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# Intestinal Characteristics, Alkaline Phosphatase and Broilers Performance in Response to Extracted and Mechanical Soybean Meal Replaced by Fish Meal

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## ABSTRACT

**An experiment was conducted to evaluate the extracted (ES) and mechanical (MS) soybean meals two levels 25% and 35% in broiler chicken feeding. Three hundred and fifty two one-day-old unsexed Lohman chickens were placed in four treatments by 4 replicates and 22 chickens in each in a 2×2 factorial arrangement. Feed intake was increased significantly in starter period by ES in comparison with MS. Feed conversion ratio was significantly improved when 25% of ES was replaced by fish meal compared with 35% of ES in starter period. Body weight was significantly higher by 25% ES replaced by fish meal in comparison with 25 and 35% MS in grower period. Metabolizable energy was declined in all options (AME, AMEn, TME and TMEn) in MS than ES. Significant depressing in jejunum length was observed by 35% in the two types of soybean meal compared with 25% mechanical soybean meal replaced by fish meal in 14 days. In more details, villous length was lower by mechanical soybean meal in comparison with ES. Alkaline phosphatase enzyme activity was significantly higher in 35% ES in comparison with 25% ES replaced by fish meal and 35% MS in 14 days. In contrast, alkaline phosphatase enzyme activity was reduced remarkably by 35% mechanical soybean meal compared with 25% extracted soybean meal replaced by fish meal in starter period. Urease activity in extracted soybean meal (0.07) was lower than mechanical soybean meal (0.22). The results of this study have shown better performance by extracted soybean meal compared with mechanical soybean meal. In spite of this, less expensively processed mechanical soybean meal (MS) at 25% level replaced by fish meal could be more economical in broiler chicken feeding.**

**Keywords**: Alkaline phosphatase, Broiler, Intestinal Characteristics, Soybean meal.

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## INTRODUCTION

Soybean meal (SBM) is the most important source of dietary protein for poultry in most of world. Variations in protein quality among samples of SBM can occur due to insufficient heating. Soybean meal quality is dependent on types of processing. The presence of anti-nutritional factors may influence the protein utilization of legumes (Arija *et al.,* 2006). Such an anti-nutritional factor (protease, trypsin inhibitor, chymotrypsin inhibitor, lectin) especially in soybean meal may affect small intestine characteristics and potential of enzyme activity and consequently broiler performance. Under-processing can leave anti-nutritional factors intact which will cause moderate to severe growth depression. During soybean meal processing, the soybeans are typically flaked prior to solvent extraction to increase the efficiency of oil removal. Heating treatments have been used to improve the nutritional quality of soybeans (Liener, 1994a, b; Bau *et al.,* 1997; Quedraogo *et al.,* 1999), but they should be

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kept to a minimum due to cost and the possibility of destroying important amino acids (Friedman *et al*., 1991) as well as reducing other nutrients availability (Van der Poel *et al*., 1995; Qin *et al*., 1998). The most common assay used to evaluate soybean meal processing is the urease index (AOCS, 1993). It could be economical if an existing quality assurance assay, such as Urease Activity (UA) determines whether the soybean meal is optimally processed. UA measurement, could be used to predict anti-nutritions such as lectin levels (Fasina *et al*., 2003).

The two types of soybean meal (extracted and mechanical) may show various reflections on intestinal compartment (length, weight or enzyme activity) and finally broiler output. In this option, shortening or blunting of villi can reduce digestive and absorptive capacity of entrocyets, increase intestinal weight and size and modulate broiler production (Puzatai, 1994). Dietary components such as the two types of soybean meal (extracted and mechanical) could influence the morphology of the brush border membrane (BBM) by altering villous height and crypt depth thereby modifying the surface area available for digestion and absorption (Sharma and Schumader, 2001).

Therefore, it is interesting to examine the effect of these two types of soybean meal on morphological and compartment characteristics of the intestine in chicken. Since processing procedure of Mechanical Soybean meal (MS) is less expensive than Extracted Soybean meal (ES) Otherwise processing procedure of Mechanical Soybean meal (MS) is less expensive than Extracted Soybean meal (ES) and may have more beneficial reactions in broiler intestine. Alkaline phosphatase enzyme could move secretion by villous tip which may be eroded by mechanical soybean meal (Fasina, *et al*., 2004). On the other hand, the extracted and mechanical soybean meal may be included at the level of 25-40% in broiler diets. At such high proportion levels, soybean meal could include high enough anti-nutritional content. to cause anti-nutritional dictions effects. In addition, under conditions prevalent in Iran, mechanical soybean meal has more benefits economically, because it needs less processing than ES. Chemical composition, metabolizable energy and broiler performance could be affected by the two types of soybean meal and need to be examined. Finally the objective of this study was to evaluate the effect of two processed types of soybean meal (extracted and mechanical) replaced by fish meal on intestinal compartments characteristics, alkaline phosphatase activity as well as metabolizable energy and broiler chicken performance.

## MATERIALS AND METHODS

### Experimental Design

An experiment was conducted in accordance with the guidelines for the care and use of animals in Agricultural Research and Teaching of Bu-Ali Sina University. Three hundred and fifty two unsexed one-day-old. Lohman chickens were allocated to four treatments (completely randomized designs) by 4 replicates and 22 chickens in each with 2×2 factorial arrangement. Two types of soybean meal (mechanical and extracted) and two levels of soybean meal (25% and 35%) replaced by fish meal were optimized as the treatment. The dietary treatments were 1) extracted soybean meal (ES) 25% replaced by fish meal, 2) mechanical soybean meal (MS) 25% replaced by fish meal, 3) extracted soybean meal (ES) 35% and, 4) mechanical soybean meal (MS) 35%. During the experiment, chickens were housed in thermostatically controlled house and had *ad libitum* access to feed and water.

### Diets, Performance and Intestinal Morphology

Two types of soybean meals (ES and MS) were obtained from Jahan Plant Oil Company. Both soybean meal types were prepared by cooking at 100°C, and oil



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extraction carried out by solvent procedure or mechanical pressure. Mechanical soybean meal (MS) was obtained by grinding the cake or chips of soybean, which is remained after removal of most oil content of soybean by a mechanical extraction process. The two types of soybean meals (ES and MS) were assessed for quality by measuring the levels of UA present in the filtered protein extracts. UA was expressed as an increase in pH units according to the procedures of the American Oil Chemists' Society (1993). The differences between the pH of the test samples and blank were used as an index of UA. Feed rations were arranged in 1-21 and 22-42 days of age (Table 1). Small intestine size and weight were assessed as compartment and percentage of body weight (BW). Body weight, feed intake, feed conversion ratio and metabolizable energy in all options (AME, AMEn, TME and TMEn) of the two soybean meals were determined by using the precision-fed rooster assay (Sibbald, 2000). Intestinal duodenum samples for enzyme assay were randomly taken at 14, 21 and 42 days of age. Eight birds in each treatment were euthanized and duodenum section of small intestine was excised. Placed on ice, tissue sections (about 4 cm long) were cut from the middle of duodenum, flushed with cold saline, weighed, placed in a vial, and stored at -20°C until analysis. Duodenum tissue samples were thawed and homogenized with 3ml of cold 0.9% saline. Homogenization was accomplished using a homogenizer (Heidolph DIAX 900) at the moderate speed setting for 30 seconds. Aliquots of homogenates were analyzed for protein concentration (Bradford, 1976) and alkaline phosphatase activity was determined spectrophotometrically by measuring the rate of para-nitrophenol formation, (micromole/milligram protein/ minute) (Forstner *et al*., 1968).

The lengths of intestinal segments (duodenum, jejunum and ileum at 14, 21 and 42 days of age) were also measured in centimeters by a clear plastic ruler. For

**Table 1**. Formulation ration and nutrient of experimental diets (%).

| | Starter (1-21days of age) | | | | Grower (21-42 days of age) | | | |
|---|---|---|---|---|---|---|---|---|
| Ingredient | Diet1 | Diet2 | Diet3 | Diet4 | Diet1 | Diet3 | Diet2 | Diet4 |
| Corn | 62.93 | 62.93 | 56.40 | 56.40 | 68.39 | 68.39 | 54.52 | 54.52 |
| ES[a] | 25 | - | 35 | - | 25 | - | 35 | - |
| MS[b] | - | 25 | - | 35 | - | 25 | - | 35 |
| Fish meal | 8.73 | 8.74 | 2.31 | 2.31 | 3.23 | 3.23 | - | - |
| Soya oil | 1.08 | 1.08 | 3.23 | 3.23 | 0.74 | 0.74 | 7.3 | 7.3 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.3 | 0.3 | 0.3 | 0.3 |
| DCP | 0.06 | 0.06 | 0.52 | 0.52 | 0.39 | 0.39 | 0.62 | 0.62 |
| Oyster shell | 1.30 | 1.30 | 1.6 | 1.60 | 1.33 | 1.33 | 1.58 | 1.58 |
| Mineral premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.15 | 0.15 | 0.19 | 0.19 | 0.12 | 0.12 | 0.18 | 0.18 |
| ME[c] (kcal $kg^{-1}$) | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| CP(%) | 21.56 | 21.56 | 21.56 | 21.56 | 18.75 | 18.75 | 18.75 | 18.75 |

[a] Extracted Soybean meal; [b] Mechanical Soybean meal, Diet 1,3, 25% replaced by fish meal and 35% ES ; 3 and 4 are 25% replaced by fish meal_and 35% MS; [c] Dicalcium phosphate, [d] Metabolizable energy, ME at starter and grower arranged based on NRC 1994.
Mineral supplementation: Mn: 64 g; Zn: 44 g; Fe: 100 g; Cu: 16 g; I: 0.64 g.
Vitamin supplementation: $B_1$: 3.3 g; $B_2$: 0.72 g; K3: 1.6 g; Vitamin E: 14.4 g; Vitamin D: 7 g; Vitamin A: 7.7 g; Pantothenic acid: 12 g; Pyridoxine: 6.2 mg, $B_{12}$: 14.4 g, Choline chloride: 440 mg.



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histological morphometric analysis of the jejunal mid epithelium, formalin-fixed jejunal tissue samples were dehydrated, embedded in paraffin, sectioned (10 µm), and stained with haematoxylin and eosin. Morphometric indices were determined on these sections by means of a computer-aided light microscopic image analyzer (Motic Images 2000 1.2, Scion Image). Measurements made included villous height (from top of villous to the crypt opening) and crypt dept (from the base of the crypt to the level of crypt opening) and are presented in Figure 1. Villous surface area (area within the villous perimeter for each bird) values used for analysis were the means from 10 adjacent, vertically oriented villous-crypt units per section.

### Statistical Analysis

Using the GLM procedure (SAS institute, 2004), data were subjected to ANOVA for completely randomized designs (CRD) with each replicate pen containing 22 chickens. Significant differences between means were determined by Duncan's multiple range tests at ($P< 0.05$) level. An additional orthogonal analysis was employed for comparison of means.

## RESULTS

The chemical analysis of the two types of soybean meals is presented in Table 2. The crude fibers (CF), ether extract (EE) and gross energy (GE) of mechanical soybean meal (MS) were higher compared with the extracted soybean meal (ES). In contrast, metabolizable energy in all options (AME, AMEn, TME and TMEn) were significantly higher ($P< 0.05$) in ES than MS (Table 3). Feed intake in ES was higher ($P< 0.05$) than MS at 21 days of age. Feed intake was increased significantly in starter period by ES in comparison with MS. Feed conversion ratio was significantly improved by 25% of ES replaced by fish meal compared with 35% of ES at 21 days of age. Body weight was significantly higher by 25% ES replaced by fish meal in comparison with 25% and 35% MS in grower period (Table 4). Urease activity (UA) in the ES and MS were 0.07$\Delta$pH and 22 $\Delta$pH, respectively. Histological parameters assessed are presented in Table 5 and Table 6. Intestinal total weight (ITW) was higher than in (MS) compared with (ES) at 14, 21 and 42 days of age. In addition, (ITW) increased significantly ($P< 0.05$) at 35% level compared with 25% level replaced by fish meal at 42 days of age. Significant differences were found in jejunum length at 21 days of age at 35% level compared with 25%

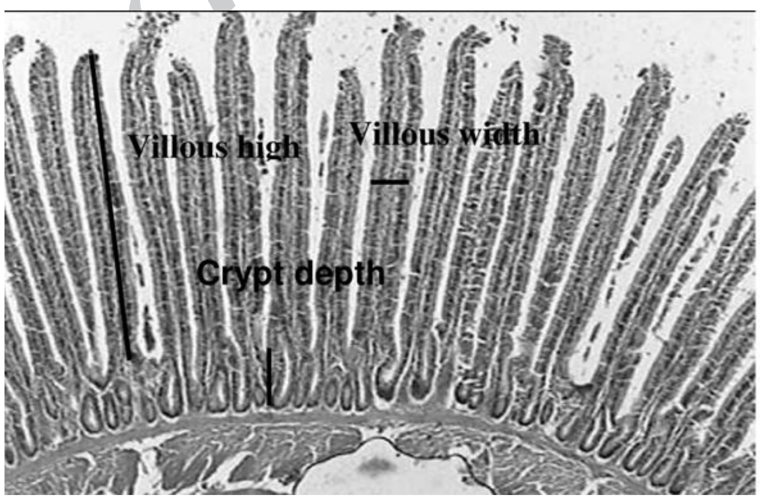


**Figure 1**. Villous height (from top of villous to the crypt opening), crypt depth (from the base of the crypt to the level of crypt opening). Scale bar is=10µm (4x microscope).



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**Table 2.** Chemical composition of two types of soybean meal (%).

| CC[a] | DM[b] | CP[c] | CF[d] | EE[e] | GE[f] | OM[g] | ASH[h] |
|---|---|---|---|---|---|---|---|
| ES[i] | 92.35 | 45.80 | 5.70 | 0.66 | 4674.98 | 84.36 | 15.64 |
| MS[j] | 96.38 | 40.20 | 7.10 | 4.41 | 5219.09 | 84.44 | 15.55 |

[a] Chemical Composition; [b] Dry Matter; [c] Crude Protein; [d] Crude Fiber; [e] Ether Extract; [f] Gross Energy; [g] Organic Matter; [i] Extracted Soybean meal, [j] Mechanical Soybean meal.

**Table 3.** Metabolizable energy of two types of soybean meal (kcal/kg).

| Treatments | AME | AMEn | TME | TMEn |
|---|---|---|---|---|
| ES | $2407^{a}$ | $2474^{a}$ | $3242^{a}$ | $3437^{a}$ |
| MS | $2150^{b}$ | $2214^{b}$ | $2982^{b}$ | $3179^{b}$ |
| *P* value | 0.0111 | 0.0113 | 0.0108 | 0.01096 |

Means with common superscripts in the same column are not significantly different (P> 0.05).
AME: Apparent Metabolizable Energy; AMEn: Appereant Metabolizable Energy corrected by nitrogen; TME: True Metabolizable Energy; TMEn: True Metabolizable Energy corrected by nitrogen; ES: Extracted Soybean meal, MS: Mechanical Soybean meal.

**Table 4.** Effect of dietary soybean meal levels on broiler performance (g).

| Day | 21 | | | 42 | | |
|---|---|---|---|---|---|---|
| Treatment | BW (g) | FI (g) | FCR | BW (g) | FI (g) | FCR |
| ES | 418.9±58.5 | $749.9^{a}$±80.9 | 1.81±0.22 | $1707.7^{a}$±59.5 | 2393.29±97.1 | $1.41^{b}$±0.07 |
| MS | 371.5±50.5 | $636.4^{b}$±67.1 | 1.72±0.23 | $1572.3^{b}$±88.1 | 2356.62±152.7 | $1.5^{a}$±0.13 |
| *P* value | 0.079 | 0.0098 | 0.094 | 0.005 | 0.582 | 0.307 |
| 25% | 402.4±56.3 | 713.2±116.8 | 1.69±0.10 | 1627.8±85.2 | 2347.27±129.0 | 1.45±0.10 |
| 35% | 388.0±48,6 | 673.04±62.5 | 1.48±0.13 | 1652.2±118.8 | 2402.54±121.9 | 1.46±0.13 |
| *P* value | 0.568 | 0.302 | 0.794 | 0.544 | 0.439 | 0.097 |
| SEM | 17.48 | 26.24 | 0.005 | 27.65 | 43.98 | 0.038 |
| 25%ES | 431.5±39.9 | $712.6^{ab}$±65.8 | $1.66^{b}$±0.15 | $1682.1^{a}$±49.5 | 2360.5±99.2 | 1.41±0.09 |
| 25%MS | 373.3±32.0 | $633.5^{b}$±24.9 | $1.71^{ab}$±0.11 | $1573.5^{b}$±81.3 | 2334.0±168.9 | 1.49±0.11 |
| 35%ES | 406.2±71.1 | $787.3^{a}$±85.0 | $1.96^{a}$±0.19 | $1733.3^{a}$±63.0 | 2424.9±69.4 | 1.40±0.06 |
| 35%MS | 369.8±51.9 | $638.8^{b}$±99.4 | $1.73^{ab}$±0.22 | $1571.2^{b}$±107.0 | 2379.2±156.6 | 1.52±0.16 |
| *P* value | 0.288 | 0.039 | 0.363 | 0.029 | 0.804 | 0.134 |
| SEM | 24.7 | 37.11 | 0.087 | 39.11 | 67.16 | 0.054 |

ES: Extracted Soybean meal; MS: Mechanical Soybean meal; FCR: Feed Conversion Ratio; BW: Body Weight. FI: Average daily feed Intake.

level replaced by fish meal. On day 14, broiler fed with 25% MS replaced by fish meal showed higher jejunum length than chickens fed with 35% of either type of soybean meals (ES and MS) (P< 0.05). Intestine weight was lower (P< 0.05) in 25% ES treatment replaced by fish meal compared with other treatments at 21 days. No significant differences were found in duodenum and ileum length at 14 days of age as well as in all segments at 21 and 42 days of age.

No significant changes were observed in villous height at 14, 21 and 42 days of age. Villous crypt depth was higher (P< 0.05) in the jejunum of birds fed with 35% MS than other treatments at 42 days of age (Table 6). Villous surface area at 42 days of age was lower (P< 0.05) in the jejunum of birds fed with 25% ES replaced by fish meal than other treatments. A significant increase (P< 0.05) was observed in the crypt depth by ES in comparison with MS at 14 days of age. The crypt depth was higher (P< 0.05) at 35% level compared with 25% replaced by fish meal at 21 days. Significant decreases (P< 0.05) in the crypt depth have been observed by 25% MS replaced by fish meal compared to 35% level of the two types of soybean meal at 14 days.

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**Table 5.** Various segments of small intestine length in chickens fed on extracted and mechanical soybean meals.

| Treatment | ES | MS | p-value | 25% | 35% | p-value | SEM | 25%ES | 25%MS | 35%ES | 35%MS | p-value | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14d | | | | | | | | | | | | | |
| ITW (g) | 6.83$^{b}$±0.78 | 6.23$^{a}$±1.38 | 0.206 | 6.73±1.11 | 6.33±1.17 | 0.39 | 0.45 | 6.30±0.9 | 6.35±1.2 | 6.15±0.6 | 7.31±1.3 | 0.296 | 0.638 |
| Duodenum(cm) | 9.80±0.82 | 10.4±0.97 | 0.102 | 10.5±0.67 | 10.16±1.17 | 0.756 | 0.34 | 9.94±0.7 | 10.16±0.6 | 9.66±0.9 | 10.66±1.2 | 0.262 | 0.979 |
| Jejunum(cm) | 42.69±3.59 | 42.63±3.55 | 0.586 | 44.06$^{a}$±3.18 | 41.31$^{b}$±3.11 | 0.006 | 1.00 | 44.25$^{ab}$±2.1 | 43.88$^{a}$±5.1 | 41.13$^{b}$±4.2 | 41.38$^{b}$±1.9 | 0.041 | 2.841 |
| Ileum(cm) | 45.31±3.79 | 45.00±3.66 | 0.825 | 45.88±3.77 | 44.44$^{a}$±4.10 | 0.426$^{b}$ | 1.67 | 45.75±1.3 | 46.00±5.1 | 44.88 ±5.3 | 44.00±2.6 | 0.843 | 2.359 |
| 21d | | | | | | | | | | | | | |
| ITW (g) | 7.04$^{b}$±1.46 | 8.16$^{a}$±0.96 | 0.027 | 7.03$^{b}$±1.44 | 8.17$^{a}$±0.96 | 0.0193 | 0.42 | 6.11$^{b}$±1.0 | 7.10$^{a}$±0.9 | 7.98$^{a}$±1.0 | 8.36$^{a}$±1.0 | 0.011 | 1.205 |
| Duodenum(cm) | 11.40±0.88 | 11.15±0.66 | 0.185 | 11.42±0.75 | 11.23±0.82 | 0.458 | 0.24 | 11.16±0.8 | 11.69±0.7 | 11.16±1.1 | 11.31±0.7 | 0.405 | 0.668 |
| Jejunum(cm) | 49.75±3.67 | 52.88±3.63 | 0.063 | 51.13±3.26 | 51.50±4.60 | 0.810 | 1.52 | 50.38±4.3 | 51.88±1.7 | 49.13±3.1 | 53.89±4.8 | 0.199 | 1.837 |
| Ileum(cm) | 56.31±4.65 | 58.38±4.65 | 0.259 | 56.81±4.44 | 57.88±4.26 | 0.553 | 1.73 | 55.63±4.9 | 58.00±3.9 | 57.00±2.6 | 58.75±5.5 | 0.625 | 2.460 |
| 42d | | | | | | | | | | | | | |
| ITW (g) | 4.47$^{b}$±0.64 | 4.99$^{a}$±0.9 | 0.347 | 4.52±0.78 | 4.93±0.82 | 0.085 | 0.22 | 4.28$^{b}$±0.6 | 4.76$^{ab}$±0.9 | 4.65$^{ab}$±0.7 | 4.22$^{a}$±0.9 | 0.069 | 0.310 |
| Duodenum(cm) | 14.44±1.86 | 14.40±0.66 | 0.468 | 14.44±1.25 | 14.03±1.52 | 0.393 | 0.46 | 14.38±1.6 | 14.50±0.8 | 13.75±2.1 | 14.31±0.5 | 0.674 | 0.649 |
| Jejunum(cm) | 66.94±5.23 | 68.81±6.59 | 0.438 | 67.37±7.36 | 68.38±4.24 | 0.676 | 2.82 | 66.50±6.2 | 68.25±8.7 | 67.38±4.5 | 69.38±4.0 | 0.841 | 3.304 |
| Ileum(cm) | 73.13±5.05 | 72.31±4.41 | 0.639 | 74.06±4.34 | 71.38±4.75 | 0.138 | 1.69 | 75.38±0.9 | 72.75±4.4 | 70.88±5.1 | 71.87±4.7 | 0.391 | 2.392 |

ES: Extracted Soybean meal, MS: Mechanical Soybean meal; ITW (g: Intestine Total Weigh, a-b, Means with common superscripts in the same column are not significantly different (P>0.05).

**Table 6.** Intestinal villi characteristics in chickens fed on extracted and mechanical soybean meals.

| Treatment | ES | MS | p-value | 25% | 35% | p-value | SEM | 25%ES | 25%MS | 35%ES | 35%MS | p-value | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14d | | | | | | | | | | | | | |
| Villi height (µm) | 770.7±94.6 | 711.8±88.4 | 0.114 | 756.5±107.4 | 728.3±79.7 | 0.671 | 37.16 | 781.1±0.78 | 728.33±117.8 | 758.6±۹۵.2 | 692.6±39.3 | 0.390 | 52.57 |
| Villi width (µm) | 93.2±9.5 | 94.3±8.3 | 0.731 | 94.1±10.2 | 93.4±7.2 | 0.803 | 2.57 | 94.3±10.9 | 93.93±10.3 | 92.00±8.3 | 94.69±9.04 | 0.876 | 3.64 |
| Crypt depth (µm) | 160.2$^{a}$±18.5 | 145.8$^{b}$±21.5 | 0.064 | 144.3$^{b}$±20.9 | 164.2$^{a}$±15.5 | 0.013 | 5.89 | 152.4$^{ab}$±17.7 | 135.03$^{b}$±21.6 | 169.14$^{a}$±16.1 | 158.52$^{a}$±13.9 | 0.031 | 8.34 |
| Villi surface area, ($mm^2$) | 0.07±0.11 | 0.07±0.01 | 0.243 | 0.03±0.01 | 0.07±0.01 | 0.518 | 0.004 | 0.075±0.01 | 0.070±0.01 | 0.700±0.01 | 0.066±0.01 | 0.584 | 0.006 |
| 21d | | | | | | | | | | | | | |
| Villi height (µm) | 848.3±117.6 | 860.3±85.1 | 0.772 | 852.1±98.6 | 857.1±103.8 | 0.865 | 38.36 | 826.3±19.4 | 865.0±22.4 | 859.4±23.1 | 854.0±12.7 | 0.971 | 54.27 |
| Villi width (µm) | 161.0±17.5 | 100.0±6.9 | 0.604 | 100.2±10.6 | 105.8±14.8 | 0.192 | 4.45 | 99.9±16.5 | 100.3±7.7 | 110.5±17.9 | 99.6±6.2 | 0.368 | 6.31 |
| Crypt depth (µm) | 168.5±23.3 | 178.4±20.8 | 0.733 | 185.1$^{a}$±20.7 | 164.1$^{b}$±18.9 | 0.049 | 8.13 | 182.0±19.4 | 186.7±22.4 | 161.7±23.3 | 167.4±12.7 | 0.175 | 23.07 |
| Villi surface area, ($mm^2$) | 0.091±0.011 | 0.086±0.01 | 0.228 | 0.086±0.02 | 0.090±0.01 | 0.681 | 0.004 | 0.084±0.01 | 0.088 ±0.03 | 0.085±0.02 | 0.095±0.03 | 0.453 | 0.006 |
| 42d | | | | | | | | | | | | | |
| Villi height (µm) | 981.2±100.2 | 991.4±97.4 | 0.639 | 965.6±85.7 | 1005.1±106.9 | 0.332 | 31.07 | 939.9±92.1 | 995.7±72.9 | 1022.6±97 | 987.7 ±119.9 | 0.320 | 48.85 |
| Villi width (µm) | 122.8±15.6 | 136.9±20.2 | 0.023 | 124.1±18.8 | 134.7±18.4 | 0.859 | 5.19 | 130.5$^{b}$±15.1 | 136.2$^{a}$±15.5 | 132.0$^{a}$±9.9 | 137.7$^{a}$±24.7 | 0.025 | 8.61 |
| Crypt depth (µm) | 186.9±20.4 | 140.0±25.2 | 0.266 | 183.2$^{b}$±16.3 | 196.9$^{a}$±26.8 | 0.0365 | 5.34 | 181.5$^{b}$±18.9 | 185.2$^{b}$±13.8 | 192.2$^{b}$±21.6 | 201.7$^{a}$±31.9 | 0.112 | 7.86 |
| Villi surface area, ($mm^2$) | 0.122±0.02 | 0.137±0.03 | 0.084 | 0.123±0.03 | 0.137±0.03 | 0.122 | 0.007 | 0.109$^{b}$±0.02 | 0.137$^{a}$±0.02 | 0.136$^{a}$±0.02 | 0.138$^{a}$±0.04 | 0.069 | 0.013 |

ES: Extracted Soybean meal, MS: Mechanical Soybean meal; a-b, Means with common superscripts in the same column are not significantly different (P>0.05).



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**Table 7.** Alkaline phosphates activity in chickens fed on extracted and mechanical soybean meal.

| Treatment | 14d | 21d | 42d |
|---|---|---|---|
| ES | 9.91 ± 3.1 | 11.05 ± 1.9 | 11.91 ± 4.1 |
| MS | 9.43± 3.0 | 12.14 ± 2.1 | 12.2 ± 2.2 |
| P value | 0.703 | 0.082 | 0.815 |
| 25% | 8.40 ± 2.8 | 12.75$^{a}$ ± 1.8 | 12.7 ± 2.6 |
| 35% | 11.0 ± 29 | 10.44$^{b}$ ± 1.7 | 11.99 ± 1.3 |
| P value | 0.058 | 0.0016 | 0.34 |
| SEM | 38.65 | 17.76 | 40.38 |
| 25%ES | 7.56$^{b}$ ± 2.3 | 10.04$^{c}$ ± 3.1 | 13.39 ± 3.2 |
| 25%MS | 9.23$^{ab}$ ± 3.1 | 10.83$^{cb}$ ± 1.9 | 11.99 ± 1.3 |
| 35%ES | 12.26$^{a}$ ± 1.9 | 12.06$^{ab}$ ± 1.5 | 10.42 ± 4.7 |
| 35%MS | 9.64$^{b}$ ± 2.1 | 13.44$^{a}$ ± 1.6 | 12.43 ± 2.6 |
| P-Value | 0.105 | 0.006 | 0.450 |
| SEM | 1.67 | 1.78 | 1.81 |

ES: Extracted Soybean meal, MS: Mechanical soybean meal; a-b: Means with common superscripts in the same column are not significantly different (P>0.05). Alkaline phosphatase activity: micromole per milligram protein in minute.

In addition, a significant increase (P< 0.05) was seen in the crypt depth by 35% MS in comparison with other treatments at 42 days of age. Alkaline phosphatase activity (ALP) was significantly (P< 0.05) increased at 25% level replaced by fish meal as compared with the 35% level at 21 days of age. ALP was significantly (P< 0.05) higher by 35% ES in comparison with 25% ES replaced by fish meal and 35% MS at 14 days of age (Table 7). Finally, alkaline phosphatase activity was significantly (P< 0.05) increased by 35% MS compared with 25% ES replaced by fish meal at 21 days of age. In contrast, no response was found at 42 days of age in this respect. Although the effects of soybean levels were removed by independent analysis method, similar reactions were found in results (P value and SEM).

## DISCUSSION

In this study, UA values in the extracted and mechanical soybean meal were (0.07 ΔpH and 0.22 ΔpH), respectively. These results are similar to those obtained by McNaughton and Reece (1980). Urease activity is used an indicator of the presence of anti-nutritional factors which would suggest that the soybean meal is under-processed. The recommended value for Urease activity is 0.02 to 0.2 unit pH change. While this has some value for detecting the under-processing of soybean meal, it cannot be used for detecting over-processing (Main and Garlish, 1995). In addition, Main and Garlish (1995) reported that the growth performance of young turkeys fed on SBM with a pH rise of 1.76 was similar to that of turkeys fed on SBM with a pH rise of 0.02. Fasina *et al*. (2003) concluded that UA of 0.03 to 0.09 units of pH change indicated that the meals were adequately processed and contained negligible lectin levels. Extracting had effects on reducing ΔUA levels in ES. McNaughton *et al*. (1981) showed that a UA of 0.19 ΔpH resulted in a poorer growth and feed conversion ratio in broilers. Batal *et al*. (2000) found that urease pH change values of 1.65 to 1.8 yielded maximum chick growth performance.

Intestinal morphological and physical attributes of chickens fed with two types of soybean meals (ES and ME) were examined in this investigation. Physical characteristics of the intestine indicated that mechanical soybean meal increased weight and length of jejunum. The intestinal

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weight, length and absorptive area, could be modified by intestine response due to change in diet (Bradford, 1996). Soybean meal which is usually incorporated at about 25-40% of broilers diets is a potential source of plant protein and especially amino acids for poultry industry. Intestine could be depressed by some anti-nutritional factors which may increase its weight and size and finally influence chicken performance. (Pusztai, 1993).

Crypt depth and villous height are useful indicators of the size of proliferate and absorptive compartments in the intestinal mucosa (Sharma and Schumacher, 2001). Soybean meal anti-nutritional factors and availability of energy and performance could be changed in the biochemical events occurring during differentiation in brush border membrane. Plants anti-nutritional factors are known to react with both the luminal content and epithelial cells because of their resistance to proteolysis breakdown in the gut. They induced a two-fold increase in small intestine length, due to increased cellular hyperplasia (increasing cells number), soybean anti-nutritional could lead to such results. Increasing intestinal length for instance, results have shown that jejunum length was increased by 25% MS replaced by fish meal in chickens 14 days of age. Intestine weight was lower in 25% ES treatment replaced by fish meal compared with other treatments at 21 days. long sentence divided into two sentences as fallowing procedure: These changes could be attributed to the high level of anti-nutritional content in MS. Consequently these modifications may be related to processing reactions which was confirmed by above studies.

Duodenum enzymes such as alkaline phosphatase are synthesized by villi attached enterocytes and are inserted into the apical membranes during the process of enterocyte differentiation (Uni, 1999). Alkaline phosphatase activity was significantly ($P<0.05$) higher when 35% ES was used in comparison with 25% ES replaced by fish meal and 35% MS at 14 days of age. This may be attributed to more enzyme activity in ES compared with MS. These results are similar to those of Hong *et al*. (1991) that reported SBM anti-nutritional factors caused reduction in brush border enzyme and destroyed villi. Fasina *et al*. (2004) showed that measuring the activities of alkaline phosohatase (ALP) in the small intestinal epithelium enabled them to assess the extent of membrance disruption caused by anti-nutritional soybean meal levels. Therefore, soybean meal products intended for feeding poultry should be routinely monitored to contain fewer anti-nutrients in processing stages. On the other hand, supplying and processing procedure of MS instead of ES in chicken ration is less expensive and could be more economical as it requires fewer processing stages.

## CONCLUSIONS

It is widely accepted that broiler production depends on intestinal potential, intestinal jejunum length and villi characteristics which were dramatically influenced by both soybean meals (ES and MS). In spite of high crude fiber of MS (7.1%), cheaper processing of mechanical soybean meal (MS) may suggest that it could be replaced by fish meal at 25% level and may be more beneficial from physiological and nutritional aspects. Finally the results of this study elucidated that 25% of MS replaced by fish meal is a good source of plant protein for broiler optimum performance with no adverse effects.

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## خصوصیات رودهای، فعالیت آنزیم آلکالین فسفاتاز و عملکرد جوجه‌های گوشتی تغذیه‌شده با کنجاله سویای استخراج‌شده و مکانیکی جایگزین شده با پودر ماهی

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## چکیده

آزمایشی جهت بررسی دو نوع کنجاله سویا استخراج شده و مکانیکی (در دو سطح۲۵٪ و ۳۵٪) در تغذیه جوجه‌های گوشتی انجام شد. سیصد و پنجاه و دو جوجه سویه لوهمن تعیین جنسیت نشده در ۴ تیمار مشتمل بر۴ تکرار و ۲۲ جوجه در هر تکرار استفاده شدند. در دوره آغازین خوراک مصرفی با کنجاله سویای استخراج شده نسبت به کنجاله سویای مکانیکی افزایش یافته بود. وزن بدن نیز به طور معنی‌داری با ۲۵٪ کنجاله سویای استخراج شده جایگزین شده با پودر ماهی در مقایسه با ۳۵٪ کنجاله سویای مکانیکی در دوره رشد افزایش یافته بود. در دوره آغازین ضریب تبدیل غذایی نیز بهبود معنی‌داری با ۲۵٪کنجاله سویای استخراج شده نسبت به کنجاله سویای مکانیکی نشان داد. انرژی قابل متابولیسم ظاهری و حقیقی و تصحیح شده بر اساس ازت، کنجاله سویای مکانیکی کاهش معنی‌داری در مقایسه با کنجاله سویا استخراج شده داشت. سطح ۳۵٪ هر دو نوع کنجاله در ۱۴ روزگی در مقایسه با تیمار ۲۵٪ کنجاله سویای مکانیکی جایگزین شده با پودر ماهی کاهش معنی‌دار در طول ژژونوم نشان دادند. در بیشتر موارد تیمارهایی که حاوی کنجاله سویا مکانیکی بودند طول ویلی آنها نسبت به تیمارهای حاوی کنجاله سویا استخراج شده کمتر بود. تیمار ۳۵٪ کنجاله سویای مکانیکی و ۲۵٪ استخراج شده نسبت به تیمار ۳۵٪کنجاله سویا استخراج شده سبب کاهش معنی‌داری در فعالیت آنزیم آلکالین فسفاتاز در ۱۴ روزگی شد. در ۲۱ روزگی تیمار ۳۵٪ کنجاله سویا مکانیکی افزایش معنی‌دار نسبت به ۲۵٪ کنجاله سویا استخراج شده در میزان آنزیم آلکالین فسفاتاز نشان داد. میزان فعالیت آنزیم اوره‌از کنجاله سویای استخراج شده (۰/۰۷) کمتر از کنجاله سویای مکانیکی (۰/۲۲) بود. نتایج این مطالعه نشان داد که به دلیل اقتصادی تر بودن عمل‌آوری کنجاله سویای مکانیکی جایگزین شده با پودر ماهی، سطح ۲۵٪ آن را می توان برای تغذیه طیور پیشنهاد داد.



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