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Effects of Meeting the Requirements in Energy and Protein, and of Systemic Inflammation on the Interval from Parturition to Conception in Dairy Cows

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ABSTRACT

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Main aim of this retrospective study was to determine the role of the level of meeting the requirements in net energy of lactation (NE_L) and utilizable crude protein at the duodenum (uCP) in weeks 3–17 postpartum on the interval from parturition until conception (IUC) in dairy cows. We compared intakes and balances of NE_L and uCP, body weight change, metabolic status, reticuloruminal pH, and serum amyloid A (SAA) as a systemic inflammation marker in 30 dairy cows differing in the IUC length (i.e., short (S; n = 8), medium (M; n = 11), and long (L; n = 11) IUC for cows confirmed pregnant within week 10 or between weeks 11 and 17 postpartum, or thereafter, respectively). Data showed that the level of meeting the requirements in NE_L and uCP in weeks 3–10 postpartum was instrumental in shortening the IUC in the cows pertaining to S IUC group ($P \leq 0.03$). As an average, during this period the S cows met 104 and 110% of their requirements in NE_L and uCP, respectively. In contrast, the M and L cows met 96 and 95% of NE_L as well as 104 and 101% of uCP requirements, respectively. The M cows showed higher milk and blood urea nitrogen ($P = 0.04$), and also lower SAA concentration ($P = 0.05$) compared to L cows. In conclusion, exceeding the requirements in both NE_L and uCP in weeks 3–10 postpartum significantly shortened the IUC to less than 10 weeks. The shorter IUC in M vs L cows went along with improved protein status and lesser systemic inflammation in week 6 postpartum in these cows.

Keywords: energy balance; cattle; early lactation; calving interval; acute phase reaction

List of abbreviations: NE_L = net energy of lactation, uCP = utilizable crude protein at the duodenum, AA = amino acids, IUC = interval from parturition until conception, PMV = protein-mineral-vitamin supplement, SAA = serum amyloid A, NEB = negative energy balance, DM = dry matter, N = nitrogen, MUN = milk urea nitrogen, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, RDP = rumen-degradable protein (RDP), RUP = rumen-undegradable protein, RNB = ruminal N balance, BCS = body condition score, BFT = backfat thickness, BUN = blood urea nitrogen, BHBA = β-hydroxybutyrate, NEFA = non-esterified fatty acids

Increased milk production in recent decades has concomitantly been associated with alarming declines in cow fertility. The excessive negative energy balance (NEB) that cows experience postpartum has long been recognized as one of the main nutritional factors for subfertility (Walsh et

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al. 2011; Drackley and Cardoso 2014). The lack of fuels during NEB is balanced by mobilization of body tissues such as fat, but also amino acids (AA) by muscle tissues which are used to support glucose production via gluconeogenesis and also milk protein synthesis (De Koster and Opsomer 2013). Excessive mobilization of body fat and depletion of key nutrients aggravates the metabolic and endocrine profile in early-lactating cows (Santos et al. 2010), hampering folliculogenesis and development of the oocyte and embryo, events which are essential for successful conception (Lucy et al. 2014); thus, extending the interval from parturition until conception (IUC) and lowering the dairy herd's reproductive efficiency (Dayyani et al. 2013).

It normally takes 6–10 weeks after parturition until cows meet their energy requirements totally through diet, although strong individual variation in the NEB level and body tissue mobilization is well known in the transition cows (Gruber et al. 2014; Humer et al. 2016). However, there is a paucity of information regarding the influence of the level of meeting the energy and nutrient needs on the reproductive performance in cows during early lactation. This retrospective study primarily aimed to determine the role of the degree of meeting the requirements in energy and protein in weeks 3–17 postpartum in cows on the IUC as a measure of reproductive efficiency. Our hypothesis stated that cows with shorter IUC meet their requirements with energy and protein for milk production through dietary sources very early after parturition.

MATERIAL AND METHODS

Animals. The trial was conducted at the Dairy Research Facilities of the Institute of Livestock Research, Agricultural Research and Education Centre Raumberg-Gumpenstein, Austria. All procedures involving animal handling and treatment were in accordance with national regulations for animal use in research and the national authority approved the study according to §9ff of Law for Animal Experiments (GZ FA10A-78Gu-19/2012). The study was conducted as longitudinal trial, as part of a larger research project (Humer et al. 2015; Gruber et al. 2017) which involved 30 dairy cows (25 Holsteins and 5 Simmentals; 12 primiparous and 18 multiparous) that were housed in a free stall.

Dietary ingredients, measurement of feed and nutrient intake, and feeding and milking procedure. All cows were monitored for exact intake of energy and nutrients from week 3 to week 17 postpartum, and for reproduction traits until confirmed pregnant. Dietary ingredients fed to cows were forages of high quality such as 15% hay, 19% corn silage, 16% grass silage, supplemented with 39% barley grain, and 11% protein-mineral-vitamin supplement (PMV) on dry matter (DM) basis. As protein source, the PMV contained soybean and rapeseed meals. The cows were fed different barleys (Humer et al. 2015; Gruber et al. 2017), but as these did not affect feed intake and overall intake of nutrients, only the specific energy and nutrient composition of barley and not barley treatment was considered in this study. Detailed information regarding the nutrient composition of all dietary ingredients is provided in Supplementary Table S1 in Supplementary Online Material (SOM). Dietary ingredients were fed separately to the cows in individual Calan gates. Cows were fed *ad libitum*, being offered 110% of the average amount of feed the individual cow consumed on the 3 preceding days (real average daily feed refusals of 5–10%). The diet was formulated according to GfE (2001) guidelines. Fresh feed was offered twice a day at 4:30 and 16:00 h. Exact intakes of dietary ingredients were measured daily by difference between the feed offered and the respective feed refusals, whereas the intake of PMV was measured electronically for each cow. All cows had free access to water. The cows were milked twice daily (at 4:00 and 15:00 h) and milk samples were analyzed for fat, protein, lactose, and milk urea nitrogen (MUN) by MilkoScan® (FOSS Electric, Denmark).

Feed sampling and chemical analyses. Feed and orts samples were collected daily from each cow and pooled for a 4-week sample. The DM was determined after oven-drying for 72 h at 55°C and then ground through a 1-mm screen. The samples were analyzed for nitrogen (N) using the Dumas method, ether extracts (EE) and ash according to the German Handbook of Agricultural Experimental and Analytical Methods (VDLUFA 2006). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were determined using the methods described by Van Soest et al. (1991), with heat stable α-amylase used in the NDF procedure and were expressed exclusive of residual ash. The concentrations of calcium

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(Ca), magnesium (Mg), and phosphorus (P) were measured using complexometric methods and the concentrations of potassium (K), sodium (Na), manganese (Mn), zinc (Zn), and copper (Cu) were determined using the atomic absorption spectroscopy according to VDLUFA (2006). The content of net energy of lactation (NE_L) of the feedstuffs was measured based on their chemical composition and the *in vivo* digestibility coefficients of individual ingredients as described by Gruber et al. (2017). The content of utilizable crude protein at the duodenum (uCP), rumen-degradable protein (RDP), rumen-undegradable protein (RUP), and the ruminal N balance (RNB) were estimated for each dietary ingredient according to GfE (2001).

Measurements of body weight, body condition score, backfat thickness, and balance of NE_L and uCP. The BW of the cows was measured daily after milking using an electronic scale integrated in the automatic feeder. The body condition score (BCS) was recorded every 3–4 weeks for each cow. In total the BCS was recorded four times in the course of the trial (for the first time during weeks 3–7 postpartum, the second time between weeks 8–10 postpartum, the third time between weeks 11–13 postpartum, and for the last time during weeks 14–17 postpartum). A scale from 1 to 5 was used, including 0.25 point intervals, where 1 = thin and 5 = extremely fat, by the same trained observer (Edmonson et al. 1989). Additionally, backfat thickness (BFT) was measured at the same time, as described by Schroder and Staufenbiel (2006), using a portable B-mode ultrasound generator (Pavo; Proxima Medical Systems GmbH, Germany) with a linear transducer and a frequency between 5.0 and 6.5 MHz. The estimated daily energy and protein balance was calculated by subtracting requirements for maintenance and milk yield (based on GfE 2001) from the respective individual measures of energy and protein intake, as outlined in detail by Gruber et al. (2014).

Reticuloruminal pH and blood analysis. The rumen pH was continuously measured from week 3 to week 10 after parturition using indwelled wireless pH-transmitting units (smaXtec animal care sales GmbH, Austria) as described recently (Humer et al. 2016). Blood samples were collected shortly before the morning feeding from the coccygeal vein at weeks 3, 6, and 13 after parturition using 10 ml serum vacutainer tubes (Vacuette®, Greiner Bio-One GmbH, Austria). Serum was obtained and analyzed for blood urea N (BUN), β-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), Ca and P with a fully automated autoanalyzer for clinical chemistry (Cobas 6000/c501; Roche Diagnostics GmbH, Austria). Concentrations of serum amyloid A (SAA) were measured using commercially available ELISA kits according to the methods described previously (Humer et al. 2015).

Estrus detection, insemination, pregnancy confirmation, and reproduction traits. Cows were monitored for estrus 3 weeks after parturition and inseminated for the first time 6 weeks after parturition. As a routine farm management procedure of estrus detection, trained personnel monitored cows through visual observation twice daily for at least 30 min. Cows were considered in estrus based on the appearance of any of the common behavioural signs of estrus such as restlessness, sniffing of vulva, frequent urination, tail raising, bellowing, and mounting behaviour. Cows displaying overt signs of estrus were artificially inseminated 6 to 12 h following signs of estrus by the farm veterinarian. Pregnancy was confirmed 4–6 weeks after the insemination using an ultrasonic device. The interval from parturition to the first service was considered the time lapse from calving day until day that cows were first inseminated. The IUC was considered the interval from parturition until the successful insemination. The number of services until the conception was also recorded as the insemination index.

Classification of IUC groups and statistical analyses. Based on the length of the IUC, cows that were successfully inseminated within 10 weeks postpartum were classified as short (S), whereas cows that got pregnant between weeks 11–17 after parturition were classified as medium (M), and cows that were confirmed pregnant thereafter were classified as late (L) IUC group. The S group involved eight cows (three primiparous and five multiparous), the M group consisted of 11 cows (five primiparous and six multiparous), whereas the L group had 11 cows (three primiparous and eight multiparous). The S group included three Simmental and five Holstein cows, the M as well as the L group involved one Simmental and ten Holsteins each.

Statistical analysis was performed using PROC MIXED of the SAS software (Statistical Analysis System, Version 9.2). The model included the fixed effects of the IUC (i.e., S, M, L), time after parturi-

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tion (i.e., day or week). Random effects were the barley treatment, breed, and parity of the cows. A possible two-way interaction between the IUC and time after parturition was initially included, but was not significant and therefore removed. The measurements taken on the same cow but at different times were considered as repeated measures in the model with a first-order autoregressive variance-covariance matrix. Comparisons between treatments were evaluated by the pdiff option. Degrees of freedom were estimated with the method of Kenward-Roger. Furthermore, a linear contrast from group S over M to L was tested. The difference between groups M and L was tested for all variables from week 11 to week 17, too. In the following, results of the investigated parameters are presented from week 3 to week 10, whereas differences between group M and L between weeks 11–17 are only provided when significant. The significance level was set at $P \leq 0.05$, and trends were considered at $0.05 < P \leq 0.10$ level.

RESULTS

Reproduction traits. The interval from parturition until the first insemination differed ($P < 0.01$) between groups, being 54 ± 6.0 days in S group, 76 ± 5.1 days in M group, and 83 ± 5.1 days in L IUC group. The IUC was 54 ± 10 days for S cows, 96 ± 9 days for M cows, and 174 ± 8 days for L cows ($P < 0.01$). This difference in IUC was also reflected in the insemination index (one service for S group, 1.73 ± 0.26 for M, and 2.73 ± 0.26 for L group ($P < 0.01$)).

DM, energy and nutrient intake. The overall DM intake in weeks 3–10 after calving did not differ between IUC groups (Table 1). The intake of PMV decreased with prolongation of the IUC, being on average 20% higher in S and M compared to L group ($P = 0.04$). No group effect for the intake of RDP, RUP, uCP as well as the RNB and NE_L was observed. The ratio of RDP to RUP was different, showing a linear increase with prolongation of the IUC from S to L ($P = 0.01$). In addition, a comparison of group M to L from week 11 to 17 after parturition revealed a tendential ($P = 0.09$) lower ratio of RDP to RUP for M (3.10) compared to L (3.15) group.

The intake of most of the investigated minerals and of trace elements (i.e., Ca, P, Mg, K, Mn, Zn, and Cu) did not differ between groups. However, S cows tended to consume on average 3.9 g more Na per day compared with L cows during the first 10 weeks of the observation period ($P = 0.07$), due to the higher PMV intake.

The level of meeting the requirements in NE_L was different in cows among the IUC groups ($P = 0.03$). As shown in Figure 1, the S cows were in lesser NEB during early lactation compared with M and

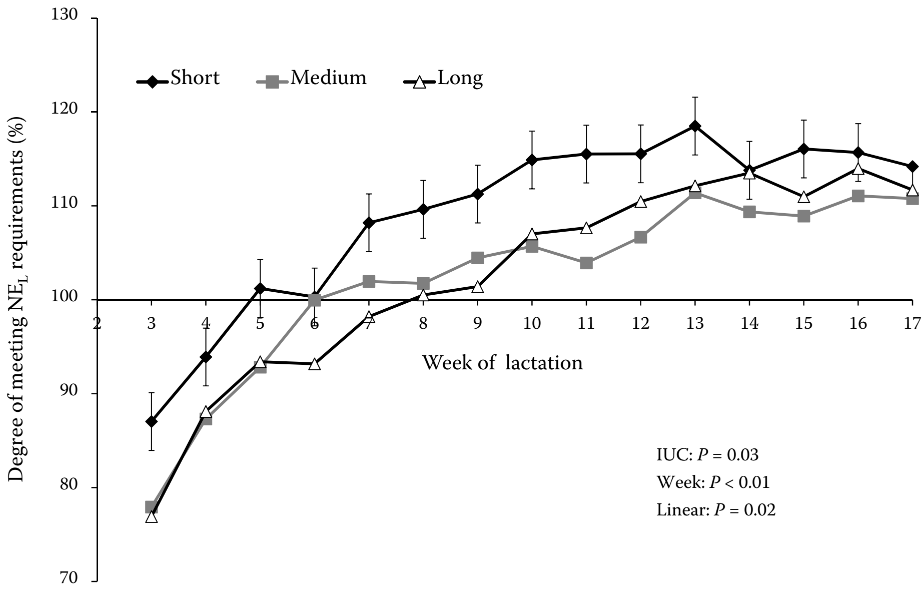

Figure 1. Degree of meeting the requirements for net energy of lactatation (NE_L) in weeks 3–17 of lactation in cows with short (< 10 weeks), medium (11–17 weeks) or long (> 17 weeks) interval from parturition until conception (IUC)

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L cows. On average, in weeks 3–10 after parturition, the S group cows had a light oversupply ($P < 0.01$) in NE_L (104%) compared to the cows of M (96%) and L (95%) groups, which both were in NEB with no difference between the groups (Table 2). Starting from week 12 postpartum, the differences in NE_L among groups diminished, and all cows were already in positive NE_L balance. No overall difference was found between M and L groups regarding meeting the NE_L requirements.

A comparably similar trend was observed between groups with respect to meeting the requirements in uCP (Figure 2). While the M and L cows were in negative uCP balance, the S cows almost met their uCP requirements through diet starting from week 3 postpartum. On average, the S group cows had a stronger oversupply ($P < 0.01$) in weeks 3–10 after parturition (110%) compared to groups M (104%) and L (101%), whereby no difference was stated between the latter two groups (Table 2).

Table 1. Daily intake of dietary ingredients, crude nutrients, and energy in weeks 3–10 of lactation in cows differing in the interval from parturition until conception (IUC)

Intakes	IUC[1]			SEM	*P*-value[2]		
	S	M	L		week	IUC	linear
Diet ingredients (kg DM/day)							
Overall diet	19.9	19.1	18.9	1.68	< 0.01	0.61	0.41
Hay	3.18	3.20	3.11	0.200	0.01	0.61	0.88
Corn silage	4.22[a]	3.83[b]	3.81[b]	0.259	0.06	0.10	0.06
Grass silage	3.37	3.30	3.35	0.180	0.04	0.78	0.51
Barley	7.08	6.77	6.96	0.433	< 0.01	0.78	0.77
Protein-mineral-mix[3]	2.14[a]	2.04[a]	1.74[b]	0.191	< 0.01	0.09	0.04
Nutrients and energy							
RDP (g/day)	2133	2060	2016	185	< 0.01	0.47	0.23
RUP (g/day)	684	656	634	57	< 0.01	0.32	0.13
RDP : RUP	3.13[b]	3.15[b]	3.20[a]	0.025	< 0.01	0.02	0.01
uCP (g/day)	2874	2751	2741	240	< 0.01	0.52	0.30
RNB (g/day)	–9.1[ab]	–5.1[a]	–14.8[b]	5.28	0.39	0.15	0.29
NE_L (MJ/day)	128	122	123	10.53	< 0.01	0.57	0.38
Ca (g/day)	123	119	114	12.93	< 0.01	0.45	0.22
P (g/day)	82.5	80.3	78.5	7.52	< 0.01	0.69	0.39
Mg (g/day)	49.6	48.4	46.9	4.22	< 0.01	0.60	0.33
K (g/day)	263	255	254	25.12	< 0.01	0.71	0.44
Na (g/day)	25.7[a]	24.8[ab]	21.8[b]	2.59	< 0.01	0.15	0.07
Mn (g/day)	1.13	1.15	1.13	0.081	< 0.01	0.93	0.98
Zn (mg/day)	852	819	778	74.6	< 0.01	0.29	0.13
Cu (mg/day)	160	159	152	14.9	< 0.01	0.56	0.37

RDP = rumen-degradable protein, RUP = rumen-undegradable protein, uCP = utilizable crude protein at the duodenum, RNB = ruminal nitrogen balance (according to GfE (2001)), NE_L = net energy of lactation, Ca = calcium, P = phosphorus, Mg = magnesium, K = potassium, Na = sodium, Mn = manganese, Zn = zinc, Cu = copper

[1]interval from parturition until conception is defined as short (S; < 10 weeks), medium (M; between 11 and 17 weeks), or long (L; > 17 weeks)

[2]effects of time after parturition in weeks 3–10, interval from parturition until conception (IUC), linear effect of increased IUC from week 3 to week 10 after parturition (linear)

[3]contained 45.48% soybean meal, 45.48% rapeseed meal, 5.04% limestone, 2.00% salt, and 2.00% mineral-vitamin supplement (provided per kg vitamin-mineral supplement: Ca 6%, P 12%, Mg 10%, Na 8%, Mn 1500 mg, Zn 5700 mg, Cu 800 mg, vitamin A 750 000 IU, vitamin D3 75 000 IU, vitamin E 3000 mg)

[a,b]different superscripts within a row represent differences between IUC groups at $P \leq 0.05$

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Table 2. Degree of meeting the requirements in energy and protein, and body condition in weeks 3–10 of lactation in cows differing in the interval from parturition until conception (IUC)

Variable	IUC[1]			SEM	*P*-value[2]		
	S	M	L		week	IUC	linear
NE_L (% of requirements)	103.7[a]	95.9[b]	95.1[b]	2.62	< 0.01	0.03	0.02
uCP (% of requirements)	110.1[a]	103.9[b]	101.0[b]	2.29	< 0.01	< 0.01	< 0.01
BW (kg)	655	649	661	62.5	0.02	0.83	0.83
BCS	3.19	3.10	3.03	0.319	0.94	0.22	0.22
BFT (mm)	12.01	10.79	10.92	1.972	0.32	0.29	0.29
BW change (kg/week)	–5.34	–14.50	–16.65	7.74	< 0.01	0.27	0.27

NE_L = level of meeting the requirement of net energy of lactation, uCP = level of meeting the requirement of utilizable crude protein at the duodenum, BW = body weight, BCS = body condition score, BFT = backfat thickness, BW change = body weight change relative to week 1 after parturition

[1]interval from parturition until conception is defined as short (S; < 10 weeks), medium (M; 11–17 weeks), or long (L; > 17 weeks)

[2]effect of time after parturition from week 3 to week 10, interval from parturition until conception (IUC), linear effect of increased IUC from week 3 to week 10 after parturition (linear)

[a,b]different superscripts within a row represent differences between IUC groups in weeks 3–10 after parturition with $P \leq 0.05$

There was no difference in the BW, BW change, BCS, and BFT between the groups (Table 2).

Milk production and ruminal pH. The groups showed no difference in milk yield in weeks 3–10 of lactation (Table 3). The concentration of fat and protein did not differ between groups in this period. A tendency towards a linear increase of lactose concentrations from group S to L was observed ($P = 0.10$). In weeks 11–17, cows of the M group showed a lower milk protein concentration compared to group L (3.3 vs 3.5%, $P = 0.04$). On the other hand, the cows of M group showed higher MUN concentrations compared to cows of L group (17.1 mg/dl vs 13.5 mg/dl, $P = 0.02$), which was also observed until week 17 of the observation period (average of weeks 11–17: 18.8 mg/dl vs 14.4 mg/dl, $P < 0.01$).

The mean reticuloruminal pH did not differ between groups (Table 3). However, the daily minimum pH ($P = 0.05$) was slightly higher in S group compared to M and L group cows. Furthermore, the time period of the pH below a threshold of

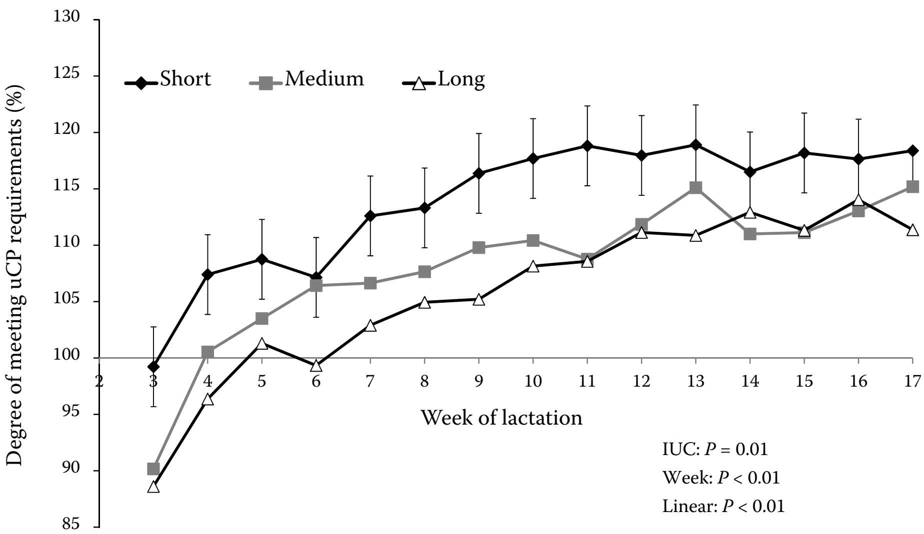

Figure 2. Degree of meeting the requirements for utilizable crude protein at the duodenum (uCP) in weeks 3–17 of lactation in cows with short (< 10 weeks), medium (11–17 weeks) or long (> 17 weeks) interval from parturition until conception (IUC)

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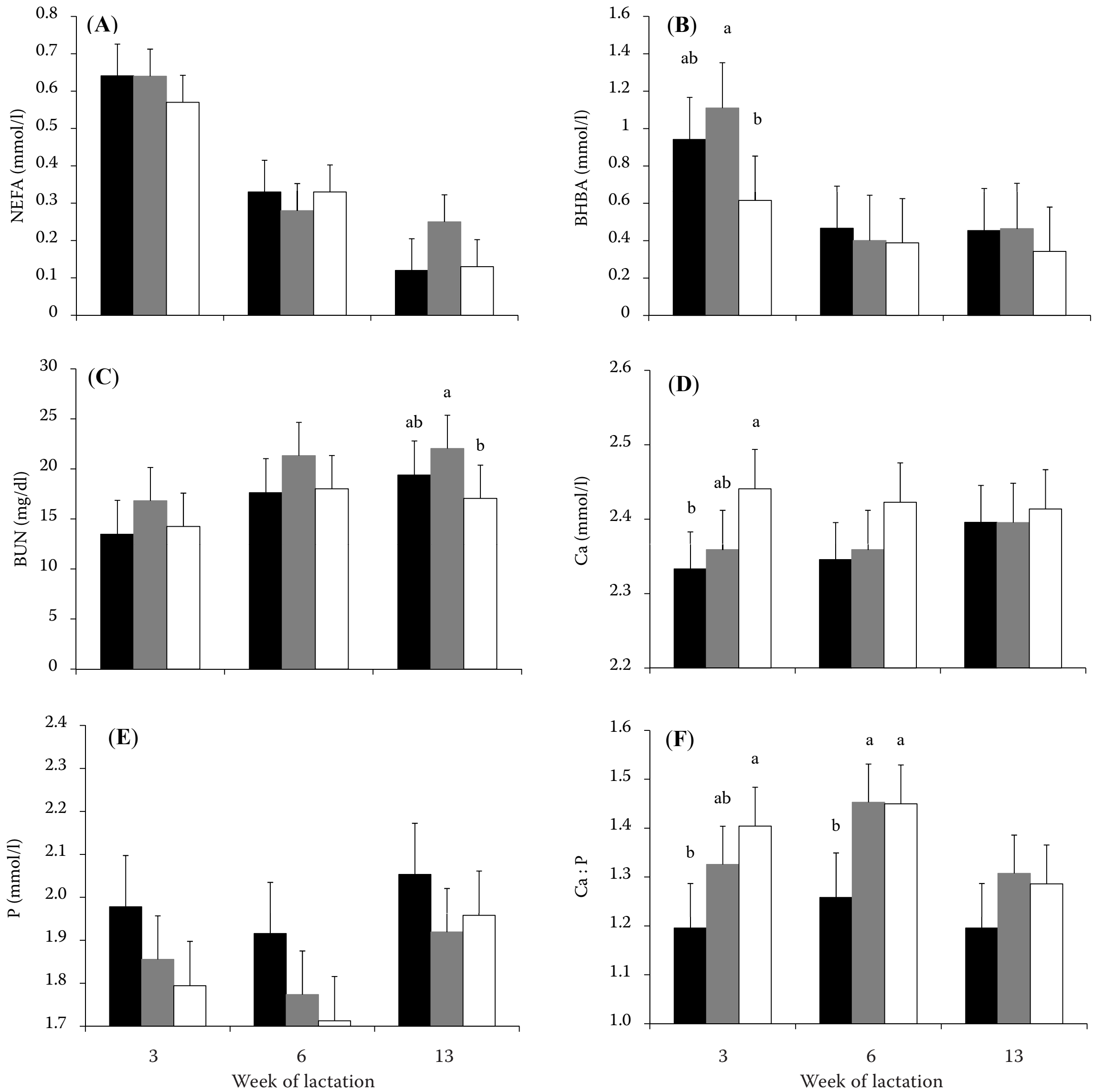

Figure 3. Serum concentrations of non-esterified fatty acids (NEFA; **A**), β-hydroxybutyrate (BHBA; **B**), blood urea nitrogen (BUN; **C**), calcium (Ca; **D**), phosphorus (P; **E**), and Ca : P ratio (**F**) in weeks 3, 6, and 13 of lactation in cows with short (< 10 weeks; black), medium (11–17 weeks; grey) or long (> 17 weeks; white) interval from parturition until conception (IUC)

data are presented as Least Squares Means ± standard errors

[a,b]statistically significant differences between IUC groups are indicated by different superscripts ($P \leq 0.05$)

5.8 was the shortest in cows pertaining to S group ($P = 0.04$).

Blood parameters. The concentration of NEFA and BHBA measured at three different time points after calving did not differ between groups (Figure 3A, B). Cows of M group showed higher BUN compared to L group ($P = 0.02$). The Ca concentrations in blood were linearly increased with increasing IUC in weeks 3 and 6 postpartum ($P = 0.04$, Figure 3D), while the opposite effect was noticed for P (Figure 3E, $P = 0.09$), which resulted in a linear increase of the ratio of Ca to P in the serum (Figure 3F, $P = 0.04$).

No difference between groups regarding SAA was found in week 3 postpartum and this acute phase protein was below the detection limit in week 13 after calving. However, an almost 13-fold higher concentration in L group was found in week 6 postpartum compared to M ($P = 0.05$) and S group ($P = 0.07$, Figure 4).

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Table 3. Milk parameters and ruminal pH in weeks 3–10 of lactation in cows differing in the interval from parturition until conception (IUC)

Variable	IUC[1]			SEM	*P*-value[2]		
	S	M	L		week	IUC	linear
Milk data							
Milk yield (kg/day)	27.3	28.9	29.1	3.41	< 0.01	0.69	0.41
Fat (%)	3.84	3.84	3.81	0.076	< 0.01	0.95	0.79
Protein (%)	3.21	3.15	3.23	0.132	< 0.01	0.72	0.88
Lactose (%)	4.74^{b}	4.81^{a}	4.80^{a}	0.066	0.07	0.19	0.10
MUN (mg/dl)	14.9^{ab}	17.1^{a}	13.5^{b}	1.55	0.02	0.02	0.36
Ruminal pH							
Mean pH	6.25	6.21	6.20	0.044	0.61	0.44	0.22
Minimum pH	5.88^{a}	5.83^{b}	5.82^{b}	0.043	0.86	0.13	0.05
Time pH < 5.8 (min/day)	28^{b}	66^{a}	49^{ab}	17.4	0.51	0.04	0.13

MUN = milk urea nitrogen
[1]interval from parturition until conception is defined as short (S; < 10 weeks), medium (M; 11–17 weeks), or long (L; > 17 weeks)
[2]effect of time after parturition from week 3 to week 10, interval from parturition until conception (IUC), linear effect of increased IUC from week 3 to week 10 after parturition (linear)
[a,b]different superscripts within a row represent differences between IUC groups from week 3 to 10 after parturition with $P \leq 0.05$

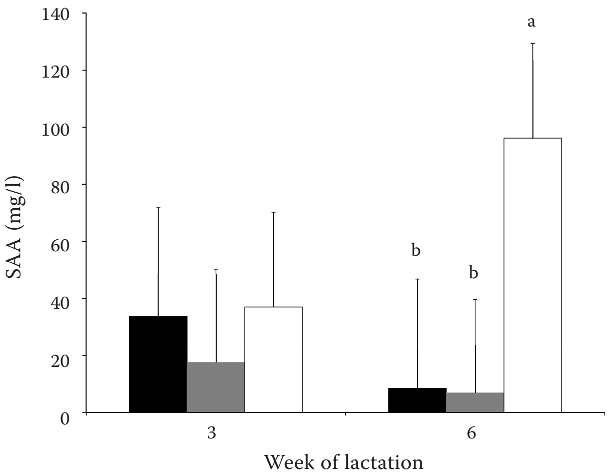

Figure 4. Concentration of serum amyloid A (SAA) in different weeks of lactation in cows with short (< 10 weeks; black), medium (11–17 weeks; grey) or long (> 17 weeks; white) interval from parturition until conception (IUC)
data are presented as Least Squares Means ± standard errors
[a,b]statistically significant differences between IUC groups are indicated by different superscripts ($P \leq 0.05$)

DISCUSSION

The study revealed that the level of meeting the energy and protein requirements during weeks 3–10 postpartum is instrumental for shortening the IUC and improving insemination index in S cows. However, because of the manifold interrelations among the factors investigated, it was not possible to clearly isolate single effects influencing the IUC length in this study. In addition, our study has a weakness with regards to monitoring and detecting the estrus, which was confirmed only by clinical observations and not by measuring progesterone changes. As such, subtle estrus that remained undetected cannot be ruled out. Therefore, variables related to estrus detection such as the time of expressing the first estrus postpartum or the number of estruses per cow should be considered with caution.

Data showed that cows of the S group, being pregnant at 54 ± 6 days postpartum, were fed 104% of energy needs for maintenance and milk production between weeks 3–10 postpartum, and these cows met their requirements in NE_L through alimentary sources earlier than M and L cows did. Especially the L cows experienced an extensive NEB which lasted until weeks 7–8 postpartum. Previous research has shown that energy deprivation is linked with impaired reproductive performance (Butler 2003; Santos et al. 2010). Because NEFA and BHBA as markers of lipid mobilization did not differ between IUC groups postpartum, the higher

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lipid mobilization *per se* was not the primary cause for the differences in fertility traits. Obviously the problem lies in the speed of recovery of energy and nutrient losses during the postpartum period and the time when deposition of the new body reserves starts. Furthermore, one has to consider that meeting 100% of the theoretical demands for energy and protein solely refer to the requirements for maintenance and milk production, whereas additional requirements for health and reproduction are not taken into account.

Regarding the degree of meeting the uCP requirements, the study showed that in weeks 3–10 postpartum the S group cows met 110% of the requirements, compared to 104% and 101% in groups M and L, respectively. In addition, M and L cows experienced longer periods in negative uCP balance. For example, while the S cows met their uCP requirements almost three weeks after parturition, it took the cows of group M one week, and cows of group L about four weeks longer to overcome the negative uCP balance. The supply of uCP postpartum affects the availability of AA whose requirements increase sharply at the onset of lactation to support milk protein synthesis (Plaizier et al. 2000). Furthermore, the contribution of AA to gluconeogenesis is another important demand during the early lactation period (Drackley et al. 2001). Thus, in addition to experiencing NEB, cows typically are also in a negative protein balance in early lactation (Plaizier et al. 2000). The more pronounced NEB in M and L cows in weeks 3–10 postpartum likely enhanced the utilization of the surplus of uCP to meet their energy requirements via glucogenic AA. This could also explain the non-significant differences in the level of circulating NEFA as well as BHBA among IUC groups, despite the marked differences in the NE_L balance.

The effects of dietary protein level and source on cow's fertility are differently discussed in the literature. Whereas high levels of CP in the diet have been reported to show a negative effect (Sinclair et al. 2014), high levels of RUP have demonstrated to improve several reproductive traits, like the number of breedings per cow, days open, and first breeding conception rates (Aboozar et al. 2012). The present results suggest a role for protein supply – both in quantitative (uCP supply) and qualitative (RDP : RUP ratio) terms of protein feeding – on reproductive traits in cows. For example, our data indicated that besides meeting and exceeding the uCP requirements, also the ratio of RDP to RUP seems to play a role in terms of IUC length among groups. The importance of feeding higher RUP : RDP levels in IUC during early lactation is likely due to limited feed intake potential of cows postpartum and the limitation in the capacity of the microbial protein synthesis in the rumen (approximately 10.1 g/MJ ME ingested), as well as because of the high requirements of uCP during early lactation (GfE 2001), thus increasing the need for higher RUP contents in the diet. On the other hand, excessive amounts of RDP degraded to ammonia by the ruminal microbes are absorbed into the blood and rapidly converted into urea in the liver. Concentrations of BUN and MUN of >19 mg/dl have been demonstrated to impair fertility rates in dairy cows (Butler et al. 1996). Overall, lower BUN and MUN concentrations were found in the group with the longest IUC compared to group M. Thus, it seems that greater MUN and BUN in M group can be interpreted as an improved protein status in those cows, suggesting that this event can even improve fertility, as long as it does not exceed a level that might lead to damage of the liver and the uterine environment. Overall, the present results suggest that the quality of the protein might be of significance in the early-lactating period. Thus, the 20% higher intake of the PMV in cows of group S and M, containing primarily high-quality protein sources such as soybean meal and rapeseed meal, likely helped in shortening the IUC in those cows.

Although all cows of the present study were clinically healthy throughout the study period, the higher SAA values observed in L cows in week 6 suggest the presence of subtle inflammatory processes during the early lactation period in these cows, which seems to have played a role for the impaired fertility traits in L cows. The SAA is an acute phase protein that is rapidly synthesized and released by bovine hepatocytes in response to inflammation or bacterial infection (Petersen et al. 2004). Because L cows had increased SAA values only during the peak of lactation (week 6) and not in week 3 or 13, the source of systemic inflammation in these cows is not clear. Interestingly, both S and M cows did not show any sign of systemic inflammation throughout the period measured. In fact, the role of inflammatory diseases occurring before breeding on reduction of fertility in dairy cows has been demonstrated (Ribeiro et al. 2013). A recent study has also im-

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plicated that inflammation reduces fertilization of oocytes and development to the morula stage, concurrent with an impaired uterine environment (Ribeiro et al. 2016).

CONCLUSION

Taken together, feeding of about 105 and 110% of the requirements in NE_L and uCP, respectively, during the early lactation is instrumental for shortening IUC length in S cows to less than 10 weeks. Furthermore, the study suggests that prevention of systemic inflammatory states during early lactation may also help shorten the IUC in cows. Further research is required to identify possible reasons for the differences in fertility in mid-lactating cows.

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