Frequency of Oestrus Signs and Pregnancy Rates in Oestrus Synchronised Kedah-Kelantan Crossbred Cows Offered Two Metabolizable Energy Levels

Azizah[1]*, A., Ahmad[2], J., Ahmad Nazri[3], A.R., Saadiah[1], J. and Yaakub[4], H.
[1] Strategic Livestock Research Centre, MARDI Serdang, Selangor, [2] Strategic Livestock Research Centre, MARDI Kluang Station, Johor, [3] Ruminant Section, Livestock Commodity Development Division, Department of Veterinary Services, Putrajaya, [4] Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor
*Corresponding author: aziamri@mardi.gov.my

Abstract

Synchronisation of oestrus in cattle can facilitate the use of artificial insemination by reducing the time needed for detection of oestrus compared to cows entering oestrus spontaneously. The purpose of the study was to determine the oestrous signs characteristics and pregnancy rates in Kedah-Kelantan crossbred cows offered two levels of metabolizable energy. The study was conducted on 30 Kedah-Kelantan crossbred cows (of mean age: 3-5 y, average body weight: 300 kg, parity: first to third). Cows were allocated into two diet groups based on maintenance energy (ME): single (1ME) and double (2ME) dietary energy requirement (0.02 Mcal /kg body weight) with crude protein level of 15% based on ARC (1980) recommendation. The results showed that the proportion of cows expressing standing oestrus in first and second oestrous cycles were 73.3 and 66.7% ($p>0.05$), and 80 and 53.3% ($p>0.05$) in 1ME and 2ME diet groups, respectively. The percentage of cows expressing oestrus was not significantly different between the first and second cycles of oestrus. There was a decrease in the percentage of cows pregnant for 1ME diagnosed at day 30 (78.6%) and day 90 (57.1%) post AI whereas cows on 2ME showed no significant difference ($p>0.05$) in percentage of pregnancy at day 30 (66.7%) and day 90 post AI (53.3%). However, the percentage of cows pregnant in the 1ME group was not significantly different compared with 2ME group ($p>0.05$). In conclusion, the diet formulated to provide twice the metabolizable energy requirement for maintenance was not able to accelerate the frequency of cows showing oestrous signs and pregnancy rate of Kedah-Kelantan crossbred cows.

Key words: Kedah-Kelantan crossbred cows, metabolizable energy, oestrous signs, pregnancy rate

Introduction

Information on oestrous characteristics and reproductive parameters related to the nutrition of the local Kedah-Kelantan (KK) cows is scarce. It is important to understand the role of nutrition, particularly energy, in determining plasma progesterone level and ovulation reaction (Gordan, 2005). Indeed, energy is also important for reproductive management such as oestrous behaviour, onset of puberty, attainment of sexual maturity, pregnancy and calving interval.

The nutrient intake and body energy reserve are major regulators of a cow's reproductive performance. It is the key factor in influencing oestrous behaviour, timing of puberty and calving intervals. Two important elements of nutrients are energy and protein. Energy is important for growth and the pubertal process (Fleck *et al.*, 1980). Energy feeds are based on high-starch feed

ingredients such as maize and broken rice, fibrous agricultural by-products such as soybean hulls, wheat middling and beet pulp, or fat sources such as soybean oils, animal and vegetables oils. Forage supplies from grazing pasture or hay provide the bulk of energy.

Energy was given to the animals for body function particularly those associated with growth, lactation, and reproduction. Factors that influence gain and composition of energy requirements include age, sex, body size, physiological state and environment (Fox, Sniffen and O'Connor, 1988; Cooke, 2010). Therefore, animals are fed with energy feeds in order to meet their requirements, while supplementation is given to the animals in order to optimize animal performance when diets are lacking in energy content.

Imbalance in the relative availability of protein and energy may reduce the efficiency of metabolism and energy status, hence may result in reduced progesterone (P_4) concentration in the blood leading to prolonged follicular growth, delayed puberty (Lemenager *et al*., 1980) and late to reach sexual maturity (Chase, 1979). These impaired reproductive events are associated with lower fertility, conception rate and increased pregnancy losses (Lemenager *et al*., 1980), probably due to poor quality of the oocytes **(**Mihm *et al.*, 2003).

Feed content and animal requirements for protein and minerals can be quantified in weight scales (Cooke, 2010). However, energy requirements of cattle is measured in reference to a known standard but not in absolutes values (Cooke, 2010). Differences in energy supplement will cause changes in follicular development. Heifers fed diet with lower energy content had delay in the chronology of development of dominant follicles (Bergfeld *et al*., 1994) and follicle recruitment (Gutierrez *et al*., 1997) which influenced the development of maturational events associated with puberty. Therefore, the objective of the study was to determine the effect of two dietary energy levels on oestrous sign characteristics and pregnancy rates in oestrus synchronised KK crossbred cows.

Materials and Methods

The study was conducted at MARDI Research Station, Kluang, Johor, Malaysia involving 30 KK crossbred cows ranging in age from 3 to 5 years, body weight from 250 to 350 kg and parity from first to third calving. The cows were randomly and equally allocated to two diet groups based on maintenance energy (ME): one dose of ME (1ME, n=15) and two doses of ME (2ME, n=15) with ME dietary energy requirement for maintenance at 0.02 Mcal /kg body weight and crude protein level of 15% based on Agricultural Research Council (ARC, 1980) recommendation. The cows underwent an adaptation period for 14 d and given the dietary treatment before oestrous synchronisation.

Synchronisation of oestrus

Synchronisation was obtained by using a controlled internal drug release (CIDR, Pharmacia & Upjohn, Australia) device containing 1.38 g of progesterone. The device was inserted into the vagina for 7 d. Intramuscular injection of 500 µg of synthetic prostaglandin analogue (Estrumate®; Schering – Plough Animal Health, Australia) was administered 2 d prior to CIDR withdrawal. Cows were observed for oestrus after 24 h after CIDR withdrawal until the cows were noticed no longer mounted by their herd mates. Cows were categorized as in oestrus when she stood while being mounted by the other herd mates.

Observation of characteristics of oestrous signs

Characteristics of oestrous signs were determined during the oestrous cycles in KK crossbred cows (a cycle after synchronised oestrous regime). Five to six cows in each treatment group were kept in a holding lot of 2.7 x 1.8 m size during this period. Cows were observed for characteristics for oestrous signs every h for 72 h which began at CIDR withdrawal. Oestrous signs were monitored by visual observation by three trained personnel. Observation was discontinued when no successful mounts were observed. Data were recorded based on the time when cows extruded mucous discharge from the vagina (termed obtained discharge), cows started to mount other cows (termed began to mount), cows started to be mounted by others (termed began to be mounted) and and time when cows were standing and ready to be mated (termed standing oestrus).

Artificial insemination and pregnancy diagnosis

Cows were first synchronised at the beginning of the study and oestrus signs were observed after CIDR was removed. Then, cows were observed in their natural oestrous cycles on the second cycle of oestrous signs after oestrous synchronisation. Cows were synchronized after the third oestrous cycle and timed AI (TAI) was then performed. TAI was performed 48-54 h after removal of CIDR for all cows. AI was carried out using frozen semen of KK bulls from National Veterinary Institute of Biotechnology (IBVK), Jerantut, Pahang. Pregnancy diagnosis (PD) was performed on days 30 and 90 post AI via ultrasonography and rectal palpation, respectively (**Martin and David,** 2002). The ultrasonography was carried out by inserting a 7.5 MHz transducer attached to the ultrasound machine (Aloka® SSD-500; Japan) transrectally in the horns of the uterus to detect the presence of fluid.

Statistical analyses

Data on number of times of cows obtained discharge, began to mount and began to be mounted were analyzed by diet groups using independent t-test. Chi square test was used to compare frequency of oestrous signs characteristics between the first and second oestrous cycle and effect of different levels of energy on pregnancy rate. All statistical analyses were performed using SPSS version 19.0 (SPSS**,** 2010).

Results and Discussion

In the first oestrous cycle there was no significant difference ($p>0.05$) between cows on 1ME and 2ME diets for obtained discharge (19.92 ± 4.59 h, 18.09 ± 3.42 h), began to mount (32.72 ± 4.57 h, 36.27 ± 7.01 h), began to be mounted (34.45 ± 6.79 h, 39.90 ± 7.55 h) and standing oestrus (32.65 ± 5.57 h, 43.65 ± 7.31 h), respectively (Table 1). However, in the second oestrous cycle, 1ME diet group showed longer ($p<0.05$) for obtained discharge (15.38 ± 5.43 h vs 12.11 ± 2.27 h) and shorter for began to mount (19.35 ± 6.19 h vs 32.86 ± 6.13 h, $p<0.05$) than 2ME diet group.

Table 1: Oestrous signs observed at the first and second oestrous cycles of cows fed different ME levels

Oestrous signs	First oestrous cycle		Second oestrous cycle	
	1ME	2ME	1ME	2ME
Obtained discharge, h	19.92 ± 4.59^{xa}	18.09 ± 3.42^{xa}	15.38 ± 5.43^{ya}	12.11± 2.27^{xb}
Began to mount, h	32.72 ± 4.57^{xa}	36.27 ± 7.01^{xa}	19.35 ± 6.19^{ya}	32.86 ± 6.13^{xb}
Began to be mounted, h	34.45 ± 6.79^{xa}	39.90± 7.55^{xa}	27.11± 5.09^{ya}	34.68 ± 4.30^{xa}
Standing oestrus, h	32.65 ± 5.57^{xa}	43.65 ± 7.31^{xa}	29.95± 5.90^{xa}	40.67 ± 4.11^{xa}

^{ab}Means within row between diets within oestrus cycle with different superscripts differ significantly at $p < 0.05$

^{xy}Means within row between oestrus cycles with different superscripts differ significantly at $p < 0.05$

In the first oestrous cycle, number and proportion of oestrus behaviour characteristics between the cows fed 1ME and 2ME diets did not differ significantly (Table 2). However, in the second cycle of oestrus more cows showed began to mount and began to be mounted categories ($p<0.05$). The expression of oestrous signs characteristics were less exaggerated in 1ME compared to 2ME. The proportion of heifers expressing standing oestrus in the first oestrous cycle was 73.3% and 80.0% for 1ME and 2ME, respectively, and in the second oestrous cycle was 66.7% and 53.3% for 1ME and 2ME, respectively.

Table 2: Number and percentage with oestrous signs observed in the first and second oestrous cycles of cows fed different ME levels

Oestrous signs	First oestrous cycle		Second oestrous cycle	
	1ME	2ME	1ME	2ME
Obtained discharge, no. (%)	14 (93.3)^{xa}	13 (86.7)^{xa}	10 (66.7)^{xa}	12 (80.0 ^{xa}
Began to mount, no. (%)	13 (86.7)^{xa}	12 (80.0)^{xa}	4 (26.7)^{ya}	12 (80.0)^{xb}
Began to be mounted, no. (%)	12 (80.0)^{xa}	12 (80.0)^{xa}	5 (33.3)^{ya}	12 (80.0)^{xb}
Standing oestrus, no. (%)	11 (73.3)^{xa}	12 (80.0)^{xa}	10 (66.7)^{xa}	8 (53.3)^{xa}

^{ab}Means within row between diets within oestrus cycle with different superscripts differ significantly at $p < 0.05$

^{xy}Means within row between oestrus cycles with different superscripts differ significantly at $p < 0.05$

There was a decrease ($p < 0.05$) in percentage of cows pregnant from 78.6% at day 30 to 57.1% diagnosed at day 90 post AI for 1ME group (Table 3), whereas there was no significant difference in percentage of cows pregnant at day 30 (66.7%) and day 90 (46.7%) post AI in the 2ME group. However, the percentage of cows pregnant for 1ME was not significantly different compared to 2ME group ($p > 0.05$).

Table 3: Effect of different dietary energy levels on the number and proportion of cows pregnant and not pregnant diagnosed using ultrasonography on day 30 and rectal palpation on day 90 post AI

Parameter	Dietary treatment	
	IME	2ME
No. of cows pregnant and % at day 30 post AI	11 (78.6)^{xa}	10 (66.7)^{xa}
No. of cows pregnant and % at day 90 post AI	8 (57.1)^{ya}	7 (46.7)^{xa}
No. of cows not pregnant (%)	3 (21.4)^{xa}	6 (42.9)^{xa}

^{xy}Means (±) within rows with different superscripts differ at $p < 0.05$.
^{ab}Means within column (dietary treatment) between number of cows pregnant at days 30 and 90 with different superscripts differ significantly at $p < 0.05$.

This study was conducted to observe the effects of varying energy levels on oestrous signs characteristics and pregnancy rate. It was observed generally the oestrous behaviour was characterized by a sequence of signs begining with extrusion of mucous discharge, followed by begining to mount other animals, being mountedby other animals and standing oestrus. Otherwise in the first oestrous cycle, the expressions of began to be mounted and standing oestrus overlapped and were not in sequence. The 1ME group expressed the oestrus sign characteristics of obtained discharge faster than ME2 group in the second oestrous cycle. This study also showed that the percentage of cows expressing oestrus was not significantly different in the first oestrous cycle of oestrous behaviour characteristics compared with the second cycle of oestrous cycle.

A significant difference in proportion of heifers detected in oestrus was also reported by Martinez *et al.* (2000) with the proportion of heifers detected in oestrus was 94% and range of 3 d, 87% and range of 1.5 d, 75% and range of 2 d and 65% and range of 1.5 d for the control, estradiol $PGF_{2\alpha}$ (E/P), GnRH and follicle ablation groups, respectively. The results were comparable to the present study with the mean proportion of cows detected in oestrus and range in the interval to oestrus of 73.3% and range of 1.35 d for 1ME and 80% and range of 1.8 d for 2ME group in first oestrous cycle of oestrus behaviour, and 66.7% and range of 1.25 d for 1ME and 53.3% and range of 1.4 d for 2ME group in second cycle of oestrous behaviour (Tables 1 and 2).

Nevertheless, the period for detection of oestrus utilized in this study should have been adequate for observing the majority of cows that expressed oestrus. Additionally, the combination of estradiol and progesterone (E/P) 48 h prior to CIDR removal should have increased the extent and synchrony of the oestrus response in these cows. The present study has shown that single dose of maintenance energy to meet the energy requirement for many physiological functions has the potential to accelerate the percentage of oestrous signs compared to cows offered double the

maintenance energy diet. Similar to the finding of Baptise *et al.* (2005), the low energy-fed heifers showed numerically greater oestrous response than high energy-fed heifers. Similar to the finding of Ferrel *et al*. (1976), it appears that the double maintenance energy diet is associated with the lower oestrous response.

The high pregnancy rate observed in heifers on a higher plane of nutrition (Patterson *et al*., 1989b) was not obtained in the present study: similar to the findings of Baptise *et al*. (2005) who found that pregnancy rate was not affected by diet or initial body weight. Therefore, the low pregnancy rate in the present study might be attributed to the inability of the short-term nutritional program used to initiate oestrous behaviour characteristics. The result obtained showed that pregnancy rate to timed AI was low. However, there was a tendency for single maintenance energy diet to have greater pregnancy rate compared to cows offered double the maintenance energy level. Although oestrus and ovulation could have been induced in pubertal heifers, the fertility of the ovulated ovum might be less than those from natural ovulations (Patterson *et al.,* 1986; Patterson *et al*., 1992). Fertility rates following melengesterol acetate synchronization (Patterson *et al*., 1989a, b) and timed AI (Kelser *et al*., 1996) were reported to be variable, and the results obtained in this study are within the reported ranges.

The age of the cows used in present study also could be a factor attributing to the findings obtained. Age ranges contribute to the higher variation on the onset of oestrus among mature cows by comparison heifers might have lower fertilization rate or subsequent embryo development when animals are inseminated at the same interval after the onset of oestrus. The results obtained in heifers and mature cows suggest that the time of AI according to the onset of oestrus is still optimal for heifers, but not for cows (Saumande *et al*., 2005).

The reduction in the percentage of pregnant cows diagnosed at days 30 and 90 might be due to foetal loss or early embryonic death. López-Gatinus *et al*. (2004) revealed 75% of pregnancy loss was recorded between days 45 and 60 of gestation, with the peak occurring conspicuously earlier in twin pregnancies. This loss could be associated with the expression of endocrine imbalance, seemingly compromising the function of the corpus luteum and its secretion of progesterone. Luteal progesterone is important to maintain pregnancy (McDougall *et al*., 2005) and to stimulate endometrial m-RNA for luteinizing hormone receptor (Weems *et al*., 2009) or endocannabinoid secretion. This situation might prolong the next oestrous cycle.

There is a tendency for cows ovulating follicles of diameter less than 12.8 mm to be less likely to support pregnancy to day 25 after insemination (Perry *et al*., 2005), and heifers ovulating follicles of less than 10.7 mm in diameter to show decreased pregnancy rate (Perry *et al*., 2007). Nonetheless, 13% of the cows that ovulated follicles of diameter less than 13.5 mm experienced late embryonic mortality between days 25 and 39. Hence, follicles of of 9 to 10 mm in diameter at the time of AI appear to be important in order to acquire ovulatory capacity and several physiological changes, including increased circulating estradiol (Sartori *et al*., 2011). However, the most important factor in achieving a positive impact on pregnancy rate outcome is that the dominant follicles must attain the physiological maturity in order to initiate a preovulatory gonadotropin surge.

Conclusion

Increased level of maintenance energy in the diet beyond the estimated metabolizable energy level required for the maintenance of cows did not affect the oestrus behaviour in cows in consecutive cycles of oestrus following oestrus synchronization. However, providing a single dose of maintenance energy has the potential to accelerate the oestrus behaviour compared to twice the dose of maintenance energy if the oestrus observations were carried out in more than two cycles.

References

Agricultural Research Council (ARC). 1980. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, Slough, 351.

Baptise, Q.S., Knights, M. and Lewins, P.E. 2005. Fertility response of yearling beef heifers after prebreeding energy manipulation, estrous synchronization and timed artificial insemination. Animal Reproduction Science, 85: 209-221.

Bergfeld, E.G.M., Kojima, F.N., Cupp, A.S., Wehram, M.E., Peters, K.E., Garcia-Winder, M. and Kinder, J.E. 1994. Ovarian folllicle development in prepubertal heifers as influenced by level of dietary energy intake. Biol. Reproduction, 51: 1051-1057.

Chase, L.E., Smith, R.D. and Sniffen, C.J. 1979. The impact of nutrition and reproduction. Proc. 11th Biennal Ruminant Health-Nutrition Conference. Syracuse, New York. Pp 1.

Cooke, R.F. 2010. Energy nutrition for cattle. Beef Cattle Library, Oregon State University, Beef cattle sciences, BEEF040: 1-4.

Ferrel, C.L., Garret, W.N. and Hinman, N. 1976. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. J. Animal Science, 42: 1477-1489.

Fleck, A T., Schalles, R.R. and Kiracofe, G.H. 1980. Effect of growth rate through 30 months on reproduction performance of beef heifers. J. Animal Science 51(4): 816-821.

Fox, D.G., Sniffen, C.J. and O'Connor, J.D. 1988. Adjusting nutrient requirements of beef cattle for animal and environmental variations. J. Animal Science, 66: 1475-1495.

Gordon, K. 2005. Animal Reproduction. Animal Science, University of Guelph, Internet ed: http://www.aps.uoguelph.ca/~gking/Ag_2350/anrepro.htm, 1-17.

Gutierrez, C.G., Oldhman, J., Bramley, T.A., Gong, J.G., Campbell, B.K. and Webb, R. 1997. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. J. Animal Science, 75: 1875-1884.

Kelser, D.J., Faulkner, D.G., Shirley, R.B., Dyson, T.S., Ireland, F.A. and Ott, R.S. 1996. Effect of interval from melengestrol acetate to prostaglandin F_2 alpha on timed and synchronized pregnancy rates of beef heifers and cows. J. Animal Science, 74: 2885-2890.

Lemenager, R.P., Smith, W.H., Martin, T.G., Singleton, W.L. and Hodges, J.R. 1980. Effects of winter and summer energy levels on heifer growth and reproductive performance. J. Animal Science, 51: 83-87.

López-Gatinus, H., Wu, Z., Satter, L.D. and Wiltbank, M.C. 2004. Effect of dietary phosphorus concentration on estrous behavior of lactating dairy cows. Theriogenology 61: 437-445.

Martin, S. and David, N. 2002. Pregnancy diagnosis in cattle. British Veterinary Association. In Practice 24: 310-317.

Martinez, M.F., Adams, G.P., Kastelic, J.P., Bergfelt, D.R. and Mapletoft, R.J.. 2000. Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology, 54 (5): 757-769.

McDougall, S., Blache, D. and Rhodes, F.M. 2005. Factors affecting conception and expression of oestrus in anoestrous cows treated with progesterone and estradiol benzoate. Animal Reproduction Science, 88: 203-214.

Mihm, M. and Bleach, E.C. 2003. Endocrine regulation of ovarian antral follicle development in cattle. Animal Reproduction Science, 78: 217-237.

Patterson, D.J., Corah, L.R. and Brethour, J.R. 1986. Effect of estrous synchronization with melengestrol acetate and prostaglandin on first service conception rates in yearling beef heifers. J. Animal Science, 63 (1): 472 (abstract).

Patterson, D.J., Kiracofe, G.H., Stevenson, J.S. and Corah, L.R. 1989a. Control of the bovine estrous cycle with melengestrol actate (MGA): a review. J. Animal Science, 67: 1895-1906.

Patterson, D.J., Kiracofe, C.H., Stevenson, J.S. and Corah, L.R. 1989b. Conception rate in Bos taurus and Bos indicus crossbred heifers after postweaning energy manipulation and synchronization of estrus with melengestrol acetate and frenprostalene. J. Animal Science, 67: 1138-1147.

Patterson, D.J., Perry, R.C., Kiracofe, C.H., Bellows, R.A., Staigmiller, R.B. and Corah, L.R. 1992. Management consideration in heifer development and puberty. J. Animal Science, 70: 4018-4035.

Perry, G.A., Smith, M.F., Lucy, M.C., Green, J.A., Parks, T.E., MacNeil, M.D., Roberts, A.J. and Geary, T.W. 2005. Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Science of the United States of America, 102(14): 5268 -5273.

Perry, G.A., Smith, M.F., Roberts, A.J., MacNeil, M.D. and Geary, T.W. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Animal Science, 85: 684- 68

Sartori, R. and Barros, C.M. 2011. Reproductive cycles in Bos indicus cattle. Animal Reproduction Science, 124(3-4): 244-250.

Saumande, J. and Humblot, P. 2005. The variability in the interval between estrus and ovulation in cattle and its determinants. Animal Reproduction Science, 85: 171-182.

SPSS, GPL Reference Guide for IBM SPSS Statistic. 2010**.** © Copyright SPSS Inc. 1989, 2010: 1-348.

Weems,Y. S., Lewis, A.W., Neuendorff, D.A., Randel, R.D., and Weems, C.W. 2009. Endocabbabinoid (ECN) receptor agonists affect cow luteal function. Prostaglandins and Other Lipid Mediators, 90:89-96.