

constitutes 1.3%, of its dry weight (Greenwood and Barton, 1965) and high crude fibre levels. This has led several workers to recommend maximum dietary levels of 10-20% for poultry (Atuahene *et al*, 1985, Adeyanju *et al*, 1978) and up to 40% in pigs, (Fleischer *et al*, 1991). Non-conventional fed ingredients like cocoa husk may have performed well as a nutrient source but not much is known on its effects on reproduction and gonadal capacity of the animal. This has prompted this study to investigate the effects of dietary cocoa husk as graded replacement for wheat offal on the testicular characteristics of mature pigs.

Materials and Methods

48 growing Large White boars with an average weight of 35 kg were randomly allocated to 6

experimental rations made up as constituted in Table 1. Ration 1 (R1) was compounded from conventional ingredients obtained from reputable suppliers with maize and maize offal as the major energy sources with no cocoa husk supplement. Rations 2,3,4,5 and 6 (R2-R6) contained 5%, 10%, 15% 20% and 25% inclusion levels of cocoa husk respectively, effectively replacing maize offal at 20%, 40%, 60%, 80% and 100%, respectively. The groundnut cake and maize levels of the diets were slightly adjusted to provide a uniform crude protein level of 18% for all the diets while crude fibre increased with increasing level of cocoa husk. The animals were provided with feed and water *ad libitum* until they attained a body weight of 60kg when they were slaughtered for evaluation of testicular characteristics

Table 1. Gross composition of experimental rations

Ingredients	R1 Control	R2	R3	R4	R5	R6
Level of cocoa pod husk (%)	0.00	5.00	10.00	15.00	20.00	25.00
Maize	52.15	51.68	51.24	50.77	50.33	49.86
Maize offal	25.0	20.0	15.0	10.0	5.00	0.00
Groundnut cake	12.35	12.82	13.26	13.73	14.17	14.64
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00
Blood meal	5.0	5.0	5.0	5.0	5.0	5.0
Bone meal	2.0	2.0	2.0	2.0	2.0	2.0
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	
Crude protein (%)	18.0	18.0	18.0	18.0	18.0	18.0
Crude fibre (%)	3.29	4.70	6.11	7.51	8.92	10.33

The right testis of each pig was removed from the scrotal sac and the tunica vaginalis communis was removed. The epididymis was freed from the body of the testis and weighed. The tunica albuginea was dissected out from the testicular coat and weighed. In addition to this, testicular volume was determined by water displacement. Thereafter, weighed samples of the testicular and epididymal tissues were

macerated and divided into two portions. One portion was homogenized in deionised water at a dilution rate of 10ml/mg of testicular and epididymal tissue, strained through a fine cloth sieve and the aliquots analysed for minerals assay. Calcium and phosphorus were analysed using Sigma kits (Sigma, 2000) and sodium, potassium, magnesium and zinc were determined by atomic absorption photometry according to

Cocoa husk inclusion in diets and testicular characteristics of pigs

procedures described by Varghese *et al.*, (1969). The other portion of testicular materials were homogenized in normal saline at 100mg/ml of saline and then filtered through 0.3mm tissue mesh. The resulting homogenate was then diluted with deionised water at a 25X dilution factor and sperm count determined using the improved Neubauer haemocytometer.

The proximate analyses of the diets were carried out according to procedures established by AOAC (1990). All results were subjected to the multi-factor analysis of variance (ANOVA) of the completely randomized block design using SPSS® (2000) statistical package and significant means separated using the Duncan's multiple range test.

Results

The results of the effects of the dietary rations on testicular and epididymal weights are summarized in Table 2. Although significant effects ($P < 0.05$) were observed in testicular weight with a mean of 150g in the control pigs and 168g in the pigs on 5% CH diet, increasing the dietary intake of CH decreased testicular weight. The weight of the tunica albuginea increased initially with increasing CH inclusion up to 10% level (17.8g) and decreased gradually with increasing CH to 10.6g at 25% CH level. The cauda epididymal weight also exhibited a similar trend. However, no significant dietary effects were observed in testicular density, and the weights of the caput and cauda epididymides.

Gonadal sperm reserves (Table 3) were increased by dietary CH rising from 2.74×10^9 in the control animals to 13.05×10^9 in the 10% CH supplemented group and falling thereafter to 3.21×10^9 at 20%CH dietary supplementation. Epididymal reserves were also similar in concentration effects to the testicular concentrations. Table 4 summarises the concentrations of cations in the testis of the pig as affected by CH supplementation. While magnesium, zinc and phosphate concentrations

were unaffected by the dietary treatments, significant effects were observed in calcium, potassium and sodium concentrations. Except for an apparently aberrant elevation in calcium level in pigs on 20% CH diet, calcium content of testicular tissue decreased with increasing CH level. Potassium and sodium however rose linearly with increasing CH incorporation up till 20% incorporation level, and declined sharply in the pigs fed the 25%CH diets. A similar trend was observed in the concentrations of calcium and sodium in the cauda epididymis (Table 5) where the mineral levels rose with increased inclusion of CH to 20% level and fell sharply at 25% inclusion. No significant effects were observed in the concentrations of the other cations in the cauda epididymis.

Discussion

The interactions between cocoa husk and testicular development were examined in this study. Faulty nutrition has long been recognized as having an injurious effect upon the male reproductive system (Perry, 1968). The results on the testicular and epididymal weights showed that cocoa husk supported normal testicular growth when incorporated at 10%-20% but significantly depressed gonadal weights beyond 20% level of incorporation. Adeyanju and Ilori (1979) found negative growth and development in pigs fed supplemental cocoa husk beyond 20% level of inclusion. Several workers have linked protein intake and nutrition to fertility in animals. Perry, (1968) found poor sperm production in underfed bulls leading to delay in puberty. However, the animals in this study were all slaughtered at a body weight of 60kg irrespective of the duration of feeding, so the effects of CH on digestibility and growth performance were not determined. The explanation for reduced sperm reserves in pigs fed high CH diets may be due to the high crude fibre and the toxic theobromine content of CH at 15% and beyond which would have reduced nutrient utilisation and hence spermatogenic activities. This confirms earlier

Adejumo

Table 2. *Effects of supplemented cocoa husk on testicular and epididymal weights (g)*

Parameters	R1 Control	R2	R3	R4	R5	R6	SEM
Level of cocoa pod husk (%)	0.00	5.0	10.0	15.0	20.0	25.0	
Testis	15.0.0 ^{ab}	168.89 ^a	150.38 ^{ab}	126.42 ^c	145.64 ^b	120.06 ^c	5.68
Tunica	11.08 ^{ab}	14.52 ^{ab}	17.8 ^a	12.1 ^{ab}	8.46 ^b	10.18 ^b	3.99
Albuginea							
Testicular density (cm ²)	0.093	2.00	0.15	0.128	0.12	0.133	0.055
Caput epididymis	9.87	15.31	8.08	7.76	10.60	10.06	5.94
Corpus epididymis	8.84	6.30	7.20	7.59	5.05	5.12	2.54
Cauda epididymis	13.02 ^{ab}	16.75 ^a	16.9 ^a	12.9 ^b	9.64 ^b	10.83 ^b	1.76

Values are means. SEM (Standard error of means)

Means along the same column differently superscripted differ significantly (P<0.05)

Table 3. *Effects of supplemented cocoa husk on testicular and epididymal sperm reserves (x 10⁹)*

Parameters	R1 Control	R2	R3	R4	R5	R6	SEM
Level of cocoa pod husk (%)	0.00	5.0	10.0	15.0	20.0	25.0	
Testis	2.74	3.27	13.05 ^a	1.85	3.21	7.30 ^b	0.58
Caput epididymis	0.90	1.63	5.64 ^a	0.78	0.66	4.12 ^a	0.71
Corpus epididymis	0.90	1.19	19.8 ^a	0.51	0.29	1.45	2.49
Cauda epididymis	2.60 ^{bc}	1.39 ^c	16.7 ^a	3.4b ^c	1.16 ^c	5.02 ^b	1.13

Values are means. SEM (Standard error of means)

Means along the same column differently superscripted differ significantly (P<0.05)

works (Dutt and Barhartt, 1959) who observed significant reduction in sperm motility, sperm concentrations and fertility of boars on low quality diets. Minerals, according to Reid (1949) are not really important to semen quality unless the animals are exhibiting deficiency symptoms. The animals in this study were in good condition and did not show any pathological symptoms of mineral deficiency due largely to the fact that

CH is also a mineral source on its own. The increase in calcium, sodium and potassium levels in the testis and epididymis with increasing CH levels may suggest a relationship between dietary minerals and testicular mineral levels. In summary, cocoa husk supported optimum testicular characteristics in mature boars at 10% level of incorporation and is tolerated without adverse effects up to 15% level. Beyond this

Cocoa husk inclusion in diets and testicular characteristics of pigs

Table 4. *Effects of supplemented cocoa husk on mineral concentration in the testis (mg dt⁻¹)*

Parameters	R1 Control	R2	R3	R4	R5	R6	SEM
Level of cocoa pod husk (%)	0.00	5.0	10.0	15.0	20.0	25.0	
Calcium	11.04 ^b	11.39 ^b	9.6 ^b	9.68 ^b	19.47 ^a	9.35 ^b	3.42
Potassium	130.80 ^b	181.90 ^a	125.0 ^b	150.0 ^{ab}	183.6 ^a	141.8 ^b	12.86
Sodium	1305 ^b	926 ^b	800 ^b	1258 ^b	1909 ^a	1402 ^{ab}	252.2
Magnesium	7.78	6.55	8.75	6.64	9.24	6.53	2.97
Zinc	1.05	1.05	1.01	0.53	1.54	1.14	0.41
Phosphate	1.12	0.41	1.37	0.74	0.94	0.86	0.59

Values are means. SEM (Standard error of means)

Means along the same column differently superscripted differ significantly (P<0.05)

Table 5. *Effects of supplemented cocoa husk on mineral concentration in the epididymis (mg dt⁻¹)*

Parameters	R1 Control	R2	R3	R4	R5	R6	SEM
Level of cocoa pod husk (%)	0.00	5.0	10.0	15.0	20.0	25.0	
Calcium	12.27 ^b	14.41 ^b	30.14 ^a	13.59	24.22 ^{ab}	10.29 ^b	4.44
Potassium	44.00	47.93	41.80	68.20	72.22	38.70	18.23
Sodium	484.00 ^{bc}	522.50 ^b	418.00 ^{bc}	616.02 ^{ab}	785.32 ^a	390.50 ^c	77.55
Magnesium	4.02	5.17	6.16	4.46	10.23	3.41	3.22
Zinc	0.45	0.95	0.66	1.08	1.65	0.61	0.57
Phosphate	0.23	1.32	0.28	0.28	0.53	0.26	0.08

Values are means. SEM (Standard error of means)

Means along the same column differently superscripted differ significantly (P<0.05)

however, significant depression in testicular and epididymal characteristics were observed with negative effects on testicular development.

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