

EFFECT OF DIETARY SUPPLEMENTED COCOA POD HUSK MEAL ON THE REPRODUCTIVE PERFORMANCE OF RABBITS

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Supporting Information

ABSTRACT: This study determined the dietary effect of cocoa pod husk meal (CPHM) on the reproductive performance of rabbits. Twelve iso-nitrogenous (16.05% CP) and iso-caloric (2500.12 Kcal kg⁻¹ ME) diets were formulated. The CPHM was included at 0, 12.5, 25 and 37.5% levels for T₁, T₂, T₃, and T₄ raw; T₅, T₆, T₇, T₈ fermented and T₉, T₁₀, T₁₁, T₁₂ hot-water treated CPHM. Sixty weaned rabbits between 5 and 6 weeks old of both sexes (30 males and 30 females) with mean initial body weight of 606.42±1.30g were used. The rabbits were randomly distributed using a completely randomized design (CRD). The animals were crossed at maturity for reproductive performance evaluation. Total protein concentrations of reproductive parts were determined. Result showed no significant dietary effect on reproductive performance. The 37.5% level recorded zero pregnancy in the raw and hot-water groups. Average gestation period ranged between 30 and 31 days. Average litter size at birth ranged 1 – 4 kittens. Average weaning weight ranged between 475 and 580.25g with the least weight in the raw group. Milk yield ranged between 205.46 and 262.94g. The sperm volume and gonadal sperm reserve recorded significant effect (P<0.05). In the raw and hot-water groups, the sperm volume decreased marginally. The protein concentration in the testes recorded higher significant (P<0.05) values in the control diet and the least value in raw group. The study concluded that fermented CPHM diets performed best at 37.5% level in terms of reproductive performance of rabbits.

Keywords: Cocoa, Gonadal sperm reserve, Kindling, Milking, Sperm volume.

INTRODUCTION

Rabbits are prolific animals that can contribute significantly to food security in sub-Saharan Africa. Currently, we are living in a world with over 925 million food insecure people (FAO, 2010) and majority of these hungry people live in poor, rural communities of Asia and African continents with crop and livestock production as their primary occupations (FAO, 2011). The present hunger and malnutrition can partly be solved through intensive rabbit production, as a means of boosting animal protein intake (Ozung, 2021) and enhancing livelihood security (Halberg and Muller, 2013). Rabbits are short cycle animals with a gestation period of one month, making them suitable for multiplication during periods of food crisis. Rabbit production can yield enormous meat and cheap animal protein. Rabbit meat is healthy as it is low in cholesterol (50g 100⁻¹g); fat (4g 100⁻¹g); energy (124Kcal 100⁻¹g) but high in protein (22g 100⁻¹g) (Aduku and Olukosi, 1990). The meat has complete amino acid profile; it has good flavour, rich in minerals and other essential nutrients as well as easily digestible.

However, rabbit farming like other livestock production enterprises is facing feeding challenges in view of the high cost of conventional feed ingredients. This development has necessitated the search for alternative feed resources in rabbit nutrition. The domestic rabbit is a pseudo-ruminant (monogastric-herbivore or hind gut fermentor) that feeds on forages, grains/ concentrates, kitchen wastes, yam and cassava peels, hay and agro by-products (Mutsami et al., 2019; Gbenge, 2022). One of the promising agro by-products that can be utilized in rabbit feeding is the cocoa pod husk. Cocoa husk is a by-product from cocoa pods obtained from cacao trees grown in the tropics. According to Olubamiwa and Akinwale (2000), 25% cocoa pod husk meal (CPHM) can partially replace maize in layers' diet with no adverse effects on egg parameters. Untreated CPHM has been added in the diets of growing swine up to 300g Kg⁻¹ without deleterious signs on body characteristics (Oddoye et al., 2010). The practice of incorporating cocoa pod husk meal in animal diets (pigs, broilers, layers, rabbits) has been reported by various researchers in previous studies (Adomako et al. 1999; Agyente - Badu and Oddoye 2005, Akanbi, 2019; Ozung, 2021).

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Extant literature has reported that sun-dried cocoa beans contain 0.7% sugar, 0.7% starch, 1.7% theobromine (an alkaloid), 6.70% protein and 50-55% pale yellow, non-drying fat known as cocoa butter. The cocoa bean testa has been used successfully in feeding trials for small ruminants without adverse effects on performance characteristics in some countries. According to [Adomako et al. \(1999\)](#), cocoa pod husks constitute 75% of the entire cocoa fruit on fresh weight basis. [Adomako \(1991\)](#) reported that cocoa beans account for less than 2.55% of the whole fruit. Cocoa pod husks contain 6-7% crude protein, 9-10% total ash, 1-8% crude fat (ether extract) and 23-33% crude fibre. Furthermore, [Sobamiwa and Longe \(1994\)](#) reported that metabolizable energy of cocoa pod husk is moderate and ranges from 2000–2100 Kcal Kg⁻¹; which is comparable to that of palm kernel cake, soybean meal, rice bran and brewers dried grain. The chemical composition of cocoa pod husk meal shows that it contains total dry matter (42.25%), crude protein (9.69%), fatty substances (0.15%), ash (SiO₂ free) (10.80%), crude fibre (33.90%), Nitrogen free extract (42.21%), glucose (1.16%), sucrose (0.18%), pectin (5.30%) and theobromine (0.20%). The processed cocoa pod husks have been reported to have low theobromine content. The crude fibre is easily digestible. It is reported that cocoa pod husk is digestible by all classes of livestock, especially ruminants. However, the high crude fibre content (21.49-34.82%) hinders its effective utilization by monogastrics ([Abiola and Tewe, 1991](#)). The constraint of high fibre content and associated poor digestibility calls for the processing of the cocoa pod husks by various methods (fermentation, hot-water treatment, urea, enzyme, fungal treatment and microbial detheobromination), so as to promote digestibility and biodegradability in animals. Furthermore, some research studies with cocoa by-products have shown that at higher levels of dietary inclusion; the alkaloids (theobromine and caffeine) have adverse effects on the reproductive potential of animals ([Adomako et al., 1999](#)). According to [Ozung et al. \(2019\)](#), these alkaloids have been reported to penetrate the placenta and blood vessels, thereby causing foetal abnormality. Therefore, this study was designed to determine the effect of different forms of cocoa pod husk meal on the reproductive performance of rabbits.

MATERIAL AND METHODS

Study location

The study was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Calabar, Calabar, Cross River State, Nigeria, West Africa. According to the GeoNames geographical database Google Earth-2021; Calabar is located at 4.9517° latitude and 8.322° longitude (in decimal degrees) with an average elevation/ altitude of 42 metres. Other workers, [Akpan et al. \(2006\)](#) reported that Calabar is located at latitude 3°N of the equator and longitude 7°E of the Greenwich meridian, with a land mass of 233.2 sq. miles (604 km²). The annual rainfall ranges between 3000 and 3500mm (average of 1,830 mm) per annum and the average daily temperature is 25° C/77° F which increases to 30° C (86° F) in August. The relative humidity is between 70 and 80%.

Collection and handling of cocoa pod husk meal (CPHM)

Freshly broken composite and discarded cocoa pod husks were obtained from the fermentation units of cocoa plantations at the rainforest zone of Etomi, Ikom, Cross River State, Nigeria. The pods were collected during the main production season in West Africa (September – March). The broken pods were washed and sun-dried to constant weight, bulked and milled with hammer mill (Model 912, Winona Attrition Mill Co., Winona, MN) to produce cocoa pod husk meal (CPHM). The resultant meal was shared into three (3) portions: The raw CPHM (RCPHM), Fermented CPHM (FCPHM) and hot-water treated CPHM (HCPHM), respectively. CPHM for the fermented treatment was thoroughly mixed with 60% water, relative to its weight as ascertained by [Bello et al. \(2015\)](#) and bagged in an air tight polythene bag. This was allowed to stay for three days under room temperature, thereafter, it was opened and shade dried to constant weight; before being packed, bagged and stored in a cool dry place until it was used for diet formulation. The final portion of CPHM was treated with hot-water that was boiled to 100° C for 15 minutes ([Adeyina et al., 2010](#)) which was later drained, shade dried and stored for later use in feed formulation.

Experimental diets

Twelve iso-nitrogenous (16.05% CP) and iso-caloric (2500.12 Kcal kg⁻¹ME) diets were formulated in line with the nutrient needs of rabbits recommended by [Aduku and Olukosi \(1990\)](#). Each treated form of CPHM was included at 0, 12.5, 25 and 37.5% levels for T₁, T₂, T₃, and T₄ (Raw CPHM), T₅, T₆, T₇, T₈ (Fermented CPHM) and T₉, T₁₀, T₁₁, T₁₂ (Hot-water treated CPHM), respectively in the experimental diets. Diet without CPHM (0%) served as control in the experiment.

Experimental rabbits and management

A total of 60 weaned mixed breed rabbits between 5 and 6 weeks old of both sexes (30 bucks and 30 does) (mating ratio of male: female was 1:1), (mean initial body weight of 606.42±1.30 g) were used in this study. The rabbits were purchased from Domino farms, Useh-Offot in Uyo, Akwa Ibom State, Nigeria. They were managed based on standard experimental protocols. On arrival at the rabbitry facility, the animals were provided with anti-stress vitalyte at 0.5 g per 75 litres of chlorine-free water. The experimental animals were housed individually in double tier wooden hutches (with wire mesh floor) measuring 65 × 65 × 65 cm (L × H × W) and raised 25cm from the ground and placed in a standard rabbitry with half walls to allow for cross ventilation.

Concrete drinking troughs and fabricated feeding troughs were provided in each cage. The rabbits adjusted for two weeks before the actual commencement of the feeding trial and within this period; they were placed on commercial pelleted grower mash and screened against ecto and endo parasites via subcutaneous injection of Ivermectin (Kepromec) at the recommended level (0.2 ml per rabbit). Thereafter, the animals were subjected to 21 weeks feeding trial and at maturity (5 months), they were crossed for reproductive performance evaluation.

Animal welfare and ethical approval

In this study, ethical approval on Animal Welfare and Rights was obtained from the University of Calabar Committee on Animal Care and Welfare based on the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition (National Health and Medical Research Council; Canberra 2013).

Experimental design

The rabbits were randomly distributed to the diets in a simple Completely Randomized Design (CRD) experiment with three processed forms of CPHM. They were twelve (12) dietary treatments with five (5) rabbits per treatment. The rabbits were distributed to the treatments after equalizing for body weight and sex.

Reproductive performance and gonadal sperm reserves

During mating, a buck and doe in a particular treatment/diet were kept together (paired) until successful mating was observed to ensure that pregnancy is achieved. The mating ratio of 1:1 (buck: doe) was maintained throughout the duration of this study. Successful mating took place when the buck summersaulted and screamed, after which they were separated. Pregnancy was diagnosed after 14 days by combining abdominal palpation with observation of body weight changes (size and weight of does) (Oguike et al., 2011). Kindling boxes were provided at the 3rd week of gestation. The following reproductive parameters were determined for the does: pregnancy rate, litter size, average birth weight, average weaning weight (at 6 weeks) and mortality. Milk yield (g⁻¹day) was estimated from the equation reported by Nguyen et al. (2000) as $1.18 \times [\text{live weight of litter at 21days} - \text{live weight at birth}]$. Reproductive parameters determined in bucks included: weights of testes and epididymes (caput, corpus and cauda). Gonadal sperm reserves were determined haemocytometrically by homogenate technique, using a modified method as described by Adejumo (2006). Daily sperm production (DSP) was obtained by dividing Gonadal sperm reserve by 3.56 (time in days of the duration of the seminiferous epithelium cycle) while total sperm reserve was calculated by multiplying semen volume by the concentration (Bitto and Egbunike, 2006; Zahraddeen et al., 2007).

Statistical analysis

Data obtained in this study were subjected to one-way ANOVA using General Linear Model for a completely randomized design (CRD). Significant means were separated using the Least Significance Difference (LSD) method (Steel and Torrie, 1980).

The experimental model used was as follows: $Y_{ij} = \mu + T_i + E_{ij}$

Where:

Y_{ij} : Observed value; μ : Overall mean value; T_i : Random effect of the i^{th} processing method of CPHM; and E_{ij} : Random residual error

RESULTS

Reproductive performance characteristics of rabbits

The reproductive performance of rabbits fed cocoa pod husk meal-based diets is summarized in Table 1. The pregnancy rate ranges from 0 - 100%. The highest inclusion level of CPHM (37.5%) recorded zero pregnancy rates in the raw and hot - water treated cocoa pod husk meal. The average gestation period ranged between 30 and 31 days across the dietary treatments. The average litter size at birth ranged from 1-4 kittens. Results further revealed that the average birth weight per kitten decreased marginally (< 2%) across dietary treatments as the levels of CPHM increased in the diets; values for kittens fed the raw CPHM were 27.85, 26.47, 25.48 and 0.00 g for 0, 12.50, 25.00 and 37.50% inclusion levels, respectively. While the fermented and hot-water treated CPHM recorded 30.51, 31.22, 29.27 and 27.17g, respectively and 30.01, 28.12, 27.98 and 0.00 g for 0, 12.50, 25.00 and 37.50 percent inclusion levels, respectively. The average weaning weight ranged between 475.00 and 580.25g across dietary treatments, with the least weight recorded in the raw CPHM group with 25.00 % inclusion level. The average weight of kittens at 21 days decreased across dietary treatments in the raw CPHM, that is; 225.25, 220.40, 200.00 and 0.00 g respectively, for diets with 0, 12.50, 25.00 and 37.50 % levels. The trend was reversed in the other processed forms of CPHM with average weight values of 212.50, 220.00, 240.00 and 250.00g for 0, 12.50, 25.00 and 37.50 % levels and 225.00, 240.00, 245.00 g for diets with 0, 12.50, 25.00 % levels, respectively with no value in 37.50%. The milk yield ranged between 205.46 and 262.94g across dietary treatments. The mortality of kittens ranged from 0.00 - 25.00%.

Table 1 - Reproductive performance of rabbits fed cocoa pod husk meal (CPHM) based diets

Parameter	Raw CPHM				Fermented CPHM				Hot-water treated CPHM				SEM
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	
	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	
No. of Does bred	2.00	3.00	3.00	3.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00	3.00	0.11
Pregnant does (day 14)	2.00	3.00	1.00	0	3.00	1.00	1.00	2.00	2.00	1.00	1.00	0	0.29
Pregnancy rate (%)	100.00	100.00	33.33	0	100.00	50.00	33.33	66.67	66.67	33.33	33.33	0	10.36
Av. Gestation length (days)	30.50	30.50	31.00	0	30.33	30.00	31.00	30.00	30.50	30.00	30.50	0	3.43
Av. Litter size at birth	4.00	3.33	1.00	0	3.00	1.00	3.00	3.50	2.00	4.00	2.00	0	0.42
Av. Birth weight (g)	27.85	26.47	25.48	0	30.51	31.22	29.27	27.17	30.01	28.12	27.98	0	3.23
Av. Wt. of kittens (g) at 21 days	225.25	220.40	200.00	0	212.50	220.00	240.00	250.00	225.00	240.00	245.00	0	25.96
No. alive at weaning	3.00	8.00	1.00	0	9.00	1.00	3.00	2.00	4.00	4.00	2.00	0	0.83
6 weeks													
Av. Weaning wt. (g)	520.00	480.00	475.00	0	580.25	570.20	566.67	560.10	512.50	510.00	520.00	0	60.38
Milk yield (g)	232.93	228.84	205.93	0	214.75	222.76	205.46	262.94	230.09	250.02	256.08	0	26.51
Mortality (%)	25.00	20.00	0	0	0	0	0	0	0	0	0	0	2.55

*Milk yield = 1.18 x [Live wt. of litter at 21 days - Live wt. at birth], equation reported by [Nguyen et al. \(2000\)](#)

Table 2 - Semen volume and gonadal sperm reserve characteristics of rabbit bucks fed cocoa pod husk meal based diets

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	SEM
	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	
Semen volume (ml)	0.87 ^a	0.53 ^c	0.50 ^c	0.47 ^c	0.67 ^b	0.57 ^c	0.43 ^c	0.47 ^c	0.57 ^c	0.47 ^c	0.37 ^d	0.33 ^d	0.08
Gonadal sperm reserve (×10 ⁶ ml ⁻¹)	611.00 ^a	575.67 ^b	510.67 ^c	498.00 ^c	520.33 ^b	558.00 ^b	401.33 ^d	369.00 ^e	313.00 ^e	390.33 ^d	377.00 ^d	366.00 ^e	19.91
Total sperm reserve (×10 ⁶ ml ⁻¹)	531.57	305.11	255.34	234.06	348.62	318.06	172.57	173.43	178.41	183.46	139.49	120.78	33.43
Daily Sperm Production (×10 ⁶ ml ⁻¹)	171.63	161.71	143.45	139.89	146.16	156.74	112.73	103.65	87.92	109.64	105.90	102.81	8.02

^{a,b,c...e} means on the same row with different superscripts are significantly different (P<0.05); SEM: Standard Error of Mean

Table 3 - Total protein concentrations in testicular and tubal fluids of rabbits fed Cocoa pod husk meal-based diets

Parameter	Raw CPHM				Fermented CPHM				Hot- water treated CPHM				SEM
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	
	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	0%	12.50%	25.00%	37.50%	
Bucks (g per 100 ml)													
Testes	0.73 ^c	0.76 ^c	0.82 ^b	0.54 ^d	1.39 ^a	1.38 ^a	1.04 ^b	0.83 ^b	0.74 ^c	0.63 ^c	0.65 ^c	0.72 ^c	0.08
Epididymis	0.89 ^a	0.64 ^c	0.53 ^d	0.32 ^e	0.76 ^b	0.64 ^c	0.70 ^b	0.72 ^b	0.68 ^b	0.58 ^d	0.56 ^d	0.34 ^e	0.05
Paired caput	0.55 ^c	0.61 ^c	0.48 ^c	0.51 ^c	1.05 ^a	1.14 ^a	0.80 ^b	0.73 ^b	0.30 ^d	0.26 ^d	0.27 ^d	0.90 ^b	0.09
Paired corpus	0.72 ^c	0.78 ^c	0.37 ^d	0.40 ^d	0.96 ^b	0.95 ^b	1.10 ^a	0.55 ^d	0.56 ^d	0.49 ^d	0.43 ^d	0.42 ^d	0.07
Paired cauda	0.85 ^a	0.71 ^b	0.83 ^a	0.29 ^d	0.88 ^a	0.90 ^a	0.34 ^d	0.39 ^d	0.43 ^c	0.73 ^b	0.47 ^c	0.48 ^c	0.07
Does (g per 100 ml)													
Ovary	0.50	0.48	0.42	0.28	2.17	1.52	0.46	0.43	0.61	0.60	0.59	0.55	0.16
Oviduct	1.04 ^c	1.01 ^b	0.68 ^d	0.60 ^d	0.91 ^c	0.73 ^c	0.56 ^d	0.58 ^d	1.72 ^a	1.71 ^a	1.25 ^b	1.27 ^b	0.12
Uterine horns	0.60	0.58	0.55	0.49	0.52	0.70	0.69	0.50	0.61	0.59	0.64	0.88	0.03
Cervix	0.29	0.22	0.37	0.27	0.63	0.69	0.72	0.59	0.95	0.83	0.83	0.85	0.07
Vagina	0.78	0.81	0.52	0.39	1.10	0.72	0.12	0.15	0.57	0.54	0.63	0.71	0.08

a, b,c,d Means on the same row with different superscripts are significantly different (p < 0.05)

Semen volume and gonadal sperm reserves of rabbit bucks

Table 2 shows the result of sperm volume and gonadal sperm reserve characteristics of rabbit bucks fed cocoa pod husk meal-based diets. The sperm volume (SV) and gonadal sperm reserve (GSR) recorded significant effect ($P < 0.05$) of dietary treatments; while results for total sperm reserve (TSR) and daily sperm production (DSP) were statistically similar. In the raw and hot-water treated cocoa pod husk meal groups, the sperm volume decreased marginally as the level of inclusion of cocoa pod husk meal increased across the diets. The values obtained for SV were 0.87, 0.53, 0.50 and 0.47ml in the raw cocoa pod husk meal group; while the fermented and hot – water treated cocoa pod husk meal groups recorded SV values of 0.67, 0.57, 0.43, 0.47ml and 0.57, 0.47, 0.37 and 0.33ml, respectively for control, 12.50, 25.00 and 37.50 % diets. Results obtained for the GSR were 611.00, 575.67, 510.67 and 498.00 ($\times 10^6 \text{ ml}^{-1}$) in the raw cocoa pod husk meal; 520.33, 558.00, 401.33 and 369.00 ($\times 10^6 \text{ ml}^{-1}$) in the fermented cocoa pod husk meal and 313.00, 390.33, 377.00 and 366.00 ($\times 10^6 \text{ ml}^{-1}$) in the hot-water treated cocoa pod husk meal, respectively for diets 0, 12.50, 25.00 and 37.50 % inclusion levels. Results for TSR were 531.57, 305.11, 255.34 and 234.06 ($\times 10^6 \text{ ml}^{-1}$) in raw cocoa pod husk meal; 348.62, 318.06, 172.57 and 173.43 ($\times 10^6 \text{ ml}^{-1}$) in fermented cocoa pod husk meal; 178.41, 183.46, 139.49 and 120.78 ($\times 10^6 \text{ ml}^{-1}$) in hot – water treated cocoa pod husk meal, respectively for 0, 12.50, 25.00 and 37.50 % inclusion levels. The values obtained for the DSP were 171.63, 161.71, 143.45 and 139.89 ($\times 10^6 \text{ ml}^{-1}$) in raw cocoa pod husk meal; 146.16, 156.74, 112.73 and 103.65 ($\times 10^6 \text{ ml}^{-1}$) in fermented cocoa pod husk meal; as well as 87.92, 109.64, 105.90 and 102.81 ($\times 10^6 \text{ ml}^{-1}$) in hot-water treated cocoa pod husk meal, respectively for diets containing 0, 12.50, 25.00 and 37.50 % inclusion levels.

Total protein concentrations in testicular and tubal fluids of rabbits

Result of the total protein concentrations in testicular and tubal fluids of rabbits fed experimental diets is summarized in Table 3. All parameters in the bucks were significantly different between dietary groups. The protein concentrations in the testes recorded higher values in the control diets across the three processing methods and the least values in the 37.50% inclusion level in the raw and fermented CPHM groups. Total protein concentrations in the testes were 0.73, 0.76, 0.82 and 0.54g 100^{-1}ml ; 1.39, 1.38, 1.04 and 0.83g 100^{-1}ml as well as 0.74, 0.63, 0.65 and 0.72g 100^{-1}ml in diets containing 0, 12.50, 25.00 and 37.50% levels, respectively for the raw, fermented and hot-water treated CPHM. The epididymis recorded total protein concentrations as 0.89, 0.64, 0.53 and 0.32g 100^{-1}ml ; 0.76, 0.64, 0.70 and 0.72g 100^{-1}ml as well as 0.68, 0.58, 0.56 and 0.34g 100^{-1}ml for 0, 12.50, 25.00 and 37.50% levels, respectively in the raw, fermented and hot-water treated CPHM. Total protein concentrations in paired caput, corpus and cauda epididymis recorded fluctuating trends across dietary treatments as the levels of CPHM increased. In the tubal fluids, the total protein concentrations recorded in the ovary decreased as the inclusion of CPHM increased in the differently processed forms of dietary treatments. Values obtained were 0.50, 0.48, 0.42 and 0.28g 100^{-1}ml in the raw CPHM; 2.17, 1.52, 0.46 and 0.43g 100ml^{-1} in the fermented CPHM as well as 0.61, 0.60, 0.59 and 0.55g 100^{-1}ml in the hot-water treated CPHM for diets containing 0, 12.50, 25.00 and 37.50% inclusion levels, respectively. Total protein concentrations in the oviduct recorded significant effect ($P < 0.05$) while the uterine horns, cervix and vagina were statistically similar and did not record particular trends among the processing methods. However, higher values were recorded in the fermented and hot-water groups compared to the raw CPHM group.

DISCUSSION

Reproductive performance of rabbits

The reproductive performance of different rabbit breeds has been reported to be an important feature that determines their efficiency in production (Fadare and Fatoba, 2018). The reproductive performance of rabbits fed cocoa pod husk meal based diets (Table 1) revealed an average pregnancy rate that dropped from 100 to 33.33% in both raw and fermented cocoa pod husk meal groups as well as 66.67 to 33.33% in hot-water treated cocoa pod husk meal group as the levels of inclusion increased up to 25.00% level across dietary treatments. The highest level of inclusion (37.50%) of cocoa pod husk meal in the raw and hot-water treated groups recorded zero pregnancy rate, while the fermented group recorded 66.67%. The pregnancy rate obtained in this study ranges from 0.00 –100%, while Ewuola and Egbunike (2010) reported 87.50-100% who fed fumonisin B₁ to male rabbits. The difference could be attributed to the age at puberty and effect of diet on the two separate studies. The litter size at birth, weight of kittens and weaning weight followed the same trend recorded for pregnancy rates in this study.

The average litter size at birth in the experimental rabbits was from 1 to 4 kittens across dietary treatments in all the processed forms of cocoa pod husk meal (CPHM). The raw CPHM group showed a regular declining trend, while the fermented and hot-water treated groups fluctuated as the levels of inclusion increased. The declining trend in reproductive performance characteristics has revealed that cocoa pod husk meal has adverse effects on the reproductive performance of rabbits. This assertion confirms the findings of EPSA (2008) on contaminants in the food chain that theobromine induces reproductive problem affecting the gonads, delayed ossifications in mice and skeletal variations in kittens of rabbits. Furthermore, the average litter size at birth recorded in this study fell below the average value (4.27-5.33) reported for New Zealand and Dutch rabbits (Akanno et al., 2004; Oseni et al., 2006) and (6.50-7.25) in cross bred rabbits reported by Ewuola and Egbunike (2010) as well as (4.23 – 6.75) reported by Fadare and Fatoba (2018) for New Zealand White, California and Palomino brown rabbits. This difference in litter size is attributable to breed or strain influence, age

at first parity (young female rabbits tend to have smaller litter than older dams), quality of feed, re - mating interval, environmental conditions and other management practices. This agrees with the findings of [Fielding \(1991\)](#) and [Olateju and Chineke \(2022\)](#) that litter size at birth is affected by genetic composition of the doe among other factors. Experimental findings by other workers show that the New Zealand breed recorded a higher mean litter size at birth (5.03) than the Dutch breed (4.67); ([Omole et al., 2007](#)). The control diets in this study had an average litter size at birth of 2 to 4 kittens, this range was closed to the average value (4.77) reported by [Fayeye and Ayorinde \(2003\)](#) for the New Zealand breed in the derived savanna zone. Also, a value of 5.33 for the same trait in California and Havana black rabbits has been reported in the humid tropical region of Nigeria ([Fadare and Fatoba, 2018](#)). Other workers have reported average litter size at birth of 6 and 7 kittens for the Japanese white and New Zealand white rabbits, respectively; with an average litter size of 8 kittens ([Casady et al., 1996](#)) as well as 6-8 kittens ([Henry et al. 2018](#)). The average gestation length observed in this study was between 30 and 31 days across dietary treatments. This fell within the normal gestation length (30 ±2 days) for rabbits ([Aduku and Olukosi, 1990](#), [Omole et al., 2007](#), [Ozung et al., 2019](#)). The normal gestation length recorded in this study suggests low neonatal loss that usually accompanies longer gestation periods. This is confirmed in the low mortality of kittens (20-25%) in the raw CPHM group and absence of mortality in the fermented and hot-water treated groups in this study. The finding confirms the submission of previous works that small litters are associated with long gestation period (33-34 days) accompanied by high mortality of kittens. The workers had suggested the use of prostaglandin F₂α as a cost-effective means of reducing the observed mortality in pregnant does having longer gestation periods. A correlation between gestation length and litter size has been suggested ([Fayeye and Ayorinde, 2003](#)).

The average birth weight of kittens obtained in this study declined as the CPHM increased in the dietary treatments; implying cocoa pod husk meal with the associated theobromine has an adverse effect on the birth weight of rabbit kittens. The values for the raw CPHM group were between 25.48 and 27.85g kitten⁻¹; while values in the fermented and hot-water treated groups were 27.17 and 31.22g kitten⁻¹; 27.98 and 30.01g kitten⁻¹, respectively. However, these values were within the range (15–30g kitten⁻¹) for birth weight reported by [Omole et al. \(2007\)](#). The average weaning weight of kittens at six weeks when compared to the values in the control diets decreased marginally across dietary treatments as the levels of CPHM increased in the raw, fermented and hot-water treated groups. The weaning weight was highest in the fermented CPHM, higher in the hot-water treated CPHM and high in the raw CPHM groups. The results have confirmed that fermentation and hot-water treatment of CPHM lower the theobromine content, which translates to better weaning weight compared to the raw CPHM with higher theobromine content and lower weaning weight. Theobromine has been reported to have negative effects on the reproductive parameters of rabbits ([EFSA, 2008](#)). The litter size recorded in this study did not adversely influence the weaning weight of kittens. This corroborates the report by [Orunmuyi et al. \(2006\)](#) that litter size at birth does not exert significant effect on weaning weight of animals. However, the mothering ability of the female rabbit (doe) decreases with increased litter size ([Fayeye and Ayorinde, 2003](#)). The milk yield of lactating does in this study ranges from 205.46-256.08g. The milk yield is a good indicator of the mothering ability of rabbit does. However, the milking ability is breed dependent with the Californian white reported to have the best milking ability compared to other breeds like the New Zealand white, Havana black, Palomino brown and Chinchilla ([Fadare and Fatoba, 2018](#)).

Semen volume and gonadal sperm reserve characteristics of rabbit bucks

The semen volume (SV) and gonadal sperm reserve (GSR) in this study had significant reduction in values with increasing levels of CPHM across treatments (Table 2). Both parameters recorded lowest values at the highest inclusion level (37.50%) of each processed form (raw, fermented and hot-water treated) of CPHM. Total sperm reserve (TSR) and daily sperm production (DSP) were statistically similar across dietary treatments, but showed gradual decline in values as the levels of CPHM inclusion increased. These observations affirmed that residual theobromine from differently processed forms of cocoa pod husk meal (especially the raw form and followed by the hot-water treated form) has deleterious effects on gonads (testes) and associated physiological processes like spermatogenesis. This observation agrees with the findings of [EFSA \(2008\)](#) that theobromine from cocoa products induces reproductive dysfunction targeting the gonads (testes) in rodents and dogs. However, the semen volume in the control diet for raw, fermented and hot-water treated CPHM was comparable to range of values (0.73-0.74ml) earlier reported by [Ewuola and Egbunike \(2010\)](#). Equally, the SV and GSR were similar in values with those earlier given by [Adams and Singh \(1981\)](#). The GSR was also comparable with the values published by [Castellini \(2008\)](#); while the TSR values were fairly higher than those reported by [Abu et al. \(2013\)](#). Also, GSR and DSP were fairly higher than the values reported by [Amao et al. \(2011\)](#). Differences in TSR, GSR and DSP could be attributed to effect of nutrition/ diet (different test materials) and age of bucks in the separate studies.

Total protein concentrations in testicular and tubal fluids

Total protein concentrations in the testicular fluids of rabbit bucks fed cocoa pod husk meal (Table 3) revealed significant (P<0.05) effect of dietary treatments in the fluids from testes, epididymis, paired caput and paired corpus. The total protein concentrations in the tubular fluids of female rabbits (does) were statistically similar except for the Oviduct. The fermented CPHM group recorded higher total protein concentrations in both testicular and tubular fluids. The total protein concentrations in fluids from the testes were of the range 0.54 -0.82g per 100ml, 0.83–1.39g per 100 ml and 0.63–0.74g per 100 ml, respectively in the raw, fermented and hot-water treated cocoa pod husk meal diets. Total protein

concentrations in the testes were least in value in the highest inclusion level (37.50%) of CPHM in the raw and fermented groups compared with the hot-water treated group. The fermented group recorded a gradual declining trend across dietary treatments as the levels of CPHM increased. The findings in this study affirmed that cocoa pod husk meal, especially the raw form with higher content of theobromine has negative effect on the total protein concentrations in the testes. This could lead to poor spermatogenesis, oligospermia and abnormal sperm cells in rabbit bucks. The total protein concentrations in the epididymis showed a marginal declining trend across dietary treatments in the raw CPHM (0.89–0.32g per 100 ml) and hot-water treated CPHM (0.68–0.34g per 100 ml), respectively while the fermented CPHM recorded a fluctuating trend that could not be ascribed to experimental diets. The total protein concentrations in the paired caput, corpus and cauda epididymis recorded fluctuating trends across dietary treatments. The testicular fluids total protein concentrations in this study are higher than the values for paired testes, paired caput, corpus and cauda epididymes reported by Bitto (2010) who fed kapok - forage combinations to rabbit bucks. These slight differences are obviously due to age (the bucks in this study were slightly older) and diet as different test ingredients were utilized in the separate studies.

The total protein concentrations in the tubal fluids of female rabbits recorded significant effect on the oviduct only, while fluids from the ovary, uterine horns, cervix and vagina showed statistically similar results in total protein concentrations. The range of values obtained in this study for fluids from the oviduct (0.56 –1.72g per 100 ml), uterine horns (0.49– 0.88g per 100 ml), cervix (0.22–0.95g per 100 ml) and vagina (0.12–1.10g per 100 ml) were fairly lower than the range of values for tubular fluids from the oviduct (0.97–1.81g per 100 ml), uterine horns (1.67–2.95g per 100 ml), cervix (0.76–0.99g per 100 ml) and vagina (0.52–1.19g per 100 ml) reported by Ozung et al. (2011) who fed varying dietary levels of cassava peel meal as replacement for maize to female rabbits. These differences in tubal fluid protein concentrations could be attributed to the effect of anti-nutrients in the diets, as it is obvious that theobromine in this study had severe adverse effects compared to cyanide in the earlier study in the total protein concentrations of tubal fluids.

CONCLUSION AND RECOMMENDATION

This study concluded that different forms of cocoa pod husk meal (CPHM) at 37.5% level of inclusion could negatively affect the reproductive performance of rabbits. The order of preference is the fermented CPHM, followed by the hot – water treated CPHM and lastly the raw CPHM, respectively. The fermented cocoa pod husk meal based diets performed best compared to other forms in terms of reproductive performance and semen characteristics as well as total protein concentrations in testicular fluids of bucks and tubal fluids of does. The study recommended that cocoa pod husk meal should be fermented before it can be incorporated in diets meant for rabbits. Hence, fermentation is the preferred method of detheobromination. The optimum level of inclusion of fermented CPHM could be up to 37.5% in the diets. Farmers using raw and hot – water treated CPHM should not exceed 25% inclusion level for fear of adverse effects of residual theobromine on reproductive performance of rabbits and semen characteristics of bucks.

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Authors' contribution

P.O. Ozung and E.I. Evans: Conceptualization, laboratory investigation, sampling, research methodology and writing of original draft; K.U. Anoh and J.A. Ubuja: Feeding trial, data collection as well as statistical analysis and table presentations; O.O.O. Kennedy and D.A. Alawa: Conceptualization, literature review, editing of manuscript and confirmation of all references.

Conflict of interests

The authors hereby declare that there is no conflict of interests in this research work.

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