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Effect of moringa leaf powder supplementation on performance and antioxidant activity in broilers under heat stress

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ABSTRACT

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An experiment was conducted to investigate the effect of Moringa leaf powder supplementation on performance and antioxidant activity to alleviate heat stress in broilers during summer months. The experiment was conducted on 240 broiler chicks (Cobb-300) and randomly categorized into six dietary treatment groups with 8 replicates with 5 birds in each replicate (6×8×5). A corn-soy basal diet (BD) was prepared as a control and BD supplemented with chlortetracycline at 0.05% (BD-C), vitamin E at 0.025% (BD-E) and *Moringa oleifera* leaf powder (MLP) at 0.25% (BD-MLP-0.25), 0.5% (BD-MLP-0.5) and 0.75% (BD-MLP-0.75) of BD were prepared. MLP supplementation at 0.5% and 0.75% levels significantly improved (P<0.05) the overall growth performance and feed efficiency, without affecting feed consumption among all treatments. Nutrient retention, carcass parameters, visceral organs weights, humoral immunity and serum biochemical parameters were not influenced by dietary variations. MLP supplementation at 0.5% and 0.75% levels showed higher (P<0.05) antioxidant enzymes (superoxide dismutase and glutathione peroxidase) activity with significant reduction in lipid peroxidation. The lowest (P<0.05) levels of lipid peroxidation was noticed in broilers fed diets containing 0.75% MLP. Thus, present study indicates that addition of MLP at 0.50 and 0.75% to the broilers diets enhanced the growth and antioxidant enzymes activity in comparison to antibiotic and antioxidant (vitamin-E) supplementation. Thus it was concluded that dietary supplementation of MLP at 0.5% level is advised for ensuring better performance and improved antioxidant enzyme activity to minimize the heat stress in broilers throughout the summer.

Keywords: Antioxidant, Broiler, Carcass, Growth, Immunity, Moringa

INTRODUCTION

Moringa oleifera is a common plant in the tropics, often referred as the horse radish tree or drumstick tree (Aregheore, 2002). It is rich in vitamins, amino acids, energy, and crude protein; has low levels of anti-nutritional components; has anti bacterial capabilities and a tendency to boost the immune system because of its main component pterygospermin (Makkar and Becker, 1996). Almost all parts of this plant, including the root, bark, gum, leaves, fruit, seeds, and seed oil, have been utilized to cure a variety of ailments (Kumar *et al.*, 2010).

Poultry farmers worldwide, in both temperate and tropical climates, are much concerned about heat stress in broiler chicken (Balnave, 2004). When the temperature rises above 30 °C, poultry experience heat stress, and this is one of the most prominent stresses that have an impact on production standards. According to Sahin *et al.* (2002a and b), heat stress in flocks of meat-type poultry frequently causes lower body weight, decreased feed intake, poor feed efficiency and high mortality. As a result of increased free radical production induced by heat stress, lipid oxidation increases, which promotes the synthesis of oxygen free radicals and causes oxidative stress in cells (Altan *et al.*, 2003).

Moringa leaves are rich in natural antioxidants like tocopherols, vitamin C, flavonoids, and other phenolic components (Siddhuraju and Becker, 2003). These natural antioxidants eliminate free radicals, activate antioxidant enzymes, and inhibit oxidases (Luqman *et al.*, 2012). There has been an increase in interest in utilizing *Moringa oleifera* to enhance the performance of poultry and ruminant farms (Abou-Sekken, 2015). Hence it is necessary to conduct an extensive investigation due to the rising popularity of *Moringa oleifera* leaves usage as a feed additive in poultry diets. In view of good nutritional and antioxidant properties of *Moringa oleifera* leaves, present investigation was made in broilers by supplementing graded levels of *Moringa oleifera* leaf powder (MLP) to study its effect on performance, slaughter parameters, serum biochemical profile, immunity and antioxidant benefits during summer.

MATERIALS AND METHODS

Birds

This experiment was conducted during summer months (April and May) on 240 broiler chicks (day-old), purchased from M/s Venkateshwara Hatcheries Pvt. Ltd, Hyderabad and divided into six dietary treatment groups

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at random with eight replicates for each treatment group and five birds in each replicate (6×8×5). From day one to 42 days of age (the marketable age), the chicks were raised in battery brooders under ideal brooding conditions. Throughout the experiment, the broiler chicks in all six groups were kept in identical housing under standard hygienic environments. All the replicate groups of chicks received ad libitum access to the respective experimental diets. All days throughout the trial, birds have unlimited access to fresh, clean drinking water. The birds received vaccinations against Marek's disease at hatchery, Lasota vaccine for Newcastle disease (ND) on 7th and 28th day, and the Georgia strain vaccine for infectious bursal disease on day 14 through ocular route.

Experimental Diets

Basal diets (BD) containing corn-soya bean meal (Table 1) were prepared for pre-starter, starter and finisher phases of broiler chicken as per the recommendation given by BIS (2007). During three phases, BD was formulated as control without any additive, whereas positive control diet (BD-C) was formulated by adding chlortetracycline antibiotic at 0.05% to BD. Another diet was formulated by adding Vitamin E at 0.025% (BD-E) to BD to compare the antioxidant effects of *Moringa oleifera* leaf powder (MLP) based diets. The MLP based diets were formulated by adding MLP at graded levels i. e. 0.25% (BD-MLP-0.25), 0.5% (BD-MLP-0.5) and 0.75% (BD-MLP-0.75) to BD.

Growth Performance

The live body weights of all birds were recorded at weekly interval and the average body weight per bird was calculated for treatment group per each dietary feeding phase. Similarly, the average feed intake (g) was calculated by recording the amount of feed consumed by each group in a day. The cumulative feed conversion ratio was calculated as total amount of feed consumed up to that particular feeding phase divided by the body weight gain recorded up to that phase.

Nutrient retention and carcass parameters

A metabolism trial of 3 days was conducted at the end of experiment with one bird from each replicate i.e. 8 birds from each group to determine the nutrients retention by following standard procedure. Samples representing feed offered, residues left and excreta voided were analysed for dry matter (DM), crude protein (CP) and ether extract (EE). The DM, CP and EE content of feed and faeces was estimated by following AOAC (2005) procedures. At the end of experiment, one bird from each replicate was selected randomly and fasted for 12hrs and slaughtered to record the carcass parameters.

Immune response, serum biochemical constituents and antioxidant enzymes

On completion of 5th week, blood samples were collected from one bird per replicate for all experimental groups. The immune response in birds was estimated by measuring antibody titer against ND vaccine by conducting Haemagglutination inhibition (HI) test in serum. The antibody titres (log_2) were measured as per standard procedure (Wegmann and Smithies, 1966). The serum biochemical constituents viz., serum glucose, total protein, albumin and cholesterol were determined by using the ErbaChem-5plus V2 clinical chemistry semi auto analyzer with commercially available diagnostic kits (M/S Excel Diagnostic Pvt. Ltd., Hyderabad, India). The antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GRx), super oxide dismutase (SOD), catalase and lipid peroxidation (LPx)

Table 1: Ingredient and Nutrient composition of basal diet of broiler chicken

Ingredients	Pre-Starter (0-14 days)	Starter (15-28 days)	Finisher (29-42 days)
Maize	54.0	56.2	60.6
Soya deoiled cake 45%	38.3	34.6	29.5
Vegetable oil	3.49	4.95	5.92
Salt	0.42	0.42	0.42
Dicalcium phosphate	1.75	1.80	1.56
Limestone powder	1.22	1.22	1.17
DL-Methionine (Degussa)	0.28	0.27	0.23
L-Lysine HCl (Azinomoto)	0.17	0.16	0.13
L-Threonine (Evonic)	0.01	0.05	0.07
L-Tryptophan	0.00	0.00	0.01
AB2D3K* (Venky s)	0.02	0.02	0.02
B complex** (Nicholas)	0.02	0.02	0.02
Choline chloride,75%	0.06	0.06	0.06
Toxin Binder (Toximar)	0.10	0.10	0.10
Trace mineral mixture***	0.12	0.12	0.12
Coccidiostat (Cygro)	0.1	0.05	0.05
Total	100	100	100
Nutrient composition			
ME (kcal/kg)	3000	3100	3200
CP (%)	23.2 (22.9)	22.1 (22.0)	20.2 (20.0)
Ca (%)	1.1 (1.0)	1.1 (0.9)	1.0 (1.2)
NPP (%)	0.46 (0.85#)	0.47 (0.82#)	0.45 (0.9#)
Digestible Lys (%)	1.31	1.22	1.00
Digestible Met (%)	0.57	0.55	0.49

*AB2D3K provided per kg diet: Vitamin A 200000 IU, Vitamin B2 25 mg, Vitamin D3 3000IU, Vitamin K 2mg.
** Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg and Niacin 15mg.
*** Trace mineral provided per kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg, Iodine 1mg and Selenium 0.1mg.
Values in the parenthesis are analysed values,
#Total Phosphorus

in serum were estimated following the methods of Paglia and Valentine (1967); Carlberg and Mannervik (1985); Madesh and Balsubramanian (1998); Luck (1963) and Ohkawa *et. al.* (1979), respectively.

Statistical Analysis

The data was analyzed using the general linear model of the Statistical Package for Social Sciences (version 15), and the means were compared using Duncan's multiple range test (Duncan, 1955). Statistical analysis of the data was carried out by using one-way analysis of variance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The mean room temperature and relative humidity recorded during experiment ranged from 32 to 42°C and 61 to 75%, respectively.

Nutrient composition of Moringa oleifera leaf powder & experimental diets

The nutrient composition of MLP used in this study is as follows, Moisture - 9.24%, Crude Protein- 21.03%, Crude Fat- 7.88%, Crude Fibre- 15.71%, nitrogen free extact-32.61% and Total Ash- 13.53%. The analyzed nutrient composition of experimental diets used during pre-starter, starter and finisher phases is as follows crude protein 22.9, 22, 20%, respectively; calcium 1.0, 0.9, 1.2%, respectively and total phosphorus 0.85, 0.82, 0.9% respectively (Table 1)

Growth performance

The feed intake (g/bird) during all three phases and overall experiment was not influenced by dietary treatments (Table 2). Body weights and FCR were not affected among treatment groups during prestarter and starter phases, while significant (P<0.05) effect was observed during finisher phase and overall experiment (Table 3 and 4). This significant (P<0.05) improvement in overall body weights and FCR was observed in birds fed diets supplemented with 0.5 and 0.75% MLP.

In the present study despite heat stress, the positive influence of MLP at 0.5 and 0.75% in broiler diets on growth performance might be due to antioxidant properties of phytochemicals present in the MLP, which may resulted in digestive organ functioning and thereby improved the overall performance of broilers. It is well attributed to higher antioxidant enzymes (superoxide dismutase and glutathione peroxidase) activity observed in this study (Table 6), which may resist deleterious effects of oxidative stress. In agreement with present study, previous studies also reported positive influence on body weights (El Tazi, 2014; Okafor *et al.*, 2014 and Ittehad *et al.*, 2022) and feed efficiency (Akhouri *et al.*, 2013; Dey and Parthasarathi, 2013 and Onunkwo and George, 2015; Ittehad *et al.*, 2022) with increased level of Moringa leaf meals (MLM) in broiler diets. Similarly, Kout *et al.* (2015) conducted a similar study in Japanese

Table 2: Effect of dietary inclusion of *Moringa oleifera* leaf powder on feed consumption (g) in broiler chicken reared under summer stress

Diet	Pre-starter(0-14 days)	Starter(15 -28 days)	Finisher(29-42 days)	Over all(0-42 days)
BD	403.7±2.12	1090.5±6.95	1901.8±27.73	3395.6±31.08
BD-C	407.6±3.34	1088.1±8.30	1829.7±23.39	3325.6±30.69
BD-E	403.1±4.60	1100.7±15.64	1929.2±46.18	3433.1±47.28
BD-MLP-0.25	398.7±6.56	1095.0±11.40	1861.8±27.63	3355.3±30.47
BD-MLP-0.5	399.0±6.17	1114.7±18.36	1895.0±34.23	3408.5±36.06
BD-MLP-0.75	409.4±5.42	1124.2±7.21	1921.7±44.59	3455.5±48.85
SEM	1.999	5.054	14.433	16.089
N	8	8	8	8
P-Value	0.578	0.242	0.344	0.181

Table 3: Effect of dietary inclusion of *Moringa oleifera* leaf powder on body weight gain (g) of broiler chicken reared under summer stress

Diet	Pre-starter(0-14 days)	Starter(15 -28 days)	Finisher(29-42 days)	Over all(0-42 days)
BD	343.6±2.46	727.0±7.37	910.8^{c}±24.76	1981.5^{c}±27.44
BD-C	342.9±2.71	727.8±9.73	996.1^{b}±18.18	$2066.7^{b\pm}$25.66
BD-E	336.9±4.60	729.4±19.06	1015.7^{ab}±25.80	2082.0^{ab}±31.11
BD-MLP-0.25	332.1±6.15	722.8±9.22	997.3^{b}±22.57	$2052.3^{c\pm}$23.44
BD-MLP-0.5	334.5±5.69	739.9±18.30	1052.8^{ab}±24.52	2127.3^{ab}±25.47
BD-MLP-0.75	342.6±3.47	754.0±5.00	1079.8^{a}±26.76	2176.6^{a}±16.18
SEM	1.826	5.112	11.447	13.195
N	8	8	8	8
P-Value	0.308	0.532	0.001	0.001

Means with different superscripts in a column differ significantly, P<0.05

quails by supplementing 0.2% MLM and observed higher body weights and best FCR. The improvement in body weight gain and FCR in present and previous studies ascribed to rich nutrient contents in Moringa and their utilization in the birds fed Moringa based diets.

In contrary, feeding on Moring leaf based diets resulted reduced body weights (Olugbemi *et al.,* 2010, Abou-Elezz *et al.,* 2011 and Gakuya *et al.,* 2014) and resulted poor FCR (Sherief *et al.,* 2012 and Gadzirayi *et al.,* 2012) in broilers. Presence of tannins in moringa (Aderinola *et al.,* 2013) can interfere with the utilization of protein and, to a lesser extent, carbohydrates and fats (Esonu *et al.,* 2001). This could account for the lower body weights and FCR found in those studies. Since leaf meals often have a bitter taste, adding MLM to broiler diets may cause reduced palatability and thus reduce feed intake ultimately, lower their body weight gains. Though feed intake did not differ across treatment groups in this study, 0.5 and 0.75% MLP groups showed improved (P<0.05) body weight gain and resulted in better feed efficiency.

Nutrient retention and slaughter parameters

In the present study, nutrient retention (DM, CP and EE) and slaughter parameters were not affected among experimental diets. Similarly, MLM supplementation up to 25 g/kg of feed did not influence nutrient utilization (Nkukwana *et al.,* 2014). The slaughter parameters viz., per cent live weight of dressed weight, abdominal fat, breast weight and thigh weights ranged from 77.12 to 79.10; 0.833 to 1.257; 21.98 to 23.66 and 21.35 to 22.38, respectively. The per cent live weight of visceral organs *viz.*, gizzard, heart, liver, spleen and thymus were also not influenced by dietary treatments and were ranged from 1.72 to 1.84; 0.47 to 0.51; 1.57 to 1.72, 0.109 to 0.130 and 0.57 to 0.67, respectively. In agreement with present study, Nkukwana *et al.* (2014) and Divya *et al.,* (2015) found no significant effects on carcass weight, dressing percentage and the relative weights of the liver, gizzard, heart and spleen in broiler fed moringa leaf meal (0.1 to 2.5% of diet). In contrary, Aderinola *et al.* (2013); Gakuya *et al.* (2014); Abou-Sekken (2015) and Ittehad *et al.* (2022) reported lowered (P<0.05) abdominal fat (%) in MLP fed birds.

Immune response and serum biochemical parameters

The humoral immune response of birds studied in terms of antibody response to CRBC (log_2 titres) did not varied significantly. However, the supplementation of MLP at 0.25, 0.5 and 0.75% in broiler diets showed more immune response by 4.5, 7.3 and 7.3 % respectively than BD fed broilers (Table 5). Our results are consistent with those of Olugbemi *et al*. (2010), Melesse *et al*. (2013), Divya *et al*. (2015) and Hossam *et al*. (2016) who all indicated that moringa leaves improved immunological responses. The antibacterial and antioxidant qualities of Moringa leaves may be the reason for the improved immunological response shown in birds on MLP-based diets (Ebenebe *et al.,* 2012 and Hassan *et al.,* 2016).

The serum biochemical parameters studied (glucose, cholesterol, total protein, albumin and globulin) did not affected among treatment groups and their levels varied from 202.98 to 208.61 (mg/dl); 138.74 to 142.51 (mg/dl); 4.11 to 4.42(g/dl); 1.81 to 2.02 (g/dl) and 2.26 to 2.45 (g/dl), respectively. As recommended for chicken by Kaneko *et al.* (1997), the serum biochemical levels were within the normal range.

Table 5: Effect of dietary inclusion of *Moringa oleifera* leaf powder on immune response of broiler chicken reared under summer stress (at 35 days of age)

Diet	HI CRBC titers (log_2)
BD	8.00±0.27
BD-C	8.50±0.27
BD-E	8.00±0.38
BD-MLP-0.25	8.38±0.46
BD-MLP-0.5	8.63±0.50
BD-MLP-0.75	8.63±0.32
SEM	0.150
N	8
P-Value	0.706

Table 4: Effect of dietary inclusion of Moringa oleifera leaf powder on feed conversion ratio in broiler chicken reared under summer stress

Diet	Pre-starter(0-14 days)	Starter(15 -28 days)	Finisher(29-42 days)	Over all(0-42 days)
BD	1.175±0.01	1.500±0.01	$2.095^{a\pm}$0.05	$1.715^{a\pm}$0.02
BD-C	1.188±0.01	1.496±0.01	$1.838^{bc\pm}$0.02	$1.609^{bcd\pm}$0.09
BD-E	1.196±0.01	1.512±0.02	$1.901^{b\pm}$0.03	$1.649^{b\pm}$0.02
BD-MLP-0.25	1.201±0.01	1.515±0.01	1.869^{bc}±0.02	1.635^{bc}±0.01
BD-MLP-0.5	1.193±0.01	1.508±0.02	$1.801^{c\pm}$0.02	1.602^{cd}±0.01
BD-MLP-0.75	1.194±0.01	1.491±0.01	$1.779^{c\pm}$0.03	1.604^{d}±0.02
SEM	0.003	0.005	0.0191	0.008
N	8	8	8	8
P-Value	0.237	0.764	0.001	0.001

Means with different superscripts in a column differ significantly, P<0.05

Antioxidant oxidants activity

The best marker of oxidative stress is LPx, it is a chain reaction triggered by free radicals (Saygili *et al.*, 2003) as its concentration rises during oxidative stress. Malondialdehyde (MDA) concentration (nmol MDA per mg protein) is a good indicator of free radical-mediated LPx. In the current investigation the highest MDA levels were found in birds fed BD and were comparable to those birds fed diets containing MLP at 0.25% and chlortetracycline (Table 6). However, significant reduction in MDA levels was observed with supplementation of MLP at 0.5 and 0.75% and vitamin-E. The lowest (P<0.05) levels was noticed in broilers fed diets containing 0.75% MLP. Thus current study indicates, as the experimental birds were exposed to heat stress the MLP inclusion at 0.50 and 0.75% in the diets of broilers resisted LPx under heat stress.

During various metabolic processes and under stressful conditions, antioxidant enzymes detoxify H_2O_2 by reducing it to H_2O and O_2 (Fridovich, 1978). In the present study, the antioxidant enzymes *viz.*, SOD and GPx concentrations were significantly influenced among dietary treatments while, GRx and CAT was not affected (Table 6). Supplementation of MLP at 0.5 and 0.75% and vitamin-E in the broilers diets resulted highest (P<0.01) SOD levels. The lowest (P<0.01) SOD levels were observed in birds fed BD, BD supplemented with chlortetracycline and MLP at 0.25%. The highest (P<0.05) GPx enzyme levels were recorded in birds fed MLP at 0.5 and 0.75%, while the GPx activity in BD-C, BD-E and MLP-1 was intermediate and comparable to above two groups. In line with previous research in broilers by Maini *et al*. (2007), Eloff (2008), Rama Rao *et al*. (2013), Habibi *et al*. (2014), Abou-Sekken (2015), and Karthivashan as al. (2015), the impact of MLP diets on lipid peroxidation and serum antioxidant enzymes was seen in the current study. Similarly, Panda *et al.* (2007) observed significant reduction in lipid peroxide in heat stressed laying hens by supplementation of vitamin E (125 mg per kg diet) suggesting antioxidant effects of vitamin E.

In the present study, heat stress might have induced the oxidative stress and enhanced MDA production. The enhanced MDA by heat stress has been well documented by Halliwell and Gutteridge (1990). In the present study supplementation of MLP significantly (P<0.01) reduced lipid peroxidation. The antioxidant potential of MLP is attributed to presence of phytochemicals such as, carotenoids, minerals, vitamins, amino acids, glycosides, sterols, alkaloids, flavonoids and phenolics (Bartov and Bornstein, 1981 and Abou-Sekken *et al.*, 2013). The antioxidant phytochemicals present in MLP supplemented diets may have contributed to the reduction in lipid peroxidation and increased activity of the antioxidant enzymes (SOD and GPx) seen in this study. This would have lessened oxidative stress and alleviated heat stress in broilers.

CONCLUSION

Broiler diets supplemented with 0.50 and 0.75% of *Moringa oleifera* leaf powder showed better growth potential, feed efficiency, and increased antioxidant enzyme activity by preventing lipid peroxidation under summer stress conditions. Therefore, it was concluded that adding 0.5% of *Moringa oleifera* leaf powder to the broiler diets will improve performance and raise the activity of antioxidant enzymes, thereby reduce the heat stress throughout the summer.

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Table 6: Effect of dietary inclusion of *Moringa oleifera* leaf powder on serum antioxidant enzyme response in broiler chicken reared under summer stress (at 35 days of age)

Diet	Lipid peroxidation (n mol MDA/ mg protein)	Superoxide dismutase (units/mg protein)	Glutathione peroxidase (units/ml)	Glutathione reductase (units/ml)	Catalase (µ mol H_2O_2/ min/mg)
BD	5.116^{a}±0.61	3.741^{b}±0.41	141.43^{b}±14.81	303.00±33.46	208.16±7.36
BD-C	5.026^{a}±0.40	4.028^{b}±0.12	162.36^{ab}±19.39	319.50±32.61	212.98±4.99
BD-E	4.216^{bc}±0.66	6.809^{a}±0.58	186.08^{ab}±25.62	354.00±40.18	221.07±5.22
BD-MLP-0.25	4.354^{ab}±0.48	4.961^{b}±0.43	183.63^{ab}±20.00	333.75±31.04	218.57±6.60
BD-MLP-0.5	3.335^{bc}±0.48	7.101^{a}±0.60	218.80^{a}±27.24	408.37±51.84	219.63±7.35
BD-MLP-0.75	2.804^{c}±0.11	7.687^{a}±0.65	225.77^{a}±10.64	435.75±62.54	226.96±5.65
SEM	0.228	0.297	8.984	18.181	2.573
N	8	8	8	8	8
P-Value	0.011	0.001	0.046	0.228	0.380

Means with different superscripts in a column differ significantly, P<0.05

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