# Daily sperm production and spermatogenic efficiency of pubertal boars fed graded levels of cassava peel-based diets

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SUMMARY

A twelve week trial was conducted to investigate the daily sperm production and spermatogenic efficiency of pubertal boars fed graded levels of composite cassava peel-based diets. Microbially fermented cassava peel meal (CPM) was incorporated at 0%. 20%, 40% and 60% level into four treatment diets that were used for the experiment. Twelve boarlings with an average initial weight of 37.12±1.04kg were randomly allotted to the four treatment diets at three boars per treatment with each boarling serving as a replicate for its treatment group. The animals were fed with these diets throughout the duration of the trial after which they were slaughtered and their testes dissected out for the determination of gonadal and extra-gonadal parameters.

Measurement of gonadal parameters revealed that the paired testis weight of boars that fed on the cassava peel diets were significantly inferior to those fed with the control diet while the paired testicular volume, volume of testicular parenchyma and paired epididymal weight followed similar trend. The right testicular circumference was highest in boars that were fed with 40% dietary inclusion of CPM. Pigs fed with 60% CPM had highest epididymal volume and the lowest epididymal density respectively. The gonadal sperm reserves were significantly different among dietary treatments and had the lowest numerical value with 60% CPM inclusion which also gave the least values for gonadal sperm reserves/gramme testis, daily sperm production and spermatogenic efficiency.

It could be concluded that the inclusion of CPM above 40% would have adverse effects on spermatogenic efficiency and by extension on the reproductive capacity of the breeding male pig.

Keywords**:** Cassava peel, fermentation, pubertal boars, sperm production

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## INTRODUCTION

The relatively high cost of the conventional feed ingredients has necessitated the concentration of research efforts on the possibility of making use of the unconventional and under-utilized feed resources in animal nutrition. These unconventional/under-utilized feed materials include cassava peels and cassava starch residues which are both by-products of cassava processing and utilization (Aro, 2010). Onyinmoyi and Okeke (2008) stated that cassava has remained a staple carbohydrate source for humans in Nigeria especially in the southern part of the country. Nigeria currently ranks as the largest producer of cassava in the world with an estimated annual production of 34-40 million metric tonnes of cassava tubers which translates to 7.2-8.0 million metric tonnes of the peels, 6.8 million metric tonnes of cassava starch residues and 2.4 million metric tonnes of leaves most of which are left to waste or rot away thereby constituting a nuisance to the environment (Aro, 2010). These alternative feed resources have been tried by several authors with varying degrees of success in livestock nutrition. The potentials of both the cassava peels and cassava starch residues have been exploited in pig's ration with the conclusion that both contain high level of anti-nutrients and fibre which have been associated with reduced growth rate and feed efficiency in pigs (Aro et al., 2010)

Fermentation as a form of biotechnology has been used for many years for modification of biological materials into useful products for man and as a method of reducing the anti-nutrient and fibre contents of feed (Campbell-Platt, 1994; Dhilon and Skirvaram, 1999). While fermentation has been able to obviate most of the nutritional challenges of cassava by-products, the same may not be said of the impact of the residual anti-nutrients in such cassava by-products on the reproductive efficiency of livestock. Feed anti-nutrients have been reported to have spermicidal effects in male animals (Oko and Hrudka, 1982; Egbunike et al., 1999; Aro, 2010). It is therefore pertinent to ascertain the appropriate level of inclusion of these unconventional ingredients in the practical livestock diets so as not unnecessarily compromise the reproductive performance of farm animals. The objective of this study therefore was determine the effects of feeding graded levels of microbially fermented cassava peels on the reproductive performance of pubertal boars.

## MATERIAL AND METHODS

### *Experimental site*

The trial was carried out at the Teaching and Research Farm (Livestock Unit) of the Federal University of Technology, Akure, Ondo State, Nigeria.

*Collection and drying of cassava peels*

The composite cassava peels used for the feeding trial were collected in the fresh form from the local gari processing factories located at Igbatoro area of Akure. The peels were subsequently sun-dried for four to seven days and packed in polythene bags prior to microbial fermentation.

*Fermentation of dried cassava peels with candidate organisms*

Two (2) kilogrammes of the dried cassava peels were weighed into plastic bowls, moistened with 100cl of water, packed and hermetically secured in nylon bags. These 2-kg packs of moistened cassava peels were then autoclaved for 15 minutes at a temperature of 121$^{o}$C at a pressure of 15lb/inch. The packs were allowed to cool to a temperature of 37$^{o}$C after which they were emptied into fermentation trays measuring 54cm x 38cm x 4cm for length, breadth and depth respectively (Fig. 1).

Figure 1: Fermentation tray with cassava peel sample undergoing fermentation.

Figure 2: Fermentation chamber with six trays drawn out to show the arrangement of trays within the chamber.

They were inoculated with the candidate organisms (*Aspergillus fumigatus, Lactobacillus delbrueckii* and *Lactobacillus coryneformis)* in a lamina flow chamber, covered with the transparent sheets and thereafter left to ferment in a fermentation chamber (Fig. 2) for five days. The fermented cassava peels were sun-dried for three days and were stored in polythene bags for subsequent use as a feed ingredient for the formulation of the experimental diets.

*Experimental diets*

Four isocaloric and isonitrogenous diets (Table 1) were formulated in which the microbially fermented cassava peels were incorporated at 0%, 20%, 40% and 60% levels in the diets. The 0% level of inclusion served as the control diet.

Table 1. Gross composition of the experimental diets.

| Ingredients | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| Maize (%) | 40.00 | 35.00 | 15.00 | 0.00 |
| Rice bran (%) | 11.00 | 7.20 | 10.00 | 0.00 |
| Palm kernel cake (%) | 26.50 | 16.50 | 14.50 | 21.30 |
| Groundnut cake (%) | 17.00 | 18.30 | 17.50 | 17.50 |
| MFCP (%) | 0.00 | 20.00 | 40.00 | 60.00 |
| Vegetable oil (%) | 2.50 | 0.00 | 0.00 | 0.00 |
| Bone meal (%) | 1.50 | 1.50 | 1.50 | 1.50 |
| Oyster shell (%) | 0.50 | 0.50 | 0.50 | 0.50 |
| Premix (vit/min) (%) | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt (%) | 0.50 | 0.50 | 0.50 | 0.50 |
| Total (%) | 100.00 | 100.00 | 100.00 | 100.00 |
| Crude protein (%) | 18.07 | 18.09 | 18.09 | 18.09 |
| ME (MJ/kg) | 12.65 | 12.60 | 12.56 | 12.62 |
| HCN concentration in mg/kg of diet | 0.00 | 5.58 | 11.16 | 16.75 |

T1 = 0% cassava peel meal (CPM); T2 = 20% cassava peel meal (CPM); T3 = 40% cassava peel meal (CPM); T4 = 60% cassava peel meal (CPM); MFCP = Microbially fermented cassava peels; ME = Metabolizable energy

*Experimental animals and their management*

Twelve 14 weeks old male pigs (boarlings) with an initial average body weight of 37.12±1.04kg were randomly allotted into four treatment diets with 0%, 20%, 40% and 60% levels of fermented cassava peel meal respectively. The animals were housed in individual pens measuring 2m x 3m equipped with permanent concrete feeders and drinkers. The animals were given shots of ivermectin® against ecto and endo-parasites before the commencement of the trial. Feeds were offered twice daily at 8.00 hours and 16.00 hours at 5% of the body weight adjusted weekly to correct for the increase in body weight. The animals were fed with these treatment diets throughout the twelve week

duration of the experiment. The onset of puberty was determined in the animal as described by Aro et al. (2011a)

*Dissection of the testis and determination of the reproductive parameters*

At the end of the experiment, all the animals were starved of feed overnight and were slaughtered the following morning during which their reproductive organs (testes, epididymides, seminal vesicles, Cowper's and prostate glands) were carefully dissected out. These samples were immediately taken to the laboratory for the determination of gonadal and extra-gonadal sperm reserves and other reproductive parameters. Briefly, the epididymides and the testes were dissected from each other and weighed. The volume of both the epididymis and the testis was determined by water displacement using normal saline. The *tunica albuginea* was dissected out of the left testis of each pig and weighed from which the testicular parenchyma weight was obtained by difference. Thereafter the testes and epididymides were homogenised using a blender each in 200ml and 100ml of normal saline respectively. The homogenates were filtered using a double ply of cheese cloth as described by Aro (2010). The filtrates were used to determine testicular and epididymal sperm count using the improved *Neubauer haemocytometer*. The testicular sperm counts were used to compute the gonadal sperm reserves from which the daily sperm production was derived from the formula:

Daily sperm production = Gonadal sperm reserves ÷ 4.37
where 4.37 is the time divisor for pigs (Egbunike, 1980).

The spermatogenic efficiency (efficiency of sperm production) was determined by dividing daily sperm production by the paired testes weight while the epididymal (extra-gonadal sperm reserves and epididymal sperm reserves/gramme epididymis were calculated from the epididymal sperm counts.

Statistical analysis: All data were subjected to one-way analysis of variance using the completely randomised design of SPSS15.0 (SPSS, 2006) statistical package. Mean separation, where applicable was done with Duncan multiple range test (DMRT) of the same statistical package.

## RESULTS

Table 2 shows the numerical values of the measured testicular parameters. The paired testis weight varied from 204.00g in T4 to 357.40g in T1. A progressive decrease in the size of the testis was therefore observed as the level of CPM increased in the diets. The same trend was obtained for the

paired epididymal density. The paired testicular volume, the paired epididymal weight and the volume of testicular parenchyma were all higher in T3 than in T2, an indication that the decrease in value recorded for animals fed 20% CPM could not have been caused by the feed anti-nutrients at that inclusion level. The paired testicular density increased from 1.05gcm$^{-3}$ in T1 to 1.65gcm$^{-3}$ in T4. The highest value for testicular circumference (20.74cm) was recorded in T3 while the least value (15.89cm) was obtained in T4. The relative weight of tunica albuginea to testicular weight showed that boars fed 60% inclusion of CPM in their diet had the thickest *tunica albuginea*.

Table 2: Gonadal measurements of boars fed graded levels of microbially fermented cassava peel meal (CPM) diets.

| Parameters | T1 | T2 | T3 | T4 | ±SEM |
|---|---|---|---|---|---|
| Paired testis weight(g) | 357.40$^{a}$ | 276.85$^{b}$ | 353.60$^{a}$ | 204.00$^{c}$ | 33.57 |
| Paired testis volume(cm$^{3}$) | 341.50$^{a}$ | 241.00$^{c}$ | 299.00$^{b}$ | 150.50$^{d}$ | 35.68 |
| Paired epididymal weight(g) | 108.60$^{a}$ | 93.10$^{b}$ | 100.05$^{ab}$ | 70.45$^{c}$ | 6.88 |
| Paired epididymal volume(cm$^{3}$) | 78.44 | 67.70 | 72.85 | 79.50 | 4.52 |
| Paired testis density(gcm$^{-3}$) | 1.05 | 1.15 | 1.19 | 1.65 | 0.12 |
| Paired epididymal density(gcm$^{-3}$) | 1.38$^{a}$ | 1.38$^{a}$ | 1.37$^{a}$ | 0.89$^{b}$ | 0.08 |
| Right testicular circumference(cm) | 17.60$^{ab}$ | 17.60$^{ab}$ | 20.74$^{a}$ | 15.89$^{b}$ | 1.03 |
| Weight of *tunica albuginea*(g) | 13.90$^{a}$ | 6.60$^{b}$ | 10.15$^{a}$ | 6.75$^{b}$ | 1.71 |
| % *tunica albuginea* | 7.75$^{a}$ | 4.96$^{b}$ | 5.53$^{b}$ | 8.45$^{a}$ | 0.88 |
| Volume of testicular parenchyma(cm$^{3}$) | 157.34$^{a}$ | 109.78$^{b}$ | 138.31$^{a}$ | 69.96$^{c}$ | 16.48 |
| Volume of *tunica albuginea*(cm$^{3}$) | 13.18$^{a}$ | 5.72$^{c}$ | 8.69$^{b}$ | 5.05$^{c}$ | 1.72 |

a,b,c,d = Means on the same row but with different superscripts are statistically (P<0.05) different. T1 = 0% cassava peel meal (CPM); T2 = 20% cassava peel meal (CPM); T3 = 40% cassava peel meal (CPM); T4 = 60% cassava peel meal (CPM)

Table 3: Gonadal and extra-gonadal sperm reserves, daily sperm production and spermatogenic efficiency of boars fed microbially fermented cassava peel meal (CPM) diets.

| Parameters | T1 | T2 | T3 | T4 | ±SEM |
|---|---|---|---|---|---|
| Gonadal sperm reserves(x10$^{9}$) | 35.85$^{a}$ | 34.48$^{a}$ | 31.75$^{b}$ | 13.06$^{c}$ | 2.88 |
| Gonadal sperm reserves/gram testis(x10$^{6}$) | 103.06$^{ab}$ | 124.16$^{a}$ | 85.49$^{b}$ | 64.02$^{b}$ | 21.53 |
| Daily sperm production(x10$^{9}$) | 8.20a | 7.87ab | 7.27b | 2.99c | 0.66 |
| Spermatogenic efficiency(x10$^{6}$) | 23.58$^{bc}$ | 28.41$^{a}$ | 19.56$^{c}$ | 14.66$^{d}$ | 4.93 |
| Epididymal sperm reserves(x10$^{9}$) | 54.95 | 46.00 | 73.97 | 50.85 | 8.34 |
| Epididymal sperm reserves/gram epididymis(x10$^{6}$) | 516.42 | 531.43 | 729.30 | 667.57 | 81.19 |

a,b,c,d = Means on the same row but with different superscripts are statistically (P<0.05) different. T1 = 0% cassava peel meal (CPM); T2 = 20% cassava peel meal (CPM); T3 = 40% cassava peel meal (CPM); T4 = 60% cassava peel meal (CPM)

Table 3 shows the sperm reserve capacity and the spermatogenic efficiency of boars fed diets containing varying levels of CPM. Values obtained for gonadal sperm reserves and daily sperm production differed ($P<0.05$) among the treatment groups and showed that the three CPM diets were inferior to the control diets as far as these two reproductive parameters are concerned. There was an increase in gonadal sperm reserves/gram testis and spermatogenic efficiency as a result of dietary inclusion of CPM at 20% level which gradually declined at 40% and 60% levels. The epididymal sperm reserves/gram epididymis showed that animals fed the CPM packed more sperm cells per gram of their epididymides than those fed the control diet.

## DISCUSSION

The decrease in paired testis weight (Table 2) observed in all the CPM diets showed that the testicular weight is negatively influenced by the dietary treatments applied. Many authors (Amao et al., 2010; Ajuomuna et al., 2011; Aro et al., 2011a) reported on the decrease in testicular weight as a result of the use of alternative feed sources of agro-industrial origin like cassava peel meal, fermented cassava tuber wastes and neem rind meal. The weight of the testis is one of the yardsticks for measuring sperm producing ability and hence reproductive efficiency of the male stock selected for breeding (Okwun et al., 1996; Aro et al., 2011b). Animals fed the diet in which CPM was incorporated at 60% level could therefore have compromised breeding ability as occasioned by their inferior testicular weight. The paired testis volume and paired epididymal weight followed the same trend as the paired testis weight. In fact, the heavier the testicle, the more the volume it will occupy in space. Testicular volume is related to the volume the testicular parenchyma will occupy *in situ* and hence the absolute volume of the seminiferous tubules (Martin et al., 1994). The larger the testicular volume, the larger the absolute volume of the seminiferous tubules and hence their capacity for spermatogenesis.

The increase in testicular density with increase in dietary inclusion of CPM is an indication of progressive decrease in both the weight and volume of the testes as the level of CPM in the diets increased. Similar observation was reported by Amao et al. (2010). The least epididymal density recorded for T4 could have been caused by its relatively small epididymal weight. Values for testicular circumference and testicular parenchyma were inferior in T4 to those of other treatments. Moser et al. (1996) reported that bulls with small scrotal circumference and by extension, small testicular circumference had higher scores for total sperm abnormalities, lower motility scores and lower percentage satisfactory scores. They also concluded that progenies from these bulls had lower weaning scrotal circumference and weaning testicular weight.

Pigs fed with 60% microbially fermented CPM could therefore have reduced breeding efficiency in terms of high sperm abnormalities and lower motility scores. The percentage weight of *tunica albugenia* to the testicular weight showed that pigs that were fed the T4 diets had more percentage of the testis as *tunica albugenia*. The implication of this is a reduction in the proportion of testicular parenchyma in boars fed this diet and hence a reduction in absolute volume of the seminiferous tubule and a concomitant decrease in reproductive efficiency of the boars (Okwun et al, 1996; Bielli et al., 1999).

Boars fed T4 diet were inferior in terms of gonadal sperm reserves, gonadal sperm reserve/gram testis, daily sperm production and spermatogenic efficiency (Table 3). All these parameters are indicators of sperm producing ability of any breeding male stock. The inclusion of microbially fermented peels at 60% level led to a significant fall in all these breeding soundness indices. The Gross composition of the diets (Table 1) showed that the level of residual cyanide at 60% inclusion of CPM was 16.75mg/kg if the diet. This would have provoked germ cell apoptosis during spermatogenesis resulting in germ cell loss. There is thus an obvious spermicidal effect of residual cyanide at 60% inclusion level. The reproductive efficiency of the boars may thus be suppressed at this concentration of residual cyanide in the diet. Similar spermicidal effect of gossypol was reported by Oko and Hrudka (1982) and Egbunike et al. (1999). The paired epididymal sperm reserve and epididymal sperm reserve/gram epididymis showed that boars fed diet with 60% CPM seemed to have a lot of sperm cells packed in their relatively smaller epididymis and could probably be sperm cells that have managed to escape the spermicidal effect of residual cyanide during spermatogenesis and are now concentrated and stored in the epididymis as a form of physiological adjustment. Aro and Adejumo (2011) reported such physiological adjustment by way of relatively bigger epididymides in polyandrous group of albino rats.

## CONCLUSIONS

Testicular weight, volume and testicular parenchyma were adversely affected by dietary inclusion of cassava peel meal (CPM) and more so at 60% inclusion level. Also at this level of inclusion, gonadal sperm reserves, gonadal sperm reserves /gram testis, daily sperm production and spermatogenic efficiency were all depressed. These indicators of reproductive efficiency in the male stock were however not adversely affected by the incorporation of 40% of CPM in the diet. It is therefore safe to conclude within the limit of this experiment that utilization CPM in the diet should not exceed 40% level so as not to compromise the reproductive efficiency of breeding boars.

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