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Influence of dietary zinc on the claw and interdigital skin of sheep

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ABSTRACT

Claw diseases like interdigital dermatitis and footrot threaten sheep health and are major welfare issues. Several studies mainly done in cattle suggested that zinc (Zn) supplementation may improve claw integrity. However, Zn supplements may differ markedly regarding Zn bioavailability. Zn bound to single amino acids has been shown to be more bioavailable than inorganic Zn sources. The aim of this study was to determine the effect of different Zn supplements on the integrity of the claw and interdigital skin of healthy sheep. At weaning 30 Merino lambs were randomly allocated to three different dietary treatments which were provided through the pelleted concentrates as follows: 1) no supplemental Zn (Zn0); 2) addition of 40 mg/kg Zn as Zn sulphate (ZnS); 3) addition of 40 mg/kg organic Zn as Zn amino acid complex (CZn). Barley straw and pelleted concentrates were given ad-libitum. The calculated Zn concentration of the total diet (roughage and concentrate) without supplemental Zn (Zn0) was 38 mg Zn/kg DM. The concentrates were formulated to meet the nutritional requirements for growing lambs and contained 207 g/kg DM crude protein and 12.4 MJ/kg DM metabolizable energy. After 8 weeks the lambs were slaughtered and the following specimens were collected: blood serum, liver, sole and coronary band of the claw, and interdigital skin. Serum and tissue Zn and copper (Cu) concentrations and claw hardness were determined. Routine pathohistology and electron microscopy were conducted. Franz diffusion cell system and Ki-67 immunostaining were used to determine the permeability of the interdigital skin and the keratinocyte proliferation in the basal layer of sole horn, coronary band and interdigital skin, respectively. The concentrations of Zn and Cu in serum and liver tissue as well as the Zn concentration in claw horn were not affected by dietary treatment. Zn0 lambs showed higher ($p < 0.05$) Cu concentrations in claw horn compared to both Zn supplemented groups. Routine pathohistology as well as electron microscopy did not show significant morphological differences between the three groups. Franz diffusion cell system proved to be a suitable method examining the interdigital skin permeability, but the group differences in this study were not significant. Dietary treatment did not affect keratinocyte proliferation in the coronary band. In the sole keratinocyte proliferation was significantly higher ($p < 0.05$) in the Zn0 group compared to CZn with ZnS being intermediate. Keratinocyte proliferation in the interdigital skin was significantly higher ($p < 0.05$) in the CZn group compared to the Zn0 with ZnS being intermediate. The results of the current experiment indicate that serum and tissue Zn concentrations and horn hardness are not affected by adding a moderate amount of Zn sulphate or Zn amino acid complex to a basal diet. However, supplemental Zn amino acid complex seems to affect keratinocyte proliferation of interdigital skin and sole of lambs. Effects on skin permeability should be retested using a higher number of animals prospectively.

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1. Introduction

Lameness is the greatest welfare and health concern in sheep [1]. Main reasons are interdigital dermatitis and footrot caused by bacterial agents like *Fusobacterium necrophorum* and *Dichelobacter nodosus*. Both bacteria affect at first the interdigital skin, expand to the interdigital wall of hoof followed by separation of the hard horn from the claw [2]. Besides treatment strategies with antibiotics against infectious agents, the question of dietary effects of trace elements like Zinc (Zn) to improve claw integrity is of increasing interest. Claw integrity is the physiological structure along with the protective and biomechanical function of the healthy claw. It ensures the functionality of interdigital skin and hoof horn to protect the interior living, sensitive epidermal cells and the dermal layer from adverse environmental noxa. Keratinocytes are the basic skin cells and undergo a progressive and complex differentiation process eventually resulting in cell death (cornification) building up the horn layer of skin or the hard horn [3]. An appropriate supply of nutrients, including vitamins, minerals, and trace elements is vital for the process of keratinization, the resulting horn quality and the overall integrity of the claw [4].

The role of Zn during the keratinization process has been reviewed by Tomlinson et al. [3] in cattle. It plays a crucial role in the differentiation and survival of keratinocytes and their unique functions [5]. Zn has traditionally been supplemented to animals' diets as inorganic salts like Zn oxide or Zn sulphate. However, there has been considerable interest in the use of chelated or organic Zn in ruminant diets. Some positive reports about improved claw integrity in cattle fed organic Zn were published in the last decades [4,6–8]. Nevertheless, the dietary factors that affect Zn bioavailability in ruminants are not clearly defined and still under discussion [9]. In the gastrointestinal tract, trace element ions from inorganic sources are released and possibly form insoluble complexes with other intestinal contents reducing their gut absorption [10]. In contrast, organic Zn supplements use amino acid absorption mechanisms across the mucosa, leading to an intracellular enrichment of Zn within the enterocytes mostly without inhibition by Zn absorption antagonists [10,11]. Responsible for the intestinal Zn uptake are members of the Zip transporter family which increase the cellular Zn concentration [12,13].

A concentration between 20–50 mg Zn/kg dry matter (DM) has been recommended for the ovine diet during growth [14,15]. Clinical Zn deficiency has occasionally been described in sheep. Affected animals show alopecia, abnormal hoof growth and lesions in the skin such as hyperkeratosis and parakeratosis [15,16]. The keratinization seemed to be reduced and apoptosis occurred in wool follicles [17]. In contrast, Zn toxicity seems to be rare and is mainly reported by excessive Zn uptake leading to anorexia, reproductive failure and weight losses [18,19]. The main outcome of chronic Zn overload seems to be copper (Cu) deficiency [20].

The aim of the following study was to determine the effects of organic and inorganic Zn supplements on the claw integrity of healthy lambs.

2. Material and methods

2.1. Animals

The project was conducted at the Agriculture School of Triesdorf (Middle Franconia, State Bavaria, Germany). Housing and feeding were done comparable to routine sheep production. A total of 30 weaned Merino lambs at the age of 2 months and an average initial body weight of 25.95 kg ($\pm$ 4.14 kg) were used. The lambs were selected from the agricultural school's pedigree flock. These lambs were selected because they had the same sire, so they were genetically similar. All lambs were vaccinated against pasteurellosis and clostridiosis (Heptavac® P Plus, MSD, Oberschleissheim, Germany) twice at an age of two weeks and were boostered 4 weeks later. Additionally, all lambs were treated with Toltrazuril (Tratol®, Zoetis, Berlin, Germany) against coccidiosis at the age of 3 weeks. Each group contained five male and five female lambs. The lambs were kept on deep litter (barley straw) in groups of 10 animals. Initially, all lambs were clinically examined and weighed. Body weights were measured at 14-day intervals and the average daily weight gain was calculated.

2.2. Feeding strategy

The lambs were randomly divided and housed in three groups. Each group was assigned to one of three dietary treatments: Concentrate A without added Zn (Zn0), concentrate B with 40 mg/kg Zn added as Zn sulphate (ZnS) and concentrate C with 40 mg/kg Zn added as organic Zn (CZn; Availa®Zn Zinc amino acid complex, Zinpro Corporation, Eden Prairie, USA).

The diets consisted of a pelleted concentrate and straw to which the lambs had free access. These feedstuffs and drinking water were provided ad libitum. Pellets were prepared with the usage of barley meal (40%), wheat meal (29.1%), extracted soybean meal (22%), calcium carbonate (5%), sugar beet molasses (2%), monocalcium phosphate (1%), sodium chloride (0.4%) and three different, individually composed vitamin premixes (0.5%). The Zn supplements were blended into the pellets at a professional feed mill. The concentrates were formulated to meet the nutritional requirements for growing lambs [21] containing

Table 1
Chemical composition (DM basis)[a] of concentrates, straw and water.

Nutrient	Concentrate A	Concentrate B	Concentrate C	Barley straw	Drinking water[b]
Dry matter; g/kg as fed	898	901	900	848	
Crude ash g/kg	68	75	76	42	
Potassium g/kg	7.8	8.5	8.4	10.5	
Calcium g/kg	13.4	15.0	15.8	2.0	69
Phosphorus g/kg	5.7	5.9	6.0	0.6	
Sodium g/kg	2.5	2.6	2.7	0.14	
Chloride g/kg	3.7	4.1	4.0	3.4	
Sulphur g/kg	1.9	1.9	1.9	0.6	
Magnesium g/kg	1.5	1.7	1.6	0.7	24
Copper mg/kg	10.3	9.3	9.3	2.6	0.09
Zinc mg/kg	38.9	67.6	62.6	28.0	0.14
Manganese mg/kg	63.0	62.0	56.0	20.0	< 0.02
Iron mg/kg	154	138	136	64	5.67
Cobalt mg/kg	0.21	0.19	0.17	0.05	

[a] Analyzed concentrations.
[b] mg/L.