

Steroids and fertility of rabbit does feed *Phyllanthus amarus* leaf meal as feed additive

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ADDITIONAL KEYWORDS

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Conception rate.
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SUMMARY

There are growing concerns on the impact of growth promoters on the reproductive physiology of animals. The utilisation of herbal supplements such as *Phyllanthus amarus* as viable alternative to synthetic growth promoters in rabbit production has been established. 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 parturition, the gestation length, litter size, litter weight, gestation weight changes were 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. In conclusion, study revealed that *Phyllanthus amarus* utilised as phyto-additives influenced the steroids of female rabbits to actualise conception. The inclusion of *Phyllanthus amarus* leaf meal up to 6% in rabbit diets enhance litter performance of rabbits does.

Esteroides y fertilidad de conejos alimentados con harina de hojas de *Phyllanthus amarus* como aditivo alimentario

RESUMEN

Existe una preocupación creciente sobre el impacto de los promotores del crecimiento en la fisiología reproductiva de los animales. Se ha establecido la utilización de suplementos a base de hierbas como *Phyllanthus amarus* como alternativa viable a los promotores de crecimiento sintéticos en la producción de conejos. En este estudio se evaluaron las hormonas reproductivas y el estado de fertilidad de conejas (conejas) alimentadas con dietas suplementadas con harina de hojas de *Phyllanthus amarus* (PALM). En una prueba de alimentación de 16 semanas, se asignaron al azar cuarenta conejas a dietas estándar para conejos (granulados) con 0, 2, 4 y 6% de inclusión de PALM como PALM 1, PALM 2, PALM 3 y PALM 4, respectivamente. En la octava y duodécima semana de la prueba de alimentación, se tomaron muestras de sangre y se analizaron los esteroides séricos utilizando un procedimiento estándar. Después de la semana 12, se introdujeron catorce machos adultos fértiles (no expuestos a PALM) para dar servicio a las hembras en un diseño completamente al azar en una proporción de 1: 3 (machos: machos) en las primeras horas del día (06:00 - 08: 00 h) sin apareamiento repetido. En el parto, se observaron cambios en la duración de la gestación, el tamaño de la camada, el peso de la camada y el peso de la gestación. El resultado obtenido reveló que el estradiol, la hormona estimulante del foliculo y las hormonas luteinizantes de las hembras vírgenes no se vieron afectados significativamente ($P > 0,05$) por las dietas basadas en PALM. Las aves alimentadas con PALM tenían un tamaño de camada y un peso de camada mayores que las alimentadas con la dieta de control. La progesterona y las hormonas estimulantes del foliculo de las hembras gestantes se vieron afectadas significativamente ($P < 0,05$) por la inclusión de PALM. En conclusión, el estudio reveló que *Phyllanthus amarus* utilizado como fitoaditivos influyó en los esteroides de las conejas para actualizar la concepción. La inclusión de harina de hojas de *Phyllanthus amarus* hasta un 6% en las dietas de conejos mejora el rendimiento de la camada de los conejos.

PALABRAS CLAVE ADICIONALES

Muérdago africano.
Hace.
Tasa de concepción.
Gonadotropina.
Rendimiento de la camada.

INFORMATION

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INTRODUCTION

The increasing incidence of drug resistance in humans and animals in recent times necessitates the identification of plants with medicinal properties and therapeutic role to replace synthetic drugs. Evaluation of medicinal properties of herbs and its extracts reveal their use to alter different reproductive functions such as facilitation of parturition, inhibition of uterine contraction, for 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 improve libido, sexual behaviour, mating performance, hasten implantation and spermatogenesis (Ajuogu et al., 2018; Ekpo et al., 2019; Iranloye et al., 2010). *Phyllanthus amarus* is a candidate medicinal herb that has been utilised in African trado-medical practice for ages. The active ingredient in *Phyllanthus* are phyllanthin and hypophyllanthin; these secondary metabolites are mainly responsible for their therapeutic or pharmacological activities (Geethangili & Ding, 2018). *Phyllanthus amarus* 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 those not pose an adverse effect on the health of the animals.

Rao & Alice (2001) reported the antifertility effect of *Phyllanthus amarus* at a dosage level of 100 mg/kg body weight of male and female mice which was attributed to changes in the hormonal profile that regulates reproductive function in animals. Similarly, Adedapo et al. (2003) reported different degrees of testicular degeneration and reduction in diameter of seminiferous tubular of rats treated with extracts of *P. amarus*. Reports of decrease in male fertility potentials after treatment of animals with anti-malaria drugs of both herbal (such as *P. amarus*) and synthetic origins, calls for caution and investigation (Ogbomade et al., 2014). It is essential to establish a safe dose that will elicit the desired therapeutic effect on the fertility of animals. This study aimed to evaluate the dietary additive effect of *Phyllanthus amarus* leaf on fertility and steroid hormones of rabbit does.

MATERIALS AND METHODS

This research was undertaken with approval from the institutional ethics committee with IACUC approval no:- FPA/EC/17/0165. Institutional and national standards (such as the National Institutes of Health guide for the care and use of Laboratory animals) for the care and use of animals for research were followed, and measures were taken to minimise pain or discomfort on the animals.

EXPERIMENTAL ANIMALS AND FERTILITY ASSESSMENT

Eight weeks-old 40 mixed bred rabbit does (with average body weight as $859.38 \pm 46g$) housed individually were used in a 16 week feeding trial. A total of ten does each were allotted per treatment in a completely randomised design into four dietary treatments. The rabbit house has natural lighting pro-

gram (12D:12L), with the dynamic ventilation system. The experimental units are made of wire mesh double sided boxes of 1.1sq. m. 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). Throughout the feeding trial, animals were fed 5% body weight, and freshwater was made available to the animals always. The weight of rabbits was monitored weekly throughout the experimental duration. At 12 weeks of feed trial, fourteen fertile adult males were introduced from the farm; the males were fed commercial rabbit pellets with NRC 1977 requirement of rabbits and were not exposed to PALM. Natural mating was conducted by moving does to buck at ratio 1:3 buck to doe in the early morning 06:00hr–08:00hr without repeated mating. At the third trimester, preparations were made for kindling box, and parity records were taken at kindling; gestation length, litter size, litter weight, gestation weight changes as outlined by Jimoh & Ewuola (2017).

STEROID HORMONE ASSESSMENT

At 10 and 15 weeks into the feed trial, fastened blood samples were collected from all bucks through the ear vein into plain sample bottles and were centrifuged at 3000 rpm for 15 min and supernatant separated as serum. 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. Hormones were assayed using Calbiotech, Inc Kit Elisa kits and its procedure; luteinising hormone Catalog No.: LH231F, progesterone Catalog No. PG129S, follicle-stimulating hormone Catalog No.: FS046F, estradiol Catalog No. ES180S.

STATISTICAL ANALYSIS

Data obtained in this study were subjected to one-way analysis of variance of general linear procedure SAS Institute Inc 2003, significant effects were detected $p < 0.05$. New Duncan's multiple range test of the same software was used to separate mean differences.

RESULTS

EFFECT OF PALM ON THE HORMONAL PROFILE OF VIRGIN RABBIT DOES

The effect of PALM on the hormonal profile of virgin rabbit does is presented in Table 2. The sex hormone of rabbit does were not significantly ($p > 0.05$) affected by *Phyllanthus amarus* leaf meal. The sex hormone of gestating does fed *Phyllanthus amarus* leaf meal is shown in Table 4. Progesterone and follicle-stimulating hormones of gestating does were signi-

ificantly ($p < 0.05$) affected by *Phyllanthus amarus* leaf meal. Luteinizing hormone of does were statistically similar across treatments. Serum progesterone and follicle-stimulating hormone tended to increase with a dosage of *Phyllanthus amarus* leaf meal. The ranges of serum hormone values obtained in gestating Does were higher than those obtained in virgin Does.

FERTILITY OF RABBIT DOES FED PHYLLANTHUS AMARUS LEAF MEAL

The fertility of rabbit Does fed *Phyllanthus amarus* leaf meal is shown in Table 3. The final weight, gestation weight changes and gestation length were not significantly ($p > 0.05$) affected by the test diets. While litter size, litter weight and average kilt weight were significantly ($p < 0.05$) influenced by *Phyllanthus amarus* leaf meal inclusion in rabbit diets. Does fed *Phyllanthus amarus* leaf meal had larger litter size and litter weight than does fed control diet. The highest values of litter size and litter weight were obtained in does fed 4% *Phyllanthus amarus* leaf meal. The average kilt weight of does on 0% and 2% *Phyllanthus amarus* leaf meal were higher than does on 4%.

DISCUSSION

Estradiol is principally responsible for the growth and development of female reproductive organs. In synergy with follicle-stimulating hormone, estradiol stimulates granulosa cell proliferation during follicular development (Alfa & Rasheed, 2008). Plants with estro-

genic property can influence pituitary action directly, 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 leaf meal does not alter ovulation, preparation of the reproductive tract for zygote implantation and the subsequent maintenance of pregnancy state, this is contrary to inhibitory roles of some plants and extract on HPG axis (Hsia et al., 2007; Egba et al., 2015). 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 supports the claims of Ogbomade et al., (2014) that increase in the follicle-stimulating hormone of Wistar rats administered oral doses of *Phyllanthus amarus*. Similarly, Obianime and Uche (2009) reported that *P. amarus* extract caused an increase in the level of testosterone in Guinea pigs. 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).

Follicle-stimulating hormone is pivotal to mammalian reproduction, instrumental in gonadal development, sexual maturity at puberty and gamete production (Simon & Nieschlag, 1995). It acts to stimulate growth and maturation of ovarian follicles by influencing granulosa cells receptors. FSH of gestating rabbit does were enhanced by PALM. PALM might

Table 1. Gross composition of experimental diet (Composición bruta de la dieta experimental).

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
PALM: <i>Phyllanthus amarus</i> leaf meal				

Table II. Effect of *Phyllanthus amarus* leaf meal on sex hormones of virgin Rabbit Does (Efecto de la harina de hojas de *Phyllanthus amarus* sobre las hormonas sexuales de conejas vírgenes)

	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	0.33±0.58	0.00±0.00	0.00±0.00	0.00±0.00	0.07	0.38
Hormone (mIU/ml)						

Different superscript denote statistically significant differences at $p \leq 0.05$, SE: standard error, P value: probability value, PALM: *Phyllanthus amarus* leaf meal

Table III. Fertility response of Rabbit does fed *Phyllanthus amarus* leaf inclusive diets (Respuesta de fertilidad de conejas alimentadas con dietas que incluían hojas de *Phyllanthus amarus*).

	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

Different superscript denote statistically significant differences at $p \leq 0.05$, SE: standard error, P value: probability value, PALM: *Phyllanthus amarus* leaf meal

Table IV. Effect of *Phyllanthus amarus* leaf meal on hormonal profile of gestating Rabbit Does (Efecto de la harina de hojas de *Phyllanthus amarus* sobre el perfil hormonal de conejas gestantes).

	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

Different superscript denote statistically significant differences at $p \leq 0.05$, SE: standard error, P value: probability value; PALM: *Phyllanthus amarus* leaf meal

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. 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.

The result of the current study indicates that the androgenic effect of PALM in rabbit diet was more pronounced in gestating does than virgin does. 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. 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). The findings in this study are not in line with reports that reduction in the levels of follicle-stimulating hormone by garlic extract; possesses an inhibitory effect on the release of the gonadotropin, hamper folliculo-

genesis and actively delay maturation of the follicle in the pre-ovulatory phase (Egba et al., 2015). This could be associated with the higher concentration of active ingredients in extract and the doses administered in the previous reports.

George et al. (2013) reported an increase in serum progesterone and estrogen of rat gavaged aqueous *Phyllanthus nururi* extract. Similarly, progesterone and estrogen of pregnant rats were enhanced by aqueous extract of *Phyllanthus amarus* at an early stage, which suggests that it hasten 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, does not adversely affect pregnancy but enhances litter productivity, unlike extracts which have been reported by previous authors. This is similar to claims by Dafaalla et al., (2015) that leaves of *R. sativus* ingestion produce increased effects on male fertility hormone and sperm quality in the adult male rat. The study reveals that the phytoconstituents of *Phyllanthus amarus* enhances growth and maturation of follicle to enhance litter size of does. Thus, in the actualisation of fast growing rabbit for meat production, the fertility and litter performance of female rabbits can be enhanced.

It is comforting to know that *Phyllanthus amarus* feed additive known to stabilize lipid and promote growth of rabbits, will enhance reproductive organ development and function to enhance breeding performance of the flock.

CONCLUSION

The study reveals that the inclusion of *Phyllanthus amarus* leaf meal up to 6% enhance the reproductive performance of female rabbits. However, for optimal litter size of rabbit does, 4% inclusion of *Phyllanthus amarus* in rabbit diets is recommended.

COMPETING INTEREST

No competing interest exists in the research outcome presented in this article

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