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Effect of Varying Levels of Whole Cottonseed Supplementation on Onset of Puberty, Response to Oestrus Synchronization with $PGF_{2\alpha}$ and Artificial Insemination in Friesian × Bunaji and Bunaji Heifers

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Abstract: Twenty Friesian × Bunaji crossbred and twenty-four Bunaji prepubertal heifers were fed 4 diets containing 0, 25, 50 and 75% whole cottonseed to determine effect of varying the level whole cottonseed on onset of puberty, response to oestrus synchronization with $PGF_{2\alpha}$ and artificial insemination. The animals were fed the diets for 140 days. At the end of the feeding trials, oestrus synchronization was carried out on all the animals using $PGF_{2\alpha}$, injected intramuscularly in 2 doses of 2 mL each given 13 days apart. Compared to animals on the control diet (0% whole cottonseed diet), there was a slight delay in onset of puberty (age at first detection of palpable ovarian structures and first oestrus) with inclusion of whole cottonseed in the diet. Following oestrus synchronization with $PGF_{2\alpha}$, interval from treatment to onset of oestrus increased. Compared to the animals on the control diet, oestrus response rates, oestrus activities (vis-à-vis number of mounts) and heat duration declined significantly ($p<0.05$) in both Friesian × Bunaji and Bunaji heifers with increasing level of whole cottonseed in the diet. Level of whole cottonseed had no effect on conception rates of Friesian × Bunaji heifers. However, there was significant decline in pregnancy rates of animals fed 25, 50 and 75% whole cottonseed diet compared to the control (averaged pregnancy rates = 100, 80.3, 40.2 and 80.3% for the Friesian × Bunaji and 66.7, 50.0, 50.0 and 50% for the Bunaji on 0, 25, 50 and 75% levels of whole cottonseed, respectively).

Key words: Whole cottonseed, heifers, puberty, oestrus synchronization, fertility

INTRODUCTION

Nutrition has been acknowledged as a modulator of reproductive function (Randel, 1990; Ferrell, 1991). The relationship between nutrition and reproduction is complex and responses are often quite variable and in consistent (O'Callaghan and Boland, 1999). Situations in which nutrition modulates reproductive functions are particularly related to retardation of growth leading to delayed puberty (Owens *et al*., 1993), initiation of cyclicity (pre-pubertal and/or during the postpartum period) and fertility (Ferguson, 1996; O'Callaghan and Boland, 1999). A measure often adapted to enhance reproductive performance of the female animals is the feeding of concentrate diets to boost nutrient supply. In most industrialized countries whole cottonseed is often used as source of energy, protein and fibre in diets of dairy cattle with reported success in improving productivity (Arieli, 1998; Rogers *et al*., 2002). The practice is gaining popularity among smallholder cattle producers in Nigeria, especially with increase in the cost of most conventional concentrates. A primary concern with the feeding of large quantities of whole cottonseed is the possibility of toxic effects of gossypol and its potential depression of fertility in dairy cattle (Lindsey *et al*., 1980; Arieli, 1998). The female ruminant seems to be relatively insensitive to the anti fertility effects of gossypol (Randel *et al*., 1992;

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Gray *et al*., 1993); however, *in vitro* data have indicated some inhibition of embryo development (Zirkle *et al*., 1988) and ovarian steroidogenesis (Gu *et al*., 1990; Lin *et al*., 1994). Randel *et al*. (1996) suggested that it is possible gossypol may affect the levels of corpus luteum progesterone content and embryo viability. Santos *et al*. (2003) studied the response of postpartum cows fed varying concentrations of gossypol in their diets to oestrus synchronization with $PGF_{2\alpha}$ and reported that oestrus response rate was higher for cows consuming high gossypol diets. Most of these studies were inconclusive as to the safe levels of whole cottonseed supplementation to growing cattle.

This study was carried out to determine the effect of feeding different levels of whole cottonseed on onset of puberty, oestrus response and fertility rates following synchronization with $PGF_{2\alpha}$ and artificial insemination in Friesian × Bunaji and Bunaji heifers. The study aimed at establishing safe levels of whole cottonseed inclusion in the diets of heifers.

MATERIALS AND METHODS

Study Site

The study was carried out at the Dairy Research Programme farm of the National Animal Production Research Institute, Ahmadu Bello University, Shika-Zaria, Nigeria. Shika lies between Latitudes 11 and 12° North and between Longitude 7 and 8° East. Mean annual rainfall in the area is 1100 mm, lasting from May to October. Mean relative humidity is about 72%, while the average daily temperature is about 25°C. The wet season is followed by a dry period (dry season), which lasts from November to April, with mean daily temperatures ranging from 14 to 36°C and mean relative humidity between 20 and 37%.

Experimental Animals and Their Management

The experimental animals consisted of the 20 Friesian × Bunaji crossbred (50:50) and 24 Bunaji prepubertal heifers. They were dewormed with Albenda 2500® (Albendazole) bolus (Eagle Chemicals Co. Ltd. Chungchongnamdo, Korea) at the rate of 5 mg kg^{-1} body weight. They were treated against ticks and other ectoparasites by twice weekly dipping in a long-walk dip containing Steladone® (Novartis Inc., Basle, Switzerland).

Experimental Feed and Feeding

Four concentrates diets formulated to contain 0, 25, 50 and 75% whole cottonseeds (Table 1) were used in this study. The heifers were weighed and randomly divided into 4 groups of 5 and 6

Table 1: Composition of experimental diets (%)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Whole cottonseed	0.000	25.000	50.000	75.000
Cottonseed cake	48.000	0.000	0.000	0.000
Wheat bran	13.700	49.300	27.300	11.600
Maize	35.300	22.700	19.700	10.400
Bone meal	2.000	2.000	2.000	2.000
Table salt	1.000	1.000	1.000	1.000
Dry Matter	96.000	96.100	96.200	96.700
Crude protein	16.000	15.400	16.300	17.800
Crude fibre	21.600	28.200	33.400	43.500
Ether extract	11.000	17.900	24.000	25.600
Neutral detergent fibre	23.400	34.500	34.600	34.800
Acid detergent fibre	24.100	28.700	31.600	41.400
Ash	11.000	10.500	6.800	6.500
ME (kcal kg^{-1})	3063.700	2831.500	3397.500	3603.100
Estimated Gossypol*	0.033	0.289	0.577	0.866

ME: Metabolizable Energy, Estimated gossypol (kg kg^{-1}); *: Estimated from Ikurior and Fetuga (1984)

animals per group for the Friesian × Bunaji crossbred and Bunaji heifers, respectively. After balancing for weight each group within breed was randomly assigned to one of four experimental diets in a randomized complete block design. They were taken through a 14 day pre-experimental period followed by a 7 day adjustment period. During the pre-experimental and adjustment the animals were fed the control diet (0% whole cottonseed diet) and diets assigned to specific groups, respectively at the rate of 1 kg $head^{-1}$ day^{-1}. The daily concentrate diet allowance was adjusted to the experimental feeding rate of 1.5% of body weight/head/day at the end of the adjustment period. Subsequently the amount of concentrate offered was adjusted fortnightly after weighing the animals. The animals were individually fed the concentrate for 3 h in the morning (between 7.00 to 10.00 am) before going out for grazing from 10:00 am to 5:30 pm. The animals grazed on natural range throughout the experimental period. Concentrate intake was measured by weighing leftovers at the end of the 3 h feeding period. Body weight changes were determined by weighing the animals fortnightly. The trial lasted for 140 days.

Rectal Palpation and Oestrus Synchronization

Prior to the oestrus synchronization and during the feeding period, the heifers were subjected to rectal palpation fortnightly by an experience theriogenologist according to the procedures of Zemjanis (1970) to monitor onset of ovarian cyclicity. The animals were visually observed for oestrus twice daily at 07:00 to 09:00 and 16:00 to 18:00 h by trained inseminators and throughout the grazing period by the grazers. Oestrus synchronization was carried out on all the 44 animals using Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), (Estrumate*, a synthetic prostaglandin analogue manufactured by Coopers Animal Health Ltd. Berkhamsted, England), containing 263 μg of Cloprostenol Sodium (Vet), equivalent to 250 μg Cloprostenol and 0.1% w/v Chlorocresol (BP). Each animal was given an initial intramuscular injection of 2 mL of the $PGF_{2\alpha}$. The procedures of $PGF_{2\alpha}$, injection and artificial insemination described by Voh Jr. *et al*. (2004) were adopted in this study. Deep-frozen semen from a single bull of proven fertility from World Wide Sire Inc., Hanford USA was used for the artificial insemination. A single experienced inseminator carried out all the inseminations using the recto-cervical method.

Data Collection, Measurements and Calculations

The parameters measured/or calculated were:

- Interval to onset of oestrus: determined as the period between $PGF_{2\alpha}$ injection and time of first mount.
- Oestrus response rate: determined as the number of animals that came into oestrus divided by the number of animals that were synchronized and expressed as a percentage.
- Heat duration: calculated as the interval between the first and last mount.
- Pregnancy rate: determined as the number of animals that became pregnant divided by the number of animals that were synchronized and expressed as a percentage.
- Conception rate: determined as the number of animals that became pregnant divided by the number of animals that came into oestrus and expressed as a percentage.

Data Management and Analysis

Data collected was computed using Microsoft Excel software (Microsoft XP). Statistical analysis was done using the General Linear Model Procedures of the Statistical Analysis System (SAS, 1987). The model used was:

$$Y_{ijk} = \mu + B_j + X_{ijk} + T_k + e_{ijk}$$

Where,

μ = Represent a mean
B = Block
X_{ijk} = Covariate
T = Treatment
e_{ijk} = Residual term

All statistical tests were done at 1 and 5% probability levels.

RESULTS

Onset of Puberty in Friesian × Bunaji and Bunaji Heifers

Table 2 shows that there were no significant ($p>0.05$) differences in the average age, weight and body condition scores at first detection of ovarian structures in Friesian × Bunaji across treatment. Friesian × Bunaji heifers on the control diet (0% whole cottonseed), were slightly younger (18 months old) and heavier (267.0 kg) at first oestrus than those on the other diets, although the differences across treatments were not significant ($p>0.05$). Among the Bunaji heifers (Table 2), average age, average weight and body condition at first detection of ovarian structures across treatments were not significantly ($p>0.05$) different. In both breeds, onset of oestrus was slightly delayed in animals fed 50% whole cottonseed diet compared to those on the other diets. Within treatments, Friesian × Bunaji heifers were significantly ($p<0.05$) younger, heavier and in better body condition at first detection of ovarian structures than Bunaji heifers. The Friesian × Bunaji heifers had significantly lower ($p<0.05$) ages, higher body weights and were in better body condition at first oestrus than the Bunaji heifers.

Response of Friesian × Bunaji and Bunaji Heifers to First Injection of PGF_{2a}

Among the Friesian × Bunaji heifers, oestrus response rates to first oestrus synchronization were similar (60%) across treatments. Onset of oestrus (interval to first mount following PGF_{2a} injection) was significantly delayed ($p<0.05$) with increase in the level of whole cotton seed in the diet (Table 3). Oestrus activities (indicated by number mounts) and heat duration declined significantly ($p<0.05$) with increase in the level of whole cottonseed in the diet. Compare to those fed the control diet (0% whole cottonseed) oestrus response rates of Bunaji heifers on 25, 50 and 75% whole cottonseed diets (Table 3), were significantly ($p<0.01$) low. Onset of oestrus following $PGF_{2\alpha}$ treatment was delayed as the level of whole cottonseed increased in the diets, although the difference

Table 2: Effect of level of whole cottonseed on onset of puberty in Friesian × Bunaji and Bunaji heifers

	Inclusion levels of whole cottonseed (%)									
	0		25		50		75			
Parameters	Frx	BJ	Frx	BJ	Frx	BJ	Frx	BJ	SEM	LOS
Age at first detection of ovarian structures (months)	18.0^{b}	24.0^{a}	20.6^{b}	24.0^{a}	19.4^{b}	24.5^{a}	19.3^{b}	24.9^{a}	1.5	**
Weight at first detection of ovarian structures (kg)	262.8^{a}	226.4^{b}	256.0^{a}	226.2^{b}	254.5^{a}	226.0^{b}	250.0^{a}	228.7^{b}	4.7	**
BCS at first detection of ovarian structures	3.5^{a}	2.5^{b}	3.5^{a}	2.5^{b}	3.5^{a}	2.5	3.8^{a}	2.5^{b}	0.1	**
Age at first oestrus (months)	19.0^{b}	30.2^{a}	22.0^{b}	32.0^{a}	21.5^{b}	33.1^{a}	20.0^{b}	32.4^{a}	1.8	**
Weight at first oestrus (kg)	267.0^{a}	237.0^{b}	260.8^{a}	235.0^{b}	265.2^{a}	225.0^{b}	265.4^{a}	235.1^{b}	6.2	**
BCS at first oestrus	3.6^{a}	2.8^{b}	3.6^{a}	2.8^{b}	3.6^{a}	2.8^{b}	3.8^{a}	2.8^{b}	0.1	*

Means within the same row in the same treatment column with different superscripts are significantly different. LOS = Level Of Significance, **: = $p<0.01$, * = $p<0.05$, ns = not significant, Frx = Friesian × Bunaji, BJ = Bunaji, BCS = Body Condition Scores

Table 3: Comparative response of Friesian × Bunaji and Bunaji heifers fed varying levels of whole cottonseed to first oestrus synchronization with $PGF_{2\alpha}$

	Inclusion levels of whole cottonseed (%)									
	0		25		50		75			
Parameters	Frx	BJ	Frx	BJ	Frx	BJ	Frx	BJ	SEM	LOS
No. treated	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0		
No. on standing heat	3.0	5.0	3.0	3.0	3.0	3.0	3.0	3.0		
Oestrus response rate (%)	60.0^{b}	83.3^{a}	60.0^{a}	50.0^{a}	60.0^{a}	50.0^{b}	60.0^{a}	50.0^{b}	3.6	**
Interval to 1st mounts	37.0^{a}	36.5^{a}	40.7^{a}	41.0^{a}	46.7^{a}	43.7^{b}	57.3^{a}	46.3^{b}	3.4	**
No. of mounts	37.0^{b}	73.0^{a}	15.0^{a}	15.0^{a}	14.0^{a}	14.0^{a}	10.0^{a}	10.0^{a}	1.4	**
Heat duration (h)	17.0^{b}	20.0^{a}	14.3^{b}	16.3^{a}	7.7^{b}	12.7^{a}	6.3^{b}	6.7^{a}	0.5	**

Means within the same row in the same treatment column with different superscripts are significantly different, LOS = Level Of Significance: ** = $p<0.01$, Frx = Friesian × Bunaji, BJ = Bunaji

in post treatment interval to onset of oestrus in animals fed 50 and 75% whole cottonseed diets was not significant ($p>0.05$). Oestrus activities vis-à-vis number of times an animal stood to be mounted and heat duration declined significantly ($p<0.01$) with increase in the level of whole cottonseed in the diets. Within treatments (Table 3), response of Friesian × Bunaji heifers to first oestrus $PGF_{2\alpha}$ injection was significantly higher ($p<0.01$) than those of the Bunaji except in animals fed 0% whole cottonseed diet. There were no significant differences ($p>0.05$) in post treatment interval to onset of oestrus and oestrus activities (number of mounts) between Friesian × Bunaji and Bunaji heifers fed 0, 25 and 50% whole cottonseed diets. However, at 75% level of whole cottonseed in the diet oestrus occurred much later in the Friesian × Bunaji than in the Bunaji heifers. Heat duration was significantly longer ($p<0.01$) in Bunaji than in Friesian × Bunaji heifers fed 0, 25 and 50% whole cottonseed diet, but not significantly different ($p>0.05$) among those fed the 75% whole cottonseed diet.

Response of Friesian × Bunaji and Bunaji Heifers to Second $PGF_{2\alpha}$, Injection and Artificial Insemination

Oestrus response rates were significantly ($p<0.01$) higher and lower in Friesian × Bunaji heifer fed the 0 and 50% whole cottonseed diets than compare to those on 25 and 75% whole cottonseed diets, respectively (Table 4). Oestrus was delayed in animals on 50 and 75% whole cottonseed compared to animals on 0 and 25% whole cottonseed; more so in those on 50% whole cottonseed diet compare to those on the 75% whole cottonseed diet. Oestrus activity was significantly ($p<0.05$) higher in Friesian × Bunaji heifers fed 0 and 75% whole cottonseed diets and lower in those on 25% whole cottonseed compare to those on the other diets. Friesian × Bunaji heifers fed 75% whole cottonseed diet remained longest in oestrus, while those on 50% whole cottonseed diets had the shortest heat period (4 to 5 h) compare to those on the other diets. Among the Bunaji heifers the highest oestrus response rate (83.0%) was recorded in animals fed 50% whole cottonseed and the least among those fed 25 and 75% whole cottonseed diets. Bunaji heifers on 0 and 75% whole cottonseed diets came into oestrus earlier than those fed 25 and 50% whole cottonseed diets. Oestrus activities (number of mounts) and heat duration were significantly ($p<0.05$) higher in Bunaji heifers fed 0% whole cottonseed diet and least among those fed 25% whole cottonseed diet had the least. Oestrus activities and heat duration increased significantly ($p<0.01$) as the level of whole cottonseed in diets increased from 25 to 75%. Within treatments, oestrus response was significantly higher ($p<0.01$) in Friesian × Bunaji than in Bunaji across treatments except among those fed 50% whole cottonseed diet (Table 4). Interval to onset of oestrus following the second $PGF_{2\alpha}$ was significantly shorter ($p<0.05$) in Bunaji heifers than in the Friesian × Bunaji heifers across treatments except among those fed 25% whole cottonseed diet. Heat duration was significantly ($p<0.01$) longer in Friesian × Bunaji than in Bunaji heifers on 25 and 75% whole cottonseed diets, while the period was longer in Bunaji heifers compared to Friesian × Bunaji heifers fed 0 and 50% whole cottonseed diets.

Table 4: Comparative response of Friesian × Bunaji and Bunaji heifers fed varying levels of whole cottonseed to second oestrus synchronization with $PGF_{2\alpha}$ and artificial insemination

	Inclusion levels of whole cottonseed (%)									
	0		25		50		75			
Parameters	Frx	BJ	Frx	BJ	Frx	BJ	Frx	BJ	SEM	LOS
No. treated	5.0	6.0	5.0	6.0	5.0	6.0	5.0	6.0		
No. on standing heat	5.0	4.0	4.0	3.0	2.0	3.0	5.0	3.0		
Oestrus response rate (%)	100.0^a	67.8^b	80.0^a	50.2^b	40.0^b	83.6^a	80.0^a	50.3^b	3.3	**
Interval to 1st mounts (h)	49.8^a	47.8^b	49.8^b	81.3^a	70.5^a	70.1^a	56.8^a	49.3^b	5.2	*
No. of mounts	43.0^b	49.2^a	8.0^b	11.3^a	16.0^b	18.8^a	43.0^a	28.6^b	4.3	*
Heat duration (h)	19.0^b	21.5^a	10.3^a	6.3^b	4.5^b	14.3^a	29.3^a	17.3^b	1.6	**
No. inseminated	5.0	4.0	4.0	3.0	3.0	2.0	4.0	3.0		
No. pregnant	5.0	4.0	4.0	3.0	3.0	2.0	4.0	3.0		
Pregnancy rate (%)	100.0^a	66.7^b	80.3^a	50.0^b	50.0^a	40.2^b	80.3^a	50.0^b	5.3	**
Conception rate (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		
Service per conception	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		

Means within the same row in the same treatment column with different superscripts are significantly different, Frx = Friesian × Bunaji, BJ = Bunaji, LOS = Level Of Significance, ** = $p<0.01$, * = $p<0.05$

Conception rates of Friesian × Bunaji heifers were similar (100%) across treatments (Table 4). However, heifers fed 0 and 50% whole cottonseed diets had the highest (100%) and lowest (40.2%) pregnancy rates, respectively compare to other treatments; pregnancy rates of heifers fed diets containing 25 and 75% whole cottonseed diet were not significantly different. Numbers of services per conception were similar (1) across treatments. Although conception rates and number of services per conception were similar across diets, Bunaji heifers fed 0% whole cottonseed diet had a significantly ($p<0.01$) higher pregnancy rate than for those on the other diets. Within treatments, conception rates were similar ($p>0.05$) in Friesian × Bunaji and Bunaji heifers across treatments. However, pregnancy rates were significantly higher ($p<0.05$) in the Friesian × Bunaji than in the Bunaji across treatments.

DISCUSSION

The result of this study shows that detection of ovarian structures and onset of first oestrus in both Friesian × Bunaji and Bunaji occurred at slightly older ages in animals fed diets with 25, 50 and 75% whole cottonseed compared to those fed 0% was diet, which seems to indicate the possible negative effect of whole cottonseed on onset of puberty. This is at variance with the reports of Williams (1989), Gambill and Humphrey (1992) and Gray *et al.* (1993). The animals in this study attained puberty at a slightly lighter weight than the average (270 kg) reported especially for the crosses between *Bos indicus* and *Bos taurus* (Mukasa-Mugerwa, 1989; Rekwot, 2000). Also the age at puberty in this study is within the range of 19 to 23.5 reported by Oyedipe *et al.* (1982), Mukasa-Mugerwa (1989) and Rekwot (2000). The low average weight gains of both Friesian × Bunaji and Bunaji heifers on the 50% whole cottonseed diet might have contributed to the delayed onset of puberty among this group of animals.

The decline in oestrus response of both Friesian × Bunaji and Bunaji heifers with inclusion of whole cottonseed in the diets following the 1st oestrus synchronization seems to indicate the possibility of whole cottonseed interfering with manifestation of oestrus activities following oestrus synchronization with $PGF_{2\alpha}$. However, the low response rates, longer post injection interval to onset of oestrus, decline in overt oestrus activities vis-a-vis the number of times an animal stood to be mounted and shorter heat duration of heifers on the 75% whole cottonseed diet agrees with the reports of Santos *et al.* (2003). The significant differences in oestrus response rates and heat duration between Friesian × Bunaji and Bunaji heifers are inline with the reported breed difference in response to oestrus synchronization with $PGF_{2\alpha}$ (Richardson *et al.*, 2002; Voh Jr. *et al.*, 2004). But the non-significant

differences in interval to onset of oestrus and number of mounts contradict the reports of these authors. Occurrence of oestrus within 48-96 h from end of $PGF_{2\alpha}$ injection in this study is in agreement with findings of Voh Jr. (1984) and Voh Jr. *et al*. (2004) and Richardson *et al*. (2002). The increase in oestrus response rates of Friesian × Bunaji heifers on 0, 25 and 75% whole cottonseed diets following the second compared to the first $PGF_{2\alpha}$ injection confirms the observation of Richardson *et al*. (2002) that expression of oestrus activities was more after the second than first $PGF_{2\alpha}$ injection. Similarly, the improved oestrus activities of both Friesian × Bunaji and Bunaji heifers on the 75% whole cottonseed diet in terms of number of time an animal stood to be mounted and heat duration agrees with Santos *et al*. (2003) who observed higher oestrus detection rates following second $PGF_{2\alpha}$ synchronization among animals consuming diets with high amounts of gossypol.

On the overall, it appears whole cottonseed did not exert a significant influence on response of both Friesian × Bunaji and Bunaji heifers to oestrus synchronization with $PGF_{2\alpha}$, even at high levels. However, considering the fact that whole cottonseed formed the largest proportion of the rejected feed at high levels, it is possible that the amount of whole cottonseed ingested might have been too small to cause any damage. The extent of expression of oestrus activities in this study was lower than reported average oestrus activities rates following oestrus synchronization with $PGF_{2\alpha}$ (Voh Jr, 1984; Richardson *et al*., 2002; Voh Jr. *et al*., 2004). On the other hand, the significant differences in the oestrus response and expression of oestrus activities between Friesian × Bunaji and Bunaji heifers further is one of the benefits derived from crossbreeding of *Bos indicus* and *Bos taurus* cattle (Mukasa-Mugerwa, 1989).

The non-significant differences in conception rates in both Friesian × Bunaji and Bunaji in this study agrees with Santos *et al*. (2003) who reported that conception rates of dairy cows were suppressed with feeding of diets that increased gossypol intake above 24.0 g day^{-1}, but no further suppression in conception rates occurred as plasma total gossypol rose to 15 μg mL^{-1}. The significantly higher pregnancy rate between of Friesian × Bunaji compared to Bunaji heifers especially among those on diets with 25, 50 and 75% whole cottonseed seems to suggest that reproductive activities in the Bunaji heifers were more sensitive to whole cottonseed than Friesian × Bunaji heifers. However, the significant reduction in pregnancy rates despite the high conception rates with increase in whole cottonseed in the diets supports the reported possibility of whole cottonseed diets having negative influence on fertility of dairy cows (Brocas *et al*., 1997; Santos *et al*., 2003). Brocas *et al*. (1997) stated that developing embryos are generally sensitive to diets that increase the plasma gossypol levels.

CONCLUSIONS

From the result of this study, it can be concluded that inclusion of whole cottonseed in the diets of Friesian × Bunaji and Bunaji heifers delayed onset of puberty. At 25 and 50% levels of whole cottonseed inclusion expression of oestrus activities following first oestrus synchronization with $PGF_{2\alpha}$ declined significantly, but improved as the level of whole cottonseed increased to 75%. Oestrus activities improved across treatments following the second oestrus synchronization with $PGF_{2\alpha}$; however, the improvement was higher with animals fed the 75% whole cottonseed diet. The significant reduction in pregnancy rates with increase in whole cottonseed in the diets despite the high conception rates is an indication of the possible adverse effect of whole cottonseed on developing embryos. Despite the delayed onset of puberty with feeding of whole cottonseed and considering the observed improvement in oestrus activities and conceptions rate at higher whole cottonseed levels, it is suggested that diets with up to 75% whole cottonseed could be used to steam up before breeding.

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