



AKADÉMIAI KIADÓ

Acta Veterinaria
Hungarica

69 (2021) 4, 354–362

DOI:
[10.1556/004.2021.00049](https://doi.org/10.1556/004.2021.00049)
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RESEARCH ARTICLE



Metabolic and production parameters of dairy cows with different dry period lengths and parities

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Received: 1 April 2021 • Accepted: 18 October 2021

Published online: 15 November 2021

ABSTRACT

To assess the effects of dry period (DP) length on metabolic, reproductive, and productive parameters, second- (SP) and third- (TP) parity cows were assigned to a traditional (9 weeks, T) or short (5 weeks, S) DP, obtaining four subgroups: second-parity cows with traditional (SPT = 8) and short (SPS = 8) DP, third-parity cows with traditional (TPT = 8) and short (TPS = 10) DP. Plasma insulin-like growth factor-I (IGF-I) and non-esterified fatty acid (NEFA) levels were assessed from 5 weeks before to 14 weeks after parturition. IGF-I concentrations were affected by parity ($P < 0.05$) and by the interaction of time and DP length ($P < 0.01$). NEFA levels were affected only by time ($P < 0.01$). S DP cows showed a shorter interval between calving and ovarian cyclicity resumption ($P < 0.01$) and a higher milk yield ($P < 0.01$) and fat and protein corrected milk ($P < 0.01$) compared with T DP cows. Decreased milk protein content was found in the SPS group compared to the SPT ($P < 0.05$) and the TPS ($P < 0.05$) group. In conclusion, a short DP length does not affect reproductive performances, except for hastening the resumption of ovarian cyclicity. A short DP appears to increase milk production and is associated with higher IGF-I levels both in the prepartum and the postpartum period.

KEYWORDS

dairy cow, dry period length, fertility, IGF-I, NEFA, milk yield

INTRODUCTION

The importance of the dry period (DP) in dairy cows has been described by many authors and mainly relies on the positive effects on the mammary epithelial component and the optimisation of milk production during the subsequent lactation (Capuco et al., 1997). The practice of drying off cows has been developed and modified mainly on the basis of the experiences of the farmers, rather than on results of planned experiments, but over decades many studies have been conducted to identify the optimal DP length on a scientific basis.

Shortening or omitting the DP was proposed as a management strategy to improve energy balance (EB) of dairy cows in early lactation (Rastani et al., 2005) and to reduce the incidence of ketosis (Santschi et al., 2011). Cows in negative energy balance (NEB) with a prolonged

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interval to first ovulation have increased circulating levels of non-esterified fatty acids (NEFA) and reduced levels of insulin-like growth factor-I (IGF-I), leading to impaired follicular growth, reduced oestradiol synthesis, and delayed ovulation (Wathes et al., 2011). The main reason for improved EB on shortening the DP is decreasing milk production without affecting postpartum dry matter intake (DMI) (Rastani et al., 2005; van Knegsel et al., 2014; O'Hara et al., 2019). The impact of a short DP on reproduction, theoretically expected to be positive due to NEB reduction, was proven only in a few studies, and consisted of shortened time to first ovulation, reduced numbers of anovulatory cows, and improved fertility (Watters et al., 2009). Other authors could not find an improvement in reproductive functions by shortening the DP (Pezeshki et al., 2008) but registered a decreased incidence of mild ketosis as a positive effect (Santschi et al., 2011). So far, most studies on the effect of DP on colostrum quality have shown no or limited reduction in colostrum volume and IgG content (Rastani et al., 2005; Shoshani et al., 2014). Overall, a complete omission of the DP does not seem to be advisable, both due to the economic implications and to the negative effects on milk production (Mantovani et al., 2010). Nevertheless, shortening the DP may increase the somatic cell count of milk (Annen et al., 2004) and it has variable effects on mastitis incidence (Pezeshki et al., 2008).

Based on literature reviews of the years 2005–2013, Santschi and Lefebvre (2014) reported that (1) multiparous cows assigned to short DP showed nonspecific milk losses, (2) no negative effects on fat content were present, (3) an increase in protein could be seen, and (4) no negative effects on colostrum or on the udder health in the subsequent lactations were observed.

It must be acknowledged that there are differences between the retrospective analysis of data and planned animal trials, clearly shown by a review in which 12 retrospective studies and 9 planned trials were compared (Bachman and Schairer, 2003). In the retrospective studies, the traditional DP appeared to be advantageous, while in the planned trials the shortened DP was found to be more appropriate (Bachman and Schairer, 2003).

Despite the numerous studies and reviews available, more recent research has been conducted with the aim to clarify the interactions between a short or omitted DP and udder health, EB, milk production and plasma metabolites (van Knegsel et al., 2013; van Hoeij et al., 2017), inflammatory biomarkers and oxidative stress (Mayasari et al., 2015), colostrum immunoglobulin content (Mayasari et al., 2015), and to evaluate the economic advantages and disadvantages of a reduced or omitted DP (Kok et al., 2017). Clearly, there is still the need to understand and define many aspects for the optimisation of short DP results. The present study aims at assessing the impact of different DP lengths on productive and reproductive performances and metabolic status in dairy cows; for this purpose, postpartum fertility, lactation performances and serum IGF-I and NEFA levels of second- and third-parity cows with a traditional and with a short DP length were compared, also in view of the well-

known association of these metabolites with postpartum uterine disease, fertility, and milk production (Butler et al., 2003).

MATERIALS AND METHODS

Ethics

The study was carried out in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes and was approved by the ethic committees belonging to the University of Milan (*n.* 34/15).

Study design

The study was performed in a commercial farm located in Northern Italy. Thirty-three Holstein-Friesian cows at second (SP, *n* = 16) and third (TP, *n* = 18) parity were selected for the study. In both groups, cows were randomly assigned to a traditional DP length of 9 weeks (*T* = 16) or to a short DP of 5 weeks (*S* = 18) before the expected date of calving. Therefore, four subgroups of cows were obtained, namely second-parity cows with traditional DP length (SPT = 8), second-parity cows with short DP length (SPS = 8), third-parity cows with traditional DP length (TPT = 8), and third-parity cows with short DP length (TPS = 10). All the cows had a previous lactation characterised by an average milk yield of 8,082 kg/305-day lactation, 3.6% fat and 3.2% protein.

During the study, cows were kept on concrete slatted floor in free-stall barns. One week before the expected day of calving they were moved to a calving box bedded with straw. A common transition diet with high fibre content but slightly below the nutrient requirements (Janovick and Drackley, 2010) was fed to the cows from the beginning of the DP until the third month of lactation. The diet consisted of a total mixed ration containing corn silage (40% as fed), alfalfa hay (14.5% as fed), alfalfa meal (14% as fed) and soybean meal (7.3% as fed) [crude protein = 17.9% dry matter (DM), ether extract = 3.2% DM, neutral detergent fibre = 41.3% DM, non-fibre carbohydrates = 32.1% DM]. The total amount of feed provided was equal in the four groups and consisted of 10 kg as fed/cow/day in the DP and 41 kg as fed/cow/day after calving. Each cow had free access to drinking water.

Sampling and clinical record

Blood samples were collected from the jugular vein once a week from 5 weeks prior to 14 weeks after calving using 10-mL vacuum tubes with 0.1 mL EDTA (Terumo Venoject, VT100STK) and were immediately centrifuged at 1,000 × *g* for 20 min. Separated plasma was stored at –20 °C until IGF-I and NEFA analysis.

Milk samples were collected from each cow twice a week from the 3rd week after parturition until the end of the study (14 weeks post-partum), and the separated whey was stored at –20 °C until progesterone (*P*₄) analysis. Whey *P*₄ profile



was used together with a clinical examination to identify cows with and without ovarian activity resumption (Comin et al., 2005).

Starting from the 3rd week after calving, the cows were examined weekly by a single practitioner by rectal palpation combined with an ultrasonographic evaluation (real-time B-mode linear array with a 7.5-MHz transducer, Esaote Pie Medical, Florence, Italy) of the ovaries and the genital tract. The resumption of ovarian cyclicity was defined as the finding of a corpus luteum by clinical and ultrasonographic examination, accompanied by the detection of a series of three consecutive values of $P_4 \geq 300 \text{ pg mL}^{-1}$ in whey. In addition, cows were clinically examined twice a day to detect oestrus. Cows in oestrus, with a pre-ovulatory follicle and normal genital tract condition (uterine tone, cervical mucus) were submitted to artificial insemination (AI) with semen of proven fertility 12 h after oestrus detection. Pregnancy diagnosis was performed by rectal palpation within 6 weeks after AI.

IGF-I, NEFA and progesterone assays

Plasma IGF-I concentrations were determined by a radioimmunoassay, as described by Renaville et al. (1993). In this method, a cryo-precipitation step was used to eliminate aggregated IGF binding proteins in plasma extracts. The sensitivity of the assay, calculated as the interpolated dose of the response to a concentration of zero minus the statistical error, was 1.8 ng mL^{-1} . Intra- and inter-assay coefficients of variation were 8.5 and 12.7%, respectively.

Enzymatic-colorimetric methods were used to determine plasma concentrations of NEFA (Wako Chemicals, Richmond, VA, USA). Intra- and inter-assay coefficients of variation were 2.7% and 5.5%, respectively. Assay sensitivity was $0.140 \text{ } \mu\text{Eq/L}$.

Progesterone was determined in whey by a direct enzyme immunoassay as described by Comin et al. (2005). The sensitivity of the method was 1.5 pg mL^{-1} , the intra- and

inter-assay coefficients of variation were 8 and 12%, respectively.

Statistical analyses

All the analyses were performed using SAS statistical package (SAS/STAT, Version V9.4, SAS Inst., Inc., NC, USA, 2013). IGF-I and NEFA values were analysed by ANOVA using a mixed procedure for repeated measurements including in the model the random effect of the animal and the fixed effects of DP length, time, parity (2nd vs. 3rd) and relative interactions. *Post hoc* pairwise contrasts were performed using Bonferroni correction.

The interval between calving and ovarian activity resumption, the interval between calving and the first AI, the interval between calving and conception (days open), and production data [milk, fat, protein, fat-corrected milk (FCM), fat- and protein-corrected milk (FPCM)] were processed by a General Linear Model including the fixed effects of DP length, parity, and their interaction. The numbers of AI/pregnancy (service/conception) were analysed using a non-parametric Kruskal–Wallis test to assess the significance among the levels of combined effect of DP length and parity. A probability level of $P < 0.05$ was considered significant.

RESULTS

Plasma IGF-I and NEFA

The length of the DP significantly increased total mean IGF-I level in the S cow group compared to the T group ($42.78 \text{ vs. } 27.56 \text{ ng mL}^{-1}$; $P < 0.0001$). Specifically, the S group showed increased IGF-I levels in the last 5 weeks before calving ($P < 0.01$) and starting from week 5 of lactation until the end of the trial ($P < 0.01$) when compared to T cows (Fig. 1). Decreased DP significantly increased IGF-I levels during the

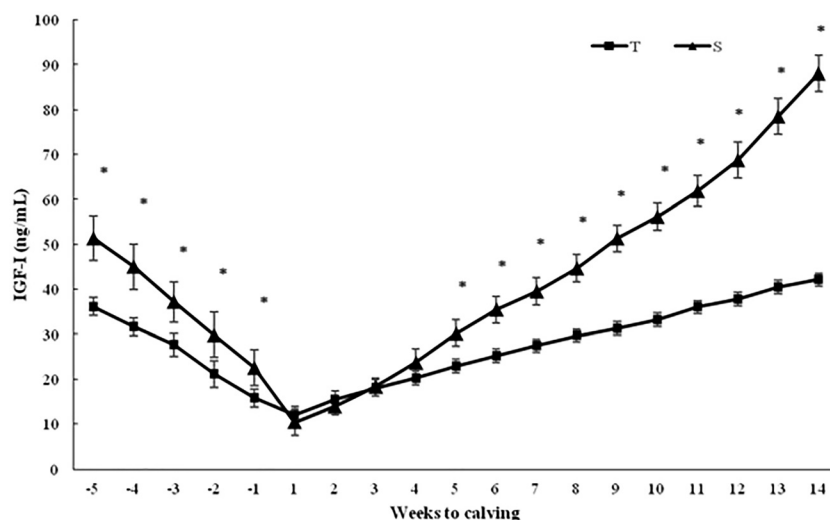


Fig. 1. Mean (\pm SD) plasma IGF-I concentrations in cows with traditional (T) or short (S) DP length (* $P < 0.01$; denotes significant differences when the interaction between DP length and sampling times was considered)



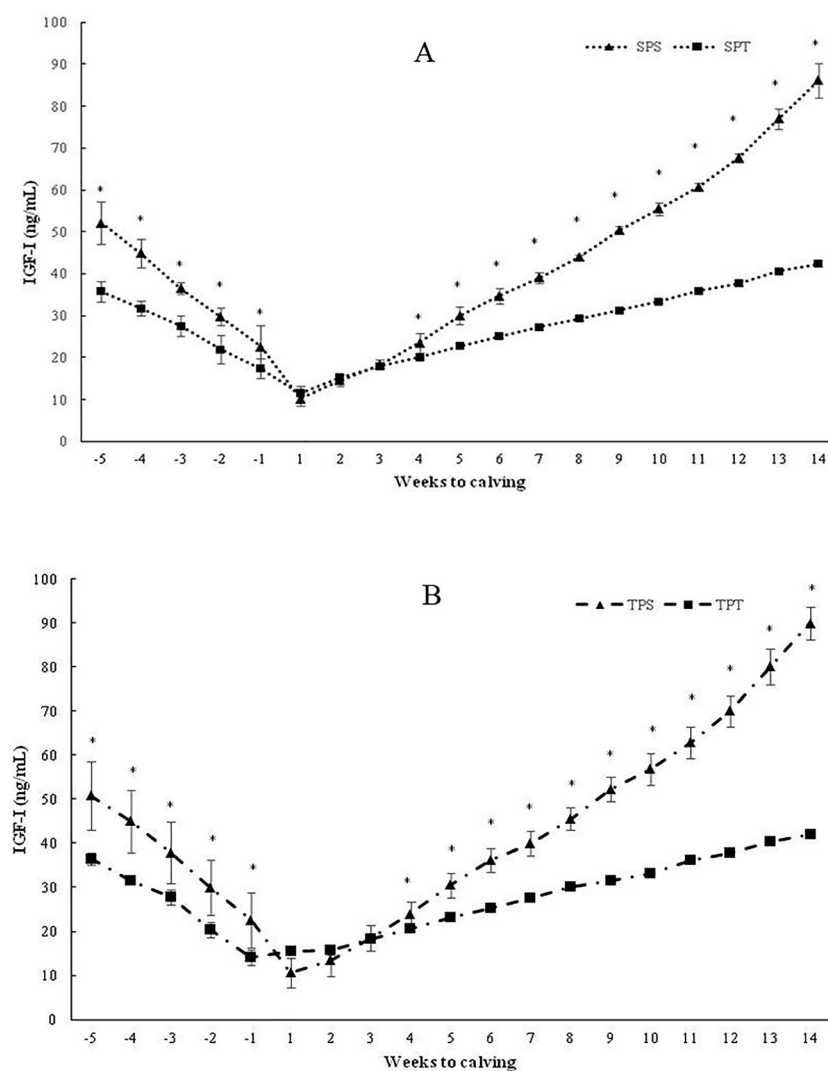


Fig. 2. Comparison between mean (\pm SD) plasma IGF-I concentrations in cows with different DP length within the same parity. Second-parity cows with traditional (SPT) and short (SPS) DP length (A); third-parity cows with traditional (TPT) and short (TPS) DP length (B). * $P < 0.0001$

whole trial in both second- (42.28 vs. 27.54 ng mL⁻¹; $P < 0.0001$) and third-parity cows (43.18 vs. 27.57 ng mL⁻¹; $P < 0.0001$) (Fig. 2A and B). No significant differences were found for traditional DP length based on parity (SPT 27.55 vs. TPT 27.57 ng mL⁻¹, respectively), while a short DP was characterised by an increased mean plasma IGF-I content during the whole trial in the TPS compared to the SPS group (42.27 vs. 43.18 ng mL⁻¹), although no significant differences were found at the different sampling days.

The length of the DP did not influence the plasma level of NEFA considering the whole experimental trial (464.42 vs. 463.70 μ Eq/L for T and S cows, respectively) or for any of the sampling days (Fig. 3). Comparing short or traditional DP length and different parities, no differences were detected for second parity in the SPT vs. SPS groups (469.12 vs. 462.69 μ Eq/L) and for third parity in TPT vs. TPS cows (459.04 vs. 464.50 μ Eq/L). Parity within traditional or shortened DP showed a trend of increased levels of NEFA in

second-parity cows compared to third-parity cows with traditional DP (469.12 vs. 459.04 μ Eq/L).

Reproductive performance

All cows but one SPT and one TPS enrolled in the study showed a normal late pregnancy, a normal spontaneous parturition of a single calf followed by placental expulsion, as well as a postpartum period without clinical abnormalities. Therefore, the statistical analysis was performed on data obtained from 31 cows.

The results of reproductive performance are reported in Table 1. The effect of DP length ($P < 0.01$) and the interaction between DP length and time ($P < 0.01$) were significant, with a mean anticipation of the resumption of ovarian activity of approximately 12 days in cows with a short DP, confirmed by the finding of a corpus luteum and by whey P4 analysis.



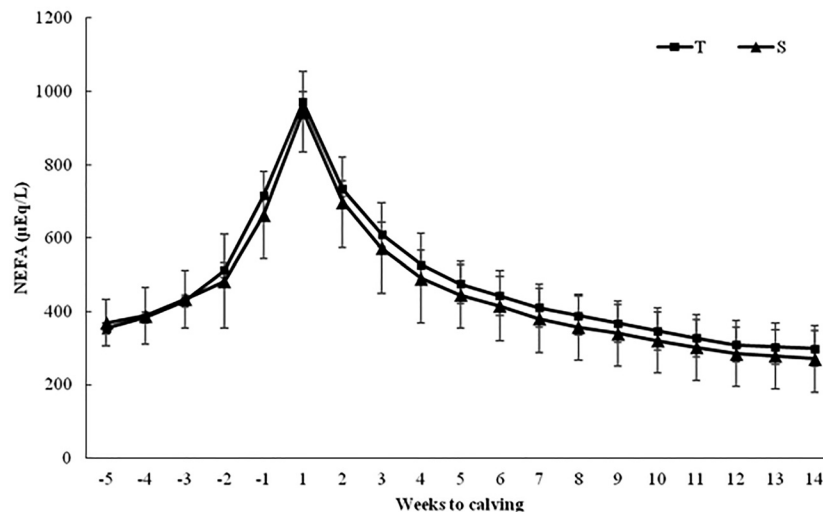


Fig. 3. Mean (\pm SD) plasma NEFA concentrations in cows with traditional (T) or short (S) DP length

No significant effects of DP length, parity or their interaction were found on calving to first AI interval, days open and service per conception in either the T or the S groups.

Lactation performance

The results of lactation performance are reported in Table 2. A shorter DP length significantly increased milk yield ($P < 0.01$) and FPCM ($P < 0.01$) but did not influence fat and protein content or FCM. The only effect of parity observed on milk parameters was a decreased crude protein content recorded in the SPS group compared to SPT ($P < 0.05$) and TPS ($P < 0.05$).

DISCUSSION

Metabolites

IGF-I levels were influenced by the interaction between DP length and time. Within the framework of IGF-I changes across the periparturient period, late pregnancy and early lactation are marked by a decrease in IGF-I concentrations, which was confirmed in the present study. The nadir of IGF-I concentration was reached in the first week after calving, as reported previously (Butler et al., 2003; Radcliff et al., 2003); after the second week, IGF-I concentrations usually increase, but they are markedly influenced by the energy status of the cows (Fenwick et al., 2008; Wathes et al., 2011), and animals with severe NEB show lower IGF-I concentrations (Fenwick et al., 2008). In the present study, cows with a traditional DP showed significantly lower IGF-I levels than S group cows, both before and after calving. O'Hara et al. (2019) found the same differences in the postpartum period, while they found the opposite before calving, with higher IGF-I levels in cows with a longer DP length. According to these results, it appears that a shorter DP certainly improves postpartum EB, while the effect on

prepartum IGF-I remains unclear. In a recent research study (De Koster et al., 2019) metabolic clusters based on blood levels of glucose, IGF-I, NEFA and β -hydroxybutyrate were used to identify dairy cows with a balanced or imbalanced metabolic profile in early lactation; DMI was consistently higher in metabolically balanced cows and lower in imbalanced cows (De Koster et al., 2019). Regardless of individual variability, the results of the present study suggest that a longer DP depresses ante-partum EB and, consequently, also the IGF-I levels, probably due to the long-term exposure to a reduced DMI (Gumen et al., 2011). The differences in IGF-I levels between different DP lengths were registered already at 5 weeks before calving, possibly due to the fact that traditional DP cows were dried off from already 4 weeks. The positive impact of a short DP on IGF-I levels in the present study perpetuates also in the postpartum period; although a similar postpartum DMI between cows with traditional (60-day) and short (30-day) DP has been reported (Rastani et al., 2005), other authors have shown that cows with a shorter DP maintain postpartum BCS better, and tend to have higher DMI immediately postpartum (Gulay et al., 2003). Parity, and the interaction between parity and DP length, had no effect on IGF-I levels in cows with a traditional DP, while higher levels were detected in third-parity cows with a short dry period compared to the respective second-parity group. These results are in contrast with other previous findings (O'Hara et al., 2019), in which younger cows had higher postpartum concentrations of IGF-I than multiparous cows (2nd vs. \geq 3rd parity). In view of the different results obtained in the present study when compared to previous research, confirmation is needed on a larger number of cows.

The plasma NEFA concentrations were not affected by DP length in the present study, contrarily to what was reported by many previous studies, in which reduced concentrations of NEFA were detected in the plasma of cows after a shorter DP (e.g., Pezeshki et al., 2007; O'Hara et al., 2019). However, a finding similar to ours was reported by

Table 1. Ovarian resumption, days to first artificial insemination (AI), days open and service per conception (mean \pm SD) of second- (SP) and third- (TP) parity dairy cows with traditional (T) or short (S) dry period length

	Dry period length		Parity		Dry period length \times Parity			
	T (<i>n</i> = 14)	S (<i>n</i> = 17)	SP (<i>n</i> = 15)	TP (<i>n</i> = 16)	SPT (<i>n</i> = 7)	SPS (<i>n</i> = 8)	TPT (<i>n</i> = 7)	TPS (<i>n</i> = 9)
Resumption of ovarian cyclicity (days)	47.1 \pm 3.23 ^A	34.9 \pm 2.75 ^B	41.9 \pm 6.88	40.0 \pm 6.73	48.8 \pm 1.72 ^A	36.0 \pm 1.55 ^B	45.3 \pm 3.56 ^A	34.0 \pm 3.32 ^B
First AI (days)	71.2 \pm 10.51	72.2 \pm 11.14	68.6 \pm 11.42	71.0 \pm 6.32	71.4 \pm 13.76	70.9 \pm 14.12	71.0 \pm 6.06	73.3 \pm 8.73
Days open (days)	104.6 \pm 20.76	107.1 \pm 24.93	100.3 \pm 17.76	103.6 \pm 15.78	103.0 \pm 23.56	107.1 \pm 29.69	106.3 \pm 19.30	107.0 \pm 21.73
Service/conception (n)	2.6 \pm 0.76	2.6 \pm 0.79	2.3 \pm 0.65	2.4 \pm 0.65	2.6 \pm 0.79	2.6 \pm 0.91	2.6 \pm 0.78	2.6 \pm 0.72

^{A,B} $P < 0.01$.

Table 2. Performances of second- (SP) and third- (TP) parity dairy cows with traditional (T) or short (S) dry period length

		Group								<i>P</i>			
		Dry period length		Parity		Dry period length × parity				SEM	Treatment	Parity	Treatment × Parity
		T	S	SP	TP	SPT	SPS	TPT	TPS				
Milk	(kg d ^{−1})	26.57 ^B	27.55 ^A	27.15	26.97	26.63	27.67	26.50	27.43	0.12	<0.01	0.15	0.68
Crude fat	(%)	3.57	3.48	3.47	3.58	3.52	3.42	3.62	3.52	0.06	0.13	0.08	0.89
Crude protein	(%)	3.32	3.29	3.29	3.32	3.36 ^a	3.22 ^b	3.28	3.35 ^a	0.04	0.35	0.46	0.02
FCM ¹	(kg d ^{−1})	23.51	24.00	23.62	23.90	23.37	23.86	23.65	24.14	1.46	0.07	0.30	1.00
FPCM ²	(kg d ^{−1})	25.24 ^B	25.82 ^A	25.42	25.64	25.21	25.63	25.28	26.00	2.01	<0.01	0.29	0.44

¹ FCM = Fat-corrected (4.0%) milk; ² FPCM = fat- (4.0%) and protein- (3.3%) corrected milk; ^{A,B} $P < 0.01$; ^{a,b} $P < 0.05$.

Rastani et al. (2005), with no differences in postpartum NEFA levels between cows assigned to 56- or 28-day DP; nevertheless, in the latter study, parity was not considered, while Pezeshki et al. (2007) found an effect of DP length on NEFA plasma levels only among primiparous cows. It is therefore reasonable to assume that discrepancies among studies may be due to considering or not the parity effect within different DP lengths. In the present study, parity (2nd vs. 3rd) was considered, and no effect on NEFA concentrations was found, but primiparous cows were not enrolled. Usually, an advantage of a reduced DP length is the reduced NEB; although the relationship between EB and plasma NEFA appears to be rather weak (Wylie et al., 2008), a less severe NEB should be expressed by reduced NEFA levels, but this effect could not be detected in the present study. A suggested NEFA cut-off value for increased risk of metabolic diseases in the postpartum period has been set to 700 $\mu\text{Eq/L}$ in early lactation (Chapinal et al., 2011), which was exceeded for cows enrolled in this study. NEFA levels were persistently higher than 600 $\mu\text{Eq/L}$ from one week before calving until two weeks after parturition, indicating that cows in the present study underwent important metabolic variations. Considering these results, and the fact that NEFA is a sign of marked mobilisation from the adipose tissue (Accorsi et al., 2005), a significantly higher NEB in the dairy cows enrolled in this study can be assumed. Milk yield is a key factor controlling EB (Pezeshki et al., 2007), and cows assigned to a short DP length in the present study showed a significantly higher milk yield compared to the traditional DP length. A higher lipolysis in the adipose tissues was likely to occur in cows with a short DP length, to support the increased milk production, and this may have counterbalanced the reduced NEB in the same cows, resulting in unchanged circulating NEFA levels. The lack of significant differences in NEFA plasma levels between cows with different parities and different DP lengths seems to suggest that the reduction of the DP from 9 to 5 weeks does not have a relevant influence on the balance of energy loss and intake.

Reproductive performance

IGF-I is a useful parameter also in the evaluation of reproductive performance of dairy cows (Konigsson et al., 2008) since it stimulates the proliferation of bovine granulosa cells *in vitro* (Spicer et al., 1993) and it is related to increased follicle diameter and oestradiol concentration *in vivo* (Simpson et al., 1994). Because IGF-I levels negatively correlate with the interval between calving and the resumption of ovarian cyclicity (Butler, 2000), higher IGF-I levels should have a positive impact on the reproductive performance of the S group. The analysis of reproductive data confirms this hypothesis, by showing an average reduction of 12 days in the interval between calving and the resumption of ovarian activity in the S compared to the T group; this difference was observed in both second- and third-parity cows. These results are consistent with those from other authors (Darwash et al., 1997; Watters et al., 2009) who found earlier ovulation in cows with a shorter

DP. A shorter DP and earlier ovulation are probably associated with a reduction in NEB during the first weeks after calving that, in the present study, was indicated by the higher IGF-I levels in the S group but not confirmed by postpartum NEFA levels, which were similar in all groups. Although an earlier postpartum first ovulation may improve general reproductive performance, no effects on the reproductive indices were detected in the present study, in agreement with the findings of another study (Royal et al., 2000). These results are in contrast with the evidence that increasing numbers of oestrous cycles before breeding correlated positively with a reduction in services per pregnancy, indicating that the earlier onset of first ovulation may increase reproductive efficiency (Darwash et al., 1997). One hypothesis is that a reduced calving to first AI interval could be more easily obtained through a better expression of oestrus than through an earlier postpartum ovulation. Still, some differences in reproductive measures in the present study probably did not reach statistical significance because of the limited number of cows (a total of 31 cows divided into four groups).

Production performance

The data about milk yield and quality show modest but significant increases for the S group. These results are in contrast with the findings of other studies (see the review by van Knegsel et al., 2013) in which cows with a short dry period produced 1.4 kg/day less milk than cows with a conventional DP, with an average milk loss of 4.5%. These previous results thus indicated that shortening the DP to about 30 days decreases milk production in the subsequent lactation. Again, a crucial point is that most studies on DP length did not differentiate between cows of different parities. Pezeshki et al. (2007) indicated that the reduction in milk production after a short DP is larger for second-parity cows compared with older cows. Moreover, both Annen et al. (2004) and Santschi et al. (2011) reported a reduction in milk production after a short DP for young cows (second parity), but not for older cows. Results from the present study are in contrast with these findings, as the slight but significant increase in milk quantity and quality was detected not only in the short DP group, including both second- and third-parity cows, but it was confirmed also when comparing different DP groups within the same parity (SPT vs. SPS and TPT vs. TPS). The improvement in production performance was therefore registered both in second- and third-parity cows. Other recent data of the literature report non-significant milk losses for multiparous cows assigned to short DP (Pezeshki et al., 2007, 2008; Santschi et al., 2011; Jolicœur et al., 2014), thus confirmation on a larger number of cows is needed. Therefore, a large variability of responses is evident when comparing production performances among cows with different DP lengths. Steeneveld et al. (2014) found that not only the parity but also daily milk production at 12 weeks before the expected calving date, and reduction in daily milk production between 16 and 12 weeks before the expected calving date, were associated with production loss



due to a short or absent DP. In conclusion, it is reasonable to suggest that these characteristics may be used to select cows for specific DP lengths in a decision-support model to aid the farmer in the choice of the economically optimal DP length for each individual cow.

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