##### SEMEN CHARACTERISTICS OF RABBIT BUCKS FED GRADED

**LEVELS OF *Newbouldia laevis* LEAF MEAL**

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Abstract

*This study evaluated the effect of Newbouldia laevis leaf meal on the semen characteristics of Rabbit bucks. Twenty four rabbit bucks of 5-6 months old weighing 1.44 kg – 1.96 kg were acclimatized and allotted randomly into four treatments: T1 (control), T2, T3 and T4. The crude protein was set at 16% with inclusion levels of 0 %, 2.5 %, 5 % and 7.5 % Newbouldia laevis leaf meal respectively for12 weeks. Semen samples were collected and evaluated fortnightly for semen volume, semen motility and semen concentration. The mean values of semen volume and semen motility of rabbit bucks was not significantly (p>0.05) different across all the groups. The mean values for semen concentration of T4 was significantly (p<0.05) different from the controls. It was concluded that Newbouldia laevis leaf meal improved the testicular functions of rabbit bucks.*

Key words: Semen characteristics, Rabbit bucks, *Newbouldia laevis* leaf meal*.*

INTRODUCTION

The utilization of herbal medicine has gradually acquired essential therapeutic role in replacing the artificial drugs for animals and humans owing to increased occurrence of their resistance to synthetic drugs (Olowosulu and Ibrahim, 2006). In Africa, especially Nigeria, numerous plants had been acknowledged to possess therapeutic and dietary importance (Egba *et al.,* 2014). In folk and conventional medicines, some herbal plants along with their extracts had been used to cure infertility in animals in their unaltered form (Vasudera and Sharma, 2007; Singh and Makkar, 2009). Many of these plants and their extracts had been documented to enhance libido, sexual behavior, mating and sperm production (Tomova *et al.,* 1981; Chauhan *et al.,* 2007), at the same time other plants balanced the level of hormone in hypothalamic-pituitary gonadal axis (Gamache and Acworth, 1998; Asuquo *et al.,* 2013) like testosterone hormone in male and follicle stimulating hormone in both male and female (Koumanov *et al.,* 1982). *Newbouldia laevis* is one of the plant species widely used in folk medicine which its therapeutics standards stood the trial of the period. Sustaining the contribution of rabbit to the already deficient per capita animal protein intake also requires ensuring increase productivity, which can be guaranteed by the use of breeding bucks

with proven fertility status. Globally, drugs and synthetic hormones worldwide have been the main stay for boosting animal’s fertility and controlling reproductive related diseases in livestock with attendant side effects both on the animals and consumers as well. However, with little or less information on the reproductive potential of *Newbouldia laevis* in animals particularly rabbits. This study therefore, was conducted with improved semen characteristics of rabbit bucks fed graded levels of *Newbouldia laevis* leaf meal*.*

MATERIALS AND METHODS

Location of the experiment

The study was carried out at the Teaching and Research Farm, of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology Minna, Gidan Kwano campus, Niger State. The location lies geographically in the North central of Nigeria within the latitude of 9˚31’18.2’’ North and longitude 9˚27’40’’ East with elevation ranging from 230-250m. It has an average annual rainfall between 1338 mm and 300c mean ambient Temperature (FUTMIN, 2012).

Experimental Animals and Plant samples

Twenty-four (24) apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) of 5.0 ± 6.0 months old of mean body weight of 1.44 – 1.96 kg used for the study were obtained from Zaria, Kaduna State, Nigeria. The rabbit bucks were screened and treated with broad spectrum medication (ivomectin, sulphadimidine and penicillin-streptomycin) against endoparasites, helminthes and other micro-organism prior to the inception of the experiment. The bucks were housed in rabbit cages, one buck per cage. Feed and water were given *ad libitum*. *Newbouldia laevis* leave were obtained from Alapa, Kwara state and Ukpo, Anambra State, Nigeria. The fresh leaves were air dried under shade, grinded, weighed and added to the feed raw materials, ground together to form the experimental diets.

Experimental Design and Diets.

The rabbit bucks were complete randomly designed and selected into four treatments of six rabbit each, designated as group T1, T2, T3 and T4. All bucks were fed diets of isonitrogenous and

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isocaloric values, consisting of maize, groundnut cake, rice offal’s, *Newbouldia laevis*, vitamin premix, palm oil, bone meal, methionine and salt, as indicated in Table 1. The diets consisted of 0, 2.5 %, 5.0 % and 7.5 % of *Newbouldia laevis* leaf meal, respectively fed over a period of 12 weeks. In the course of feeding, semen samples were collected fortnightly for evaluation.

Semen collection

Semen samples were collected using a specially designed artificial vagina (AV) for rabbits consisting of a short plastic cylinder, latex condom used as a liner, whose end was cut off to allow both ends opened. A rubber band was used to fix the line on the cylinder at one end, then glycerol was administered into the space between the cylinder and the rubber liner and the other end of the cylinder was fixed with another rubber band to assemble the AV. The assembled AV was placed in a beaker of warm water at 40˚C, the warm water caused expansion of the glycerol within the liner and also provided the necessary pressure and temperature. Trace of water was cleaned from the AV, a short test tube was attached at one end of the AV and the other end lubricated with non- perfumed petroleum jelly for easy penetration. To collect the semen from the bucks, it was ensured that the collector was properly gloved and a rabbit doe was introduced to the buck’s cage to serve as a teaser. The buck was watched closely and as it mounted the doe, the AV was placed gently at the vulva of the doe, so as to direct the penis into the AV for penetration and eventual ejaculation.

Table1. Gross composition of experimental diet

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Ingredients | T1 | T2 | T3 | T4 |
| Maize | 36.25 | 34.89 | 34.13 | 33.36 |
| GNC | 22.00 | 22.50 | 22.93 | 23.37 |
| Maize offal | 15.00 | 15.00 | 15.00 | 15.00 |
| Rice bran | 20.50 | 18.86 | 16.69 | 14.52 |
| Palm oil | 1.00 | 1.00 | 1.00 | 1.00 |
| NBLM | 0.00 | 2.50 | 5.00 | 5.00 |
| Bone meal | 4.00 | 4.00 | 4.00 | 4.00 |
| Lysine | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Vit. Premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100 | 100 | 100 | 100 |
| Calculated values |  |  |  |  |
| Crude protein | 16.00 | 16.00 | 15.99 | 16.00 |
| Energy | 2544.23 | 2489.40 | 2437.27 | 2403.41 |
| (Kcal/MEKg) |  |  |  |  |
| Fibre | 11.59 | 11.39 | 11.00 | 11.01 |

KEY:-T1: (control diet), T2: (2.5 % Inclusion of *Newbouldia laevis* leaf meal), T3: (5 % Inclusion of *Newbouldia laevis* leaf meal), T4: (7.5 % Inclusion of *Newbouldia laevis* leaf meal), GNC (Groundnut Cake), NBLM (*Newbouldia laevis* leaf meal).

Data Collection

Data were collected from the ejaculate for semen evaluation according to the method described by Zemjanis, (1970). This involved the visual or gross evaluation of the semen immediately after semen collection. Volume of semen was measured directly from the calibrated tube used for the collection. The three colour categories of milky, creamy and watery designated 1, 2 and 3 were used for scoring colour as described by Zemjanis, (1970). Semen pH was determined by dipping a litmus paper into the ejaculate and corresponding colour changes were observed and recorded.

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Gross motility was examined as quickly as possible after collection, by putting a droplet of the semen sample on a pre-warmed glass slide, coverslip and examined at ×10 magnification while spermatozoa concentration was determined using haemocytometer. Micropipette was used to aspirate 0.1ml of semen and diluted with 1ml of water. The exterior of the pipette was wiped to remove any adhering semen. Two drops of diluted semen were allowed and the third one was drop on the haemocytometer then a slip is used to cover it. The haemocytometer was then examined using a light microscope at ×40 magnification and the sperm cells were counted in five Thomas squares of the chamber (i.e. four corner and the centre squares).

RESULTS

Proximate composition of *Newbouldia laevis* leaf meal.

The proximate composition of the *Newbouldia laevis* leaf meal is hereby presented in Table 2. The proximate composition shows dry matter of 89.40 %, crude protein of 29.75 %, crude fibre of 8.50

%, ash of 11.50 %, ether extract of 9.00 % and NFE of 41.25 %. Table 2: Proximate composition of *Newbouldia laevis* leaf meal.

|  |  |
| --- | --- |
| Parameter | Proximate composition (%) |
| Dry matter | 89.40 |
| Crude protein | 29.75 |
| Crude fibre | 8.50 |
| Ash | 11.50 |
| Ether extract | 9.00 |
| Nitrogen free extract (NFE) | 41.25 |

Proximate composition of the experimental diets.

The proximate analysis of the experimental diets is hereby presented in Table 3. The crude protein of the diet ranges between 15.48 - 15.60 in T1 and (T2 and T3) respectively. The crude fibre of the diets was between 15.00 - 12.00 from T3 and T1 respectively.

Table 3: Proximate composition of the experimental diets

Parameter (%) Treatments

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | T1 | T2 | T3 | T4 |
| Dry matter | 91.40 | 93.80 | 94.20 | 92.20 |
| Crude protein | 15.48 | 15.60 | 15.60 | 15.00 |
| Crude fiber | 12.00 | 14.00 | 15.00 | 14.50 |
| Ether extract | 4.00 | 4.50 | 5.50 | 3.50 |
| Ash | 5.00 | 6.50 | 5.00 | 6.50 |
| Nitrogen free | 54.92 | 53.20 | 53.30 | 53.30 |
| extract |  |  |  |  |

KEY:-T1: (control diet), T2: (2.5 % Inclusion of *Newbouldia laevis* leaf meal), T3: (5 % Inclusion

of *Newbouldia laevis* leaf meal), T4: (7.5 % Inclusion of *Newbouldia laevis* leaf meal).

Semen characteristics of rabbit bucks fed graded levels of *Newbouldia laevis* leaf meal diets. Semen characteristics of rabbit bucks fed graded levels of *Newbouldia laevis* leaf meal diets is shown in Table 4. The results showed the mean values of volume, gross motility and concentration of the semen of rabbit bucks fed with diets of 0, 2.50, 5.00 and 7.50% graded levels of *Newbouldia laevis* leaf meal. The mean volume of the rabbit bucks showed no significant difference (p>0.05) throughout the time of the experiment across all the groups but an increased ejaculate was observed between T1 (0.61±0.01), T2 (0.63±0.01), T3 (0.66±0.02) and T4 (0.69±0.05) as the inclusion level of *Newbouldia laevis* leaf meal increases. There was no significant (p>0.05) difference in gross motility amongst all the group of the experimental animal. Similarly, to the semen volume, there was concomitant increase in the mean gross spermatozoa motility as the level of inclusion of *Newbouldia laevis* leaf meal increased. However, the semen concentration of rabbit bucks showed a significant difference (p<0.05) while the semen colour and semen pH showed no significant (p>0.05) among treatment groups.

Table 4: Semen characteristics of rabbit bucks fed graded levels of Newbouldia laevis leaf meal.

|  |  |  |
| --- | --- | --- |
| Parameter (%) | Treatments |  |
|  | T1 | T2 | T3 | T4 | SEM | LS |
| Volume(ml) | 0.61±0.01 | 0.63±0.01 | 0.66±0.02 | 0.69±0.05 | 0.02 | NS |
| Motility (%) | 63.01±1.16 | 63.40±1.42 | 66.73±2.30 | 69.14±4.02 | 1.28 | NS |
| Concentration (x106/ml) | 86.60±7.06c | 137.48±3.50b | 137.72±3.42b | 152.25±2.66a | 3.66 | \* |
| Colour | 1.07 | 0.80 | 1.00 | 1.07 | 0.06 | NS |
| pH | 6.72 | 7.02 | 6.92 | 6.66 | 0.07 | NS |

a,b,c Means along the same row with the same superscript are not significantly (p<0.05) different, SEM- Standard error of mean , LS- level of significance, \* -significant difference, T1: (control diet), T2: (2.5 % Inclusion of *Newbouldia laevis* leaf meal), T3: (5 % Inclusion of *Newbouldia laevis* leaf meal), T4: (7.5 % Inclusion of *Newbouldia laevis* leaf meal).

DISCUSSION

The mean semen volume values for all the groups were not significantly different (P>0.05), although, there was a progressive increase in semen volume across the treatment groups. The increase in volume may be due to some phytochemical constituents (flavonoids and steroidal

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saponin) contained in the plant (Morakinyo *et al*., 2008). This outcome observed was similar to an increase in sperm functions (semen volume inclusive) in rats treated with extract of *Kigellia africana* (which belongs to the family *Bignoniaceae* as *Newbouldia laevis*) (Azu *et al*., 2010). Similarly, the mean semen motility values were not significantly (P>0.05) different across all the treatment groups, though they were higher than the control values. This could as well be due to flavonoids and saponin phytochemical components of the *Newbouldia laevis* leaf meal. This agrees with Robak and Gryglewski (1988) and Morakinyo *et al.,* (2008) who reported that phytochemical constituents in *Newbouldia laevis* leaf possess fertility enhancing activities and stimulatory effect on androgen production which might contribute to increased testosterone level consequently resulting in increased semen motility, thus leading to improvement of spermatogenesis and fertility. Mean semen concentration values were rather significantly higher across the treatment groups compared to the control group. This may be due to the effect of some phytochemical constituents contained in the *Newbouldia laevis* leaf meal (flavonoid and saponin), which have been reported to be abundant in the plant especially flavonoids (which is an effective aromatase inhibitor) in the plant as reported by Azu *et al.,* (2010). The cytochrome P-450 aromatase is required for the conversion of androgen to estrogens and hence aromatase inhibitor would decrease the contraction of estrogen and maintain a higher level of testosterone, (Morakinyo *et al.,* 2008 and Azu *et al.,* 2010) which in turn increases semen concentration, improves spermatogenesis and fertility of the rabbit bucks.

CONCLUSION

From the findings of this work, it can be concluded that semen concentration values were increased significantly (p<0.05) in the *Newbouldia laevis* leaf diet treated groups (T2 = 137.48±3.50

×106/mL, T3 = 137.72± 3.42 ×106/mL and T4 = 152.25±2.66 ×106/mL), which may be responsible for its use as a fertility enhancer by the natives.

More so, 7.5% inclusion level of *Newbouldia laevis* leaves showed higher testicular functions (T4 bucks had better sperm motility (69.14±4.02%) and sperm volume (0.69±0.05mL) than T3 = (66.73±2.30%) and (0.66.±0.02mL), T2 (63.40±1.42%) and (0.63±0.01mL) and T1 that had

(63.01±1.16 %) and (0.61±0.01mL)) respectively.

Based on the results obtained it is recommended that inclusion of *Newbouldia laevis* at 7.5 % improved the sperm concentration of rabbit bucks and should be considered in their feed

formulation especially for breeders.

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