

CHARACTERIZATION OF THE RELATIVE ABUNDANCE OF DUODENAL MRNA RELATED TO GENES INVOLVED IN COPPER ABSORPTION IN DIFFERENT BREEDS OF SHEEP

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Abstract

A deeper understanding of mechanisms for copper assimilation and metabolism is necessary across multiple animal species, given prevalent copper deficiencies in some regions and species, together with known sensitivities to copper intake in other species. This work represents the first stage of a comparative approach, starting with domestic sheep. The objective of this experiment was to characterize relative expression of mRNA related to genes involved in Cu absorption in the duodenum of different breeds of sheep. Opportunistic liver and small intestine samples were collected from three harvested market weight Lincoln and Suffolk wether lambs. Each breed was from an independent production facility but received diets that were similar in crude protein (CP), energy (NEg), and copper (Lincoln diet; CP = 14.8 %; NEg = 0.70 Mcal/kg; Cu = 6.82 mg/kg DM; Suffolk diet: CP = 14.4 %; NEg = 0.74 Mcal/kg; Cu = 6.67 mg/kg DM). Although individual animal feed intake was not determined at each location, both breeds of sheep were of similar body weight and age at the time of slaughter. A 5 cm sample of the duodenum (15 cm aboral from the pyloric sphincter) was collected from each lamb at the time of slaughter. Samples were immediately excised, sliced open, and washed with a PBS solution. The mucosal lining of the intestine was scraped with a scalpel blade to collect enterocytes. The scraped enterocytes were placed into a conical tube containing RNAlater™ (Thermo Fisher Scientific Inc., Waltham, MA 02451). Liver tissue, from the center of the right lobe of the liver, was also collected from each lamb, washed with PBS solution, and stored in a conical tube until liver Cu concentrations could be determined. All intestinal and liver samples were stored at 4°C until transported back to the laboratory. Total enterocyte RNA was extracted and purified using TRI reagent and RNeasy (QIAGEN, Redwood City, CA 94063). Primers for antioxidant 1 (ATOX1), Cu transporting alpha-polypeptide Atpase (ATP7A), Cu transporting beta-polypeptide Atpase (ATP7B), and Cu transporter1 (CTR1), were designed for real time PCR analysis. Liver Cu concentrations were lower ($P < 0.05$) in Lincoln (225.3 ± 23.1 mg Cu/kg DM) compared to Suffolk lambs (342.1 ± 31.2 mg Cu/kg DM). The relative expression of ATOX1 ($P < 0.05$), ATP7B ($P < 0.01$), and CTR1 ($P = 0.04$) were lower in Lincoln compared to Suffolk lambs. These data may indicate that Cu absorption from the duodenum may be different in different breeds of sheep. Further research examining the impact of dietary Cu dose on genes involved in Cu absorption in different breeds of sheep as well as identification and quantification of proteins involved in Cu absorption, is warranted. Given these potential breed differences within a single species, multi-species sampling and similar comparisons are also warranted.