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Influence of Energy Balance on Reproductive Performance and Milk Production of Dairy Cows at Pre-partum and Early Lactation Periods

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Abstract: The objective of this study was to evaluate the influence of energy balance in dairy cows at pre-partum and early lactation periods on their subsequent reproductive and productive performance. High producing, Ayrshire cows were randomly selected from the heavy pregnant, dry cow herd of Ambewela dairy farm in Up-Country, Sri Lanka. The serum NEFA (non esterifie fatty acid) and milk BHBA (beta hydroxyl butyric acid) concentrations were used as indicators of energy status of the cows. Days for the first AI, open days and conception rate were used to evaluate the reproductive performance. Sub-clinical ketosis (milk BHBA≥200 µmol/L) was recorded among 25 and 31.25% cows at 5 days pre-partum and during 100 days post-partum periods, respectively. Further, they took significantly longer period to reach the first AI and showed significantly greater ($p<0.0001$) open days compared to the cows those did not show signs of sub-clinical ketosis (milk BHBA<100 µmol/L) during the period. Cows those recorded≥200 µmol/L BHBA level in milk during the lactation period from 10 to 60 days took longest period for the first AI and had the highest open days. Only 36% cows were pregnant at 100 days post-partum. Further, none of the cows showed sub-clinical ketosis during 100 days post-partum was pregnant. They had higher circulating NEFA levels at 5 days pre-partum and 10 days post-partum periods and significantly lower ($p<0.05$) in milk production compared to their pregnant counterparts. This study indicated that the magnitude and duration of the prepartum energy status (i.e., negative energy balance) has a detrimental effect on subsequent reproductive and productive performances in high producing dairy cows.

Keywords: BHBA, Conception rate, NEFA, open days, Postpartum, Pre-partum

INTRODUCTION

Maintenance of high reproductive efficiency in cows is vital to ensure the profitability of dairy cattle farming. Despite implementation of an efficient AI programme, the reproductive efficiency of dairy cattle herds in Sri Lanka is reported to be well below the acceptable level (Abeygunawardane *et al*., 1995; Abeygunawardena, 1998). The energy status of dairy cows particularly during early and peak lactation periods can influence their subsequent reproductive performance (Butler *et al*., 1981; Jorritsma *et al*., 2005; McDougall *et al*., 2005). During these periods, many high producing dairy cows experience Negative Energy Balance (NEB) as insufficient dietary intake does not meet the nutrient demand for high milk production (Gerloff, 2000; Hayirli *et al*., 2002). Therefore, better understanding of the energy state of high producing dairy cattle during early and peak lactation periods is vital to achieve greater reproductive performance. Non esterifies fatty acids (NEFA) and Beta-Hydroxy Butrate (BHBA) levels in serum and milk are commonly used biomarkers of energy balance dairy cattle (Geishauser *et al*., 2000; Rukkwamsuk *et al*., 1999a). The present study examines the influence of energy status of dairy cows at pri-partum and early lactation transitional period on their subsequent reproductive performance and milk production in Ambewela dairy farm in Up-Country, Sri Lanka.

MATERIALS AND METHODS

The study was conducted during four months period (April-June, 2010) at Ambewela dairy cattle farm. The farm situated at 1, 848 m above MSL in Nuwara-Eliya district of Up-Country, Sri Lanka (6° 53'N, 80° 48′E), which experiences 2050 mm mean annual rainfall and 15.5°C mean annual temperature. High producing Ayrshire breed cows were randomly selected from the heavy pregnant, dry cow herd. Blood samples (10 mL) were collected from coccygeal vein at 5 days before calving (pre-partum) and 10, 40, 60, 80 and 100 days after calving (post-partum) from each cow

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into plain, redtop vacutainer (9.00 am-11.00 am) and handled according to the recommendations (Stokol and Nydam, 2005). Enzymatic colorimetric method (acyl-coenzyme a synthetase-acyl-coenzyme an oxidase; Wako NEFA C test) was performed to determine the serum NEFA level using a commercially available kit (Wako Chemicals USA Inc., Richmond, VA). Cows were hand milked twice a day (4.00 am and 4.00 pm) and yields were recorded. Milk samples (10 mL) were collected from morning milking from each cow into Micro Centrifuge (MC) tubes on 10, 40, 60, 80 and 100 days of the lactation. The level of BHBA in milk was semi-quantitatively determined using Sanketopaper also known as Ketolac test (Sanwa Kagaku Kenkyusho Company, Nagoya, Japan), through the reaction which nitroprusside changes colour from white to pink or purple. Results of the test were denoted as 0, 50, 100, 200, 500 and 1000, indicating BHBA level in milk at <50, 50 to 99, 100 to 199, 200 to 499, 500 to 999 and≥1000 mmol/L, respectively.

Cows were individually inspected twice a day during the period of the experiment (from 5 days pre-partum until 100 days post-partum). During the post-partum, cows at heat were detected and inseminated artificially (AI) at the second heat and thereafter (i.e., if they come to heat again). Number of days from calving to every heat was recorded. Pregnancy was Diagnosed (PD) by rectal palpation technique and confirmed by ultrasound technique by a veterinarian at 100 days post-partum. Number of days from calving to conception (i.e., open days) and conception rate were computed for each cow. It has been reported that milk BHBA level is a useful indicator of the energy status of dairy cows (Reist *et al*., 2002). Further, cows having <100 mmol/L BHBA in milk are normal and those having 200 mmol/L are at sub-clinical status of ketosis (Dirksen and Breitner, 1993). Therefore, cows included in the present experiment were divided in to 3 groups (i.e., <100, 100≤199 and 200≤ μmol/L) according to their maximum milk BHBA level recorded during the study period. Statistical analyses were carried out using version 9.1 of SAS software (2002–2003). Data were subjected to analysis of variance (ANOVA) procedures and the influence of energy status of cows on days for first AI, open days and conception rate was tested. Duncan's multiple range tests was used to separate means. Correlation coefficient was estimated between serum NEFA level and milk yield.

RESULTS

Table 1 presents the frequency distribution of cows according to milk BHBA level in pre-partum and post-partum periods. There was a significant (p>0.05) association between stage of gestation cycle (i.e., pre-partum or post-partum period) and milk BHBA level. Pregnancy (i.e., pregnant or not pregnant) as detected at 100 days pos-tpartum and milk BHBA level was also significantly associated. Ultrasound technique confirmed that only 36.00% cows were pregnant at 100 days post-partum period. The effect of milk BHBA level on reproductive performance of cows is shown in Table 2. Cows those recorded the highest milk BHBA level (200≤ μmol/L) took significantly (p<0.05) longer

Table 1: Frequency distribution of cows according to milk BHBA level in pre-partum and post-partum periods

Milk BHBA level (μmol/L)	Cows*		Pregnant cows*	
	Pre-partum[1]	Post-partum[2]	Pre-partum[1]	Post-partum[2]
< 100	31.25	31.25	40.00	40.00
100 ≤ 199	43.75	37.50	57.14	66.67
200 ≤	25.00	31.25	0.00	00.00

*Significant (p<0.05) association.[1], At 5 days pre-partum period.[2], Until 100 days post-partum period

Table 2: Maximum milk BHBA concentration and reproductive performance of cows

BHBA concentration[1] (μmol/L)	Day in milkat first service[1,2]	Open days[1,2]	Conception rate[1,2]
<100	54±3.3[a]	54±4.7[a]	1.7±0.67[a]
100≤199	50±3.3[a]	64±10.5[a]	1.6±0.40[a]
200≤	76±3.2[b]	164±18.2[b]	2.5±0.50[a]

[1]: Mean±SE; [2]: Means within a column followed by different superscripts are different at p<0.05

Table 3: Pre-partum and post-partum serum NEFA concentration and pregnancy[1] of cows

Period	Serum NEFA concentration[2,3] (mEq/L)		
	Overall	Pregnant cows	Non-pregnant cows
Pre-partum period (days)			
5	0.58±0.073[a]	0.35±0.152[a]	0.69±0.197[b]
Post-partum period (days)			
10	0.47±0.049[a,b]	0.34±0.124[a]	0.54±0.108[b]
40	0.42±0.070[a,b]	0.38±0.091[a]	0.45±0.263[a]
60	0.34±0.066[b]	0.33±0.173[a]	0.34±0.720[a]
80	0.37±0.071[b]	0.36±0.205[a]	0.38±0.233[a]
100	0.37±0.083[b]	0.33±0.260[a]	0.40±0.180[a]

[1]Pregnancy was diagnosed at 100 days post-partum period.[2], Mean±SE[3], Means within a row followed by different superscripts are different at p<0.05

Table 4. Milk production of pregnant and non-pregnant cows[1]

Days in milk	Milk production (kg/ day)[2,3]	
	Pregnant cows	Non-pregnant cows
10	19.2±1.30[a]	15.7±3.03[b]
40	24.3±3.11[a]	21.7±2.60[a]
60	24.7±2.73[a]	20.7±3.43[a]
80	22.2±3.00[a]	19.7±3.52[a]
100	19.9±2.20[a]	17.3±4.03[a]

[1]: Pregnancy was diagnosed at 100 days post-partum period.[2]: Mean±SE., [3]: Means within a row followed by different superscripts are different at p<0.05

period to reach the second heat where they were serviced. They also had greater open days compared to those recorded lower milk BHBA levels. However, there was no significant ($p>0.05$) effect of milk BHBA level on the conception rate of the cows.

Table 3 presents the relationship between serum NEFA concentration during pre-partum and post-partum periods and pregnancy. The serum NEFA concentration remained at low level (33-38 mEq/L) in pregnant cows throughoutthe study period. Further, the levels were found to be significantly ($p<0.05$) lower on 5 days pre-partum and on 10 days post-partum periods in pregnant cows compared to their non-pregnant counterparts. Mean milk productions of pregnant and non-pregnant cows are revealed in the Table 4. Cows those detected as pregnant by 100 days post-partum, had recorded significantly ($p<0.05$) greater milk production at 10 days in milk compared to their non-pregnant counterparts. However, milk production was not significantly ($p>0.05$) different afterwards between pregnant and non-pregnant cows.

DISCUSSION

This study was conducted to investigate the influence of energy status of pre-partum, transition and early lactating dairy cows on subsequent reproductive and productive performances. Serum NEFA and milk BHBA concentrations were used as indicators of energy status while postpartum period to reach the first AI, conception rate and open days were used to assess the reproductive performances. Milk production up to 100 days in milk was also assessed. Cows on the state of NEB derive energy from glycerol by mobilizing body fat reserves which leads to increase the concentration of NEFA in blood. Resultant NEFA further oxidizes to derive additional energy or esterifies into triglycerides in the liver leading to increase the level of ketone bodies such as BHBA in both blood and other body secretions (Baird, 1982; Rukkwamsuk *et al*., 1999b). Therefore, increased levels of NEFA and BHBA in blood and body secretions indicate breakdown of body fat reserves (lipolysis). Further it indicates the ability of liver to oxidize NEFA and store triacylglycerol (Baird, 1982; Rukkwamsuk *et al*., 1999a).

During the post-partum study period the BHBA level in milk was rapidly increased and decreased with increasing milk production in all cows. The threshold level of milk BHBA for detection of sub clinical ketosis in dairy cows is 200 µmol/L (Geishauser *et al*., 2000). Milk BHBA level indicated that percentage of cows undergoing sub-clinical ketosis was greater during the post-partum period compared to pre-partum period (Table 1). Further, nearly one third (i.e., 31.25%) of the lactating heard is on a state of NEB during their early lactation. The present results agree with the reported finding that cows are at high risk of sub-clinical ketosis within the first 2 months of postpartum period (Duffield *et al*., 1998). Incidence of sub-clinical ketosis in dairy cattle herds ranges from 6.9% to 14.1% during the initial 2 months of the lactation (Dohoo and Martin, 1984; Duffield *et al*., 1997; Nielen *et al*., 1994). Although, the incidence of sub-clinical ketosis found in the present study was little greater than the above report the incidence is not as high as 34% that has been reported elsewhere (Duffield *et al*., 1998; Kauppinen, 1983). The greatest serum NEFA concentration occurred 5 days before parturition (Table 3) and this result concurs with the finding that plasma NEFA concentration is highest at calving (Grummer, 1995). Cows those produced milk containing BHBA concentration greater than 200 µmol/L during the initial 60 days of the lactation has spent significantly longer ($p<0.001$) period to reach the first AI as well as to conceive compared to those produced milk containing lower BHBA concentrations (Table 2). During the initial stage of the postpartum period animals undergo a recovery period which is vital to reestablish the activity of hypothalamic-pituitary-ovarian system and the reproductive track (Wathes *et al*., 2003; Zurek *et al*., 1995). The physiological state of energy deficiency impairs hypothalamic responsiveness to circulating estradiol-17β, resulting reduced GnRH pulse frequency and concomitant reduction in pulsatile secretion of LH required for follicular maturation and ovulation (Dawuda *et al*., 2002; Jolly *et al*., 1995). Therefore, the state of NEB of the early lactating cows which evident by producing milk containing BHBA greater than 200 µmol/L had caused to delay the postpartum ovarian development and initiation of ovarian cyclicity. Non-pregnant cows showed significantly ($p<0.05$) greater serum NEFA levels on 5 days pre-partum and on 10 days post-partum periods (Table 3). The result agrees with previous studies which reveal detrimental effects of elevated pre and post partum NEFA levels on conception (Ospina *et al*., 2010). In-vitro maturation of follicles in media conditions analogous to those follicular fluid concentrations of glucose and BHBA as experienced during sub-clinical ketosis significantly reduced the ability of fertilized ova to become morula and hatched blastocysts (Leroy *et al*., 2005, 2006). They have suggested a direct toxic effect of BHBA and NEFA on maturation of ova. State of NEB causes to reduce estrogen receptors in the brain thus less responsiveness of the central nervous system to estradiol inhibits estrous behavior (Hileman *et al*., 1999). Further, state of NEB lower the fertility of growing follicles leading to lower the conception rate

(Diskin *et al*., 2003). As investigated in a previous study (Dawuda *et al*., 2002) longer open days have been resulted in cows experienced NEB in the present study. Out of the 18.75% cows found in the state of sub-clinical ketosis at peak lactation, 50% cows were found to produce milk containing greater than 200 μmol/L of BHBA at tenth day of postpartum period. These cows took longer time to reach the first AI and had longer open days. Additionally, none of the cows showed signs of sub-clinical ketosis (i.e. milk BHBA level ≥200 μmol/L) were pregnant at 100 days post-partum (Table 1). Therefore, it is clear that the magnitude and duration of NEB during pre-partum and post-partum periods are associated with the poor reproductive performance in dairy cows in Ambewela farm, Sri Lanka. These findings support the earlier findings on the relationship between energy balance and number of days spent to reach postpartum ovulation in dairy cows (Butler, 2000; Butler *et al*., 1981). Pregnant cows recorded significantly ($p<0.05$) greater milk production only at 10 days in milk compared to their non-pregnant counterparts (Table 4). Lower milk production in non-pregnant animals may have been attributed by their poor energy status, indicated by higher NEFA and BHBA concentrations during this period. This result confirms the findings that showed negative effects of energy deficiency at pre-partum and early lactation periods in high producing dairy cows on milk production (Dohoo and Martin, 1984; Duffield *et al*., 1998). Other than utilizing body reserves which caused to increase serum NEFA and milk BHBA levels cows increase feed intake in order to face the problem of energy deficiency during the post-partum period. It may be a reason for poor correlation ($r = 0.3262$; $p>0.01$) between serum NEFA level and milk yield after 10 days post-partum. Our findings further confirmed those of Reist *et al*. (2003)and Walsh *et al*. (2007) that NEB as measured by NEFA or BHBA has stronger association with reproductive performance than milk production. The study also reveals that concentration of serum NEFA and milk BHBA positively correlate with the subsequent reproductive performances of high producing dairy cows. Therefore, both serum NEFA and milk BHBA tests can effectively be used to evaluate energy status and predict reproductive performances of high producing dairy cattle.

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ABBREVIATIONS

NEFA, non esterified fatty acid; BHBA, beta hydroxyl butyric acid

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