

EFFECT OF SUPPLEMENTING MORINGA (*Moringa oliefera*) AND LETTUCE (*Lactuca sativa*) LEAVES ON HAEMATOLOGY AND SERUM BIOCHEMISTRY OF MONGREL RABBIT

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Abstract

An experiment was conducted to determine the effect of supplementation of moringa and lettuce leaves on heamatology and serum biochemistry of mongrel rabbit which was carry out at the Rabbitry unit of the teaching and research farm in the department of Animal science, ADUSTECH Wudil, Kano State. The supplementary diets comprised of four treatments and replicated four times T_1 with 0%, T_2 with 5% lettuce, T_3 5% Moringa and T_4 the combination of 5% lettuce and 5% moringa respectively. The animals were allotted to the four dietary treatments in a Completely Randomized Design, which replicated four times. Experimentation period lasted for 6 weeks and experimental diet fed 3% of the experimental animals body weight. Blood sample with (EDTA) and without anticoagulant were collected from all treatments which was used to determine hematological and serum indices. The laboratory results were analyzed using analysis of variance (ANOVA) in a statistical analysis system (SAS, 2002) version 6th statistical software, difference between treatment means were separated at 5% level of probability using Duncan multiple range test (DMR). Results regarding hematological attributes revealed that, there were statistically significant ($P < 0.05$) difference across all the treatments values recorded on RBC, except T_2 (3.12) which shows no significant ($P > 0.05$) difference. Similarly significant ($P < 0.05$) differences were observed across all the treatment values recorded on WBC except T_1 (75.0) which shows no significant ($P < 0.05$) difference. Moreover significant ($P > 0.05$) difference was observed across all the treatments values recorded on monocytes, except T_2 (5.63). While serum chemistry revealed that, there were statistically significant ($P > 0.05$) differences observed across all the treatments values recorded on chlorine (CL-) except T_4 (103.00). Similarly significant ($P < 0.05$) difference was observed across all the treatments values recorded on glucose fast except T_4 (4.26). However significant ($P < 0.05$) difference observed across 2 treatments T_3 (0.54) and T_4 (0.18) on HDL. It concluded that lettuce and moringa oliefera leaves supplementation to mongrel rabbit as 5% and 10% combination (lettuce and moringa oliefera) leaves had significantly affected blood metabolites, especially RBC and WBC and LFT enzymes of mongrel rabbits respectively.

Keywords: Moringa, Lettuce, Heamatology, Serum biochemistry, Mongrel Rabbit

Introduction

Rabbit (*Oryctolagus cuniculus*) belongs to the family Leporidae (rabbits, hares) of the order Lagomorpha (leporids, picas). Once classified as a rodent, the rabbit was given a separate order because of dentition differences, chiefly the incisors. Lagomorphs have 2 pairs of upper incisors (they are born with 3 upper pairs but lose the outer pair early). The 2nd pair of upper incisors is

smaller and is located immediately behind the 1st. Closer ties exist between lagomorphs and artiodactyls than between lagomorphs and rodents. Other differences, many of which will be presented in this series, justified the suggestion by paleontologists that lagomorphs departed from the main eutherian line early in phylogeny (Romer 2020).

The American Rabbit Breeders Association recognizes over 100 breeds of rabbits. Three breeds are commonly used in the laboratory: the Dutch Belted (1.5 to 2 kg); the New Zealand White (5 to 6 kg); and the Flemish Giant (8 to 9 kg). In the wild, *O. cuniculus* dig burrows, unlike most other rabbits and hares. These burrows can be very extensive, combining with neighboring burrows to form warrens that may occupy 2 or more acres of land. Essentially nocturnal, rabbits may girdle trees and destroy plants at night and retire to their burrows in the daytime. Domesticated rabbits released in the field have been known to develop burrows. Rabbits can be frightened easily, leading to widely varying responses. Emotional stress can cause a fall in body temperature. The rat can mimic the rabbit in this phenomenon. In most endotherms, including the human, emotional stress tends to cause an increase in body temperature. Fright causes marked stimulation of the autonomic nervous system. Renal ischemia can develop. If the rabbit is then given water by stomach tube, water intoxication, convulsions, and rapid death can result. Compulsive water drinking of psychogenic origin can be brought on by raucous noise (rock music; children playing) and may cause diabetes insipidus. (Iversen *et al.*, 2022). Rabbits made hypoxic have reduced kidney function. Another fright reaction may be splanchnic vasoconstriction, shunting blood to the head and the heart. Emotional stress may cause a leucopenia in rabbits (in cats, it causes a leucocytosis. (Nice and katz 2006). As in other mammals, stress in rabbits can also cause cardiomyopathy (Weber and vanderwatt 2003) and gastric lesions. (Iversen *et al.*, 2022). Rabbits must be handled with great care. In picking up a rabbit, obtain a firm grip over the scruff of the neck with 1 hand and support the animal with the other so that the rabbit will not kick. The skeleton of the rabbit is relatively fragile. The rabbit skeleton weighs about one-half that of a cat of the same body weight. Broken backs can occur in rabbits when handlers are not careful. The objective of the study is to determine the effect of supplementation of moringas (*Moringa oliefera*) and lettuce (*Lactuca sativa*) leaves on Hematology and serum biochemistry of mongrel rabbit.

Lettuce (*Lactuca sativa*) belongs to Asteraceae family, tribe Cichorieae, it has many medicinal values (Mou, 2011).Lettuce has some benefits mainly due to the presence of natural antioxidant compounds (vitamins A, C and E, carotenoids, polyphenols) alongside significant amounts of fiber and certain minerals (Al-Shmgani *et al.*, 2017). Furthermore, lettuce has sedative, analgesic, hypoglycemic, antifungal as well as antioxidant properties. Cabbage waste also contains vitamins that are important for the health of rabbits. For example, one cup of chopped raw green cabbage provides around 50% of the recommended daily intake of vitamin C for humans, which is also essential for rabbits. Vitamin C is necessary for the synthesis of collagen, a protein that provides structural support to tissues such as skin, bone, and cartilage. Vitamin C also functions as an antioxidant, protecting cells from oxidative stress. In addition to vitamin C, cabbage waste is a good source of vitamin K, which is necessary for blood clotting and bone metabolism. Cabbage waste also contains potassium, an electrolyte that helps to maintain fluid balance and regulate blood pressure. Finally, cabbage waste contains calcium, which is essential for the growth and maintenance of bones (Adamu *et al.*, 2009).

Moringa Olifera belongs to a family Moringaceae which known to have high content of protein (Olugbemi *et al.*, 2010) and low ant nutritional factors (Farinu, 2010). A high extent of this protein is conceivable accessible for digestion due to low content of acid detergent insoluble

protein and high content of pepsin soluble nitrogen (Farinu, 2010). Moreover, protein of *Moringa Oleifera* is reported to be a good source of essential amino acids that allegedly boost immunity (Olugbemi *et al.*, 2010). Therefore, *Moringa Oleifera* can be used as a dietary supplement in poultry (Mahajan *et al.*, 2007).

Materials and Method

Experimental Site

The experiment was conducted at the Rabbitry unit of the Teaching and Research Farm of the Department of Animal Science, Aliko Dangote University of Science and Technology, Wudil, Kano State, Nigeria. The area lies between the longitude and latitude of 8⁰ 51" East and 11⁰ 49" North at an altitude of 403 meter above sea level (Olofin *et al.*, 2008).

Source and preparation of lettuce (*Lactuca sativa*) and moringas leaves (*moringa oliefera*) leaves material

Fresh lettuce (*Lactuca sativa*) material was purchased from Wudil market and carry it directly to the experimental rabbit at different level of supplementation (0%, lettuce 5%, moringa 5%, moringa 5% and lettuce 5%) morning and evening respectably. While the moringa leaves were purchased from Gaya research farm and allowed to dried on under shade. The dried moringa and lettuce leaves were used to formulate diet at different levels (0% and 15%). Sample of the leaves were taken to the laboratory for phytochemical and proximate composition analysis using standard procedure of Ahemen *et al.* (2013). Four (4) treatments were used, each treatment consists of 1 rabbit and replicated 4 times which is equals to 16 rabbits.

Experimental Animals and their Management

Sixteenth (16), grower rabbits of 10 - 12 weeks with average body weight of 1,320 g \pm 20 g was used for the study. Rabbits were housed individually in a wire-mesh cage in an indoor pen under similar management and environmental conditions.

The experimental rabbit was treated against internal and external parasites using Ivermectin injection (Kepromec®). They were also given anti-coccidial as prophylaxis using Embazin - Forte at dose rate of 30 g / 50 litres of drinking water for 3 days, and Oxytetracycline powder (Samoxine). The rabbits were initially fed control diets *ad libitum* for one (1) week of physiological adjustment. Fresh, clean, and cool water was supplied *ad libitum* throughout the experimental period.

Experimental design and procedure

Four (4) treatments(T₁, T₂, T₃ and T₄) were fed in a completely Randomized design (CRD) at different levels of supplementation (T₁=M0%L0%, T₂=M0%L5%, T₃= M5%L0%, and T₄=M5%L5%) with four (4) rabbit per treatment which was replicated four (4) times.

Haematology and Serum chemistry

Blood samples with (EDTA) and without anticoagulant was collected from marginal ear vein before slaughter. From each treatment, the blood samples were collected four times. Blood samples collected with EDTA was used to determine packed cell volume (PCV), red blood cell counts (RBC), white blood cell (WBC) counts platelets, haematocrit value and hemoglobin concentration (Hb) in blood samples. The PCV, RBC, WBC and Hb values were determined

using the Wintrobe's microhaematocrit, improved Neubauer haemocytometer and cyanomethaemoglobin method respectively (Coles, 1986). The mean corpuscular hemoglobin (MCH) was calculated according to Bush (1991).

Blood samples collected without anticoagulant was subjected to serum Procurement which was then used to determine the biochemical components. Creatinine, high density lipoproteins (HDL) low density lipoproteins (LDL), triglycerides, electrolyte (sodium, phosphorus, chlorine) and liver enzymes (Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)). Serum glucose and urea was estimated by methods described by while total cholesterol was determined by colorimetric enzyme method as outlined by Bush (1975). Similarly, serum total protein (TP), albumin (AL) and globulin concentration was determined by Coles, (1986) and Biuret reactions (Bush, 1975).

Statistical Analysis

The experimental data collected was analyzed using analysis of variance (ANOVA) in statistical analysis system (SAS, 2002) version 6th statistical software. Differences between treatment means were separated at ($P < 0.05$) using Duncan Multiple Range Test (Silva and Azevedo, 2009).

Result and Discussion

Blood investigation is a method for evaluating clinical and wellbeing status of animals and humans. Agreeing to Oyedemi *et al.* (2010) the valuation of hematological factors could be beneficial to study the harmful effect of some substances present in plant supplementation on the blood composition of various animals and humans. It also reveals the physical and functional response of the organisms in its surroundings Ahemen *et al.* (2013). The present work is a novel one as it targets apparently healthy mongrel rabbit as models for study of supplemented with moringa and lettuce leaves on various hematological and serum chemistry attributes. The results of hematological parameters in our study depicted that supplementation of moringa and lettuce leaves increased in chlorine, creatinine, glucose fast, total cholesterol HDL, IDL and AST for serum and that of heamatology WBC, monocyte and RBC compared to the control group.

Table 1: Haematological Indices of Mongrel Rabbits Supplemented with Moringa (*Moringa oliefera*) and Lettuce (*Lactuca sativa*) Leaves

Parameters	Treatments				SEM
	T1 (0%)	T2 (5L %)	T3 (5M %)	T4 (5% L5%M)	
Hemoglobin (G/dl)	11.433	11.73	12.53	12.43	0.311
Packed cell volume (%)	33.40	36.00	28.33	35.66	1.563
Red blood cell (x/10)	2.24 ^{ab}	3.12 ^a	2.26 ^b	2.20 ^b	0.187
MCV (fl)	135.48	141.83	144.6	148.56	2.904
MCHC (g/dl)	289.00	336.00	335.00	307.00	13.29
MCH (pg)	42.46	42.76	42.83	42.00	0.463
White blood cell (x10%)	75.80 ^a	45.63 ^{ab}	42.76 ^b	45.20 ^{ab}	0.237
Neutrophils (%)	12.03	12.66	12.66	12.83	0.399
Lymphocytes (%)	83.66	79.66	85.33	74.33	3.146
Monocytes (%)	3.67 ^b	5.63 ^a	3.66 ^b	3.00 ^c	0.403
Platelets (x10%)	131.67	124.00	119.67	124.67	4.304

^{abc} = means in the same row with different superscript are significantly different ($P > 0.05$); RBC, WBC, and Monocytes SEM – Standard error of mean.

However, a significant increase was noticed for T3 with moringa supplementation which was supplemented 500 mg/kg of *M. oleifera*. The higher level of supplementation of combination of lettuce and moringa examined in this research is similar to the results obtained earlier in a work by Osman, Shayoub, and Babiker, (2012) conducted to assess the effect of *M. Oleifera* and lettuce leaves on blood parameters and body weights of albino rats and rabbits. These results are also in line with the previous study conducted on rabbits El-Gindy, Zeweil, and Hamad, (2017). These increase levels of moringa and lettuce supplementation for rabbits could be attributed to an increased erythropoiesis and an increased bone marrow functioning by Ikwunze *et al.* (2016). In contrast, no significant change in supplementation of lettuce and moringa was observed by Ahemen *et al.* (2013) and Jiwuba *et al.* (2016) who studied physiological responses of rabbits supplemented of lettuce and moringa in their diets on hematological and serum biochemical indices of growing rabbits. The results of this study are related to the previous researches but differences in the results were also observed as compared to many studies. The differences in the results of this study from previous may be due to the different breeds of rabbits used in different studies, difference in climate or difference in dosage forms of Moringa and lettuce supplementation. The red blood cells were significantly lower in all Moringa and lettuce supplementation to treatment groups as compared to the control group in our study. The red blood cells in fact, represents the total number of dividing cells in contrast to the number of cells being analyzed and is the percentage of cells undergoing mitosis. A decreased number in Moringa and lettuce supplementation groups in our study is a beneficial effect of Moringa as illustrated previously Sharayu and Asmita, (2017). The chlorine was significantly higher in T2 (96.33) as compared to control and all other treatment groups. Similar results have been reported for anemic human patients by (Suzana *et al.*, 2017) and for albino rabbits supplemented with Moringa Ofem *et al.* (2015). Though an increase in this haematological parameter is considered as some kind of nutrient deficiency Constantino, (2013), however, in our study its increase can be valued in respect to increase in Moringa supplementation to rabbits. Similar results have been reported for growing rabbits fed graded levels of *M. oleifera* leaf meal (MOLM) (Ahemen *et al.*, 2013, Ewuola, *et al.*, 2012). The results examined were similar to the results obtained by Nuhu, (2010) conducted to evaluate the effect of MOLM on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. The non-significant effect of Moringa leaf on ALT, ALP and ALP is an indicator that the treatments have no untoward effect on the health status of the rabbits. However, the results of our study were in contrast to the earlier findings in which physiological responses of rabbits supplemented with lettuce and moringa levels on hematological and serum biochemical indices of growing rabbits were studied Jiwuba *et al.* (2016). The difference in the results of this study may be due to the different breeds of rabbits, difference in climate or difference in dosage forms of Moringa and lettuce supplementation. The results of our finding showed that creatinine was significantly higher in T3 (supplemented with 300mg/ (kg) live weight of Moringa and lettuce leaves) and lower in T2 (supplemented with 500mg/ kg of Moringa and lettuce leaves) as compared to control and group treatment 1. However, all the values were within the reference ranges of healthy rabbits described in previous study by Melillo, (2007). The result of our study is in agreement with the previous study conducted in Egypt on the treatment of *Ulva lactuca* (sea lettuce) by Jiwuba *et al.* (2016). The result of present study was in agreement with the results of study performed in Nigeria on rats Achuba, Ubogu, and Ekute, (2016) and on broiler chicken by Tijani *et al.* (2016). The creatinine is produced from muscle creatine and is excreted through the kidneys at a constant rate. An increase beyond the reference values is an indicator of impaired kidney disease. However, as the values of present study were within the reference range for

rabbits, hence, the supplementation of moringa and lettuce with 300mg/kg Moringa could be attributed to no stress or dehydration in rabbits throughout the seasons.

Table2. Serum indices of mongrel rabbit supplemented with Drumsick (*moringa olienfera*) and lettuce (*Lactuca sativa*) leaves

Parameters	Treatments				SEM
	T ₁ (0%)	T ₂ (5L %)	T ₃ (M %)	T ₄ (5L% 5M %)	
Urea (mml/L)	5.90	5.66	18.80	5.13	3.815
Sodium (mml/L)	136.00	135.00	131.00	136.33	1.205
Potassium (mml/L)	3.15	2.90	3.16	6.86	0.815
HCO ₃ (mml/L)	25.00	25.66	25.33	22.00	0.638
Chlorine (mml/L)	97.66 ^{ab}	96.33 ^{ab}	94.33 ^{ab}	103.00 ^a	1.206
Creatinine (mml/L)	88.33 ^a	75.00 ^a	58.33 ^b	76.66 ^a	2.488
Glucose fast (mml/L)	3.60 ^{ab}	4.05 ^{ab}	3.36 ^b	4.26 ^a	0.131
Total col (mml/L)	6.9 ^a	6.27 ^a	5.4 ^b	5.11 ^b	0.112
HDL (mml/L)	0.99 ^a	1.03 ^a	0.54 ^c	0.81 ^b	0.022
TRIG (mml/L)	1.35	1.34	1.41	1.32	0.638
LDL (U/L)	4.44 ^a	3.99 ^b	3.97 ^b	4.26 ^{ab}	0.063
ALP (U/L)	124.00	187.33	197.33	1157.00	27.17
ALT (U/L)	32.67.	51.33	44.30	61.01	8.862
AST (mml/L)	10.67 ^b	59.33 ^a	37.67 ^{ab}	33.00 ^{ab}	8.026
BIL (mml/L)	112.13	14.00	13.86	13.86	0.433
BIL DIRECT (U/L)	9.00	5.30	4.33	4.90	0.776
Total protein (g/L)	109.00	101.67	94.33	101.00	4.373
Albumen (g/L)	60.66	60.66	52.16	52.66	1.434
Globulin (g/L)	43.66	46.86	44.77	53.36	2.076

^{abc}= Means in the same row with different superscript are significantly different (P>0.05); Chlorine, Creatinine, Glucose fast, Total col, HDL, LDL, and AST. SEM – Standard Error of Mean.

Conclusion

Base on the result obtained from the study its concluded that; Lettuce and moringa leaves supplementation to mongrel rabbit, at 5% level had significantly affected blood metabolites of grower mongrel rabbits. Also combinations of moringa and lettuces have increases in red and white blood cell respectively.

Recommendation

Its recommended that; farmers should supplemented lettuce and moringa leaves for mongrel rabbits on blood metabolites at 5% levels without detrimental effect on their health.

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