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HISTOMORPHOMETRIC ANALYSIS OF COWS HOOF SOLE HORN

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ABSTRACT

The aim of the study was to perform histomorphometric analysis of the sole horn in the thoracic limbs and pelvic limbs of primiparous and multiparous cows. During hoof trimming, samples of the sole horn were collected from the fore and hind legs of 30 (15 primiparous and 15 multiparous) cows of the Holstein-Friesian breed. Once prepared, the histological slides were analysed using Multiscan software. The bovine sole horn was subjected to histomorphometric analysis, taking account of the number of horn tubules per 1.5 mm^2, diameter of the horn tubules, and distances between the horn tubules. In both primiparous and multiparous cows, a significantly greater diameter of the horn tubules was observed in the fore legs, 51.60 and 48.62 vs. 42.42 and 39.88 µm. In second and third lactation cows, the number of horn tubules in the sole horn was significantly greater in the fore legs, 31.00 per 1.5 mm^2 vs. 22.50 per 1.5 mm^2 (pelvic limbs). There were no significant differences in the histological structure of the sole horn between the primiparous and multiparous cows.

Key words: cows, hoof horn, histomorphometric analysis

INTRODUCTION

Intensive genetic selection for performance traits has considerably increased the mean milk yield of the cows [Oltenacu and Broom 2010, Miglior et al. 2017]. This was accompanied by a deterioration of functional traits (fertility, health, longevity) of dairy cows [Stefański et al. 2014, Egger-Danner et al. 2015], resulting in higher incidence of lameness caused by digit and interdigital space diseases. Lameness results in reduced milk yield and fertility problems [Krpálková et al. 2019, O'Connor et al. 2020, Logroño et al. 2021, Jagusiak et al. 2023].

After mastitis and reproductive problems, leg and hoof diseases are the third most common cause of dairy cows being culled from the herd; they generate substantial economic losses [Koefler 2001, Żukowski 2008, Skrzypek et al. 2016] and efforts are made to minimize them. In order to do that, it is important to know the exact reasons for hoof diseases [Mendes et al. 2013]. The pathological processes in bovine digits are due to improper feeding [Bergsten 2003, Becker et al. 2014, Langova et al. 2020, Queiroz et al. 2021], housing system and insufficient physical activity [Bergsten and Herlin 1996, Bergsten 2003, König and Liebich 2014] and especially improper hoof care [Vermun 2005].

The condition of the hooves is determined by environmental and physiological factors, including the housing system, diet, milk yield, age, body condition, period of lactation, and genetic factors. Essential to hoof health is the quality of the hoof horn, which also includes its histological structure [Hulsen 2017, İzci et al. 2019]. The bovine sole horn is tubular in structure, and the number of horn tubules per unit area, their diameter and the spaces between them determine hoof horn strength and hardness, thereby influencing the health condition of bovine hooves [Mülling et al. 1999, Nowicki 2001].

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Hoof horn is made up of keratinized epidermal cells. The horn of the external and middle layer, the horn of the sole and the horny part of pad are tubular in structure. The horn of the sole is separated from that of the wall by the white line. It is part of the wall segment and consists of the laminar horn and the tubular horn. The composition of the epidermal horn by three different structures results in the three-layered appearance of the white line [Wang et al. 2020, Wang et al. 2021].

The outermost, thin layer is adjacent to the coronary horn and is easily distinguished from the surrounding horn by its lighter colour. The middle part of the white line is formed by the horn laminae and the crest horn filling the spaces between the laminae. The terminal horn fills the spaces between the laminae [König and Liebich 2014]. The white line lies between the hard coronary wall horn and the more pliable sole horn and runs from the heel bulb around the abaxial claw, to the toe and then along the axial wall where it extends proximally and becomes non weight bearing. It allows some elasticity and mobility within the hoof. The white line consists of terminal horn tubules embedded in intertubular horn in the inner part (next to the sole horn) and horny lamellae in the outer part (next to the claw horn). The intertubular horn consists of keratinized squame cells cemented together. Horn turnover is more rapid in the white line than in the other horny structures. This commonly results in incomplete keratinization, particularly at the axial and abaxial terminations, and therefore reduced horn quality and hardness, which leaves the structure more susceptible to damage and vascular disturbances [Budras et al. 1996].

Tubular horn consists of parallel horn tubules (formed by a cortex and a medulla), which are separated by the cells of the amorphous intertubular horn. The horn tubules are produced by the papillae of the corium, and the intrtubular horn is made by the matrix between the papillae [Budras et al. 1989, Budras et al. 1996]. A cross-section of the horn tubules shows the central part – the medulla, and the peripheral part – the cortex. The cortex cells are more pigmented, tightly layered, while those in the medulla are loose. In the microscopic image, horn tubules are either oval or round [Empel 1984, Nowicki 1999, König and Liebich 2014]. Histological examination consists in calculating the number of horn tubules per unit area, their diameter and the spaces between them in the microscopic image [Dietz and Prietz 1981, Mülling et al. 1999]. There is a relationship between the histological structure of the horn and the incidence of hoof diseases. Hinterhofer et al. [2007] found that, in cattle with chronic laminitis, the low horn quality was attributable to the malformed tubular and lamellar structure of the diseased dermis. The hoof horn strength and hardness depend on the number of horn tubules per unit area – the more the tubules, the stronger the horn. According to Dietz and Prietz [1981], the hardest corner of the anterior claw wall contained 79 tubes on 1 mm of horn, the weaker plantar horn 16, and the weakest pads even less.

There are few studies on the microstructure of the bovine hoof horn [Budras et al. 1989, Singh et al. 1992, Mülling et al. 1994, Budras et al. 1996, Mülling et al. 1999, Franck et al. 2006, Knott et al. 2007, Danscher et al. 2010, Döpfer et al. 2011, Mendes et al. 2013, Assis et al. 2017]. Apart from normal anatomical structure and zoometric measurements, the model of a structurally normal hoof should account for hoof horn quality, based on histomorphometric measurements of its structure.

The research hypothesis was to check whether the parityof the cows affects changes in the structure of the hoof horn. Since the most common cause of lameness in cows is hoof disease, with 80% of these diseases occurring on the rear outer or the front inner hoof, we decided to see if there were also differences in the structure of the fore and hind claws.

The aim of the present study was to perform histomorphometric analysis of the sole horn in the fore and hind legs of primiparous and multiparous cows.

MATERIAL AND METHODS

Hoof tissue from thoacic and pelvic limbs was collected for histological examination from 30 dairy cows (15 primiparous and 15 multiparous cows) of the Polish Holstein-Friesian breed (Black-and-White variety) with an average milk yield of 9000 kg. Animals were selected from a herd of 120 cows. The selection criteria were: the age of the cows (primiparous, 1st lactation and multiparous, 2nd and 3rd lactation, 8 and 7 cows, respectively), lactation period (40–200 days of milking), cows' condition (2.75–3.25 points on the scale BCS, Body Condition Scoring), health condition based on the analysis of the treatment book (cows healthy within the last 3 months), hoof health based on the locomotion score (very good condition, no lameness). Animals were housed in a 2-row tie-stall barn with a central feed bunk and with two dunging passages along the walls. The stalls (175 × 120 cm) were separated by metal partitions (90 cm long). Animals were divided into two groups (primiparous and multiparous cows). Cows were fed TMR diets and were milked twice daily. The ration of fodder was composed with: maize silage, lucerne and grass haylage, straw and concentrate (soybean meal, rapeseed meal, triticale, maize, mineral-vitamin supplements). The cow stalls were littered with straw. Hoof correction in cows was performed once a year. Preparations for dry disinfection were used prophylactically. They were used once a week by sprinkling the lying part of stand.

During hoof trimming performed with an angle grinder and knife, horn samples separated from the hoof (1 × 3 cm) were fixed in vials containing 10% formalin neutralized with $CaCO_3$ for 48 hours. The samples were cut into sections of approx. 1.0 × 1.0 cm and softened in formic acid for 24 h. The fixed and softened sections were dehydrated, cleared and infiltrated with paraffin in a tissue processor (Thermo Shandon) and then embedded in paraffin blocks using a Medite embedding station.

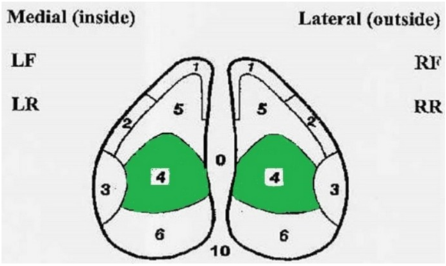

Fig. 1. International foot map [Greenough and Vermunt 1991]; locations where the horn samples were taken in green colour

The paraffin blocks with a hoof horn tissue were cut into sections 7 µm thick on a rotary microtome (Thermo Shandon). Sections were mounted on glass slides and placed in an oven (38°C) for 24 h. After dewaxing, the slides were stained with hematoxylin and eosin (HE) as topographic staining.

The microscopic images of the hoof horn tissue were saved on a computer disk using a Delta Optical Evolution 300 microscope equipped with a ToupCam™digital camera. Next, MultiScan 18.03 software was used to calculate the number of horn tubules per 1.5 mm^2, and to measure the diameter of the horn tubules (µm) and the distances between the horn tubules (µm). A total of 240 claws (30 cows x 4 feet x 2 claws) were analyzed.

The results were statistically analysed using Statistica 13.1 PL. The normal distribution of the traits was checked with the Shapiro-Wilk W test. Statistical analyzes were performed using the one-factor analysis of variance. Arithmetic means (x) and standard deviation (SD) were calculated. The comparison of means was completed with a confidence interval of 95% using Tukey's test.

RESULTS AND DISCUSSION

The results for the histological examination of the bovine sole horn are shown in Fig. 2.

The microscopic images allowed for calculating the number of horn tubules per unit area, their diameter and the distances between them. Table 1 presents histological structure of the sole horn from the examined primiparous and multiparous cows. Comparison of the primiparous and multiparous cows showed significant ($P < 0.05$) differences in the analysed parameters, namely the diameter of the horn tubules (47.17 µm vs. 43.25 µm) and the distances between the horn tubules (204.81 µm vs. 186.63 µm). In a similar study, Rabelo et al. [2015] showed that the number of horn tubules per 1 mm^2 decreased, while the diameter increases with the age of the animals.

Teter et al. [2014] provided conclusive evidence for the relationship between cows' age and hoof health. Claw horn disruption lesions constitute a noninfectious subset of lameness-causing diseases and include sole ulcers, sole hemorrhage, and white line diseases. These lesions have a high rate of reoccurrence, delayed detection of lameness increases the risk of more severe lameness. This risk increases as the cow ages. Lameness during one lactation is thought to impact on future lactations making it especially important to identify and treat lameness as early as possible particularly in first lactation animals [Newsome et al. 2016]. Ambient humidity also affects the quality of the hoof horn. Horn rot and digital dermati-

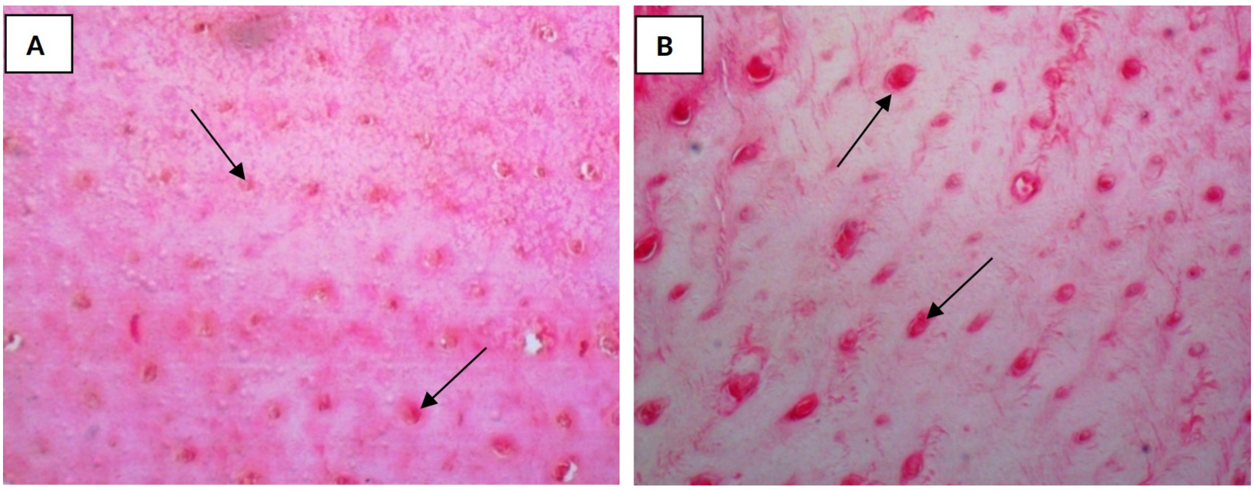

Fig. 2. Histological slides of the sole horn in a primiparous cow (A) and multiparous cow (B) with visible horn tubules (arrows). Stained with hematoxylin and eosin, 40x magnification

Table 1. Histological structure of the sole horn in primiparous and multiparous cows

Group of cows	Number of horn tubules/1.5 mm^2	Diameter of horn tubules, µm	Distance between horn tubules, µm
Primiparous	25.30 ±3.52	47.17[a] ±3.95	204.81[a] ±11.12
Multiparous	26.75 ±3.43	43.25[b] ±7.92	186.63[b] ±13.08

[a, b] significant differences at $P < 0.05$.

Table 2. Comparison of the histological structure of the sole horn in thoracic and pelvic limbs

Limbs	Number of horn tubules/1.5 mm^2	Diameter of horn tubules, µm	Distance between horn tubules, µm
Thoracic	25.80 ±4.04	50.41[a] ±5.07	192.17 ±11.56
Pelvic	25.67 ±3.50	41.85[b]±7.42	203.75 ±12.38

[a, b] significant differences at $P < 0.05$.

Table 3. Histological structure of the sole horn in thoracic and pelvic limbs with regard to the age of the cows

Group of cows	Limbs	Number of horn tubules/1.5 mm^2	Diameter of horn tubules, µm	Distance between horn tubules, µm
Primiparous	Thoracic	22.33 ±5.24	51.60[a] ±4.24	204.52 ±12.98
	Pelvic	26.57 ±4.51	42.42[b] ±5.17	204.94 ±15.52
Multiparous	Thoracic	31.00[a] ±6.01	48.62[a] ±14.07	173.65[a] ±16.10
	Pelvic	22.50[b] ±1.52	39.88[b] ±11.85	199.62[b] ±20.75

[a, b] significant differences at $P < 0.05$ between thoracic and pelvic limbs according to the age of the cows.

tis are mainly caused by humidity, anaerobic conditions caused by adherence of manure and strong microbial action [Wieczorek 2018].

Table 2 compares histological structure of the bovine sole horn in thoracic and pelvic limbs. The number of horn tubules per 1.5 mm^2 in the front and rear hooves was similar (25.80 µm vs. 25.67 µm per 1.5 mm^2). The front compared to rear hooves exhibited significantly ($P < 0.05$) greater diameters of the horn tubules (50.41 µm vs. 41.85 µm) and distances between the horn tubules (192.17 µm vs. 203.75 µm).

A study by Skrzypek et al. [2016] with tethered Holstein-Friesian cows showed that 95% of digit diseases occurred in rear hooves and only 5% in front hooves, with diseases such as sole ulcer, foot rot, and interdigital hyperplasia occurring only in rear hooves. In the tie-stall system, rear hoof horn quality and health is determined, in particular, by the constant presence of contamination and the associated excess humidity [Rabelo et al. 2015]. A study conducted in the USA showed that cows with claw horn lesions were 1.7 times more likely to be culled [Machado et al. 2010].

The results of our study are in line with the findings of Dietz and Prietz [1981], who demonstrated that the sole horn of German Black-and-White cattle contained 24 tubules in conversion to 1.5 mm^2. Similar results were obtained by Rosochowicz et al. [1986] who compared the number of the sole horn tubules in Black-and-White bulls, Holstein-Friesian bulls and the crosses of these breeds with Jersey [Franck et al. 2006].

Among the multiparous cows, front hooves contained an average of 8.5 more horn tubules per 1.5 mm^2 than rear hooves, and in the primiparous cows this difference was half as small (4.24 horn tubules per 1.5 mm^2) (Table 3). Also the horn tubule diameter was greater ($P < 0.05$). For both the primiparous and multiparous cows, significantly smaller diameter of the horn tubules was observed in the rear hooves (by 9.18 µm and 8.94 µm, respectively). In both the front and rear hooves, distances between the horn tubules were greater in primiparous cows (204.52 µm and 204.94 µm) than in multiparous cows (173.65 µm and 199.62 µm, respectively).

According to Dietz and Prietz [1981], the hoof horn strength and hardness increases with the increasing number of horn tubules per unit area. Furthermore, Pijl [2006] reports that pathological lesions are much less frequent in the thoracic limb hooves compared to the pelvic limb hooves.

Some cows remain unaffected throughout their lives, which may suggest a genetic role for disease susceptibility or resistance in these animals [Orsel et al. 2018].

CONCLUSIONS

This is still a future-oriented method as it needs to be fine-tuned for details and principles of the analysis, especially in larger populations. Its usefulness and accuracy may be increased in the case of more detailed hoof examinations, taking into account the division of outside and inside hooves according to the international hoof map. This is of particular importance because in many bull selection indices this trait is essential (e.g. in the Polish PF index it has a weighting of 30%). In our study, we found visible differences in the histological structure of the hoof horn between primiparous and multiparous cows. Based on the results, multiparous differ from primiparous for having lower density of tubules in the soles of pelvic compared with thoracic limbs. In both groups, a significantly higher diameter of the horn tubules was found in the thoracic limbs.

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ANALIZA HISTOMORFOMETRYCZNA ROGU RACICY KRÓW

STRESZCZENIE

Celem pracy była analiza histomorfometryczna rogu podeszwy racic krów (w kończynach piersiowych i miednicznych) pierwiastek i wieloródek. W trakcie korekcji racic od 30 krów (15 pierwiastek i 15 wieloródek) rasy polskiej holsztyńsko-fryzyjskiej pobrano próbki rogu podeszwy racicy z kończyn przednich i tylnych. Przygotowane preparaty histologiczne analizowano przy pomocy programu Multiscan. Przeprowadzono analizę histomorfometryczną rogu podeszwy racicy bydła, uwzględniając: liczbę rureczek rogowych na powierzchni 1,5 mm^2, średnicę rureczek rogowych oraz odległości między nimi. Zarówno u krów pierwiastek, jak i wieloródek, istotnie większą średnicę rureczek rogowych stwierdzono w kończynach piersiowych. U krów w II i III laktacji istotnie większą liczbę rureczek rogowych w rogu podeszwy racicy odnotowano w kończynach piersiowych. Może to świadczyć o lepszych warunkach higienicznych utrzymania kończyn piersiowych krów w porównaniu z miednicznymi w oborach uwięziowych. Nie stwierdzono istotnych różnic w budowie histologicznej rogu podeszwy racicy między krowami pierwiastkami i wieloródkami.

Słowa kluczowe: krowy, róg racicy, analiza histomorfometryczna

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