A literature research on the etiology of zinc deficiency in South American camels and other mammals including humans

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1 Introduction

Zinc-responsive dermatosis is related to keratinization disorders caused by a deficient zinc status. Twenty percent of the entire body zinc stores are found in the skin, with the epidermis holding more than six times the amount of the dermis. Zinc levels tend to be higher in tissues with increased proliferation, which further suggests that zinc plays a significant part in the keratinization of the epidermis. (Aggett and Comerford 1995)

Zinc-responsive dermatosis has been described in many species including cattle, sheep, goats, pigs, rats, dogs, South American camels and humans. To diagnose zinc responsive dermatosis, the veterinary practitioner should always obtain a detailed dietary and production history, perform the entire physical examination and take appropriate skin and blood samples.

In humans, this condition is known as Acrodermatitis enteropathica and has numerous resemblances with the disease in dogs and cattle. However, depending on species and severity of the zinc deficiency, clinical symptoms incline to vary. The first clinical symptom tends to be anorexia which can be attributed to the decreased sense of smell and taste. Other symptoms of zinc deficiency embrace growth retardation, decreased fertility, wound healing disorders and typical parakeratotic skin lesions. (Colombini 1999) Most tissues with a fast turnover, like dermal cells and mucosal cells are most susceptible to deficient zinc levels. (Aggett and Comerford 1995)
Zinc has three different functions in living organisms, including regulatory, structural and catalytic functions. (Coffee 2001) Zinc is part of catalytic sites in numerous different metalloenzymes. The first zinc-dependent metalloenzyme was described by Keilin and Mann in 1939. (Keilin and Mann 1940) Today, well studied zinc metalloenzymes include the carbonic anhydrase, the alcohol dehydrogenase, alkaline phosphatase and the RNA polymerase. In the 1980s it has been described that zinc is a structural component of regulatory proteins in genes. Today hundreds of zinc finger sequences have been made apparent in bacteria, viruses, insects and mammals. Zinc finger proteins play an important role in the building and maintaining numerous off all tissues including the dermis. Zinc further has important regulatory functions as it moves through gated membrane channels as an ionic signal. Zinc is also stored and released from nerve cells in the cortex of the cerebrum. Once zinc is located intracellularly, it can alter cell functions by interacting with zinc-dependent proteins. (Frederickson et al. 2005) (Maverakis et al. 2007)

Four different stages have been defined when a diet is not able to meet the need for certain elements such as zinc. 1) Depletion; this is when the stores in the body are becoming diminished. 2) Deficiency; occurs when the concentrations of the mineral, which is typically kept within defined limits, falls. 3) Dysfunction; when the functions of the mineral in the body or tissue become rate limited. 4) Disorder; when the animals become clinically abnormal and show poor performance levels. (Suttle 2010)

The main physiological loss of zinc occurs in the intestine due to secretions from the pancreas. The loss consists of approximately 34 g of zinc/kg/day in humans. Zinc is also reabsorbed over the kidney with an estimated loss of around 7.5 g of zinc/kg/day. This value was also obtained from studies conducted in humans. Zinc is further lost to shedding or release of epidermis, hair, sweat, semen and bleeding. (Maverakis et al. 2007)
The aim of this literature research was to gain a deeper insight into the different etiologies of zinc deficiency in South American camels and other animal species including humans. The study should further answer following questions:

- Are there minimal dietary zinc concentrations which must be received by llamas and alpacas to avoid dietary zinc deficiency?
- Dietary zinc deficiency, hereditary and immunological based zinc deficiencies have been elucidated in certain species: Is there any evidence for hereditary causes responsible for zinc deficiency in South American camels as described in other animal species including humans?
- To extent does the immune system interact with the zinc metabolism, further causing hypozincaemia in llamas and alpacas?
2 History of zinc deficiency

First reports on the importance of zinc in organisms were documented in 1869 by Raulin, a student of Louis Pasteur. He found that Aspergillus niger (causes black mold), a fungus, needed zinc for its ability to grow. These findings were confirmed by two scientists in 1911. In 1914, similar results were presented in maize whilst conducting an experiment using hydroponic methods. Due to the lack of replicability in terms of the experiment, it took another ten years until Sommer and Lipman managed to obtain consistent results. They showed that sunflowers and barley needed zinc for development and growth. These findings resulted in zinc being recognized as crucial for higher plants. In 1905 Mendel and Bradley were the first to discover that zinc plays an important role in animals by finding zinc in the respiratory pigment (hemosycotyn) of snails. (Nielsen 2012) It took approximately another 30 years until Wilbert Todd, Edwin Hart and Conrad Elevehjem were able to establish the essentiality of zinc for animals by showing that zinc played an important role for the development and general condition of the rat. Their experiments took place at the University of Wisconsin and were conducted in 1934. (Todd et al. 1980)

In 1955, the supplementation of zinc was reported to improve parakeratosis in swine. Especially young, fast-growing pigs fed diets with high levels of calcium or low levels of Zn, tend to develop crusty lesions in the region of the head, further the distal limbs can also be affected. Further clinical signs of zinc deficiency in pigs are restrained growth and the failure to gain weight. The lesions resolved completely after oral zinc supplementation. Only in the 1960s, zinc supplementation was found to improve the clinical symptoms of parakeratosis in cattle and sheep. (Nielsen 2012)
3 Zinc deficiency in different animal species including humans

3.1 South American Camels

South American camelids which are held as farm or zoo animals often show typical cutaneous lesions with an etiology which is still unclear. Zinc deficiency or disorders concerning the process of keratinization/inflammation due to supraphysiological systemic zinc concentrations are made responsible. (Rosychuk 1994) Commonly only a few animals in a herd are affected, suggesting a possible innate cause of this disease. (Pugh et al. 2004)

Skin lesions consist of scales, papules, and plaques with crusts, they are mostly seen in hairless regions such as the ventral abdomen, perineum, medial part of the thighs, medial forelegs and the axillae. Furthermore, the periocular area and the bridge of the nose including the muzzle may be affected. For a lot of cases, pruritus is absent or only very mild. Studies suggest that the affected animals typically are between 1 and 2 years old, male, and have predominately dark fleeces. (Rosychuk 1994) Findings in humans show that colored hair has more zinc compared to white hair. (Sturaro et al. 1994) Clauss et al. found out that llamas and alpacas with white hair were significantly less affected by cutaneous lesions in comparison to colored animals. Fowler and Rosychuk both presented similar results, that colored animals were more vulnerable to cutaneous lesions compared with non-colored animals. This shows that llamas and alpacas with colored fleece and therefore higher contents of zinc and other minerals in their hair, have increased demands on the mineral metabolism, which make them more susceptible to deficiency diseases such as zinc deficiency. (Clauss et al. 2004)

Sheep, yaks, musk oxen and South American camelids produce fleece and wool. Wool production in llamas and alpacas can increase the demand for minerals such as zinc and therefore is also listed as a cause of zinc deficiency. (White et al. 1994) (Neathery et al. 1973) (Grace and Clark 1991)
The typical histological findings include diffuse orthokeratotic hyperkeratosis and a moderate perivascular dermatitis with plasma cells, macrophages lymphocytes, and at times eosinophil infiltration. (Scott et al. 2011) When evaluating the histological findings, it is important to be aware of the fact that the periarteriolar cellular infiltrates which normally suggest the event of an inflammation, are physiological in South American camels. (Pugh et al. 2004)

Figure 1: Histology of the skin of an alpaca with zinc deficiency (Scott et al. 2011)

In a retrospective study comprising 68 alpacas (Vicugna pacos) with skin diseases, Scott et al. found out that 8% had zinc responsive dermatosis. Further, a study on zinc levels in alpacas and llamas was conducted in Germany by Clauss et al. The animals were given commercial feed for camelids and hay which had a deficient mineral content. Around 25% of the animals had skin alterations on the nose and ears. Animals with dark fleeces were more likely to suffer from zinc responsive dermatosis. The affected animals were all female and either pregnant or had already given birth that year. Different species show decreased body zinc levels during pregnancy, which can be explained by the increased requirements of the fetus during late gestation. In herds of poor mineral supply, deficiencies concern pregnant females first. This stands in contrast to the findings of Rosychuk, who claims that male animals are more often affected by zinc responsive dermatosis. Clauss et al. found no difference in zinc serum concentration when comparing animals with and without cutaneous lesions. In this herd, zinc supplementation of the diseased animals was effective. Further, fleece colour, sex or breed (Huacaya vs Suri) had no influence on the zinc serum concentrations. Therefore, they were not able to distinguish between affected and nonaffected by only measuring and interpreting the zinc serum concentrations. (Clauss et al. 2004) Pechova et al. further states that age had no effect on the zinc plasma level. Similar results were described by Smith et al. when measuring zinc serum concentrations. (Pechová et al. 2017)
Another study on South American camelids was performed in the United Kingdom. Data on population, husbandry and diseases including skin diseases were collected. The research work clearly demonstrates that more than 50 percent of all questionnaires were answered positive for skin diseases, with 81.4 %, the majority of all skin diseases were found to be by alpacas. Signs of skin disease were reported by 111 of 217 respondents (51.1 %), and 317 (9.0 %) animals were reported to have been affected. The numbers ranged from one to 43 per unit; 258 of them (81.4 %) were alpacas, 55 (17.3 %) were llamas and four (1.3 %) were guanacos, and they constituted 9.5 %, 7.6 % and 6.4 % of their respective populations. The study consisted of 15 different diagnosis, in 23 cases the cause seemed to be due to zinc deficiency and in 19 cases ectoparasites were made responsible for the skin alterations. The other 13 causes of skin disease were far less common. According to the results, zinc deficiency is the most common cause of skin disease, followed by ectoparasites. Furthermore, sex and age of the animals were elaborated. The collected data shows that most animals affected were female and over 18 months of age, with 79 % of the affected animals being female and 90 % over 18 months of age. (D'Alterio et al. 2006)
The therapy consists of oral zinc supplementation, in severe cases 2 to 4 g of zinc methionine per day is required and in less severe cases, a daily dosage of 1 g zinc sulphate is indicated. (Pugh et al. 2004) When animals are treated, it is recommended to decrease the amount of calcium by avoiding high dosages of calcium supplements and alfalfa hay. (Rosychuk 1994) The lesions tend to improve after 8 to 12 weeks. (Clauss et al. 2004) The Kansas State University published a study comparing the digestibility of organic and inorganic zinc in alpacas and llamas. Zinc sulphate was used for the inorganic group and zinc methionine and zinc proteinate for organic zinc supply. At the hands of this study, it became clear that zinc methionine had the strongest therapy effect. (Linden et al. 2007) If zinc supplementation is not successful, systemic antibiotics or systemic glucocorticoids can be applied, although glucocorticoids can cause abortion during late gestation in South American camels. In some cases, ending the feed of concentrated forage and mineral mixtures and only feeding hay resolved the cutaneous lesions. To dissolve the top layers of the parakeratotic skin alterations, propylene glycole (mixed with water to obtain an end concentration of 50-70 %) is sprayed on the skin lesions. This procedure should be repeated twice a week. If the animal develops a secondary infection, antibiotics should be consulted after obtaining the antibiogram results. Further, Chlorhexidin 3 % shampoo can be applied 1 to 2 times per week in addition to antibiosis. (Zanolari et al. 2018)
3.2 Cattle, Calves and Small Ruminants

The clinical signs of zinc deficiency in cattle, sheep and goats are very similar. (Scott 1988) Next to primary zinc deficiency which can be due to management problems or inadequate forage, there is a secondary zinc deficiency which occurs when zinc supplies are altered due to numerous dietary elements like calcium, copper, molybdenum and cadmium (zinc-antagonists). Furthermore, zinc deficiency due to genetical disorders have been described in Holstein-Friesian calves and phenotypically similar cutaneous lesions in Fleckvieh calves which have no connection to impaired zinc absorption in the small intestine.

Induced zinc deficiency by feeding deficient diets have been already been described by Miller and Miller in 1962. Young Holstein calves were feed a diet containing only 2,7 mg of Zinc per kg of body weight. These calves showed a reduction in weight gain, severe parakeratosis and oedematous swelling around the fetlock joint. Calves which were fed on 40 mg of zinc per Kg of body weight showed no pathological clinical symptoms. (Nielsen 2012)

The Bovine Hereditary Zinc Deficiency (BHZD), is a congenital disease, normally seen in Holstein-Friesian calves. (Machen et al. 1996) Most publications state an autosomal recessive gene which causes this deficiency, and some suggest that only calves related on both parental sides to a Dutch bull ("Adema 21 van de Woundhoeve", born in 1946), show these typical clinical signs of zinc deficiency. Therefore, BHZD is also known under the name Adema disease. The Adema disease effects the general condition of the animal, the digestive system and the skin, including mucosal membranes. The first person who described this disease was McPherson in 1964. Due to similar clinical signs of zinc deficiency in cattle the disease was associated to low zinc levels in the animal. Studies on the genetics of these cows later revealed the congenital component of this disease. (Kroneman et al. 1975) Today we know that in Holstein-Friesian cattle, BHZD is caused by a splice-site variant in SLC39A4, which prevent the physiological uptake of zinc in the duodenum and jejunum. Calves are born healthy and start showing typical symptoms after 1 to 2 months. Calves develop a dry, scaly coat with general loss of health. In certain areas the animal shows hair loss and hyperkeratotic crusts around the ear, eyes, muzzle and legs. Due to the impaired immune system, affected animals are more susceptible to infections of any kind. This leads to an increased chance of calf diseases such as pneumonia and enteritis. BHZD is lethal if left untreated. (Yuzbasiyan-Gurkan and Bartlett 2006)
The pathological examination shows a hypoplasia of the thymus and parts of other lymphatic systems. Further examinations revealed that the zinc serum concentrations are subnormal, also the alkaline phosphatase levels in the serum were decreased. Studies have shown that the intestinal uptake of zinc is reduced whilst the rate of elimination over the kidney stays normal. The daily supplementation of zinc restores all clinical appearances of BHZD. (Weismann and Flagstad 1976)

A similar disease is described in Fleckvieh and is due to a nonsense mutation in the phospholipase D family member 4 coding gene. These findings were made using genome-wide association analysis, autozygosity mapping and analysis of whole-genome sequencing data. (Jung et al. 2014)

Crusting and scaling of skin with adhesion of the hair manifested around the eyes, muzzle, extremities and above the sternum (Figure 2 A, B). Calves had erosions and ulcerations on the oral mucosa and suffered from interdigital skin erosions (Figure 2 C). Furthermore the animals had recurring diseases such as pneumonia and diarrhoea and were impoverished in both height and weight. (Jung et al. 2014)

Figure 2: Clinical symptoms in Fleckvieh calves (Jung et al. 2014)
Figure 3 illustrates a hematoxylin and eosin (H&E) stained pathohistological slide, of an effected Fleckvieh calf. The skin shows a chronic dermatitis with crusting and bacterial colonization. Numerous intracorneal aggregations of serum such as ulcerations and epidermal necrosis can be seen. In multiple sections the epidermis showed signs of oedema. Furthermore, the dermis and epidermis were infiltrated by high amounts of neutrophils. Along with oedema, the dermis also shows moderate infiltration of lymphocytes and plasma cells. (Jung et al. 2014)

Figure 3: Pathohistological slide of skin alterations in Fleckvieh calves (Jung et al. 2014)

In a contemporary study, eight calves with skin lesions and bad general health were identified in a Fleckvieh population. The clinical and pathological findings showed remarkable similarities to diseased individuals of Friesian descent and humans with zinc deficiency. These disorders are due to mutations in SLC39A4 and can be treated with oral zinc supplementation. Despite zinc supplementation, the Fleckvieh calves showed no clinical improvement. Mutations in SLC38A4 were excluded as the cause of this disease, posing a different aetiological background to this disease observed in Fleckvieh calves. Common ancestors were revealed during the pedigree inspection of the Fleckvieh calves, suggesting a hereditary background. Due to the anamnesis, the clinical and pathological exam, the calves were temporarily diagnosed with BHZD. (Jung et al. 2014)
To determine the disease associated region, genotypes of diseased calves were obtained using the Illumina BovineHD Beadchip. 777,962 SNPs (single nucleotide polymorphism) were contrasted with 1,339 genotypes of healthy animals. An association signal was noted on chromosome 21. Autozygosity mapping of diseased calves showed a common segment of homozygosity encircling 1.023kb. This region consists of 17 genes, with two genes coding for zinc transporters in the gastro-intestinal tract (CRIP1, CRIP2). One affected calf was re-sequenced and the results showed a nonsense mutation (p.W215X) in a phospholipase encoding gene (PLD4). To verify the connection between the impaired function of PLD4 and the typical skin lesions, genotypes of the mutation (p.W215X) from 3650 animals were obtained. No healthy animal was homozygous, while all diseased animals were homozygous for the defect allele. This allele has an approximate frequency of 1.1% in Fleckvieh. In toto, it seems that a false function of PLD4 is responsible for the skin lesions and impaired general condition in Fleckvieh calves. The abbreviated PLD4 protein may be retained or after transcription, degraded due to nonsense mediated mRNA decay. If the shortened protein is retained, the function is severely compromised due to the lack of domains needed for enzyme activity. Further, the oral supplementation of zinc did not improve the clinical symptoms of these Fleckvieh calves, which confirms that zinc metabolism is not causal for this disease. (Jung et al. 2014)
Besides the genetic disorders mentioned above, perinatal zinc deficiency is described in cattle, which is due to increased zinc transportation from the placenta to the foetus and from the blood to the mammary gland. Bulls, heifers and calves divided into different age groups and fed basic diets without mineral supplementation showed sufficient zinc serum concentrations. Animals in the phase of late pregnancy or at the start of lactation, fed on the same diet, showed insufficient zinc serum levels. A study was conducted showing that zinc levels of most calves were notably higher compared to those of their dams. Likewise calves on farms whose dams had mean zinc concentrations below 10 µmol/l had sufficient zinc serum concentrations. These results indicate a sufficient placental transfer of zinc to the fetus. (Pavlata et al. 2012)

Feeding sufficient amounts of microelements during gestation are crucial to meet the needs of the dam. Although the zinc status does not only depend on dietary allowance but also on the capability of digestion through the intestine and storage in various organs, which both can be altered by the interaction with other food components. (Enjalbert et al. 2006) Phytate for example is present in proteins of soy and can decrease the intestinal absorption of zinc. Large amounts of calcium and other elements such as copper and iron may also have a negative effect on the intestinal absorption of zinc. The combination of excess calcium and high amounts of phytate in feed is said to have an even greater effect on decreasing intestinal absorption of zinc. The deficiency of essential fatty acids (EFA) have proven to impair intestinal zinc absorption and the supplementation of EFA are documented to enhance zinc absorption. (Cunnane 1982)

Zinc deficiency during pregnancy and early rearing can have a negative influence on the health, production and reproduction of the animal. (Abdelrahman and Kincaid 1993) (Pavlata et al. 2012) A retrospective study conducted in France showed that the odd ratios for productive disorders were high in zinc deficient animals, especially for milk production on dairy farms. This can be attributed to the loss of appetite, which is the first sign of zinc deficiency. Enjalbert describes poor growth in calves in herds of zinc deficient dams which can be related to the decreased milk production due to lack of appetite and therefore lower feed efficiency. (Enjalbert et al. 2006)
The relationship between zinc status and the risk of retained placental membranes was examined. In this study animals with deficient zinc serum levels showed an increased risk of retained fetal membranes which therefore increased the risk of metritis. Campbell and Miller also explored this relationship but did not observe a decreased incidence of retained placental membranes after zinc supplementation in dairy cattle. However, the diet contained more than 1 g of zinc/day and plasma zinc levels were sufficient, suggesting that all the cows used in their experiment had adequate zinc statuses and would therefore not benefit from zinc supplementation. (Campbell and Miller 1998)

The important factors which cause postparturient hypocalcaemia are known but there are always cases were normal therapy does not apply. Heilig et al. described that cows with milk fever had lower zinc serum levels compared to non-diseased animals. This can be explained due to the diverse function of zinc in bone metabolism and calcium mobilisation in the pathogenesis of milk fever. Due to these findings, the zinc status in cattle should be regularly monitored and further be part of prophylactic and therapeutic treatment of postparturient hypocalcaemia. (Heilig et al. 2018)
Negative effects on the immune system due to low zinc concentrations have been documented in heifers. (Engle et al. 1997) Increased humoral immunity in calves after oral zinc supplementation has also been documented. (Prasad and Kundu 1995) In the study of Enjalbert, the relationship between infectious diseases and zinc statuses was analysed. It was found that animals with low zinc serum levels were at increased risk for metritis and mastitis in adults and diarrhoea in calves. (Enjalbert et al. 2006)

In contrast, studies conducted in lambs, show that minor zinc deficiency has no effect on the humoral and cellular immunity. (Droke and Spears 1993) Although lambs which were fed on a severe zinc deficient diet (3.7 mg of Zn/kg) had a decreased blastogenic response to PHA, which is a t cell mitogen, but an enhanced reaction to PWM, a beta and t cell dependent mitogen. Lambs showed a higher percentage of neutrophils and a lower percentage of lymphocytes in the peripheral blood. The inflammatory reaction to PHA which was administered intradermally was indifferent in zinc adequate and zinc deficient lambs. But as mentioned above, the immune response of lambs fed on marginally zinc deficient diets (8.7 mg of Zn/kg) did not differ from those which received adequate diets (44 mg of Zn/kg). (Droke and Spears 1993)

Similarly, according to Spears, there is no definite association between the zinc status and immunity in steers as the supplementation of zinc did not elevate the in vitro lymphocyte response to PWM or PHA stimulation, nor was there an in-vivo cellular response after PHA was administered intradermally. Furthermore, zinc supplementation had no effect on the number of antibodies after IBRV vaccination. The addition of 150 or 300 mg of Zinc per kg of body mass to a diet which already contained 65 mg of Zn/kg showed no change in mitogen induced blastogenesis, bactericidal and phagocytic activity in neutrophils and the production of IL-2 by lymphocytes. Nonetheless, calves on a diet with 17 mg of Zn/kg had a decreased induration reaction after the intradermal injection of PHA compared to animals which received zinc supplementation. (Spears 2000)

These findings contrast with the results obtained in humans and rats in which already minor zinc deficiency can reduce the immune response. (Fraker et al. 1984)
Congenital cases regarding zinc responsive dermatosis in goats are very rare. Induced zinc deficiency by feeding deficient diets have been described by Nelson, Reuter and Schulze. (Krametter-Froetscher et al. 2005)

As mentioned above, most clinical manifestations of zinc deficiency in goats are very similar to those in other ruminants. Anorexia, weight loss, depression, alopecia, dry and hard skin, with erosions and fissures on the hind limbs, head, neck and scrotum are described. Other cutaneous lesions are typically found around the eyes, ears, mouth and nose. In all areas, crusting of the skin is very prominent. Other symptoms are hypoplastic testicles, increased epithelial growth of the dental pad and therefore decreased feed intake. Not all animals show pruritus. (Singer et al. 2000)

Two independent cases of zinc deficiency which are not related to deficient zinc diets in dairy goats are documented. The clinical symptoms were very similar to those described above. Pathohistological examinations showed parakeratotic and orthokeratotic hyperkeratosis. The first serum zinc measurements were low in both goats with 461 µg/l and 521 µg/l. During early stages of oral zinc supplementation, mild skin alterations persisted. Throughout prolonged zinc therapy these lesions finally resolved. Once oral zinc supplementation was stopped, the typical parakeratotic skin lesions reappeared in both animals. One goat (case 2) had two kids, one of which showed moderate skin alterations at the age of eight months together with low zinc serum levels (434 µg/l). This case further reinforces the suspicion of a hereditary zinc malabsorption as known in Holstein Fresian calves. The clinical and histological findings of these goats also match syndrome 1 hereditary zinc deficiency described in nordic dog breeds. The other offspring had no skin lesions and was also normal in zinc serum with a concentration of 530 µg/l. This represents the first descriptive study of hereditary malabsorption of dietary zinc in goats. In order to overcome these symptoms a lifelong oral zinc supplementation is mandatory. (Krametter-Froetscher et al. 2005)
Figure 4 represents a pathohistological image of a skin biopsy obtained from a goat (case 1 in the study of Reinhild Krametter-Froetscher, Simone Hauser and Walter Baumgartner). The image shows massive orthokeratotic hyperkeratosis and epidermal hyperplasia with perivascular inflammatory infiltration.

Figure 4: Pathohistological slide of skin alterations in a goat (case 1) (Krametter-Froetscher et al. 2005)

Naturally occurring cases of zinc deficiency in sheep are very rare, although adults are less affected when compared to lambs. The importance of zinc for sheep regarding all age groups has been evaluated in different experimental studies. Sheep which suffer from zinc deficiency show a bad nutritional state, wool and weight loss, depression, alopecia, dry and hard skin, with erosions and fissures. These skin lesions are typically located in the region of the head (nose, ears, upper lip, periocular) and proximal from the claw pending towards the hook joint. Due to secondary infections of the skin in these areas, sheep sometimes show signs of pruritus. Malformation of the developing horn due to impaired horn growth has also been documented. Male sheep can further show testicular atrophy become oligozoospermic or even azoospermic. The aetiology in sheep seems to be the same as in other species. Since only a few members of a flock showed clinical signs of zinc deficiency, an innate cause to this disease is being argued. (Behrens et al. 2001)
Clinical symptoms of parakeratosis and immunological disfunctions in Holstein- Frisian calves resolved after oral zinc supplementation. The daily dosage during rearing is 5-10 mg of zinc carbonate (ZnCO₃)/kg of body weight. After weaning, the concentration is normally increased to 10-15 mg/kg of body weight of ZnCO₃ because the bioavailability of zinc in dry animal food is less than in milk. The same effects are obtained when treated with zinc sulphate, zinc oxide and zinc acetate. During consequent treatment the general health and cutaneous lesions improve and completely resolve after 3 to 4 weeks. After another two weeks, the affected areas are fully covered by hair again. With this therapy, calves can be fattened until they are sold to the slaughter house. Once oral supplementation is stopped, the clinical symptoms resolve after a period of 3 to 4 weeks. Primary and secondary zinc deficiency in cattle is treated by increasing the amount of zinc in the forage. Either feed is altered to achieve 250 mg Zn/kg of dry matter or calves each receive 50 mg of zinc and adults each obtain 250-500 mg of zinc to resolve the cutaneous lesions. Zinc can be applied as zinc sulphate, zinc oxide or zinc chloride. In less severe cases, once can provide the animals with mineral mixtures containing salt or salt licks with 1-2 % of zinc. (Dirksen et al. 2002)

The treatment in goats consists of either a 1 % zinc sulphate drench or 250 mg to 1 g of orally applied zinc sulphate. This treatment should be carried out for 2 to 4 weeks, depending on the severity of the skin lesions. A zingosel bolus (Telsol Ltd) is also used in goats and sheep. The bolus contains zinc, selenium and cobalt. (Matthews 1999) In sheep the most common treatment is the daily oral application of 50 mg zinc (0.07 g zinc oxide, 0.1 g zinc carbonate or 0.2 g zinc sulphate). After a few days improved clinical symptoms can be observed. (Behrens et al. 2001)
3.3 Canine zinc deficiency

Many cases regarding skin changes due to low zinc levels have been seen in the past, but due to improvements in breeding and changes in quality of commercial diets, zinc deficiency has become less frequent. A case study revealed that the age at which the lesions become apparent lies between 6 months and 10 ½ years with 41 % developing the lesions with 18 months of age. (Colombini 1999)

Due to the clinical symptoms, zinc responsive dermatosis in dogs is classified into two syndromes. Syndrome 1 is most commonly found in Siberian Huskies and Alaskan Malamutes but has also been described in Doberman Pinschers and Great Danes. (DACVD) The cause of this disease is due to a genetic defect which decreases the intestinal absorption of zinc. Ongoing breeding should be prevented in affected animals due to the familial background of this disease. These typical cutaneous lesions become apparent during puberty but can also be triggered by illness, estrus or pregnancy during adulthood. Most cases have been reported between September and April. Furthermore, no gender predilection seems to exist. (Colombini 1999)

Syndrome 1 includes focal erythema and alopecia; these lesions can develop to scaly and crusted skin alterations. The eyes, nose, mouth and ears are typical regions were these lesions occur. Other areas such as the perianal region, footpads and points of pressure located on the limbs can also be affected. Initially lesions are found to be unilateral, although the clinical symptoms become symmetrical at later stages of the disease. Although the sebaceous glands produce excess sebum, the hair coat seems dry and dull. Chronic cases of zinc responsive dermatosis show hyperpigmentation and footpads tend to become hyperkeratotic. (Thoday 1989) Secondary infections with bacteria and Malassezia increase the severity of the lesions. The animal becomes more susceptible to these infections due to the breakdown of epithelial barriers and impaired function of the immune system. Dogs further showed pruritus which contributes to the development of skin lesions. (Colombini 1999)
Syndrome 2 is described in fast growing puppies which are fed diets with low amounts of zinc or diets containing excess minerals (calcium) or phytate, which have a negative effect on the absorption and metabolism of zinc. (Miller and Muller 2013) All breeds can be affected, and the range of clinical signs are highly variable. Some are unaffected whilst others show signs of depression, anorexia, impaired growth, fever and lymphadenopathy. The dermatological disorders are very similar to those observed in Syndrome 1, with pressure points on the limbs and the head being predominantly involved. Furthermore, hyperkeratosis on the footpads and on the planum cutaneum of the nose with secondary fissuring are described. (Colombini 1999)

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<td>Periorbital region</td>
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<td>Pinna</td>
<td>6</td>
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<tr>
<td>Dorsum of nose</td>
<td>8</td>
</tr>
<tr>
<td>Lateral muzzle</td>
<td>8</td>
</tr>
<tr>
<td>Lips</td>
<td>7</td>
</tr>
<tr>
<td>Footpads</td>
<td>5</td>
</tr>
<tr>
<td>Ventral mandible</td>
<td>5</td>
</tr>
<tr>
<td>Dorsum of head</td>
<td>4</td>
</tr>
<tr>
<td>Limbs</td>
<td>3</td>
</tr>
<tr>
<td>Perianal region</td>
<td>2</td>
</tr>
<tr>
<td>Sternum</td>
<td>1</td>
</tr>
<tr>
<td>Lateral thorax</td>
<td>1</td>
</tr>
<tr>
<td>Ventral abdomen</td>
<td>1</td>
</tr>
<tr>
<td>Scrotum</td>
<td>1</td>
</tr>
<tr>
<td>Axilla</td>
<td>1</td>
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Figure 5: Distribution of skin lesions in 17 northern breed dogs (Colombini 1999)

The table above illustrates the distribution of lesions in 17 northern breed dogs which are affected by zinc responsive dermatosis. The periorbital region is the most affected area followed by the pinna, dorsum of the nose, lateral muzzle, lips, footpads, ventral mandible, dorsum of the head, limbs, perianal region, sternum, lateral thorax, ventral abdomen, scrotum and the axilla.
Perivascular dermatitis, epidermal hyperplasia, spongiotic parakeratosis and round purulent crusts (Fig. 6) are histological findings found in both Syndromes. (Colombini 1999)

Figure 6: Pathohistological slide of skin alterations in dogs (Colombini 1999)

For completeness, lethal Acrodermatitis in Bull terriers, which is not truly a zinc responsive dermatosis is included. It is an inherited autosomal recessive disorder. The pathogenesis of this diseases is still unknown, but an impaired cellular zinc metabolism is being suspected. (Smits et al. 1991)

Experimental results suggest that a deficient copper status might also be a primary cause or predilection for this disorder. Dogs are not able to use zinc from the small intestine and develop a cell-mediated immunodeficiency. Clinically the animals show impaired growth, gastrointestinal disorders and systemics infections. The typical skin lesions include fissures on the footpads, pododermatitis, pyoderma and hyperkeratosis. Lesions tend to develop at an age of 6 weeks and are most commonly found in areas of high friction and near body openings. The primary histological finding is parakeratotic hyperkeratosis. Acrodermatitis does not respond to parenteral nor oral supplementation of zinc and many animals die before the age of 1 year. The parents and siblings of diseased Bull Terriers should not be used for breeding purposes, as they could be carrying these traits as well. (Colombini 1999)
The most important differential diagnosis for canine zinc responsive dermatosis are dermatophytosis (ringworm), demodicosis, pemphigus foliaceus (autoimmune skin disease), mucocutaneous pyoderma, systemic lupus erythematosus and the superficial necrotic dermatitis. (Miller and Muller 2013)

A great amount of evidence suggests that zinc has an important role in the protection of skin against oxidative damage induced by free radicals. The expression of heat shock proteins (HSP27, 72 73 and 90), copper and zinc superoxide dismutase (SOD), metallothionein (MT), active caspase 3 and Ki-67 antigen were investigated in healthy canine skin and from 8 dogs which had zinc responsive dermatosis. All four HSPs showed increased cytoplasmic immunostaining in the diseased epidermis. The expression of copper and zinc SOD was indifferent to that in normal skin. The immunoreactivity of MT was present in both the nucleus and cytoplasm of basal cell in healthy skin but was absent in the diseased epidermis. Caspase 3 activation was also absent in the epidermis of dogs with zinc responsive dermatosis but had a high Ki-67 index (3.5 to 9 times as much compared to healthy animals). The results of this study show that cellular response to stress is part of the pathogenesis of skin alterations in zinc responsive dermatosis in dogs. The decreased metallothionein immunoreactivity in the diseased epidermis may be due to low zinc concentrations, due to the susceptibility to oxidative tissue damage. On the other hand, high levels of HSPs expression in the skin of diseased dogs may guarantee protection against potentially dangerous stimuli, also contributing to the prevention of apoptosis and to regulate the cell cycle in growing keratinocytes. (Romanucci et al. 2011)
A retrospective study performed by Colombini and Dunstan demonstrates that approximately 88% of the dogs had reversed clinical symptoms 3 to 210 days after initiating zinc supplementation. Zinc sulfate is most commonly used for zinc supplementation. Since the amount of zinc in different medications varies, the elemental amount of zinc is used to calculate the dosage. An appropriate starting dosage is 1 mg (elemental zinc) per kilogram body weight per day. If there is no response after one month of treatment using the initial dose, the dose should be increased every 3 to 4 weeks by 50%. If no improvement is seen when treated orally, zinc sulfate can also be administrated intravenously. Recommended is 10-15 mg/kg of body weight of zinc sulfate, diluted (1:1) with NaCl. (Colombini 1999)

If zinc is not supplemented on a regular basis, lesions tend to recur within 14 to 60 days, meaning that under normal circumstances a lifelong therapy is required. Changes in therapy schemes such as dosage or time of supplementation, can influence the clinical image and even result in the return of lesions. (Degryse et al. 1987) These lesions tend to have the same distribution but are less severe compared to the initial symptoms. The first sign of recurrence has often been described to be pruritus. (Degryse et al. 1987) As a result of dosage change, reappearance of the disease may occur. Returning to the initial dosage, usually rapidly resolves these new lesions. However, cases have been described were dogs were kept on the same dose of zinc for a long period of time and randomly redeveloped these typical lesions. The symptoms tend to resolve within 14 days without any further treatment. Change in dosage or frequency to induce a faster resolution of the recurrent symptoms has no therapeutic value. (Colombini 1999)

Puppies with Syndrome 2 respond to corrected diets and lesions usually resolve entirely after 14 to 40 days. Yet zinc supplementations are given to replace diminished zinc stores and help promote the overall recovery. Only a few cases that have been described were puppies that required zinc supplementation until they reached maturity. (Colombini 1999)

Adding glucocorticoids to the regime improves the effects of zinc therapy due to its anti-inflammatory effect on the skin and improved zinc absorption in the gastrointestinal tract. In addition, tetracycline and lincomycin have been used for the treatment of zinc responsive dermatosis. The benefit of using antibiotics is due to its anti-inflammatory and antibacterial properties. Topical medication such as antibacterial and antiseborrhoic shampoos are also commonly applied. (Colombini 1999)
The supplementation of essential fatty acids (EFA) in the treatment of canine zinc responsive dermatosis is said to be beneficial. For both syndromes the pre- and post-prandial level of serum triglycerides were significantly lower in diseased compared to healthy dogs. It has been documented that low serum EFA levels decrease intestinal zinc absorption and that the supplementation of EFAs elevates zinc absorption. Subsequently, the treatment ameliorates the symptoms caused by zinc deficiency. (Cunnane 1982)
3.4 Human zinc deficiency

Acrodermatitis enteropathica is an autosomal recessive zinc deficiency disorder causing typical skin lesions in humans with no predilection for sex or race. Clinical signs are skin manifestations with alopecia and diarrhoea. These skin lesions which are characterised by zinc deficiency are considered as Acrodermatitis. Typical predilection areas are periorificial, acral and anogenital. (Ogawa et al. 2018)

Acrodermatitis enteropathica has an incidence of 2 per million children and occurs worldwide. In contrast, in 2002, the World Health Report stated that, dietary zinc deficiency is a disease which poses problems in developing countries. In sub-Saharan Africa and Southeast Asia, low dietary zinc intake affects more than a third of the population. In these countries altered physical growth due to zinc deficiency affects around 40 % of children aged between 1 and 3 years. Supplementing zinc has shown to reduce these morbidities and infant mortalities in general. (Shrimpton et al. 2005) In comparison, the malnourished, alcoholics, vegetarians and premature infants are at risk of zinc deficiency in developed countries. Another group at risk are people with malabsorption syndromes like Crohn’s disease, celiac sprue and short bowel syndrome. (Maverakis et al. 2007)

The disorder seems to be similar to the Adema disease in cattle of Friesian descent and (Kroneman et al. 1975) was first described by Brandt in 1936. (Weismann and Flagstad 1976) The defective gene which is responsible for encoding the zinc transporter, known as Zip4, has been identified as SLC39A4. On biochemical basis, zinc transporters represent transmembrane proteins which are encoded by two SLC (solute-linked-carrier) gene families, SLC 30 (ZnT) and SLC39 (Zip). Human zinc transporter genes are labelled as SLC30A and SLC39A followed by a number representing the order of reveal. ZnT and Zip have different functions in the cellular homeostasis of zinc, ZnT are responsible for lowering the intracellular zinc concentration by promoting efflux or transporting zinc into vesicles. Zip transporters in contrast, promote influx of zinc or release zinc from intracellular vesicles, therefore increasing the intracellular concentration of zinc. Till now 9 ZnT and 15 Zip transporters have been found in human beings. Most of zinc homeostasis takes place in the small intestine (primarily the jejunum) with zinc transporters being expressed according to the amount of zinc intake. When dietary zinc is deficient, the intestinal zinc absorption is increased by the expression of more transmembrane transporters, decreasing any further zinc losses. (Liuzzi and Cousins 2004) Once zinc absorption into the enterocytes has happened, ZnT family
members are responsible for the efflux of zinc into the circulation. In the bloodstream, zinc binds to albumin and get dispersed by the portocaval system. 85 % of zinc is stored in the skeletal muscle and bone. (Maverakis et al. 2007) Further, ZIP is not only essential for the intestinal uptake of zinc but also for the reuptake from the exocrine pancreas. (Maares and Haase 2016)

Another important aspect of Acrodermatitis enteropathica is the reduced immune function. The interaction of zinc in different immune mechanisms have been studied extensively in humans. Results reveal that already moderate zinc deficiencies can promptly lower antibody and cell mediated immune responses, which thus, reduces the resistance to all kinds of diseases. Atrophy of lymphatic organs such as the thymus has also been described. (Maares and Haase 2016)

The lesions are symmetrical and demarcated and vary from bullae to verrucous plaques in the mucocutaneous areas and distal regions of the legs and arms. As in cattle, symptoms are very diverse. Paronychia, alopecia, stomatitis, conjunctivitis and mental disorders are described. altered growth, respiratory and gastro-intestinal infections are often present. Symptoms begin after the child has been weaned or close after birth if not breast-fed. Infants which are born premature are especially vulnerable to acquired zinc deficiency, due to low zinc stores in muscle and bone and an increased demand for zinc. Further, during the last 2 ½ months of pregnancy most of the zinc is transferred from the mother to the foetus. Acquired zinc deficiency is most commonly seen in infants which were fed on milk with low amounts of zinc but has also been described in premature infants which received adequate diets and milk with normal zinc levels. (Stapleton et al. 1995) (Maverakis et al. 2007)

Without treatment septicaemia or bronchopneumonia can become fatal. At first, mother milk was the only therapy. In the early 1950s treatment with halogenated hydroxyquinolines began. The mechanisms behind hydroxyquinoline are not fully understood but result in an increased serum zinc concentration. This was a lifesaving treatment but was associated with strong side effects, damaging the visual functions of the eye. Histological examinations showed that the Paneth cells had abnormal inclusions before and after hydroxyquinoline therapy. (Maverakis et al. 2007)
Further, patients sometimes show clinical signs of deficiency dermatitis although the zinc status is within the physiological range. This can be explained due to the tight zinc plasma regulation, which contains only 0.1% of the entire body zinc. Therefore, a measurement of the exact zinc status is very difficult.

Pathognomonic for the histological exam in humans with deficiency dermatitis is the cytoplasmic vacuolization, ballooning, degeneration and necrosis of keratinocytes in the stratum granulosum and stratum spinosum of the skin. This pathohistological image is referred to as fully developed necrolysis of the epidermis. (Maverakis et al. 2007)

Figure 7: Pathohistological slide of skin alterations in humans with Acrodermatitis enteropathica (Maverakis et al. 2007)
Zinc supplementation ideally starts at 3 mg/kg per day of elemental zinc (zinc sulphate has 50 mg of elemental zinc per 220 mg) in Acrodermatitis enteropathica. Zinc levels in plasma or serum and enzymes dependent on zinc are recommended to be monitored up to 4 times a year to adjust the dosage of zinc sulphate appropriately. Depending on the individual requirements lie at around 3 mg/kg per day to regulate the genetic disorder of zinc metabolism. Clinical recovery becomes apparent with days or weeks, even before a change in serum zinc can be seen. Due to the misfunctioning Zip4 transporters in the enterocytes, the supplemented zinc will be absorbed paracellularly. In acquired zinc deficiency, therapy should start with 0.5 to 1 mg/kg per day of elemental zinc. Malnourished patients further need to obtain a multinutrient replacement to guaranty fast recovery.

A study was conducted to examine methods for increasing intestinal zinc uptake. As mentioned above, ZIP4 transporter in the small intestine is needed for zinc absorption. An overexpression of these ZIP4 protein would increase the absorption of zinc and by that the cellular zinc concentrations, implying that food components which are able to increase ZIP4 could possibly raise zinc absorption in the small intestine. In this experiment, mice Hepa cells (mouse liver cells) were used, which regulate mZip4 (mouse Zip4) transporters (identical to those in intestinal enterocytes), to find food components which boost the number of ZIP4. The ZIP4- targeting strategy was used to identify two soybean extracts that decreased the endocytosis of mZip4 in response to zinc uptake. The results further showed that these extracts can increase the number of apical mZip4 in canine kidney cells (transfected and polarized Caco2 and Madin-Darby) and two apical mZip4 AE mutants. Through the process of purifying soybean components, soyasaponin Bb was determined to elevate mZip4 protein and zinc concentrations in Hepa cells. Verifying that soyasaponin Bb can increase cell ZIP4 in human cells. The results of this study therefor suggest that ZIP4 targeting can represent a new method of improving zinc uptake in humans. (Hashimoto et al. 2015)

Zinc therapy may decrease copper absorption in the gastrointestinal trac. This interaction has been proven in the study of the Wilson´s disease, were this effect is wanted to reduce the copper serum concentration. Further side effects are gastric irritation with haemorrhages and vomiting. Overdoses of zinc sulphate can cause fatal multiorgan failure. (Brocks et al. 1977)
Zinc is needed to avoid impaired immune function. The differentiation of nonimmune cells and lymphocytes rely on sufficient zinc supplies. Low zinc levels in mice have shown to increase the number of monocytes and neutrophils, on the other hand lymphopoiesis and erythropoiesis become deprived. (King and Fraker 2002) Dubben et al. found out that during differentiation of myotubes and adipocytes, MT, which is a protein that binds zinc, was up-regulated and the MT bound zinc was translocated to the nucleus of the according cells. Via X-ray fluorescence it further became apparent that cellular zinc was redistributed to the nucleus during PMA (phorbolmyristate acetate, a Protein-kinase C activator) induced differentiation of HL-60 cells. (Dubben et al. 2010) The cell line HL-60 (acute myeloid leukaemia cell line) is a common used in vitro system for studying the differentiation into monocytes induced by PMA or 1.25 D3. (Collins 1987)

During the administration of 1 alpha, 25-dihydroxyvitamin D3 changes in gene expressions were observed with a reduction of Zip zinc transporters in HL-60 cells. This resulted in a decrease in intracellular-free zinc concentrations. After amplifying the effect of 1.25 D3 with zinc chelator TPEN or zinc depleted cell culture medium, the expression of CD11b and CD14 (surface markers) of monocytes was even stronger. Furthermore, the functions concerning monocytes, such as phagocytosis, TNF-alpha secretion and oxidative burst were enhanced by the presence of TPEN. cAMP is said to enhance the differentiation of monocytes. Dubben et al. was able to show that zinc inhibits adenylate cyclase which is needed for the synthesis of cAMP. (Dubben et al. 2010)
Fraker and King observed that monocytes and granulocytes increased strongly in bone marrow of zinc deficient mice, (Fraker and King 2004) whilst erythropoiesis and lymphopoiesis were reduced. (King and Fraker 2002) These results were explained by increased glucocorticoid levels in zinc deficient mice. Glucocorticoids are suspected to cause apoptosis in lymphoid cells and elevate the number of neutrophils and monocytes in bone marrow. (Trottier et al. 2008) Dubben et al used a zinc deficient cell culture medium and showed that low zinc levels alone can cause an amplified differentiation of the monocytic lineage, stating that there must be another mechanism next to increased glucocorticoid levels. In contrast Dubben et al found no evidence of zinc being involved in the differentiation of granulocytes. Therefore the effect of zinc concentration may only be relevant for monocytes. (Dubben et al. 2010)

Low zinc blood levels are part of the acute-phase response. (Gabay and Kushner 1999) Zinc accumulates in the liver, caused by IL-6 induced Zip 14 up regulation and increased expression of MT in liver cells. The biological reasoning seems to be that hypozincaemia inhibits the growth of bacteria and regulates the processes of gluconeogenesis. Furthermore, it can be assumed that, the increased hepatic concentration of zinc plays an important role in the synthesis of acute phase proteins. (Liuzzi et al. 2005)
Humann-Ziehank et al. performed a study to show the response of zinc, iron, copper and selenium concentrations in twenty piglets when infected with *Actinobacillus pleuropneumoniae*. Humann-Ziehank et al. observed a decrease in serum zinc concentrations in APP infected piglets. These findings have also been described by other authors. Recent studies show that zinc deficient pigs show an enhanced spread of infection, causing inflammation and therefore worsen respiratory symptoms. In this experiment, the pigs were fed with sufficient amounts of zinc. The serum and liver zinc concentrations were all within reference ranges. The exact mechanism is not yet fully understood, although decent explanations have been made by Haase and Rink: 1) Zinc is sequestered to deprive the pathogens of important nutrients. 2) macrophages increase the intracellular concentration of zinc to intoxicate the microorganisms. 3) low zinc levels result in an increased differentiation of monocytes. Another hypothesis is that zinc is required by the liver and is therefore redistributed, serving a role in energy metabolism and the synthesis of acute phase proteins. Furthermore the combination of increased zinc and metallothionein levels in the liver may be required for protection against oxidative stress which may occur during inflammation. (Humann-Ziehank et al. 2014) (Haase and Rink 2014)
5 Diagnostic procedures

The diagnosis is based on the patient’s anamnesis, clinical signs during the physical examination, results of the skin biopsy, clinical chemistry of the blood, ruling out other differential diagnoses and the reaction to zinc therapy. The most used indicator of zinc status in animals are blood zinc levels. Plasma analyses are of greater diagnostic importance when compared to serum assays because erythrocytes contain large quantities of zinc and due to haemolysis the zinc concentrations are increased significantly. (Rosychuk 1994) Other indicators such as bone and hair samples have been considered as well. Bones have been used to evaluate the zinc status in animals since they are the main storage site, with the main disadvantage being that they can only be sampled post-mortem. Further, hair zinc levels can be measured, but are not considered as a sensitive diagnostic method. (Enjalbert et al. 2006) Blood zinc levels need to be interpreted with care due to the various factors which contribute to its contamination. Butyl rubber in the stoppers of tubes and syringes are rich in zinc and may cause contamination. Haemolysis can change the zinc concentration in the blood and polystyrene containers and brown paper bags can also contaminate the sample. (also relevant for feed samples, when zinc deficiency is suspected).

Likewise, the animal should not suffer general illness. Zinc levels tend to decrease during inflammatory processes. The exact mechanism has not yet been completely elucidated, although interleukin 6, an acute phase mediator and a TH-2 (T-helper type 2) cytokine seem to increase the amount of ZIP14 zinc transporter in mice liver. The up regulation of ZIP14 is thought to occur due to the temporary hypozincaemia during the acute phase response. (Liuzzi et al. 2005) Further, due to hepatic and renal diseases, hypothyroidism, glucocorticoid administration and neoplastic diseases, zinc concentrations in the blood might be falsely altered. (Pugh et al. 1999) Physiological circumstances like age, environmental temperature and stress can also alter the results of zinc measurements via blood samples. Blood sampling itself requires fixation, this causes stress for the animal and can therefor alter the results of the blood test. To minimize the stress, Holasová et al. recommends to monitor the trace elements over hair samples. (Holasová et al. 2017)
Pechova et al. evaluated the zinc plasma concentration in 299 alpacas and claims that the reference range for zinc plasma concentrations in alpacas lies between 1.56 and 8.01 μmol/l with a range of 3.54 μmol/l. These values correspond with results of Bechert and Smith which claim a reference range between 2.45 and 5.66 μmol/l with a mean of 3.5 μmol/l. Johnson states a reference range between 4.6 and 7.7 μmol/l for llamas which were fed on a suitable diet. In contrast, Fowler describes a wider reference range for alpacas with values between 3 and 14.6 μmol/l. A study by Cebra et al. examined 846 alpacas and 195 llamas. They showed that the range of zinc in serum for llamas is 3.21-17.29 μmol/l and for alpacas 2.90-31.37 μmol/l. (Pechová et al. 2017) Generally, South American camels have lower zinc serum concentrations when compared with ruminants. The reference ranges mentioned by Herdt and Hoff for cattle are 9.2-29.0 μmol/l and 8.4-18.3 μmol/l for sheep. Suttle et al. claims that zinc serum concentrations which are lower than 6.12-9.18 μmol/l indicate zinc deficiency in cattle, sheep and goats. These cut off values have also been established by Cebra et al. in alpacas (2.29-3.06 μmol/l) and llamas (2.60-3.36 μmol/l). Pechova et al. found similar results when measuring zinc in blood plasma of alpacas (1.79-2.72 μmol/l). (Pechová et al. 2017) Another study conducted in Austria, examined the zinc concentrations in both male and female llamas and alpacas. Male alpacas showed zinc serum concentrations between 1.92 and 4.05 μmol/l, female alpacas had higher average concentrations with values between 2.04 and 4.27 μmol/l. Male llamas had zinc serum concentrations between 2.10 and 5.96 μmol/l and female llamas had lower average concentrations with values between 2.09 and 5.08 μmol/l. The study of stanitznig et al. was able to demonstrate that there are statistically significant differences (p<0.001) between the zinc concentrations in llamas and alpacas, stating that the zinc serum levels of llamas and alpacas should be assessed separately. (Stanitznig et al. 2018) Furthermore, a study on South American camels showed correlations between zinc levels in liver and plasma zinc and liver zinc and serum zinc. Further, serum zinc and plasma zinc correlated well. (Pugh et al. 2004)
For Humans, blood samples should be taken before breakfast via a trace element free tube. To prevent false results, no zinc supplements should be received on the day of the testing. Other factors like storage will not affect the results. The blood plasma or serum zinc levels should be >70 g/dL before breakfast. Following meals throughout the day will decrease the measured value. When the serum zinc concentration falls beneath 50 g/dL the suspected diagnosis of Acrodermatitis enteropathica can be made. In addition to the blood work the zinc status in red blood cells and hair can be investigated. These parameters can be beneficial for the diagnosis of human zinc deficiency, although standardized cut off values for the normal limits are not yet fully established. (Maverakis et al. 2007)

The start of hair analysis dates to the early 1960s. Although significant developments only began around 1990. Medical research expanded the knowledge of histological and physiological functions of the hair, further improving methods to measure trace element concentrations in these samples. The World Health Organization and the Global Environmental Monitoring Systems of the United Nations Environment Programme even mention hair as a non-invasive biomarker. An experiment conducted in humans revealed that concentration of trace elements is higher in hair samples in comparison to blood samples. Hair provide evidence of the intracellular trace element concentration. (Holasová et al. 2017) Kempson and Lombi claim that hair samples are useful to evaluate because the sample is easy to attain, it is robust, and its treatment and preparation is very simple since no preservation is needed. (Kempson and Lombi 2011) This means that the assessing of individuals and populations can be very convenient using this method. Deeming and Weber already identified a correlation between the zinc concentrations in hair and bones and testes of rates in 1977. (Holasová et al. 2017) Ikemoto et al. further determined that zinc levels in hair correlate with the zinc concentration in liver and kidney in Caspian seals. (Ikemoto et al. 2004) Jacob, Klevay and Logan in contrast, claim that there is no significant correlation between the zinc status in the liver and hair of rats and that the zinc concentration in hair samples does not resemble the zinc status of the body. (Jacob et al. 1978) (Taylor 1986)
The zinc concentration in hair of South American camelids was found to be within 86.1-211.3 mg/kg of dry matter with a mean of 134.4 mg/kg of dry matter. Patkowska-Sokola et al. reported values between 75 and 88.8 mg/kg of dry matter in sheep wool. (Patkowska-Sokola et al. 2009) Values for cattle were broader with 125 to 427.4 mg/kg of dry matter of zinc. (Cygan-Szczegielniak et al. 2014) The mean value for goat hair was found out to be 97.9 ± 10.1 mg/kg of dry matter. (Pavlata et al. 2011) Cats and dogs had very similar results with 238.9 mg/kg of dry matter and 243 mg/kg of dry matter. (Skibniewska et al. 2011) (Chyla and Zyrnicki 2000)

The measurement of the concentration concerning zinc dependent enzymes is another method of defining the zinc status in Humans. Alkaline phosphatase, lymphocyte 5-nucleotidase and copper-zinc superoxide dismutase have all been considered in different studies, yet there is no agreement as to which enzyme is the most suitable to represent the body’s zinc status. The Alkaline phosphatase test is widely spread, therefore, worth using. Zinc regulated proteins like erythrocyte metallothionein concentrations can also be measured. Finally, albumin in serum can be evaluated because zinc concentrations tend to decline during hypoalbuminemia given zinc binds albumin in the blood. (Maverakis et al. 2007)
6 Dietary zinc recommendations

McDowell claims that the critical forage zinc concentration for ruminants held on pastures is 30 mg/kg of dry matter. Treating meadows with fertilizers which contain zinc increases the concentration of zinc in forages. Under certain circumstances, for example economic reasons or extensive range conditions, the fertilisation of forages becomes impossible. Salt licks holding between 1 and 2 % of zinc then usually provide enough zinc intake for grazing animals. (Nielsen 2012) Further, legumes have higher available amounts of zinc then grasses. Cereal grains contain even less zinc when compared with grasses. (Pugh et al. 2004) The present references for goats and sheep vary according to age and size. The zinc requirements based on information by the National Research Council, for lambs range from 21 to 38 mg/kg of dry matter and for ewes, between 30 and 45 mg/kg of dry matter. Goat kids require 15 to 23 mg/kg of dry matter, gestating does from 26 to 48 mg/kg of dry matter and lactating goats require between 40 and 70 mg/kg of dry matter. (Smith and Sherman 1994) A zinc pellet which is applicated into the rumen has been developed, which releases zinc over a period of six weeks in sheep. (Nielsen 2012)

Van Saun claimed that the minimum zinc requirement for alpacas and llamas during growth and maintenance is 0.53 mg/kg of body weight. (Van Saun 2006) During pregnancy and lactation, the amount of zinc is increased and lies at 0.67 mg/kg of body weight. These reference values were deduced from nutrient recommendations in cattle, sheep, and goats. The zinc requirement for lactating cows is 40 mg of Zn/kg of dry matter and 30 mg of Zn/kg of dry matter for none lactating cattle. Based on this data, Van Saun suggests that forage zinc concentration need to lie between 35 and 54 mg of Zn/kg of dry matter for South American camels. The evaluation of mineral content in forage is an important preventive measure against deficiency diseases in South American camels. (Pechová et al. 2017)
Guidelines have been established by the National Research Council (NRC) for canine diets, according to their age and physical activity. The maintenance for zinc in adult dogs lies at 9.7 mg per 1000 kcal of metabolized energy per day. Animals in growth and adults during pregnancy, lactation or diseased dogs require 2 to 3 times the daily amount recommended by the NRC. (Colombini 1999)

Medical research on Acrodermatitis enteropathica in humans has established different zinc dosages according to the age. The amount for infants aged between 0 and 6 months is 2 mg of zinc per day, 7 months to 36 months require 3 mg of zinc per day, 4 to 8 years require 5 mg of zinc per day, 9 to 13 years require 8 mg of zinc per day and boys which are older than 13 years and men need 11 mg of zinc per day. Girls and women between 14 and 18 require 9 mg of zinc per day and women older than 18 years need 8 mg of zinc per day. Pregnant girls and women aged between 14 and 18 years have a dietary allowance of 12 mg of zinc per day and women older than 18 years require 11 mg of zinc per day. (Maverakis et al. 2007)

On average, cow milk contains between 3 and 5 mg of zinc per litre. The zinc levels of human breast milk rapidly decline after birth. Two weeks postpartum the zinc concentration is 4 mg per litre of milk. After two months postpartum the concentration is 2 mg/l and after 6 months only 1.2 mg of zinc per litre of milk is left. (Krebs et al. 1995) Hence, after 5 to 6 months postpartum, feeding human milk without supplementation can cause zinc deficiency in infants. Since the bioavailability of zinc in human breast milk is much higher than that of cow milk, human milk remains a good source of zinc for children aged between 1 and 6 months. Many studies exist which have tried to clarify why the bioavailability of zinc in human breast milk is much higher compared to cow milk. Through gel chromatography the authors managed to show that zinc in cow milk is associated with a high molecular weight. In contrast, human milk is associated with a low molecular weight. This suggest that zinc is bound to different molecules in human milk compared to cow milk. The increased zinc bioavailability in human breast milk can also be linked to the digestibility of protein, which is lower due to increased casein amounts in cow milk compared to human milk. (Maverakis et al. 2007)
By looking back at the initial question (Are there minimal dietary zinc concentrations which must be received by llamas and alpacas to avoid dietary zinc deficiency?), it is now possible to confirm that there are defined ranges of dietary zinc allowance for llamas and alpacas declared in certain studies. Van Saun et al. (2006) claimed that the minimum zinc requirement during growth and maintenance is 0.53 mg/kg of body weight and 0.67 mg/kg of body weight for pregnant llamas and alpacas. These values originate from nutrient recommendations in cattle, sheep and goats. Since the reference values for ruminants are uniformly identical, it can be expected that they are valid for llamas and alpacas due to our profound knowledge on the physiological conditions in these animals. Forage zinc concentrations have also been studied thoroughly in ruminants. The zinc requirement for lactating cows is 40 mg of Zn/kg of dry matter and 30 mg of Zn/kg of dry matter for none lactating cattle. Similar values are described for goats and sheep. (Chapter 6) Based on this data, Van Saun et al. (2006) also suggested that forage zinc concentrations needed to lie between 35 and 54 mg of Zn/kg of dry matter for South American camels. Further stating that the evaluation of zinc levels in forage are crucial to prevent animals from developing zinc-responsive dermatosis. Animals that are kept in pasture all year round are more at risk when compared to animals living in stables with only limited access to pastures. The zinc content of their feed on the respective pasture should therefore be checked on a regular basis. Animals kept in the stable are less at risk of developing zinc-responsive dermatosis, since they typically only attain commercial feed which guarantee to meet the minimal requirements of llamas or alpacas. Smith et al. conducted a study in llamas, using forage with a zinc concentration of 22 mg/kg of dry matter. Animals showed clinical signs of zinc responsive dermatosis. The inadequate zinc intake was made responsible for the diseased llamas since the suggested zinc forage level for llamas lies significantly above, with values between 35 and 45 mg/kg of dry matter. (Pechová et al. 2017) Thus, the question is partially answered and therefore there still is need for further research to define these reference values more precisely.
Today we know that in Holstein-Friesian cattle, Bovine Hereditary Zinc Deficiency (BHZD) is caused by a splice-site variant in SLC39A4, which prevent the physiological uptake of zinc in the duodenum and jejunum. The same accounts for humans (Acrodermatitis enteropathica) and dogs (Syndrome 1). The only evidence for a hereditary cause of zinc deficiency in South American camels is the fact that commonly only a few animals in a herd are affected and show these typical parakeratotic skin lesions. These herds need to be closely evaluated and examined on possible innate causes of this disease. Therefore, the second question (Is there evidence for hereditary causes responsible for zinc deficiency in South American camels as described in other animal species including humans?) is not yet answered and medical research and development in this area is needed.

The third question is: To what extent does the immune system interact with the zinc metabolism, further causing hypozincaemia in llamas and alpacas? As mentioned in chapter 4, zinc levels tend to decrease during inflammatory processes (acute phase response). The exact mechanism has not yet been completely understood, although interleukin 6, an acute phase mediator and a TH-2 (T-helper type 2) cytokine seem to increase the amount of ZIP14 zinc transporter in the liver, increasing the zinc concentration in mice liver cells and at the same time causing a temporary hypozincaemia. Humann-Ziehank et al. further described decreasing zinc serum concentrations in APP (Actinobacillus pleuropneumoniae) infected piglets, which contributes to the hypothesis that inflammatory processes decrease blood zinc levels. To this day, there are no studies which examine the impact of inflammatory processes on the zinc plasma or zinc serum concentration in South American camels nor in any other ruminant.

Despite the marked similarities between the clinical signs of zinc deficiency in Holstein-Friesians suffering from BHZD and the phenotypic presence of the Fleckvieh calves described in the study of Jung et al. (2014), there are no apparent connections between PLD4 and the metabolism of zinc. Further, the oral supplementation of zinc did not improve the clinical symptoms of these Fleckvieh calves, which confirms that zinc metabolism is not causal for this disease. Based on these results the diagnosis of a deficient zinc metabolism in Fleckvieh calves is no longer valid. (Jung et al. 2014)
A similar lethal illness when compared to BHZD in calves was described in bull terriers showing skin lesions and growth retardation. (Jezyk et al. 1986) Studies showed that both calves and bull terriers inherited this condition as an autosomal recessive disorder. Further, there seems to be many parallels with Acrodermatitis enteropathica in human new-borns, which suffer from periorificial dermatitis, diarrhoea and alopecia. As in cattle hypoplasia of the thymus and other lymphatic tissues occurred. Furthermore, patients had low levels of arachidonic acid and the metabolism of amino acids (tryptophan) appeared to be disturbed. Also alkaline phosphatases in the serum and bone were low, but rapidly increased after zinc supplementation. (Weismann and Flagstad 1976)

![Figure 8: Zinc deficiency in cattle versus Acrodermatitis enteropathica in humans (Weismann and Flagstad 1976)](image)

Figure 8 shows that the symptoms between Acrodermatitis enteropathica and the Adema disease (Bovine Hereditary Zinc Deficiency) are very similar. Abnormal Paneth cells have only been described in Humans, therefore, zinc deficiency in humans can be caused by altered Paneth cell function. The hypoplasia of lymphoid tissue and increased risk of bacterial infections in both diseases again indicates that zinc has an important role in the function of the immune system. (Weismann and Flagstad 1976)
In comparison to Bovine Hereditary Zinc Deficiency in calves, Acrodermatitis enteropathica in humans and lethal Acrodermatitis in bull terriers, syndrome 1 (a hereditary zinc deficiency) occurs in dogs which are at least half a year old. According to Colombini, 59 % of all dogs are older than 2 years when they start to develop these typical skin lesions. Forty percent of the dogs with zinc responsive dermatosis were reported to show pruritus. (White et al. 2001) These observations go in hand with the clinical symptoms in sheep, in which the areas of parakeratotic skin lesions become infected and animals therefore show signs of pruritus. In contrast, goats presented no signs of pruritus. The case study by Krametter-Froetscher shows that the symptoms displayed by the goats were very similar to those of syndrome 1 in Nordic dog breeds. In dogs the reappearance of skin lesions after long term zinc supplementation has been documented. This was not observed by any other species. Goats showed re-emerging parakeratotic skin lesions in early stages of zinc supplementation in the study of Krametter-Froetscher. This was explained by the fact that these animals were integrated into another herd and therefore were temporary exposed to stress.

Sheep and goats with zinc responsive dermatosis are reported to show parakeratotic, orthokeratotic or even a combination of both. In cattle and dogs, the histology reviled diffuse parakeratotic hyperkeratosis. Skin-biopsies from South American camels with zinc-responsive dermatitis showed keratinization abnormality which were diffuse and mainly orthokeratotic. (Scott et al. 2011)

Usually inorganic zinc is used to treat zinc responsive dermatosis in ruminants. Krametter-Froetscher states that zinc oxide is the most frequently used substrate. There is a hand full of different studies which examine the bioavailability of zinc sulphate and zinc oxide. Experiments in sheep, swine, and chicken have revealed that zinc sulphate had a greater bioavailability compared to zinc oxide. In contrast, Rojas and Sandoval found no significant difference in the bioavailability of inorganic zinc using sheep in their experiment. (Krametter-Froetscher et al. 2005) The Kansas State University published a study comparing the digestibility of organic and inorganic zinc in alpacas and llamas. The study revealed that organic zinc in form of zinc methionine is the most effective way of battling zinc responsive dermatosis in South American camels. (Linden et al 2007)
According to Nielsen, the essentiality of zinc for plants and mammals was discovered more than 75 years ago. The first report on zinc deficiency in an agriculturally important animal (swine) occurring under farm conditions was 47 years ago. (Nielsen 2012) Due to intensive medical research over the past 20 years, different causes for zinc deficiency have been made apparent, including effects of the immune system on the zinc status of the body. To this day, there are no publications which systematically evaluate factors that predispose South American camels to the condition of zinc responsive dermatosis. To obtain a clearer and more distinct image of the combinations of different causes leading to low body zinc levels and its imminent clinical symptoms, medical research and development in this area needs to be invested.
9 Methods and Materials

For this literature search diverse special literature as well as the University Library (UB) of the University of Veterinary Medicine Vienna and its virtual library were used (specialist journals, scientific database information system).
10 Summary

The first clinical symptom of zinc deficiency in humans and animals tends to be anorexia followed by growth retardation, wound healing disorders and typical parakeratotic skin lesions. All organisms necessitate a constant source of zinc in their diet due to their limited stores of accessible zinc. The requirement for zinc depends on the function of the animal and the contents of its diet. Next to dietary zinc deficiency, congenital causes of zinc deficiency have been described in animals and humans. In Holstein Frisian calves an autosomal recessive gene has been made responsible for this disease. In humans a defective gene which is responsible for encoding the zinc transporter, known as Zip4, has been identified as SLC39A4. Another aspect is the interaction of zinc with the immune system. Low zinc blood levels are part of the acute-phase response as zinc accumulates in the liver, caused by IL-6 induced Zip 14 up regulation and increased expression of MT (zinc binding protein) in liver cells. Regarding all species, this physiological process could play an important role in the development of zinc deficiency. In the existing literature it is reported that male South American camels aged between 1 and 2 years with dark fleece have the highest probability to suffer from skin changes caused by zinc deficiency. The therapy for both animals and humans is oral zinc supplementation. Usually inorganic zinc (zinc sulphate) is used to treat zinc responsive dermatosis in ruminants. Studies have revealed that organic zinc in form of zinc methionine is the most effective way of battling zinc responsive dermatosis in South American camels. To this day, there are no publications which systematically evaluate factors that predispose South American camels to the condition of zinc responsive dermatosis.
11 Zusammenfassung

12 References


