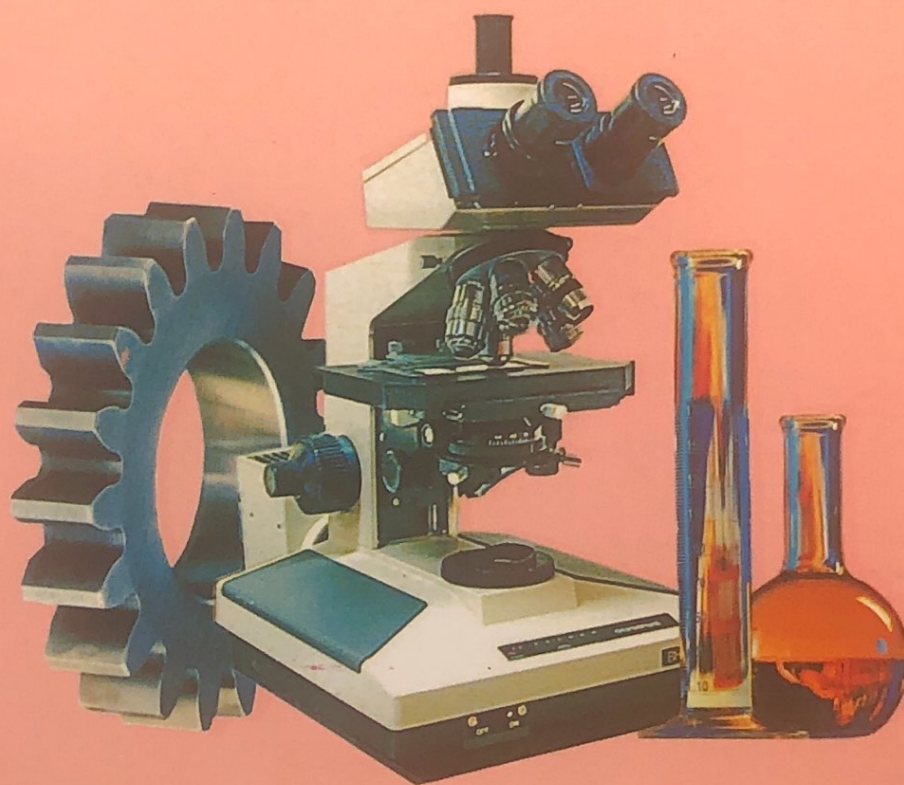


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Effects of Varying Levels of *Mucuna Pruriens* Extract on Reproductive Performance of West African Dwarf Bucks

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Abstract

Infertility in farm animals is a major public health concern that manifests as either one or more pathological conditions of either male or female origin. This is often addressed with the use of synthetic hormonal substances which rather than solving the problem also has a negative effect on man due to the consumption of residual hormones. This led to the search for plant products that could replace the synthetic hormones at reduced cost. Ethanolic extracts of *Mucuna pruriens* were randomly administered subcutaneously to thirty West African Dwarf bucks between 1 to 2 years old in a five treatment trial. The animals in treatment A and B received 0.01ml/kg body weight normal saline and sildenafil-citrate respectively while animals in treatments C, D and E were given 0.01, 0.02 and 0.03ml/kg BW of *Mucuna pruriens* extract respectively for seven days. The result showed that ethanolic extract of *Mucuna pruriens* helps to improve libido, reduce blood pressure and increase serum testosterone with optimum performance exhibited at 0.02ml/kg BW of extract administration.

Keywords: *Mucuna pruriens*, Semen quality, West-African Dwarf goats, Libido, Subcutaneous.

Introduction

Plants are sources of feed to ruminants to supply both nutrients and non-nutritional substances to grazing animals. Grazing animals are known to select the right type of forage depending on their physiological needs (Hutchings *et al.*, 2003). Plants

contain primary metabolites (sugar, fats and amino-acids) which supply needed nutrients and secondary metabolites (glycosides, steroids, alkaloids, terpenes, peptides, phenols, tannins, lignins, etc) which exert physiological effects on the body (Mendonca- Filho, 2006). The maintenance of health and reproductive performance of man and animal depend on many of these plant metabolites.

The reproductive performance of male animals depends on the sperm output which is usually affected by various factors such as species, season, age, scrotal size, frequency of ejaculation as well as the degree of sexual preparation. The degree of sexual preparation on the other hand is influenced by the production of testosterone (male sex hormone) by the interstitial cell (Leydig cells) of the testes. Various plants have been said to improve or increase testosterone production in man or rats by various researchers among which is *Mucuna pruriens* (Kumar *et al.*, 1994; Misra and Wagner, 2007).

Mucuna pruriens contains L-3,4-dihydroxyphenyl alanine (L-Dopa) a precursor to the neurotransmitter dopamine, an important brain chemical involved in mood, sexuality and movement (Molloy *et al.*, 2006). Its different preparations (from the seeds) are used in the management of several free radical mediated diseases such as rheumatoid arthritis, diabetes, atherosclerosis, Parkinsonism, nervous disorder, male infertility and ovulation in women (Kumar *et al.*, 1994; Rajeshwar *et al.*, 2005; Molloy *et al.*, 2006). The effect of dopamine on sexual functions has been reported with a typical dose for a man being 15g of ground seeds mixed with cow's milk (Giuliano *et al.*, 2001). It also has antidepressant properties when consumed. The seeds contain a high concentration of levodopa, a direct precursor of the neurotransmitter dopamine which has been shown in large amounts (30g dose) to be as effective as pure levodopa /carbidopa in the treatment of Parkinson's disease (Manyam *et al.*, 2004). The hair lining the seed pods contains 5-hydroxytryptamine (serotonin) which causes severe itching (erythema), so the hairs are common ingredient in itching powder. It has been used in Siddha medicines for quite a long time for improving sexual function, but not much work is available on its use in ruminant animals as a result of the peculiar nature of microbial degradation of substances in the rumen.

This present work is aimed at determining the reproductive function of *Mucuna pruriens* extracts in West African Dwarf bucks.

Materials and Method

Preparation of extract

Mucuna pruriens seeds were bought in the market in Ilorin, Kwara State, Nigeria. The seeds were air-dried, picked to remove dirt, ground to powder and packaged in cellophane bag for extraction. The extraction of the active substance of the *Mucuna pruriens* seed was carried out with a modified method of Swain (Swain, 1999) with 96% ethanol at 75- 80°C in a soxhlet apparatus for four hours. At the end of the extraction, the liquid extract was filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in a vacuum dessicator at 30°C for 25minutes using a rotary evaporator (Gallenkamp, UK) to obtain a dark brown mass. The

resulting residue (extract) was transferred to a hot air oven where it was dried to a constant weight at 45°C. Subsequently, 5g of the extract was dissolved in 250ml of saline water to obtain appropriate concentrations suitable to achieve a 0.5ml dose by volume of extract which was later administered subcutaneously to the bucks for a period of seven days.

Management of animals

Thirty West African Dwarf bucks of roughly the same age of between 1 to 2 years were purchased within Ilorin metropolis. These bucks were quarantined for 14 days. During this period, the bucks were given oxytetic^R, vitamin and ivermectin^R injection to relieve them from stress and external and internal parasites. The bucks were vaccinated against *peste des petite ruminantium* (PPR) infection before the commencement of the experiment. The bucks were weighed, their heart beats and pulse rates as well as rectal temperatures were taken and recorded for the period of the experiment. Blood samples were collected before, during and after the experiment. Semen samples were also collected with the use of electroejaculator on the third and sixth days of experiment. Animals were housed in individual pens with slatted floor. Wooden troughs and plastic bowls for feeding and watering respectively were provided.

Feed was formulated to meet the basic nutrient requirement of the bucks and they were fed 500g per day throughout the experimental period. *Panicum maximum*, *Gliricidia sepium* and water were provided *ad-libitum*.

Experimental procedure

The experiment follows a completely randomized design. Animals were randomly assigned into five treatments of six animals per treatment. Animals in treatments A and B received 0.01ml/kg bodyweight normal saline and sildenafil citrate respectively while animals in treatment C, D and E were given 0.01, 0.02, and 0.03ml/kg body weight extract respectively for seven days. The injection was given in the morning subcutaneously.

Statistical analysis

Data on performance indices (Red Blood Cell, White Blood Cell, Packed Cell Volume and Haemoglobin), biochemical parameters (Total protein, Albumin and Serum testosterone), sperm-count and sperm morphology were analyzed using the analysis of variance (ANOVA) procedure following a completely randomized model with the COSTAT program. The levels of significance were determined using the Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Table 1 shows the haematological, biochemical and physiological parameters of the experimental animals. Groups B, C, D and E treated with 0.01ml Sildenafil-citrate, 0.01, 0.02 and 0.03ml/kg BW of *Mucuna pruriens* extracts respectively, showed regular display of sexual activity (aggressiveness), mounting and intromission from

the second day of administration. The blood pressure was significantly different ($p < 0.05$) in all the groups; pulse rates and rectal temperatures were slightly different but not significantly. Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Erythrocyte counts (RBC), white blood cell count (WBC), total protein and albumin, though significantly different, seem to follow a definite trend. They increase as the level of extract was increased, to reach the maximum at 0.02ml/kg BW extract after which they reduced.

The effect of *Mucuna pruriens* extract on reproductive performance of West African Dwarf bucks (Tables 2) showed that Spermatozoa concentration was significantly different ($p < 0.05$) with increase in the extract dose. Sperm motility, though significantly different ($p < 0.05$) reduced with increasing extract doses; however, it reduced as the dosage of extract was increased above 0.02ml/kg BW. Percent live-dead ratio of spermatozoa was also significantly different ($p < 0.05$) in all the treatments. It was significantly reduced as doses were increased beyond 0.02ml/kg BW. Semen pH was not significantly affected by the extract doses used, though it slightly increased with increasing extract doses. Percent live-spermatozoa on the other hand was significantly different ($p < 0.05$) in all the treatments, it reduced when doses were above 0.02ml/kg BW. Percent sperm-abnormality was also significantly different ($p < 0.05$), it increased with increasing extract dose. Serum testosterone was significantly different ($p < 0.05$), increasing with increased extract dose.

The blood pressure though significantly different ($p > 0.05$) was observed to reduce with increase in *Mucuna pruriens* extract. The systolic and diastolic blood pressure of groups C and D were close to the control, A. Sexual excitement in animals may likely increase the pumping of blood by the heart, with a resultant increase in pulse-rate and rectal temperature (Duncan *et al.*, 1994), as this was not the case with the test substance, it seems it may likely help to reduce pressure on the heart (Criddle *et al.*, 2003; Damiani and Rossoni, 2003). This supports its use in the treatment of Parkinson's disease (Kumar *et al.*, 1994; Rajeshwar *et al.*, 2005) and corroborated by the report of Molloy *et al.* (2006) that *Mucuna pruriens* contains L- dopa which is a precursor to the neurotransmitter dopamine (an important brain chemical involved in mood, sexuality and movement) Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Erythrocyte counts (RBC), white blood cell count (WBC), total protein and albumin seem to follow a definite trend and were significantly different. They all increased as the level of extract was increased, to reach the maximum at 0.02ml/kg extract after which they reduced. It could be observed that increase in the extract administration increase RBC and Hb, while PCV, TP and albumin were slightly increased, though not significantly, supporting previous work that they heighten arousal and increase sexual activity to a moderate level and sustain it for a longer time (Giuliano *et al.*, 2001; Molloy *et al.*, 2006). The reduced WBC shows that it does not have a negative effect on the body in the ranges at which it was used. This supports previous work that it inhibits the specific chemical reactions induced by superoxides and hydroxyl radicals and that it contains hypoglycaemic principles which may be both organic and mineral, which seems to act indirectly by the release

of insulin or by a direct insulin-like action (Manyam et al., 2004; Schalm et al., 2010).

The Sperm count of all the animals was significantly different ($p < 0.05$). Sperm count increased with increasing doses of extract administered. That the extract increase sperm count lend support to its use as sexual stimulant or aphrodisiac, either wholly or in combination with other herbal plants in humans (Evan, 2009). *Mucuna pruriens* (Mp), has been used in improving male sexual performance and infertility due to the presence of L-3, 4 dihydroxyphenyl alanine (L-DOPA) (Molloy et al., 2006). Sperm motility was significantly different ($p < 0.05$), though it significantly reduced with increasing extract doses. This could be due to the enhanced activity of the sperm cells that led to their burning-out quickly. This could be attested to by the live-to-dead ratio. The extract doses affected the live-to-dead ratio as the number of living cells was significantly reduced with increasing dosage. This could be attributed to the effects of the extract in improving cell motility that led to cells burning out readily and leading finally to the death of cells. Though, this could improve the progressive movement of the sperm cells to the oviduct in natural mating, the best dose of the extract will be between 0.01 to 0.02ml/kg BW. The extract doses did not have significant effect on the pH of semen. Substances that do not have detrimental effect on an animal or change intracellular or extracellular fluid may not likely have significant effect on semen pH as semen pH is determined by the combined effect of prostate secretions (acidic) and seminal vesicles (alkaline). The acceptable semen pH ranges from 7.2 to 7.8. A higher pH may indicate infection of the prostate while a lower pH signifies abnormalities of seminal vesicles, vas deferens or epididymis (Ilesanmi, 2009). The percentage of live spermatozoa was significantly different; it increased with increase in extract doses from 0.01 to 0.02ml/kg BW after which it significantly reduced. This shows that the extract could improve sexual performance as well as reduce male infertility as reported by previous researchers (Watson, 1993; Kumaravelu et al., 1996; Yang et al., 2004 and Kaphle et al., 2006).

Percent Sperm abnormality was also significantly different ($p < 0.05$) in this study. The highest was in treatment E. This shows that sperm abnormality increases with increasing extract dose. The quality of spermatozoa as indicated by sperm abnormality is known to be the best indicator of fertility in males (Ilesanmi, 2009 and Okukpe et al., 2012). Testicular sperm is infertile and must undergo maturation changes in the epididymis to acquire fertilizing capacity. The capacity for fertility and motility are acquired in the corpus epididymidis. The most obvious morphological change as spermatozoa move from the caput to the cauda epididymis is the movement of the cytoplasmic droplet from the neck of the sperm down the tail and its eventual loss. As sperm output depends on age, species, breed, season, scrotal size, frequency of ejaculation and degree of sexual preparation (Osinowo, 2006), the frequency of ejaculation and degree of sexual preparation may likely have negative impact on the sperm morphology as more low quality sperm cells are likely to have been voided from the caput epididymis – low quality as a result of the short time for capacitation before it was ejaculated.

Serum testosterone was significantly different ($p < 0.05$) in animals administered Mp extracts with those given 0.2ml/kg BW having the highest testosterone. This supports the work of previous authors that *Mucuna pruriens* increases serum testosterone, hence its use in improving male sexual disorders such as psychogenic impotence and unexplained infertility (Yang *et al.*, 2004 and Kaphle *et al.*, 2006). The presence of L-3, 4-dihydroxyphenyl alanine (L-DOPA) helps to improve mood, sexuality and movement (Molloy *et al.*, 2006). This also corroborates the report of Kumar *et al.* (1994) that seed powder of *Mucuna pruriens* helps against stress, increases semen secretion and acts as a restorative and invigorating tonic (aphrodisiac) in diseases characterized by weakness or loss of sexual power.

Conclusion

In conclusion it was observed that ethanolic extract of *Mucuna pruriens* seeds administered subcutaneously at 0.02ml/kg body weight could be used to enhance reproductive performance of West African Dwarf bucks in terms of increased libido and semen output as well as sperm concentration. The mechanism by which the extracts influence such reaction could be researched.

TABLE 1. EFFECT OF *Mucuna pruriens* (Mp) ON HAEMATOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF WEST AFRICAN DWARF BUCKS.

Parameters	A 0.1ml of saline	B 0.1ml of Sc	C 0.1ml of Mp extract	D 0.2ml of Mp extract	E 0.3ml of Mp extract	± SEM
Hb , g/dl	6.10 ^c	8.10 ^c	11.30 ^b	11.90 ^b	13.31 ^a	0.01
PCV, %	18.00 ^c	24.00 ^b	31.50 ^a	32.00 ^a	32.50 ^a	0.02
RBC, ×10 ⁶ mm ³	2.10 ^c	3.10 ^{ab}	3.00 ^b	3.50 ^{ab}	3.80 ^a	0.06
WBC, ×10 ³ mm ³	6.80 ^c	8.20 ^a	8.20 ^a	8.45 ^a	7.70 ^b	0.20
Total protein, g/dl	53.00 ^c	62.00 ^b	76.50 ^a	78.30 ^a	81.00 ^a	0.08
Albumin, g/dl	36.00 ^d	35.00 ^d	42.84 ^c	51.32 ^b	57.40 ^a	0.05
Blood pressure, mmHg	118 ^b /6 ^b	134 ^a /85 ^a	104 ^c /64 ^b	101 ^c /65 ^b	96 ^d /60 ^b	6.38/4.73
Pulse-rate, beat/min.	68.30	69.30	71.30	69.00	71.30	69.67
Rectal temp., °C	38.20 ^b	38.50 ^{ab}	39.10 ^a	38.70 ^{ab}	38.50 ^{ab}	0.19

Means with different superscript on the same row are significantly different ($P < 0.05$). SEM- Standard Error of Means, Mp- *Mucuna pruriens*, Sc- Sildenafil- citrate, PCV- Packed cell volume, RBC- Red blood cell, Hb- Haemoglobin, WBC- White blood cell.

TABLE 2: EFFECT OF *Mucuna pruriens* (Mp) ON REPRODUCTIVE FUNCTION OF WEST AFRICAN DWARF BUCKS.

Parameters	A 0.1ml of saline	B 0.1ml of Sc	C 0.1ml of Mp extract	D 0.2ml of Mp extract	E 0.3ml of Mp extract	± SEM
Sperm count, 10 ⁹ /ml	1.38 ^b	1.65 ^{bc}	1.80 ^{bc}	2.30 ^{ac}	2.65 ^a	0.19
Motility, %	65.00 ^a	40.50 ^{bcd}	53.40 ^{ad}	56.10 ^{ac}	35.30 ^{bcd}	6.98
Live-dead ratio	4.70 ^a	3.16 ^{ab}	4.20 ^{ab}	5.41 ^a	2.20 ^b	0.68
Semen pH	6.90	7.10	7.10	6.90	7.00	0.09
Live spermatozoa, %	81.50 ^a	75.30 ^{ac}	80.40 ^a	83.50 ^a	68.50 ^{bc}	2.58
Sperm abnormality, %	7.33 ^b	12.33 ^a	11.63 ^a	8.46 ^b	14.36 ^a	1.13
Serumtestosterone, g/dl	1.20 ^{bc}	1.40 ^{bc}	1.50 ^{bd}	2.50 ^a	1.80 ^{acd}	0.22

a, b, c, d - Means with different superscript on the same row are significantly different ($P < 0.05$). SEM- Standard Error of Means, Mp- *Mucuna pruriens*, Sc- Sildenafil- citrate.

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