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Original Article

Effect of Different Thermal and Non-Thermal Processing Methods on Chemical Composition, Quality Indicators and Apparent Nutrient Digestibility of Full-Fat Soybean

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ABSTRACT

The aim of this research was to study the effect of thermal and non-thermal processing methods on chemical composition, quality indicators and apparent digestibility of soybean nutrients. For this, four processing methods (unprocessed, extruded, thermal, and gamma radiation) were applied on two soybean varieties (Katol & Caspian). Rations were formulated based on kinds of soybean (processed and unprocessed). The experiment was continued using 600 male Ross broiler chickens during 3 feeding phases (starter, grower, and finisher). Traits were measured and analyzed with the Lsmeans procedure by SAS software. The results showed the interaction effects of soybean varieties and processing methods were significant on the crude fiber, calcium, and phosphorus ($p<0.05$). The effect of soybean variety was significant on the crude protein, crude fiber, and phosphorus ($p<0.05$). Also, the effect of processing methods was significant on the dry matter, crude fiber, and phosphorus ($p<0.05$). The effect of soybean variety, processing methods, and their interaction were significant on urease activity, KOH protein solubility, and Protein dispersibility index ($p<0.05$). The results of the current study showed that the best soybean variety and the processing method is the Caspian and thermal, respectively.

INTRODUCTION

The world production of processed feed is about one billion tons per year. A large part of the feed is processed in a form or another. Furthermore, even if the complete feed is not produced, many feed components are processed using feed processing techniques before the feed is consumed. As a result, the feed processing effect on the feed nutritional value is important to take into consideration. Before the particle size reduction and digestibility of feed ingredients increase, they are ground (Wondra *et al.*, 1995). Also, maybe feed ingredients are processed by thermal method to reduce the effect of the anti-nutritional factors, but heating effects on energy and nutrient digestibility may be negative or positive (Herkelman *et al.*, 1992). Examples of processing techniques include expander processing, pelleting, and extrusion (Lundblad *et al.*, 2011). The soybean is by far the most important oilseed crop in the world. Its crude protein content with the high content of essential amino acids and the oil content is 38% and 18%, respectively. Also, the fiber content of soybean is low. Soybean is used as a protein source in animal feed (Garnsworthy and Wiseman, 2009). If the soybean oil is not extracted, it is known as full-fat soybean. There are important points in the use of full-fat soybeans in chicken rations. Other than eliminating the oil extraction cost, the high-energy rations can be formulated (Simovic *et al.*, 1972).

The use of raw soybeans in chicken rations was limited by the presence of heat-labile trypsin inhibitors and other anti-nutritional factors (Offiong, 1984). These factors make growth depression and pancreatic hypertrophy in many animals (Birk, 1988). It was shown that growth depression is caused by the combined effect of essential amino acids loss (especially amino acids contained sulfur) and decreased intestinal proteolysis (caused by trypsin inhibitors) in raw soybeans (Tan-Wilson & Wilson, 1986). To improve the nutritional value of full-fat, soybean should be treated by heat before using it in the chicken ration. Some of the heat processing methods of full-fat soybean are extrusion, micronization, and jet Sploding (Marty & Chavez, 1993). Researchers documented that with the increase in the cooking duration of soybean, apparent digestibility and retention of dietary dry matter, nitrogen, and ether extract increased (Vandergrift *et al.*, 1983.; Balloun, 1980; and Kaankuka *et al.*, 1996). A study reported that ileal digestibility of dry matter, gross energy, and nitrogen were greatest and lowest for extruded and roasted soybean, respectively (Kim *et al.*, 2000). The nutritional quality of soybean can be increased by processing methods of toasting, cooking, extruding, salt treatment, fermentation, germination pressure cooking, cooking, soaking, and urea treatment (Akande & Fabiyi, 2010; Milczarek *et al.*, 2017).

Therefore, this study aimed to investigate the effect of different thermal and non-thermal processing methods on chemical composition, quality indicators and apparent digestibility of nutrients of soybean.

MATERIALS AND METHODS

Chicken

In the present study, 600 male broiler chickens of Ross 308 were randomly distributed into 8 treatments with 5 replicates per treatment, in a total of 15 birds per treatment. The experiment started with one-day-old chicks, which were randomly assigned into groups with similar mean body weights, with a starting average weight of 42 g. The experimental rations were maintained until the age of 42 days. Birds were given ad libitum access to water and feed. The chickens were fed the isocaloric and isonitrogenous starter, grower and finisher rations formulated according to Aviagen recommendations for Ross 308 broiler chickens. The basal ration is presented in table 1. The amount used of processed and unprocessed soybean varieties in the starter, grower, and finisher rations were 5, 10 and 15%, respectively. In each ration, soybean meal is replaced with the full-fat soybean.

Table 1 – Composition of starter, grower and finisher rations (%).

Ingredient	(starter) 1 to 10 days	(grower) 11 to 24 days	(finisher) 25 to 42 days
Corn	56.80	65.20	66.76
Soybean meal	36.00	29.40	28.16
Soybean oil	3.00	1.91	1.31
Sodium chloride	0.44	0.42	0.41
Limestone	1.36	1.05	1.05
Dicalcium phosphate	1.66	1.32	1.35
DL-methionine	0.14	0.10	0.11
L-Lysine HCL	0.10	0.10	0.10
Min + Vit premix	0.50	0.50	0.50
Calculated content			
ME (kcal/g)	2950	3000	3050
Crude protein (%)	22.68	20.69	19.06
Linoleic acid (%)	1.22	1.14	0.95
Crude fiber (%)	3.36	3.33	3.23
Calcium (%)	1.02	0.86	0.81
Available P (%)	0.49	0.43	0.40
Sodium (%)	0.19	0.18	0.17
Arginine (%)	1.28	1.09	0.97
Lysine (%)	1.24	1.05	0.92
Met + Cys (%)	0.92	0.80	0.72
Threonine (%)	0.81	0.70	0.62
Tryptophan (%)	0.20	0.17	0.15

L-Lysine HCL= L-Lysine Hydrochloride. Min + Vit premix= mineral and vitamin premix supplied per kilogram of diet= vitamin A: 10000 IU, vitamin D3: 9790 IU, vitamin E: 121 IU, vitamin B12: 20 µg, riboflavin: 4.4 mg, calcium pantothenate: 40 mg, niacin: 22 mg, choline: 840 mg, biotin: 30 µg, thiamin: 4 mg, zinc sulfate: 60 mg, copper sulfate: 100 µg, selenium (sodium selenate): 0.2 mg, iodine: 1 mg, manganese oxide: 60 mg. ME= Metabolizable energy. Met= Methionine. Cys= Cysteine.

Processing methods of soybean rations

All thermal and non-thermal processing was performed at the Nutrition Laboratory of the agriculture faculty of Islamic Azad University, Qaemshahr Branch. For the heat processing, samples of 1.5 kg were placed into the center of trays and then distributed homogeneously over the tray's surface to achieve uniformity during treatment. After the thermal processing, the soybean was cooled in a different tray. Then, it was transferred into plastic bags and stored at a suitable temperature (25°C, 60% humidity). In this experiment two soybean varieties were used (Katol & Caspian). The soybean samples were wet extruded at 145, 155 and 165°C for 15s by an extruder system (Yemmak Co. Turkey). Soybean seeds were irradiated with the 1, 2, 4, and 10 kGy doses of gamma radiation. The dose rate was 228Gy/min (Štajner *et al.*, 2007).

Apparent nutrient digestibility

On day 15 a digestibility trial was carried out on the chickens. Weighed quantities of rations were supplied, and excreta were collected over 72 h in plastic sheeting placed under the wire mesh of the cage by the total collection method. Excreta samples were oven dried (70ºC for 20 h), weighed, ground and stored in airtight Kilner jars (Apata, 2008). Duplicate samples of rations and dried excreta were analyzed for proximate components using Association of official analytical chemists (1990) methods. Then, the values were used to compute apparent nutrient digestibility (such as dry matter, crude ash, crude fat, crude fiber, and crude protein).

With the combination of factors' levels under study, eight treatments were studied as follows:

1) control, un-processed soybean with Katol soybean, 2) extruded soybean with Katol soybean, 3) thermal soybean with Katol soybean, 4) gamma soybean with Katol soybean, 5) control, un-processed soybean with Caspian soybean, 6) Extruded soybean with Caspian soybean, T) thermal soybean with Caspian soybean and 8) Gamma soybean with Caspian soybean.

Quality indicators

Urease activity was determined according to the AOCS (1997) official procedure. Two hundred milligrams of full-fat soybean sample was incubated in 10.0 ml of phosphate buffered urea solution at 300 C for 30 minutes, after which the increase in pH was recorded from 7.00.

KOH protein solubility determination

Protein solubility was determined in potassium hydroxide as follows: 1.5 g (± .001 g) of a full-fat soybean sample, ground in a Udy mill (Udy Corporation, Boulder, CO), so it would pass through a 0.5 mm screen, mixed with 75 ml of 0.2% (0.036 normal, pH= 12.5) potassium hydroxide, then stirred for 20 min on a magnetic plate. The mixture was centrifuged at 2700 rpm for 15 min. The supernatant was decanted, avoiding centrifugation, and filtered through glass wool. About 40 mL were recovered in a 50 mL beaker. Fifteen milliliters, in duplicate from a single filtrate, was transferred to Kjeldahl tubes giving a 0.3-g aliquot of the original sample (1.5 g x 15 mL per 75 ml). Twelve-and-a-half ml of concentrated H2SO4, 2 Kjeltabs, and 2 ml of 30 % H2O2 were added to each tube. Total nitrogen was determined by the Kjeldahl method, and the protein content was calculated. For the original samples, the crude protein content was also determined. Protein solubility was determined as a percentage of the total protein soluble in the 2 % solution of potassium hydroxide (Araba & Dale, 1990).

Protein dispersibility index (PDI) determination

The PDI for the full-fat soybean was determined according to method AOCS (1997). For this, a 20 g sample was weighed, and processed for 10 min at 8500 rpm in distilled water (pH= 7.0) at 25°C. The mixture was centrifuged for 10 min at 1400 rpm and room temperature (24°C), and the supernatant nitrogen was determined. Results were expressed as the percentage of dispersible protein respect to the protein content of the original sample.

Statistical analysis

The main and interactions effects of the soybean varieties (Katol and Caspian) and its processing methods (unprocessed, extruded, thermal, and gamma radiation) were studied in a 2×4 factorial experiment in form of completely randomized design. Data analysis was done with the Lsmeans procedure by SAS package (2001).

The statistical model was as follows:

$y_{ijk}= \mu + A_i+ B_j+ (AB)_{ij}+ e_{ijk}$

Where y_{ijk}= the value of each observation, μ= mean effect, A_i= effect of soybean varieties (Katol and Caspian), B_j= processing methods of soybean (unprocessed, extruded, thermal, and gamma radiation), $(AB)_{ij}$= interaction effect of soybean varieties and processing methods of soybean, and e_{ijk}= residual effects.

RESULTS AND DISCUSSION

As shown in table 2, the effect of soybean variety was significant on crude protein, crude fiber, and phosphorus ($p<0.01$). Also, the effect of processing methods was significant on dry matter, crude fiber, and phosphorus ($p<0.01$). The interaction effect of soybean variety and processing methods was significant on crude fiber, calcium, and phosphorus ($p<0.05$). The highest and the lowest amount of crude fiber were in Katol soybean without processing and extruded Caspian soybean, respectively. The highest and lowest calcium was in thermal Katol soybean and extruded Caspian soybean respectively. The highest and lowest phosphorus was in thermal Caspian soybean and extruded Katol soybean respectively. In soybean variety, the highest and the lowest crude protein and phosphorus content were found in Caspian and Ketal, and the highest and lowest amount of raw fiber was found in Katol and Caspian, respectively. The highest and the lowest amount of dry matter and phosphorus were observed in thermal processing and gamma as well as the highest and the lowest crude fiber, respectively, without processing and heat treatment, respectively. Results of Maidala *et al.* (2013) and Milczarek *et al.* (2017) showed in their studies that there was an increase in crude protein, crude fiber and crude fat of differently processed soybeans. There was a decrease in the nitrogen-free extract, calcium, and phosphorus of differently processed soybean. The chemical compositions and functional properties of the full-fat soybean were influenced by processing and soybean variety. Suitable processing techniques may enhance the utilization of soybean (Ukwuru, 2003). When comparing Full-fat soybean (FFSB) with the extruded FFSB product, it seems likely that the high residence time during micronization in combination with the lack of any extra moisture may have resulted in more severe destruction of protein as expressed by the lower available lysine (Zarkadas & Wiseman, 2005).

Table 2 – Effect of experimental treatments on chemical composition of ration's soybean (%).

Treatments	Dry matte	Ash crude	Crude protein	Crude fiber	Crude fat	Calcium	Phosphorus
Soybean variety							
Katol	92.18	5.63	35.19[b]	9.16[a]	20.72	0.23	0.53[b]
Caspian	92.25	5.94	37.38[a]	8.19[b]	21.05	0.22	0.55[a]
p Value	0.90	0.11	0.00	0.00	0.66	0.86	0.00
SEM	0.19	0.04	0.10	0.03	0.13	0.14	0.00
Processing method							
Unprocessed	90.70[b]	5.99	36.34	10.72[a]	20.35	0.24	0.55[a]
Extruded	93.35[a]	5.84	36.04	7.69[c]	21.67	0.22	0.53[b]
Thermal	94.22[a]	5.55	36.42	7.63[c]	21.17	0.23	0.56[a]
Gamma radiation	90.60[b]	5.77	36.35	8.67[c]	20.35	0.24	0.53[b]
p Value	0.00	0.39	0.14	0.00	0.53	0.36	0.00
SEM	0.25	0.08	0.14	0.07	0.20	0.21	0.01
Interaction of soybean variety × processing method							
Katol × Unprocessed	91.65	5.94	35.26	11.10[a]	20.56	0.23[ab]	0.54[c]
Katol × Extruded	92.80	5.62	34.64	8.26[b]	21.35	0.20[b]	0.51[d]
Katol × Thermal	93.88	5.57	35.56	8.21[b]	20.45	0.25[a]	0.54[c]
Katol × Gamma radiation	90.50	5.40	35.30	9.08[ab]	20.55	0.24[a]	0.53[c]
Caspian × Unprocessed	91.43	6.07	37.58	9.31[ab]	21.43	0.23[a]	0.56[ab]
Caspian × Extruded	93.00	6.00	37.00	7.00[b]	20.00	0.23[ab]	0.55[bc]
Caspian × Thermal	94.65	5.52	37.28	7.06[b]	21.90	0.21[ab]	0.57[a]
Caspian × Gamma radiation	90.70	6.15	37.40	8.27[b]	20.16	0.23[ab]	0.53[c]
p Value	0.23	0.43	0.67	0.00	0.77	0.05	0.02
SEM	1.13	0.67	0.21	0.09	0.32	0.37	0.07

[a,b] Means within a row with different superscripts differ significantly; $p<0.05$. SEM: Standard error of the mean. *p* Value = probability value. SEM= standard error of mean.

The effect of processing methods was significant on apparent nutrient digestibility factors such as crude protein, ether extract, and ash ($p<0.05$) (Table 3). Interaction effect of soybean variety and processing methods was significant on dry matter, crude protein, and ether extract ($p<0.05$) (Table 3). The results of a study on broiler chickens showed that the apparent digestibility of nutrients was affected by the dietary treatments in raw and roasted soybeans (Rocha *et al.*, 2014), that was consistent with the results of this study. Parsons *et al.* (1992) evaluated the effects of over-heating on the availability of soybean meal amino acids in broilers and found that the true digestibility of several amino acids decreased as autoclaving time increased from 0 to 40 min. In a study, digestibility of dry matter, Neutral Detergent Fiber (NDF), and crude protein did not differ in ground soybean and roasted soybean (Andrade *et al.,* 2015). Zhang *et al.* (1993) reported that extruding significantly increases the digestibility of soybeans. Lichovnikova *et al.* (2004) showed that extrusion processing significantly increased the apparent digestibility of crude fat and crude protein in the poultry. Extrusion can not only reduce anti-nutrients in full-fat soybean to a large extent, but also, using the high pressure it produces, can pressure the seed and, by breaking down, digestible nutrients, and the metabolizable energy of the seeds to improve (Perilla *et al.,* 1997). In general, heating improves the digestibility of proteins by inactivating enzyme inhibitors and denaturing the protein that may expose new sites for enzyme attack (Camire *et al.*, 1990). Danicke *et al.* (1998) found that increasing the temperature of the thermal treatments resulted in an increase in crude fat digestibility. Dahlin and Lorenz

(1993) observed a beneficial effect of extrusion on protein digestibility of cereal-grain in vitro. The result of a study showed that roasted full-fat soybeans had no effect on nutrient digestibility of dry matter. crude protein, and crude fiber, which was consistent with the results of this study (Hamilton & McNiven, 2000).

Table 3 – Effect of experimental treatments on apparent nutrient digestibility of soybean (%).

Treatments	Dry matter	Crude protein	Crude fiber	Ether extract	Ash
Soybean variety					
Katol	79.74	79.07	84.62	31.42	67.14
Caspian	79.22	78.58	84.04	31.84	67.93
p Value	0.61	0.53	0.35	0.31	0.81
SEM	0.41	0.29	0.27	0.08	0.44
Processing method					
Unprocessed	77.96	71.37[c]	84.70	28.54[c]	67.65[ab]
Extruded	80.82	83.22[a]	84.30	33.07[a]	69.02[a]
Thermal	78.93	82.76[a]	84.22	33.09[a]	66.01[b]
Gamma radiation	80.22	77.94[b]	84.11	31.82[b]	65.47[b]
p Value	0.22	0.00	0.90	0.00	0.04
SEM	0.50	0.38	0.30	0.19	0.43
Interaction of soybean variety × processing method					
Katol × Unprocessed	78.24[ab]	71.90[d]	85.24	28.81[c]	68.32
Katol × Extruded	81.71[a]	83.25[a]	84.21	32.77[ab]	67.60
Katol × Thermal	75.94[b]	81.92[ab]	83.50	32.71[ab]	68.00
Katol × Gamma radiation	81.14[a]	78.78[bc]	85.31	31.39[b]	64.71
Caspian × Unprocessed	77.68[ab]	70.84[d]	84.17	28.27[c]	66.99
Caspian × Extruded	79.69[ab]	82.97[a]	84.64	33.30[ab]	68.78
Caspian × Thermal	80.23[ab]	83.39[a]	84.45	33.54[a]	65.74
Caspian × Gamma radiation	79.29[ab]	77.11[c]	82.92	32.25[ab]	66.22
p Value	0.00	0.00	0.27	0.03	0.70
SEM	0.65	0.53	0.49	0.31	0.56

[a,b] Means within a row with different superscripts differ significantly; $p<0.05$. SEM: Standard error of the mean. *p* Value= probability value. SEM= standard error of mean.

The effect of soybean variety, processing methods, and interaction of them were significant on urease activity, KOH protein solubility, and protein dispersibility index ($p<0.05$) (Table4). Protein quality indicators show that some of the samples may be over-processed or less processed. According to Batal *et al.* (2000), the protein differentiation index (PDI) of 45% for soybeans is suitable for thermal processing, which results in good growth performance in broiler chickens. Batal and *et al.* (2000) reported that the PDI index was an appropriate response to heat soybean, while Dudley-Kash (2001) stated that the PDI index could show better soybean quality than other indicators.

In conclusion, the thermal method and the use of soybean Caspian improved the quality indicators of soybean measured in this study. The apparent digestibility of dry matter, crude protein and crude fiber in the extruded process showed better results. The thermal processing in comparison with others has the best result on soybean, especially on Caspian variety. In general, Caspian soybeans and thermal processing methods are recommended for replacing soybean meal in broiler chickens.

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Table 4 – Effect of experimental treatments on quality indicators of soybean.

Treatments	Urease activity	KOH protein solubility (%)	Protein dispersibility index (%)
Soybean variety			
Katol	86.07[a]	0.92[a]	36.99[a]
Caspian	84.82[b]	0.75[b]	34.85[b]
p Value	0.00	0.00	0.00
SEM	0.11	0.00	0.09
Processing method			
Unprocessed	94.52[a]	1.99[a]	59.15[a]
Extruded	81.59[c]	0.11[d]	22.53[c]
Thermal	78.60[d]	0.15[c]	20.05[d]
Gamma radiation	87.06[b]	1.08[b]	30.93[b]
p Value	0.00	0.00	0.00
SEM	0.15	0.00	0.07
Interaction of soybean variety × processing method			
Katol × Unprocessed	95.52[a]	2.18[a]	60.65[a]
Katol × Extruded	82.58[b]	0.13[b]	24.08[b]
Katol × Thermal	79.60[ab]	0.16[b]	18.41[b]
Katol × Gamma radiation	86.56[b]	1.22[ab]	34.83[b]
Caspian × Unprocessed	89.35[ab]	1.22[ab]	52.14[ab]
Caspian × Extruded	80.19[b]	0.10[b]	20.89[b]
Caspian × Thermal	77.61[c]	0.13[b]	19.70[b]
Caspian × Gamma radiation	87.56[ab]	0.94[ab]	31.05[b]
p Value	0.01	0.00	0.00
SEM	0.23	0.06	0.10

[a,b] Means within a row with different superscripts differ significantly; $p<0.05$. *p* Value = probability value. SEM= standard error of mean. KOH = potassium hydroxide.

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