

## EFFECT OF SUPPLEMENTED DIETS OF SCENT LEAF AND BITTER LEAF ON HORMONAL LEVELS AND REPRODUCTIVE PERFORMANCE OF RABBITS

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### ABSTRACT

In an 8-week feeding trial, 12 rabbits (Chinchilla X New Zealand White; 4-months old; averaged 1578g body weight) were used to investigate the effect of supplemented diets of Scent leaf and Bitter leaf on hormonal levels and reproductive performance of rabbits. The dietary supplement of Scent leaf and Bitter leaf were added at 10 g/kg each in the feed. The rabbits were housed individually and randomly assigned to three dietary treatments (n=4/group; at 2:2 bucks to does ratio) including a Control diet, diet 2 (Control+Scent leaf), and diet 3 (Control+Bitter leaf). Before mating, blood samples were collected from all the experimental animals for hormonal analysis. The reproductive performance was measured. Results of the hormonal levels were analysed using two-way randomised block ANOVA while the reproductive performance data were subjected to statistical analysis using one-way analysis of variance (ANOVA) in completely randomized design. The hormonal levels of rabbits fed Scent leaf and Bitter leaf diets revealed that serum luteinising hormone concentration was affected by sex of the rabbit ( $p<0.05$ ) not the supplemented diets ( $p>0.05$ ) but there was a significant supplemented diet  $\times$  sex interaction ( $p<0.05$ ). Serum testosterone concentration was unaffected by both the supplemented diets ( $p>0.05$ ) and rabbit sex ( $p>0.05$ ) but there was a significant supplemented diet  $\times$  sex interaction ( $p<0.05$ ). Under the reproductive performance, supplemented *Ocimum gratissimum* and *Vernonia amygdalina* diets significantly ( $p<0.05$ ) increased does' live weight before mating, sexual receptivity, conception rate, litter size at birth and litter weight at birth compared with the control. The study concludes that *Ocimum gratissimum* and *Vernonia amygdalina* inclusion in rabbit diet is capable of stimulating serum luteinising hormone and testosterone concentration thereby improve does' sexual receptivity, conception rate, litter size at birth. As herbal plants, they may be used to influences hypothalamic-pituitary-gonadal axis in rabbits.

### INTRODUCTION

The rabbit has some outstanding attributes that promotes its production. This includes good growth rate, ability to convert forages into high quality meat, short gestation length, early sexual maturity and ability to rebreed several times within a year (Apori et al., 2015). Recently research efforts have been targeted towards enhancing the reproductive performance of farm animals through scientific approaches involving the nutritional and physiological manipulations (Bindari et al., 2013). Nutritional factors have an important effect on the reproductive system and its endocrine glands are tightly connected and regulated by dietary factors (Panth et al., 2018). Reproductive processes in animals are under the influence of gonadotropic and steroid hormones regulated by the hypothalamus. The two crucial roles of an adult testis are the production of spermatozoa (fertility) and the secretion of testosterone which is needed for the expression of secondary sexual characteristics (virility). These functions depend on stimulation by the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are stimulated by hypothalamic gonadotrophin-releasing hormone (GnRH). Testosterone is secreted by the Leydig cells under LH stimulation and is essential for promoting spermatogenesis. Also, testosterone stimulates the development, growth and activity of reproductive parts and triggers the development of male secondary sex characteristics. While FSH has a role in the development of the immature testis (Orth 1993). The progesterone causes the hypothalamus to trigger LH production by the pituitary. Oestrogen is the steroid hormone responsible for the female menstrual cycle and is involved in the inter-play between FSH and LH. The identification of efficacious herbal plants that influences the hypothalamus pituitary gland axis may provide alternative treatment for infertility or as a contraceptive and perhaps ultimately lead to more effective medicine. A number of recent studies have identified plants that have fertility enhancement (Lans et al., 2018), psychopharmacological properties improving sexual behaviour (Singh et al.,

2013). For instance, *Eurycoma longifolia* has been reported to boost serum testosterone concentration, have profertility effects in animals (Chan et al., 2009) and man (Tambi et al., 2012) and has aphrodisiac properties (Asiah et al., 2007). Scent leaf (*Ocimum gratissimum*) and Bitter leaf (*Vernonia amygdalina*) are useful in the improvement of the nutritional quality of rabbit feeds due to their richness in alkaloids, tannins, phytates, flavonoids, terpenoids and saponin (Oladosu-Ajayi et al., 2017). The study assesses the effect of supplemented diets of Scent leaf and Bitter leaf on hormonal levels and reproductive performance of rabbits.

## MATERIALS AND METHODS

The institutional committee of Alvan Ikoku Federal College of Education (AIFCE), Owerri, Imo State, Nigeria approved the animal study and its experimental protocols, which was performed in accordance to the standard guidelines of the Institution's Animal Scientific procedures. The dietary supplement of Scent leaf and Bitter leaf were added at 10 g/kg each. The rabbits were housed individually and randomly assigned to three dietary treatments (n=4/group; at 2:2 buck to doe ratio) including a Control diet, diet 2 (Control+Scent leaf), and diet 3 (Control+Bitter leaf) as the feeding trial lasted for 8 weeks. Before mating the does, blood samples were collected from all the does for hormonal analysis. The reproductive performance was measured. The data values were presented as mean  $\pm$  SEM (standard of error of mean) from replicated experiments. Differences at  $p < 0.05$  were considered statistically significant. The reproductive performance data were subjected to statistical analysis using one-way analysis of variance (ANOVA) in a completely randomized design while the hormonal levels were analysed using two-way randomised block ANOVA using Genstat 18th Edition (Hemel Hempstead, United Kingdom (UK)). A  $3 \times 2$  factorial design was used with the different supplemented diets (3) and rabbit gender (2) used as factors. The main effects and their interaction were included in each statistical model.

## RESULT AND DISCUSSION

The hormonal levels of rabbits fed Scent leaf (*Ocimum gratissimum*) and Bitter leaf (*Vernonia amygdalina*) diets is presented in Table 1. Serum luteinising hormone concentration was affected by rabbit gender ( $p < 0.05$ ) but not supplemented diets of Scent leaf and Bitter leaf ( $p > 0.05$ ) however, there was a significant in supplemented diets and gender interaction ( $p < 0.05$ ; Table 1). This showed in the absence of supplemented diets of Scent leaf and Bitter leaf, serum luteinising hormone concentration was decreased by rabbit gender. In contrast, in the presence of supplemented diets of Scent leaf and Bitter leaf, serum luteinising hormone concentration was similar in male and female rabbits. Serum testosterone concentration was unaffected by both supplemented diets of Scent leaf and Bitter leaf ( $p > 0.05$ ) and rabbit gender ( $p > 0.05$ ) however, there was a significance in supplemented diets and gender interaction ( $p < 0.05$ ; Table 1). Serum testosterone concentration was decreased by supplemented diets of Scent leaf and Bitter leaf. In contrast, in the presence of rabbit gender, serum testosterone concentration was similar in supplemented diets of scent leaf and Bitter leaf. The reproductive performance of rabbits fed Scent leaf (*Ocimum gratissimum*) and Bitter leaf (*Vernonia amygdalina*) diets is presented in Table 2. Supplemented *Ocimum gratissimum* and *Vernonia amygdalina* diets significantly ( $p < 0.05$ ) increased does' live weight before mating, sexual receptivity, conception rate, litter size at birth and litter weight at birth compared with the control.

Scent leaf and Bitter leaf might have different effects on reproductive physiology of both male and female rabbits. Hormonal balance is required for effective reproduction in animals. Hypothalamic-pituitary-gonadal axis regulates luteinising hormone and testosterone biosynthesis which are associated with enhancement of sexual desire, motivation and performance (Swaney et al., 2012). The supplemented diets of Scent leaf and Bitter leaf improve the serum luteinising hormone and testosterone concentration observed in the present study. These suggest that Scent leaf and Bitter leaf supplemented diets enhanced the sexual activity of the male rabbits. The significant increase in serum luteinising hormone and testosterone levels in Scent leaf and Bitter leaf-treated animals when compared with the control contributes to the sex-dependent performance. It is well established that testosterone promotes spermatogenesis. The increased level of testosterone observed in male rabbits might be associated with improved spermatogenesis. There was an associated higher gonadotropin levels (LH) in the male rabbits, this is possibly due to the positive feedback control exerted by testosterone levels. There was significant difference observed in sexual receptivity in this study and

this could be traced to oestrus in the does that brought the action of gonadotropic hormones (LH and testosterone) on their gonads leading to the synthesis and release of oestrogen which is responsible for initiation and control of oestrus behaviour in female animals. Under the regulation by gonadotropic hormones, ovarian follicles mature and oestrogen secretions exert their biggest influence; the female then exhibits sexually receptive behaviour, a situation that may be signalled by visible physiologic changes (Geoffrey et al., 2007). It is suspected that the supplemented diets of Scent leaf and Bitter leaf used in this experiment might have stimulated the anterior pituitary gland bringing about the release of the gonadotropic hormones. The conception rate as observed in this study tend to decline with the supplemented diets of Scent leaf and Bitter leaf when compared to the control group. This decline in conception rate is suspected to be associated with an inhibitory action by the pituitary gland preventing further release of gonadotropins in connection with a negative feedback mechanism initiated as a result of an increased level of the gonadotropins in the blood. A lower conception rate observed in the control group could be associated with a lower sexual receptivity recorded in the control group during this study. The average litter size and weight observed in this study tended to increase following the supplemented diets of Scent leaf and Bitter leaf suggesting that the hormone may have influenced the number of ova shed during ovulation through its influence on the synthesis and secretion of the gonadotropins and steroid hormones. The maximum potential litter size depends on number of ova released (Herbert, 1998).

**Table 1: Hormonal levels of rabbits fed Scent leaf (*Ocimum gratissimum*) and Bitter leaf (*Vernonia amygdalina*) diets**

Parameter	Diet			Gender		SEM	P-value		
	1	2	3	Male	Female		T	G	T x G
Oestrogen (pg/ml)	83.30	98.00	89.30	93.60	89.7	9.28	ns	ns	ns
FSH (mUI/ml)	1.90	2.89	1.79	2.15	2.24	0.94	ns	ns	ns
LH (mUI/ml)	1.05	1.20	0.96	1.43 <sup>a</sup>	0.95 <sup>b</sup>	0.13	ns	<0.01	<0.05
Progesterone (ng/ml)	6.21	5.21	5.32	5.32	5.58	0.80	ns	ns	ns
Testosterone (ng/ml)	0.24	0.22	0.21	0.25	0.21	0.02	ns	ns	<0.05

a, b: Mean in the same row with different superscripts are significantly ( $p < 0.05$ ) different. SEM: standard error of mean. ns - not significant; FSH - Follicle Stimulating Hormone; LH - Luteinising Hormones.

Diet 1: Basal diet with no treatment (control)

Diet 2: Basal diet with bitter leaf (10g/kg)

Diet 3: Basal diet with scent leaf (10g/kg)

**Table 2: Reproductive performance of rabbits fed Scent leaf (*Ocimum gratissimum*) and Bitter leaf (*Vernonia amygdalina*) diets**

Parameters	Diet 1	Diet 2	Diet 3	SEM	P-value
Does' live weight before mating (kg)	1927.00 <sup>c</sup>	2298.00 <sup>a</sup>	2123.00 <sup>b</sup>	186.00	0.02
Sexual receptivity (0.5)	3.00 <sup>c</sup>	5.00 <sup>a</sup>	4.00 <sup>b</sup>	1.00	0.04
Conception rate (%)	50.00 <sup>b</sup>	100.00 <sup>a</sup>	50.00 <sup>b</sup>	50.00	0.02
Gestation length (days)	31.00	30.00	31.00	0.50	0.85
Litter size at birth (Number)	3.00 <sup>c</sup>	6.00 <sup>a</sup>	7.00 <sup>a</sup>	2.00	0.05
Litter weight at birth (g)	48.00 <sup>a</sup>	47.00 <sup>a</sup>	41.00 <sup>b</sup>	3.50	0.05

a, b: Mean in the same row with different superscripts are significantly ( $p < 0.05$ ) different. SEM: standard error of mean., Diet 1: Basal diet with no treatment (control), Diet 2: Basal diet with bitter leaf (10g/kg) , Diet 3: Basal diet with scent leaf (10g/kg)

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