Performance and histological studies on West African Dwarf bucks fed air-dried *Moringa oleifera* leaf-based diets

Short title: performance of WAD goats fed varying levels of *Moringa oleifera* leaf meal-based diets

Abstract

Context Although air-drying has been acclaimed to have reduced the some antinutritional factors in *Moringa oleifera* leaf . It will not be conclusive to adjudge this method as the panacea without scaling its effects on the tissues of the leftover phytochemicals. Aim: An eighty four-day feeding trial was carried out to determine the performance and histology of WAD bucks on certain organs of the body fed air-dried *Moringa oleifera* leaf based diets. Method: Twenty (20) West African Dwarf bucks of about six (6) to eight (8) months of age weighing 7.66 kg were randomly assigned to four (4) treatments containing 0% (control), 5%, 10% and 15% of air-dried *Moringa oleifera* leaf meal (MOLM) on weight basis in a Completely Randomised Design. Key results:Dry matter intake was in the range of 382 – 391 g/day while the highest crude protein intake of 32.69g/day was observed in 0 % MOLM inclusion level. The average feed intake per day ranged between 0.44 and 0.64 kg per buck. 10 % and 15 % inclusion of MOLM recorded the highest final weight of 9.32 kg and weight gain of 1.76 kg respectively. Conclusion: The results on the histological examination of the kidney, liver, and small intestine of the bucks fed control diet showed no visible lesion at Haematoxylin and Eosin Bar=100µm. Photomicrograph of bucks in 5% showed lymphocytic infiltration of the lamina propria of the small intestine, mild diffuse centrilobular areas of necrosis of the hepatocytes at Haematoxylin and Eosin Bar=200µm and tubular degeneration in the kidney. Also there was tubular epithelial regeneration of the kidney and all cells were normochromic. Implication: *Moringa oleifera* leaf meal at 15 % posed little or no threat to WAD bucks.

Keywords*: Haematoxylin,* l*esion,* necrosis, *Photomicrograph, weight gain* .

Introduction -

Moringa oleifera Lam. (Moringaceae) is native to the southern part of the Himalayas in northwest India and the most widely cultivated species of the genus Moringa (Osewa *et al*., 2013). It has become naturalized in many tropical countries of Africa (Mustapha, 2013). The common English names are: Moringa, drumstick tree, horseradish tree and benzoil tree. Locally, in Nigeria, it is known as 'Zogale-gandi' in Hausa, 'Ewe igbale' in Yoruba and 'Okweoyibo' in Igbo: it is also known as "Miracle tree", (Ijeoma *et al*., 2012*;* Mustapha, 2013). Studies have shown that *Moringa oleifera* plays an important role in animal's voluntary intake and performance by providing nutritional, medicinal and therapeutic value as its numerous functions (Kakengi *et al*., 2007, Al-kharusi *et al*., 2009; Sanusi, 2017). The crude protein content (circa 25%) in the air dried leaves is high (Reyes-Sánchez *et al*., 2006). About twenty potentially harmful compounds commonly found in several fodder plants are present in Moringa plants such as soluble calcium oxalates (Noonan and Savage, 1999; Radek and Savage, 2008), mimosine, cyanogenic glycosides, moringine, moringinine, condensed tannins and alkaloids. The use of forage trees and shrubs has been limited and ad libitum feeding of these forages is rarely used in livestock feeding. However, the presence of various anti-nutritional compounds and their deleterious effects in animals has also been discussed (Ghosh *et al*., 2008). These molecules can cause digestive problems, side effects and the formation of kidney stones not only in animals but also in human beings (Finkielstein and Goldfarb, 2006). Although air-drying has been acclaimed to have reduced the said antinutritional factors. It will not be conclusive to adjudge this method as the panacea without scaling its effects on the tissues of the leftover phytochemicals. Hence, the aim of this study was to determine the performance and histology of WAD bucks on certain organs of the body fed air-dried *Moringa oleifera* leaf based diets.

Study area

The study was carried out at the Kalahari Unit of the Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Ogun State, which is located in the tropical rainforest zone in Nigeria within 7°13'47.4''N, 3°23'43.4''E. Seasonal distribution of rain is approximately 163 mm (14.6%) in the early dry season and 110 mm (9.97%) in the late dry season (Jan-March) (October – December) (OORBDA, 2012).

Harvesting and processing of *Moringa oleifera* leaves

Moringa oleifera leaves of Nigerian ecotype were obtained in Odeda Local Government, Ogun state. The harvested Moringa leaves were air-dried by spreading on a tarpaulin or cemented floor in a roofed and well ventilated room . The leaves were frequently turned until they were crispy to touch while retaining their greenish colouration. The leaves were then hand-milled to obtain a product herein referred to as Moringa leaf meal (MOLM) which was stored in sacs until needed.

Management and feeding of experimental animals

Twenty (20) West African Dwarf bucks of about six (6) to eight (8) months of age weighing 7.66±0.22 kg were randomly assigned to four (4) treatments of five (5) replicates each that received experimental diets containing 0 % (control), 5%, 10% and 15% of air-dried *Moringa oleifera* leaf meal on weight basis. The experiment lasted for a duration of 98 days (14 days of adjustment period, 84 days for the trial). Prior to the start of the experiment, flock treatment was carried out during which they were dewormed. The animals were managed under intensive system. 5 kg of the experimental diets were offered at 08:00 hours every morning and water was given *ad libitum.*

Table:1 Experimental diets

Experimental Diets	0 %	5 %	10 %	15 %
MOLM (kg)	0.00	5.00	10.00	15.00
Concentrate (kg)	100.00	95.00	90.00	85.00
Total (kg)	100.00	100.00	100.00	100.00

Specimen collection and histology

After the feeding trial period, one buck per treatment was slaughtered and parts of the organs such as liver, kidney, heart, small intestine were dehydrated in graded levels of alcohol (about 70% - 80%) in ascending order to remove the water content. After the dehydration, the tissues were cleared in xylene impregnated with paraffin wax and sectioned at 5 microns thickness using rotary microtone. The sections were floated on a paraffin water bath maintained at a temperature of 2-3 ^{0}C below melting point of the paraffin wax 56 ^{o}C after which the sections were dried on a slide dryer maintained at a temperature of 3 ^{o}C higher than the melting 58-59 ^{0}C point of the paraffin wax used. After proper drying, the sections were stained with haematoxylin and eosin (H and E) and mounted using histomount. Photography of the desired sections was made for further observation.

Statistical analysis

All investigated parameters were based on completely randomized design using the procedure of Statistical Analysis Software 9.1 (SAS, 2003) and treatment means were compared using Duncan's procedure (Duncan, 1955) of the same software. The statistical model focused

primarily on inclusion level effect as the main treatment. The following model was used: Y_{ij} = μ + M_J + e_{ij}; where Y_{ij} is the dependent, continuous variable; μ is the overall mean; M_j is the fixed effect of the *j*th inclusion of air-dried *Moringa oleifera* leaves (j = 0%, 5%, 10% 15%) and e_{ij} is the residual error.

Results

The proximate composition of experimental diets is shown in Table 2.0. Dry matter content was in the range of 83.4 and 88.3 % while the highest crude protein was 8.54 % in 0% MOLM. The values nitrogen free extract , acid detergent fibre and hemicellulose in 15 % MOLM were 46.0, 21.2 and 15.2 % respectively.

Table 2.0: Proximate composition of experimental diets (% DM)

Parameter	0%	5%	10%	15%
Dry Matter	88.35	83.47	84.66	83.35
Crude protein	8.54	8.17	7.25	7.93
Crude fibre	6.44	9.01	7.57	6.80
Ether extract	14.23	8.66	2.51	6.67
Ash	15.51	24.07	18.66	16.44
Nitrogen free extract	43.63	33.56	48.67	46.00
Neutral detergent fibre	49.17	37.31	22.00	36.55
Acid detergent fibre	32.38	11.47	11.59	21.27
Hemicellulose	16.79	25.84	10.41	15.28

Nutrient intake of West African Dwarf goat fed *Moringa oleifera* leaf meal-based diets is shown in Table 3.0. The lowest dry matter intake of 366.8 g/day was observed in bucks fed 10 % MOLM. The highest crude protein, ether extract, neutral detergent fibre and acid detergent fibre intakes were 32.6, 61.5, 140 and 297 g respectively in bucks fed 0 % MOLM. Total feed intake was in the range of 53.4 to 36.7 kg. Bucks fed 0, 5 10 and 15 % MOLM had initial body weight of 7.18, 8.08, 8.04 and 7.34 kg respectively while total weight gain were 1.58, 0.92, 1.28 and 1.76 kg respectively.

Table 3.0: Nutrient Intake of West African Dwarf Goat fed *Moringa oleifera* leaf meal -Based Diets

Parameters (g)	**Air-dried *Moringa oleifera* leaf meal inclusion levels**				
	0%	**5%**	**10%**	**15%**	**SEM**
Dry Matter	382.90	382.59	366.88	391.39	15.73
Crude Protein	32.69	31.25	26.59	31.03	1.80
Ether Extract	61.57[a]	39.69[b]	10.88[c]	34.43[b]	6.87
Ash	59.51[c]	92.09[a]	68.46[b]	71.16[b]	2.61
Neutral Detergent Fibre	140.41[a]	52.43[c]	50.69[c]	109.76[b]	14.69
Acid Detergent Fibre	297.29[a]	231.19[b]	46.39[c]	193.41[b]	35.10

[a,b,c] means within a row with different superscript are different ($p<0.05$) ns means not significantly different ($p>0.05$)

The histological examination of the kidney fed control 0% *Moringa oleifera*) showed mild diffuse degeneration while 5% MOLM inclusion in diet showed tubular degeneration and necrosis but also showed tubular epithelial regeneration. The kidney of the animals in Treatment three and Treatment four fed 10% *Moringa oleifera* and 15% *Moringa oleifera* inclusion in

diets both showed tubular epithelial regeneration. The section of the hepatocyte of 0% *Moringa oleifera* showed no visible lesion while hepatocyte with 5% and 10% MOLM in diet showed mild diffuse centrilobular areas of necrosis respectively. The hepatocyte of animals in fed 15% *Moringa oleifera* inclusion in diet showed focal areas of necrosis with mononuclear cells infiltration. The small intestine of the 0% *Moringa oleifera* showed no visible lesion while 5%, 10%, and 15% MOLM all showed lymphocytic infiltration of the lamina propia.

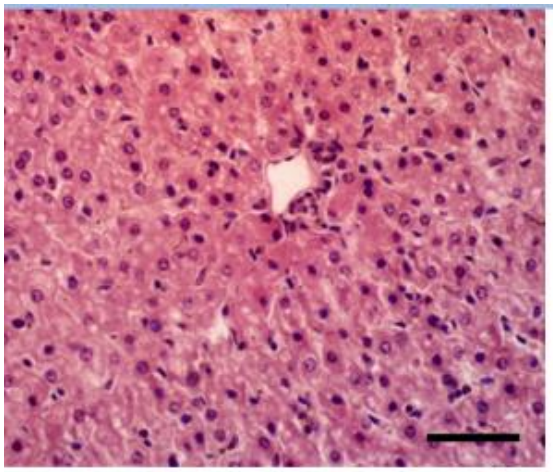

Fig. 1: Photomicrograph of liver section of 0% MOLM showing no visible lesion H &E Bar=100µm

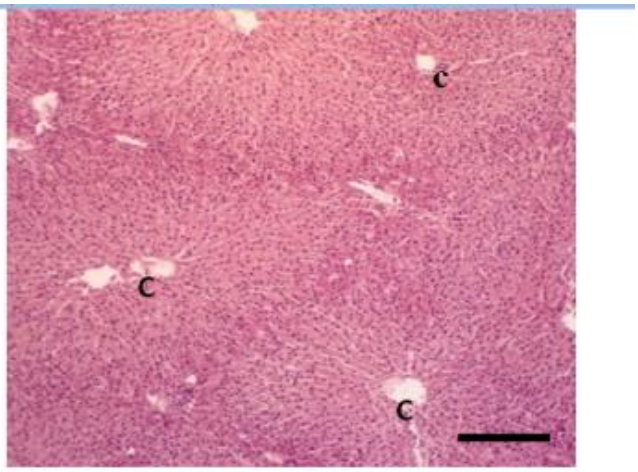

Fig. 2: Photomicrograph of liver section of 5% MOLM showing mild diffuse centrilobular areas of necrosis (C) H &E Bar=200µm

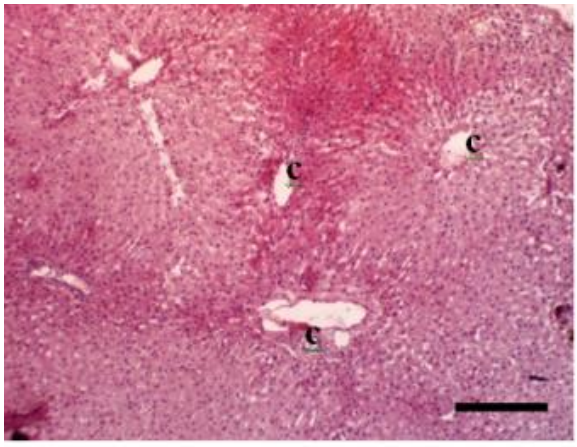

Fig. 3: Photomicrograph of liver section of 10% MOLM showing mild diffuse centrilobular ares of necrosis H &E

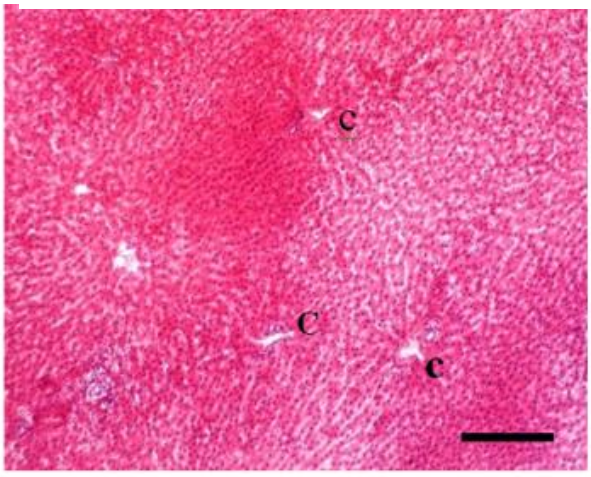

Fig. 4: Photomicrograph of liver section of 15% MOLM showing focal areas of necrosis with mononuclear cells

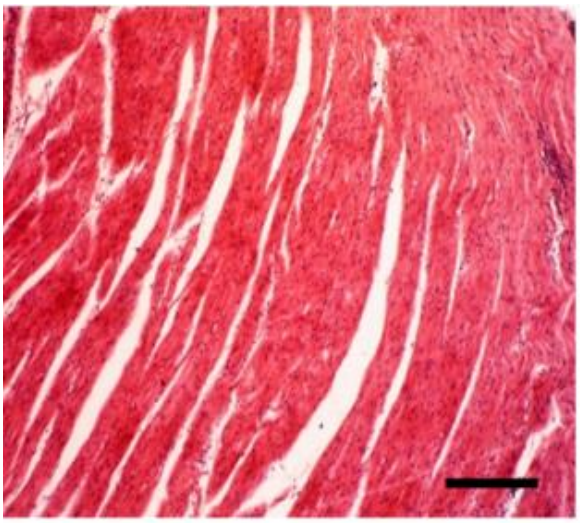

Fig. 5: Photomicrograph of kidney section of 0% MOLM showing no visible lesion H &E Bar=100µm

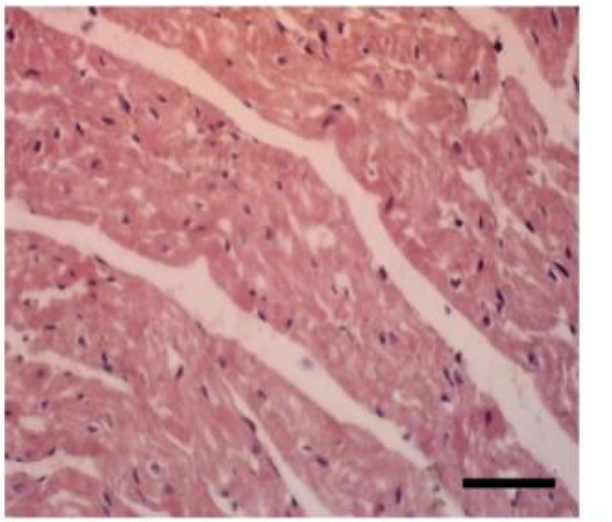

Fig. 6: Photomicrograph of kidney section of 5% MOLM showing no visible lesion H &E Bar=100µm

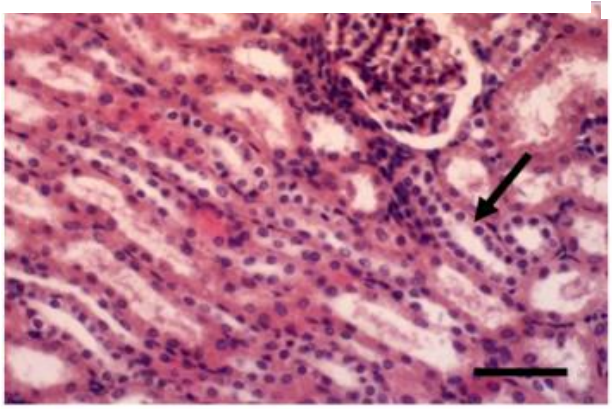

Fig. 7: Photomicrograph of kidney section of 10% MOLM showing tubular epithelial regeneration (arrow)H &E Bar=100µm

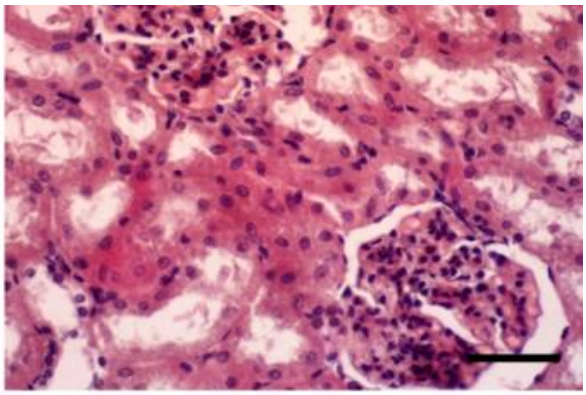

Fig. 8: Photomicrograph of kidney section of 15% MOLM showing tubular epithelial degeneration and necrosis with mild regeneration of epithelial cells H &E Bar=100µm

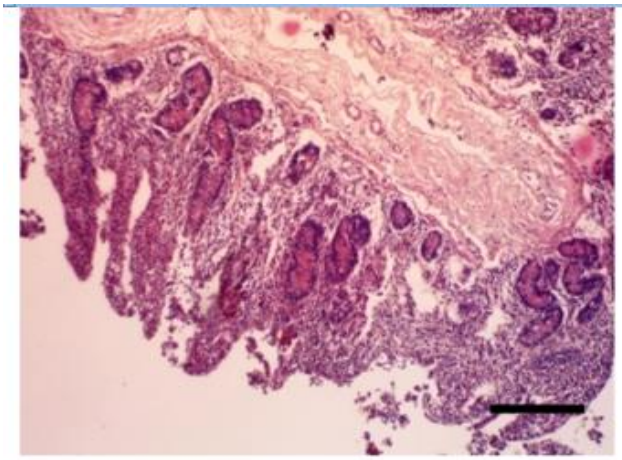

Fig. 9: Photomicrograph of small intestinal section of 0% MOLM showing no visible lesion. H &E Bar=100µm

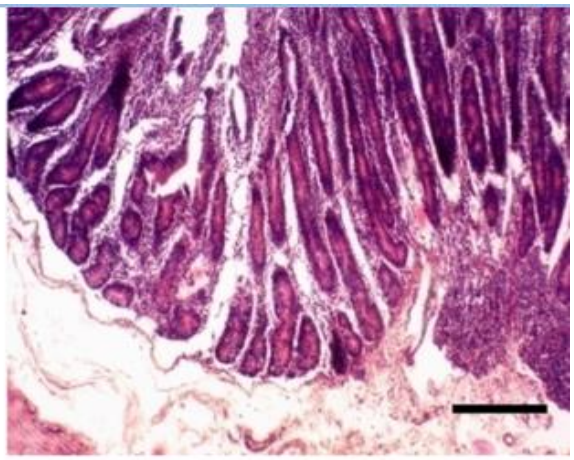

Fig. 10: Photomicrograph of small intestinal section of 5% MOLM showing lymphocytic infiltration of the lamina propia H &E Bar=200µm

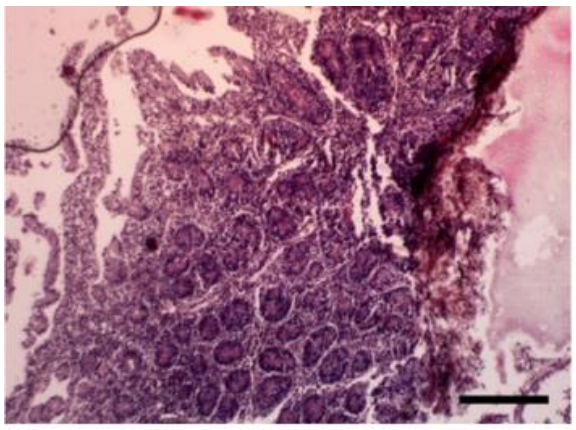

Fig. 11: Photomicrograph of small intestinal section of 10% MOLM showing lymphocytic infiltration of the lamina propia H &E Bar=200µm

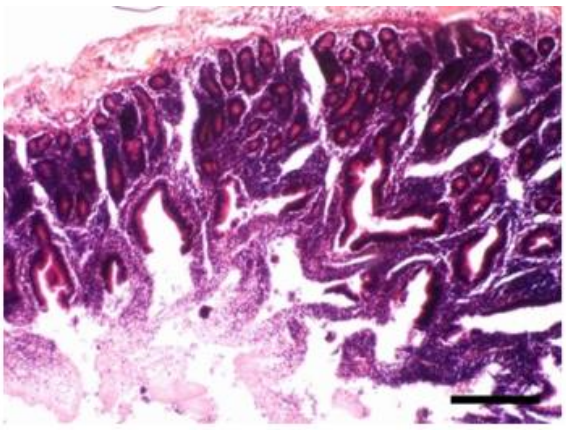

Fig. 12: Photomicrograph of small intestinal section of 15% MOLM showing lymphocytic infiltration of the lamina propia H &E Bar=200µm

Discussion

Inclusion of *Moringa oleifera* leaf meal with other ingredients resulted in a rich blend of good-quality feed. It was reported that the inclusion of M. *stenopetala* leaves with other feedstuffs improved dry matter intake and nitrogen retention capacity (Gebregiorgis *et al.*, 2012). The crude protein of *Moringa oleifera* has been reported by Sanusi (2017) to be of better quality for ruminants because of its high content of by-pass protein (McNeill *et al.*, 1998).

Neutral detergent fibre and acid detergent fibre fractions of the diets were within the same range irrespective of the processing involved. The fat content ranged between 2.51 and 14.2. It has been adduced that fat content adduced to influence the acceptability and palatability of the feed. The dry matter intake as was similar ($p > 0.05$) among treatments with 15%, 5%, 0%, 10% having 391, 382, 382 and 366 respectively. These values compared with the range of 291 – 313 g/animal/day which was reported by Ukanwoko *et al*. (2009) for West African Dwarf goats fed cassava peels-cassava leaf meal based diets. These values fell within dry matter intake recommended levels for small ruminants (NRC, 1985). This superiority stems mainly from the balanced and complete nutrient profile of the ingredients Asaolu *et al*. (2010) reported DMI values ranging from 54.6 to 59.6 g/kg-0.75 for West African Dwarf goats on groundnut hay basal diets, and offered Moringa and bamboo foliages as supplements. The observed high DMI of the experimental diets by the goats indicate the adaptability of the goats to the test ingredient. This trend of feed intake observed by the bucks could be as a result of the palatability of the feed and acceptability, because the higher the palatability of a feed, the more the animal consumes the feed, this was also observed by Asaolu *et al*. (2012). Masafu (2006) also described feed intake as a measure of diet appreciation, selection and consumption by an animal. The mode of feed presentation can stimulate the appetite and feed intake thus the high value of

intake for the diets. This can be deduced that more of a diet with high crude protein would be consumed compared with that of low crude protein content (Taiwo and Anosa, 1995*).*

There was little variability between the experimental diets with regards to their chemical composition. The crude protein (CP) content of the MOLM-based diets (5%, 10% and 15 %) compared favourably with that of the control diet (0% MOLM) which was in a range of 7.25 g/Kg to 8.54 g/Kg. This was within the normal requirement of goats (Sanusi, 2017). The CP of the control diet was slightly higher than those of other diets and tended to decrease with increasing levels of MOLM in the diet and stepped up in 15%. Apori (1988) suggested a range of 7% to 12% CP which he believed to be adequate for WAD goats. Protein forms the structure of most body organs and tissues, if less protein is present in the ration, then less quantity of tissues will be deposited as flesh in the body. A low protein diet was found to reduce the capacity for protein synthesis in tissues such as liver and skeletal muscles (Oduguwa, 2000). Crude protein intake was highest for 5% and lowest for 0% MOLM --between the treatments ($p < 0.05$). Similar observations had been earlier reported by Arigbede (2007). Animals fed at 5% level of inclusion had the highest crude protein intake this could be due to variation in the level of inclusion. Feed intake was described by Masafu (2006) as a measure of diet appreciation, selection and consumption by an animal. (Becker 1995) reported that the crude protein content of *Moringa oleifera* to be of better quality for ruminants because of its high content of by-pass protein (47% versus 30% and 41% for *Gliricidia sepium* and *Leucaena leucocephala* respectively). Higher proportions of by-pass protein have been reported to result in faster weight gains in livestock (McNeill *et al*., 1998).

The control diet showed no visible lesion, damage, degeneration or marked regeneration in all organs except for the kidney which showed mild degeneration this might be as a result of the standard and quality of the feedstuff materials of the feed. The histopathological examination of all the kidneys of the experimental animals fed 5 % *Moringa oleifera* inclusions suffered

from tubular degeneration of the kidney, but the degeneration diminished as *Moringa oleifera* inclusion increased leading to tubular epithelial regeneration. This might be as a result of the cytokinin-type hormone present in the *Moringa oleifera* as reported by Foidl *et al*. (2001) which accelerates cell growth. Necrosis especially at the centrilobular areas accompanied with cellular infiltration by the mononuclear cells of the hepatocytes which were focal at 15% of the *Moringa oleifera* was similar to the ones observed by Odetola *et al*. (2012) when rabbits were fed with graded levels of whole kenaf. The heart is the main organ responsible for pumping of blood and an important index of physiological, pathological and nutritional status in the animal (Ewuola *et al*., 2004) to all parts of the body showed no sign of damage. It was observed that there were cases of lymphocytic infiltration of the lamina propria of the intestine across the treatments. This suggests that *Moringa oleifera* has immunogenic effect which can help to fight pathogens or diseases in the gastro intestinal tract. This will help the animal to have a healthy gastro intestinal tract, aids digestion, nutrient uptake and absorption. The level of mildness of the necrosis in the liver which is not severe could be as a result of the medicinal effect of Moringa and the nutrient density.

Conclusion

Inclusion of *Moringa oleifera* in concentrates enhanced better nutrient utilization of West African dwarf bucks. The results obtained in the present study confirms a positive effect of using *Moringa oleifera* inclusion in feeds for goats. The response in terms of feed intake and growth indices show that the Treatment diets can serve as sustainable feeds that will overcome dry season weight losses and poor performance in terms of health wise of the goats.

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Declaration of interest statement:

The authors here-by declare no conflict of interest

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