M., KABEL M. A., HENDRIKS W. H., GERRITS W. J. (2014): Separation of digesta fractions complicates estimation of ileal digestibility using marker methods with Cr_2O_3 and cobalt-ethylenediamine tetraacetic acid in broiler chickens. Poultry Science 93, 2010-2017.
WANG L., PIAO X. L., KIM S. W., PIAO X. S., SHEN Y. B., LEE H. S. (2008): Effects of *Forsythia* suspensa extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. Poultry Science 87, 1287-1294.
ZAHID R., DURRANI F. R. (2007): Biochemical, hematological, immunological and growth promotant role of feed added milk thistle (*silybum marianum*) in broiler chicks. MSC. (hons) Thesis submitted to The University of Agriculture, Peshawar, Pakistan.
ZHAO X., JORGENSEN H., EGGUM B. (1995): The influence of dietary fibre on body composition, visceral organ weight, digestibility and energy balance in rats housed in different thermal environments. British Journal of Nutrition 13, 687-699.

Received 20 January 2021; accepted for publication 17 March 2021