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Effects of levels of *Moringa oleifera* leaf extract dietary inclusion on carcass characteristics and testicular measurement of rabbit bucks

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Abstract

In developing nations, Moringa oleifera leaf is used for nutritional, medicinal purposes and it is rich in minerals and vitamins. There is little information on its use for rabbit production and management. Therefore, there is need to focus attention on its status in rabbit to achieved good productivity. In this study, Moringa oleifera leaf extract (MOLE) was used to assess the effect on carcass yield, gonadal and extra-gonadal sperm reserve of rabbits buck. Four diets were formulated to contain MOLE inclusion levels at 0 (control), 25, 50 and 75mL. Twenty eight grower rabbit bucks of mixed breeds aged 10-11 weeks were used for the experiment. They were randomly allotted to four groups of seven animals per treatment with three animal per replicate. At the end of feeding trial, three rabbits per treatment were randomly selected, weighed and slaughtered for carcass and testicular sperm reserve evaluation. The results revealed higher ($p<0.05$) bled weight (2041.73g; 2063.83g), live weight (2156.50g; 2206.25g) and dressing % (66.57; 65.55) in treatments 1 and 2, respectively than treatment 3 and 4 in carcass yields. Abdominal fat (46.05g; 53.18g) and caecum (24.75g; 26.15g) followed similar trends. Gonadal and extra-gonadal sperm reserve were significantly ($p<0.05$) higher in treatments 1 and 2 for right (27.18; 28.12)($x10^6$)/g and paired (69.49;73.30)($x10^6$)/g testis, left (45.38; 57.21)($x10^6$)/g, right (31.11; 39.11)($x10^6$)/g and paired (76.49; 96.32)($x10^6$)/g caudal epididymis than other treatment groups hence, produced positive effects at 25mL level. Inclusion above 25mL depressed productive and reproductive performance, as such it can be safely useat 25mL without any detrimental effects.

Keywords: Rabbit, *Moringa oleifera*, leave extracts, carcass, gonadal sperm reserve

Effets des niveaux d'extrait de feuille de moringa oléifères inclusion alimentaire sur les caractéristiques de la carcasse et la mesure testiculaire des daims de lapin

Résumé

Dans les pays en développement, la feuille MoringaOleifera est utilisée à des fins alimentaires nutritionnelles et médicinales et elle est riche en minéraux et en vitamines. Il y a peu d'informations sur son utilisation pour la production et la gestion de lapin. Par conséquent, il est nécessaire de concentrer l'attention sur son statut chez le lapin pour obtenir une bonne productivité. Dans cette étude, l'extrait de feuille de MoringaOleifera

(MOLE) a été utilisé pour évaluer l'effet sur le rendement de la carcasse, la gonadale et la réserve de sperme extra-gonadal de lapins Buck. Quatre régimes ont été formulés pour contenir des niveaux d'inclusion MOLE à 0 (contrôle), 25, 50 et 75 ml. Vingt-huit daims de lapin de producteurs de races mixtes âgées de 10 à 11 semaines ont été utilisées pour l'expérience. Ils ont été alloués au hasard à quatre groupes de sept animaux par traitement avec trois animaux par réplication. À la fin du procès d'alimentation, trois lapins par traitement ont été sélectionnés au hasard, pesés et abattus pour une évaluation de la carcasse et de la réserve de sperme testiculaire. Les résultats ont révélé supérieur (P <0,05) poids saigné (2041,73 g; 2063,83g), poids vivant (2156.50g; 2206,25 g) et pansement% (66.57; 65,55) dans des traitements 1 et 2, respectivement que le traitement 3 et 4 de la carcasse rendement. Graisse abdominale (46.05g; 53,18g) et caecum (24,75 g; 26.15g) ont suivi des tendances similaires. Les réserves de sperme gonadal et extra-gonadal étaient significativement (p <0,05) plus élevées dans les traitements 1 et 2 pour le droit (27.18; 28.12) (x106) / g et jumelé (69,49; 73,30) (x106) / g testtis, à gauche (45,38; 57.21) (x106) / G, droite (31.11; 39.11) (x106) / g et jumelé (76,49; 96.32) (x106) / gépididymis caudal que d'autres groupes de traitement, ont donc produit des effets positifs au niveau de 25 ml. L'inclusion au-dessus de 25 ml de performance productive et reproductive déprimée, en tant que telle, elle peut être utilisée en toute sécurité 25 ml sans aucun effet néfaste.

Mots-clés: Lapin, MoringaOleifera, Extraits de feuille, Carcasse, Réserve de sperme de Gonadal

Introduction

The need to explore available feed resources such as leaf from tropical trees is calling for attention as some of these materials have been reported to contain substances that influence growth and development of farm animals (Ayssiwede *et al.*, 2011; Alikwe *et al.*, 2013). Leaf extracts have appetizing and digestion-stimulating properties and antimicrobial effects (Langhout, 2000). The recommended policy is to identify and use locally available feed resources to formulate diets that are as balanced as possible (Guèye and Branckaert, 2002). Green plants leaf are used in the ration of the livestock to reduce the cost of production (Ayssiwede *et al.*, 2011) and enhance reproductive performance (Adeyemi *et al*., 2014) of farm animals, thus increasing overall livestock production when used as a feed component or additive. *Moringa oleifera* Lam belongs to monogeneric family of shrubs and tree, moringaceae and commonly known as "Drumstick". The seeds have no dormancy period, so they can be planted as soon as they are mature and they will retain the ability to germinate for up to one year (Fuglie, 1999). Phytochemical screening of the leaf indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins in the leaf extracts (Patel *et al*., 2014). The saponin content of the leaves was 80 g kg having nohaemolytic activity and a phytate content of 21 g /kg (Makkar and Beeker, 1996). The presence of this substance in feed ingredients may produce various responses in farm animals based on the ability of the animals to utilize the feed components. The efficiency of feed utilization can be expressed in organ development in terms of weight and functions. A reduction or increase in the size of an organ maybe an indication of injury or diseased condition. Several research findings on leaf meal and its effects on organs have been reported

(Amata and Bratte, 2008; Oguewu *et al.,* 2007; Olafadehan, 2011 and Sese *et al.,* 2014). However, there is paucity of information on the effect of Moringa oleifera leaf extracts on carcass and testicular morphometry of rabbit bucks. Thus, this study was aimed at determining the effect of *Moringa oleifera* leaf extract dietary inclusion on carcass characteristics and testicular measurement of rabbit bucks.

Materials and methods

Experimental site and location

The experimental site wasthe Rabbitary Unit of Research Farm of the Federal University of Agriculture Makurdi Livestock Teaching and Research Farm Makurdi, Benue State. It is geographically located on latitude 7.73 degrees North of the equator and longitude 8.53 degrees East of the prime meridian on the map of the world. The state has a tropical sub humid climate with two distinct seasons, wet and dry which last for seven months between April and October while the dry season is between November and March. Temperatures are generally very high during the day particularly in March and April. Along the river valleys these high temperatures in addition to relative humidity, produce inclement/debilitating weather conditions. Makurdi records maximum and minimum daily temperatures of 35°Cand 21°C in summer and 37°C and 16°C in winter respectively (https://www.maps-streetview.com/Nigeria/Makurdi).

Experimental animals and their management

A total of 28 grower rabbit bucks (mixed breeds) 10-11 weeks of age were used for the experiment. They were sourced within Makurdi metropolis. An adjustment period of two weeks was allowed, followed by 65 days of experimental period. Upon arrival, anti-stress (StresRoa®) were given at a dose 0f 20mL per 12 liters of water for two days after which they were given dewormers (Mebenzole (Zodex®)) at a dose of 100g per 5 liter of water. They were housed in an individual cages, measuring 60cm L × 55cm W × 140cm H and 55cm HAG (height above the ground), 60cm L × 57cm W × 120cm H and 45cm HAG; 60cm L × 57cm W × 115cm H and 49cm HAG with a roof over to prevent the direct impact of sun rays and minimize ambient temperatures. Drinkers and feeders were provided in each cage, well fitted to prevent feed and water wastages.

Experimental diet and design

Four experimental diets were formulated using maize, full fat soyabean, rice offal, palm kernel cake, bone meal, salt and premix as a single basal diet irrespective of the treatment (Tables 1 and 2). Treatments T1 served as control and rabbits were given cool drinking water without MOLE whereas in T_2, T3 and T4, they were given 25mL: 100mL, 50: 100mL and 75:100mL, respectively. Twenty eight rabbit bucks were randomly selected, weighed and assigned to four treatment groups, seven per treatment and each served as a replicate in a completely randomized designed.

Table 1: Experimental diet fed to rabbit bucks

Ingredient	Quantity (kg)
Maize	44.1
Full fat soya bean	23.9
Rice offal	20.0
Premix	0.2
Palm kernel Cake	9.5
Salt	0.3
Bone ash	2.0
Total	**100**

Table 2: Analyzed composition of experimental diets

Nutrient	Composition
Crude protein	17.50
Crude fibre	9.40
Ether extract	10.40
Ash	6.50
Nitrogen free extract	56.20
Dry matter	91.95

Preparation of MOLE

The Moringa oleifera leaves were sourced from Wuse in Nasarawa State. Care was taken to obtain only fresh leaves. They were washed free from sand and other contaminants, allowed to drain such that, the water from the washing was negligible. A clean mortar and pestle was used to pound the leaves. Only the leaves with twigs were pounded with no water added to it. After pounding, the extract was gotten by squeezing in muslin (cheese cloth). The extract was then be served to the animals at different level of inclusion with, 25:100mL for T_2, 50:100mL for T_3 and 75:100mL for T_4 of Extract: Water respectively.

Measured parameters

Carcass yield

Sixteen rabbits made up of four animals from each treatment group were randomly selected fasted for 12 hours and slaughtered for carcass evaluation at the end of the experiment feeding period. Each animal was stunned, bled, dressed by flaying and eviscerated. The dressed weight and the weight of heart, lungs, liver, spleen and kidneys were recorded using an electronic weigh master digital scale. Dressing percentage was determined by dividing the dressed weight by the live weight and multiplied by one hundred (DP (%) = DW/LW x 100).

Internal organ weight

The small intestine and caecum (without appendix) were measured. The stomach, small intestine, caecum and kidney were weighed. Water or blood that may have adhered to the liver and kidney was removed with filter paper. *Animal were weighted on weekly bases to get the weekly weight. During the period of termination,* (Aduku and Olukosi 1990); animals were starved of feed for twelve (12) hours but had access to adequate drinking water. Each rabbit were weighed before slaughtering. Evisceration and singeing was done as outlined byOluremi *et al.* (2006).

Testicular morphometry

The reproductive organs were obtained from three rabbit per treatment immediately after slaughter and evaluated as reported by Bitto and Egbunike (2006a). The testes and epididymides were dissected, trimmed free of fat and adhering connective tissues. Each testis, epididymal sections and ductus deference were separated and measured.

Statistical analysis

Data obtained from this study were subjected to the analysis of variance in aCompletely Randomized Designed and where significant difference(s) occur, the means were separated using the Duncan Multiple Range Test (DMRT) as outline by Steel and Torrie (1980).

Results and discussion

The result of carcass measurement of rabbit bucks fed *Moringa oleifera* leaf extract is presented in Table 3. Treatments 1 and 2 were significantly ($p<0.05$) higher compare to treatments 3 and 4 in dressing %, live and bled weight with the exception of eviscerated and singed weight. The significantly ($p<0.05$)increase at 25mL of MOLE could support earlier reports that *Moringa oleifera* is of high nutritional

value (Makkar and Becker, 1996; Anwar *et al.*, 2007). It is also correlate with the statement of Subadra and Monica (1997) and Faye (201)1 that amino acids, vitamins and minerals particularly iron increase carcass yield of rabbit bucks. Fadi *et al.*(2010) reported an increase in carcass weight when animals kept in captivity where energy expenditure is minimal.

Table 3: Carcass yield of rabbit bucks on different levels of inclusion of MOLE

Different inclusion levels of *Moringa oleifera* leaf extracts						
Parameters	T1(0mL)	T2(25mL)	T3(50mL)	T4(75mL)	SEM	P-value
Eviscerated weight (g)	1388.35	1503.55	1453.65	1336.25	84.15	0.162
Bled weight (g)	2041.73^{ab}	2063.83^{a}	1900.03^{b}	1901.50^{b}	80.90	0.021
Singed weight (g)	1071.53	1165.30	1063.03	1041.10	63.15	0.082
Live weight (g)	2156.50^{a}	2206.25^{a}	1987.25^{b}	1986.25^{b}	25.11	0.050
Dressing %	66.57^{a}	65.552^{a}	60.399^{b}	59.532^{b}	2.11	0.046

NS= Not significantly different (*p>0.05)*; * = significantly different (*p<0.05*) SEM = Sum of error mean; a,b = Values in the same row with different superscripts differ significantly (*p<0.05*)

The result of visceral organ weights of male rabbits fed MOLE is presented in Table 4. There was no significant (p>0.05) effect of diet on all the parameters studied except caecum and abdominal fat. Treatment 5 was significantly (p<0.05) higher compare to other treatments in the caecum content. This could be resulted from the changes in bacterial loads. Amber *et al.*(2014) found that addition of some probiotics in rabbit diets reduced number of total bacterial count in the caecum content of rabbits which is in support to the statement of Siddhuraju and becker (2003) that MOLE have potential prebiotics and antioxidant phyto-chemicals such as chlorogenic and caffeic acids. Variations observed in abdominal fat with significantly(p>0.05) higher value in treatments 3 and 4 compare to other treatments could be correlated with the findings of Amber *et al*. (2014) who observed differences in abdominal fats when poultry birds treated with prebiotics. The author further stated that no clear mechanisms have been reported responsible for the reduction of lipids synthesis by prebiotics and herb oligosaccharides. It may be due to increase in beneficial bacteria such as Lactobacillosis that decrease the activity of acetyl- CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis (Toghyani *et al.,* 2011).

The result of testicular weight, sperm reserve and epididymal sperm reserve of rabbit bucks fed *Moringa oleifera* leaf extract is presented in Table 5. There was no significant (p<0.05) difference except sperm reserve and epididymal sperm reserve. Testicular sperm reserve of rabbit bucks in treatments 1 and 2 were significantly (p<0.05) higher than other bucks in right and paired testis caput. Reduced testicular sperm reserve observed in treatments 3 and 4 may be due to the toxic effect of some anti- nutritional components of the moringa leaf such as tannin Makker and Becker (1997). Ewuola and Egbunike (2010) reported tendency of reduced sperm reserve production when an animal is exposed to feed that contain potentially toxic substance. However, the significant increase in the testicular sperm reserves observed in rabbit bucks fed T1 and T2 MoLE could be attributed to the beneficial effect of *Moringa oleifera* leaf in stimulating spermatogenesis and subsequent sperm cell formation in the testes. The leaf has been reported to contain phytochemicals like β- carotene and

tocopherol, which enhances sperm formation (Nambiar, 2001 and Adeyemi *et al*., 2014). Similarly, the significant increase in the epididymal sperm reserve of rabbit fed T1 and T2 than those on T3 and T4 in left, right and paired caudal epididymis could be attributed to the treatment effect. This indicates that the leaf is best use as supplement at lower inclusion level to prevent accumulation of its inherent anti- nutrient in the animal. Also, it will encourage proper utilization of its antioxidants and other beneficial phytochemicals Coppin (2008). This in turn helps to mitigate reactive oxygen species and free radicals induced by hot climate, which affect reproductive efficiency of male rabbits in the tropics (Afolabi *et al*., 2013; Cajuday and Pocsidio 2010). Reduction in sperm production was observed with increase in the inclusion level of MoLE above 25mL though sperm production of rabbits administered 25mL MoLE compared favourably with that of bucks on control indicating that the use of the leaf meal extract at this level did not adversely altered the nutritional quality of the diet at the expense of the sperm production in the rabbit bucks. This also agrees with the earlier report of no adverse effect of the crude moringa leaf extract on the blood profile and health status of gestating and lactating does Ewuola *et al*. (2015).

Table 4:Visceral and endocrine organ weight of rabbit bucks on different levels of inclusion of MOL

Different levels of inclusion *Moringa oleifera* leaf extracts						
Parameters	**TI(0mL)**	**T2(25mL)**	**T3(50mL)**	**T4(75mL)**	**Pvalue**	**SEM**
Spleen	0.53	0.63	0.48	0.45	0.16	0.271
Liver	39.18	37.95	38.53	37.55	1.30	0.144
Heart	3.60	4.15	4.30	3.95	0.75	0.155
Lungs	8.90	9.58	7.88	7.25	1.21	0.344
Pancrease	10.18	13.90	9.15	13.73	2.41	0.081
Left kidney	4.08	4.32	4.53	4.48	0.29	0.121
Right kidney	4.00	4.30	4.43	4.45	0.48	0.064
Adrenal left	0.20	0.18	0.13	0.20	0.06	0.132
Adrenal right	0.20	0.15	0.15	0.15	0.03	0.167
Trachae	0.75	0.53	0.55	0.75	0.51	0.110
Bladder	2.75	2.93	4.23	2.63	1.20	0.084
Abdominal fat	46.05^{ab}	53.18^{a}	42.98^{b}	38.45^{b}	3.59	0.051
Oesophagus	1.50	1.78	1.48	1.43	0.58	0.112
Stomach	14.38	16.23	16.68	13.50	1.74	0.071
Small intestine	29.33	33.45	26.38	27.95	3.73	0.532
Caecum	24.75^{a}	26.15^{a}	20.58^{b}	18.35^{b}	1.19	0.032
Colon	23.88	24.40	22.33	23.85	1.14	0.142

NS= Not significantly different ($p>0.05$); * = Significantly different ($p<0.05$) SEM = Sum of error mean ; a,b = Values in the same row with different superscripts differ significantly ($p<0.05$)

Table 5: Effect of MOLE levels on gonadal and extra-gonadal sperm reserve of rabbit bucks

Different inclusion levels of moringa oleifera leaf extracts						
Parameters	T1(0mL)	T2(25mL)	T3(50mL)	T4(75mL)	SEM	P values
Testis weight (g)						
Left testis	0.15	0.18	0.17	0.21	0.062	0.132
Right testis	0.13	0 .15	0.15	0.18	0.031	0.110
Paired testis	0.28	0.33	0.32	0.39	0.121	0.112
TSR ($x10^6$)/g						
Left testis	42.31	45.18	41.10	38.16	3.56	0.081
Right testis	27.18^{ab}	28.12^{a}	24.06^{b}	23.11^{b}	2.01	0.052
Paired testis	69.49^{a}	73.30^{a}	65.16^{b}	61.27^{b}	1.96	0.041
ESR ($x10^6$)/g						
Left caput	10.87	11.00	8.14	9.40	1.54	0.062
Right caput	7.13	8.11	5.10	4.36	1.96	0.311
Paired caput	18.00	19.11	13.24	13.76	3.01	0.124
Left corpus	5.01	6.11	3.67	4.03	1.49	0.075
Right corpus	3.21	4.32	4.11	3.01	0.76	0.144
Paired corpus	8.22	10.43	7.78	7.04	1.75	0.851
Left caudal	45.38^{ab}	57.21^{a}	39.11^{b}	38.10^{b}	5.94	0.046
Right caudal	31.11^{a}	39.11^{a}	20.23^{b}	23.05^{b}	4.02	0.017
Paired caudal	76.49^{ab}	96.32^{a}	58.33^{b}	16.06^{c}	9.94	0.034

NS= Not significantly different ($p>0.05$); * = significantly different ($p<0.05$) ; TSR = Testicular sperm reserve; ESR= Extra-gonadal sperm reserve; SEM = Sum of error mean; a,b = Values in the same row with different superscripts differ significantly ($p<0.05$)

Conclusion

The study showed that *Moringa oleifera* leaf extracts produced positive effects at 25mL level on carcass, gonadal and extra-gonadal sperm reserve of rabbit bucks. Inclusion above25mLdepressed productive and reproductive performance as it can be safely use at 25mL without any detrimental effects.

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