Effects of aqueous seed extract of *Moringa oleifera* (L) on haematology, gonadotropins and testosterone profiles of Yankasa rams

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**Introduction**

It is adequately reported that reproductive health and performance of farm animals are largely dependent on their nutritional status. Evidence from literature revealed that nutritional factors are the most imperative, possibly because of their direct effect on enhancement of reproductive performance as well as their potential to moderate the effects of other factors (Rekwot et al., 1987; Alabi, 2005; Kheradmand et al., 2006). Herbal therapy has been reported to alleviate male infertility, irrespective of the aetiology (Anthony et al., 2006). A large number of plants have been tested for their potential fertility regulatory properties (Bhatia et al., 2010). Some medicinal plants are widely used as aphrodisiac to mitigate sexual dysfunction or as fertility enhancing agents (Fatoba et al., 2013). They provide a boost of nutritional value, thereby enhancing sexual performance and libido (Yakubu et al., 2007; Sumalatha et al., 2010).

Many factors such as infectious and non-infectious diseases, drug treatments, chemotherapy, toxins, air pollution, and inadequate vitamin intake may have harmful effects on spermatogonial production (Mosher and Pratt, 1991; Iliyasu et al., 2014). Stress is known to compromise reproductive function particularly, oxidative stress resulting from the production of oxygen free radicals in excess of the antioxidant capacity in stressed tissues. All oxidizing radical are potential agents of oxidative stress (Ayobami et al., 2013). The main antioxidant enzymes in mammals are superoxide dismutase (SOD), catalase and glutathione peroxidase, which are all endogenous. While, substances like vitamins A, C, flavonoids and carotenoids are examples of exogenous antioxidants usually found in food (Asma et al., 2005; Musa-Azara et al., 2014). *Moringa oleifera* (Horseradish tree or Drumstick tree or Magic tree) is a medicinal plant that belongs to the family Moringaceae. The plant is well recognized in India, Pakistan, Bangladesh and Afghanistan (Mughal et al., 1999). *M. oleifera* is a small or medium sized tree grow up to 10 m tall, with thick, soft, corty, deeply fissured bark, growing mainly in semiarid, tropical and subtropical areas around the globe (Asma et al., 2005). The leaves and the pods are known to have a high content of protein, minerals and vitamins (Council of Scientific and Industrial Research, 1962). *Moringa oleifera* leaves (Asma et al., 2005) seeds (Lalas...
and Tsaknis, 2002) and roots (Faizi et al., 1998; Ashok and Pari, 2003) have been reported as excellent sources of antioxidants. The stem bark has been used as abortifacient and antioxidant (Ghasi et al., 2000) in livestock. The roots of *M. oleifera* were shown to possess antilithic, rubefacient, vesicant, carminative, anti-inflammatory, laxative and fertility regulatory activities (Asma et al., 2005). Similarly, the roots of the plant possess stimulatory effect in paralytic affections, cardiac stimulant and also act as circulatory tonic. It is recommended in the management of rheumatism, lower back and kidney pains (Padmarao et al., 1996).

Infertility is one of the foremost reproductive health problems in livestock production industries (Isidori et al., 2006). The management of male infertility in animals usually involves the use of spermatogenic drugs such as Clomiphene (Clomid®). However, prescription drugs like these are very expensive and beyond the reach of poor resource farmer. This led to gradual shift to herbal therapy and its acceptance among the herdsmen, lead to indiscriminate use of the medicinal plants. Herbal practitioners take advantage and claim to have cured of numerous kinds of diseases (Anthony et al., 2006; Varsha et al., 2013).

Testicular tissue is often characterized with high metabolism and cell replication, hence prompt to oxidative stress, which affects the tissue thereby causing damage. There is the need, therefore, to increase antioxidant capacity of the testicular organs to combat oxidative stress (Varsha et al., 2013). Testicular oxidative stress has been reported to be a common attribute that underlying causes of male infertility (Canto et al., 2003), which propose that, there may be benefits to develop a better and cheaper anti-oxidant therapy for small ruminants. Therefore, the present study was designed to investigate the role of aqueous seeds extract of *Moringa oleifera* administered orally, on haematological parameters, and some reproductive hormones of Yankasa rams.

**Materials and Methods**

**Plant collection and identification**

Plant with fruits was collected at the harvesting period (November-March) from Anguwan Yusif, Sabongari Local Government Area of Kaduna state. The plant was authenticated and assigned with Voucher number 0571 by a taxonomist at the Herbarium Unit, Department of Biological Sciences, Faculty of Science, ABU, Zaria, Nigeria.

**Aqueous extraction of Moringa oleifera seed**

The seeds were obtained from the fruits that were dried under shade for 14 days to ease the shedding of the seeds. The dried seeds were made into powdered form (40 g) which was weighed using a weighing balance (MAX-J 200W19 Max Weight® California, USA) and transferred into one litre beaker. Three hundred millilitre (300 ml) of distilled cold water at room temperature was added to the powder and allowed to stand for 48 hours with vigorous shaking at 6 hours interval. Thereafter it was heated on a water bath at 60°C for 3 hours. One hundred millilitres (100 ml) of warm water at 60°C was added continuously to the residue and subsequently filtered. The procedure was repeated three times at 10-15 mins interval and then the filtrate was evaporated to dryness on a water bath at 60°C. The liquid extract was concentrated to dryness in vacuo at 40°C using a rotary evaporator. The dried extract was stored at 4°C until required for use as described by Varsha et al. (2013).

**Acute toxicity study**

The acute toxicity effects of the aqueous extract of the seeds of *M. oleifera* were determined by the method described by Lorke (1983). The experiment was carryout in two phases. In phase I trial, nine rats were randomly divided into three groups of three rats each. Groups 1, 2 and 3 were given *M. oleifera* aqueous seeds extracts at dose rates of 10, 100 and 1000 mg/kg respectively, orally. In the second phase (II) three rats were placed at random into three groups of one rat each. Group 1, 2 and 3 were giving *M. oleifera* aqueous seeds extracts at dose rates of 1600, 2900 and 5000 mg/kg. The median lethal dose (LD₅₀) of *M. oleifera* aqueous seeds extract as an indication of its acute toxic effect was determined by taking the geometric mean of the highest dose that did not produce death and the lowest dose that produced death (Okumu et al., 2016).

**Experimental animals**

Twenty-five, apparently healthy Yankasa rams with an average body weight of 18-20 kg were purchased from Kafur central market, Kafur Local Government Area of Katsina State, Nigeria. The animals were examined to ensure that they were in good physical health. Also, the animals were acclimatized for two weeks during which blood and faecal samples were collected for laboratory investigations. The animals were kept under intensive management at the experimental pen of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria, and the animals were fed on basal diet of *Digiteria smuttsi* groundnut leaves supplemented with beans husks. Salt lick and water were provided ad libitum. All experimental protocols were approved by Ahmadu Bello University Committee on Animal Use and Care with clearance number ABUCAUC/2017/029.

**Experimental design**

The Yankasa rams were divided randomly into five groups (A, B, C, D and E). Group E served as untreated control, while Groups A-D were given different doses 1000, 2000, 3000, 4000 mg/kg of *M. oleifera* aqueous seed extract respectively. The extract was administered orally in the morning between 7:00 and 9:00 AM daily for 5 months (March, April, May, June and July). Body weight was taken once every week using weighing balance throughout the experimental period. Blood samples were collected once every week to evaluate haematological parameters and sera were also harvested to assay for gonadotropins and testosterone concentrations.

**Blood collection for haematological parameters**

For haematological studies 2 ml of blood were collected aseptically via jugular vein using 5 ml syringe (Zarinject® Zaria, Nigeria) of 21 gauge hypodermic needles, once a week and decanted to EDTA bottles.
Determination of packed cell volume (PCV)
The packed cell volume (PCV) was determined as described by Schalm et al. (1975) using the microhaematocrit method. The PCV is the ratio of the volume occupied by the red blood cells to the volume of whole blood. The ratio was measured after appropriate centrifugation and expressed as a decimal fraction as described by Yusuf et al. (2018).

White blood cell count
Total white blood cell (TWBC) and total red blood cell (TRBC) count was done using haemocytometer as described by Schalm et al. (1975) and Dacie and Lewis (1975).

Serum for gonadotropins and testosterone profiles
To obtain sera for gonadotropins and testosterone assay, 3 ml of blood were collected aseptically via jugular vein, three times at 30 minutes interval between 7:00 and 8:00 AM once every week. The blood was decanted into labelled plain test tubes and allowed to stand for 15-30 minutes before centrifuged at 3000 x g for ten minutes. Thereafter, the harvested serum was decanted into a microtubc and stored at -20°C until evaluated.

Testosterone and gonadotropins assay
Sera obtained were analysed for reproductive hormones (FSH, LH and Testosterone) using their respective commercial ELISA kits (Accubind© New Jersey, United State, ELISA kit Mi- crowells product code 425-300; 625-300 and 3725-300 respectively). The assay was done using enzyme-linked immuno sorbent assay (ELISA) technique as described by each manufacturer’s manual. The instructions and recommendations contained in the manual were strictly adhered.

Statistical analysis
All the data were analysed using special package of statistical analysis graph pad version 5, GraphPad. (2000). Data generated were presented as mean ± SEM. Data from individual groups were subjected to one-way ANOVA and Post hoc test Turkeys to determine their differences. Values of p < 0.05 were considered significant.

Results
There was significant (p < 0.05) increase in packed cell volume (PCV) in dose depended manner among groups treated with M. oleifera aqueous seed extract compared to the control group. Similar findings were observed in total red blood cell and white blood cell count as presented in Table 1.

There was episodic fluctuation of testosterone concentration among the experimental animals, with significant (p < 0.05) increase in the testosterone concentration was optimum by 7:00 AM as it progressively decreased by 8:00 AM as presented in Figure 1.

Table 1. Effects of M. oleifera aqueous seed extract on haematological parameters of Yankasa rams post oral administration

<table>
<thead>
<tr>
<th>Group/months</th>
<th>MOASE (mg/kg)</th>
<th>PCV (%)</th>
<th>TRBC (×10^12/L)</th>
<th>TWBC (×10^12/L)</th>
<th>Hb(g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1000</td>
<td>22.8 ± 0.2</td>
<td>4.1 ± 1.0</td>
<td>4.3 ± 1.0</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>21.1 ± 1.0</td>
<td>5.0 ± 0.8</td>
<td>5.1 ± 0.8</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>21.0 ± 0.3</td>
<td>5.0 ± 0.7</td>
<td>5.3 ± 0.5</td>
<td>4.9 ± 0.7</td>
</tr>
<tr>
<td>D</td>
<td>4000</td>
<td>22.1 ± 0.5</td>
<td>4.7 ± 1.2</td>
<td>9.4 ± 0.6</td>
<td>4.5 ± 1.5</td>
</tr>
<tr>
<td>E</td>
<td>10 (H2O)</td>
<td>23.4 ± 0.3</td>
<td>4.8 ± 0.9</td>
<td>7.8 ± 0.3</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>April</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1000</td>
<td>21.7 ± 0.3</td>
<td>5.4 ± 0.1</td>
<td>5.7 ± 0.3</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>20.6 ± 1.3</td>
<td>5.9 ± 0.7</td>
<td>8.0 ± 1.2</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>22.5 ± 0.7</td>
<td>4.2 ± 1.5</td>
<td>8.3 ± 0.5</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>4000</td>
<td>22.6 ± 0.3</td>
<td>5.2 ± 0.4</td>
<td>9.6 ± 0.1</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td>E</td>
<td>10 (H2O)</td>
<td>23.7 ± 0.9</td>
<td>5.2 ± 0.8</td>
<td>9.7 ± 0.9</td>
<td>6.1 ± 0.9</td>
</tr>
<tr>
<td>May</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1000</td>
<td>29.7 ± 0.3</td>
<td>5.6 ± 0.3</td>
<td>11.7 ± 0.9</td>
<td>11.7 ± 0.7</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>30.6 ± 1.3</td>
<td>5.9 ± 0.5</td>
<td>18.0 ± 1.4</td>
<td>11.2 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>37.5 ± 0.7</td>
<td>6.1 ± 0.5</td>
<td>18.3 ± 0.5</td>
<td>17.9 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>4000</td>
<td>43.6 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>10.6 ± 0.7</td>
<td>19.5 ± 0.6</td>
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<tr>
<td>E</td>
<td>10 (H2O)</td>
<td>24.7 ± 0.9</td>
<td>5.2 ± 0.3</td>
<td>9.7 ± 0.3</td>
<td>10.1 ± 0.9</td>
</tr>
<tr>
<td>June</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>1000</td>
<td>30.2 ± 0.3</td>
<td>5.1 ± 0.7</td>
<td>11.3 ± 0.3</td>
<td>11.9 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>31.8 ± 1.0</td>
<td>5.5 ± 0.1</td>
<td>18.2 ± 0.5</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>36.0 ± 0.3</td>
<td>5.5 ± 1.2</td>
<td>18.1 ± 0.6</td>
<td>12.4 ± 0.7</td>
</tr>
<tr>
<td>D</td>
<td>4000</td>
<td>41.4 ± 0.5</td>
<td>5.5 ± 1.2</td>
<td>11.8 ± 0.6</td>
<td>17.9 ± 0.5</td>
</tr>
<tr>
<td>E</td>
<td>10 (H2O)</td>
<td>25.4 ± 0.2</td>
<td>4.9 ± 0.7</td>
<td>9.7 ± 0.1</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>July</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1000</td>
<td>30.4 ± 0.2</td>
<td>5.2 ± 0.6</td>
<td>12.4 ± 0.9</td>
<td>10.3 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>31.3 ± 0.5</td>
<td>5.5 ± 0.5</td>
<td>18.7 ± 0.7</td>
<td>11.2 ± 0.4</td>
</tr>
<tr>
<td>C</td>
<td>3000</td>
<td>36.1 ± 0.3</td>
<td>5.8 ± 0.3</td>
<td>18.9 ± 0.3</td>
<td>11.7 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>4000</td>
<td>41.3 ± 0.7</td>
<td>5.2 ± 0.1</td>
<td>10.1 ± 1.1</td>
<td>18.2 ± 0.7</td>
</tr>
<tr>
<td>E</td>
<td>10 (H2O)</td>
<td>25.3 ± 0.1</td>
<td>5.0 ± 0.4</td>
<td>10.4 ± 0.5</td>
<td>10.9 ± 0.3</td>
</tr>
</tbody>
</table>

Means in the same columns for each parameter with different superscript alphabets between the groups are statistically (p < 0.05) different.

MOASE – Moringa oleifera aqueous seed extract; PCV – packed cell volume; TRBC – total red blood cell; TWBC – total white blood cell; Hb – haemoglobin.
There was sharp increase in follicle stimulating hormone FSH concentration in group A by 7:00AM compared to the other groups treated with Moringa oleifera aqueous seed extract at the same time. However, the FSH concentration continued to fluctuate with a significant (p < 0.05) difference at different time interval compared to the control group as presented in Figure 2. There was variation in the concentration of luteinizing hormone (LH) with high concentration observed in group A by 7:00 AM. At 7:30AM, there was progressive increase in LH concentration among groups A, B and C treated with Moringa oleifera aqueous seed extract with a significant (p < 0.05) difference in group compared to the control group E. Thereafter, there was progressive decrease in LH concentration among the groups at 8:00 AM as presented in Figure 3.

**Figure 1.** Effects of graded dose of Moringa oleifera aqueous seed extract on Testosterone concentration (ng/ml) of Yankasa rams (p < 0.05) MOASE – Moringa oleifera aqueous seed extract; H2O – water.

**Figure 2.** Effects of graded dose of Moringa oleifera aqueous seed extract on Follicle stimulating hormone FSH (ml U/ml) of Yankasa rams (p < 0.05) MOASE – Moringa oleifera aqueous seed extract; FSH – follicle stimulating hormone; H2O – water.
logical parameters of Yankasa rams particularity to our findings were a significant fluctuation of
ary to our findings were a significant fluctuation of

Sudanese goats (Babeker and

It was noteworthy that several scholars have reported that M. oleifera dried leaves and seed supplementation have a high tendency for a return of PCV to normal level following an infection through compensatory accelerated production, with PCV value above 32 % as documented by Ojo et al. (2019) to be normal for circulatory system in sheep.

However, toward the end of the study, a low feed intake was observed among the treated groups, this might have contributed to the decrease in PCV, which may induce oxidative stress and nutritional imbalance that can trigger haemolysis. Konlan et al. (2012) reported that TWBC is one of the indicators that offer explanation for defence mechanism of livestock. Although the TWBC values in the present study are within physiological ranges earlier reported for West African Dwarf WAD goats (Yusuf et al., 2019) and Sudanese goats (Babeker and Elmansoury, 2013). WAD goats and sheep seem to possess protective system when fed with M. oleifera dried leaves and seed, which provide a potent and rapid defence against any infectious agent and this is probably the physiological basis for the adaptation of these plants by many farmers across ecological zone of the globe characterized by high prevalence of diseases and poor nutritional plants as reported by Opara et al. (2010).

Substances from some medicinal plants have been reported (Yusuf et al., 2018) to solve fertility problems in rams. Rams fed with marijuana experience decrease in testosterone levels which might affect semen characteristics and lead to infertility (Naidu et al., 2020). This is contrary to the findings in the present study were testosterone concentrations significantly increased in the early hours of the day and subsequently decreases later in the day (Perumal et al., 2020). It also agrees with the findings reported by (Chinju et al., 2020) who reported increase in testosterone profile in bulls fed high protein diet. Anabolic steroid has been reported by (Lozano et al., 2017) to decrease FSH level in rats this is contrary to our findings were a significant fluctuation of FSH were observed and this might be attributed to the ability of

### Discussion

Nutritional plants are acknowledged to improve haematological indices and hormonal function, which are prerequisite and necessary to support optimum reproductive performance. In this study, *Moringa oleifera* aqueous seed extract have significantly improved the haematological parameters of Yankasa rams particularly the packed cell volume (PCV), total red blood cell (TRBC) and total white blood cell (TWBC). The observation from this study is in agreement with the findings reported by Varsa et al. (2013), who reported polycythaemia in rats fed with *M. oleifera* aqueous seed extract for twenty-one day. This can be attributed to the nutritional quality of the seed extract and its ability to enhance erythropoiesis. The increase in RBC and WBC demonstrate the ability of *M. oleifera* aqueous seed extract to combat anaemia and support immunity for optimum defence of the body system Elzein et al. (2018). It was also reported by several scholars that *M. oleifera* seed has the potential of providing antimicrobial activities and enhancement of blood production (Mayo et al., 2016). Anaemia is a consistent feature of poor nutritional management associated with disease and oxidative stress that causes damage to the membrane of erythrocytes and associated components as reported by Abdullahi et al. (2019) in guinea pigs.

It was noteworthy that several scholars have reported that *M. oleifera* dried leaves and seed supplementation resulted in significant increase in PCV and RBC at the end of their study on Sudan Nubian goat, similar observation was seen in the present study post supplementation with only *M. oleifera* aqueous seed extract which offered Yankasa rams a better plane of nutrition. Such high PCV values have been regarded as signs of good health often associated with high productivity in animals as reported by Addass et al. (2010), Ikhiimiya and Imasuen (2007) reported that goats fed on *Moringa oleifera* dried leaves and

#### Figure 3. Effects of graded dose of *Moringa oleifera* aqueous seed extract on Luteinizing hormone LH (mIU/ml) of Yankasa rams (p < 0.05)

MOASE – *Moringa oleifera* aqueous seed extract; LH – luteinizing hormone; H2O – water.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LH (mIU/ml)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MOASE1000mg/kg)</td>
<td>45 ± 3.2</td>
<td>7:00</td>
</tr>
<tr>
<td>B (MOASE2000mg/kg)</td>
<td>35 ± 2.8</td>
<td>7:30</td>
</tr>
<tr>
<td>C (MOASE3000mg/kg)</td>
<td>25 ± 2.1</td>
<td>8:00</td>
</tr>
<tr>
<td>D (MOASE4000mg/kg)</td>
<td>15 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>E (H20MOASE10mL/kg)</td>
<td>10 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

*p > 0.05
the extract to supplement required nutrient that can boost secretion of reproductive hormone for optimum performance as reported by Varsha et al. (2013). Increase luteinising hormone was reported by (Martin et al., 2017) in rats fed with low dose of Moringa oleifera seed and agree with our finding were high level of LH was observed in group treated with low dose of the extract. This can be attributed to the low dose of saponins present in the seed constituents which was enough to improve LH secretion (Varsha et al., 2013).

Conclusion

Based on the results of this study, it can be concluded that M. oleifera aqueous seed extract is a multi-purpose plant supplement with nutritional and therapeutic uses, which has the potential to reduce the dependence on expensive conventional source of haematopoietic supplements and hormonal therapeutics for small ruminants. M. oleifera aqueous seed extract administered orally to ruminants have been found to improve animal performance by increased PCV, TWBC TRBC, and reproductive hormones of Yankasa rams in Zaria.

Conflict of interest

No conflict of interest was encountered throughout the research period.

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