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Short communication: Supplementation of colostrum and milk with 5-hydroxy-L-tryptophan affects immune factors but not growth performance in newborn calves

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ABSTRACT

In ruminants, colostrum is the main source of immunoglobulins for the newborn animal, conferring immune protection until the immune system becomes active and able to synthesize its own immunoglobulins. Serotonin (5-HT), a biogenic amine derived from tryptophan, has stimulatory effects on many physiological processes, including components of the innate (mastocytes, eosinophils, and natural killer cells) and adaptive (T and B lymphocytes) immune systems. Based on the known effects of 5-HT on the immune system, we hypothesized that increased concentrations of 5-HT, through administration of its precursor 5-hydroxy-L-tryptophan (5-HTP), may positively affect development of the calf's immune system and therefore support health and growth performance during the first weeks of life. Eighteen calves were randomly assigned to 1 of 2 experimental groups (control and 5-HTP), resulting in n = 9 per treatment group. Both groups received 2 colostrum meals from a common pool of colostrum. Thereafter, calves were fed milk replacer twice daily for 30 d. In the 5-HTP group, colostrum and milk replacer were supplemented with 1.5 mg of 5-HTP/kg of birth weight during the first 15 d after birth. Body weight was recorded at birth and on d 5, 10, 15, and 30 after birth. Blood samples were collected every morning (0800 h) before feeding from birth until d 5 and then on d 7, 9, 11, 13, 15, and 30 after birth. Serum 5-HT concentrations were increased as a consequence of the 5-HTP supplementation. Plasma immunoglobulin G concentrations did not differ between groups throughout the experimental period. The blood mRNA abundance of several factors related to the innate and adaptive immune system [nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), serum amyloid A-1 (SAA1), chemokine C-C motif ligand 5 (CCL5), cyclooxygenase 2 (PTGS2), haptoglobin (HP), and IL-1β] were increased in calves supplemented with 5-HTP. Supplementation of 5-HTP did not affect any of the measured metabolites (fatty acids and glucose) or minerals (calcium and magnesium) or milk feed intake, feed conversion ratio, and growth. In conclusion, 5-HTP supplementation induced an increase of 5-HT concentrations in blood and caused an increase in mRNA abundance of several factors related to the innate and adaptive immune systems, which might increase the protection of the calf against external agents.
Key words: serotonin, calves, immune system, colostrum

Short Communication

The first few days after birth are among the most critical in the life of the calf, in which morbidity and mortality are the greatest (Mee, 2013). In ungulates, the transfer of immunoglobulins from the dam to the fetus through the placenta is limited. Therefore, colostrum is the main source of immunoglobulins in these species, providing immune protection until the immune system of the newborn animal becomes active and efficient (Erhard et al., 1999; Baumrucker and Bruckmaier, 2014; Hernández-Castellano et al., 2015b). Colostrum contains a complex mixture of proteins that actively participate in the protection of the neonate against pathogens and other postpartum environmental challenges (Blum and Hammon, 2000; Bendixen et al., 2011; Hernández-Castellano et al., 2015a), with IgG being the most abundant bioactive component (Butler, 1973; Hernández-Castellano et al., 2016). Serotonin (5-hydroxytryptamine, **5-HT**) is a neurotransmitter synthesized from 5-hydroxy-L-tryptophan (**5-HTP**), a hydroxylated derivative of the amino acid tryptophan, which contributes to the regulation of physiological processes in the central nervous system such as those related to mood or appetite. Most of the peripheral 5-HT is synthesized by the enterochromaffin cells in the gut (Gershon et al., 1965); however, 5-HT is also

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synthesized by mammary epithelial cells (Hernandez et al., 2009) and by platelets (Lesurtel et al., 2006). Peripheral 5-HT participates in many physiological processes including calcium homeostasis (Weaver et al., 2016; Hernández-Castellano et al., 2017a) and the modulation of glucose and lipid metabolism (Sugimoto et al., 1990; Watanabe et al., 2014; Laporta et al., 2015). Additionally, 5-HT is involved in regulating several pathways of the innate immune system including the adhesion and chemotaxis of mastocytes in humans and mice (Kushnir-Sukhov et al., 2006), the chemotaxis of eosinophils in humans (Boehme et al., 2004), and the stimulation of cytolytic activity and proliferation of natural killer cells in humans (Evans et al., 2008; Hernandez et al., 2010). Serotonin also affects the adaptive immune system by stimulating the proliferation of T and B lymphocytes (Serafeim et al., 2002; León-Ponte et al., 2007). Based on the diverse effects of 5-HT on the immune system, we hypothesized that supplementation of colostrum and milk with 5-HTP may positively affect development of the calf's immune system and affect diverse metabolic pathways during the first weeks of life.

The present study was approved by the Cantonal Committee on Animal Experiments (Canton of Fribourg, Switzerland) and all experimental procedures followed Swiss law of animal protection. Eighteen newborn singleton dairy calves from the experimental herd of the Agroscope Institute for Livestock Science research farm (Posieux, Switzerland) were used in this study. At birth (d 0), animals were randomly assigned to 1 of 2 experimental groups: control and 5-HTP). During the first 2 h after birth, calves were dried, weighed, and ear tagged. Both groups were bottle-fed from a common pool of bovine colostrum containing 13.47% protein, 4.92% fat, 3.01% lactose, and a total IgG concentration of 53.7 mg/mL. Colostrum chemical composition was determined at the Suisselab AG Zollikofen (Zollikofen, Switzerland). The total IgG concentration in the pool of colostrum was measured using a commercial ELISA kit specific to bovine IgG (Bethyl Laboratories, Montgomery, TX). Each animal received 4 L of colostrum equally divided in 2 colostrum meals at 2 and 12 h after birth (2 L/meal). After the 2 colostrum meals, calves in both groups were fed using nipple buckets twice daily (0800 and 1800 h) with a commercial milk replacer, at 16%, specially formulated for calves (UFA 207 plus, UFA, Herzogenbuchsee, Switzerland; 95.5% DM, 23.6% CP, and 22.7% fat, air-dry powder basis) until d 30 after birth. In each meal, calves were fed until refusal, when the remaining milk replacer was measured and discarded. Colostrum and milk replacer for the 5-HTP group were supplemented with 1.5 mg of 5-HTP/kg of birth weight during the first 15 d following birth. From birth to d 15 after birth, daily individual feed intake was recorded. From d 15 to 30, both groups were fed with the same milk replacer without 5-HTP supplementation. Body weight was recorded at birth and on d 5, 10, 15, and 30 after birth. Calves were housed in individual calf hutches, providing a covered space of $1.7 \times 1.20 \times 1.50$ m and an uncovered space of $1.50 \times 1.50 \times 1.50$ m. Animal health status was monitored during the experimental period (for diarrhea, parasites, or fever), and all animals were found to be healthy throughout the experimental period.

Blood samples were collected every morning (0800 h) before feeding from birth through d 5 and then on d 7, 9, 11, 13, 15, and 30 after birth. Blood samples were taken by puncture from the jugular vein using vacuum tubes for serum and plasma (containing K_3-EDTA) collection. After sampling, tubes were stored either on wet ice for 15 min (plasma tubes) or at room temperature for 2 h (serum tubes) and then centrifuged at $2,500 \times g$ for 20 min at 4°C. Plasma and serum were then aliquoted and stored at −80°C. Additional blood samples for RNA isolation were collected at birth (d 0) and on d 15 and 30 after birth using PAXgene blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland). After sampling, the tubes were stored at 4°C for 12 h, stored at −20°C for 24 h, and finally stored at −80°C until RNA extraction. The concentration of IgG_1 and IgG_2 in plasma was measured using commercial ELISA kits specific to bovine IgG_1 and IgG_2, respectively (Bethyl Laboratories) with some modifications as described by Lehmann et al. (2015). Glucose and fatty acids (**FA**) plasma concentrations were measured using commercial kits from Randox (GL364 and FA115, respectively; Randox Laboratories Ltd., Schwyz, Switzerland). Total serum calcium concentrations were determined using a commercial kit from Diatools (DIA00461, Diatools AG, Villmergen, Switzerland). Serum magnesium concentrations were determined using a commercial kit from Randox (MG531, Randox Laboratories Ltd.). Serum serotonin concentrations were determined using a commercial 5-HT ELISA kit (IM1749, Beckman Coulter GmbH, Sinsheim, Germany). Extraction of total RNA was performed using PAXgene Blood RNA kit (PreAnalytiX) according to the manufacturer's instructions. The purity and quantity of RNA was measured using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Reverse transcription of 1 μg of total RNA was performed with the RNA-dependent DNA polymerase Moloney Murine Leukemia Virus Reverse Transcriptase RNase H Minus, Point Mutant (MMLV-RT; Promega Corp., Madison, WI) and random hexamer primers (Invitrogen, Leek, the Netherlands). The mRNA abundance of housekeeping genes (GAPDH and ubiquitin) and target genes