

EFFECT OF COLD AQUEOUS EXTRACT OF *Moringa oleifera* LEAVES ON REPRODUCTIVE PARAMETERS AND RELATIVE ORGAN WEIGHT IN COCKERELS

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A total of forty eight (48). 16 weeks old (1.825 ± 0.06 kg) cocks were selected to assess the effect of cold aqueous extract of *Moringa oleifera* (MoALE) leaves on relative organ weight, semen quality and reproductive hormone of cocks. The cocks were randomly assigned to four treatments (n=12) at 0.00, 0.25, 0.50 and 0.75gramme of the extract per litter of water in a completely randomized design experiment over an eight week experimental period. The result of water quality analysis of moringa leaf extract showed that its vitamin C percentage was 12.73%. There were no significant differences ($p > 0.05$) in the relative organ weight (heart, kidney, gizzard, liver and testes) and hormonal profiles (Testosterone, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH)). However, there was significant ($p < 0.05$) increase in the sperm concentration ($148.00-162.00 \times 10^6/ml$) with increase in extract administration. This study suggests that MoALE can be alternative source to improve sperm production and sexual functioning in cocks and oral administration of MoALE depict no detrimental effect on vital organs in the body.

Key word: Moringa leaf extract, cock, Reproductive hormone, sperm

Medicinal herbs can alleviate infertility in male animals irrespective of the disease condition surrounding the situation (Anthony *et al.*, 2006) and to a large extent, many plants have been shown to have positive regulatory potentials on male and female fertility (Bhatia *et al.*, 2010).

Several plants are exclusively used in folk medicine as aphrodisiac to relieve sexual dysfunction and to boost fertility by

improving sperm concentration and motility thereby enhancing libido in male animals (Sumalatha *et al.*, 2010).

Moringa oleifera is one of such medicinal plant with such useful potentials whose tree can be used as food for human or livestock (Hsu, 2006). *Moringa* leaves and green fresh pod are rich in Carotene and ascorbic acid. It also has a good Amino acid profile. (Makkar and Becker, 1996). The various parts of the plant have been shown to contain water-soluble vitamins which include; vitamin B1(thiamine), vitamin B2 (riboflavin), vitamin B3 (nicotinic acid), and also vitamin C (ascorbic acid). The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, pro vitamin A as beta-carotene, vitamin K, manganese and protein, among other essential nutrients.(Peter, 2008).

Most of these minerals and vitamins present in *Moringa* are crucial nutrients in the reproductive system for the normal hormone functioning, sperm production and movement (Ogunlesi *et al.*, 2009). Minerals and vitamins in moringa that have been shown to improve sperm counts and motility include zinc, selenium, vitamin B12, antioxidants such as vitamin C and vitamin E (Sinclair 2000). *Moringa* is rich in vitamin C and have been shown to improve sperm quality and prevents sperm agglutination thereby causing progressive motility (Glenville, 2008).

The growing interest for more knowledge concerning reproductive functions and improvement using natural plants to enhance fertility for successful application of artificial insemination in animals necessitate this investigation on the effect of cold

aqueous extract of *Moringa oleifera* leaves on reproductive responses and semen characteristics of cocks.

MATERIALS AND METHODS

The research was carried out at the Poultry section of the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

Preparation of the extract

Moringa leaves were obtained from the Moringa plantation farm, University of Ilorin, Ilorin Nigeria. The leaves with twigs were cured at ambient temperature in the laboratory over a fourteen day period. The dried leaves were later removed over a platform by shacking the twigs. The leaves were later collected and milled using a blending machine. The milled moringa leaves was then weighed using a sensitive weighing scale into 0.25, 0.50 and 0.75 gramme per litre of water respectively. The solution was allowed to stay over one hour period after which it was sieved to remove the residues. The moringa solutions were then analyzed for pH, Electrical Conductivity, Total hardness, Total Dissolved Solid (TDS), Total Suspended Solid (TSS) and vitamin C using the methods of Association of Official Analytical Chemist (AOAC, 1990).

Experimental animals and management

Forty eight (48) sixteen weeks old cocks (weighing 1.82 ± 0.06 kg) were used for this experiment. The birds were weighed using top loading weighing balance and randomly allocated into four treatments of three replicates and four birds per replicate before the commencement of the experiment. The four treatments of 0.00, 0.25, 0.50 and 0.75grammes of the leaves per litre of water was prepared on daily basis. The extract solution was orally administered daily in an eight week experimental trials. The birds were housed in battery cages and were fed diet containing 17% C.P and 2700 kcal/kg M.E (Table 1).

Table 1: Composition of diet

INGREDIENT	% composition
Maize	50.00
Wheat offal	20.00
PKC	13.00
GNC	9.00

Soya bean meal	4.00
Fish meal	1.00
Bone meal	1.00
Oyster shell	1.00
Lysine	0.30
Methionine	0.15
Premix	0.30
Salt	0.25
Total	100.00

Nutrient composition of diet

Crude protein %	17.02
Metabolizable energy(kcal/kg)	2700.52
Calcium%	0.88
Phosphorus%	0.76

Semen Analysis

Semen of the cocks were collected and analyzed for the quality and characteristics. The semen was collected from the cocks by the abdominal massage according to Hafez (1978). The semen was subjected to microscopic examinations and physical evaluations. Sperm motility was determined by placing a drop of the semen on a clean glass slide under the cover slip and viewed on a binocular microscope. Sperm motility was determined by counting both motile and immotile spermatozoa per unit area and Sperm concentration was determined using the haemocytometer.

Hormonal Assay

Blood samples were collected from two birds per replicate for hormonal assay. The blood samples were collected from the wing vein using a sterilized disposable syringe and needle into sterilized plain bottle. Luteinizing hormone, follicle stimulating hormone and testosterone in the blood were analyzed using immunoenzymometric assay IEMA/ELISA with microplate reader(Rayto RT-2100C)

Organ Analysis

Two birds per replicate were sacrificed by cervical dislocation and dissected immediately. The heart, liver, gizzard kidney and testes were removed and weighed on a microbalance .

Statistical analysis

Data obtained from this investigation were subjected to analysis of variance ANOVA in a completely randomized design and

significant means were separated using Duncan multiple range test (Steel and Torres 1980; Duncan, 1955).

RESULTS AND DISCUSSION

The result of water quality analysis (Table 2) shows that the cold aqueous *Moringa oleifera* extract (MoALE) significantly ($p < 0.05$) increased the values of the pH, electrical conductivity, total hardness, total solids and vitamin c. This suggests that vitamin C in moringa is soluble and bioavailable which may explain the increase in sperm concentration of cocks since

vitamin C has been reported to improve semen quality (Glenville, 2008).

Table 3 shows the effect of MoALE on the relative organs weight of the cocks. The result reflected that the organs (heart, kidney, gizzard, liver and testes) were not significantly ($p > 0.05$) affected by the extract administration. This implies that the MoALE does not have detrimental effect on both vital and reproductive organs of the body. This report agrees with Ayotunde *et al.*, (2011) and ,Zanu *et al.*, (2012) that inclusion of moringa leaves meal in the diets of animals had no adverse effect on the

Table 2: Comparative study of water quality analysis of Moringa leaves extract. Treatments (g/l)

PARAMETERS	0g/l	0.25g/l	0.50g/l	0.75g/l	SEM
pH value	6.63 ^a	6.65 ^{ab}	6.90 ^b	6.95 ^b	0.06
Electrical conductivity(μ s)	0.02 ^a	0.04 ^{ab}	0.06 ^{bc}	0.08 ^c	0.01
Total Dissolved Solids(mg/l)	0.40	0.62	0.81	1.02	0.15
Total Suspended Solids(mg/l)	0.80	0.90	1.00	1.30	0.16
Total hardness(mg/l)	0.35 ^a	1.00 ^b	1.30 ^{bc}	1.60 ^c	0.16
Total solids(mg/l)	1.20 ^a	1.55 ^b	2.10 ^c	2.29 ^c	0.08
Vitamin C (%)	0.03 ^a	5.03 ^b	8.95 ^c	12.48 ^d	0.98

a,b,c,d: means values along the same row with different superscripts are significantly different ($P < 0.05$).

Table 3: The Effect of aqueous Moringa leaves extract on the Relative Organs Weights of cocks

PARAMETERS(g/100g body weight)	TREATMENT (g/l)				SEM
	0g/l	0.25g/l	0.50g/l	0.75g/l	
HEART	0.577	0.613	0.513	0.540	0.048
KIDNEY	0.330	0.213	0.177	0.163	0.084
GIZZARD	3.100	2.847	2.517	2.500	0.207
LIVER	1.313	1.377	1.180	1.207	0.095
TESTES	1.025	1.113	1.153	1.160	0.207

Table 4: The Effect of aqueous Moringa leaves extract on the hormonal profile of cocks

PARAMETERS	TREATMENT				SEM
	0g/l	0.25g/l	0.50g/l	0.75g/l	
TESTOSTERONE(ng/ml)	7.77	10.30	10.42	10.49	0.48
LUITENIZING HORMONE(ng/ml)	7.49	8.85	10.14	11.77	0.44
FOLLICLESTIMULATING HORMONE(ng/ml)	8.11	8.52	8.73	9.01	0.60

Table 5: The Effect of aqueous Moringa leaves extract on semen quality

PARAMETERS	TREATMENT				
	0g/l	0.25g/l	0.50g/l	0.75g/l	SEM
Volume(ml)	0.41	0.45	0.36	0.39	0.05
Sperm Concentration(x10 ⁶ /ml)	148.00 ^a	152.50 ^b	158.75 ^c	162.00 ^c	11.06
Motile sperm cells (%)	72.25	74.75	73.75	71.25	23.54
Immotile sperm cells (%)	27.75	25.25	26.25	28.75	0.38
Normal morphology (%)	86.50	85.50	87.25	87.75	2.09
Abnormal morphology (%)	13.50	14.50	12.75	12.25	0.69

a,b,c: means values along the same row with different superscripts are significantly different (P<0.05).

relative organ weight of birds in the treatment groups compared to the control. Also, the weights of kidney and liver recorded in this study were in agreement with the report of Nuhu,(2010) that inclusion of moringa leaves in the diets of rabbit was not found to increase or reduce the organ weights of the animals. This is an indication that the administration of MoALE was not toxic to the cocks. According to Ahamefule et al, (2008), liver and kidney are used as evidence of toxicity in animal.

Table 4 shows the hormonal profile of cocks administered MoALE. There was no significant (p>0.05) change in the hormonal profile of cocks across the treatments.. However, the observed values of testosterone and FSH is suggestive of MoALE's ability to maintain sexual aggressiveness and sperm production in cocks. Testosterone is a male hormone that is responsible for agility and aggressiveness in male,

Semen characteristics and assessment are important indications of reproductive potentials of breeding cocks.

Table 5 shows the effect of MoALE on semen characteristics. There was a significant increase (p<0.05) in sperm concentration of the cocks with increase in extract concentration. This could have resulted from the inhibition of 6-beta-hydroxylation of testosterone by vitamin C present in moringa aqueous extracts which produces androgenic effect by enhancing sexual drive through increased serum and testicular testosterone levels (Cajuday and Pocsidio, 2010). It is believed that increase

in sperm concentration might lead to higher fertility (Oyeyemi *et al.*, 2008). There was no significant change(p>0.05) in sperm morphology observed in this experiment. This is an indication that MoALE did not affect sperm morphology negatively and it corroborates the reports of normal sperm morphology in rabbits (Abu *et al.*, 2013) and Albino rat (Fatoba *et al.*, 2013) given *Moringa oleifera* leaves.

In conclusion, the oral administration of MoALE up to 0.75g/l can be said to support sexual aggressiveness and libido in cocks and improvement in cock fertility and it is therefore recommended.

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