



## Association among calving season and measures of energy status, resumption of ovulation and subclinical endometritis in early lactating dairy cows

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### Abstract

A total of 65 multiparous cows that calved during the hot season (n = 21), temperate season (n = 23) or cold season (n = 21) were used to investigate the relationships between heat stress, seasonal changes, metabolic traits, body condition score (BCS), resumption of ovulation and occurrence of subclinical endometritis (SE). Cows were monitored for the first 7 weeks of lactation by ultrasonographic examination. Blood sampling and BCS evaluations were performed on a weekly basis. Plasma progesterone (P4) and blood metabolites related to energy status including  $\beta$ -hydroxybutyrate, nonesterified fatty acids, total cholesterol, blood glucose and blood urea nitrogen were analyzed. Resumption of ovulation postpartum was confirmed by the first detection of a corpus luteum or a rise in P4  $\geq$  1 ng/ml. Moreover, subclinical endometritis was diagnosed by brush cytology on day  $40 \pm 2$  of lactation and cows having polymorphonuclear cell percentages (PMN%)  $\geq 5$  were considered SE positive. As expected, mean temperature humidity index differed (P < 0.001) among hot (75-85), temperate (50-65) and cold seasons (35-45). The BCS in cows that calved during the hot season was lower (P < 0.001) when compared with those that calved during the cold and temperate seasons. The percentage of cows that resumed ovulation in the first 45 days of lactation was greater (P < 0.05) for those that calved during the hot season (90%) than the temperate season (60%). The concentration of NEFA was greater (P < 0.05) for cows that calved during the cold season than the hot season during different weeks postpartum. The concentration of glucose in weeks 4 to 7 postpartum was greater (P < 0.01) for cows that calved during the temperate season when compared to cows that calved during the hot season. There was no difference in the prevalence of SE according to calving season. The percentage of PMN tended (P < 0.1) to be greater in cows with SE during the temperate season than during the hot season. In conclusion, calving season may influence BCS, blood metabolites and resumption of ovulation whereas no association with prevalence of subclinical endometritis was observed.

**Keywords:** calving season, endometritis, metabolites, ovulation.

### Introduction

High yielding dairy cows have been selected to produce large amounts of milk through their ability to mobilize fat and muscle to maintain milk yield during early lactation (Kadokawa and Martin, 2006). During that period, nutrient requirements for milk synthesis increase dramatically and the cow is unable to satisfy her energy demand with enough feed intake (Konigsson *et al.*, 2008). Therefore, most cows enter a period of negative energy balance (NEB) during the peripartum period that lasts several weeks (Taylor *et al.*, 2003; Konigsson *et al.*, 2008). This condition of NEB is associated with a loss of body condition and alterations in blood metabolite concentrations and hormone profiles which in turn influence fertility (Pryce *et al.*, 2001; Taylor *et al.*, 2003), particularly the resumption of ovarian activity during the early postpartum period (Wathes *et al.*, 2007).

Environmental temperature, radiant energy, relative humidity and wind speed are all components that influence the degree of heat stress (De Rensis and Scaramuzzi, 2003). Therefore, heat stress gives rise to more conditions than those in the animal thermal neutral zone. The temperature-humidity index (THI) incorporates the effects of both ambient temperature and relative humidity. This index is widely used in hot areas worldwide to assess the impact of heat stress on dairy cows (Hahn, 1969; Fuquay, 1981). Despite this, published literature evaluating the direct effects of THI and temperature on the energy balance status of dairy herds is sparse (Wheelock *et al.*, 2010). In this study, we examined the impact of some climate variables such as temperature and THI on the energy metabolic indicators of high producing dairy cows during the early postpartum period and its subsequent effect on the resumption of ovulation and subclinical endometritis.

The calving interval for high-producing dairy cows is economically most favorable at 13 to 14 months (Dijkhuizen *et al.*, 1997) and cows should conceive within 85 days after parturition. This requires normal cyclicity within a few weeks after calving (Opsomer *et al.*, 2000). The onset of normal restoration of ovarian cyclicity is one of the most important events for a modern high-yielding cow to regain her maximum breeding capacity following parturition. A large study stated that up to 49% of high-yielding dairy cows suffer

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from ovarian dysfunction postpartum (Opsomer *et al.*, 2000), resulting in lower conception rates and longer calving intervals (Shrestha *et al.*, 2004; Walsh *et al.*, 2007). However, it is imperative to identify possible etiological factors that may predispose to or result in the occurrence of these disturbances.

An alteration in blood metabolites characterizes NEB. An excessive demand for glucose postpartum induces high peripheral blood nonesterified fatty acid (NEFA) concentration and ketone bodies (Herdt, 2000).  $\beta$ -hydroxybutyrate (BHBA) is the predominant form of ketone body in the blood and its higher concentration is a clue for fatty acid concentration. Serum metabolic profiles in high yielding postpartum cows are reflected in the follicular fluid and, as a result, may affect the quality of both the oocyte and granulosa cells (Leroy *et al.*, 2004), with a subsequent delayed resumption of ovulation.

The aim of the present study was to characterize the energy balance, body condition score, incidence of resumption of ovulation and subclinical endometritis early postpartum during different calving seasons in high yielding Holstein cows.

## Materials and Methods

### Animals

The study was carried out during 2007 and 2008 on a producing herd of 198 Holstein-Friesian dairy cows at the National Livestock Breeding Center, Iwate Station (Morioka, Japan). This area has a warm climate with four distinct seasons, with peak summer temperatures reaching 34°C and winter minimum temperatures reaching -10°C. Throughout the year, cows were kept in free-stall barns bedded with rice straw. Cows were fed according to National Research Council - NRC (1988) to meet their production requirements. They were group-fed a total mixed ration consisting of alfalfa, timothy and oat hay, corn, tofu, ground, wet beet pulp, cottonseed and soybean (roasted and meal), with approximately 13.7% CP (Crude protein) and 66% TDN (Total digestible nutrients) for close-up cows, and 17.5% CP and 73% TDN for lactating (35 kg of milk/day) cows. Cows were machine-milked twice daily and the 305 day milk production was 11,299  $\pm$  1,452 kg. The age of the cows ranged from 3 to 7 years. A total of 65 multiparous cows which calved during the hot season (May - September, n = 21; parity = 2.2  $\pm$  0.8; Mean  $\pm$  SD), temperate season (March - April, October - November, n = 23; parity = 2.0  $\pm$  0.8) and cold season (December - February, n = 21; parity = 2.3  $\pm$  0.5) were studied for energy status, resumption of ovulation and incidence of subclinical endometritis during 45 days postpartum. All animals in the study were selected based on a clinically normal status of the genital tract with no history of dystocia, caesarean section, retained fetal membranes, acute mastitis or periparturient paresis

prior to the beginning of the study. No antimicrobial or hormonal treatments were given to any of the cows during the 45 day study.

### Transrectal ultrasound examination of the ovaries

All cows were subjected to ultrasonographic (US) examination to monitor the first ovulation with subsequent formation of luteal tissue and uterine involution. The ovaries and uteri were examined weekly from weeks 3 to 7 postpartum using a real-time B-mode mobile US unit with a 5/7.5 MHz linear array transducer (Agroscan; ECM Noveko International Inc., Angouleme, France). Resumption of ovarian activity was considered when a series of at least two consecutive plasma samples showed P4 concentrations  $\geq$ 1 ng/ml. In the present work, every time P4 was  $>$ 1 ng/ml a CL was visualized, and every time P4 was  $<$ 1 ng/ml no CL was visualized. The postpartum interval for resumption of ovulation (PORI) was defined as the interval from calving to the first sample day that plasma progesterone concentration was  $\geq$ 1 ng/ml.

### Diagnosis of subclinical endometritis

On day 40  $\pm$  2 postpartum, the diagnosis of subclinical uterine inflammation was carried out by endometrial cytology (EC) according to Senosy *et al.* (2009). Cytological evaluation (brush cytology) was used to estimate the polymorphonuclear cell percentage (PMN%) by counting a minimum of 200 PMN and endometrial cells at 400X magnification for quantitative assessment of endometrial inflammation. Mononuclear cells (lymphocytes and macrophages) were not included in counting. Cows having  $\geq$ 5% PMN were considered to have subclinical endometritis, whereas cows with  $<$ 5% PMN were considered to have a healthy uterus.

### Blood sampling and body condition scoring

Blood samples were obtained on a weekly basis from weeks 2 to 7 postpartum by puncture of the coccygeal blood vessel using evacuated heparinized tubes. Agitation of the cows and long durations of sampling were avoided since stress may interfere with endocrine and metabolic variables. Blood samples were centrifuged at 2000 x g and plasma was harvested and stored at -30°C until analysis. Body condition scoring (Ferguson *et al.*, 1994) was estimated by the same observer from weeks 2 to 7 postpartum.

### Progesterone and biochemical analyses

Plasma progesterone concentrations were measured by time-resolved fluorescence immunoassay (TR-FIA) kits (DELFLIA Progesterone Reagents, Wallac Oy, Turku, Finland) according to the manufacturer's protocol and a previous method (Senosy *et al.*, 2009).



In each sample, the concentrations of BHBA, NEFA, total cholesterol (T-chol), glucose and blood urea nitrogen (BUN) were measured on an Operationally Enhanced Random Access analyzer (Accute, Toshiba Medical Systems, Toshiba, Japan) using kinetic enzymatic kits (Roche Diagnostics, Japan; NEFA-HA Wako test kit, Autokit-3HB Wako test kit for BHBA, Glucose HK Rikitech test kit, BUN Rikitech test kit and Total cholesterol [TCII] Rikitech test kit) according to the manufacturer's instructions. The ranges of measurements were as follows: NEFA up to 2.00 mEq/l, BHBA 1.17 to 3000  $\mu\text{mol/l}$ , glucose 2 to 750 mg/dl, BUN 0.5 to 150 mg/dl and T-chol 5 to 800 mg/dl. The intra- and inter-assay coefficients of variation were below 5%.

#### *Meteorological variables*

Climate data such as daily mean and maximum temperature (T) and mean and minimum relative humidity were recorded inside the stalls every hour during the study period using an automated device (SK-L200II series; SK-L200THII  $\alpha$  for temperature and humidity, Sato Keiryoki MFG. CO., LTD, Japan). The device was located 3.5 m above the ground. The mean temperature humidity index (THI) was fitted to the following equations (Hahn, 1969):

$$\text{Mean THI} = \{(0.8 \times \text{mean T})\} + \{(\text{mean RH}(\%)/100) \times (\text{mean T} - 14.4)\} + 46.4$$

T: air temperature; RH: relative humidity expressed in decimals.

#### *Statistical analyses*

The primary goal of this study was to investigate the relationship among calving season and blood metabolites (BHBA, NEFA, T-chol, glucose and BUN), BCS, resumption of ovulation and subclinical endometritis. The general linear models ANOVA for repeated measures (SPSS Version 16.0; SPSS Inc., Chicago, IL, USA) were used to determine main effects of group and week, and their one-way interactions. The data of PMN% was log transformed in order to be normally distributed. Comparisons between proportions of animals resuming ovarian activity was performed by a Chi square test using pairwise comparisons of the proportions. Calving season and age were considered main effects, while interval to resumption of ovarian activity, blood metabolic traits including BHBA, NEFA, T-chol, glucose, BUN, BCS, average milk yield,

prevalence of subclinical endometritis and PMN% were considered dependent variables. Data are presented as mean  $\pm$  SEM, while proportions of cows resuming ovulation and with subclinical endometritis were categorical data. Probability values with  $P \leq 0.05$  were considered significant and  $0.05 < P \leq 0.1$  were considered as tending toward significance.

## **Results**

### *Temperature humidity index, milk production, age and body condition score*

The calculated means of THI during the cold, temperate and hot seasons varied ( $P < 0.001$ ) between 35-45, 50-65 and 75-85, respectively. Average milk production in cows 305 days postpartum tended ( $P = 0.07$ ) to be greater in cows that calved in the temperate season ( $12,067.3 \pm 1,383$  kg) when compared to those that calved during the cold ( $10,845.3 \pm 1,479.3$  kg) and hot seasons ( $11,285 \pm 1,648.5$  kg). Milk production during the first 45 days postpartum was  $1,685.2 \pm 215.6$  kg,  $1,683.8 \pm 237.2$  kg and  $1,635.3 \pm 238.8$  kg in cows calving during the temperate, hot and cold seasons, respectively. There was no difference in age between the animals that calved during different seasons. BCS was lower ( $P < 0.001$ ) in cows that delivered during the hot season when compared to those that calved during the cold and temperate seasons at different time points postpartum. Furthermore, body condition score loss during later times was less extensive in the animals that calved during the hot season when compared to those that calved during both the cold and temperate seasons. There was no difference between the BCS of cows that calved during the cold and temperate seasons (Fig 1).

### *Resumption of ovarian cyclicity*

Based on the first detection and weekly detection of both luteal tissue formation and a progesterone level  $\geq 1$  ng/ml, the percentage of animals that resumed ovarian cyclicity within 45 days postpartum was higher ( $P < 0.05$ ) in cows that calved during the hot season (90%) than those that calved during the temperate season (60.9%; Table 1). There was no difference ( $P > 0.05$ ) in PORI among the different groups of cows that calved during the hot ( $32.3 \pm 7.3$  days), temperate ( $32.0 \pm 7.8$  days) and cold seasons ( $34.3 \pm 7.8$  days; Table 1).

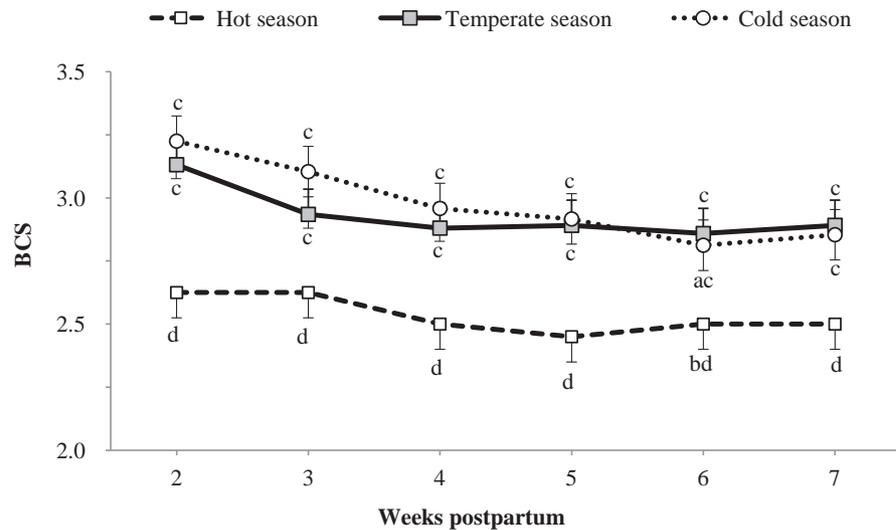


Figure 1. Body condition score (BCS) in animals calved during the hot, temperate and cold seasons. Values with different superscripts between groups within the same week were significant (<sup>ab</sup>P < 0.01; <sup>cd</sup>P < 0.001). There were significant main effects of week and group (P < 0.001), whereas their interaction was not significant.

Table 1. Effect of calving season on restoration of ovarian cyclicality, occurrence of subclinical endometritis at day 40 ± 2, polymorphonuclear cell percentage (PMN %) and postpartum resumption of ovulation interval (PORI, Mean ± SEM).

	Hot season (n = 21)	Temperate season (n = 23)	Cold season (n = 21)
Percentage of resumed cows	90 (19/21) <sup>a</sup>	60.9 (14/23) <sup>b</sup>	66.7 (14/21) <sup>ab</sup>
Incidence of subclinical endometritis	42.9 (9/21)	30.4 (7/23)	33.3 (7/21)
PMN% in positive animals	6.3 ± 0.9	18.1 ± 15.9	12.2 ± 9.1
PORI (days)	32.3 ± 7.3	32.0 ± 7.8	34.3 ± 7.8

<sup>a,b</sup> Within rows, values with different superscripts were different (P < 0.05).

#### Metabolic trait changes

There was no significant difference among groups in relation to BHBA concentrations during different weeks postpartum (Fig. 2A). At week 5 postpartum, BHBA concentration tended (P = 0.06) to be higher in cows that calved during the temperate season (619.7 ± 136.5 μmol/l) than cows that calved during the hot season (523.8 ± 148.5 μmol/l). NEFA concentration was higher in cows that calved during the cold season when compared to those that calved during the hot season at week 2 (P < 0.05), week 3 (P < 0.001), week 4 (P < 0.05), week 5 (P < 0.05), and week 6 postpartum (P < 0.01; Fig. 2B). Moreover, NEFA concentration was higher in animals that calved during the temperate season at weeks 3 (P < 0.01), 4 (P < 0.05) and 6 postpartum (P < 0.01) when compared to those that calved during the hot season (Fig. 2B). Blood glucose concentrations were higher in cows that calved during the temperate season than those animals that calved during the hot season at week 4 (P < 0.01), week 5 (P < 0.05), week 6 (P < 0.05) and week 7 postpartum (P < 0.01; Fig 2C). There was no significant difference among groups regarding total cholesterol concentration

during different weeks postpartum (Fig. 2B). The concentration of BUN was higher in cows that calved during the temperate season than cows that calved during the hot season at week 2 (P < 0.05), 3 (P < 0.01), 4 (P < 0.01), 5 (P < 0.01), 6 (P < 0.01) and 7 (P < 0.01) postpartum (Fig. 2E). Furthermore, BUN concentration in cows that calved during the cold season was higher (P < 0.05) at weeks 4 and 6 postpartum when compared with those that calved during the hot season. On the other hand, BUN concentration in cows that calved during the cold season was lower (P < 0.05) than of cows that calved during the temperate season (Fig. 2E).

#### Polymorphonuclear cell percentage (PMN%) and incidence of subclinical endometritis

The prevalence of a cytological diagnosis of endometritis was 7/21 (33.3%), 7/23 (30.4%) and 9/21 (42.9%) during the cold, temperate and hot seasons, respectively. Polymorphonuclear cell percentage (PMN%) tended (P < 0.1) to be greater in cows with subclinical endometritis that calved during the temperate season (18.1 ± 15.9%) when compared to those that calved during the hot season (6.3 ± 0.9%; Table 1).

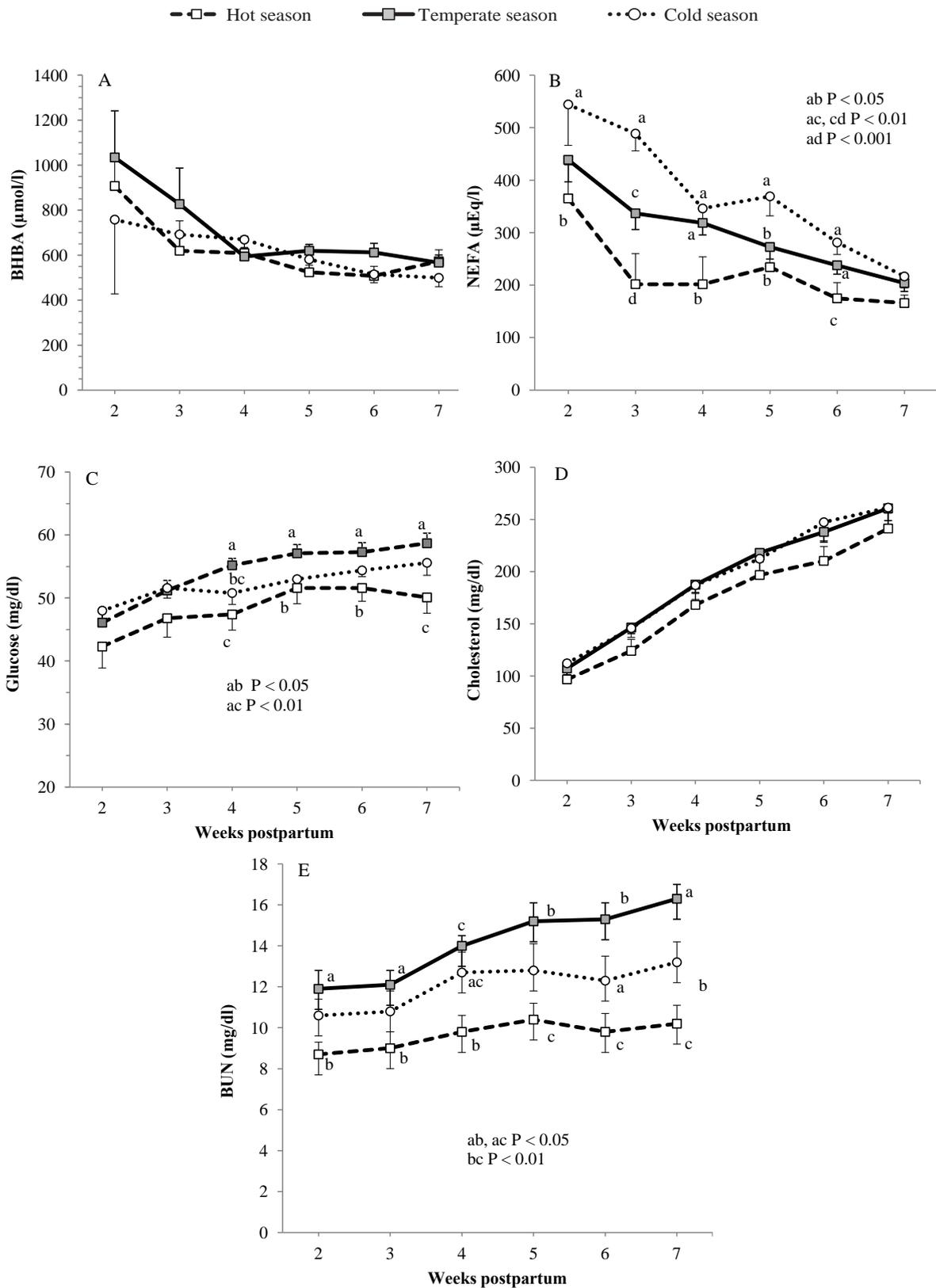


Figure 2. Blood metabolic traits in cows calved during the hot, temperate and cold seasons, including (A)  $\beta$ -hydroxybutyrate (BHBA), (B) nonesterified fatty acids (NEFA), (C) blood glucose, (D) total cholesterol (T-chol), and (E) blood urea nitrogen (BUN) during weeks 2, 3, 4, 5, 6, and 7 postpartum. Values with different superscripts between groups within the same week were significant. There were significant main effects of week and group ( $P < 0.05$ ), whereas their interaction was not significant.



## Discussion

The present study investigated the association among calving season and measures of energy status, resumption of ovulation and subclinical endometritis during early lactation in dairy cows. It has been generally believed that heat stress can aggravate NEB due to declined dry matter intake, with the purpose of avoiding an excessive heat addition of digestion and to control metabolic heat production (Collier *et al.*, 1992; Blackshaw and Blackshaw, 1994). In the present study, dairy cows that calved during hot months had a lower BCS when compared to the cold and temperate month calving cows. Earlier studies confirmed our findings and stated that BCS was lower in cows during the summer when compared to cows during the winter at 6 weeks postpartum (Jonsson *et al.*, 1997; Shehab-El-Deen *et al.*, 2010). Changes in BCS give an indirect assessment of fertility loss as a loss of one unit of BCS has been associated with a reduction in conception rates and, hence, lower fertility rates (Butler, 2000). This greater loss of BCS is due to a reduced dry matter intake (Collier *et al.*, 1992; Blackshaw and Blackshaw, 1994). Other studies showed that a hot climate significantly contributes to reduced milk production indirectly through its effect on feed intake (Mayer *et al.*, 1999; Payne and Wilson, 1999; West, 2003). These results are in accordance with ours in which average milk production at 305 days of lactation tended to be lower in animals that calved during the summer season when compared to those that calved during temperate weather. Moreover, Ray *et al.* (1992) concluded that milk production was depressed for cows calving during the summer and fall when compared to those calving during winter and spring.

A higher proportion of cows ovulated had calved during the cool season when compared with cows that calved during the hot season (Kornmatitsuk *et al.*, 2008). Conversely, our study revealed that a greater percentage of cows that calved during the hot season resumed their ovarian cyclicity when compared to those that calved during temperate months. Our observation was also reported by Hansen (1997), who found that conception rates remained lower during autumn when the climate is temperate and the cows are no longer exposed to heat stress. Moreover, cows exposed to heat stress suffered from impaired steroidogenesis in medium and large sized follicles 3 weeks later (Roth *et al.*, 2001). Furthermore, thecal cells appeared to be consistently susceptible to heat stress and expressed a carryover effect on androgen production in both types of follicles. This observation may be explained by the fact that ovarian follicles are susceptible to heat stress (Badinga *et al.*, 1993; Wolfenson *et al.*, 1995) and by the fact that it takes about 40–50 days for small, antral follicles to develop into large, dominant follicles (Lussier *et al.*, 1987). Thus, exposure to summer heat stress during the early stages of follicular development

may impair later follicular function and decrease fertility during autumn.

It has been widely accepted that heat stress can aggravate NEB, predominantly due to a reduced dry matter intake in order to prevent an excessive heat increment of digestion and to control metabolic heat production (Collier *et al.*, 1992; Blackshaw and Blackshaw, 1994). NEFA was higher during the summer than during winter (Shehab-El-Deen *et al.*, 2010). These results were contrary to our results as NEFA was higher during winter than summer. Therefore, higher NEFA during winter may be not due to heat stress but a low plane of nutrition (Rhoads *et al.*, 2009; Schwartz *et al.*, 2009), as low temperatures may affect or lower food intake. Increased plasma NEFA concentrations are a classic glucose-sparing mechanism that animals on a low plane of nutrition implement to maximize milk synthesis. Serum glucose concentrations change all throughout the year and are affected by the month of the year (Ndlovu *et al.*, 2009). This study reported that serum glucose concentrations were highest in October and started to decline in January or February. These findings were comparable to our findings. Moreover, Shehab-El-Deen *et al.* (2010) found that blood glucose was lower during summer months when compared to winter months at different weeks postpartum, from week 1 to week 6. The possible mechanisms by which heat stress reduces plasma glucose concentrations include reduced DMI (Abilay *et al.*, 1975; Roman-Ponce *et al.*, 1981; Flamenbaum *et al.*, 1995), altered metabolic hormone secretion and the partitioning of energy to thermoregulation (Finch, 1986).

Subclinical inflammation of the endometrium takes place in the absence of signs of endometritis, as determined by cytology (LeBlanc and Duffield, 2002). Hence, the first reaction of the uterus against foreign bodies is a neutrophilic influx into the endometrium and uterine lumen (Klucinski *et al.*, 1995). In the present study, PMN% tended to be higher in cows with subclinical endometritis that calved during the temperate season when compared to those that calved during the hot season. This is a clue that heat stress and season may play a role in PMN% in cytologically diagnosed subclinical endometritis. These results suggested that heat stress interferes with innate and acquired immune status (do Amaral *et al.*, 2011). Therefore, heat stress has an adverse effect on the uterine environment and endometrial function including the production of heat-shock proteins by the endometrium and an increased production and release of prostaglandin F<sub>2α</sub> from the endometrium (Wolfenson *et al.*, 2000).

In conclusion, calving season and heat stress can influence the pattern of some blood metabolites, such as NEFA and blood glucose. Moreover, cows that calved during hot months were thinner at calving. Resumption of ovarian cyclicity was lower during the temperate season, which is an indication of the carry



over effect of heat stress during summer season on the subsequent season (autumn). There was no influence of calving season on the incidence of subclinical endometritis, while there was an effect on the PMN% on positively diagnosed animals between the hot season and the temperate season.

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