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Original Research Article

The Effect of Substitution of Extruded Soybean Meal (ESM) on Growth Performance, Carcass Characteristics, Immune Responses, Biochemical Variables of Blood, and Nutrient Digestibility of Ileal in Broiler Chickens

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Abstract: This study aimed to investigate the effect of substitution of extruded soybean meal (ESM) (Digesta™) on growth performance, carcass characteristics, immune responses, biochemical variables of blood, and nutrient digestibility of ileal in broiler chickens (Ross 308). 225 chicks were randomly distributed among 5 trial groups (3 replicates of 15 chickens). The dietary treatments included different levels (0, 25, 50, 75, and 100%) of diet replacing ration of soybean meal with ESM which were fed to birds during a 42-day experimental period. The results showed that chickens fed diets 75% or 100% ESM had the highest ($P < 0.001$) daily overweight rates and also had the best FCR. The results showed that the treatments had a significant effect on carcass parts. Replacement of 50% of soybean meal with ESM resulted in the decreased ventricular area and increased percentage of chest compared to the control group ($P < 0.05$). Replacement of 75% soybean meal with ESM increased the antibody titer against the influenza virus in comparison with other experimental groups ($P < 0.01$). The serum triglycerides, glucose, and uric acid (UA) were decreased by the replacement of soybean meal with ESM ($P < 0.05$), but the total protein showed an increase by the increasing ESM to 100 % ($P < 0.01$). Substitution of 75% and 100% of ESM in diets decreased the activity of enzyme aspartate aminotransferase ($P < 0.01$) and increased triiodothyronine hormone (T3) ($P < 0.05$). The use of ESM in all substitution levels increased the carcass protein content ($P < 0.001$) and improved ileal digestibility of crude fat ($P < 0.01$). Also, the highest ileal digestibility coefficients of protein were observed in the chickens fed diets 75 % or 100% ESM. In conclusion, the results of this experiment indicate that the extruding process of SBM has a beneficial effect on growth performance and nutrient digestibility in broiler chicks.

Keywords: Antibody titer, broilers, digestibility, performance, soybean meal.

INTRODUCTION

A transformation of current animal production towards a more sustainable operation is vital to face nutritional challenges and there is an urgent demand for more sustainable high-quality feed sources (Leeson and Summers, 2005; Sayed *et al*., 2019). In fact, it is documented that feed represents the major cost of poultry production, constituting up to 70 percent of the total. Approximately, 95 percent of total feed cost is used to meet energy and protein needs, up to 4 percent for vitamin and mineral requirements, and up to 2 percent for feed additives (Ravindran, 2010; De Bruyn, 2017; Mwaniki and Kiarie, 2018). After the ban of the use of animal products as protein sources in the livestock diets, producers were forced to utilize soybeans in poultry diets (Bingol *et al*., 2016). Soybeans have a suitable amino acids profile, and also contain 18-22% of oil with good quality mainly with a high content of linoleic acids (Foltyn *et al*., 2013). Soybean meal is the most used vegetable protein in animal feed because of its high protein content and also balanced amino acid profile, but anti-nutritional factors decrease its utilization and digestibility (Sudaryono, 2006; Rada *et al*., 2017; Alagawany *et al*., 2018). Protease inhibitor, lectin, antivitamins, saponins, tanins, non-starch oligosaccharides and polysaccharides, and phytate are factors in the soybeans which negatively affect animal performance (Dourado *et al*., 2011; Nabizadeh, 2018). The researchers studied the effect of different heat treatments on the anti-nutrients in soybeans and soybean meal. Autoclaving (Anderson-Haferman *et al*., 1992), roasting (Bos, and

Flikweert, 1996), jet splodig (Clarke and Wiseman, 2005), micronizing (Clarke and Wiseman, 2007), microwave (Mateos *et al*., 2003), extruding (Perilla *et al*., 1997) and expanding (Mateos *et al*., 2003) are some of the most important and most commonly used processes. All these methods use heat and pressure to reduce the effect of antinutritional substances present in soybeans, but the thermal treatment should be used cautiously to avoid destroying other essential nutrients (Chunmei *et al*., 2010; Rada *et al*., 2017). Subuh *et al*. (2002) mentioned that extruding is the best method for processing soybeans and including extruded soybean in the diet improves the growth performance of broilers. They concluded that the extruded soybeans could substitute soybean meal without any unfavorable effects on growth performance, mortality rate, and carcass characteristics. This study aimed to examine the effects of SBM replacement by different levels of ESM in diets on growth performance, immunity, and nutrient digestibility, and serum metabolites of broilers.

MATERIALS AND METHODS

Birds, experimental treatments

The experiment was conducted in a modern commercial farm located in Ardestan city, Isfahan province, Iran. The trial lasted 42 d, pens with dimensions of 1.2×1.5 m and 1 m height, were randomly assigned to five dietary treatments with each having five replicates of 15 birds. Before the experiment, the poultry rearing unit was thoroughly cleaned and rinsed using pressurized. The hall was gasified after drinker and feeder installation and 24 h before starting the experiment. A total of 375 1 d-old male chickens (Ross-308), from a commercial hatchery, were selected and divided into 5 trial groups (3 replicates of 5 chickens). The study was conducted in a completely randomized design. The average body weight (BW) of broilers was 42.7 g. Chickens were vaccinated following the standard vaccination schedule, and to reduce the stress caused by vaccination to birds, 24 h before and after vaccination, a multi-electrolyte solution was added in drinking water. The lighting program for the broilers is illustrated in table 1. The three-stage diet is tailored to the proposed pattern for the Ross 308 strain (including 0-10, 11-24, and 25-42 days). The experimental diets included the different levels of extruded soybean meal (ESM) (Digesta™) (25%, 50%, 75%, and 100%) and also the control group which included the common soybean meal. Extruders vary in design, performance, and price. In this research, the extruding process was done at a temperature of 150 to 160 degrees Celsius for 15 to 20 seconds as suggested by Pathak (2010), acknowledged to be effective in reducing the levels of anti-nutrients. The feed ingredients and nutrient analysis of the basal diets are presented in table 2 to table 5.

Table-1: Lighting programs for broiler

Weeks	1	2	3	4	5	6
Light exposure (h)	22	20	20	20	22	23
Darkness(h)	2	4	4	4	2	1

This lightening program is adopted from the Ross Broiler Pocket Guide-Aviagen (2014)

Table-2: Basic chemical composition and anti-nutritional factors (ANF) of soybean meal (SBM) and its extruded form

Items	**SBM**	**Extruded SBM**
Composition		
Dry matter (%)	89.63	89.95
Crude protein (%)	44.47	44.58
Crude fiber (%)	6.56	6.24
Ether extract (%)	0.89	0.92
Total ash (%)	5.94	5.88
ANF		
Trypsin-inhibitor (units/g)	16,800	2,400
Urease activity (Δ pH units)	0.23	0.00

Table-3: Feed ingredients and nutrient analysis of the basal diet, 1-14 day of age (%, unless mentioned)

Ingredients	**C[1]**	**T1[2]**	**T2[3]**	**T3[4]**	**T4**
Corn	52.09	54.21	56.15	57.96	59.61
SBM	41.62	29.91	19.14	9.20	-
Extruded SBM	-	9.97	19.14	27.59	35.42
Soybean oil	1.94	1.50	1.10	0.73	0.39
Limestone	1.18	1.18	1.18	1.18	1.18
DCP	1.92	1.93	1.94	1.95	1.96
NaCl	0.24	0.24	0.24	0.24	0.24
SOD BICARB	0.15	0.15	0.15	0.15	0.15
Mineral premix*	0.25	0.25	0.25	0.25	0.25

Vitamin premix**	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.21	0.22	0.23	0.24	0.24
L-Lys HCl	0.12	0.14	0.16	0.18	0.20
L-Taurine	0. 03	0. 05	0. 07	0. 08	0. 10
ME (Kcal/kg)	2900	2900	2900	2900	2900
CP (%)	22.50	22.50	22.50	22.50	22.50
EE (%)	4.85	4.53	4.23	3.95	3.70
Met (%)	0.57	0.57	0.57	0.57	0.57
Met + Cys (%)	0.93	0.93	0.93	0.93	0.93
Lysine (%)	1.35	1.35	1.35	1.35	1.35
Threonine (%)	0.90	0.90	0.90	0.90	0.90
Calcium (%)	1.00	1.00	1.00	1.00	1.00
nPP (%)	0.48	0.48	0.48	0.48	0.48
Sodium (%)	0.15	0.15	0.15	0.15	0.15

[1]Control diet = ratio including soybean meal, [2]Treatment 1 = 25 % replacement of soybean meal with the extruded soybean meal (digesta), [3]Treatment 2 = 50 % replacement of soybean meal with the extruded soybean meal (digesta), [4]Treatment 3 = 75 % replacement of soybean meal with the extruded soybean meal (digesta), [5]Treatment 4 = 100 % replacement of soybean meal with the extruded soybean meal (digesta).

Abbreviations: SBM = soybean meal. DCP = di-calcium phosphate. NACL = sodium chloride.
SOD BICARB = Sodium bicarbonate. L-Lys HCl = L-Lysine hydrochloride. ME = metabolizable energy. CP = Crude Protein. EE = Ether Extract. Met = Methionine. Cys = Cysteine. nPP = Non-phytate Phosphorus

*Supplied per Kg of mixture: 1,081 mg trans-retinol; 20 mg cholecalciferol; 4 mg α-tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate acid; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.

** Supplied per Kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium; 1 mg excipient.

Table-4: Feed ingredients and nutrient analysis of the basal diet, 11-24 day of age (%, unless mentioned)

Ingredients	**C[1]**	**T1[2]**	**T2[3]**	**T3[4]**	**T4**
Corn	57.88	59.73	61.40	62.97	64.39
SBM	36.12	25.95	16.61	7.98	-
Extruded SBM	-	8.65	16.61	23.95	30.74
Soybean oil	1.87	1.49	1.15	0.82	0.53
Limestone	1.15	1.15	1.15	1.16	1.16
DCP	1.80	1.81	1.82	1.83	1.84
NaCl	0.24	0.24	0.24	0.24	0.24
SOD BICARB	0.15	0.15	0.15	0.15	0.15
Mineral premix*	0.25	0.25	0.25	0.25	0.25
Vitamin premix**	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.18	0.18	0.19	0.20	0.21
L-Lys HCl	0.10	0.12	0.14	0.15	0.17
L-Taurine	0. 01	0. 03	0. 04	0. 05	0. 07
ME (Kcal/kg)	2960	2960	2960	2960	2960
CP (%)	20.50	20.50	20.50	20.50	20.50
EE (%)	4.99	4.71	4.46	4.22	4.00
Met (%)	0.51	0.52	0.52	0.52	0.53
Met + Cys (%)	0.85	0.85	0.85	0.85	0.85
Lysine (%)	1.20	1.20	1.20	1.20	1.20
Threonine (%)	0.80	0.80	0.80	0.80	0.80
Calcium (%)	0.95	0.95	0.95	0.95	0.95
nPP (%)	0.45	0.45	0.45	0.45	0.45
Sodium (%)	0.15	0.15	0.15	0.15	0.15

[1]Control diet = ratio including soybean meal, [2]Treatment 1 = 25 % replacement of soybean meal with the extruded soybean meal (digesta), [3]Treatment 2 = 50 % replacement of soybean meal with the extruded soybean meal (digesta), [4]Treatment 3 = 75 % replacement of soybean meal with the extruded soybean meal (digesta), [5]Treatment 4 = 100 % replacement of soybean meal with the extruded soybean meal (digesta).

Abbreviations: SBM = soybean meal. DCP = di-calcium phosphate. NACL = sodium chloride.
SOD BICARB = Sodium bicarbonate. L-Lys HCl = L-Lysine hydrochloride. ME = metabolizable energy. CP = Crude Protein. EE = Ether Extract. Met = Methionine. Cys = Cysteine. nPP = Non-phytate Phosphorus

*Supplied per Kg of mixture: 1,081 mg trans-retinol; 20 mg cholecalciferol; 4 mg α-tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate acid; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.

** Supplied per Kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium; 1 mg excipient.

Table-5: Feed ingredients and nutrient analysis of the basal diet, 25-42 day of age (%, unless mentioned)

Ingredients	**C**[1]	**T1**[2]	**T2**[3]	**T3**[4]	**T4**
Corn	63.54	65.08	66.52	67.82	69.05
SBM	30.61	22.01	14.08	6.77	-
Extruded SBM	-	7.34	14.08	20.31	26.07
Soybean oil	1.85	1.53	1.23	0.96	0.71
Limestone	1.14	1.15	1.15	1.15	1.15
DCP	1.74	1.75	1.76	1.77	1.78
NaCl	0.24	0.24	0.24	0.24	0.24
SOD BICARB	0.15	0.15	0.15	0.15	0.15
Mineral premix*	0.25	0.25	0.25	0.25	0.25
Vitamin premix**	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.16	0.17	0.18	0.18
L-Lys HCl	0.08	0.09	0.11	0.12	0.13
L-Taurine	-	-	0. 01	0. 03	0. 04
ME (Kcal/kg)	3020	3020	3020	3020	3020
CP (%)	18.50	18.50	18.50	18.50	18.50
EE (%)	5.17	4.94	4.72	4.52	4.33
Met (%)	0.47	0.47	0.47	0.47	0.47
Met + Cys (%)	0.78	0.78	0.78	0.78	0.78
Lysine (%)	1.05	1.05	1.05	1.05	1.05
Threonine (%)	0.71	0.70	0.70	0.70	0.70
Calcium (%)	0.92	0.92	0.92	0.92	0.92
nPP (%)	0.43	0.43	0.43	0.43	0.43
Sodium (%)	0.15	0.15	0.15	0.15	0.15

[1]Control diet = ratio including soybean meal, [2]Treatment 1 = 25 % replacement of soybean meal with the extruded soybean meal (digesta), [3]Treatment 2 = 50 % replacement of soybean meal with the extruded soybean meal (digesta), [4]Treatment 3 = 75 % replacement of soybean meal with the extruded soybean meal (digesta), [5]Treatment 4 = 100 % replacement of soybean meal with the extruded soybean meal (digesta).

Abbreviations: SBM = soybean meal. DCP = di-calcium phosphate. NACL = sodium chloride.
SOD BICARB = Sodium bicarbonate. L-Lys HCl = L-Lysine hydrochloride. ME = metabolizable energy. CP = Crude Protein. EE = Ether Extract. Met = Methionine. Cys = Cysteine. nPP = Non-phytate Phosphorus

*Supplied per Kg of mixture: 1,081 mg trans-retinol; 20 mg cholecalciferol; 4 mg α-tocopherol acetate; 800 mg menadione; 720 mg thiamine; 2,640 mg riboflavin; 4,000 mg niacin; 12,000 mg calcium pantothenate acid; 1,200 mg pyridoxine; 400 mg folic acid; 6 mg cyanocobalamin; 40 mg biotin; 100,000 mg choline; 40,000 mg antioxidant.

** Supplied per Kg of mixture: 39,680 mg manganese; 20,000 mg iron; 33,880 mg zinc; 4,000 mg copper; 400 mg iodine; 80 mg selenium; 1 mg excipient.

Growth performance, carcass traits

The initial weight of each bird was taken before being allotted into treatments and also at the end of the experiment. The birds were given a known kg of feed in the morning and the left-over were weighed in the evening and recorded. The feed conversion ratio was measured weekly, as an index of feed utilization for each treatment group and calculated as the ratio of feed intake to weight gain. At the end of the study, the birds were killed via cervical dislocation, and the abdominal fat, livers, and gizzards, and other internal organs were separated to weigh, the gizzard was weighed after opening and removal of feed residues, and expressed as a percentage of total body weight (grams per 100 g). The thymus, bursa, and spleen were immediately removed, dry, and weighed (g) for each individual. Then the carcasses were submitted to cuts to measure the weight of carcass components as affected by the treatments.

Lymphoid organs and Immunity

Chicken's immune system lacks lymph nodes and has the bursa of Fabricius, which plays a crucial role in the immune system (Zhang *et al.*, 2019). The thymus and spleen are also important in the defense system of the body (Sharideh *et al.*, 2019). Antibody level measured using serum samples of each bird. Hemagglutination inhibition test was used to detect Influenza and Newcastle, which is a reliable indicator of the immune status of poultry, while the indirect enzyme-linked immunosorbent assay was used for evaluation of antibody titers against Bronchitis and Gumboro (De Herdt *et al.*, 2005; Morrow, 2008; Miller and Koch, 2013; Collett, 2013).

Blood biochemical and metabolic parameters

At the end of the experiment, blood samples were collected, using 2- mL syringes and 25-gauge detachable needles. The whole blood was then centrifuged at 4500 rpm for 5 minutes and serum samples were stored at -20 ° C. In the following, the samples were analyzed using Zist Shimi Co. kits for measuring Triglycerides, Cholesterol, LDL, HDL, Glucose, TP, and UA (Azadmanesh and Jahanian, 2014; Mirfendereski and Jahanian, 2015). The hormone analysis of T3 and T4 levels were measured using radioimmunoassay gamma counter described by Azadmanesh and Jahanian. (2014).

Nutrient digestibility

Apparent Ileal Digestibility of crude protein, crude fat, and ash was determined using chromic oxide or insoluble ash (Celite). The marker was added to the diets from the day- 37 and for 5 days. On day 42, the birds were slaughtered and the ileum removed and digesta flushed from the ileum using distilled water and a syringe. The precision was taken to get adequate digesta samples from the birds for chemical analysis. The samples were frozen (— 20°C), freeze-dried, finely ground, and frozen (— 20°C) before the chemical analysis (Kadim and Moughan, 1997).

Statistical analysis

This experiment was conducted in a completely randomized design (CRD) with 300 male chickens (Ross-308) allocated to five treatments and three replicates. Data were analyzed using the General Linear Models (GLM) procedures of SAS software (SAS, Version 9.1, SAS Institute). The data analysis formula was as follows: $Yij = \mu + ti + \varepsilon ij$, where Yij is the observation, μ is the general mean, ti is the effect of treatments and εij is the error. Duncan's multiple-range test and analysis of variance were used to compare the means.

RESULTS AND DISCUSSION

Growth performance, carcass traits

The results of the growth rate are shown in Table 6. The results illustrated that chickens fed diets had the replacement of 75 and 100% soybean meal with ESM had the highest ($P < 0.001$) daily overweight rates compared to other groups. Jahanian and Rasouli (2016) investigated the efficacy of extrusion of inadequately processed SBM on the performance of broiler chicks and stated that extrusion of SBM could improve its nutritive value for broiler chicks. Soybean proteins have a high biological value, relatively balanced amino acid, and a high content of unsaturated fatty acids, however, anti-nutritional factors affect their digestibility (Leeson and Summers, 2005). Thus, extrusion is a reliable processing technique for SBM, which increases ileal amino acid digestibility. Woodworth *et al*. (2001) stated that extruded SBM has greater digestible and metabolize energy than solvent-extracted SBM, and will enhance the growth rate and stimulate feed intake. The results of the feed intake are shown in Table 6. The results revealed that in the growth stage, the use of the replacement level of 50% increased the feed intake of birds significantly ($P < 0.05$) compared to other experimental groups, which could be also justified by the palatability of the diet. Hancock and Behnke (2001) mentioned that extrusion processing of diet improves its palatability. The heat treatment methods of the feed materials, if done correctly, can positively influence the digestibility of feedstuff and also can increase the palatability of the diet for the birds (Pathak, 2010; Patience, 2012; Almeida *et al*., 2014; Vaga *et al*., 2017).

In confirmation of these statements, Said (1996) reported on resource utilization that extruding technology could increase the digestibility and palatability of many agricultural and livestock wastes, making it possible to use them as alternative feed sources.

Table-6: Growth rate (GR), Feed intake (FI), and Feed conversion ratio (FCR) measurement in different stages (g)

Treatments	1-14 d GR	1-14 d FI	1-14 d FCR	15-28 d GR	15-28 d FI	15-28 d FCR	29-42 d GR	29-42 d FI	29-42 d FCR	1-42 d GR	1-42 d FI	1-42 d FCR
C^1	23.23^b	32.90	1-14 d	60.60	101.94^b	1.68	72.36^{bc}	163.28	2.25	52.06^b	99.37	1.91
T^1	23.80^b	33.76	1.42	61.13	102.83^{ab}	1.68	72.71^{bc}	164.30	2.26	52.54^b	100.29	1.91
T^2	24.09^{ab}	32.75	1.42	61.67	103.89^a	1.69	72.16^c	162.83	2.26	52.64^b	99.82	1.91
T^3	25.20^a	33.78	1.36	61.89	101.93^b	1.65	74.04^a	163.61	2.21	53.71^a	99.77	1.86
T^4	25.30^a	33.47	1.34	63.29	102.49^b	1.62	73.51^{ab}	163.52	2.23	54.03^a	99.83	1.85
SEM	0.0071	0.0894	1.32	0.2167	0.0227	0.1252	0.0214	0.7930	0.0761	0.0002	0.4766	0.0014
P.Value	0.411	0.315	0.0091	0.804	0.426	0.020	0.415	0.825	0.014	0.274	0.342	0.011

Means within each column with no common superscript differ significantly at $P < 0.05$.

[1]Control diet = ratio including soybean meal, [2]Treatment 1 = 25 % replacement of soybean meal with the extruded soybean meal (digesta), [3]Treatment 2 = 50 % replacement of soybean meal with the extruded soybean meal (digesta), [4]Treatment 3 = 75 % replacement of soybean meal with the extruded soybean meal (digesta), [5]Treatment 4 = 100 % replacement of soybean meal with the extruded soybean meal (digesta).

The results of the feed conversion ratio (FCR) are shown in Table 6. As can be seen, the use of substitution levels of 75% and 100% of ESM instead of soybean meal, respectively, influenced the daily growth rate of the birds, resulting in an improvement (P <0.01) of FCR in the first stage of the experiment. However, in the growth and final stages, dietary treatments did not have a significant effect on FCR, although, numerically (P = 0.08), the highest changes in feeding at the end of the experiment were allocated to birds fed with a diet containing 75% replacement of ESM instead of soybean meal. The use of substitution levels of 75% and 100% of ESM instead of soybean meal produced the best FCR.

The observed improvement in feed conversion ratios in chickens fed with high levels of digestion is highly related to the positive effect of this method on the digestibility of amino acids (Bandegan *et al*., 2010; Jahanian and Rasouli, 2016). The effect of different levels of ESM replacement on the relative weights of internal organs (liver and pancreas) and carcass characteristics of broilers at 42 days of age is shown in Table 6. The use of ESM in the diet decreased the relative weight of the liver but this effect was not statistically significant. The use of ESM in the diet decreased the relative weights of the pancreas in birds fed diets containing high levels of ESM (P <0.01). Also, the substitution of at least 50% diet soybean meal with ESM reduced abdominal fat (P <0.01) and the lowest amount of abdominal fat was in birds fed diets containing 75% replacement level. The cause of the relative weight loss of the pancreas can be due to the removal of inhibitors in soybean by the extrusion process. In this regard, some reports (Wilcke *et al*., 1979; Perilla *et al*., 1997) have shown that the relative weight of the pancreas in soybean-fed chickens was significantly higher than in the control group birds.

The results reveal the positive effects of extruded soybean meal on the relative weight of the breasts, thighs, and on the percentage of broiler carcasses which is largely due to the increased bioavailability of amino acids from this protein source. Numerous studies have shown that proper heat treatment, especially the extrusion process, can significantly increase the digestibility of amino acids (Woodworth *et al*., 2001; Oryschak *et al*., 2010ab; Drulyte & Orlien, 2016). It has been found that the supply of certain essential amino acids such as lysine, which have been suggested as a limiting factor for muscle growth such as breast (Leeson & Summers, 2001; Leeson & Summers, 2005), can improve the relative weight of ready-to-cook carcasses and its economic components.

The lower lipid content in broilers fed diets containing high levels of ESM may be related to higher digestibility and uptake of protein and amino acids in these birds (Woodworth *et al*., 2001; Lichovnikova *et al*., 2004; Amornthewaphat *et al*., 2005; Ayoade *et al*., 2012; Jahanian and Rasouli. 2016). Consuming higher digestible diets in terms of amino acids and providing adequate quantities of restrictive amino acids will reduce abdominal fat. Also, in a diet with higher digestibility, the microbial population responsible for fermentation of carbohydrates such as Lactobacillus bacteria will increase which will indeed positively affect the health of animals (Alleman *et al*., 2000; Leeson & Summers, 2005; Arija *et al*., 2006).

Lymphoid organs and Immunity

Table-7: Relative weights of carcass characteristics, internal organs and lymphatic organs, as affected by the different treatments at the end of the experiment

Treatments	Liver	Pancreas	AF	Breast	Thigh	Carcass		BF	Thymus	Spleen
C^1	2.28	0.26^a	1.73^a	21.97^b	19.16^b	62.34^b		0.108	0.137	0.113^{bc}
T^1	2.19	0.25^{ab}	1.67^{ab}	22.46^{ab}	19.66^{ab}	62.48^b		0.100	0.136	0.112^c
T^2	2.02	0.24^b	1.57^{bc}	22.81^a	20.02^{ab}	62.93^{ab}		0.096	0.141	0.125^a
T^3	2.07	0.24^b	1.51^c	23.17^a	19.23^b	63.75^a		0.104	0.146	0.123^{ab}
T^4	2.03	0.23^b	1.55^{bc}	22.90^a	20.75^a	63.53^a		0.109	0.132	0.120^{abc}
SEM	0.1285	0.0072	0.0095	0.0378	0.0424	0.0213		0.6699	0.3090	0.0381
P.Value	0.080	0.005	0.043	0.261	0.375	0.326		0.007	0.005	0.003

Means within each column with no common superscript differ significantly at $P < 0.05$.
Abbreviations: AF: Abdominal fat. BF= Bursa of Fabricius.

The relative weight of lymphatic organs in broiler chickens is presented in Table 7. As it is shown only spleens are affected by the different treatments. Sharideh *et al.* (2019) mentioned that the immune response of broiler is influenced by many factors including lymphatic organs. The spleen is the largest peripheral lymphoid organ in chickens, and it plays a significant role in immune responses and its development is closely related to immune function maintenance (Zhang *et al*., 2019). The use of substitution levels of 50% and 75% of ESM instead of soybean meal increased the relative weight of the spleens. Cui *et al.* (2004) demonstrated that nutrients could an impact on the weight spleen in chickens. The increased weight of the spleens under the effects of ESM could be related to the higher amino acid availability of these diets.

To investigate the effect of using ESM on immune system responses, antibody production titers against various viral antigens (including Influenza, Newcastle, Bronchitis, and Gamboro) were studied.

Table-8: Antibody titer level against different virus antigens in broiler chickens as affected by the different treatments at the end of the experiment

Treatments	Influenza (Log2)	Newcastle (Log2)	Bronchitis (Log10)	Gumboro (Log10)
C^1	3.50^b	4.40	3.93^c	5.71
T^1	4.20^b	4.50	4.25^{bc}	5.87
T^2	4.30^b	4.00	4.77^{ab}	5.46
T^3	5.20^a	4.90	4.95^a	6.27
T^4	4.30^b	4.60	5.13^a	5.57
SEM	0.0055	0.2925	0.0046	0.4830
P.Value	0.268	0.283	0.210	0.321

Means within each column with no common superscript differ significantly at $P < 0.05$.

As can be seen in Table 8, the use of different levels of ESM did not affect the antibody production titers to Newcastle disease and Gamboro virus, but 75% of ESM replacement increased the antibody titer against influenza virus. Also, the use of at least 50% ESM significantly increased ($P < 0.01$) the antibody titers against Bronchitis.

It has been found that heat treatment modulates starch structure, protein, and fiber in feedstuffs and increases the availability of digestive enzymes to their substrates, which in turn increases digestibility and nutrient uptake (Ostergard *et al.*, 1989). Higher nutrient digestibility can lead to greater access to the immune system to nutrients, which naturally results in increased responses of the system to various antigens.

This may be one of the reasons for the increase in antibody production against Influenza and Bronchitis in response to increased levels of ESM in the diet. Why the antibody titer against Newcastle and Gamboro was not affected by the experimental treatments, but the influenza titer was changed, is probably related to the age of the bird at the time of antigen induction (immune system stimulation). In other words, at an early age, the bird's sensitivity to the supply of essential amino acids is greater, and because the extruding process can lead to increased digestibility of protein and amino acids, thereby making the bird more accessible to the amino acids at an early age. It enhances immune responses (especially the antibody titer against the flu).

On the other hand, the extruding process by effectively removing or reducing the number of inhibitors such as lectins and trypsin inhibitors (Dragan *et al.*, 2008; Jahanian & Rasouli, 2016) can be effective in improving immune responses. It has been reported that crude soybeans contain inhibitors such as lectins and trypsin inhibitors (Michele *et al.*, 1999), and weaker anti-nutritional substances such as saponins, tannins, oligosaccharides, and hemoglobin (Dourado *et al.*, 2011) that these inhibitors collectively reduce feed intake, slow growth performance, and decreasing nutrient digestibility (Palacios *et al.*, 2004; Valencia *et al.*, 2009).

It is worth noting that the response of different immune responses (immune responses) to dietary changes depends on the type of stimulus antigen, the age of induction, the age of blood sampling, the type of basal diet and so many other factors.

In this connection, it has been shown that any decrease in nutrient digestibility and absorption can further affect the immune system. Thus, increasing the digestibility and absorption of nutrients (such as protein and fat, also seen in the present study) can help meet the needs of the immune system and the development of lymphatic organs (Klasing, 1998).

Blood biochemical and metabolic parameters

Table-9: Blood serum biochemical parameters and metabolic parameters (mg/dL), as affected by the different treatments at the end of the experiment

Treatments	Triglycerides	Cholesterol	LDL	HDL	Glucose	TP[1]	UA[2]	ALT (U/L)	AST (U/L)	T3 (ng/mL)	T4 (ng/mL)
C[1]	38.34^{a}	128.5	27.71	70.18	275.8^{a}	5.21^{c}	5.26^{a}	25.6	^{a}210.6	^{c}4.61	8.77
T[1]	32.82^{b}	132.1	23.83	76.25	237.6^{b}	5.43^{c}	5.15^{a}	26.6	^{b}192.0	^{bc}5.13	9.89
T[2]	35.30^{ab}	127.5	24.48	75.02	256.2^{ab}	5.54^{bc}	4.65^{b}	22.3	^{ab}198.0	^{bc}5.06	10.08
T[3]	33.74^{b}	147.4	24.03	72.97	239.6^{b}	6.02^{ab}	4.65^{b}	24.4	^{b}185.0	^{a}5.87	10.12
T[4]	32.09^{b}	131.6	23.86	71.69	235.0^{b}	6.34^{a}	4.57^{b}	21.8	^{b}187.4	^{ab}5.57	9.83
SEM	0.0428	0.1430	0.5940	0.4480	0.0394	0.0025	0.0121	0.2010	0.0074	0.0104	0.3174
P.Value	1.429	5.262	1.797	2.495	9.798	0.187	0.154	1.580	4.695	0.220	0.493

Means within each column with no common superscript differ significantly at $P < 0.05$.

Abbreviations: LDL = Low-density lipoprotein. HDL = High-density lipoprotein. TP = Total Protein. UA = Uric Acid. ALT = Alanine Transaminase. AST = Aspartate Aminotransferase. T3= Triiodothyronine Hormone. T4= Thyroxine Hormone

[1]Control diet = ratio including soybean meal, [2]Treatment 1 = 25 % replacement of soybean meal with the extruded soybean meal (digesta), [3]Treatment 2 = 50 % replacement of soybean meal with the extruded soybean meal (digesta), [4]Treatment 3 = 75 % replacement of soybean meal with the extruded soybean meal (digesta), [5]Treatment 4 = 100 % replacement of soybean meal with the extruded soybean meal (digesta).

Blood serum biochemical parameters are presented in Table 9. The highest concentrations of serum triglycerides were in birds fed the control diet (without ESM). Replacement levels of 25, 75, and 100% ESM significantly reduced serum triglycerides ($P < 0.05$). In general, Soyabean could reduce the blood triglycerides because it contains isoflavonoids which leads to the lowering of triglycerides (Yousef *et al*. 2003), and the extra effects of the extruding are because of its impact on that the amino acid composition of protein which causes some changes in cholesterol and triglycerides metabolism (Alsaftli *et al*., 2015).

Although the experimental treatments did not have a significant effect on blood serum cholesterol, low-density lipoprotein, and high-density lipoprotein concentrations, blood glucose concentration was significantly ($P < 0.05$) higher in the birds fed the control diet.

Using different levels of extruded soybean meal in the diet followed by a linear relationship increased ($P < 0.01$) total blood protein. Also, the use of at least 50% ESM instead of soybean meal in the diet caused a significant decrease ($P < 0.05$) in blood serum uric acid concentration.

The reason for the decrease in blood glucose levels in response to the use of ESM in the diet is unclear, however, probably due to the consumption of blood glucose reserves for protein synthesis and higher metabolism in these birds. But an increase in total blood protein levels can be attributed to an increase in digestibility and uptake of amino acids (Ayoade *et al*., 2012) and the provision of a proper pattern of amino acids for protein synthesis in the body. This proper pattern of amino acids, in addition to stimulating and enhancing protein synthesis, will also reduce the rate of production and excretion of uric acid (Leeson and Summers, 2001). In support of the present results, Dehghani-Tafti and Jahanian. (2016) reported that a decrease in dietary protein levels (and an appropriate amino acid imbalance) increased serum uric acid concentrations.

To evaluate the body's metabolic responses to the use of extruded soybean meal in the diet, the activity of Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) enzymes, as well as the concentrations of Triiodothyronine Hormone (T3) and Thyroxine Hormone (T4) hormones, were measured.

Blood serum metabolic parameters (mg/dL) are shown in Table 9, the use of ESM in the diet significantly ($P < 0.01$) reduced the activity of AST, and the lowest enzyme activity was assigned to birds fed diets with a 75% replacement level. In contrast, ALT activity was not affected by the experimental treatments. The decrease in AST activity in response to replacing high levels of ESM in the diet is probably due to the higher digestibility of protein and amino acids than an extruded meal (Amornthewaphat *et al*., 2005; Jahanian and Rasouli, 2016). Thus, extruded soybean meal can provide the bird with a more optimal pattern of amino acids in high quantities and make them useful for protein synthesis in the body. If this were to happen, the body would naturally need less amino acid conversion and consequently, the activity of the enzymes involved in trans-amination would be reduced (Bhagavan, 2002).

The results show that the concentration of T4 was not affected by the use of extruded soybean meal in the diet, but replacing 75 and 100% diet soybean meal with ESM significantly ($P < 0.05$) increased the concentration of T3 in comparison with the control group.

In agreement with the present results, Newkirk and Classen (2002) reported that the use of processed canola meal (roasting) increased the serum level of T3 in broiler chickens at 39 days of age. Recent researchers have found that the blood serum levels of T3 were higher in chickens than in chickens. In contrast, chick T4 was lower in chickens at 19 and 39 days of age than the chickens, which is in agreement with the changes in these two hormones in the present study.

Nutrient digestibility

The effect of using extruded soybean meal on ileum nutrient digestibility is presented in Table 4-4. The use of 75 and 100% ESM replacement levels instead of soybean meal in the diet significantly (P <0.01) increased the ileum digestibility of crude protein compared to the control group. Also, all dietary replacement levels in the diet increased crude fat ileum digestibility compared to the control group (P <0.01), and the highest crude ileum digestibility was observed in birds fed the diet based on a 75% diet level. There was no significant difference between the experimental groups in terms of ileum ash digestibility. The extruding process is known as a food processing technology that has been able to effectively increase the nutritional value of nutrients such as canola, linseed, pea, and soybean crude for broilers and pigs (Kim *et al*., 2000; Thacker *et al*., 2005; Htoo *et al*., 2008). Extrusion increases nutrient digestibility through denaturation which accomplishes high temperatures and pressures (Oryschak *et al*., 2010a), both would increase the exposure of enzyme-susceptible sites on the protein (Camire, 2001; Singh *et al*., 2007). Heat reduces anti-nutritional factors (Yáñez *et al*., 2019), and in extruding the feed is exposed to a high temperature with pressure for a short period, which may increase nutrient digestibility through accelerating physical disruption of cell walls (Meng *et al*., 2005). Hancock and Behnke (2001) also suggested that the extruding process improves the energy efficiency and palatability of the diet. In agreement with the present results, Woodworth *et al*. (2001) reported that diets containing extruded soybean meal increase ileal digestibility of the amino acids and metabolizable energy in pig's diet. Gracia *et al*. (2003) also found that applying heat treatment to barley grains increased the digestibility of dry matter, organic matter, starch, fat, and energy in broilers. The extruding improves enzyme susceptibility and digestion of feed ingredients which results in an increase in starch and protein (Jahanian, and Rasouli. 2016). Clarke and Wiseman (2007) reported that extruding soybean seed at 90, 110, 130 and 160 ° C resulted in a linear decrease in trypsin inhibitory activity and has positive impacts on ileum nutrient digestibility. Soybean processing to improve its digestibility needs to be properly controlled, because both under- and overheating may have reverse results by reducing the utilization of its nutrients (Felix, 2019), and extruding in this experiment proved to be a beneficial technique. However, part of the positive effect of ESM replacement on ileal digestibility of protein and fat can be attributed to the possible improvement of intestinal morphological parameters. In this regard, it has been reported that heat treatment, and in the particular extrusion of nutrients, can increase the height of the small intestine villi and, consequently, provide a higher level of absorption to increase nutrient uptake (Foltyn *et al*., 2013; Xie *et al*., 2013).

CONCLUSION

Using extruded soybean meal (ESM) (Digesta™) at 75 and 100% replacement levels instead of soybean meal can improve the growth performance components in broilers and had a positive effect on carcass characteristics, by reducing the amount of abdominal fat and increasing the percentage of carcass economical components (ie, thighs and muscles). The extruded soybean meal affects the diet's amino acid balance and improves nutrient digestibility, increases total serum protein levels, and reduces blood uric acid concentration. This can have a beneficial effect on reducing the environmental pollution. The use of extruded soybean meal in the diet has also the potential to improve immune responses in broilers. To conclude, the extruding technique has proven to be an effective and recommendable technique to improve soybean meal quality to be used in the poultry diet.

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REFERENCES

- Alagawany M, Attia AI, Ibrahim ZA, Mahmoud RA & El-Sayed SA. (2017). The effectiveness of dietary sunflower meal and exogenous enzyme on growth, digestive enzymes, carcass traits and blood chemistry of broilers. Environmental Science and Pollution Research, 24:12319-12327.
- Alleman F, Michel J, Chagneau AM & Leclercq B. (2000). The effects of dietary protein independent of essential amino acids on growth and body composition in genetically lean and fat chickens, British Poultry Science, 41: 214-218.
- Almeida FN, Htoo JK, Thomson J & Stein HH. (2014). Effects of heat treatment on apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Animal Feed Science and Technology, 187: 44-52.
- Alsaftli ZA, AL-Saadi MA, Subuh AM. (2015). Effect of using extruded full-fat soybean on performance and carcass characteristics in female Turkeys, Asian Journal of Animal Sciences, 9: 198-207.

- Amornthewaphat N, Lerdsuwan S & Attamangkune S. (2005). Effect of extrusion of corn and feed form on feed quality and growth performance of poultry in a tropical environment, Poultry Science, 84: 1640-1647.
- Anderson-Haferman JC, Zang Y, Parsons CM, Hymowitz T, (1992). Effect of heating on the nutritional quality of kunitztrypsin-inhibitor-free and conventional soybeans for chickens. Poultry Science, 71: 1700-1709.
- Arija I. 2006). Nutritional evaluation of raw and extruded kidney bean (Phaseolus vulgaris L. Pinto) in chickens diets. Poultry Science, 85: 635-644.
- Ayoade DI, Kiarie E, Woyengo TA, Slominski BA & Nyachoti, CM. (2012). Effect of a carbohydrase mixture on ileal amino acid digestibility in extruded full-fat soybeans fed to finishing pigs. Journal of Animal Science, 90: 3842-3847.
- Azadmanesh V & Jahanian R. (2014). Effect of supplemental lipotropic factors on performance, immune responses, serum metabolites and liver health in broiler chicks fed on high-energy diets. Animal Feed Science and Technology, 195: 92-100.
- Bandegan A, Kiarie E, Payne RL, Crow GH, Guenter W & Nyachoti CM. (2010). Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. Poultry Science, 89: 2626-2633.
- Bhagavan NV. 2002). Medical Biochemistry. 4^{th} ed. Harcourt/Academic Press, London, UK.
- Bingol NT, Dede S, Karsli MA, Deger Y, Kilinc DK & Cetin S. (2016). Effects of the Replacement of Soybean Meal with Pea as Dietary Protein Source on the Serum Protein Fractions of Broilers. Brazilian Journal of Poultry Science, 18: 639-43.
- Bos C & Flikweert S. (1996). Effect on broiler performance of increasing amounts of fullfat soybeans in the diet. In 2 nd International Fullfat Soya Conference. Budapest, Hungary. pp: 266-275.
- Camire ME. (2001). Extrusion and nutritional quality. Pages 108– 130 in Extrusion Cooking: Technologies and Application. R. Guy, ed. Woodland Publishing, Cambridge, MA.
- Chunmei G, Hongbin P, Zewei S & Guixin Q. (2010). Effect of soybean variety on anti-nutritional factors content, and growth performance and nutrients metabolism in rat. International Journal of Molecular Sciences, 11: 1048-1056.
- Clarke E & Wiseman J. (2005). Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. Animal Feed Science and Technology, 121: 125-138.
- Clarke E & Wiseman J. (2007). Effects of extrusion conditions on trypsin inhibitor activity of full fat soybeans and subsequent effects on their nutritional value for young broilers. British Poultry Science, 48: 703-712.
- Collett SR. 2013). Principles of disease prevention, diagnosis, and control introduction. In: Swayne DE (Eds). Diseases of poultry. 13th ed. Oxford, UK: Wiley-Blackwell; 4-60.
- Cui H, Xi P, Junliang D, Debing L & Guang Y. (2004). Pathology of lymphoid organs in chickens fed a diet deficient in zinc. Avian Pathology, 33: 519–524.
- De Bruyn J. (2017). Healthy chickens, healthy children? Exploring contributions of village poultry- keeping to the diets and growth of young children in rural Tanzania. Doctor of Philosophy thesis submitted to the University of Sydney, 1 December 2017.
- De Herdt P, Jagt E, Paul G, Van Colen S, Renard R, Destrooper C & van den Bosch G. (2005). Evaluation of the enzyme-linked immunosorbent assay for the detection of antibodies against infectious bursal disease virus and the estimation of the optimal age for IBDV vaccination in broilers. Avian Pathology, 34: 501-504.
- Dehghani-Tafti N & Jahanian R. (2016). Effect of supplemental organic acids on performance, carcass characteristics, and serum biochemical metabolites in broilers fed diets containing different crude protein levels. Animal Feed Science and Technology, 211: 109-116.
- Dourado LRB, Pascoal LAF, Sakomura NK, Costa FGP & Biagiotti D. (2011). Soybeans (Glycine max) and soybean products in poultry and swine nutrition. In: Krezhova D. (ed.): Recent Trends for Enhancing the Diversity and Quality of Soybean Products. InTech Publishing, Rijeka, Croatia, 175-190.
- Dragan VP, Palic DL, Slavica AS & Olivera, MD. (2008). Quality control of full-fat soybean using urease activity: Critical assessment of the method. BIBLID, 39: 47-53.
- Drulyte D & Orlien V. (2019). The effect of processing on digestion of legume proteins. Foods, 8: 224-230.
- Felix PA, Menezes Souza CM, Bastos TS, Bill Kaelle GC, Oliveira, CG & Maiorka A. (2019). Digestibility of raw soybeans in extruded diets for dogs determined by different methods. Journal of Animal Science, 11: 4598-4607.
- Foltyn M, Rada V, Lichovníková M, Šafařík I, Lohniský A & Hampel D. (2013). Effect of extruded full-fat soybeans on performance, amino acids digestibility, trypsin activity, and intestinal morphology in broilers. Czech Journal of Animal Science, 58: 470-478.
- Gracia MI, Latorre MA, García M, Lázaro R & Mateos GG. (2003). Heat processing of barley and enzyme supplementation of diets for broilers. Poultry Science, 82: 1281-1291.

- Hancock JD & Behnke KC. (2001). Use of ingredient and diet technologies (grinding, mixing, pelleting, and extruding) to produce quality feeds for pigs. Pages 469–492 in Swine Nutrition. 2nd ed. A. J. Lewis and L. L. Southern, ed. CRC Press, Boca Raton, FL.
- Htoo JK, Araiza BA, Sauer WC, Rademacher M, Zhang Y, Cervantes M & Zijlstra RT. (2008). Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. Journal of Animal Science, 85: 3303-3312.
- Jahanian, R & Rasouli E. (2016). Effect of extrusion processing of soybean meal on ileal amino acid digestibility and growth performance of broiler chicks. Poultry Science, 95: 2871-2878.
- Kadim IT & Moughan PJ. (1997). Development of an ileal amino acid digestibility assay for the growing chicken-effects of time after feeding and site of sampling. British Poultry Science, 38: 89-95.
- Kim IH, Hancock JD, Hines RH & Gugle TL. (2000). Roasting and extruding affect nutrient utilization from soybeans in 5 and 10 kg nursery pigs. Asian-Australasian Journal of Animal Sciences, 13: 200-206.
- Klasing KC. (1998). Nutritional modulation of resistance to infectious disease. Poultry Science, 77: 1119-1125.
- Leeson S & Summers JD. (2001). Scott's Nutrition of the Chickens. 4th ed. Uni. Book, Guelph, Ontario, Canada.
- Leeson S & Summers JD. (2005). Commercial Poultry Nutrition. 3rd ed. Nottingham University Press, Nottingham, UK.
- Lichovnikova M, Zeman L, Kracmar S & Klecker D. (2004). The effect of the extrusion process on the digestibility of feed given to laying hens. Animal Feed Science and Technology, 116: 313-318.
- Mateos GG, Latorre MA & Lazaro R. (2003). Processing Soybean. American Soybean Association, Madrid, Spain.
- Meng X, Slominski BA, Nyachoti CM, Campbell LD & Guenter W. (2005). Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poultry Science, 84: 37-47.
- Michele WD, Parsons CM & Hymowitz T. (1999). Nutritional evaluation of lectin-free soybeans for poultry. Poultry Science, 78: 91-95.
- Miller PJ & Koch G. Newcastle Disease. (2013). In: Swayne DE (Eds). Diseases of poultry. 13th ed. Oxford, UK: WileyBlackwell; 68-107.
- Mirfendereski E & Jahanian R. (2015). Effects of dietary organic chromium and vitamin C supplementation on performance, immune responses, blood metabolites, and stress status of laying hens subjected to high stocking density. Poultry Science, 94: 281-288.
- Morrow C. (2008). Laboratory investigation to support health programs and disease diagnosis. In: Pattison M, McMullin PF, Bradbury JM, *et al*. (Eds) Poultry diseases. 6th ed. London, UK: Saunders Elsevier; 39-47.
- Mwaniki ZN & Kiarie E. (2018). Standardized ileal digestible amino acids and apparent metabolizable energy content in defatted black soldier fly larvae fed to broiler chickens. Canadian Journal of Animal Science. Canadian Journal of Animal Science, 99: 211-217.
- Nabizadeh A. (2018). Effects of soybean meal substitution with isolated soya protein in pre-starter diet on immune system and performance of broiler chickens. Journal of Animal and Feed Sciences. 27: 263-267.
- Newkirk RW & Classen HL. 2002). The Effects of toasting canola meal on body weight, feed conversion efficiency, and mortality in broiler chickens. Poultry Science, 81: 815-825.
- Oryschak M, Korver D, Zuidhof M & Beltranena E. (2010). Nutritive value of single-screw extruded and nonextruded triticale distillers dried grains with solubles, with and without an enzyme complex, for broilers. Poultry Science, 89: 1411-1423.
- Oryschak M, Korver D, Zuidhof M, Meng X & Beltranena E. (2010). Comparative feeding value of extruded and nonextruded wheat and corn distillers dried grains with solubles for broilers. Poultry Science, 89: 2183-2196.
- Ostergard K, Bjorck I & Vainionpaa J. (1989). Effects of extrusion cooking on starch and dietary fiber in barley. Food Chemistry, 34: 215-227.
- Palacios MF, Easter RA, Soltwedel KT, Parsons CM, Douglas MW, Hymowitz T & Pettigrew JE. (2004). Effect of soybean variety and processing on growth performance of young chicks and pigs. Journal of Animal Science, 82: 1108-1114.
- Pathak N. (2010). Textbook of Feed Processing Technology. 2nd rev. ed. IBDC Publishers, Lucknow, India.
- Patience JF. 2012). Feed Efficiency in Swine. Wageningen Academic Publishers, Wageningen, The Netherlands.
- Perilla NS, Cruz MP, De Belalcuzar, F & Diaz GJ. (1997). Effect of temprature of wet extrution on the nutritinal value of full fat soybeans for broiler chickens. British Poultry Science, 38: 412-416.
- Rada V, Lichovnikova M & Safarik I. (2017). The effect of soybean meal replacement with raw full-fat soybean in diets for broiler chickens. Journal of Applied Animal Research, 45:112-117.
- Ravindran V. (2010). Poultry Feed Availability and Nutrition in Developing Countries – Alternative Feedstuffs for use in Poultry Feed Formulations. Food and Agriculture Organization of the United Nations Poultry Development Review; 2010. [Online] Available from: http://fao.org/docrep/013/al706e/ al706e00.pdf

- Said NW. (1996). Extrusion of alternative ingredients: an environmental and a nutritional solution. Journal of Applied Poultry Research, 5: 395-407.
- Sayed WAA, Ibrahim NS, Hatab MH, Zhu F, Rumpold BA. (2019). Comparative Study of the Use of Insect Meal from Spodoptera littoralis and Bactrocera zonata for Feeding Japanese Quail Chicks. Animals, 9: 136.
- Sharideh H, Zhandi M, Zaghari M, Akhlaghi A, Hussaini S M H, Yousefi A R. (2019). The Iranian Journal of Veterinary Research, 20: 120-125.
- Singh VJ, Rausch KD, Yang P, Shapouri HR, Belyea L & Tumbleson ME. (2001). Modified dry-grind ethanol process. Public. No. 2001-7021. University of Illinois at Urbana-Champaign, Urbana, IL.
- Subuh AMH, Motl MA, Fritts, CA & Waldroup, PW. (2002). Use of various ratios of extruded fullfat soybean meal and dehulled solvent extracted soybean meal in broiler diets. International Journal of Poultry Science, 1: 9-12.
- Sudaryono A. (2006). Use of Azolla (Azolla pinnata) Meal as a substitute for defatted Soybean Meal in diets of Juvenile Black Tiger Shrimp (Penaeus monodon). The Journal of Coastal Development, 9: 145-154.
- Thacker PA, Willing BP & Racz VJ. (2005). Performance of broiler chicks fed wheat-based diets supplemented with combinations of non-extruded or extruded canola, flax and peas. Journal Animal Veterinary Advances, 4: 902-907.
- Vaga M, Hetta M & Huhtanen P. (2016). Effects of heat treatment on protein feeds evaluated in vitro by the method of estimating utilisable crude protein at the duodenum. Journal of Animal Physiology and Animal Nutrition, 101: 1259-1272.
- Valencia DG, Serrano MR, Lazaro R, Jiménez-Moreno E & Mateos GG. (2009). Influence of micronization (fine grinding) of soya bean meal and full-fat soya bean on the ileal digestibility of amino acids for broilers. Animal Feed Science and Technology, 150: 238-248.
- Wilcke HL, Hopkins DT & Waggle D H. (1979). Soya Protein and Human Nutrition. Academic Press, New York, NY, USA.
- Woodworth JC, Tokach MD, Goodband RD, Nelssen JL, O'Quinn PR, Knabe DA & Said N W. (2001). Apparent ileal digestibility of amino acids and the digestible and metabolizable energy content of dry extruded-expelled soybean meal and its effects on growth performance of pigs. Journal of Animal Science, 79: 1280-1287.
- Xie XX, Meng QX, Liu P, Wu H, Li SR, Ren LP, Li XZ. (2013). Effects of a mixture of steam-flaked corn and extruded soybeans on performance, ruminal development, ruminal fermentation, and intestinal absorptive capability in veal calves. Journal of Animal Science, 91: 4315-4321.
- Yáñez JY, Woyengo TA, Jha R, Van Kempen TATG, Zijlstra RT. (2019). Nutrient digestibility of soybean products in grower-finisher pigs. Journal of Animal Science, 97: 4598-4607.
- Yousef MI, El-Demerdash FM, Kamel KI & Al-Salhen KS. (2003). Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology, 189: 223-234.
- Zarkadas LN & Wiseman J. (2005. Influence of processing of full fat soya beans included in diets for piglets. I. Performance. Animal Feed Science and Technology, 118: 109-119.
- Zhang Q, Sun X, Wang T, Chen B, Huang Y, Chen H & Chen Q. (2019). The Postembryonic Development of the Immunological Barrier in the Chicken Spleens. Journal of Immunology Research, 4: 1-10.

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