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Scanning Electron Microscopic Investigation of Rumen Papillae in Sheep that Died from Acidosis

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ABSTRACT
Objective: In this reseach aimed to observe changes in the rumen papillae using SEM in cases of rumen acidosis, which is prevalent among sheeps. Rumen acidosis is a common issue in sheeps, resulting from uncontrolled excessive intake of easily fermentable carbohydrates. In severe patient, it can lead to passsing away within a day. **Materials and Methods:** For this work, rumens were collected from sheep diagnosed with acidosis and slaughtered Samples were coated with gold and examined using a Jeol JVM 5000 microscope at 5-15 kV. **Results:** SEM findings revealed balloon-like cells on the surface of the rumen papillae in animals with acidosis. It was noted that there were more dead keratinized cells compared to healthy animals. No deep cellular damage or parakeratosis was observed in the papillae. Although no serious deterioration in the ruminal epithelium was found, cells exhibited alterations from their normal form. The study observed cellular damage and anatomical changes at the cellular level due to rumen acidosis, and the findings were presented. **Conclusion:** Overall, this study highlights the significant impact of rumen acidosis on the morphology of sheep papillae. Monitoring these changes through SEM can provide valuable insights into the pathophysiology of rumen acidosis and help in developing more effective management strategies for this common disease in sheep farming.
Keywords: Acidos, Rumen, Scanning Electron Microscopy, Sheep.

Asidozdan Ölen Koyunlarda Rumen Papillalarının Taramalı Elektron Mikroskobik İncelenmesi

ÖZ
Amaç: Bu çalışmada koyunlarda yaygın olarak görülen rumen asidozu olgularında SEM ile rumen papillalarındaki değişimlerin gözlenmesi amaçlanmıştır. Rumen asidozu koyunlarda yaygın görülen, kolay fermente olabilen karbonhidratların kontrolsüz aşırı alımı sonucu oluşan bir sorundur. Şiddetli hastalarda bir gün içinde ölüme yol açabilir. **Gereç ve Yöntem:** Bu çalışma için asidoz tanısı konulan ve kesilen koyunlardan rumen örnekleri toplandı. Örnekler altınla kaplandı ve 5-15 kV'da Jeol JVM 5000 mikroskobu kullanılarak incelendi. **Bulgular:** SEM bulguları asidozlu rumen bulgularında papillalarının yüzeyinde balon benzeri hücreler olduğunu gösterdi. Sağlıklı hayvanlarla karşılaştırıldığında daha fazla ölü keratinize hücre olduğu kaydedildi. Papillalarda derin hücresel hasar veya parakeratoz gözlenmedi. Ruminal epitelde ciddi bir bozulma bulunmamasına rağmen hücreler normal formlarından değişiklikler gösterdi. Çalışmada rumen asidozu nedeniyle hücresel düzeyde hücresel hasar ve anatomik değişiklikler gözlemlendi ve bulgular sunuldu. **Sonuç:** Genel olarak, bu çalışma rumen asidozunun koyun rumen papillalarının morfolojisi üzerindeki önemli etkisini vurgulamaktadır. Bu değişikliklerin SEM aracılığıyla izlenmesi, rumen asidozunun patofizyolojisi hakkında değerli bilgiler sağlayabilir ve koyun yetiştiriciliğinde bu yaygın hastalık için daha etkili yönetim stratejilerinin geliştirilmesine yardımcı olabilir.
Anahtar Kelimeler: Asidoz, Koyun, Rumen, Taramalı Elektron Mikroskobu (SEM).

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INTRODUCTION

The rumen epithelial is a important part of the immune arrangement in sheeps (Penner et al., 2011). Uncontrolled concentrated feeding, which forms the basis of acidosis, origines harm of the rumen mucosa in animals. (Liu et al., 2013). Rapidly fermentable carbohydrates accelerate the fermentation process of acid production. Acid accumulation in the rumen increases the likelihood of impaired rumen epithelial barrier function and thus exposure to toxins (Beauchemin et al., 2008). Damage in this area can be visualized histologically and by SEM (Steele et al., 2009). In Türkiye, sheep are mostly raised in non-agricultural areas, pastures and meadows, and contribute to the economy by converting natural vegetation into meat, milk and wool.

Sheep, which are ruminants, digest hay-like feeds in their forestomachs through microbial fermentation. This is enhanced by different mucosal structures that vary greatly in shape according to their feeding habits. Many studies have revealed the anatomical and histological structure of the forestomach mucosa in ruminants (Loe et al., 1959; Yamamoto et al., 1998). Such animals have been classified into three categories according to their feeding preferences: pasture-feeders, concentrate-feeders and inter mediate-type feeders (Hofmann, 1982). Domesticated ruminants are either grazers or inter mediate-type eaters. Pasture-feeders generally digest low-quality roughage, while inter mediate-type feeders select leaves and young plants with high energy content if available (Hofmann, 1982). High-energy feeding causes a deteriorated epithelial erosion in the rumen. Multiple systemic symptoms such as rumenitis and liver abscess are observed in high-energy diets (Liu et al., 2013). It has been defined that papillae are under the impact of dietary replace and feeding period. It has been noticed that the number of papillae increases significantly in groups fed with concentrate compared to those fed with dry feed (Gäbel, 1987). It has also been observed that the shape of the papillae changes basing on the food. While small, finger-shaped papillae were noticed in the group fed with hay, large, leaf-shaped and tongue-shaped papillae with a wide surface were observed in animals fed with concentrate (Ahmed et al., 2013). In general, it has been reported that the development and growth of rumen papillae is highly related to diet. Physically and chemical stimuli, short-chain fatty acids, the animal's age, and the timing of weaning have been identified as factors directly influencing the size and shape of papillae. (Ahmed et al., 2013; Swan & Groenewald, 2000; Zitnan et al., 1999). It has been presented that papillae are largest and densest in the ventral part of the rumen, in the parts most exposed to feed. It has been observed that the total of papillae per cm^2 mucosa grows importantly in concentrated feeding compared to straw feeding. As the duration of concentrated feeding increases, the papillae ruminis take the form of leaves and elongate (Ahmed et al, 2013; Gäbel et al., 1987). It has also been noticed that papilla growth depends on short-chain fatty acids originating from feed ingredients (Brownlee, 1956). It is recognized that ruminal surface experience proliferative or reductive actions basing on the type of feed. Ruminal mucosa gradually decreases in the number and volume of ruminous papillae when fed energy-poor diets, but increases intensively with high-energy diets (Dirksen, 1985).

When the average length and width of ruminous papillae were compared between the concentrate and straw-fed groups, it was reported that the papillae were strikingly higher in the concentrate-fed group (Ahmed et al., 2013). SEM findings indicated that in the views of rumen papillae, recess and dead keratinized cells along the surface were more pronounced in the concentrate-fed animals than in the straw-fed animals. Deep cellular harm were noticed in the surface cells of rumen papillae in goats fed concentrate. During the straw diet, rumen papillae sections, nuclei, mitochondria and intercellular connections were normal. Cellular necrosis and cellular corrosion were observed in all cell layers in goats fed with concentrate feed. It has been reported that high energy diet causes serious deterioration in ruminal epithelium, especially in subacute ruminal acidosis during feeding, and significant epithelial cell damage and cell erosion (parakeratosis) have been detected (Liu et al., 2013; Steele et al., 2009). In addition, in such cases where the rumen epithelium is disrupted, this layer becomes permeable to endotoxin and can cause poisoning (Liu et al., 2013). It has been reported that morphologically rich deep ridges and grooves are observed in SEM images of rumen papillae taken from all cattle. Heterogeneous microflora including abundant bacteria and protozoa has been reported to be detected in deep ridges and grooves, especially in the roughage-based diet. In additional magnification, keratinized squamous cells of the stratum corneum layer were found to be prominent in the high energy diet. While the microbial flora decreases, peeling of dead keratinized cells was reported to be prominent (Steele et al., 2009). It has been reported that sheep fed a high energy density diet have darker brown ruminus papillae in the ventral part of the rumen compared to sheep fed a low energy density diet (Joshua et al., 2018).

In this regard, this study aimed to reveal the morphological status of the mucosal morphology structures of the rumen from the forestomachs of sheep that died of acidosis.

MATERIALS AND METHODS

Four female and adult Akkaraman sheep, a breed native to Turkiye that died of acidosis, were used in the study. Sections taken from the ventral part of the rumen of animals that died of acidosis were fixed in

the laboratory for SEM examinations and morphological findings were obtained. For SEM specimens were fixed in 5% glutaraldehyde in phosphate buffer (pH 7.3), followed by fixation in 1% osmium tetroxide (OsO4) at 40 °C for 2 hours. Material specimens were processed through alcohol series and critical point dried. Samples were coated with gold and examined using a Jeol JVM 5000 microscope at 5-15 kV (JSM 6390LV, JEOL, Germany).

Ethical considerations

Since the study was conducted on dead material, ethics committee approval is not required.

RESULTS

Finger-shaped structures called papillae ruminis and areas called pila ruminis were observed macroscopically on the rumen surface of our materials. Ruminal papillae (Figure 1) were seen all over the ruminal mucosa except for the rumen columns called pila ruminis. The ruminal columns called pila ruminis and their vicinities were reduced in both number and length. It was observed that the papillae were densely distributed throughout the ruminal surface outside of this. Their color was determined as dark brown. Ruminal papillae were plump. They varied greatly in shape from short tongue-shaped to long and wide leaf-like shapes, depending on their location. Short tongue-shaped papillae ruminis were mostly noticed on the dorsal and lateral walls of the rumen, while long and wide leaf-like papillae were found in the ventral part. No grooves called primary or secondary were observed on the papilla rumenis.

Cells on the rumen surface were observed at different magnifications (Figure 2). In scanning electron microscopic magnification (x34), a granular shape was seen on the surface of the papilla ruminis (Figure 1). Polygonal cells were noticed on the entire inner mucosa of the rumen at 10 kV and 130 magnification. The cells were mostly determined to be quadrangular, pentagonal and hexagonal (Figure 2). It was determined that each side of the cells had different lengths. It was determined that these lengths varied between 8.5 µm and 11.4 µm. In the 130 magnifications in the SEM images, swollen intermediate balloon-type cells were seen on the rumen surface. In the spacimen taken from the rumen ventral wall, cell harm was detected in the root parts of the papilla at 34 magnification. It was determined that these erosions were observed in each papilla ruminis and occupied approximately one-third of the area. This was determined to cover a considerable area in terms of volume (Figure 1).

DISCUSSION

In the study, the color of the rumen papillae was determined to be brown. Studies have shown that changes in papilla color are dependent on diet (Ahmed et al., 2013; Atalgin et al., 2023; Dyce et al., 2010). Ahmet et al. (2013) reported that papillae obtained from sheep fed with straw were light brown. However, in the 6 and 12 weeks of concentrated feed group, dark brown colored papillae were observed

It was shown that the forestomach of sheep dying of acidosis had papillae of various shapes and sizes. In the imaging performed with SEM, many papillae were detected, topographically different in number and shape (Anderson et al., 1987; Tamate et al., 1979).It was shown that these differences in papilla development were partially related to feeding habits, as in our results. These studies noticed in animals with known nutritional position revealed wide differences, especially in the rumen, according to the written Works (Ahmed et al., 2013; Atalgin et al., 2023; Dyce et al., 2010; Loe et al., 1959; Sander et al., 1959; Weyrauch & Schnorr, 1979).

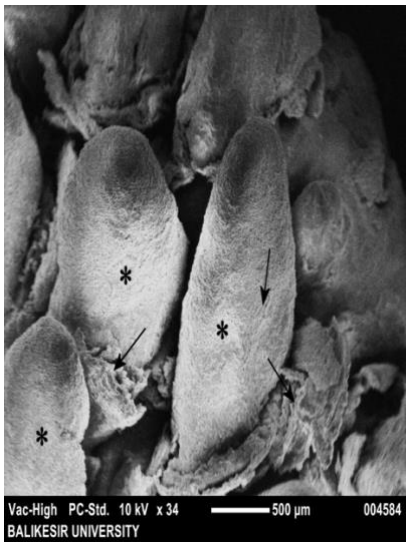

Figure 1. Ruminal papillae (asterisks), cell debris (arrows).

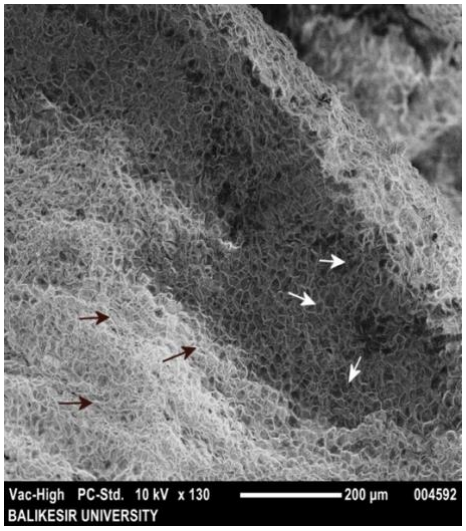

Figure 2. Balloon type cells (arrows).

(Ahmed et al., 2013; Dyce et al., 2010). These evidences are generally consistent with the ends of our work.

A study reported that the entire rumen surface was covered with polygonal cells (Scott & Gardner, 1973). This finding is consistent with our findings. Scott and Gardner (1973) reported that cell boundaries were clearly seen. The existing boundaries were also observed in our study. Angela García (2012) reported in her study that the ruminal wall was seen as a smooth surface and there were no signs of keratinization or desquamation, this view is not fully consistent with our study. Necrotic areas were observed in our study and cell debris was detected.

Along with our results, most researchers (Ahmed et al., 2013; Penner et al., 2011) reported that there was cellular necrosis, parakeratosis and cell debris in the papillae ruminis of animals fed with concentrate feed. However, in one study (Scott & Gardner, 1973; Penner et al., 2011; Ahmed et al., 2013), it was stated that this shedding is a feature of the rumen epithelium and that this shedding is normal. In our study, necrotic cell residues were observed in the epithelial cells of the ruminus papilla in acidotic animals. These results contradict the results of this literature, except for normal cell death. Tamata et al. (1979) defined primary and secondary grooves in the ruminal papilla in sheep, resulting from the longer stay of concentrated feeds in this area. Yamamoto et al. (1994) and Scott and Gardner (1973) also mentioned these structures. Atalgın et al., (2023) stated in his study that the ruminus papilla contains two different types of grooves in terms of length and size, and that these grooves are shaped by the longer stay of concentrated feeds in this area. Although it was stated that the primary groove is located in the middle along the length of the papilla and the small secondary ones are shallow shaped, and the secondary grooves cut the primary groove, no groove was observed in our study. Mahes et al. (2014) detected smaller pointed and blunt protrusions such as secondary papillae between the large and small dense papillae on the ventral wall of the rumen in their study. However, such secondary papillae were not observed in our study.

Cytologically, Schnorr and Vollmerhaus (19682), Hofmann and Schnorr (1982) defined ceratinized cells as flat, balloon and intermediate type cells. It was determined that the cells seen in our study mostly resembled balloon type cells.

Steele et al. (2011) stated that heterogeneous microflora such as bacteria and protozoa were observed in SEM images, especially in hay-based diets. However, such microflora was not observed in our study, but the presence of this flora is thought to be directly related to the sample preparation technique. In the study of Atalgin (2023), it was observed that the ruminus papillae were not full. Atalgin (2023) reported that the ruminus papillae in his study were relatively weaker than our study and had grooves on them, which is consistent with this data, whereas in our study, the ruminus papillae were observed to be quite full.

CONCLUSION

As a result, in this article, the rumen of sheep with acidosis was investigated electron microscopically using SEM. Rumen papillae and cells were visualized. It was observed that the papillae were dark brown. According to the literature, it was determined that the papillae were more swollen and full in shape. It was observed that the cells in the rumen mucosa were mostly balloon-type cells. Epithelial debris and cell necrosis were detected. It was determined that these erosions were observed in the ventral part of each ruminus papillae and were approximately one-third of the area. Although it was reported theoretically (Ahmed et al., 2013; Penner et al., 2011), this finding was shown by scanning electron microscopy. It is thought that an acidotic animal will be exposed to necrotic areas and a deteriorated epithelial erosion, depending on the degree of the disease. As a result, the presence of cellular erosion formed by necrotic cells in the ruminous papilla of acidotic animals has been demonstrated.

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: ŞHA; **Material, methods and data collection:** ŞHA; **Data analysis and comments:** ŞHA; **Writing and corrections:** ŞHA.

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Ethical Approval

Since the study was conducted on dead material, ethics committee approval is not required.

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