Postpartal resumption of ovarian activity in dairy cows: Implications for herbage-based feeding systems in Switzerland

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Postpartale Wiederaufnahme der Ovaraktivität bei Milchkühen: Auswirkungen auf grasbasierte Fütterungssysteme in der Schweiz

Die Milchproduktion in der Schweiz basiert hauptsächlich auf Grasfütterung mit einer geringen Kraftfutterergänzung. Die vorliegende Studie untersuchte die Auswirkungen einer ausschliesslich auf Gras basierenden Diät mit (C) und ohne Kraftfutter (nC) auf die Lutealaktivität, die Milchproduktion und den Stoffwechselstatus bei 23 multiparen Holstein-Milchkühen mit frühzeitiger oder verzögerter Wiederaufnahme der Ovarzyklizität post partum (pp). Die Kühe wurden retrospektiv entweder einer Gruppe mit früher (bis 25 Tage pp, EOV) oder verzögerter Wiederaufnahme der Ovaraktivität (>30 Tage pp, DOV) zugeordnet, was je nach Konzentratfütterung zu vier Untergruppen führte: DOV-C, DOV-nC, EOV-C, EOV-nC. Die Milchprogesteron (P4)-Konzentration wurde alle 3 Tage gemessen und verschiedene Metaboliten wurden in wöchentlichen Blutproben analysiert. Eine Wiederaufnahme der Ovaraktivität wurde zwischen 19–25 Tage pp in EOV und zwischen 30–60 Tagen pp in DOV festgestellt. In DOV-C begann die Wiederaufnahme der Zyklizität tendenziell früher (38,3 ± 1,7 Tage pp) als in DOV-nC (45,2 ± 6,3 Tage pp; P = 0,10). Unabhängig von der Gruppierung trat die Ovaraktivität später bei Kühen mit höheren β-Hydroxybutyrat (BHB)-Konzentrationen im Plasma auf (P < 0,05). Die Milch-P4-Konzentration beim ersten Anstieg > 1 ng/ml unterschied sich nicht zwischen den Gruppen (P > 0,05), aber die Milch-P4-Peaks während des Experiments waren bei EOV höher als bei DOV (P < 0,05). DOV-nC-Kühe zeigten in den ersten 3 Wochen pp die negativste Energiebilanz mit gleichzeitig niedrigster Plasmaglucose und höchsten Konzentrationen an nicht veresterten Fettsäuren und BHB. Die Konzentrationen an insulinähnlichem Wachstumsfaktor-1 waren bei Kühen ohne Kraftfutterergänzung niedriger, ohne Zu-

Summary

Milk production in Switzerland is mainly based on herbage feeding with little input of concentrates. The present study investigated the effects of a solely herbage-based diet with (C) and without concentrate (nC) supplementation on luteal activity, milk production and metabolic status in 23 multiparous Holstein dairy cows with early or delayed resumption of ovarian cyclicity post partum (pp). Cows were retrospectively assigned either to a group with early (until d 25 pp, EOV) or delayed resumption of ovarian activity (>d 30 pp, DOV), resulting in four subgroups depending on concentrate feeding: DOV-C, DOV-nC, EOV-C, EOV-nC. Milk progesterone (P4) concentration was measured every 3 d, and different metabolites were analyzed in weekly blood samples. Resumption of ovarian activity was detected between d 19 and 25 pp in EOV, and between d 30 and 60 pp in DOV. In DOV-C, resumption of cyclicity tended to start earlier (38.3 ± 1.7 d pp) compared to DOV-nC (45.2 ± 6.3 d pp; P = 0.10). Independent of grouping, the ovarian activity occurred later in cows with greater plasma β-hydroxybutyrate (BHB) concentrations (P < 0.05). Milk P4 concentration at the first rise > 1 ng/mL did not differ between groups (P > 0.05), but milk P4 peaks during the experiment were higher in EOV compared to DOV (P < 0.05). Cows of DOV-nC experienced the most negative energy balance during the first 3 weeks pp with concomitantly lowest plasma glucose and highest concentrations of non-esterified fatty acids and BHB. Insulin-like growth factor-1 concentrations were lower in cows without concentrate supplementation, but not related to EOV or DOV (P = 0.61). Overall, concentrate supplementation caused an earlier onset of luteal activity in cows in herbage-based feeding systems. Resumption of ovarian activity in cows with DOV was further delayed if energy and nutrient supply were limited.

Key words: first ovulation, herbage feeding, metabolism, reproduction, resumption ovarian activity

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sammenhang zu EOV oder DOV (P=0,61). Insgesamt bedingte die Kraftfutterergänzung einen früheren Beginn der Lutealaktivität bei Kühen in grasbasierten Fütterungssystemen. Die Wiederaufnahme der Ovaraktivität bei Kühen mit DOV wurde weiter verzögert, wenn Energie- und Nährstoffversorgung limitiert waren.

Schlüsselwörter: erste Ovulation, Grasfütterung, Stoffwechsel, Fortpflanzung, Wiederaufnahme der Ovaraktivität

Introduction

Herbage-based feeding systems for dairy cows aim at maximizing nutrient use from herbage for milk production. Ideally, the lactation curve and thus nutrient demand of cows coincide with the growth pattern of herbage. Therefore, most calvings take place in late winter/early spring prior to the time until sufficient herbage is available to best meet energy and nutrient requirements for lactation. However, milk production and concomitantly nutritional requirements of dairy cows have tremendously increased during the last years. Hence, exclusive herbage feeding without concentrate supplementation limits milk production and aggravates the negative energy balance (NEB) in high-yielding dairy cows during early lactation.[37] It has been shown that a more severe energy deficiency following parturition reduces reproductive performance with an increased duration until successful conception.[4,20]

The maintenance of a seasonal calving rhythm is characteristic for pasture-based milk production worldwide. Theoretically, cows need to be inseminated before 85 d post partum (pp) to ensure a calving interval of maximal 365 d.[22] However, this strict adherence of farm management implies that cows not getting timely pregnant will be culled. Insufficient fertility is one of the major culling reasons in early lactating dairy cows, particularly as consequence of the NEB pp,[2] and of major importance in farms with seasonal calving. In general, an early resumption of ovarian activity after parturition is crucial for early insemination. The metabolic status in early lactation is known to affect the onset of luteal activity in dairy cows. Although a number of studies showed the impact of inadequate nutrition and energy status on ovarian cyclicity,[1,4,9,10,23,34] only few studies specifically investigated the resumption of ovarian activity in herbage-based feeding systems. Therefore, the objective of the present study was to evaluate the effects of a solely herbage-based diet with and without concentrate supplementation on luteal activity, milk production and metabolic status in cows with early or delayed resumption of ovarian cyclicity after parturition.

Materials and Methods

Animals

The experimental study was carried out in accordance with the Swiss Federal Law on Animal Protection and was approved by the Committee of Animal Experiments of the Canton Fribourg, Switzerland (approval no. 2012_12_FR). The study design and feeding regimen were described in detail elsewhere.[37]

In short, 23 multiparous Holstein dairy cows (parity no. (mean ± SD): 3.2 ± 1.3, range: 2–6) either received a sole fresh herbage diet (n=10, average milk production of the previous lactation (mean ± SD): 7416 ± 1263 kg, range: 5168–9225 kg) or were supplemented with additional concentrate (n=13, average milk production of the previous lactation: 7569 ± 1930 kg, range: 4679–10808 kg) to meet their energy and nutrient requirements during the period from week 3 before until week 8 pp. Fresh herbage (5.75 ± 0.49 MJ NEL/kg DM) was cut daily and fed ad libitum, whereas the protein-rich concentrate (up to 2.0 kg/d; 8.1 ± 0.1 MJ NEL/kg DM; based on corn, barley, and corn gluten) and energy-rich concentrate (up to 5.0 kg/d; 8.1 ± 0.1 MJ NEL/kg DM; based on corn kernels and barley) were supplied according to the individual milk yield of the animals. All diets were provided indoors and the consumed amounts recorded in feeding troughs equipped with electronic balances and transponder feeding stations, respectively. Composition and nutritive value of the herbage and concentrates were reported earlier.[37] After parturition, milking was performed twice daily in a milking parlor (at 05:30 and 16:30), and body weight was measured automatically after each milking. Energy balance (EB) for individual animals was calculated on a weekly basis as the difference between energy intake via feed and energy requirements for maintenance and milk production (based on body weight, milk yield and milk composition).[37]

Sampling and analysis of milk, blood, and saliva

For the determination of milk gross composition (i.e., fat and protein), milk samples from one evening and the following morning milking were pooled once weekly. The milk samples were analyzed by infrared spectroscopy (Combi-Foss FT 6000, Foss, Hillerød, Denmark) in the laboratory of Suisselab AG (Zollikofen, Switzerland). Progesterone (P4) in skim milk was analyzed by ELISA.21 For this purpose, separate milk samples (approximately 10 mL) were obtained from the morning milking three times per week (Monday, Wednesday, Friday), centrifuged and the fat layer removed. The detection limit of P4 in milk was 0.2 ng/mL. Once weekly throughout the study, blood was sampled from the jugular vein after the morning milking and before feeding into evacuated tubes with EDTA (Vacuette, Greiner Bio-One, St. Gallen, Switzerland). Blood samples were cooled on wet ice, centrifuged for 20 min (2,500 × g, +4 °C), and the plasma harvested and stored at –20 °C until analysis. Concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and urea were measured on an auto-analyzer (Cobas Mira, Hofmann-La Roche, Switzerland) using commercial enzymatic kits. Insulin-like growth factor (IGF)-1 in plasma was determined by radioimmunoassay.[33]

Grouping of animals and statistical analysis

Resumption of ovarian cycle was assumed when milk P4 concentrations reached once levels > 1 ng/mL indicating luteal activity.[5] For statistical evaluations, cows were assigned either to a group with early resumption of ovarian activity (until d 25 pp, EOV, n = 12) or delayed resumption of ovarian activity (> d 30 pp, DOV, n = 11). Concomitantly, subgroups were created depending on the feeding regimen, i.e. herbage feeding with (C) or without (nC) concentrate supplementation: DOV-C (n = 6), DOV-nC (n = 5), EOV-C (n = 7), EOV-nC (n = 5). All data presented are means ± SEM, except where stated as SD. Statistical analysis was carried out using the software SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Pearson correlation coefficients among fertility traits and metabolic parameters were calculated using the CORR procedure. In MIXED models, group (EOV, DOV), experimental week, feeding (C, nC), and the interactions group × week and group × feeding were considered as fixed effects and the individual cow as repeated subject. Parity number and the previous lactation yield were included as covariates in the statistical model. Due to lacking significance, parity number was removed from the final evaluations. Group differences over time were detected by the Bonferroni t-test with P-values < 0.05 considered to be significant, while trends were assumed at 0.05 ≤ P ≤ 0.10.

Results

Characteristics of luteal activity

Resumption of ovarian activity was detected between d 19 and 25 pp in EOV (22.1 ± 2.1 d, mean ± SD), and between d 30 and 60 pp in DOV (41.5 ± 9.6 d, mean ± SD). In DOV-C, resumption of ovarian activity tended to start earlier (38.3 ± 1.7 d pp) compared to DOV-nC (45.2 ± 6.3 d pp; P = 0.10), whereas resumption of ovarian activity in EOV-C and EOV-nC did not differ (22.6 ± 0.9 vs. 21.4 ± 0.9 d pp; P = 0.77). Independent of grouping, time until resumption of ovarian activity was longer in cows with greater plasma BHB concentrations (P < 0.05) and in cows with lower milk P4 (P < 0.001).

Duration until resumption of ovarian activity pp was negatively correlated with the maximal milk P4 concentrations observed during the experimental period (r = –0.62; P < 0.0001).

Milk P4 concentration at the first rise of P4 > 1 ng/mL did not differ between EOV and DOV (P > 0.05), but milk P4 peaks observed during the experiment were higher in EOV compared to DOV (2.84 ± 0.24 ng/mL vs. 1.94 ± 0.25 ng/mL; P < 0.05).

Relationships of performance and metabolism with the resumption of ovarian activity

Dry matter intake and milk yield did not differ between EOV and DOV (P > 0.05; Figures 1 A and C). Furthermore, we observed no interaction between feeding of concentrate and EOV and DOV, respectively (P > 0.05; Figures 1 A and C). Energy balance was associated with grouping according to EOV and DOV (P < 0.05; Figure 1 B). Omission of supplementary concentrate resulted in a more pronounced negative EB in both EOV and DOV (Figure 1 B). Cows assigned to DOV-nC experienced the most negative EB during the first 3 weeks of lactation (Figure 1 B). Milk fat content closely reflected group differences as observed for EB. Concentrate depletion was followed by increased milk fat contents, whereby greatest milk fat contents were observed in DOV-nC during the first 6 weeks pp (Figure 1 D). No group differences were observed for milk protein content (P = 0.26, data not shown).

The omission of concentrate resulted in decreased plasma glucose concentrations in EOV and DOV with lowest glucose concentrations in DOV-nC during the first 5 weeks pp (Figure 2 A). In contrast, plasma NEFA concentrations showed an inverse pattern. Concentrations of NEFA in plasma were highest in DOV-nC compared to the other groups during the first 4 weeks of lactation (Figure 2 B). Similarly, DOV-nC had greater plasma BHB concentrations compared to DOV-C, EOV-nC, and EOV-C (Figure 2 C). Plasma concentrations of IGF-1 were lower in cows without concentrate supplementation, but not related to EOV or DOV (P = 0.61; Figure 2 D). Urea concentration in plasma did not differ between groups (P = 0.66), nor did we detect an interaction between concentrate feeding and EOV/DOV (P = 0.24; data not shown).

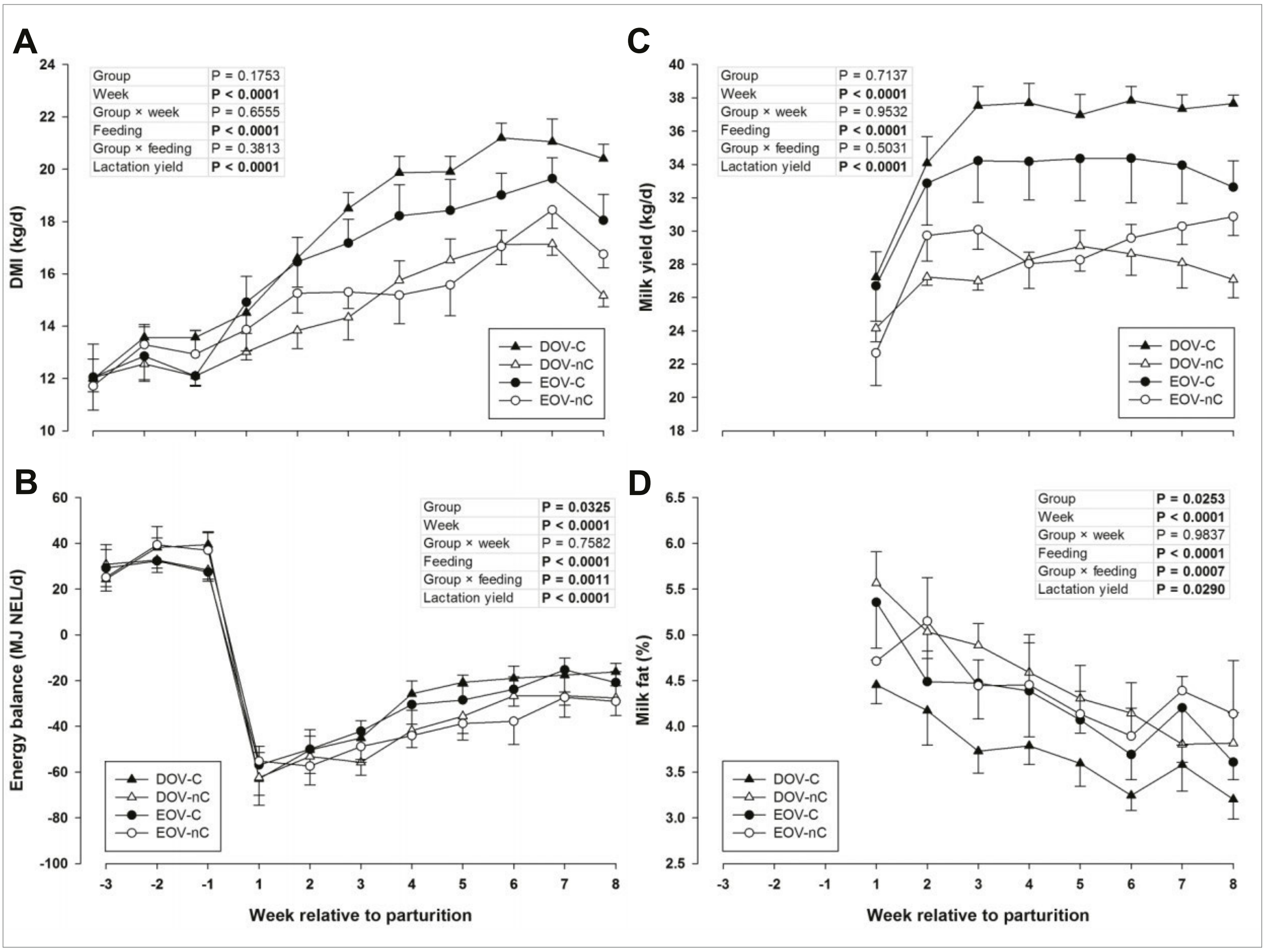

Figure 1: Dry matter intake (DMI, Figure 1 A), energy balance (Figure 1 B), milk yield (Figure 1 C) and milk fat (Figure 1 D) in 23 dairy cows with early (EOV) and delayed (DOV) resumption of ovarian activity. Animals in both groups (EOV and DOV) were either fed a herbage-based diet with (EOV-C, DOV-C) or without supplementary concentrate (EOV-nC, DOV-nC). Data represent means ± SEM.

Discussion

An early resumption of ovarian cyclicity after parturition is a prerequisite for high fertility and early insemination.[14,28,31] However, not in all cows the developing dominant follicles ovulate, which results in a delayed start of ovarian activity and in a subsequently reduced reproductive performance.[13,14] The interval between calving and resumption of ovarian cyclicity in cows of the present study was similar to earlier studies.[5,12]

Several studies could show that the extent of the NEB after parturition,[1,10,19,20,31] but also during the dry period5 affects the resumption of ovarian activity. Furthermore, the protein deficiency accompanying the NEB affects fertility after parturition, too.[10] In the present study, concentrate withdrawal resulted in a lower total DMI in both EOV and DOV, as well as in a reduced milk yield. Consequently, energy balance was more negative in groups without supplementary concentrate. The effects of the (calculated) energy status on resumption of ovarian activity, however, cannot be fully clarified as the observed reductions in milk yield may not be interpreted in the context of an alleviation of the NEB, but must be considered an adaptation to a catabolic situation despite the priority of lactation during early lactation. Nevertheless, a number of processes associated with the NEB (e.g., mobilization of adipose tissue, elevated plasma concentrations of NEFA and BHB, low levels of IGF-1) have been shown to exert direct and indirect effects on reproductive performance and are assumed to be causative for inhibition of luteinizing hormone (LH) secretion, development and ovulation of the follicle, etc.[4,27,30,32,36]

Elevated concentrations of metabolic markers in plasma like NEFA and BHB along with an increased milk fat content reflect the degree of a NEB in early lactation.[7] In the present study, concentrate omission in herbage fed dairy cows increased respective concentrations of

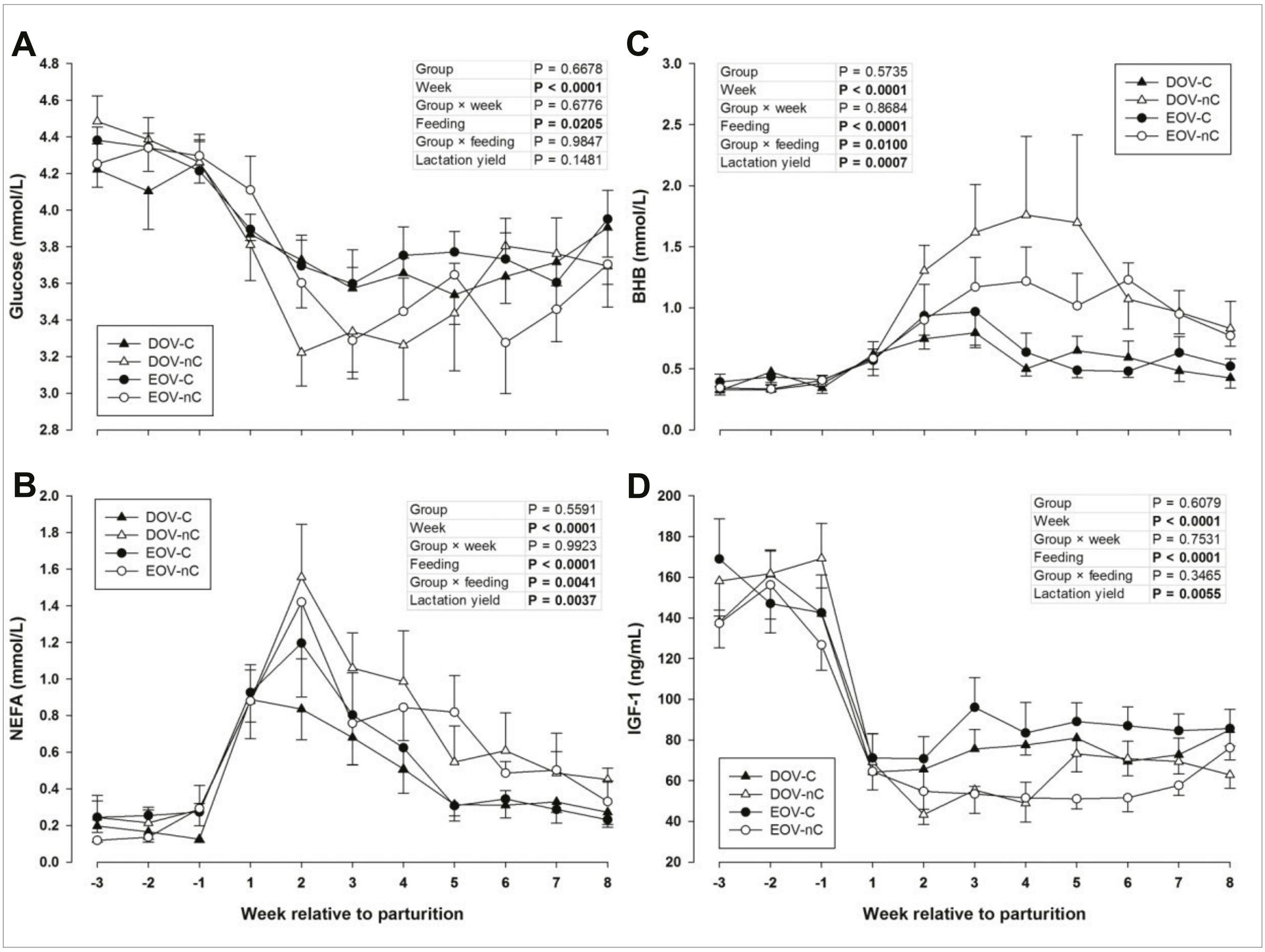

Figure 2: Plasma concentrations of glucose (Figure 2A), non-esterified fatty acids (NEFA, Figure 2B), β-hydroxybutyrate (BHB, Figure 2C), and insulin-like growth factor (IGF)-1 (Figure 2D) in 23 dairy cows with early (EOV) and delayed (DOV) resumption of ovarian activity. Animals in both groups (EOV and DOV) were either fed a herbage-based diet with (EOV-C, DOV-C) or without supplementary concentrate (EOV-nC, DOV-nC). Data represent means ± SEM.

NEFA, BHB, and milk fat, particularly in cows with a delayed commencement of ovarian activity. In agreement with our findings, fewer cows of another study were observed with resumption of ovarian cyclicity when they were ketotic.[29] It was therefore not surprising that cows with greater BHB concentrations required more time until resumption of ovarian activity and had less milk P4. A similar finding was reported on the relationship between circulating BHB and serum P411 and milk P424 concentrations, respectively. Earlier studies demonstrated that low P4 concentrations are related to the NEB, and in turns are associated with low conception rates along with increased embryonic death.[6,9,34] However, when animals of the present study were grouped into EOV and DOV, no group differences in terms of the start of ovarian activity were detected for plasma concentrations of NEFA and BHB, i.e., delayed resumption of ovarian activity cannot exclusively be attributed to differences in feeding or metabolic stress. This result is consistent with observations from a field study where the start of cyclicity was not different despite differences in metabolic status.[26] Nonetheless, it needs to be emphasized that high concentrations of NEFA and BHB during the early lactation period have a negative impact on the immune system, conception success, and were partly shown to have toxic effects on the oocyte consequently resulting in poor reproductive performance.[8,16,17,25] Hence, the supplementation of additional concentrate would improve energy balance and reduce the need to mobilize body reserves.

Plasma IGF-1 concentrations closely reflect the energy status of dairy cows, are indicative for nutrient partitioning towards the lactating mammary gland[3,8] and conception rate.[23] IGF-1 plays a significant role in follicular growth, mediation of follicle stimulating hormone (FSH) and LH effects up to oocyte development and maturation.[15,18] Dairy cows with reduced IGF-1 concentrations took longer to resume ovarian activity as less of the first dominant follicles came to ovulation.[1,23,36] Confirming our results, Wathes et al.[35] did not see an effect of circulating IGF-1 concentrations and the time to the rise of P4, although we could show that concentrate omission further reduced plasma IGF-1 concentrations.

Besides the negative impact of an energy deficiency on the commencement of luteal activity, further adverse consequences of low glucose and elevated plasma concentrations of NEFA and BHB for follicle and oocyte quality were identified,[15,36] which are beyond the scope of the present study. In particular high-yielding dairy cows showed greater BHB concentrations when no additional concentrate was supplied.[37] The feeding of supplementary concentrate in herbage-based feeding systems, however, clearly improved the metabolic status of cows potentially benefitting fertility of dairy cows.

Conclusions

Cows without supplementary concentrate experienced a more pronounced energy deficiency and had greater circulating concentrations of NEFA and BHB. Consequently, resumption of ovarian activity was further delayed by concentrate omission. Thus, concentrate supplementation is important not only to limit the energy deficiency but also for a fast resumption of luteal activity in cows in herbage-based feeding systems. Concentrate supplementation to support general health is most effective in cows with a high genetic production potential. Potential adverse carry-over effects and consequences of concentrate omission for follicular and oocyte quality and finally reproductive performance are likely, but cannot be conclusively clarified by the present study because the experimental period covered only the first two months of lactation not following insemination success.

Acknowledgements

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Reprise postpartale de l'activité ovarienne chez les vaches laitières: implications pour les systèmes d'alimentation à base d'herbe en Suisse

En Suisse, la production laitière est principalement basée sur une alimentation à base d'herbe avec peu d'apport en concentrés. La présente étude a examiné les effets d'un régime alimentaire exclusivement à base d'herbe avec (C) et sans (nC) supplémentation de concentrés sur l'activité lutéale, la production laitière et le statut métabolique de 23 vaches laitières Holstein multipares ayant une reprise précoce ou retardée de la cyclicité ovarienne post-partum (pp). Les vaches ont été assignées rétrospectivement soit à un groupe avec une reprise précoce (jusqu'à 25 jours pp, EOV), soit retardée de l'activité ovarienne (>30 jours pp, DOV), résultant en quatre sous-groupes en fonction de la supplémentation en concentrés: DOV-C, DOV-nC, EOV-C, EOV-nC. La concentration de progestérone (P4) dans le lait a été mesurée tous les 3 jours et différents métabolites ont été analysés dans des échantillons sanguins hebdomadaires. La reprise de l'activité ovarienne a été détectée entre le jour 19 et 25 pp en EOV et entre le jour 30 et 60 pp en DOV. En DOV-C, la reprise de la cyclicité avait tendance à commencer plus tôt ($38{,}3 \pm 1{,}7$ jour pp) par rapport à DOV-nC ($45{,}2 \pm 6{,}3$ jours pp; $P = 0{,}10$). Indépendamment du groupe, l'activité ovarienne est survenue plus tard chez les vaches avec des concentrations plasmatiques de β-hydroxybutyrate (BHB) plus élevées

Ripresa post-partum dell'attività ovarica nelle vacche da latte: implicazioni per i sistemi di alimentazione a base di erba in Svizzera

La produzione di latte in Svizzera si basa principalmente su un'alimentazione a base di erba con un minimo apporto di foraggio concentrato. Il presente studio ha esaminato gli effetti di una dieta esclusivamente a base di erba con (C) o senza integrazione di foraggio concentrato (nC) sull'attività luteale, la produzione di latte e lo stato metabolico in 23 vacche da latte multipare di razza Holstein con ripresa precoce o ritardata della ciclicità ovarica post partum (pp). Le vacche sono state assegnate retrospettivamente a un gruppo con ripresa precoce (fino a 25 giorni pp, EOV) o ritardata (>30 giorni pp, DOV) dell'attività ovarica, che ha portato a quattro sottogruppi a seconda dell'alimentazione a foraggio concentrato o no: DOV-C, DOV-nC, EOV-C, EOV-nC. La concentrazione di progesterone nel latte (P4) è stata misurata ogni 3 giorni, e diversi metaboliti sono stati analizzati in campioni di sangue settimanali. La ripresa dell'attività ovarica è stata rilevata tra 19 e 25 giorni in EOV, e tra 30 e 60 giorni pp in DOV. In DOV-C, la ripresa della ciclicità tendeva ad iniziare prima ($38{,}3 \pm 1{,}7$ giorni pp) rispetto a DOV-nC ($45{,}2 \pm 6{,}3$ giorni pp; $P = 0{,}10$). Indipendentemente dal gruppo, l'attività ovarica è subentrata più tardi nelle vacche con maggiori concentrazioni plasmatiche di β-idrossibutirrato (BHB) ($P < 0{,}05$). La concentrazione di P4 nel latte al non differiva tra i gruppi al primo incremento >1 ng/

(P<0,05). La concentration de P4 dans le lait ne différait pas entre les groupes lors du premier pic de>1ng/ml (P>0,05), tandis que les pics de P4 dans le lait mesurés tout au long de l'expérience étaient plus élevés dans le groupe EOV par rapport au groupe DOV (P<0,05). Les vaches de DOV-nC ont présenté le bilan énergétique le plus négatif au cours des 3 premières semaines pp avec simultanément la glycémie plasmatique la plus faible et les concentrations d'acides gras non estérifiés et de BHB les plus élevées. Les concentrations de facteur de croissance analogue à l'insuline-1 étaient plus faibles chez les vaches sans supplémentation en concentrés, mais n'étaient pas liées au groupement EOV ou DOV (P=0,61). Dans l'ensemble, la supplémentation en concentrés chez des vaches affouragées à base d'herbe a abouti à une reprise de l'activité lutéale plus précoce. Lors d'une limitation de l'apport énergétique et nutritionnel, la reprise de l'activité ovarienne chez les vaches présentant un DOV était encore davantage retardée.

Mots clés: première ovulation, alimentation en herbe, métabolisme, reproduction, reprise de l'activité ovarienne

ml (P>0,05), ma i picchi di P4 nel latte durante l'esperimento erano più elevati in EOV che in DOV (P<0,05). Le vacche DOV-nC hanno mostrato il bilancio energetico maggiormente negativo durante le prime 3 settimane pp con il glucosio plasmatico più basso in concomitanza con le più alte concentrazioni di acidi grassi nonesterificati e BHB. Le concentrazioni del fattore di crescita insulino-simile 1 erano inferiori nelle vacche senza integratore di foraggio concentrato, ma non in relazione a EOV o DOV (P=0,61). Nel complesso, l'integrazione di foraggio concentrato ha causato un inizio precoce dell'attività luteale nelle vacche con alimentazione a base di erba. Quando l'apporto di energia e di nutrienti veniva limitato, la ripresa dell'attività ovarica nelle vacche in DOV era ulteriormente ritardata.

Parole chiave: prima ovulazione, alimentazione con erba, metabolismo, riproduzione, ripresa dell'attività ovarica

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