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Ruminant Nutrition

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Differences in copper and selenium metabolism between Angus (*Bos taurus*) and Brahman (*Bos indicus*) cattle

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Abstract

A 2-yr study was conducted at the Range Cattle Research and Education Center, University of Florida – Institute of Food and Agricultural Sciences (IFAS) (Ona, FL), to evaluate differences in the metabolism of Cu and Se of Angus (*Bos taurus*) and Brahman (*Bos indicus*) cattle. Thirty-two pregnant beef cows (n = 8 Brahman and 8 Angus/yr) were enrolled in the study in the first trimester of gestation. This study consisted of three phases: 1) restriction (day 0 to 90), 2) supplementation (day 91 to 150), and 3) calving. During all three phases, cows were individually fed and housed in partially covered drylot pens. During the restriction and supplementation phases, cows were provided a 1.5 kg/d of a grain-based concentrate supplement, which was fortified with flowers of S (50 g of supplemental S/cow daily; restriction phase) or Cu and Se (100 and 3 mg/d of Cu and Se, respectively; supplementation phase). Blood and liver samples were collected from all cows at 30 d intervals and from both cows and calves within 24 h of calving. Colostrum and milk samples were collected at calving and 7 d after birth. All data were analyzed using the MIXED procedure of SAS, where cow and calf were the experimental unit. During the restriction phase, a breed × day effect (P = 0.03) was observed where Brahman had greater liver Cu concentration than Angus cows in all sampling days. For liver Se concentration, a tendency (P = 0.07) for a breed effect was observed where Angus cows tended to have greater liver Se concentration than Brahman. During the supplementation phase, breed (P < 0.001) and day (P < 0.01) effects were observed, where Brahman cows had greater liver Cu concentration than Angus. For liver Se concentration, a day effect (P < 0.001) was observed, where liver Se concentration increased (P < 0.001) from day 90 to 120 and remained unchanged (P = 0.86) until day 150. At calving, no effects of breed (P = 0.34) were observed for liver Cu concentration of cows; however, Brahman calves tended (P = 0.09) to have greater liver Cu concentration than Angus calves. For Se liver concentration at calving, Angus cows tended (P = 0.07) to have greater liver Se concentration than Brahman cows; however, no breed differences (P = 0.70) were observed for liver Se concentration of calves at birth. In summary, substantial differences in multiple indicators of Cu and Se status were observed between Angus and Brahman cattle, implying that Angus and Brahman cattle possibly have different mechanisms to maintain adequate Cu and Se status.

Key words: antioxidant, cows, long-term supplementation, maternal transfer, newborn calves, sulfur

Abbreviations

CP	ceruloplasmin
GPx	glutathione peroxidase
RBC	red blood cells
SOD	superoxide dismutase

Introduction

Copper and Se are trace minerals required for the optimum performance of animals. Selenium is an important component in antioxidant molecules, while Cu is required for the function of more than 30 enzymes. The lack of these trace minerals in the diet can lead to Cu and Se deficiency, which are the most common trace mineral deficiencies for grazing cattle around the world (McDowell and Arthington, 2005), resulting in reduced performance, impaired immune response, and reproductive failure (McDowell, 1996; Spears, 2000).

In order to avoid such mineral deficiencies, a mineral supplementation strategy is required, which can be accomplished by indirect (i.e., pasture and/or soil fertilization) or direct (i.e., mineral licks, mixtures, drenches, boluses, and injections) methods of supplementation. The target intake of mineral supplements is based on the established dietary requirements of cattle (NASEM, 2016). These dietary requirements are a function of the metabolic requirement and endogenous or inevitable losses of each particular mineral and the efficiency in which a mineral is absorbed from the diet. The requirements for most of the minerals are not constant and are impacted by a number of dietary and physiological factors that affect either absorption or metabolic demand (Spears, 2002). Among the physiological factors, age, sex, production stage (maintenance, growth, reproduction, and lactation), and production level are usually the factors with the highest consideration for the estimation of the nutritional requirements. However, one factor that is sometimes overlooked is the impact of genetics.

The effect of breed on mineral metabolism has been observed in previous studies focused on *Bos taurus* breeds (Angus, Simmental, and Charolais; Ward et al., 1995; Mullis et al., 2003; Pogge et al., 2012; Fry et al., 2013). Given the differences in trace mineral metabolism found among *B. taurus* breeds, it would be logical to expect even greater differences when comparing breeds within different subspecies. Langlands et al. (1980) reported that *Bos indicus* cattle (Afrikaner and Brahman breeds) had greater blood Se concentration and greater whole blood glutathione peroxidase (**GPx**) activity than *B. taurus* cattle (Hereford × Shorthorn). In addition, Dermauw et al. (2014) reported that *B. taurus* (Holstein × Friesian × Abyssinian Highland Zebu) became severely Cu deficient, whereas *B. indicus* cattle (Abyssinian Highland Zebu) maintained plasma Cu concentration just below the threshold value for deficiency after 11 wk consuming a Cu-deficient diet.

These initial studies reveal that cattle genetics do impact Cu and Se metabolism, and *B. indicus* breeds may be more tolerant to deficiency when consuming diets inadequate in Cu and Se. In the United States, Angus and Brahman breeds represent the most widely used *B. taurus* and *B. indicus* breeds, respectively, in the commercial beef industry. Therefore, we hypothesized that Brahman cattle will be less susceptible to Cu and Se deficiency than Angus cattle when fed a diet low in Cu and Se and supplemented with high S. Thus, the objective of this study was to identify potential differences in metabolism of Cu and Se in purebred Angus and Brahman cattle.

Material and Methods

A 2-yr study (2017 and 2018) was conducted at the Range Cattle Research and Education Center (**RCREC**), University of Florida – IFAS (Ona, FL), to identify potential differences in the metabolism of Cu and Se in purebred Angus (*B. taurus*) and Brahman (*B. indicus*) cattle. All procedures were similar between years and approved by the Institutional Animal Care and Use Committee of the University of Florida (protocol no. 201609623).

Animals, diets, handling, and study design

Thirty-two mature pregnant beef cows ($n = 8$ Brahman and 8 Angus/year) were enrolled in the first trimester of gestation over two consecutive years. Cows were obtained from three source herds in southern Florida, including the RCREC, University of Florida – IFAS (Ona, FL; Brahman and Angus); Kempfer Cattle Company (St. Could, FL; Brahman); and Hilliard Brothers (Clewiston, FL; Angus). All the cows enrolled in the study were managed as a single herd at the RCREC for a year before the beginning of the study.

The study consisted of three phases: 1) restriction, 2) supplementation, and 3) calving. During all three phases, cows were individually fed and housed in partially covered drylot pens. Ground grass hay (*Cynodon dactylon and Hemarthria altissima*) was provided continuously in amounts to ensure ad libitum consumption. During the restriction phase (day 0 to 90) and supplementation phase (day 91 to 150), cows were provided 1.5 kg/d of a grain-based concentrate supplement

Table 1. Chemical composition of pasture, hay, and grain supplemented to the cows during restriction, supplementation, and calving phases[1]

Item	Hay	Concentrate	Hay	Concentrate
	------ Year 1 ------		---- Year 2 -VV----	
crude protein (CP), %	3.95	26.2	13.6	25.1
acid detergent fiber, %	33.8	22.3	33.5	26.8
neutral detergent fiber, %	74.8	36.2	68.1	41.5
nonfiber carbohydrates, %	11.3	.	8.40	.
total digestible nutrients, %	53.0	76.0	55.0	74.0
Ca, %	0.13	0.90	0.37	1.10
P, %	0.12	0.52	0.29	0.52
Mg, %	0.22	0.27	0.27	0.26
K, %	0.26	1.49	1.75	1.44
Na, %	0.04	0.15	0.02	0.07
Fe, mg/kg	77.0	164	70.0	199
Zn, mg/kg	17.0	44.5	22.0	52.0
Cu, mg/kg	4.5	8.0	7.5	9.0
Mn, mg/kg	9.5	22.5	13.0	29.5
Mo, mg/kg	0.1	3.0	0.2	2.5
S, %	0.1	0.2	0.3	0.4
Se, mg/kg	0.01	0.2	0.1	0.2

[1]Hay and concentrate samples were collected monthly for each year, dried, and ground to pass a 4-mm screen. Hay and concentrate samples were analyzed for wet chemistry in duplicate at Dairy One Forage Laboratory (Ithaca, NY).

(Table 1). In the restriction phase, the supplement was fortified with flowers of S to deliver 50 g of supplemental S/cow daily. In the supplementation phase, S was removed from the supplement, and Cu (Cu sulfate) and Se (sodium selenite) were incorporated into the formula to provide 100 and 3 mg/d of Cu and Se, respectively. At the end of the supplementation phase, cows were moved to a bermudagrass pasture (*C. dactylon*), where they remained in a single group until approximately 30 d before the expected calving date. During this period and also during the individual housing phase, cows were provided free-choice access to water and white stock salt without added minerals. Approximately 30 d before the estimated calving, all cows were returned to the initial pen assignments. During the calving phase, cows were provided the same supplement previously provided during restriction and supplementation but without added minerals. Seven days after calving, cows and calves were removed from the study.

In year 1, two Angus cows aborted, one during the restriction phase and one during the supplementation phase, resulting in data removal from the study. During the calving phase in year 1, one Angus calf was stillborn. In this case, the cow's data remained in the study without its calf data. Lastly, in year 1, two Brahman cows had retained placentas. In both cases, all cow and calf data were included with the exception of cotyledon tissue data. In year 2, one Brahman cow was removed from the study due to disposition problems. During the calving phase, two Brahman calves were stillborn. In both cases, the cow's data remained in the study without calf data. These outcomes resulted in 14 Angus cows, 15 Brahman cows, 13 Angus calves, and 13 Brahman calves for the final 2-yr study dataset.

Blood sampling, liver biopsies, cotyledon, colostrum, and milk collections

Liver tissue was collected from all cows at 30 d intervals (days 0, 30, 60, 90, 120, and 150). A final liver tissue sample was collected from both cows and calves within 24 h of calving. Liver tissue was collected using biopsy procedures by a trained technician as previously described (Arthington and Corah, 1995). Two liver tissue samples were collected between the 11th and 12th intercostal space using a Tru-Cut biopsy needle (CareFusion; 14 gauge × 15 cm, Becton Dickinson, Vernon Hills, IL). For each sample, three to four core tissue samples were collected. The first liver sample was immediately placed on ice and was later sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI). The second liver sample was wrapped in aluminum foil and immediately submerged in liquid nitrogen for snap freezing. This liver sample was stored at –80 °C until RNA extraction could be completed.

Blood samples were collected from the jugular vein of all cows at 30 d intervals (days 0, 30, 60, 90, 120, and 150) and from both cows and calves within 24 h of calving. Blood samples were collected into commercial blood collection tubes (Vacutainer; 10 mL, Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. Aliquots of whole blood were collected prior to blood centrifugation at 1,200 × *g* for 30 min at 4 °C for plasma and red blood cells (**RBC**) collection. Plasma, whole blood, and RBC samples were frozen at –20 °C on the same day of collection.

Immediately after calving, the placentas of all cows were retrieved for cotyledon collection as described by Marques et al. (2016). Following cotyledon dissection from the placenta, two samples were collected. One sample was composed of four to five cotyledons, which were washed with double-distilled water, placed in an aluminum pan, dried at 60 °C for 72 h, ground, and stored at –20 °C until shipping to a commercial laboratory for the determination of Cu and Se concentrations (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI). The second sample consisted of a single cotyledon, which was vigorously washed with double-distilled water, wrapped in aluminum foil, and submerged in liquid nitrogen. These samples were stored at –80 °C until RNA could be extracted from the samples. Colostrum and milk samples were collected within 24 h after calving and on day 7 after calving. Pooled samples were collected into 50 mL polypropylene tubes (Falcon, 50 mL; Thermo Fischer Scientific, Pittsburg, PA) by hand milking from all quarters (approximately 10 mL each). Samples were immediately placed on ice and stored at –20 °C until further analysis.

Laboratory analysis

Copper and Se concentrations of plasma, whole blood, colostrum, and milk were analyzed by inductively coupled plasma mass spectrometry (**ICP-MS**; ng/mL; Perkin-Elmer Corp., Norwalk, CT) at the Department of Soil & Water Science, University of Florida (Gainesville, FL), as described by Ranches et al. (2017). Samples, blanks, and standards (SRM2976; National Institute of Standard and Technology, Gaithersburg, MA) were analyzed in triplicate. Intra-assay coefficient of variation (CV) was 7.1%, and inter-assay CV was 20.0%. Liver and cotyledon samples were analyzed similarly with an ICP-MS, at the Animal Health Diagnostic Laboratory, Michigan State University (Lansing, MI).

Superoxide dismutase (**SOD**; U/mL) activity was measured in erythrocyte lysate using a commercial kit (No. 706002, Cayman Chemical; Ann Arbor, MI), where the total SOD activity was measured using tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical. The intra-assay CV was 4.1%, and the inter-assay CV was 3.0%.

Plasma ceruloplasmin (**CP**) oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974). CP concentration is expressed as mg/dL as described by King (1965). Intra-assay CV was 10.5% and inter-assay CV was 5.1%.

Glutathione peroxidase (nmol/min/mL) activity was measured in erythrocyte lysate using a commercial kit (No. 703102; Cayman Chemical, Ann Arbor, MI) with activity measured according to the decrease in absorbance at 340 nm (Epoch Spectrophotometer; Biotek Instrument, Winooski, VT). The rate of decrease in the absorbance is directly proportional to the GPx activity in the sample. The inter-assay CV was 12.8% and the intra-assay CV was 9.8%.

Primer design, RNA extraction, and gene expression analysis

Thirty-five genes were selected based on previous literature (Papp et al., 2007; Fry et al., 2013; Dermauw et al., 2014; Gladyshev et al., 2016; Qazi et al., 2018) and using the GenBank (National Center for Biotechnology Information [NCBI]) sequence database for genes related to the metabolism of Cu (n = 15; Table 2) and Se (n = 20; Table 3). Primers were designed and manufactured by Fluidgim Delta Gene assays (Fluigdim; San Franciso, CA) and validated in the liver and cotyledon tissues of Angus and Brahman cows prior to being used in the study. Additionally,

Table 2. Gene identification, accession number, and proposed function of genes related to Cu metabolism[1]

Gene	Approved name	Accession number	Proposed function[2]
A2M	*Alpha-2-macroglobulin*	NM_001109795.1	Cu-binding protein
ALB	*Albumin*	NM_180992.2	Cu transporter
ATOX1	*Antioxidant 1 copper chaperone*	NM_001130758.1	Cu chaperone activity
ATP7A	*ATPase copper transporting Alpha*	NM_001192852.1	Cu chaperone activity
CCS	*Copper chaperone for superoxide dismutase*	NM_001046187.2	Cu chaperone activity
CP	*Ceruloplasmin*	NM_001256556.1	Cu transporter
COMMD1	*Copper metabolism domain containing 1*	NM_001046384.2	Cu ion binding, homeostasis
CUTC	*CutC copper transporter*	NM_001206896.1	Cu transporter
LOX	*Lysyl oxidase*	NM_173932.4	Cu ion binding
MT1A	*Metallothionein-1A*	NM_001040492.2	Metal ion binding
MT2A	*Metallothionein-2A*	NM_001075140.1	Metal ion binding
MT3	*Metallothionein 3*	NM_001113304.2	Cu ion binding
MT4	*Metallothionein 4*	NM_001114859.1	Metal ion binding
SLC31A2	*Copper uptake protein 2*	NM_001034556.1	Cu transporter
SOD1	*Superoxide dismutase 1*	NM_174615	Scavenges superoxide ion

[1]Primers were designed and manufactured by Fluidgim Delta Gene assays (Fluigdim, San Franciso, CA). Primers were validated in the liver and cotyledon tissues prior to being used in the study. Additionally, four primers of housekeeping genes (*GAPDH*, *TATABP*, *ACTB*, and *SDHA*) were validated in the same tissues.
[2]Proposed functions were compiled from published literature on the specific genes of interest (Papp et al., 2007; Fry et al., 2013; Dermauw et al., 2014; Gladyshev et al., 2016; Qazi et al., 2018).

Table 3. Gene identification, accession number, and proposed function of genes related to Se metabolism[1]

Gene	Approved name	Accession number	Proposed function[2]
DIO1	*Iodothyronine deiodinase 1*	NM_001122593.2	Thyroid hormone metabolism (Se required)
DIO3	*Iodothyronine deiodinase 3*	NM_001010993.3	Thyroid hormone metabolism (Se required)
GPX1	*Glutathione peroxidase 1*	NM_174076.3	Antioxidant (cytosolic enzyme)
GPX2	*Glutathione peroxidase 2*	NM_001163139.2	Antioxidant (epithelium-specific gastrointestinal glutathione)
GPX3	*Glutathione peroxidase 3*	NM_174077.4	Antioxidant (extracellular compartments)
GPX4	*Glutathione peroxidase 4*	NM_174770.4	Reduce phospholipid and cholesterol hydroperoxides directly
SELENOF	*Selenoprotein F*	NM_001034759.2	Not known; possible quality control of protein folding
SELENOH	*Selenoprotein H*	NM_001164092.1	Response to redox status and antioxidant defense
SELENOI	*Selenoprotein I*	NM_001075257.2	Lipid and protein metabolism
SELENOK	*Selenoprotein K*	NM_001037489.3	Antioxidant (endoplasmic reticulum and plasma membrane)
SELENOM	*Selenoprotein M*	NM_001163171.2	Possible redox function
SELENON	*Selenoprotein N*	NM_001114976.2	Not known; possible role in muscle metabolism
SELENOO	*Selenoprotein O*	NM_001163193.2	Not known; possible redox activity
SELENOS	*Selenoprotein S*	NM_001046114.3	Degradation process of misfolded proteins in the endoplasmic reticulum
SELENOT	*Selenoprotein T*	NM_001103103.2	Antioxidant and glucose homeostasis, hormone production
SELENOV	*Selenoprotein V*	NM_001163244.2	Not known; possible redox activity
SELENOW	*Selenoprotein W*	NM_001163225.1	Not known; possible role in muscle metabolism
SEPHS2	*Selenophosphate synthetase 2*	NM_001114732.2	Catalyzes the conversion of selenide to selenophosphate
TXNRD1	*Thioredoxin reductase 1*	NM_174625.4	Reduction of thioredoxins; redox homeostasis
TXNRD2	*Thioredoxin reductase 2*	NM_174626.3	Reduction of thioredoxins; redox homeostasis

[1]Primers were designed and manufactured by Fluidgim Delta Gene assays (Fluigdim, San Franciso, CA). Primers were validated in the liver and cotyledon tissues prior to being used in the study. Additionally, four primers of housekeeping genes (*GAPDH*, *TATABP*, *ACTB*, and *SDHA*) were validated in the same tissues.
[2]Proposed functions were compiled from published literature on the specific genes of interest (Papp et al., 2007; Fry et al., 2013; Dermauw et al., 2014; Gladyshev et al., 2016; Qazi et al., 2018).

four primers of housekeeping genes (*GAPDH*, *TATABP*, *ACTB*, and *SDHA*) were validated in the same tissues.

Total RNA was extracted from the liver (days 0, 90, 150, and calving) and cotyledon samples (at calving) using TRIzol Reagent (Invitrogen; Carlsbad, CA) following the procedure suggested by the manufacturer. After RNA extraction, samples were purified using RNeasy Mini Kit (Qiagen; Germantown, MD). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop 2000; Thermo Fisher Scientific, Minneapolis, MN) at 260 and 260:280 nm ratio, respectively. RNA extracted samples were equalized to 45 ng/uL and stored at –80 °C until further processing.

Gene expression analyses were performed using BioMark real-time polymerase chain reaction (PCR) system (Fluigdim; San Franciso, CA) for high-throughput microfluidic real-time PCR amplification at the CFAR Laboratory at Miller School of Medicine, University of Miami. Target-specific pre-amplification after reverse transcription was performed on all samples using

the Preamp and Reverse Transcription Master Mix (Fluidigm) for 20 cycles. The procedure for real-time RT-PCR using the BioMark HD system (Fluidigm; San Francisco, CA; Spurgeon et al., 2008) was conducted as follows: primer sets and samples were loaded on an integrated fluidic circuit plate and placed into a controller that prepares the nano-volume reactions. Real-time RT-PCR was carried out on the BioMark HD system. A total of 40 PCR cycles were performed using EvaGreen (Bio-Rad; Hercules, CA) chemistry on the 96.96 dynamic array integrated fluidic circuit developed by the manufacturer. Cycle threshold (**Ct**) values were calculated by the Fluidigm real-time PCR analysis software. The cutoff for undetectable genes was set at Ct > 29.

The geometric mean of the four housekeeping genes was calculated, and relative expression to the geometric mean of the housekeepers was calculated for the 35 genes of interest using the $2^{-\Delta Ct}$ method.

Statistical analyses

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.4). For all analyses, cow and calf were considered the experimental unit for each variable. The model statement for cow liver mineral concentration and blood variables included the fixed effects of breed, day, and the interaction. For these variables, year was included in the random statement. Day was included in the repeated statement and cow was used as the subject. Compound symmetry was the covariance structured used, as it generated the lowest Akaike information criterion. Identical models were used for the restriction and supplementation phases; however, the model used for the restriction phase contained days 0, 30, 60, and 90, whereas the model used for the supplementation phase contained days 90, 120, and 150. The model statement for colostrum, milk, cotyledon mineral concentration, liver mineral concentration at calving, and all calf-related data included the fixed effects of breed. Year was included in the random statement. Compound symmetry was the covariance structure used, as it generated the lowest Akaike information criterion.

Gene expression data analysis was conducted using calculated $2^{-\Delta Ct}$ values for all the genes of interest. Tests were conducted individually for each sampling day, as they represent a single time point of a specific phase of the study (restriction, supplementation, and calving); therefore, the model only included the effect of breed. Gene expression data are presented as the relative expression to housekeeping genes.

Data were separated using PDIFF if a significant preliminary *F*-test was detected. Significance was set at $P \leq 0.05$ and tendencies if $P > 0.05$ and ≤ 0.10.

Results

Liver mineral concentrations

Restriction phase

Effects of breed × day ($P = 0.03$), breed ($P < 0.01$), and day ($P < 0.01$) were observed for liver Cu concentration during the restriction phase. Liver Cu concentration increased ($P < 0.01$) from day 0 to 30, followed by a decrease ($P < 0.001$) from day 30 to 60, and remained constant ($P = 0.17$) until day 90. Brahman cows had greater ($P < 0.001$) liver Cu concentration than Angus cows during the restriction phase (Table 4).

Effects of breed ($P = 0.07$) and day ($P < 0.001$) were observed for liver Se concentration during the restriction phase. No effects of breed × day ($P = 0.39$) were observed. Liver Se concentration was constant ($P = 0.15$) from day 0 to 30, decreasing ($P < 0.001$) from day 30 to 60, and then remained unchanged ($P = 0.31$) until day 90 (Table 5). Liver Se concentration of Angus cows tended ($P = 0.07$) to be greater than Brahman cows during the restriction phase (0.69 vs. 0.59 mg/kg DM, respectively; SEM = 0.081).

Table 4. Liver Cu concentration of Angus and Brahman cows during the restriction phase

	Treatment			
Day of the study[1]	Angus	Brahman	Largest SEM	*P*-value
	---- mg/kg DM ----			
0	24.5[a]	137[bc]	23.45	<0.001
30	45.5[a]	212[a]	23.45	<0.001
60	34.9[a]	102[c]	23.83	0.02
90	39.9[a]	153[b]	22.83	<0.001

[1]Liver tissue was collected from cows at 30 d intervals, by a trained technician. Immediately after collection, liver samples were placed on ice. Liver samples were sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[a–c]Means with different superscripts within a breed differ ($P \leq 0.03$).

Supplementation phase

Effects of breed ($P < 0.001$) and day ($P < 0.01$) but no effects of breed × day ($P = 0.37$) were observed for liver Cu concentration during the supplementation phase. Liver Cu concentration increased ($P = 0.04$) from day 90 to 120 and remained unchanged ($P = 0.44$) until day 150 (Table 6). Brahman cows had greater ($P < 0.001$) liver Cu concentration than Angus cows (45.5 vs. 191 mg/kg DM, respectively; SEM = 25.50).

No effects of breed × day ($P = 0.32$) or breed ($P = 0.53$) were observed for liver Se concentration during the supplementation phase. However, a day effect ($P < 0.001$) was observed, where liver Se concentration increased ($P < 0.001$) from day 90 to 120, and then remained unchanged ($P = 0.86$) until day 150 (Table 6).

Calving

No effects of breed ($P = 0.34$) were observed for liver Cu concentration of cows at calving. Angus cows tended ($P = 0.07$) to have greater liver Se concentration than Brahman cows at calving (Table 7). Brahman calves tended ($P = 0.09$) to have greater liver Cu concentration than Angus calves at birth with no effects ($P = 0.70$) of breed observed for liver Se concentration of calves at birth (Table 8).

Plasma mineral concentrations

Restriction phase

Brahman cows had greater ($P < 0.001$) plasma Cu concentration than Angus cows, but no effects of breed × day ($P = 0.31$) or day ($P = 0.21$) were observed for plasma Cu concentration (864 and 1,023 ng/mL for Angus and Brahman cows, respectively; SEM = 75.3).

Effects of breed ($P = 0.05$) and day ($P < 0.001$), but not breed × day ($P = 0.69$), were observed for plasma Se concentration during the restriction phase. Plasma Se concentration was greatest on day 0 and decreased ($P < 0.001$) on day 30, followed by a slight increase ($P < 0.01$) from day 30 to 60, and then remained unchanged ($P = 0.11$) through day 90 (Table 5). During the restriction phase, plasma Se concentration was greater

Table 5. Effect of Cu and Se restriction on indices of Cu and Se status in Brahman (n = 8) and Angus (n = 8) cows

Item	Day 0	Day 30	Day 60	Day 90	SEM
Liver Se[1], mg/kg DM	0.67[ax]	0.74[a]	0.55[b]	0.60[b]	0.037
Plasma Se[2], ng/mL	82.2[a]	68.3[c]	76.2[b]	71.1[b,c]	2.36
Whole blood Cu[2], ng/mL	768[a]	689[a,b]	628[b]	611[b]	44.9
SOD[3], U/mL	3.50[b]	3.90[a]	4.07[a]	3.90[a]	0.107
Plasma CP[4], mg/dL	15.6[a]	11.6[b]	16.2[a]	15.8[a]	1.40

[1]Liver tissue was collected from cows at 30 d intervals and at calving by a trained technician. Immediately after collection, liver samples were placed on ice. Liver samples were sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[2]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. An aliquot of whole blood was collected prior to sample centrifugation. Plasma samples were obtained after centrifugation of blood at 1,200 × g for 30 min at 4 °C. Copper and Se concentrations in plasma and whole blood were analyzed by ICP-MS (ng/mL; Perkin-Elmer Corp., Norwalk, CT) at the Department of Soil & Water Science, University of Florida (Gainesville, FL), as described by Ranches et al. (2017).
[3]SOD (U/mL) activity was measured in erythrocyte lysate using a commercial kit (No. 706002, Cayman Chemical; Ann Arbor, MI). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical.
[4]Plasma samples were obtained after centrifugation of blood at 1,200 × g for 30 min at 4 °C. Plasma CP oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974).
[a–c]Values with different superscripts differ (P < 0.05) within item and across days.
[x]Liver Se concentration on day 0 tended to be greater (P = 0.10) than day 90.

Table 6. Effect of Cu and Se supplementation on indices of Cu and Se status in Brahman (n = 8) and Angus (n = 8) cows

Item	Day 90	Day 120	Day 150	SEM
Liver Cu[1], mg/kg DM	90.5[b]	125[a]	136[a]	15.47
Liver Se[1], mg/kg DM	0.60[b]	1.11[a]	1.12[a]	0.057
Plasma Se[2], ng/mL	71.1[b]	96.7[a]	98.5[a]	2.10
Whole blood Se[2], ng/mL	148[a]	160[b]	200[c]	5.4
GPx[3], nmol/min/mL	458[b]	505[a]	481[a,b]	13.2

[1]Liver tissue was collected from cows at 30 d intervals and at calving, using biopsy procedures by a trained technician. Immediately after collection, liver samples were placed on ice. Liver samples were sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[2]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. An aliquot of whole blood was collected prior to blood sample centrifugation. Plasma samples were obtained after centrifugation of blood at 1,200 × g for 30 min at 4 °C. Copper and Se concentrations in plasma and whole blood were analyzed by ICP-MS (ng/mL; Perkin-Elmer Corp., Norwalk, CT) at the Department of Soil & Water Science, University of Florida (Gainesville, FL), as described by Ranches et al. (2017).
[3]GPx (nmol/min/mL) activity was measured in erythrocyte. Activity was measured according to the decrease in absorbance at 340 nm. The rate of decrease in the absorbance is directly proportional to the GPx activity in the sample.
[a–c]Values with different superscripts differ (P < 0.05) within item and across days.

(P < 0.001) for Angus cows than Brahman cows (88.2 vs. 60.6 ng/mL, respectively; SEM = 5.01).

Supplementation phase

Effects of breed × day (P < 0.01), breed (P < 0.001), and day (P < 0.001) were observed for plasma Cu concentration during the supplementation phase. Plasma Cu concentrations were unchanged (P ≥ 0.70) for Angus cows during the supplementation phase, while plasma Cu concentration of Brahman cows increased (P < 0.001) from day 90 to 120, and remained unchanged (P = 0.42) through day 150. Plasma Cu concentration did not differ between Angus and Brahman cows on day 90 but was greater (P < 0.001) for Brahman cows on days 120 and 150 compared with Angus cows (Table 9).

Effects of breed (P < 0.001) and day (P < 0.001), but not breed × day (P = 0.34), were observed for plasma Se concentration during the supplementation phase. Plasma Se concentration increased (P < 0.001) from day 90 to 120 and remained unchanged (P = 0.22) through day 150 (Table 6). During the supplementation phase, plasma Se concentration was greater (P < 0.001) for Angus vs. Brahman cows (103 vs. 73.1 ng/mL, respectively; SEM = 2.50).

Calving

No effects (P = 0.16) of breed were observed for plasma Cu concentration at calving. Plasma Se concentration was greater (P < 0.01) for Angus cows compared with Brahman cows at calving (Table 7). Brahman calves had greater (P < 0.01) plasma Cu concentration than Angus calves at birth, whereas Angus calves tended (P = 0.08) to have greater plasma Se concentration than Brahman calves at birth (Table 8).

Whole blood mineral concentrations

Restriction phase

A tendency (P = 0.08) for a day effect was observed for whole blood Cu concentration during the restriction phase, but no effects of breed (P = 0.72) or breed × day (P = 0.11) were observed. No differences were observed in whole blood Cu concentration from day 0 to 30 (P = 0.25), from day 30 to 60 (P = 0.34), and from day 60 to 90 (P = 0.79). However, whole blood Cu concentration was less on days 60 and 90 than on day 0 (Table 5).

No effects of breed × day (P = 0.47), breed (P = 0.31), or day (P = 0.17) were observed for whole blood Se concentration of Angus and Brahman cows during the restriction phase (pooled average: 156 and 147 ng/mL for Angus and Brahman cows, respectively; SEM = 6.20; data not shown).

Supplementation phase

Effects of breed × day (P = 0.01) and day (P < 0.001), but not breed (P = 0.90), were observed for whole blood Cu concentration during the supplementation phase. The whole blood Cu concentration of Angus cows did not (P = 0.91) change during the supplementation phase. In contrast, whole blood Cu concentration of Brahman cows increased from day 90 (P = 0.04) to 120 and from day 120 to 150 (P < 0.01). Angus cows tended (P = 0.07) to have greater whole blood Cu concentration than Brahman cows on day 90; however, no breed differences (P ≥ 0.12) were observed on days 120 and 150 (Table 10).

An effect of day (P < 0.001) was observed for whole blood Se concentration during the supplementation phase, but no effects

Table 7. Liver, blood, colostrum, and milk Cu and Se concentrations and blood enzymatic activities of samples collected from cows at calving

Item	Treatment		Largest SEM	P-value
	Angus	Brahman		
Liver Cu[1], mg/kg DM	86.1	135	36.21	0.34
Liver Se[1], mg/kg DM	0.60	0.44	0.062	0.07
Plasma Cu[2], ng/mL	943	1,037	47.2	0.16
Plasma Se[2], ng/mL	49.1	35.0	3.21	<0.001
Whole blood Cu[2], ng/mL	649	604	23.9	0.16
Whole blood Se[2], ng/mL	116	102	7.1	0.16
SOD[3], U/mL	3.52	3.05	0.100	<0.01
Plasma CP[4], mg/dL	21.6	21.8	1.60	0.91
GSH-Px[5], nmol/min/mL	510	502	19.5	0.76
Cotyledon Cu[6], mg/k DM	7.47	5.12	1.025	0.12
Cotyledon Se[6], mg/kg DM	0.74	0.77	0.034	0.55
Colostrum Cu[7], ng/mL	163	278	21.3	<0.001
Colostrum Se[7], ng/mL	88.6	79.5	7.91	0.45
Milk Cu[7], ng/mL	120	353	33.1	<0.001
Milk Se[7], ng/mL	38.9	53.6	7.13	0.14

[1]Liver tissue was collected from cows at 30 d intervals and at calving, by a trained technician. Immediately after collection, liver samples were placed on ice. Liver samples were sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[2]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. An aliquot of whole blood was collected prior to blood sample centrifugation. Plasma samples were obtained after centrifugation of blood at 1,200 × *g* for 30 min at 4 °C. Copper and Se concentrations in plasma and whole blood were analyzed by ICP-MS (ng/mL; Perkin-Elmer Corp., Norwalk, CT) at the Department of Soil & Water Science, University of Florida (Gainesville, FL), as described by Ranches et al. (2017).
[3]SOD (U/mL) activity was measured in erythrocyte lysate using a commercial kit (No. 706002, Cayman Chemical; Ann Arbor, MI). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical.
[4]Plasma samples were obtained after centrifugation of blood at 1,200 × *g* for 30 min at 4 °C. Plasma CP oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974).
[5]GPx (nmol/min/mL) activity was measured in erythrocyte. Activity was measured according to the decrease in absorbance at 340 nm. The rate of decrease in the absorbance is directly proportional to the GPx activity in the sample.
[6]Immediately after calving, placentas of all cows were retrieved for cotyledon collection. Cotyledons were washed with double-distilled water and placed in an aluminum pan, dried at 60 °C for 72 h, ground, and stored at –20 °C until sent to a commercial laboratory for the determination of Cu and Se concentrations (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[7]Colostrum samples were collected within 24 h after calving and milk samples were collected on 7 d after calving. Pooled samples were collected into 50 mL polypropylene tubes by hand milking from all quarters (approximately 10 mL each). Samples were immediately placed on ice and stored at –20 °C until the determination of Cu and Se concentrations (Department of Soil & Water Science, University of Florida, Gainesville, FL).

of breed × day ($P = 0.73$) or breed ($P = 0.45$) were observed. Whole blood Se concentration increased ($P \leq 0.04$) from day 90 to 120 and from day 120 to 150 (Table 6).

Calving

No effects of breed ($P \geq 0.15$) were observed for whole blood Cu or Se concentration of cows at calving or calves at birth (Tables 7 and 8, respectively).

Superoxide dismutase activity

Restriction phase

Effects of breed ($P < 0.001$) and day ($P < 0.001$), but not breed × day ($P = 0.16$), were observed for erythrocyte SOD activity during the restriction phase. There was an increase ($P = 0.01$) in erythrocyte SOD from day 0 to 30 with no change ($P \geq 0.32$) from day 30 to 90 (Table 5). Angus cows had greater ($P < 0.001$) erythrocyte SOD activity than Brahman cows (4.30 vs. 3.35 U/mL, respectively; SEM = 0.109).

Supplementation phase

Effects of breed × day ($P = 0.05$), breed ($P = 0.01$), and day ($P < 0.001$) were observed for erythrocyte SOD activity during the supplementation phase. Erythrocyte SOD activity did not change ($P = 0.99$) from day 90 to 120 and decreased from ($P = 0.05$) day 120 to 150 for both breeds. Angus cows had greater ($P \leq 0.05$) erythrocyte SOD activity than Brahman cows on days 90 and 120 (Table 11).

Calving

Angus cows had greater ($P < 0.01$) erythrocyte SOD activity at calving than Brahman cows (Table 7). No effects of breed ($P = 0.21$) were observed for erythrocyte SOD activity of calves at birth (Table 8).

Plasma CP

Restriction phase

Effects of breed ($P < 0.01$) and day ($P < 0.001$), but not breed × day ($P = 0.36$), were observed for plasma CP concentration of cows during the restriction phase. Plasma CP concentration decreased ($P < 0.001$) from day 0 to 30, followed by an increase ($P < 0.001$) from day 30 to 60, and then remained unchanged ($P = 0.64$) until day 90 (Table 5). Angus cows had greater ($P < 0.01$) plasma CP concentration than Brahman cows during the restriction phase (16.2 vs. 13.4 mg/dL, respectively; SEM = 1.37).

Supplementation phase

Effects of breed × day ($P < 0.01$) and day ($P = 0.05$), but not breed ($P = 0.36$), were observed for plasma CP concentration of cows during the supplementation phase. Plasma CP concentration was unchanged ($P \leq 0.66$) in Angus cows during the supplementation

Table 8. Calf liver and blood Cu and Se concentration and blood enzymatic activities of samples collected at birth

	Treatment			
Item	Angus	Brahman	Largest SEM	P-value
Liver Cu[1], mg/kg DM	243	345	69.8	0.09
Liver Se[1], mg/kg DM	1.13	1.07	0.109	0.70
Plasma Cu[2], ng/mL	253	361	24.6	<0.01
Plasma Se[2], ng/mL	40.5	32.3	3.01	0.08
Whole blood Cu[2], ng/mL	489	441	24.7	0.16
Whole blood Se[2], ng/mL	117	134	8.7	0.15
SOD[3], U/mL	2.35	2.30	0.030	0.21
Plasma CP[4], mg/dL	4.44	5.27	0.591	0.25
GPx[5], nmol/min/mL	542	468	19.4	<0.001

[1]Liver tissue was collected from calves within 24 h after birth, using biopsy procedures by a trained technician. Immediately after collection, liver samples were placed on ice. Liver samples were sent to a commercial laboratory for mineral analysis (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI).
[2]Blood samples were collected from the jugular vein of calves within 24 h after birth. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. An aliquot of whole blood was collected prior blood samples centrifugation. Plasma samples were obtained after centrifugation of blood at 1,200 × *g* for 30 min at 4 °C. Copper and Se concentrations in plasma and whole blood were analyzed by ICP-MS (ng/mL; Perkin-Elmer Corp., Norwalk, CT) at the Department of Soil & Water Science, University of Florida (Gainesville, FL), as described by Ranches et al. (2017).
[3]SOD (U/mL) activity was measured in erythrocyte lysate using a commercial kit (No. 706002, Cayman Chemical; Ann Arbor. MI). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical.
[4]Plasma samples were obtained after centrifugation of blood at 1,200 × *g* for 30 min at 4 °C. Plasma CP oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974).
[5]GPx (nmol/min/mL) activity was measured in erythrocyte. Activity was measured according to the decrease in absorbance at 340 nm. The rate of decrease in the absorbance is directly proportional to the GPx activity in the sample.

Table 9. Plasma Cu concentration of Angus and Brahman cows during the supplementation phase

	Treatment			
Day of the study[1]	Angus	Brahman	Largest SEM	P-value
	------ ng/mL ------			
90	902[a]	996[b]	131.2	0.18
120	919[a]	1,185[a]	131.2	<0.001
150	929[a]	1,219[a]	131.2	<0.001

[1]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. An aliquot of whole blood was collected prior to sample centrifugation.
[a,b]Means with different superscripts within a breed differ ($P < 0.001$).

Table 10. Whole blood Cu concentration of Angus and Brahman cows during the supplementation phase

	Treatment			
Day of the study[1]	Angus	Brahman	Largest SEM	P-value
	----- ng/mL DM -----			
90	656[a]	562[a]	50.4	0.07
120	647[a]	648[b]	51.1	0.98
150	703[a]	782[c]	50.4	0.12

[1]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. Plasma samples were obtained after centrifugation of blood at 1,200 × *g* for 30 min at 4 °C.
[a–c]Means with different superscripts within a breed differ ($P \leq 0.04$).

phase. In contrast, plasma CP concentration of Brahman cows increased ($P < 0.01$) from day 90 to 120 remained unchanged ($P = 0.53$) through day 150. Angus cows had greater ($P = 0.01$) plasma CP concentration than Brahman cows on day 90 with no differences ($P \geq 0.33$) on days 120 and 150 (Table 12).

Calving

No effects of breed ($P = 0.91$) were observed for plasma CP concentration of Angus and Brahman cows at calving (Table 7). No effects of breed ($P = 0.25$) were observed for plasma CP concentration of Angus and Brahman calves at birth (Table 8).

Glutathione peroxidase activity

Restriction phase

No effects of breed × day ($P = 0.41$), breed ($P = 0.45$), or day ($P = 0.31$) were observed for erythrocyte GPx activity of cows during the restriction phase (pooled average: 450 and 487 nmol/min/mL for Angus and Brahman cows, respectively; SEM = 12.2).

Supplementation phase

An effect of day ($P = 0.03$) was observed for erythrocyte GPx activity of cows during the supplementation phase, but no effects of breed × day ($P = 0.60$) or breed ($P = 0.42$) were observed.

Table 11. Erythrocyte SOD activity of Angus and Brahman cows during the supplementation phase

	Treatment			
Day of the study[1]	Angus	Brahman	Largest SEM	P-value
	------ U/mL ------			
90	4.15[a]	3.60[a]	0.162	<0.01
120	4.00[a]	3.60[a]	0.162	0.05
150	3.00[b]	3.15[b]	0.193	0.38

[1]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. SOD activity was measured in erythrocyte lysate using a commercial kit (No. 706002, Cayman Chemical; Ann Arbor, MI). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% of dismutation of the superoxide radical.
[a,b]Means with different superscripts within a breed differ ($P \leq 0.05$).

Table 12. Plasma CP concentration of Angus and Brahman cows during the supplementation phase

	Treatment			
Day of the study[1]	Angus	Brahman	Largest SEM	P-value
	----- mg/dL -----			
90	18.2[a]	13.9[b]	3.33	0.01
120	18.7[a]	17.7[a]	3.33	0.52
150	16.8[a]	18.4[a]	3.33	0.33

[1]Blood samples were collected from the jugular vein of cows at 30 d intervals. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection. Plasma CP oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974).
[a,b]Means with different superscripts within a breed differ ($P \leq 0.01$).

Erythrocyte GPx activity increased ($P < 0.01$) from day 90 to 120 and did not change ($P = 0.16$) from day 120 to 150 (Table 6).

Calving

No effects of breed ($P = 0.76$) were observed for erythrocyte GPx activity of cows at calving (Table 7). Angus calves ($P < 0.01$) had greater erythrocyte GPx activity than Brahman calves at birth (Table 8).

Cotyledon, colostrum, and milk mineral concentrations at calving

No effects of breed were observed for cotyledon Cu ($P = 0.12$) or Se ($P = 0.55$) concentration of cows at calving (Table 7). Brahman cows had greater ($P < 0.001$) colostrum Cu concentration than Angus cows. No breed effects ($P = 0.45$) were observed for colostrum Se concentration (Table 7). Brahman cows had greater ($P < 0.001$) milk Cu concentration than Angus cows. No breed effects ($P = 0.14$) were observed for milk Se concentration of Angus and Brahman cows (Table 7).

Gene expression analysis

Among the 35 genes evaluated in this study, 9 genes related to Cu and 13 genes related to Se metabolism were or tended to be differentially expressed between Angus and Brahman cattle at one of the time points analyzed in cow liver tissue (days 0, 90, 150, and calving), cotyledon tissue, or calf liver tissue (Table 13).

No breed differences were observed in the gene expression of liver samples on day 0 ($P = 0.93$) or at calving ($P = 0.94$).

On day 90, GPX3 ($P < 0.001$) and SELENOP ($P < 0.01$) gene expression in the liver tissue was greater for Angus cows than Brahman cows.

On day 150, gene expression in the liver tissue of genes MT1A ($P = 0.02$), GPX3 ($P = 0.02$), SELENOH ($P = 0.03$), SELENON ($P = 0.02$), and SELENOP ($P < 0.01$) was greater for Angus vs. Brahman cows. However, gene expression of A2M ($P = 0.03$) was greater for Brahman vs. Angus cows, and gene expression of SLC31A2 ($P = 0.10$) and SELENOO ($P = 0.08$) in the liver tissue tended to be greater for Brahman cows than Angus cows.

Gene expression in the cotyledon tissue of genes ATP7A ($P < 0.001$), SELENOT ($P = 0.02$), and SEPHS2 ($P = 0.02$) was greater for Angus than Brahman cows. However, gene expression of A2M ($P = 0.04$), CP ($P = 0.02$), MT1A ($P = 0.03$), MT2A ($P = 0.04$), MT4 ($P = 0.02$), DIO1 ($P = 0.02$), and SELENOM ($P = 0.04$) in the cotyledon tissue was greater for Brahman than Angus cows, while gene expression of COMMD1 ($P = 0.08$) and MT3 ($P = 0.06$) tended to be greater for Brahman than Angus cows.

Gene expression in the calf liver tissue of genes GPX4 ($P = 0.07$) and SELENOF ($P = 0.08$) tended to be greater for Angus calves than Brahman calves. However, gene expression in the calf liver tissue of genes MT3 ($P < 0.01$), SLC31A2 ($P = 0.02$), and SELENOO ($P < 0.001$) was greater for Brahman calves vs. Angus calves, while COMMD1 ($P = 0.07$), TXNRD2 ($P = 0.06$), and SELENOT ($P = 0.08$) tended to be greater for Brahman calves than Angus calves.

Discussion

Liver Cu concentration of Angus and Brahman cows did not change during the restriction phase indicating that the S supplementation strategy was not sufficient to create Cu deficiency (liver Cu ≤ 40 mg/kg DM; Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI). Although liver Cu concentration did not change during the restriction phase, Angus cows had consistently lesser liver Cu concentration than Brahman cows during this phase. The final outcome of the restriction phase for liver Cu concentration was not as expected but may be partially explained by the lack of Mo in the diet to foment the action of S as a Cu antagonist through the formation of thiomolybdates in the rumen (Manson et al., 1982). In contrast, Se deficiency (liver Se ≤ 0.6 mg/kg DM; Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI) was achieved during the restriction phase for both breeds by day 90. Liver Cu and Se concentrations were restored for both breeds during the supplementation phase, and the differences between the two breeds for Cu concentration were maintained, whereas no breed differences were observed for Se during this phase.

Differences in mineral tissue accumulation between breeds have been previously reported in the literature but comparing other cattle breeds. Fry et al. (2013), working with Angus and Simmental cows, reported that Angus cows had greater liver Cu concentration than Simmental cows after 112 d of S and Mo supplementation, while consuming a Cu-deficient diet, suggesting that Simmental cattle are more susceptible to Cu deficiency and less tolerant of Cu antagonists. The

Table 13. Gene expression of genes related to Cu and Se metabolism on liver and cotyledon samples[1]

Item	Angus	Brahman	SEM	*P*-value
Cow liver—Day 90[2]				
Selenium				
GPX3	1.13	0.195	0.2566	<0.001
SELONOP	6.53	2.33	0.739	<0.01
Cow liver—Day 150[2]				
Copper				
A2M	51.4	75.1	6.03	0.03
MT1A	1.91	0.736	0.2749	0.02
SLC31A2	0.048	0.078	0.0089	0.10
Selenium				
GPX3	0.760	0.066	0.1569	0.02
SELENOH	0.118	0.091	0.0059	0.03
SELENON	0.008	0.005	0.0007	0.02
SELENOO	0.061	0.076	0.0044	0.08
SELENOP	12.6	5.89	1.071	<0.01
Cotyledon[3]				
Copper				
A2M	225	230	59.0	0.04
ATP7A	0.172	0.092	0.0110	<0.001
CP	0.308	0.369	0.0698	0.02
COMMD1	0.187	0.237	0.0129	0.08
MT1A	0.512	0.629	0.0957	0.03
MT2A	5.27	5.57	1.298	0.04
MT3	0.018	0.033	0.0052	0.06
MT4	0.002	0.003	0.0004	0.02
Selenium				
DIO1	0.238	0.308	0.0586	0.02
SELENOM	0.166	0.238	0.0279	0.04
SELENOT	0.206	0.108	0.0210	0.02
SEPHS2	0.143	0.173	0.0388	0.02
Calf liver—Birth[2]				
Copper				
MT3	0.012	0.030	0.0039	<0.01
COMMD1	0.095	0.123	0.0081	0.07
SLC31A2	0.049	0.073	0.0095	0.02
Selenium				
GPX4	0.763	0.469	0.0580	0.07
SELENOO	0.029	0.055	0.0039	<0.001
TXNRD2	0.026	0.035	0.0030	0.06
SELENOF	0.332	0.237	0.0263	0.08
SELENOT	0.181	0.226	0.0130	0.08

[1]Gene expression data are presented as the relative expression to the geometric mean of the housekeeping genes using calculated using the $2^{-\Delta Ct}$ method.
[2]Liver tissue was collected from all cows at 30 d intervals (days 0, 30, 60, 90, 120, and 150) by a trained technician. A final liver sample was collected from both cows and calves within 24 h of calving. Immediately after collection, the sample was wrapped in aluminum foil and submerged in liquid nitrogen for snap freezing. The sample was stored at −80 °C until RNA extraction could be completed.
[3]After calving, the placentas of all cows were retrieved for cotyledon collection as described by Marques et al. (2016). Following cotyledon dissection from the placenta, two samples were collected. The sample for gene expression analysis consisted of a single cotyledon, which was vigorously washed with double-distilled water, wrapped in aluminum foil, and submerged in liquid nitrogen. These samples were stored at −80 °C until RNA could be extracted from the samples.

same rationale could be applied to the current study, where Angus cattle may be more susceptible to Cu deficiency and less tolerant to Cu antagonists than Brahman cows. However, the homeostatic regulation of Cu should be considered, and perhaps Angus cows have better maintenance of Cu homeostasis, while Brahman cows may indiscriminately accumulate Cu. To further support this rationale, in the current study, although no breed differences were observed in cotyledon Cu concentration, liver Cu concentration of Brahman calves only tended to be greater than Angus calves and both were within the range of adequacy (newborns: 125 to 650 mg/kg; Herdt and Hoff, 2011). Further, gene expression of MT1A was greater in liver samples of Angus cows when compared with Brahman cows on day 150. It has been demonstrated, in rodents and pigs, that gene expression of MT1A increases during Cu loading to protect cells from redox activity, therefore, suggesting that Angus cows had an upregulated mechanism to manage Cu excess (Bauerly et al., 2004; Fry et al., 2012).

Fry et al. (2013) who previously reported differences in Cu metabolism between Angus and Simmental cattle reported no differences in cotyledon Cu concentration between Angus and Simmental cows. In that study, cows that were considered Cu deficient, regardless of breed, had less cotyledon Cu concentration than cows considered Cu sufficient, suggesting that low status of Cu will result in reduced Cu transfer through the placenta. In the current study, although Angus cows had a reduced Cu status compared with Brahman cows, this difference was not sufficient to impact Cu maternal transfer.

When inducing Cu deficiency in Zebu and Holstein × Friesian cows, Dermauw et al. (2014) reported no differences in liver Se concentration between the two breeds; however, greater kidney Se concentration was observed in Zebu vs. Holstein × Friesian cows. Although it seems that Angus and Brahman cows differ in the accumulation of Se in the liver when challenged with a diet rich in antagonists, this difference did not impact the transfer of Se to calves as no breed differences were observed for cotyledon or liver Se concentration of calves at birth.

Interestingly, during the supplementation phase, no changes were observed for plasma Cu concentration of Angus cows; however, Brahman cows had increasing plasma Cu concentration with advancing days of supplementation. This response suggests a potential for better Cu homeostatic control for Angus cows as plasma Cu concentration will only decline when the liver Cu stores are exhausted (Claypool et al., 1975; Herdt and Hoff, 2011). The differences observed in Cu and Se plasma concentration of cows were reflected in the calves at birth, where Brahman calves had greater plasma Cu concentration than Angus calves, and Angus calves had greater plasma Se concentration than Brahman calves. Dermauw et al. (2014) reported that plasma Cu concentration of Holstein × Friesian cows indicated severe Cu deficiency, whereas plasma Cu concentration of Zebu cows was just below the threshold for deficiency (adequate: 700 to 900 ng/mL; Kincaid, 1999) after 11 wk of Mo supplementation while consuming a Cu-deficient diet. Although plasma Cu concentration of cows in the current study was not considered severely deficient, both studies present the same response where *B. taurus* or *B. taurus*-influenced cows had lesser plasma Cu concentration than *B. indicus* cows. Fry et al. (2013) also reported differences in plasma Cu concentration between Angus and Simmental cows, where Angus cows

had greater plasma Cu concentration than Simmental cows. Although the authors did not report a time effect for each sampling day during the Mo and S supplementation period, it seems that plasma Cu concentration of Angus cows remained fairly constant, which is similar to the response observed in the current study. Furthermore, Mullis et al. (2003) working with Angus and Simental heifers that were supplemented with different Cu concentrations (0, 7, or 14 mg Cu/d) reported that Cu supplementation generally did not increase plasma Cu concentration in Angus heifers, while Simmental heifers had increased plasma Cu concentration with increasing levels of Cu supplementation. The authors of that study concluded that Angus heifers may have a lesser minimal Cu requirement than Simmental heifers. A similar rationale could be applied to the current study, where Angus cows may have a reduced Cu requirement compared with Brahman cows. Regarding plasma Cu concentration of calves at birth, Ward et al. (1995) reported that Angus calves had greater plasma Cu concentration than Simmental calves. The authors suggested that because Cu status was greater for Angus vs. Simmental dams in their study, there was an opportunity for greater transfer and storage of Cu in the Angus vs. Simmental calves. A similar rationale could be applied to the current study; where at the time of calving, Brahman cows had greater plasma Cu concentration than Angus cows resulting in Brahman calves having greater plasma Cu concentration than Angus calves.

The majority of Cu found in plasma is bound to CP (90% to 95%), making it an important indicator of Cu status (Cousins, 1985). Plasma CP concentration has been reported to be responsive to Cu supplementation or the lack of it (Arthington et al., 2003). Further, CP is an acute-phase protein, which is responsive to an array of insults, such as inflammation, bacterial infection, and physical injury (Carroll and Forsberg, 2007). Therefore, the use of plasma CP concentration as an indicator of Cu status should be viewed with caution, especially in long-term studies where cows are confined and subject to frequent handling. The differences in CP concentration observed in this study, might not be related to Cu status but possibly breed-impacted differences in acute-phase signals between Angus and Brahman cattle. Mullis et al. (2003) previously reported breed differences for plasma CP concentration, where Angus steers had greater plasma CP concentration than Simmental steers throughout a 149-d study, while steers were supplemented with Fe. Similarly, Angus cows had greater plasma CP concentration than Simmental heifers, and Charolais heifers had intermediary plasma CP concentration between Angus and Simmental cows when supplemented with Fe (Ward et al., 1995). Plasma CP concentrations at calving were the greatest values observed in the current study, which is in agreement with Hussein et al. (2012) and Trevisi et al. (2012) who reported that dairy cows had greater plasma CP concentration during the periparturient period illustrating the impact of calving on the acute-phase reaction (Koets et al., 1998). No differences were observed for plasma CP concentration of calves at birth; however, no differences should be expected as fetus and newborns tend to accumulate Cu in the liver, synthesizing primarily apo-CP within the first days of life (Hellman and Gitlin, 2002). CP synthesis will increase as the calf ages as demonstrated by Bertoni et al. (2009). Plasma CP concentration observed for the calves in this study is most likely due to the colostrum transfer (Hernández-Castellano et al., 2015), which has greater CP concentration within the first 12 h after birth (Albera and Kankofer, 2009).

Plasma Se concentration is not the best indicator of Se status, as plasma Se concentration is sensitive to short-term dietary intake, which results in rapid fluctuations with small changes in intake (Herdt and Hoff, 2011). However, it is certainly an indication of differences in the Se metabolism between Angus and Brahman cows. Additionally, Angus cows had greater relative expression of GPX3 and SELENOP on days 90 and 150 in the liver samples, when compared with Brahman cows. Both GPX3 and SELENOP are considered to be Se transporter proteins and had recently been reported in mouse models to contribute to maternal–fetal Se transfer (Burk et al., 2013). Although placental classification differs between cattle and rodents, it is possible that both GPX3 and SELENOP are also involved in the maternal transfer of Se in ruminants. In contrast to the current study, Dermauw et al. (2014) working with Zebu and Holstein–Friesian heifers did not observe breed differences in plasma Se concentration. However, Pogge et al. (2012) reported differences in plasma Se concentration between Angus and Simmental steers, where Angus steers had greater plasma Se concentration than Simmental steers following the administration of injectable trace minerals.

According to Herdt and Hoff (2011), the regulation of blood Cu concentration is not well understood but is presumably under homeostatic control at the level of the hepatic Cu stores. Furthermore, liver Cu concentration and whole blood concentration have been reported to be correlated (López-Alonso et al., 2006). During the restriction phase, whole blood Cu concentration tended to decrease over time, but, interestingly, during the supplementation phase, the whole blood Cu concentration of Angus cows did not change while the whole blood Cu concentration of Brahman cows increased. To the knowledge of the authors, the effect of cattle breed on the whole blood Cu concentration has not been previously evaluated.

Although SOD carries approximately 60% of Cu in erythrocytes, Kincaid (1999) suggests that SOD is not a sensitive indicator of Cu status since activity does not decrease with deficient intakes of Cu until after plasma Cu and CP activities have declined. During the restriction and supplementation phases, as well as at calving, Angus cows had greater SOD activity than Brahman cows. However, this difference was not observed for calves at birth. SOD is a powerful antioxidant and responds to the production of reactive oxygen species in the body (Spears and Weiss, 2008). Furthermore, pregnancy and parturition increase oxygen requirement, which increases the production of oxygen reactive species (Sordillo, 2005). It has been demonstrated in humans that SOD activity decreases as pregnancy progresses (Patil et al., 2007) as observed for the cows in the current study. The production of reactive oxygen species was not measured in the current study, but it is possible that the observed breed differences in SOD activity are in fact simply due to the differences in response to the production of reactive oxygen species in both breeds.

According to Herdt and Hoff (2011), whole blood contains both the erythrocyte and plasma Se fraction of blood, and the contribution of erythrocyte Se to whole blood Se in cattle is roughly 60%. Erythrocyte Se is primarily associated with the GPx enzyme, and, therefore, the behavior of whole blood Se concentration is expected to be similar to GPx activity. Langlands et al. (1980) reported breed differences for whole blood Se concentration among cattle that were born and managed as a single herd. In contrast to the current study, in their study, Brahman heifers had greater whole blood Se concentration than Africander and Hereford × Shorthorn heifers. In the same study, they reported that Braham × Hereford had greater whole blood Se concentration than Hereford, Friesian × Hereford, and Simmental × Hereford heifers.

The GPx found in erythrocytes is formed during erythropoiesis; therefore, erythrocyte GPx activity for the determination of Se status will represent the long-term supplementation of Se and not the immediate intake of Se (Herdt and Hoff, 2011). During the restriction phase, which lasted 90 d, no changes in GPx activity were observed. However, during the supplementation phase, both breeds had increasing GPx activity, but no breed differences were observed during supplementation or calving. Additionally, GPx activity was greater in Angus vs. Brahman calves at birth. Although no breed differences were observed in GPx activity between cows, gene expression of SELENOH was greater for Angus cows in the liver samples on day 150 compared with Brahman cows. The function of SELENOH is not fully known; however, it has been proposed to participate in the regulation of glutathione synthesis (Qazi et al., 2018), which could explain the differences in GPx activity observed for calves. In contrast to the current study, Langlands et al. (1980) reported that Africander and Brahman heifers had greater GPx activity than Hereford × Shorthorn. However, Pogge et al. (2012), who previously reported differences in plasma Se concentration of Angus and Simmental steers, did not observe differences in GPx activity between the two breeds following the administration of an injectable mineral containing Se.

According to Suttle (2010), colostrum and milk Cu concentrations are around 0.15 mg/mL and will decrease as lactation progresses as observed by Kume and Tanabe (1993). In the current study, colostrum Cu concentration was less in Angus vs. Brahman cows. In addition, colostrum Cu concentration decreased by 24% in Angus cows from colostrum to milk samples. Differently, Brahman cows had a 26% increase in Cu concentration from colostrum to milk, which was in contrast to Salih et al. (1987), who reported decreasing Cu concentration from colostrum to milk of Brahman cows. Interestingly, Ward et al. (1995), when feeding either a Cu-deficient or Cu-adequate diet to Angus and Simmental heifers, reported that milk Cu concentration was low (approximately 1 mg/kg of DM) and not affected by the dietary Cu treatments, suggesting that Cu status does not influence Cu concentration in the milk. Although colostrum and milk are not the main route of Cu excretion, the increase in Cu concentration from colostrum to milk observed for Brahman was not expected and could be considered as an alternate excretion route to reduce the risk of Cu overload. A dilution factor could also explain the breed differences observed for Cu concentration in colostrum and milk; however, a minimum of a 5-kg difference in colostrum and milk production would be necessary, between the two breeds, for this dilution effect. Brown and Brown (2002) reported differences in milk production less than 5 kg/d between Angus and Brahman cows while managed on forage diets. Although breed differences were observed for colostrum and milk Cu concentration, no breed differences were observed for colostrum or milk Se concentration.

Gene expression of genes related to the metabolism of Cu and Se in cattle has not been widely published in the literature. In the current study, gene expression data of liver and cotyledon tissue revealed differences in the relative expression of genes related to Cu and Se metabolism between Angus and Brahman cattle. In the cotyledon, the Cu-related genes, such as *COMMD1*, *MT1A*, *MT2A*, *MT3*, and *MT4*, were greater expressed in the Brahman cows, except for ATP7A and CP, which were greater expressed in the Angus cows. Interestingly, ATP7A, which encodes for the ATP7A protein and is related to Cu homeostasis in mammals, was greater expressed in the cotyledon of Angus cows and may be related to the maternal transfer of Cu to the fetal circulation (Fontaine and Mercer, 2007). Among the differentially expressed genes, COMMD1 had greater expression in the cotyledon of Brahmans cows and the liver of Brahman calves at birth compared with Angus. Several studies support the role of COMMD1 in copper homeostasis, and mutation of COMMD1 has been reported to affect biliary Cu excretion leading to Cu accumulation (Klomp et al., 2003; Vonk et al., 2008). In contrast to the current study, Fry et al. (2013) reported that COMMD1 was upregulated in the fetus within cows fed a Cu-deficient diet during pregnancy when compared with the fetus of cows fed a Cu-adequate diet.

Regarding Se metabolism, expression of cotyledon SELENOM and SEPHS2 was greater in Brahman cows, while cotyledon SELENOT expression was greater in Angus cows. At birth, liver GPX4 and SELENOF expression was greater in Angus calves, while liver SELENOO, TXNRD2, and SELENOT expression was greater in Brahman calves. The gene SEPHS2 encodes for selenophosphate synthetase 2 that catalyzes the formation of monoselenophosphate and has been demonstrated by Zhou et al. (2009) in the liver of pigs to not be influenced by dietary Se. In that study, the authors also reported that gene expression of liver GPX4 of pigs was not impacted by dietary Se.

In summary, substantial differences in multiple indicators of Cu and Se status and metabolism were observed between Angus and Brahman cows. Data from this study imply that Brahman cows, compared with Angus, are more efficient at maintaining adequate Cu status when consuming a Cu-inadequate diet with high S. In contrast, Angus cows, compared with Brahman cows, are more efficient at maintaining adequate Se status when consuming a Se inadequate diet with high S. Furthermore, Brahman cows may be particularly susceptible to Se, but not Cu, deficiency. Angus cows appear to be better adapted to low Cu nutrition, as witnessed by adequate enzyme function with measures of limited Cu status. These findings provide important breed-related information when assessing cattle supplementation strategies for the two most deficient trace minerals in warm-season forages. More research on this topic is warranted to further understand the mechanisms responsible for the observed differences in Cu and Se status in Angus and Brahman cattle.

Conflict of interest statement

No conflict of interest, financial, or otherwise is declared by the authors.

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