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Feed incorporation of MOS and *Ocimum sanctum*: Effects on antioxidant and biochemical, profile of New Zealand white rabbits

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Abstract
The present study was conducted on weaned broiler rabbits having similar body weights, which were divided into four groups with ten rabbits in each group for a period of 42 days to study the effect of inclusion of mannan oligosaccharides (MOS), oxytetracycline and *Ocimum sanctum* supplements in the basal diet on antioxidative enzymes status and biochemical profile in Newzealand white rabbits. The study revealed that the catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were significantly (p<0.05) increased in *Ocimum sanctum* supplemented group, whereas catalase and SOD were increased significantly (p<0.05) in MOS and Oxytetracycline groups. However, MDA levels were significantly (p<0.05) decreased in MOS and *Ocimum sanctum* supplemented groups. Among several biochemical parameters observed under the study ALT and AST were exhibited significant (p<0.05) decrease in T1 (MOS) and T3 (*Ocimum sanctum*) groups, whereas T2 (Oxytetracycline) group exhibited similar range with control group. Total protein, albumin levels were significantly (p<0.05) increased in T1 and T3 groups, whereas T2 group exhibited similar range with control group. Glucose and cholesterol exhibited significant (p<0.05) decrease in all treatment groups compared to the control group. Creatinine levels were significantly (p<0.05) decreased in T1 and T3 groups. Bilirubin exhibited significant (p<0.05) decrease in all groups compared to the control group. A significantly (p<0.05) increased levels of chloride was recorded in T1 and T3 groups.

Keywords: Antioxidative enzymes, Biochemical profile, MOS, Oxytetracycline, *Ocimum sanctum*, Rabbit

1. Introduction
In rabbit production, a high mortality due to stress is the major concern. The growth rate of broiler rabbits is rapid hence put them under the lot of stress, which results in poor performance and high mortality. Oxidative stress is a major concern during the period of rapid growth in broiler rabbits. This is produced due to formation of excess molecules of reactive oxygen species (ROS) owing to peroxidation leading to build up of lipid peroxides (Kim *et al.*, 2010) [15]. The oxidative stress induced by reactive oxygen species (ROS) is a major contributor to a variety of diseases. Antioxidants are chemicals that delay or prevent the oxidation of substrates such as protein, lipids, carbohydrates and other cellular organelles including DNA (Kurien and Scofield 2006) [18]. Stress mitigation for enhanced growth rates in domestic animals is a major concern across the globe. Among several mechanisms of reducing the oxidative stress, one of the common methods is dietary supplementation with several additives that act as anti-stressors. The common additives to combat oxidative stress through dietary supplementation are prebiotics, growth promoting antibiotics and herbal compounds. Mannan oligosaccharides (MOS) are a group of prebiotics produced from the outer cell wall of yeast *Saccharomyces cerevisiae* which are made up of the mannan element, which protects the gut mucosal receptors by washing out the harmful pathogens resulting in optimum growth and gain weight of the animals (Ayyat *et al.*, 2018; Abdel-Hamid and Farahat 2015) [7, 1]. MOS act as free radical scavengers to reduce oxidative stress (Bozkurt *et al.*, 2012) [9]. The antibiotic oxytetracycline acts by interfering with bacteria's ability to grow or proliferate (Kahsay *et al.*, 2013) [14]. Oxytetracycline exhibits antioxidant properties by its free radicals scavenging activity (Kładna *et al.*, 2012) [16]. Phytogenic feed additives are defined as herbal ingredients added to feed to improve animal's performance, health and production. They serve to improve the flavour, taste and feed utilization and there by animal performance (Krieg *et al.*, 2009) [17]. Tulasi (*Ocimum sanctum*) is one of the herbal ingredients added to the diets for better growth by reducing the oxidative stress (Varaprasad Reddy *et al.*, 2014a) [28].

Hence, a study was conducted to observe the effects of dietary supplementation of MOS, oxytetracycline and *Ocimum sanctum* on antioxidative enzymes status and biochemical profile in Newzealand white rabbits.

2. Materials and Methods

The present research work was carried out at college of veterinary science, SVVU, Tirupati. All the experimental procedures were reviewed and approved by the Student Advisory Committee and Institute Animal Ethics Committee. Prebiotic MOS, antibiotic oxytetracycline used in this experiment was procured commercially in powder form, whereas herbal powder was prepared from freshly collected and shade dried Tulasi (*Ocimum sanctum*) leaves. These powder supplements were added separately to the basal ration as presented in experimental design (Table 1). Ingredients and chemical composition, proximate analysis of basal ration was presented in Table 2 and Table 3 respectively.

2.1 Experimental design and animals

The experiment was conducted in twenty-four Newzealand white male rabbits of around one month age and were assigned into four homogeneous treatment groups randomly (C1, T1, T2 and T3) each with six rabbits. The trail was conducted for a period of 42 days (6 weeks) in order to investigate the effect of MOS, oxytetracycline and *Ocimum sanctum* supplementation on antioxidative enzymes status and biochemical profile in New Zealand white rabbits.

2.2 Prebiotic, antibiotic and herbal preparations

Prebiotic (Mannan oligosaccharides) (MOS) used in this experiment was procured in the form of powder from Jilariya chemphar company, Gujarat. An antibiotic oxytetracycline powder was procured from Medibios laboratories Ltd., Thane. Tulasi (*Ocimum sanctum*) leaves were collected freshly from local gardens, thoroughly washed, shade dried and made it into powder. The basal ration used for the experiment was procured from VRK Nutritional Solutions, Maharashtra.

2.3 Blood sample preparation

Blood samples were collected from rabbits on 42nd day of experimental period from the ear vein of rabbits into both ethylene diamine tetra acetic acid (EDTA) coated vacutainer tubes and clot activator vials for serum collection. After collection, blood in EDTA vials was used for hemogram and hemolysate preparation. Initially the centrifugation of blood samples was done at 3000 rpm for 15 minutes for separation of plasma and buffy coat. After centrifugation, plasma and the buffy coat were aspirated and removed. The resulting erythrocyte sediment was washed thrice with 0.9% w/v NaCl solution, each time mixing and then centrifuging the suspension at 2000 rpm for 5 min and then supernatant was discarded. The washed red cells were lysed by adding 4 parts of chilled distilled water to give stock hemolysate solution (25% v/v) which was quickly stored at -20 ℃. The hemolysate was used within 2 days to estimate the antioxidant enzymes [catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)] status and lipid peroxidation [malondialdehyde (MDA)] levels. The clotted blood was centrifuged at 2500 rpm for 5 minutes to separate serum and stored at -20˚C for further analysis. The biochemical parameters were estimated by Mispa VIVA semi-automatic biochemistry analyzer Agappe, using standard procedure by the kits supplied by Erba. The concentration of MDA was measured to estimate the lipid peroxidation by the method of Niehaus and Samuelsson (1968) [20], Catalase activity was measured by the method of Beers and Sizer (1952) [8], Superoxide dismutase (SOD) activity was measured according to the method of Misra and Fridovich (1972) [19] and glutathione peroxidase (GSH-Px) activity was assayed by the method of Rotruck *et al.*, (1973) [21].

2.4 Statistical analysis

The results obtained were subjected to analysis through software (version 22.0, SPSS 2013) [26] by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test with a significance at $p<0.05$ (Duncan 1955) [11]. All the statistical procedures were done as per Snedecor and Cochran (1994) [25].

3. Results and Discussion

3.1 Antioxidant enzyme status

Antioxidant enzyme profile on 42nd day of experiment was presented in table 4. SOD exhibited a significant ($p<0.05$) increase in all treatment groups compared to control group. SOD levels were significantly ($p<0.05$) higher in T3 group compared to T1 and T2 groups. Catalase exhibited a significant ($p<0.05$) increase in all treatment groups compared to control group. Among treatment groups, T3 exhibited a significantly higher value compared to T1 and T2 groups. GSH-Px levels were significantly ($p<0.05$) higher in T3 group, while there was no significant ($p<0.05$) change in T1 group and T2 group compared to control group. MDA levels exhibited a significant ($p<0.05$) decrease in T1 and T3 groups compared to control group.

Supplementation of *Ocimum sanctum* in our experiment has shown superior results than control and other treatment groups i.e. MOS, oxytetracycline supplemented groups. The antioxidant enzyme (catalase, SOD and GSH-Px) levels were significantly ($p<0.05$) higher along with a significant ($p<0.05$) decrease in MDA levels. Whereas, MOS supplemented group has shown better results than control and oxytetracycline supplemented groups, with significantly ($p<0.05$) higher values of catalase and SOD along with decreased MDA levels. But, oxytetracycline supplemented group has shown better results than control group with significantly ($p<0.05$) higher levels of catalase and SOD enzymes.

An increased SOD levels in present study with *Ocimum sanctum* supplementation was in agreement with Sethi *et al*., (2004) [22] and Jyothi *et al.*, (2007) [13] who reported similar results in rabbits. Whereas, Varaprasad Reddy *et al*., (2009) [30] reported a significant increase in SOD levels in *Ocimum sanctum* supplemented group in broiler chicken. Significant decrease ($p<0.05$) in MDA levels with *Ocimum sanctum* supplementation was in line with findings of Sethi *et al*., (2004) [22] and Varaprasad Reddy *et al*., (2007) [31] who reported a decrease in MDA levels with *Ocimum sanctum* supplementation in rabbits and broiler chicken respectively. Similarly, higher Catalase and GSH-Px levels observed in the present study in *Ocimum sanctum* supplemented group were in agreement with Varaprasad Reddy *et al*., (2009) [30] who reported similar results in broiler chicken. To achieve superior body weights, broiler rabbits in their growth period require a stronger cellular metabolism. This results in severe oxidative stress at the cellular level, which leads to damage of cellular

organelles thereby destroy cells and tissues by attacking macromolecules like protein, DNA and lipids (Kurien and Scofield, 2006) [18]. However, the present study exhibited higher levels of antioxidative enzymes namely catalase, SOD and GSH-Px, whereas, significantly decreased MDA levels in *Ocimum sanctum* supplemented group to scavenge ROS and free radicals which are produced more during the rapid growth. Since, *Ocimum sanctum* possessed significant antioxidant activity and also acted as free radical scavenger by preventing oxidative damage to the cells (Subramanian *et al.*, 2005) [27]. This may be due to the active ingredient Eugenol and total thiols in *Ocimum sanctum*, which was demonstrated to have significant antioxidant properties and effectively reduced lipid peroxidation and oxidative stress-induced damage. (Sethi *et al.*, 2004) [22].

The SOD levels were significantly (p<0.05) higher in the present study with dietary supplementation of MOS were in agreement with Attia *et al.*, (2017) [4] and Zheng *et al.*, (2018) [32] who reported significantly increased levels of SOD and GSH-Px with supplementation of MOS in poultry and sheep respectively. Similarly, Zheng *et al.*, (2018) [32] reported significant decrease in MDA levels in MOS supplemented group compared to control group in sheep. These findings are in agreement with the present findings. We predicted that the MOS enhanced gut microbes, which further generated some bioactive substances that could potentially protect against oxidative damage by stimulating the levels of antioxidant enzymes. In this experiment, the levels of catalase and SOD enzymes in oxytetracycline supplemented group were increased significantly (p<0.05), whereas, no significant (p<0.05) change in the GSH-Px activity and MDA levels. On the contrary, a significant increase in MDA levels with oxytetracycline supplementation in growing rabbits was reported by Abdel-Samad *et al*., (2021) [2]. This may be due to the variation of dose of the antibiotic given and experimental period.

Though all the treatments increased the antioxidative enzymes, the reduction of MDA levels in T1 and T3 groups exemplifies the additive effect of MOS and *Ocimum sanctum* on antioxidation at cellular level apart from priming effect. Further the higher values of antioxidative enzymes and reduced levels of MDA in *Ocimum sanctum* supplemented group (T3) signifies a higher potential for *Ocimum sanctum* as antioxidant among the three.

3.2 Biochemical profile

The effect of supplementation of MOS, oxytetracycline and *Ocimum sanctum* on different biochemical parameters were presented in the Table 5. Among several biochemical parameters set for the study, ALT and AST were exhibited significant decrease in T1 and T3 groups. Total protein, albumin significantly increased in T1 and T3 groups. Glucose, cholesterol and bilirubin exhibited significant decrease in all groups compared to the control group. Creatinine levels were significantly decreased in T1 and T3 groups. Calcium levels were significantly decreased in T1 group but similar range with control group. Phosphorus levels were similar in range compared to control group. Significantly (p<0.05) increased levels of chloride in T1 and T3 groups were recorded.

The results obtained in the present study with respect to ALT, AST and albumin were in close agreement with the reports made by Attia *et al.*, (2013) [6] but differed with respect to glucose, cholesterol, total protein and globulin levels that were same with control group in growing rabbits. This difference may be due to either variation in the dietary supplementation of MOS or experimental protocol. The results obtained in the present study with respect to total protein, albumin and globulin levels were conflicting with that of Attia *et al.*, (2014) [5]. They reported decrease in the total protein, albumin and globulin levels with simultaneous increase in the glucose, cholesterol, urea and AST levels in growing rabbits. This may be due to dose of MOS used and time period of experiment. The results obtained in the present study were in close agreement with the reports made by Attia *et al.*, (2017) [4] in broiler chicks. Whereas, Abdel-Hamid and Farahat (2015) [1] reported no change in cholesterol and decrease in albumin in rabbits which was contrast to our results. This may be due to different estimation methods and/or sample size. The lower values of ALT and AST in prebiotic (MOS) supplemented group indicates the hepato protective nature of MOS. Supplementation of MOS exhibited hypoglycemic and hypocholestremic effect within physiological range. BUN and creatinine levels were within the physiological range and indicate normal renal function (Slunnil, 1974) [24]. Low bilirubin levels imply improved protection against oxidative damage (Aliyu *et al.*, 2007) [3].

Abdel-Samad *et al*., (2021) [2] reported a significant increase in ALT, AST and creatinine; a decrease in the total protein, albumin and globulin concentration in oxytetracycline supplemented group in growing rabbits. The above findings were contradictory with our present results. These difference may be due to dose of the antibiotic given and experimental period. Results in the present study with respect to glucose with supplementation of *Ocimum sanctum* are in agreement with Sethi *et al.*, (2004) [22] who reported similar results in rabbits, whereas, ALT and AST levels are in agreement with Varaprasad Reddy *et al*., (2014b) [29] who reported similar results in broiler chicken. The lower values of ALT and AST indicate the hepato protective nature of *Ocimum sanctum*. Serum globulin and cholesterol levels are in agreement with Buba *et al*., (2016) [10] in growing rabbits. They also reported no significant change in the total protein when supplemented with *Ocimum sanctum* to rabbits. This disparity may be due to amount of *Ocimum sanctum* given, experimental period and experimental breeds. The serum calcium and phosphorus levels are in contrast to the findings of Shah and Barai (2016) [23] who reported significantly increased serum calcium and phosphorus levels. *Ocimum sanctum* exhibited hypoglycemic and hypocholestremic effect within physiological range. Constituents in *Ocimum sanctum* have stimulatory effect on physiological pathway of insulin secretion in reducing the glucose production (Hannan *et al.*, 2006) [12]. Cholesterol levels decreased significantly in all groups when compared to the control. Flavonoids may involved in reducing cholesterol levels in the *Ocimum sanctum* supplemented group (Buba *et al*., 2016) [10]. Among the several biochemical parameters observed under the study, ALT, AST, total protein and albumin levels were on positive side in MOS and *Ocimum sanctum* supplemented groups exemplifying their roles in hepatoprotection. Further, *Ocimum sanctum* (T3) had a higher protective nature on the kidney among the three treatments owing to a higher reduction of creatinine levels. All the treatments could reduce circulatory glucose and cholesterol, further among all the treatment groups, *Ocimum sanctum* exhibited a better hypoglycemic and hypocholestremic effect compared to MOS and oxytetracycline.

Table 1: Experimental design

Group	No of Animals	Treatment
C1	06	Basal diet
T1	06	Basal diet + Mannan oligosaccharide (1.0g/ Kg feed)
T2	06	Basal diet + Oxytetracycline hydrochloride powder (250mg/Kg feed)
T3	06	Basal diet + Tulasi (*Ocimum sanctum*) leaf powder (50g/Kg feed)

Table 2: Composition of basal ration for rabbits during experimental period (for 100kg feed)

S. No	Ingredient	Level of addition (Kg)
1	Legume (Lucerne)	50.00
2	Maize	34.50
3	Wheat bran	5.00
4	Soyabean meal	10.00
5	Salt and Vitamin- mineral mixture	0.50
	Total	100.00

Table 3: Proximate analysis of basal ration as per *Indian Council of Agricultural Research* standards (ICAR, 2013).

Nutrient	Requirement
Moisture (%)	8.85
Crude protein (%)	18.24
Crude Fibre (%)	12.80
Fat (%)	3.60
Acid detergent fiber (ADF) (%)	16-18
Calcium (%)	1.26
Phosphorus (%)	0.67
Total lysine (%)	0.70
Energy (K cal/kg)	3030

Table 4: Antioxidative enzyme profile of experimental groups

Parameter	C1	T1	T2	T3
SOD (units/mg protein)	3.75 ± 0.15^{a}	6.22 ± 0.63^{b}	6.15 ± 0.43^{b}	8.42 ± 0.41^{c}
Catalase (units/mg protein)	1.91 ± 0.18^{a}	2.42 ± 0.14^{b}	3.11 ± 0.11^{c}	3.82 ± 0.22^{d}
GSH-Px (units/mg protein)	109.55 ± 4.86^{a}	112.29 ± 4.18^{a}	105.50 ± 4.64^{a}	142.90 ± 8.84^{b}
MDA (µg/ml hemolysate)	4.42 ± 0.55^{c}	2.98 ± 0.27^{ab}	4.04 ± 0.45^{bc}	2.48 ± 0.39^{a}

Means with different superscript(s) in each row differ significantly ($p<0.05$)

Table 5: Biochemical profile of experimental groups

Parameters	C1	T1	T2	T3
ALT (IU/L)	67.96 ± 2.51^{b}	51.73 ± 2.20^{a}	62.95 ± 3.43^{b}	46.96 ± 1.12^{a}
AST (IU/L)	79.73 ± 4.97^{b}	51.69 ± 1.21^{a}	78.57 ± 4.32^{b}	45.72 ± 0.83^{a}
Total protein (g/dl)	6.02 ± 0.33^{a}	6.99 ± 0.23^{b}	6.67 ± 0.36^{ab}	6.97 ± 0.10^{b}
Albumin (g/dl)	4.53 ± 0.17^{a}	5.30 ± 0.31^{b}	5.10 ± 0.26^{ab}	6.97 ± 0.10^{b}
Globulin (g/dl)	1.48 ± 0.31^{a}	1.69 ± 0.28^{a}	1.90 ± 0.19^{a}	1.45 ± 0.15^{a}
Glucose (mg/dl)	144.13 ± 4.64^{c}	115.10 ± 3.27^{a}	127.77 ± 2.98^{b}	106.95 ± 3.61^{a}
Cholesterol (mg/dl)	66.90 ± 1.37^{c}	53.14 ± 2.85^{b}	51.25 ± 0.69^{ab}	46.28 ± 2.60^{a}
BUN (mg/dl)	45.55 ± 0.99^{a}	51.52 ± 1.18^{b}	42.16 ± 1.41^{a}	43.05 ± 1.98^{a}
Creatinine (mg/dl)	1.74 ± 0.06^{a}	1.71 ± 0.07^{a}	1.57 ± 0.08^{a}	1.77 ± 0.06^{a}
Bilirubin (mg/dl)	0.16 ± 0.007^{b}	0.13 ± 0.006^{a}	0.11 ± 0.006^{a}	0.13 ± 0.01^{a}
Calcium (mg/dl)	12.82 ± 0.63^{a}	11.49 ± 0.35^{a}	12.09 ± 0.57^{a}	13.58 ± 0.70^{a}
Phosphorus (mg/dl)	5.78 ± 0.21^{a}	6.25 ± 0.25^{a}	6.18 ± 0.20^{a}	5.93 ± 0.12^{a}
Chloride (mEq/l)	85.21 ± 0.73^{a}	95.40 ± 0.79^{b}	86.04 ± 3.46^{a}	92.16 ± 0.51^{b}

Means with different superscript(s) in each row differ significantly ($p<0.05$)

4. Conclusions

It was concluded from the present study that the dietary supplementation of *Ocimum sanctum* performed better with significantly ($p>0.05$) increased antioxidant enzymes i.e. catalase, SOD and GSH-Px along with significantly ($p>0.05$) lower MDA levels to combat the oxidative stress in Newzeland white rabbits. Among the several biochemical parameters set for the study, ALT, AST, total protein and albumin levels were on positive side in T1 and T3 groups.

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