Biogenic amines: concentrations in serum and skeletal muscle from late pregnancy until early lactation in dairy cows with high versus normal body condition score

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Complete List of Authors:	Hosseini Ghaffari, Morteza; University of Bonn, Institute of Animal Science, Physiology and Hygiene Group Sadri, Hassan; University of Bonn, Institute of Animal Science, Physiology and Hygiene Group; University of Tabriz, Department of Clinical Sciences Schuh, Katharina ; University of Bonn, Institute for Animal Science Physiology & Hygiene; University of Applied Sciences Bingen, Animal Nutrition Dusel, G. ; University of Applied Sciences Bingen, Animal Nutrition Frieten, Dörte Frieten; University of Applied Sciences Bingen, Animal Nutrition Koch, Christian; Educational and Research Centre for Animal Husbandry, Hofgut Neumühle, Prehn, Cornelia; Helmholtz Zentrum München, German Research Center for Environmental Health Adamski, J.; Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg Sauerwein, Helga; Rheinische Friedrich Wilhelms Universitat Bonn, Institute for Animal Science, Physiology & Hygiene Unit;
Key Words:	body condition score, biogenic amines, transition cows

Interpretive summary: Biogenic amines: concentrations in serum and skeletal muscle from late pregnancy until early lactation in dairy cows with high versus normal body condition score. *By Ghaffari et al.* Biogenic amines (BA) have been well studied in dairy products, but their physiological functions and roles in dairy cattle remain to be clarified. We characterized the BA profile in serum and skeletal muscle (*M. semitendinosus*) of cows with high or normal BCS at dry-off. In both groups, serum concentrations of most BA followed time-related changes during the transition from late gestation to early lactation but those of muscle remained unchanged. Overconditioning at dry-off was associated with elevated serum concentrations of carnosine, alpha-aminoadipic acid, hydroxyproline, and putrescine postpartum, suggesting that adiposity may be linked to the postpartum metabolism of these BA.

RUNNING HEAD: BIOGENIC AMINES IN TRANSITION COWS

Biogenic amines: concentrations in serum and skeletal muscle from late pregnancy until early lactation in dairy cows with high versus normal body condition score

M. H. Ghaffari[1], H. Sadri[2*]†, K. Schuh[1,3], G. Dusel[3], Dörte Frieten[3], C. Koch[4], C. Prehn,[5] J. Adamski,[5,6,7] and H. Sauerwein[1]

[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, 53115 Bonn, Germany

[2]Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, 516616471 Tabriz, Iran

[3]Department of Life Sciences and Engineering, Animal Nutrition and Hygiene Unit, University of Applied Sciences Bingen, 55411 Bingen am Rhein, Germany

[4]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, 67728 Muenchweiler an der Alsenz, Germany

[5]Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 München-Neuherberg, Germany

[6]Lehrstuhl für Experimentelle Genetik, Technische Universität München, 85350 Freising-Weihenstephan, Germany

[7]German Center for Diabetes Research (DZD), 85764 München-Neuherberg, Germany

*Corresponding author: sadri@tabrizu.ac.ir

†H. Sadri was a visiting scientist at the Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, 53115 Bonn, Germany at the time the research was done.

ABSTRACT

Biogenic amines (BA) are a class of nitrogenous compounds, involved in a wide variety of physiological processes, but their role in transition cows is poorly understood. Our objectives were to describe the longitudinal changes of BA in serum and in skeletal muscle during the transition period and to characterize temporal responses of BA in relation to body condition score (BCS) of periparturient dairy cows. Fifteen weeks before calving, 36 multiparous Holstein cows were assigned to two groups (n = 18 per group) that were fed differently to reach either high [(HBCS; 7.2 NEL MJ/kg dry matter (DM)] or normal BCS (NBCS; 6.8 NEL MJ/kg DM) at dry-off. The targeted BCS and back fat thickness (BFT) at dry-off (HBCS: >3.75 and >1.4 cm; NBCS: <3.5 and <1.2 cm) were reached. Thereafter, both groups were fed identical diets. Blood samples and muscle (*M. semitendinosus*) biopsies were collected at d -49, +3, +21, and +84 relative to parturition. In serum and skeletal muscle, BA concentrations were measured using a targeted metabolomics assay. The data was analyzed as a repeated measure using the MIXED procedure of SAS. The serum concentrations of most BA [i.e., creatinine, taurine, carnosine putrescine, spermine, alpha-aminoadipic acid (alpha-AAA), acetylornithine (Ac-Orn), kynurenine, serotonin, hydroxyproline (t4-OH-Pro), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA)] fluctuated during the transition period, while others [i.e., spermidine, phenylethylamine] did not change with time. The muscle concentrations of BA remained unchanged over time. Creatinine had the highest concentrations in the serum, while carnosine had the highest concentration amongst the muscle BA. The serum concentrations of creatinine (d +21), putrescine (d +84), alpha-AAA (d +3), and t4-OH-Pro (d +21) were or tended be higher for HBCS compared with NBCS post partum. The serum concentrations of SDMA **(**d -49**)** and Ac-Orn (d +84) were or tended be lower for HBCS compared with NBCS, respectively. The serum

kynurenine/tryptophan ratio was greater in HBCS than in NBCS (d +84). Compared to NBCS, HBCS had lower muscle concentrations of carnosine, but those of t4-OH-Pro were higher (d -49). In both serum and muscle, the ADMA concentrations were greater in HBCS than in NBCS (d -49). No correlation was found between serum and skeletal muscle BA. This study indicates that overconditioning of dairy cows may influence serum and muscle BA concentrations in the periparturient period.

Key words: body condition score, biogenic amines, transition cows

INTRODUCTION

Biogenic amines (**BA**) are basic nitrogenous compounds formed by microbes, plants, and animals mainly through decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Medina et al., 2003). In un-fermented food, the presence of BA indicates microbial spoilage and is thus undesired. With respect to dairy foods, accumulation of BA in cheese, in particular, histamine, tyramine, putrescine, and, to a lesser extent cadaverine are considered as a risk for consumers` health (Linares et al., 2011). However, BA also comprise compounds that are physiological part of endogenous regulation processes, e.g., catecholamines such as adrenaline or dopamine, functioning as hormones and neurotransmitters. Through the activation of trace amine-associated receptor 1, some BA (comprising phenylethylamines, e.g., tyramine) also interact in transmission in dopaminergic, adrenegic, and serotonergic neurons in the central nervous system (Miller, 2011).

Biogenic amines, in particular polyamines (spermidine and spermine, as well as their precursor putrescine) have been related to beneficial effects on human health, because of their anti-

oxidant and anti-inflammatory attributes (Lagishetty and Naik, 2008). They are involved in essential cellular functions, including cell proliferation and differentiation (Kalac and Krausová, 2005) and in adipogenesis (Vuohelainen et al., 2010). Several studies have also pointed to a role of polyamines in glucose utilization, insulin sensitivity, and fat oxidation (Lockwood and East, 1974; Sadasivan et al., 2014). However, the concentrations of polyamines generally decline with aging in most organisms and this has been associated with a number of age-related health disorders in humans (Handa et al., 2018). It has been suggested that polyamines may increase longevity via stimulating the autophagic turnover of cytoplasmic organelles or aged proteins (Madeo et al., 2010). Our knowledge about BA in ruminants is mostly limited to dietary BA (Steidlová and Kalač, 2002, 2003), and the ruminal concentrations of BA in dairy cows (Ametaj et al., 2010; Saleem et al., 2012) or their effects on digestive function (Phuntsok et al., 1998; Wang et al., 2013). In contrast, the BA concentrations in blood or tissues of dairy cattle are hardly known. The BA present in serum of cattle originate from cellular syntheses and α-decarboxylation of specific amino acids in feed and digesta during ruminal fermentation (Bailey et al., 2002; Wang et al., 2013). Previous reports have suggested that ruminal microorganisms extensively metabolize dietary amines, and thus less exogenous amines can potentially be absorbed than contained in the diet (Phuntsok et al., 1998). Recently, Huber et al. (2016) found that elevated plasma concentrations of anti-inflammatory and anti-oxidative BA (i.e., carnosine, sarcosine, and spermidine) of transition cows were associated with increased productive lifespan.

As reported previously from this animal experiment (Schuh et al., 2019), cows calving with high BCS were metabolically challenged during early lactation because of a more severe negative energy balance and high mobilization of body reserves mainly lipids and to some extent proteins. In addition, high BCS at calving was associated with compromised antioxidative capacity,

reflected by numerically higher values of derivatives of reactive oxygen metabolites (dROM) as well as lower values of total ferric reducing antioxidant power (FRAP). In view of anti-oxidant and anti-inflammatory attributes of BA (Lagishetty and Naik, 2008), we hypothesized that over-conditioning at calving may be accompanied by altered BA profiles in blood of transition cows, in particular of polyamines. We also hypothesized that the serum concentrations of BA would correlate with those in skeletal muscle during the extensive mobilization of body reserves in early lactation. Therefore, we quantified the BA concentrations in blood serum and also in skeletal muscle tissue of Holstein cows during late gestation and early lactation using a targeted metabolomics approach and investigated whether over-conditioning at calving altered these compared with normal-conditioned cows.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

The experiment was conducted at the Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler a.d. Alsenz, Germany. All animal experiments were performed in accordance with the German Animal Welfare Act and were approved by the local authority for animal welfare affairs [Landesuntersuchungsamt Rheinland-Pfalz, (G 14-20-071)] Koblenz, Germany. The basic set-up of the trial with the performance results as well as the data of "classical" variables assessed in blood serum was already described by Schuh et al. (2018). In brief, 36 multiparous German Holstein cows were classified 15 weeks before their expected calving date as either normal-conditioned (**NBCS**; n = 18; average parity: 2.42 ± 1.84, mean ± SD) or over-conditioned (**HBCS**; n = 18; average parity: 3.37 ± 1.67, mean ± SD) cows. From week

15 to 7 before the anticipated calving date, NBCS cows were fed a low-energy ration [6.8 NEL (MJ/kg of DM)], while HBCS cows were fed a high-energy ration [7.2 NEL (MJ/kg of 138 DM)] (Supplemental Table S1) as described previously (Schuh et al., 2019) to reach different targets for body condition score (BCS) and back fat thickness (BFT) at dry-off (HBCS: > 3.75 and > 1.4 cm; NBCS: <3.5 and <1.2 cm). Cows were initially pre-selected from the entire herd (150 lactating cows) by their history of body condition, i.e., using BCS and BFT records from the preceding lactation. The preselected cows were also stratified for comparable milk yields (NBCS: 10,361 ± 302 kg; HBCS: 10,315 ± 437 kg). During the dry period and the subsequent lactation, all cows were fed the same diet as total mixed ration (TMR). The diets were formulated according to the recommendations of the German Society of Nutrition Physiology (GfE, 2001). Both BCS and BFT were continuously monitored by one person every two wk during the entire period of the trial (15 wk ante partum to 12 wk post partum).

Sampling and Laboratory Analyses

Feed sampling was carried out as described previously (Schuh et al., 2019). The nutrient composition of the feed samples was analyzed according to the official recommendations of the Association of German Agricultural Analytic and Research Institutes (Naumann and Bassler, 2004). Samples were analyzed for DM, crude ash, CP, utilizable CP, crude fat, crude fiber, ADF, NDF, and NFC, whereas the minerals Ca, P, Mg, Na, and K were analyzed by x-ray fluorescence analysis. The energy content of the diet (ME and NE_L) was calculated according to GfE (2009).

Blood samples were collected from the *Vena caudalis mediana* before the morning feeding on d -49 (SD = 5.3), +3 (SD = 1.6), +21 (SD = 1.8), and +84 (SD = 1.7) relative to calving. After clotting for 45 min at room temperature and subsequent centrifugation (10 min, 2,000 × *g*), the serum was obtained and stored at -20 °C until analysis. Also, biopsies from *M. semitendinosus*

were collected on the same days of blood sampling. The animals were sedated by intravenous injection of Xylazine (20 mg/mL, 0.1 mL/100 kg BW; CP-Pharma Handels GmbH, Burgdorf, Germany) and fixed in a headlock. The biopsy area was cleaned, shaved, and disinfected with 70% isopropyl alcohol. Muscle samples were obtained under local anesthesia with procaine hydrochloride (20 mg/mL, 8 mL per biopsy; Richter Pharma AG, Wels, Austria) by a 12 G × 20 cm Core Tissue Biopsy Needle with a Bard Magnum® biopsy instrument (Bard Inc., Tempe, AZ). Thereafter, oxytetracycline hydrochloride was applied on the skin (25 mg/mL, EngemycinTM, MSD Animal Health Innovation GmbH, Schwabenheim an der Selz, Germany) and a ketoprofen injection (100 mg/mL, 3 mL/100 kg BW; Streuli Pharma AG, Uznach, Germany) was given to prevent infection and pain. Tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C until analysis.

The BA concentrations in serum and skeletal muscle were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) measurements through targeted metabolomics using the Absolute*IDQ*™ p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria). This kit was validated according to the European Medicines Agency guidelines (EMEA Quality guidelines), which implies a proof of reproducibility within a given error range. All analyses were performed in the Helmholtz Zentrum München (GmbH), German Research Center for Environmental Health, Genome Analysis Center. In case of serum, 10 µL of the thawed sample were applied directly to the assay. In case of muscle, frozen samples were homogenized and extracted using homogenization tubes with ceramic beads (1.4 mm) and a Precellys 24 homogenizer with an integrated cooling unit (PEQLAB Biotechnology GmbH, Erlangen, Germany). For this, 3 µL of dry ice cooled mixture of ethanol/phosphate buffer (85/15 v/v) were added to each mg of frozen muscle tissue. After centrifugation, 10 µL of the homogenate

supernatant were applied to the well plate of the p180 kit. The assay procedures of the Absolute*IDQ*™ p180 Kit, the detailed description of the tissue preparation and the metabolite nomenclature were described in detail elsewhere (Zukunft et al., 2013 and 2018). Sample handling was performed by a Hamilton Microlab STAR™ robot (Hamilton Bonaduz AG, Bonaduz, Switzerland) and an Ultravap nitrogen evaporator (Porvair Sciences, Leatherhead, U.K.), beside standard laboratory equipment. Mass spectrometric analyses were done on an API 4000 triple quadrupole system (Sciex Deutschland GmbH, Darmstadt, Germany) equipped with a 1200 Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) controlled by the software Analyst 1.6.1. Data evaluation for quantification of metabolite concentrations and quality assessment were performed with the Met*IDQ*™ software package, which is an integral part of the Absolute*IDQ*™ Kit. Internal standards were used as a reference for the calculation of metabolite concentrations. The concentrations of the serum samples are given in µmol/L, the concentrations of the tissue samples in pmol/mg tissue and also in µmol/L for the homogenates. The LOD was set to three times the values of zero samples (PBS for serum, ethanol/phosphate buffer for tissue homogenate).

Statistical Analyses

Cows were blocked according to expected calving dates. A repeated-measures model was fitted to data using the Proc MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) with first-order autoregressive covariance structure. Before analysis, all data were tested for normality of distribution by evaluating the Shapiro–Wilk statistic using the UNIVARIATE procedure of SAS. Where appropriate, data were transformed using a log10 transformation. The model consisted of treatment, time (sampling date), treatment × time interaction, block, and parity as the fixed effects, and cow as the random effect. The Tukey-Kramer adjustment was applied to

account for multiple comparisons. The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.10$. The correlations between the BA in the serum vs. skeletal muscle were analyzed collectively for all the studied time-points in GraphPad Prism (version 7.01, GraphPad Software, La Jolla, CA).

RESULTS

This study was a part of a larger study designed to investigate the performance and metabolic responses of cows differing in the mobilization of body reserves. The experimental approach to obtain such differences, i.e., a pre-selection of cows 15 wk before calving in combination with a feeding regime aiming to support these differences until dry-off, was successful as detailed by Schuh et al. (2018). In brief, both BCS and BFT were greater in HBCS than in NBCS cows at enrollment (d -105 ante partum) and were maintained until d 105 post partum (Figure 1A, B). The HBCS cows also lost more BCS and BFT until d 105 post partum (Figure 1C, D).

In the bovine samples tested herein, the metabolite coverage of the targeted metabolomics approach was used to measure 21 BA. In total 14 BA were detected in serum (Figure 2A) and 14 BA in muscle tissue (Figure 2B). Out of these 14 BA, 13 were identical between serum and muscle, but histamine was only detected in muscle samples, whereas symmetric dimethylarginine (SDMA) was limited to the serum samples. Dopamine, DOPA, nitrotyrosine, methionine-sulfoxide, and cis-4-hydroxyproline were not found in either sample.

Biogenic amines levels

Serum creatinine was the most abundant BA in the serum of cows in this study (Figure 2A). The creatinine concentrations ranged between 81.0 and 99.5 µmol/L in serum (Figure 3A) and 597

223 to 704 pmol/mg tissue in skeletal muscle (Figure 4A). The circulating creatinine concentrations
224 increased on d +3 in both groups and tended to be greater ($P = 0.08$) in HBCS than in NBCS cows
225 at d +3; however, the muscle creatinine concentrations did not differ between the treatments.
226 Taurine was the second most abundant BA in both serum and skeletal muscle (Figure 2A, B).
227 The taurine concentrations ranged between 52.9 and 76.1 µmol/L in serum (Figure 3B) and 862
228 and 1157 pmol/mg tissue in muscle (Figure 4B). The taurin concentrations in both serum and
229 muscle were not different between groups. Changes with time were limited to serum showing an
230 increase ($P = 0.01$) during the postpartum period.
231 The serum concentrations of carnosine were greater ($P < 0.05$) in HBCS than in NBCS cows at
232 d -49, d +21, and d +84 with concentrations ranging between 7.40 and 13.5 µmol/L (Figure 3C).
233 The serum carnosine concentration decreased after calving (d +3), and subsequently increased to
234 a higher level at d +84 ($P < 0.001$). In skeletal muscle, carnosine was the most abundant BA (Figure
235 2B) with concentrations ranging between 5330 and 7135 pmol/mg tissue. The carnosine
236 concentrations in muscle (Figure 4C) were greater ($P < 0.05$) in NBCS than in HBCS cows at d -
237 49, but remained unchanged from late pregnancy to early lactation.
238 In this study, polyamines (putrescine, spermidine, and spermine) were found at very low
239 concentrations in the serum of cows and ranged between 0.04-0.05 (Figure 3D), 0.02-0.03 (Figure
240 3E), and 0.02-0.04 µmol/L (Figure 3F), respectively. The serum concentrations of putrescine
241 increased on d +84 ($P = 0.04$) and were greater ($P < 0.05$) in HBCS than in NBCS cows. For
242 spermidine, no treatment effects were observed, and the serum concentrations remained unchanged
243 from late pregnancy to early lactation. The serum concentrations of spermine were not affected by
244 the treatments but showed an increase at d +21 ($P = 0.01$). The serum concentrations of spermine
245 in both groups increased ($P = 0.01$) on d +21. In skeletal muscle, the concentrations of polyamines

were similar for both groups ranging between 1.16-4.21, 1.35-2.91, and 3.36-4.02 pmol/mg tissue for putrescine, spermidine, and spermine, respectively.

The serum concentrations of alpha-aminoadipic acid (alpha-AAA; Figure 3G), acetylornithine (Ac-Orn; Figure 3H), kynurenine (Figure 3I), and serotonin (Figure 3J) ranged between 1.8-3.6, 3.7-5.9, 2.6-4.9, and 8.9-16.2 µmol/L, respectively. The serum concentrations of alpha-AAA were greater (d +3, $P = 0.01$; d +84, $P = 0.08$) in HBCS than in NBCS cows. At d -49, the serum concentrations of Ac-Orn tended to be lower ($P = 0.08$) in HBCS than in NBCS cows. The serum concentrations of serotonin did not differ between the treatments, but increased at d +21 (Figure 3J; $P < 0.001$). The concentrations of alpha-AAA (Figure 4G), Ac-Orn (Figure 4H), kynurenine (Figure 4I), and serotonin (Figure 4J) in muscle averaged 12.6, 9.5, 3.1, and 0.27 pmol/mg tissue, respectively, without group effects. The alpha-AAA muscle concentrations fluctuated around calving ($P = 0.01$). The muscle concentrations of Ac-Orn, kynurenine, and serotonin remained unchanged from late pregnancy to early lactation.

Phenylethylamine (PEA) was detected at low concentrations in serum and muscle of transition cows. For PEA, no treatment effects were observed in serum (Figure 3K; average 0.03 µmol/L) and its levels remained unchanged from late pregnancy to early lactation. In muscle tissue, the PEA concentrations ranged between 0.022-0.035 pmol/mg tissue and were greater ($P < 0.05$) in NBCS than in HBCS cows at d +21 (Figure 4K).

The concentrations of hydroxyproline (t4-OH-Pro) ranged between 10.1-20.2 µmol/L in serum (Figure 3L) and 16.2-29.6 pmol/mg tissue in muscle (Figure 4L). The serum concentrations of t4-OH-Pro were greater in HBCS than in NBCS cows in early lactation (d +3, d+21, and d +84, $P < 0.05$) and increased from d -42 until d +3 but decreased to a lesser level at d +84 ($P < 0.001$).

The concentrations of t4-OH-Pro in muscle did not differ between the treatments and the concentrations remained unchanged from late pregnancy to lactation.

Asymmetric dimethylarginine (ADMA) concentrations ranged between 0.48-0.85 µmol/L (Figure 3M) and 0.48-0.73 pmol/mg tissue in muscle (Figure 4M). The concentrations of ADMA were greater in HBCS than in NBCS cows in serum ($P < 0.05$) and muscle (trend, $P = 0.08$) at d -49. Changes with time were limited to serum showing a decrease during the postpartum period ($P < 0.001$). Symmetric dimethylarginine (SDMA, Figure 3N) ranged between 0.65-0.88 µmol/L and the conecentrations were greater in NBCS than in HBCS at d -49. The serum concentrations of SDMA were greater during the postpartum period than at d -49 ($P = 0.001$). In muscle tissue, histamine concentrations ranged between 18-28 pmol/mg tissue (Figure 4N). No treatment effects were observed for histamine in muscle, and its concentrations remained unchanged from late pregnancy to early lactation.

The ratio of kynurenine to tryptophan was also calculated (Figure 5). The serum kynurenine/tryptophan ratio was higher (P < 0.05)? in HBCS than in NBCS cows only at d +84 (Figure 5A). The muscle kynurenine/tryptophan ratio was similar between the treatments, and the ratio remained unchanged from late pregnancy to early lactation (Figure 5B).

Correlations between the BA concentrations in serum and in muscle

No significant correlations were found between the serum and muscle BA concentrations across all time points (Figure 6).

DISCUSSION

In this study, we analyzed serum BA profiles relative to their counterparts in skeletal muscle of both NBCS and HBCS cows during late gestation, and early lactation and our results confirmed a complex relationship between adiposity and serum and muscle BA in periparturient dairy cows. Out of the 14 BA detected in serum, creatinine, putrescine, alpha-AAA, t4-OH-Pro, ADMA, and SDMA were different in NBCS versus HBCS cows. In muscle tissue, despite differences in the concentrations of carnosine, PEA, t4-OH-Pro, and ADMA (trend) between HBCS and NBCS cows, most of the BA remained unchanged during the late gestation and early lactation.

The serum creatinine concentrations measured in this study confirmed previously published results in periparturient cows, showing a decrease during the postpartum period (Pires et al., 2013). Creatinine production is continuous, and its excretion is proportional to body muscle mass. Creatinine in serum can be used as an indicator of muscle protein breakdown under conditions of normal kidney function (Phillips et al., 2003), and the decrease of serum creatinine likely reflects skeletal muscle breakdown (Bruckmaier et al., 1998). The postpartal reduction in the serum creatinine levels in this study might be partially explained by altered kidney function of the cows (Perrone et al., 1992), when the onset of lactation is not followed by an increase in glomerular filtration rate (Maltz and Silanikove, 1996). The postpartum decrease of creatinine in serum observed in our study, and the group differences indicate that creatinine could discern the greater mobilization of body reserves in HBCS cows during the postpartum period. Further research has reported a trend for lower plasma creatinine and a greater 3-MH: creatinine ratio in cows with low BCS as compared to medium and HBCS cows, pointing to less muscle mass but more intense mobilization of muscle protein in lean cows (Pires et al., 2013). Indeed, in our study, the increase of lipolysis was verified in all animals by increasing concentrations of NEFA in serum, whereby the HBCS cows had higher levels (Schuh et al., 2019).

Taurine can be derived from the diet or from endogenous production of methionine and cysteine mainly in the liver but also in other tissues (Craig, 2004), including adipose tissue (Ueki and Stipanuk, 2009). Taurine has multiple physiological functions: in muscle it facilitates Ca-dependent excitation-contraction, and as organic osmolyte it contributes to the regulation of cell volume (Spriet and Whitfiled, 2015). It is crucial for bile acid formation and participates in the defense against oxidative stress, in particular in inflammation associated with oxidative stress (Marcinkiewicz and Kontny, 2014; Spriet and Whitfiled, 2015). The antioxidant role of taurine has been attributed to its ability to scavenge reactive oxygen species (ROS) and to reduce the end products of lipid peroxidation (Balkan et al., 2002; Başaran-Küçükgergin et al., 2016). Taurine has been shown to alleviate liver lipid accumulation, at least in non-ruminants (Vailati-Riboni et al., 2016) and would thus be expected to increase in the circulation of cows during the postpartum period. It is well documented that oxidative stress increases several fold through an excessive release of ROS in dairy cows after parturition (Bernabucci et al., 2005; Sordillo and Raphael, 2013). In this study, an increase in the concentration of ROS [determined via the dROM method (detection of reactive oxygen metabolites)] was observed during the postpartum period (Schuh et al., 2019). Given the relation between taurine metabolism and obesity and insulin resistance (Anuradha and Balakrishnan, 1999) with decreased blood concentrations in diabetic patients (Ito et al., 2012), we expected lesser concentrations in HBCS than in NBCS cows. However, the lack of differences between the groups is not in support of body fat influencing taurine in the circulation of dairy cows.

The carnosine concentrations in serum measured in this study confirmed previously published results in dairy cows, showing a significant increase during the postpartum period (Huber et al., 2016; Zhang et al., 2017). Carnosine is an endogenous dipeptide (β-alanine and L-histidine)

primarily synthesized in skeletal muscle, which has the highest concentrations in the body (Boldyrev et al., 2013). Carnosine can provide anti-oxidative and anti-inflammatory protection towards ROS formation (Boldyrev et al., 2013). Here, we found lower muscle carnosine levels in HBCS than in NBCS cows on d -49. Synthesis of carnosine in skeletal muscle is potentially regulated by insulin action (Gualano et al., 2012), which could be an alternative explanation why carnosine content was reduced in the muscle of HBCS cows. However, in the current study, no such changes were observed in the serum concentrations of insulin in HBCS cows (Schuh et al., 2019).

Polyamines (PA; putrescine, spermidine, and spermine) play major roles in diverse physiological processes, including protein synthesis and function, protection against oxidative damage, and gene expression (Pegg, 2014); however, limited data are available about the role of individual PA in these processes in the ruminant. In this study, we observed that the circulating concentrations of the two main polyamines (spermidine, and spermine) were similar between the groups, but the serum concentrations of putrescine were greater in HBCS than in NBCS cows. Elevated putrescine levels in HBCS cows may suggest a link between body condition and activity of ornithine decarboxylase (Dong et al. 2018). The levels of individual PAs in tissue are maintained and buffered by complex physiologic regulatory mechanisms (Rea et al., 2014). The concentrations of PA in muscle were reportedly increased by anabolic stimuli (Lee and MacLean, 2011). The trend for higher levels of muscle spermidine during the postpartum period might indicate a higher activity of ornithine decarboxylase, suggesting a shift towards an anabolic status.

Hepatic Lys catabolism is catalyzed by a bifunctional protein complex, aminoadipic semialdehyde synthase (AASS) which contains lysine ketoglutarate reductase and saccharopine dehydrogenase activities (Markovitz and Chuang, 1987; Papes et al., 1999). Both of these enzymes

are sensitive to dietary supply of Lys in the diet (Blemings et al., 1990; Foster et al., 1993). Alpha-AAA is an intermediate in the degradation of lysine (Tucker et al., 2017) and thus the observed increase in the serum alpha-AAA concentrations in HBCS cows might be due to increased hepatic Lys catabolism. This might be due to the greater serum concentrations of Lys in HBCS observed on d -49 (results now shown), though the observed differences largely disappeared from d +3 onwards, i.e., after receiving the identical diets. Lysine is exclusively a ketogenic AA; the products of Lys catabolism can be used for the synthesis of ketone bodies, beta-hydroxybutyrate and acetoacetate. As reported previously from this animal experiment (Schuh et al., 2019), the postpartal increase in the serum BHBA concentrations was largely limited to HBCS cows and thus hyperketonaemia (BHBA > 1.2 mmol/l) was more frequent in HBCS cows compared to NBCS cows. Thus, we speculate that elevated alpha-AAA and BHBA levels in HBCS cows might be, at least in part, related to the activation of Lys degradation pathway. However, without measuring AASS protein abundance or activity in the present study, the exact link between α-AAA, BHBA, and hepatic Lys catabolism is unclear.

Acetylornithine has been observed in the serum of dairy cows in recent omics studies (Humer et al., 2016. Dervishi et al., 2018); however, little information appears to be available concerning its role in ruminant metabolism. Acetylornithine is an intermediate metabolite of ornithine metabolism (Zhang et al., 2017). It is also a precursor of ornithine that enters the urea cycle and participates in gluconeogenesis in dairy cows (Dervishi et al., 2018). Here, we found an increase in serum Ac-Orn levels during the postpartum period, suggestion that the hydrolysis of Ac-Orn and subsequent production of ornitine may be influenced by the metabolic status of the cow.

Kynurenine, a metabolite of tryptophan, is synthesized via the kynurenine pathway in the liver and many other tissues (Fallarino et al., 2003). It has been reported that the kynurenine pathway can be induced by intracellular enzymes [tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO)] during the immune responses mediated by proinflammatory cytokines (Hüther et al., 2016). In human patients, the systemic inflammation related to obesity was found to be associated with the development of the metabolic syndrome by induction of the tryptophan-kynurenine pathway. Overweight/obese adults had increased kynurenine serum levels and an increased kynurenine/tryptophan ratio. Here, we observed a higher ratio of kynurenine/tryptophan in the serum of HBCS cows only on d +84 after parturition. The elevated serum kynurenine concentrations during the postpartum period in the current study could be related to activation of hepatic TDO and extrahepatic IDO (Hüther et al., 2016). Zhang et al. (2017) suggested that the ratio of kynurenine/tryptophan, along with other metabolites, could be used as early predictive serum biomarkers for the risk of ketosis in transition dairy cows.

The serum serotonin concentrations measured in this study confirmed previously published results in dairy cows, showing a significant increase during the postpartum period (Moore et al., 2015; Kessler et al., 2018). Serotonin is a monoamine synthesized from L-tryptophan via a short metabolic pathway in the central nervous system and many peripheral tissues, including mammary gland and gastrointestinal tract (Mawe and Hoffman, 2013; Hernández-Castellano et al., 2017). Serotonin plays important roles in a wide range of biological functions, including regulation of energy metabolism and calcium homeostasis in dairy cows (Laporta et al., 2013, 2015). So far, comparable data regarding muscle serotonin concentrations in cattle are not available in the literature. In this study, we observed that the circulating levels of PEA were similar between the groups, but muscle concentrations of PEA were lesser in HBCS compared with NBCS cows on d

+21. Phenylethylamine is an endogenous neuroamine and is synthesized by the action of aromatic amino acid decarboxylase on phenylalanine (Irsfeld et al., 2013). Phenylethylamine was detected at low concentrations in the serum and muscle of transition cows, which might be due to the high turnover rate and a very brief endogenous pool half-life of PEA (Pei et al., 2016). Phenylethylamine has been found in bovine mammary epithelial cells (Fusi et al., 2008) and liver (Suzuki et al., 1980) of cows; however, no reports exist about the PEA content in other tissues of ruminants and its potential roles that would allow for a discussion about their function.

Hydroxyproline is synthesized by hydroxylation of the amino acid proline that is extensively metabolized in the liver and can be derived from several different tissue sources such as breakdown of collagen during the involution of the uterus post partum (Kaidi et al., 1991). In our study, we found a trend towards greater serum t4-OH-Pro concentrations in HBCS than in NBCS cows during early lactation along with greater t4-OH-Pro levels post partum. However, no association between the concentrations of t4-OH-Pro in serum and excessive lipolysis in early lactation cows was observed (Humer et al., 2016). Asymmetric dimethylarginine and SDMA are both methylated analogs of L-arginine, and are recognized as endogenous inhibitors of nitric oxide synthase (Vallance et al., 1992). The observed changes in the serum ADMA concentrations in this study were partially in line with previously published results, showing that serum ADMA concentrations decrease from 8 wk ante partum until 4 wk post partum in healthy cows (Zhang et al., 2017).

In the current study, we showed that changes in BA concentrations in serum during the transition period in dairy cows were not parallel in muscle tissue. The BA concentrations in muscle were unchanged during the late gestation and early lactation, with the exception of alpha-AAA and spermine which fluctuated around calving in skeletal muscle. These data suggest that tissues

424 other than skeletal muscle are likely source of changes in the serum BA during the periparturient
425 period of dairy cows. Moreover, the skeletal muscle compartment as a whole seems to maintain
426 stable concentrations of BA, reflecting an adaptive mechanism to prevent abrupt changes in
427 metabolism of cows.

428

429 **CONCLUSION**

430 We herein demonstrated that some BA change in serum with time relative to parturition. The
431 serum concentrations of some BA (e.g., alpha-AAA, t4-OH-Pro, putrescine) during postpartum
432 were higher in HBCS cows compared to NBCS cows, suggesting that adiposity may be linked to
433 the postpartum metabolism of these BA. In muscle tissue, despite differences in the levels of some
434 BA (carnosine, PEA, and t4-OH-Pro) between treatments, most of BA remained unchanged during
435 late gestation and early lactation. Moreover, in contrast to our hypothesis, no correlations were
436 found between serum BA and their muscle counterparts in both groups, suggesting that tissues
437 other than skeletal muscle are contributing to the systemic alterations in BA during late gestation
438 and early lactation in dairy cows.

439

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Legend of the Figures

Figure 1. Changes of body condition score (BCS, A), back fat thickness (BFT, B), BCS loss (C), and BFT loss (D) of normal- (NBCS) and high-conditioned (HBCS) cows during the experimental period (n = 18 per treatment). Data for BCS, BFT, BCS loss, and BFT loss are from Schuh et al. (2019).

Figure 2. Concentration of biogenic amines in (**A**) serum and in (**B**) skeletal muscle of dairy cows postpartum (d +21).

Figure 3. Longitudinal changes of biogenic amines in serum of normal- (NBCS) and high-conditioned (HBCS) cows during the experimental period (n = 18 per treatment). Symbols indicate a significant difference (*$P \leq 0.05$) or a trend (†$P < 0.10$) between groups at a given time-points. Data are presented as means ± SEM.

Figure 4. Longitudinal changes of biogenic amines in muscle normal- (NBCS) and high-conditioned (HBCS) cows during the experimental period (n = 18 per treatment). Symbols indicate a significant difference (*$P \leq 0.05$) or a trend (†$P < 0.10$) between groups at a given time-points. Data are presented as means ± SEM.

Figure 5. Longitudinal changes of kynurenine/tryptophan ratio in (**A**) serum and (**B**) muscle of normal- (NBCS) and high-conditioned (HBCS) cows during the experimental period (n = 18 per treatment). Asterisks indicate a significant difference ($P < 0.05$) between groups at a given time-point. Data are presented as means ± SEM.

Figure 6. Correlations of biogenic amines in transition cows between serum vs. skeletal muscle (n = 36) across all time-points.

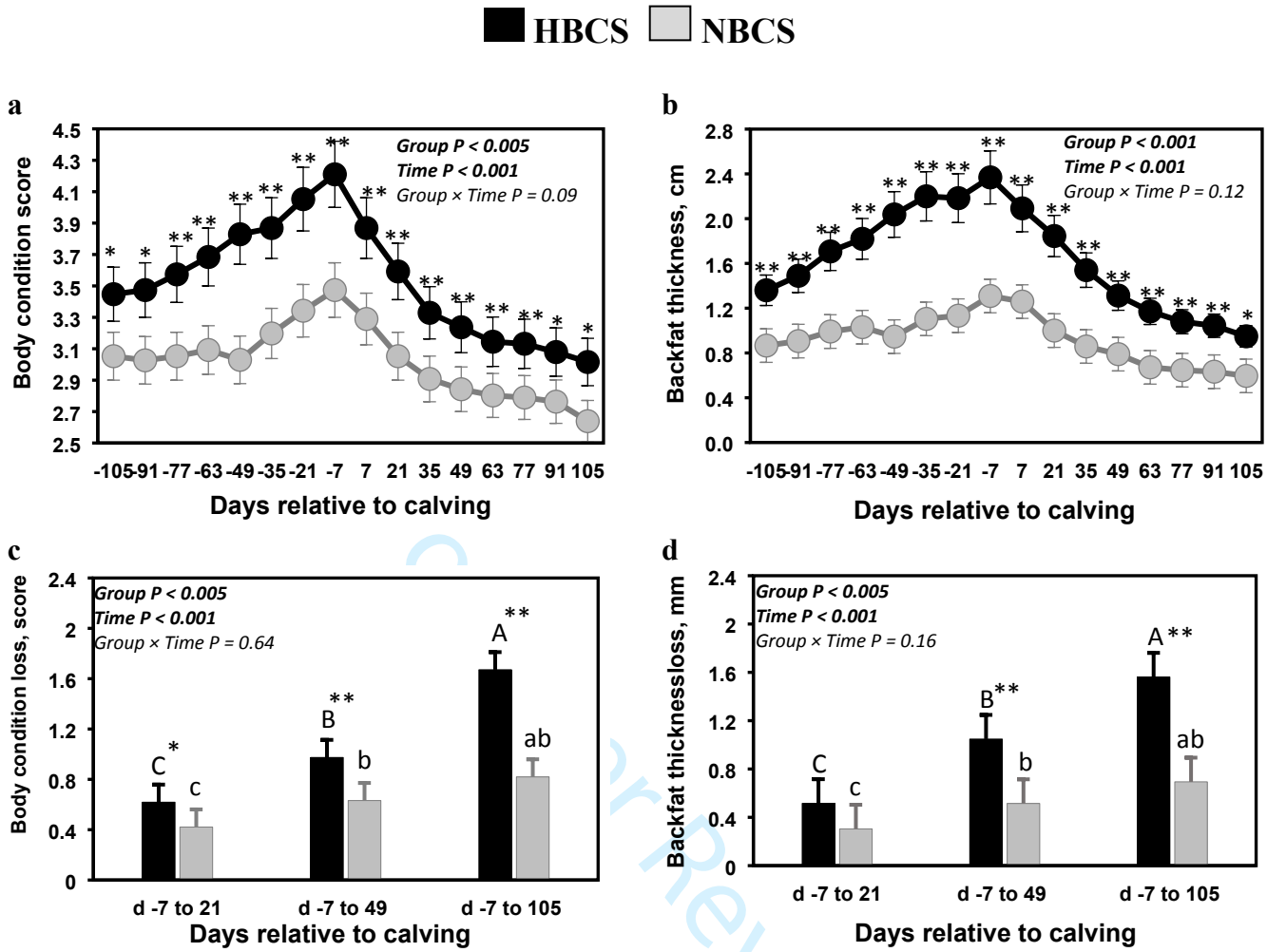
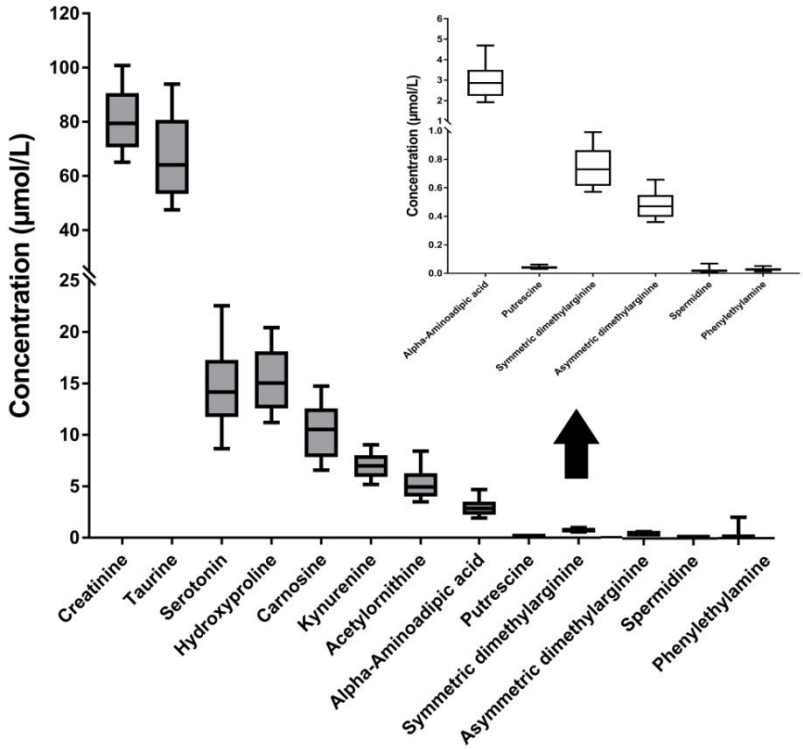


Figure 1.

(A)



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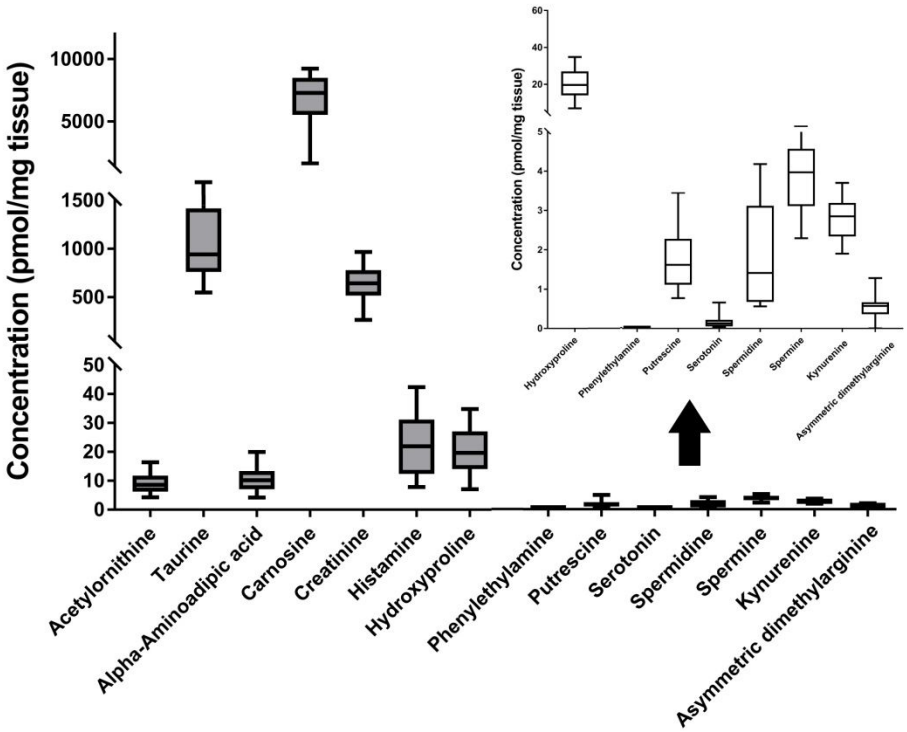
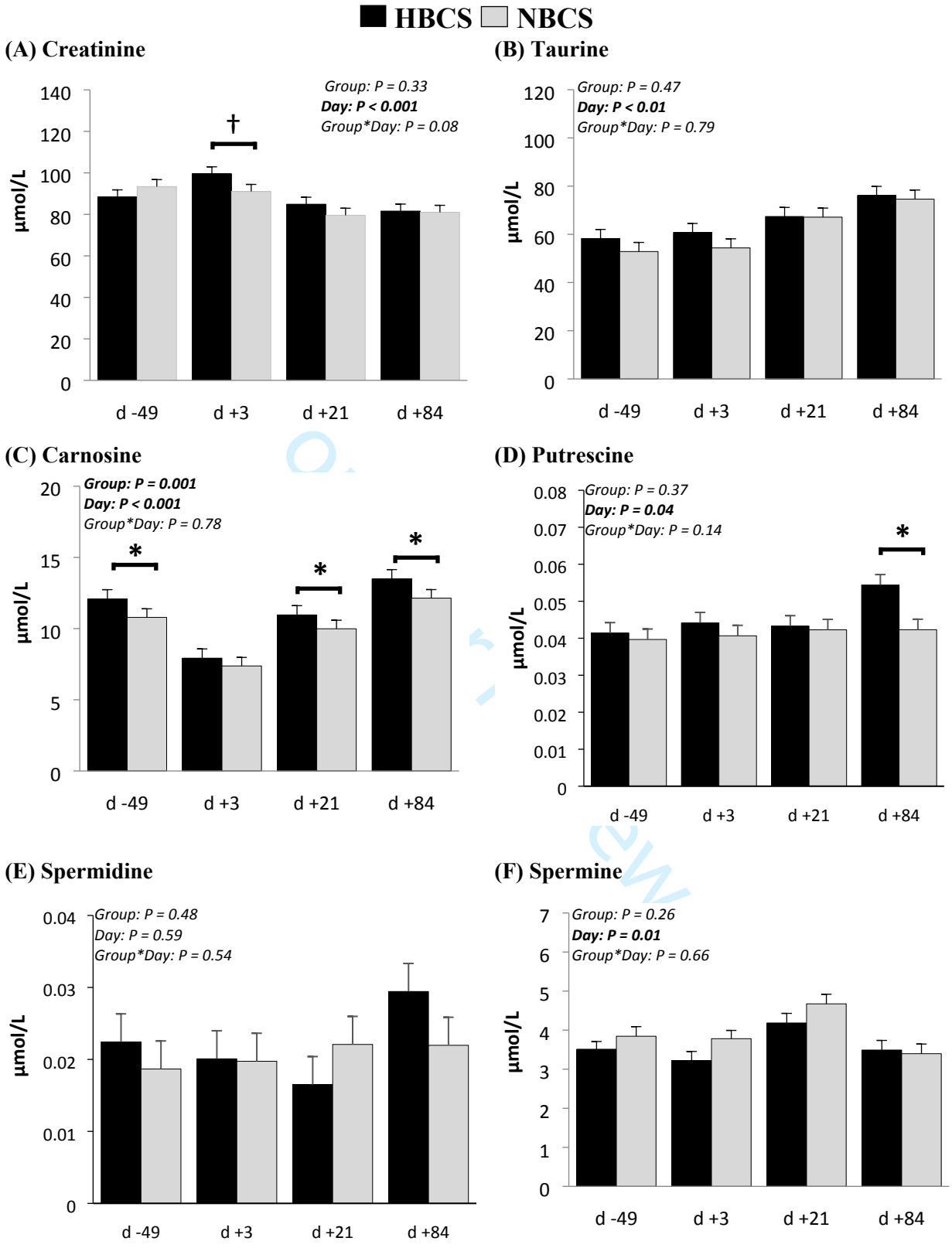


686 **Figure 2.**



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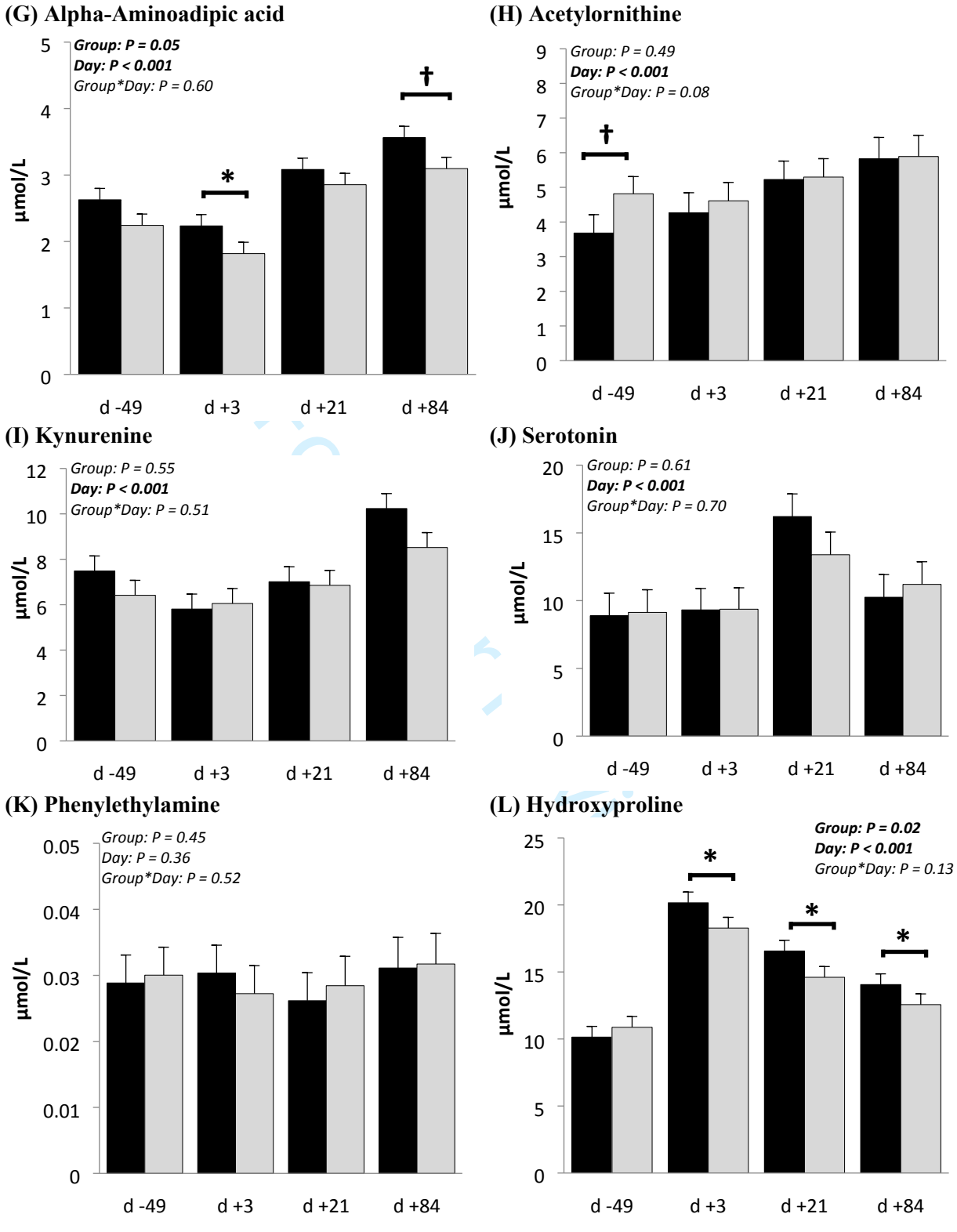

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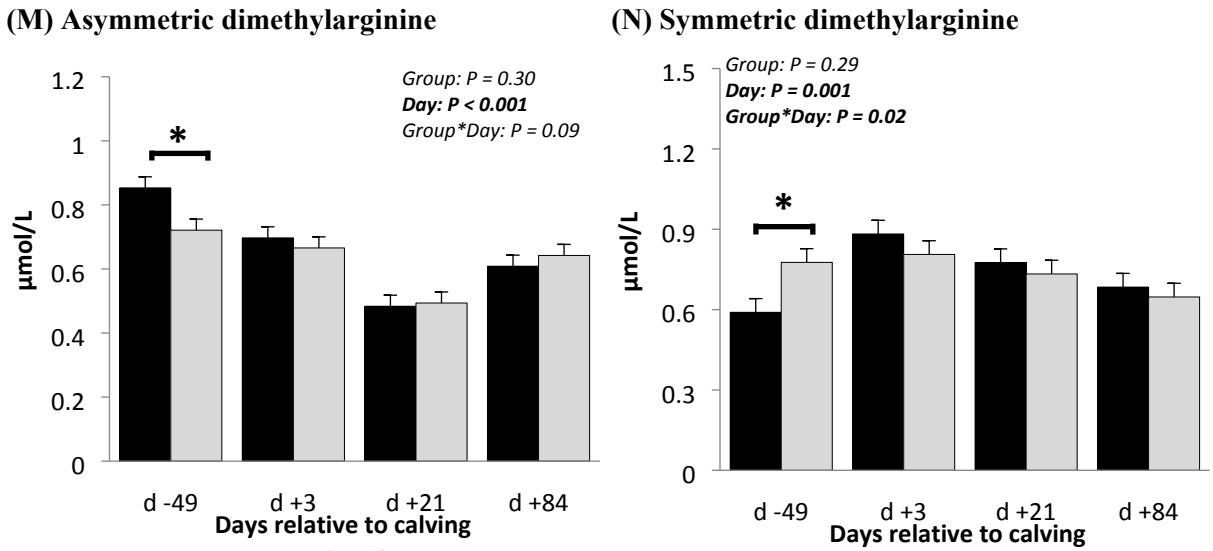
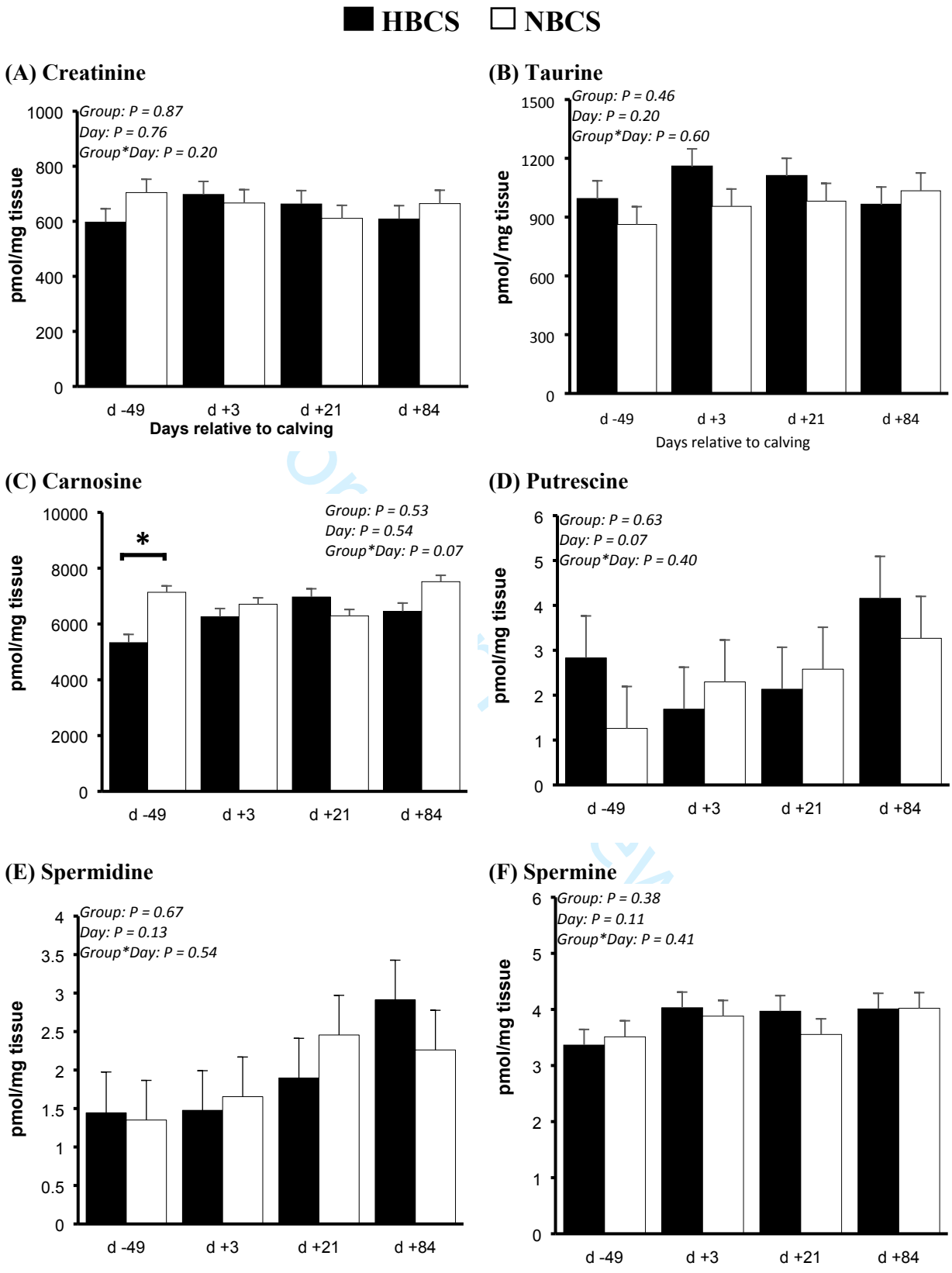


Figure 3.



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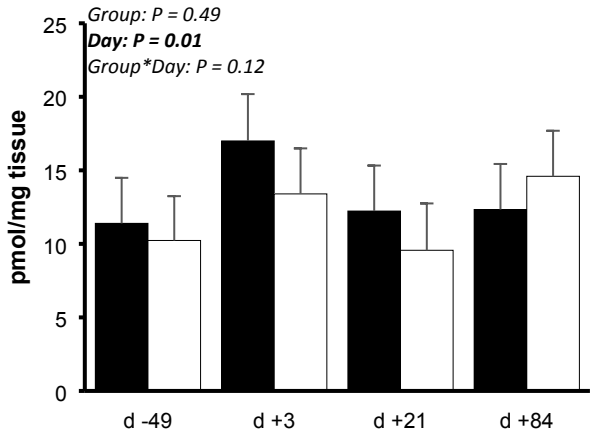
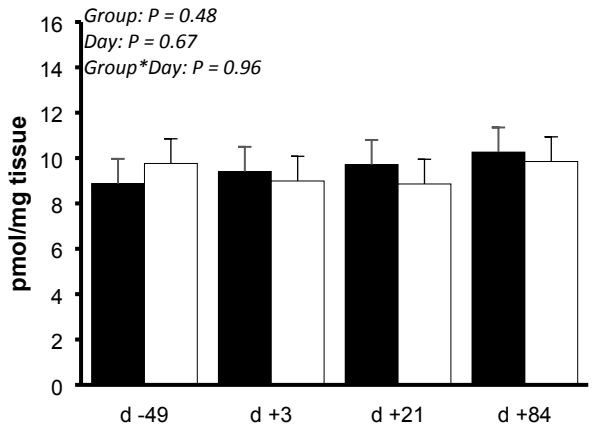
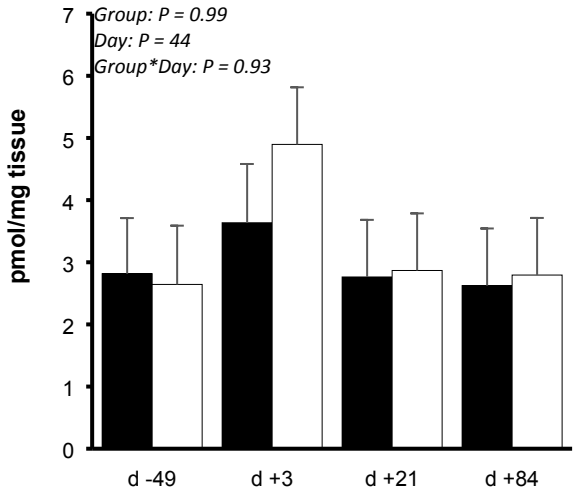
(G) Alpha-Aminoadipic acid



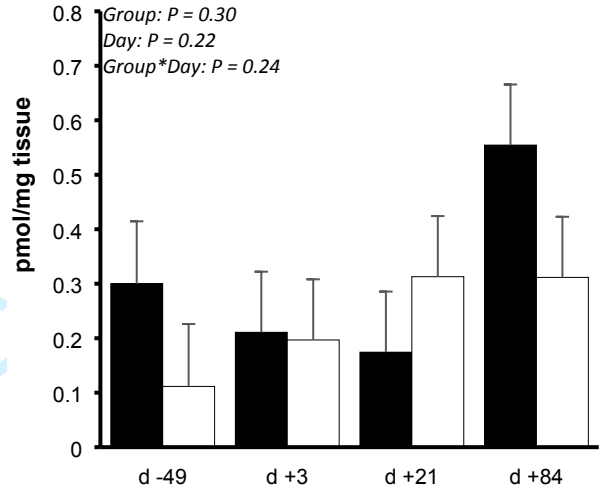
(H) Acetylornithine



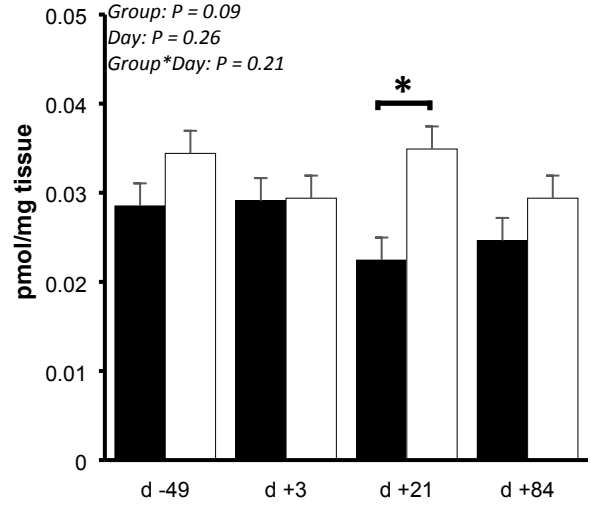
(I) Kynurenine



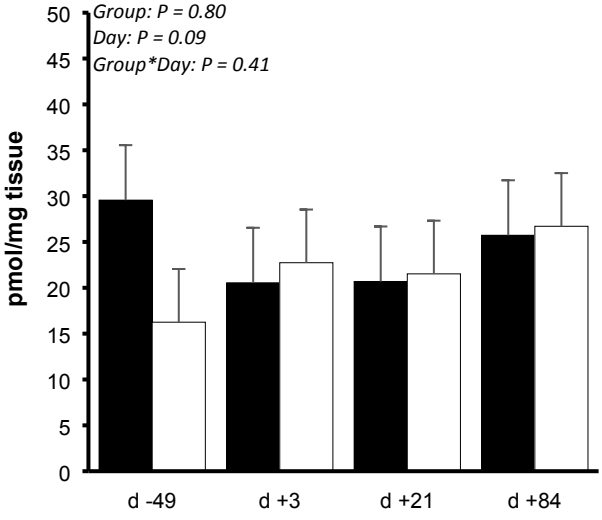
(J) Serotonin



(K) Phenylethylamine



(L) Hydroxyproline



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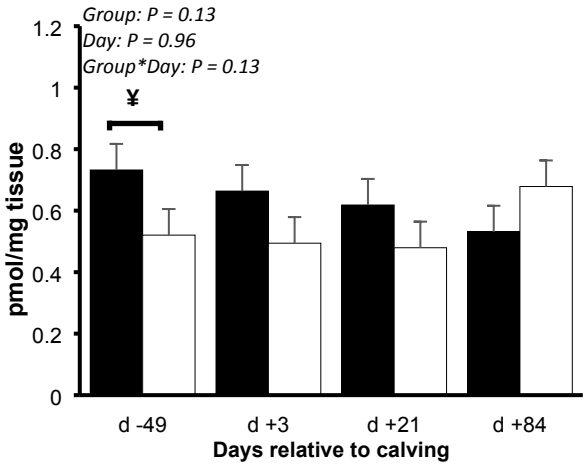
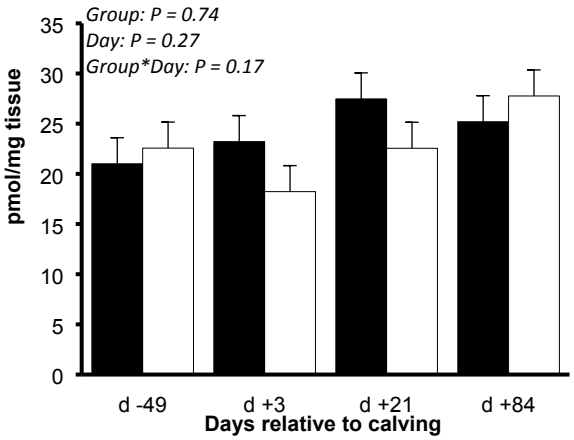
(M) Asymmetric dimethylarginine



(N) Histamine



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714 **Figure 4.**

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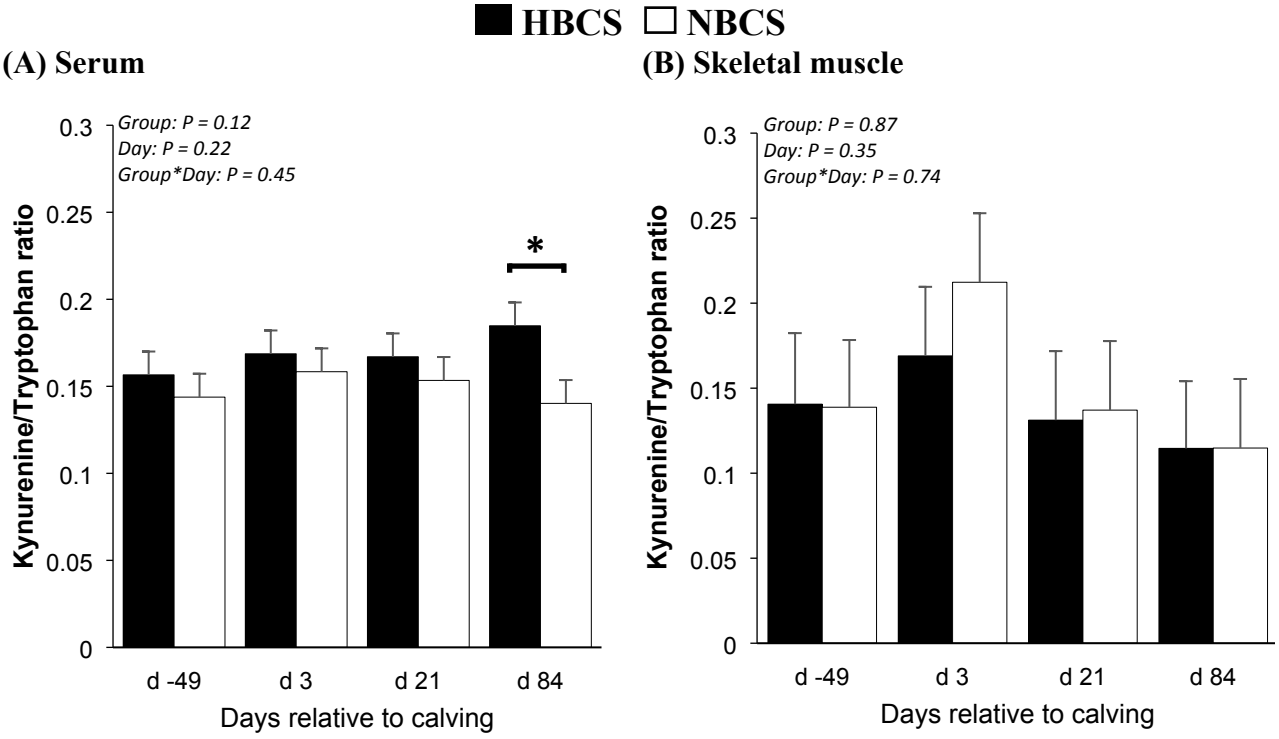

Figure 5.

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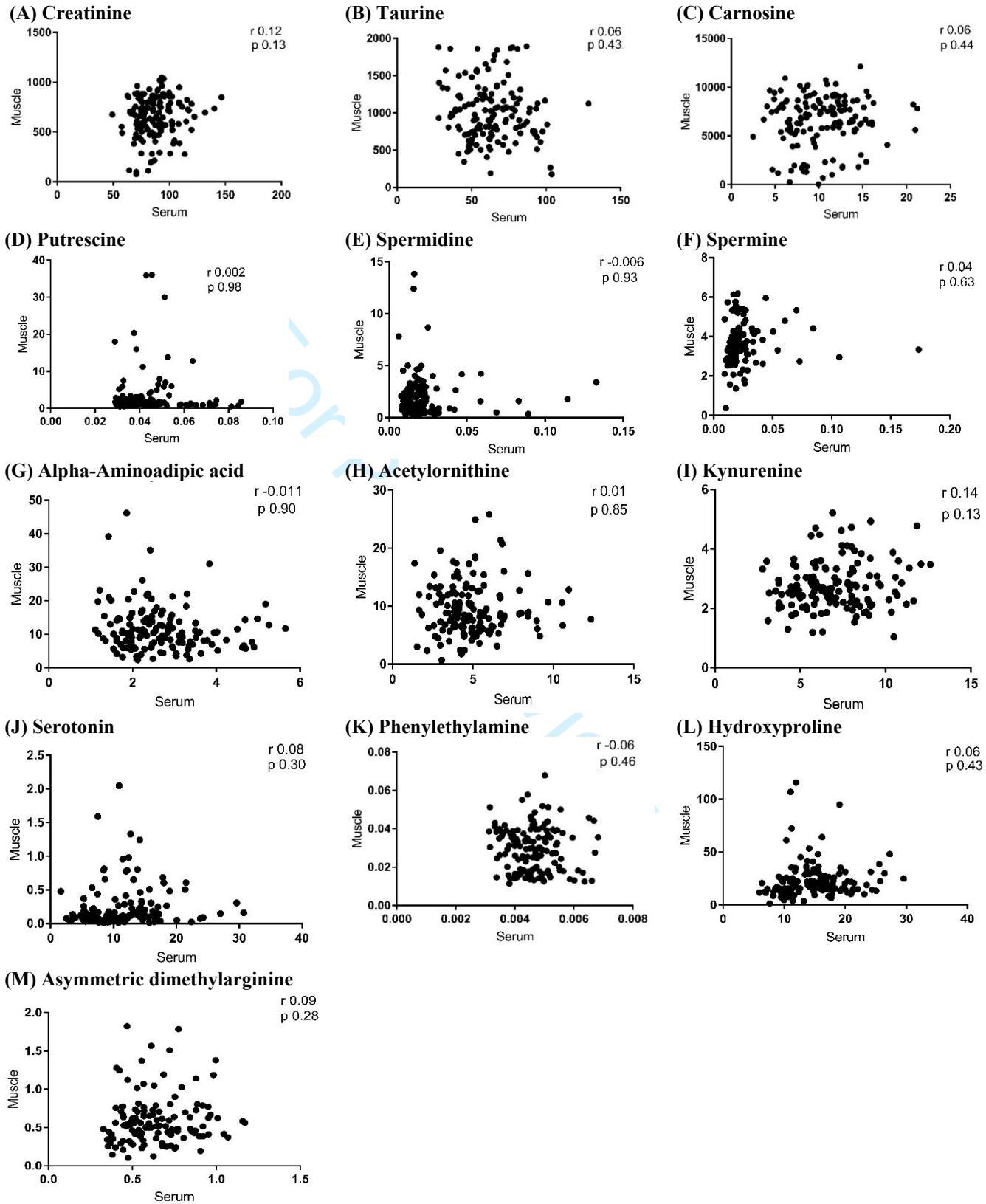

750 **Figure 6.**

751 **Supplementary files**

752 **Table S1:** Ingredient composition and chemical composition (% of DM, unless otherwise
753 noted) of rations during the observation period for cows of the HBCS and NBCS group

	Late lactation		Dry period	Early lactation
	15 to 7 weeks *a.p.*		week 7 *a.p.* to parturition	1 to 14 weeks in milk
Item	HBCS	NBCS	HBCS / NBCS	HBCS / NBCS
Ingredient				
Grass silage	22.4	32.0	32.0	22.4
Corn silage	20.7	32.0	32.0	20.7
Pressed beet pulp silage	12.5	-	-	12.5
Hay	5.5	5.4	5.4	5.5
Straw	2.3	4.1	4.1	2.3
Vitamin and mineral mix[1]	0.4	0.7	0.7	0.4
Concentrate[2]	36.2	25.8	25.8	36.2
Analyzed chemical composition				
ME (MJ/kg of DM)	10.8	10.6	10.6	10.8
NE_L (MJ/kg of DM)	7.2	6.8	6.8	7.2
Crude protein (g/kg of DM)	170	157	157	170
Utilizable CP (g/kg of DM)	156	149	149	156
NDF[†] (g/kg of DM)	359	382	382	359
ADF[‡] (g/kg of DM)	204	223	223	204
NFC [#] (g/kg of DM)	402	360	402	360
Ruminal N balance (g/d)	3.4	2.3	2.3	3.4

754 [1]Contained (DM basis): 9.0% Ca, 9.0% P, 9.0% Na, 10% Mg, 10,000 mg/kg of Zn, 6,000 mg/kg of Mn,
755 1,500 mg/kg of Cu, 60 mg/kg of Co, 200 mg/kg of I, 53 mg/kg of Se, 1000 kIU/kg of vitamin A, 150 kIU/kg
756 of vitamin D_3, 6 kIU/kg of vitamin E.
757 [2]Concentrate portion consisted of barley (25% of DM), corn grain (31% of DM), soybean meal (18% of
758 DM), and canola meal (26% of DM)
759 [†]NDF, Neutral Detergent Fiber
760 [‡]ADF, Acid Detergent Fiber
761 [#] NFC, Non-Fiber Carbohydrate
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