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Moringa Oleifera Leaf Meal (MOLM) with Cocktail Enzyme Supplementation on Immunity, Serum biochemical and Antioxidant Activity of Layers

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ABSTRACT

The objective of the present study was to evaluate the effect of *Moringa oleifera* leaf meal (MOLM) on immunity status, antioxidant and serum biochemical parameters of layers. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50 replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups: T_1 (Control) = Corn-soybean meal Basal diet (BD); T_2= 5% MOLM in BD diet without enzyme supplementation; T_3= 7.5% MOLM without enzyme supplementation; T_4= 10% MOLM in BD diet without enzyme supplementation; T_5= 10% MOLM with enzyme supplementation. The results revealed that the cell mediated immune response was significantly (P<0.01) higher in T_5 as compared to other treatments, whereas no significant difference was observed in the Humoral immune response among the birds fed diets with or without inclusion of moringa leaf meal. There was no significant difference was recorded in protein values among T_1, T_2 and T_5, however, lower protein values were observed in T_3 and T_4 but not significantly differ with T_1. There was no significant difference in albumin content among the treatments. Lower (P<0.01) cholesterol level was noticed in T_5 and higher (P<0.01) cholesterol level was observed in T_1. The calcium and phosphorus levels in serum were higher (P<0.01) in T_4 and T_5 as compared to other treatments, with the lowest (P<0.01) calcium and phosphorus levels recorded in T_1. The glutathione peroxidase activity was significantly higher (P<0.01) in the birds fed Moringa leaf meal diets as compared to the control. The lipid peroxidation and RBC catalase activity was inversely proportional to inclusion levels of MOLM in diets. Thus, 10% *Moringa oleifera* leaf meal with or without enzyme supplementation could be used for improving immunity and antioxidant status of layers.

Key words: Antioxidant activity, Cholesterol, Calcium, Immunity, Moringa leaf meal

INTRODUCTION

Moringa oleifera commonly referred to as the drumstick tree is a plant from the Moringaceae family and it is the most widely cultivated species of the genus Moringa. The tree is often called 'multipurpose' due to the fact that all parts including the leaves, pods, seeds, flowers, fruits and roots are edible (Orwa *et al.,* 2009). The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in Moringa leaves (Atawodi *et al.,* 2010). Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in Moringa leaves (Siddhuraju and Becker, 2003). Yang *et al.* (2006) and Jung *et al.* (2010) reportedd that *M.oleifera* was among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals, processing properties, ease of growing, and also on palatability, stability and shelf life of meat products. Akinola and Ovoter (2018) reported that *Moringa oleifera* leaf meal favoured the good cholesterol, HDL of the fresh eggs at levels of 0.5 and 1.0% inclusion in layers. Hossam *et al.* (2016) recorded increased antioxidant activity, immunity of chicks with Moringa leaf extract. Therefore, this study was designed to evaluate the effect of *Moringa oleifera* leaf meal on immunity status, antioxidant and serum biochemical parameters of layers.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Experimental Station, Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. Two hundred, 34-week-old BV 300 White Leghorn layer birds were randomly allotted to 50

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replicates with 4 birds in each replicate and these replicates were in turn allotted to 5 dietary groups. The birds were raised in cages under uniform management and fed the respective diets from 34th to 49th weeks of age. Birds were fed the experimental diet at 120 g/bird per day but this amount was adjusted with regard to the level of their production throughout the experimental periods (NRC, 1994). Leaf was harvested from young Moringa oleifera trees of about four years of age from forage farm under supervision of CVR food products. The harvested leaves from the trees were spread out on a concrete floor and allowed to dry for a period of three days under shade and aerated conditions, then run through a hammer mill sieve with a size of five mm to produce the leaf meal.

Five treatment diets included T_1 (Control) = Corn-soybean meal Basal diet (BD); T_2= 5% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_3= 7.5% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_4= 10% *Moringa oleifera* leaf meal in BD without enzyme supplementation; T_5= 10% *Moringa oleifera* leaf meal in BD with enzyme supplementation was formulated to which MOLM substitutes SBM (Table 1). Cocktail of enzymes including (Cellulase- 1,50,255 CMCU/G, Phytase- 2,225 FYT/G, Beta mannanase- 2,00,000 IU/

Table 1. Proportion (%) of ingredients used for formulating experimental diets

Ingredients (%)	T_1	T_2	T_3	T_4	T_5
Maize	59.8	56.5	54.9	53.4	53.4
SBM	21.5	19.8	18.915	17.915	17.915
DORB	7	7	7	7	7
Moringa	0	5	7.5	10	10
Limestone	10	10	10	10	10
DL-Methionine (Degussa)	0.16	0.16	0.16	0.16	0.16
Salt	0.15	0.15	0.15	0.15	0.15
Vitamin Premix*(Venky s)	0.05	0.05	0.05	0.05	0.05
Trace mineral mixture**	0.1	0.1	0.1	0.1	0.1
Choline Chloride,75%	0.05	0.05	0.05	0.05	0.05
Toxin binder (Toximar)	0.05	0.05	0.05	0.05	0.05
Di-calcium phosphate	1.09	1.09	1.09	1.09	1.09
Enzyme (MAXIZYME EX)	0	0	0	0	0.025
L-Lysine HCl (Azinomoto)	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
Calculated Nutrient composition*					
CP(%)	15	15	15	15	15
ME (kcal/kg DM)	2500	2500	2500	2500	2500
Calcium (%)	4	4	4	4	4
Available Phosphorus (%)	0.34	0.34	0.34	0.34	0.34
Lysine (%)	0.68	0.68	0.68	0.68	0.68
Methionine (%)	0.32	0.32	0.32	0.32	0.32
Sodium (%)	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.22	0.22	0.22	0.22	0.22

*Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B_2 25 mg, Vitamin D_3 3000IU, Vitamin K 2 mg.Riboflavin 25 mg, Vitamin B_1 1 mg, Vitamin B_6 2 mg, Vitamin B_{12} 40 mg and Niacin 15 mg; **Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg and Selenium 0.1 mg.

G, Protease- 7,00,115 IU/G, Alpha Amylase- 70,000 U/G, Lipase- 18,000 U/G, Xylanase- 2,80,000 IU/G, Arabinase- 2120 IU/G, Beta Galactosidase- 25,000 IU/G) was added in T_5. The Treatment diets were formulated to be iso-caloric and iso-nitrogenous, to meet the ME and CP requirement of laying hens according to NRC (1994).

Layers were vaccinated against ND by subcutaneous route at 47 wks of age with Lassota vaccine (Indovax Pvt., Ltd., Hyderabad, India). After 14 days, blood was collected and serum was separated. Subsequently, micro-hemagglutination activity of serum was estimated and the antibody titers (log_2) measured following the standard procedure (Wegmann and Smithies., 1966). A phytohemagglutinin-P (PHA-P) injection assay (Cheng and Lamont 1988) was used to evaluate *in vivo* T-cell-mediated immune response of laying hens. At the end of experiment, blood sample were collected aseptically from wing vein (one bird from each replicate) in vacutainers and kept in incubator at room temperature for serum collection. The serum was used for estimation of cholesterol, total protein, albumen, globulin and antioxidant activity by using spectrophotometer with commercially available kits (Coral Clinical Systems, Goa).

Data were analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989) and comparison of means were done using Duncan (1955) using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

There was no significant difference observed in the humoral immune response among the birds fed diets with or without inclusion of Moringa leaf meal (Table 2). However, the cell mediated immune response was significantly ($P<0.01$) higher in T_5 when compared to other treatments and control. Moringa leaves contain compounds such as protein, flavonoids, tannin, *etc.* and these compounds are efficiently utilized by the enzymes in T_5 which might have contributed to give better immune response. More immunity levels were observed in birds fed MOLM based diets may be attributed to antimicrobial and antioxidant properties of Moringa leaves (Ebenebe *et al.,* 2012; Hassan *et al.,* 2016). Melesse *et al.* (2013) reported that *Moringa oleifera* leaves have a beneficial effect in enhancing the immune responses. Eze *et al.* (2013) reported increase in total and differential cell numbers and haemagglutination inhibition (HI) titre in the chicken fed *Moringa oleifera* against Newcastle disease (ND). Chollom *et al.* (2012) demonstrated *M. oleifera* seed extract have nutritional value as well as strong antiviral activity against NDV *in-ovo*. Hossam *et al.* (2016) found that, Moringa leaf extract acts as antibacterial, growth promoter,

Table 2. Effect of dietary inclusion of *Moringa oleifera* leaf meal on immune parameters in layers

Diet	HI	CMI
T_1	6.30	159.8^b
T_2	6.70	163.8^b
T_3	6.90	164.2^b
T_4	6.90	169.3^b
T_5	7.10	223.7^a
P-Value	0.330	0.000
N	10	10
SEM	0.125	4.994

^{a,b} Means with different superscripts in a column differ significantly ($P<0.05$); T_1= Corn-soybean meal Basal Diet (BD) ; T_2= 5% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_3= 7.5% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_4= 10% *Moringa oleifera* leaf meal in BD without enzyme supplementation ; T_5= 10% *Moringa oleifera* leaf meal in BD with enzyme supplementation. P value = probability value; N = number of replicates (10 birds in each replicate); SEM= Standard Error Mean. HI= Humoral immunity, CMI=Cell Mediated Immunity.

antioxidant and have beneficial effect on immunity and hemato-biochemical parameters. Moringa leaves are rich in Vitamin A which might have improved the function of immune system. Similarly, increased immunity *Moringa oleifera* leaf meal was also reported by Divya *et al.* (2015).

Dietary inclusion of *Moringa oleifera* leaf meal in layer diets had shown a significant effect in serum biochemical profile except in albumin (Table 3). Lower cholesterol levels and higher calcium and phosphorus in serum were noted in birds fed Moringa leaf meal diets. However, there was an increase in the total protein level in serum at 5 % MOLM and then decrease as the MOLM content increased and again it was hiked at 10% MOLM with enzyme supplementation. The increase in the protein level in 5% MOLM fed birds might be due to increase in digestibity. As MOLM was increased, the protein digestibility was decreased, might be due to higher fibre level (10.4%) in Moringa leaf meal. Whereas, the increased protein in 10% with enzymes supplementation was due to action of protease enzyme. Total serum protein has been reported as an indication of the protein retained in the animal body (Esonu *et al.,* 2001). The relatively greater total serum protein content of birds receiving dietary MOLM might be an indication of the good protein and/or quality of the leaf meal. The result of the total protein of the present study tallied with the report of Hassan *et al.* (2016) who reported difference in total protein of the blood in broilers that were fed the same leaf meal.

Fahey *et al.* (2005) stated that Moringa is a good source of phytochemicals (natural plant chemicals) such as glucosinolates, alkaloids and isothiocyanates that provides cholesterol lowering activities. The present study had shown the lower cholesterol activity with the increase of the Moringa leaf meal in the diets. It supported the findings of Ghasi *et al.* (2000), Nabila *et al.* (2015) and Yassmine *et al.* (2017) and Olugbemi *et al.* (2010) that moringa leaf has hypocholesterolemic properties. Dey and Parthasarathi (2013) noted the decrease in total cholesterol as the MOLM increased in the diet. The cholesterol reducing action was also reported by Ashong and Brown (2011), may be attributed to a bioactive phytoconstituent alpha sitosterol, a plant sterol with a structure similar to cholesterol present in Moringa leaves (Ghasi *et al.,* 2000). Moringa leaves powder of the present study was having 10 % crude fibre and increase in fiber may also be responsible for less absorption of cholesterol from the intestinal tract of the birds.

The serum calcium and phosphorus levels were higher in T_4 and T_5 since Moringa leaf meal is the rich source of calcium and phosphorus. The proximate composition of the present study had shown calcium of 2.5% and phosphorus of 0.36% which might attribute to the increase of calcium and phosphorus levels in serum as compared to other treatments.

The glutathione peroxidase activity was

Table 3. Effect of dietary inclusion of *Moringa oleifera* leaf meal on serum biochemical parameters in layers

Diet	Protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Phosphorus (mg/dl)	Calcium (mg/dl)
T_1	4.630^{ab}	1.967	176.8^{a}	5.224^{c}	16.13^{c}
T_2	5.007^{a}	1.634	140.5^{b}	5.352^{bc}	18.74^{b}
T_3	4.325^{b}	1.791	133.7^{b}	5.662^{b}	19.21^{b}
T_4	4.212^{b}	1.505	134.9^{b}	6.230^{a}	20.17^{a}
T_5	5.103$_{a}$	1.491	112.0^{c}	6.301^{a}	20.29^{a}
P-Value	0.022	0.114	0.001	0.001	0.001
N	10	10	10	10	10
SEM	0.108	0.067	3.569	0.087	0.230

Table 4. Effect of dietary inclusion of *Moringa oleifera* leaf meal on antioxidant parameters in layers

Diet	GPx	LPO	RBC Catalase
T_1	800[b]	4.662[a]	82.00[a]
T_2	1381[a]	4.424[a]	77.40[a]
T_3	1599[a]	3.453[b]	77.35[a]
T_4	1198[a]	2.567[c]	55.44[b]
T_5	1553[a]	1.623[d]	47.76[b]
P-Value	0.001	0.000	0.000
N	10	10	10
SEM	72.74	0.168	3.151

significantly higher (P<0.01) in the birds fed Moringa leaf meal included diets as compared to the control (Table 4). Lipid peroxidase enzyme activity was significantly higher (P<0.01) for T_1 and T_2, and the least (P<0.01) activity was noticed in T_5. There was a decreasing trend in the lipid peroxidation from T_1 to T_5 with increase in Moringa levels in the diet. Catalase activity was significantly higher (P<0.01) in T_1, T_2 and T_3 as compared to T_4 and T_5. The results of the current study regarding glutathione peroxidase activity are similar to the findings of Wei *et al.* (2016) who noted the increase of glutathione peroxidase activity in the Moringa leaf meal included diets. Abdulaziz *et al.* (2015) reported that the phenols and flavonoids of Moringa leaf meal play an important role to cure and even prevent oxidative damages caused by free radicals. The increased glutathione peroxidase activity in Moringa leaf meal included diets as compared to control diet might be due to the presence of flavonoid groups such as quercetin and kaempferol and their antioxidant activity is higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in Moringa leaves (Siddhuraju and Becker, 2003). In the present study, the RBC catalase activity was more in control birds that implies presence of more free radicals. The phenols and flavonoids of Moringa leaf meal prevented the oxidative damages caused by free radicals and decreased the stress of animals, which was noticed in 10% *Moringa oleifera* leaf meal included diets. The antioxidant activity of phenols was mainly due to their redox properties which played an important role in neutralizing free radicals, quenching singlet and triplet oxygen molecule (Gupta and Abu Ghannam, 2010).

The decrease in lipid peroxidation indicates role of *M. oleifera* leaves as an antioxidant. lipid peroxidation is a process involved in degradation of structural components of cell membranes and reactive oxygen species (ROS) accumulation is known to induce lipid peroxidation and oxidative stress. The influence of MOLM diets on lipid peroxidation and serum antioxidant enzymes in present study are in congruence with Rama Rao *et al.* (2013) and Habibi *et al.* (2014) in broilers. The antioxidant potential of MOLM is attributed to presence of phytochemicals such as, carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics.

CONCLUSION

It can be concluded that supplementation of 10% *Moringa oleifera* leaf meal with enzyme supplementation increased the immune status, antioxidant activity, serum protein, calcium and phosphorus levels and lowered the serum cholesterol levels of layers. Thus, 10% *Moringa oleifera* leaf meal with or without enzyme supplementation could be safely included for improving immunity and antioxidant status of layers.

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