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Effects of Monk's Pepper (*Vitex agnus-castus*) on Utero-ovarian Morphometry, Reproductive Performance of Rabbit Does, and Pre-Weaning Performance of their Bunnies

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Abstract

THIS study investigated the effects of dietary inclusion of monk's pepper (*Vitex agnus-castus*) on the utero-ovarian morphometry, reproductive performance of rabbit does, and pre-weaning growth of their bunnies. A total of 24 healthy rabbit does aged four months and averaging 2.2 ± 0.3kg body weight, were randomly assigned to four dietary treatment groups in a completely randomized design. The control group (T1) received a basal diet without Vitex agnus-castus, while treatments T2, T3, and T4 had inclusions of *Vitex agnus-castus* fruit powder at 2, 4, and 6 g/kg, respectively. Each treatment was replicated thrice, with two does per replicate. The experiment lasted 16 weeks. At the end of the 4-week feeding period, reproductive organs were excised post-mortem for morphometric evaluation. Ovarian and uterine dimensions were measured as indicators of reproductive tract morphometry. Reproductive parameters assessed included litter size and litter weight at birth and weaning, and pre-weaning growth performance of the bunnies. Results showed that 4 g/kg *Vitex agnus-castus* (T3) significantly ($p<0.05$) improved litter size, and total litter weight at birth and weaning. Similarly, T3 does had the highest mean weights of ovaries and uterine structures, and enhanced uterine horn and body lengths. While 6 g/kg (T4) significantly ($p<0.05$) improved individual bunny weight during the pre-weaning phase, it reduced litter size and total litter weight, suggesting dose-dependent antagonistic effects. These findings indicate that *Vitex agnus-castus* at 4 g/kg optimally enhanced fertility and maternal function in rabbit does, offering a promising, low-cost phytogenic alternative for mitigating reproductive inefficiencies in tropical regions.

Keywords: Morphometry, phytoestrogens, rabbit does, reproductive performance, *Vitex agnus-castus*.

Introduction

Livestock remains a cornerstone of global food security, contributing significantly to nutritional, economic, and livelihood outcomes [1]. In Nigeria, traditional livestock species have failed to meet the rising demand for animal protein, contributing to widespread protein-energy malnutrition [2]. Rabbit (*Oryctolagus cuniculus*) farming presents a promising alternative due to the animal's high reproductive potential, short gestation period, and efficient feed conversion [3, 4]. Rabbit meat is particularly rich in calcium and phosphorus, high in digestible protein and B-vitamins while also being lower in fat and cholesterol compared to beef and pork, making it a healthier source of animal protein [5]. It also requires less land and capital investment compared to conventional livestock [6]. Despite these advantages, rabbit production in tropical

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climates has faced significant constraints because reproductive efficiency in rabbits is highly susceptible to environmental stressors, particularly heat stress, which alters hormonal balance and impairs ovarian function [7]. High ambient temperatures above 35°C negatively affect ovarian function, hormone secretion, and embryonic development, often leading to reduced litter sizes, irregular estrus, increased fetal mortality and compromise the overall productivity of rabbit does [8, 7, 9]. To mitigate this, the use of phytogenic feed additives with antioxidant and hormone-modulating properties has gained interest as a natural, cost-effective, and safe alternative to synthetic growth promoters and antibiotics [10, 11].

Vitex agnus-castus, also known as monk's pepper, is a perennial shrub of the Verbenaceae family, traditionally used to manage gynecological disorders in humans due to its phytoestrogenic and luteinizing hormone-modulating activities and antioxidant properties [12, 13]. Phytochemical analyses of *V. agnus-castus* have identified iridoid glycosides (e.g., agnuside), flavonoids (e.g., apigenin, casticin), and diterpenoids, which exhibit estrogenic and dopaminergic properties [14, 15]. These properties potentially support its role in enhancing reproductive performance in livestock.

Although some studies have demonstrated positive effects of *V. agnus-castus* on reproductive performance in rodents [16, 17], there remains limited empirical evidence on its application in rabbits. This study, therefore, investigates the effect of graded dietary levels of *V. agnus-castus* fruit powder on ovarian morphometry, reproductive performance of rabbit does, and the growth performance of their bunnies during the pre-weaning phase, providing insights into its potential as a natural fertility enhancer under tropical climatic conditions.

Material and Methods

Study Location and Duration

The experiment was conducted in natural tropical environmental settings at the Rabbit Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, located in Nsukka Local Government Area, Enugu State (latitude 6◦ 51′39″ N and longitude7◦ 21′39″ E). The study lasted for 16 weeks, from January to April 2024.

Experimental Animals and Design

Twenty-four (24) clinically healthy, sexually mature rabbit does aged four months and with an average body weight of 2.20 ± 0.3 kg were used in this study. Upon arrival, animals were allowed to acclimatize for one week under uniform management conditions. They were then weighed individually using an electronic digital scale and randomly assigned to four dietary treatment groups (T1–T4), with each group comprising six animals. Each treatment group was further replicated three times, with two animals per replicate, in a completely randomized design (CRD).

Diet Formulation and Monk's Pepper Preparation

All groups received a commercial grower mash containing 16% crude protein and 2,650 kcal/kg metabolizable energy (Supreme Feeds®). The monk's pepper (*V. agnus-castus)* fruits were sourced from Ondo State, Nigeria, authenticated by a botanist, air-dried at ambient temperature, ground, and was incorporated into the diet at 0 (control), 2, 4, and 6 g/kg of feed for Treatments T1–T4, respectively. Feed and water were provided ad libitum throughout the 16-week experimental period.

Management Practices

Animals were housed in individual wire mesh cages, with *ad libitum* access to clean drinking water and feed. Standard biosecurity and hygiene protocols were maintained throughout the experimental period.

Data Collection

Reproductive Performance

Parameters calculated included litter size at birth and weaning, total and average litter weight at birth and weaning.

Pre-Weaning Growth Performance

Bunnies were weighed at birth by means of a weighing scale and weekly thereafter for six weeks.

Ovarian Morphometric Evaluation

At the end of the experiment, two (2) does were randomly selected from each treatment group and humanely sacrificed via cervical dislocation under light anesthesia, in accordance with institutional ethical standards and the American Veterinary Medical Association (AVMA) guidelines [18]. The reproductive tract was exteriorized through a mid-ventral abdominal incision. The ovaries, uterine horns, and uterine body were carefully excised, trimmed of adherent tissues, and subjected to morphometric analysis. Organ weights were determined using a digital precision balance (M-METTLER, M411L), while lengths were measured using a digital vernier caliper and flexible measuring tape and recorded to the nearest 0.1 cm.

Statistical Analysis

Data generated from the experiment were subjected to one way analysis of variance (ANOVA) using Statistical Product and Service Solutions (IBM SPSS Statistics) version 23. Results were presented as Mean ± SE. Significantly different means were separated using Duncan's New Multiple Range Test [19], and accepted at 5% level of probability.

Results

Effect of Monk's Pepper (Vitex agnus Castus) on the Reproductive performance of Rabbit Does

Table 1, shows the effect of Monk's Pepper (*Vitex agnus Castus*) on the reproductive performance of rabbit does. Dietary inclusion with *V. agnus-castus* significantly ($p<0.05$) affected litter size, average litter weight at birth and total litter weight at birth and weaning. The T3 does (4 g/kg) had the highest ($p<0.05$) average litter size at birth (7.07) and total litter weight at birth (344.83g), compared to T4 (3.80 g and 174.89 g, respectively). Average litter weight at birth was highest in T2 (52.00 g), and lowest in T4 (43.72 g) does. Average litter weight at weaning was highest in T4 (615.87 g), while T2 (379.45 g) bunnies had the lowest. Total litter weight at weaning was highest at T3 (2611.07 g) and lowest at T4 (1807.17 g).

The effect of Monk's pepper (Vitex agnus castus) on the growth and pre-weaning performance of the bunnies

Table 2, shows the effect of Monk's pepper (*Vitex agnus castus)* on the growth and pre-weaning performance of the bunnies. There were no significant ($p>0.05$) differences in birth weight across treatments. However, T4 bunnies showed significantly higher body weights from week 4 (303.87 g) through week 6 (593.04 g). T2 had the highest weight at week 1 (108.42g), while T3 and T4 gained progressively more weight in later weeks.

The effect of Monk's pepper (Vitex agnus castus) on the ovarian and uterine morphometry of rabbit does

Table 3, shows the effect of Monk's pepper (*Vitex agnus castus)* on the ovarian and uterine morphometry of rabbit does. Dietary inclusion of *V. agnus-castus* exerted a statistically significant ($p < 0.05$) effect on ovarian morphometric parameters in rabbit does (Table 3). The group receiving 4 g/kg of Vitex (T3) demonstrated superior outcomes across all measured indices. Specifically, T3 recorded the highest mean weights of the right and left ovaries (17.42g and 16.93g, respectively), as well as the greatest uterine body and horn weight (17.50 g). Uterine structural dimensions were also enhanced in T3, with the longest right and left uterine horns (36.00cm and 33.85cm, respectively), and the longest uterine body (14.40 cm). These values were significantly higher compared to the control group (T1: 0 g/kg), while T2 (2 g/kg) and T4 (6 g/kg) exhibited moderate improvements.

Discussion

The results demonstrate that dietary inclusion of *V. agnus-castus* significantly enhances reproductive performance in rabbit does, especially at 4 g/kg feed. The improved litter size and weight at birth observed in T3 are consistent with the findings of Adamu et al. [17] and Ezekwe et al. [20], who reported enhanced fertility and implantation rates following *V. agnus-castus* supplementation in female rodents. Similarly, Ghany et al. [21] found that *V. agnus-castus* supplementation improved reproductive traits such as conception rate, litter size, and weight in rabbits. This improvement is attributed to the plant's estrogenic compounds, particularly apigenin and casticin, which bind selectively to estrogen receptor-β [14, 22]. The increase in total litter size and weight may also be linked to improved uterine receptivity and embryo viability, potentially mediated by increased luteinizing hormone (LH) and progesterone levels [23]. However, at the highest inclusion level (6 g/kg), reproductive outcomes were suppressed, suggesting possible overstimulation or negative feedback on hypothalamic-pituitary-gonadal axis [24].

Improved feed utilization leads to increased weight gain and better pre-weaning performance [25]. Interestingly, pre-weaning growth trends showed that T4 (6 g/kg) bunnies, exhibited superior individual growth, particularly after week 3. This may be due to reduced intra-litter competition for milk because the bunnies were fewer in number and the potential galactagogue effects of *V. agnus-castus* on maternal milk production [21]. The presence of iridoid glycosides and flavonoids like catalpol, aucubin and apigenin have shown to stimulate appetite, improve nutrient digestibility and metabolism in both does and their offspring [25]. Sirotkin et al. [26] reported that apigenin may indirectly influence prolactin levels. Increased prolactin helps stimulate milk production for nursing litters. When consumed during gestation and lactation, apigenin's effects are thought to program the hypothalamic-pituitary-adrenal axis in offspring, improving their postnatal development. The effect of *V. agnus-castus* on the bunnies at birth through to week 6 supports the claims of İnal et al. [27], who reported that essential oils present in monk's pepper contain monoterpenes such as limonene, caryophyllene and sabinene. These aids in digestion, have anti-microbial effects, improves the secretion of hormones involved in growth and development, and protects the gut from pathogens that can stunt growth. By enhancing digestion, nutrient utilization, and immunity, the constituents in *V. agnus-castus* promoted weight gain and improved growth performance in rabbit bunnies. However, these findings underscore the dosage-dependent efficacy of *V. agnus-castus* in rabbit production. Moderate inclusion (4 g/kg) optimizes reproductive performance, while higher levels may favor growth at the expense of reproductive indices.

Our study demonstrated that dietary supplementation with *V. agnus-castus* significantly enhanced ovarian morphometric parameters in rabbit does, with the 4 g/kg inclusion level (T3) producing

the most pronounced effects. These improvements included increased ovary weights, uterine horn lengths, and uterine body dimensions, which are critical indicators of reproductive health and functionality. Estrogen is a key regulator of female reproductive physiology. It is a steroid hormone primarily produced by the ovaries and it plays a crucial role in regulating various aspects of the female reproductive system. It stimulates the growth and development of ovarian follicle leading to an increase in ovarian weight [28]. The observed increases in ovarian weight and uterine dimensions may be attributed to the phytoestrogenic properties of *V. agnus-castus.* Phytoestrogens, such as apigenin, vitexin, and casticin bioactive compounds present in Vitex have been shown to bind to estrogen receptors and mimic endogenous estrogen activity [29, 14]. Estrogen also promotes endometrial proliferation, and increased vascularization of reproductive tissues which manifests as increases in the length and weight of the uterine horn and body [30]. The significantly higher uterine horn and body measurements observed in T3 align with findings from Promprom et al. [31], who reported increased uterine weight and vaginal weight and endometrial thickness in *Vitex pinnata* treated rats. These morphological enhancements suggest that Vitex may exert its reproductive benefits by modulating the hypothalamic-pituitary-gonadal axis, thereby enhancing the secretion of gonadotropins such as LH and FSH, which stimulate ovarian follicle development and uterine growth [32, 33].

Interestingly, while both 2 g/kg (T2) and 6 g/kg (T4) inclusion levels produced moderate improvements over the control, the 4 g/kg dose (T3) yielded the highest values across all parameters. This suggests a dose-dependent effect up to a threshold beyond which the efficacy may plateau or decline, possibly due to receptor saturation or negative feedback mechanisms [34].

Conclusion

The findings of this study demonstrated the efficacy of *V. agnus-castus* (monk's pepper) as a natural dietary supplement for enhancing reproductive performance in rabbit does. The 4 g/kg inclusion level optimally enhanced ovarian structure, improved litter size and total litter weight at birth and weaning, suggesting enhanced fertility and maternal function. While a higher dose (6 g/kg) improved individual bunny weight during the pre-weaning period, it concurrently led to reductions in litter size and total litter weight, indicating potential dose-related antagonistic effects on reproduction. Given the increasing demand for natural growth enhancers and reproductive aids in animal production, especially in heat-stressed tropical regions, *V. agnus-castus* at 4 g/kg offers a promising, low-cost phytogenic alternative.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study followed the regulations of the Animal Welfare and Ethics Committee, Department of Animal Science, University of Nigeria, Nsukka (UNN/C025ARO12.12.01.2024) in ensuring minimal pain and discomfort to the specimen used in this study.

TABLE 1. Effect of Monk's Pepper (*Vitex agnus Castus*) on the Reproductive performance of Rabbit Does.

Parameters	Treatment				P Value
	T1 (0g/kg)	T2 (2g/kg)	T3 (4g/kg)	T4 (6g/kg)	
Average Litter Size at Birth	5.38 ± 0.12^{b}	5.29 ± 0.48^{b}	7.07 ± 0.13^{a}	3.80 ± 0.41^{c}	0.00**
Total Litter Weight at Birth (g)	281.08 ± 12.00^{b}	254.35 ± 11.67^{b}	344.83 ± 12.80^{a}	174.89 ± 18.96^{c}	0.00**
Average Litter Weight at Birth (g)	51.88 ± 0.97^{a}	52.00 ± 3.91^{a}	48.49 ± 1.06^{ab}	43.72 ± 1.28^{b}	0.04*
Average Litter Size at Weaning	3.81 ± 0.67^{b}	4.14 ± 0.64^{ab}	5.82 ± 0.44^{a}	3.30 ± 0.26^{b}	0.00**
Total Litter Weight at Weaning (g)	2321.45 ± 110.52^{ab}	2153.50 ± 109.76^{ab}	2611.07 ± 197.22^{a}	1807.17 ± 150.99^{b}	0.04*
Average Litter Weight at Weaning (g)	416.28 ± 8.24^{b}	379.45 ± 9.70^{b}	472.09 ± 17.38^{b}	615.87 ± 96.96^{a}	0.02*

*Significant (P<0.05), ** significant (P<0.01), NS – not significant.

^{abc} : means on the same column with different superscripts are significantly different ($p\leq0.05$).

TABLE 2. The effect of Monk's pepper (*Vitex agnus castus)* on the growth and pre-weaning performance of the bunnies.

Parameter	Treatment				P value
	T1 (0g/kg)	T2 (2g/kg)	T3 (4g/kg)	T4 (6g/kg)	
Birth Weight (g)	51.35±1.34	52.00±2.79	48.49±1.03	43.72±1.23	0.24^{NS}
Week 1	$73.82±2.70^{b}$	$108.42±8.84^{a}$	$79.95±2.27^{b}$	$68.07±3.95^{b}$	0.00**
Week 2	$119.35±4.80^{c}$	$170.10±12.71^{a}$	$145.60±3.73^{b}$	$142.09±13.89^{bc}$	0.00**
Week 3	$163.09±3.78^{b}$	$165.81±7.82^{b}$	$184.61±4.98^{ab}$	$187.25±9.08^{a}$	0.03*
Week 4	$218.20±3.57^{b}$	$228.97±10.08^{b}$	235 22±6.17^{b}	$303.87±27.68^{a}$	0.00**
Week 5	$296.81±3.92^{b}$	$295.17±13.19^{b}$	$327.91±8.21^{b}$	$446.07±43.60^{a}$	0.00**
Week 6	$407.35±9.10^{b}$	$385.39±21.80^{b}$	$447.31±14.10^{b}$	$593.04±56.06^{a}$	0.00**

*Significant (P<0.05), ** significant (P<0.01), NS – not significant.

^{a,b,c} – Means in the same row with different superscripts are significant at 5% (* P < 0.05.)

TABLE 3. Effect of dietary inclusion of *V. agnus-castus on* utero-ovarian morphometry of rabbit does

Parameter	Treatment				p-value
	T1 (0g/kg)	T2 (2g/kg)	T3 (4g/kg)	T4 (6g/kg)	
Right ovary weight (g)	$11.30±0.46^{c}$	$14.34±0.49^{b}$	$17.42±0.49^{a}$	$12.96±0.19^{b}$	0.001**
Left ovary weight(g)	$10.37±0.58^{d}$	$14.78±0.50^{b}$	$16.93±0.20^{a}$	$12.25±0.46^{c}$	0.004**
Uterine body and horn(g)	$9.30±0.40^{c}$	$15.05±0.02^{b}$	$17.50±0.80^{a}$	$12.80±0.46^{b}$	0.001**
Length of Right horn(cm)	$23.85±1.06^{c}$	$29.65±0.66^{b}$	$36.00±0.00^{a}$	$26.50±0.28^{c}$	0.005**
Length of left horn(cm)	$23.00±0.00^{c}$	$29.50±0.86^{b}$	$33.85±1.35^{a}$	$26.10±0.34^{bc}$	0.005**
Length of uterine body(cm)	$10.25±0.43^{c}$	$12.75±0.43^{b}$	$14.40±0.23^{a}$	$11.50±0.29^{bc}$	0.004**

$^{a,b,c,\ d.}$ – Means in the same row with different superscripts are significant at 5 or 1 % (P < 0.05* significant ; P < 0.01** highly significant)

g-grams, cm-centimetres

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