

# HAEMATO-BIOCHEMICAL PARAMETERS IN POULTRY LAYERS FED ON PROCESSED BLACK SOYBEAN SUPPLEMENTED DIET

Jyoti Joshi and Anshu Rahal<sup>1</sup>

Department of Animal Nutrition, College of Veterinary and Animal Sciences  
G.B. Pant University of Agriculture and Technology, Pantnagar-263 145 (Uttarakhand) India

## ABSTRACT

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An experiment was conducted to study the effect of feeding black soybean on haemato-biochemical parameters in poultry layers. Hundred poultry layers of twenty weeks age were selected and randomly divided into five treatment groups viz. T<sub>1</sub>: Control; T<sub>2</sub>: 0.2% raw black soybean; T<sub>3</sub>: 0.2% germinated black soybean; T<sub>4</sub>: 0.2% roasted black soybean and T<sub>5</sub>: 0.2% cow urine treated black soybean. A twelve week feeding trial was conducted in a completely randomized block design. At the end of third phase (32 weeks) blood collection was done to analyze effect of feeding black soybean on haemato-biochemical parameters. Highly significant ( $P \leq 0.01$ ) difference was observed in serum cholesterol. Significant differences ( $P \leq 0.05$ ) were observed among the different treatment groups in cholesterol content of egg and in serum glucose. It was concluded that feeding black soybean @ 0.2% has effect on cholesterol content and helps in lowering glucose.

**Key words:** Black soybean, haemato-biochemical, poultry layers

## Introduction

Black soybean commonly known as Kalabhata is cultivated in hilly terrain of Uttarakhand and is well known for its nutritional value. People consume this legume in different forms after processing in their daily diet. But this crop still does not form part of the diet of their livestock and poultry due to myths that it will affect animal productivity and health. In this experiment attempt was made to study impact of supplementation of processed black soybean on haemato-biochemical parameters in poultry layers.

## Materials and Methods

In order to study effect of supplementation of black soybean on haemato-biochemical parameters, hundred layers (20 weeks age) belonging to same hatch were selected from Instructional Poultry Farm, Nagla, College of Veterinary and Animal Sciences, Pantnagar (Uttarakhand) for feeding trial which was conducted in completely randomized design. All the birds were individually weighed and randomly allocated into 5 treatment groups each with two replicates of 10 laying hens each. Feed used for experimental layer was per BIS specification (1992). Black soybean was collected from the market of Haldwani. Black soybean was divided into 4 groups and then treated individually. The group T<sub>1</sub> served as control in which feed was provided with no supplementation of black soybean while T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were the treated groups in which raw black soybean, germinated black soybean, roasted black soybean and cow urine treated black soybean was added, respectively in ground form at 2 g/kg to layer ration. The proximate analysis of experimental diet was done per AOAC (2005). The experimental birds from different treatment groups were reared in individual cages in California cage system under similar housing and managemental conditions. Sixteen hours light was provided to the birds during the experimental period. By the end of phase III (32 week) about 6 ml of blood was collected aseptically from three laying birds of each replicate from the wing vein using sterilized disposable syringes and

needles and transferred it in sterile glass test tubes under sterile conditions. One part (2 ml) of the collected blood was transferred to vials containing anticoagulant EDTA for analysis of haematological traits viz. haemoglobin concentration, packed cell volume, total erythrocyte count and total leukocyte count and the remaining 4 ml was transferred to clean, dry and sterilized glass tube and kept in slanted position undisturbed at room temperature for 3 to 4 h for clot formation. The serum sample was collected using micro pipette and stored in eppendorf tube at -20°C till further use.

## Haematological parameters

Haemoglobin concentration was estimated as per the method defined by Sharma and Singh (2000) using Sahli's haemoglobinometer with acid haematin method. Packed cell volume (PCV) was estimated with the help of micro haematocrit method as prescribed by Sharma and Singh (2000). Total erythrocytes count (TEC) was enumerated using Neubauer's counting chamber as described by Jain (1986). Enumeration of total leucocyte count was conducted with Neubauer's counting chamber (Jain, 1986).

## Serum biochemical studies

Serum samples were used for study of certain biochemical parameters, protein profile, and some health status related parameters. Estimation of glucose and enzymes were done within 24 hours of collection. Glucose estimation was performed by enzymatic GOD-POD method using Span Diagnostic Kit at wavelength 505 nm against blank reagent (Sacks, 1998). Total cholesterol concentration in serum was estimated spectrophotometrically with the help of Erba Diagnostic Kit with enzymatic CHOD-PAP method at wavelength 505 nm (Tietz, 1998). Concentration of total protein in serum was estimated by Modified Biuret End Point Assay method using Erba Diagnostic Kit at 540 nm wavelength (Johnson *et al.*, 1999). Albumin concentration in the serum was estimated by bromocresol green, end point assay method with the help

<sup>1</sup>Corresponding author, email: anshurahal@rediffmail.com

of Erba diagnostic kit at 630 nm wavelength.

Serum calcium estimation was performed by O-Cresolphthalein Complexone (OCPC), end point assay method using Erba Diagnostic Kit at wavelength 578 nm against blank reagent (Moorehead and Briggs, 1974). Serum phosphorus estimation was performed by ammonium molybdate, end point assay method using Erba diagnostic kit at wavelength 340 nm against blank reagent (Wang *et al*, 1983).

All statistical analysis was done with the help of SPSS procedure and the data obtained during the experiment was further evaluated using one way analysis of variance (ANOVA) (Snedecor and Cochran, 1994).

## Results and Discussion

The results of proximate analysis of experimental diet are depicted in Table 1. The dry matter content of layer diet in treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 89.38, 89.08, 89.00, 89.18 and 89.45 per cent, respectively. The crude protein content of layer diet of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> was 20.82, 22.17, 22.09, 22.47 and 22.67 per cent, respectively. The crude protein % of T<sub>1</sub> group differed significantly ( $P \leq 0.05$ ) from T<sub>4</sub> and T<sub>5</sub> group, while non-significant difference was found between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>; T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>; T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>. The ether extract of layer diet of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> was 3.12, 3.01, 2.49, 3.50 and 2.69 per cent, respectively. The ether extract of T<sub>4</sub> group differed significantly ( $P \leq 0.05$ ) from T<sub>2</sub>, T<sub>3</sub>, T<sub>5</sub> and that of T<sub>3</sub> from T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub>. The ash content of layer diet of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> was 16.37, 15.95, 16.80, 18.28 and 18.67 percent, respectively. Ash content of T<sub>4</sub> and T<sub>5</sub> differed significantly ( $P \leq 0.05$ ) from T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. No significant difference was found among different experimental diets in terms of DM and CF%.

The haematological parameters of experimental layers have been presented in Table 2. Non significant differences were observed in values of haemoglobin in different treatment groups although higher values were noted in raw and treated black soybean groups. The haemoglobin values of different groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 9.28, 9.32, 9.28, 9.33 and 9.30, respectively. Similar results were obtained by Sada *et al*. (2013). He observed non-significant difference in the haemoglobin level between the control and soybean supplemented groups. According to Alada *et al*. (2004), rats fed 25% to 75% concentration of soybean in the diets, steadily but significantly increased the concentration of haemoglobin in the treated groups. Packed cell volume (PCV) average values of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 27.67, 28.33, 27.83, 28.0 and 27.16, respectively. Non significant differences were observed in PCV values of different treatment groups. However, highest and lowest PCV values were recorded in T<sub>2</sub> (28.33) and T<sub>5</sub> (27.16), respectively. Similar results were obtained by Sada *et al*. (2013). He observed non-significant difference in the packed cell volume (PCV) between the control and soybeans supplemented groups. The average total erythrocyte count (TEC) of different groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 2.67, 2.65, 2.63, 2.65 and 2.65 ( $\times 10^6/\mu\text{l}$ ), respectively. Non-significant difference was observed in total erythrocyte count among different treatment groups. Similar results were obtained by Sada *et al*. (2013). He observed non-significant difference in the total erythrocyte count (TEC) between the control and

soybeans supplemented groups.

The average total leucocyte count (TLC) ( $\times 10^3/\mu\text{l}$ ) of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 26.00, 23.17, 25.67, 25.50 and 24.50, respectively. Non significant difference was observed in total leucocyte count among treatment groups. Highest value of TLC was recorded in T<sub>1</sub> group, followed by T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>2</sub>. The average mean corpuscular volume (MCV) (%) of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 103.76, 106.76, 105.97, 105.61 and 102.51, respectively. Non-significant differences were observed among the groups. The average mean corpuscular haemoglobin (MCH) of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 34.81, 35.10, 35.34, 35.20 and 35.10, respectively. Non-significant differences were observed in MCH in treatment groups. Non-significant differences were observed in mean corpuscular haemoglobin concentration (MCHC) in treatment groups. The average MCHC (%) of different treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 33.56, 32.92, 33.35, 33.36 and 34.23, respectively.

The serum biochemical parameters of experimental layers have been presented in Table 3. Significant differences were found in serum glucose among the treatment groups. The average values of serum glucose (mg/dl) of the different groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 214.02, 183.03, 178.71, 165.84 and 167.50 (mg/dl), respectively. The values of serum glucose decreased significantly in treated groups compared to control. The maximum reduction was observed in T<sub>4</sub> followed by T<sub>5</sub>, T<sub>3</sub>, and T<sub>2</sub>. Lo *et al*. (1986) reported that supplementation of soy fibre in the diets of patients with IGT (Impaired glucose tolerance) significantly reduced fasting blood glucose level.

The soybean fibre includes pectins, galactomannans and arabinogalactans having high viscosity responsible for delayed gastric emptying and glucose absorption which limits the rate of intra-luminal diffusion of glucose to the absorption surface (Holt *et al*, 1979; Madar, 1983). Similar results were observed by Chang *et al*. (2008). He reported decrease in serum glucose in diabetic patients when they were given their pills along with roasted soybean powder. Chen *et al*. (2010) also reported that soybean oligosaccharides significantly reduced abnormal blood glucose. According to Pollak and Kritchevsky, 1981, phytosterols have a hypocholesterolaemic effect through their action on intestinal cholesterol absorption and/or synthesis. In the present study highly significant differences ( $P \leq 0.05$ ) were found between T<sub>1</sub> and T<sub>4</sub> while no significant difference was found between T<sub>3</sub> and T<sub>5</sub>. Serum cholesterol (mg/dl), decreased significantly ( $P \leq 0.01$ ) among the raw and black soybean treated groups. Maximum serum cholesterol was recorded in control group (191.74 mg/dl) which was significantly higher compared to supplemented groups. Maximum reduction was observed in the group T<sub>4</sub> (168.73 mg/dl) which was significantly different from other treated groups T<sub>2</sub>, T<sub>3</sub> and T<sub>5</sub> (185.84, 178.46, 176.40 mg/dl). Isoflavones possesses weak oestrogenic activity in biological systems.

Isoflavones in soybeans decrease serum cholesterol via 'oestrogenic' effects (Potter, 1995; Anthony *et al*, 1996). According to Sugano and Koba (1993), the indigestible fraction of soy protein lowers the serum and liver cholesterol concentration while Potter (1995) also suggested that hypocholesterolaemic action of whole soy protein or protein hydrolysates was attributable due to the presence of

Table 1: Proximate composition of experimental diets

Parameters (%)	T <sub>1</sub> (Control)	T <sub>2</sub> (0.2% RBS)	T <sub>3</sub> (0.2% GBS)	T <sub>4</sub> (0.2% RoBS)	T <sub>5</sub> (0.2% CUTBS)
Dry matter	89.38±0.39	89.08±0.31	89.00±0.25	89.18±0.44	89.45±0.48
Crude protein**	20.82 <sup>b</sup> ±0.18	22.17 <sup>ab</sup> ±0.60	22.09 <sup>ab</sup> ±0.39	22.47 <sup>a</sup> ±0.27	22.67 <sup>a</sup> ±0.24
Ether extract	3.12 <sup>ab</sup> ±0.17	3.01 <sup>bc</sup> ±0.01	2.49 <sup>d</sup> ±0.17	3.50 <sup>e</sup> ±0.05	2.69 <sup>cd</sup> ±0.03
Ash	16.37 <sup>bc</sup> ±0.04	15.95 <sup>c</sup> ±0.01	16.80 <sup>b</sup> ±0.01	18.28 <sup>a</sup> ±0.01	18.67 <sup>a</sup> ±0.48
Crude fibre	5.59±0.08	5.64±0.05	5.52±0.01	5.57±0.05	5.59±0.02

Means bearing different superscript in a row differ significantly (P≤0.05)

Table 2: Means ± S.E. of haematological parameters of laying hens

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	SEM	P value (0.05)
Hb (g/dl)	9.28 ±0.05	9.32 ±0.01	9.28 ±0.01	9.33 ±0.13	9.30 ±0.03	0.023	0.974
PCV (%)	27.67 ±0.33	28.33 ±1.00	27.83 ±0.16	28 ±1.00	27.16 ±0.16	0.254	0.783
TEC (%)	2.67 ±0.01	2.65 ±0.02	2.63 ±0.007	2.65 ±0.01	2.65 ±0.01	0.006	0.493
TLC (%)	26.00 ±0.33	23.17 ±1.16	25.67 ±2.00	25.50 ±0.16	24.50 ±0.16	0.490	0.414
MCV (%)	103.76 ±1.76	106.76 ±4.77	105.97 ±0.90	105.61 ±4.23	102.51 ±0.24	1.12	0.842
MCH (%)	34.81 ±0.01	35.10 ±0.26	35.34 ±0.03	35.20 ±0.26	35.10 ±0.26	0.126	0.846
MCHC (%)	33.56 ±0.58	32.92 ±1.23	33.35 ±0.25	33.36 ±0.71	34.23 ±0.33	0.276	0.772

Means bearing different superscript in a row differ significantly (P≤0.05)

saponins. Similar results were obtained by Kwon *et al.* (2007). He found that black soybean anthocyanins significantly reduced the levels of serum triglyceride and cholesterol (P<0.05). Byun *et al.* (2010) also concluded that plasma total cholesterol in the black soybean group were significantly lower than that in the normal diet with casein group. Non-significant differences were found in serum total protein in different treatment groups. The average serum protein (g/dl) of treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 5.30, 5.25, 5.31, 5.30 and 5.32 g/dl, respectively.

Higher values of serum total protein were noted in group fed cow urine treated black soybean. According to Alada *et al.* (2004), rats fed 25% to 75% concentration of soybean in the diets, steadily but significantly increased the concentration of total plasma protein in the treated groups. Non-significant differences were found in serum albumin in different treatment groups. The average serum albumin (g/dl) of treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 1.85, 1.87, 1.87, 1.89 and 1.83 g/dl, respectively. Highest value of total albumin was recorded in T<sub>2</sub> and lowest in T<sub>5</sub>. According to Alada *et al.* (2004), rats fed 25% to 75% concentration of soybean in the diets, steadily but significantly increased the concentration of plasma albumin in the treated groups. Non-significant differences were found in serum globulin different treatment groups. The average serum globulin of treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 3.45, 3.38, 3.44, 3.41 and 3.49 g/dl, respectively. Non-significant differences were found in A:G in different treatment groups. The average values of serum albumin: globulin of treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 0.54, 0.55, 0.54, 0.56 and 0.52,

Table 3: Means ± S.E. of serum bio-chemical parameters of laying hens

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	SEm	P value (0.05)
Glucose (mg/dl) *	214.02 <sup>a</sup> ±1.37	183.03 <sup>b</sup> ±0.54	178.71 <sup>bc</sup> ±8.38	165.84 <sup>c</sup> ±2.98	167.50 <sup>bc</sup> ±3.19	5.962	0.003
Cholesterol (mg/dl) **	191.74 <sup>a</sup> ±1.77	185.84 <sup>b</sup> ±0.59	178.46 <sup>c</sup> ±0.89	168.73 <sup>d</sup> ±0.59	176.40 <sup>c</sup> ±1.18	2.666	0.000
Total protein (g/dl)	5.30 ±0.05	5.25 ±0.06	5.31 ±0.03	5.30 ±0.01	5.32 ±0.005	0.015	0.777
Total albumin (g/dl)	1.85 ±0.02	1.87 ±0.01	1.87 ±0.01	1.89 ±0.02	1.83 ±0.03	0.010	0.371
Globulin	3.45 ±0.3	3.38 ±0.05	3.44 ±0.01	3.41 ±0.03	3.49 ±0.03	0.017	0.308
A:G	0.54 ±0.001	0.55 ±0.003	0.54 ±0.002	0.56 ±0.01	0.52 ±0.01	0.005	0.175
Calcium (mg/dl)*	21.15 <sup>ab</sup> ±1.15	19.70 <sup>b</sup> ±1.03	23.44 <sup>a</sup> ±0.17	22.41 <sup>ab</sup> ±0.57	21.32 <sup>ab</sup> ±0.46	0.492	0.109
Phosphorus (mg/dl)*	3.47 <sup>ab</sup> ±0.14	3.19 <sup>b</sup> ±0.14	3.47 <sup>ab</sup> ±0.14	3.74 <sup>a</sup> ±0.13	3.47 <sup>ab</sup> ±0.14	0.074	0.234

Means bearing different superscript in a row differ significantly (P≤0.05)

respectively. Significant differences were found in serum calcium between T<sub>2</sub> and T<sub>3</sub> group, whereas non-significant differences were found between T<sub>1</sub>, T<sub>4</sub> and T<sub>5</sub> group. The average value of calcium (mg/ml) of treatment group T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 21.15, 19.70, 23.44, 22.41 and 21.32, respectively. In serum phosphorus significant differences were found between T<sub>2</sub> and T<sub>4</sub> group, whereas non-significant differences were found between T<sub>1</sub>, T<sub>3</sub> and T<sub>5</sub> group. The average value of phosphorus (mg/ml) of treatment group T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were 3.47, 3.19, 3.47, 3.74 and 3.47, respectively. It may be concluded that black soybean can be recommended for feeding poultry layers @ 0.2% in their ration.

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