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Reproductive performance of Wistar rats fed *Acacia colei* seed-based diets

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Abstract

Weanling male and female Wistar rats were fed diets incorporating 0%, 20% and 40% *Acacia colei* seed flour (*Leguminosae*), to raise three generations of animals. Weight gain decreased with increasing levels of acacia incorporation (crude protein content 12.6%) in first generation animals. Mating, 13 weeks after weaning, resulted in 80%, 60% and 0% pregnancy in female rats, average litter size of 5 and 5.5; and survival rate of 83% and 94% by rats fed 0%, 20% and 40% acacia diets, respectively. Increasing the protein content to 18% reversed the reproductive failure in the 40% acacia group: pregnancy rate was 80%, 40% and 71%; mean litter size was 8.5, 12 and 3.8 in rats fed 0%, 20% and 40% acacia diets, respectively. Growth rate was fastest in the 0% acacia group second-generation rats after the first 64 days. Mating this second generation resulted in 82%, 70% and 83% pregnancy over two matings for animals on 0%, 20% and 40% acacia diets, respectively. Weights of reproductive and other internal organs were little affected by diet-type indicating that incorporation of acacia into rat diet would not affect reproduction except for the low quality of its protein.

Keywords: Famine food; Dietary protein; Growth; Reproduction

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1. Introduction

Seeds of some 50 dry-zone Acacia species were a significant seasonal component of the diet of the Aborigines of Australia (Harwood, 1994). The Australian species of *Acacia colei* [*A. colei*] (Maslin and Thomson, 1992) [*Leguminosae*] is planted for windbreaks and fuelwood production in parts of the Sahelian region of tropical Africa. *A. colei* plant displays good growth, survival and seed production in the Maradi district of southern Niger (Harwood, 1994). Therefore, this species may offer the prospect of a significant new food source in areas where hunger, malnutrition and starvation are a common occurrence (FAO, 1995). There were serious failures of the staple crops—millet and sorghum and resulting food shortages have occurred in the Maradi region (mean annual rainfall below 600 mm) of southern Niger Republic in 1973–75, 1984, 1998, 1994, 1996 and 2000 as a result of drought and other factors such as pest attack. During these periods, rural populations of Maradi eat the chaff of sorghum and millet, the bark and leaves of trees and about anything that could fill the stomach.

People around Maradi became aware of the food potential of *A. colei* seeds in 1990 and began to develop palatable foods which incorporate acacia seed flours into traditional recipes. In 1992, Commonwealth Scientific and Industrial Research Organisation's Australian Tree Seed Centre, (CSIRO-ATSC) with funding support from the Australian Centre for International Agricultural Research (ACIAR), coordinated a detailed nutritional and safety evaluation of the seeds of *A. colei* as a prelude to their possible incorporation into the local diets as staple or famine foods.

Analysis of *A. colei* seed indicated that it had an average protein efficiency ratio and that known toxic factors such as cyanide, trypsin and chymotrypsin inhibitors, toxic amino acids like djekolic acid derivatives and lathyrogens were absent or at levels below those which would cause concern (Evans et al., 1977; Kortt, 1985; Harwood, 1994; Falade et al., 2005). The result of crude protein analysis of traditional and acacia-modified diets showed a marked advantage of incorporating acacia into the traditional diets through the increase in the content of their crude protein, lipid, minerals and most of the vitamins (Adewusi et al., 2003).

A safety bioassay using 20 rats of both sex per treatment at 12.8% protein content in the diet for 13 weeks indicated that *A. colei* seed flour incorporated in millet-based diets at a level of 20% improved growth rates and animals remained in good health (Adewusi et al., unpublished data). Supplementation with 0.2% DL-methionine improved the protein efficiency ratio (PER) and net protein retention (NPR) of rats fed 42–50% acacia from 0.91 and 2.1–1.7 and 2.9, respectively (Adewusi et al., unpublished data). However, seeds with very good nutritional values may contain compounds exhibiting contraceptive properties such as cotton seed meal, which contains gossypol (Fong, 1985; Nwoha, 1995). Histological observations of rats fed 40% acacia diet for 13 weeks indicated a gross distortion in the structure of the testis (Adewusi et al., unpublished results). This worrying result may have been an artefact due to unavoidable delay in tissue processing for histological examination. There has been no study on the reproductive performance of animals fed acacia-incorporated diets. Since acacia seeds only served as seasonal components of the Aboriginal diets, the study was conducted as part of the safety evaluation program before the introduction of this "old food" to the people of Maradi. This study is especially germaine at a period of acute famine in the arid region of Niger Republic with a record of heavy mortality in human and animals and a call for food aid to the international community. The result of this investigation would also assist other arid regions of the world prone to food shortage and starvation as a result of drought. Detailed histological and histochemical findings of the study will be presented elsewhere.

2. Materials and methods

2.1. Preparation and analysis of acacia flour

A. colei seeds were collected from several trees around Maradi, Niger Republic and transported to Ile-Ife, Nigeria in two 50 kg lots. The seeds were cleaned of debris, twigs and resinous matter and milled using a local milling machine. The flour was then sieved, using a sieve with aperture size of 0.6 mm, to remove the larger fragments of the seed coats. Crude protein ($N \times 6.25$) of the sieved flour was determined by the microKjeldahl method (AOAC, 1984). Oil was determined by soxhlet extraction of samples using petroleum ether b.p. 60–80 °C (AOAC, 1984). Ash was determined by the standard AOAC (1984) method and dietary fibre was estimated by the method of Lee et al. (1992). The crude protein content of ground and sieved *A. colei* seed flour was 21.4%, the ether extract 12.1% and the dietary fibre 29.1%, respectively.

2.2. Bioassay procedure

This was a good laboratory practise study (GLP) following essentially the Organisation for Economic Cooperation and Development (OECD) guidelines. Weanling litter mate Wistar strain rats, from the colony of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, weaned at 28 days, were randomly allocated to three groups with ten female and five male rats in each group. The average weight of female rats was 84 g and the males about 72 g at the beginning of the study. The animals were acclimatized to the cages in three groups of five males each per group and six groups of five females each per group, for 4 days during which time they were given commercial chow (Bendel Feed Mill Ltd, Benin Nigeria). Subsequently, they were placed on diets incorporating 0%, 20% and 40% (w/w) of acacia, respectively (compositions shown in Table 1). The diets were balanced

Table 1
Composition of initial diets containing 0%, 20% and 40% (w/w) *Acacia colei* seed flour and 12.6% crude protein

Acacia incorporation (%)	0	20	40
Ingredients (%)			
Red sorghum	62.9	53.9	44.0
Sugar	14.0	14.0	14.0
Acacia seed flour	—	20.0	40.0
Casein	9.3	4.0	—
Cellulose	7.8	4.6	—
Vitamin/mineral mix	1.0	1.0	1.0
Oil	5.0	2.5	1.0
Crude protein content (%)	12.6	12.6	12.6

Table 2
Composition of improved diets containing 18% crude protein and incorporating 0%, 20% and 40% acacia seed flour

		Level of acacia incorporation (%)		
		0	20	40
Ingredients (%)	CP (%)			
Red sorghum	8.74	46.0	46.0	46.0
Sugar	—	5.0	5.0	5.0
Acacia seed flour	21.4	—	20.0	40.0
Casein	76.0	18.4	12.8	7.2
Cellulose	—	16.6	5.8	—
Corn starch	—	6.7	5.5	—
Vitamin	—	0.5	0.5	0.5
Mineral mix	—	2.0	2.0	2.0
Oil	—	4.8	2.4	—
CP content (%)		18.0	18.0	18.0
CP—crude protein				

with respect to oil and dietary fibre according to AOAC (1984) and had a crude protein content of 12.6% contributed solely by casein and sorghum for the control; the same protein content of the 20% acacia diet was contributed by casein, sorghum and acacia while that of the 40% acacia diet was supplied by the acacia seed flour and sorghum only. All diets and water were provided ad libitum. Due to the lack of pregnant F_0 females (0/10) at 40% acacia, improved diets each containing 18% crude protein were formulated (Table 2) and provided for all the treatments.

2.2.1. Reproduction

The F_0 rats were fed for 15 weeks and then mated two female rats to a male on the same diet to produce the F_1 generation. The mating lasted for 5 days to ensure mating and conception, after which the female rats were removed. To determine whether toxicity or nutritional deficiency was the cause of the infertility in the 40% acacia treatment group (F_0), three male and female rats from each treatment group were sacrificed at week 24 of the study for weight, histological and histochemical examination of organs. The remaining F_0 rats were fed the improved diet containing 18% crude protein for 60 days and again mated as before. It was necessary to wait for a long period of time (60 days) to ascertain the effect (if any) of the improved diet. All male and female rats were weighed on a weekly basis during the pre-mating, mating, gestation and lactation periods. Similarly, food consumption was measured on a weekly basis and clinical signs of adults and offspring were monitored and recorded. In addition, reproductive performance; judged by the number of pregnancies, litter size, weight of pups at birth and at weaning and their survival; was recorded. The second generation of rats (F_{1b}) was weaned at 21 days onto the improved control and test diets. Six female and three male rats were randomly selected for further breeding to produce third generation (F_{2a}). Mating procedure, at 16 and 26 weeks after birth, was as described for the F_0 generation. The mating scheme of the study is outlined in Fig. 1.

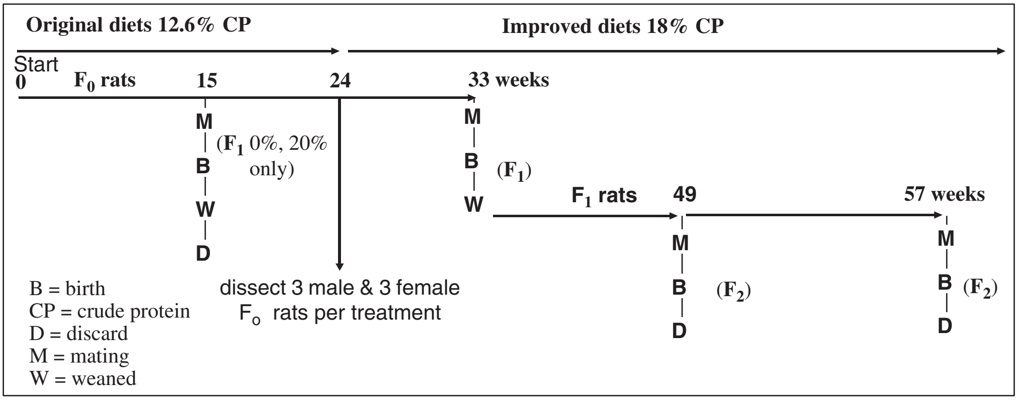

Fig. 1. Mating and sampling scheme for the trial.

3. Results and discussion

3.1. Growth of F_0 rats

In the first 15 weeks of the feeding trial, rats fed the control diet gained weight faster than those fed 20% (w/w) acacia supplemented diet while the least growth rate was in rats fed with 40% (w/w) acacia diet (Table 3). Casein, included in the control and 20% acacia diets, is an animal protein and expected to be a superior source of protein to acacia seed. In addition, feed intake during this period was lowest in rats fed with 40% acacia diet. These factors would account for the differences in the growth rate observed. Palatability could be another factor but this was not assessed since the presence of 10% sucrose in the diet was expected to increase palatability and possibly mask the effect of acacia added to the diet. Feed conversion efficiency defined as the weight of feed needed for a 1 g increase in the weight of animals, was also lowest in rats fed 40% acacia diet and highest in those on the control diet (Table 3).

3.2. First F_0 mating

Eight and six females in the control and 20% acacia diets became pregnant after the first mating commencing at week 15, and had an average of 5.0 and 5.5 offspring, respectively. Females on 40% acacia diet were not pregnant. The infertility in rats fed 40% acacia diet could be due to the toxicological effect of the treatment or simply due to nutritional effects of the diet and resulting smaller size of the animals at mating. The litters were weaned after 34 days with 83% and 94% survival rate. Weanlings weighed on the average 38.4 and 29.4 g for the control and 20% acacia-fed treatments, respectively. Throughout the feeding period (63 days), the litters on the control diet grew faster than those on the 20% acacia diet such that the average weight of the rats on the control diet was 107.6 g while that of rats on 20% was 79.5 g. It was not possible to determine whether the difference in growth rate was sex dependent or not since sex separation was not carried out until after 63 days when the reproductive organs of the rats were visible. Out of 22 rats on the control diet, 12 were female and 10 male, while out of 22 rats on the 20% acacia diet, 18 were male and

Table 3
Mean food intake, average weight gain, pregnancy and reproductive performance of F_0 rats fed 0%, 20% and 40% (w/w) acacia incorporated diets for 15 weeks*

Acacia incorporation (%)	0	20	40
Female rats			
Number of rats	10	10	10
Food consumption (g/kg bw)	5506 ± 329	5766 ± 503	6698 ± 1142*
Feed intake (g/rat)	985 ± 23.9	914 ± 26.2*	780 ± 28.1**
Weight gain (g)	96 ± 6.1	76 ± 9.6*	36 ± 12.8**
Feed conversion efficiency[a]	10.3 ± 0.3	12.0 ± 0.3*	21.7 ± 0.8**
Weight range before mating (g)	170–200	145–185	90–150
Number falling pregnant	8	6	0
Number of offspring/rat	5.0 ± 1.1	5.5 ± 0.8	0.00*
Sex ratios (male: female)	10:12	18:4	—
Male rats			
Number of rats	5	5	5
Feed consumption (g/kg bw)	5193 ± 364	6041 ± 865	6417 ± 989*
Feed intake (g/rat)	1195 ± 22	1081 ± 21*	772 ± 19**
Weight gain (g)	158 ± 5	109 ± 14*	52 ± 12*
Feed conversion efficiency[a]	7.6 ± 0.1	9.9 ± 0.2*	14.8 ± 0.4**
Weight range before mating (g)	210–255	155–210	105–160

All diets contain 12.6% crude protein content.
Values with different asterisks within the same row are significantly different from each other and the control at $P > 0.05$.
[a]g feed/g weight gain.

only 4 female. At this point (63 days), this generation was discarded. However, this result called for a more thorough investigation of the possible presence of steroidal-like compounds in acacia seed, which may affect the sex and or the viability of the offspring.

3.3. Study of the internal organs of F_0 rats

Adult body and organ weights are presented in Table 4. Mean weight of the liver in absolute value decreased, with increase in acacia incorporation in both male and female rats but this change essentially disappeared when expressed as a percentage of body weight. Kidney weight decreased in the male rats fed 20% and 40% rats compared to the control but the difference, which was only apparent in female rats on 40% acacia, also disappeared when expressed as a percentage of body weight. Mean weight of the heart decreased in both females and males compared to control group, however this difference disappeared when expressed as a percentage of body weight. In the testis, the mean weight tended to decrease at increasing level of acacia incorporation in the diet. Acacia diet increased the weight of the testis in a dose-dependent manner when expressed as a percentage of body weight. This may be a consequence of the presence of endocrine disruptors though estrogen mimics such as phytosterols and isoflavonoids were not determined in this study. The possibility of a natural male contraceptive in acacia seed could therefore not be ruled out. The ovary was not weighed since a preliminary study did not indicate any histological effect of acacia incorporation at 40% in the diet of female

Table 4
Organ weights of F_0 generation rats after 24 weeks*

Levels of acacia incorporation (%)	0	20	40
Female animals ($n = 3$)			
Body weight (g)	163±3.4	173±7.8	135±1.8*
Liver (g)	5.7±0.2	5.5±0.1	4.2±0.1*
(% of body weight)	3.5±0.1	3.2±0.1	3.1±0.04
Kidney (g)	0.49±0.01	0.52±0.00*	0.41±0.02**
(% of body weight)	0.3±0.01	0.3±0.00	0.3±0.02
Heart (g)	0.44±0.00	0.37±0.01	0.39±0.02
(% of body weight)	0.3±0.00	0.2±0.04	0.3±0.01
Male animals ($n = 3$)			
Body weight (g)	283±7.8	178±14.6*	118±3.4**
Liver (g)	8.1±0.3	3.9±0.3*	3.7±0.1*
(% of body weight)	2.9±0.0	2.2±0.2*	3.1±0.1
Kidney (g)	0.66±0.01	0.38±0.03*	0.37±0.01*
(% of body weight)	0.23±0.01	0.21±0.01	0.31±0.01*
Heart (g)	0.62±0.01	0.36±0.03*	0.37±0.00*
(% of body weight)	0.22±0.02	0.20±0.01	0.31±0.00
Testis (g)	1.4±0.02	1.2±0.02*	1.1±0.02*
(% of body weight)	0.47±0.01	0.63±0.01*	0.93±0.02**

Values with different asterisks within the same row are significantly different from each other and the control at $P > 0.05$.

rats. Mokady et al. (1989) using Charles River CD strain of rats reported the liver weight as 2.93–3.08% of the body weight of the male and 3.26–3.74% for female rats fed an experimental *Dunaliella bardawil* (algae) diet for 1 year. This is in general agreement with the values reported herein. Whereas, our values for kidney weight, as a percentage of body weight, were much smaller than 0.78–0.86% for control and 0.60–0.79% reported by Mokady et al. (1989) for rats fed *D. bardawil* for 1 year. The testis weight, as percent body weight, increased significantly with the increase in the level of acacia (Table 4). However, the result of the histology and histochemical examination of the internal organs indicated that the acacia diets did not affect the cellular structure of the reproductive organs of the rats (Adewusi et al., unpublished results). The earlier observation of a gross distortion in the histology in the testis of rats fed 40% acacia diet could therefore be an artefact as speculated earlier.

3.4. Second F_0 mating

Of the remaining, re-mated F_0 rats on the improved 18% protein diet, four of the five female rats on the control diet became pregnant and delivered 34 offspring. Two of the five female rats on the 20% acacia diet became pregnant and had 24 offspring while five of the seven rats on 40% acacia diet delivered 19 offspring (Table 5). The litter size per pregnancy (Table 5) was lowest in the animals fed 40% acacia diet, presumably due to the lower quality of the protein content of this feed. It is well known that protein and energy intake affect reproductive performance (Leathem, 1958; FAO/WHO, 1991). However, the weight of pups at birth was highest in rats fed with 40% acacia diet, followed by those on 20%

Table 5
Reproductive performance of first generation (F_0) female rats fed improved 0%, 20% and 40% (w/w) acacia-incorporated diets for 9 weeks

Level of acacia incorporation (%)	0	20	40
Number of rats	5	5	7
Average weight at mating (g)	207 ± 10.5	194 ± 7.3*	156 ± 9**
Number falling pregnant	4	2	5
Litter size/rat	8.5 ± 0.9	12 ± 1*	3.8 ± 0.2**
Weight at birth (g/pup)	5.8 ± 0.4	6.0 ± 0.6	6.8 ± 0.9
Weight at 7 days (g/pup)	9.6 ± 1.0	10.0 ± 1.1	16.4 ± 1.5*
Survival rate at weaning (%)	97	83	95

Values with different asterisks within the same row are significantly different from each other and the control at $P > 0.05$.

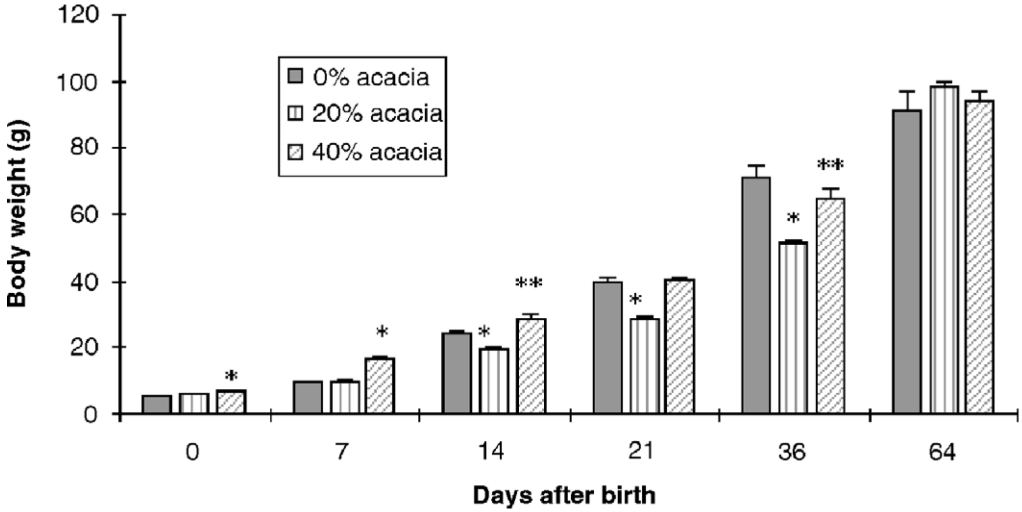

Fig. 2. Growth of F_1 rats (litters obtained after improved diets) from birth to sex separation at 64 days.

and least for rats on the control diet. The high birth weight of the pups from the 40% diet could be due to the small size of the litters, but this could not hold for the animals fed with 20% acacia, which had a larger number of litters per pregnant rat than the control group but the same average litter weight. The rate of survival up to weaning at 21 days was 97% in the control and 95% in the 40% acacia diet fed rats and lower at 83% in the 20% acacia diet.

The growth performance of the F_1 generation is presented in Fig. 2. Growth rate was faster on postnatal day 0, 7 and 14 at 40% than the controls. Growth rate at postnatal day 21 was similar for the control and 40% groups. At postnatal day 36, the growth rate at 40% was less than the controls. However, at postnatal day 64 the growth at 40% was slightly faster than the controls. The growth rate at 40% was higher than the growth rate of the 20% animals at the aforementioned postnatal days, with the exception of postnatal day 64; whereby the growth rate at 20% was faster than both the controls and 40%

groups. From then on, control male and female rats grew faster than those on the 20% acacia diet which in turn grew faster than those on 40%. It is difficult to ascribe a specific reason for the differences in growth rate before postnatal day 74 except that the dams on 40% had a smaller number of litters (with bigger weight) to nurse compared to the other treatment groups thus giving them an advantage earlier in life.

3.5. F_1 mating

All six female rats (F_1) on the control diet became pregnant but one female died before parturition. The cause of death could not be ascertained but may have been connected with labour. Two out of four female rats on the 20% acacia diet and five out of six female rats on the 40% acacia diet became pregnant representing 50% and 83% respectively. All animals delivered their litters but most of these litters were cannibalized. From our

Table 6
Reproductive performance of female rats following the second mating of second generation (F_1) rats

Level of acacia incorporation (%)	0	20	40
Number of rats	5	4	6
Average weight at mating (g)	230±5.5	210±10*	189±4.5**
Number falling pregnant	3	4	4
Average litter size/rat	4.3±0.4	7.5±0.6*	7.8±1.0*
Weight at birth (g/pup)	5.2±0.8	4.3±0.5	3.7±0.7*

Values with different asterisks within the same row are significantly different from each other and the control at $P>0.05$.

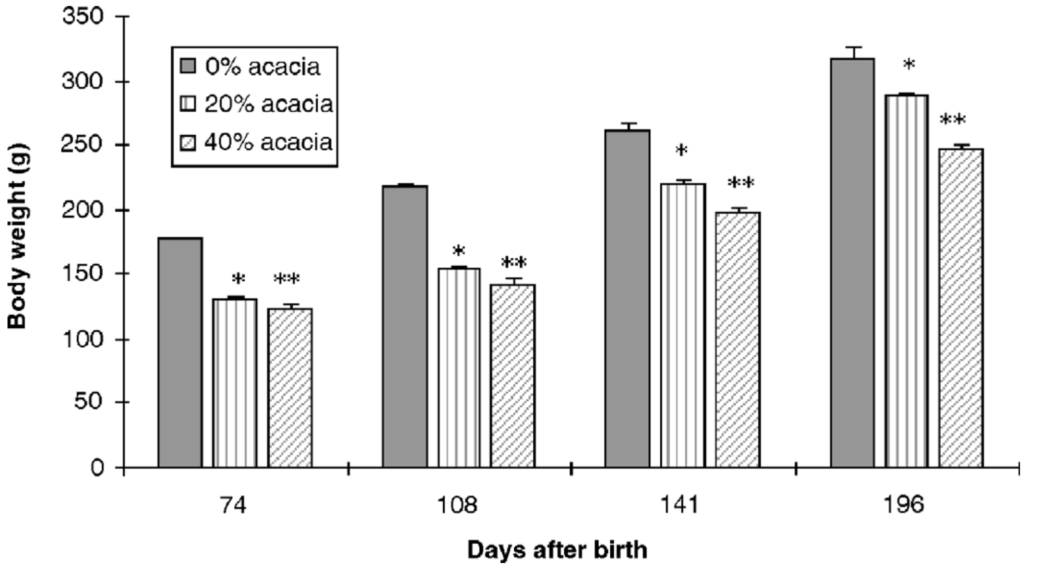

Fig. 3. Growth of F_1 male rats (litters obtained after improved diets) after sex separation.

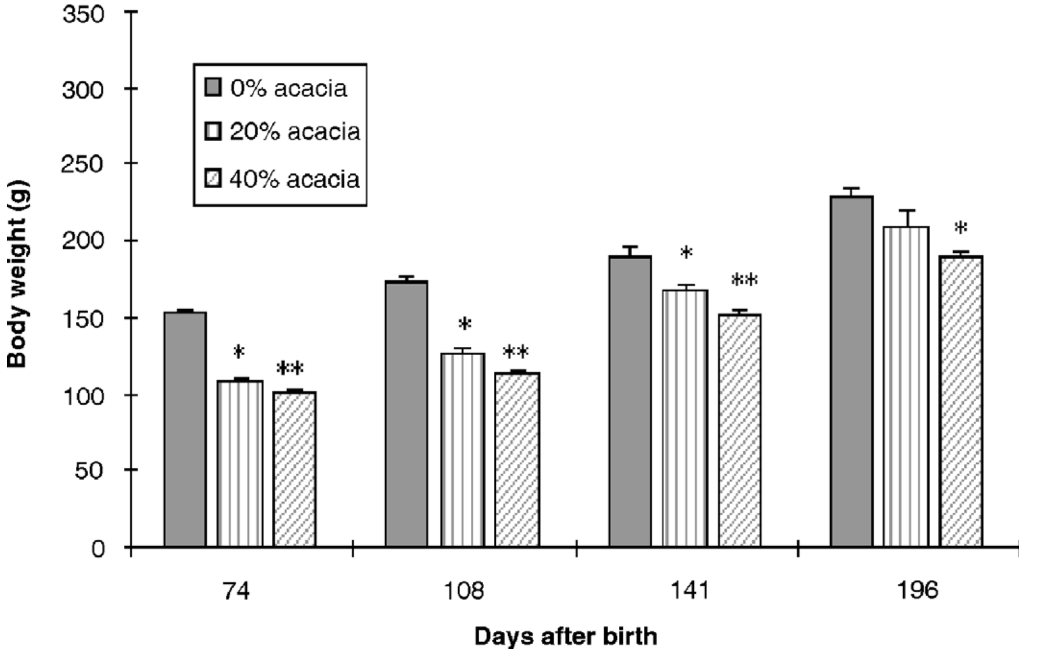

Fig. 4. Growth of F_1 female rats (litters obtained after improved diets) after sex separation.

experience, rats feed on their young and on each other when undernourished or starved for longer than 48 h. Both conditions were absent in the present study.

These litters were discarded and the F_1 animals re-mated after a 56-day period to stabilize the animals in case of an error in diet formulation or handling. Three out of the remaining five rats on the control diet, all four rats on 20% acacia diet and four out of six rats on 40% acacia diet became pregnant. The results presented in Table 6 indicated that the three rats on the control diet delivered 13 pups, an average of 4.3 pups per rat. The animals on the 20% acacia diet all became pregnant and had a mean litter size of 7.5 while four out of six of those on the 40% acacia diet became pregnant and had a mean litter size of 7.8. Average weight of pups at birth was highest in the control diet, presumably due to the low litter size delivered by this group while the average weight was lowest in the offspring from the 40% acacia fed rats (Figs. 3 and 4).

Rats on the three diets remained healthy and active throughout the course of the experiment, and there was no observed morbidity except for some hair loss in a few of the animals fed on 40% *Acacia*.

4. Conclusion

The trial reported here showed that laboratory rats can grow and reproduce successfully on sorghum-based feeds incorporating high proportions of *A. colei* seed flour. At 12.6% crude protein in the diet, rats fed a diet incorporating 20% acacia reproduced successfully, while rats fed 40% acacia with a crude protein level of 12.6% could not reproduce. Weights of reproductive and other organs were little affected by diet type except the testis, when expressed as percent of body weight. Increasing the crude protein content of the diet to 18% enabled reproduction in the 40% acacia fed rats which was probably a reflection of the inferior quality of the *Acacia* protein.

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