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PATHOLOGICAL EFFECT OF REPLACEMENT OF SOLVENT EXTRACTED SOYBEAN MEAL WITH EXPELLER EXTRACTED SOYBEAN MEAL IN BROILERS

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Soybean meal is an important source of protein in the diet of poultry bird. However, it has antinutritional factors which have to be taken care. Therefore, the study was carried out to determine the effect of replacement of solvent extracted soybean meal (SBM) with expeller extracted soybean meal on pathology of various organs along with serum AST, ALT, Urea and Creatinine. The study was carried out on day-old broiler chicks (n=180), which were kept and randomly divided in to 5 groups (A, B, C, D and E). Birds of these groups were given treatment by replacing 0%, 25%, 50%, 75% and 100% of solvent extracted SBM with expeller extracted SBM, respectively. The trial lasted for 21 days. Birds were fed iso-nitrogenous and iso-caloric diets. All the birds from each group were slaughtered. The results indicate that the effect of expeller extracted soybean meal on kidney, liver and pancreas were severe in birds given 100% expeller extracted SBM. However, where 100% solvent extracted SBM was used the changes were less severe. While, the changes in intestine were more severe in birds given 100% solvent extracted SBM. The results on serum creatinine and urea supported histological changes seen in kidney in respective groups. However, the results of serum AST and ALT did not correspond well, as in liver, more severe changes of necrosis were noted in birds given 100% expeller extracted SBM, while the values of these enzymes were the lowest. It is concluded from the present study that birds kept on 100% expeller extracted and solvent extracted soybean meal showed effect on various body organs, while 50% of both the solvent extracted and expeller extracted soybean meal in combination gave better results.
Keywords: Solvent extracted soybean meal, Expeller extracted soybean meal, Broilers, Serum enzymes.

INTRODUCTION

In animal feed, the primary protein source is the soybean. In many cultured fish, soybean meal has been tested with varying degree of success as substitute of fish meal (Tomas *et al*., 2005). Soybean meal has high protein and has an excellent amino acid profile that complements the corn, the basic energy source in the poultry diet (Navicha *et al*., 2017).The source of vegetable protein in the chicken diet is soybean (Swick, 2004).The needs of vital amino acids and protein of broiler chicken is fulfilled by soybean which is used as major nutrients in the diet. Soya protein can affect immune response and act as antitumor factor (Nabizadeh, 2018). New processing technologies have been introduced for expulsion of harmful variables present the soybean. There are two ways by which the harmful substances are removed from the soybean meal, such as expeller extraction and solvent extraction (Powell *et al*., 2011). The solvent extraction method results in soybean meal containing less than one percent residual oil content (Newkirk, 2010). For Maillard reaction that affects nutritive value of meal, especially in terms of amino acids availability the high moisture and temperature treatments during solvent extraction provides ideal conditions (Newkirk, 2010). Expeller extraction is used as less effective and simple extraction with less capital investment and high in energy and high in oil contents (> 6 %) (Pacheco *et al*., 2014). The availability of soy protein is made possible by giving heat treatment. The most important methods of heating are boiling in water, microwave and autoclave: these treatments destroy the anti-nutritional factors, particularly trypsin inhibitor, phytates, tannins, anti-vitamins, goitrogens, lectins, protease inhibitors and lectins (Habiba, 2002). However, availability of important nutrients is also reduced due to heat treatment. Decrease in amino acid digestibility and reproductive performance has been observed in broilers and roosters due to replacement of soybean meal

with full fat soybean meal. The diet containing 10, 20 and30% solvent extracted soybean meal change the thickness of mucus membrane, with abundance of goblet cells (Bansemer *et al.,* 2011). There is hypertrophy of pancreas and the gut enteritis induced by raw soybean meal is less severe in rainbow trout, while severe in Atlantic salmon fish (Sealey *et al*., 2009). There is need to have data on use of solvent extracted soybean meal and expeller extracted soybean meal in broilers, therefore, the present study was planned to determine the effects of gradual replacement of solvent extracted soybean meal with expeller extracted soybean meal on gross and histopathology changes in some organs of broiler along with effect on serum AST and ALT levels. It was hypothesized that the birds fed with expeller extracted soybean meal will show positive effects in broiler and thus could be added as complete birds' diet.

MATERIALS AND METHODS

***Experimental Feed*:** The two broiler feeds including expeller extracted and solvent extracted soybean meals were produced at the Hi-Tech Edible Oil Mills, Sahiwal. These feeds were processed to determine the meal quality including the proximate analysis, solubility in KOH and the pepsin digestibility.

***Experimental design*:** The experiment was divided into different groups and different feed ingredients were distributed to each group as presented in Table 1.

A total of 180, day-old broilers were procured from the commercial hatchery. Birds were vaccinated against ND, IB, and IBD following the local recommendations. Chicks were reared and assigned to 5 treatments having 4 replicates, each containing 9 birds. The duration of the trial was 21 days and birds were fed iso-nitrogenous and iso-caloric containing 20% crude protein and ME 3100 kcal/kg diet.

***Sample Collection*:** The blood samples were collected from the jugular vein, after slaughtering, in sterile test tubes on 21st day, for serum separation. Samples from visceral organs including liver, kidney, heart, pancreas and intestine were also collected after slaughtering and kept in 10% buffered formalin for histopathological studies.

***Serum Biochemistry*:** Serum was separated from the blood after allowing the blood to clot for approximately sixty minutes. The amount of Aspartate aminotransferase (AST) (IU/L) and Alanine amino transferase (ALT) (IU/L) were determined by using the commercially available kits obtained from Merck Company having Catalog # 5.17530.0001 and 5.17520.0001, respectively. Blood urea concentration was measured by the using commercially available kit (Merck, France) having catalog# 5.17550.0001. Serum creatinine was measured by the use of commercially available kit (Merck, France) having catalog# 5.17610.0001.

***Gross and histopathology*:** Different organs including liver, heart, lungs, kidneys, intestine and proventriculus were examined grossly to determine the hemorrhages, change in color, size, shape, consistency and any other abnormality in the birds treated with soybean meal. Histopathology was carried out by fixing the tissues of different body organs in 10% buffered formalin. Tissues were further processed for dehydration, embedding, cutting, mounting and staining by

Table 1.Composition of different experimental feed ingredients in percentage.

Ingredients	Group A	Group B	Group C	Group D	Group E
Maize	35.00	35.00	35.00	35.00	35.00
Rice tips	27.00	27.00	27.00	27.00	27.00
Rice polishing	1.20	1.20	1.20	1.20	1.20
Molasses	1.10	1.10	1.10	1.10	1.10
Corn gluten (CP 60%)	1.60	1.60	1.60	1.62	1.62
Fish meal (CP 61%)	5.00	5.00	5.00	5.00	5.00
Solvent extracted SBM (CP 45%)	**22.88**	**17.16**	**11.44**	**5.72**	**0.00**
Expeller extracted SBM	**0.00**	**6.50**	**13.00**	**19.50**	**26.00**
Soybean oil	**3.12**	**2.34**	**1.56**	**0.78**	**0.00**
DCP	1.30	1.30	1.30	1.30	1.30
Limestone	0.90	0.90	0.90	0.90	0.90
L-Lysine HCl	0.23	0.23	0.23	0.23	0.23
DL-Methionine	0.15	0.15	0.15	0.15	0.15
L-Threonine	0.10	0.10	0.10	0.10	0.10
Common salt	0.15	0.15	0.15	0.15	0.15
*Vitamin mineral **premix**[a]	0.27	0.27	0.27	0.27	0.27
****Celite®** [b]	1.00	1.00	1.00	1.00	1.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

*Each vitamin mineral premix[a] carried 20 mg of vitamin (A, D3, B1, B2, B12 and H), 100 mg of vitamin E, 40 mg of vitamin K3, 300 mg of vitamin C, 30 mg of vitamin (B3, B5 and Folic acid), 250 mg of (Ferrous sulphate, Manganese sulphate and zinc sulphate).**Celite®[b] contained acid insoluble ash from Celite Corporation, Lompoc, California, USA.

following the protocol as described by Bancroft and Gamble (2008).

***Statistical Analysis*:** The data thus obtained were analyzed by the analysis of variance technique(ANOVA)and to compare the means of different groups Duncan's Multiple range test (DMR) (P<0.05) was used. All the data were analyzed by a statistical software SAS (2004).

RESULTS

***Gross Pathology*:** Grossly, no lesions were observed in birds of group C, while only mild congestion in liver, kidney, heart, pancreas and intestine was seen in birds of group A, B, D and E.

Histopathology Liver: The microscopic changes including congestion, atrophy, degeneration and inflammation in liver were seen in birds of group A. Hepatic cells showed mild degenerative changes in birds of group B and C. Degenerative and necrotic changes with pyknotic nuclei were observed in birds of group D and E.

Kidney: Kidney showed mild degenerative and necrotic changes with vacuolar cytoplasm and pyknosis in group A, while similar but milder changes were observed in birds of group B. Mild vacuolar degeneration in cytoplasm of renal tubular epithelial cells was seen in birds of group C. The birds of group D and E showed glomerular dilatation and moderate necrotic changes in renal epithelial cells.

Intestine: The microscopic changes in intestine showed necrotic and inflammatory changes with increased accumulation of polymorphonuclear leukocytes in the lamina propria and submucosa in birds of group A. There predominance of goblet cells was seen in birds of group B. Mild inflammatory cells along with sloughing of intestinal epithelial cells were seen in birds of groups C, D and E.

Pancreas: The pancreatic changes seen under microscope were almost similar in group A to D, except that the epithelial cells were taller in birds of group A and tallness of the cells decreased gradually from groups A to D. however, there was severe necrosis seen in epithelial lining of the pancreatic follicles in birds of group E.

Cardiac Muscles: The heart muscle did not show striking histopathological changes except in birds of group A, where milder degree of degenerative changes were present in birds group of E.

***Serum Biochemistry*:** Mean (±SE) values for AST, ALT, serum urea and creatinine concentrations in broilers of different groups have been presented in Table 2.

At day 21 of the experiment, the values of AST and ALT were higher in birds of group A treated with 100% solvent extracted soybean meal, while lower (P<0.05) values were observed in birds of group B, C, D and E treated with 100% expeller extracted soybean meal.

The values of serum creatinine were significantly (P<0.05) increased in birds of group E, while in all the other groups the values were non-significantly different from group A. The level of blood urea was significantly (P<0.05) high in birds of group D and E as compared to the group A.

DISCUSSION

An important part of soybeans is soy protein which is a rich source of dietary protein. Soy protein is viewed as a complete protein in that it has an abundant amount of all vital amino acids in addition to a few different micronutrients with a nutritive value generally equal to that of high biological value of animal protein (Janardhanan *et al*., 2003; Abdullah *et al*., 2019). In most of the world, soybean is the most essential source of protein for aquaculture and poultry. Among the plant-based proteins, soy protein is important; because it contains isoflavones. Quality of soybean meal depends upon the processing. Apart from proteins, the soybean meal also contains anti-nutritional factors (Arija *et al.,* 2006). It has been reported that there is moderate to extreme growth depression in fish due to the presence of anti-nutritional factors such as trypsin inhibitor, protease, lectin and chymotrypsin inhibitor (Garg *et al*., 2002). Solvent or expeller extraction methods can be used for production of soybean meal with reduction in levels of these anti-nutritional factors. Solvent extracted soybean meal contains less oil but more crude protein than expeller extruded soybean meal. Presently, the expeller process is still the most commonly used industrial method in Nigeria but, solvent extraction is the most common method of extracting soybean oil today; leaving less than 1% fat (Powell *et al.,*2011).To enhance the nutritious quality of

Table 2. AST, ALT, Serum creatinine and Urea concentration of chicks administered with varying level of solvent extracted soybean meal, expeller extracted soybean meal and oil (Mean ±SD).

Group	Treatment	AST(IU/L)	ALT(IU/L)	Serum Creatinine (mg/dl)	Urea (mg/dl)
A	22.88% SESBM,0% EESBM & 3.12% oil	286.02±23.9	47.15±2.36	5.36±0.68	8.95±1.26
B	17.16% SESBM, 6.5% EESBM & 2.34% oil	208.84±6.36*	33.32±2.33*	4.71±1.11	10.14±1.07
C	11.44% SESBM, 13.0%; EESBM & 1.56% oil	129.25±15.1*	24.21±0.99*	5.06±1.00	9.90±0.56
D	5.72% SESBM, 19.5%; EESBM & 0.78% oil	102.98±8.80*	17.64±2.21*	6.41±0.54	12.39±1.04*
E	0% SESBM, 26.0% EESBM & no oil	67.11±8.85*	9.78±1.97*	7.82±1.03*	11.99±1.11*

Figures (Mean ±SD) with asterisk are significantly different than control at (P<0.05)

soybeans, heating treatments can be used. Heat treatment of soybeans can improve protein digestibility and reduce the activity of trypsin inhibitors. Overheating, may reduce the nutritional value of soy protein and reduce or destroy the availability of certain heat sensitive amino acids. The extracted soybean meal may have the effect on broiler performance and intestine compartment. The effect includes blunting and shortening of villi, decreases in absorption and digestion tendency of the enterocyte, enhance the intestinal size and weight (Uran *et al*., 2008). Soybean meal can cause inflammation in intestine which is linked with the presence of saponins in soybean meal. Enteritis in distal part of intestine and reduction incapacity of enterocytes with shortening of mucosal folds can occur due to soybean meal (Uran, 2008).

Grossly, no observable changes were noted in birds received 50% of both solvent extracted soybean meal and expeller extracted soybean meal during present study. However, congestion was noted in various internal organs when 100% expeller extracted, or 100% solvent extracted soybean meal was used. Similarly, Beukovic *et al*. (2012) reported that heat treated soybean meal affect internal organs.

The histological changes in liver in birds received 100% solvent extracted soybean meal showed congestion and atrophy of hepatocytes with very mild inflammatory reaction. While changes in birds received 75-100% expeller extracted soybean meal showed degenerative and necrotic changes in hepatocytes. When 50% of both the expeller extracted and solvent extracted soybean meals were used in combination, no histological changes in liver hepatocytes were noted, which suggest some counter balancing effect when equal amount of solvent extracted, or expeller extracted soybean meal is used. However, when higher percentage of solvent extracted and expeller extracted soybean meals were used, the pathological changes in liver were observed. Kosif *et al*. (2010) reveled that there was congestion in central vein, portal vein and sinusoids due to soybean oil.

The histopathological changes in kidneys were more severe including necrosis of epithelial cells of renal tubules with pyknotic nucleus and eosinophilic cytoplasm with sloughing of epithelial cells in birds given 75-100% expeller extracted soybean meal. Such changes were also noted in birds given 100% solvent extracted soybean meal, though the degree of severity was less in these birds. Similarly, inflammatory changes and severe necrosis in intestine were noted in birds given each 100% solvent extracted and expeller extracted soybean meals. However, the intestinal epithelial cells were tall and had reasonably high number of goblet cells in birds of other groups. The inflammatory changes in intestine were also reported by Uran (2008), which were related with presence of saponin in soybean meal as causative factor. But why the changes were very mild when both the solvent extracted and expeller extracted soybean meals were used in combination need, further investigations. Bansemer *et al.* (2011) revealed that diet containing 10, 20 and30 % solvent extracted soybean meal change the thickness of mucus membrane with abundance of goblet cells.

The histopathological changes of pancreas were very severe with necrosis in follicular cells in birds given 100% expeller extracted soybean meal, while pancreatic changes were very mild in group given 100% solvent extracted soybean meal which were not present in other groups. These results indicate that the effect of expeller extracted soybean meal on kidney; liver and pancreas were severe in birds given expeller extracted soybean meal. Similarly, where 100% solvent extracted soybean was used the changes were less severe as compared to the expeller extracted soybean meal. However, the changes in intestine were otherwise and were more severe in birds given 100% solvent extracted soybean meal than other groups. These results suggest that the changes in different organs vary with the use of solvent extracted or expeller extracted soybean meal.

The results on serum creatinine and urea supported histopathological changes seen in the kidney in respective groups. However, the results of serum AST and ALT did not correspond well with the histopathological changes seen in liver, as more severe changes of necrosis were noted in birds given 100% expeller extracted soybean meal, while the values of these enzyme were lowest. It may be due to reason that as changes were more severe the enzymes have long before been leaked during degenerative process and have become low when changes advanced to necrosis. This is strengthened by the finding in bird fed 100% solvent extracted soybean meal which showed highest value of these enzymes, where degenerative changes were still present and changes were not advanced to necrosis in most of the cells, an indication of leakage of enzymes occurring from degenerative cells.

***Conclusions*:** It is concluded from the present study that birds kept on 100% expeller extracted and solvent extracted soybean meal showed effects on various body organs, while 50% of both the solvent and expeller extracted soybean meal in combination did not show gross and histopathological changes. Results of serum urea and creatinine were high in birds showing histopathological changes of damage in kidney corresponded to severity of damage, however, serum AST and ALT level corresponded with degenerative changes and were high when degenerative changes were present but were low when necrotic changes in hepatocytes were present.

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