Effects of Gestational Status on Apparent Absorption and Retention of Copper and Zinc in Mature Angus Cows and Suffolk Ewes**

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ABSTRACT : Six mature purebred Angus cows (3.5±1.5 years of age, 631.36±8.63 kg), three pregnant (250±7 days pregnant) and three non-pregnant and eight mature Suffolk ewes (5±1.2 years of age, 68.18±2.3 kg) four pregnant (115±5 days pregnant) and four non-pregnant were utilized in a five-day metabolic trial to determine the effects of gestational status (pregnant vs. non-pregnant) on apparent absorption and retention of copper (Cu) and zinc (Zn). Animals were selected based on body weight, age, and gestational status, and randomly assigned to metabolic crates for total fecal and urine collection. Animals were allowed to acclimate to their new environment for seven days. Pregnant and non-pregnant cows and ewes were then paired (within a species) by body weight and pair-fed throughout the 5 day collection period. Copper and Zn intakes were similar for pregnant and non-pregnant animals within a species. Apparent absorption of Cu ($p<0.06$) and Zn ($p<0.04$) were higher in pregnant cows relative to non-pregnant cows. Pregnant cows also had a higher apparent retention of Cu ($p<0.05$) and Zn ($p<0.06$) relative to non-pregnant cows. Pregnant ewes had a higher ($p<0.01$) apparent absorption and retention of Zn compared to non-pregnant ewes. However, apparent absorption and retention of Cu were similar for pregnant and non-pregnant ewes. These data indicate that certain physiological and/or metabolic parameters are altered in pregnant cows and ewes consuming an alfalfa-based diet that enhance the apparent absorption and retention of certain trace minerals. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 515-518)***

Key Words : Copper, Zinc, Gestation, Cow, Ewe

INTRODUCTION

Animals require trace elements for proper growth, immune function, and reproduction (Underwood and Suttle, 1999). Typically, foraged-based diets consumed by grazing ruminants contain inadequate concentrations of copper (Cu) and zinc (Zn) to meet the animal's requirement. Therefore, Cu and Zn are supplemented to avoid deficiencies.

The majority of Cu and Zn consumed by an animal will be excreted due to a relatively low absorption rate. The increase in supplemental Cu and Zn in order to prevent deficiencies, combined with low absorption rates can lead to heavy metal contamination of the soil and groundwater in areas where livestock are concentrated. This facilitates the need for examining certain factors that affect the ability of the animal to absorb and retain trace minerals.

While many factors have been studied, there has been little research examining the effects of gestational status (pregnant vs non-pregnant) on trace mineral absorption in the bovine and ovine. Earlier research has indicated that Zn concentrations increase in bovine conception products (placenta, placental fluids, and fetus) during the last trimester of pregnancy (Hansard et al., 1968). Furthermore, human studies have indicated that pregnant women tend to absorb and retain more Zn than non-pregnant women (Swanson and King, 1982). Similar results regarding Cu and Zn absorption have been reported in pregnant rats (Mills, 1980). Therefore, the objective of this study was to determine how gestational status affects apparent absorption and retention of Cu and Zn in pregnant (last trimester of gestation) vs. non-pregnant mature Angus cows and Suffolk ewes.

MATERIALS AND METHODS

Animals

Six mature purebred Angus cows (3.5±1.5 years of age, 631.36±8.63 kg), three pregnant (250±7 days pregnant) and three non-pregnant and eight mature Suffolk ewes (5±1.2 years of age, 68.18±2.3 kg) four pregnant (115±5 days pregnant) and four non-pregnant were utilized in a five-day metabolic trial. Prior to the initiation of the present experiment, the Colorado State University Animal Care and Use Committee approved care, handling, and sampling of all animals described herein.

Animals used in this experiment were obtained from our resident instructional herds at the Agricultural Research, Development, and Education Center (ARDEC; Colorado State University, Fort Collins, CO). Six cows and eight sheep were selected based on body weight, age, and gestational status. Cows and sheep were maintained in separate group pens (20 m×60 m and 10 m×15 m, respectively; equipped with automatic waterers), with

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ad libitum access to a 100% alfalfa hay diet (basal alfalfa hay diet contained 7.6 mg Cu/kg DM, 23.0 mg Zn/kg DM, 315.0 mg Fe/kg DM, 0.23%, and 1.8 mg Mo/kg DM) prior to use in this experiment. During this time cows were halter broke prior to introduction to the metabolic crates, for animal and handler safety. After the cows became accustomed to handling, cows and sheep were housed in a climate controlled metabolism building. All cows and sheep were placed in digestion crates (2 m×3 m, 1 m×2 m, respectively) and acclimated to their new surroundings for seven days. Each digestion crate contained a self-waterer (water contained: 0.01 mg Cu/L, 0.1 mg Zn/L, 0.01 mg Fe/L, 0.03% S, and 0.02 mg Mo/L), a rubber matted floor, and a feed bin. Animals were haltered to the front of the digestion crates. This prevented the animals from turning around, but allowed animals easy access to water and feed, as well as the ability to stand up and lay down. Animals were fed a 100% alfalfa diet, daily, in amounts to allow ad libitum access to feed throughout the day. Feed intakes were recorded daily.

After the seven-day acclimation phase all animals were weighed. Animals in the pregnant and non-pregnant groups were paired to one another (within a species) by bodyweight and pair-fed throughout the remainder of the trial. Pair feeding was implemented to normalize energy intake.

Total collection phase

All animals were fed a 100% alfalfa diet, daily, in amounts relative to that of their pair-fed counterpart. Total feces and urine were collected for five days. Cow urine was diverted into stainless steel containers to avoid mixing with the feces. Total urine volume was recorded daily and a 150 ml aliquot was retained for Cu and Zn analysis each day. For the ewes, feces were collected using a screen, which caught fecal material and allowed urine to pass through for collection. Total urine volume was recorded daily and a 100 ml aliquot was retained for Cu and Zn analysis each day. For all animals, fecal samples were collected daily and 10% of the daily fecal output obtained. Prior to taking a 10% fecal sample, the 24 h fecal sample collected was thoroughly mixed to obtain an accurate representative fecal sample. Representative daily samples of urine and feces were immediately labeled and frozen at -20°C. After the five-day collection, animals were returned to their appropriate pastures.

Analytical porocedures

Fecal sub-samples were later thawed at room temperature, and dried at 100°C for 48 h in a forced-air drying oven. Immediately following removal from the ovens, all samples were allowed to cool in desiccators, prior to being weighed for % dry matter. The dried fecal samples were then ground in a Thomas Wiley mill (model 4), through a 1 mm screen. A 5 day composite of dried feces was created for each animal by taking a 10% aliquot of each day's sample. Urine samples were thoroughly mixed and composited for each animal. Feed and water samples were also collected and analyzed for Cu and Zn.

Cow and ewe fecal, feed, water, and urine samples were analyzed for Cu and Zn concentrations via atomic absorption spectrophotometry (Varian Model 1275; as described by Engle et al., 1997). Urine samples were diluted with deionized water to a dilution of 1:6 and analyzed. Feed and fecal samples were placed into crucibles and ashed overnight. Each sample was diluted with 10 ml of 3.6 N nitric acid and placed in an Ultrasonic Cleaner (Cole Palmer Model 8845-4) for 5 minutes. Samples were then diluted with deionized water to a dilution of 1:5. The dilution mixture was then vortexed vigorously and then analyzed.

Calculations

Apparent absorption of Cu and Zn was calculated by subtracting the total amount (mg) of fecal Cu or Zn excreted by each animal (over the 5 d collection phase), from the total amount (mg) of Cu or Zn consumed by each animal (over the 5 d collection phase: [mg of mineral consumed -mg of mineral in feces]=apparent absorption). Apparent absorption is a general indicator of the amount of a specific nutrient absorbed form the gastrointestinal tract. Apparent retention of Cu and Zn was calculated by subtracting the sum of Cu or Zn excreted in the feces and urine of each animal, from the total amount (mg) of Cu or Zn consumed by each animal (over the 5 d collection phase: [mg of mineral consumed–(mg of mineral in feces+mg of mineral in urine)]=apparent retention). Apparent retention is a general indicator of the amount of a specific nutrient retained in the animal's body.

Statistical analysis

Data were analyzed by least squares ANOVA for a randomized complete block design using the using the GLM procedure of SAS (1988).

RESULTS

Cattle

Effects of gestational status on apparent absorption and retention of Cu and Zn are shown in Table 1. Copper and Zn intakes were similar for both the pregnant and non-pregnant groups due to experimental design. The concentration of Cu in the feces of the non-pregnant cows was greater ($p<0.10$) than that of the pregnant cows. There was no difference in the concentration of Cu excreted in the urine between the non-pregnant and pregnant cows. Therefore, apparent absorption ($p<0.06$) and retention

Table 1. Effect of gestational status on apparent absorption and retention of copper and zinc in Angus beef cows

Item	Pregnant	Non-pregnant	SEM	P<
DM intake, kg/d	12.9	12.9	-	-
Fecal DM, kg/d	4.7	4.4	0.5	0.23
DM digestion, %	64.2	65.1	1.1	0.31
Copper				
Intake, mg/d	88.9	88.9	-	-
Excretion, mg/d				
Fecal copper	81.0	86.0	1.8	0.10
Urinary copper	1.1	1.2	0.1	0.28
Apparent absorption, mg/d	7.9	2.9	1.8	0.06
Apparent retention, mg/d	6.8	1.7	3.1	0.05
Zinc				
Intake, mg/d	220.3	220.3	-	-
Excretion, mg/d				
Fecal zinc	198.4	208.3	4.1	0.08
Urinary zinc	4.6	4.2	0.3	0.37
Apparent absorption, mg/d	21.9	12.0	3.8	0.04
Apparent retention, mg/d	17.3	7.8	4.1	0.06

Table 2. Effect of gestational status on apparent absorption and retention of copper and zinc in ewes

Item	Pregnant	Non-pregnant	SEM	P<
DM intake, kg/d	1.59	1.59	-	-
Fecal DM, kg/d	0.57	0.62	0.04	0.37
DM digestion, %	0.64	0.61	0.02	0.42
Copper				
Intake, mg/d	4.40	4.40	-	-
Excretion, mg/d				
Fecal copper	3.55	3.75	0.25	0.60
Urinary copper	1.27	1.66	0.24	0.29
Apparent absorption, mg/d	0.85	0.65	0.25	0.60
Apparent retention, mg/d	-0.42	-1.01	0.36	0.29
Zinc				
Intake, mg/d	30.67	30.67	-	-
Excretion, mg/d				
Fecal zinc	24.67	28.30	0.45	0.01
Urinary zinc	0.40	0.72	0.14	0.17
Apparent absorption, mg/d	6.00	2.37	0.45	0.01
Apparent retention, mg/d	5.91	1.65	0.47	0.01

($p<0.05$) were higher for pregnant relative to non-pregnant cows. Fecal Zn concentrations were higher ($p<0.08$) and urinary Zn concentrations similar ($p>0.10$) in non-pregnant relative to pregnant cows, thus, apparent absorption ($p<0.04$) and retention ($p<1.06$) of Zn were greater for pregnant cows.

Sheep

Copper and Zn intakes were similar in both the pregnant and non-pregnant ewes due to experimental design (Table 2). Fecal and urine Cu concentrations were similar ($p>0.10$) for pregnant and non-pregnant ewes, therefore, apparent absorption and retention did not differ between treatment groups. Fecal Zn concentrations were higher ($p<0.01$) in non-pregnant ewes relative to pregnant ewes. There was no difference in urinary Zn concentration between pregnant and non-pregnant ewes. Apparent absorption ($p<0.01$) and retention ($p<0.01$) of Zn was greater in pregnant ewes relative to their non-pregnant counterparts.

DISCUSSION

Altered metabolism of trace minerals during pregnancy has been demonstrated in beef cattle. Using crossbred beef heifers, Hansard et al. (1968) reported that a total of 5.8 mg Zn was transferred to the fetus during the first 90 days of gestation. As gestation progressed, fetal Zn increased thirteen times between the first and second trimester and seven times during the third trimester. However, there were no effects of stage of pregnancy on fecal Zn excretion patterns. In another experiment, Hansard et al. (1968) reported that Zn^{65} values tripled in fetal liver and bone from pregnant cows slaughtered after 24, 96, and 168 h post-intravenous dosing, while maternal liver Zn^{65} values decreased at each period. These data indicate that maternal Zn metabolism is altered by fetal growth and as the fetus increases in size, fetal demand for Zn increases. The aforementioned data may help explain the reason for the increase in apparent absorption and retention of Zn observed in pregnant (last trimester of gestation) cattle and sheep relative to their non-pregnant counterparts. Increasing absorption and retention of Zn may allow for a greater supply of Zn to the fetus.

Swanson and King (1982) reported similar results involving pregnant and non-pregnant women used in a confined metabolic study. Pregnant women excreted less Zn in their feces than their non-pregnant counterparts, indicating that pregnant women had a greater mean Zn absorption. However, pregnant women did excrete numerically more Zn in their urine. Furthermore, using a stable isotope of Cu contained within the diet, Turnland et al. (1983) determined the amount of Cu absorbed from the diet of pregnant and non-pregnant women. Pregnant women consuming plant proteins absorbed more Cu than their non-pregnant counterparts. In addition, Mills (1980) reported that in the rat, the efficiency at which Cu and Zn are absorbed doubles during the last stages of pregnancy to meet maternal demand. These results are in agreement with results from the present cow study.

It is unclear as to why apparent absorption and retention of Cu was increased in pregnant cows relative to their non-pregnant counterparts but not between pregnant and non-pregnant sheep. It has been well documented that sheep are more sensitive to excess dietary Cu relative to cattle (Underwood. 1977). Feeding young weaned Holstein bull calves dietary Cu concentrations ranging from 0 to 900 mg Cu/kg DM for 98 days had no adverse effects on performance or health (Felsman et al., 1973). However, based on Cu toxicity reports in sheep flocks. Adamson et al., (1969) suggested that the maximum dietary Cu concentration for growing sheep ranges form 20-30 mg of Cu/kg DM. It should be noted however, that dietary compounds other than Cu (i.e. Zn. S. Mo. Fe) can influence the maximum tolerable dietary Cu concentrations of sheep (Bremner et al., 1976). The drastic differences between the maximum tolerable dietary Cu concentrations between sheep and cattle indicate a potential difference in factors that regulate absorption and metabolism of Cu between species.

Mehra and Bremner (1984) reported differences in lysosomal bound Cu and cytosolic Cu bound to metallothionine in sheep hepatocytes relative to other animal species, indicating a difference in Cu metabolism post-absorption. Furthermore, it has been reported that hepatic Cu concentrations in pregnant (last trimester of gestation) cows are typically lower than that of their fetus, whereas hepatic Cu concentrations in pregnant (last trimester of gestation) sheep are typically higher than that of their fetus (Pryor, 1964). This metabolic modification by the ewe (increasing maternal liver Cu stores during parturition) is most likely a protective mechanism in order to prevent a possible Cu overload in newborn sheep.

These data, in conjunction with differences in maximum tolerable dietary concentrations of Cu between sheep and cattle as well as alterations in hepatic Cu metabolism between sheep and other animal species, indicate a potential difference in factors that regulate absorption and/or metabolism of Cu between sheep and other animal species. This could help to explain the aforementioned differences between apparent absorption and retention of Cu in pregnant cattle and sheep relative to their non-pregnant counterparts. Perhaps, during gestation, Cu absorption is not increased in sheep as observed in pregnant beef cattle, in order to prevent fetal Cu toxicity.

The present experiments indicate that gestational status (pregnant vs non-pregnant) alters the apparent absorption and retention of certain trace minerals during the last trimester of gestation. Further research is required to determine the metabolic mechanisms that enable pregnant cows and ewes to enhance apparent absorption and retention of certain trace minerals as well as the animal's specific metabolic requirement for both maintenance and fetal development. In addition, research to determine if breed differences exist relative to pregnancy and trace mineral absorption is warranted.

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