



## EFFECT OF DIFFERENT DRY PERIOD DURATION ON MILK COMPONENTS AND SERUM METABOLITES, AND THEIR ASSOCIATIONS WITH THE FIRST CONCEPTION RATE IN MULTIPAROUS HOLSTEIN DAIRY COWS

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### Summary

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The aims of the present study were to evaluate the effects of different dry period (DP) lengths on milk fat to protein ratio (FPR) and metabolic status – blood leptin, adiponectin and non-esterified fatty acids (NEFA) concentrations in dairy cows, and their associations with the result of the first timed artificial insemination (TAI). Cows were blocked either to short DP (SDP; 30±2 days; n=72) or conventional DP (CDP; 60±2 days; n=76). Milk FPR was calculated at 30 and 60 days in milk (DIM). Body condition score (BCS) was recorded at –60, –30, calving, and 60 DIM. Blood samples were obtained at –60, –30, –7, calving, +7, +30, and +60 DIM for serum metabolites measurement. TAI was implemented between 65–75 DIM for all cows. Milk FPR and its changes were statistically analysed using an independent sample *t* test. To assess the impact of time, the pattern of BCS, and serum metabolites on the result of the first AI, repeated measure ANOVA was used. Only FPR-30 DIM revealed significant difference between pregnant and non-pregnant cows in SDP group ( $P<0.01$ ). Reduced BCS loss was observed in the SDP group and followed by slightly higher probability of pregnancy at first AI ( $P=0.19$ ). Leptin was not altered by shortening the DP ( $P\geq 0.1$ ). Significant differences were observed in blood adiponectin prepartum ( $P<0.001$ ) and at +7 DIM ( $P<0.01$ ), as well as in NEFA at +7 and +30 DIM between the two groups ( $P<0.05$ ). Pregnant cows following the first AI had significantly high postpartum leptin concentrations ( $P<0.05$ ), high prepartum adiponectin ( $P\leq 0.001$ ), and lower NEFA at +7 DIM ( $P<0.01$ ) in the SDP group. In conclusion, shortening the dry period caused reduced BCS loss postpartum and variations in serum metabolites that favoured the possibility of pregnancy at first AI.

**Key words:** cow, dry period, milk, metabolite

## INTRODUCTION

The time between pregnancy without lactation and lactation without pregnancy is accompanied by massive increase in energy requisition to maintain postpartum milk production which is not entirely matched with a concurrent increment in dry matter intake (DMI) (Lucy, 2001). This leads to negative energy balance (NEB) characterised by altered ovarian function (Waldmann *et al.*, 2006), decreased estrus expression, increased days open and increased calving to first ovulation interval (Butler, 2003).

In NEB arising from postpartum lipolysis, the percentages of milk fat increase and milk protein decrease (De Marchi *et al.*, 2014), so increased fat to protein ratio (FPR) is an expected outcome. A correlation between energy levels and milk components by measuring different criteria such as FPR, protein to fat ratio, milk protein concentrations and milk yield has been shown in some studies (Reist *et al.*, 2002). After calving, higher milk fat concentration is associated with increased fat mobilisation due to lipolysis and lower milk protein concentration is related to a decrease in DMI. FPR is frequently used as a diagnostic tool to estimate nutritional imbalance, NEB, metabolic disorders, and abomasal displacement (Eicher, 2004). Therefore, alteration in milk FPR could be an indicator of cows' adapting potential to meet demands of milk production and reproductive performance during postpartum period (Loeffler *et al.*, 1999).

Since NEB is accompanied by less DMI and accordingly body condition score (BCS) loss postpartum, serum non-esterified fatty acids (NEFA) concentrations have been proposed as an excellent index for NEB (Ospina *et al.*, 2010). Periparturient period is accomplished with high energy demand, elevation of adipose

lysis and high concentrations of NEFA which cause reduction in P/AI at first AI (Garverick *et al.*, 2013) and also conception risk (CR) till 150 days in milk (DIM) (Westwood *et al.*, 2002). This is mediated by a delay in resuming ovarian activity postpartum (Butler & Smith, 1989). Furthermore, the extent of postpartum BCS loss can increase anovulatory condition in dairy cows at the end of the voluntary waiting period (VWP) (Santos *et al.*, 2004). Therefore, reproductive efficiency may be adversely influenced by postpartum NEB through increasing anovular cows in the herd. It should be considered that severe BCS loss from calving to the first AI is related to poor reproductive performance (Santos *et al.*, 2009).

Leptin, primarily synthesised in adipocytes, is adversely associated with serum NEFA concentrations and curbs feed intake (Block *et al.*, 2001). Moreover, increasing evidence show that leptin concentration positively affects reproductive efficiency and recently, it has become an interesting research focus on investigating an endocrine link between reproduction and nutrition in dairy cows. Despite a vast majority of research carried out the biology of leptin in different species (Spicer, 2001), much remains to be elucidated regarding leptin concentration and its impressibility by particularly DP fluctuations in dairy cows before and after parturition, and how reproductive performance could be affected by different leptin levels in cows after peak milk yield.

Besides producing leptin, adipose tissue secretes another factor influencing fertility of dairy cows named adiponectin whose quantity in blood plasma is high compared with other hormones (Maillard *et al.*, 2010). Serum adiponectin concentration increases postpartum, but during

distinct lactational stages remains constant (Ohtani *et al.*, 2012). Also, this metabolite's effects are exerted by NEB (Mailard *et al.*, 2010). Recently, it was indicated that serum adiponectin concentration reached maximum level during late pregnancy in dairy cows, then it decreased around parturition and at last, over the next two months after calving, its concentration reached the levels of late pregnancy (Giesy *et al.*, 2012).

The implementation of traditional DP of ~60 days in optimising fertility efficacy is controversial (Grummer & Rastani, 2004), since some studies illustrated that shorter DP and persistent milk production are accompanied with better energy balance (EB), metabolic profile, animal health, and efficient reproductive performance in ongoing lactation period (Rastani *et al.*, 2005). In spite of large sample sizes in some recent studies, distinct DP spans were not randomly included in them (Grummer & Rastani, 2004).

Information regarding the effect of short DP on postpartum milk FPR and blood metabolites such as serum leptin, adiponectin and NEFA concentrations at pre- and postpartum periods is scarce, and their effects on pregnancies per AI at first AI, in most cases, are disputable. Moreover, the majority of studies have focused on measuring serum metabolites during the postpartum period, not prepartum. Hence, more research may be warranted to understand the potential influence of these associations. Therefore, the aims of the present study were to assess the effect of 60-day and 30-day DP on milk FPR, BCS and metabolic status, including leptin, adiponectin and NEFA concentrations before and after parturition in Holstein multiparous dairy cows, and then to investigate their associations with the re-

sult of the first TAI.

## MATERIALS AND METHODS

The Committee for Research and Animal Experiments of Islamic Azad University approved the experimental protocol. Milk components and serum metabolites were measured in central diagnostic laboratory of Large Animal Hospital, Faculty Of Veterinary Medicine, Islamic Azad University, Tehran, Iran.

### *Animals*

The present study was conducted on 148 multiparous Holstein dairy cows of a commercial dairy herd in Tehran, Iran with total population of 15,200 cows and 6,340 lactating cows. All cows were kept in free-stall barns with washed sand for bedding throughout the year without access to pasture. All cows were milked three times daily and had ad libitum access to feed and water. All cows were dried-off abruptly and then all quarters received a commercially available intramammary antimicrobial labelled for use in dry cows according to herd standard protocol. Only healthy cows free of any clinical internal disease (e.g. pneumonia, diarrhoea, lameness), reproductive disorders e.g. dystocia, retained foetal membrane (RFM), metritis, clinical endometritis, and metabolic diseases (e.g. hypocalcaemia, ketosis) during the interval two months before and after calving were selected for the present study.

### *Study design*

Briefly, multiparous Holstein cows (parity: 2–5) were assigned randomly in 2 different DP groups: CDP; 60±2 days; n=76 and SDP; 30±2 days; n=72. All of them were followed till recording the first P/AI outcome. In both groups, milk fat

and protein percentages were measured at 30±2 and 60±2 DIM, then their ratios were calculated as FPR-30 DIM and FPR-60 DIM, respectively. Serum leptin and adiponectin concentrations were measured at -60 (in CDP group), -30 (in SDP group), -7, 0, +7, +30 and +60 DIM (0 = calving). NEFA concentration was determined only at -30, -7, 0, +7 and +30 DIM. BCS was also recorded at the time of dry-off in both groups, calving day and +60 DIM. Pregnant or non-pregnant groups were divided after pregnancy diagnosis in individuals inseminated between days 65- 5 DIM (the first P/AI).

#### *Milk sampling*

All samples were obtained by trained personnel. The following steps were performed on milk sample collection: 1) washing all teats with clean water in each cow, 2) drying teats with towel, 3) teat scrubbing with cotton swab from front to rear, 4) discarding the first three squirts, 5) collecting milk sample from rear to front into a sterile capped 30-mL tube, 6) recording the number of each cow on it, 7) transfer to refrigerator, and 8) analysis within 24 h of collection. This process was repeated for each cow on days 30±2 and 60±2 DIM. Gerber and Kjeldahl methods were used for measurement of milk fat and protein, respectively, by using the Eco Milk analyzer system (EON Trading LLC, USA Company). FPR-30 and FPR-60 DIM were calculated in both CDP and SDP groups.

#### *Blood sampling*

A subset of 25 samples were collected randomly in each group. All blood samples were collected from the jugular vein in the morning immediately before the first feeding at mentioned dates, except for calving day in which blood samples

were collected immediately after calving. Collected blood samples were incubated in 37 °C to allow clotting and then centrifuged at 1500×g for 20 min at 4 °C within 40 min after sampling. Serum samples were then collected and stored at -30 °C until further analysis.

Commercial ELISA kit (GmbH, Pharmaceuticals, Germany) was used for leptin measurement. Intra-assay and inter-assay coefficient of variability (CV) were 5.4% and 6.8%, respectively. Adiponectin was measured by commercial ELISA kit (Bovine Adiponectin, CUSABIO Biotech Co. Ltd., China). The inter-assay and intra-assay CV were 3.4% and 4.7%, respectively. The sensitivity of the test was 1.562 µg/mL.

Serum NEFA concentrations were determined by using an enzymatic colorimetric method (NEFA C, Wako Chemicals GmbH, Neuss, Germany).

#### *Determination of BCS*

Evaluation of BCS was done by the same expert technician at initiation of dry-off (days 60 and 30 prepartum in CDP and SDP groups, respectively), calving day and 60 DIM on a 5-scale system (from 1=very thin to 5=extremely fat) (Edmonson *et al.*, 1989). Spinal column, ribs, spinous processes, tuber sacrale, tuber ischia, anterior coccygeal vertebra and thigh regions were considered in determination of BCS.

#### *Reproductive performance*

Reproductive activity was year-round and VWP was considered approximately 60 days postpartum. The fertility programme was Presynch-Ovsynch (PGF-14d-PGF-10d-GnRH-7d-PGF-1d-PGF-1d-GnRH-16h-TAI) for all multiparous dairy cows and all of them were subjected to TAI. In fact, there was no insemination by heat

detection. The hormones injected for all cows were Cystoreline (GnRH di-acetate, CEVA SANTE ANIMALE, Libourne, France) and Enzaprost (Natural PGF, Dinoprost, CEVA SANTE ANIMALE, Libourne, France). All hormonal injections were between 08:00–10:00 AM. TAI was between 65–75 DIM in all cows with conventional, frozen-thawed sperms. A real-time B-mode ultrasound scanner (Mini-Scan; BCF Technology Ltd., Livingston, UK) equipped with a 5 MHz linear-array transducer was used for pregnancy diagnosis at day 35±3 post-AI.

#### Nutrition

All dairy cows studied in the present study were fed a total mixed ration (TMR) prepared in accordance with National Research Council (NRC, 2001). The ration included primarily alfalfa hay, corn, beet pulp, corn silage, soybean, cotton seed and barley. Cows in the CDP group were fed a dry-cow ration from 60 days until 21 days before calving, then they shifted to a parturition ration, whereas cows in the SDP group received the parturition diet for the whole duration of their DP. All cows were fed the early lactation period

ration after parturition.

#### Statistical analysis

SPSS software (v. 24; Armonk, NY: IBM Corp (2016), USA) was used for analysing data. The normal distribution of milk FPR, BCS and serum metabolites were assessed by Kolmogorov-Smirnov or Shapiro-Wilk tests. Data for the CDP and SDP were analysed separately. Milk FPR at both CDP and SDP groups and its changes in pregnant and non-pregnant dairy cows were statistically analysed by an independent sample *t* test. To assess the impacts of time and the pattern of BCS and serum metabolites in CDP and SDP groups on the result of the first AI, repeated measure ANOVA was applied. The result of AI in each DP was analysed by Chi-square test. Significance was defined as  $P \leq 0.05$ . Mean ± standard deviation (SD) values were used for expression of the results.

## RESULTS

Mean milk FPR-30 DIM and FPR-60 DIM in CDP group were 1.13±0.2 and

**Table 1.** Mean postpartum milk fat to protein ratio (FPR) and percent of pregnant/non-pregnant dairy cows at two different dry period (DP) lengths. Data are presented as mean ± SD

Result of the 1 <sup>st</sup> AI <sup>1</sup>	Number (%) of cows	FPR-30 DIM <sup>2</sup>	P	FPR-60 DIM <sup>3</sup>	P
<i>Conventional dry-off period (60 days)</i>					
Pregnant	32 (42.1%)	1.17 ± 0.21	0.17	1.09 ± 0.16	0.56
Non-pregnant	44 (57.9%)	1.11 ± 0.21		1.12 ± 0.23	
<i>Short dry-off period (30 days)</i>					
Pregnant	39 (54.2%)	1.30 ± 0.20	0.005	1.02 ± 0.22	0.44
Non-pregnant	33 (45.8%)	0.99 ± 0.14		1.06 ± 0.19	

<sup>1</sup>AI: artificial insemination, <sup>2</sup>FPR-30 DIM: fat to protein ratio at day 30 postpartum; <sup>3</sup>FPR-60 DIM: fat to protein ratio at day 60 postpartum; independent sample *t* test was used to compare milk FPR means.

**Table 2.** Mean BCS and serum concentrations of leptin, adiponectin and NEFA in late pregnancy and early lactation of multiparous dairy cows. Data are presented as mean  $\pm$  SD, n=25

Days relative to calving	Conventional dry-off period (60 days)		Short dry-off period (30 days)	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
<i>Body condition score</i>				
-60	3.73 $\pm$ 0.29	3.64 $\pm$ 0.44	–	–
-30	3.50 $\pm$ 0.29	3.55 $\pm$ 0.39	3.52 $\pm$ 0.23	3.49 $\pm$ 0.70
0	3.36 $\pm$ 0.34	3.50 $\pm$ 0.37	3.43 $\pm$ 0.36	3.69 $\pm$ 0.39
+60	2.99 $\pm$ 0.37	3.11 $\pm$ 0.38	3.27 $\pm$ 0.30	3.37 $\pm$ 0.33
<i>Leptin (ng/mL)</i>				
-60	3.11 $\pm$ 0.42	2.79 $\pm$ 0.44	–	–
-30	2.93 $\pm$ 0.47	2.46 $\pm$ 0.39	3.82 $\pm$ 0.57	3.13 $\pm$ 0.40
-7	2.80 $\pm$ 0.43	1.98 $\pm$ 0.37	3.29 $\pm$ 0.57	2.73 $\pm$ 0.35
0	2.50 $\pm$ 0.27	1.56 $\pm$ 0.25	2.94 $\pm$ 0.52	2.37 $\pm$ 0.35
+7	1.96 $\pm$ 0.20	1.34 $\pm$ 0.23	2.41 $\pm$ 0.44	1.87 $\pm$ 0.36
+30	1.86 $\pm$ 0.23	1.36 $\pm$ 0.23	2.46 $\pm$ 0.43	1.96 $\pm$ 0.42
+60	1.86 $\pm$ 0.24	1.46 $\pm$ 0.23	2.55 $\pm$ 0.41	1.98 $\pm$ 0.38
<i>Adiponectin (<math>\mu</math>g/mL)</i>				
-60	3.01 $\pm$ 0.15	2.84 $\pm$ 0.28	–	–
-30	2.95 $\pm$ 0.12	2.74 $\pm$ 0.21	3.83 $\pm$ 0.63	2.78 $\pm$ 0.23
-7	2.56 $\pm$ 0.13	2.37 $\pm$ 0.22	3.46 $\pm$ 0.56	2.52 $\pm$ 0.21
0	3.18 $\pm$ 0.16	2.0 $\pm$ 0.14	3.30 $\pm$ 0.21	2.13 $\pm$ 0.20
+7	3.79 $\pm$ 0.16	2.36 $\pm$ 0.08	3.99 $\pm$ 0.34	2.67 $\pm$ 0.30
+30	4.10 $\pm$ 0.51	2.76 $\pm$ 0.17	3.72 $\pm$ 0.41	3.05 $\pm$ 0.28
+60	4.07 $\pm$ 0.43	2.77 $\pm$ 0.22	4.06 $\pm$ 0.40	3.12 $\pm$ 0.28
<i>Non-esterified fatty acids (mmol/L)</i>				
-30	0.12 $\pm$ 0.04	0.13 $\pm$ 0.04	0.14 $\pm$ 0.1	0.09 $\pm$ 0.03
-7	0.19 $\pm$ 0.02	0.40 $\pm$ 0.07	0.16 $\pm$ 0.02	0.37 $\pm$ 0.06
0	0.54 $\pm$ 0.05	0.61 $\pm$ 0.05	0.43 $\pm$ 0.03	0.53 $\pm$ 0.03
+7	0.65 $\pm$ 0.05	0.72 $\pm$ 0.05	0.53 $\pm$ 0.03	0.66 $\pm$ 0.04
+30	0.55 $\pm$ 0.05	0.61 $\pm$ 0.05	0.51 $\pm$ 0.05	0.51 $\pm$ 0.02

<sup>a,b</sup>: numbers with different superscripts in the same rows differ significantly (P<0.05).

1.11 $\pm$ 0.2, respectively and 1.16 $\pm$ 0.2 and 1.04 $\pm$ 0.2 in SDP group, respectively. Mean milk FPR-30 DIM and FPR-60 DIM in both pregnant and non-pregnant dairy cows following the first AI postpartum at two different DP are illustrated in Table 1. Only FPR-30 DIM revealed significant difference between pregnant and non-pregnant cows in the SDP group (P<0.01).

After drying the cows off, the BCS decreased gradually to reach nadir level postpartum at 60 DIM. The trend of BCS fluctuations was significant only at postpartum period between CDP and SDP groups (P<0.001). However, the level of BCS loss between calving to 60 DIM was more prominent at CDP compared with SDP group (P<0.001). The percentages of pregnant cows in SDP group (54.2%)

were slightly higher than the CDP group (42.1%) (P=0.19).

Serum leptin concentration decreased after cessation of milking at both groups until the day of calving. After parturition, the decline continued until day 7 postpartum at SDP and day 30 postpartum at CDP group, then started to increase gradually at both groups. Within SDP group, the value of serum leptin concentration was significantly different throughout the experiment (P<0.01). On the other hand, this value demonstrated significant difference only at prepartum period at CDP group (P<0.01). However, t test showed no significant difference between CDP and SDP groups at none of the sampling points. Pregnant cows following the first AI had greater leptin concentrations throughout the study in CDP and SDP groups. The leptin values revealed significant variations at 7 (P=0.01), 30 and 60 DIM (P<0.05) in SDP compared with CDP group. However, non-pregnant cows in SDP group showed only significant difference at 30 DIM compared with counterparts in the CDP group (P < 0.05) (Table 2).

Serum adiponectin concentration at CDP group reached a nadir level 7 days before calving, and after that remained steady until parturition and then increased dramatically until day 30 postpartum.

However, adiponectin concentration at SDP group reached the lowest level at parturition and then increased. Fluctuations of adiponectin concentration throughout the study were not significant. Significant differences were observed between CDP and SDP groups prepartum (P<0.001) and on day 7 postpartum (P<0.01). Pregnant cows following the first AI had greater adiponectin concentrations throughout the study in CDP and SDP groups, but statistically significant difference was only observed prepartum in the SDP group (P≤0.001) whereas non-pregnant cows had only high adiponectin concentrations at day 7 after calving (P<0.01; Table 2).

Plasma NEFA concentrations increased after dry-off and peaked at day 7 postpartum and then decreased gradually in both CDP and SDP groups. Within CDP group, this serum metabolite was significantly different except for calving day and +30 DIM (P<0.01). On the other hand, this value illustrated significant difference at calving and all postpartum samplings in SDP group (P<0.01). In addition, the t test showed significant difference only at 7 and 30 DIM between CDP and SDP groups (P<0.05). Pregnant cows following the first AI demonstrated lower NEFA concentrations throughout the experiment in both CDP and SDP groups

**Table 3.** Distribution of pregnant and non-pregnant dairy cows following the first AI in different dry periods using Chi-Square test (P=0.19)

	Pregnant	Non-pregnant	Total
Dry-off period 60 days	32 (42.1%)	44 (57.9%)	76 (100%)
Dry-off period 30 days	39 (54.2%)	33 (45.8%)	72 (100%)
Total	71 (48%)	77 (52%)	148 (100%)

(Table 2), but statistically significant difference was observed only on day 7 postpartum of SDP group ( $P < 0.05$ ). Nevertheless, the lowest NEFA concentration was recorded at day -30 relative to parturition and 30 DIM in SDP group which were significantly different from CDP group at same time intervals ( $P < 0.05$ ).

Although the percent of pregnant cows in SDP group was slightly higher, the length of drying period showed no statistically significant effect on the outcome of AI (Table 3).

## DISCUSSION

Initiation of a challenging period after calving, in which cows experience NEB, is associated with economic loss if managed improperly in dairy herds. Thus, appropriate management of prepartum period can affect the health, productivity and reproduction indices of dairy cows in the coming lactation period.

Body reservoir mobilisation is positively related to higher milk fat, lower milk protein, and then increased interval to first ovulation and lower P/AI at first AI (Butler & Smith, 1989). Accordingly, precise analysis of first AI outcome could be possibly applicable by utilisation of milk FPR rather than milk components individually (Podpecan *et al.*, 2010). Despite lack of effect on milk fat, decrease in DP leads to a slightly increase in milk protein concentrations at early lactation (van Knegsel *et al.*, 2013).

The present study revealed significant difference in FPR-30 DIM between pregnant and non-pregnant dairy cows at short DP which is comparable with the study of Loeffler *et al.* (1999) who also showed remarkable correlations between FPR changes and fertility. They took into account FPR index as a reliable predictor of

CR at first AI. Milk fat percentage, not protein, can significantly alter FPR (Podpecan *et al.*, 2010). They considered 1.1 as a valuable cut-off for FPR to determine more than 90% of cows with calving to conception interval (CCI) below 120 days. This cut-off was also obtained in our experiment. Non-pregnant cows at first AI had FPR less than 1.1 in both 30 and 60 DIM. Buttchereit *et al.* (2010) examined the clinical value of FPR in EB status assessment from 11 to 305 DIM. They found that the greatest energy deficiency was associated with the greatest FPR in early lactation. This result is in parallel with the milk FPR calculated on 30 and 60 DIM in the present study that decreased over time in conventional DP. Toni *et al.* (2011) showed that milk FPR and energy status had a reverse association and the former could be used as a practical tool to predict energy status. However, De Vries & Veerkamp (2000) showed fluctuations in milk fat contents during 60 days of lactation were the most reliable predictor of energy imbalances. Since NEB could not exert a significant effect on milk protein contents and FPR, they reported these two variables may not lead to a standard assessment of energy status.

Despite contradicting scientific findings, milk fat and protein percentages are routinely measured in dairy herds. Thus, by keeping these data in mind and calculation of FPR, veterinarians could serve it as an appropriate indicator for assessing the postpartum EB in dairy cows along with other precise and direct tests.

Moreover, reduced BCS from parturition to 60 DIM in the present study demonstrated that cows given short DP experienced slightly less BCS loss postpartum and probably less NEB than cows with longer DP, presumably because of



greater feed intake. Decrement in BCS loss in short DP was accompanied by slightly higher P/AI at first AI, but it was not statistically significant. Similarly, distinct DP durations could not affect preparturient BCS and subsequent P/AI (Kalem *et al.*, 2017) and the other studies illustrated that decline or even absence of DP did not bring about any alteration in postpartum BCS (Pezeshki *et al.*, 2007). However, Butler & Smith (1989) reported a positive relation between losing BCS units and reduction of P/AI at first AI. Some other parallel studies reported reduction in BCS loss after parturition through shortening DP (Gulay *et al.*, 2003; Rastani *et al.*, 2005). Recently it was reported that P/AI at first AI decreased in tandem with BCS decline (Carvalho *et al.*, 2014) and that it can increase anovulatory status in cows (Santos *et al.*, 2009).

This study clearly showed high serum concentrations of leptin during late pregnancy because of next lactation period characterised by high energy intake, followed by the lowest level at the first week of lactation presumably suggestive of difference in energy status and fat mobilisation with variable severities. Hoggard *et al.* (2001) proposed the placenta as a source of leptin production in which reduced level in preparturition and even early postparturition period was assigned to placental repulsion, but in our study the decline started at first sampling date in both groups before parturition. Although the decline from parturition until +7 DIM in the current study could be justified by the expulsion of the foetal membrane, it was likely due to decline in DMI in late pregnancy as an influencing factor for leptin concentration adjustment. Underlying mechanisms involved in leptin regulation before parturition remains to be clari-

fied, and feed intake can be easily affected by abrupt reduction in leptin levels at this time. As it was demonstrated in the present study, despite recovering energy status along with progressive lactation, the serum concentrations of leptin remained low in that other factors may become more effective (Block *et al.*, 2001). Such factors in dairy cows could be: 1) food intake or fat supplies alterations; 2) hormonal effects on adipose tissue activity during different stages from pregnancy to lactation; and 3) leptin amounts produced by placenta. In the present study, short DP was accompanied by slightly high mean serum leptin concentration before and after parturition. During NEB, adipocytes may secrete lower levels of leptin similar to fasting or underfeeding condition (Henry *et al.*, 2001). As shown by van Knegsel *et al.* (2013) NEB intensity could be decreased by shortening DP, so the amount of leptin seems to be incremented. Extended decline in leptin levels after calving is accompanied by a long interval to the first ovulation in cows (Kadokawa *et al.*, 2000). Hence, it is postulated that reproduction efficiency and leptin production are regulated by common physiological events, as the present study showed pregnant cows following the first AI to have higher serum leptin levels compared with non-pregnant cows. In ruminants, less information is available, a link between leptin concentration and establishing a successful pregnancy may still explain hormonal changes performed by NEB during the postpartum period. On the other hand, Kokkonen *et al.* (2005) showed that body fatness in early lactation, rather than energy status, affected serum leptin concentrations. Chelikani *et al.* (2004) proposed another issue referring to physiological regulation of lactation and reported that serum leptin con-

centration was significantly reduced by fasting in lactating cows compared with dry cows. Overall, the decreased secretion of leptin postpartum is a likely outcome of combined impacts of alterations in EB, BCS, and physiological changes needed to prepare for lactation.

Another metabolite produced by adipocytes and involved in different metabolisms of fatty acids and glucose, is adiponectin (Yamauchi *et al.*, 2001). NEB may alter serum adiponectin concentration during late pregnancy and early lactation. In the present study, serum adiponectin concentrations changed contrastingly during the whole study period in high-producing dairy cows. All cows experienced a decline in adiponectin concentration after dry-off until parturition, then in the calving time this metabolite reached the lowest level and at last underwent an increase after parturition. In parallel with these findings, Mielenz *et al.* (2013) reported decrease in adiponectin levels from day 21 precalving followed by extreme decline on day 1, and thereafter experienced the peak levels on day 14 after calving. Moreover, adiponectin concentrations were shown to increase after the 3<sup>rd</sup> week postpartum in healthy cows (Kafi *et al.*, 2015). Greater concentrations of prolactin, growth hormone (GH), and glucocorticoids may result in reduction in adiponectin concentration around parturition (Brochu-Gaudreau *et al.*, 2010). At the end of the DP, adiponectin concentration decreased which in turn reduced fatty acid oxidation, leading to recruitment of fatty acids to other metabolic pathways (like triglyceride formation) and lipolysis cessation (Brochu-Gaudreau *et al.*, 2010). Thus, lower serum levels of adiponectin prepartum may impact on surrounding tissues by augmenting lipolysis, increasing gluconeogenesis and declining glucose

uptakes (Singh *et al.*, 2014). In consideration of these effects, decreased adiponectin concentrations provide mammary glands with nutrients essential to enhance milk production postpartum (Singh *et al.*, 2014). Furthermore, it was showed that AdipoR1 mRNA levels increased at peak lactation in bovine mammary tissues (Ohtani *et al.*, 2011), as it was showed in the current study, high levels of serum adiponectin corresponded with high milk production period. These reports suggest the NEB during lactation decreased adiponectin mRNA levels in adipose tissues and serum adiponectin concentrations. As previously discussed, the severity of NEB was decreased in short DP (van Knegsel *et al.*, 2013), as we demonstrated an increase in serum concentrations of adiponectin at precalving period and +30 days postcalving in short vs. conventional DP. There is more evidence that adiponectin had dramatic effects on hypothalamic-pituitary-gonadal axis and even a direct impact on embryo (Richards *et al.*, 2012; Syriou *et al.*, 2018). Favourably, our findings elucidated that non-pregnant cows following the first AI had less adiponectin concentration in comparison with pregnant cows.

As earlier noted, during the pericalving period characterised by different intensities of energy imbalances, feed intake decreased which in turn led to depletion of liver glycogen storage; therefore, NEFA and ketone concentrations will be incremented (Saco *et al.*, 2008). In the current study, last week of pregnancy and the day of calving were associated with increased NEFA concentrations followed by maximum level at day +7 postpartum. Then, all cows until +30 DIM experienced reduced NEFA concentrations. Transition time which is referred to 3 weeks pre- and postcalving highly depends on lipid me-

tabolism and is accompanied by increasing NEFA concentrations around parturition that is used as an indicator of NEB in dairy cows (Beever, 2006). Rastani *et al.*, (2005) showed that shortening the DP, rather than conventional one, could improve energy condition postcalving and decrease postpartum NEFA levels that was seen similarly in the present study. It would also be worthy to consider that the lower appetite, increased fat depletion and subsequent increased plasma NEFA could be explained by inflammation occurring around calving and just after that. The intensity of reduced DMI periparturition could be a superior indicator of metabolic health of postpartum cows rather than actual level of feed intake (Bicalho *et al.*, 2017). Hammon *et al.* (2006) suggested that increased plasma NEFA levels preparturition can be attributed to uterine disease and neutrophil dysfunction. We also demonstrated that non-pregnant cows have high NEFA concentrations before calving. Our data further confirmed that pregnant cows following the first AI had low levels of NEFA throughout the study compared with non-pregnant cows. More NEB within postpartum period followed by increased NEFA concentrations have been shown to accompany decreased P/AI at first AI (Ospina *et al.*, 2010). The effect high NEFA concentrations may have on the oviduct and uterus should be taken into account, because *in vitro* experiments illustrated that fertilisation, cleavage, and embryonic growth after maturation were diminished in high concentrations of NEFA (Jorritsma *et al.*, 2004).

## CONCLUSION

Our data demonstrated that short DP has: 1) significant impact only on milk FPR-30 DIM, 2) reduced BCS loss in early lacta-

tion, 3) increased leptin concentrations numerically pre- and postcalving, 4) increased adiponectin concentrations precalving and only at day 30 postcalving, and 5) decreased postpartum NEFA concentrations. Moreover, the observations arising from the present study provide evidence that pregnant cows following the first TAI have relatively high leptin concentrations, high adiponectin concentrations and low NEFA concentrations. Collectively, the above findings suggest that the intensity of EB that happens during early postpartum period may have a remarkable impact on the probability of pregnancy at first AI. Postpartum monitoring of milk FPR, as an easy and inexpensive tool, and blood metabolites, as accurate diagnostic tools, could practically be used to recognise cows at risk for low-fertility and enable targeted strategies to improve nutritional status and reproductive outcomes. More research is warranted to elucidate underlying molecular mechanisms and precise associations among different mentioned variables and energy balance which has profound effect on fertility.

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