1.

Early prepartum classification of high versus normal body condition in dairy cows leads to pronounced metabolic differences post partum

Frühzeitige präpartale Einteilung von Milchkühen anhand der Körperkondition, führt zu erhöhten metabolischen Unterschieden post partum

*Schuh K., Koch C., Dusel G., Frahm J., Dänicke S., Sauerwein H., Sadri H. – Bonn/Bingen a. R./Münchweiler a. d. Alsenz/Braunschweig

For dairy cows overconditioning before calving is well known to result in excessive lipolysis with elevated blood concentrations of free fatty acids (NEFA) and ketone bodies and thus an increased risk for metabolic diseases. For experimental purposes feeding above energy requirements during the dry period is mostly used to induce overcondition, but did not always result in significant elevations of body condition or circulating NEFA concentrations, respectively [1-2]. The success of such elevated feeding regimen to cause overconditioning seems to be more probable if cows were preselected according to their body condition at drying off [2-3], thus indicating a genetic or epigenetic component for accumulating body fat. With this background we aimed to group dairy cows according to their body condition well before the dry period, i.e. during late lactation, and to feed them for enhancing or maintaining their high or normal body condition score (BCS) when still lactating, respectively, but not when dry, to test whether the difference would be sustained into lactation.

Methods: Fifteen weeks (wk) before the anticipated calving, 38 multiparous, pregnant Holstein Frisian cows were allocated in either a high (HBCS) or normal (NBCS) BCS-group according to their actual BCS and back fat thickness (BFT); while pre-selected for their history (previous lactation) of body condition. From then on, HBCS and NBCS cows were fed with either a high energy diet (7.1 NEL MJ/kg DM) or a low energy diet (6.6 NEL MJ/kg DM), respectively, until dry off. Thereafter, all cows received the same ration. Individual dry matter intake (DMI) was recorded with an electronic feeding system from d 21 ante partum (ap) until d 100 postpartum (pp), while feed was always offered ad libitum. During the entire study, BCS and BFT were obtained biweekly, while body weight (BW) before calving was recorded weekly. After calving, milk yield and BW were recorded daily; milk composition was analyzed weekly. In addition, weekly blood samples were collected from d 49 ap to d 84 pp. The serum concentrations of NEFA, ß-hydroxybutyrate (BHB), and glucose were quantified using an automatic analyzing system, based on a photometric measurement (Eurolyser, Type VET CCA, 5020 Salzburg, Austria). The data were analyzed by the mixed model procedure (SPSS, version 21.0) with Bonferroni correction. Calculated correlations were made using Pearson. Level of significance was set at $p \leq 0.05$. The data are presented as mean ± SE.

Results: Early allocation of cows to either a HBCS or a NBCS group led to significant differences in BCS and BFT ($p < 0.001$), that were maintained over the whole observation period. Considerable losses of BCS and BFT ($p \leq 0.001$) were observed in HBCS (BFT: 11 ± 0.9 mm) cows 7 wk after calving compared to NBCS cows (BFT 5 ± 0.8 mm). Accordingly, energy balance (EB) was more negative in HBCS cows ($p < 0.05$) several wk around calving. Contrastingly, DMI was higher in NBCS cows ($p < 0.05$) 3 wk before and 2-4 wk after calving. The serum concentrations of NEFA were increased in HBCS ($p < 0.05$) in the first six wks after calving. Additionally, BHB concentrations were greater ($p < 0.05$) in HBCS cows from 2 to 5 wk pp, peaking in wk 3 (HBCS: 1.6 ± 0.8 mmol/L; NBCS: 1.1 ± 0.5 mmol/L). Thereby, BHB increased more than 98 % for HBCS cows compared to wk 1 ap. The EB and NEFA values were inversely related ($p = 0.001$; R^2=-0.30), and NEFA and BHB were positively associated ($p < 0.001$; R^2=0.30). Similarly, serum glucose concentrations were positively correlated with BCS and BFT. However, milk yield, energy-corrected milk and milk components showed only weak differences between the groups.

Conclusion: Differences in BCS evolving during lactation and boosted by feeding in late lactation were shown to be maintained until the subsequent lactation, even in absence of differential feeding during the dry period. Group differences were confirmed by NEFA and BHB concentrations. Thus, allocating pre-selected late lactation-cows to a high or a low energy diet before dry off, is an appropriate approach to achieve target BCS at calving.

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**University of Bonn, Institute of Animal Science, Physiology and Hygiene Unit, Katzenburgweg 7-9, 53115 Bonn, Germany, kschuh@uni-bonn.de*

2.

Effects of elevated BHB concentrations on glucose metabolism in dairy cows before and after parturition

Auswirkungen von erhöhten BHB-Konzentrationen auf den Glukosestoffwechsel von Milchkühen vor und nach der Abkalbung

*Gross J., Zarrin M., Grossen-Rösti L., Bruckmaier R. – Bern/Yasouj

Recent studies in mid- and late lactating dairy cows showed that beta-hydroxybutyrate (BHB) infusion had considerable impact on glucose metabolism, and immune response during intramammary lipopolysaccharide challenge. The objective of the present study was to infuse BHB during the dry period and after parturition to investigate the effects of elevated plasma BHB concentrations on metabolism and endocrine changes in transition dairy cows. The hypothesis tested was that regulation of glucose metabolism changes at different physiological stages and an additional elevation of BHB concentration alters glucose concentration.

Methods: Multiparous Holstein cows in wk -2 (a.p.; n = 6) and wk +2 (p.p.; n = 8) relative to calving were infused (4 h from 0800 to 1200 h) with a BHB solution to increase plasma BHB concentration to 1.5 to 2.0 mmol/L (HyperB), and the same period on the next day without any infusion was assigned as control treatment (CON). Blood samples were taken 1 h before the start of infusion as reference samples and every 30 min during the following 6 h (4 h infusion and 2 h after the stop of infusion) in HyperB and on the control day, and analyzed for glucose, BHB, insulin, and glucagon concentrations. Statistical analysis was performed with SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA). Differences in basal concentrations of plasma metabolites and endocrine parameters between a.p. and p.p., and between control and infusion d in wk -2 and +2 relative to calving were evaluated using the MIXED procedure of SAS with time points (a.p., p.p.) and paired experimental days (HyperB, CON) as fixed effects. The individual cow was used as repeated subject in the statistical model.

Results: Plasma BHB concentration reached 1.7 ± 0.1 mmol/L (a.p.), and 1.6 ± 0.2 mmol/L (p.p.) in HyperB compared with 0.6 ± 0.1 mmol/L, and 0.6 ± 0.0 mmol/L in CON, respectively. The 4-h average BHB infusion rate was 12.4 ± 1.0 and 13.3 ± 0.9 µmol/kg BW/min in wk -2 and +2, respectively. BHB infusion caused a decrease of plasma glucose concentrations compared with pre-infusion levels both before and after parturition, which was not different between a.p. and p.p. infusion though basal glucose concentrations were different before and after calving (3.7 ± 0.1 vs. 3.2 ± 0.2 mmol/L). BHB infusion increased plasma insulin a.p. but not p.p. despite higher basal insulin concentration before than after parturition (29.0 ± 8.4 vs. 5.8 ± 0.8 µU/mL).

Conclusions: These findings show that effects of hyperketonemia on plasma glucose concentrations are independent of lactational stage, but endocrine adaptation to hyperketonemia differs before and after parturition. We assume that BHB is a metabolic key regulator in early lactating dairy cows, and may affect glucose concentration by further pathways beyond regulation via insulin and glucagon, such as gluconeogenesis and altered lipolysis.

*University of Bern, Veterinary Physiology, Vetsuisse Faculty, Bremgartenstrasse 109a, 3012 Bern, Switzerland, josef.gross@vetsuisse.unibe.ch

3.

Effects of intensive milk feeding and butyrate supplementation on the somatotropic axis in German Holstein calves

Einfluss einer intensiven Milchfütterung und einer Buttersäurezusatz auf die somatotrope Achse bei Kälbern der Rasse Deutsche Holstein

*Frieten D., Gerbert C., Koch C., Dusel G., Eder K., Mielenz B., Hoeflich A., Hammon H. – Münchweiler a. d. Alsenz/ Bingen/Giessen/Dummerstorf

In traditional feeding programmes calves are reared with restricted amounts of milk or milk replacer (MR), not able to exploit their full growth potential (1). Intensified colostrum and milk feeding maturates the somatotropic axis indicated by a reduced growth hormone/insulin-like growth factor (IGF)-I ratio in blood plasma and an elevated IGF-I gene expression in the liver (2). In addition, butyrate supplementation in calves induces accelerated feed efficiency and growth (3). Therefore we hypothesised that the combining effect of intensive MR feeding and butyrate supplementation stimulates energy metabolism and the somatotropic axis in preweaning calves.

Methods: 64 German Holstein calves (n=32 for male and female, respectively) were studied between June 2014 and June 2015 from birth until wk 11 of life. Calves were allocated to one of four feeding groups after measurement of birth weight and colostrum intake (2.5 kg from their dam). Subsequent feeding with transition milk from their dams was supplied *ad libitum* (Adlib; max. 25 L/d, n=32) or in restricted amounts (Res; 6 L/d, n=32) until d 4. Afterwards Adlib and Res groups were subdivided (n = 16/group) to MR feeding at 12.5 % dry matter with (ResB+; AdlibB+) or without 0.24 % Ca-/Na-butyrate (ResB-; AdlibB-) from d 4 on. Gradually weaning took place from wk 9 to 10, whereas 2 L/d of MR were offered until the end of trial. Calves were housed in an open straw-bedded stable with an automatic feeding system and had free access to water, hay and concentrate. Measurements of feed intake were performed daily and body weight was determined weekly. Blood samples for analysing IGF-I, IGF binding proteins (IGFBP), insulin and glucose were taken after birth, on d 2, 4 and 7, then weekly or biweekly (IGFBP) until wk 11 of life. Liver samples were taken on d 50 ± 2 (mean ± SD) and at the end of the study to measure gene expression of the somatotropic axis. Data were analysed by the Mixed Model of SAS with feeding regimen, butyrate supplementation, time, and respective interactions as fixed effects.

Results: Except for the first colostrum intake (2.5 ± 0.09 kg) liquid feed consumption was much greater in Adlib than in Res groups ($P < 0.001$). Res had a greater concentrate intake ($P < 0.001$), but lower weight gain ($P < 0.001$) throughout the trial. Plasma concentrations of IGF-I, IGFBP-3, insulin and glucose were greater ($P < 0.01$) and plasma concentration of IGFBP-2 was lower ($P < 0.05$) in Adlib than in Res. Butyrate supplementation depressed ($P < 0.05$) plasma IGF-I from wk 1 - 4 and 9. On d 50, abundance of the hepatic form of the growth hormone receptor and of IGF-I was greater ($P < 0.01$) and mRNA abundance of IGFBP-2 was lower in Adlib than in Res. At the end of the study, IGFBP-2 mRNA abundance was greater in Adlib than in Res. Butyrate increased hepatic IGFBP-2 mRNA abundance at the end of the study.

Conclusions: *Ad libitum* MR feeding stimulated the systemic and hepatic somatotropic axis, which mirrored the greater growth rate during the intensive MR feeding. Butyrate supplementation did not stimulate growth performance but partly depressed the IGF/IGFBP system.

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*Education and Research Centre for Animal Husbandry, Berlinstrasse 109, 55411 Münchweiler an der Alsenz, Greenland, d.frieten@th-bingen.de

4.

Organ and epithelial growth in the gastrointestinal tract of Holstein calves fed milk replacer *ad libitum* and supplemented with butyrate

Organ- und Epithelwachstum im gastrointestinalen Trakt bei Kälbern der Rasse Holstein mit ad libitum Milchfütterung und Butyratzusatz

*Gerbert C., Frieten D., Koch C., Dusel G., Eder K., Zitnan R., Hammon H. – Münchweiler a. d. Alsenz/Bingen/Giessen/Kosice/Dummerstorf

Previous studies have shown that an elevated milk feeding intensity during the first weeks of life play an important role for growth and development in preweaning calves (1,2). Furthermore, butyric acid (BA) is known to stimulate intestinal cell growth and maturation, especially in the small intestine (3). We hypothesized that a combination of intensive milk replacer (MR) feeding and BA supplementation expedites the development of the gastrointestinal tract as well as organ growth in preweaning calves.

Methods: German Holstein male calves (n=32) were studied from birth until d 81 (±2) of life. All calves received 2.5 kg colostrum during 2 hours after birth from their dams. For the first three days of life transition milk was offered twice a day and from day 4 onwards calves were fed MR (12.5 % dry matter) in amounts of either 6 l/d (Res; n=16) or *ad libitum* (Adlib; max. 25l/d; n=16) for 8 wk. In both feeding groups half of the calves (n=8/group) were fed MR with 0.24% Ca-/Na-BA (ResB+; AdlibB+) or same MR with no BA supplement (ResB-; AdlibB-). From wk 8 to wk 11 MR was linearly reduced in all calves to 2 kg MR/d. Hay, water and concentrate were offered *ad libitum*. At the end of the trial calves were slaughtered and liver, pancreas and kidney fat were weighted. The forestomaches were emptied, washed and weighted and the length of the small and large intestine was determined. Mucosa samples of the rumen (atrium, ventral sac, ventral blind sac) and small intestine (duodenum, proximal, middle and distal jejunum, ileum) were taken for histomorphometric measurement of rumen papilla and intestinal villus and crypt size. Data were analyzed by the Mixed Model of SAS with feeding regimen, BA supplementation, rumen/intestinal segment, and respective interactions as fixed effects and calf as random effect.

Results: Body weight at slaughter and kidney fat weight were greater ($P < 0.05$) but pancreas weight was lower ($P < 0.01$) in Adlib than in Res. The small intestine was 3.8 m longer ($P < 0.05$) in AdlibB- than in ResB-. No treatment differences were found for rumen papilla size, but villus circumference, surface and height in duodenum, proximal jejunum and ileum were greater ($P < 0.01$) in Adlib than in Res. Villus circumference, surface and height was greater ($P < 0.05$), except in duodenum, in B+ than in B-. Crypt depth was larger ($P < 0.05$) in Adlib than in Res in duodenum and proximal jejunum and was reduced ($P < 0.02$) by BA in ileum. The villus height/crypt depth ratio increased by *ad libitum* feeding and by BA and was greatest ($P < 0.05$) in AdlibB+ throughout the small intestine.

Conclusions: Intensive milk feeding increased body weight and small intestinal growth, whereas BA, partly together with intensive milk feeding, stimulated intestinal mucosal growth. These findings indicated an elevated maturation and absorbing capacity of the small intestine by intensive milk feeding and BA supplementation. Increased pancreas weight in Res calves may have compensated for insufficient nutrient supply. Ruminal mucosa was not affected by milk feeding regimen suggesting that intensive milk feeding did not impair rumen development.

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*Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, 67728 Münchweiler a. d. Alsenz, c.gerbert@web.de