Effects of Varying Energy Levels on Follicular Development and Time of Ovulation in Kedah-Kelantan Crossbred Cows

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Abstract

Nutrition element such as energy can influence reproductive performance, and the difference in energy provided will lead to changes in follicular development in cows. Therefore a study was conducted to evaluate the effect of varying energy levels on the size of preovulatory follicles and ovulation time of Kedah-Kelantan (KK) crossbred cows. The cows were assigned into two treatment groups: 1M in which the cows were fed to meet their full daily metabolizable energy requirement above maintenance (n=15), and 2M in which the cows were fed twice the level for daily metabolizable energy requirement above maintenance (n=15). The diet contained protein level ranging from 13-15%. Cows were inserted intravaginally with controlled internal drug releasing device (CIDR), containing 1.38 g of progesterone for 7 days. Intramuscular injection of 25 mg prostaglandin was administered 2 days prior to CIDR removal. Each cow had their ovaries scanned at 6-hour intervals from the time of CIDR removal until ovulation (loss of dominant follicles). The day of ovulation at the beginning of emergence of new follicles was designated as Day 0. The ovaries were then scanned at 2-day intervals to determine the follicular wave development (FD). No significant difference (P>0.05) in preovulatory follicle size of 10.5 ± 0.07 mm and 11.1 ± 0.06 mm and ovulation time of 92.0 ± 3.63 h and 89.8 ± 4.08 h of cows fed the two energy levels of 1M and 2M, respectively. The number of follicular waves, length of oestrous cycle, growth and regression rates of dominant follicules and post-synchronized progesterone concentration were not significantly different (P>0.05) between 1M and 2M energy diets. The length of oestrous cycle was 20.6 ± 0.49 days, and mean growth and regression rates of dominant follicules were 1.4 ± 0.02 mm per day and 1.7 ± 0.03 mm per day, respectively. The cows in the present study were in active oestrus cycle evidenced from the presence of active corpus luteum as indicated by the serum concentration of progesterone of 1.3 ± 0.19 ng/ml and 1.5 ± 0.31 ng/ml in the 1M and 2M groups, respectively. The findings from this study seemed to suggest that cows that were offered higher levels of energy above their maintenance requirement would not develop preovulatory follicle of larger size, increase the diameter of the dominant follicles, reduce the number of follicular waves, and the time of ovulation to occur earlier. It may be concluded that the energy levels offered in this study were not sufficiently adequate to alter and affect the follicular development, particularly the preovulatory follicle diameter and time of ovulation of KK crossbred cows in a tropical condition.

Keywords: Energy, follicular development, ovulation, KK cattle

Introduction

There is scarcity of information on follicular changes in Kedah-Kelantan (KK) cattle and crosses produced from its crossing with other breeds of cattle. Mature KK cows weigh about 300 kg (Ariff *et al.,* 1986) and have their first calves at an average age of 42 months. The existence of follicular development differences among breeds of cattle is important in order to establish the common ovulation parameters and adjust management procedures in the breeding of zebu cows, especially those husbandry practices related to the feeding of the cows.

Optimal feeding is an important factor in ensuring that the development of the follicles attains dominant size (Murphy *et al*., 1991; Bergfeld *et al*., 1994; Rhodes *et al*., 1995). Reduction in feed intake may contribute to negative energy balance which may lead to an abnormality and irregular follicle growth (McDougall *et al*., 2005). Moreover, long-term reduction in dietary intake may cause insufficient circulating luteinizing hormone (LH) that affects the growth, persistence and ovulation of the dominant follicle (DF) (Murphy *et al*., 1991; Rhodes *et al*., 1996). Energy available to an animal is important for growth and pubertal process (Fleck *et al.*, 1980). Mammals of different size have different energy requirement hence they are subjected to different energy constraints to perform reproduction (Haresign *et al*., 1984).

The acute restriction in energy intake three to six days before ovulation has been reported to decrease the growth rate and maximum size of the preovulatory DF (Mackey *et al*., 1997). Angus-Hereford crossbred beef cows weighing 378 kg fed to lose 1% of their body weight per week with the level of feeding restricted to 40% of their recommended daily maintenance requirement showed maximum dominant follicular diameter of 10.4 ± 0.9 mm compared to 15.7 ± 0.9 mm for the cows fed the full energy requirement (Bossis *et al*., 1999). Feeding cows less than the full daily energy requirement also resulted in a decrease in growth rate (Diskin *et al*., 1999). However when nutritional supply in beef heifers was increased from 1 to 1.8 folds of the metabolizable energy (ME), growth rate and maximum diameter of successive DFs showed an increase until the resumption of ovulation (Diskin *et al*., 1999).

It was hypothesized that cows offered higher levels of ME above their maintenance requirement would develop preovulatory follicle of larger size, increase the diameter of the dominant follicles, reduce the number of follicular waves, and initiate earlier time of ovulation compared to cows offered lower ME level. Therefore, a study was conducted to evaluate the effects of ME level on follicular development and time of ovulation in KK crossbred cows in a tropical condition.

Materials and Methods

The experiment was conducted at MARDI Research Station, Kluang, Johor, Malaysia. Thirty KK crossbred cows aged 3 to 5 years, with body weight about 250 to 350 kg, and average body condition score of 4 (1=emaciated, 4=moderate, 8=overweight) were used in the study. The cows were randomly and equally allocated into two treatment groups: 1M in which the cows were fed to meet their full daily ME requirement above maintenance (n=15), and 2M in which the cows were fed twice the level for daily ME requirement above maintenance (n=15). The diet contained protein level ranging from 13-15%. The ME requirement was calculated based on net energy for maintenance requirement of cattle as recommended by the Agricultural Research Council (ARC, 1980). The cows in each treatment were kept in holding pens with a concrete floor. The cows underwent an adaptation period of 14 days prior to been fed the treatment diets before oestrus synchronisation.

Synchronisation of Oestrus

Synchronisation was obtained by using controlled internal drug releasing device (CIDR) containing 1.38 g of progesterone (Pharmacia & Upjohn, Australia). The device was inserted into the vagina for 7 days. Intramuscular injection of 25 mg prostaglandin (Estrumate®; Schering – Plough Animal Health, Australia) was administered 2 d prior to CIDR removal. The cows were observed for oestrus after 24 h post-CIDR withdrawal until the cows refused to be mounted by their herd mates. The cows were categorized as in oestrus when they remained standing when mounted.

Ultrasonography and Follicular Mapping

Each cow from the two treatment groups had their ovaries scanned using a 7.5 MHz linear array transrectal transducer (Aloka Japan) attached to a portable ultrasound (Aloka®, SSD-500) at 6-h intervals from the time of CIDR removal until the time of ovulation. Ovulation was predicted by loss of dominance as described by Pursely *et al*. (1995). The day of ovulation at the beginning of emergence of new follicles was designated as Day 0. The time of ovulation was calculated by subtracting the day of the DF was not observed from the day when the last DF was observed on the ultrasonography monitor.

The ovaries were then scanned at 2-d intervals to determine the follicular wave development (FD). The number of follicles greater than 4 mm in diameter was counted, and the size measured and mapped from both ovaries on the follicle map. The DF of a wave was determined as the one that grew to at least 8 mm in diameter and exceeded the diameter of all other follicles in the wave following the procedure of Ginther *et al*. (1989). Measurement was done on both sides of the interface follicular wall by taking the mean of the two diameter measurements of the follicle. The data of the 2 ovaries of each animal were combined. Maximum diameter and growth rate of the DF of the first, second and third waves were recorded.

Following the classification of Wael (2003) a follicular wave was categorized into anovulatory or ovulatory with the largest follicle (≥ 8 mm) attaining a diameter of its subordinate follicles. Each DF was then classified into growing, static or regressing phase. The 2- and 3-follicular wave interovulatory intervals were classified as first wave with a dominant anovulatory follicle and second wave with dominant ovulatory follicle. The third wave was described as having a dominant ovulatory follicle.

Blood Collection and Progesterone Assays

During each ultrasound examination,10 ml blood was collected from each cow through jugular venipunture into plain tubes (Vacutainer®, Becton Dickinson Limited, England) using a hypodermic disposable needle (4.57 mm x 0.38 mm) for progesterone hormone analyses. The blood was then kept at room temperature for 1 h and stored in a refrigerator at 4°C for 24 h. Serum was obtained from all blood samples by centrifugation at 700 g for 20 min. The serum was decanted and kept in small bottles before it was frozen and stored in a freezer at –20°C. The serum samples were analysed for their progesterone concentration using radio immunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, CA 90045) and a gamma counter unit. The RIA kits contained progesterone antibody-coated tubes and buffered deionized iodine 125 (^{125}I). The sensitivity of the assay was determined as 0.02 ng/ml. The inter- and intra-assay coefficients of variations for progesterone were 6.5 and 12.9%, respectively.

Statistical Analyses

Data were analyzed using independent samples t-test for the differences between the two treatment groups using SPSS package for windows release 19.0.

Results and Discussion

The cows in the 1M treatment group had 66.7% showing 3-follicular wave and 33.3% 2-follicular wave pattern of FD whereas cows fed 2M diet showed 40.0 and 60 % having 2- and 3-follicular waves, respectively (Table 1). The 1M group had a higher number of new follicles which emerged in the first follicular wave compared to 2M group (4.0 ± 0.49 and 2.8 ± 0.8, respectively, P< 0.05). However, the day and size when new follicles emerged were similar in both groups (1.5 ± 0.16 d and 1.2 ± 0.33 d; and 5.2 ± 0.01 mm and 5.2 ± 0.01 mm in 1M and 2M groups, respectively; P> 0.05; Table 2).

Table 1: The number (and percentage) of cows with 2- or 3-follicular waves fed diets with different energy levels

	Energy level[1]	
	1M	2M
N/ Parameter	15	15
2 waves	5[a] (33.3)	6[a](40.0)
3 waves	10[a] (66.7)	9[a] (60.0)

[a]Means (±) within row with different superscripts are significantly different (p<0.05).
[1]Energy level = 1M: Single maintenance energy diet;
2M: Double maintenance of energy diet.

Table 2: Follicular parameters characteristic of first follicular wave during oestrous cycle of cows fed with different energy level diets

Follicular parameters	Energy level[1]	
	1M	2M
No. of follicles at emergence	4.0 ± 0.49[a]	2.8 ± 0.28[b]
Size of follicle at emergence (mm)	5.2 ± 0.00[a]	5.2 ± 0.01[a]
Day of follicle emergence	1.5 ± 0.10[a]	1.2 ± 0.26[a]

[ab]Means (±) within row with different superscripts are significantly different (p<0.05).
Day of ovulation = Day 0
[1] Energy level = 1M: Single maintenance energy diet; 2M: Double maintenance of energy diet.

Table 3: Day of maximum diameter, size of dominant follicles and ovulatory follicles during oestrous cycle of cows fed diets with different energy levels

	Energy levels[1]	
	1M	2M
a) First follicle wave		
Day at maximum diameter	7.1 ± 0.96[a]	3.0 ± 0.64[a]
Maximum diameter of dominant follicle (mm)	12.3 ± 0.60[a]	12.7 ± 0.69[a]
b) Second follicle wave		
Day at maximum diameter	15.2 ± 1.05[a]	15.9 ± 0.75[a]
Maximum diameter of dominant follicle (mm)	11.8 ± 0.07[a]	12.9 ± 0.06[a]
c) Third follicle wave		
Day at maximum diameter	18.4 ± 0.50[a]	19.5 ± 0.96[a]
Maximum diameter of ovulatory follicle (mm)	12.3 ± 0.75[a]	14.2 ± 0.75[a]
Size of preovulatory follicle (mm)	10.5 ± 0.07[a]	11.1 ± 0.06[a]
Ovulation time (h)	92.0 ± 3.63[a]	89.8 ± 4.08[a]

[a]Means (±) within a row with different superscripts are significantly different ($p < 0.05$).
Day of ovulation = Day 0
[1]Energy level = 1M: Single maintenance energy diet; 2M: Double maintenance of energy diet

Table 4: Mean and standard error mean (Mean±SE) of oestrous cycle length, follicle growth rate, regression rate of follicles and progesterone concentration of cows fed with different energy level diets

Parameters	Energy level[1]	
	1M	2M
N	15	15
Oestrous cycle length (days)	20.8 ± 0.61[a]	20.3 ± 0.38[a]
Follicle growth rate (mm/day)	1.6 ± 0.02[a]	1.3 ± 0.02[a]
Follicle regression rate (mm/day)	1.6 ± 0.03[a]	1.8 ± 0.03[a]
Progesterone concentration (ng/ml)	1.3 ± 0.19[a]	1.5 ± 0.3 [a]

[a]Means (±) within a row with different superscripts are significantly different ($p < 0.05$).
[1]Energy level = 1M: Single maintenance energy diet; 2M: Double maintenance of energy diet.

The two energy level diets did not affect the preovulatory follicle diameter (10.5 ± 0.07 and 11.1 ± 0.06 mm for 1M and 2M groups, P>0.05, respectively; Table 3), and ovulation time (92.0 ± 3.63 and 89.8 ± 4.08 h (p>0.05), respectively; Table 3). The intervals of days when the follicles achieved dominance in the first, second and third wave of follicular development were not significantly different (P>0.05) between 2- and 3-follicular waves for both two energy level diets.

The number of waves, length of oestrous cycle, growth and regression rate of DF, and post-synchronized progesterone concentration indicated no diferences between 1M and 2M (P>0.05; Table 4) with the mean oestrous cycle of 20.6 ± 0.49 d, and mean growth and regression rate of DFs of 1.4 ± 0.02 mm per day and 1.7 ± 0.03 mm per day, respectively. The cows were considered cycling due to the presence of active corpus luteum indicated by the serum concentration of progesterone of 1.3 ± 0.19 ng/ml and 1.5 ± 0.31 ng/ml in the 1M and 2M groups, respectively.

The results of this study supported the hypothesis that antral follicles develop in a wave-like pattern. However, the results found that no significant reduction in the number of follicular and no increase in the diameter of the dominant follicles with higher levels of dietary energy provided to the cows. It was possible that the level of ME provided was not sufficient enough to trigger the positive effect on follicular development or there might be the presence of other environmental factors such as physiological state of the cows which had not been completely controlled.

In the present study, transrectal ultrasonographic monitoring of the ovarian follicular wave development revealed energy levels of 1M and 2M did not result in the KK crossbred cows to have 2- or 3-follicular wave of FD. Only 63.3% of the cows predominantly had 3-follicular waves of FD. The findings were similar to those reported by Sirosis *et al*. (1988), Savio *et al*. (1988) and Wael (2003). Wael (2003) reported that 71.4% of native cows showed predominance of 3-follicular waves, however, Sirosis *et al*. (1988) and Savio *et al*. (1988) showed that only 20% of cows had 2-follicular waves during each oestrous cycle and the remainder had 3-follicular waves.

Murphy *et al*. (1991) concluded that the low dietary intake tended to increase the proportion of oestrous cycle with three dominant follicles. We suggest that level of energy intake does not regulate the incidence of 3- to 2- follicular wave in order to shorten the duration of oestrous cycle, or it could not shift from 3- to 2-follicular wave development. The predisposing factors to the high incidence of the 2- or 3-follicular waves might be based on the concentration of gonadotropic hormones particularly FSH (Wael, 2003). Genetic predisposition or uncontrolled environmental conditions could also play an important role in regulating the incidence of 2- or 3-follicular waves within one oestrous cycle, through influences on follicular development and the level of their oestrogen secretion.

The comparison of number, size and day of follicle at emergence showed that diet 1M had a higher number of new follicles that emerged compared to 2M at the first follicular wave post synchronisation. However, the size of ovulatory follicle of present study was smaller compared to Diskin (1999) (12.7 mm). Murphy *et al*. (1991) found that maximum dominant follicular diameter was 11.8 ± 0.1 mm, 13.7 ± 0.2 mm and 13.2 ± 0.3 mm of Hereford-Friesian crossbred beef heifers fed 0.7, 1.1 or 1.8% of their body weight as dry matter per day during weeks 6 to 9 of dietary restriction with level of feeding relative to 0.6M, respectively.

However, Bossis *et al.* (1999) found that in Angus-Hereford crossed beef cows fed to lose 1% of body weight per week with the level of feeding relative to 0.4M showed maximum dominant follicular diameter of 10.4 ± 0.9 mm compared to the 1M of 15.7 ± 0.9 mm.

Evans (2003) treated heifers with oestradiol benzoate and reported the maximum diameter of the ovulatory follicle in the emergence was smaller (11.7 ± 1.2 mm) compared to heifers in the control group (13.1 ± 0.1 mm). Difference in heifers treated at dominance showed a trend towards smaller ovulatory follicles in oestradiol benzoate treatment, but did not alter the stage of follicle development. In order to achieve the oestrous cycle of 20.6 ± 0.49 d, follicles have to grow at the rate of 1.4 ± 0.02 mm per d, and regress at the rate of 1.7 ± 0.03 mm per d. The present study revealed the follicles took about 20.5 d to attempt the maximum size of 10.8 mm before they ovulated at 90.9 h after CIDR removal.

Conclusions

It is concluded that there is no pattern of increased follicle diameter with an increase in dietary energy intake in the KK crossbred cows fed 1M and 2M ME diets. The two levels of diet energy of 1M and 2M did not alter the follicular development in terms of shifting the 3- to 2-follicular waves, increase the preovulatory follicle diameter, shorten the ovulation time, increase the preovulatory follicle diameter and affect the time of ovulation of KK crossbred cows in a tropical condition.

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