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# USEFULNESS OF CLINICAL, HISTOPATHOLOGICAL AND SOME BIOCHEMICAL AND MINERAL INVESTIGATIONS IN DIAGNOSIS OF BOVINE HYPERKERATOSIS IN DAIRY CATTLE

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**Abstract**

Bovine hyperkeratosis is a polyetiologic disease that is increasingly widespread at high milk yielding dairy farms. Clinical manifestation is characterized by focal skin lesions with distinct borders. A clinical study and observation were carried out on 26 Holstein-Friesian dairy cows. During the initial phase of the condition, the skin of the affected cows was itchy, hence the animals tended to lick the skin lesions, or else rub against surrounding objects, with the resultant formation of single spots of raised coat and skin flaking resembling dandruff. These changes appeared on the posterolateral upper sides of the pelvic limbs and around the vulva. Distinct thickening of the wrinkled skin was observed as a result of excessive growth of the epidermis. The superficial part of the skin on major portions of the lesions was dry, corrugated and covered with numerous scales. The epidermis was dry, thickened and rough, with cracks showing the reddened dermal layer. When touched, the animals reacted as if in pain. The disease generally progressed into a chronic condition. In the studied cases, histopathological examination confirmed hyperkeratosis with widened hair follicle infundibulums filled with keratin, the swelling of sweat glands, epithelial atrophy of sweat glands, infiltration of inflammatory cells between and around blood vessels, and massive expansion of keratinized layers of the epidermis.

The content of both calcium and magnesium as well as copper, zinc, iron and manganese in grain, roughage, mineral mixtures and in other feeds met the requirements of dairy cows. Blood biochemistry profiles revealed only slightly lower serum calcium values, while zinc values were

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within the reference range. However, the zinc concentration in skeletal muscles and in the skin was reduced. The mean serum alkaline phosphatase (ALP) activity in the tested animals was also somewhat decreased. Adverse environmental factors such as direct skin contact with faeces and urine as well as zinc deficiency in the cows' tissues were significant factors in the formation of skin lesions characteristic for hyperkeratosis.

**Key words:** dairy cattle, the environment, zinc, hyperkeratosis.

### PRZYDATNOŚĆ BADAŃ KLINICZNYCH, HISTOPATOLOGICZNYCH I NIEKTÓRYCH BIOCHEMICZNYCH ORAZ MINERALNYCH W DIAGNOSTYCE HIPERKERATOZY U BYDŁA MLECZNEGO

**Abstrakt**

Hiperkeratoza bydła jest chorobą polietiologiczną, występującą coraz częściej w gospodarstwach bydła mlecznego o wysokiej wydajności. Objawy kliniczne zlokalizowane na skórze mają ograniczony charakter. Badania i obserwacje przeprowadzono na 26 krowach mlecznych, rasy czarno-białej Na początku rozwoju choroby zaobserwowano świąd, intensywne oblizywanie się i lekkie łzawienie oraz ocieranie o otaczające przedmioty, co powodowało powstawanie pojedynczych ognisk nastroszenia sierści oraz łuszczenia się naskórka, przypominającego łupież. Zmiany pojawiały się na tylnogórnej stronie kończyn miednicznych i wokół sromu. W wyniku nadmiernego rozrostu naskórka rogowego obserwowano wyraźne zgrubienie pomarszczonej skóry. Skóra na znacznej powierzchni ogniska była sucha, pofałdowana, pokryta licznymi łuskami. Naskórek był suchy, zgrubiały, szorstki, popękany aż do widocznej czerwono zabarwionej skóry właściwej. Podczas dotyku zwierzęta odczuwały bolesność. Przebieg choroby miał charakter przewlekły. Badaniem histopatologicznym potwierdzono hiperkeratozę. Zaobserwowano poszerzone lejki mieszków włosowych wypełnionych keratyną, poszerzenie gruczołów potowych, zanik nabłonka gruczołów potowych, naciek komórek zapalnych między naczyniami krwionośnymi, naciek komórek zapalnych wokół naczyń krwionośnych oraz masywne poszerzenie warstwy rogowaciejącej naskórka.

Zawartość zarówno wapnia i magnezu oraz miedzi, cynku, żelaza i manganu w granulowanej paszy treściwej oraz w mieszance mineralnej, jak i w pozostałych paszach stosowanych w żywieniu krów pokrywała ich zapotrzebowanie. Jednak wyniki badań biochemicznych wykazały, że stężenie wapnia w surowicy krów było zaniżone, zaś cynku mieściło się w granicach wartości referencyjnych. Natomiast stężenie cynku w mięśniach szkieletowych i w skórze było obniżone. Średnia aktywność ALP w surowicy badanych krów była także dość niska.

Niekorzystne czynniki środowiskowe (kontakt bezpośredni skóry z kałem i moczem) oraz niedobór cynku w tkankach krów stanowiły istotną przyczynę powstawania zmian chorobowych skóry, charakterystycznych dla hiperkeratozy.

**Słowa kluczowe:** bydło mleczne, środowisko, cynk, hiperkeratoza.

# INTRODUCTION

Skin disease can be looked upon as a manifestation of the impact of environmental factors and nutrition (especially mineral nutrition) on dairy cattle. Hyperkeratosis is counted as one of major diseases in cattle. Initially, the aetiology of the disease was unknown and that is why parakeratosis was called the X-disease (Hagan 1950). Later, many studies showed that the disease can be induced by the prolonged feeding of cows with alfalfa hay, containing calcium phosphate $Ca(HPO_4)_2$. Subsequently, parakeratosis was observed in cattle as a result of long-term nourishment with commercial foodstuffs and feeds with an experimentally reduced fatty acid content. At that time, decreased concentrations of vitamin A were also reported in blood samples from cattle. Then, general hyperkeratosis was recognized in Germany, in areas where highly chlorinated naphthalenes were used in industry. Among highly chlorinated naphthalenes, tetrapenta-hepta-chlornaftalen can be found in wood protectants, some motor oils and commercial feeds contaminated with industrial lubricants (Gregory et al. 1954). These naphthalenes can evoke the disease even several years after wood impregnation. Similar effects have been attributed to some insecticides and iodine preparations (Foret 2005). Exposing cattle to elevated emissions of arsenic can also lead to general hyperkeratosis. Causes of local hyperkeratosis include persistent pressure on specific body areas like joints or keeping animals on hard or damp surfaces for long periods of time. Typical hyperkeratotic lesions can also accompany a hereditary "fish-scale disease of cattle" (ichthyosis), also called "calf armour", and fetal hyperkeratosis (Fi 2003, Testoni 2006, Jong Ki et al. 2007). Although many reports have appeared on hyperkeratosis, the aetiology and pathogenesis are not completely understood yet. It is believed that, like other skin diseases, it is a result of many exogenous and endogenous factors, including viruses and free radicals (Yukiko et al. 2007). It should be emphasized that the immune and antioxidant systems especially make use of the germicidal properties of oxidants by producing oxidative forms of compounds, which is an essential step in the oxidative burst mechanism. Phagocytes activated in this way produce both reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxides ($\bullet O_2$-), nitric oxides (NO•) and nitric ions (OONO-). These highly reactive compounds are involved in the cytotoxic response of phagocytes and, despite being obviously advantageous, they can also lead to cell and tissue damage in cattle. It is this nonspecific character of oxidants that enables them to destroy almost every cell, which is an effect that may be seen in hyperkeratosis. The consequences of the condition primarily include abnormal protein metabolism and excessive production of keratin or excessive keratin retention (ichthyosis). Initially, hyperkeratosis is characterized by the reddening of the affected area. Later, the skin thickens due to increased proliferation of keratinocytes, causing hypertrophy of the epidermal stratum corneum (O´Toole, Fox 2003).

In the early stage of the condition, cows can be seen intensely licking and slightly tearing the skin. In some cases, loss of appetite may occur. The disease progresses with a slow weight loss, diarrhoea, reddening of the oral mucosa and nose, followed by the appearance of small, round ulcerations. If the disease does not progress into a systemic form, the body temperature remains between 37.5 and 39.5°C. Otherwise, the temperature rises and roughly thickened and keratinized lesions appear, with a tendency to spread around the back, ears, sides of the thorax, and on the medial sides of the pelvic limbs. Sometimes these lesions resemble warts, then change gradually to scale-like growths. Excessive epidermal keratinization causes substantial thickening of the wrinkled skin, with formation of cracks, sometimes giving the skin surface a reticular character. Lesions occur on the surface of the skin. The illness follows a more severe course in heifers than in adult cattle, with subsequent reproduction problems, including miscarriages, and reduced milk yield.

Bovine ichthyosis (also called "calf armour" and fetal hyperkeratosis) can occur in two forms. The fetal form, which leads to spontaneous abortions or damage to the reproductive system of the mother, and the congenital form, mostly affecting bulls, with lesions only on certain parts of the body. The affected fetus may be aborted, may be born less viable or dead, or may die within two weeks of birth. The skin is dry, thickened and rough, and cracked to the extent that the red-colored dermal layer is visible. The presence of numerous cracks gives the skin a "fish scale" appearance, and when the animals are touched it looks as if they feel pain. The mucous membranes are bright red, the borders between the skin and mucous membranes are curled outwards, and the ears seem to be shorter and thickened. Lesions are most often diffuse, but single focal lesions should not be ruled out. Sometimes, gesttation is prolonged.

In general, the course of hyperkeratosis is chronic, although this is also dependent on the age of cattle. In younger animals, the disease is more severe, with a possible fatal outcome, while in older animals, the condition is milder with possible spontaneous healing of the skin.

The nature of anatomo-pathological changes depends on the duration of the disease. Inflammation is observed first, followed by hyperkeratosis of the skin and mucous membranes of the mouth, oesophagus, rumen, reticulum, bile ducts, gall bladder and the vagina. Histopathologic changes of various types can also occur in the testes and epididymides. Keratosis can reach the subcutaneous tissue. In addition, we can observe interstitial glomerulonephritis, cirrhosis of the liver, pancreatic degeneration, keratosis of hair follicles, brittle hair and hair loss. Large areas of the skin surface becomes dry, wrinkled and covered with numerous scales.

As the disease progresses, excessive and incomplete keratinization of epidermal cells occurs as well as the formation of intercellular bridges through excessive thickening of the stratum corneum. Hyperkeratosis may take on the orthokeratotic form, where the cells are devoid of nuclei, or the parakeratotic form, with the presence of nucleated cells (Jelinek, Tachezy 2005). If present, the nuclei of the stratum corneum cells are shrunken and may take the form of rods. In the course of chronic dermatoses, the aforementioned forms of parakeratosis are not generally diagnosed. In the "fish scale" form, only the previously described clinical signs can be seen, and no other changes have been identified. However, in histopathological examination, other signs can be recognized, including distinct keratosis of the superficial epidermal layers, the skin glands and hair follicles as well as papillary formations on the dermis.

The diagnosis is based on history, clinical examination and laboratory tests, which mainly include histopathological examinations; in cases of secondary infections, microbiological tests are also made, including isolation and identification of both bacteria and fungi. The differential diagnosis should focus mainly on foot-and-mouth disease, parakeratosis and dandruff. However, in the differential diagnosis of parakeratotic disseminated hyperkeratosis, seborrhoea, dermatitis caused by zinc and vitamin A deficiency and thallium poisoning should be considered.

The prognosis may be favourable if treatment is initiated in the early stages of the disease, and unfavourable when the disease process is well underway. The prognosis for the "fish scale" disease is unfavorable. For disease prevention, it is very important to monitor constantly a cows' herd with respect to general welfare, including proper nutrition, and to eliminate exposure to highly chlorinated naphthalenes and arsenic compounds. In addition, it is vital to eliminate from the herd individuals afflicted with the hereditary bovine "fish scale" disease (Jong-Ki et al. 2007).

Before treatment, the affected area should be washed with soap and warm water and then thoroughly dried. Substances with keratolytic properties (containing salicylic acid), anti-inflammatory, antibacterial, antifungal and astringent products as well as vitamin A are used. Farmers should be advised to change the animals' diet by reducing the use of commercial feed and increasing homegrown feeds rich in vitamins and minerals (antioxidants). The "fish scale" disease is very difficult to treat and generally has an unfavourable outcome (Wright, Spears 2004).

The purpose of this article is to present a review of the general concept of skin hyperkeratosis formation in dairy cattle as influenced by environmental factors, the concept of clinical pathology with respect of aetiological diagnosis, as well as mineral nutrition.

## MATERIAL AND METHODS

The study involved 26 Holstein-Friesian (HF) dairy cows aged 3-6 years. The cows were in a transition period (21 days before to 21 days after parturition). The cows were divided into 2 groups (experimental-1 and control-2). The average annual yield of the cows was 6200 ± 100 kg of milk per year, with a milk fat content of 3.39%. The body condition score (BCS) ranged from 3 to 3.2 before parturition to 2.9 to 3.1 afterwards. In the spring (April), the breeder reported to the veterinarian that during the previous 10 months some animals had been observed to manifest progressive changes of the skin around the back, ears, sides of the chest, around the base of the tail and around the vulva, with concomitant weight loss and reduced appetite. These changes had been preceded by the occurrence of itching, intense licking and rubbing against surrounding objects, as well as slight tearing. Each of the 26 cows was individually examined clinically, with particular attention paid to the skin and according to generally accepted standards. No obvious lesions were found in 23 cows (group II), although three cows (group I) had macroscopically visible lesions previously described by the breeder

These lesions were of a chronic nature. Analysis of the cows' feeding routine showed that they were fed according to the accepted NRC standards. A daily ration was balanced with respect to protein and energy content. In the winter season, daily rations consisted of homegrown maize silage and hay, while in the summer cows ate pasture grass. During both seasons, the roughage was supplemented with granules containing a mineral mixture. Based on data regarding the history of potential occurrence of environmental toxic agents, including chlorinated naphthalenes, such a threat was excluded. Samples were taken from group I cows, from the periphery of skin lesions, for further parasitological, bacteriological and mycological studies. These examinations were conducted in accordance with the relevant standards. Full thickness skin biopsy samples, approximately 1 $cm^2$, were taken from the gluteal region of cows in both group 1 (experimental – 3 cows) and 2 (control – 23 cows). These samples were divided into three parts. One was fixed in 10% neutralized formalin, embedded in paraffin and sliced with a Zeiss Hyrax M55 rotary microtome into 4μm sections. The sliced specimens were placed on glass slides and stained with hematoxylin-eosin stain. Another part of the full-thickness skin biopsies was used for determinations of the calcium, magnesium, copper, zinc and iron content. The remaining part of the biopsies was weighed and dried for 72 hours at 37°C. Subsequently, the concentration of the aforementioned elements was determined in the tissue dry weight. For 7 days after calving, each morning before the morning feeding, blood samples for biochemical and mineral analysis were drawn from the external jugular vein from all cows in groups I and II . Blood samples were collected into tubes without an anticoagulant to obtain serum. The concentrations of calcium, magnesium, copper, zinc and iron in the blood, in the full thickness skin biopsies and in the roughage (mineral mixture containing granules) were made using the flame atomic absorption method (Perkin-Elmer 1100) after prior mineralization in an Ethos 900 microwave system (Milestone). The ALP in serum was determined by spectrophotometry using Point equipment.

After the testing period, the barn conditions were improved through daily use of dry bedding consisting of rye and oat straw. Also, for 6 months, a higher dose of micronutrients was introduced in the form of a supplement (KenoBiotyn) in a therapeutic dose.

The results were statistically analyzed using Student *t*-test.

# RESULTS

### Clinical examination results

The results of the clinical examination, biochemical and histopathological findings are presented in Figures 1-5 and Tables 1-2.

Of the 26 dairy cows in the transition period (from 3 weeks before to 3 weeks after parturition), 11.5% had clinically evident skin lesions around the back, ears, the sides of the chest, the pelvic limbs, around the vulva and at the base of the tail. The hair was raised, and there were small white scales and scaly skin evident (Figure 1). No scratching was seen in the examined

Fig.1. A clinical picture of a cow with hyperkeratosis

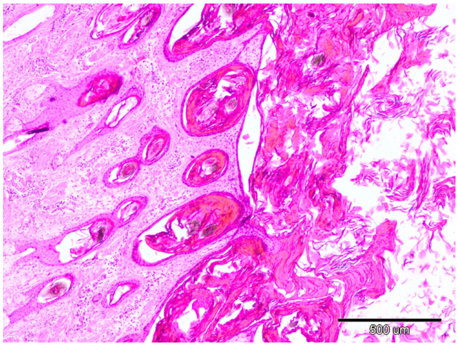


Fig. 2. Massive distension of the folicular infundibula with keratyn, hyperkeratosis

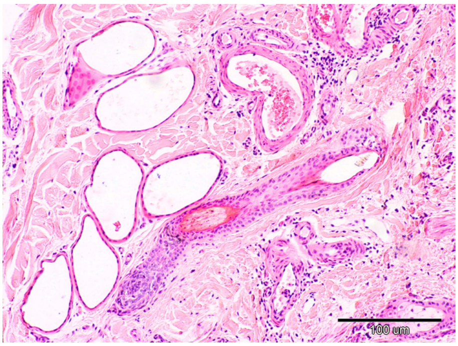


Fig. 3. Significant dilatation of sweat gland, atrophic epithelial cells layer of sweat gland. Inflammatory infiltration among blood vessels

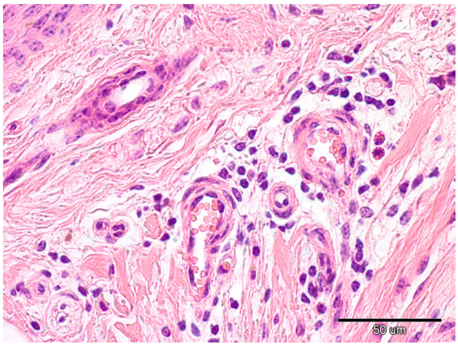


Fig. 4. Inflammation infiltration among blood vessels, composed maliny of mononucleus cells

animals, even though this sign was reported during the interview with the breeder. Vital signs were normal in both groups, cows with no skin lesions (group II) and with lesions (group I). The average body temperature of the animals was 38.5±0.5°C, the average heart beat rate was of 55.0±5.8 $min^{-1}$, and the average respiratory rate was 28.5±3.1 $min^{-1}$. The accessible lymph

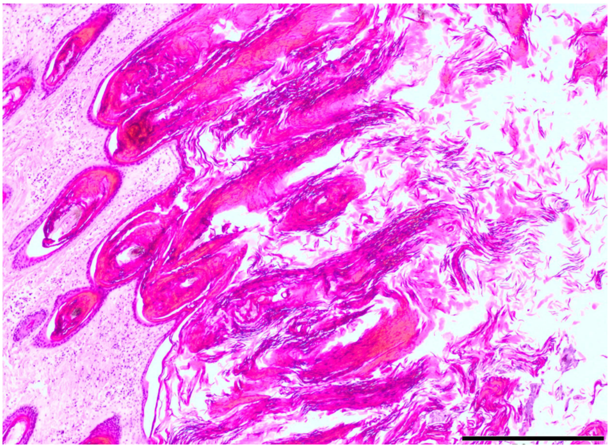

Fig. 5. Massive distension of the keratinized epithelium layer

nodes on both sides of each animal were of normal size, consistency and temperature upon palpation. The mucous membranes and conjunctiva were of normal colour with no signs of dehydration.

## The concentration of macro- and microelements in feed

The concentration of calcium, magnesium, copper, zinc and iron in the feed is shown in Table 1.

Table 1

Mineral content in feed for dairy cows

| Type of feed | Calcium | Magnesium | Copper | Zinc | Iron |
|---|---|---|---|---|---|
| | (g $kg^{-1}$ d.m.) | | (mg $kg^{-1}$ d.m.) | | |
| Granules | 8.37±0.15 | 3.89±0.06 | 15±2.35 | 212.0±18.7 | 210.0±18.7 |
| Mineral mixture | 12.6±0.8 | 7.8±0.72 | 560.5±76.9 | 4393.4±495 | 7043.4±179.0 |

Key: $\bar{x}$ – means , ±SD – standard deviation (15 samples).

## Parasitological and microbiological examination

Parasitological examination of the cows with and without skin lesions did not reveal the presence of ectoparasites. Likewise, bacteriological and mycological examinations did not reveal the presence of pathogenic bacteria, or any dermatophytes or their spores, in direct smears. The microbial flora of predominantly Gram positive bacteria was seen in the samples, consisting of bacilli and spherical forms. Haemolytic *Streptococcus* sp. L, coagulase-negative *Staphylococcus* and *Bacillus* sp. were grown on bacteriological substrates.

### Histopathological examination of the skin samples

The microscopic structure of full-thickness skin samples taken from the cows in group II with no macroscopically visible lesions was normal, including the epidermis, dermis, hair follicles and sweat glands. Conversely, the microscopic structure of full-thickness skin samples taken from the cows in group I, with macroscopic skin lesions, revealed massive areas of keratinized epidermis and widening of hair follicle infundibulums, which were filled with keratin. There was significant widening of the sweat glands as well as atrophy of the epithelium of these glands. Infiltration of primarily mononuclear inflammatory cells around the blood vessels was also noted (Figures 2-5).

### Blood microelements and biochemistry results

There were statistically significant ($P \leq 0.05$) lower levels of serum calcium (by 192%), magnesium (20%) and zinc (27.7%), as well as higher concentrations of serum iron (by 48.5%) in the cows with skin lesions compared to the cows without dermatological changes. No statistically significant differences were noted in the serum concentrations of copper in the cows from groups I and II ($P > 0.05$) – Table 2. Alkaline phosphatase activity in blood samples taken from the cows with skin lesions (group I) was slightly lower than in samples taken from the healthy animals, but the difference was not statistically significant.

### Concentration of microelements in skin biopsy samples

In cows with dermatological changes (group I), significantly lower ($P \leq 0.01$) concentrations of calcium, magnesium and zinc in full-thickness skin biopsies were seen compared to the concentrations of these elements in skin samples taken from the animals without skin lesions (group II). The concentrations of calcium, magnesium, copper and zinc in the skin of animals in group I were lower than in group II by 287.7 to 14.9%. The concentration of iron in the full-thickness skin biopsies from the animals with dermatological changes was statistically significantly higher ($P \leq 0.05$) by about 62% compared to the concentration of this element in the skin taken from the animals without dermatological lesions (Table 2).

## DISCUSSION

Rapid increase of milk yield in dairy cows that has occurred during the last 20 years has also contributed to negative trends in the health of these animals. Therefore, monitoring levels of mineral concentrations in animal tissues is important for assessing the effect of contamination on animal health and safety of products of animal origin in human nutrition. Unfavorable changes can occur especially during the transition period, covering a period

Table 2

Characteristics of selected indicators of metabolism in biological material derived from dairy cows

| Type of biological materials | ALP | | Ca | | Mg | | Cu | | Zn | | Fe | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (U $l^{-1}$) | | (mmol $l^{-1}$) serum (g $kg^{-1}$ d.m.) minerals in muscles, skin | | | | (µmol $l^{-1}$) serum (mg $kg^{-1}$ d.m.) minerals in muscles, skin | | | | | |
| | groups I and II | | | | | | | | | | | |
| | experimental (*n*-3) | control (*n*-23) | experimental (*n*-3) | control (*n*-23) | experimental (*n*-3) | control (*n*-23) | experimental (*n*-3) | control (*n*-23) | experimental (*n*-3) | control (*n*-23) | experimental (*n*-3) | control (*n*-23) |
| Serum | 36.8±2.8 | 38.5±10.1 | 0.66**±0.14 | 1.93±0.23 | 0.90*±0.28 | 1.08±0.23 | 15.53±2.28 | 16.4±1.25 | 15.58*±1.33 | 19.9±3.4 | 35.5*±5.9 | 23.9±5.7 |
| Muscles | - | - | 16.1*±4.2 | 21.5±3.5 | 30.0**±4.2 | 37.8±3.4 | 0.412*±0.11 | 0.9.4±0.236 | 29.5***±6.8 | 42.4±30.4 | 42.2*±5.8 | 35.6±5.6 |
| Skin | - | - | 26.5*±3.1 | 35.3 ±26.5 | 16.1*±1.7 | 21.5±2.5 | 1.94±0.98 | 2.13±0.41 | 23.6***±7.98 | 1.56±13.2 | 17.2*±1.3 | 27.9±2.59 |

Key: $\bar{x}$ – means , ±SD – standard deviation, * statistically significant differences ($P \leq 0.05$) between control and experimental group, ** statistically significant differences ($P \leq 0.01$) between control and experimental group, ***statistically significant differences ($P \leq 0.001$) between control and experimental group.

of three weeks before parturition to about three weeks post-parturition (Kandylis 1999, Nowak et al. 2009). These changes include mainly a reduction in oxidative stress resistance and immunity (Breen et al. 2006) in dairy cows, increased predisposition to dermatological, infectious and invasive diseases, as well as metabolic disorders in relation to reproduction, an increased number of somatic cells in milk, changes in the chemical composition of milk, mastitis, and foot diseases (Erdman 1993, Cisowski 2001, Bernabucci et al. 2005). The final result of a higher disease incidence in a herd is a shortened lifespan of cows (up to 2-3 lactations), increased mortality and premature culling of breeding cows. This in turn decreases the profitability of milk production and discourages farmers from breeding dairy cattle (Afzaal et al. 2004, Gaëtan et al. 2006). Proper nutrition during the drying-up period determines the profitability of milk production in subsequent cycles. This is particularly important as the maintenance of cows during the antenatal period has not been researched as thoroughly as in the postpartum period. The drying-up period is a very difficult time in the production cycle of an animal due to some enormous hormonal and metabolic changes. These changes are aimed at supplying intermediate transitional minerals needed to meet the new requirements during the postpartum period, especially with respect to energy management (Goff, Horst 1993, Goff et al. 1995, Twardoń et al. 2006, Jones et al. 2009, Wócik et al. 2009). The metabolic and hormonal changes that occur during the transition period prepare the body for both the labour and lactation (Castillo et al. 2005, Fűrll et al. 1996). In terms of both nutrition and well-being, the decisive factor is the reduction of the risk of many bovine diseases, including hyperkeratosis, reniforcement of the immune status, increased resistance to oxidative stress and improved profitability of milk production.

Observations have shown that the stalls in which the cows were kept were too long, resulting in the accumulation of faeces in the furthermost area. Each day during the long hours of rest, the cows were forced to have the most caudal portions of their bodies in direct contact with faeces and urine. That situation would last from the beginning of the cowshed period (late autumn) to spring. As a result of persisting high humidity and direct exposure of the skin to faeces and urine, 8 of the animals exhibited syndroms of itching. Intense licking and slight tearing heralded the disease. Rubbing against surrounding objects resulted in the formation of focal areas of raised hair and skin flaking. Small white scales composed of horny skin cells were observed on the surface of the skin. This initial stage of the disease looked like dandruff. The internal body temperature was in the range of 37.5 to 39.5°C. Rough, keratinized calluses, showing a tendency to spread, appeared on the posterior side of the upper portion of the pelvic limbs and around the vulva. Sometimes the lesions resembled scales. As a result of the excessive growth of the epithelial stratum corneum, there was marked thickening of the wrinkled skin as well as formation of cracks that sometimes gave the skin surface a reticular-like nature. These lesions were localized on the surface of the skin. The skin on most of the surface of the lesions was dry, wrinkled, and covered with numerous scales. The epidermis was dry, thickened, rough, with deep fissures showing the underlying red-colored dermal layer. When touched, the animals exhibited reactions as if in pain. The disease had a chronic character, although it was somewhat dependent on the age of a cow. In younger animals, the disease was slightly more acute, whereas in older animals it was milder. There were even some instances of spontaneous healing of the skin. All of the environmental factors mentioned above, in which the cows were housed, adversely affected their health, causing skin damage. Similar observations have also been made by other authors (HRISTOV et al. 2008**,** ALAM et al. 2010 ).

Macro- and microminerals play important roles in bodily functions. Cattle require 10 microminerals. The requirements for seven of the 10 microminerals, including copper, zinc, iron, manganese, selenium, cobalt and iodine, have been established. Table 1 and the estimated values it comprises show that the content of calcium, magnesium, copper, zinc, iron and manganese in granulated feeds, mineral mixtures as well as in other feeds supplied to the cattle met their requirements. In other words, absolute mineral deficiencies, which quite frequently lead to skin changes in cows in Poland, were not a direct cause of hyperkeratosis in that case. However, the results in Table 2 indicate that the concentration of serum calcium in cows was somewhat decreased at 0.66 mmol $l^{-1}$, and was in the upper range of values characteristic for cows with postpartum shock (0.62-1.75 mmol $l^{-1}$) **(**SOBIECH et al. 2010). This may indicate an excessively alkaline diet caused by high levels of strong cations. Regardless of calcium metabolism disturbances, alkaline diet may predispose to skin disease. A recent study has also shown that “anionic salts” had a positive effect on mineral metabolism and antioxidant status of dairy cows (KLECZKOWSKI et al. 2011). The microbiological examination revealed normal body surface and environmental flora.

In this study, magnesium could affect the prooxidative-antioxidative status in dairy cows as well as their health, which was demonstrated by the earlier study. The average serum concentration of magnesium in cows was 0.90 mmol $l^{-1}$. Variations in Mg concentrations can be observed during different months throughout the year. The highest concentration of about 0.9 mmol $l^{-1}$ is seen from January to April and from July to December, while the lowest one occurs from May to June. The serum concentration of Mg in the studied cows was typical for April and did not differ from the reference values.

Copper is an important micromineral of many enzyme systems essential for the normal prooxidative-antioxidative status, immunity and healh of skin. In addition, copper affects the activity of lysyl oxidase, which in turn depends on the biosynthesis of collagen and elastin present in the skin (BARSZCZ et al. 2009). Tables 1 and 2 show that the serum copper concentrations (15.53 mmol $l^{-1}$) were within the reference values (10.2-17.3 mmol $l^{-1}$). The concentration of this trace element was similar (0.412 mg $kg^{-1}$) in skeletal muscles compared to reference values (about 0.64 mg $kg^{-1}$) as well as in the skin (1.94 mg $kg^{-1}$) compared to the reference values (about 2.0 mg $kg^{-1}$) (KLECZKOWSKI et al. 2011).

Zinc is required for the function of a large number of enzymes, therefore its nutritional deficiency contributes to the formation of numerous clinical symptoms. Among the many factors that predispose ruminants to zinc deficiency are an increased calcium and phosphorus intake, a diet rich in legumes, high phosphorus grain supplements without increased microminerals, and increased soil fertilizers, especially those containing nitrogen and phosphorus. The most important symptoms include skin damage around the nostrils, eyes, lips, anus, vulva, and at the base of the ears, around the back of the larynx, neck, outer thighs, on the lower limbs and folds of the joints, the skin of the udder and scrotum. Initially, one can observe skin peeling as well as raised and clumped-up hair. Pruritus causes the animal to lick and intensely rub at surrounding objects, leading to the formation of wounds and infection. The hair falls out and there is inflammation. Fissures and wounds form as a consequence of the numerous skin lumps and cracks, sometimes limiting the ability of the animals to move. There is an inflammation of the oral and nasal mucous membranes, swelling of the gums and drooling. Sometimes the only lesion seen in cows is eczema at the base of the tail. There may be a seasonal pattern in the clinical signs, particularly during the drying-up period. Sometimes ulcers of the abomasum and alopecia occur. Histologically, hyperkeratosis of the epidermis was observed with multiple deformities, loss of granulosa cells and hypertrophy of basal layer of the epidermis. The results obtained show that the serum concentrations of zinc in cows (15.58 mmol $l^{-1}$) were within the reference values (10.8--20.0 mmol $l^{-1}$). However, the zinc concentration in skeletal muscle was 29.5 mg $kg^{-1}$ (reference value of about 70 mg $kg^{-1}$). This shows that the content of this trace element in bovine tissues was reduced. Serum zinc concentrations of 4.9-6.1 mg $l^{-1}$ were reported in sheep and goats in a herd where anorexia, wool eating, alopecia, hyperkeratosis and parakeratosis were observed. This demonstrates the strong homeostatic mechanisms that work in order to maintain at all costs an appropriate level of this micronutrient in the blood. However, the distribution of zinc in tissues differed from adequate values. Perhaps some of the reasons of reduced zinc levels in diseased tissues were undesirable environmental conditions, including long-term local pressure on some body parts as well as contact with urine and feces. Also, complex interactions between zinc and iron, and between zinc and copper, which may alter the distribution of zinc in tissues, must be kept in mind (AL-SAAD et al. 2010). Hyperkeratosis is primarily characterized by the presence of damaged skin cells. Histopathological changes also confirm the occurrence of lesions typical for parakeratosis (Figures 2-5). This results from the fact that zinc is involved in numerous biochemical life processes as well as in the replication of skin cells. In addition, zinc is responsible for cell oxidation and sequestration of free radicals (WRIGHTT, SPEARS 2004, MARKIEWICZ et al. 2007).

Stress is thought to increase susceptibility to skin diseases, which in part is due to increased endogenous glucocorticosteroid concentrations. Glucocorticosteroids have an immunosuppressive effect resulting from their interference with interleukin-1.4, leading to a blunted acute phase of inflammation. Hyperferremia, also induced by glucocorticosteroids, may as well contribute to the mechanism of stress-induced susceptibility to infection. Exogenous glucocorticosteroids in cattle have been related to increased serum iron concentrations, therefore it may be assumed that hyperferremia contributes to bacterial infections in this species (Mueller, Pahi 2000, Taira et al. 2008, Sordillo, Aitken 2009). Oxidative stress is a major factor in inflammatory, malignant and skin diseases in cattle. The cellular and total body iron status influences oxidative stress-mediated cell damage as a result of an excess iron ($Fe^{2+}$) pool, which produces harmful free radicals (hydroxyls) via the Fenton reaction (Kaplovitz 2000, Koichi, Kiyotaka 2008).

The serum iron concentration in the cows was 35.5 mmol $l^{-1}$ (reference range 30.67-36.1 mmol $l^{-1}$), whereas the muscle iron concentration was 42.2 mg $kg^{-1}$ (reference range of about 56 mg $kg^{-1}$) and the skin iron concentration was 17.2 mg $kg^{-1}$. These results indicate that the iron serum concentrations were within the reference values.

Alkaline phosphatase (ALP) is a zinc-dependent enzyme located in many cell membranes, where it is involved in the transport of metabolites across cell membranes. A particularly high content of alkaline phosphatase is found in osteoblasts, which explains its increased activity in young, growing animals. High levels of alkaline phosphatase are also present in hepatocytes, renal tubules and intestinal epithelial cells. The enzyme is excreted into bile, therefore its concentration increases in cases of obstruction of bile outflow from the liver to the intestines as a result of urolithiasis or cancer. Blood ALP activity in cattle ranges from 41.0 to 116.0 U $l^{-1}$. The bone fraction makes up 50-60% of the total enzyme activity, the liver fraction - 10-20% of the total enzyme activity, and the intestinal fraction – 30% of the total enzyme activity. The average serum AP activity of the tested cattle was rather low, at 36.8 U $l^{-1}$, which might have resulted from an inadequate level of animal welfare and nutritional conditions (Alam et al. 2010).

## CONCLUSIONS

Deficiency of dietary zinc and adverse environmental factors had recognizable influence on the cows, which exhibited different clinical signs. Significant changes were noticed in blood biochemical values and biochemical and histopathological results of tissue studies that were characteristic for hyperkeratosis. After diagnostic tests revealed these problems, the animal welfare was improved through everyday use of a desiccant bedding, consisting of rye and oat straw, in individual stalls.

An increased supply of micronutrients (KenoBiotyn) was also given for a period of 6 months in a preparation which consisted of 25 000 mg of zinc, 2 500 mg of copper, 12 500 mg of manganese in the form of a chelate and 1 000 mg of biotin. Yeast (*Saccharomyces cerevisiae*) was used as a medium. The KenoBiotyn preparation was used for 6 months in a therapeutic dose of 100 g/cow.

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