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Full Length Research Paper

Effect of *Moringa oleifera* leaf extract on the haematological and serum biochemistry of rabbits reared in a semi-humid environment

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This research is aimed at improving the productive and pathological picture of rabbits reared in a semi-humid environment using *Moringa oleifera* leaf extract (MOLE). Twenty four mixed- breed rabbits, having an average weight of 700g, were used in this study. They were randomly divided into four equal treatments (6 rabbits each) and gavaged with various concentrations of MOLE. Treatment 1 (control) was given 0ml MOLE/kg body weight, treatment 2 (30 ml MOLE/kg body weight), treatment 3 (60 ml MOLE/kg body weight) and treatment 4 (90 ml MOLE/kg body weight). Results showed that *M. oleifera* leaf extract at all doses produced significant ($p<0.05$) changes in the blood levels of Packed Cell Volume (PCV), haemoglobin and white blood cell (WBC) count when compared to the control group. Rabbits given 30 of MOLE/kg body weight caused significantly ($p<0.05$) increased the recorded values of alkaline phosphatase (ALP). However, MOLE at tested dose of 90ml MOLE/kg body weight produced a significantly ($p<0.05$) lowered value in the serum level of alkaline phosphatase (ALP) with a non-significant change ($p>0.05$) observed in other serum parameters across the treatments. The total antioxidant capacity (TAC) value of rabbits increased consistently with increased MOLE concentration, while MDA values were not significantly influenced across the treatments. It can be concluded that (MOLE) can be used at 90ml MOLE/kg body weight to reduce lipid peroxidation and enhance oxidative status of rabbits in a semi-humid environment.

Key words: Gavaged, production, *Moringa oleifera*, rabbit, reproduction.

INTRODUCTION

Since ancient times, medicinal plants have been used for the control and treatment of human and livestock ailments (Ganesan and Bhatt, 2008). Such medicinal plants include, *Aloe ferox V* (Mwale et al., 2014), *Telfaria occidentalis* (Dada and Abiodun, 2014) and *Moringa oleifera* (Ojo et al., 2015). The effects of any feed ingredient including medicinal plants on the haematological factors of the livestock are of immense assistance in deciding whether or not such a feed ingredient will be safe as feedstuff (Mitruka and

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Rawnsley, 1997). Oleforuh-Okoleh et al. (2015) stated that certain hematological factors such as packed cell volume, red blood cell, hemoglobin, etc., can be associated with certain production traits and serve as means of assessing clinical and nutritional health status of animals. It has also been documented that high packed cell volume (PCV) and high hemoglobin content (Hb) are associated with high feed conversion ratio (Mitruka and Rawnsley, 1997), while high percentage of white blood cells especially lymphocytes are associated with the ability of the animals to perform well under very stressful conditions. Medicinal plants, however, contain some toxins that have multi-system effects, such as acute kidney injury accompanied by hepatitis and colitis (Swanepoel et al., 2008). In some cases, however, medicinal plants do not have harmful effects on haematological and serum biochemical parameters (Jaouad et al., 2004; Oduola et al., 2007). According to Ewuola and Egbunike (2008), some medicinal plants are basically used as feed supplements, or for medicinal purposes thereby becoming involved in a cascade of physiological reactions, that may lead to alteration of haematological and serum biochemical parameters. This could result from the toxic substances that might be present in the plants in cases of lowering or elevating the haematological and biochemical values. It could also act as non-toxic invaluable compounds that maintain the values within the expected reference ranges for chickens (Simaraks et al., 2004). In view of this, the toxicological effects of *M. oleifera* on haematological and serum biochemical parameters and the effect on the oxidative status of rabbits naturally reared in a semi-humid environment were evaluated.

MATERIALS AND METHODS

This study was carried out at the Rabbitry section of the Teaching and Researc arm, College of Agriculture, KwaraStateUniversity, Ilorin, in Kwara State. The study lasted for 9 weeks.

Preparation of *M. oleifera* leaves extract (MOLE)

Fresh leaves of *M. oleifera* were collected early in the morning at Ita-alamu area of Ilorin, Kwara State. The *M. oleifera* leaves were manually removed from the stem, cleaned and made free of sand and other impurities using distilled water. The fresh leaves were blended into powdered using an electric kitchen blender. Finely pulverized *M. oleifera* leaves weighing 300 g was poured into a 2.5 L macerating flask and 1.5 L of distilled water added. The resulting mixture was thoroughly homogenized and sieved with a cheese cloth and then filtered using whatman filter paper (24 cm). Resulting filtrate was stored in the freezer (4 or -20°C) till use.

Experimental animals and management

Twenty four (24) grower rabbits of mixed breed rabbits aged9 months old, with average initial body weight ranging from 600 to 800 g were used for the experiment. The experimental animals were randomly assigned to four (4) groups comprising 6 animals per treatment in a completely randomized design. Each treatment was replicated thrice having 2 animals per replicate. The animals were allowed 10 days of acclimatization in the Teaching and Research Farm before data collection commenced. Prior to the data collection, the animals were given oxytetracycline (5%) intramuscularly twice, vitamin B complex intramuscularly and ivomec through subcutaneous route of administration. Treatment one (1) served as the control given 0 ml *M. oleifera* leaf extract /kg body weight of animal, while rabbits in treatments 2, 3 and 4 received the MOLE extract at 30 ml MOLE/kg body weight, 60 ml MOLE/kg body weight and 90 ml MOLE/kg body weight, respectively via gavaging method of administration. Feed and water were given *ad libitum*. The experimental design was completely randomised design (CRD).

Table 1. Chemical compositions of *Moringa oleifera* leaves.

Item	*M. oleifera* leaves (MOL)
Dry matter (%)	91.78
Moisture content	8.22
Crude protein (%)	28.43
Crude fat (%)	6.40
Crude fibre	9.15
Total ash (%)	9.09
Nitrogen free extract (%)	46.93%

Chemical analysis

Proximate analysis of experimental diets was carried out in a reputable chemical analysis labouratory using the method described by Association of Analytical Chemist (A.O.A.C, 1990).

Blood collection

2 ml blood sample was collected from the marginal ear vein of 12 rabbits which were comfortably restrained prior to the blood collection. The blood samples were collected in heparinised tubes, temporarily stored in crushed ice and transported to a reputable laboratory for analysis. Malondialdehide (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). Total antioxidant capacity of serum sample was determined according to the method described by Baydar et al. (2007).

Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) in a completely randomized design, while significant means were separated using Duncan's new multiple test of software (SAS, 2003).

RESULTS

Chemical analysis

The results of proximate analysis in Table 1 showed that Moringa leaves had an appreciable crude protein content (28.43%), crude fibre (9.15%), ash (9.09%), dry matter (91.78%), nitrogen free extract (NFE) (46.93%), but low

Table 2. Haematological parameters of rabbits fed MOLE.

Parameter	T_1 (0%)	T_2 (30%)	T_3 (60%)	T_4 (90%)
PCV (%)	32.00^{a}±2.52	25.50^{a}±1.50	22.33^{ab}±2.33	26.67^{ab}±2.40
HB (g/dl)	10.30^{a}±1.14	8.30^{ab}±0.30	7.33^{b}±0.58	8.66^{ab}±0.80
RBC ($\times10^{6}$)	5.16±0.58	4.12±0.06	3.56±0.34	4.25±0.47
WBC ($\times10^{3}$)	5.82^{b}±2.24	4.70^{bc}±4.00	4.25^{c}±6.64	8.05^{a}±3.3
Platelet ($\times10^{3/}$l)	88.67± 2.5	88.50±3.5	67.33±5.9	93.33±6.6
Lymphocytes (%)	62.00±4.35	66.00±0.00	68.33±2.73	67.00±1.15
Neutrophils (%)	33.67±4.33	29.50±0.50	28.67±2.3	29.67±0.6
Monocytes (%)	2.33±0.33	2.00±1.00	1.67±0.33	1.33±0.33
Eosinophils (%)	2.00±0.57	2.50±0.50	1.33±0.35	1.67±0.88

$^{a-b}$Means bearing different superscript in the same row differ significantly($P < 0.05$). T_1 = Control, T_2 = 30 ml of *M. oleifera* leaf extract (MOLE) /kg body weight, T_3 = 60 ml of *M. oleifera* leaf extract (MOLE) /kg body weight, T_4 = 90 ml of *M. oleifera* leaf extract (MOLE) /kg body weight; SEM, Standard error of means. NS = No significant different; S = Significant different; MDA = Lipid peroxidation; TAC = Total antioxidant capacity; K = potassium level.

content of ether extract (6.40%). Dry matter content of MOLE in this study was lower than the values reported by Mutayoba et al. (2011) who reported dry matter values of 93.7%. The crude protein (CP) value of MOLE obtained in this study was higher than the value reported by Olugbemi et al. (2010) which was 27.44%, although Mutayoba et al. (2011) recorded a higher (30.65%) crude protein value. The crude fat and ash values (6.40 and 9.09%) observed in this study were higher than the values 2.11 and 7.93% reported by Ogbe et al. (2011/2012). Crude fibre value of 9.15% reported in this study was higher than 5.43% which was reported in the study conducted by Sodamade et al. (2013). These differences in values of MOLE have been observed in the previous studies stated to be due to differences in the soil type, climatic conditions, stage of maturity and their genetic make-up.

Haematological analysis

The haematological parameters of rabbits given the varying concentrations of MOLE are presented in Table 2. Results showed that *M. oleifera* at all doses administered to the animals produced significant ($p>0.05$) change in the blood levels of packed cell volume, haemoglobin and total white blood cell count when compared to the control group. The lowest value of PCV was observed in rabbit group given 60ml MOLE/kg body weight (22.33±2.33%), while the highest value was recorded in the control group with 0ml MOLE/kg body weight (32.00 ±2.52%). 60ml MOLE/kg body weight significantly decreased the HB value compared with the control value (10.30±1.14 g/dl). MOLE administration at 90ml MOLE/kg body weight significantly lowered the WBC value at 60 ml MOLE/kg body weight (4.25×10^{3} ± 6.64) while the highest value was observed in rabbits given 90 ml MOLE/kg body weight (8.05×10^{3}±3.3). The RBC, platelet, lymphocytes, neutrophil, monocyte and eosinophil were not significantly affected among experimental groups.

Serum biochemical analysis

On the other hand, treatment of the animals with 30 ml of MOLE/kg body weight caused significantly ($p<0.05$) increased value of ALP. However, *M. oleifera* at tested dose of 90ml /kg body weight produced a significantly ($p<0.05$) lowered value in the serum level of ALP with a non-significant change ($p>0.05$) observed in the serum levels of albumin, globulin, albumin: globulin ratio (A: G ratio), total protein, total bilirubin, cholesterol, creatinine, blood urea nitrogen (BUN), aspartate transaminase, alanine amino transferase, calcium and sodium levels when compared to the control group (Table 3).

Table 4 shows the result of serum lipid peroxidation (MDA), total antioxidant capacity (TAC) of rabbit bucks fed *M. oleifera* leaf extract (MOLE). The values recorded for MDA ranged from 1.46 to 0.31 u/l with the highest value recorded in rabbits given no MOLE (control 0 ml MOLE /kg body weight) while the lowest value was observed in rabbits given 90 ml MOLE /kg body weight, although no significant ($p>0.05$) difference was observed in the MDA value across the treatments. The TAC recorded was significantly influenced by the MOLE administration. The TAC value of rabbits increased consistently with an increased MOLE concentration. The highest value (40.81 µg/ml) was recorded in rabbits given 90 ml MOLE/kg body weight, while the lowest value (28.32 µg/ml) was observed in the control group which received no MOLE.

DISCUSSION

The study investigated the effect of aqueous extract of *M.*

Table 3. The serum biochemical parameters of rabbits given oral administration of MOLE.

Parameter	T_1 (0%)	T_2 (30%)	T_3 (60%)	T_4 (90%)
Albumin(g/dl)	3.53±0.20	3.60±0.20	4.00±0.31	4.03±0.23
Globulin(g/dl)	4.50±0.35	4.75±0.15	5.17±0.27	5.00±0.10
A:G ratio	0.73±0.03	0.70±0.00	0.77±0.06	0.73±0.03
Total protein(g/dl)	8.03±0.54	8.35±0.35	9.17±0.33	9.03±0.23
Total Bilirubin(mg/dl)	0.30±0.11	0.15±0.05	0.23±0.06	0.20±0.00
Cholesterol(mg/dl)	20.67±6.66	19.50±1.56	25.67±8.98	23.0±5.50
Creatinine(mg/dl)	1.30±0.40	1.55±0.25	2.06±0.33	2.03±0.22
BUN(mg.dl)	14.10±0.40	15.45±0.95	15.90±0.73	15.93±0.33
AST(iu/l)	23.67±0.67	33.0±8.00	18.0±2.65	44.30±14.81
ALT(iu/l)	149.67±61.29	113.50±0.50	162.37±11.5	140.3±24.90
ALP(iu/l)	36.33^{ab}±0.88	39.0^{a}±1.00	33.0^{ab}±1.00	32.33^{b}±2.90
Ca(mg/dl)	13.07±1.34	12.70±0.90	13.07±1.59	13.67±1.47
Na(mg)	119.0±4.35	112.50±2.50	116.00±3.05	118.00±4.60

$^{a-b}$Means bearing different superscript in the same row differ significantly($P < 0.05$); T_1 = Control, T_2 = 30 ml of *M. oleifera* leaf extract (MOLE) /kg body weight, T_3 = 60 ml of *M. oleifera* leaf extract (MOLE)/kg body weight, T_4 = 90 ml of *M. oleifera* leaf extract (MOLE) /kg body weight; SEM, Standard error of means. NS = No significant different; S = Significant different; MDA = Lipid peroxidation; TAC = Total antioxidant capacity; K = potassium level.

Table 4. Lipid peroxidation, total antioxidant capacity of rabbit bucks fed MOLE.

Parameter	T_1	T_2	T_3	T_4	Std. Mean Error	P-value
MDA (u/l)	1.46	0.37	0.59	0.31	0.21^{NS}	0.126
TAC (µg/ml)	28.32^{a}	33.36^{ab}	38.96^{b}	40.81^{b}	1.84^{S}	0.009

$^{a-b}$Means bearing different superscript in the same row differ significantly($P < 0.05$); T_1 = Control, T_2 = 30 ml of *M. oleifera* leaf extract (MOLE) /kg body weight, T_3 = 60 ml of *M. oleifera* leaf extract (MOLE) /kg body weight, T_4 = 90 ml of *M. oleifera* leaf extract (MOLE) /kg body weight; SEM, Standard error of means. NS = No significant different; S = Significant different; MDA = Lipid peroxidation; TAC = Total antioxidant capacity; K = potassium level.

oleifera on haematology, serum biochemical indices and oxidation status of rabbits reared in a semi-humid environment. Results of the present study showed clearly that oral administration of MOLE to rabbits affected significantly some of the studied traits. According to Oyedemi et al. (2011), the assessment of haematological parameters could be used to reveal the deleterious effect of some chemicals in plant extracts on the blood constituents of animals. It also reflects the physiological responsiveness of the animal to its internal and external environments (Esonu et al., 2001). Observation showed that MOLE had no significant influence on the Hb, RBC and PCV values of rabbits given 30 and 90 ml MOLE/kg body weight. This shows that MOLE can be used for rabbits under heat stress without any pathological deviation from the normal. However, the recorded values for rabbits in T3 showed as a depression which perhaps could be as a result of other factors different from the experimental materials.

The present findings indicated that environmentally-induced heat stress increased the level of lipid peroxidation as reflected by the high value of malondialdehyde (MDA) in rabbits under treatment 1(0 ml of MOLE/kg body weight), in addition, heat stress also depleted the antioxidant capacity of the birds as recorded in the low level of TAC (Ismail et al., 2013). *M. oleifera leaf extract* (MOLE) decreased the incidence of lipid peroxidation as shown by the decreasing MDA values in treatments 2 (30 ml MOLE/kg body weight), 3 (60 ml MOLE/kg body weight) and 4 (90 ml MOLE/kg body weight). This is in agreement with the result of Luqman et al. (2012) who observed a lowered MDA level in mice given aqueous fruit extract of *M. oleifera*. Increased activity of antioxidant enzymes was observed in rabbit group given 30, 60 and 90 ml of MOLE/kg body weight in a dose-dependent manner. This result is similar with the results obtained by Luqman et al. (2012) who recorded total antioxidant capacity of *M. oleifera* extracts which increased with an increase in MOLE concentration. It can therefore be concluded that administration of MOLE at 90 ml /kg body weight can be used to enhance the antioxidant capacity of rabbits reared in a semi-humid

environment without any adverse effect on the blood and serum biochemistry of the test animals. Further studies can be done to evaluate use of MOLE at a higher concentration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests**.**

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