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The Effect of Protease Enzyme and Replacement Soybean Meal by Cotton Seed Meal on Protein Efficiency and Performance in Broiler Chickens

Research Article

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ABSTRACT

This study was conducted to investigate the effect of replacing soy bean meal (SBM) by cottonseed meal (CSM) with or without of protease enzymes, on performance, in-vitro protein digestibility, carcass characteristics, gastrointestinal morphology and hematological parameters in broilers. A total 450 one-day-old mixed sex broiler chickens Ross 308 were managed in 6 treatments, 5 replicates and 15 chickens in each in a 2 × 3 factorial arrangement with completely randomized design (CRD). Treatments consist of: treatment 1 (0% CSM, -protease), treatment 2 (0% CSM, +protease), treatment 3 (20% CSM, -protease), treatment 4 (20% CSM, +protease), treatment 5 (30% CSM, -protease), treatment 6 (30% CSM, +protease). The results have shown that there were no significant affected on feed intake, body weight gain and feed conversion ratio by various treatments in starter period (P>0.05). Digestive organs and body weight were not affected by treatments at 27 days of age. Breasts weight was significantly increased by 20% replacement SBM with CSM (P<0.05). Protein digestibility was increased significantly by protease enzymes and *in vitro* method. Serum cholesterol levels reduced by enhanced levels of replacement CSM (P<0.05). There was concluded that no adverse effects were found by replacing 30% SBM with CSM on performance status, as well as improved in vitro protein digestibility.

KEY WORDS broiler chicken, cottonseed meal, protease enzyme, soy bean meal.

INTRODUCTION

Soybean meal (SBM) is a predominant protein source in formulation poultry diets. When the soybean meal price increases, poultry producers seek some unconventional protein sources such as cottonseed meal used as a replacement by soybean meal in poultry diet (Liu *et al.* 2006). Now in regarding to soybean meal price in poultry industry most of researchers in throughout the world try to identified and find out an alternative protein source. However, it is difficult to have a consistent supply of good quality soybean meal in poultry production at a range of reasonable price (Xiong *et al.* 2016). Cottonseed meal (CSM) is a by-product of process to extract oil from cotton seeds arid contains 40 or 44% crude protein depending on the extent of hull separation prior to the oil extraction process (Heidarinia and Malakian, 2011). It is a potential alternative of SBM in poultry diet (Nagalakshmi *et al.* 2007). When broilers were fed cottonseed meal based diets, the presence of anti-nutritional factors such as gossypol disturbed the absorption of nutrients and reduces amino acid digestibility. This is may increase the need for protease enzyme demand to more nutrient digestibility and optimize the growth in poultry (Blevins *et al.* 2010). Also, high demands of amino acids by the broiler chicken could be met by increasing protein contents or added exogenous protease enzyme (Nazemzadeh *et al.* 2017). On the other hand, Gossypol, a potentially toxic agent, has limited the use of cottonseed meal, as an attractive protein source for poultry feed. The toxic effects of gossypol may be alleviated by supplementation of soluble iron salts that could be bind by gossypol (Shawrang *et al.* 2011). Enzyme supplementation has become increasingly popular in animal feeds in recent years, the most commonly used are proteases (Kaczmarek *et al.* 2014).

Consequently, it has been suggested that the immature of the digestive system in young chickens may result in the poor utilization of dietary nutrients (Jin *et al.* 1998). The nutrient digestion rather than the ability of absorption has been indicated to be the primary limiting factor (Parsons, 2004). Based on SBM price, this study was focus to CSM as an alternative protein source and protease enzyme on nutrient digestibility and broiler performance. This study aimed was to investigate the effect of replacing soy bean meal (SBM) by cottonseed meal (CSM) with or without of protease enzymes, on performance, *in vitro* protein digestibility, carcass characteristics, gastrointestinal morphology and hematological parameters in broilers.

MATERIALS AND METHODS

The experiment was conducted at poultry farm in the Faculty of Agricultural in, Bu-Ali Sina University, Hamedan, Iran. A total 450 one-day-old mixed sex broiler chickens Ross 308 with initial body weight of 38.86 ± 7.2 gram were designed by 6 treatments, 5 replicates and 15 chickens in each in a 2 × 3 factorial arrangement with completely randomized design (CRD). Treatments includes: treatment 1 (0% CSM, without protease), treatment 2 (0% CSM, with protease), treatment 3 (20% CSM, without protease), treatment 4 (20% CSM, with protease), treatment 5 (30% CSM, without protease), treatment 6 (30% CSM, with protease). Ferrous sulfate (0 or 200 g/ton) was used for disable gossypol cottonseed meal and protease enzyme 200 g/ton (ton; 75000 PROT/g; Ronozyme® ProAct) in the diet. Experimental diets were considered in starter, grower and finisher periods (1 to 10, 11 to 24 and 25 to 42 days of age respectively).

Diets were formulated according to Ross 308 recommend in 2014. All of the diets were formulated based on standardized ileal digestible amino acids (SID) and ideal protein (Table 2, 3 and 4). Diet, in mash form, and water were provided in *ad libitum* consumption. The feed intake, body weight and feed conversion ration were considered on a weekly period.

Chemical analysis

The dry matter, crude (ash, fiber, protein and fat) content of corn, corn gluten meal (CGM), SBM and CSM were analyzed in the Feed Analysis Laboratory using proposed by the Association of Official Analytical Chemists, AOAC methods (AOAC, 2000) as shown in Table 1. Acid detergent fiber (ADF), Neutral detergent fiber (NDF) and the amino acid profile of corn, CGM, SBM and CSM were analyzed respectively by method of Van Soest *et al.* (1991) and Near Infrared Reflectance Spectroscopy (NIRs) (Table 1). The exogenous protease enzyme was used (Ronozyme ® ProAct) produced by nutritional products DSM, Parsippany, NJ, USA and the commercial product had a protease activity of 75.000 U/g and the unit of activity was defined as the amount of enzyme that released 1 µmol of p-nitroaniline from 1 mM substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 0.9 and temperature 37 ˚C.

Statistical analysis

A completely randomized design (CRD) was applied. Data were analyzed by the GLM procedure (SAS, 2004). Means were separated by Duncan's multiple range tests. Treatments differences were considered significant at (P<0.05).

RESULTS AND DISCUSSION

The results of broiler chickens performance were presented in Table 6. There was no significant effect on performance parameters in the entire period by interaction between cottonseed meal and protease enzyme (P>0.05). Improved feed conversion ratio (FCR) was found by protease enzyme in broiler diet. The feed conversion ratio was significantly lower by interaction between CSM and protease enzyme in comparison without enzyme treatment (P=0.053). In addition, the European broiler index (EBI) increased by control diet and 20% replacing SBM with CSM without enzymes (P<0.05). As shown in Tables 6 and 7, no significant differences were found on relative weight of carcass, breast, leg, heart, liver, bursa of Fabricius, between treatments (P>0.05). Proventriculus and pancreas weight were significantly increased by 30% replacing SBM with CSM compared with control and 20% replacing (P<0.05).The highest weight of gizzard was observed by 30% replacing SBM with CSM compared with control diet (P<0.05). *In vitro* dry matter digestibility was decreased with higher levels of dietary CSM (P<0.05). Protein digestibility were increased by 30% replacing SBM with CSM with enzyme in comparison to all treatment's groups (P<0.05) (Table 8). As shown in Table 8, no significant differences were observed in the albumin and total protein in blood plasma of broiler chicken in different treatments (P>0.05).

Table 1 Chemical compositions of dietary ingredients (%; as-is)

Item	Corn	GCM	SBM	CSM
Dry matter	90.34	94.86	91.86	89.89
Crude protein	8.15	56.64	46.82	41.17
Crude fiber	2.60	2.50	4.30	9.80
Ether extract	3.70	1.51	1.50	2.00
Total ash	1.20	1.80	6.70	7.80
NDF	11.30	6.10	11.50	21.20
ADF	3.70	4.50	6.50	15.80
Amino acid[1]				
Lysine	0.241	1.082	2.699	1.934
Methionine	0.166	1.538	0.605	0.731
Methionine + cystine	0.347	2.587	1.283	1.527
Cystine	0.181	1.031	0.665	0.787
Threonine	0.303	2.049	1.745	1.522
Valine	0.399	2.662	2.099	2.046
Tryptophan	0.060	0.314	0.604	0.563
Arginine	0.367	1.991	3.145	5.107
Isoleucine	0.293	2.362	1.988	1.468
leucine	1.055	9.706	3.345	2.718
Histidine	0.236	1.222	1.177	1.320
Phenylalanine	0.419	3.675	2.215	2.443

[1] SID amino acid measured by NIRS.
NDF: neutral detergent fiber; ADF: acid detergent fiber; GCM: gluten corn meal; SBM: soy bean meal and CSM: cotton seed meal.

Table 2 Components of experimental diets and their chemical analysis (%, as-is basis) (starter period from 1 to 10 days)

Ingredient[2]	Dietary treatments[1]					
	1	2	3	4	5	6
corn	57.29	59.08	57.21	59.72	57.30	59.55
SBM	33.35	31.14	26.30	24.59	22.8	21.24
GCM	3.00	3.00	3.00	3.00	3.00	3.00
CSM	-	-	6.66	6.23	10	9.33
Soybean oil	1.69	1.36	1.96	1.63	2.12	1.77
DCP	1.87	1.89	1.84	1.84	1.81	1.83
Oster shell	1.15	1.15	1.18	1.18	1.21	1.21
NaCl	0.33	0.33	0.33	0.33	0.33	0.33
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.32	0.32	0.33	0.3	0.32	0.3
L-Lysine HCl,	0.36	0.35	0.49	0.47	0.55	0.53
Threonine	0.13	0.13	0.19	0.18	0.2	0.2
Iron sulfate	0.01	0.01	0.01	0.01	0.02	0.02
Enzyme	-	0.02	-	0.02	-	0.02
Calculated analysis[5]						
AMEn (kcal/kg)	2950	2950	2950	2950	2950	2950
CP (%)	22.62	22.62	22.62	22.62	22.62	22.62
Ca (%)	0.94	0.94	0.94	0.94	0.94	0.94
AP (%)	0.47	0.47	0.47	0.47	0.47	0.47
Lysine (%)	1.31	1.31	1.28	1.29	1.27	3.46
Methionine (%)	0.66	0.65	0.64	0.64	0.64	2.75
Methionine +cysteine (%)	0.96	0.97	0.95	0.95	0.94	0.95
Threonine (%)	0.85	0.85	0.85	0.85	0.85	0.85
Tryptophan (%)	0.22	0.21	0.21	0.20	0.20	0.20
Determined analysis (%)						
DM	91.55	91.53	91.22	91.6	91.43	91.26
Total ash	6.75	6.45	6.55	6.4	6.45	6. 5
CP	21.21	21.42	22.42	22.25	21.52	22.36

[1] 1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
[2] SBM: soybean meal; GCM: gluten corn meal; CSM: cottonseed meal; DCP: dicalcium phosphate and NaCl: common salt.
[3] Mineral premixes supplied per kilogram of diet: Mn ($MnSO_4 \cdot H_2O$): 66 mg; Zn (ZnO): 66 mg; Fe ($FeSO_4 \cdot H_2O$): 33 mg; Cu ($CuSO_4 \cdot 5H_2O$): 6 mg; I: 0.9 mg; Co (CoSO4·H2O): 0.2 mg and Se (Na_2SeO_3): 0.2 mg.
[4] Vitamin premixes supplied per kilogram of diet: vitamin A (retinyl acetate): 10000 IU; vitamin D_3: 2750 IU; vitamin E (α-tocopheryl acetate): 20 IU; Menadione: 3.0 mg; Thiamin: 2.5 mg; Riboflavin: 6.0 mg; Pyridoxine: 2.5 mg; vitamin B_{12}: 12 µg; Folic acid: 1.5 mg; Niacin: 20 mg; Ca-pantothenate: 15 mg and Biotin: 80 µg.
[5] AMEn: apparent metabolizable energy corrected for nitrogen; CP: crud protein; Ca: calcium; AP: available phosphate and DM: dry matter.

Table 3 Components of experimental diets and their chemical analysis (%, as-is basis) (from 11 to 24 days)

Ingredient[2]	Dietary treatments[1]					
	1	2	3	4	5	6
corn	60.78	63.31	60.72	63.22	60.64	63.14
SBM	31.85	29.64	25.1	23.37	21.75	20.27
GCM	1.00	1.00	1.00	1.00	1.00	1.00
CSM	-	-	6.36	5.92	9.55	8.86
Soybean oil	2.37	2.05	2.64	2.31	2.78	2.44
DCP	1.62	1.63	1.59	1.6	1.57	1.58
Oster shell	1.04	1.05	1.08	1.08	1.1	1.1
NaCl	0.29	0.29	0.29	0.29	0.29	0.29
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.26	0.24	0.27	0.25	0.27	0.25
L-Lysine HCl	0.20	0.20	0.32	0.32	0.39	0.37
Threonine	0.08	0.07	0.12	0.11	0.14	0.13
Iron sulfate	0.01	0.01	0.01	0.01	0.02	0.02
Enzyme	-	0.02	-	0.02	-	0.02
Calculated analysis[5]						
AMEn (kcal/kg)	3000	3000	3000	3000	3000	3000
CP (%)	20.81	20.81	20.81	20.81	20.81	20.81
Ca (%)	0.84	0.84	0.84	0.84	0.84	0.84
AP (%)	0.42	0.42	0.42	0.42	0.42	0.42
Lysine (%)	1.15	1.15	1.12	1.13	1.12	1.11
Methionine (%)	0.55	0.54	0.55	0.54	0.55	0.54
Methionine + cysteine (%)	0.83	0.83	0.83	0.83	0.83	0.83
Threonine (%)	0.74	0.74	0.74	0.74	0.74	0.74
Tryptophan (%)	0.20	0.20	0.19	0.19	0.19	0.19
Determined analysis (%)						
DM	91.1	91.48	91.75	91.13	91.8	91.46
Total ash	6.01	5.91	5.46	5.6	5.55	5.43
CP	19.62	20.12	20.35	20.19	20.22	20.15

[1] 1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
[2] SBM: soybean meal; GCM: gluten corn meal; CSM: cottonseed meal; DCP: dicalcium phosphate and NaCl: common salt.
[3] Mineral premixes supplied per kilogram of diet: Mn ($MnSO_4 \cdot H_2O$): 66 mg; Zn (ZnO): 66 mg; Fe ($FeSO_4 \cdot H_2O$): 33 mg; Cu ($CuSO_4 \cdot 5H_2O$): 6 mg; I: 0.9 mg; Co (CoSO4·H2O): 0.2 mg and Se (Na_2SeO_3): 0.2 mg.
[4] Vitamin premixes supplied per kilogram of diet: vitamin A (retinyl acetate): 10000 IU; vitamin D_3: 2750 IU; vitamin E (α-tocopheryl acetate): 20 IU; Menadione: 3.0 mg; Thiamin: 2.5 mg; Riboflavin: 6.0 mg; Pyridoxine: 2.5 mg; vitamin B_{12}: 12 µg; Folic acid: 1.5 mg; Niacin: 20 mg; Ca-pantothenate: 15 mg and Biotin: 80 µg.
[5] AMEn: apparent metabolizable energy corrected for nitrogen; CP: crud protein; Ca: calcium; AP: available phosphate and DM: dry matter.

Also, plasma cholesterol levels of broiler were decreased significantly by 30 and 20% SBM replacement with CSM compared with control diet (P<0.05). No significant effect was found on villus height, villus thickness, villus height to crypt depth ratio in the jejunum by different levels replacement of SBM with CSM and protease enzyme in the diet (P>0.05) (Table 9).

Results of this study have shown there is no significant differences in performance by treatments.

Similarly, Azman and Yilmaz (2005) have reported 20% replacing CSM with SBM no significant differences in body weight, daily weight gains, daily feed intake and feed conversion ratios.

Cotton seed meal, in spite of undesirable properties of gossypol, was one of the protein sources that could be used as replacement with soybean meal in diet without any adverse effects on the performance efficiency of broiler chickens (Tang *et al.* 2012).

Table 4 Components of experimental diets and their chemical analysis (%, as-is basis) (from 25 to 42 days)

Ingredient[2]	Dietary treatments[1]					
	1	2	3	4	5	6
Corn	61.58	64.22	61.99	64.6	62.18	64.78
SBM	30.87	28.54	23.93	22.13	20.46	18.93
GCM	-	-	-	-	-	-
CSM	-	-	6.17	5.71	9.26	8.56
Soybean oil	3.85	3.51	4.03	3.67	4.13	3.76
DCP	1.45	1.46	1.41	1.43	1.4	1.41
Oster shell	0.97	0.98	1.01	1.01	1.02	1.03
NaCl	0.27	0.27	0.27	0.27	0.27	0.27
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.24	0.22	0.25	0.23	0.25	0.23
L-Lysine HCl	0.14	0.14	0.26	0.26	0.32	0.31
Threonine	0.06	0.06	0.1	0.09	0.12	0.11
Iron sulfate	0.01	0.01	0.01	0.01	0.02	0.02
Enzyme	-	0.02	-	0.02	-	0.02
Calculated analysis[5]						
AMEn (kcal/kg)	3050	3050	3050	3050	3050	3050
CP (%)	18.59	18.59	18.59	18.59	18.59	18.59
DCAD	235	235	230	230	225	225
Ca (%)	0.76	0.76	0.76	0.76	0.76	0.76
AP (%)	0.38	0.38	0.38	0.38	0.38	0.38
Lysine (%)	1.02	1.02	0.99	1.01	0.99	0.99
Methionine (%)	0.53	0.52	0.52	0.51	0.51	0.51
Methionine + cysteine (%)	0.79	0.79	0.78	0.79	0.78	0.78
Threonine (%)	0.66	0.66	0.66	0.66	0.66	0.66
Tryptophan (%)	0.19	0.18	0.18	0.18	0.17	0.17
Determined analysis (%)						
DM	91.66	92.13	92.53	91.68	92.61	93.08
Total ash	5.84	5.42	6.21	6.01	5.59	5.92
CP	18.56	19.12	18.25	19.23	18.47	18.69

[1] 1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
[2] SBM: soybean meal; GCM: gluten corn meal; CSM: cottonseed meal; DCP: dicalcium phosphate and NaCl: common salt.
[3] Mineral premixes supplied per kilogram of diet: Mn ($MnSO_4 \cdot H_2O$): 66 mg; Zn (ZnO): 66 mg; Fe ($FeSO_4 \cdot H_2O$): 33 mg; Cu ($CuSO_4 \cdot 5H_2O$): 6 mg; I: 0.9 mg; Co ($CoSO4 \cdot H2O$): 0.2 mg and Se (Na_2SeO_3): 0.2 mg.
[4] Vitamin premixes supplied per kilogram of diet: vitamin A (retinyl acetate): 10000 IU; vitamin D_3: 2750 IU; vitamin E (α-tocopheryl acetate): 20 IU; Menadione: 3.0 mg; Thiamin: 2.5 mg; Riboflavin: 6.0 mg; Pyridoxine: 2.5 mg; vitamin B_{12}: 12 µg; Folic acid: 1.5 mg; Niacin: 20 mg; Ca-pantothenate: 15 mg and Biotin: 80 µg.
[5] AMEn: apparent metabolizable energy corrected for nitrogen; CP: crud protein; Ca: calcium; AP: available phosphate and DM: dry matter.

Table 5 Chemical analysis of protease enzyme

Calculated chemical analysis	Amount
Metabolizable energy	2079 kcal
Crude protein	47 %
Crude fiber	8.9 %
Calcium	17.0 %
Available phosphorus	32.0 %
Sodium	04.0 %
Potassium	22.1 %
Iron	009.0 %

The highest EBI were observed by 20% replacing SBM with CSM without enzymes. As well as no significant response was found on relative weight of carcass, breast and thigh by treatments. The breast, thigh, wings, neck and back were not differences by experimental diets contain CSM levels (Elangovan *et al.* 2006). Since these organs were muscular and required to high quality protein for growth, in contrast protein digestibility the CSM is lower than soybean meal (80.9% *vs.* 91.67%).

Table 6 Effects of dietary cotton seed meal and protease enzyme on performance of broiler chickens

Dietary factors	Growth performance						
	BWG (g/bird)	FI (g/bird)	FCR (g/g)	EBI	BW (g/bird)	Breast (g/bird)	Leg (g/bird)
Protease enzyme							
With enzyme	2447.43	3905.33	1.569	369.05	2483.50	599.39	446.89
Without enzyme	2379.59	3874.42	1.628	351.30	2407.78	588.83	436.50
SEM	36.23	78.75	0.02	9.23	62.81	23.37	16.32
P-value	0.7015	0.0884	0.2106	0.0784	0.2512	0.6597	0.5364
CSM levels %							
0	2391.19	3824.42	1.601	358.62	2479.42	ab610.25	447.42
20	2434.79	3921.92	1.611	365.89	2463.17	a618.50	450.25
30	2414.56	3923.29	1.624	356.02	2394.33	b553.58	427.42
SEM	44.37	96.44	0.03	11.30	76.93	28.63	19.98
P-value	0.5214	0.6329	0.7412	0.6714	0.5207	0.0851	0.4823
CSM levels × protease enzyme %							
1	3801.9	2468.7	1.540^{b}	387.3^{a}	2559.2	625.50	459.83
2	3846.9	2313.7	1.662^{a}	329.31^{b}	2399.7	595.00	435.00
3	3885.9	2413.06	1.610^{ab}	362.65^{ab}	2406.00	613.17	437.00
4	3958.00	2456.52	1.613^{ab}	369.13^{a}	2520.30	623.83	463.50
5	3956.1	2417.09	1.636^{ab}	350.09^{ab}	2371.00	548.83	417.32
6	3890.5	2412.03	1.611^{ab}	361.94^{ab}	2417.7	558.33	437.50
SEM	136.4	62.75	0.04	15.99	108.79	40.49	28.27
P-value	0.8052	0.2548	0.0536	0.0231	0.1436	0.8148	0.2282

BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; EBI: European broiler index and CSM: cottonseed meal.
1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of dietary cotton seed meal and protease enzyme on organs relative weight (% of live body weight) of broiler chicks

Dietary factors	Proventriculus	Gizzard	Pancreatic	Heart	Liver	Bursa of Fabricius	Spleen
Protease enzyme							
With enzyme	9.175	32.07	5.63	11.64	48.33	1.58	2.82
Without enzyme	9.177	30.17	5.74	12.04	47.08	1.44	2.62
SEM	0.45	1.39	0.31	0.7	2.52	0.17	0.17
P-value	0.0102	0.0372	0.0305	0.8335	0.9092	0.2584	0.1367
CSM Levels %							
0	8.49^{b}	28.55^{b}	5.26^{b}	11.59	48.42	1.52	2.47
20	8.7^{b}	31.25^{ab}	5.43^{b}	12.12	47.59	1.31	2.75
30	10.34^{a}	33.6^{a}	6.36^{a}	11.81	47.08	1.7	2.94
SEM	0.55	1.7	0.38	0.87	3.09	0.22	0.22
P-value	0.9971	0.1971	0.7206	0.5867	0.6299	0.4527	0.2650
CSM levels × protease enzyme %[1]							
1	8.73	29.33	5.48	11.45	49.24	1.66	2.77
2	8.26	27.76	5.05	11.73	47.61	1.38	2.17
3	8.36	30.55	5.32	12.03	45.96	1.18	2.86
4	9.03	31.94	5.55	12.21	49.22	1.44	2.65
5	9.77	34.96	5.86	11.27	46.51	1.64	3.05
6	10.91	32.22	6.86	12.36	47.65	1.76	2.82
SEM	0.77	2.41	0.54	1.23	4.37	0.32	0.30
P-value	0.2663	0.9347	0.2805	0.7232	0.6569	0.3145	0.4029

CSM: cottonseed meal.
1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of dietary cotton seed meal and protease enzyme on protein and dry matter digestibility (%) and blood parameters

Dietary factors	Dry matter (%)	Protein (%)	Cholesterol (g/d)	Albumin (g/d)	Total protein (g/d)
Protease enzyme					
With enzyme	74.75	69.04[b]	132.28[a]	1.38	3.22
Without enzyme	73.49	75.26[a]	94.06	1.28	3.32
SEM	1.00	0.67	21.54	0.13	0.18
P-value	0.2334	< 0.0001	0.0506	0.3549	0.5149
CSM levels %					
0	75.69[a]	73.70[a]	169.24[a]	1.31	3.38
20	74.15[ab]	70.22[b]	95.09	1.30	3.13
30	72.53[b]	72.54[a]	75.18	1.38	3.31
SEM	1.23	0.82	17.59	0.11	0.15
P-value	0.0733	0.0038	0.0022	0.8194	0.4084
CSM levels × protease enzyme %					
1	73.63	73.98[b]	86.13	1.44	3.27
2	71.43	73.42[b]	104.05[b]	1.19	3.50
3	75.27	66.77[c]	70.82	1.17	3.11
4	73.03	73.68[b]	79.54	1.43	3.15
5	75.36	66.39[c]	115.16[a]	1.28	3.25
6	76.01	78.70[a]	107.32[a]	1.48	3.37
SEM	1.74	1.16	30.47	0.18	0.26
P-value	0.4311	< 0.0001	0.1243	0.0317	0.5844

CSM: cottonseed meal.
1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 9 Effect of dietary cotton seed meal and protease enzyme on jejunum morphology in broiler chickens at 42 days of age (μm)

Dietary factors	Villus height	Villus thickness	Villus depth	Villus height × villus depth	Villus height / crypt depth
Protease enzyme					
With enzyme	800.01	136.06	213.18	114337	4.07
Without enzyme	817.00	121.14	218.23	100779	4.61
SEM	72.6	16.74	27.17	0.6	0.56
P-value	0.6404	0.2409	0.8221	0.4414	0.4011
CSM levels %					
0	839.95	123.9	218.00	108747	4.26
20	817.68	115.83	239.28	99682	4.13
30	761.54	147.54	187.26	114118	4.68
SEM	81.99	20.5	33.28	0.73	0.7
P-value	0.6306	0.2412	0.4205	0.7832	0.8147
CSM levels × protease enzyme %					
1	854.4	131.33	227.91	120130	4.11
2	825.5	116.46	208.09	97364	4.42
3	841.1	104.70	299.58	87850	3.91
4	798.2	125.11	189.04	109541	4.32
5	721.2	159.59	227.27	112841	3.65
6	788.4	139.51	160.59	114969	5.37
SEM	125.74	28.99	47.07	1.04	0.99
P-value	0.5912	0.9313	0.0561	0.7808	0.3165

CSM: cottonseed meal.
1: Control without enzyme; 2: Control with enzyme; 3: 20% replacing CSM with SBM without enzyme; 4: 20% replacing CSM with SBM with enzyme; 5: 30% replacing CSM without SBM without enzyme and 6: 30% replacing CSM with SBM without enzyme.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Therefore no significant improved in breast and thigh growth were shown by CSM as lower protein source (Nie *et al.* 2015; Jazi *et al.* 2017). The tissues of the target organs (heart, liver and bursa of Fabricius) were also affected by treatments. In this study, proventriculus, pancreas and gizzard weight were significantly increased by CSM levels which may be due to high fiber content.

Dietary structural components, such as fiber, have a attracted considerable attention as they may be increased large, well-developed and improved gizzard function (Kheravii *et al.* 2017). Therefore, gizzard was able to grind feed particles more completely and pancreatic enzyme secretion elevated by increased release of cholecystokinin (Amerah *et al.* 2007). Decreased dry matter *in vitro* digestibility, impairs and decreased the absorption of nutrients by anti-nutritional factors such as gossypol and cyclopropenoid fatty acids with higher levels of dietary CSM (Liu *et al.* 2006). The highest protein digestibility was observed by 30% replacing SBM with CSM with enzyme but no correspond with study of Cheng and Hardy, (2002). Also, some researches have noted that CSM reduced amino acid digestibility, this may be increased the demand of dietary synthetic amino acid supplementation in poultry performance.

Mireles-Arriaga *et al.* (2015) have reported that ideal digestibility of protein increased by protease enzyme supplementation. Reduction plasma cholesterol by 30 and 20% SBM replacement with CSM in the diet may be due to its high fiber content (9.8% crude fiber). Reduces intestinal transit time and increased excretion of sterols (increased secretion of bile) by fiber binding with bile salt in the gastrointestinal tract.

This could affects cholesterol metabolism and ultimately leads to the lower plasma cholesterol levels (Mateos *et al.* 2012; Taheri *et al.* 2016). In the study of Elangovan *et al.* (2006), cottonseed meal levels have no significant response on plasma protein which supported these results. No significant differences were observed in intestinal morphology by treatments. These results were in contrast with the results of some researches, which indicated fermented cottonseed meal increased villus height and villus height to crypt depth ratio.

CONCLUSION

In conclusion, no differences were found on performance by replacement of different levels of soybean meal with cottonseed meal in broiler chicken diet. Due to the low price of CSM up to 30% of cotton seed meal could be used in broiler chicken diet. Improved protein digestibility was shown by 30% replacement of SBM byCSMwith200 g/ton protease enzyme reaction.

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