

SEX HORMONES AND FERTILITY OF RABBIT DOES FED *PHYLLANTHUS AMARUS* LEAF MEAL AS FEED SUPPLEMENT

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ABSTRACT

The reproductive hormones and fertility status of female rabbits (does) fed diets supplemented with *Phyllanthus amarus* leaf meal (PALM) were assessed in this study. In a 16 week feeding trial, forty rabbits were randomly allotted to standard rabbit diets (pelletized) with 0, 2, 4, and 6 % of PALM inclusion as PALM 1, PALM 2, PALM 3 and PALM 4, respectively. At the eighth and 12th weeks of the feeding trial, blood was sampled and assayed for serum steroids using standard procedure. After the 12th week, fourteen fertile adult bucks (not exposed to PALM) were introduced to service the does in a completely randomised design at ratio 1:3 (buck: does) in the early hours of the day (06:00 – 08:00 hr) without repeated mating. At kindling; gestation length, litter size, litter weight, gestation weight changes were also observed. The result obtained revealed that estradiol, follicle-stimulating hormone and luteinising hormones of virgin does were not significantly ($P > 0.05$) affected by PALM based diets. Does fed PALM had larger litter size and litter weight than does fed the control diet. Progesterone and follicle-stimulating hormones of gestating does were significantly ($P < 0.05$) affected by PALM inclusion. The study concluded that including up to 6% leaf meal of *Phyllanthus amarus* in rabbit diets will enhance litter performance of rabbits does.

Keywords: African mistletoe; does; conception rate; gonadotropin; litter performance

INTRODUCTION

The increasing incidence of drug resistance in humans and animals in recent times has necessitated the identification of plants with medicinal properties and therapeutic role to replace synthetic drugs. Evaluation of medicinal properties of herbs and their extracts reveal their possible uses in altering different reproductive functions such as facilitation of parturition, inhibition of uterine contraction and the alleviation of menstrual cramps (Ogbomade *et al.*, 2014). Several herbal plants and their extracts have been reported to enhance fertility and balance sex hormones in male and female animals as well as improve libido, sexual behaviour, mating performance, hasten implantation and spermatogenesis (Iranloye *et al.*, 2010). *Phyllanthus amarus* (known as *Eyin olobe* in Yoruba) is a candidate medicinal herb that has been utilised in African trado-medical practice for ages. It is used traditionally to remediate illnesses, diseases and organ damage, male and female infertility (James *et al.*, 2009). Jimoh *et al.* (2020) reported that 4 % inclusion of *Phyllanthus amarus* in rabbit diet acts as a growth promoter and does not pose any adverse effect on the health of animals.

However, there are contrasting scientific reports on the effect of *Phyllanthus amarus* on reproductive functions. Rao and Alice (2001) and Adedapo *et al.* (2003) reported adverse effects of *Phyllanthus amarus* at certain dosages, contrary to positive effects reported by Obianime and Uche (2009), George *et al.* (2013) and Ogbomade *et al.* (2014). It is therefore essential to establish a safe dose that will elicit the desired therapeutic effect on the fertility of animals. This study evaluated the dietary additive effect of *Phyllanthus amarus* leaf meal on the fertility and steroid hormones of rabbit does.

MATERIALS AND METHODS

Eight weeks-old 40 mixed bred rabbit does (with average body weight as 859.38 ± 46 g) were used in a 16 week feeding trial. A total of 10 does each were allotted per treatment in a completely randomised design into four dietary treatments. Fresh *Phyllanthus amarus* leaves were obtained from the farm premises and shade-dried to constant weight before milling. The leaf meal was referred to as *Phyllanthus amarus* leaf meal (PALM) and incorporated into the respective diets such as PALM 1, 2, 3 and 4 consisting of 0, 2, 4 and 6% PALM inclusion respectively. The respective diets were pelletized to contain dry matter (87.87 %), crude protein (16.44 %), energy (2717.86 Kcal/kg), ether

extract (3.34 %), crude fibre (16.48%), lysine (11.01 %), methionine (0.75 %), calcium (1.60%) and phosphorus (0.45 %).

Table 1: Gross composition of experimental diets

Ingredient	PALM1	PALM 2	PALM3	PALM4
Maize	25.00	25.00	25.00	25.00
Wheat offal	4.80	4.80	4.80	4.80
Rice bran	6.00	4.00	2.00	0.00
Brewer dried grain	5.00	5.00	5.00	5.00
Soyabean meal	17.00	17.00	17.00	17.00
Groundnut haulms	40.00	40.00	40.00	40.00
Methionine	0.40	0.40	0.40	0.40
Lysine	0.10	0.10	0.10	0.10
Bone meal	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Vegetable oil	0.20	0.20	0.20	0.20
PALM	0.00	2.00	4.00	6.00
Total	100.00	100.00	100.00	100.00
Nutrient analysis				
Dry Matter (%)	87.87	86.07	84.27	82.47
Crude Protein (%)	16.44	16.20	15.96	15.72
Metabolisable Energy (kcal/kg)	2717.86	2660.66	2603.46	2546.26
Ether Extract (%)	3.34	3.09	2.84	2.59
Crude Fibre (%)	16.48	16.23	15.98	15.73
Lysine (%)	1.01	1.00	0.99	0.98
Methionine (%)	0.75	0.74	0.74	0.73
Calcium (%)	1.60	1.60	1.60	1.60
Phosphorus (%)	0.45	0.44	0.43	0.42

Throughout the feeding trial, animals were fed based on 5% body weight, and fresh water was provided *ad libitum*. The weight of rabbits was monitored weekly throughout the experimental duration. At the 12th week, fourteen fertile adult male rabbits (bucks) were introduced from the farm; the males were fed commercial rabbit pellets with NRC 1977 requirements of rabbits and were not exposed to PALM. Natural mating was carried out by moving does to buck at ratio 1:3 buck to doe in the early hours of the day (06:00 – 08:00hr) without repeated mating. At the third trimester, preparations were made for kindling boxes, and parity records were taken at kindling such as gestation length, litter size, litter weight, gestation weight changes. At the 10th and 15th weeks into the feeding trial, fasted blood samples were collected from all does and serum obtained. Serum hormonal assay was conducted on samples. Samples collected at ten weeks (pre-mating) were assayed for estradiol, luteinising hormone and follicle-stimulating hormone. Serum samples collected at 15 weeks (third trimester of gestation) were assayed for progesterone, luteinising hormone and follicle-stimulating hormone using Calbiotech, Inc Kit Elisa kits. Data obtained was subjected to one-way analysis of variance of general linear procedure of SAS Institute Inc. 2003. The New Duncan's multiple range test of the same software was used to separate significant means.

RESULTS AND DISCUSSION

The sex hormones of rabbit does were not significantly ($P>0.05$) affected by *Phyllanthus amarus* leaf meal based diets (Table 2). Plants with estrogenic property can directly influence pituitary action, by peripheral modulation of luteinising hormone and follicle-stimulating hormone. PALM did not influence serum levels of FSH, LH and Estradiol of virgin rabbits does. This suggests that the leaf meal does not alter ovulation, preparation of the reproductive tract for zygote implantation and the subsequent maintenance of pregnancy state. This finding is contrary to inhibitory roles of some plants and extracts on the Hypothalamic - Pituitary Gonadal (HPG) axis (Hsia *et al.*, 2007).

Table 2: Effect of *Phyllanthus amarus* leaf meal based –diets on sex hormones of virgin rabbit does

Hormone	PALM1	PALM 2	PALM3	PALM4	SE	P value
Estradiol (ng/ml)	16.00±5.20	13.75±2.87	12.50±0.71	13.00±2.00	0.85	0.59
Lutenizing Hormone (mIU/ml)	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.58	0.10	0.17
Follicle Stimulating Hormone (mIU/ml)	0.33±0.58	0.00±0.00	0.00±0.00	0.00±0.00	0.07	0.38

Female rabbits (does) fed *Phyllanthus amarus* leaf meal based – diets had larger litter size and litter weight than does fed the control diet (Table 3). The highest values of litter size and litter weight were obtained in does fed diets with 4 % *Phyllanthus amarus* leaf meal. The average kitt weight of does on 0 and 2 % *Phyllanthus amarus* leaf meal based – diets were higher than does fed 4%. The 4% inclusion of PALM enhanced the litter size of the animals. This is contrary to claims that aqueous extract of *Phyllanthus amarus* hasten implantation but does not sustain the resulting pregnancy as it causes abortion. This is because it has an abortifacient property which does not support the traditional claim that it can treat sterility but may ease difficult childbirth (Iranloye *et al.*, 2010).

Table 3: Fertility response of rabbit does fed *Phyllanthus amarus* leaf based - diets

Parameter	PALM 1	PALM 2	PALM3	PALM4	SEM
Initial weight (before mating, kg)	2.00	2.00	2.08	2.01	0.02
Final weight (at kindling, kg)	2.23	2.23	2.25	2.13	0.05
Gestational changes (g/day)	7.74	5.80	0.00	2.68	1.48
Litter size (Kitts)	1.67 ^b	2.25 ^{ab}	4.50 ^a	3.50 ^{ab}	0.45
Gestational length (days)	31.33	31.50	31.50	30.75	0.28
Litter weight (g)	62.97 ^b	79.39 ^b	148.74 ^a	132.28 ^a	18.04

*Means on the same row with different superscripts are significantly different (P < 0.05)

SE: standard error, P value: probability value

Progesterone and follicle-stimulating hormones of gestating does were significantly (P < 0.05) affected by *Phyllanthus amarus* leaf meal based diets (Table 4). Luteinizing hormone of does were statistically similar across dietary treatments. Serum progesterone and follicle-stimulating hormone tended to increase with increasing dosage of *Phyllanthus amarus* leaf meal. The ranges of serum hormone values obtained in gestating Does were higher than those obtained in virgin Does. The result of this study indicated that the androgenic effect of PALM in rabbit diets was more pronounced in gestating does than virgin does. The steroid hormones of virgin does were not affected by PALM inclusion; however, during gestation, the inclusion of PALM influenced progesterone and FSH. Progesterone and FSH activities peak at 6 % PALM inclusion in rabbit does. This supported the claims of Ogbomade *et al.*, (2014) who reported increase in the follicle-stimulating hormone of Wistar rats administered oral doses of *Phyllanthus amarus*.

Table 4: Effect of *Phyllanthus amarus* leaf meal based – diets on hormonal profile of gestating Rabbit Does

Parameter	PALM 1	PALM 2	PALM 3	PALM 4	SEM	P value
Progesterone (ng/ml)	22.75±14.48 ^a	15.00±9.20 ^b	20.50±4.36 ^{ab}	25.75±13.07 ^a	3.38	0.02
Lutenizing Hormone (mIU/ml)	0.25±0.50	0.25±0.50	0.00±0.00	0.25±0.50	0.10	0.35
Follicle Stimulating Hormone (mIU/ml)	0.25±0.50 ^b	0.00±0.00 ^b	0.25±0.50 ^b	1.25±1.89 ^a	0.26	0.04

*Means on the same row with different superscripts are significantly different (P < 0.05)

SE: standard error, P value: probability value

Follicle-stimulating hormone is pivotal to mammalian reproduction, instrumental in gonadal development, sexual maturity at puberty and gamete production (Simon and Nieschlag, 1995). It stimulates the growth and maturation of ovarian follicles by influencing granulosa cells receptors. FSH levels of gestating rabbit does were enhanced by PALM. PALM might have exerted its effect on the anterior pituitary or the hypothalamus, due to its regulatory role in the secretion of follicle-stimulating hormone. The increase observed in the level of this hormone would enhance conception in female animals. LH activities were not affected by PALM inclusion in rabbit diets; this is contrary to a dose-dependent decrease in luteinising hormone in Wistar rats (Ogbomade *et al.*, 2014). Luteinizing hormone surge stimulates ovulation of mature follicles in the ovary. Any substance capable of altering its release could provoke disruption of ovulation by influencing the number of mature follicles. It is evident that the dose of *Phyllanthus amarus* does not affect LH in both virgin and gravid does. George *et al.* (2013) reported an increase in serum progesterone and estrogen of rats gavaged aqueous *Phyllanthus niruri* extract. Similarly, progesterone and estrogen of pregnant rats were enhanced by aqueous extract of *Phyllanthus amarus* at an early stage, which suggests that it hastens implantation. However, the dosage does not sustain the resulting pregnancy as it causes abortion (Iranloye *et al.*, 2010). The result of this study suggests that leaf meal of *Phyllanthus amarus* is safe and does not adversely affect pregnancy but enhances litter productivity, unlike the extracts which have been reported by previous authors.

CONCLUSION

The study concluded that including *Phyllanthus amarus* leaf meal up to 6 % in rabbit diets will enhance reproductive performance. However, to optimise litter size of rabbit does, 4 % inclusion of *Phyllanthus amarus* in rabbit diets is hereby recommended.

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