Effects of old pawpaw leaf-based diets on the kinetics of spermatogenesis and gonadal sperm storage and production potentials in the domestic chicken

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Abstract

The effects of dietary inclusion of dried old pawpaw leaves on spermatogenesis, sperm storage and production potentials of the domestic chicken were evaluated using 50 White Rock cockerels of 12 weeks of age. The dietary levels of pawpaw leaves were 0, 3.75, 7.50, 11.25 and 15.00% and feeding was for 13 weeks. Liveweight was not affected by the dietary inclusion of pawpaw leaves unlike absolute testis weight and paired testes weight that were higher in the diet with 7.50% pawpaw leaves than those with higher dietary levels of pawpaw leaves. Spermatogenesis was stable with treatment and stage 5 of the cycle of seminiferous epithelium was the most frequent occurring stage and lasted for 16.32 hours. Sperm production and gonadal sperm storage potentials were significantly higher in the diet with the highest level of dried pawpaw leaves (15.00%). The results imply that the papain in the dried leaves may have a differential influence on liveweight and testicular development but any concluding statement will await further studies.

Introduction

It is indeed in the interest of animal producers to continuously search for other feed resources for which there is no competition between man and his livestock for survival (Egbunike and Ikpi, 1988; Egbunike, 1997). Nigeria is known to have a lot of agro-industrial byproducts and crop residues (Egbunike and Ikpi, 1988) and non-conventional feed resources. Thus a proper evaluation of these alternative feed resources will reduce the pressure on the ever expensive conventional feed ingredients and hence result in the attainment of feed surplus for monogastric animals.

So far, some work has been done on the use of seeds/kernels of mangoes (*Magnifera indica*) (Odunsi and Farinu, 1997), whole palm kernel (Oruwari, Sese and Mgbere, 1995), full-fat sunflower seed (Adeniyi and Ologhobo, 2000), full-fat extruded soybean (Nworgu and Egbunike, 1999; Nworgu et al, 2000), neem (*Azadirachta indica*) kernels (Sokunbi, Adisa and Egbunike, 1999), jackbean (*Canavalia ensiformis*) (Esonu, Udedibie and Imo, 2001; Ologhobo, Mosenthin and Alaka, 2003), limabean (Ologhobo et al, 2003) and bambara groundnut (*Voandzea subterranean* (L) thours) (Anyanwu et al, 2003) in animal production. Also, there have been studies on mango leaves (Agunbiade et al, 2001), cassava and its byproducts (Tewe, 1983; Tewe and Egbunike, 1988; Esonu and Udedibie, 1993; Ogbonna and Ige, 2002), pawpaw leaves, seeds and peels (Egbunike, Sadiku and Okebiorun, 2000), leaves of *Centrosema pubesens* and *Cynodom dactylon* (Nworgu et al, 1999), leaves of *Gliricidia sepium, Fleminga macrophyllum, Leucaena leucocephala, Tithonia diversifolia* and *Azadirachta indica* (Gbadamosi et al, 2002) and *A. indica* leaves (Sokunbi et al, 2002) among others. All these studies have resulted in varied levels of success/failures.

The plant pawpaw (*Carica papaya*) has the potential of being utilized as a cheap source of supplements for livestock feeds as the leaves, seeds and peels have a high protein content with minerals and vitamins (Oyenuga, 1968; Ihekeronye and Ngoddy, 1985) except that they contain a proteolytic enzyme, papain (Bersin, 1935), which has embryotoxic and teratogenic effects (Singh and Devi, 1978). In our earlier study (Egbunike et al, 2000) we showed that dietary inclusion of pawpaw leaves, seeds and peels at about 50/50 respectively

with rat pellets adversely affected sperm production and storage potentials and epididymal transit in the rat. We therefore decided to reduce the dietary levels of dried old pawpaw leaves to see their effects, if any, on the kinetics of spermatogenesis and sperm storage and production potentials of adult cocks.

Materials and methods

Location and climate

This study was carried out in the Teaching and Research Farm, University of Ibadan located at latitude 07° 20'N and longitude 03° 50'E with a semi-hot equatorial climate (Egbunike and Steinbach, 1979) from September, 1997 to January, 1998 in the late dry season.

Animals and management

50 White Rock cockerels, 12 weeks old, were procured and used for the study. They were housed singly in standard battery cages in an open-sided poultry house, placed on commercial growers' mash (Livestock Feeds, Ltd.) for one week and then randomly assigned to five treatment groups in which soybean meal was replaced by sundried old pawpaw leaves at 0, 25, 50, 75 and 100% levels designated as treatments 1, 2, 3, 4 and 5 respectively (Table 1). Thus these diets contained 0, 3.75, 7.50, 11.25 and 50.00% dried pawpaw leaves, respectively. Feed and water were supplied *ad libitum* while all necessary vaccinations and medication were administered as and when due

Table 1: Percentage composition of experimental diets

	Diets				
	Control 1 (0%)	2 (3.75%)	3 (7.50%)	4 (11.25%)	5 (15.00%)
Maize	40.00	40.00	40.00	40.00	40.00
Soybean meal	15.00	11.25	7.50	3.75	0
Old Pawpaw leaves	0	3.75	7.50	11.25	15.00
Fish meal	1.50	1.50	1.50	1.50	1.50
Palm kernel cake	17.00	17.00	17.00	17.00	17.00
Wheat offal	16.00	16.00	16.00	16.00	16.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	7.00	7.00	7.00	7.00	7.00
Salt	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Determined composition (%)					
Crude protein	19.69	19.03	18.38	17.94	17.28
Crude fibre	5.48	5.63	5.78	5.93	6.08
Ether extract	1.85	1.53	1.82	1.89	1.55
Ash	20.50	19.00	14.50	16.50	16.50
Nitrogen free extracts	43.42	45.85	49.64	50.10	50.07
Metabolizable energy (kcal/kg)	2579.70	2649.90	2720.10	2790.30	2860.00

All animals were sacrificed at 26 weeks of age i.e. after being on the experimental diets for 13 weeks. Thereafter the testes were dissected, weighed and processed routinely for histology (Egbunike et al, 1980) and homogenization (Egbunike et al, 1976).

Testicular analyses

Spermatogenesis was determined by estimating the frequency of occurrence of the stages of the seminiferous epithelial cycle as already described by Swierstra and Foote (1963) in 100 seminiferous tubules from two slides per animal while the gonadal sperm storage was determined from the homogenates according to Egbunike et al (1976). The sperm production capacity was then determined from the gonadal sperm according to Amann (1970).

Statistical analyses

Data collected were subjected to analyses of variance (Steel and Torrie, 1980) and the means were compared using Duncan's Multiple Range test (Duncan, 1955).

Results

The percentage composition of the experimental diets is as shown in Table 1. There were no significant changes in the proximate composition of the diets although the crude protein content tended to decrease from 19.69% in the control diet (Treatment 1) to 17.28% in the diet in which soybean meal was completely replaced by dried old pawpaw leaves (Treatment 5). A reversed trend was also noticed in the metabolizable energy of the diets.

Although body weight of the birds remained stable with treatment at the end of the study, absolute testis weight was significantly ($P<0.05$) affected with the animals on the diet in which 50% of the soybean was replaced by dried pawpaw leaves (Treatment 3) being superior to those with higher levels of pawpaw leaves but not the control birds (Table 2). Paired testes weight was higher ($P<0.05$) in treatment 3 birds than the others while testes weight relative to body weight was unaffected by treatment. Right testes did not differ from the left in weight.

Feeding of these prepubertal birds for 13 weeks on pawpaw leaf-based diets did not affect the kinetics of spermatogenesis in the birds (Fig. 1). Data of the cocks on pawpaw leaf-based diets were then pooled to obtain the frequencies of occurrence of the stages of the cycle of the seminiferous epithelium for the group. Stage 5 was the most frequent of the eight stages of the cycle of seminiferous epithelium while stage 4 was the least with duration lasting for 16.32 and 9.12 hours, respectively (Table 3).

Treatment significantly ($P<0.05$) affected the sperm storage and production potentials of the birds. Generally, sperm storage and production rates were highest ($P<0.05$) in birds on diets in which pawpaw leaves completely replaced soybean (Treatment 5) and least in those in treatment 2 (Table 4).

Table 2: Morphometric characteristics of testis of cocks fed pawpaw leaf-based diets (Means ± Sem)

Components	Diets				
	Control 1 (0%)	2 (3.75%)	3 (7.50%)	4 (11.25%)	5 (15.00%)
Body weight (kg)	2.33±0.13^{a}	2.10±0.08	2.33±0.07	2.22±0.04	2.23±0.02
Right testis (g)	9.84±0.60	10.70±0.30^{a}	12.17±0.14^{a}	10.04±2.06^{a}	7.39±0.85^{b}
Left testis (g)	10.49±1.08^{ab}	11.06±1.11^{ab}	12.19±0.43^{a}	9.82±1.08^{b}	7.26±0.07^{c}
Paired testes (g)	20.33±0.07^{b}	21.76±0.47^{ab}	24.36±0.20^{a}	19.86±1.04^{b}	14.65±0.48^{c}
Paired testes (%)	0.87±0.07	1.04±0.08	1.05±0.05	0.89±0.13	0.66±0.06

abc: Values along the same row with different superscripts are significantly different ($P<0.05$).

Table 3: Frequencies of occurrence and duration of the stages of the cycle of seminiferous epithelium of domestic chickens fed pawpaw leaf-based diets

Stages	% Occurrence (means ± Sem)	Duration *	
		h	days
1	14.00±0.49	13.44	0.56
2	10.31±0.39	9.84	0.41
3	10.55±0.50	10.08	0..42
4	9.06±0.66	9.12	0.38
5	16.94±0.05	16.32	0.68
6	13.38±0.42	12.72	0.53
7	13.51±0.66	12.96	0.54
8	12.25±0.43	11.76	0.49

*The length of one cycle of seminiferous epithelium is calculated (De Reviers, (1968).

Table 4. Sperm storage and production potentials of cocks fed pawpaw leaf-based diets (Means ± SEM)

Parameters	Diets				
	Control 1(0%)	2 (3.75%)	3 (7.50%)	4 (11.25%)	5 (15.00%)
1. *Sperm storage ($x10^9$)*					
Right testis	0.24 ± 0.12^{c}	0.15 ± 0.01^{c}	0.21 ± 0.05^{c}	0.28 ± 0.06^{b}	0.87 ± 0.07^{a}
Left testis	0.67 ± 0.14^{b}	0.32 ± 0.03^{c}	0.58 ± 0.20^{b}	0.30 ± 0.01^{b}	1.04 ± 0.04^{a}
Paired testes.	0.91 ± 0.08^{bc}	0.47 ± 0.02^{c}	0.79 ± 0.09^{bc}	0.58 ± 0.04^{b}	1.91 ± 0.10^{a}
2. *Sperm production ($x10^9$)*					
Right testis	0.12 ± 0.06^{c}	0.07 ± 0.01^{c}	0.11 ± 0.03^{c}	0.54 ± 0.12^{b}	0.45 ± 0.04^{a}
Left testis	0.35 ± 0.07^{b}	0.17 ± 0.02^{c}	0.30 ± 0.01^{b}	0.57 ± 0.07^{b}	0.54 ± 0.02^{a}
Paired testes	0.47 ± 0.04^{bc}	0.24 ± 0.01^{c}	0.41 ± 0.05^{bc}	1.11 ± 0.08^{b}	0.99 ± 0.05^{a}

abc: Values along the same row with different superscripts are significantly different ($P<0.05$).

Discussion

The nutrient composition of the experimental diets is within the normal range for breeders and hence were considered adequate for breeders in the tropics as judged from the recommendations of Oluyemi and Roberts (1980).

The stability of the body weight across the treatment groups after 13 weeks of treatment is an indication that the diets promoted normal growth in the birds. However, it is worthy to note that this growth was not reflected in the development of the testes. In fact birds in treatment 3 (i.e. 7.50% pawpaw leaves) had the highest paired testes weight while birds with higher dietary levels of pawpaw leaves had the least. The paired testes weight in the first three diets but not the last two were similar to those reported by Egbunike and Oluyemi (1979) and Nkanga and Egbunike (1990a). As earlier observed in poultry (Clulow and Jones, 1982; Nkanga, 1989) there was no disparity in weight between the left and right testes.

As regards the kinetics of spermatogenesis, eight unique patterns indicating eight stages of the cycle of seminiferous epithelium observed previously by Nkanga (1989) and Nkanga and Egbunike (1990b) were identified and evaluated in relation to the treatments. That this was not affected by the feeding regime may be as a result of the presence of several stages in one cross section of the testis (Nkanga and Egbunike, 1990b) which may have influenced their evaluation. However, it is worthy to note that stage 5 of the cycle of seminiferous epithelium still remained the most abundant in agreement with Nkanga (1989) and Jimoh (1998) and lasted for 16.32 hours. This stage contains only one generation of elongating spermatids and two generations of spermatogonia, types A an B (Nkanga and Egbunike, 1990b).

Daily sperm storage potential ranged from 0.47×10^9 in treatment 2 to 1.91×10^9 in treatment 5 while daily sperm production was from 0.24×10^9 in treatment 2 to 0.99×10^9 in treatment 5. These values are lower than those reported by Nkanga and Egbunike (1990b) probably due to differences in cock strains. It was also showed by these authors that there was a loss of 23.24% in sperm production values of exotic cocks determined by homogenization method. The significant treatment effects on sperm storage and production potentials especially in treatments 4 and 5 in spite of the apparent similarities in liveweight may be construed to imply that the papain in the leaves (Bersin, 1935) after drying, has a selective influence on liveweight and testicular development. Hence, while treatment enhanced feed intake and so growth rate (Akorede, 1998), testicular growth was not sustained. So far, we cannot fathom why sperm storage and production potentials were positively influenced by the increasing levels of pawpaw leaves and hence papain in the diet especially as our earlier findings (Egbunike et al, 2000) showed that dietary inclusion of pawpaw leaves has some adverse effects on these parameters. Any conclusion will thus await further studies.

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