EFFECT OF SYNCHRONIZING THE RATE OF DIETARY ENERGY AND NITROGEN RELEASE ON REPRODUCTIVE PERFORMANCE IN BRAHMAN-THAI NATIVE CROSSBRED BEEF CATTLE

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Abstract

The objective of this research was to determine the effect of synchronizing the rate of dietary energy and nitrogen release on fertility in Brahman-Thai native crossbred beef cattle. Sixteen Brahman-Thai native crossbred beef cattle weighing an average of 276.31 $\pm$ 34.29 kg were housed individually. The animals were divided into two groups, each group balanced by heifers and cows. Prior to formulation of the dietary treatments, feed ingredients were analyzed for chemical composition and the nylon bag technique was used to analyze feed ingredients for degradability. The treatments were organized in two levels of a synchrony index (low, 0.39 and high, 0.74) and assigned randomly to one of two groups of animals. The results indicate that blood urea nitrogen decreased ($P<0.01$) when the synchrony index increased. First service conception rate, hematology, diameter of reproductive organ, pre-insemination estrous cycle length and post-insemination progesterone concentration were not different ($P>0.05$) as the levels of the synchrony index increased, while body weight gain increased ($P<0.05$) as levels of the synchrony index increased.

Keywords: Beef cattle, energy, nitrogen, fertility, synchrony index

Introduction

Feeding excess degradable intake protein can affect early embryonic development, embryonic survival rate and reduce reproductive efficiency in ruminants (Robinson, 1996; Butler, 1998). Cows fed excess ruminally degradable protein resulted in increased blood urea, altered uterine fluid composition, decreased uterine pH and reduced conception rates (Elrod and Butler, 1993; Elrod *et al*., 1993; Melendez *et al*., 2000), and increased plasma ammonia (Sinclair *et al*., 2000b).

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Plasma progesterone concentrations were reportedly lower in cows fed high dietary protein than cows fed low dietary protein (Sonderman and Larson, 1989). Ferguson *et al.* (1993) reported that a blood urea nitrogen concentration exceeding 20 mg% was associated with reduced conception rate in lactating cows. Butler *et al.* (1996) also reported that plasma urea nitrogen and milk urea nitrogen concentrations > 19 mg% were associated with approximately a 20 percentage point decrease in the pregnancy rate in lactating dairy cows. Ruminants fed synchronous diet had decreased blood urea nitrogen (Shabi *et al.*, 1998; Chumpawadee *et al.*, 2004a) and avoided excessively high levels of plasma ammonia (Sinclair *et al.*, 2000a). Therefore, synchronizing the rate of dietary energy and nitrogen release is a possible method to avoid excessive blood urea nitrogen and excessive high levels of plasma ammonia, leading to improved reproductive efficiency.

Information on the effect of synchronizing the rate of degradation of dietary energy and nitrogen release on the reproductive performance in beef cattle is very limited. Therefore, the aim of this study was to determine the effect of synchronizing the rate of degradation of dietary energy and nitrogen release on fertility in Brahman-Thai native crossbred beef cattle.

Materials and Methods

In situ Degradability Characteristics of Feedstuffs

The feedstuffs were collected from various feed mills and organizations (Kantharavichai Dairy cooperatives, Khonkaen Dairy cooperatives, Mahasarakham University feed mill, Khon Kaen University feed mill, Numhenghoad feed supplier, Chareon Esan commercial feed mill and Songserm Kankaset feed supplier) in the Northeast of Thailand. All feedstuff samples (Table 1) were ground through a 1 mm screen for an *in situ* degradability study and chemical analysis. The feedstuff samples were analyzed for dry matter (DM), crude protein (CP) and ash (AOAC, 1990) and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (van Soest *et al.*, 1991).

Ruminal degradation determined by nylon bag technique (Ørskov and McDonald, 1979) was carried out after a two weeks adaptation period in two Brahman-Thai native crossbred beef steers (250 ± 15 kg BW., fitted with permanent rumen cannula). Steers were offered rice straw *ad libitum* and received concentrate at 0.5% BW. The concentrate consisted of 49.80% cassava chip, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0% mineral mix and 8.30% sugarcane molasses.

Approximately 5.0 g (fresh matter) of each tested feed was accurately weighed into the nylon bag with a mean pore size of 45 μm (Shabi *et al.*, 1998). The bag plus the sample were placed into the rumen 30 minutes after the morning meal and retrieved after periods of 2, 4, 6, 12, 24 and 48 h. After removal from the rumen, bags were rinsed in pipe line fresh water and washed by hand under tap water until the water became clear. After washing, the bags were placed into a hot-air force-dry oven at 65°C for 48 h and weighed. To determine the content of water soluble material, bags representing 0 h degradation also underwent the same washing procedure as the incubated bags. Dried residues of each incubation time from each steer were pooled, DM, organic matter (OM) and CP analyzed; then DM, OM and CP disappearance values were calculated as the difference between the weight of nutrients before and after incubation of each sample. The degradability data obtained for OM and N for each feed were fitted to the equation $P = a + b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979), where P is the amount degraded at time *t*, *a* is the rapidly soluble fraction, *b* is the potentially degradable fraction; *c* is the rate of degradation of fraction *b*.

Urea and sugarcane molasses were also included in the database. It was assumed that 95% of urea N was degraded in the first hour after feeding, with the remaining 5% of urea N degraded at a rate (c) = 0.5/h (Sinclair *et al.*, 1995) and 100% of N and organic matter of molasses was degraded in the first hour post feeding.

Diet Formulation and Synchrony Index

The synchrony index of OM to N was calculated as follows:

Synchrony index =

$$\frac{25 - \sum_{1-24} \frac{\sqrt{\left[(25 - hourlyN/OM)^2\right]}}{24}}{25}$$

where 25 = 25 g of N per kg OM truly digested in the rumen. A synchrony index of 1.0 represents perfect synchrony between N and the energy supply throughout the day whilst values < 1.0 indicate the degree of asynchrony according to Sinclair *et al*. (1993).

The computer program described previously (Sinclair *et al*., 1993) was used which written to calculate dietary OM and N supply to the rumen and contains the database of raw material proximate analysis, fiber composition and degradation characteristics obtained from the *in situ* degradability experiment (Tables 1 - 2). The program requires inputs of the proportion of each constituent in the diet, total dry matter intake per day (DMI), the time of feeding during the day and the outflow rate of solids (k) from the rumen. The formulation assumed that the animals were fed in two equal meals at 07.00 and 19.00 h, had a DMI of 2.0% BW and had a ruminal outflow rate of 0.05/h.

Using the computer program, two diets were formulated to have a similar metabolizable energy (ME), crude protein (CP), rumen

Table 1. Chemical composition of feedstuffs used for feed formulation in the experiment

Feedstuffs	DM (%)	CP	Ash	NDF	ADF	ADL
		%DM basis				
Rice straw	91.50	3.00	13.64	72.13	53.28	4.89
Ground corn	92.20	8.53	1.69	13.25	3.63	0.41
Cassava chip	93.40	1.89	2.01	6.93	6.35	1.87
Rice bran	91.70	14.26	6.31	20.29	8.12	2.61
Kapok seed meal	91.01	28.09	8.91	42.50	29.49	16.34
Soybean meal	91.31	47.24	7.12	12.84	8.26	0.10

Where DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

Table 2. Degradation characteristics of organic matter and nitrogen of feedstuffs*

	OM degradation				N degradation			
	a	b	c	a + b	a	b	c	a + b
Rice straw	0.09	0.75	0.014	0.84	0.28	0.57	0.004	0.85
Ground corn	0.36	0.63	0.024	0.99	0.29	0.45	0.051	0.74
Cassava chip	0.77	0.22	0.033	0.99	0.60	0.19	0.065	0.79
Rice bran	0.40	0.36	0.176	0.76	0.36	0.42	0.156	0.78
Kapok seed meal	0.37	0.22	0.057	0.59	0.10	0.61	0.264	0.71
Soybean meal	0.34	0.65	0.045	0.99	0.10	0.89	0.038	0.99

*$P = a + b\,(1-e^{-ct})$ Where a = the rapidly soluble fraction b = the potentially degradable fraction which in time t, c = the rate of degradation of fraction b, e = the natural logarithm

degradable protein (RDP) and rumen degradable organic matter (Table 3), but differed in the synchrony index of 0.39 (low) and 0.74 (high), respectively.

Feeding Trials

Sixteen Brahman-Thai native crossbred beef cattle weighing an average of 276.31 $\pm$ 34.29 kg were housed individually in pens. Animals were divided into two groups (8 replications per treatment), each group balanced by heifers and cows. The diet contained two levels of synchrony index treatment (low, 0.39 and high, 0.74) and was assigned randomly to one of the two groups of animals. The animals were fed at the rate of 2.0 %BW in two equal meals at 07.00 h and 19.00 h. Clean water and mineral lick were offered and available at all times. This experiment was carried out at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Thailand, from September 1, 2003 to November 28, 2003.

The roughage and concentrate were sampled every three weeks and the composited analyze for DM, CP and ash content (AOAC, 1990), and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest *et al*., 1991).

The animals were weighed at the beginning and at the end of the experiment. The estrous cycles of cows and heifers were synchronized by giving two injections of PG $F_{2\alpha}$ (25 mg, Lutalyse™ Dinoprost tromethamin, Phamacia N.V./S.A. Puurs- Belgium, MI) administered 11 days apart. The first injection was given 4 days before the first day of feeding the experimental diets. Cows and heifers were monitored for estrous activity four times per day for 30 min by farm personnel while they worked in the barn, beginning approximately 19 days after the second $PGF_{2\alpha}$ injection (i.e., approximately day 16 of the synchronized estrous cycle). Approximately 12 h after observed spontaneous standing estrus (i.e., 20 to 28 days after the second injection of $PGF_{2\alpha}$), each animal was inseminated by one technician using semen from a single ejaculate of an active AI sire. Occurrence of pregnancy to that one insemination was determined by rectal palpation of the uterus approximately 45 days after insemination. Blood samples were taken from the jugular vein twice weekly from the initiation of the experiment until insemination and then were sampled once weekly until the end of the experiment. Plasma was stored at -20°C until it was assayed for progesterone by RIA (ImmuChem™ Coated tube Progesterone ^{125}I RIA Kit, MP Biochemicals, ICN Phamaceuticals, Inc.).

On day 38 of the experiment, blood samples were collected at 4 h post-feeding from the jugular vein using heparinised vacutainers (two tubes, each tube containing 10 ml of the sample) of each animal. The first tube was gently inverted a couple of times, then kept in an icebox and later centrifuged at 3,000 RPM for 15 min. The plasma was then transferred into storage tubes and labeled with the date and animal identification. The plasma samples were kept at-20°C until analyzed for BUN (BMG's urea reagent, Boehringer Mannheim, Indiana polis, IN). The second tube was taken to determine heamatocytology. On the same day, determination's of the cervix, uterine horn and ovary diameter sizes by rectal palpation and an estimate of the diameter size were made.

Statistical Analysis

Conception rate data were analyzed by a chi-square test of association. The other parameters (BUN, hematology, diameter of reproductive tract and plasma progesterone) were subjected to group T-test procedure of SAS (1996). Significance was shown at P<0.05 unless otherwise noted.

Results and Discussion

Chemical Composition and Degradability Characteristics

Chemical composition and degradability characteristics of feed ingredients used in the experiment are shown in Tables 1 - 2, respectively. The feed ingredients varied widely in terms of composition and degradability characteristics.

Crude protein content ranged from 1.89% for cassava chip to 47.24 % for soybean meal. Ash content ranged from 1.69% for ground corn to 13.64% for rice straw. Cell wall content ranged from 6.93% for cassava chip to 72.13% for rice straw. The rapidly soluble fractions (a) of OM and N were highest in the cassava chips. These results were similar to thos reported by Nitipot and Sommart (2003) who studied the in vitro gas production techniques. They reported that the rate and extent of degradation of cassava chip was higher than that in ground corn, broken rice and other industrial by-products of local feed resources. Sommart *et al*. (2000) suggested that cassava and urea are known to be readily degraded in the rumen and thus may provide rumen synchrony when fed to animals. They also reported that, because cassava chip is available locally in Thailand and is inexpensive, it is the best potential rumen fermentable energy feed source for beef and dairy cattle. The potentially degradable fraction (b) of OM was highest in rice straw and soybean meal. The rate of degradation of fraction b (c) of OM was highest in rice bran.

Chemical composition analyses of the two diets are presented in Table 3. Both diets had a similar chemical composition. The rations of CP, ash, and NDF content were approximately 10.74%, 9.23%, and 52.66%, respectively.

Blood Urea Nitrogen

Blood urea nitrogen (BUN) concentrations at 0, 3, 6, and 9 h post-feeding are presented in Table 4. Blood urea nitrogen concentrations decreased ($P<0.001$) in animals offered the high synchrony index diets. The results disagreed with Sinclair *et al*. (2000a), who found that plasma

Table 3. Diet composition and chemical composition by calculation and analysis

Ingredient	Synchrony Index	
	Low (0.39)	High (0.74)
Rice straw	54.82	54.84
Cassava chip	8.79	16.90
Rice bran	13.92	4.97
Ground corn	7.91	2.97
Soybean meal	-	13.49
Kapok seed meal	8.90	1.98
Salt (NaCl)	0.50	0.50
Urea	1.00	0.20
Mineral mix	0.50	0.50
Molasses	3.65	3.65
Total	100.00	100.00
Chemical composition		
DM, %	92.53	93.74
Ash, %	9.01	9.45
CP, %	10.66	10.83
NDF, %	54.37	50.96
ADF, %	34.15	30.95
ADL, %	4.29	3.01
ME*, Mcal/kg	2.91	2.26
Rumen degradable OM*, %	44.06	47.51
Rumen degradable N*, %	6.96	6.64

* calculated values

urea levels were unaffected by a synchronous treatments in temperate fed basal diet. Urea is a small molecule that equilibrates between the reproductive tract and plasma (Duby and Trischler, 1986). Deleterious effects of high blood urea have been demonstrated on sperm viability (Duby and Trischler, 1986) and embryo survival both *in vitro* (Dasgupta *et al*., 1971) and *in vivo* (Saitoh and Takahashi, 1977). In addition, feeding high rumen degradable protein diets altered uterine pH in heifers (Elrod and Butler, 1993), hormonal patterns during the estrous cycle and early pregnancy (Berardinelli *et al*., 2001), and thus have a direct effect on gametes (Canfield *et al*., 1990) and embryo survival (Melendez *et al*., 2000; Berardinelli *et al*., 2001). Recently, in this experiment it was found that beef cattle fed a diet containing a higher synchrony index had a lower BUN; this would indicate that a synchronous diet increased N utilization efficiency. Synchronizing the rate of dietary energy and nitrogen release is a possible way to avoid excess blood urea nitrogen and excessively high levels of plasma ammonia, leading to improved reproductive efficiency. However, Ferguson *et al*. (1993) reported that a blood urea nitrogen concentrations exceeding 20 mg% was associated with a reduced conception rate in lactating cows. Since the BUN concentration in this experiment was lower than 20 mg%, BUN would not affect the fertility in beef cattle.

Hematology

The effects of the synchrony index on hematological values are shown in Table 4. Hematocrit, total white blood cell count, heterophyl, eosinophyl, basophyl, lymphocyte and monocyte were not significantly different (P>0.05) between the treatments. The results indicated that the synchrony index in the diets had no effect on hematological values. Hematological values are widely used in veterinary medicine for detecting various metabolic and nutritional disorders in cattle. The hematological values in this experiment are within a normal range when compared with the reference values of Jain (1993); therefore all animals in this experiment are healthy.

Table 4. Blood urea nitrogen and hematological values of beef cattle fed a diet a containing synchrony index of 0.39 and 0.74

Parameter	Synchrony index		SEM	p-value
	Low (0.39)	High (0.74)		
Blood urea nitrogen, mg%				
0 h	15.52	8.37	0.34	0.003
3 h	16.98	12.22	0.83	0.001
6 h	16.91	12.47	0.82	0.002
9 h	13.47	9.54	0.82	0.010
Average (0-9 h)	15.41	10.65	0.38	0.001
Hematocrit,%	29.25	31.62	0.74	0.57
Total white blood cell count, x 10^3cell/ml	16.60	15.16	0.68	0.96
Heterophil, %	37.0	33.62	2.58	0.93
Eosinophil, %	8.0	11.50	1.18	0.54
Basophil, %	0.0	0.14	0.06	0.35
Lymphocyte, %	54.37	54.00	2.73	0.57
Monocyte, %	0.62	0.75	0.19	0.65

Palpation Diameter of Cervix, Uterine Horn and Ovary

Rectal palpation diameters of the reproductive organs in the experiment are shown in Table 5. Cervix, uterine horn, left ovary and right ovary diameters were not significantly different (P>0.05) between the treatments. The results indicate that the synchrony index diets had no effect on rectal palpation diameters of the reproductive organs. In order to understand the normal structures of the ovary, uterine horn or cervix, the normal diameter of the cervix and uterine horn range from 2 to 7 and 3 to 4 cm, respectively (Morrow, 2004), ovary diameter range from 1.27 to 1.90 cm (Sorensen and Berverly, 2004). Examination of the reproductive organs of cattle via rectal palpation is possible because of several anatomical features of the cow (Hansen, 2004). Anatomical structures palpated on the cow's reproductive organs can indicate the stage of the estrous cycle and pregnancy status.

The cervical and uterine diameters of beef cattle fed the high synchrony and low synchrony index diets were lower than those reported previously (Morrow, 2004). The diameter of ovaries in beef cattle fed the high synchrony index diet are normal, while in beef cattle fed the low synchrony index diet they are lower than previous references. It is possible that synchronous diet can increase the nutrient supply (Sinclair *et al*., 1993) and lead to an increased ovary diameter.

First Service Conception Rates, Pre-insemination Estrous Cycle Lengths and Body Weight Gains

The first service conception rates, pre-insemination estrous cycle lengths and body weight gains are shown in Table 5. The first service conception rates were not significantly different (P>0.05) between the treatments. Ferguson *et al*. (1993) suggested that a blood urea concentration exceeding 20 mg% was associated with fertility in dairy cows. The reasons for this parameter were not significant; it possible that as the BUN concentration in the present experiment was lower than 20 mg%, BUN was not detrimental to the reproductive system. Several researchers such as Elrod and Butler, (1993); Elrod *et al*. (1993); Melendez *et al*. (2000); Berardinelli *et al*. (2001) have suggested that dairy cows fed excess ruminally degradable protein showed increased blood urea, altered uterine fluid composition, decreased uterine pH and thus, reduced conception rates.

Table 5. Rectal palpation diameters of cervix, uterine horn, left ovary and right ovary; first service conception rates, pre insemination estrous cycle lengths, and body weight gains of beef cattle fed a diet containing a synchrony index of 0.39 and 0.74

Parameter	Synchrony index		SEM	p-value
	Low (0.39)	High (0.74)		
Cervix, cm	1.23	1.56	0.11	0.39
Uterine horn, cm	1.40	1.75	0.13	0.15
Left ovary, cm	1.12	1.46	0.15	0.11
Right ovary, cm	1.10	1.46	0.16	0.83
First service conception rate, %	50.00 (4/8) [1]	62.5 (5/8)	0.40	0.83
Pre insemination estrous cycle length, d	22.00	20.87	0.36	0.92
Body weight gain, (kg/d)	0.13	0.36	0.08	0.03

[1] Number of pregnant cows/Number of animals in the group

Moreover cows fed excess dietary protein had a low first breeding pregnancy rate (McCormick *et al*., 1999). A synchronous diet is a possible way to avoid excess blood urea nitrogen and excessivelly high levels of plasma ammonia. Nevertheless, as most rations of beef cattle do not contain excess ruminally degradable protein, beef cattle do not have this problem. A synchronous diet is valuable for nutrient supply, particularly protein supply (Sinclair *et al*., 1993)

The pre-insemination estrous cycle lengths of cattle did not differ (P>0.05) between cattle fed either the low synchrony index diet or the high synchrony index diet, and averaged 22.00 and 20.87 d, respectively. Berardinelli *et al.* (2001) suggested that the estrous cycle lengths of ewes did not differ between ewes fed either the high protein or control diet. The estrous cycle in cattle was relative to patterns of follicular growth and estrogen secretion. This cycle pattern is followed at approximately 21 days intervals. The normal estrous cycle lengths in cattle range from 18 to 22 days (Elrod and Butler, 1993). In the present study, the estrous cycle lengths are in the normal ranges, from 20.87 to 22.00 days. Therefore, the synchrony index diet did not affect the estrous cycle.

The body weight gains were affected (P<0.05) by the synchrony index diet. The beef cattle fed the higher synchrony index gained more weight. The results agree with Witt *et al.* (1999) and Chumpawadee *et al.* (2004b), who reported that a higher synchrony index diet was associated closely with increased microbial protein synthesis and nutrient digestibility, which enhanced body weight gains.

Plasma Progesterone

Plasma progesterone concentrations are shown in Figure 1 - 2. The progesterone concentrations post-insemination for pregnant animals and all animals were not significantly different (P>0.05). The results indicate that the synchronous diet did not affect to post-insemination progesterone concentrations, while pre-insemination progesterone concentrations at 0, 7, 14 and 28 days of the experiment for pregnant animals and all animals were significant different (P<0.01). The results show that at 0 and 28 days of the experiment, animals fed the low synchrony index diet had higher plasma progesterone than animals fed the high synchrony index diet, while at 7 and 14 days of the experiment animals fed the low synchrony index diet had lower plasma

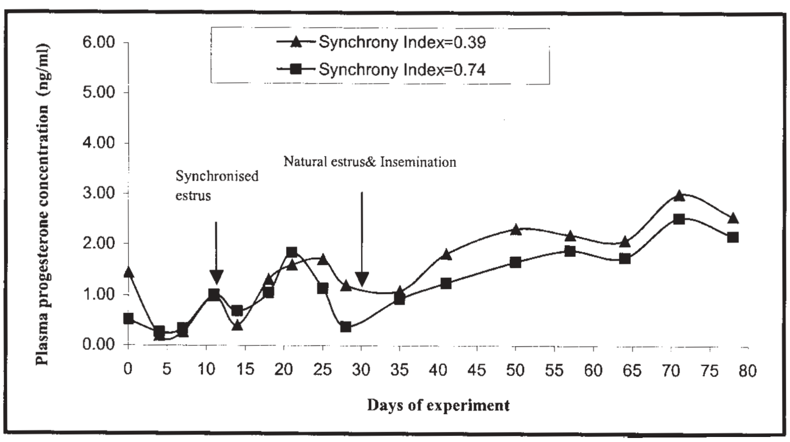

Figure 1. Plasma progesterone concentration from all cattle (pre-insemination and post-insemination) fed a diet containing a synchrony index of 0.39 (n = 8) and 0.74 (n = 8)

progesterone than animals fed the high synchrony index diet. Fertility of cattle inseminated at a natural estrus is related to the endocrine profile before and after insemination. The progesterone level is most important in pregnant animals to maintain the pregnant status, while high concentrations of progesterone in the blood during the luteal phase of the estrous cycle preceding insemination were associated with higher conception rates (Britt and Holt, 1988). Progesterone concentrations were lower (Jordan and Swanson, 1979; Folman *et al.*, 1981; Jordan *et al.*, 1983; Sonderman and Larson, 1989) or not affected (Blauwiekel *et al.*, 1986; Carroll *et al.*, 1988; Elrod and Butler, 1993) in cows fed a high protein diet. High blood urea concentration may alter uterine pH (Elrod and Butler, 1993) and progesterone production (Butler *et al.*, 1996) resulting in an undesirable uterine environment for embryo survival. In the present study animals fed the low synchrony index diet had elevated blood urea nitrogen, but there was no effect on post-insemination plasma progesterone concentrations, whereas at 0, 7, 14 and 28 days pre-insemination, it was significantly different with the treatment. However progesterone concentrations in the present study are in the normal range, when compared to Randel (1990).

Conclusion

Blood urea nitrogen was decreased by increasing the levels of the synchrony index. First service conception rates, hematology, diameter of reproductive organs, pre-insemination estrous cycle lengths and post-inseminated progesterone concentrations were not different as levels of the synchrony index increased. Therefore, synchronizing the rate of degradation of dietary energy and nitrogen release did not affect the fertility in Brahman-Thai native crossbred beef cattle.

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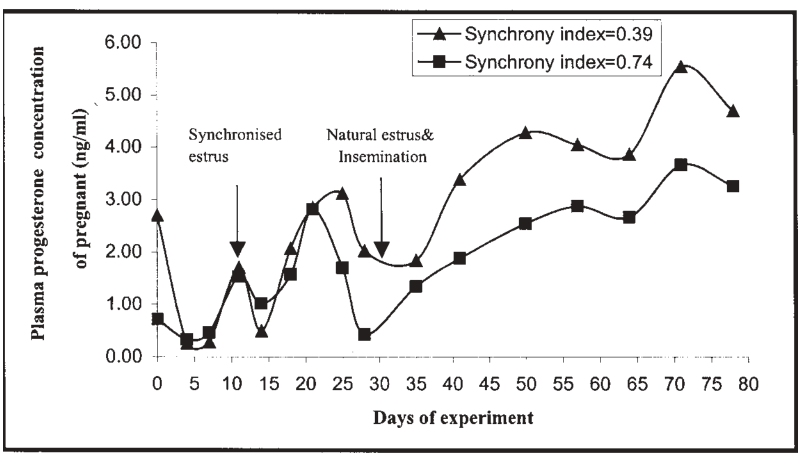

Figure 2. Plasma progesterone concentration in pregnant cattle (pre-insemination and post-insemination) fed a diet containing a synchrony index of 0.39 (n = 4) and 0.74 (n = 5)

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