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Original article

Effect of leucaena and gliricidia leaf meals on the seminal characteristics, testis weights and seminiferous tubule diameters of rabbits

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Abstract – Eighteen crossbred rabbit bucks aged 8–10 months were placed on one of three diets containing leucaena (LLM), gliricidia (GLM) leaf meals both included at 20% of dry matter, or a control diet (CTL). Semen volume for the CTL group was 0.71 ± 0.02 mL, which was higher (P<0.05) than the values for the LLM (0.57 ± 0.02 mL) and GLM (0.58 ± 0.01 mL) groups. The spermatozoa concentration values obtained were (110.3 ± 3.5) × 10^6 per mL, (103.5 ± 4.1) × 10^6 per mL and (94.2 ± 3.4) × 10^6 per mL for the CTL, LLM and GLM groups respectively. Seminiferous tubular diameters were significantly (P<0.05) wider in the control group (234 ± 21.3 μm) than in the other two groups, which were similar (P>0.05). There were indications of mild degenerations in some samples from the leucaena and gliricidia groups. These results indicate that the inclusion of leucaena and gliricidia leaf meals at 20% in rations for mature rabbit bucks could cause mild depressive effects on semen production and quality.

rabbit / semen / forage tree legume

Résumé – Effet de l'incorporation de feuilles de leucaena ou de gliricidia dans l'alimentation des lapins sur les caractéristiques séminales, le poids des testicules et le diamètre des tubules séminifères. Dix-huit lapins mâles croisés âgés de 8 à 10 mois ont été répartis en trois groupes et ont reçu soit un régime supplémenté avec des feuilles de leucaena (LLM) ou de gliricidia (GLM) broyées, les deux incorporés à un taux de 20 % de matière sèche, soit un régime témoin (CTL). Le volume de sperme du groupe témoin a été de 0,71 ± 0,02 mL, volume plus élevé (P<0,05) que celui du groupe LLM (0,57 ± 0,02 mL) et du groupe GLM (0,58 ± 0,01 mL). La concentration en spermatozoïdes a été, respectivement, de (110,3 ± 3,5) × 10^6 par mL, (103,5 ± 4,1) × 10^6 par mL et (94,2 ± 3,4) × 10^6 par mL pour les groupes CTL, LLM et GLM. Le diamètre des tubules séminifères a été significativement plus élevé pour le groupe témoin (234 ± 21,3 μm) que pour les deux autres groupes, pour lesquels les diamètres ont été similaires (P>0,05). Des signes bénins de dégénérescence ont été observés dans quelques échantillons provenant des groupes LLM et GLM. Ces résultats suggèrent que l'incorporation de feuilles de leucaena ou de gliricidia à 20 % de matière sèche dans l'alimentation de lapins mâles adultes pourrait provoquer des effets délétères bénins sur la production et la qualité du sperme.

lapin / sperme / fourrage de légumineuse arbustive

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1. INTRODUCTION

Forage tree legume feeding has been advocated and is being adopted by small to medium scale livestock farmers in the tropics to boost the nutritional regimes of their animals [12]. In studies involving leucaena and gliricidia leaf meals for doe rabbits, Herbert [5, 6] observed that the reproductive performance of females was depressed by the inclusion of high levels of the leaf meals in the diets, especially during gestation and lactation.

Various reports involving male animal species other than rabbits being fed leucaena indicate some negative effects on reproductive performance. Depressed performances have been observed in bulls [8], rats [13] and fish [14]. Jones et al. [8] observed that withdrawing bulls from the leucaena pasture that they had been feeding on also returned them to normal reproductive functions in subsequent seasons. There is little information on the effects of leucaena and gliricidia leaf meals in rabbit buck diets on their reproductive performance. The present study was designed with the main objective of determining the effect of incorporating leucaena and gliricidia leaf meals in the diets of breeding rabbit bucks on semen quality and testicular histology.

2. MATERLALS AND METHODS

Eighteen New Zealand White × Dutch-belted crossbred rabbit bucks aged between 8 months and 10 months (mean = 9.3 months) were used in the experiment. The animals were divided into three groups of 6 rabbits each. Care was taken when placing the animals into groups in order to balance the groups such that there were no significant differences between them on basis of weight. The groups were then randomly assigned to three experimental diets: control, no leucaena, no gliricidia leaf meals (CTL); 20% leucaena leaf meal (LLM) and 20% gliricidia leaf meal (GLM).

Fresh leucaena *(Leucaena leucocephala,* Lam De Witt) and gliricidia *(Gliricidia sepium,* Jacq) leaves were collected and sundried for 5–7 days, milled and incorporated into rations at a 20% level of inclusion. The diets were formulated to be isonitrogenous having a crude protein content of 17.0%. The composition of the diets is given in Table I. The animals were individually caged, while water and feed were provided ad libitum. Body weights were taken every fortnight and records of daily feed intake were kept throughout the 10-week experimental period.

An artificial vagina (AV) described by Herbert and Adejumo [7] was used for the semen collection. The animals were ejaculated twice weekly (once each on Mondays and Thursdays) for 10 weeks. Semen volume was read off the collection tube and recorded in millilitres. Individual sperm motility and spermatozoa massal motility were determined on freshly collected semen on a warm stage at 37 °C. The samples were diluted with physiological saline solution (PSS), and observations were made at 400× magnification. Individual spermatozoa motility was scored subjectively under a microscope and recorded in percentages while spermatozoa massal motility was scored on a scale of 0–4 (no motility – high mass movement). Spermatozoa counts were carried out on semen diluted with PSS using the haemocytometer method. Total spermatozoa per ejaculate was derived by calculation. The animals were weighed and slaughtered at the end of the 10th week. At slaughter, the pairs of testes were quickly removed, weighed and the weights were recorded in grams. The testes were fixed in aqueous Bouin fluid. Tissue samples were taken from the equatorial regions of the testes, washed in 50% and 70% alcohol before being embedded in paraffin wax [9]. After embedding, tissue samples were cut at 5 microns using a microtome. Staining was done with haematoxylin-eosin. Two slides were prepared per testis.

Table I. Nutrient composition of leucaena and gliricidia leaf meal diets fed rabbit bucks.

Component	CTL	LLM	GLM
Ingredients (%):			
Maize	36.6	35.6	32.3
Brewer dried grains	54.9	37.6	40.3
Groundnut cake	1.72	0.00	0.66
Fish meal	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00
Vitamin/mineral premix	0.25	0.25	0.25
Salt	0.50	0.50	0.50
Dried leucaena leaf meal	0.00	20.0	0.00
Dried gliricidia leaf meal	0.00	0.00	20.0
	100	100	100
Chemical Composition (in % of DM):			
Crude protein	17.7	17.4	17.3
Crude fibre	12.1	13.2	14.2
Ether extract	2.9	2.5	2.1
Ash	6.8	10.5	11.1

CTL: control diet; LLM: leucaena leaf meal; GLM: gliricidia leaf meal.

The slides were read for histopathological indicators in order to observe possible degenerative changes on the testicular structure. Histometric measurements were taken on selected slides to generate data on seminiferous tubule diameters. This was carried out using a Zeiss microscope fitted with an ocular micrometer previously calibrated with a stage micrometer according to the procedure described by Majumdar [9]. Three tubules were measured in each slide. Measurements were taken twice on each tubule, the second perpendicular to the first. The tubular diameter was calculated as the average of the two measurements.

Statistical differences between treatment groups were determined with the analysis of variance test using the computerised statistical analysis system (SAS) [2]. The experimental model was of the type: $Y = \mu + Ti + e$ (T = treatment). When differences were observed between treatments, the means were compared using the Duncan test.

3. RESULTS

The bodyweights of the animals at slaughter were 2060 ± 43 g, 2063 ± 115 g and 1940 ± 71 g for the CTL, LLM and GLM groups respectively, which were similar ($P>0.05$). Average feed intakes over the experimental period were 93.3 ± 5.8 g, 89.8 ± 2.7 g and 90.6 ± 3.8 g for the CTL, LLM and GLM groups respectively, which were similar ($P>0.05$).

The results of the seminal characteristics observed in this study are shown in Table II. Semen volumes recorded for the LLM and GLM groups were similar, but lower than that obtained for the CTL group ($P<0.05$). Spermatozoa concentration in the CTL group was higher ($P<0.05$) than for the GLM group but similar to that of the LLM group.

Individual spermatozoa motility and spermatozoa massal motility were significantly ($P<0.05$) higher for the CTL than for the

Table II. Effect of inclusion of leucaena and gliricidia leaf meal diets on the seminal characteristics, testis weights and seminiferous tubule diameters of rabbits.

Variable	CTL	LLM	GLM
Semen volume (mL)	0.71±0.02[a]	0.57±0.02[b]	0.58±0.01[b]
Spermatozoa concentration ($\times 10^6$ per mL)	110±4[a]	104±4[ab]	94±3[b]
Total spermatozoa ($\times 10^6$)	78±2[a]	59±2[b]	54±3[b]
Individual spermatozoa motility (%)	67.5±0.5[a]	62.1±0.1[ab]	60.5±0.5[b]
Spermatozoa mass motility (0–4)	2.8±0.01[a]	2.5±0.03[b]	2.3±0.03[b]
Testis weight (g)	6.7±0.2	6.4±0.3	6.4±0.2
Seminiferous tubule diameter (μm)	234±21[a]	209±13[b]	204±14[b]

CTL: control diet; LLM: leucaena leaf meal; GLM: gliricidia leaf meal.
[a, b]: Means on the same row bearing different superscripts are significantly different ($P<0.05$).

treated animals, except that individual motility did not differ ($P>0.05$) between the CTL and the LLM groups (Tab. II).

Examination of slides from the different groups of animals studied indicated there was the presence of spermatogenic cells in all the sections viewed. Fully formed spermatozoa could be identified at the luminal sections of the seminiferous tubules. In the LLM group, some of the testes showed signs of interstitial congestion. There were also a few cases of degeneration of the seminiferous epithelium involving a number of primary spermatocytes. However, these signs were not pronounced and could be regarded as incidental. Some of the observations mentioned for the LLM group were also observed in a few of the samples examined in the GLM group.

Testis weights did not differ significantly ($P>0.05$) between groups (Tab. II). Histometric observations revealed significant ($P<0.05$) differences between the groups in their seminiferous tubule diameters. The animals on the CTL had a mean value which was higher than that observed for the LLM and GLM groups (Tab. II).

4. DISCUSSION

The depression in the reproductive performance of rabbit bucks experienced in this study as a result of feeding leucaena and gliricidia have been reported for other animal species. Rahman [13] has reported various effects of leucaena on the reproductive characteristics of male and female rats. He observed that, in male rats, the seminiferous tubules are desquamated while there are degenerative changes on spermatogenic cells, leading to low sperm counts in animals fed high doses of leucaena leaf meals. Similarly, Rahman et al. [14] observed that leucaena has depressive effects on live spermatozoa proportions in fish.

In this study, both the volume and the concentration of semen output were depressed by the feeds containing leucaena and gliricidia. Toxic effects have not been linked with gliricidia feeding in recent studies [1, 3, 6, 11]. In fact, these studies have recommended the feeding of gliricidia to small ruminants [1, 3, 11] and rabbits [6]. However, the conditions observed in gliricidia-fed animals were similar to those of the leucaena group indicating that the tannins and other polyphenols contained in gliricidia may have similar depressive effects on spermatozoa production like those of mimosine and 3,4-dihydroxypyridone contained in leucaena. Shandilya et al. [15] demonstrated the depressive effect of gossypol on the reproductive performance of male monkeys *(Macaca fascicularis).*

Nutrition has long ago been shown to affect the secretory functions of the accessory sex glands, the products of which make up the seminal plasma [10]. In the stallion and the boar, the large volumes of semen produced are known to be the result of massive secretions by the accessory sex glands [4]. Even though the mechanism of action of the substances contained in these plants has not been clearly demonstrated, it is likely that there is some action on the structural material of the basement membrane of the seminiferous tubule causing it to disintegrate. Degeneration of spermatocytes can be linked to the effect of mimosine in leucaena and polyphenols (including tannins, isoflavones, etc.) in gliricidia on cell division and DNA synthesis. The works of Tsai and Ling [16, 17, 18] have highlighted similar effects. There may have been the inhibition of the proliferation of spermatogenic cells within the epithelium of the seminiferous tubules, thus reducing spermatozoa production. Rahman [13] observed the disappearance of spermatozoa in the seminiferous tubules of rats fed on leucaena leaves. He also observed that the epithelia of the seminiferous tubules were degenerated, desquamated and devoid of spermatozoa. However, the observations in the present study reveal that all the testes sections examined had spermatogenic cells and fully formed spermatozoa in their seminiferous tubule epithelia. This indicates that the treatments used in this study did not elicit adverse effects on the primary reproductive organ of male rabbits to the extent reported by Rahman [13] for rats, perhaps because the level of the test material included in this study was lower than that in the study by Rahman [13] or as a result of species effect.

The similar testis weights show that there was no testicular atrophy as a result of leucaena and gliricidia feeding to mature rabbit bucks.

5. CONCLUSION

The quantity and quality of semen produced in this study were lower for the treated animals than for the control. Though the histopathological effects observed on the testes could occur without leucaena and gliricidia feeding, their association with depressed spermatozoa production and semen output in the animals receiving the leaf meals is a source of concern and should be given adequate attention, while recommending leaf meals of forage tree legumes for the diets of breeding rabbit bucks.

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