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Effect on blood biochemical, serum total immunoglobulin and blood antioxidant profile of rabbits fed probiotic supplemented diets

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Abstract
Newly weaned rabbits are more susceptible for colonization of various microbes in gastrointestinal track. The study was taken up in the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India). Sixty weaned rabbits of almost same age (28 days old) and similar initial live body weight were selected and distributed into four groups (T_1, T_2, T_3 and T_4 groups) of fifteen each. The diets were given in pelleted form and *ad libitum* throughout the trials. The pellet feed for the T_1 group was not supplemented with probiotics while the pellet feed for groups T_2, T_3, and T_4 was supplemented with *Lactobacillus plantarum* (Lp), *Saccharomyces cerevisiae* (Sc), and a combination of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Lp + Sc), respectively. The microbial population was 10^6 CFU/g pellet feed. The result indicates significantly ($p<0.05$) decreased serum cholesterol level and non-significantly increased serum total immunoglobulin level were found in probiotics supplemented groups. Improved blood antioxidant property found in probiotics supplemented groups.

Keywords: Rabbit, probiotic, blood, cholesterol, immunoglobin

Introduction
Probiotics are defined as monocultures or mixed cultures of live microorganisms; when consumed, they exert a beneficial influence on animal health by quantitative and qualitative effects on the intestinal microflora or even modification of the immune system (FAO/WHO, 2001; Reid, 2016) [6, 11]. Probiotic are reported as an alternative to antibiotic for growth promotion. Raising rabbits in an intensive system can cause many environmental and physiological stresses, mainly during the weaning period. The establishment of healthy, stable and diverse digestive tract microflora is of great significance for rabbits to resist intestinal diseases. Proper function of the mucosa-associated immune system relies on the presence of intestinal bacteria. The use of probiotics had many potential benefits such as modified host metabolism, immune-stimulation, anti-inflammatory reactions, exclusion and killing of pathogens in the intestinal tract, reduced bacterial contamination on processed broiler carcasses, enhanced nutrient absorption and performance, and ultimately decreased human health risk (Edens, 2003; Patil *et al*., 2015) [2, 11]. This study was conducted to assess the effect of probiotics feeding on various blood parameters of rabbit.

Materials and Methods
The present study was conducted to evaluate the effect of probiotic supplementation in rabbit feed in terms of biochemical, immunological and antioxidant parameters of blood, in weaned rabbits. A total 60 number of weaned (28 days old) Soviet Chinchilla male and female rabbits with nearly similar initial live body weight were used for the study. The rabbits were divided into four groups (T_1, T_2, T_3, and T_4 groups) with fifteen (8 males + 7 females) rabbits in each group. The feeding experiments on weaned rabbits was carried out for 60 days at the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India).

Rabbits were housed individually under similar housing and management conditions in wire mesh cages (180 × 240 × 180 mm) inside an asbestos-roofed animal shed. The diets were given in pelleted form and *ad libitum* throughout the trials. The pellet feed for the T_1 group was not supplemented with probiotics while the pellet feed for groups T_2, T_3, and T_4 was supplemented with *Lactobacillus plantarum* (Lp), *Saccharomyces cerevisiae* (Sc), and combination of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Lp+Sc) respectively. The freshly cultured broth media of individual probiotics was incorporated as 100 milliliters per kilogram of feed during the pellet feed formulation. Pellets were assessed for respective microbial populations (10^6 CFU/g Pellet) after 0, 7 and 15 days interval. Blood samples were collected from the animals on the final day of experimental feeding by heart puncture into tubes containing anticoagulant (sodium heparin) or without anticoagulant for different analyses. The blood samples were transported to the laboratory in an ice bath. Serum was harvested from whole blood after clotting at 37 ºC for 4 h followed by centrifugation at 500×g for 20 min. Serum was stored at -20 ºC until further analysis. After centrifugation the supernatant (*i.e.* serum) was made in two aliquots; one for serum enzymes and another for total protein, cholesterol, albumin, and serum glucose estimation. All the estimations were done in duplicate. All the blood biochemical parameters were estimated by using Accurex Biomedical Pvt Ltd, India. Total immunoglobulin in serum sample was estimated by zinc turbidity method (Mc Ewan and Fisher, 1970) [8]. For the estimation of blood antioxidant enzyme activity blood was centrifuged at 2000 rpm for 10 min. After that plasma was removed from centrifuge tube and then 1 ml of chilled normal saline was added in the sediment and again it was centrifuged at 2000 rpm for 20 min at 4 °C, after that supernatant along with buffy coat was removed and again 1 ml of chilled normal saline was added. Same step was repeated for 3 times. After removal of supernatant, equal amount of normal saline was added in the tube as much it contained sediment and was mixed well. Lastly hemolysate was made by adding 200 µl RBC suspension into 1800 µl EDTA stabilizing solution (2.7 mM, pH-7 EDTA and 0.7 mM beta-mercaptoethanol). Hemolysate was kept in -20 °C for further analysis. The total antioxidant capacity (TAC), total oxidative status (TOS) and oxidative stress index were determined manually following the procedures described by Erel (2004) [4] and Erel (2005) [5]. Superoxide dismutase activity was determined manually following the procedure described by Marklund and Marklund (1974) [9]. The experimental data's were analyzed using ANOVA using SPSS-16 software. The Significance of mean values among different dietary treatments were tested by Tukey's test.

Results and Discussion

Table 1: Effect of probiotics on blood biochemical parameters and serum total immuoglobin (n=6)

Particulars	T_1	T_2	T_3	T_4	SEM	P-Value
Hemoglobin g/dl	11.60	11.25	11.84	11.59	0.183	0.784
Glucose mg/dl	83.36	94.91	92.46	90.21	3.048	0.624
Triglyceride mg/dl	97.30	97.74	98.57	99.25	1.626	0.979
Cholesterol mg/dl	71.34^{b}	66.49^{ab}	64.87^{ab}	58.85^{a}	1.576	0.032
Total protein g/dl	4.24	4.02	4.09	4.35	0.109	0.727
Albumin g/dl	2.93	2.65	2.51	2.87	0.073	0.147
Globulin g/dl	1.31	1.36	1.57	1.47	0.093	0.771
Albumin/Globulin	2.39	2.08	1.81	2.14	0.146	0.607
Serum Total Immunoglobulin mg/ml	11.53	12.34	12.40	12.22	0.291	0.725

Results of blood biochemical parameters were presented in Table 1. The findings of this study revealed no effect of probiotics supplementation on blood components including hemoglobin, serum glucose, triglyceride, total protein, albumin, globulin, and ratio of albumin and globulin. However, cholesterol level was affected *($p \leq 0.05$).* The cholesterol-lowering effect was found in the probiotics supplemented groups (T_2, T_3, and T_4) as compared to the no-probiotics supplemented group (T_1). Results of total immunoglobulin were insignificant numerical increased in probiotic supplemented groups compared with the no-probiotic group. Abdel-Azeem *et al.* (2009**)** [1] reported that cholesterol content in serum blood were reduced significantly ($p \leq 0.01$ or 0.05) for rabbits fed a diet containing 400 mg Bioplus 2B/kg feed as compared to the other groups. El-Shafei *et al.* (2019**)** [3] also reported a similar result of cholesterol-lowering effect and increase serum immunoglobulin effect in probiotics supplemented groups.

Table 2: Effect of probiotic on blood antioxidants (n=6)

Parameters	T_1	T_2	T_3	T_4	SEM	P-Value
TAC (µM Trolox Equi.)	3896.11	3443.33	3511.48	4041.11	136.869	0.352
TOS (µM H_2O_2 Equi.)	94.85^{b}	56.83^{a}	39.88^{a}	60.35^{a}	5.345	0.000
OSI (Per cent)	2.54^{b}	1.68^{a}	1.15^{a}	1.53^{a}	0.158	0.007
SOD (U/g)	60.13^{a}	79.92^{ab}	87.81^{b}	84.69^{b}	3.593	0.017

Mean value of various antioxidants were presented in table 2. The results showed that, all probiotics supplemented groups recorded non-significant differences in total antioxidant capacity (TAC). Significant ($p \leq 0.05$) differences were observed in total oxidative status (TOS), super oxide dismutase (SOD) activity and oxidative stress index (OSI). Highest TOS was reported in no-probiotics supplemented group as compared to probiotic supplemented groups. OSI was also indicated low TOS in probiotic supplemented groups. SOD activity was reported high in probiotic supplemented group than no-probiotic supplemented group. Ghoneim *et al.* (2016) [7] also reported microsomal SOD activity was elevated in liver, kidneys, and skeletal muscles of probiotics-fed animal. Shah *et al.* (2018) [12] also reported the superoxide dismutase increased ($p \leq 0.05$) in probiotic supplemented group compared to the control groups.

Conclusion and Acknowledgement

In conclusion, it's fair to state that the inclusion of probiotics (Lp. and Sc.) in rations for weaned rabbits showed lowering serum cholesterol, increased serum total immunoglobulin and improved SOD activity in blood. The authors are thankful to the Director of ICAR-Central Sheep and Wool Research Institute, Avikanagar (Rajasthan), and Dean of Post Graduate Institute of Veterinary Education and Research, Jaipur (Rajasthan) for the support and fund provided to carry out the research.

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