Short communication: Circulating serotonin is related to the metabolic status and lactational performance at the onset of lactation in dairy cows

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) affects many physiological functions because it is involved in glucose and lipid metabolism, calcium homeostasis, and regulation of lactation in dairy cows. This study aimed to examine physiological differences in serum 5-HT concentrations (high vs. low) and their association with metabolic status and milk production at the onset of lactation. Twelve multiparous Holstein dairy cows were milked within 4 h of calving, and blood and milk samples were collected at the first 6 subsequent milkings after parturition and at the evening milkings on d 5, 8, 10, and 14. Cows were retrospectively divided into 2 groups (6 cows/group): low serum 5-HT (LSS) and high serum 5-HT (HSS) according to their calculated areas under the curve (AUC) for serum 5-HT for the entire experimental period (cut-off: 46,000 ng/mL × 324 h). Concentrations of 5-HT, free fatty acids (FFA), β -hydroxybutyrate (BHB), glucose, calcium, and IGF-1 were measured in blood. Milk was analyzed for fat, protein, lactose, and 5-HT concentrations. Milk yield was recorded at each milking and energy-corrected milk yield was calculated. Serum 5-HT concentrations were higher in HSS than in LSS [AUC (ng/mL × 324 h): 57,830 ± 4,810 vs. 25,005 ± 5,930]. The amount of energy-corrected milk was lower in HSS than in LSS. The HSS group produced less colostrum and had decreased milk yield, specifically during the first 6 milkings. Concentrations of FFA, BHB, and glucose in plasma did not differ between groups. Concentrations of IGF-1 in serum were elevated in HSS compared with LSS throughout the experiment. Total circulating calcium concentrations in serum tended to be higher in HSS than in LSS. Milk fat and protein yields were decreased in HSS compared with LSS. Milk 5-HT decreased overall during the experimental period, with LSS maintaining higher 5-HT concentrations than HSS until d 14 of

lactation. In conclusion, cows with high serum 5-HT concentrations showed a reduced metabolic load at the onset of lactation, concomitantly lower milk yield, and a reduced energy output via milk.

Key words: serotonin, metabolism, dairy cow, onset of lactation, calcium

Short Communication

To avoid hypocalcemia and other associated periparturient diseases (e.g., ketosis), robust regulation of calcium homeostasis around parturition is crucial. Recent studies revealed serotonin (5-hydroxytryptamine; **5-HT**) to be a key regulator of calcium homeostasis in dairy cows (Laporta et al., 2015; Weaver et al., 2016; Hernandez, 2017). In addition, in various studies conducted in mice and sheep, 5-HT increased plasma glucose as well as insulin concentrations (Sugimoto et al., 1990; Watanabe et al., 2014); in contrast, Sumara et al. (2012) observed increased lipolysis, resulting in elevated plasma free fatty acid (**FFA**) levels. Moreover, a role for 5-HT as a regulator of lactation has been proposed (Matsuda et al., 2004; Marshall et al., 2010; Collier et al., 2012).

So far, most recent investigations conducted in transition dairy cows have been based on manipulated 5-HT concentrations by infusion of a 5-HT precursor (Weaver et al., 2016; Hernández-Castellano et al., 2017). The objective of this study was to investigate whether differences in physiological 5-HT serum concentrations (without exogenous manipulation) are associated with alterations in calcium homeostasis, metabolic status, and milk production in dairy cows during the first 14 d after parturition. We hypothesized that higher circulating serum 5-HT concentrations are accompanied by a reduced metabolic load at the onset of lactation in dairy cows.

The present experiment followed Swiss law on animal protection and was approved by the Committee of Animal Experiments, Canton of Fribourg, Switzerland (permit no. 2013-01-FR). Twelve multiparous Holstein dairy cows were studied during the first 2 wk of lactation. Cows were housed in a freestall barn at the

Received February 22, 2018.

Accepted August 10, 2018.

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research station Agroscope (Posieux, Switzerland). Approximately 7 d before parturition, cows were moved to straw-bedded calving pens and transferred back to the herd on d 2 after calving. Cows were machine milked twice daily at 0530 and 1630 h, and milk yield of individual milkings was recorded. For all our sampling events until d 14 of lactation, cows were separated from the herd shortly before milking, milked with the same bucket unit in the calving pens, and transferred back to the herd immediately after blood sampling and milking.

The diet of dry cows consisted of hay and fresh herbage *ad libitum* and 0.5 kg of mineral supplementation daily until calving. In the first week of lactation, cows were fed roughage, 0.3 kg/d of a mineral mix (including 107.7 ± 4.8 g/kg DM of calcium) for lactating cows, 2 kg/d of protein-rich concentrate (based on soybean meal, 8.06 ± 0.14 MJ of NE_L /kg of DM, 189 ± 16 g/kg DM of available CP at the duodenum), and 0.5 kg/d of a cereal-based energy concentrate (based on wheat and barley, 8.11 ± 0.12 MJ of NE_L /kg of DM, 81 ± 7 g/kg DM of available CP at the duodenum), which was supplied in an amount of 2 kg/d in the second week of lactation. Each cow received 2 oral calcium boli (390 g net weight each, immediately after parturition and at 4 h after parturition; 142 g of calcium/kg as provided by 24% $CaCO_3$ and 13.6% $CaCl_2$; Calci-For, Multiforsa AG, Auw, Switzerland). To prevent suckling, calves were separated directly after calving from their dams.

Blood and composite milk sampling was performed 4 h after parturition (± 15 min due to close monitoring of calving time by staff and cameras during day and night) and thereafter twice daily at regular milking times (0530 and 1630 h) for the next 5 milkings. Additional samples were taken during the afternoon milking on d 5, 8, 10, and 14 postpartum. Blood samples were collected from the jugular vein 20 min after the start of milking using evacuated tubes (1 EDTA-coated tube and 1 serum tube containing a clot activator; Vacuette, Greiner Bio One, Kremsmünster, Austria). The EDTA samples were placed on ice until centrifugation and the serum samples were allowed to clot for 30 min at room temperature before centrifugation at $2,500 \times g$ (20 min, $4^\circ C$) to harvest plasma and serum, respectively. Aliquots of serum (analysis of 5-HT, calcium, and IGF-1) and milk samples (analysis of 5-HT) were stored at $-80^\circ C$, whereas plasma and further milk samples (for analysis of other factors) were frozen at $-20^\circ C$.

Plasma glucose concentrations were measured using a commercially available assay from bioMérieux (Geneva, Switzerland; #61269). Free fatty acids and BHB concentrations in plasma were assessed using the commercially available kits #FA 115 and RB 1007, respectively (both from Randox Laboratories Ltd., Schwyz, Switzerland). Serum calcium concentrations

were analyzed with a commercially available assay from DiaSys Diagnostic Systems (#CALC AS, Holzheim, Germany). Serum 5-HT concentrations were measured with a commercial ELISA kit (Serotonin ELISA Fast Track, #BA E-8900; Rocky Mountain Diagnostics, Colorado Springs, CO) according to the manufacturers' instructions. Intra- and interassay coefficients of variation (CV) for 5-HT were <5 and $<15\%$, respectively. The analysis of 5-HT was performed in serum, which allowed the platelets to release most of their contents. Although 5-HT is synthesized in various tissues of the body, the measured circulating 5-HT in serum reflects the overall current 5-HT synthesis. Insulin-like growth factor-1 in serum was measured by RIA as described previously by Vicari et al. (2008). Intra- and interassay CV of IGF-1 RIA were $<10\%$.

Average concentrations of fat, protein, and lactose in milk samples were measured by a FTS Infrared Milk Analyzer (Bentley Instruments Inc., Chaska, MN; Milchprüfing Baden-Württemberg e.V. Ravensburg, Germany). Energy-corrected milk yield was calculated according to Sjaunja et al. (1990). Milk concentrations of 5-HT were analyzed using a commercial ELISA kit as described above. Intra- and interassay CV for 5-HT were <5 and $<15\%$, respectively.

For comparison of individual serum 5-HT profiles defined by the scheduled sampling events covering the experimental period during the first 14 DIM, the area under the curve (AUC) of serum 5-HT concentrations was calculated for each cow using the trapezoidal rule. Cows were retrospectively divided into 2 groups based on their circulating 5-HT concentrations according to the median of the determined AUC. Six cows each were assigned to the high serum 5-HT at a cut-off value of $46,000 \text{ ng/mL} \times 324 \text{ h}$ [HSS; AUC of 5-HT ($\text{ng/mL} \times 324 \text{ h}$): $57,830 \pm 4,810$; range from 53,719 to 73,674] and the low serum 5-HT group [LSS; AUC of 5-HT ($\text{ng/mL} \times 324 \text{ h}$): $25,005 \pm 5,930$; range from 7,522 to 38,142]. Parities were 3.7 ± 0.2 for LSS and 3.5 ± 0.6 for HSS; and milk yield of the previous lactation was $8,733 \pm 392$ in LSS and $8,803 \pm 1,022$ kg in HSS (mean $\pm$ SEM). Parity number and previous lactation yield did not differ between LSS and HSS.

Statistical analysis was performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC). Data presented are means $\pm$ standard error of the means, except where noted to be standard deviations. All data were checked for normal distribution and log-transformed if normal distribution could not be assumed. The MIXED procedure was used to evaluate the effects of high and low serum 5-HT concentrations on blood metabolites, IGF-1, milk yield and milk composition, with time, group, and time $\times$ group interaction as fixed effects and the individual cow as repeated subject. Parity was

originally included as a fixed effect in the model but consequently removed due to lack of power because only a relationship to calcium concentrations at the day of calving was found. Differences between groups (HSS and LSS) were estimated with the Tukey-corrected *t*-test at *P*-values < 0.05. Group differences at individual sampling events were assessed with the TTEST procedure of SAS. Significant effects were considered at *P* < 0.05 and tendencies toward significance were assumed up to *P* ≤ 0.10.

Based on the grouping according to AUC of serum 5-HT concentrations, cows assigned to the HSS group had elevated 5-HT concentrations [AUC of 5-HT (ng/mL × 324 h): 57,830 ± 4,810] compared with the LSS group [AUC of 5-HT (ng/mL × 324 h): 25,005 ± 5,930; *P* < 0.0001]. Differences in endogenous 5-HT concentrations between groups were persistent throughout the experimental period (*P* < 0.0001, Figure 1A). Grouping according to circulating 5-HT concentrations was associated with increased serum IGF-1 concentrations in HSS cows compared with LSS cows during the entire study period (*P* < 0.001, Figure 1B).

Plasma concentrations of FFA tended to decrease over time in both groups (Figure 2A). In contrast, plasma BHB concentrations increased steadily during the experiment, whereas plasma glucose concentrations decreased markedly from the first to the fourth milking (Figures 2B and 2C). Concentrations of FFA, BHB, and glucose in plasma were not associated with serum 5-HT concentrations (Figures 2A, 2B, and 2C, *P* > 0.05). Total calcium concentrations in serum increased from the third milking to d 5 postpartum and remained at a similar concentration until d 14 postpartum (Figure 2D). Although grouping into HSS and LSS was not related to calcium concentrations in serum, HSS tended toward increased calcium concentrations compared with LSS (*P* = 0.12).

Grouping by circulating 5-HT concentration identified increased milk yield (*P* < 0.05) and increased ECM (*P* < 0.01) in LSS cows compared with HSS cows (Figures 3A and 3B). Colostrum yield and ECM obtained at the first milking were decreased in HSS cows compared with LSS cows (*P* < 0.05 and *P* < 0.01, respectively). Milk yield for the first consecutive 6 milkings, spanning the period with a marked rise in milk production, was higher in LSS cows than in HSS cows (*P* < 0.05). Thereafter, milk yield did not differ between groups.

Milk fat and protein yields were increased in LSS cows compared with HSS cows (*P* < 0.01, data not shown) whereas lactose yield did not differ between groups (*P* = 0.17, data not shown). Milk 5-HT concentrations decreased over time in both LSS and HSS cows (Figure 3C). Higher milk 5-HT concentrations were

observed for LSS cows compared with HSS cows during the entire study period (*P* < 0.001).

In cows receiving repeated 5-hydroxy-L-tryptophan infusions before parturition, resulting in a 3- to 6-fold increase in serum 5-HT concentrations (reaching 5-HT concentrations comparable to those of the HSS cows of the present study), milk production at the beginning of lactation was not altered (Weaver et al., 2016; Hernández-Castellano et al., 2017). However, the colostrum yield in Hernández-Castellano et al. (2017) was decreased in cows treated with 5-hydroxy-L-tryptophan. In agreement with those authors, we observed decreased colostrum yield in cows with higher basal 5-HT concentrations. Moreover, HSS cows in

Figure 1. Serotonin (5-hydroxytryptamine, 5-HT; A) and IGF-1 (B) concentrations in blood of cows with low serum 5-HT (LSS) and high serum 5-HT (HSS) at the first 6 consecutive milkings after parturition (p.p.) and on d 5, 8, 10, and 14 of lactation. Differences for serum 5-HT concentrations between HSS and LSS at individual sampling events are indicated by ** (*P* < 0.01), * (*P* < 0.05), and + (0.05 ≤ *P* ≤ 0.10). Data are presented as means ± SEM.

the present study produced less milk during the first 2 wk of lactation, particularly from the second to the sixth milking postpartum. The reduced colostrum yield in HSS cows could be related to a prolonged effect of 5-HT on colostrum synthesis (Hernández-Castellano et al., 2017). Different mechanisms have been proposed to explain the effects of 5-HT on milk production, such as a reduction of mammary blood flow due to the vasoconstrictive effect of 5-HT on blood vessels (Busk et al., 1999). However, based on previously published results (Kessler et al., 2014), milk yield during the first 5 d of lactation is not related to subsequent milk production. Therefore, a reduced increase in milk yield during the first 3 d of lactation associated with the concomitant higher circulating 5-HT concentrations might be beneficial by potentially reducing metabolic load and therefore reducing the risk of developing health disorders. The relation of different physiological 5-HT

concentrations with long-term lactational performance needs to be clarified.

In the present study, cows with increased 5-HT concentrations tended to have elevated circulating calcium concentrations, except on the day of calving. These results are in agreement with Moore et al. (2015), who also detected only weak correlations between serum calcium concentrations and 5-HT concentrations between d 6 and 10 postpartum. In our study, parity additionally affected serum calcium concentrations, but only on the day of calving. Hence, potentially higher calcium concentrations as observed in cows with increased 5-HT concentrations might alleviate (subclinical) hypocalcemia and reduce incidence of associated diseases (e.g., retained placenta, ketosis; Rodríguez et al., 2017).

Different serum 5-HT concentrations in the present study were not associated with circulating FFA or BHB in early lactation, confirming observations from other

Figure 2. Plasma concentrations of free fatty acids (FFA; A), BHB (B), and glucose (C) and serum calcium concentration (D) in cows with low or high serum serotonin (5-hydroxytryptamine, 5-HT; LSS or HSS) at the first 6 consecutive milkings after parturition (p.p.) and on d 5, 8, 10, and 14 of lactation. Differences for plasma FFA, BHB, and glucose and serum calcium concentrations between HSS and LSS at individual sampling events are indicated by * ($P < 0.05$) and + ($0.05 \leq P \leq 0.10$). Data are presented as means $\pm$ SEM.

Figure 3. Milk yield (A), ECM (B), and 5-hydroxytryptamine (5-HT, serotonin) concentration (C) in milk in cows with low serum 5-HT (LSS) and high serum 5-HT (HSS) at the first 6 consecutive milkings after parturition (p.p.) and on d 5, 8, 10, and 14 of lactation. Differences for milk yield, ECM, and 5-HT concentration in milk between HSS and LSS at individual sampling events are indicated by ** ($P < 0.01$), * ($P < 0.05$), and + ($0.05 \leq P \leq 0.10$). Data are presented as means $\pm$ SEM.

studies (Hernández-Castellano et al., 2017; Weaver et al., 2017). Although recent findings demonstrated that elevated 5-HT improved glucose homeostasis (Sumara et al., 2012; Watanabe et al., 2014; Laporta et al., 2015), our study did not reveal higher plasma glucose concentrations in cows showing higher endogenous 5-HT.

Cows in the HSS group in the present study exhibited higher serum IGF-1 concentrations than cows in the LSS group. Reduced IGF-1 concentrations were associated with lower milk yield and reduced concentrations of FFA and BHB during periods of energy shortage as at the onset of lactation (Lucy et al., 2009; Grala et al., 2011; Gross and Bruckmaier, 2015). Hence, cows with higher circulating 5-HT concentrations displaying higher IGF-1 concentrations could have an improved capacity to cope with the metabolic load imposed by the onset of lactation with a temporarily lower milk yield.

Whereas earlier studies revealed no obvious relationship between milk fat content and increased exposure of the mammary gland to 5-HT (Hernandez et al., 2008, 2011), the present study identified reduced fat and protein yields, resulting in reduced energy output via milk, in cows with elevated circulating 5-HT during the entire experimental period.

Serotonin is not only present in blood, but is also detectable in milk, at least during the first week of lactation (Moore et al., 2015). We clearly emphasize the extremely low 5-HT concentrations in milk compared with 5-HT concentrations in blood. In our study, 5-HT concentrations were highest in colostrum and decreased thereafter to low levels in mature milk, with LSS showing higher concentrations in milk during the first 2 wk of lactation. Likewise, higher 5-HT concentrations in milk were found in prepartum milked colostrum of cows with low circulating 5-HT around parturition (Laporta et al., 2014). Although these observations on the inverse pattern of 5-HT in serum versus milk are characterized by manifold (200 to 400 times) higher concentrations in serum, further research is warranted to investigate the biological relevance of the marginal 5-HT amounts secreted into colostrum and milk.

In conclusion, cows with higher 5-HT concentrations showed lower milk production and reduced energy output via milk during the colostrum period compared with cows with low serum 5-HT concentrations. We further observed that increased circulating 5-HT concentrations were associated with increased IGF-1 concentrations and likely higher circulating calcium concentrations. However, because of the small number of animals enrolled, the expected improved metabolic situation during the very first days of lactation in cows with elevated 5-HT concentrations requires further investigation in a larger population to determine effects on long-term

animal health and lactational performance. Moreover, we confirmed the inverse relationship between serum and milk 5-HT concentrations, despite the tremendous differences in their magnitude.

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