Trop J Anim Sci. 3 (2) : 77-84 (2000)
ISSN 1119-4308

SPERM PRODUCTION AND RELATED PHENOMENA IN RATS FED DIETS CONTAINING PAWPAW PEELS, SEEDS AND LEAVES

G.N. Egbunike, M.O. Sadiku and O.E. Okebiorun

Animal Physiology Laboratory, Dèpartment of Animal Science, University of Ibadan, Ibadan, Nigeria

Target audience: Animal scientists, Livestock farmers, Veterinarians

ABSTRACT

This study undertaken to see the effects of dietary inclusion of pawpaw peels, seeds and leaves on sperm production and related phenomina in rats using four diets as follows: In diet 1 and 2 50% of rat pellets were replaced by pawpaw leaves ands seeds respectively, while in diet 3 40% of rat pellets was replaced by unripe pawpaw peels and dies 4 was 100% rat pellet and served as control.

The control and pawpaw seed-based diets supported a continuous positive growth unlike the other two diets. Also organ weights, sperm reserves of the control rats were generally superior to those of rats on the other diets except the seed-based diets. However, sperm moved faster through the epididymis of rats fed the leaf-based diet while the movement of sperm through the epididymis of rats on pawpaw peel- and seed-based diets was delayed compared to the sperm transit in the control group. The results have shown that dietary inclusion of pawpaw leaves, seeds and peels has some adverse effects on sperm production and storage potentials and epididymal transit in the rat.

Key words: Pawpaw peels, seeds and leaves; sperm production; rats.

DESCRIPTIONOF PROBLEM

Within the past three decades the interest of Nigerian animal producers, like those elsewhere, has been centred mostly on the search for cheaper feed ingredients that are always available and have no competition with mans' dietary demands (1,2). Although some agro-industrial byproducts and plants have been used so far with varied levels of success, the plant, pawpaw (*Carica papaya*, Linn), seems to be a promising cadidate especially as the peels of its fruit whether ripe or unripe as well as its seeds and dried leaves can be utilized as cheap sources of supplements for livestock feeds especially as they have a high protein content in addition to minerals and vitamins (3) and pawpaw leaves were shown to be similar to banana and cassava leaves as regards their effects on basic haematological parameters (4).

However. every part of pawpaw produces a latex which contains papain. a proteolytic enzyme (5). which can remove the zona pellucida of mouse eggs (6). causes premature closure of epiphyses (7). has anti-implantation activity (8) and has embryotoxis and teratogenic effects (9). Also. Morimatsu et al (10) suggested that papain-hydrolysis of pork produced peptides that have hypochlesteroleamic effects through their interference with the steroid absorption process.

So far. to the best of our knowledge. there has been no work on the interaction of pawpaw parts or papain on testicular function. It is therefore in light of the above that we decided to investigate the effects of pawpaw peels. seeds and leaves on sperm production. sperm reserves and sperm transit time through the epididymis in the rat.

MATERIALS AND METHODS

Animal and Management

The adult rats of Wistar strain used in this study were housed in caged with wood shavings as bedding. They were fed and given clean water *ad libitum*. There were four diets As follows: diet 1 contained 50% dried pawpaw leaves and 50% rat pellets (Pfizer): diet 2 contained 50% dried pawpaw seeds and 50% rat pellets; diet 3 contained 40% unripe pawpaw peels and 60% rat pellets and diets 4 was 100% rat pellets (this served as control). These combinations were based on the nutrient composition of the pawpaw parts resulting from theproximate analyses using the AOAC (11) methods. These are shown in Table 1 while the proximate composition of the experimental diets is no Table 2. All the animal were weighed weekly.

Table 1. Dry matter. crude protein and energy levels of pawpaw leaves. unripe peels and seeds and rat pellets (means ± sem)

Materials	Dry matter (%)	Crude protein (%)	Energy (Kcal/g)
Pawpaw leaves	99.50	26.25 ± 0.02	2.18 ± 0.08
Unripe pawpaw peels	99.50	18.45 ± 0.05	1.72 ± 0.01
Pawpaw seeds	99.50	20.56 ± 0.05	2.83 ± 0.41
Pfizer pellets	88.00	21.00 ± 0.21	2.15 ± 0.05

Sampling and Histometric Analyses

After the rats had been fed for six weeks they were all stunned and decapitated. Thereafter the testes. epididymides. seminal vesicles, livers, adrenal glands. and prostate glands were all dissected and weighed immediately. The testes and epididymal regions were then homogenized according to Egbunike et al (12) and the sperm producing potential of the rats determined by dividing the gonadal sperm reserves by the divisor for rats as proposed by Amann et al (13). The time of transit of sperm through the epididymis was determined by the method already outlined by Amann et al (13).

Statistical Analyses

Data were subjected to Students' tests while chi-squire tests were used on the data on growth rates for the assessment of significance (14).

Table 2. Proximate composition of experimental diets

Parameters (%)	Diets			
	1(Pellets + Leaves)	2 (Pellets + Seeds)	3 (Pellets + Peels)	4 (Pellets)
Moisture	22.30	9.69	12.71	12.00
Ether extracts	4.29	16.42	2.92	3.50
Crude fibre	8.15	11.62	9.30	6.00
Crude protein	31.72	28.62	26.25	21.00
Total ash	10.95	7.47	7.51	6.82
Nitrogen free Extracts	20.77	26.14	41.31	50.68

RESULTS AND DISCUSSION

The growth rates of all the animals are as summarized in Figure 1. There were two growth patterns with rats on control diet and those on pawpaw seed-based diet showing a continuous positive growth while those on pawpaw peel-baseddiet showed an apparently neutral growth and those on pawpaw leaf-based diet exhibited a negative growth. Thus the overall mean growth rates were 91.23 ± 1.58, 123.14 ± 4.03, 98.96 ± 2.14 and 123.35 ± 5.08% for the diets with pawpaw leaves, seeds and peels and control diets, respectively. The control and pawpaw seed-based diets were highly significantly different ($P<0.01$) from the other two diets, which were different ($P<0.05$) from each other.

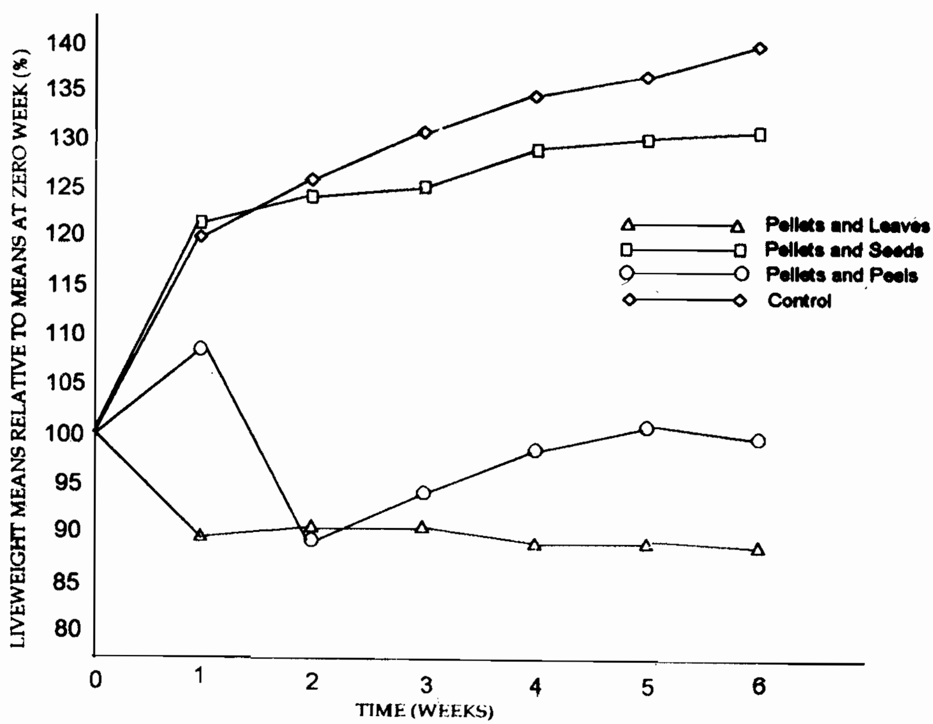

Fig. 1 Comparative growth rate of adult male rats fed diets containing pawpaw leaves, seeds and

The paired testes of rats on control diets were significantly ($P<0.05$) heavier than those of rats on pawpaw peel-based diets which were however not different from those on the other two diets (Table 3). The weights of the epididymides and seminal vesicaels of control rats were more than those of the others on the diets containing pawpaw parts. As regards prostate glands, the weights from control rats were more ($P<0.05$) than those on the diet with pawpaw peels but not those on diets with pawpaw leaves and seeds while the livers from control rats were heavier ($P<0.05$) than those on pawpaw leaves and peels. The paired kidneys and adrenals were unaffected by the treatment.

Testicular sperm reserves of control rats were significantly ($P<0.05$) higher than those of rats in the other diets while those in the caput, corporal and cauda epididymal regions and the paired epididymides of the control rats were superior to all the others exept those on pawpaw seed-based diets (Table 4). The daily sperm production potential (DSP) and the efficiency of sperm production DSP/g testis parenchyma) are summarized in Figure 2. Mean DSP of the rats ranged from 159.25 ± 34.76 to $218.90 \pm 36.03 \times 10^6$ while DSP/g testis parenchyma ranged from 121.63 ± 29.81 to $136.05 \pm 27.49 \times 10^6$. Treatment means were not significantly different although the control rats appeared to be superior.

Table 3. Absolute organ weight of male rats fed diets containing pawpaw leaves, seeds and unripe peels and control diets (means ±SEM)

	Diets			
Organs (g)	1(pellets +Leaves)	2(pellets + Seeds)	3(Pellets +Peels)	4(Pellets)
Paired testes	1.45 ± 0.10^{ab}	1.43 ± 0.05^{ab}	1.32 ± 0.21^{b}	1.78 ± 0.13^{a}
Paired adrenals	0.02 ± 0.001	0.02 ± 0.001	0.03 ± 0.005	0.03 ± 0.009
Paired kidneys	1.0 ± 0.08	0.07 ± 0.06	0.99 ± 0.06	1.16 ± 0.11
Paired	0.48 ± 0.06^{b}	0.49 ± 0.07^{b}	0.45 ± 0.08^{b}	0.63 ± 0.02^{a}
epididymides	0.35 ± 0.15^{b}	0.37 ± 0.08^{b}	0.34 ± 0.14^{b}	0.67 ± 0.03^{a}
Seminal vesicles	0.15 ± 0.01^{a}	0.10 ± 0.02^{ab}	0.09 ± 0.02^{b}	0.19 ± 0.02^{a}
Prostate gland	6.36 ± 0.77^{b}	7.48 ± 0.90^{ab}	6.20 ± 1.19^{b}	8.18 ± 0.41^{a}
Liver				

ab: Value on the same row with different superscripts differ significantly ($P<0.05$)

Epididymal transis time of sperm in these rats was significantly ($P<0.05$) influenced by the dietary treatments (Table 5). On the whole, spermatozoa spent less time ($P<0.05$) in the epididymis of rats fed pawpaw leaf-based diet than those on pawpaw seed- and peel-based diets, which however were similar to the control rats. Generally, sperm spent a mean total of 10.17 ± 1.33 days in the epididymis with 6.10 ± 0.89 days (59.98%) spent in storage in the cauda epididymis.

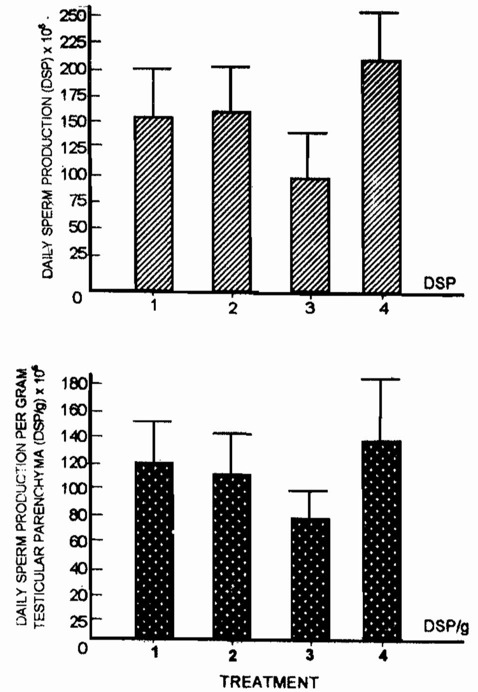

Fig. 2. Daily Sperm production (DSP) and efficiency of spermatogenesis (DSP/g testis parenchyma) of rats fed diets containing pawpaw leaves, seeds and peels (means ± sem).

(Graph)

That pawpaw seed-based diet was able to support a positive growth like the control diets unlike the pawpaw peels- or leaf-based diets despite the differences in the crude protein content of the diets is interesting. Although the digestion of the feeds was not studied, the

Table 4. Testicular and epididymal sperm reserves of rats fed diets containing pawpaw leaves, seeds and unripe peels and control diets ($x19^8$, means ± sem)

Regions	Diets			
	1 (pellets + Leaves)	2 (pellets + Seeds)	3 (Pellets +Peels)	4 (Pellets)
Testes	9.71 ± 2.12^{ab}	9.97 ± 2.72^{ab}	6.11 ± 2.60^{b}	13.35 ± 2.20^{a}
Caput epididymides	3.41 ± 1.67^{b}	7.57 ± 2.05^{a}	3.16 ± 1.78^{b}	6.42 ± 0.82^{a}
CorpuS epididymides	0.30 ± 0.12^{b}	0.40 ± 0.10^{b}	0.44 ± 0.20^{b}	0.84 ± 0.27^{a}
Cauda epididymides	6.38 ± 2.70^{c}	9.61 ± 3.68^{ab}	8.59 ± 4.24^{bc}	14.39 ± 4.85^{a}
Paired epididymides	10.09 ± 2.70^{b}	17.07 ± 4.02^{a}	12.19 ± 2.95^{b}	21.65 ± 5.22^{a}

abc: Value on the same row differently superscripted differ significantly ($P<0.05$).

results suggest that three was little or no papain in the pawpaw seeds as compared to contents in the peels and leaves. Das (15) in his study had established that adult male rats fed with 0.2 ml of a suspension containing 20 mg of pawpaw seed powder daily for eight weeks had a serious reduction in body weight gain while Singh and Devi (9. 16) showed that administration of crude papain into regnant rats at 370 mg/kg be resulted in severe growth retardation and reduced elongation of the humerus. radius and ulna of the developing foetus.

Similarly. the response of the paired testes weight, epididymides. seminal vesicles, prostates. livers and kidneys to the diets tends to suggest the presence of papain at differing levels. However. peels and leaves appear to be more implicated than the seeds. The digestives effects of papain may have affected 5-α-reductase activity in the prostate and seminal vesicles and hence affect the growth rate of these organs (17). This is confirmed by the results of Akorede (18) showing that growth rate tended to decrease with increasing levels of pawpaw leaves.

As would be expected. gonadal and extragonadal sperm reserves as well as sperm production potential followed the same trend as the paired testes and epididymal weights as per the dietary treatments. It is worthy to note that Das (15) had observed that papaya seed powder had no effect on spermatogenesis. However, on further analysis. it was observed that the sperm storage capacity of the epididymis was big enough to accommodate 6.31, 10.54, 12.19 and 9.84 days' production of sperm in the pawpaw leaf-, seed- and peel-based and control diets. respectively. Although we have no data on the physiology of the semen in rats, these results suggest that the semen of rats on pawpaw leaf-based diet could contain less mature and active sperm cells than the others.

Our results on epididymal transit time showed that sperm moved faster through the epididymis of rats fed pawpaw leaf-based diet while their movement was delayed in rats fed pawpaw peel- and seed-based diets compared to the control group (Table 5). Whether this is through any effects of these dietary treatments on androgen-dependent activities of the epididymis will await further studies but it is known that sperm

Table 5. Sperm epididymal transit time in rats fed diets containing pawpaw leaves. seeds and unripe peels and control diet (days; means ± SEM).

Dietary Treatment	Epididymal regions			
	Caput	Corpus	Cauda	Total
Pellets + Leaves	2.46 ± 0.96^b	0.21 ± 0.07^b	3.82 ± 1.18^b	6.48 ± 1.61^a
Pellets + Seeds	4.88 ± 0.52^a	0.32 ± 0.11^{ab}	5.82 ± 1.60^{ab}	11.02 ± 2.03^a
Pellets + Peels	4.17 ± 2.87^a	0.54 ± 0.33^a	8.06 ± 3.43^a	12.77 ± 6.33^{ab}
Pellets (Control)	3.29 ± 0.80^{ab}	0.45 ± 0.17^a	6.68 ± 1.64^a	10.42 ± 2.62^{ab}
Means ± SEM	3.70 ± 0.52	0.38 ± 0.07	6.10 ± 0.89	10.17 ± 2.62^{ab}

ab: Treatment values with different superscripts differ significantly ($P<0.05$).

Transit is accompanied by changes in the metabolic profile of sperm and hence the capacity for motility couple with the loss of the droplet of spermatid cytoplasm from the cell tail and a modification of the rostral region of the acrosome (19, 20). The results of this study have shown that the inclusion of pawpaw leaves, seeds or peels in rat feeds has some adverse effects on sperm production and storage potentials and sperm epididymal transit in the rat. However, the seeds appear to be less potent than the peels and leaves.

REFERENCES

1. Egbunike, G.N. and Ikpi, A.E. (1988). Can agroindustrial byproducts and crop residues save The Nigerian Livestock Industry? In: Proc. PANESE/ARNAB Workshop on Utilissation of Research Results in Forage and Agricultural byproduct material as Animal Feed Resources in Africa" (Dzowela, B.H., Said, A.N. Wendem-Angenehu, Asrat and Kategile, J.A., eds), December 5-9, 1988, Lilongwe, pp 477-487.

2. Egbunike, G.N. (1997). What is Animal Science and how can Nigeria get out of malnourishment? In: Proc. 2nd Ann. Confr. Anim. Sci. Assoc. Negeria, Sept 16-17, 1997, Ikeja, Lagos, pp 1.12.

3. Oyenuga, V.A. (1968). Nigeria's foods and feeding stuffs: Their chemistry and nutritive value. University of Ibadan Press, Ibadan.

4. Oteku, I.T., Arijeniwa, A and Egharevba, O.I. (1999). Effect of age and tropical leaf types on heamatological parameters of growing New Zealand white rebbits. In: Proc. 4th Ann. Confir. Anim. Sci. Assoc. Nigeria, Sept. 14-16, 1999, Ibadan, pp 148-149.

5. Bersin, T. (1935). Cited in Adverses in Enzymology and Related Subjects in Biochemistry (F.F. Nord, ed), Fordham University, New York, 1957, 19, 267-334.

6. Gwatkin, R.B.L. (1964). Effects of enzymes and acidity on the zona pellucida of the mouse egg before and after ferilization. J. Reprod. Fert. 7:99.

7. Spicer, S.S. and Bryant, J.H. (1958). Systematic effects in rabbits receiving injection of papain and chondroitin sulphate. Am. J.Path. 34:61-67.

8. Grag, S.K, Saksena, S.K. and Chaudhury, R.R. (1970). Antifertility screeningof plant parts .VI. effect of five indigenous plants on early pregnancy in albino rats. Indian J. Med. Res. 58: 1258-510.

9. Singh, S. and Devi, S. (1978). Teatogenic and embryotoxic effects of papain in raat. Indian J. Med. Res. 67: 499-510.

10. Morimatsu, F., Ito, M., Budijanto, S., Watanabe, I., Furukawa, Y. and Kimura, S. (1996). Plasma cholesterol-suppressing effect of papain-hydrolyzed pok meat in rat fed hypercholesterolemic diet. J. Nutr. Sci. and Vitaminology 42: 145-153.

11. A.O.A.C. (1990). Official methods of analysis. Association of Official Analytical Chemists (15th ed), Arlington, VA.

12. Egbunike, G.N., Holtz, W., Endell, W. and Smidt, D. (1975). Reproductive capacity of German Landrace boars. I. Gonadal and extragonadal sperm reserves. Zuchtygiene 10: 184-187.

13. Amann. R.P.. Johnson. L.. Thomson. D.L.. Jr.. and Pickett. B.W. (1976). Daily spermatozoa production. epididymal spermatozoa reserves and transit time to the spermatozoa through the epididymis of the rhesus monkey Biol. Reprod. 15: 586-592

14. Steel. R.G.D. AND Torrie. J.H (1980). Principels and Procedures of Statistics: A biometrica: Approach (2nd ed). McGraw-Hill Book Co. New York.

15. Das. R.P. (1980). Effect of papaya seed kon the genital organs and fertility of rats. Indian J. Exp Biol. 18: 408-409.

16 Singh. S. and Devi. S. (1980). Effect of papain on foetal chodrogenesis. Result male of material administration of the enzyme in the rats. Indian J. Exp. Biol. 18: 953-957.

17. Nalbadov. A.V. (1976). Reproductive Physiology in Mammals and Birds. W.H. Freeman and company. San-Francisco (3rd ed).

18. Akorede. A.A. (1998). Effects of old pawpaw leaf diet on sperm reserves and semen characteristics in the chicken. M. Sc. Dissertation. University of Ibadan. Ibadan.

19. ~~Bedford. J.~~M. (1975). Maturation. transport and fate of spermatozoa in the epididymis. In: Handbook of physiology: Endocrinology. Vol. V.. (R.O> Greep and D.H. Hamilton. eds). American Physiological Society. Washington. D.C.. pp 303-317.

20. Bedford. J.M. (1975). Evolution of sperm maturation and storage functions of the epididymis. In: The Spermatozoon (D.W Fawcett and J.M Bedford. eds). Urban and Schwarzenberg. Baltimore/Munich. pp 7-34