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Effects of chemicals (minerals) and yeast (probiotic) on the haematobiochemical profile of rabbit

K.Shrivastava*, R.R.Jha

Department of Chemistry, Ranchi University, Ranchi, Jharkhand, (INDIA)

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ABSTRACT

In recent years the inorganic constituents in biological system have been receiving increasing attention. About 30 elements are recognized as essential to life[4]. Some are required in bulk, or at least in macroscopic amount, essentially in all forms of life. The important elements are H, Na, K, Mg, Ca, C, N, O, P, S and Cl. The others occur in trace quantities, although Fe, Cu and Zn are at the top end of this "trace" scale and are apparently, also essential to all, or at least most forms of life. One of the major roles played by metallic element in biochemistry is in metaloenzymes and in the enzyme catalysis. The role of the metal atoms in enzymic catalysis is currently an active subject of research. The trace elements unquestionably play an important role in Pathology and Physiology of biological system. Therefore, the effect of probiotic bacterial culture and mineral mixture individually or in combination was studied in 24, apparently healthy weaned rabbits of 6 weeks of age. At 6th week of age, all the rabbits were randomly divided into five groups. Each group was having 6 rabbits. The differences among groups were non-significant. Group G_1 was considered as control group maintained on basal diet without probiotic and mineral mixture. G_2 and G_3 groups were supplemented with 2% and 4% mineral mixture, respectively along with basal diet. Group G_4 was supplemented with 60g probiotic along with basal diet whereas G_5 group was supplemented with 60g probiotic and 2% mineral mixture along with basal diet. The present results showed that rabbits of G_5 and G_4 groups had significantly higher total serum protein values compared with the other dietary treatments. Glucose level was observed to be the highest in the G_5 group followed by G_4 group. The total serum cholesterol level was reduced significantly in treatment groups as compared to control group suggesting that these feed supplements may reduce the cholesterol level and may be helpful in preventing atherosclerosis or other cardiac diseases. The highest level of lipid in blood of rabbit was observed in G_5 group. The AST, ALT and ALP in serum of the rabbits did not differ significantly among treatment groups and control group indicating that these feed supplements have no adverse effect on liver, kidney, heart or other vital organs. Variation in the level of Sodium and Potassium in serum was non significant among treated group and control group. They were in their physiological level indicating that supplementation of probiotics/ yeast culture and mineral mixture did not change the ratio of these minerals in the blood. It further supported that these feed supplements have no adverse effect on kidney. A reduction in the cholesterol level despite increase in total lipid suggested that their effect on lipid metabolism might be leading to lower the blood level of unwanted saturated fatty acid while increasing the level of unsaturated essential fatty acids which can be concluded as most important positive effect of these feed supplements.

INTRODUCTION

Rabbit is one of the most important laboratory animals which is widely used for various experimental works. It is therefore, imperative to know the base value of various biochemical parameters of serum of rabbit blood, so that the comparison could be made with the test value. The role of minerals in living organisms is an emerging area of strong interest for producers, feed manufactures and scientists because trace elements

unquestionably play an important role in Pathology and Physiology of biological system. Adequate mineral intake and absorption is required for a variety of metabolic functions including immune response, reproduction and growth. Mineral supplementation strategies quickly become complex because of differences in trace mineral status of all livestock. Sub clinical mineral deficiencies pose greater economic threat than acute mineral deficiency. Interference in absorption through intestine is a major cause of sub clinical mineral deficiency if there is no dietary insufficiency. In this respect it is to be noted that digestive process is very complex and fragile in rabbits due to caecal microbial fermentation in them because of great variation in adoptability in such microflora. That is why rabbits are rather sensitive to enteric diseases and especially when they are exposed to negative impacts, e.g. weaning or heat stress, causing high losses. To get rid of this problem, antibiotics were used to check growth of harmful organisms which cause inhibition of these innocuous useful micro floras through competitive inhibition. However, because of the general intention to limit antibiotics in animal feed as growth promoter concerning side-effects, resistance and recent public perception about healthy food, new alternatives to antibiotics are needed[14]. Probiotic that contain yeast, live bacteria or bacterial spores can also prevent enteric diseases of rabbits. Instead of growth promoters with antibiotics that kill some of the rabbit's own gastrointestinal flora, Probiotic promote gut colonization and stabilize eubiosis by competitive growth against harmful microorganisms, reducing the intestinal pH with production of lactic acid and encouraging digestion by producing enzymes and vitamins. These functions strengthen the animal's own non-specific immune defense[5]. Dietary administered probiotic bacteria decreased the frequency of *E. coli* translocation[11] and were effective in preventing the growth of *E. coli* O157:H7 in the intestine of neonatal rabbits[20]. Hamrany et al.[7] found a dose dependent positive effect of a probiotic bacterium on *E. coli* occurrence in the caecum and small intestine in young rabbits. Term "probiotics" in translation from Greek means "for life". In many authors', scientists' opinion, probiotics-living micro organisms or their fermented products, which decompose undesirable food matter, increase digestion of feed. Therefore such probiotical preparations and their compositions added to feed "widen" the system of fermented digestion. Mineral play important role in haematology and help in the formation of haemoglobin and maturation of RBC in animals besides bone formation metabolic activator and overall growth of animal species. Therefore, effect of probiotic bacterial culture and mineral mixture individually or in combination was studied in 24, weaned rabbits of 6 weeks of age.

TABLE 1 : Composition of rations of different treatment groups given to the rabbits.

Ingredients	G1	G2	G3	G4	G5
Maize (%)	30	30	30	30	30
Groundnut cake (%)	26	26	26	26	26
Wheat bran (%)	43	43	43	43	43
Common salt (%)	1	1	1	1	1
Mineral mixture (%)	-	2	4	-	2
Probiotics (g/100 kg)	-	-	-	60	60

MATERIALS AND METHODS

A feed trial was conducted on 24 apparently healthy weaned rabbits of 6 weeks of age for a period of 7 weeks under farm system of management. At 6th week of age, all the rabbits were randomly divided into five groups. Each group was having 6 rabbits. The differences among groups were non-significant.

Group G_1 was considered as control group maintained on basal diet without probiotic and mineral mixture. G_2 and G_3 groups were supplemented with 2% and 4% mineral mixture, respectively along with basal diet. G_4 was supplemented with 60g probiotic along with basal diet whereas G_5 group was supplemented with 60g probiotic and 2% mineral mixture along with basal diet. Composition of rations of different treatment groups of rabbit is presented in TABLE 1. The compositions of mineral mixture and probiotic are presented in TABLE 2 and 3 respectively.

The rabbits were fed *ad lib* given in the morning after weighing and subsequently the consumptions of feed were recorded after subtracting the left over feed in the feeder and wastage. The ration was analyzed for proximate principles[1]. Blood samples were collected directly from heart of the experimental animals taking all aseptic precautions into two sets of sterile glass tubes.

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TABLE 2 : Composition (per kg) of mineral mixture (Agrimin high power powder) given to the rabbits.

Sl. No.	Ingredients	Amount
1	Cobalt	150mg
2	Copper	1200mg
3	Iodine	325mg
4	Iron	5000mg
5	Magnessium	6000mg
6	Manganese	1500mg
7	Potassium	100mg
8	Selenium	10mg
9	Sodium	5.9mg
10	Sulphur	0.922%
11	Zinc	9600mg
12	DL-metionine	1920mg
13	L-LysineMono-hydrochloride	4400mg
14	Phosphorus	12%
15	Calcium	24%

TABLE 3 : Composition of probiotic powder (Biovet YC powder) given to the rabbits.

Sl. No.	Ingredients	Amount
1	Lactobacillus sporogenes	30,000 million c.f.u.
2	Live yeast culture Saccharomyces cerevisiae SC-47	125,000 million c.f.u.
3	Alpha amylase	5g

One set with anticoagulant using potassium salt of ethylene diamine tetra-acetic acid (EDTA) and another without anticoagulant for serum collection.

The total serum protein was estimated by using phenol reagent according to the method of Lowery et al.[12]. Blood glucose was estimated by Nelson-Somogy method[15]. Total cholesterol in blood serum was estimated by the method of Ziatkis et al.[22]. The total serum lipid estimation was done by using the method of Marsh and Weinstein[13]. The total phospholipid in the serum was estimated by method of Post and Sen[17]. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in the serum was assessed by Reitman and Frankle[18] method. Sodium (Na^+) and Potassium (K^+) was estimated by conventional method using flame photometer.

RESULTS AND DISCUSSION

The mean values of total serum protein, blood glucose, total serum cholesterol, total serum lipid and phospholipids are presented in TABLE 4. The present results showed that rabbits of G_5 (supplemented with 2% mineral mixture and 60g probiotic) and G_4 (supplemented with 60g probiotic) had the higher ($P<0.05$) total serum protein values (5.19±0.25 and 4.87±0.13g/dl respectively) compared with the other dietary treatments. The total plasma protein in rabbit ranged from 4.36±0.09 to 5.19±0.25g/dl. Increased serum total protein has been reported to be directly responsive to protein intake and protein quality improved by supplementation of probiotics[3]. It is also due to the Zn that influence at the cellular level for the synthesis of nucleic acids and protein synthesis[21]. The glucose level in rabbit blood ranged from 131.42±1.27 to 151.37±2.84 mg %. Glucose level was observed to be the highest in the G_5 (151.37±2.84) group of rabbits (supplemented with 2% mineral mixture and 60g probiotic) followed by G_4 (142.81±1.42) group, supplemented with 60g probiotic and G_3 group (137.54±2.20). However, the control group G_1 did not differ significantly from G_2 and G_3 groups. Increase in level of glucose in probiotic & mineral treated group suggest increased digestion of carbohydrate & their absorption through intestine which might be due to more fermentative action of microflora present in probiotics. The total serum cholesterol level was reduced significantly in treatment groups as compared to control group. The serum cholesterol level was observed to be the highest in control group G1 66.09±1.37 mg %. The treated groups G_2, G_3, G_4 and G_5 did not differ significantly among themselves (TABLE 4). Our observations corroborated with data published by Gudev et al.[6], Kannan et al.[9], and Onifade et al.[16], who stated that there was a decrease in plasma cholesterol for chicks fed with diets containing yeast and different probiotics. Feeding of calcium carbonate to rabbits also prevent the elevation of serum cholesterol[8]. A number of probiotics are known to metabolize bile salts, bile acids and prevent reabsorption and recirculation of bile acids into blood. In this way, Probiotics could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids. Use of probiotics lead to increased excretion of deconjugated bile acids. Cholesterol is precursor of bile acid, hence more mol-

TABLE 4 : Average haematobiochemical profiles of rabbit at 13th week of age raised on different types (composition) of feed.

Parameters	G_1	G_2	G_3	G_4	G_5
TSP(g/dl)	4.36 ± 0.09^{c}	4.41 ± 0.09^{c}	4.54 ± 0.08^{bc}	4.87 ± 0.13^{a}	5.19 ± 0.25^{a}
Blood Glucose (mg%)	135.20 ± 1.55^{cd}	131.42 ± 1.27^{c}	137.54 ± 2.20^{bd}	142.81 ± 1.42^{b}	151.37 ± 2.84^{a}
Cholesterol (mg%)	66.09 ± 1.37^{a}	44.25 ± 0.92^{b}	46.48 ± 1.01^{b}	44.88 ± 0.52^{b}	47.51 ± 1.56^{b}
Lipid (mg ml^{-1})	4.93 ± 0.16^{b}	4.83 ± 0.26^{b}	5.12 ± 0.23^{b}	5.73 ± 0.16^{ab}	6.58 ± 0.69^{a}
Phospholipids (mg lecithin ml^{-1} serum)	131.65 ± 1.76^{c}	127.36 ± 4.12^{c}	135.01 ± 1.57^{bc}	150.74 ± 3.30^{a}	141.60 ± 2.58^{b}

Each value is the average of 6 observations. Mean under the same superscript in a row did not differ significantly.

TABLE 5 : Average AST, ALT, LDH, ALP, Sodium and Potassium in serum of rabbits at 13th week of age raised on different types (composition) of feed.

Parameters	G_1	G_2	G_3	G_4	G_5
AST (μM pyruvate released min^{-1} L^{-1} serum)	12.36±0.48	13.01±0.58	12.83±0.48	17.95±0.56	13.09±0.45
ALT (μM pyruvate released min^{-1} L^{-1} serum)	13.62±0.81	14.89±0.76	14.71±0.77	14.98±0.31	15.09±0.30
LDH (UL^{-1} min^{-1})	37.99 ± 1.03^{a}	39.55 ± 1.15^{a}	37.35 ± 1.27^{a}	32.99 ± 0.94^{b}	38.22 ± 1.36^{a}
ALP (μMp_iL^{-1} min^{-1} serum)	35.34±0.91	34.85±0.77	35.80±0.44	33.59±0.72	35.28±0.48
Sodium (mEq/L)	46.76±1.76	45.79±1.16	46.12±2.26	45.34±1.22	47.22±1.34
Potassium (mEq/L)	15.02±0.48	15.06±0.72	14.46±0.99	13.90±0.88	14.77±0.57

Each value is the average of 6 observations. Mean under the same superscript in a row did not differ significantly.

ecules are spent for recovery of bile acids[2]. As a result of increased synthesis of this acid, it appears that the level of serum cholesterol gets reduced. Klaver and Van Der Meer[10] suggested that coprecipitation with bile acids might be of importance for decreasing of serum cholesterol concentrations. The highest level of lipid in blood of rabbit was observed in the treated group G_5 (6.58±0.69mg ml^{-1}) suggesting that these feed supplements may reduce the cholesterol level and may be helpful in preventing atherosclerosis or other cardiac diseases. However the treated group G_2, G_3 and G_4 did not differ significantly from control group (TABLE 4) which is suggestive of more absorption of lipid through intestine after increased digestion.

Average values AST, ALT, LDH, ALP, Sodium and Potassium in serum of rabbits at 13th week of age raised on different types (composition) of feed are presented in TABLE 5. The AST, ALT and ALP in serum of the rabbits did not differ significantly among treatment groups and control group. These enzymes are located intracellularly in the body including liver, heart, kidney etc. Their level in blood is increased when there is membrane damage in these cells due to degenerative changes. Hence, normal level of these enzymes in blood in probiotic treated rabbits suggests that it has no adverse effect on the cells vital organs. The present result corroborates well with the findings of Singh et al.[19]. Variation in the level of Sodium and Potassium in serum was non significant among treated group and control group. They were in their physiological level indicating that supplementation of probiotics/ yeast culture and mineral mixture did not change the ratio of these minerals in the blood.

A reduction in the cholesterol level despite increase in total lipid suggested that its effect on lipid metabolism might be leading to lowering the blood level of unwanted saturated fatty acid while increasing the level of unsaturated essential fatty acids which can be concluded as most important positive effect of these feed supplements.

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