Evaluation of the nutritional value of sunflower meal and its effect on performance, digestive enzyme activity, organ weight, and histological alterations of the intestinal villi of broiler chickens

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Primary Audience: Nutritionists, Researchers

SUMMARY

Two experiments were conducted to evaluate the AME of sunflower meal (SFM) and the use of various levels of SFM on the performance, digestive enzyme activity, organ weight, and histological alterations of intestinal villi of broilers chickens. In experiment 1, the AME_n of SFM was determined. The extrapolation value for the AME_n of SFM at 100% inclusion was 1,219 kcal/kg. In experiment 2, the effects of various levels of SFM (0, 70, 140, and 210 g/kg) on chick performance, blood parameters, digestive enzyme activities, and morphological measurements of intestinal villi were tested. Body weight gain, feed intake, and FCR were improved ($P < 0.05$) when up to 140 g of SFM was used. However, 210 g of SFM had a negative effect on performance ($P < 0.05$). Relative weights (g of organ/kg of BW) of the gastrointestinal tract and gizzard increased ($P < 0.05$). The activities of digestive enzyme (protease and α-amylase) were not influenced by treatment. Among the blood parameters, concentration of high-density lipoprotein increased significantly and that of low-density lipoprotein decreased ($P < 0.05$). Villus height was decreased and crypt depth was increased in both the duodenum and jejunum with increasing levels of SFM ($P < 0.05$). Therefore, up to 140 g of SFM/kg of diet can be used in broiler diets without adverse effects on performance or other parameters.

Key words: blood parameter, broiler, digestive enzyme, histology, metabolizable energy, sunflower meal

2012 J. Appl. Poult. Res. 21:293–304
http://dx.doi.org/10.3382/japr.2011-00396

DESCRIPTION OF PROBLEM

Sunflower (*Helianthus annuus*) is an oilseed cultivated worldwide for oil extraction because of its great capability for adaptation to different climate and soil conditions [1]. The by-product rendered by the oil industry, sunflower meal (**SFM**), is used as an alternative source of

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protein in animal and poultry nutrition. Its CP content, which ranges from 29 to 45%, depends on the dehulling and oil extraction process, in inverse relation to the fiber content (32 to 14% of CF). The use of SFM in broiler diets has been restricted to less than 150 g/kg because of its high fiber, low energy, and lysine contents [2, 3]. Several authors have shown that supplementing limiting amino acids and oil to SFM-based diets can increase SFM inclusion levels up to 200 to 350 g/kg without affecting broiler performance [4–7]. Arija et al. [8] and Suresh et al. [9] reported no adverse effects from including up to 50 and 120 g/kg, respectively, of sunflower seed hulls in broiler diets. Therefore, on the basis of data from the literature, it is possible to incorporate greater concentrations of SFM in broiler diets. Because many studies have resulted in contradictory conclusions and, to our knowledge, no information is available on the use of SFM on digestive enzyme activities and histological alterations of the intestinal villi of broiler chickens, the current study was designed with the following objectives: 1) to determine the ME content of SFM (cultivated in north of Iran) and 2) to evaluate the effects of increasing levels of SFM on performance, blood parameters, carcass traits, digestive enzyme activities, and histological alterations of the intestinal villi of broiler chickens

MATERIALS AND METHODS

A batch of local SFM (cultivated in the north of Iran) was obtained from a commercial supplier and used in both experiments. The test material was analyzed in duplicate for CP (N × 6.25), CF, and EE by AOAC procedures [10]. For DM determination, samples were dried in a forced-air oven at 105°C for 6 h. Dietary and fecal gross energy for experiment 1 were determined by adiabatic bomb calorimetry [11], using benzoic acid as an internal standard. These bird studies were performed using protocols approved by the Animal Care Committee of the Ferdowsi University of Mashhad.

Experiment 1

This experiment was conducted to determine the AME_n value of SFM with a multilevel assay that included 3 dietary inclusion levels. A maize-soybean meal basal diet (Table 1) was prepared in mash form and formulated to meet the nutrient requirements of broiler chickens (2 to 3 wk of age) recommended by the NRC [12]. The SFM was incorporated into the basal diets at 3 concentrations (70, 140, and 210 g/kg). The 4 experimental diets, which contained 3 g of chromium oxide/kg as an indigestible marker, were evaluated in a balance trial to determine the AME content.

One-day-old male chicks (Ross 308) were housed in floor pens, exposed to light for 24 h/d, and fed a standard broiler diet for 2 wk. Feed and water were provided for ad libitum consumption. At 1 d of age, the temperature was set at 33°C and then reduced by 2°C/wk. On d 10, 80 birds were placed at random in 16 cages (5 birds in each cage), with 4 replicates per dietary treatment. On d 15, feed was withheld for 4 h, and the birds then received their respective experimental diets from 15 to 21 d of age. During the last 3 d, excreta samples from each cage were collected and stored at −20°C. After being thawed, excreta were homogenized, dried, and ground through a hammer mill provided with a 1-mm screen. Diets and excreta were analyzed for DM, CP, chromium oxide, and gross energy.

Experiment 2

The objective of this experiment was to study the effects of various levels of SFM on performance, blood parameters, carcass characteristics, and histological alterations of broilers. Four isocaloric and isonitrogenous broiler starter and finisher diets were formulated to contain 0, 70, 140, and 210 g of SFM/kg (Tables 2 and 3). The starter diets (2,900 kcal/kg of ME; 208.6 g of CP/kg) were fed from 0 to 3 wk. From 4 to 7 wk, the finisher diets (3,000 kcal/kg of ME; 187.5 g of CP/kg) were used. All the diets were calculated to meet the requirements of broiler chicks recommended by the NRC [12]. A total of 176 one-day-old male commercial broiler chicks (Ross 308) were individually weighed. Chicks were weight sorted and randomly distributed into 4 treatments, with 4 replicates (11 chicks) for each treatment. Lights were on continuously for the first 3 d posthatching, after which a 23L:1D lighting schedule was maintained for

Table 1. Composition and nutritive content of the basal diet and diets with increasing levels of sunflower meal (SFM), experiment 1

	Level of SFM in diet, %			
Item	0	7	14	21
Ingredient, % of diet (as fed)				
Maize	61.95	57.43	52.9	48.37
Soybean meal (43% CP)	33.88	31.40	28.93	26.46
SFM	—	7	14	21
Sodium chloride	0.4	0.4	0.4	0.4
Limestone	1.47	1.47	1.47	1.47
Dicalcium phosphate	1.35	1.35	1.35	1.35
DL-Methionine	0.15	0.15	0.15	0.15
Chromium oxide	0.3	0.3	0.3	0.3
Vitamin-mineral premix[1]	0.5	0.5	0.5	0.5
Calculated nutritive value, %				
ME, kcal/kg	2,937	2,858	2,770	2,680
CP	20.11	20.74	21.38	22.02
Lysine	1.02	1.02	1.01	1.01
Methionine + cysteine	0.95	0.89	0.94	0.99
Threonine	0.8	0.8	0.99	1.08
Tryptophan	0.29	0.32	0.35	0.37

[1]Vitamin-mineral premix provided the following per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 2,500 IU; vitamin E, 18.75 mg; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; riboflavin, 6 mg; vitamin B_{12}, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg; copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg; salinomycin, 60 mg; chlortetracycline, 0.1 g; choline chloride, 2.0 g; ethoxyquin, 0.3 g.

the duration of the experiment. Feed and water were provided ad libitum. At 1 d of age, the temperature was set at 33°C and subsequently reduced by 2°C/wk.

Weekly BW gain (**BWG**) and feed intake (**FI**) of each pen were recorded. At 28 d of age, 4 birds per treatment (1 from each replicate) were randomly selected and killed by cervical dislocation, and blood was collected by heart puncture. Serum was separated and analyzed for concentrations of high-density lipoprotein (**HDL**), low-density lipoprotein (**LDL**) [13], triglycerides [14], total serum protein [15], and calcium [10]. The inorganic phosphorus concentration of serum was measured using the phosphomolybdic acid method [16]. Serum was also analyzed for determination of alkaline phosphatase by using the protocol provided by the kit manufacturer [17].

Digestive enzyme activities (protease and α-amylase) were determined in the ileal digesta of broiler chicks at 4 wk of age (1 bird from each replicate). The ileum from Meckel's diverticulum to 4 cm above the ileocecal junction was dissected, and the contents were aseptically collected in screw-capped sterile specimen vials and placed in a freezer at −20°C until required. Four hundred milligrams of ileal content was quickly weighed into test tubes kept on ice, and 6 mL of ice-cold physiological saline (9 g of sodium chloride/L) was added and centrifuged at 2,000 × *g* for 10 min at 4°C. Portions of the supernatant fractions containing enzymes were assayed for protease and amylase activities according to the procedure of Najafi et al. [18, 19].

In addition, at 28 d of age, 1 bird from each replicate was randomly selected and killed by cervical dislocation, and the following segments were removed: 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; and ileum, the segment from Meckel's diverticulum to the ileocecal junction, 10 cm of the first section of which was taken for microscopy. The samples were flushed with physiological saline and fixed in 10% formalin. Cross sections for each intestinal sample (10 villi per bird per segment) were prepared after staining with hematoxylin and eosin by using standard paraffin embedding procedures [20]. Villus height was measured from the tip of the villus to the villus-crypt junction;

Table 2. Composition and nutrient contents of the basal diet and diets with increasing levels of sunflower meal (SFM) in the starter phase (d 1 to 21 posthatch), experiment 2

	Level of SFM in diet, %			
Item	0	7	14	21
Ingredient, % of diet (as fed)				
Maize	61.26	59	54.25	50
Soybean meal (43% CP)	34.3	26.46	22.11	17.26
SFM	—	7	14	21
Corn gluten meal (60% CP)	—	3	3	3
Animal-vegetable oil	0	0	2	4
Limestone	1.47	1.47	1.47	1.47
Dicalcium phosphate	1.8	1.7	1.7	1.7
Sodium chloride	0.47	0.47	0.47	0.47
Vitamin-mineral premix[1]	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	—	0.2	0.3	0.4
Calculated nutritive value, %				
ME, kcal/kg	2,900	2,900	2,900	2,900
CP	20.86	20.86	20.86	20.86
Ether extract	2.6	2.7	4.64	6.64
CF	2.69	4.05	5.45	6.85
Calcium	1.05	1.02	1.02	1.02
Available phosphorus	0.47	0.44	0.44	0.44
Sodium	0.2	0.2	0.2	0.2
Lysine	1.24	1.23	1.23	1.23
Methionine + cysteine	0.9	0.91	0.92	1.01
Threonine	0.8	0.87	0.93	0.99
Tryptophan	0.25	0.29	0.30	0.32

[1]Vitamin-mineral premix provided the following per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 2,500 IU; vitamin E, 18.75 mg; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; riboflavin, 6 mg; vitamin B_{12}, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg; copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg; salinomycin, 60 mg; chlortetracycline, 0.1 g; choline chloride, 2.0 g; ethoxyquin, 0.3 g.

crypt depth was defined as the depth of the invagination between adjacent villi. Villus width was measured at the bottom of the villi. The slides were evaluated using an Olympus microscope coupled to a camera (20×) [21] and computer image analysis software for morphometric research [21]. At the end of the experiment, 1 bird from each replicate (close to the mean BW of the replicate) was selected and slaughtered to study the relative weights (based on BW) of the liver, abdominal fat, gizzard, thigh, breast, and gastrointestinal tract (from the beak to the cloaca).

Calculations and Statistical Analysis

The AME was calculated as follows [22]: ME (kcal/kg) = dietary gross energy × [1 − (diet chromium oxide/excreta chromium oxide) × (excreta gross energy/diet gross energy)]. The correction of AME to zero nitrogen retention (AME_n) was based on a factor of 8.22 kcal/g of retained nitrogen. The AME_n value of SFM was calculated using the following equation: $AME_n = (AME_n\ T - \alpha \times AME_n\ B)/b$, where T is the test diet, α is the proportion of the basal diet in the test diets, B is the basal diet, and b is the proportion of SFM in the test diets [23].

Statistical analyses were performed using the GLM procedures of SAS [24]. Data generated from experiment 1 were subjected to ANOVA to identify variation produced by the inclusion level of SFM; regression analysis was also used to establish dietary changes as a function of the inclusion level of SFM. Experiment 2 was conducted using a completely randomized design. These data were subjected to ANOVA according to the GLM procedure of SAS [24]. Orthogonal polynomial contrast statements were used to test for linear or quadratic dietary energy effects. A significance level of $P \leq 0.05$ was used during analysis.

Table 3. Composition and nutrient contents of the basal diet and diets with increasing levels of sunflower meal (SFM) in the finisher phase (d 22 to 42 posthatch), experiment 2

	Level of SFM in diet, %			
Item	0	7	14	21
Ingredient, % of diet (as fed)				
Maize	66.92	65.70	60.5	56
Soybean meal (43% CP)	29.3	20.5	16.60	12
SFM	—	7	14	21
Corn gluten meal (60% CP)	—	3	3	3
Animal-vegetable oil		0	2	4
Limestone	1.4	1.35	1.35	1.35
Dicalcium phosphate	1.45	1.4	1.4	1.4
Sodium chloride	0.35	0.35	0.35	0.35
Vitamin-mineral premix[1]	0.5	0.5	0.5	0.5
DL-Methionine	0.08	0.05	0.05	0.05
L-Lysine	—	0.15	0.25	0.35
Nutrient composition, %				
ME, kcal/kg	3,000	3,000	3,000	3,000
CP	18.75	18.75	18.75	18.75
Ether extract	2.78	2.91	4.86	6.82
CF	2.61	3.96	5.38	6.78
Calcium	0.93	0.9	0.9	0.9
Available phosphorus	0.4	0.38	0.38	0.37
Sodium	0.15	0.15	0.15	0.15
Lysine	1.09	1.02	1.03	1.02
Methionine + cysteine	0.73	0.75	0.78	0.80
Threonine	0.74	0.78	0.84	0.90
Tryptophan	0.25	0.26	0.27	0.28

[1]Vitamin-mineral premix provided the following per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 2500 IU; vitamin E, 18.75 mg; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; riboflavin, 6 mg; vitamin B_{12}, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg; copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg; salinomycin, 60 mg; chlortetracycline, 0.1 g; choline chloride, 2.0 g; ethoxyquin, 0.3 g.

RESULTS AND DISCUSSION

The nutrient composition of the SFM used in this study is presented in Table 4. Values for CP and EE were similar to those reported by Zatari and Sell [25], whereas CF content was higher, probably because of the processing conditions during the extraction procedure. Additionally, hull content, preconditioning, dehulling, cooking, and solvent extractions determined the subsequent nutritive value of SFM.

AME

Table 5 shows AME_n data (kcal/kg) for the experimental diets. An increasing inclusion rate of SFM decreased the AME_n of the diets. To further assess this trend, the dietary AME_n values were regressed against the inclusion level of SFM by using linear and quadratic models. The linear component was highly significant, whereas the quadratic component was not significant. Therefore, the energy contribution of SFM to the diets was additive, and the inclusion rate did not alter the use of other dietary ingredients. By using the AME_n values determined for the basal diet and the basal diet containing a given amount of SFM, the AME_n (kcal/kg) of this feed was calculated by the difference (Table 5). The AME_n values obtained for diets in the experi-

Table 4. Determined analysis of sunflower meal[1] (as-fed basis)

Chemical composition	Amount, %
Moisture	12
CP	30
Ether extract	2.5
CF	21.2
Calcium	0.21
Gross energy, kcal/kg	4,104

[1]Values are the means of 2 samples tested.

ment reported here were regressed on level of SFM in the basal diet to estimate the AME_n content in SFM. The equation derived from fitting a linear model was as follows: $y = 2{,}957 - 1.735x$; $R^2 = 0.736$ ($P < 0.05$). An estimate of the AME_n of SFM was obtained by extrapolation from the equation, where 1,000 g/kg of SFM in the diet gave a value of 1,219 kcal/kg. The low value of AME_n of SFM in this study may be related to CF content. The cell walls of grains and oilseeds can serve as a physical barrier for digestive enzymes and nutrients contained within the cells and can either prevent entirely or delay digestion of nutrients in the last part of the duodenum [26]. Not only the total fiber content, but also the physical and chemical structure of fibrous polysaccharides and their anatomical arrangement within each specific ingredient can affect the accessibility of enzymes for digestion of nutrients. Protein and many other nutrients are "encapsulated" to variable degrees inside fibrous structures, and they remain less available for digestion by the proteases and other endogenous enzymes of the bird. These effects may decrease the AME_n value of seed meals. In our experiment, the CF of the diets increased with an increasing level of SFM; thus, the AME_n value of the diets decreased. Mandal et al. [27] determined values of 1,458, 1,458, and 1,481 kcal/kg of AME_n in SFM for cockerels, guinea fowl, and quail, respectively.

Growth Performance

The FI increased quadratically ($P < 0.01$) with increasing levels of dietary SFM during the grower (22- to 42-d) and finisher (43- to 49-d) periods as well as overall (Table 6). In addition, FI tended to increase quadratically ($P = 0.0673$) at the starter phase with the treatments. The BWG responded quadratically ($P < 0.01$) with increasing levels of dietary SFM (Table 6). In the grower and finisher phases, FCR improved quadratically with increasing levels of SFM ($P < 0.01$). The improved performance of broilers fed lower levels of SFM compared with the performance of control birds was related to cellulose content of the diet. It has been shown that SFM has 220 g/kg of cellulose [28]. These results are in accordance with the results of Rama Rao et al. [29], who showed that FI was significantly higher in broilers fed 170 and 340 g of SFM/kg of diet compared with those fed the basal diet, and BWG was significantly higher for chickens fed 340 g of SFM/kg of the diet. They also determined that total replacement of soybean meal with SFM resulted in similar FI but a significant decrease in BWG. This is in agreement with the results of Ibrahim and Zubeir [5], who reported that a high-fiber (230.5 g of CF) SFM could be included at up to 300 g/kg of broiler diet with no adverse effects on growth rate or FE. This confirms the findings of Jacob et al. [30], who showed that replacing a portion (80 g/kg) of imported soybean meal in broiler diets with SFM had no significant effect on growth rate or FE. Zatari and Sell [25] reported that up to 100 g/kg of SFM can be used in diets without adversely affecting growth or FCR up to 7 wk of age in broiler chickens. In addition, Elangovan et al. [31] showed that BWG, FI, nutrient retention, and carcass characteristics of quails did not vary significantly ($P > 0.05$) when SFM was increased in the diets.

Digestive Enzyme Activity

The activities of neither protease nor α-amylase in chick digesta were significantly affected (Table 7). In the literature reviewed, we found no information on the determination of digestive enzyme activities of broilers fed SFM. In our experiment, only α-amylase activ-

Table 5. Nitrogen-corrected AME[1] of diets with increasing levels of sunflower meal (SFM), and of SFM determined by difference and regression analysis, experiment 1

Item	AME_n of diets, kcal/kg	AME_n of SFM, kcal/kg
Level of SFM, g/kg		
0	2,995[a]	—
70	2,792[b]	1,879.16
140	2,687[c]	1,687.08
210	2,625[d]	1,827.81
SEM	39.42	

[a–d]Values with a common letter do not differ significantly ($P < 0.05$).

[1]AME_n determinations were made based on 4 cages of 5 birds each per treatment. Linear regression equation: $y = 2957 - 1.735x$; $R^2 = 0.736$, where y is AME_n (kcal/kg) and x is dietary inclusion level of SFM (g/kg).

Table 6. Effect of sunflower meal (SFM) on performance parameters of chickens (1 to 49 d of age), experiment 2[1]

	FI, g/bird				BWG, g/bird				FCR			
Item	1 to 21 d	22 to 42 d	43 to 49 d	1 to 49 d	1 to 21 d	22 to 42 d	43 to 49 d	1 to 49 d	1 to 21 d	22 to 42 d	43 to 49 d	1 to 49 d
SFM, %												
0	879.1	2,807.5	1,128.7	4,829.1	465.7	1,180.1	488.7	2,027.5	1.88	2.37	2.30	2.38
7	943.8	2,938.3	1,231.0	5,025.8	513.5	1,282.7	521.4	2,288.2	1.83	2.29	2.37	2.20
14	955.7	3,011.1	1,308.4	5,291.3	528.2	1,382.4	556.4	2,471.9	1.81	2.18	2.35	2.14
21	908.3	2,799.5	1,158.8	4,981.3	507.3	1,275.7	497.9	2,273.3	1.79	2.19	2.53	2.19
SEM	27.87	48.52	19.86	66.47	9.17	26.81	17.53	43.43	0.051	0.044	0.069	0.039
					P-value							
Orthogonal contrast												
Linear	NS	NS	NS	0.03	<0.001	<0.001	NS	<0.001	NS	<0.001	NS	<0.001
Quadratic	0.067	<0.001	<0.001	<0.001	<0.001	<0.001	<.001	<0.001	NS	<0.001	<0.001	0.01

[1]Values are least squares means; n = 4. FI = feed intake; BWG = BW gain.

ity tended ($P = 0.0624$) to be significantly modified by treatments (Table 7). Increasing dietary fiber content can increase the production of saliva, gastric juices, hydrochloric acid, and pepsin [32]. Graham and Aman [33] noted a similar increase in pancreatic flow, accompanied by a closely related increase in electrolyte production. Thus, activities of lipases and amylases may increase.

The presence of chlorogenic acid (a group of phenolic compounds) in amounts of 10 to 40 g/kg [34] in the sunflower kernel could justify the negative effect on the growth of the birds. In the literature reviewed, we found information on the determination of digestive enzyme activity of broilers fed SFM. In our experiment, the linear decrease ($P = 0.12$) in protease activity may have been caused by the presence of chlorogenic acid because this material has been shown to inhibit the activity of trypsin by 30% [35].

Organ Weight

There were no effects ($P > 0.01$) of increasing levels of SFM on relative weights (g/kg of BW) of the breast and abdominal fat (Table 8). Similarly, Reddy [7], Ramesh Kumar [36], and Rama Rao et al. [29] reported no effect of feeding SFM on the relative weights of breast and abdominal

Table 7. Effect of increasing levels of sunflower meal (SFM) on digestive enzyme activities in the digesta of broiler chicks[1] at 28 d of age

Item	α-Amylase,[2] units	Protease,[3] units
Level of SFM, %		
0	267	4,129
7	196	4,149
14	242	4,082
21	273	4,056
SEM	24.7	39.2
	P-value	
Orthogonal contrast		
Linear	NS	NS
Quadratic	0.062	NS

[1]Values are least squares means; n = 4.

[2]One unit of enzymatic activity is defined as the amount of enzyme required to produce 1 µmol of glucose per minute under assay conditions.

[3]One unit of protease activity on azocasein was defined as the amount of enzyme required to produce an absorbance change of 1.0 at 440 nm/min at 55°C and pH 8.

Table 8. Effects of feeding different levels of sunflower meal (SFM) on relative organ weights of chicks at 49 d of age (% of BW)[1]

Item	Breast	Thigh	Gastrointestinal tract	Liver	Gizzard	Abdominal fat
Level of SFM, %						
0	20.74	9.35	11.23	2.97	2.24	1.38
7	21.02	9.57	12.31	2.54	2.54	1.51
14	22.25	9.78	12.44	2.20	2.86	1.69
21	20.5	9.32	12.78	2.62	2.89	1.43
SEM	0.912	0.094	0.086	0.10	0.095	0.091
	P-value					
Orthogonal contrast						
Linear	NS	NS	<0.001	NS	<0.001	NS
Quadratic	NS	<0.001	0.0731	<0.001	NS	NS

[1]Values are least squares means; n = 4.

fat in broilers. Relative weights of the thigh and liver were quadratically ($P < 0.01$) increased and decreased, respectively ($P < 0.01$), as SFM levels increased in the diets. Likewise, relative weights of the gastrointestinal tract and gizzard were linearly increased ($P < 0.01$) as dietary levels of SFM increased (Table 8). The higher levels of fiber in the SFM-based diets might be responsible for hypertrophy of these organs, as was evident in previous studies in broilers on high-fiber diets [37–40].

Blood Parameters

Cholesterol, calcium, and protein concentrations did not demonstrate a linear or quadratic response to increasing levels of SFM (Table 9). However, glucose and phosphorous concentrations linearly increased as the dietary SFM levels increased ($P < 0.05$). Triglyceride and HDL concentrations increased quadratically ($P < 0.01$) with an increasing level of SFM. In addition, LDL concentration decreased quadratically as dietary levels of SFM increased ($P < 0.01$). However, there was no increase in alkaline phosphatase activity, either linear or quadratic, in response to increasing levels of SFM ($P > 0.05$). Because a higher dietary fiber content is known to reduce dietary fat utilization by deconjugation of bile salts [41, 42], which might have reduced fat absorption through the gut, the body fat (liver fat) might have been utilized for the metabolic needs and thereby increased the HDL concentration in serum. The reduced triglyceride concentration in the serum of broilers fed a higher level of SFM (210 g/kg of diet) also supports this hypothesis. A similar trend was observed in the experiments of Rama Rao et al. [29, 43], in which the serum concentrations of LDL and triglycerides decreased in birds receiving high-fiber diets.

Intestinal Morphology

A quadratic response was observed for villus heights of the duodenum and jejunum with increasing levels of SFM ($P < 0.01$; Table 10). Villus height of the ileum did not exhibit a linear or quadratic response. A quadratic response to increasing levels of dietary SFM was observed for crypt depth of the duodenum and jejunum ($P < 0.01$). However, no effect on crypt depth of the ileum, either linear or quadratic, was due to increasing levels of SFM. A quadratic response to increasing levels of SFM was observed for villus width of the duodenum ($P < 0.01$). With increasing levels of SFM, villus width of the jejunum decreased linearly ($P < 0.05$). The villus height-to-crypt depth ratios of the duodenum and jejunum responded quadratically to increasing levels of SFM ($P < 0.01$). However, this parameter was not significant for the ileum. In the literature reviewed, we found no evidence of the effect of SFM on the histology of broiler chickens. The structure of the intestinal mucosa can reveal some information on gut health. Stressors that are present in the digesta can lead to relatively rapid changes in the intestinal mucosa

Table 9. Effects of increasing levels of sunflower meal (SFM) on blood parameters of broiler chickens at 28 d of age[1]

Item	Glucose, mg/dL	Cholesterol, mg/dL	Triglycerides, mg/dL	Calcium, mg/dL	Phosphorous, mg/dL	Alkaline phosphatase, U/L	HDL,[2] mg/dL	LDL,[3] mg/dL	Protein, g/dL
Level of SFM, %									
0	268	102	68.75	13.2	7.3	111	81	36.0	3.52
7	265	105	126.75	12.7	8.2	123	72	26.2	3.35
14	275	116	138.25	13.3	8.6	117	85	22.7	3.35
21	290	112	116.50	13.7	8.8	97	91	20.5	3.35
SEM	7.44	5.05	8.17	0.34	0.37	3.01	2.91	1.08	0.11
	P-value								
Orthogonal contrast									
Linear	0.045	0.087	<0.001	NS	<0.01	NS	<0.01	<0.001	NS
Quadratic	NS	NS	<0.001	NS	NS	NS	<0.01	<0.01	NS

[1]Values are least squares means; n = 4.

[2]High-density lipoprotein.

[3]Low-density lipoprotein.

Table 10. Effects of sunflower meal (SFM) on the morphology of different sites of the small intestine[1]

	Level of sunflower meal, %					*P*-value	
Site	0	7	14	21	SEM	Linear	Quadratic
Villus height, μm							
Duodenum	1,010.09	1,132.88	989.32	988.28	16.48	<0.01	<0.01
Jejunum	407.17	536.1	435.9	398.52	11.73	<0.001	<0.001
Ileum	226.36	230.85	180.55	235.6	28.02	NS	NS
Crypt depth, μm							
Duodenum	185.47	142.22	188.33	194.05	1.57	<0.001	<0.001
Jejunum	115.58	106.86	80.18	119.33	7.97	NS	<.01
Ileum	109.39	102.98	76.54	91.28	10.46	NS	NS
Villus height:crypt depth							
Duodenum	5.44	7.96	5.25	5.09	0.104	<0.001	<0.001
Jejunum	3.63	5.04	5.44	3.04	0.250	NS	<0.001
Ileum	2.24	2.24	2.35	2.60	0.313	NS	NS
Villus width, μm							
Duodenum	200.95	152.61	148.40	198.05	8.35	NS	<0.001
Jejunum	136.83	118.52	123.85	102.6	9.23	0.0363	NS
Ileum	147.34	138.49	150.23	143.41	15.49	NS	NS

[1]Values are least squares means; n = 4.

because of the close proximity of the intestinal contents to the mucosal surface. One possible hypothesis about changes in intestinal morphology, such as shorter villi and deeper crypts, has been associated with the presence of toxins [20]. Shortening of the villus decreases the surface area for nutrient absorption. The crypt can be regarded as a villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue [44]. Demand for energy and protein for gut maintenance is higher compared with other organs. A fast-growing broiler devotes about 12% of the newly synthesized protein to the digestive tract [44]. Any additional tissue turnover will increase nutrient requirements for maintenance and will therefore lower the efficiency of the bird. A shortening of the villus and a large crypt can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, diarrhea, reduced disease resistance, and lower overall performance. In the present study, the villus heights of the duodenum and jejunum were quadratically affected as dietary levels of SFM increased. These results are in accordance with the results for performance. There was improvement in the performance of broiler chickens fed SFM up to 140 g/kg of the diet. However, the performance parameters of birds fed a high level of SFM in the diet (210 g/kg) decreased. The crypt is the area where stem cells divide to permit renewal of the villus; a large crypt indicates fast tissue turnover and a high demand for new tissue.

In previous studies, antinutritional factors in SBM, such as trypsin inhibitor (**TI**) and soybean globulins, had an adverse effect on the morphology and function of the digestive tract in animals [45, 46]. It is known that TI interfered with the proper functioning of trypsin and chymotrypsin, leading to abnormal intestinal morphology [47]. Zarkadas and Wiseman [48] demonstrated a negative correlation between the TI level in soybean meal and villus height in weaned piglets. Additionally, many have suggested that the morphological changes observed in young animals and poultry are due to transient hypersensitivity to antigenic components of soybean diet [49, 50]. Antigenic materials in soybean proteins are associated with villus atrophy, increased crypt cell mitosis, and crypt hyperplasia, and thereby cause malabsorption syndrome [51–53]. Therefore, in our experiment, histological alterations may result from some antinutritional factors, such as chlorogenic acid, in SFM.

In addition, changes in small intestinal mucosa may be caused indirectly by the viscous characteristics of nonstarch polysaccharides [54]. Malathi and Devegowda [28] determined that SFM contains 110.01, 220.67, 40.92, and 410.34 g/kg of pentosan, cellulose, pectin, and

nonstarch polysaccharides, respectively. Pectin is a nonstarch polysaccharide that is not readily digested by the endogenous gut enzymes of broilers. Sakata [55] demonstrated that an increase in bacterial activity in the gastrointestinal tract was associated with a change in the morphology of the gut wall. They attributed this to the presence of the high level of pectin found in dates [56].

CONCLUSIONS AND APPLICATIONS

1. The AME_n of local SFM (cultivated in the north of Iran) obtained was 1,219 kcal/kg.
2. Increasing levels of SFM in the diet quadratically affected FI (in the grower and finisher phases), but BWG (in the starter and grower phases) and FCR (in the grower phase) were linearly affected.
3. A quadratic response was observed in the relative weights of the thigh and liver with increasing levels of SFM.
4. Triglyceride and HDL concentrations increased and LDL concentration decreased as dietary levels of SFM increased.
5. Sunflower meal can be used in broiler chick diets at levels up to 140 g/kg, and its fiber content had no significant effect on nutrient intake.

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Acknowledgments

Special thanks are extended to the Excellent Center of Animal Sciences in Ferdowsi University of Mashhad (Mashhad, Iran) for financial help. The authors also thank the Segol Factory (Nishabour, Iran) for supplying the SFM used in this study.