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Influence of diet variability on gonadal development in heterogeneous rabbit stock during rainy season in southwest Nigeria

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Abstract
In Nigeria, the rabbit stocks are mainly heterogeneous with limited information on their developmental potentials as influence by varying nutritional and climatic factors. This study assessed the effect of diet variations on testicular and ovarian development in heterogeneous rabbit stock during rainy season in Southwest Nigeria. Sixty-six heterogeneous adult rabbits (55 does and 11 bucks) were mated to obtain the kits used for this study. The rabbits were managed during a period (April to October) characterized by cold condition in Nigeria and fed concentrate diet throughout gestation till the kits were weaned at 4–5 weeks old. At weaning, the rabbits were randomly assigned to three diets- diet 1 (100% concentrate), diet 2 (50% concentrate and 50% fresh *Moringa oleifera* leaves), and diet 3 (25% concentrate and 75% fresh *Moringa oleifera* leaves) and monitored till puberty. The weight and histological assessment of the ovary and testes was done at 4, 8, 12 and 16weeks old. Data obtained was analysed using one way Analysis of Variance and means separated using Duncan Multiple Range test. The results obtained showed higher testes and ovary weights in rabbits fed diets 1 and 2 compared to those fed diet 3. The number of germ cells, follicles with lumen and granulosa cells in the ovaries from 4 to 12 weeks old were similar among the groups. The number of primordial cells, seminiferous tubules diameter, tubules with lumen and spermatogonia count were also not adversely altered by the diets however lower values were recorded in the testes of rabbits fed diet 3. Histological assessments at 16 weeks showed a significant ($p < 0.05$) impact of diets on the testes and ovaries of the rabbits at this age and consequently on puberty attainment. This suggests that concentrate diet with readily available plants such as *Moringa oleifera* leaves fed at a ratio of 1:1 may promote gonadal development in heterogeneous stock of rabbits thus minimizing feeding cost, enhance climate-friendly rabbit farming and achieve optimum gonadal development during rainy season.

Key words: Histology, *Moringa oleifera*, Ovary, Temperature-Humidity Index, Testes

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Introduction

Rabbit farming is emerging as a strategic component of livestock development in Nigeria due to its low start-up cost and potential to contribute to food security. However, poor reproductive performance, particularly under tropical conditions, limits its productivity (Ebeid *et al*., 2023). The productive capacity and gonadal development of rabbits involve a timed sequence of events from foetal life and to maturity (Marco-Jimenez *et al*., 2014). This lays a foundation for

understanding sexual maturation and fertility in mammals. A knowledge of the physiological development of gonads prior to puberty is crucial for effective breeding programs and reducing reproductive failures. The Nigerian population of rabbits is highly heterogeneous with no distinct record of its reproductive potential as influenced by various factors. Breed differences influence ovarian indices and hormonal profiles in prepubertal does (Jimoh and Ewuola, 2021); and the processes within the testes or ovaries are greatly influenced by environmental temperature (Krishnan *et al.,* 2017; Mutwedu *et al*., 2021) with subsequent impact on puberty attainment. The onset of reproductive cycle marks the attainment of puberty, and it is influenced by nutrition (West *et al*., 2024). A synergistic effect of forages with concentrate diets support growth and facilitates overall health and productivity of rabbits; and forages not only have the potential to support the health of rabbits but also reduces feed costs, as they can be sourced locally (Ikyume *et al*. 2019, Adeyemi *et al*., 2024). *Moringa oleifera* is one of such plants that has been adopted in feeding rabbits in various forms due to its nutritive value, drought resistance potential and availability. Evaluating gonadal development in the Nigerian heterogeneous stock of rabbits as influenced by varying diets under cold environmental conditions is crucial for understanding rabbit reproduction and optimizing breeding programs. This study therefore assessed the sole and synergistic effect of *Moringa oleifera* and concentrate diet on the development of the ovary and testis in heterogeneous stock of rabbits during the rainy season in southwest Nigeria.

Materials and Methods

Sixty-six heterogeneous adult rabbits (55 does and 11 bucks) were mated to obtain the kits used for this study. The rabbits were managed during rainy season (April to October) characterized by cold condition in Nigeria and fed concentrate diet throughout gestation till the kits were weaned at 4–5 weeks old. At weaning, the rabbits were randomly assigned to three diets- diet 1 (100% concentrate), diet 2 (50% concentrate and 50% fresh *Moringa oleifera* leaves), and diet 3 (25% concentrate and 75% fresh *Moringa oleifera* leaves). The weight and histological assessment of the ovary and testes was done at 4, 8, 12 and 16weeks old. The weights were measured using a digital weighing scale and recorded in grams. The rabbits were slaughtered, and the ovaries were carefully removed, weighed and fixed in a 10% formalin solution for preservation and subsequently subjected to histological assessment as described by Suvarna *et al*., (2019). The number of germ cells, number of developing follicles, follicles with lumen and granulosa cells in the ovaries; and primordial cells count, seminiferous tubules diameter, tubules with lumen and spermatogonia count in the testes were assessed as seen under the microscope (HE x100, x400). Data obtained was analyzed using one way Analysis of Variance and means separated using Duncan Multiple Range test of SAS software.

Results and Discussion

Higher ovary weights were recorded in rabbits fed diets 1 and 2 compared to those fed diet 3 (Table 1). For all the parameters assessed, value recorded at four weeks when the kits were fed similar diet was recorded as baseline. The number of germ cells, number of developing follicles, follicles with lumen and granulosa cells in the ovaries from 4 to 12 weeks old were similar among the groups. The diets had significant ($p < 0.05$) effect on the ovarian parameters assessed at 16 weeks old; female rabbits fed diet 3 had significantly lower values for ovary weight, number of germ cells, number of developing follicles compared to those fed diet 1. This implies that concentrate and moderate quantity of fresh *Moringa oleifera* diets as fed in diet 1 and 2 is suitable for efficient ovarian activities compared to diet 3. Testes weight was higher in rabbits fed diets 1 and 2 compared to those fed diet 3 (Table 2). The number of primordial cells, seminiferous tubules diameter, tubules with lumen and spermatogonia count were also not adversely altered by the diets however lower values were recorded in the testes of rabbits fed diet 3. Histological assessments at 16 weeks showed a significant ($p < 0.05$) impact of diets on the testes of the rabbits at this age as rabbits fed diet 1 had wider seminiferous tubule. Increased lumination within

the seminiferous tubule indicates maturing testicles (Ahmed *et al*., 2012). This implies that the diets had significant effect on testicular development based on its impact on seminiferous tubule which directly influences spermatogenesis. The result from this study corroborates the findings of Ikyume *et al*. (2019) and Ayoola *et al*. (2024) who reported impacts of forage and/or concentrates on performance, health and reproduction in rabbits.

Table 1: Ovarian parameters of growing rabbits fed different diets during rainy season

Week	Baseline	Diet 1	Diet 2	Diet 3	SEM	p
			Paired Ovary Weight (g)			
4	N/A					
8		0.039	0.038	0.030	0.001	0.064
12		0.180^{a}	0.143^{ab}	0.071^{b}	0.031	0.014
16		0.227^{a}	0.162^{b}	0.145^{b}	0.025	0.032
			Number of Germ cell per ovary			
4	1214					
8		1731.00	1686.75	1403.75	115.89	0.289
12		1923.33	1378.50	1588.00	412.32	0.745
16		4631.71^{a}	4078.25^{ab}	3955.50^{b}	207.99	0.011
			Number of developing follicles per ovary			
4	162.33					
8		296.75	305.25	255.25	81.29	0.232
12		360.67^{a}	297.25^{ab}	281.57^{b}	42.26	0.009
16		460.75^{a}	397.25^{ab}	358.75^{b}	8.28	<.001
			Number of Follicles with lumen per ovary			
4	0					
8		29.50^{ab}	33.25^{a}	20.00^{b}	2.49	0.003
12		35.67^{a}	32.00^{b}	32.50^{b}	1.16	0.001
16		40.25	39.25	40.00	1.74	0.068
			Number of Granulosa Cells per ovary			
16		2186.50	1903.00	2058.25	72.88	0.053

a,b,c – means within the same row are significantly different at a probability level (p) of 0.05; SEM – Standard Error of mean; Diet 1 (100% concentrate); Diet 2 (50% concentrate and 50% fresh Moringa oleifera leaves); Diet 3 (25% concentrate and 75% fresh Moringa oleifera leaves)

Table 2: Testicular parameters of growing rabbits fed different diets during rainy season

Week	Baseline	Diet 1	Diet 2	Diet 3	SEM	p
			Paired Testes weight (g)			
4	0.109					
8		0.19	0.18	0.15	0.001	0.635
12		0.54^{a}	0.26^{b}	0.17^{b}	0.013	0.032
16		0.62^{a}	0.40^{ab}	0.25^{b}	0.011	0.017
			Germ Cells Count per testis			
4	993.83					
8		1008.80	1347.50	1349.00	79.87	0.129
12		1359.80	1288.30	1315.00	40.40	0.799
16		1944.00	1413.00	1570.80	104.72	0.092
			Number of Seminiferous tubules with lumen per testis			
4	200.33					
8		214.00	258.00	249.00	61.94	0.612
12		236.75	254.00	268.75	11.33	0.559
16		375.00^{a}	255.25^{b}	264.50^{b}	80.26	0.044
			Average diameter of Seminiferous Tubules (µm)			
4	245.30					
8		239.80	274.61	286.20	18.96	0.628
12		378.58^{a}	251.81^{b}	248.95^{b}	25.08	0.034
16		1011.80^{a}	568.70^{b}	584.00^{b}	145.22	0.027
			Spermatogonia count			
4	620.00					
8		664.30	87.001	981.50	66.00	0.134
12		976.75	935.50	974.00	29.22	0.841

16	1265.30	1012.80	1094.30	55.55	0.169

a,b,c – means within the same row are significantly different at a probability level (p) of 0.05; SEM – Standard Error of mean; Diet 1 (100% concentrate); Diet 2 (50% concentrate and 50% fresh Moringa oleifera leaves); Diet 3 (25% concentrate and 75% fresh Moringa oleifera leaves)

Conclusions and Outlook

Concentrate and fresh leaves of *Moringa oleifera* diets can support gonadal development in the heterogeneous stock of rabbits in Southwest Nigeria. With this practice, feeding cost can be minimized and climate-friendly rabbit farming promoted. However, based on the impact of the diets on other performance indices in rabbit breeding, feeding concentrate and fresh leaves of *Moringa oleifera* ratio 1:1 might be more beneficial than ratio 1:3.

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