İstanbul Üniv. Vet. Fak. Derg.
40 (1), 41-52, 2014
Araştırma Makalesi

J. Fac. Vet. Med. Istanbul Univ.
40 (1), 41-52, 2014
Research Article

Investigation of Manure-Induced Physicochemical Changes of the Claw Horn in Dairy Cattle

Toncho PENEV[1], Aleksandar ILIEV[1], Chonka MITEVA[1], Yurii MITEV[1], Penka VALKOVA[2], Krassimira UZUNOVA[3]*

[1]*Trakia University, Faculty of Agriculture, Department of Applied Ecology and Animal Hygiene, 6000 Stara Zagora, Bulgaria*
[2]*Trakia University, Faculty of Agriculture, General Laboratory of Science and Investigation, 6000 Stara Zagora, Bulgaria*
[3]*Trakia University, Faculty of Veterinary Medicine, Department of Animal Hygiene, Ethology and Welfare, 6000 Stara Zagora, Bulgaria*

**Corresponding Author: Toncho PENEV Trakia University, Faculty of Agriculture, Department of Applied Ecology and Animal Hygiene, 6000 Stara Zagora, Bulgaria*
e-mail:penevtoncho@yahoo.com

Geliş Tarihi / Received: 08.10.2012

ABSTRACT

The study was conducted on three claw horn areas (walls, soles and heels) in dairy cows. Claw horn samples were collected from cows at first lactation without signs of lameness. The changes in the hardness, the fat content and the swelling under the influence of manure from intensively reared cows were evaluated. The hardness and fat content were determined by the beginning of the experiment and at 7-day intervals for 28 days stay in manure. The swelling of hoof zones was done by placing samples in graduated cylinders filled with manure and distilled water for 48 h. The hardness of claw walls decreased most significantly during the first 14 experimental days (3.75 Shore A units). For the same period, fat content of this claw zone underwent the most significant reduction. In the sole area, the hardness decreased most intensely during the first 14 days – by 8.15 Shore A units which was in agreement with claw fat reduction in this area. A similar trend was observed in heels, where the hardness decreased most considerably until the 7th day by 3.85 Shore A units and between days 7 and 14 – by 4.45 Shore A units. The respective fat content reduction was by 0.75 and 2.79 mg/g DM. The significant loss of claw horn fat predisposes to water penetration and a strong swelling of keratin. The strongest and most prolonged swelling was detected in the heel area, which enlarged their volume until the 48th experimental hour up to 8 cm^3, and where fat content decreased the most compared to other studied claw horn zones.

Key Words: Manure mass, claw horn, keratin, fat, softening, swelling

ÖZET

GÜBREYE MARUZ BIRAKILAN SÜTÇÜ SIĞIRLARIN TIRNAK YAPILARINDA MEYDANA GELEN FİZİKOKİMYASAL DEĞİŞİMLERİN İNCELENMESİ

Bu çalışma sütçü sığırlarda üç farklı tırnak bölgesinde (duvar, taban, ökçe) yürütülmüştür. Tırnak örnekleri birinci laktasyonunda topallık göstermeyen sığırlardan toplanmıştır. Entansif yetiştirilen ineklerde tırnağın sertliği, yağ içeriği ve şişme gibi özelliklerinde gübreye bağlı şekillenen değişimler incelenmiştir. Sertlik ve yağ içeriği çalışmanın başında ve tırnağın gübre içinde kaldığı 28 gün boyunca 7 şer gün aralıklarla ölçülmüştür. Tırnak yapısındaki şişme düzeyleri, örneklerin 48 saatlik bir süreyle su ve gübre ile doldurulmuş dereceli silindire bırakılmaları sonrası belirlenmiştir. Tırnak duvarındaki sertlik araştırmanın ilk 14 günü içerisinde önemli derecede azalmıştır (3,75 Shore A birimi). Aynı periyotta, tırnağın yağ içeriğindeki en belirgin düşüş şekillenmiştir. Taban bölgesinde ise sertliğin en yoğun şekilde düştüğü ilk 14 gün boyunca 8,15 Shore A birimlik azalma bu bölgedeki yağ düşüşüyle uyumludur. Benzer şekilde ökçe bölgesinde de düşüşün en yoğun olduğu 7. güne kadar 3,85 shore A birimlik, 7. ve 14. günler arasında ise 4,45 Shore A birimlik bir düşme gözlemlenmiştir. Yağ içeriğindeki düşüşler ise sırasıyla; 0,75 ve 2,79 mg/g DM şeklindedir. Tırnak bölgesindeki yağ düzeyinin belirgin olarak azalması, tırnağı su penetrasyonuna ve keratin dokusunun aşırı şişmesine yatkın kılar. En şiddetli ve uzun süren şişlik ökçe bölgesinde saptanmıştır. Bu bölgede deneyin 48. saatine kadar hacim 8 cm^3'e çıkmış ve yağ içeriği çalışmada incelenen diğer tırnak bölgelerine kıyasla daha fazla azalmıştır.

Anahtar Kelimeler: Gübre, tırnak, keratin, yağ, yumuşama, şişme

Introduction

Lameness and hoof diseases are among the commonest problems in cattle farms, resulting in reduction of milk yields, lower fertility and shorter service period (Collick et al., 1989; Coulon et al., 1989). According to some epidemiological studies, hoof diseases are highly prevalent during the first three lactation months (Offer et al., 2000; Whay et al., 1997). Numerous factors in the puerperium, as hormonal changes, production system, locomotion and feeding, could induce lameness and influence hoof health in cattle (Clarkson et al., 1996). The impaired synthesis and enhanced degradation of keratin (the structural protein of hooves) due to impaired macroarchitectonics of keratin molecules, which is responsible for the mechanical resistance of hooves, could induce locomotory disorders of a various extent (Vermunt and Greenough, 1995), most commonly manifested with lameness.

The primary hygiene factor in animal rearing facilities, with negative impact on claw horn, is manure, as it causes softening and swelling of keratin and makes it highly vulnerable to wearing (Bonser et al., 2003; Gregory, 2004; Gregory et al., 2006).

Manure has also an effect on fat content of hoof horn. The role of fat for keratin resistant is essential, as fat is mainly distributed in the intercellular space of keratinocytes (Elias and Meonon, 1991; Golden et al., 1989). The authors provided proofs that ceramides are the fats determining the physical properties of keratin. The research of Higuchi and Nagahata (2001) and Higuchi et al. (2004; 2005) demonstrated that fats and factors influencing their amount in the hoof capsule are important for the water content of the horn. They found out that the claw horn with high water content was softer. Thus, such hooves are highly susceptible to wear and mechanical injury of the corium. In their later research, Higuchi et al. (2009) gave evidence for a statistically significant reduction of horn hardness ($P<0.05$) and elasticity ($P<0.01$) after placement in ammonia and hydrogen sulfide solutions (in concentrations similar to those in manure) as compared to claw horn immersed in water for 12, 24 and 48 hours. They observed that ceramide content of hoof horn samples immersed in ammonia and hydrogen sulfide solution decreased compared to baseline concentrations. Higuchi et al. (2009) suggested that the changes in the physical properties of claw horn (softening and swelling) caused by ammonia and hydrogen sulfide were consequent to occurring biochemical alterations in cattle manure.

The present investigation aimed to establish the physicochemical changes (swelling and softening) occurring in claw horn under the influence of manure, obtained from cattle reared under intensive production systems. An important part of the experiment was the analysis of fact content in the same claw horn zones after its 4-week stay in manure.

Materials and Methods

The investigation was performed on 20 clinically sound hooves from cows in their first lactation, subject to emergency slaughter at several slaughterhouses. Two samples of the horn were obtained from each hoof from three areas (walls, soles and heels) with a size of 30x15x6 mm, according to the method of Baggott et al. (1988) (Figure 1).

One set of samples were used for determination of claw horn hardness, and the other set – for analysis of fat content and claw horn swelling.

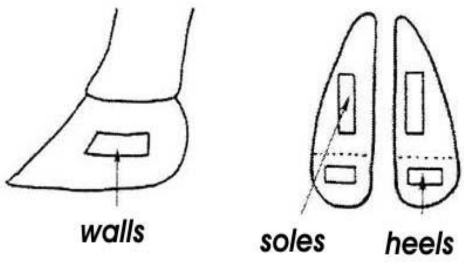

Figure 1. Claw horn areas (walls, soles, heels) included in the investigation (Baggott et al., 1988).

Şekil 1. Çalışmaya dahil edilen tırnak bölgeleri (Duvar, Taban, Ökçe) (Baggott ve ark., 1988).

Determination of claw horn hardness

Claw horn hardness (walls, soles and heels) was determined by means of a durometer (‘Zwick Shore A’ ASTM D2240 DIN 53505; ISO 868), with a scale within the range of 0 to 100 Shore A units (Figure 2). The measurements were done at the lab of Medina Med – Stara Zagora.

Figure 2. Durometer, “Zwick Shore A” for determination of claw horn hardness.

Şekil 2. Tırnak sertliğini ölçmek için kullanılan durometre “Zwick Shore A”.

The horn hardness was determined immediately after collection of samples. Afterwards, samples were placed in manure for 28 days. The claw horn hardness was periodically determined at 7-day intervals until the end of the experiment. The manure mass when horn samples were immersed was prepared by mixing faeces and urine at a ratio 2:1. The manure mass was replaced twice per week. By the end of each week, samples were removed, dried and the hardness was measured. The mean hardness of all measurements for the group (walls, soles and heels) were calculated.

Determination of hoof horn fat content

Each horn samples was grinded to fine particles. Bulk samples from each of the three horn zones were used for analysis of fat content.

The fat content as determined in the beginning of the study and at 7-day intervals until the 28th day by the method of Soxhlet on Soxtec 2050 fat extraction system.

Determination of claw horn keratin swelling

The swelling of keratin and the change in sample weight were determined from the beginning to the 48th has followed:

Perforations 1 mm in diameter were made on plastic cylinders. At every 1 cm^2 area of the cylinder, 5-6 perforations were made. Two grams of a sample were placed in each cylinder, it was pressed with a piston and its volume in cm^3 was measured.

Four samples were prepared from each horn zone (walls, soles and heels) two of which were placed in manure, and the other two – in distilled water. The manure and water levels were to the samples' surface. The changes in the weight and volume of samples were detected by the 12th, 24th and 48th hour of their immersion.

Data were statistically processed with STATISTICA 6 software with Repeated measures Anova.

Results and Discussion

The results from claw horn hardness evaluation in the different zones (walls, soles and heels) showed that the initial hardness of hoof walls was 98.85 Shore A units (Figure 3). After the stay in manure, their hardness decreased and the duration of stay had a statistically significant influence between days 7 and 14 ($P<0.05$). During the first 7 days of stay, the hardness in this zone did not change considerably. After the 14^{th} day, hoof hardness decreased by 0.10 Shore A units to 95.00 Shore A (day 21) and 94.50 Shore A (day 28), without significant differences from day 14 to day 28.

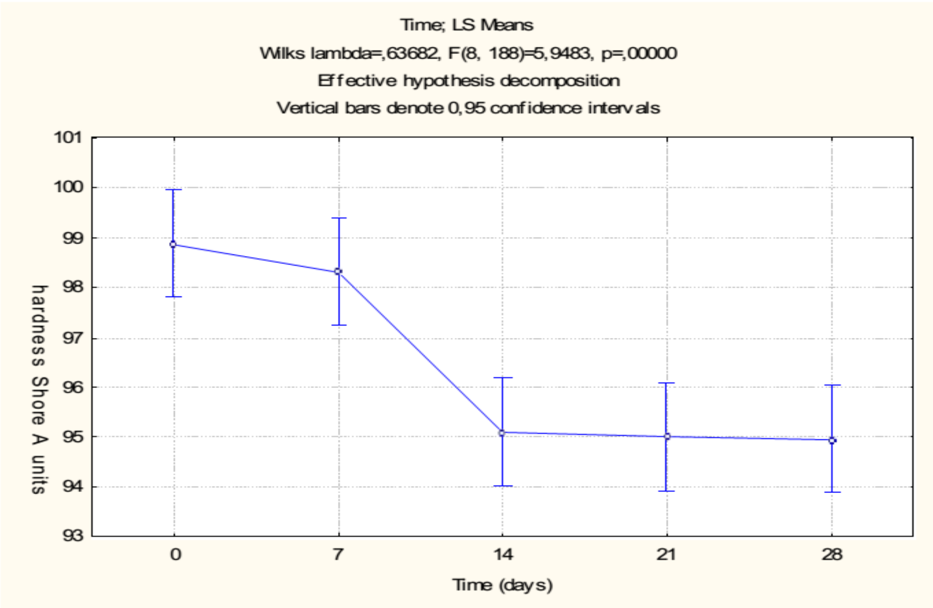

Figure 3. Effect of duration of stay in manure mass on claw horn hardness in the region of hoof walls.

Şekil 3. Gübre içerisinde kalma süresinin tırnak duvarı sertliği üzerine olan etkisi.

Claw horn walls, as hardness is concerned, are the most resistant hoof zone. Our data confirmed the reports of Higuchi et al. (2009), who demonstrated that hoof walls were the hardest zones and least prone to softening than the other two evaluated zones. According to authors, claw horn hardness decreased as early as after a 6-hour stay in ammonia solution. In our experiment, a statistically significant softening occurred after 7-day stay in natural manure mass, which came in support of data of Gregory (2004) and Gregory et al. (2006).

Claw sole harness decreased statistically significantly ($P<0.05$) from the beginning until the 14^{th} day of the experiment (Figure 4).

Sole hardness decreased from a baseline value of 95.00 Shore A units to 90.65 Shore by the 7^{th} day and 86.85 Shore A units by the 14^{th} day ($P<0.05$). Afterwards, there was no substantial softening of claw horn in the sole region.

In the heel region, a considerably softening of claw horn has occurred over the first experimental week ($P<0.05$) (Figure 5).

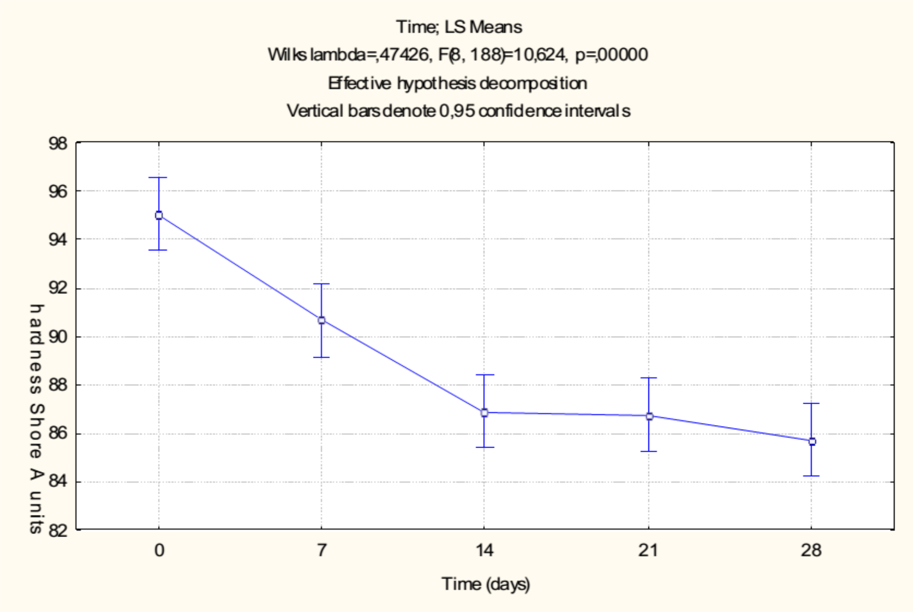

Figure 4. Effect of duration of stay in manure mass on claw horn hardness in the sole region.

Şekil 4. Gübre içerisinde kalma süresinin taban sertliği üzerine olan etkisi.

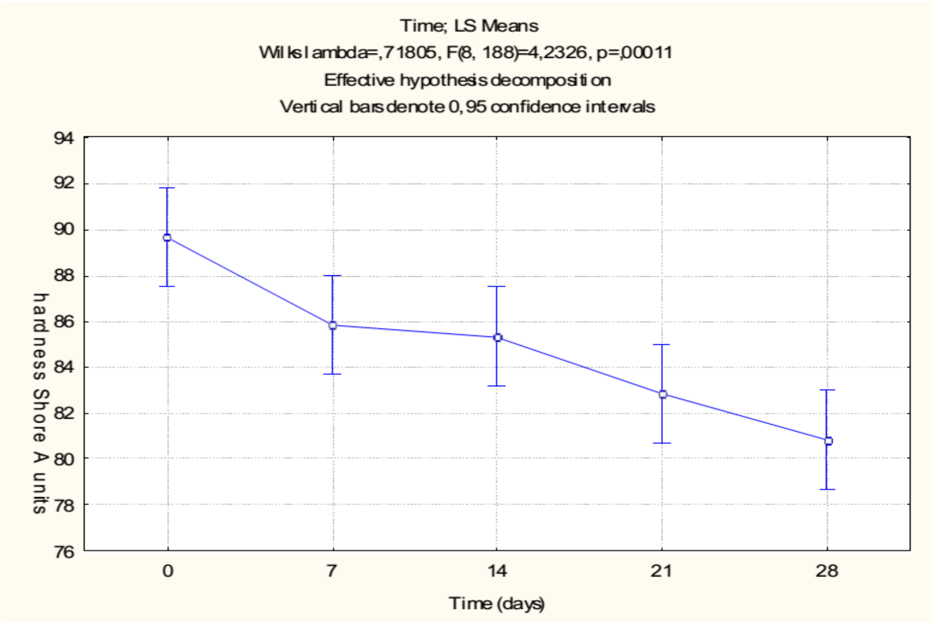

Figure 5. Effect of duration of stay in manure mass on claw horn hardness in the heel region.

Şekil 5. Gübre içerisinde kalma süresinin ökçe bölgesi üzerine olan etkisi.

Heel hardness decreased from 89.65 Shore A units in the beginning to 85.8 Shore A units ($P<0.05$). The results established a more intensive reduction of hardness after the 14th day, with statistically significant differences between values measured on day 14 and day 28 ($P<0.05$).

In the three studied areas, claw horn hardness decreased considerably over the 28-day period vs baseline. These results are alarming as claw horn needs 3 months to undergo a complete repair and to be replaced by new horn (Vermunt and Greenough, 1995). This requires supplementation of dairy cows' ration with components such as biotin, which improve claw horn strength (Higuchi et al., 2003).

Our experimental design is as close as possible to natural conditions in cattle farms, with regard to the constant contact between hooves and the aggressive manure mass. The continuous stay of claw horn in manure mass predisposed keratin, which functions as a hydrophilic gel to absorb some water, to swell and to become softer (Mertin and Lippold, 1997).

Crude fat content (CF) of claw horn walls showed a substantial reduction ($P<0.05$) until the 14th day of the study from 8.97 mg/g DM in the beginning to 8.01 mg/g DM on day 14 (Figure 6). The comparison to hardness data in this zone shown on Figure 3 demonstrated that hoof wall hardness was related to fat content of horn. After the 14th day, no significant decrease in claw horn crude fat in this zone has occurred.

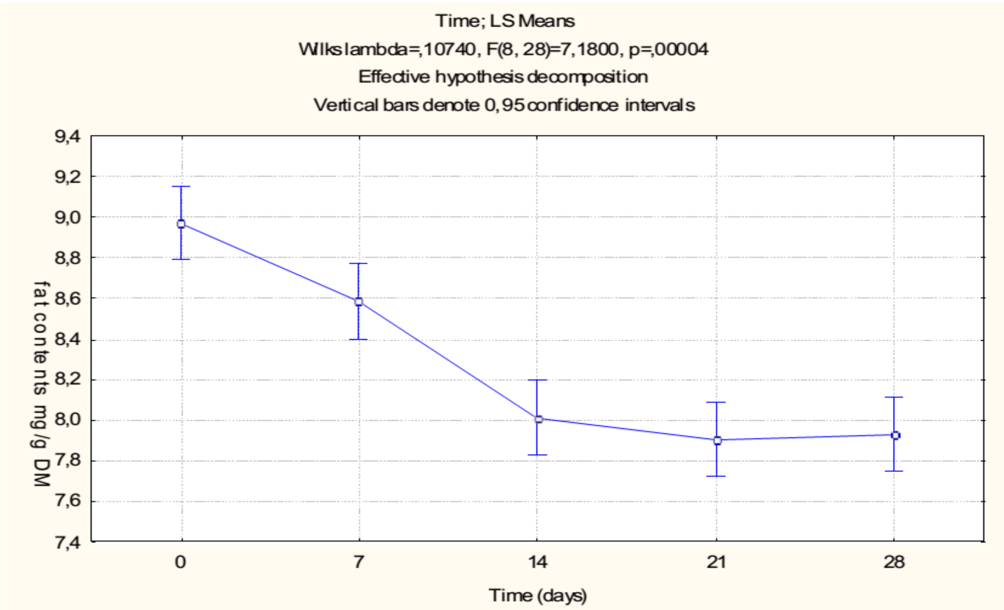

Figure 6. Effect of duration of stay in manure mass on crude fat content of claw horn in the region of hoof walls.

Şekil 6. Gübre içerisinde kalma süresinin tırnak duvarı ham yağı üzerine olan etkisi

Crude fat content in the sole region is depicted on Figure 7. Over the entire experimental period, it decreased considerably ($P<0.05$), most intensively during the first 2 experimental weeks by 11.97 mg/g DM. This confirmed before mentioned hypothesis that the loss of crude fat from claw horn was related to loss of hardness and strength.

In the heel regions, the most intensive decrease in crude fat content occurred between days 14 and 21 of the experiment ($P<0.05$; Figure 8). Claw horn hardness in this area (Figure 5) showed a considerable reduction in hardness during the first 7 days and after the 14th day ($P<0.05$), corresponding to fat loss from the heel region.

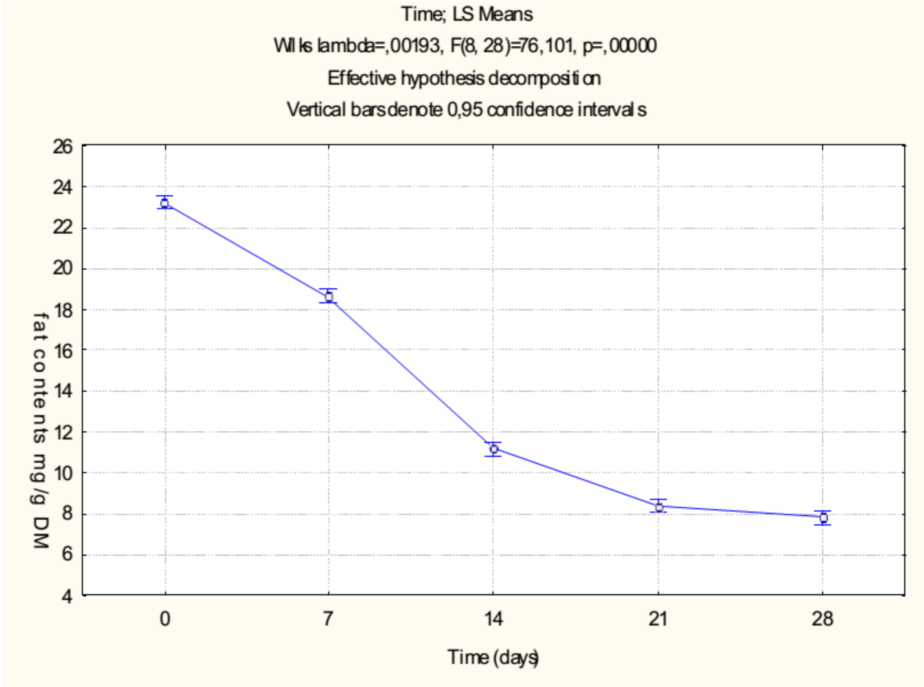

Figure 7. Effect of duration of stay in manure mass on crude fat content of claw horn in the sole region.

Şekil 7. Gübre içerisinde kalma süresinin taban ham yağı üzerine olan etkisi

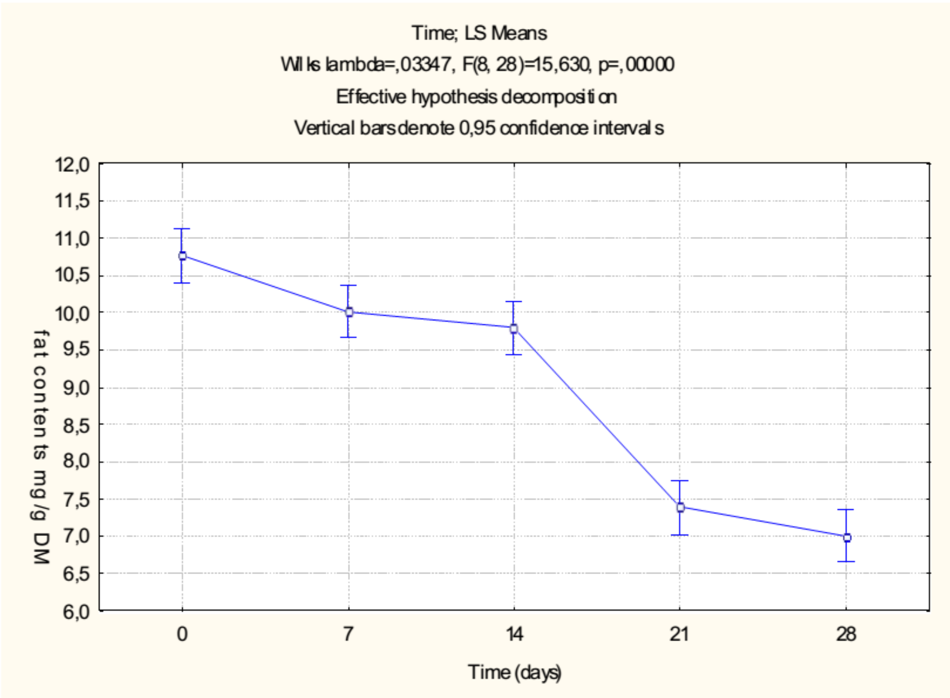

Figure 8. Effect of duration of stay in manure mass on crude fat content of claw horn in the heel region.

Şekil 8. Gübre içerisinde kalma süresinin ökçe bölgesi ham yağı üzerine olan etkisi

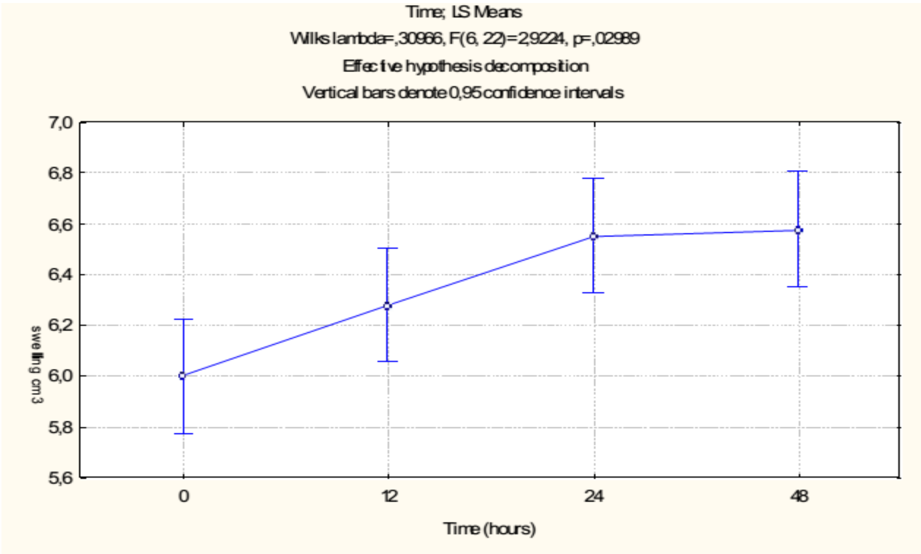

Figure 9. Effect of duration of stay in water on claw horn swelling in the region of hoof walls.

Şekil 9. Su içerisinde kalma süresinin tırnak duvarındaki şişmeye olan etkisi.

According to these data, manure mass exerted a negative effect on fat content of claw horn of cattle. Under the influence of alkaline substances in manure mass, claw horn fat decreased significantly and horn became permeable to water. It swelled, became softer and highly susceptible to various kinds of injury, manifested clinically by lameness (Higuchi et al., 2004; 2009).

The determination of claw horn swelling in water and manure mass in the wall region exhibited substantial swelling until the 24th hour of the study (P<0.05) (Figures 9 and 10).

The horn of hoof walls swelled up to 6.3 cm^3 after a 12-hour stay in water and up to 6.6 cm^3 in manure mass. Until the 24th hour, the swelling extent attained 6.5 cm^3 (Figure 9), and 7 cm^3 (Figure 10), respectively. After that time, claw horn volume did not change.

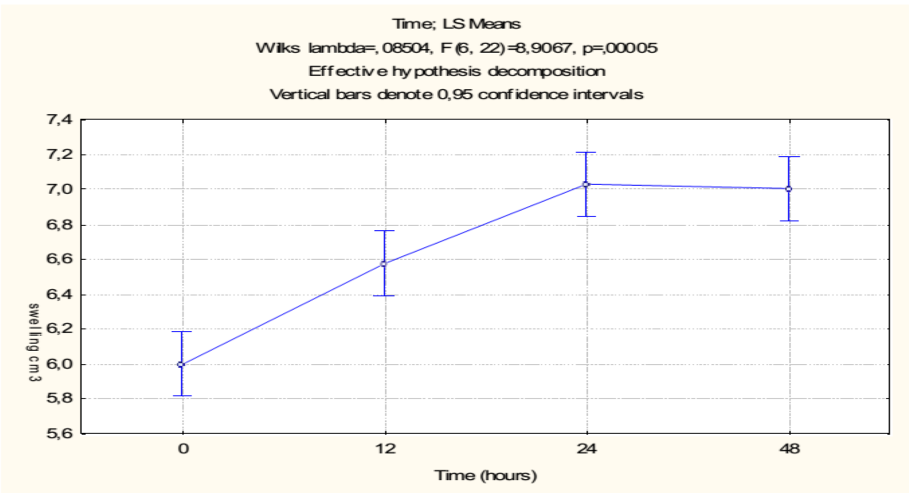

Figure 10. Effect of duration of stay in manure mass on claw horn swelling in the region of hoof walls.

Şekil 10. Gübre içerisinde kalma süresinin tırnak duvarındaki şişmeye olan etkisi.

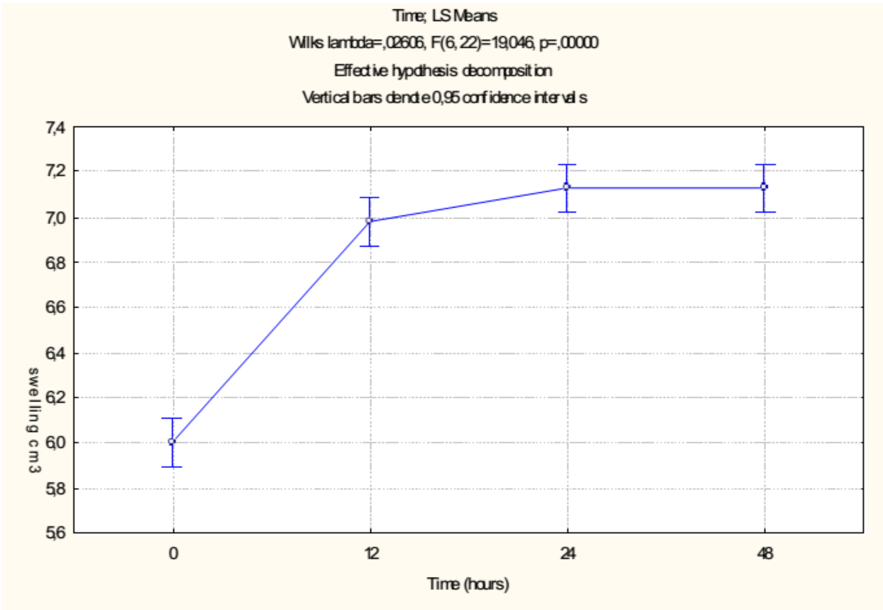

Figure 11. Effect of duration of stay in water on claw horn swelling in the sole region.

Şekil 11. Su içerisinde kalma süresinin taban bölgesindeki şişmeye olan etkisi.

Samples obtained from the sole region also increased their volume when placed in water and manure mass. In water, the swelling continued up to hour 24 and attained 7.1 cm^3 (Figure 11), with substantial change in volume until the 12^{th} hour ($P<0.05$). In manure mass, sole horn swelled significantly until the 12^{th} hour ($P<0.05$) (Figure 12). This difference came in support of the opinion of Gregory (2004) and Gregory et al. (2006), that manure was a powerful factor facilitating water penetration and claw horn swelling.

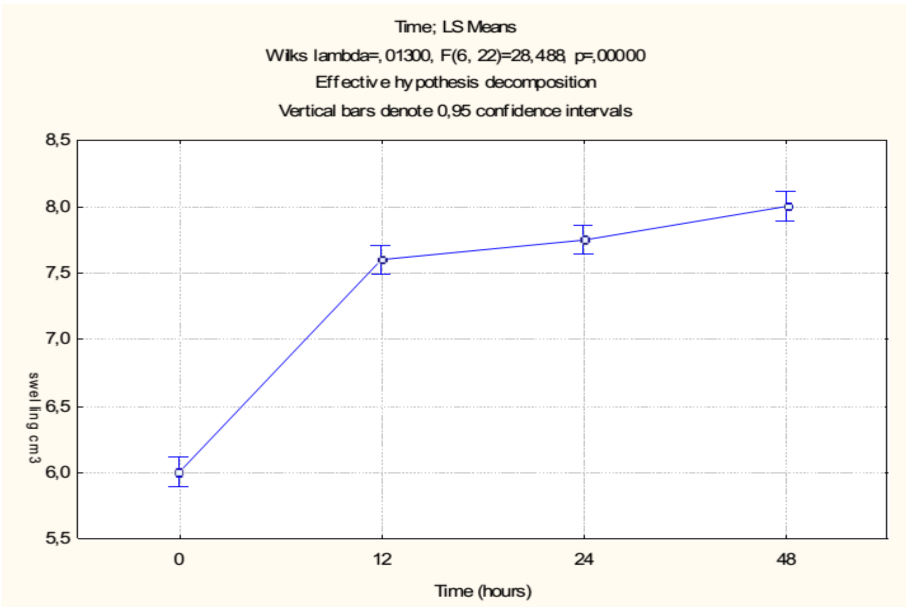

Figure 12. Effect of duration of stay in manure mass on claw horn swelling in the sole region.

Şekil 12. Gübre içerisinde kalma süresinin taban bölgesindeki şişmeye olan etkisi.

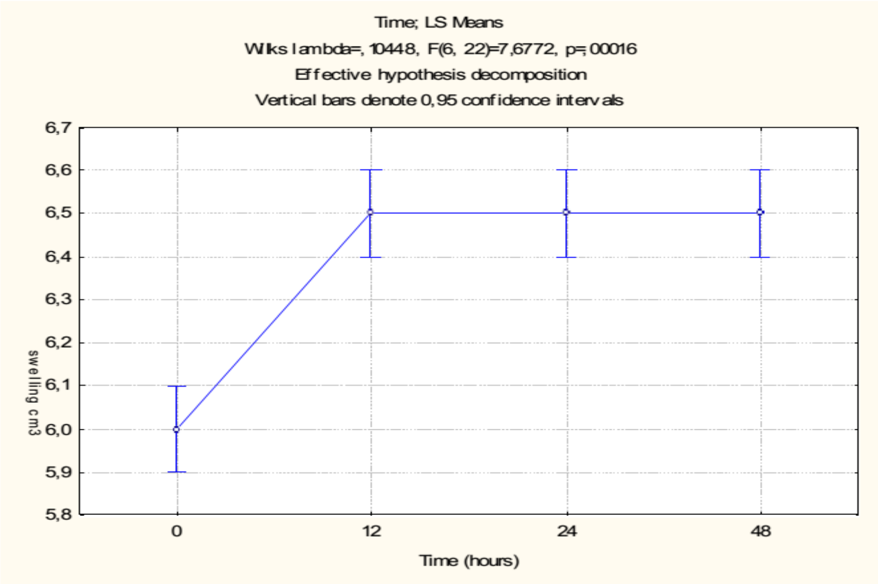

Figure 13. Effect of duration of stay in water on claw horn swelling in the heel region

Şekil 13. Su içerisinde kalma süresinin ökçe bölgesindeki şişmeye olan etkisi

The stay of heels in distilled water resulted in significant swelling until the 12th hour ($P<0.05$) (Figure 13). The samples placed in manure mass swelled up to hour 24, with statistically significant difference until the 12th experimental hour ($P<0.05$) (Figure 14). The analysis of claw horn from this area showed that manure mass had stronger effect compared to water, provoking a stronger and continuous swelling of heel horn.

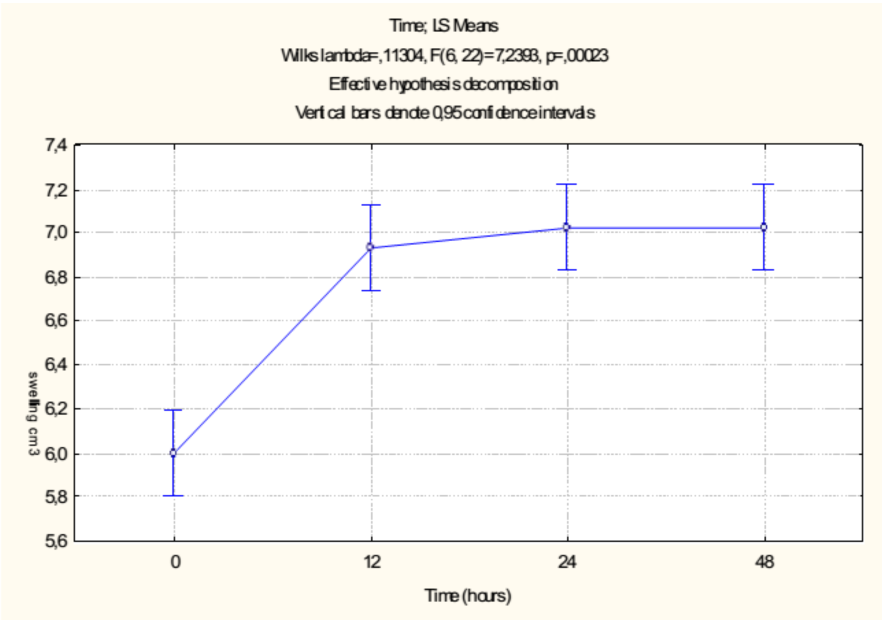

Figure 14. Effect of duration of stay in manure mass on claw horn swelling in the heel region.

Şekil 14. Gübre içerisinde kalma süresinin ökçe bölgesindeki şişmeye olan etkisi.

Under the influence of manure mass, cows' hooves (walls, soles and heels) increased their volume (swelled) at a higher extent as under the effect of distilled water. Manure mass degrades the hydrogen and disulfide bonds of keratin and thus, provokes molecular unfolding swelling and fluid absorption (Gregory et al., 2006). When keratin is swollen and in a hydroxylic state, it is predisposed to wearing and thinning and hence, makes hooves prone to damage (Bonser et al., 2003).

The present experiment detected the strongest swelling in sole claw horn samples, placed in manure mass. This claw horn area has the highest crude fat content compared to both walls and heels. This allowed us concluding that when fat is extracted from the horn, the hydrogen and disulfide bonds of keratin molecule are damaged, resulting in penetration of fluid and swelling of claw horn.

Conclusion

The claw horn fats were extracted as a result of the alkaline pH of manure mass. The latter predisposed the claw horn to water absorption, swelling and lack of mechanical resistance (softening) as evidenced from the studies on the hardness of the three studied claw horn zones (walls, soles and heels). It could be therefore concluded that manure in dairy cattle farms with intensive production systems was an essential technological and hygiene factor for the appearance of lameness. The occurring lameness should be interpreted not only as a clinical sign of hoof and foot disease, but also as a sign of physicochemical changes in claw horn which requires actions for improvement of claw horn strength and resistance.

REFERENCES

Baggott, D.G., Bunch, K.J., Gill, K.R., 1988. Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. The British Veterinary Journal 144, 534-542.

Bonser, R.H.C., Farrent, J.W., Taylor, A.M., 2003. Assessing the frictional and abrasion-resisting properties of hooves and claws. Biosystems Engineering 86, 253-256.

Clarkson, M.J., Downham, D.Y., Faull, W.B., Hughes, J.W., Manson, F.J., Merritt, J.B., Murray, R.D., Russell, W.B., Sutherst, J.E., Ward, W.R., 1996. Incidence and prevalence of lameness in dairy cattle. The Veterinary Record 138, 563-567.

Collick, D.W., Ward, W.R., Dobson, H., 1989. Association between types of lameness and fertility. Veterinary Record 125, 103-106.

Coulon, J.B., Landais, E., Garel, J.P., 1989. Pathology and productivity of the lactating cow: interrelations based on the level of lactation. Annales de Recherches Veterinaires 20 (4), 443-459.

Elias, P.M., Meonon, G.K., 1991. Structural and lipid biochemical correlates of the epidermal permeability barrier. Advances in Lipid Research 24, 1-26.

Golden, G.M., Guzec, D.B., Kennedy, A.H., McKie, J.E., Potts, R.O., 1989. Stratum corneum lipid phase transition and water barrier properties. Biochemistry 26, 2382-2388.

Gregory, N., Craggs, L., Hobson, N., Krogh, C., 2006. Softening of cattle hoof soles and swelling of heel horn by environmental agents. Food and Chemical Toxicology 44, 1223-1227.

Gregory, N.G., 2004. Swelling of cattle heel horn by urine. Australian Veterinary Journal 82, 161-163.

Higuchi, H., Kurumado, H., Mori, M., Degawa, A., Fujisawa, H., Kuwano, A., Nagahata, H., 2009. Effects of ammonia and hydrogen sulfide on physical and biochemical properties of the claw horn of Holstein cows. The Canadian Journal of Veterinary Research 73, 15-20.

Higuchi, H., Maeda, T., Kawai, K., Kuwano, A., Kasamatsu, M., Nagahata, H., 2003. Physiological changes in the concentrations of biotin in the serum and milk and in the physical properties of the claw horn in Holstein cows. Veterinary Research Communications 27, 407-413.

Higuchi, H., Maeda, T., Nakamura, M., Kuwano, A., Kawai, K., Kasamatsu, M., Nagahata, H., 2004. Effects of biotin supplementation on serum biotin levels and physical properties of samples of solar horn of Holstein cows The Canadian Journal of Veterinary Research 68, 93-97.

Higuchi, H., Nagahata, H., 2001. Relationship between serum biotin concentration and moisture content of the sole horn in cows with clinical laminitis or sound claws. Veterinary Record 148, 209-210.

Higuchi, H., Nakamura, M., Kuwano, A., Kasamatsu, M., Nagahata, H., 2005. Quantities and types of ceramides and their relationships to physical properties of the horn covering the claws of clinically normal cows and cows with subclinical laminitis. The Canadian Journal of Veterinary Research 69, 155-158.

Mertin, D., Lippold, B.C., 1997. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. Journal of Pharmacy and Pharmacology 49, 30-34.

Offer, J.E., McNulty, D., Logue, D.N., 2000. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. Veterinary Record 147, 105-109.

Vermunt, J.J., Greenough, P.R., 1995. Structural characteristics of the bovine claw: horn growth and wear, horn hardness and claw conformation. British Veterinary Journal 151, 157-180.

Whay, H.R., Waterman, A.E., Webster, A.J.F., 1997. Associations between locomotion, claw lesions and nociceptive threshold in dairy heifers during the peripartum period. Veterinary Journal 154, 155-161.