

Original Research Article

Dietary Cocoa Pod Husk Meal Could Influence the Reproductive Tract Morphometry of Rabbits

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Abstract: The reproductive tract morphometry of rabbits fed dietary cocoa pod husk meal was evaluated in this study, with 72 animals (36 bucks and 36 does) having mean body weight of 606.42±1.32g. Twelve *iso-nitrogenous* (16.24% CP) and *iso-caloric* (2,517.08 Kcal/kg ME) diets were formulated containing four levels of raw, fermented and hot – water treated cocoa pod husk meal (CPHM) at 0, 12.5, 25 and 37.5 percent levels respectively. The rabbits were randomly assigned to the diets using a completely randomized design and thereafter subjected to eleven weeks feeding trial. Results showed that the reproductive tract morphometry of rabbit bucks showed significantly ($P < 0.05$) declining effect in the absolute weight of paired testes, paired epididymes and length of caput epididymes between dietary treatments in the raw CPHM, while the fermented and hot – water treated forms recorded significant ($P < 0.05$) effect also but without a regular pattern. On the other hand, the female rabbits did not record any significant ($P > 0.05$) effect of diets. This study concludes that cocoa pod husk meal could be included in rabbit diets up to 25%. The fermented and hot- water treated diets showed superiority in the reproductive tract morphometry vis- a-vis reproductive efficiency of rabbits.

Keywords: Buck, cocoa, doe, rabbit, reproduction.

INTRODUCTION

Traditionally, ruminants and non-ruminants are the main sources of domestic meat. However, rabbits and other micro livestock species are emerging in many developing countries like Nigeria as potential sources of animal protein. The inadequacy of animal protein coupled with stiff competition between man and animals for agricultural produce call for enhancing the diversification and productivity of livestock and this has culminated in resurgence of interest in rabbit production (Oyadeyi, *et al.*, 2011). The renewed interest in rabbit production by researchers and farmers in Nigeria has necessitated research into alternative feed resources that are readily available, under - utilized agro by - products or agro - industrial wastes to substitute or supplement the highly demanded conventional cereals (Agunbiade *et al.*, 2000). The immediate challenge therefore is for Animal Scientists/ researchers to promote the utilization of cheap and affordable alternative feed resources that are rich in nutrients and are well adapted to tropical climatic conditions. One of the promising agro by - products that can be utilized in rabbit feeding trials is

the cocoa pod husk meal (Ozung *et al.*, 2017). Cocoa pod husks constitute 75 percent of the entire cocoa fruit on fresh weight basis. The proximate composition of the differently processed forms of cocoa pod husk meal as earlier reported by Ozung, *et al.* (2016) showed that the raw, fermented and hot –water treated cocoa pod husk meal had the following average ranges for dry matter (85.28 - 94.60%), crude protein (7.70 - 9.37%), ether extract (8.83 – 11.68%), ash (9.30 – 10.85%) and crude fibre (53.37 – 61.80%). Cacao is free from acutely toxic compounds; the nitrogen content contains two alkaloids namely theobromine and caffeine. These alkaloids are water soluble and harmful symptoms such as excessive stimulation of kidneys, heart and smooth muscles have been associated with the intake of excess theobromine while caffeine has been noted to increase sensory properties. These alkaloids have been reported to be detrimental to animals, while they may have desirable effects in human beings, they cannot be efficiently metabolized by animals and can cause cardiac and nervous system malfunctions and if consumed in high doses may lead to death.

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Furthermore, current research in these alkaloids is centred on their potential reproductive toxicities. Caffeine and theobromine have been reported to pass through the placenta and blood vessels, thereby causing foetal abnormality by adversely affecting growth. Nutritional and growth performance studies with cocoa pod husk meal have been carried out on poultry and small ruminants by other researchers, but there is paucity of research findings on the effect of raw, fermented and hot – water treated cocoa pod husk meal on the reproductive tract morphometry vis- a- vis reproductive potential of rabbits, hence the main objective of this present study.

MATERIALS AND METHODS

The study was carried out at the rabbitry unit of the Teaching and Research Farm, University of Calabar, Calabar, Cross River State, Nigeria. Freshly broken composite cocoa pod husks were obtained from the fermentation units of the Cocoa Research Institute of Nigeria (CRIN) sub - station at Ajassor, Ikom LGA of Cross River State. The broken pods were washed and sun - dried for two weeks, bulked and milled with hammer mill 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 percent water, relative to its weight and bagged using an air tight polythene bag. This was allowed to stay for three (3) days under room temperature, thereafter, it was opened and shade dried for five (5) days; before being packed, bagged and stored in a cool dry place until it was used. 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. Each processed form of CPHM was included at 0, 12.5, 25 and 37.5 percent levels for T₁, T₂, T₃, and T₄ (RCPHM), T₅, T₆, T₇, T₈ (FCPHM) and T₉, T₁₀, T₁₁, T₁₂ (HCPHM), respectively in the experimental diets. Seventy two (72) weaned mixed breed rabbits between 5 and 6 weeks old of both sexes (36 bucks and 36 does), (average initial body weight of 606.42±1.30g) were used in a completely randomised design in this study which lasted for 11 weeks. They were twelve (12) iso-caloric and iso- nitrogenous dietary treatments with six (6) rabbits (3 bucks and 3 does) per treatment (each rabbit served as a replicate). At the 11th week, four rabbits (2 bucks & 2 does) per treatment were sacrificed and dissected while the respective reproductive tracts were removed immediately after slaughter, trimmed of fats and tissues and subjected to morphometric analysis. All weights were determined using an electronic precision balance (Kerro model), while linear measurements were obtained with a measuring tape and glass rule, respectively. Data obtained in this study were subjected to one – way analysis of variance with significant means separated using Least Significance Difference (LSD) option (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Results of the reproductive tract morphometry of male rabbits (bucks) and female rabbits (does) are summarised in Tables 1 and 2, respectively. The paired testicular and epididymal weight and length of caput epididymis recorded significant ($P < 0.05$) effect of dietary treatments, implying that residual theobromine has negative effect on these important male reproductive organs (gonads). The paired testicular and epididymal weight showed significantly drastic decline in values (8.48 – 5.12 g and 6.63 – 1.77 g, respectively) across dietary treatments in the raw CPHM group; while the fermented and hot – water treated groups did not follow regular trends that could be ascribed to dietary / processing effect of CPHM (Table 1). The control diet in the raw and fermented groups respectively, produced fairly heavier testes than the CPHM based diets, implying that the residual theobromine depressed testicular growth. As testes weight is related to sperm production (Medvev and Turchanov, 1999), the significant effect of diets on the absolute weights of paired testes observed in this study may imply higher sperm production rates in rabbits fed control diet only; this observation is in consonance with the separate findings of Bitto & Aroh (2006). In all parameters of the reproductive tract, it was observed that raw CPHM group recorded the least values in weight and length at the highest inclusion (37.50 percent) level, compared with the control diet and other dietary treatments and processing methods. The observations have confirmed that inclusion of higher cocoa pod husk meal levels and associated theobromine has toxic effect on the gonads and reproductive structures of rabbit bucks. This finding agrees with the observations of other researchers that theobromine and other compounds like caffeine, theophylline, diketopiperazine and pyrazine cause reproductive toxicity targeting the testes, precipitating oligospermia and metabolic disarrangement in animals and sometimes death, especially in rodents (EFSA, 2008). The average testes volume obtained in this study ranges from 1.25 - 4.25 ml across dietary treatments, which is fairly higher than the range of values (1.47 – 1.80 ml) reported by Abu *et al.*, (2013) who fed *Moringa oleifera* leaf meal (MOLM) to rabbit bucks. This difference in testes volume could be attributed to the age, live weight, size of testes of bucks and dietary effect in the separate studies.

The reproductive tract morphometry of female rabbits (does) fed cocoa pod husk meal based diets (Table-2) revealed no significant effect of diets. Parameters (respective weight and length of reproductive tract, paired oviducts, uterine horns, cervix and vagina) did not follow a trend that could be ascribed to the effect of diet; but absolute weight of the whole reproductive tract, paired ovaries, paired oviduct, uterine horns, cervix and vagina were fairly higher than the respective values reported by Ogbuwu *et al.*, (2010) who fed neem leaf meal to female rabbits. The

weight differences could be attributable to the effect of different test materials used in the separate studies on these reproductive organs. The length of oviduct and uterine horns were comparable to the findings of Ogbuewu *et al.* (2010) but length of cervix and vagina were lower than the values earlier given by these researchers. However, when compared with values in the control diet for the raw, fermented and hot – water treated CPHM, the weight and length of vagina decreased with increasing levels of inclusion for the raw CPHM group only. This observation revealed that the higher the raw CPHM level in the diet of rabbits, the lower the weight and shorter the length of the vagina. This affirms that theobromine has an adverse or toxic effect on certain reproductive parts/organs in rabbits. Research has shown that heavier weight and longer length of the vagina aid intromission and successful kindling in rabbits (Frandsen, 1986). The respective weight and length of the whole reproductive tract, oviduct, uterine horn, cervix and vagina in this study were comparable with the findings of Ozung, Bitto and Ikurior (2011) who fed cassava peel meal based diets to female rabbits; while the length of these parameters are higher than the values reported by Ojebiyi *et al.*, (2010) for rabbit does. The raw CPHM group showed obvious reduction in length of the reproductive tract, oviduct, uterine horn, cervix and vagina at the highest level (37.50 percent) of inclusion compared to other CPHM groups. This means that female rabbits exposed to high levels of raw CPHM diets might be at risk of reproductive failure due to inherent theobromine.

CONCLUSION

Within the experimental conditions of this study, it is therefore concluded that cocoa pod husk meal (CPHM) could be included in rabbit diets up to 25% level. The fermented and hot- water treated cocoa pod husk meal (CPHM) diets showed superiority in the reproductive tract morphometric parameters over the raw CPHM diets and are recommended to farmers without fear of compromising the reproductive tract morphometry vis- a-vis reproductive efficiency of rabbits in the tropics.

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Table-1: Reproductive tract morphometry of rabbit bucks fed cocoa pod husk meal based diets

Parameter	RCPHM				FCPHM				HCPHM					
	T ₁ 0%	T ₂ 12.50%	T ₃ 25.00%	T ₄ 37.50%	T ₅ 0%	T ₆ 12.50%	T ₇ 25.00%	T ₈ 37.50%	T ₉ 0%	T ₁₀ 12.50%	T ₁₁ 25.00%	T ₁₂ 37.50%	S. E. M	
Pubertal Bucks (Average weight of parameters):														
Average live weight of buck (g)	2050.00 ^{bc}	2400.00 ^a	1850.00 ^d	2175.00 ^{bc}	2475.00 ^a	1950.00 ^c	2275.00 ^b	2275.00 ^b	2325.00 ^{ab}	2300.00 ^{ab}	1875.00 ^d	1550.00 ^e	48.12	
Paired testes (g)	8.48 ^b	8.00 ^c	7.05 ^c	5.12 ^d	5.90 ^d	6.36 ^d	11.00 ^a	8.99 ^b	9.18 ^a	8.74 ^{ab}	10.96 ^a	7.75 ^c	0.53	
Paired epididymis (g)	6.63 ^a	5.99 ^a	2.20 ^c	1.77 ^d	5.70 ^a	3.38 ^b	2.02 ^c	2.30 ^c	1.48 ^d	3.31 ^b	2.03 ^c	1.02 ^d	0.55	
Caput epididymis (g)	0.42	0.58	0.31	0.35	0.69	0.81	0.56	0.47	0.52	0.50	0.33	0.31	0.05	
Corpus epididymis (g)	1.81	0.44	0.20	0.22	0.24	0.15	0.11	0.14	0.17	0.15	0.11	0.10	0.14	
Cauda epididymis (g)	0.73	0.14	0.08	0.17	0.55	0.08	0.09	0.06	0.11	0.12	0.04	0.02	0.06	
Testes volume (ml)	2.00	2.25	2.00	1.25	1.75	3.75	4.25	2.25	4.00	3.50	4.25	3.75	0.32	
Gonadosomatic index (GSI) (%)	0.41	0.33	0.38	0.45	0.24	0.33	0.48	0.40	0.40	0.38	0.59	0.73	0.05	
Relative weight of reproductive organs (% live weight of bucks):														
Paired testes	0.41	0.33	0.38	0.24	0.24	0.33	0.48	0.40	0.40	0.38	0.59	0.50	0.03	
Paired epididymis	0.32	0.25	0.12	0.08	0.23	0.17	0.09	0.10	0.06	0.14	0.11	0.07	0.02	
Caput epididymis	0.02	0.02	0.02	0.02	0.03	0.04	0.03	0.02	0.02	0.02	0.02	0.02	0.002	
Corpus epididymis	0.09	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.007	
Cauda epididymis	0.04	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.003	
Linear measurements (cm):														
Testicular length	6.75	4.75	3.75	4.00	6.50	6.50	6.50	8.50	7.75	7.25	6.75	5.50	0.42	
Entire epididymis length	17.50	12.50	12.25	9.00	10.50	7.25	7.50	8.05	7.50	8.75	7.75	8.00	0.73	
Caput epididymis length	4.75 ^a	2.75 ^c	2.25 ^c	1.75 ^c	3.25 ^b	2.50 ^c	2.00 ^c	1.80 ^c	1.75 ^c	2.75 ^c	1.75 ^c	1.25 ^d	0.24	
Corpus epididymis length	7.75	6.75	5.50	5.25	4.25	2.75	3.50	3.25	3.75	3.50	3.00	3.75	0.31	
Cauda epididymis length	5.00	3.00	4.50	2.00	3.00	2.00	2.00	3.00	2.00	2.50	3.00	3.00	0.28	

^{a,b,c,...,e} means on the same row with different superscripts are significantly different (P < 0.05)

HCPHM: Hot -water treated Cocoa Pod Husk Meal

FCPHM: Fermented Cocoa Pod Husk Meal

Table-2: Reproductive tract morphometry of rabbit Does fed cocoa pod husk meal based diets

Parameter	RCPHM				FCPHM				HCPHM				S. E. M
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂	
Av. live weight of Doe (g)	2500.00 ^a	1825.00 ^d	2250.00 ^b	2100.00 ^c	1975.00 ^c	2225.00 ^b	2050.00 ^c	2000.00 ^c	1800.00 ^d	1900.00 ^d	1400.00 ^e	1450.00 ^e	91.25
Weight of Rep. tract (g)	13.21	11.70	13.24	12.25	18.22	17.01	18.55	18.06	17.31	15.10	11.66	16.31	0.77
Weight of Paired ovaries (g)	0.25	0.21	0.41	0.35	0.51	0.86	0.80	0.70	0.66	0.61	0.51	0.24	0.06
Weight of Paired oviducts (g)	1.60	1.52	2.54	2.22	4.43	4.24	2.54	1.78	4.79	4.55	4.15	1.17	0.40
Weight of uterine horns (g)	1.25	1.23	3.38	4.54	6.86	6.28	3.36	4.82	5.61	5.60	4.75	3.67	0.52
Weight of cervix (g)	2.00	1.55	3.22	3.41	2.69	2.50	1.58	2.31	3.48	3.85	3.60	1.06	0.27
Weight of vagina (g)	1.41	1.30	1.01	0.67	1.44	1.79	1.36	1.08	1.75	1.61	1.25	0.89	0.10
Relative weight of reproductive part (% live weight of Doe):													
Reproductive tract	0.53	0.64	0.59	0.58	0.92	0.32	0.42	0.40	0.96	0.80	0.83	0.44	0.06
Paired ovaries	0.01	0.01	0.02	0.02	0.03	0.04	0.04	0.04	0.04	0.03	0.04	0.02	0.003
Paired oviducts	0.06	0.08	0.11	0.11	0.22	0.19	0.12	0.09	0.27	0.24	0.30	0.08	0.02
Uterine horns	0.05	0.07	0.15	0.22	0.35	0.28	0.16	0.24	0.31	0.30	0.34	0.25	0.03
Cervix	0.08	0.08	0.14	0.16	0.14	0.11	0.08	0.02	0.19	0.20	0.26	0.07	0.02
Vagina	0.06	0.07	0.04	0.03	0.07	0.08	0.07	0.05	0.10	0.08	0.09	0.06	0.01
Linear measurements of reproductive part (cm):													
Whole rep. tract length:	25.75	23.25	25.50	17.75	30.25	25.00	26.00	30.50	26.25	22.25	23.50	24.25	0.98
Length of oviduct	9.25	7.75	9.50	6.25	11.75	10.00	10.25	11.25	7.25	7.25	6.25	7.75	0.54
Length of uterine horn	7.25	6.25	6.25	4.25	9.75	7.50	9.75	11.75	8.25	6.00	11.00	13.75	0.80
Length of cervix	6.50	5.75	6.50	5.00	4.75	3.50	3.75	4.50	7.50	5.50	4.00	4.75	0.35
Length of vagina	3.00	3.25	3.00	2.00	3.75	2.75	2.25	2.50	4.50	3.50	2.25	2.50	0.21

^{a,b,c,...e} means on the same row with different superscripts are significantly different (P < 0.05)

RCPHM: Raw Cocoa Pod Husk Meal

FCPHM: Fermented Cocoa Pod Husk Meal

HCPHM: Hot -water treated Cocoa Pod Husk Meal, S.E. M: Standard Error of Mean