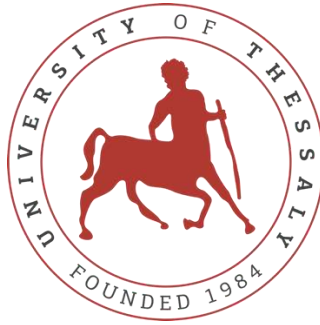


UNIVERSITY OF THESSALY



SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY MEDICINE

CLINICAL VETERINARY MEDICINE DEPARTMENT

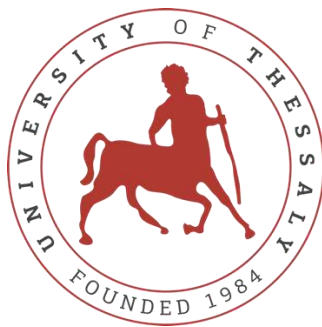
ADVANCING THE UNDERSTANDING OF RUMINAL ACIDOSIS

A thesis submitted for the degree of Doctor of Philosophy by

Nikolaos M. Voulgarakis, DVM

Supervisor: Georgios Christodoulopoulos, Professor

Karditsa, 2024



UNIVERSITY OF THESSALY

SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY MEDICINE

CLINICAL VETERINARY MEDICINE DEPARTMENT

ADVANCING THE UNDERSTANDING OF RUMINAL ACIDOSIS.

Postgraduate student:

Nikolaos M. Voulgarakis, DVM

Supervisor:

Georgios Christodouloupoulos, Professor, Department of Animal Science, Agricultural University of Athens, Greece

Advisory Committee:

Vasileios G. Papatsiros, Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

Dimitra Psalla, Professor, Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece

Examination Committee:

Georgios Christodouloupoulos, Professor, Department of Animal Science, Agricultural University of Athens, Greece

Vasileios G. Papatsiros, Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

Dimitra Psalla, Professor, Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece

Georgios C. Fthenakis, Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

Labrini V. Athanasiou, Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

Konstantinos Katsoulis, Assistant Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

Dimitrios A. Gougoulis, Assistant Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

The research described in this PhD thesis was conducted at the Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece and it was financially supported by the financial account of the Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly (Karditsa, Greece) through the Research Committee of the University of Thessaly (code: 2557, Scientific Responsible: Manolis Saridomichelakis).

The histological examinations were performed by the **Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece**, Stavrou Voutyra str, Thessaloniki, GR 54627, Greece. Contact person: Assoc. Professor Dim. Psalla, Tel:+302310999931, Email: dpsalla@vet.auth.gr

ACKNOWLEDGEMENTS

The present experimental study investigates the effects of diet on the feedlot cattle and dairy sheep industries in Greece. It aims to explore the frequency of Subacute Ruminant Acidosis (SARA) and its impacts on milk production, with a special focus on diagnosing SARA using colorimetry facilitated through a mobile application for rapid and reliable diagnosis in routine practice.

This doctoral thesis was conducted at the Clinic Medicine of the Department of Veterinary Medicine at the School of Health Sciences, University of Thessaly. The experimental research was approved by the Ethical Committee for the Use of Animals (ECUA) under protocol numbers 151 and 152 (dated 16-05-2021 for feedlot cattle and dairy sheep, respectively). During the experimentation, the housing and handling of the animals adhered to the "Good Farm Practice Guidelines-GFP," and no changes were made to their diet and treatment.

The completion of this thesis would not have been possible without the support and contributions of many individuals, to whom I owe my deepest gratitude.

First and foremost, I would like to express my heartfelt thanks to Professor Georgios Christodouloupoulos for his unwavering support and confidence in me throughout this journey. His tireless enthusiasm, assistance, experience, and invaluable advice were crucial in the completion of this doctoral thesis.

I extend my gratitude to Professor Vasileios Papatsiros, a member of my advisory committee, for his valuable guidance and co-supervision. His advice and suggestions significantly contributed to my education in Clinic Medicine, particularly in Farm Animal Medicine.

I am deeply grateful to Professor Dimitra Psalla, another member of my advisory committee, for both her co-supervision and the opportunity to collaborate with the Laboratory of Pathology at Aristotle University of Thessaloniki. Her guidance was instrumental in the experimental aspects of this thesis.

Special thanks to Professor Manolis Saridomichelakis, the current director of the Clinic of Animal Medicine at the Veterinary School of the University of Karditsa. His role in providing essential consumables through the clinic's service program was pivotal for my research.

I also wish to thank Associate Professor Dimitrios Gougoulis for his unwavering support, invaluable contribution, and guidance in both my research and the writing of this thesis. His assistance in the statistical analysis and interpretation of results was greatly appreciated.

To Dr. Georgios Papakonstantinou, my colleague and now Doctor of Clinic Medicine, thank you for your unwavering support both academically and personally.

I am grateful to all members of the Clinic Medicine of the Department of Veterinary Medicine at the University of Thessaly, both scientific and technical staff, for their support and help.

To my siblings, nieces, nephews, friends, and all my loved ones who supported me from the very beginning of this journey, thank you for always being by my side.

A special thanks to the individual who accompanied me at the inception of my doctoral research and remained a steadfast companion, supporting me throughout the development of this dissertation.

Lastly, I want to express my profound gratitude to my mother, Anastasia, for her constant support in my studies and life. And to my father, Moschos, who, though not physically

present, instilled in me the values, knowledge, and experiences that have been fundamental to my success. I owe them immeasurable gratitude for their love and education.

Nikolaos M. Voulgarakis

University of Thessaly

June 2024

TABLE OF CONTENTS

ACKNOWLEDGEMENTS5

INTRODUCTION.....9

REFERENCES.....15

CHAPTER I.....23

REVIEW OF LITERATURE.....23

1. RUMINAL ACIDOSIS.....24

2. ACUTE RUMINAL ACIDOSIS (ARA) 25

3. SUBACUTE RUMINAL ACIDOSIS (SARA).....30

4. DIAGNOSTIC APPROACH OF RUMINAL ACIDOSIS.....37

5. TREATMENT OF RUMINAL ACIDOSIS.....41

6. PREVENTION OF RUMINAL ACIDOSIS.....42

7. CONCLUSION.....47

REFERENCES.....49

CHAPTER II.....66

CAN COMPUTERIZED RUMEN MUCOSAL COLORIMETRY SERVE AS AN EFFECTIVE FIELD TEST FOR MANAGING SUBACUTE RUMINAL ACIDOSIS IN FEEDLOT CATTLE? 66

1. INTRODUCTION.....67

2. MATERIALS AND METHODS.....68

3. RESULTS.....76

4. DISCUSSION.....79

5. CONCLUSIONS.....80

REFERENCES.....82

CHAPTER III.....86

SUBACUTE RUMEN ACIDOSIS IN GREEK DAIRY SHEEP: PREVALENCE, IMPACT, AND COLORIMETRY MANAGEMENT.....86

1. INTRODUCTION.....87

2. MATERIALS AND METHODS.....89

3. RESULTS.....94

4. DISCUSSION.....98

5. CONCLUSIONS.....101

REFERENCES.....103

GENERAL CONCLUSIONS.....110

SUMMARY.....113

ΠΕΡΙΛΗΨΗ.....116

ANNEX I.....119

NIKOLAOS VOULGARAKIS' CV.....119

ANNEX II.....126

PUBLICATIONS FROM PHD DISSERTATION.....126

INTRODUCTION

Ruminal acidosis, introduced in 1959 by Krotkova, is a ruminant nutritional disorder. It's known by various names (Dehkordi and Dehkordi, 2011). This disorder emerges after consuming feed high in carbohydrates and low in digestive fiber (Mahmood et al., 2013; Snyder and Credille, 2017). It's defined by a rapid reduction in ruminal pH due to extensive accumulation of small-chain fatty acids (SCFA) (Kawas et al., 2007; Bramley et al., 2008). Rumen acidosis has two types: acute (ARA) and subacute (SARA), classified by pH reduction and clinical signs (Aschenbach et al., 2011; Reis et al., 2014). ARA occurs commonly in feedlots, while SARA is observed in dairy farms (Kleen et al., 2003; Krause and Oetzel, 2006).

In cattle, a rumen pH of 5.0-5.6 indicates SARA, while a pH below 5.0, nearing 4.5, signifies ARA (Owens et al., 1998; Krause and Oetzel, 2006). Small ruminants have pH levels below 5.2 in ARA and between 5.2 and 5.8 in SARA (Church, 1991; Commun et al., 2009). In bovine medicine, various pH thresholds have been proposed to define SARA. Several authors agree on values below 5.6 for at least 3 hours a day (Petzold et al., 2014; Bilal et al., 2016). Likewise, in small ruminants, SARA manifests with a pH range between 5.0 and 5.6 or between 5.5 and 6.0 for at least 3 hours (Jiang et al., 2014; Shen et al., 2019a; Taghipoor et al., 2020).

Consuming large amounts of rapidly fermentable carbohydrates with insufficient fiber leads to SARA. It's characterized by low ruminal pH (5.0-5.5), increased VFA (150-225 mM), and varying lactic acid (10-40 mM) (Calsamiglia et al., 2012; Li et al., 2014). In subacute acidosis, clinical signs are subtle, primarily low feed intake, resulting in a low mortality rate. The incidence of SARA causes microbial changes, including higher levels of *Lactobacillus* spp., lactic acid-producing and utilizing bacteria, and reduced or absent ciliated protozoa (Nagaraja and Titgemeyer, 2007).

SARA is common in intensive livestock systems (Lopez-Campos et al., 2010; Gao and Oba, 2014). In dairy cattle, it's estimated at 11-26%, with 11-20% in early lactation and 18-26% in mid-lactation (Kitkas et al., 2013; Atkinson, 2014; Stefanska et al., 2016). On farms with high SARA history, rates can reach 48.2%-65.3% at different milk production stages (Kitkas et al., 2019). In beef feedlot cattle, can report SARA anytime, with pH <5.5 and prevalence from 14% to 42% (Nagaraja and Lechtenberg, 2007). Primiparous cows have higher SARA rates (29% vs. 19% in multiparous cows) (Kitkas et al., 2013; Oetzel, 2017). For detailed SARA epidemiology, see our review (Voulgarakis et al., 2023).

Lira et al. (2013) conducted an extensive survey in northeastern Brazil within the realm of sheep farming, revealing that ruminal acidosis occurred at a rate of 13.9% in goats and 12.4% in sheep. Similarly, a cross-sectional study conducted on 19 dairy sheep farms in Argentina by Suarez and Buseti (2009) reported that 42.1% of the surveyed flocks encountered ruminal acidosis.

The clinical signs of SARA emerge after a delay of onset time or disguise by other common diseases, such as lameness and considerable deterioration of body condition (Colman et al., 2015). Several clinical signs have been presented in the affected by SARA ruminants which are presented extensively in our published comprehensive review (Voulgarakis et al., 2023).

In SARA-affected ruminants, the rumen mucosa is reported grey to dark brown (Ploger et al., 2012). SARA's major impacts include parakeratosis, epithelial thickening, and detachment (Steele et al., 2009). Parakeratosis persists, and the epithelium, stratum corneum, stratum granulosum, and stratum spinosum may weaken in SARA cases (Steele et al., 2011; Zhang et al., 2020). Necropsies may reveal liver and subcutaneous abscesses, pulmonary bacterial emboli, peritonitis, and inflammation in organs like kidneys, lungs, heart valves, and joints (Kleen et al., 2003; Abdela, 2016).

Feeding a High Concentrate (HC) diet with 60% concentrate can boost milk quality and yield in the short term. However, it also raises the risk of SARA and lowers rumen pH (Abaker et al., 2017; Shen et al., 2019b). SARA decreases milk production and milk fat concentration but doesn't impact protein and energy levels (Chang et al., 2015; Xu et al., 2015). According to the literature, is estimated an approximate decrease of 2.7-3 kg per cow per day in milk production, a decrease from 37 to 34 g/kg (a 0.3% decrease) in milk fat, and a decrease from 29 to 28 g/kg (a 0.12% decrease) in true protein (Xu et al., 2016).

A milk fat to milk protein ratio below 1 in animals fed an HC diet can serve as a valuable diagnostic indicator of SARA in ruminants (Enemark et al., 2002; Giger-Reverdin et al., 2014). In bovine medicine, Cook et al. (2005) suggest that the suspicion of SARA should arise when 10% of cows exhibit individual milk fat percentages of 2.5 or less.

In the USA dairy industry, SARA's cost estimate, which considers milk production losses, reduced production efficiency, and high culling rates, can reach as high as 1.12 USD per cow per day. The economic losses attributed to SARA are substantial, ranging from 500 million to 1 billion dollars per year across the entire USA dairy industry (Enemark, 2008; McCann et al., 2016). Furthermore, the financial impact of decreased milk fat and milk protein, excluding early culling and veterinary expenses, is estimated to be between 400-475 dollars per cow (Abdela, 2016).

In USA beef industry, Stock (2000) notes a financial loss of 9.40 dollars per feedlot steer, primarily because of acidosis impacting the reduction in final cattle weight. Additionally, SARA-related reduced dry matter intake (DMI) results in decreased growth of beef calves, leading to losses of 10-13 dollars per animal (Stock and Britton, 1996).

Various diagnostic tests are conducted for both the evaluation and prevention of ruminal acidosis. These tests include rumination activity, biochemical profiles, blood analysis, milk analysis, feces, and urinalysis, all of which are essential for managing SARA (Oetzel, 2017; 11

Snyder and Credille, 2017). However, the most common diagnostic method for SARA remains the measurement of rumen pH using different methods of rumen fluid extraction (Kleen et al., 2003; Oetzel, 2017; Voulgarakis et al., 2024a).

According to the scientific literature, several accepted protocols have been recommended for the diagnosis of SARA. However, the main standard protocol for evaluating SARA involves sampling rumen pH from twelve cattle via rumenocentesis. These samples should be collected following specific guidelines, rules, timelines, and conditions, which are extensively described in our comprehensive review (Voulgarakis et al., 2014a). The scientific community has proposed two cut-off points for SARA diagnosis: pH ≤ 5.5 (indicative of SARA) and pH > 5.8 (indicative of non-SARA). A herd is considered SARA-positive if at least 3 out of 12 samples have ruminal pH ≤ 5.5 (Kleen et al., 2003; Oetzel, 2017). In small ruminants, a rumen pH between 5.2 and 5.8 for a duration of 3 hours per day is proposed for SARA diagnosis (Ma et al., 2018; Wang et al., 2020).

According to the literature, several diagnostic methods have been used for rumen fluid collection, including rumenocentesis, oral stomach tube, rumen cannula, and modern telemetric systems, which are electronic devices known as 'smart pills' or 'smart boluses.' However, each diagnostic method has its advantages and disadvantages for rumen fluid collection (Voulgarakis et al., 2024a). Several authors have proposed rumenocentesis and the measurement of pH with a portable pH meter as the gold standard diagnostic method for rumen fluid collection (Morgante et al., 2009; Chaudhry et al., 2018).

Besides measuring rumen pH, several tests have been suggested for SARA diagnosis, with varying levels of practical application. Automated sensors like microphones, accelerometers, and laser interferometry can detect rumination activity, potentially indicating SARA in cattle (Oetzel, 2017; Hamilton et al., 2019). Rumen temperature ($> 39.2^{\circ}\text{C}$ to 41°C) can rise in an acidotic rumen (pH 5.6 to 5.0), serving as a diagnostic tool for ruminal acidosis (AlZahal et al., 2012).

al., 2008; Reis et al., 2018). Additionally, low blood pH (7.1 - 7.44), low urine pH (7.8 - 8.3), and low stool pH (<6.0) can use as potential indexes for diagnosing subacute rumen acidosis (Li et al., 2012; Danscher et al., 2015; Chaudhry et al., 2018). Various milk fat percentage protocols are recommended for SARA investigation. However, a low milk fat value can't solely indicate SARA due to various factors affecting milk fat reduction. Milk fat values should be regularly assessed, at least weekly, for a more accurate evaluation of SARA status (Kleen et al., 2003; Lean et al., 2007; Enemark, 2008; Voulgarakis et al., 2024a).

In our studies, both beef cattle and dairy sheep participated in the investigation of subacute ruminal acidosis in an extended area of central Greece, specifically Thessaly. Moreover, the prevalence of SARA is still unknown for these host species in Greece. Additionally, we investigated the usage of new potential diagnostic examination, such as colorimetry on rumen mucosa, for diagnosing SARA. Furthermore, our investigation produced two publications and one preprint (Voulgarakis et al., 2024b; Voulgarakis et al., 2024c; Voulgarakis et al., 2024d) providing answers to research questions such as:

- I. the assessment of rumen mucosa color, especially regarding the presence of darker color in ruminants affected by SARA and its relation with rumen pH (Voulgarakis et al., 2024b, Voulgarakis et al., 2024c, Voulgarakis et al., 2024d),
- II. the effect of rumen pH on the layers of rumen epithelium, specifically reporting the thickness of non-keratinized epithelium in ruminants affected by SARA (Voulgarakis et al., 2024b, Voulgarakis et al., 2024c, Voulgarakis et al., 2024d),
- III. the use of colorimetry on rumen mucosa for diagnosing SARA, particularly Red (RGB color system) and color lightness (L*, CIELAB color system), revealing the most significant relations with rumen pH and the layers of rumen epithelium, mainly with non-keratinized epithelium thickness (Voulgarakis et al., 2024b, Voulgarakis et al., 2024c, Voulgarakis et al., 2024d),

- IV. the impacts of the presence of subacute ruminal acidosis on milk components and the relations among them with rumen pH, color components, and layers of ruminal epithelium (Voulgarakis et al., 2024c, Voulgarakis et al., 2024d),
- V. the prevalence of SARA in the Greek Dairy Sheep industry (Voulgarakis et al., 2024c, Voulgarakis et al., 2024d).

References

- Abaker JA, Xu TL, Jin D, Chang GJ, Zhang K, Shen XZ (2017) Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J Dairy Sci* 100:666–678.
- Abdela N (2016) Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. *Achiev Life Sci* 10:2:187-196.
- AlZahal O, Kebreab E, France J, Froetschel M, McBride BW (2008) Ruminal temperature may aid in the detection of subacute ruminal acidosis. *J Dairy Sci* 91:(1):202–7.
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2011) Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89:1092–1107.
- Atkinson O (2014) Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract* 22:(1):1-9.
- Bilal MS, Abaker JA, Aabdin ZU, Xu T, Dai H, Zhang K, Liu X, Shen X (2016) Lipopolysaccharide derived from the digestive tract triggers an inflammatory response in the uterus of mid-lactating dairy cows during SARA. *BMC Vet Res* 12:(1):284.
- Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND (2008) The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. *J Dairy Sci* 91:308–321.
- Calsamiglia S, Blanch M, Ferret A, Moya D (2012) “Is subacute ruminal acidosis a pH related problem? Causes and tools for its control”. *Anim Feed Sci Technol* 172:(1-2):42–50.
- Chang G, Zhuang S, Seyfert HM, Zhang K, Xu T, Jin D, Guo J, Shen X (2015) Hepatic TLR4 signaling is activated by LPS from digestive tract during SARA, and epigenetic mechanisms contribute to enforced TLR4 expression. *Oncotarget* 6:36.

- Chaudhry AS, Khan MA, Khan MS, Ashraf K, Saleem MH, Khan NU, Usman T, Ahmad I (2018) Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J Anim Plant Sci* 28:(1):80-84:1018-7081.
- Church DC (1991) *Livestock Feeds and Feeding*, third ed. Prentice-Hall Inc., USA, 349 pp.
- Colman E, Waegeman W, De Baets B, Fievez V (2015) Prediction of subacute ruminal acidosis based on milk fatty acids: a comparison of linear discriminant and support vector machine approaches for model development. *Comput Electron Agric* 111:179–85.
- Commun L, Mialon MM, Martin C, Baumont R, Veissier I (2009) Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J Anim Sci* 87:3372–3379.
- Cook NB, Oetzel GR, Nordlund KV (2005) Clinical forum—subacute ruminal acidosis—a problem in UK dairy herds?. *UK Vet* 10:40–46.
- Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC (2015) Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Vet Scand* 57:(1):39.
- Dehkordi AJ, Dehkordi ZK (2011) Occurrence of metabolic alkalosis in rumen lactic acidosis: a review article. *Comp Clin Pathol* 20:(1):1–3.
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet J* 176:1:32-43.
- Enemark JMD, Jørgensen RJ, Enemark P (2002) Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Vet ir Zootech* 20:(42):16-29.

- Gao X, Oba M (2014) Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. *J Dairy Sci* 97(5):3006-16.
- Giger-Reverdin S, Rigalma K, Desnoyers M, Sauvant D, Duvaux-Ponter C (2014) Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *J Dairy Sci* 97:4367–4378.
- Hamilton AW, Davison C, Tachtatzis C, Andonovic I, Michie C, Ferguson HJ, Somerville L, Jonsson NN (2019) Identification of the Rumination in Cattle Using Support Vector Machines with Motion-Sensitive Bolus Sensors. *Sensors* 19(5):1165.
- Jiang XY, Ni YD, Zhang SK, Zhan YS, Shen XZ (2014) Identification of Differentially Expressed Proteins in Liver in Response to Subacute Ruminal Acidosis (SARA) Induced by High-concentrate Diet. *Asian-Australas J Anim Sci* 27:8:1181-1188.
- Kawas JR, Garcia-Castillo R, Fimbres-Durazo H, Garza-Cazares F, Hernandez-Vidal JFG, Olivares-Saenz E, Lu CD (2007) Effects of sodium bicarbonate and yeast on nutrient intake, digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. *Small Rumin Res* 67:149-156.
- Kitkas GC, Valergakis GE, Karatzias H, Panousis N (2013) Subacute ruminal acidosis: prevalence and risk factors in Greek dairy herds. *Iran J. Vet. Res.* 14:183-189.
- Kitkas GC, Valergakis GE, Kritsepi-Konstantinou M, Gelasakis A, Arsenos G, Kalaitzakis E, Panousis (2019) Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation. *J Hell Vet Med* 70:1551-1560.
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): A review. *J Vet Med A Physiol Pathol Clin Med* 50:406–414.

- Krause KM, Oetzel GR (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim Feed Sci Technol* 126:215–236.
- Krotkova AP (1959) Effect of sugar beet on acidosis in the ruminant. *Zhivotnovodstvo* 7:63-64.
- Lean IJ, Annison F, Bramley E, Browning G, Cusack P, Farquharson B, Little S, Nandapi D (2007) Ruminal Acidosis- aetiopathogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR) A V Association ed Blackwell Publishing Asia Pty Ltd Carlton Vic Australia pp. 1-60.
- Li F, Cao Y, Liu N, Yang X, Yao J, Yan D (2014) Subacute ruminal acidosis challenge changed in situ degradability of feedstuffs in dairy goats. *J Dairy Sci* 97:5101–5109.
- Li S, Gozho G, Gakhar N, Khafipour E, Krause DO, Plaizier JC (2012) Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can J Anim Sci* 92:353-364.
- Lira MAA, Simões SVD, Riet-Correa F, Pessoa CMR, Dantas AFM, Miranda Neto EG (2013) Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui Vet Bras* 33:(2):193-198.
- Lopez-Campos O', Bodas R, Prieto N, Giraldez FJ, Perez V, Andres S (2010) Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim* 4:6:958–964.
- Ma N, Abaker JA, Bilal MS, Dai H, Shen X (2018) Sodium butyrate improves antioxidant stability in sub-acute ruminal acidosis in dairy goats. *BMC Vet Res* 14:275.
- Mahmood AK, Khan MS, Khan MA, Kahn MA, Bilal M, Farooq U (2013) Lactic Acidosis in Goats: Prevalence, Intra-ruminal and Haematological investigations. *J Anim Plant Sci* 23:(6):1527-1531.

- McCann JC, Luan S, Cardoso FC, Derakhshani H, Khafipour E, Loor JJ (2016) Induction of Subacute Ruminal Acidosis Affects the Ruminal Microbiome and Epithelium. *Front Microbiol* 7:701.
- Morgante M, Giancesella M, Casella S, Ravarotto L, Stelletta C, Giudice E (2009) Blood gas analyses, ruminal and blood pH, urine and faecal pH in dairy cows during subacute ruminal acidosis. *Comp Clin Path* 18:229–232.
- Nagaraja TG, Lechtenberg KF (2007) Acidosis in feedlot cattle. *Vet Clin Food Anim Pract* 23:333-350.
- Nagaraja TG, Titgemeyer EC (2007) Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci* 90(E. Suppl.):E17-E38.
- Oetzel GR (2017) Diagnosis and Management of Subacute Ruminal Acidosis in Dairy Herds. *Vet Clin North Am Food Anim Pract* 33:3:463-480.
- Owens FN, Secrist DS, Hill WJ, Gill DR (1998) Acidosis in cattle: a review. *J Anim Sci* 76(1):275-86.
- Petzold M, Meyer U, Spilke J, Danicke S (2014) Using rumen probes to examine effects of conjugated linoleic acids and dietary concentrate proportion on rumen pH and rumen temperature of periparturient dairy cows. *J Anim Physiol Anim Nutr* 98:785–796.
- Ploger S, Stumpff F, Penner GB, Schulzke JD, Gabel G, Martens H (2012) Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann N Y Acad Sci* 1258: 52–59.
- Reis LF, Araujo CASC, Sousa RS, Minervino AHH, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto Júnior RA, Mori CS, Ortolani EL (2018) Prevention of acute ruminal lactic acidosis in sheep by probiotic or monensin supplementation: clinical aspects. *Semin Cienc Agrar* 39:4:1575-1584.

- Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Ortolani EL (2014) Comparison of Rumen Fluid pH by Continuous Telemetry System and Bench pH Meter in Sheep with Different Ranges of Ruminal pH. Hindawi Publishing Corporation Scientific World Journal Volume, Article ID 195782, 4 pages.
- Shen Y, Zhao F, Yu L, Yang W, Wang M, Wang H (2019a) Starch sources and concentration in diet of dairy goats affected ruminal pH and fermentation, and inflammatory response. *Anim Prod Sci* 59:9:1640-1647.
- Shen YZ, Ding LY, Chen LM, Xu JH, Zhao R, Yang WZ, Wang HR, Wang MZ (2019b) Feeding corn grain steeped in citric acid modulates rumen fermentation and inflammatory responses in dairy goats. *Anim* 13:2:301–308.
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33:(3):451-461.
- Steele MA, AlZahal O, Hook SE, Croom J, McBride BW (2009) Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet Scand* 51:39.
- Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K, McBride BW (2011) Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am J Physiol Regul Integr Comp Physiol* 300:6:1515-1523.
- Stefańska B, Nowak W, Komisarek J, Taciak M, Barszcz M, Skomial J (2016) Prevalence and consequence of subacute ruminal acidosis in polish dairy herds. *J Anim Physiol Anim Nutr* 101(4):694-702.
- Stock R (2000) Acidosis in cattle: an overview. In: *Proceedings of the 33rd Annual Convention of the American Association of Bovine Practitioners*, Rapid City USA pp. 30-37.

- Stock R, Britton RL (1996) Acidosis. NebGuide, University of Nebraska–Lincoln. October 1995 <[http://www.ianr.unl.edu/pubs/ animaldisease/g1047.htm](http://www.ianr.unl.edu/pubs/animaldisease/g1047.htm)> (assessed 27.11.99).
- Suárez VH, Buseti MR (2009) Health management practices and disease prevalence in dairy sheep systems in Argentina. *Pesqui Vet Bras* 29(11):931-937.
- Taghipoor M, Delattre M, Giger-Reverdin S (2020) A novel modelling approach to quantify the response of dairy goats to a high-concentrate diet. *Sci Rep* 10:20376.
- Voulgarakis N, Athanasiou L, Psalla D, Gougoulis D, Papatsiros V, Christodoulopoulos G. (2024a) Ruminant Acidosis Part II: Diagnosis, Prevention and Treatment. *Journal of the Hellenic Veterinary Medical Society*, 74(4):6329–6336.
- Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Angelidou-Tsifida M, Papatsiros V, Athanasiou L, Christodoulopoulos, G (2023) Ruminant Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease. *Journal of the Hellenic Veterinary Medical Society*, 74(3):5883–5891.
- Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Katsoulos PD, Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodoulopoulos G (2024b) Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle? *Vet Res Commun*, 48(1):475-484.
- Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou GI, Katsoulis K, Angelidou-Tsifida M, Athanasiou LV, Papatsiros VG, Christodoulopoulos G (2024c) Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management. Preprints, 2024061041. <https://doi.org/10.20944/preprints202406.1041.v1>
- Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou GI, Katsoulis K, Angelidou-Tsifida M, Athanasiou LV, Papatsiros VG, Christodoulopoulos G (2024d) Subacute Rumen

- Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management. *Animals*, 14(14), 2061. <https://doi.org/10.3390/ani14142061>
- Wang Y, Liu J, Huang J, Chang G, Roy AC, Gao Q, Cheng X, Shen X (2020) Sodium butyrate attenuated iE-DAP induced inflammatory response in the mammary glands of dairy goats fed high-concentrate diet. *J Sci Food Agric* 101(3):1218-1227.
- Xu C, Shen T, Yang W, Yu H, Gao S, Huang B (2016) The effect of subacute ruminal acidosis of dairy cows on productivity, digestibility and greenhouse gas emission. *J Agric Sci* 8:92.
- Xu T, Tao H, Chang G, Zhang K, Xu L, Shen X (2015) Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet Res* 11:52.
- Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, Wang HR (2020) Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 103:1931–1943.

CHAPTER I

Review of literature

1. Ruminal Acidosis

Ruminal acidosis has been introduced in the literature since 1959 with several names, such as rumen overload, toxic indigestion, grain engorgement, grain overload, grain poisoning, and acute indigestion (Krotkova et al., 1959; Dehkordi and Dehkordi, 2011). It is classified into two basic types, acute and subacute (also called latent or subclinical) based on the degree of ruminal pH reduction in the rumen and the clinical manifestations (Aschenbach et al., 2011; Reis et al., 2014). Acute Ruminal Acidosis (ARA) is usually observed in feedlots with a significant morbidity and mortality rate compared to subacute ruminal acidosis (SARA) which is frequently recorded in dairy farms and compromises farm profitability due to treatment costs, reduced herd productive and early culling (Kleen et al., 2003; Krause and Oetzel, 2006; Snyder and Credille, 2017).

Ruminal acidosis is considered to be one of the most frequent metabolic disorders especially for SARA, many authors have highlighted the considerable economic losses mainly due to reduced milk production and low body condition (Krause and Oetzel, 2006; Chaudhry et al., 2018). From an animal welfare perspective, SARA is considered a source of further complications in animal health due to consequent pathological situations such as abdominal pain and depression (Oetzel, 2003; Wang et al., 2019).

Several diagnostic examinations are applied not only for ruminal acidosis evaluation but also for prognosis and preventative interventions (Snyder and Credille, 2017). Rumination activity, biochemical profiles, blood-gas analysis, milk analysis, feces, and urinalysis provide crucial data for SARA management. Ruminal acidosis prevention is performed at the herd level with interventions in nutrition preparation and management and further to ancillary dietary or not supplementations such as buffers, probiotics, yeast, plant-derived extracts with antimicrobial properties, antibiotics, vaccination, and rumen fluid transformation (Oetzel, 2017).

The aim of this chapter is to present a comprehensive overview of the etiology, pathophysiology, and clinical presentation of ruminant acidosis in cattle and small ruminants, as well as the current diagnostic methods, management interventions, and treatment protocols reported over the last 30 years (1990-2020).

Definition of Ruminal Acidosis

Ruminant acidosis is a ruminant nutritional disorder that emerge due to the consumption of feed with high carbohydrate and low digestive fiber content (Mahmood et al., 2013; Snyder and Credille, 2017). The pathophysiological definition of ruminal acidosis is the rapid reduction of ruminal pH due to extensive accumulation of small chain fat acids (SCFAs) (Kawas et al., 2007; Bramley et al., 2008).

Types of Ruminal Acidosis

As mentioned, two main types of ruminal acidosis, ARA and SARA, have been suggested by the majority of the researchers.

In bovine medicine, physiological rumen pH varies between 6 and 7 for animals on a roughage diet (Gruenberg and Constable, 2009). In small ruminants, normal ruminal pH ranges between 5.8 and 6.8 (Jasmin et al., 2011; Wang et al., 2019). A range of ruminal pH at 5.0-5.6 is suggestive for SARA. Ruminal pH below 5.0, approaching 4.5 or lower, characterizes the ARA (Krause and Oetzel, 2006). In small ruminants, rumen pH decreases below 5.2 in cases of ARA and between 5.2 and 5.8 in cases of SARA (Church, 1991; Commun et al., 2009).

2. Acute Ruminal Acidosis (ARA)

Acute Ruminal Acidosis called clinical ruminal acidosis or acute ruminal lactic acidosis (ARLA). Ruminal pH value lower than 5 is suggested as the main diagnostic feature for ARA (Commun et al., 2009; Kleen and Cannizzo, 2012). In bovine, ARA is characterized by a large

accumulation of lactic acid (50- 150 mM) (Blanch et al., 2009). In small ruminants during ARA, concentrations of lactic acid varying around 34 mM and total Volatile Fatty Acids (VFAs) around 94 mM (Wiryawan and Brooker, 1995).

In addition, several changes evoke in microbial populations, such as the decreased population of Gram-negative bacteria and lactic acid-utilizers, the increased population of Gram-positive bacteria (*Streptococcus bovis*, *Lactobacillus* spp.), the increased population of lactic acid-producers and the absence or reduction of ciliated protozoa. Furthermore, other microbial toxic products are present in cases of ARA such as ethanol, amines, and endotoxins (Wiryawan and Brooker, 1995; Nagaraja and Titgemeyer, 2007).

During ARA, blood profile is also altered with blood pH recorded below 7.35 (normal range 7.35- 7.45), lactic acid increased (especially the D-lactic acid), bicarbonate decreased <20 mEq/L and packed cell volume (PCV) increased >40% (Nagaraja and Titgemeyer, 2007).

It has been well established that ARA causes damage to the epithelial barrier integrity in the ruminoreticulum and/or abomasum, which allows toxins (Lipopolysaccharide- LPS), biogenic amines and bacteria, such as *Fusobacterium necrophorum*, to infiltrate across ruminal epithelium (Tadepalli et al., 2009; Minuti et al., 2014).

Epidemiology

The acute form is considered rarer and mainly accidental compared to the subacute form (Vasconcelos and Galyean, 2008; Giger-Reverdin, 2018). The incidence of ARA varies between 0.3-3% during lactation, with a considerably higher rate during the 1st- month post-calving (Krause and Oetzel, 2006; Lean et al., 2007).

Acute ruminal acidosis emerges after the significant consumption of rapidly fermentable carbohydrates such as starch, corn, wheat and barley in ruminants unadapted to a high-concentrate diet. The administration of wheat flour at a dose of 50 g/kg of body weight (BW)

in sheep and a dose of 80-100 g/ kg BW in goats can cause ARA (Aslan et al., 1995; Lettat et al., 2010). Similarly, ruminal acidosis can be provoked due to the unintended gross consumption of sorghum flour amounts especially for goats at a dose of 50 g/kg BW (Nour et al., 1998). Further, ARA is observed in the early feeding period when newly feedlot cattle are introduced to a primarily concentrate-based ration and stepped up too rapidly (Krause and Oetzel, 2006).

Feeding practices with a pasture of low neutral detergent fiber (NDF) and high non-structural carbohydrate (NSC) may induce acidosis (Wales et al., 2001). Diets that are deprived of forage (<10% of total Dry Matter Intake, DMI) significantly increase the risk of ruminal acidosis (Enemark et al., 2002). Ruminal acidosis may be invoked from the ingestion of small particle size (diameter less than 1.78mm) (Yansari et al., 2004; White et al., 2014). ARA is more frequent when animals are grouped than alone due to the psychological competition for overconsuming (Smith, 2009).

A great risk of acidosis for sheep is recorded when animals gain access to cropping regions especially after harvest. This risk is attributed to the availability of large amounts of grain, either remaining after harvest or split during the handling, in combination with the lack of any adaption stage. Similar issues are provoked by the rapid introduction of sheep to crops, particularly brassicas such as rapes, kales or turnips, that are highly digestible (Lean et al., 2007).

Clinical Signs

Acute ruminal acidosis emerges as one of the most significant causes of increased morbidity and mortality in ruminant production systems (Plaizier et al., 2017).

The affected ruminants are presented with tachypnea, shallow respiration, diarrhea, profuse salivation, nasal discharge, abdominal respiration, and bilateral abdominal dictation. Behaviour alterations are manifested by anorexia, weakness, depression, lethargy, staggering,

recumbency, flank watching, and lameness (Meyer and Bryant, 2017; Pupin et al., 2017). Clinical and laboratory testing reveal pyrexia in early stage and hypothermia in later stages, tachycardia, dehydration, congestion of mucous membranes and vessels of sclera, pneumonia, metabolic acidosis, hypocalcaemia, severe toxemia, acidified urine, and ruminal atony (Snyder and Credille, 2017; Reis et al., 2018a).

Moreover, central nervous system symptoms may be presented such as ataxia, blindness, head pressing, opisthotonos, altered gait, teeth grinding, incoordination, tremors, seizures, prostration, and coma. The recumbent ruminants are perceived with their heads tucked in their flanks and similar presentation of parturient paresis (Meyer and Bryant, 2017; Sabes et al., 2017). Ruminants with a peracute form of acidosis emerge dead with few or no obvious clinical signs (Snyder and Credille, 2017).

In bovine medicine, profuse, watery foul-smelling or fetid diarrhea can be presented with a gray color and undigested grains or the presence of blood due to the increased passage and decreased digestibility (Meyer and Bryant, 2017; Snyder and Credille, 2017). In small ruminant medicine, diarrhea could be watery with yellowish color and acidic odor or greenish or soupy or just watery feces (Minuti et al., 2014; Pupin et al., 2017).

Pathoanatomical lesions

The hallmark of macroscopic examination of ruminant epithelium in ARA is easy detachment of ruminal papillae accompanied by patchy areas of sloughed papillae and multifocal erosion or ulceration (Ismail et al., 2010). In ARA and repeated ruminal acidosis episodes parakeratosis is also observed. Parakeratosis is the thickening of the stratum corneum and emerges with the dark color of ruminal mucosa (Enemark, 2008; Lopez-Campos et al., 2010). The ruminal papillae mucosa appears brown, friable, and easily detached. Parakeratosis often results in rumenitis due to impaired epithelial barrier caused by the decreased ruminal pH

(Steele et al., 2009). Rumen content with undigested grain and sour smell and profuse amounts of fluid in the whole gastrointestinal tract are common findings in bovine with ARA (Meyer and Bryant, 2017). Nodules on papillae in both dorsal and ventral ruminal sacs, hyperaemia of papillae in atrium ruminis, epithelial necrosis, dyskeratosis, and acute superficial ulcers have been referred in ARA cases, as well (Aslan et al., 1995; Fanning et al., 2018). On the other hand, Odongo et al. (2006) reported a reduced number of papillae on the ventral sac of the rumen during ruminal acidosis in lambs.

Ruminal fluid during ARA (intraruminal lactate concentration >30 mg/100 ml or 3.3 mmol/L) presents several aspects such as milky/greenish-brown or gray or yellow to orange color, sticky/sour or putrid odor and frothy consistency with contained pods and fragments. Laboratory analysis reveals watery or thin viscosity, no or fast flotation/sedimentation, pH 5.2-3.8, methylene blue reduction (MBR) test >5 min, reduced glucose fermentation test, absence or reduced protozoal population and dominance of gram-positive bacteria (Snyder and Credille, 2017; Ribeiro et al., 2020).

At the microscopical level, the most prominent lesions seen in rumen epithelium after ARA and repeated ruminal acidosis episodes are increased apoptosis of ruminal epithelium and parakeratosis (Steele et al., 2009; Minuti et al., 2014). Attenuation of the cell defense system due to acidosis is suspected to be the mechanisms that render epithelial cells more vulnerable to cellular damage and apoptosis (Steele et al., 2009; Hollmann et al., 2013). Ruminal parakeratosis is characterized by the accumulation of keratinized, nucleated squamous epithelial cells. Moreover, the cellular adhesion in the stratum corneum and stratum spinosum is weakened after ARA episodes. Large gaps among stratum corneum and stratum granulosum lead to microbial colonization of rumen epithelium and consequent infiltration of inflammatory cells (Steele et al., 2009). In small ruminants, parakeratosis in the rumen, omasum and reticulum is referred to as the main lesion with mild to severe detachment of the mucosa from the

submucosa, ballooning degeneration of mucosal cells with cellular debris, swollen and vacuolated epithelia with intraepithelial pustules, necrosis, and presence of neutrophils (Bacha et al., 2017; Pupin et al., 2017).

Additionally, LPS, endotoxins, and histamine release evoke endotoxic shock, cardiovascular collapse, rumen ulceration, mycotic or bacterial rumenitis, renal failure, liver abscess, laminitis, enterotoxaemia, systemic acidemia, and thromboembolic respiratory disease (Snyder and Credille, 2017; Ribeiro et al., 2020). Lungs are characterized by congestion with severe diffuse edema (Lira et al., 2013). Liver failure, muscular weakness, and further rhabdomyolysis are aggravated due to recumbency during ARA (Constable, 2010; Fartashvand and Haji-Sadeghi, 2018).

Economic impacts

ARA emerges as a financial significant loss in the beef production industry, where the estimated treatment cost is 10 dollars/animal (Snyder and Credille, 2017). In Australia, the financial losses are estimated at 9 million dollars/year in beef feedlots (Shu et al., 2000).

A survey over six years about mortality and morbidity in feedlot cattle presents that SARA and ARA emerge with 3-7% morbidity and 14-36% mortality (Edwards, 1996). Rahman et al., (2014) reported morbidity rates at 10-50% in goats with ARA. The fatality rates are 90% for untreated animals and 30-40% for treated animals (Oliveira et al., 2009).

3. Subacute Ruminal Acidosis (SARA)

It is also called Subclinical Ruminal Acidosis or Latent Acidosis. The consumption of huge amounts of rapidly fermentable carbohydrates combined with inadequate fiber induce SARA, which is characterized by a low ruminal pH (5.0-5.5), an increase in VFA (150-225 mM) and a fluctuating lactate acid (10- 40 mM) (Calsamiglia et al., 2012; Li et al., 2014a). In the subacute form of acidosis, the clinical signs are not obvious. Several microbial changes are

observed during the incident of SARA such as an increase of *Lactobacillus* spp., lactic acid-producing bacteria and lactic-acid utilizers, and the absence (or) reduction of ciliated protozoa (Nagaraja and Titgemeyer, 2007).

For definition of SARA in bovine medicine several cut-off values have been suggested based on the duration of decreased pH, such as 6.0, 5.8, 5.6, and 5.5, with the majority to agree with values below 5.6 for a period of 3h/day (Petzold et al., 2014; Bilal et al., 2016).

Several authors demonstrated as the main characteristic of subacute ruminal acidosis, in small ruminants, short periods with a pH range between 5.0-5.6 (Shen et al., 2019b). In caprine medicine, a range of 5.5 to 6.0 is used as rumen pH cut-off in SARA definition for at least 3 hours (Jiang et al., 2014; Taghipoor et al., 2020).

Furthermore, SARA is based on the presence of butyrate or propionate or both acids. The butyric form of SARA is defined as a consequence of increased corn consumption (1.2% of BW) and characterized by a ruminal pH among 5.5-5.8, an increased butyrate percentage (from 13 to 22% of total VFA), the total value of VFA at 107 mM and a reduction of lactate concentration (<5 mM) (Doreau et al., 2001; Lettat et al., 2012). According to Lettat et al., (2010), the propionic form of SARA in small ruminants is caused by increased consumption of beet pulp (1.2% of BW) and is characterized by a mean ruminal pH among 5.8-6.2, a propionate percentage at 35%, a total VFA concentration at 110 mM and lactate concentration less than 10 mM. However, in bovine, a propionic form of SARA has as its characteristics, a mean pH of 5.67, a total VFA concentration at 114 mM, and a lactate concentration of less than 3 mM (Khafipour et al., 2009).

Epidemiology

SARA emerges commonly in intensive livestock production systems (Lopez-Campos et al., 2010; Gao and Oba, 2014). Episodes of low ruminal pH and consequent SARA in lactating

cows are recorded among calving and 5 months post-calving (Kleen et al., 2013). However, the beef feedlot cattle could be exposed anytime to SARA, from the onset of their gathering into the feedlots until culling time, with pH <5.5 and prevalence of 14 to 42% (Nagaraja and Lechtenberg, 2007).

In dairy cattle, the occurrence of SARA is estimated at 11-26% with 11-20% in early and 18-26% in mid-lactation (Kitkas et al., 2013; Atkinson, 2014; Stefanska et al., 2016). A higher SARA frequency was recorded at a farm with history of high SARA prevalence in early- and mid-lactation [48.2%, 53.8% and 65.3% at 30, 90 and 150 days in milk (DIM), respectively] (Kitkas et al., 2019). The early lactation period is at high risk due to the diminished size and reduced absorption ability of rumen epithelium/papillae, fewer adapted rumen microflora and the rapid introduction to high energy-dense diets (DeVries et al., 2009). During mid-lactation, SARA emerges due to management errors such as automatic feeding mis-adjustment, wrong calculation of components in the preparation of total mixed ration (TMR), inconsistency in feeding time- schedule, and insufficient feed bunk space per cow (Kleen et al., 2003, Kitkas et al., 2013).

In sheep farming, similar findings were presented by Lira et al., (2013) in an extended survey in north- eastern Brazil, where ruminal acidosis emerged at a frequency of 13.9% in goats and 12.4% in sheep. A cross-sectional survey of 19 dairy sheep farms in Argentina reported that 42.1% of flocks challenged presented ruminal acidosis (Suarez and Buseti, 2009).

Primiparous cows present a higher frequency of SARA (29% than 19% of multiparous cows) and occurrence earlier in lactation. The above emerged because primiparous cows are not yet capable of managing their feed intake during the consumption of a high-energy diet and are not able to have access to feed bunk for small and frequent meals due to socially domination (Oetzel, 2017; Kitkas et al., 2013).

Clinical signs

The clinical signs of SARA emerge after a delay of onset time or disguise by other common diseases, such as lameness and considerable deterioration of body condition (Colman et al., 2015).

The affected by SARA ruminants are usually presented with intermittent diarrhea, poor body condition and reduced or variable feed intake, and rumination. Behaviour alterations comprise mild transient anorexia, alternating mood, depression, and weakness. Clinical and laboratory examination present signs of dehydration, rumen stasis, abdominal pain, feces alterations, laminitis, metabolic disorders, lower fecal and urinary pH, reduced milk yield, and reduced fat and protein concentration in milk (Snyder and Credille, 2017; Shen et al., 2019a). In bovine, continuous tail swishing, cud drooping during rumination, and increased colic incidence are presented as further clinical signs of SARA (Abdela, 2016; Meyer and Bryant, 2017).

Feces may be presented brighter and of yellowish color with the sweet-sour smell. The consistency may be loose and frothy with fibrin casts, undigested particles (1-2 cm) and whole cereal grains. Significant body fecal soiling, especially in the hindquarters has been reported in the case of SARA (Meyer and Bryant, 2017; Oetzel, 2017).

Furthermore, SARA impact on bovine reproduction consists of infertility, abortions, stillbirths, premature births and being prone to environmental mastitis (Abdela, 2016; Snyder and Credille, 2017).

Pathoanatomical lesions

Macroscopic examination of the rumen wall in SARA reveals grey to dark brown mucosa and is considered a typical sign of parakeratosis and/or hyperkeratosis (Ploger et al., 2012). The major impacts of SARA are parakeratosis, thickening and detachment of epithelium

and rumenitis (Steele et al., 2009). While parakeratosis is considered a persistent finding, the thickness of the total epithelium, stratum corneum, stratum granulosum and stratum spinosum may appear debilitated during SARA cases as well (Steele et al., 2011; Zhang et al., 2020). The pathogenetic mechanisms responsible for these lesions are similar to those pertaining to ARA.

Furthermore, necropsy besides rumen lesions may reveal liver abscesses, subcutaneous abscesses, pulmonary bacterial emboli, peritonitis, or inflammation in organs such as kidneys (pyelonephritis), lungs (pneumonia), heart valves (endocarditis) and joints (arthritis) (Kleen et al., 2003; Abdela, 2016).

Economic impacts

According to literature cost estimate of SARA in the USA dairy industry, based on milk production losses, reduction efficiency of milk production, premature and high culling rate, rises up to 1.12 USD/ day/cow. Economic losses of SARA are estimated up to 500 million - 1 billion dollars/year in total USA dairy industry (Enemark, 2008; McCann et al., 2016).

For the beef USA industry, Stock, (2000) reports a financial loss of 9.40 dollars/feedlot steer due to acidosis impact on the reduction of final cattle weight. Furthermore, the reduced DMI due to SARA leads to the decreased growth of beef calves with losses of 10 - 13 dollars/animals (Stock and Britton, 1996).

Effect of SARA on milk production

Administration of the HC diet (High Concentrate diet; concentrate 60%) may increase milk quality and milk yield in a short duration of time, but also may reduce rumen pH and increase the risk of SARA in dairy ruminants (Abaker et al., 2017; Shen et al., 2019a). SARA reduces milk production and milk fat concentration but has no effect on protein and energy levels (Chang et al., 2015; Xu et al., 2015). On the other hand, short-term SARA has no effect on milk fat or protein concentration (Li et al., 2012a; Tian et al., 2017).

A ratio of milk fat: milk protein lower than 1 in animals feed with an HC diet, could be a useful diagnostic sign of SARA in ruminants (Enemark et al., 2002; Giger-Reverdin et al., 2014). According to Cook et al., (2005) in bovine medicine, the incidence of SARA should be suspected when 10% of cows have individual milk fat percentages of 2.5 or less. Also, Danscher et al., (2015) reported a reduction of milk fat content at 4.14% during SARA.

The estimated reduction in milk production, milk fat and true protein is 2.7-3 kg/cow/day, from 37 to 34 g/kg (0.3% points) and 29 to 28 g/kg (0.12%), respectively (Xu et al., 2016). However, the financial losses of reduced milk fat and milk protein are estimated at 400 - 475 dollars/cow without taking into account the early culling and veterinary expenses (Abdela, 2016).

The increase in milk Somatic Cell Count (SCC) is reported as a sign of SARA due to reduced milk production (higher cell concentration) and due to the greater inflammatory status in cases of HC diets (Shen et al., 2019a; Antanaitis et al., 2020).

Welfare implications

Several authors reported that ruminants affected with acidosis will be distinguished due to their feeding behavior, which is dependent on daily DMI, cumulative intake patterns, fractional intake rate, sorting behavior, and chewing behavior (Calsamiglia et al., 2012; Giger-Reverdin, 2018).

During SARA a higher rate of saturation or an aversion to diet can be mainly presented by reduced concentrated feed intake and increased roughage intake (Nocek, 1997; Giger-Reverdin et al., 2014). The “off-feed” periods during acidosis decrease and alter feeding intake patterns (Calsamiglia et al., 2012).

Ruminants present different patterns in dealing with ruminal acidosis based on chewing behavior for buffering rumen pH, which explains why several animals suffer acidosis more than

others in a herd with the same diet. During SARA, the chewing/kg Dry Matter (DM) duration and saliva production is decreased (Giger-Reverdin et al., 2014). It is noteworthy that goats can browse rather than graze, which allows them to choose their feed in a feedstuff variety during SARA (Desnoyers et al., 2008).

Animals with acidosis prefer to nibble or eat small amounts of food. This can be explained as an effort to increase the daily intake rate and further reduce the movement of high rapidly fermentable carbohydrates amount into the rumen during acidosis (Giger-Reverdin, 2012). The total time of eating, chewing, and ruminating is decreased in goats with HC diets, due to the reduction of NDF (Maekawa et al., 2002; Desnoyers et al., 2008).

The episodes of ruminal acidosis may be manifested by a slightly total effect on cow behavior such as standing time, lying time, or feeding time (DeVries et al., 2009). Brzozowska et al. (2013) report that SARA causes discomfort which stimulates the cow to increase feeding time due to an increase in both meal frequency and duration. Lying time and rumination during SARA is decreased (by 1h/day) and standing time is increased. Furthermore, high acidosis risk (HR) cows sort their ratio to a greater level than low acidosis risk (LR) cows (DeVries et al., 2009).

Goats with SARA demonstrate a position curled up as a sign of sickness or pain mainly in the afternoon, especially after feeding hours (Desnoyers et al., 2008). On the other hand, sheep are more aggressive and less responsive, and they are devoting less time to recumbency and more time to standing with the head up posture and ears pricked up during SARA (Commun et al., 2012).

Water or salt consumption may be higher during the acidosis challenge. Salt supplementation in water causes 1.4 times more drinking water during acidosis (Commun et al., 2012).

4. Diagnostic approach of Ruminal Acidosis

Sampling protocol and rumen pH values

ARA diagnosis is considered straightforward when the rumen pH is between 4.0 and 5 for a period of 6 to 24 hours after induction of acidosis (Kirbas et al., 2014; Reis et al., 2018b).

On the contrary several but non unanimously accepted protocols have been recommended for diagnosis of SARA. In cattle twelve (12) samples collected by rumenocentesis is considered to be an optimum sample number for SARA evaluation. The samples should be collected within 2-4h after concentrate meal supplementation in herds or within 5-8h in grass only or total mixed ration (TMR) fed herds. The above mentioned protocol should be performed at 45-150 days in mid-lactation or among 2 and 180 lactation days. There are two reported cut-off points, $\text{pH} \leq 5.5$ (positive) and $\text{pH} > 5.8$ (negative) for SARA diagnosis. Several authors report that if at least 3/12 samples have ruminal $\text{pH} \leq 5.5$ then herd is considered SARA positive. In any other situation, (prevalence of low ruminal pH between 16.7-33.3%) additional sampling or another diagnostic index of SARA is recommended (Kleen et al., 2003; Oetzel, 2017).

In small ruminants, several authors proposed slightly different limits for SARA diagnosis with rumen pH between 5.2 to 5.8 for a duration of 3 h/d (Ma et al., 2018; Wang et al., 2020).

Sampling of rumen content and pH measurement

The standard diagnostic method is rumen fluid extraction via rumenocentesis and measurement of pH with portable pH meter (Morgante et al., 2009; Chaudhry et al., 2018). The most important disadvantages of rumenocentesis are the limited volume of rumen fluid collected and the estimation of ruminal pH alone without any other data such as the color, the odor, and the microflora populations (Mialon et al., 2012). Moreover, abscess or haematoma

formation or septic peritonitis are among the reported complications of the procedure (Aceto et al., 2000; Penner et al., 2009).

Oral stomach tube has also been used to collect ruminal fluid samples for pH determination. However, is not reliable, as pH varies along the ruminal areas reached by the tube, saliva contamination (alkaline pH values), sampling in the fiber mat of the rumen and time of sampling in relation to feeding (Lean et al., 2007; Lee et al., 2019).

In ruminants, rumen cannula is proposed for collecting reliable samples of ruminal fluid, but it is only used in research (Nocek, 1997). However, welfare issues have been raised regarding this practice and there are a number of practical constraints, such as frequently cleaning, repeated removal and replacement of cannula cover and monitor rumen cannula sites, restricting their utilize in field situations (Lean et al., 2007; Tajik and Nazifi, 2011).

Bench pH-meter is the most commonly used technic for evaluation of rumen pH (Sato et al., 2012; Zhang et al., 2020). Despite being more practical, the test paper strips for pH evaluation do not provide reliable results due to the color of ruminal fluid which may conflict with the interpretation of the color change on the strip and also the large fluctuations of pH (Kirbas et al., 2014; Oetzel, 2017).

It is worth noted that collection of rumen samples with different techniques in same animal show different pH values (Duffield et al., 2004; Al Zahal et al., 2007; Bramley et al., 2008). Comparing pH values in samples collected by rumenocentesis vs stomach tube, several authors report a mean difference in rumen pH from 0.28 to 1.1 with the lower values detected by rumenocentesis (Nordlund et al., 1995; Garrett et al., 1999; Enemark et al., 2004). Also, samples by rumenocentesis present 0.28 units lower ruminal pH than ruminal cannula (Garrett et al., 1999).

Telemetric systems

In recent years, telemetric systems are used for continuously measurements of rumen pH and temperature. Telemetric systems consist of a submersible electrode, a data logger, a transfer cable and specific software. The electrodes are mainly placed in the ventral sac of the rumen. The continuous telemetry measurement system evaluates rumen fluid pH every 5 minutes and has a sensitivity of 0.01 pH units. Telemetry units present several limitations such as rapid fluctuation in pH values, bulky size, rumen fistula insertion of pH sensor and necessity of retrieval for calibration and battery replacement (Oetzel, 2017; Reis et al., 2018b).

Electronic devices that are called ‘smart pills’ or ‘smart boluses’ belong also to the category of telemetric systems. Such devices are administered once, are completely maintenance-free, and measure direct, insightful values, including rumen pH, with the highest accuracy inside your cows, in the reticulum. Specific readout devices with integrated Internet connection read out the data from the ‘smart boluses’. SARA is included in the diagnostic possibilities of such devices (Kilic, 2011).

Ancillary diagnostic tests

In addition to measuring the rumen pH, many other tests have been proposed to diagnose SARA and predict the onset of ARA, and some have been successful even without having yet been widely applied in practice.

The detection of rumination activity via automated sensors can be used as a possible SARA indicator in cattle (Oetzel, 2017). Cattle that are ill feed less consequently ruminate less, thus; the estimation of ruminating time could be used as a health index. A range of methods using microphones, accelerometers and laser interferometry have been evaluated to derive this information (Hamilton et al., 2019).

The fat percentage in milk is also considered as evidence of absence of SARA in a herd, but the case of low value could not be used as an index of SARA due to more causative agents of milk fat reduction. Milk fat values must be evaluated frequently (at least once a week) for better evaluation of SARA status (Enemark, 2008). Acidosis should be further investigated if more than 10 % of cows in a herd test have a higher milk protein than milk fat or a rapid fall in milk fat of 0.3-0.5 % in a week or a sudden fall in milk protein of > 0.3% in a week occurs (Kleen et al., 2003; Lean et al., 2007).

Rumen temperature can be used as a new diagnostic tool for the evaluation of ruminal acidosis. Particularly ruminal temperature can be increased above 39.2°C and reach up to 41°C with ruminal pH values among 5.6 and 5.0. During ARA, ruminal temperature can reach maximum value of 40.5°C with pH value of 4.2 (AlZahal et al., 2008; Reis et al., 2018b).

Blood pH is also used in the diagnosis of acidosis. Low blood pH (7.1-7.44) is reported in all forms of acidosis (Li et al., 2012b; Chaudhry et al., 2018). The affected animals with ARA or SARA present a reduction of plasma pH, base excess, total CO₂⁻ content (TCO₂) and bicarbonate and an increased anion gap (Sabes et al., 2017; Snyder and Credille, 2017). The standard base excess (SBE) values are between 14 and 16 mmol/l in case of acidosis induction (Aslan et al., 1995).

Ruminal lactic acid can also be used as another acidosis index. The normal ruminal lactic acid values are between 0-5 mmol/l, whereas during ARA are increased at 50-150 mmol/l (Nagaraja and Titgemeyer, 2007; Henning et al., 2010). On the other hand, the lactic acid concentration is increased by less than 1 mM during SARA (Sun et al., 2018).

In addition, urine pH measurement has been used in the diagnosis of ruminal acidosis. In ruminants, decreased urine pH values of 5.3-6.7 have been reported during ARA (Patra et al., 1993; Sabes et al., 2017). Urine pH during SARA ranges from 7.8 to 8.3 (Li et al., 2012b; Danscher et al., 2015). Moreover, when the net acid-base excretion (NABE) is <83 mmol/l, the

inorganic phosphate concentration is >5.7 mmol/l and the urine pH is <7.8 , the cow should be considered as subacute acidotic (Furll, 2014).

Stool pH <5 is indicative of ARA, while when it falls below 6.0 it is compatible with SARA (Li et al., 2012b; Minuti et al., 2014; Danscher et al., 2015; Chaudhry et al., 2018). Investigation of acidosis is recommended if more than 15% of a herd is scouring. In farm routine, the stool scoring of at least 25 fresh manure samples in the field using the 1-5 scoring system is also recommended and taking action if more than 5/25 samples are score 2 or less (Lean et al., 2007).

Furthermore, values of acute phase proteins (APPs) have been proposed as indicators of ruminal acidosis by many researchers (Gonzalez et al., 2010; Zebeli et al., 2012; Nikvand et al., 2020).

5. Treatment of Ruminal Acidosis

In ARA cases, treatment strategy should aim to adjust ruminal pH through prevention of lactic acid accumulation, correction of electrolyte losses, adequate circulating blood volumes and normal gastrointestinal motility (Radostits et al., 1994). Recumbent ruminants are usually of poor prognosis. However, severely affected ruminants should be euthanized for welfare reasons (Meyer and Bryant, 2017).

A hypertonic saline solution (HSS) (7.2%, dose of 4 ml/kg over a period of 10 minutes) should use to compensate for a considerable volume deficit. However, the results of the above solution are of short duration and should be followed by oral or intravenous fluids infusion (5-10L/liter of HS infusion). For clinical acidosis, 5% Sodium bicarbonate solution (NaHCO_3) is recommended at 5 L over 30 minutes for a 450 kg/animal, followed by 1.3% NaHCO_3 infusion at a volume of 150 ml/kg of body weight (BW) over 6 to 12 hours. Another solution of NaHCO_3 (8.4%) is used nowadays for a shorter treatment at a rate of 5 ml/kg BW over a time of 10 to 20

minutes. NaHCO₃ administration should be followed by isotonic intravenous or oral fluid (Snyder and Credille, 2017).

The therapeutic protocol for repairing rumen microflora requires the removal of acidic rumen content, the infusion of rumen buffers and the transformation of rumen fluid. The transformation of acidic rumen fluid can be achieved by either rumenotomy or rumen lavage. Rumen lavage is applied by repeated flushing of the rumen with warm water via a large-bore stomach tube in mild acidosis cases (Snyder and Credille, 2017).

Rumenotomy or rumen lavage should be followed by infusion of rumen fluid from a donor with a normal diet. Regarding the optimum amount of rumen fluid for the infusion, several authors propose the 3-10 L for cattle heavier than 400 kg of BW and 2-4 L for smaller ones (Snyder and Credille, 2017).

The ruminal fluid transfaunation (RFT) is considered as a routine procedure in ruminant production systems (DePeters et al., 2014). Healthy RFT provides rapid recovery of rumen fluid color, odor, and consistency, unaffected fermentative activity of rumen microflora and rapid return of protozoa population (Pereira et al., 2018). RFT relieves the damage of rumen epithelium morphology and function due to an increase of total Volatile Fatty Acids (VFA), acetate, propionate, and butyrate concentrations, reduction of lactate and Lipopolysaccharide (LPS) concentrations and thus the restoration of rumen epithelial integrity (Liu et al., 2019).

6. Prevention of Ruminal Acidosis

Nutrition

An adequate intake of neutral detergent fiber (NDF) and acid detergent fibre (ADF) is necessary for keeping ruminal pH within the normal interval (Lean et al., 2007). A minimum of 25% NDF in dry matter of a total diet for dairy cattle (Li et al., 2014b). Likewise, in small

ruminant, a proportion 30% of NDF is proposed (Lean et al., 2007; Jaramillo-Lopez et al., 2017).

Management

A considerable management issue is the bunk space for easily feeding of all animals without excessive competition. The social issues among cows, such as domination, could be vanished if the bunk space is 0.38-0.46 m/cow (Lean et al., 2007; Snyder and Credille, 2017).

For the prevention of SARA, a further nutritional management strategy could include meal size and frequency. The frequency of meals fluctuates between 8 and 12 meals/day, but there is a significant animal-to-animal variation as well as day-to-day variation in meal frequency and size (Meyer and Bryant, 2017).

Dietary Buffers

The dietary buffers cannot eliminate ruminal acidosis but they can minimize or prevent it (Enemark, 2008). The oral administration of buffers is used in affected animals, which are not treated with rumenotomy or lavage (Snyder and Credille, 2017).

a) Sodium Bicarbonate (SB)

Sodium bicarbonate (SB) addition in feedstuffs increases rumen pH and palatability and prevents rumen epithelium from damage by the proliferation of the acid-tolerant lactobacilli during lactic acidosis episodes (Kleen et al., 2003; Dai et al., 2017). In dairy cattle rations, SB, at a dose of 150g/day, increases dry matter intake (DMI), milk production and milk fat percentage (Enemark, 2008). In bovine diets based on corn silage, a dose of 200-300g SB/animal/day is proposed (Lean et al., 2007).

b) Sodium Butyrate (NaB)

Sodium butyrate (NaB) is used as an anti-inflammatory agent to prevent SARA (Bilal et al., 2017). NaB supplement administration evokes the reduction of LPS and pro-inflammatory cytokine concentrations (Aabdin et al., 2018). Administration of 10 g NaB with or without 10 g SB in a high concentrate (HC) diet keeps steady ruminal pH and prevents LPS accumulation (Li et al., 2018; Ma et al., 2018).

c) Various buffers

Magnesium hydroxide ($\text{Mg}(\text{OH})_2$) is recommended at a dose of 1g/kg of BW which is diluted in a volume of water adequate for entering throughout the rumen (Snyder and Credille, 2017).

Magnesium oxide (MgO) raises rumen pH, milk production and milk fat percentage at a dose of 0.4-0.8% dry matter (DM) on corn silage and pasture-based diets. The rumen pH is increased after the administration of 0.5% MgO and 1.5% SB to both low and high-forage diets. A dose of 30-45g MgO /animal/day is proposed for acidosis prevention (Lean et al., 2007).

In addition, the administration of limestone (CaCO_3) at 1-2% in diet can keep steady rumen pH between 5.7 and 6.0 and ameliorate DMI in fattening lambs and cattle (Jaramillo-Lopez et al., 2017).

Probiotics (Direct-Fed Microbials DFM) and yeast

Several direct-fed-microbials (DFM) are used as feed additives for acidosis prevention, such as *Enterococcus faecium*, *Lactobacillus plantarum*, and yeast *Saccharomyces cerevisiae*, via intraruminal administration in the concentration of 10^5 cfu/ml. In lactating cows, 5g *Saccharomyces cerevisiae*/day (10^{10} cfu) can improve the average ruminal pH after one week of administration (Bach et al., 2007). In addition, the combination of *Enterococcus faecium* and

yeast at a dose of 5×10^9 cfu/day has been proven to increase the ruminal digestion of forage DM and DM consumption in early lactation cows (Enemark, 2008).

Probiotic yeasts have a symbiotic relationship with lactic acid-consuming bacteria [Selenomonas ruminantium (Sel ruminantium), Megasphaera elsdenii (Me elsdenii)] (Kleen et al., 2003; Henning et al., 2010). Moreover, the combination of Sel ruminantium subsp lactilytica and Me elsdenii could prevent lactic acidosis due to lactate fermentation by Me elsdenii and to glucose-lactate fermentation by Sel ruminantium subsp lactilytica. The above combination contributes to the growth stimulation of other rumen bacteria and the consequent reduction of Streptococcus bovis (Str bovis) domination (Wiryawan and Brooker, 1995).

Flavonoids, Essential Oils, and Antioxidants

Alternative strategies are been used for acidosis prevention, such as plants with antimicrobial properties and plants with similar results on ruminal fermentation as in-feed antibiotics (Durmic et al., 2012). β -sitosterol (BSS), one phytosterol (plantsterol), has been proven to relieve inflammatory response and control ruminal fermentation after high-grain diets consumption (Xia et al., 2020). Additional, Naringin, a type of citrus flavonoid with proven antioxidant properties, reduces the discomfort that might be associated with damage to the ruminal wall in cases of subclinical acidosis (Lopez-Campos et al., 2010).

Flavonoids and essential oils are defined as plant-derived compounds (Puvača et al., 2022). Flavonoids ameliorate fermentation and increase the population of lactate-consuming bacteria in the rumen. Essential oils from Allium sativa (garlic), Origanum vulgare (oregano), Capsicum annum (pepper), Yucca schidigera (mojave yucca), and Cinnamomum cassia (cinnamon) present antimicrobial ability, pH buffering and anti-inflammatory response (Jaramillo-Lopez et al., 2017; Oetzel, 2017; Puvača, 2022). Particularly, a mixture of

cinnamaldehyde (0.18 g/d) and eugenol (0.09g/day) could reform the rumen microbial fermentation in beef heifers-fed high-grain diets (Jaramillo-Lopez et al., 2017).

Antimicrobials

Several antimicrobials have approved for the control of lactate-producing bacteria and liver abscess in feedlot cattle, as inhibitors of Gram-positive bacteria (Nurmeiliasari et al., 2017). The main antimicrobial categories that have been used are ionophores (lasalocid, monensin) and non-ionophores (virginiamycin, tylosin) (Kleen et al., 2003).

a) Ionophore Antibiom

In bovine, monensin ameliorates the ruminal pH due to the inhibition of most lactate-producing bacteria in favor of lactate-consuming bacteria (Oetzel, 2017). Monensin is added at 250-300 mg/animal/day in dairy cattle for at least two days before the carbohydrate challenge for effective control of acidosis (Lean et al., 2007).

In small ruminants, monensin is used in high doses (22-33 mg/kg of total DMI) for ruminal pH control (Meissner et al., 2010; Lammie et al., 2016). However, Miranda Neto et al., (2011) reported that monensin usage (33 mg/kg DM) do not help to prevention of ARA in goats.

Lasalocid (0.66-1.32 mg/kg BW) is also used as a feed additive in the worldwide cattle industry. This antibiotic provides a higher rumen pH and lowers L and D- lactate concentrations in dairy cattle (Lean et al., 2007; Meyer and Bryant, 2017).

b) Non-Ionophore Antimicrobials

In small ruminants, virginiamycin (VM) administration (30-80 mg/animal per day) reduces the proliferation of lactic acid-producing bacteria during the fermentation of grain (Jassim et al., 2003; Hutton et al., 2010). Virginiamycin, could be added to the diet of bovines

at 20 ppm or could be combined with ionophore antimicrobials e.g., monensin 20 ppm and VM 20 ppm (Lean et al., 2007).

Administration of tylosin (150 mg/animal/day) combined with monensin or other ionophores antibiotics presented similar beneficial results with virginiamycin (Lean et al., 2007).

The usage of antibiotics can potentially provoke negative effects, such as depressed ruminal fermentation and antimicrobial resistance and public health concerns due to antimicrobial residues while production costs are also increased (Wiryawan and Brooker, 1995). Due to this concerns limitations and bans of antibiotics usage have been applied by several countries (Lammie et al., 2016).

Vaccines

Immunization with intramuscular injection of a live *Str. bovis* vaccine (*Str. bovis* strain SB-5) followed by a booster dose at 28 days has been reported to reduce the risk of lactic acidosis (Enemark, 2008; Jaramillo-Lopez et al., 2017).

7. Conclusion

Ruminal acidosis emerges as the most important nutritional disorder in ruminant intensive farming. Especially SARA causes significant economic losses due to milk fat depression and liver abscesses. The diagnosis of SARA in practice remains questionable.

Prevention plays a key role in controlling the epidemiologic occurrence and the consequent financial losses of SARA. Prevention should be based mainly in equilibration of providing diet with or without additions of dietary additives such as probiotics, buffers and minerals. Antibiotics should be considered as the ultimate solution with great concern on antimicrobial resistance issues.

Veterinarians should try to comprehend the mode of feed assimilation, energy accumulation, and pH regulation in ruminants. It is the only path to achieving desirable productivity goals with an appreciation of animal needs and welfare.

References

- Aabdin ZU, Bilal MS, Dai H, Abaker JA, Liu X, Benazir S, Yan J, Shen X (2018) NOD1/NF- κ B signaling pathway inhibited by sodium butyrate in the mammary gland of lactating goats during sub-acute ruminal acidosis. *Microb Pathog* 122:58-62.
- Abaker JA, Xu TL, Jin D, Chang GJ, Zhang K, Shen XZ (2017) Lipo- polysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J Dairy Sci* 100:666–678.
- Abdela N (2016) Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. *Achiev Life Sci* 10:2:187-196.
- Aceto H, Simeone AJ, Fergusson JD (2000) Effect of rumenocentesis on health and productivity in dairy cows. *J Anim Sci* 78:(1)(Abstr. 162).
- AlZahal O, Kebreab E, France J, Froetschel M, McBride BW (2008) Ruminal temperature may aid in the detection of subacute ruminal acidosis. *J Dairy Sci* 91:(1):202-7.
- Alzahal O, Kebreab E, France J, McBride B (2007) A Mathematical Approach to Predicting Biological Values from Ruminal pH Measurements. *J Dairy Sci* 90:3777-85.
- Antanaitis R, Juozaitienė V, Malašauskienė D, Televičius M (2020) Inline Reticulorumen pH as an Indicator of Cows Reproduction and Health Status. *Sensors (Basel)* 20:(4):1022.
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2011) Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89:1092-1107.
- Aslan V, Thamsborg SM, Jorgensen RJ, Bassel A (1995) Induced Acute Ruminal Acidosis in Goats Treated with Yeast (*Saccharomyces cerevisiae*) and Bicarbonate. *Acta Vet Scand* 36:65-77.

- Atkinson O (2014) Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract* 22:(1):1-9.
- Bach A, Dinare's M, Devant M, Carre X (2007) Associations between lameness and production, feeding, and milking attendance of Holstein cows milked with a robotic milking system. *J. Dairy Res* 74:52-57.
- Bacha FB, Pupin RC, Leal PV, Carvalho NM, Franco GL, Ítavo CCBF, Riet-Correa F, Lemos RAA (2017) Experimental poisoning by *Enterolobium contortisiliquum* in sheep. *Pesqui Vet Bras* 37:(1):23-30.
- Bilal MS, Abaker JA, Aabdin ZU, Waheed U, Xu T, Dai H, Junfei G, Shen X (2017) High grain diet triggers inflammation in the goat uterus: A comprehensive regulation diet modulates the immune response. *Int J Agric Biol* 19:34–40.
- Bilal MS, Abaker JA, Aabdin ZU, Xu T, Dai H, Zhang K, Liu X, Shen X (2016) Lipopolysaccharide derived from the digestive tract triggers an inflammatory response in the uterus of mid-lactating dairy cows during SARA. *BMC Vet Res* 12:(1):284.
- Blanch M, Calsamiglia S, DiLorenzo N, DiCostanzo A, Muetzel S, Wallace RJ (2009) Physiological changes in rumen fermentation during acidosis induction and its control using a multivalent polyclonal anti-body preparation in heifers. *J Anim Sci* 87:1722-1730.
- Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND (2008) The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. *J Dairy Sci* 91:308-321.
- Brzozowska AM, Sloniewski K, Oprzadek J, Sobiech P, Kowalski ZM (2013) Why are dairy cows not able to cope with the subacute ruminal acidosis?. *Pol J Vet Sci* 16:4:813-821.
- Calsamiglia S, Blanch M, Ferret A, Moya D (2012) “Is subacute ruminal acidosis a pH related problem? Causes and tools for its control”. *Anim Feed Sci Technol* 172:(1-2):42-50.

- Chang G, Zhuang S, Seyfert HM, Zhang K, Xu T, Jin D, Guo J, Shen X (2015) Hepatic TLR4 signaling is activated by LPS from digestive tract during SARA, and epigenetic mechanisms contribute to enforced TLR4 expression. *Oncotarget* 6:36.
- Chaudhry AS, Khan MA, Khan MS, Ashraf K, Saleem MH, Khan NU, Usman T, Ahmad I (2018) Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J Anim Plant Sci* 28:(1):80-84:1018-7081.
- Church DC (1991) *Livestock Feeds and Feeding*, third ed Prentice-Hall Inc USA 349 pp.
- Colman E, Waegeman W, De Baets B, Fievez V (2015) Prediction of sub- acute ruminal acidosis based on milk fatty acids: a comparison of linear discriminant and support vector machine approaches for model development. *Comput Electron Agric* 111:179-85.
- Commun L, Mialon MM, Martin C, Baumont R, Veissier I (2009) Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J Anim Sci* 87:3372-3379.
- Commun L, Silberberg M, Mialon MM, Martin C, Veissier I (2012) Behavioural adaptations of sheep to repeated acidosis challenges and effect of yeast supplementation. *Anim* 6:2011-2022.
- Constable PD (2010) Grain overload in ruminants. *The Merck Veterinary Manual* 10th ed (Khan C, Ed) Merck &Co Inc Whitehouse USA pp:200-203.
- Cook NB, Oetzel GR, Nordlund KV (2005) Clinical forum-subacute ruminal acidosis-a problem in UK dairy herds?. *UK Vet* 10:40-46.
- Dai H, Liu X, Yan J, Aabdin ZU, Bilal MS, Shen X (2017) Sodium butyrate ameliorates high-concentrate diet-induced inflammation in the rumen epithelium of dairy goats. *J Agric Food Chem* 65:596-604.

- Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC (2015) Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Vet Scand* 57:(1):39.
- Dehkordi AJ, Dehkordi ZK (2011) Occurrence of metabolic alkalosis in rumen lactic acidosis: a review article. *Comp Clin Pathol* 20:(1):1-3.
- DePeters EJ, George LW (2014) Rumen transfaunation. *Immunol Lett* 162:69-76.
- Desnoyers M, Duvaux-Ponter C, Rigalma K, Roussel S, Martin O, Giger-Reverdin S (2008) Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. *Anim* 2:12:1802-1808.
- DeVries TJ, Beauchemin KA, Dohme F, Schwartzkopf-Genswein KS (2009) Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feeding, ruminating, and lying behaviour. *J Dairy Sci* 92:5067-5078.
- Doreau M, Ollier A, Michalet-Doreau B (2001) An atypical case of ruminal fermentations leading to ketosis in early lactating cows. *Rev Med Vet (Toulouse)* 152:301-306.
- Duffield T, Plaizier J, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J, McBride B (2004) Comparison of Techniques for Measurement of Rumen pH in Lactating Dairy Cows. *J Dairy Sci* 87:59-66.
- Durmic Z, Hutton P, Murray K, Vercoe P (2012) Inclusion of selected levels of Australian native plant *Eremophila glabra* in fermentation substrate can influence events leading to rumen lactic acidosis in in vitro and in vivo carbohydrate-challenged systems. *Anim Feed Sci Technol* 178:57-66.
- Edwards A (1996) Respiratory diseases of feedlot cattle in central USA. *Bovine Practitioner* 0:30:5-7.
- Enemark JMD (2008) The monitoring, prevention and treatment of sub- acute ruminal acidosis (SARA): A review. *Vet J* 176:1:32-43.

- Enemark JMD, Jørgensen RJ, Enemark P (2002) Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Vet ir Zootech* 20:(42):16-29.
- Enemark JMD, Jorgensen RJ, Kristensen NB (2004) An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. *Vet Res Commun* 28(8):687-709.
- Fanning JP, Hynd PI, Cockcroft PD (2018) The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. *Small Rumin Res* 162:57-62.
- Fartashvand M, Haji-Sadeghi Y (2018) Evaluation of serum cardiac bio- markers in sheep with acute lactic acidosis. *J Hell Vet Medical Soc* 68:(2):219-224.
- Fürll M (2014) Möglichkeiten zur praktischen Diagnostik von Pansenfermentationsstörungen bei Kühen. *Tierärztl Umsch* 69:315-322.
- Gao X, Oba M (2014) Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. *J Dairy Sci* 97(5):3006-16.
- Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR (1999) Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. *J Dairy Sci* 82:1170-1178.
- Giger-Reverdin S (2018) Recent advances in the understanding of sub- acute ruminal acidosis (SARA) in goats, with focus on the link to feeding behaviour. *Small Rumin Res* 163:24-28.
- Giger-Reverdin S, Lebarbier E, Duvaux-Ponter C, Desnoyers M (2012) A new segmentation-clustering method to analyse feeding behaviour of ruminants from within-day cumulative intake patterns. *Comput Electron Agric* 83:109-116.
- Giger-Reverdin S, Rigalma K, Desnoyers M, Sauvant D, Duvaux-Ponter C (2014) Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats:

- Relationships between behavioral and physiological parameters and effect of between-animal variability. *J Dairy Sci* 97:4367-4378.
- Gonzalez FHD, Ruiperez FH, Sanchez JM, Souza JC, Martinez-Subiela S, Ceron JJ (2010) Haptoglobin and serum amyloid A in subacute ruminal acidosis. *Rev Med Vet Zoot* 57:159-167.
- Gruenberg W, Constable P (2009) Function and Dysfunction of the Ruminant Forestomach. in *Current Veterinary Therapy Elsevier Inc* pp. 12-19.
- Hamilton AW, Davison C, Tachtatzis C, Andonovic I, Michie C, Ferguson HJ, Somerville L, Jonsson NN (2019) Identification of the Rumination in Cattle Using Support Vector Machines with Motion-Sensitive Bolus Sensors. *Sensors* 19(5):1165. <https://doi.org/10.3390/s19051165>.
- Henning PH, Horn CH, Steyn DG, Meissner HH, Hagg FM (2010) The potential of *Megasphaera elsdenii* isolates to control ruminal acidosis. *Anim Feed Sci Technol* 157:13-19.
- Hollmann M, Miller I, Hummel K, Sabitzer S, Metzler-Zebeli BU, Razzazi-Fazeli E, Zebeli Q (2013) Downregulation of Cellular Protective Factors of Rumen Epithelium in Goats Fed High Energy Diet. *PLoS ONE* 8(12):e81602.
- Hutton PG, Durmic Z, Vercoe PE (2010) Investigating *Eremophila glabra* as a bioactive agent for preventing lactic acidosis in sheep. *Anim Prod Sci* 50:449-453.
- Ismail M, Mahmoud A, Nasr M, Badr Y (2010) Clinical and laboratory studies on experimentally induced acute ruminal lactic acidosis in male goats. *Alex J Vet Sci* 31:53-62.
- Jaramillo-Lopez E, Itzá-Ortiz M, Peraza-Mercado G, Chávez J (2017) Ruminal acidosis: Strategies for its control. *Austral J Vet Sci* 49:139-148.

- Jasmin BH, Boston RC, Modesto RB, Schaer TP (2011) Perioperative ruminal pH changes in domestic sheep (*Ovis aries*) housed in a biomedical research setting. *J Am Assoc Lab Anim Sci* 50:(1):27-32.
- Jassim ARAM, Gordon GLR, Rowe JB (2003) The effect of basal diet on lactate-producing bacteria and the susceptibility of sheep to lactic acidosis. *Anim* 77:459-469.
- Jiang XY, Ni YD, Zhang SK, Zhan YS, Shen XZ (2014) Identification of Differentially Expressed Proteins in Liver in Response to Sub- acute Ruminal Acidosis (SARA) Induced by High-concentrate Diet. *Asian-Australas J Anim Sci* 27:8:1181-1188.
- Kawas JR, Garcia-Castillo R, Fimbres-Durazo H, Garza-Cazares F, Hernandez-Vidal JFG, Olivares-Saenz E, Lu CD (2007) Effects of sodium bicarbonate and yeast on nutrient intake, digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. *Small Rumin Res* 67:149-156.
- Khafipour E, Krause DO, Plaizier JC (2009) Alfalfa pellet-induced sub- acute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. *J Dairy Sci* 92:1712-1724.
- Kilic U (2011) Use of Wireless Rumen Sensors in Ruminant Nutrition Research. *Asian J Anim Sci* 5:46-55. DOI: 10.3923/ajas.2011.46.55.
- Kirbas A, Baydar E, Kandemir FM, Dorman E, Kizil O, Yildirim BA (2014) Evaluation of serum cardiac troponin I concentration in sheep with acute ruminal lactic acidosis. *Vet Arh* 84:(4):355-364.
- Kitkas GC, Valergakis GE, Karatzias H, Panousis N (2013) Subacute ruminal acidosis: prevalence and risk factors in Greek dairy herds. *Iran J. Vet. Res.* 14:183-189.
- Kitkas GC, Valergakis GE, Kritsepi-Konstantinou M, Gelasakis A, Arsenos G, Kalaitzakis E, Panousis (2019) Effects of ruminal pH and subacute ruminal acidosis on milk yield and

- composition of Holstein cows in different stages of lactation. *J Hell Vet Med* 70:1551-1560.
- Kleen JL, Cannizzo C (2012) Incidence, prevalence and impact of SARA in dairy herds. *Anim Feed Sci Technol* 172:4-8.
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): A review. *J Vet Med A Physiol Pathol Clin Med* 50:406-414.
- Kleen JL, Uppang L, Rehage J (2013) Prevalence and consequences of subacute ruminal acidosis in German dairy herds. *Acta Vet Scand* 55:48.
- Krause KM, Oetzel GR (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim Feed Sci Technol* 126:215-236.
- Krotkova AP (1959) Effect of sugar beet on acidosis in the ruminant. *Zhivotnovodstvo* 7:63-64.
- Lammie SL, Hughes JM (2016) Antimicrobial resistance, food safety, and one health: The need for convergence. *Annu Rev Food Sci Technol* 7:287-312.
- Lean IJ, Annison F, Bramley E, Browning G, Cusack P, Farquharson B, Little S, Nandapi D (2007) Ruminal Acidosis- aetiopathogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR) A V Association ed Blackwell Publishing Asia Pty Ltd Carlton Vic Australia pp. 1-60.
- Lee M, Jeong S, Seo J, Seo S (2019) Changes in the ruminal fermentation and bacterial community structure by a sudden change to a high-concentrate diet in Korean domestic ruminants. *Asian-Australas J Anim Sci* 32:1:92-102.
- Lettat A, Nozière P, M Silberberg, Morgavi P, Berger C, Martin C (2010) Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. *J Anim Sci* 88:3041-3046.

- Lettat A, Nozière P, Silberberg M, Morgavi DP, Berger C, Martin C (2012) Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. *BMC Microbiol* 12:142.
- Li F, Cao Y, Liu N, Yang X, Yao J, Yan D (2014a) Subacute ruminal acidosis challenge changed in situ degradability of feedstuffs in dairy goats. *J Dairy Sci* 97:5101-5109.
- Li F, Yang XJ, Cao YC, li X, Yao JH, Li Z, Sun FF (2014b) Effects of dietary effective fiber to rumen degradable starch ratios on the risk of sub-acute ruminal acidosis and rumen content fatty acids composition in dairy goat. *Anim Feed Sci Technol* 189.
- Li L, He ML, Wang K, Zhang YS (2018) Buffering Agent via Insulin-Mediated Activation of PI3K/AKT Signaling Pathway to Regulate Lipid Metabolism in Lactating Goats. *Physiol Res* 67:753-764.
- Li S, Gozho G, Gakhar N, Khafipour E, Krause DO, Plaizier JC (2012) Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can J Anim Sci* 92:353-364.
- Lira MAA, Simões SVD, Riet-Correa F, Pessoa CMR, Dantas AFM, Miranda Neto EG (2013) Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui Vet Bras* 33:(2):193- 198.
- Liu J, Li H, Zhu W, Mao S (2019) Dynamic changes in rumen fermentation and bacterial community following rumen fluid transplantation in a sheep model of rumen acidosis: implications for rumen health in ruminants. *FASEB J* 33:8453-8467.
- Lopez-Campos O', Bodas R, Prieto N, Giraldez FJ, Perez V, Andres S (2010) Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim* 4:6:958-964.

- Ma N, Abaker JA, Bilal MS, Dai H, Shen X (2018) Sodium butyrate improves antioxidant stability in sub-acute ruminal acidosis in dairy goats. *BMC Vet Res* 14:275.
- Maekawa M, Beauchemin KA, Christensen DA (2002) Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *J Dairy Sci* 85(5):1165-75.
- Mahmood AK, Khan MS, Khan MA, Kahn MA, Bilal M, Farooq U (2013) Lactic Acidosis in Goats: Prevalence, Intra-ruminal and Haematological investigations. *J Anim Plant Sci* 23(6):1527-1531.
- McCann JC, Luan S, Cardoso FC, Derakhshani H, Khafipour E, Loores JJ (2016) Induction of Subacute Ruminal Acidosis Affects the Ruminal Microbiome and Epithelium. *Front Microbiol* 7:701.
- Meissner HH, Henning PH, Horn CH, Leeuw KJ, Hagg FM, Fouché G (2010) Ruminal acidosis: A review with detailed reference to the controlling agent *Megasphaera elsdenii* NCIMB 41125. *S Afr J Anim Sci* 40(2).
- Meyer NF, Bryant TC (2017) Diagnosis and Management of Rumen Acidosis and Bloat in Feedlots. *Vet Clin North Am Food Anim Pract* 33(3):481-498.
- Mialon MM, Deiss V, Andanson S, Anglard F, Doreau M, Veissier I (2012) An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anesthesia. *Vet J* 194:55-59.
- Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Jahan N, Bani P (2014) Experimental acute rumen acidosis in sheep: Consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. *J Anim Sci* published online July 1.

- Miranda Neto EG, Silva STG, Mendonça CL, Drummond ARF, Afonso JAB (2011) Aspectos clínicos e a bioquímica ruminal de caprinos submetidos à acidose láctica experimental e suplementados ou não com monensina sódica. *Pesqui Vet Bras* 31:416-424.
- Morgante M, Giancesella M, Casella S, Ravarotto L, Stelletta C, Giudice E (2009) Blood gas analyses, ruminal and blood pH, urine and faecal pH in dairy cows during subacute ruminal acidosis. *Comp Clin Path* 18:229-232.
- Nagaraja TG, Lechtenberg KF (2007) Acidosis in feedlot cattle. *Vet Clin Food Anim Pract* 23:333-350.
- Nagaraja TG, Titgemeyer EC (2007) Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci* 90(E. Suppl.):E17-E38.
- Nikvand AA, Nouri M, Razi Jalali M, Teimory Azadbakht MR (2020) Effects of activated charcoal and zeolite on serum lipopolysaccharides and some inflammatory biomarkers levels in experimentally induced subacute ruminal acidosis in lambs. *Turk J Vet Anim Sci* 44:830-837.
- Nocek JE (1997) Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005-1028.
- Nordlund KV, Garrett EF, Oetzel GR (1995) Herd based rumenocentesis: a clinical approach to the diagnosis of subacute rumen acidosis. *Compendium Contin Educ Pract Vet* 17:8:48-56.
- Nour MMS, Abusamra MT, Hago BED (1998) Experimentally induced lactic acidosis in Nubian goats: Clinical, biochemical and pathological investigations. *Small Rumin Res* 31:7±17.
- Nurmeiliasari N, Priyanto R, Astuti DA, Salundik, Takahashi J (2017) Utilization of Rumen Mechanical Stimulator as Pseudo Fiber in Ruminant to Minimize Metabolic Problem. *Wartazoa* 27:2:067-080.

- Odongo NE, Alzahhal O, Lindenger MI, Duffield TF, Valdes EV, Terrell SP, McBride BW (2006) Effects of mild heat stress and grain challenge on acid-base balance and rumen tissue histology in lambs. *J Anim Sci* 84:447-455.
- Oetzel GR (2003) Subacute ruminal acidosis in dairy cattle. *Advances in Dairy Cattle* 15:307-317.
- Oetzel GR (2017) Diagnosis and Management of Subacute Ruminal Acidosis in Dairy Herds. *Vet Clin North Am Food Anim Pract* 33:3:463- 480.
- Oliveira DM, Medeiros JMA, Assis ACO, Neves AB, Galiza GJN, Simões SVD, Dantas AFM, Riet-Correa F (2009) Acidose láctica ruminal agu-da em caprinos. *Ciênc Anim Bras* 117-122.
- Patra RC, Lal SB, Swarup D (1993) Physicochemical alterations in blood, cerebrospinal fluid and urine in experimental lactic acidosis in sheep. *Res Vet Sci* 54:217-220.
- Penner GB, Aschenbach JR, Gäbel G, Rackwitz R, Oba M (2009) Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to sub-acute ruminal acidosis in sheep. *J Nutr* 139:1714-1720.
- Pereira PFV, Romão FTNAM, Penzeti EM, Sanches JFZ, Curti JM, Flaiban KKMC, Lisboa JAN (2018) Value of transfaunation for the treatment of induced ruminal lactic acidosis in goats and sheep. *Pesqui Vet Bras* 38:(4):670-678.
- Petzold M, Meyer U, Spilke J, Danicke S (2014) Using rumen probes to examine effects of conjugated linoleic acids and dietary concentrate proportion on rumen pH and rumen temperature of periparturient dairy cows. *J Anim Physiol Anim Nutr* 98:785-796.
- Plaizier JC, Li S, Tun HM, Khafipour E (2017) Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Front Microbiol* 7:2128.

- Ploger S, Stumpff F, Penner GB, Schulzke JD, Gabel G, Martens H (2012) Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann N Y Acad Sci* 1258: 52-59.
- Pupin RC, Leal PV, Lima SC, Melo GKA, Pott A, Araújo MA, Barros CSL, Lemos RAA (2017) *Enterolobium contortisiliquum* is a cause of acute ruminal acidosis in sheep. *Toxicon* 126:90-95.
- Puvača N (2022) Bioactive Compounds in Dietary Spices and Medicinal Plants. *JATEM* 5:704-711.
- Puvača N, Tufarelli V, Giannenas I(2022) “Essential Oils in Broiler Chicken Production, Immunity and Meat Quality: Review of *Thymus vulgaris*, *Origanum vulgare*, and *Rosmarinus officinalis*” *Agriculture* 12(6):874.
- Radostits OM, Blood DC, Gay CC (1994) *Veterinary Medicine* 8th ed., Bailliere Tindall, London.
- Rahman MdM, Islam MS, Adam GO, Alam MdR, You MJ (2014) Prevalence of ruminal lactic acidosis and clinical assessments of four therapeutics in goats of Bangladesh. *J Vet Clin* 31:0-6.
- Reis LF, Araujo CASC, Sousa RS, Minervino AHH, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto Júnior RA, Mori CS, Ortolani EL (2018a) Prevention of acute ruminal lactic acidosis in sheep by probiotic or monensin supplementation: clinical aspects. *Semin Cienc Agrar* 39:4:1575-1584.
- Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Ortolani EL (2014) Comparison of Rumen Fluid pH by Continuous Telemetry System and Bench pH Meter in Sheep with Different Ranges of Ruminal pH. *Hindawi Publishing Corporation Scientific World Journal* Volume, Article ID 195782, 4 pages.

- Reis LF, Sousa RS, Oliveira FLC, Rodrigues FAML, Araújo CASC, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Minervino AHH, Ortolani EL (2018b) Comparative assessment of probiotics and monensin in the prophylaxis of acute ruminal lactic acidosis in sheep. *BMC Vet Res* 14:9.
- Ribeiro ACS, Conceição AI, Soares GSL, Correia FR, Oliveira-Filho JP, Soaresd PS, Mendonça CL, Afonso JAB (2020) Hemogasometry, cardiac biomarkers and blood metabolites in goats with experimentally induced acute ruminal lactic acidosis. *Small Rumin Res* 191:106187.
- Sabes AF, Girardi AM, Fagliari JJ, Oliveira JA, Marques LC (2017) Serum proteinogram in sheep with acute ruminal lactic acidosis. *Int J Vet Sci Med* 5:1:35-40.
- Sato S, Ikeda A, Tsuchiya Y, Ikuta K, Murayama I, Kanehira M, Okada K, Mizuguchi H (2012) Diagnosis of subacute ruminal acidosis (SARA) by continuous reticular pH measurements in cows. *Vet Res Commun* 36:201-205.
- Shen Y, Zhao F, Yu L, Yang W, Wang M, Wang H (2019a) Starch sources and concentration in diet of dairy goats affected ruminal pH and fermentation, and inflammatory response. *Anim Prod Sci* 59:9:1640- 1647.
- Shen YZ, Ding LY, Chen LM, Xu JH, Zhao R, Yang WZ, Wang HR, Wang MZ (2019b) Feeding corn grain steeped in citric acid modulates rumen fermentation and inflammatory responses in dairy goats. *Anim* 13:2:301-308.
- Shu Q, Gill HS, Leng RA, Rowe JB (2000) Immunization with *Streptococcus bovis* vaccine administered by different routes against lactic acidosis in sheep. *Vet J* 159:262-269.
- Smith BP (2009) Large animal internal medicine: diseases of the alimentary system. In: Murray MJ, Guard C editors. *Large animal internal medicine* 4th ed New York (NY): Mosby.
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33:(3):451-461.

- Steele MA, AlZahal O, Hook SE, Croom J, McBride BW (2009) Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet Scand* 51:39.
- Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K, Mc- Bride BW (2011) Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am J Physiol Regul Integr Comp Physiol* 300:6:1515-1523.
- Stefańska B, Nowak W, Komisarek J, Taciak M , Barszcz M, Skomial J (2016) Prevalence and consequence of subacute ruminal acidosis in polish dairy herds. *J Anim Physiol Anim Nutr* 101(4):694-702.
- Stock R (2000) Acidosis in cattle: an overview. In: *Proceedings of the 33rd Annual Convention of the American Association of Bovine Practitioners*, Rapid City USA pp. 30-37.
- Stock R, Britton RL (1996) Acidosis. *Neb Guide*, University of Nebraska-Lincoln. October 1995 <[http://www.ianr.unl.edu/pubs/ animaldisease/g1047.htm](http://www.ianr.unl.edu/pubs/animaldisease/g1047.htm)> (assessed 27.11.99).
- Suárez VH, Buseti MR (2009) Health management practices and disease prevalence in dairy sheep systems in Argentina. *Pesqui Vet Bras* 29(11):931-937.
- Sun YY, Cheng M, Xu M, Song LW, Gao M, Hu HL (2018) The effects of subacute ruminal acidosis on rumen epithelium barrier function in dairy goats. *Small Rumin Res* 169:1-7.
- Tadepalli S, Narayanan SK, Stewart GC, Chengappa MM, Nagaraja TG (2009) *Fusobacterium necrophorum*: A ruminal bacterium that invades liver to cause abscesses in cattle. *Anaerobe* 15:36-43.
- Taghipoor M, Delattre M, Giger-Reverdin S (2020) A novel modelling approach to quantify the response of dairy goats to a high-concentrate diet. *Sci Rep* 10:20376.

- Tajik J, Nazifi S (2011) Serum Concentrations of Lipids and Lipoproteins and Their Correlations together and with Thyroid Hormones in Iranian Water Buffalo (*Bulbalus bulbalis*). *Asian J Anim Sci* 5:196-201.
- Tian P, Luo Y, Li X, Tian J, Tao D, Hua C, Geng Y, Ni Y, Zhao R (2017) Negative effects of long-term feeding of high-grain diets to lactating goats on milk fat production and composition by regulating gene expression and DNA methylation in the mammary gland. *J Anim Sci Biotechnol* 8:74.
- Vasconcelos JT, Galyean ML (2008) ASAS centennial paper: contributions in the Journal of Animal Science to understanding cattle metabolic and digestive disorders. *J Anim Sci* 86:1711-1721.
- Wales WJ, Williams YJ, Doyle PT (2001) Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial ryegrass pastures. *Aust J Exp Agric* 41:465-71.
- Wang K, Zheng M, Ren A, Zhou C, Yan Q, Tan Z, Zhang P, Yi K (2019) Effects of High Rice Diet on Growth Performance, Nutrients Apparent Digestibility, Nitrogen Metabolism, Blood Parameters and Rumen Fermentation in Growing Goats. *Kafkas Univ Vet Fak Derg* 25:(6):749-755.
- Wang Y, Liu J, Huang J, Chang G, Roy AC, Gao Q, Cheng X, Shen X (2020) Sodium butyrate attenuated iE-DAP induced inflammatory response in the mammary glands of dairy goats fed high-concentrate diet. *J Sci Food Agric* 101(3):1218-1227.
- White BH, Clayton EH, Friend MA (2014) The effect of mechanical processing of wheaten straw on intake and rumen parameters following grain feeding in sheep. *Anim Prod Sci* 54:1455-1459.
- Wiryawar KG, Brooker JD (1995) Probiotic control of lactate accumulation in acutely grain-fed sheep. *Aust J Agric Res* 46:1555-68.

- Xia G, Sun J, Fan Y, Zhao F, Ahmed G, Jin Y, Zhang Y, Wang H (2020) β -Sitosterol Attenuates High Grain Diet-Induced Inflammatory Stress and Modifies Rumen Fermentation and Microbiota in Sheep. *Anim* 10:171.
- Xu C, Shen T, Yang W, Yu H, Gao S, Huang B (2016) The effect of sub- acute ruminal acidosis of dairy cows on productivity, digestibility and greenhouse gas emission. *J Agric Sci* 8:92.
- Xu T, Tao H, Chang G, Zhang K, Xu L, Shen X (2015) Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet Res* 11:52.
- Yansari AT, Valizadeh R, Naserian A, Christensen DA, Yu P, Shahroodi FE (2004) Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. *J Dairy Sci* 87:3912-3924.
- Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W (2012) Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *J Dairy Sci* 95:1041-1056.
- Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, Wang HR (2020) Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 103:1931-1943.

CHAPTER II

RESEARCH

Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle?

1. Introduction

High-concentrate diets find common usage in both dairy and beef industries to enhance production efficiency. Nonetheless, this practice can precipitate ruminal acidosis (RA) due to the rapid reduction of ruminal pH (Plaizier et al., 2012; Abaker et al., 2017). RA manifests as acute (ARA) or sub-acute (SARA) categories based on pH reduction and clinical manifestations (Aschenbach et al., 2011). Within intensive livestock farming, SARA is among the important nutritional disorders due to its potential for substantial welfare and economic repercussions. These encompass the deterioration of milk quality and quantity, diminished feed intake, weight loss, formation of liver abscesses, and elevated culling rates (Kleen et al., 2003; Krause and Oetzel, 2006; Enemark, 2008).

The task of diagnosing and confirming SARA proves to be intricate due to the absence of pathognomonic symptoms and precise diagnostic techniques (Nocek, 1997; Tajik and Nazifi, 2011; Snyder and Credille, 2017). Field diagnosis of SARA primarily relies on measuring rumen fluid pH, whereby a rumen pH below 5.5–5.8 indicates the presence of SARA (Krause and Oetzel, 2006; Plaizier et al., 2008; Humer et al., 2018). However, this pH measurement is subject to various influencing factors, including the method of measurement, sampling location, and timing of sampling (Neubauer et al., 2017; Villot et al., 2017).

Numerous researchers have noted a connection between the gray to dark discoloration of the rumen wall epithelium during necropsies of ruminants or culling and subacute and acute rumenitis (Enemark, 2008). Nonetheless, an accurate interpretation of this coloration presents a challenge. Visual interpretation can be susceptible to bias, whereas instrumental colorimetric methods lack the capability for remote analysis and are particularly sensitive to the size of the colorimeter and spectrophotometer apertures (Yancey and Kropf, 2008). Offering a practical and dependable alternative, computerized Red, Green, and Blue (RGB) analysis has found application in studies within the food industry (Chmiel et al., 2012; Silva et al., 2022).

This study seeks to establish a correlation between rumen epithelial color and SARA status in feedlot cattle, as determined through long-term periodic evaluations of rumen pH. The primary goal of this study was to assess the viability of computerized rumen colorimetry as a diagnostic tool for SARA within the context of herd management for feedlot cattle.

2. Materials and Methods

2.1. Selection of farms

A total of 21 beef farms located in Thessaly, central Greece, were preselected for the study. These farms raise a breed of beef known as the “Greek Red Breed,” an adapted variant of the Limousin breed. The final selection was based on their ration and three-year medical history, extracted from clinical case records at the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly. The 21 pre-selected beef farms were divided into 2 categories: farms with no previous history of ARA (2 farms) and farms with a history of ARA (19 farms). Among the latter, 3 subgroups were formed based on the percentage of digestive fiber in their ration: (i) 5 farms with less than 15% Neutral Detergent Fiber (NDF); (ii) 8 farms with 15–20% NDF; and (iii) 6 farms with 20–25% NDF. The study encompassed all farms with no prior history of ARA (Category I, 2 farms) and a random selection of 3 farms with a history of ARA from each of the 3 subgroups (Category II, 3 farms), using computer-generated random numbers. Table 1 presents a breakdown of the feed composition for the 5 farms included in the study. There were no modifications to the dietary composition over the course of the 12-month trial period.

Table 1 Ingredients, Chemical Composition, and Forage-to-Concentrate Ratio (F:C) in Total Mixed Rations for Farm 1 and Farm 2 (Category I), and Farms 3, 4, and 5 (Category II)

Item	Farm 1 Diet	Farm 2 Diet	Farm 3 Diet	Farm 4 Diet	Farm 5 Diet
Ingredients (% of DM)					
Alfalfa silage	20.0	15.0		20.0	19.0
Corn Silage	15.0	10.0	20.0	5.0	11.0
Wheat straw	5.0	15.0			
Ground Corn	25.0	20.0	60.0	40.0	40.0
Ground wheat			5.0		7.5
Ground barley	15.0	18.0		20.0	10.0
Soybean meal	12.0	14.5	12.0	12.5	10.0
Wheat Bran	5.0	5.0			
Vitamins and minerals	3.0	2.5	3.0	2.5	2.5
F:C	40:60	40:60	20:80	25:75	30:70
Chemical composition					
ME (Mcal/kg DM)	0.25	0.24	0.27	0.26	0.26
CP (% of DM)	16.1	15.2	14.0	16.3	15.2
NDF (% of DM)	31.9	34.4	14.8	22.2	23.2
ADF (% of DM)	18.3	20.7	9.1	12.2	12.9

ME = Metabolizable energy, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

2.2. Experimental design

In each of the 5 participating beef farms, 15 cattle aged 8–10 months were subject to periodic monitoring every 2 weeks for rumen pH over the course of 1 year before slaughter (from September 2021 to August 2022). Inclusion criteria encompassed a body weight range of 220–246 kg (with an initial body weight of 233 ± 13 kg, $n = 75$) and an absence of prior medical history. Measurement of rumen pH involved sampling the rumen content after esophageal tubing, specifically 3 h after the morning administration of concentrated feed. The average pH of all measurements taken throughout the year (24 measurements per animal) was designated as pH_{farm}. Each animal was distinguished with distinct national registration numbers affixed to plastic ear-tags on both ears. All animals received uniform preventive medication, including vaccination and antiparasitic drugs.

The 15 monitored cattle on each farm were co-housed with an additional 4–12 cattle of the same age range (8–10 months at the onset of monitoring). All calves within these enclosures were slated for slaughter in September 2022, attaining an age of 20–22 months. During the

slaughterhouse phase, samples of rumen wall and rumen fluid were procured from the monitored animals designated for regular production cycle slaughter. It is important to note that no animals were slaughtered exclusively for experimental purposes.

At the time of slaughter, all 75 monitored animals were sampled. Directly following evisceration, samples of rumen content and rumen wall were collected. The pH of rumen fluids was measured, the color of the rumen epithelium was visually assessed, and a digital gross image of the rumen wall epithelium was captured. The recorded pH measurement at this juncture was denoted as pH_{abattoir}.

A total of 40 abdominal wall samples (20 from Category I and 20 from Category II) were submitted for histological examination at the Pathology Laboratory, Veterinary School, Aristotle University of Thessaloniki. The 40 rumen samples submitted were sourced from the initial 20 follow-up animals from each farm category sent to the slaughterhouse.

2.3. Sample size justification

According to the literature, the range of SARA prevalence is relatively wide from 14 to 42% (Nagaraja and Lechtenberg, 2007). In Greece, there is limited information about the prevalence of SARA (Kitkas et al., 2013). An average value of 22% prevalence was selected in the sample size calculation enhanced by the fact that animals were partly selected by farms with a previous history of ARA. Therefore, a sample size of 66 animals was calculated with precision 0.1 and confidence level of 95% to which 10% were added for any sample depreciation (Sergeant, 2018). Based on this calculation, the 75 samples investigated in this study were considered an adequate sample size.

2.4. Esophageal tubing (Rumen scoop)

Rumen fluid sampling was conducted using the Rumen Scoop (Flora, profs-products.com, Futtermittelüberwachung Bayern Regierung von Oberbayern, Sachgebiet 56, 80,534 München) (Geishauser et al., 2012). This all-metal device featured a 2.7-meter-long hose with a head and handle on each end. The head, functioning as a fluid scoop, was a cylindrical container measuring 3 cm in diameter and 21 cm in length, with a 40 mL capacity and side openings controlled by a screw mechanism.

To collect the rumen fluid, the head was inserted through the esophagus into the rumen for 10 to 20 s, allowing the fluid to be naturally drawn into the head without applying a vacuum. Subsequently, the handle activated the closing mechanism of the openings, and the device was carefully withdrawn. The head was then unscrewed and emptied. The gathered rumen fluid was immediately analyzed for pH using a digital portable pH meter (HI-2002 Edge pH Meter, Hanna instruments).

2.5. Abattoir work protocol

Prior to slaughter, cattle were subjected to an approximate 3-hour fasting period, during which they underwent a comprehensive physical examination. They were marked with spray paint on the fleece for monitoring from slaughter to evisceration. After evisceration, each rumen was re-marked with a labeled cable before entering the gut room, where it was separated by slaughterhouse staff, following the procedure described by Jonsson et al., (2020).

The rumen fluid sample was obtained through aspiration, using a 16G 105-mm needle and a 50 mL syringe, from the eviscerated rumen. Subsequently, it was drained into a sterile glass container with a capacity of 100 mL. The pH of the rumen content was promptly measured

using the same digital portable pH meter mentioned earlier (HI-2002 Edge pH Meter, Hanna instruments).

The procedure for sampling the rumen wall involved specific steps. Firstly, the rumen was completely emptied of its contents. Next, the reticulorumen was oriented left-facing, with the reticulum in the front, the large rumen behind, the dorsal ruminal sac cranially, and the ventral ruminal sac caudally, with the esophagus positioned in front and dorsally. The location for the incision was determined based on standard measurements (Fig. 1), and a 5×5 cm square of the rumen wall was extracted for sampling. Subsequently, the sample was thoroughly washed with water to remove any visible residues of the rumen content. After cleaning, the sample epithelium was visually assessed, and was graded based on its color using a three-tiered score: (a) white or pale white (score 0), (b) grey (score 1), and (c) dark grey or black (score 2) (Fig. 2). Finally, a digital gross image was obtained following the standard procedure and the biopsy sample was placed in a container with a formalin solution for histological examination.

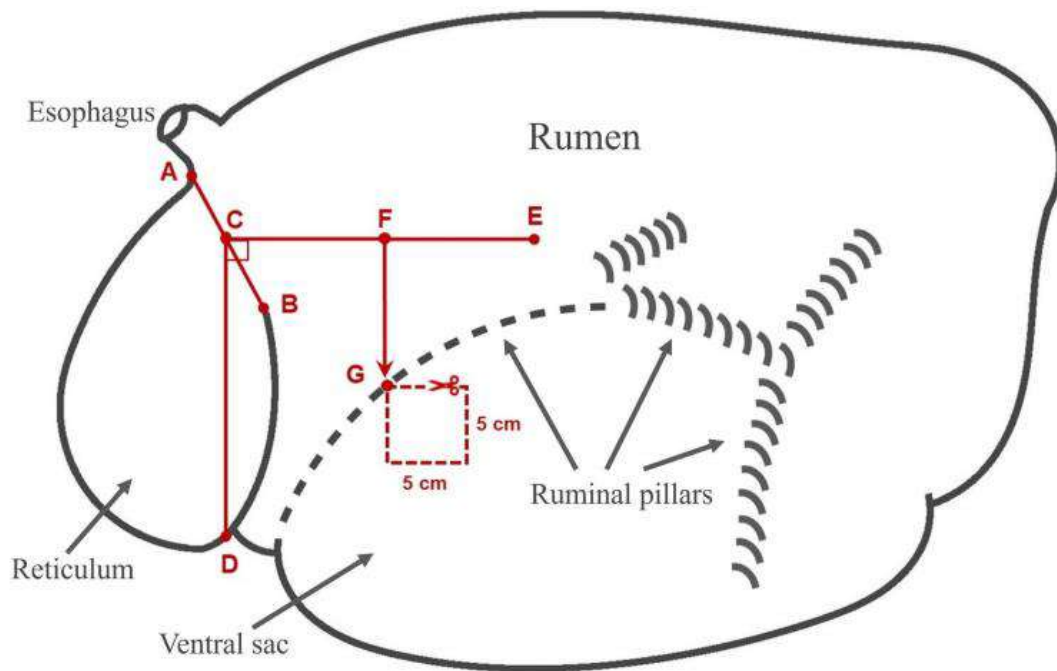


Fig. 1 The procedure for sampling the rumen wall was performed following specific standard seven steps: (i) Commence by drawing a straight line between point A, the lower point of esophageal adhesion, and point B, the dorsal-caudal reticulorumen adhesion point. (ii) Locate the midpoint of line AB and designate it as point C. (iii) Draw a vertical line crossing point C and mark point D, where the vertical line intersects the edge of the reticulum. Measure the distance CD. (iv) Find point E in a manner that ensures the distance from C to E is equal

to the distance from C to D and also ensures that angle DCE forms a right angle. (v) Identify the midpoint of line CE and label it as point F. (vi) Determine the point on the rumen pillar where a vertical line from point F intersects with the ventral pillar and mark it as point G. (vii) We now have the sampling region defined: it will be a square in the ventral ruminal sac, with the cranial side extending from the FG and point G as its upper-left corner (cranial-dorsal corner). The square should have dimensions of 5 cm x 5 cm



Fig. 2 A three-tiered scale for visually assessing the rumen epithelium: (a) white/pale white (score 0); (b) grey (score 1); (c) dark grey or black (score 2)

2.6. Image acquisition and color measurement

For image acquisition, the collected samples were placed on a specific desk, and digital gross images were taken. A NIKON COOLPIX L330 digital camera with a 60-mm macro lens was used, with standard lighting conditions and settings. The images were saved in raw format.

The quantification of rumen discoloration was performed through software applications, namely RGB (RGB Color Detector, 2022) and CIELab (CIE, 1986). Within the RGB application, computations were made for color components encompassing Red, Green, and Blue (RGB), as well as Hue, Saturation, and Value (HSV), alongside Hue, Saturation, and Lightness (HSL). The determination of color components L (color lightness), a (redness-greenness of the color), and b (yellow-blueness of the color) was carried out utilizing CIELab (CIE, 1986). For every rumen wall sample, the average values of these color components were computed from 9 distinct spot measurements, serving to facilitate subsequent statistical analysis. It is important to highlight that while the L, a, and b values are technically denoted as L*, a*, and b* respectively, for the sake of simplicity, this text omits the asterisks.

To distinguish between Category I and Category II, the color difference Delta E (ΔE) was calculated using the average values of L, a, and b color components. The following equation was used:

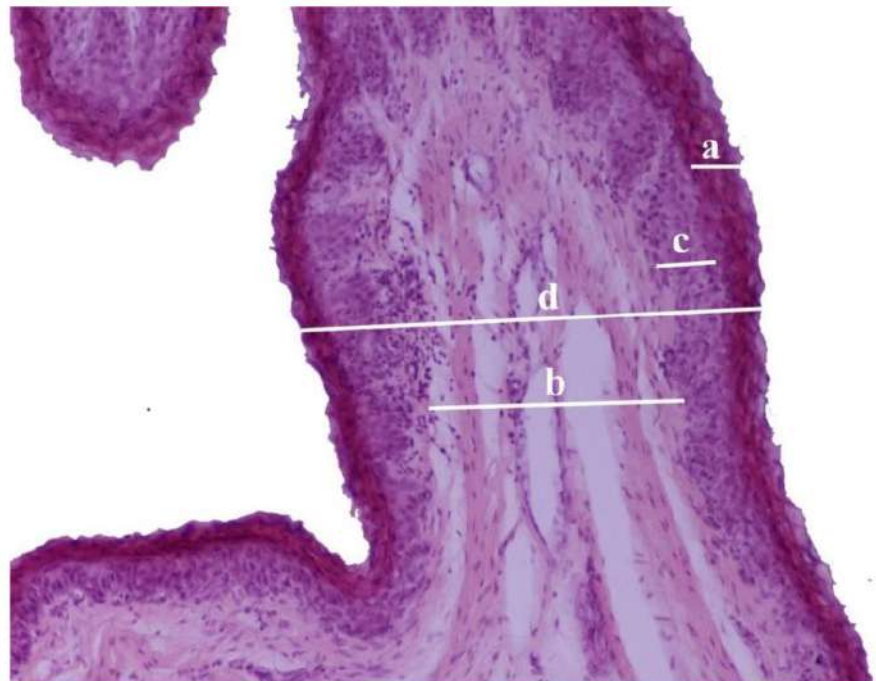
$$\Delta E = \sqrt{(L_I - L_{II})^2 + (a_I - a_{II})^2 + (b_I - b_{II})^2}$$

Where ΔE = color difference; L_I , a_I , b_I = mean values of the color components determined for Category I, and L_{II} , a_{II} , b_{II} = mean values of the color components determined for Category II. ΔE is measured on a scale from 0 to 100, where 0 indicates less color difference, and 100 indicates complete distortion (Brainard, 2003).

2.7. Histological examination

For histological examinations, the rumen wall samples were cut into 1×1 cm particles and fixed in 10% formalin buffer for 48 to 72 h. Afterward, they were dehydrated using alcohol solutions and routinely embedded in paraffin (Paraplast Plus®, Kendall, England). Dewaxed 3–5 μm -thick sections were stained with hematoxylin and eosin (H&E) for histopathological evaluation. From each sample, randomly selected fields with a x10 objective were captured with a microscope-coupled camera, and ImageJ software was used to measure stratum corneum thickness, connective tissue width, non-keratinized epithelium thickness, and total epithelium thickness in each photomicrograph (Fig. 3).

Fig. 3 Measurements of the beef ruminal papillae: (a) Stratum corneum thickness. (b) Width of the connective tissue. (c) Thickness of the non-keratinized epithelium. (d) Total epithelium thickness. (Hematoxylin-eosin staining; magnification x10)



2.8. Statistical analysis

Data on continuous variables were checked for normal distribution, and appropriate log transformations were applied to address skewness. A t-test was conducted to assess differences in pH values, color measurements, and rumen epithelium characteristics between the two animal categories. The mean pH status was calculated by averaging the measurements taken every 2 weeks for the continuously monitored animals. The chi-square test for independence, a non-parametric statistical procedure, was used to compare visual (categorical) data. Data analysis was performed using IBM SPSS Statistics version 29.0.0.0. (241), and statistical significance was set at either the 0.001 or 0.05 level.

3. Results

All animals successfully completed the study, and prior to sampling at the slaughterhouse, all animals underwent clinical examination with no evidence of acute acidosis or other clinical disease recorded.

The monitored animals had a pH_{farm} range from 5.27 to 6.15, with a mean value of 5.80 (Standard Error [SE] = 0.020). Animals from farms with previous cases of ARA had a pH range of 5.27 to 5.90, with a mean value of 5.72 (SE = 0.023). In contrast, animals from farms without ARA cases had a pH range of 5.70 to 6.15, and their mean pH was 5.93 (SE = 0.021, $p < 0.001$).

Similarly, in the abattoir, the mean $\text{pH}_{\text{abattoir}}$ value of Category I samples was 5.97 (SE = 0.023), which was statistically higher than the mean value of Category II samples (5.74, SE = 0.026, $p < 0.001$) (Table 2).

Table 2 Comparison of pH_{farm} , pH_{abattoir} , and color components (mean $\pm$ SD) between two categories of farms: Category I (without SARA) and Category II (with SARA) farms

	Category I (n = 30)	Category II (n = 45)	p value	95% Confidence Interval of the difference
pH_{farm}	5.93 (0.12)	5.72 (0.15)	< 0.001***	0.14 to 0.28
pH_{abattoir}	5.97 (0.13)	5.74 (0.18)	< 0.001***	0.16 to 0.30
L	48.1 (11.7)	29.62 (10.3)	< 0.001***	13.3 to 23.5
a	-2.9 (9.1)	0.55 (5.1)	0.420	0.13 to 6.7
b	14.5 (13.2)	0.77 (5.1)	< 0.001***	9.36 to 18.0
Red	118.1 (31.0)	71.4 (23.0)	< 0.001***	34.1 to 59.1
Green	115.3 (29.4)	70.1 (23.5)	< 0.001***	32.9 to 57.4
Blue	89.9 (33.8)	69.5 (23.8)	0.003*	7.2 to 33.7
RGB-SUM	323.3 (85.0)	211.1 (69.0)	< 0.001***	76.6 to 147.8
HSV	20.1 (26.6)	19.9 (16.4)	0.968	9.7 to 10.1
HSL	40.1 (13.5)	25.6 (11.5)	< 0.001***	8.7 to 20.3

*Significant difference at $p \leq 0.05$

***Significant difference at $p \leq 0.001$

L: color lightness

a: redness-greenness of the color

b: yellow-blueness of the color

HSV: Hue, Saturation and Value

HSL: Hue, Saturation, and Lightness

3.1. Color measurements

The chi-square test for visual examination showed significant differences, with a higher percentage of grey and black discoloration in Category II compared to Category I ($p = 0.044$) (Table 3).

Table 3 Comparison of the visual examination results between Category I (Farms without SARA) and Category II (Farms with SARA) [number of samples (percentage)]

	Category I (n = 30)	Category II (n = 45)	p value	Pearson Chi-Square
White/Pale White	27 (90.0%)	29 (64.4%)	0.044*	6.225
Grey	2 (6.7%)	10 (22.2%)		
Dark Grey/Black	1 (3.3%)	6 (13.3%)		

*Significant difference at $p \leq 0.05$

The average value of the L component in Category I samples was 48.1 units (SE = 2.14), which was significantly ($p < 0.001$) higher than the value for Category II (29.6 units, SE = 1.53); a similar trend was observed for the b component. However, there was no significant difference in the average value of the a component (Table 2).

The color difference (ΔE) between Category I and Category II rumen wall samples was calculated using the L, a, and b color components. A significant color difference ($\Delta E = \sim 13.2177$) was found between the 2 categories (Table 2).

Concerning the color measurement using the RGB Analysis system, Category I samples exhibited significantly higher mean values for Red, Green, Blue, and RGB-SUM in comparison to Category II samples. Additionally, significant differences were observed for the L value, but not for the V value (Table 2).

3.2. Rumen epithelium histopathology and histomorphometry

Histological examinations showed no pathological findings in the rumen epithelium samples, and there were no signs of parakeratosis. In samples with the darkest coloration, the dark color was limited to the keratinized layer.

Based on the results, Category II exhibited significantly greater thickness in 2 key measurements: Non-keratinized epithelium thickness (mean 131.5, SE = 9.94) and Total

epithelium thickness (mean 278.1, SE = 12.37), in contrast to Category I, where these measurements were lower (mean 88.3, SE = 5.48 and mean 237.1, SE = 9.40, respectively). In contrast, no significant disparities were observed in stratum corneum thickness and connective tissue width (Table 4).

Table 4 Comparison of histological measurements in the layers of the ruminal epithelium (mean $\pm$ SD) among rumen samples from Category I (without SARA) and Category II (with SARA) farms

	Cat- egory I (n=20)	Category II (n=20)	p value	95% confi- dence interval of the difference
Stratum corneum (μ m)	28.8 (10.1)	30.0 (10.7)	0.72	-5.4 to 7.8
Connective tissue (μ m)	120.1 (30.2)	116.7 (32.0)	0.73	-23.3 to 16.5
Non-keratinized epithelium (μ m)	88.3 (24.5)	131.5 (44.4)	0.001***	20.2 to 66.2
Total epithelium (μ m)	237.1 (42.1)	278.1 (55.3)	0.012*	9.5 to 72.5

*Significant difference at $p \leq 0.05$

***Significant difference at $p \leq 0.001$

4. Discussion

SARA is defined as increased periods of rumen pH under 5.6–5.8 (Kleen et al., 2003; Krause and Oetzel, 2006). The animals in Category II showed a mean ruminal pH value lower than 5.8, indicating the presence of subacute ruminal acidosis (SARA). In contrast, animals in Category I had a mean ruminal pH value above 5.8, indicating the absence of SARA.

Sampling for pH measurements using the Rumen Scoop has generated concerns about its accuracy among veterinary clinicians. Nevertheless, this device has proven its precision in specific research studies, as exemplified by Geishauser et al., (2012), and has consistently delivered reliable results in our clinical practice. Additionally, taking into account animal

welfare concerns, it's crucial to highlight that esophageal tubing stands as the sole non-invasive method for collecting rumen fluid samples. Therefore, for our current research, we have chosen this method, giving precedence to animal welfare considerations.

The mean values of color components, regardless of the evaluation method (RGB or CIELab), were significantly lower in Category II samples, indicating the presence of dark coloration in the ruminal epithelium. This finding was consistent with the results of Alhidary et al. (2016), where a correction of the ratio in lambs resulted in more light-colored epithelium. Other researchers have observed grey to dark ruminal epithelium due to parakeratosis resulting from lowered pH and increased accumulation of volatile fatty acids (VFAs) in rumen fluid (Enemark, 2008; Steele et al., 2009, 2011). However, in our study, we observed an increase in non-keratinized rumen epithelium in Category II animals without parakeratosis. The darkening of the rumen epithelium's color should be attributed to the effect of rumen pH on the keratinized layer of the epithelium.

The animals in Category II had increased non-keratinized epithelium thickness and total epithelium thickness compared to Category I. The thickness of the non-keratinized epithelium showed the strongest correlation with the average pH_{farm}. It is possible that the lower rumen pH may increase the rate of replacement of the keratinized epithelium, leading to an increase in the non-keratinized epithelium to correspond to the need of producing keratinized layers.

5. Conclusions

This study affirms the potential utility of computerized rumen colorimetry in investigating subacute ruminal acidosis (SARA) in beef cattle. The availability of portable computing devices equipped with color analysis software offers a convenient, swift, and dependable means of assessing the chromatic variation in rumen epithelium. This assessment can be conducted

during exploratory necropsy or at the abattoir, rendering it a valuable resource for monitoring the effectiveness of management, nutritional, and preventive interventions within a beef farm setting. To fully explore the prospective application of computerized rumen colorimetry as a diagnostic instrument for live animals, further research is warranted, involving the utilization of a gastroscope.

References

- Abaker JA, Xu TL, Jin D, Chang GJ, Zhang K, Shen XZ (2017) Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J Dairy Sci* 100:666–678. <https://doi.org/10.3168/jds.2016-10871>
- Alhidary I, Abdelrahman MM, Alyemni AH, Khan RU, Al-Mubarak AH, Albaadani HH (2016) Characteristics of rumen in Naemi lamb: morphological changes in response to altered feeding regimen. *Acta Histochem* 118:331–337. <https://doi.org/10.1016/j.acthis.2016.03.002>
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2011) Ruminant Nutrition Symposium: role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89:1092–1107. <https://doi.org/10.2527/jas.2010-3301>
- Brainard DH (2003) 5-Color appearance and Color Difference Specification. In: Shevell SK (ed) *The Science of Color*, 2nd edn. Elsevier, Optical Society of America, Amsterdam, Boston, pp 191–216
- Chmiel M, Slowinski M, Dasiewicz K, Florowski T (2012) Application of a computer vision system to classify beef as normal or dark, firm, and dry. *J Anim Sci* 90:4126–4130. <https://doi.org/10.2527/jas.2011-5022>
- CIE (1986) Colorimetry: official recommendations of the international commission on illumination. CIE Publ. No. 15.2. CIE Central Bureau, Vienna
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): a review. *Vet J* 176(1):32–43. <https://doi.org/10.1016/j.tvjl.2007.12.021>
- Geishauser T, Linhart N, Neidl A, Reimann A (2012) Factors associated with ruminal pH at herd level. *J Dairy Sci* 95:4556–4567. <https://doi.org/10.3168/jds.2012-5380>
- Humer E, Aschenbach JR, Neubauer V, Kröger I, Khiaosaard R, Baumgartner W, Zebeli Q (2018) Signals for identifying cows at risk of subacute ruminal acidosis in dairy

- veterinary practice. *J Anim Physiol Anim Nutr* 102:380–392. <https://doi.org/10.1111/jpn.12850>
- Jonsson NN, Ferguson HJ, Koh-Tan HH, McCartney CA, Cernat RC, Strachan EM et al (2020) Postmortem observations on rumen wall histology and gene expression and ruminal and caecal content of beef cattle fattened on barley-based rations. *Animal* 14:1447–1460. <https://doi.org/10.1017/S1751731119002878>
- Kitkas GC, Valergakis G, Karatzias H, Panousis N (2013) Subacute ruminal acidosis: prevalence and risk factors in greek dairy herds. *Iran J Vet Res* 14:183–189. <https://doi.org/10.22099/IJVR.2013.1679>
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): a review. *J Vet MedAPhysiol Pathol Clin Med* 50:406–414. <https://doi.org/10.1046/j.1439-0442.2003.00569.x>
- Krause KM, Oetzel GR (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: a review. *Anim Feed Sci Technol* 126:215–236. <https://doi.org/10.1016/j.anifeedsci.2005.08.004>
- Nagaraja TG, Lechtenberg KF (2007) Acidosis in feedlot cattle. *Vet Clin Food Anim Pract* 23:333–350. <https://doi.org/10.1016/j.cvfa.2007.04.002>
- Neubauer V, Humer E, Kröger I, Braid T, Wagner M, Zebeli Q (2017) Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J Anim Physiol Anim Nutr* 102(1):343–349. <https://doi.org/10.1111/jpn.12675>
- Nocek JE (1997) Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005–1028. [https://doi.org/10.3168/jds.S0022-0302\(97\)76026-0](https://doi.org/10.3168/jds.S0022-0302(97)76026-0)

- Plaizier JC, Krause DO, Gozho GN, McBride BW (2008) Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Vet J* 176:21–31. <https://doi.org/10.1016/j.tvjl.2007.12.016>
- Plaizier JC, Khafipour E, Li S, Gozho GN, Krause DO (2012) Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Anim Feed Sci Technol* 172:9–21. <https://doi.org/10.1016/j.anifeedsci.2011.12.004>
- RGB Color Detector (2022) Available from: [com.TheProgrammer](http://com.TheProgrammer.com). RGB Color Detector (Version 3.0.7). Accessed 10 Oct 2022
- Sergeant ESG (2018) Epitools Epidemiological Calculators. Ausvet. Available at: <http://epitools.ausvet.com.au>. Accessed 15 August 2021
- Silva EPE, Moraes EP, Anaya K, Silva YMO, Lopes HAP, Andrade Neto JC et al (2022) Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. *PLoS ONE* 7(2):e0263714. <https://doi.org/10.1371/journal.pone.0263714>
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33:451–461. <https://doi.org/10.1016/j.cvfa.2017.06.003>
- Steele MA, AlZahal O, Hook SE, Croom J, McBride BW (2009) Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet Scand* 51:39. <https://doi.org/10.1186/1751-0147-51-39>
- Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K et al (2011) Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am J Physiol Regul Integr Comp Physiol* 300:1515–1523. <https://doi.org/10.1152/ajpregu.00120.2010>
- Tajik J, Nazifi S (2011) Serum concentrations of lipids and lipoproteins and their correlations together and with thyroid hormones in Iranian Water Buffalo (*Bubalus bubalis*). *Asian J Anim Sci* 5:196–201. <https://doi.org/10.3923/ajas.2011.196.201>

- Villot C, Meunier B, Bodin J, Martin C, Silberberg M (2017) Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal* 12:481–490. <https://doi.org/10.1017/S1751731117001677>
- Yancey JWS, Kropf DH (2008) Instrumental reflectance values of fresh pork are dependent on aperture size. *Meat Sci* 79:734–739. <https://doi.org/10.1016/j.meatsci.2007.11.006>

CHAPTER III

RESEARCH

Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management

1. Introduction

In high-yield livestock farming, rumen acidosis is a common clinical issue (Penner et al., 2009; Xia et al., 2020). It occurs in two forms: acute, characterized by a rumen pH below 5.0, and sub-acute (SARA), with a pH consistently lower than 5.8 for at least four hours per day (Church, 1991; Commun et al., 2009; Chaudhry et al., 2018). In contrast to acute rumen acidosis, SARA does not cause acute rumenitis, and any potential mucosal degenerative changes either do not occur or are reversible. However, SARA still leads to economic losses due to reduced milk production and lower milk fat content (Chaudhry et al., 2018; Voulgarakis et al., 2023).

Feeding high-concentrate diets with low percentages of dietary fiber for extended periods increases the risk of subacute rumen acidosis, negatively affecting both milk production and fat concentration (Desnoyers et al., 2008; Xu and Ding, 2011; Xu et al., 2015). The subsequent lower rumen pH disrupts fermentation and bacterial synthesis, leading to increased production of long-chain fatty acids (LCFA), which hinders milk fat synthesis in the udder (Li et al., 2012; Kitkas et al., 2019).

Several hematobiochemical parameters have been suggested as diagnostic markers for SARA in small ruminants, but none have proven entirely accurate (Chaudhry et al., 2018; Voulgarakis et al., 2024a). Rumen fluid pH measurement is the gold standard, with a pH between 5.2 and 5.8 for more than four hours per day indicating SARA in intensive small ruminant livestock farming (Chaudhry et al., 2018; Voulgarakis et al., 2024a).

Rumen acidosis, marked by rumen wall discoloration, especially in subacute rumenitis, manifests as a gray to dark discoloration (Enemark, 2008; Lopez-Campos et al., 2010; Andres et al., 2018; Fanning et al., 2018). In cattle, the objective estimation of color using spectrophotometers or colorimeters through Red, Green, and Blue (RGB) analysis has emerged

as a potential method for assessing various health issues (Chmiel et al., 2012; Silva et al., 2022; Voulgarakis et al., 2024b).

The sheep farming in Greece is a significant component of the country's agricultural landscape, with approximately 7.3 million animals across 83,000 holdings (Eurostat 2021, Papanikolopoulou et al. 2023). Sheep farming in Greece is distinguished by its high dairy specialization, with 93% of sheep dedicated to milk production. The primary use of this milk is for the production of Feta cheese, a Protected Designation of Origin product and the most important export of the Greek primary agriculture (Pulina et al. 2017). Despite its importance, the Greek sheep population has seen a decline of over 10% since 2010. This reduction has been marked by a decrease in the number of small and medium-sized holdings, which have been increasingly replaced by fewer, larger operations housing more than 500 ewes each. These larger enterprises predominantly employ an intensive indoor production system, relying heavily on imported concentrate feeds, supplemented by locally produced hay and sometimes ensilage. The current sheep population mainly consists of cross-breeds between various indigenous sheep and foreign breeds such as mainly Lacaune and secondary Friesian, which especially Lacaune cross adapt well to the intensive farming methods now prevalent in Greece.

While SARA has been extensively studied in other livestock, research on this topic in dairy sheep is limited (Atkinson, 2014). This study aims to address this gap by evaluating the prevalence and impact of SARA in Greek dairy sheep farming, and by assessing the effectiveness of computerized rumen colorimetry —a tool developed by the authors in a previous publication (Voulgarakis et al. 2024b) — for managing and detecting SARA in these farms.

2. Materials and Methods

2.1. Selection of Farms

Forty-two dairy sheep flocks, each consisting of 500-1000 milking ewes, were carefully selected to participate in this study. These farms are situated in Thessaly, a central region of Greece. Inclusion criteria required farms to be within reach of the Department of Clinical Veterinary Medicine, Faculty of Veterinary Science, University of Thessaly, Greece. Farms also needed to have crossbreed ewes of Lacaune with the local Karagouniko breed, maintain a stable ration composition throughout the entire milking period, and be confirmed as free of paratuberculosis and ovine progressive pneumonia, as evidenced by the Department's archives. The ration composition on the 42 farms consisted of a specified amount of complementary concentrate mixture given per head and cereal hay provided for ad libitum consumption. Throughout the 9-month trial period, the complementary feed remained unchanged.

2.2. Experimental Design

The selected farms were monitored every two months for rumen pH throughout the entire milking period, spanning approximately 9 months. Five visits were conducted at each farm during the first 10 days of the 1st, 3rd, 5th, 7th, and 9th months post-partum.

At each visit, six ewes were randomly chosen and assessed for rumen pH, ensuring they had not been previously selected for rumen pH measurement to prioritize animal welfare. Rumen fluid sampling took place four hours after the morning administration of concentrated feed. Simultaneously, milk samples were gathered from the bulk milk tank during morning visits. Additionally, data on daily milk yield was sourced from the farmers' records.

Furthermore, between July and August 2021, during the culling of aged ewes from the above 42 farms, rumen sampling was performed in slaughterhouses. Due to economic

89

constraints, a total of 280 ewes were included in the sampling, with at least 6 ewes from each farm.

2.3. Sample size justification

According to the existing literature, the prevalence of subacute rumen acidosis (SARA) in dairy sheep is approximately 12% (Lira et al., 2013). To ensure a desired level of accuracy with a margin of error (e) of ± 0.05 and a 95% confidence interval ($Z = 1.96$), the estimated sample size (n) was calculated using the formula $n = (Z^2 \times P \times (1-P)) / e^2$, yielding a value of 162. This study exceeded this calculated sample size requirement during both farm and slaughterhouse sampling phases.

2.4. Ethical approval

The study adhered to established animal welfare regulations (Directive 98/58/EC) and obtained approval from the Ethics Committee of the Faculty of Veterinary Science at the University of Thessaly, Greece (Approval No. 152/16 May 2021). No modifications were made to the animals' diet or treatment for experimental purposes during the entire duration of the study.

2.5. Clinical and laboratory procedures

2.5.1 Rumenocentesis and rumen pH measurement

Rumenocentesis, conducted without sedation, was employed for the collection of rumen fluid samples, following established methodologies from prior studies (Mialon et al., 2012). The procedure included an external abdominal puncture using a 16G 105-mm needle (Intralune

PP, Écouen, France) in the left abdominal region, specifically targeting the ventral sac of the rumen and aspirating the fluid with a 50 mL syringe (Morgante et al., 2007; Chaudhry et al., 2018). The point for rumenocentesis was identified at the intersection of two lines: one horizontal, passing through the stifle joint, and one vertical, passing through the midpoint between the base of the last rib and the hip bone. The obtained rumen fluid underwent immediate pH determination, utilizing a digital portable pH-meter (HI-2002 Edge pH Meter, Hanna instruments Leighton Buzzard, United Kingdom).

2.5.2 Milk Analyses

To maintain sample quality, milk samples were refrigerated at 4°C during transportation to the laboratory. On the same day of collection, the milk composition, including fat, protein, lactose, and total solid content, was analyzed using an infrared analysis technique with the assistance of an FTIR interferometer (Milkoscan FT6000 Analyzer, Foss Electric, Hilleroed, Denmark). The annual average values for the mentioned milk components were calculated based on the five measurements in each farm, along with the monthly milk yield, following basic arithmetic procedures (Johnson and Kuby, 2011).

2.5.3 Rumen wall Sampling and image acquisition

The sampling procedure for the rumen wall followed a standardized protocol as detailed by Voulgarakis et al. (2024b), ensuring that the same anatomical area of the rumen wall was sampled each time. This standardized protocol, developed specifically for this purpose, was based on certain measurements and was created by the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly. Finally, a 5 cm x 5 cm square section of the rumen wall was excised for sampling. After excision, the sample underwent thorough washing with water to eliminate any visible remnants of rumen content.

For image acquisition, the collected samples were placed on a specific desk in 35-37 mm distance between the lens and ruminal epithelium surface, and digital gross images were taken. A digital camera (NIKON COOLPIX L330, Tokyo, Japan) was used with an EF-S 60-mm macro lens (NIKKOR Wide-Angle Optical Zoom Lens 21×, Nikon Corporation, Tokyo, Japan) with standard lighting conditions [halogen lighting (4 matt bulbs, 18 W each, color temperature of 2800 K, color rendering index of 90 to 100)] and settings (manual operation mode with an f/3.5 aperture, 1/30 sec shutter speed, and ISO range on 160). The images were saved in raw format (Voulgarakis et al., 2024b). The sample was then placed in a container with a 10% formalin solution for subsequent histological examination.

2.5.4 Rumen Wall Image Color Analysis

The quantification of rumen mucosa color utilized software applications, specifically RGB Colour Detector 3.0.7 (2022) and CIELab 1.0 (CIE, 1986).

In the RGB application, the analyzed color components included Red, Green, and Blue (RGB), Value (V), Lightness (L), Hue, Saturation, and Value (HSV), and Hue, Saturation, and Lightness (HSL). Additionally, the summary of the Red, Green, and Blue color components was computed and labeled as RGB-SUM (Chmiel et al., 2012). Using the CIELab software 1.0, the L (color lightness), a (redness-greenness of the color), and b (yellow-blueness of the color) color components were assessed (CIE, 1986).

For processing and analyzing the image, a 1 cm × 1 cm area within the region of interest was randomly selected from each sample. For each rumen wall sample, average values of these color components were calculated from nine distinct spot measurements, and the resulting average value was employed for subsequent statistical analysis. It is essential to note that, although the L, a, and b values are technically denoted as L*, a*, and b*, respectively, for simplicity, this text omits the asterisks.

2.5. 5 Histological Examination

For the histological examinations, the rumen wall samples were cut into 1 cm × 1 cm size particles. Tissues were fixed in 10% formalin buffer for 48 to 72 hours. The processed tissues were dehydrated by immersion in a series of alcohol solutions and embedded in paraffin sections (Paraplast Plus®, Kendall, England). Afterwards, 4 µm-thick sections were obtained via the semi-automatic precision microtome (CUT 5062, SLEE medical GmbH, Nieder-Olm, Germany), dewaxed and stained with hematoxylin and eosin (H&E), for histopathological evaluation. From each sample, randomly selected fields with a ×10 objective were captured using a microscope (Nikon eclipse 50i, Tokyo, Japan) -coupled camera (Nikon, Digital sight DS-L1, Tokyo, Japan). ImageJ software 1.53 was utilized to measure the thickness of the stratum corneum, connective tissue, non-keratinized epithelium, and total epithelium in each photomicrograph (Figure 1).

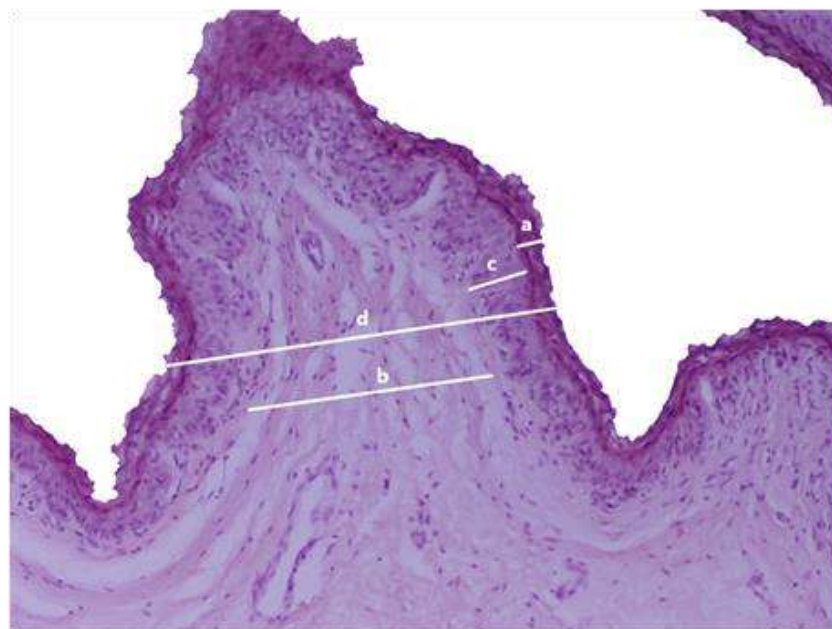


Figure 1. Measurements of the ewe rumen papillae: (a) Stratum corneum thickness. (b) Width of the connective tissue. (c) Thickness of the non-keratinized epithelium. (d) Total epithelium thickness. (Hematoxylin-eosin staining; magnification x10).

2.6. Statistical Analysis

The consistency of rumen pH measurements over time in each farm was examined using the Intraclass Correlation Coefficient (ICC). Continuous variables in the dataset, including pH values, milk parameters (such as yield, fat, protein, lactose, and total solids content), color measurements, and characteristics of the rumen epithelium, were checked for normal distribution. When necessary, log transformations were applied to address any observed skewness in the data. To explore potential differences in these variables among farms with low, moderate, and high SARA prevalence, a One-way Analysis of Variance (ANOVA) was conducted using IBM SPSS Statistics version 29.0.0.0 (241). The results were presented using the mean and standard deviation (SD) as measures of central tendency and variability, respectively. The analysis was performed using IBM SPSS Statistics version 29.0.0.0 (241), with a significance level of 0.05 used to determine statistical significance.

3. Results

All animals involved in the study completed the entire monitoring period without any adverse effects on their welfare.

The 42 farms were categorized into three groups based on the number of rumen pH measurements below 5.8 out of 30 measurements conducted in each farm [$n = 5 \text{ visits} (\times) 6 \text{ ewes each visit} = 30 \text{ measurements per farm}$]. The categories and the number of farms in each category are as follows:

- Category 1: Farms with a low prevalence of SARA, including 17 farms (41% of the total), with each farm exhibiting only 0-2 cases of pH <5.8 in the 30 measurements.
- Category 2: Farms with a moderate prevalence of SARA, comprising 19 farms (45% of the total), with each farm showing 3-9 cases of pH <5.8 in the 30 measurements.

- Category 3: Farms with a high prevalence of SARA, consisting of 6 farms (14% of the total), with each farm having 10-14 cases of pH <5.8 in the 30 measurements.

Table 1 shows the chemical composition and feeding amount per head of concentrate mixtures used on farms. Although there are arithmetic differences between the three farm categories, these differences were not statistically significant.

Table 1. Chemical composition and feeding amount per head (mean $\pm$ SD) of the complementary concentrate mixture used on farms.

Chemical Composition	Category 1 ⁱ (n = 17)	Category 2 ⁱⁱ (n = 19)	Category 3 ⁱⁱⁱ (n = 6)	<i>p</i> -Value
ME (Mcal/kg DM)	2.65 $\pm$ 0.07	2.66 $\pm$ 0.07	2.66 $\pm$ 0.08	0.96
CP (% of DM)	16.06 $\pm$ 0.97	16.05 $\pm$ 0.97	16.50 $\pm$ 1.38	0.72
NDF (% of DM)	14.47 $\pm$ 0.80	14.42 $\pm$ 0.77	14.33 $\pm$ 1.21	0.95
ADF (% of DM)	5.94 $\pm$ 0.83	6.11 $\pm$ 0.81	6.17 $\pm$ 0.75	0.77
Feeding Amount (g/day)	1.682 $\pm$ 107	1.705 $\pm$ 118	1.758 $\pm$ 150	0.43

ⁱ Farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA; ME = metabolizable energy; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; DM = dry matter.

Throughout the farming sampling period, the mean pH values of the participating farms, with six randomly selected ewes each time, ranged from 5.73 to 5.93, yielding a mean pH of 5.83 (Standard Deviation SD=0.06). Specifically, in Category 1 farms, the pH range was between 5.87 and 5.93, with a mean pH of 5.89 (SD=0.01). In Category 2, the pH range was from 5.78 to 5.84, with a mean pH of 5.81 (SD=0.02). Lastly, in Category 3, the pH range was recorded between 5.73 and 5.76, with a mean pH of 5.74 (SD=0.01). The sample size for each category was consistent. Post hoc analysis using Bonferroni adjustment revealed significant differences among all possible pairwise comparisons between the three farm categories ($p < 0.001$) (Table 2).

In the abattoir, post hoc analysis revealed significant differences in the color components among all possible pairwise comparisons of the three farm categories, with the exception of the

a component across all farm categories and the b component between Category 1 and Category 2 ($p < 0.001$) (Table 3).

Table 2. Comparison of rumen pH, milk yield, and milk components (mean $\pm$ SD).

Farm Sampling Period	Category 1 ⁱ (n = 17)	Category 2 ⁱⁱ (n = 19)	Category 3 ⁱⁱⁱ (n = 6)	p-Value
Rumen pH	5.89 $\pm$ 0.01	5.81 $\pm$ 0.02	5.74 $\pm$ 0.01	<0.001
Milk yield (liters/ewe/milking period)	412.0 $\pm$ 1.1	414.5 $\pm$ 2.1	415.1 $\pm$ 0.6	0.143
Milk fat content (%)	5.92 $\pm$ 0.14	5.69 $\pm$ 0.11	5.65 $\pm$ 0.06	<0.001
Milk protein content (%)	5.72 $\pm$ 0.10	5.72 $\pm$ 0.12	5.83 $\pm$ 0.05	0.064
Milk lactose content (%)	4.71 $\pm$ 0.09	4.81 $\pm$ 0.14	4.95 $\pm$ 0.16	0.001
Milk total solids' content (%)	10.87 $\pm$ 0.23	11.06 $\pm$ 0.31	11.73 $\pm$ 0.40	<0.001
Milk fat/milk protein ratio	1.04 $\pm$ 0.02	0.99 $\pm$ 0.02	0.96 $\pm$ 0.01	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA.

Table 3. Comparison of color components (mean $\pm$ SD) in rumen mucosa samples.

Color Components	Category 1 ⁱ (n = 111)	Category 2 ⁱⁱ (n = 124)	Category 3 ⁱⁱⁱ (n = 45)	p-Value
L	39.93 $\pm$ 5.24	30.59 $\pm$ 4.92	21.98 $\pm$ 6.92	<0.001
a	0.66 $\pm$ 4.03	1.01 $\pm$ 4.34	2.28 $\pm$ 4.04	0.091
b	9.54 $\pm$ 5.80	9.53 $\pm$ 4.67	4.49 $\pm$ 6.92	<0.001
Red	100.94 $\pm$ 14.33	78.38 $\pm$ 9.98	58.41 $\pm$ 12.34	<0.001
Green	93.38 $\pm$ 12.69	71.16 $\pm$ 13.14	51.60 $\pm$ 16.13	<0.001
Blue	78.92 $\pm$ 15.15	57.53 $\pm$ 14.52	46.33 $\pm$ 17.81	<0.001
RGB-SUM	273.23 $\pm$ 36.99	207.08 $\pm$ 32.36	156.34 $\pm$ 43.06	<0.001
HSV	0.40 $\pm$ 0.06	0.33 $\pm$ 0.06	0.24 $\pm$ 0.05	<0.001
HSL	35.47 $\pm$ 4.85	26.82 $\pm$ 4.08	20.44 $\pm$ 5.57	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA; L: color lightness unit ranged from 0 to 100. a: redness/greenness of the color unit ranged from -128 to 127. b: yellowness/blueness of the color unit ranged from -128 to 127. Red, Green, and Blue unit ranged from 0 to 255 in an 8-bit per channel representation. RGB-SUM is labeled as the summary of the Red, Green, and Blue color components; HSV: hue, saturation, and value. Value unit is a percentage (0% to 100%). HSL: hue, saturation, and lightness. Lightness unit is a percentage (0% to 100%).

3.1. Milk analysis

Milk fat content varied across farm categories, with Category 1 having the highest average fat content and Category 3 the lowest, as shown in Table 2. Post hoc analysis indicates significant differences between Category 1 and Category 2, as well as between Category 1 and

Category 3 ($p < 0.001$). As a direct consequence of these fat differences, there were also variations in the milk fat-to-milk protein ratio, with Category 1 having the highest ratio and Category 3 the lowest. Milk yield and milk protein did not vary between the farm categories; however, small but statistically significant differences were found in the concentration of lactose and the total solids content (Table 2).

3.2. Colour Measurement

Pairwise comparisons of mean L^* component values among all categories (1, 2, 3) revealed statistically significant differences ($p < 0.001$). Specifically, Category 1 exhibited the highest mean L value, whereas Category 3 reported the lowest mean L value, as reported in Table 3. Significant differences were also observed in the mean values of the b component between Category 1 and Category 3, as well as between Category 2 and Category 3, as shown in Table 3. However, no statistically significant differences were detected in the mean values of the a component across all possible comparisons (Table 3).

Regarding the color measurement using the RGB Analysis system, the mean values of Red, Green, Blue, and RGB-SUM were found to be significantly higher in Category 1 when compared to the mean values of Category 2 and Category 3 samples. Similarly, Category 2 exhibited significantly higher mean values compared to Category 3. Furthermore, significant differences were observed for the HSV value and HSL value among all possible comparisons, with Category 1 having the highest values compared to Category 2 and Category 3. Lastly, Category 2 revealed significantly higher mean values compared to Category 3 (Table 3).

3.3. Ruminal epithelium histopathology and histomorphometry

Histological analyses revealed no abnormal findings in the rumen epithelium samples, and parakeratosis was not observed. The darkest coloration was confined solely to the keratinized layer in those samples.

According to the statistical analysis, Category 3 exhibited significantly higher thickness in non-keratinized epithelium thickness and total epithelium thickness in contrast to Category 2 and Category 1, as shown in Table 4. Additionally, the non-keratinized epithelium thickness and total epithelium thickness were significantly higher in samples of Category 2 compared to samples of Category 1. However, no significant differences were observed in stratum corneum thickness and connective tissue width in any of the possible comparisons (Table 4).

Table 4. Comparison of histological measurements in the layers of the ruminal epithelium (mean $\pm$ SD).

Ruminal Epithelium	Category 1 ⁱ (n = 15)	Category 2 ⁱⁱ (n = 15)	Category 3 ⁱⁱⁱ (n = 15)	p-Value
Stratum corneum (μm)	33.67 $\pm$ 12.83	43.07 $\pm$ 24.97	41.67 $\pm$ 16.25	0.342
Connective tissue (μm)	103.27 $\pm$ 39.35	135.00 $\pm$ 61.32	148.13 $\pm$ 63.83	0.090
Non-keratinized epithelium (μm)	80.13 $\pm$ 14.46	116.47 $\pm$ 28.64	166.67 $\pm$ 21.41	<0.001
Total epithelium (μm)	217.07 $\pm$ 51.69	294.53 $\pm$ 61.26	356.47 $\pm$ 74.08	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA.

4. Discussion

This research represents the first attempt to investigate the epidemiology of SARA in dairy sheep farms in Greece. The findings strongly indicate that SARA is a common disorder in the modern Greek dairy sheep industry. These results are unsurprising given the typical nutritional management practices on farms. Ewes were provided with a complementary concentrate mixture that exceeded 1,500 g per day throughout the milking period (see Table 1) and were also offered cereal hay ad libitum. Typically, ewes consume the allotted amount of the complementary concentrate mixture promptly, but the consumption of cereal hay often depends

on its quality, leading to variable intake and potentially failing to meet the animals' daily fiber requirements. It is known that feeding more than 500 g of cereals daily increases the risk of ruminal acidosis in sheep; therefore, the recommendation is that if more than 500 g is fed to a ewe, it should be in the form of a total mixed ration (TMR) rather than as a complementary feed (Povey et al., 2018).

The complementary concentrate mixtures used on the farms did not show significant differences in their chemical composition or feeding amounts (see Table 1). This was expected, as feeding practices and ration formulation in modern Greek sheep farming are quite homogeneous. The lack of differences in the concentrate mixtures among the investigated farms underscores the causative role of hay in the cases of SARA documented in this research.

In this study, farms with a higher number of SARA cases (Categories 2 and 3) exhibited lower milk fat content. Lower ruminal pH is attributed to alterations in rumen fermentation and changes in the composition of rumen bacteria and fermentation patterns, leading to increased production of specific LCFA. These LCFA, once absorbed, can inhibit milk fat synthesis in the udder (Li et al., 2012; Kitkas et al., 2019). Multiple studies, including those by Tao et al. (2015), Zhang et al. (2020), Li et al. (2012), and Kitkas et al. (2019), have consistently observed lower milk fat percentages in high-concentrate groups compared to low-concentrate groups in both dairy cows and goats. To the best of our knowledge, this research is the first to document the same effect in dairy ewes.

This research proposes computerized measurement of rumen epithelium color as a promising method for monitoring SARA status. This technique can be easily applied during the slaughter of aged culled ewes, providing health advisors with reliable data on SARA status in the farm. Currently, rumen pH measurement is considered the gold standard for diagnosing SARA in ruminants (Plaizier et al., 2008; Humer et al., 2018). However, pH measurement can be influenced by various factors, including the method of measurement, sampling location, and

timing (Neubauer et al., 2017; Villot et al., 2017). Beyond pH measurements, diagnostic indicators such as milk fat percentage and the milk fat-to-protein ratio have been proposed for detecting SARA in dairy cows. Rapid weekly drops in milk fat of 0.3-0.5% or a milk fat-to-protein ratio below 1 suggest potential SARA cases (Kleen et al., 2003; Cook et al., 2005; Lean et al., 2007; Giger-Reverdin et al., 2014). However, these indexes have not been established for the dairy sheep industry, partly due to ongoing genetic improvements in dairy sheep breeds. Additionally, chronic diseases such as progressive pneumonia and Johne's disease are associated with drops in milk fat (Christodouloupoulos, 2006, McKenna et al., 2006).

Existing literature indicates that the prevalence of SARA in dairy sheep is approximately 12%, but this study was conducted in Brazil (Lira et al., 2013). This study exceeded the calculated sample size requirement based on this figure to account for the lack of extensive data from Greece and other Mediterranean countries.

The mean color values, measured by RGB and CIELab systems, were significantly lower in Category 3 samples compared to Categories 1 and 2, indicating darker discoloration of the ruminal epithelium. Extensive research and reviews on this topic, both in dairy and fattening lambs, have shown that feeding highly fermentable feed, which leads to reduced ruminal pH and increased accumulation of volatile fatty acids and lactic acid, is associated with a gray to dark coloration (Abdelrahman et al., 2005; Enemark, 2008; Lopez-Campos et al., 2010; Alhidary et al., 2016; Andres et al., 2018; Fanning et al., 2018). Similar results were reported in feedlot cattle by Voulgarakis et al. (2024b). Unfortunately, to the best of our knowledge, no research data from dairy ewes have been published yet. Other factors contributing to the darkening of the ruminal mucosa include pelleting, fiber particle size, dietary iron levels, and the buffering system (Hamada et al., 1969; Alhidary et al., 2016). However, the generally accepted conclusion among sheep practitioners is that rumen pH plays a decisive role in the darkening of the keratinized layer in the ruminal epithelium.

Histological findings showed that the darkening of the rumen epithelium's color was due to the effect of rumen pH on the keratinized layer rather than parakeratosis. This is a notable finding as previous research attributed rumen epithelium darkening to parakeratosis (Kleen et al., 2003).

Furthermore, an increase in the thickness of ruminal epithelium layers, particularly non-keratinized and total epithelium, was observed in animals from farms with a high incidence of SARA. Steele et al. (2012) observed increased total epithelial thickness and stratum corneum thickness in animals fed a high-grain diet. Bo Trabi et al. (2020) found that animals fed a low-grain non-pelleted TMR concentrate had higher total epithelium and stratum corneum thickness compared to those fed high-grain non-pelleted TMR concentrate or high-grain TMR in pelleted form. Wang et al. (2022) reported increased total epithelium and stratum corneum thickness in animals fed a ground corn high-grain diet compared to a whole corn high-grain diet. No significant difference in stratum corneum thickness among the categories, but Category 3 samples had a greater length of the stratum corneum compared to Category 1 samples. Garcia Diaz et al. (2018) reported higher total epithelium and stratum corneum thickness, but lower connective tissue width and non-keratinized thickness, in animals fed a grain-based diet compared to those on an additive-supplemented grain-based diet. Lower rumen pH may increase the rate of replacement of keratinized epithelium, stimulating non-keratinized epithelium growth to produce keratinized layers (Voulgarakis et al., 2024b).

5. Conclusions

SARA is a prevalent condition in the modern dairy sheep industry of Greece, significantly affecting farms with higher milk yields. This study demonstrates that SARA not only impacts production metrics but also induces notable changes in the rumen epithelium,

detectable through rumen colorimetry concerning the color of the epithelium. The discoloration in the rumen epithelium, observed as a darker hue, is attributed to the acidified rumen content's effect on the upper layers of the epithelium, rather than the presence of parakeratosis as previously suggested.

The use of computerized rumen colorimetry in ewes slaughtered as part of the replacement stock offers a promising management tool for assessing the SARA status of a farm. By implementing this technique, farmers and health advisors can gain reliable insights into the prevalence of SARA and make informed decisions to optimize feeding strategies and overall herd health.

Overall, the findings highlight the need for continued research and the development of comprehensive feeding practices that balance high energy intake with adequate dietary fiber. Incorporating concentrate mixtures into a TMR may provide a viable solution, mitigating the risk of SARA while supporting optimal milk production and animal well-being in dairy sheep farms.

References

- Abdelrahman MM, Alhidary I, Alyemni AH, Khan RU, Bello AR, Al-Saiady MY, Amran RA (2005) Effect of alfalfa hay on rumen fermentation patterns and serum biochemical profile of growing Naemi lambs with ad libitum access to total mixed rations. *Pak. J. Zool.* 49, 15–19.
- Alhidary I, Abdelrahman MM, Alyemni AH, Khan RU, Al-Mubarak AH, Albaadani HH (2016) Characteristics of rumen in Naemi lamb: Morphological changes in response to altered feeding regimen. *Acta Histochem* 118(4), 331-7.
- Andrés S, Jaramillo E, Bodas R, Blanco C, Benavides J, Fernández P, González EP, Frutos J, Belenguera A, López S, Giráldez FJ (2018) Grain grinding size of cereals in complete pelleted diets for growing lambs: Effects on ruminal microbiota and fermentation. *Small Rumin Res* 159, 38–44.
- Atkinson O (2014) Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract* 22(1), 1-9.
- Bo Trabi E, Seddik HE, Xie F, Wang X, Liu J, Mao S (2020) Effect of pelleted high-grain total mixed ration on rumen morphology, epithelium-associated microbiota and gene expression of proinflammatory cytokines and tight junction proteins in Hu sheep. *Anim. Feed Sci. Technol.* 263, 114453.
- Chaudhry AS, Khan MA, Khan MS, Ashraf K, Saleem MH, Khan NU, Usman T, Ahmad I (2018) Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J Anim Plant Sci* 28(1), 80-84:1018-7081.
- Chmiel M, Slowinski M, Dasiewicz K, Florowski T (2012) Application of a computer vision system to classify beef as normal or dark, firm, and dry. *J Anim Sci* 90(11), 4126-30.

- Christodouloupoulos G (2006) Maedi–Visna: Clinical review and short reference on the disease status in Mediterranean countries, *Small Rumin Res* 62(1–2), 47-53.
- Church DC (1991) *Livestock Feeds and Feeding*, 3rd ed.; Publisher: Prentice-Hall Inc.: Hoboken, NJ, USA, pp. 349.
- CIE. (1986) *Colourimetry: Official recommendations of the international commission on illumination*. CIE Publ. No. 15.2. CIE Central Bureau, Vienna.
- Commun L, Mialon MM, Martin C, Baumont R, Veissier I (2009) Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J. Anim Sci.* 87, 3372–3379.
- Cook NB, Oetzel GR, Nordlund KV (2005) Clinical forum–subacute ruminal acidosis–a problem in UK dairy herds? *UK Vet* 10, 40–46.
- Desnoyers M, Duvaux-Ponter C, Rigalma K, Roussel S, Martin O, Giger-Reverdin S (2008) Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. *Anim* 2(12), 1802–1808.
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet J* 176(1), 32-43.
- Eurostat 2021. Available online: <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220517-2> (accessed on 7 July 2024).
- Fanning JP, Hynd PI, Cockcroft PD (2018) The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. *Small Rumin Res* 162, 57-62.
- Garcia Diaz T, Ferriani Branco A, Jacovaci FA, Cabreira Jobim C, Bolson DC, Pratti Daniel JL (2018) Inclusion of live yeast and mannanoligosaccharides in high grain-based diets for sheep: Ruminal parameters, inflammatory response and rumen morphology. *PLoS ONE* 13(2), e0193313.

- Giger-Reverdin S, Rigalma K, Desnoyers M, Sauvant D, Duvaux-Ponter C (2014) Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *J Dairy Sci* 97, 4367–4378.
- Hamada T, Maeda S, Kameoka K (1969) Effects of minerals on formation of colour in the rumen epithelium of kids. *J Dairy Sci* 53, 588–591.
- Humer E, Aschenbach JR, Neubauer V, Kröger I, Khiaosa-Ard R, Baumgartner W, Zebeli Q (2018) Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J Anim Physiol Anim Nutr (Berl)* 102(2), 380-392.
- Johnson RR, Kuby PJ (2011) *Elementary Statistics* 11th (eleventh) Edition, Boston, MA, USA.
- Kitkas G, Valergakis G, Kritsepi-Konstantinou M, Gelasakis A, Arsenos G, Kalaitzakis E, Panousis N (2019) Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation. *J Hellenic Vet Med Soc* 70, 1551-1560. 10.12681/jhvms.20861.
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): A review. *J. Vet. Med. A Physiol Pathol Clin Med* 50, 406–414.
- Lean IJ, Annison F, Bramley E, Browning G, Cusack P, Farquharson B, Little S, Nandapi D (2007) *Ruminal Acidosis- aetiopahtogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR), Melbourne, Australia, pp 1-60.*
- Li S, Gozho GN, Gakhar N, Khafipour E, Krause DO, Plaizier JC (2012) Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can J Anim Sci* 92, 353-364.

- Lira MAA, Simões SVD, Riet-Correa F, Pessoa CMR, Dantas AFM, Miranda Neto EG (2013) Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui Vet Bras* 33(2), 193-198.
- Lopez-Campos O', Bodas R, Prieto N, Giraldez FJ, Perez V, Andres S (2010) Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim* 4(6), 958–964.
- McKenna SL, Keefe GP, Tiwari A, VanLeeuwen J, Barkema HW (2006) Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. *Can Vet J* 47(11), 1089-99.
- Mialon MM, Deiss V, Andanson S, Anglard F, Doreau M, Veissier I (2012) An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anaesthesia. *The Veterinary Journal* 194(1), 55-59.
- Morgante M, Stelletta C, Berzaghi P, Ganesella M, Andrighetto I (2007) Subacute rumen acidosis in lactating cows: An investigation in intensive Italian dairy herds. *J Anim Physiol Anim Nutr* 91, 226–234.
- Neubauer V, Humer E, Kröger I, Braid T, Wagner M, Zebeli Q (2017) Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J Anim Physiol Anim Nutr* 102(1), 343–349.
- Papanikolopoulou V, Vouraki S, Priskas S, Theodoridis A, Dimitriou S, Arsenos G (2023) Economic Performance of Dairy Sheep Farms in Less-Favoured Areas of Greece: A Comparative Analysis Based on Flock Size and Farming System. *Sustainability* 15, 1681.

- Penner GB, Aschenbach JR, Gäbel G, Rackwitz R, Oba M (2009) Epithelial Capacity for Apical Uptake of Short Chain Fatty Acids Is a Key Determinant for Intraruminal pH and the Susceptibility to Subacute Ruminal Acidosis in Sheep. *J Nutr* 139(9), 1714-1720.
- Plaizier JC, Krause DO, Gozho GN, McBride BW (2008) Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet J* 176, 21–31.
- Povey G, Stubbings L, Phillips K (2018) Feeding the ewe A manual for consultants, vets and producers, 2nd ed.; Publisher: AHDB Agriculture and Horticulture Development Board Middlesbrough Business Park Siskin Parkway East, Coventry, England, pp. 1-64.
- Pulina G, Milán MJ, Lavín MP, Theodoridis A, Morin E, Capote J, Thomas DL, Francesconi AHD, Caja G (2017) Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *J. Dairy Sci* 101, 6715–6729.
- RGB Color Detector (2022) Available from: [com.TheProgrammer.RGBColorDetector](https://www.dreamcatcher-ai.com/TheProgrammer/RGBColorDetector) (Version 3.0.7). Accessed 10 Oct 2022
- Silva EPE, Moraes EP, Anaya K, Silva YMO, Lopes HAP, Neto JCA, Oliveira JPF, Oliveira JB, Rangel AHN (2022) Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. *PLoS One* 17(2), e0263714.
- Steele MA, Greenwood SL, Croom J, McBride BW (2012) An increase in dietary non-structural carbohydrates alters the structure and metabolism of the rumen epithelium in lambs. *Can J Anim Sci* 92, 123-130.
- Tao H, Chang G, Xu T, Zhao H, Zhang K, Shen X (2015) Feeding a High Concentrate Diet Down-Regulates Expression of ACACA, LPL and SCD and Modifies Milk Composition in Lactating Goats. *PLoS ONE*, 10(6), e0130525.
- Villot C, Meunier B, Bodin J, Martin C, Silberberg M (2017) Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal* 12, 481–490.

- Voulgarakis N, Athanasiou L, Psalla D, Gougoulis D, Papatsiros V, Christodoulopoulos G (2024a) Ruminant Acidosis Part II: Diagnosis, Prevention and Treatment. *J Hellenic Vet Med Soc* 74(4), 6329–6336.
- Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Angelidou-Tsifida M, Papatsiros V, Athanasiou L, Christodoulopoulos G (2023) Ruminant Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease. *J Hellenic Vet Med Soc* 74(3), 5883–5891.
- Voulgarakis N, Gougoulis DA, Psalla D, Papakonstantinou GI, Katsoulos PD, Katsoulis K, Angelidou-Tsifida M, Athanasiou LV, Papatsiros VG, Christodoulopoulos G (2024b) Can Computerized Rumen Mucosal Colorimetry Serve as an Effective Field Test for Managing Subacute Ruminant Acidosis in Feedlot Cattle? *Vet Res Commun* 48, 475-484.
- Wang L, Qi W, Mao S, Zhu W, Liu J (2022) Effects of whole corn high-grain diet feeding on ruminal bacterial community and epithelial gene expression related to VFA absorption and metabolism in fattening lambs. *J Anim Sci* 100(3), skac056.
- Xia G, Sun J, Fan Y, Zhao F, Ahmed G, Jin Y, Zhang Y, Wang H (2020) β -Sitosterol Attenuates High Grain Diet-Induced Inflammatory Stress and Modifies Rumen Fermentation and Microbiota in Sheep. *Anim* 10, 171.
- Xu T, Tao H, Chang G, Zhang K, Xu L, Shen X (2015) Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet Res* 11, 52.
- Xu Y, Ding Z (2011) Physiological, biochemical and histopathological effects of fermentative acidosis in ruminant production: a minimal review. *SJAR* 9(2), 414-422.

Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, Wang HR (2020) Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 103(2), 1931-1943.

GENERAL CONCLUSIONS

The studies carried out during this PhD thesis led to the following conclusions:

Chapter II general conclusions

- The study observed significant differences in color components (L, b, Red, Green, Blue, and RGB-SUM) between farms without SARA (Category I) and farms with SARA (Category II). Category II samples had lower mean values, indicating darker rumen epithelium coloration.
- Visual examination of the rumen epithelium revealed more frequent grey and black discoloration in samples from the farms with SARA (Category II).
- The darker coloration is attributed to the impact of low rumen pH on the keratinized layer, not parakeratosis.
- Histopathological examination found no pathological findings or signs of parakeratosis in the rumen epithelium. However, Category II samples showed significantly thicker non-keratinized epithelium and total epithelium compared to Category I. This suggests a higher rate of replacement of the keratinized epithelium, leading to an increase in the non-keratinized epithelium in response to the need to produce keratinized layers due to low rumen pH.
- There were no significant differences in stratum corneum thickness or connective tissue width between the two categories.
- Computerized rumen colorimetry, utilizing portable devices and color analysis software, proved effective for assessing chromatic variation in the rumen epithelium during necropsy or at the abattoir.

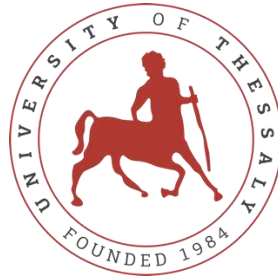
- Further research with a larger sample size and the use of a gastroscope is necessary to validate computerized rumen colorimetry as a diagnostic tool for live animals.

Chapter III conclusions

- SARA is common in the modern dairy sheep industry in Greece, particularly affecting farms with higher milk yields. SARA cases were identified in 19 farms (45%, n=42).
- Farms with a higher incidence of SARA tend to produce higher milk yields but exhibit lower milk fat content. The lower milk fat is likely attributed to alterations in rumen fermentation and fatty acid profiles caused by SARA.
- SARA induces notable changes in the rumen epithelium, specifically causing darker discoloration attributed to the acidified rumen content's effect on the upper layers of the epithelium, rather than parakeratosis.
- Similar results to feedlot cattle were reported for dairy ewes in color measurements. Farms with smallest incidence of SARA showed the highest color values, indicating lighter color, while farms with highest incidence had the lowest, indicating darker color.
- The histopathological examination revealed no abnormal findings or parakeratosis in rumen epithelium samples. Farms with highest incidence of SARA showed the highest non-keratinized and total epithelium thickness.
- The use of computerized rumen colorimetry in ewes slaughtered as part of the replacement stock offers a promising management tool for assessing the SARA status of a farm. By implementing this technique, farmers and health advisors can gain reliable insights into the prevalence of SARA and make informed decisions to optimize feeding strategies and overall herd health.

- This study is the first to investigate the epidemiology of SARA in dairy sheep farms in Greece, filling a gap in existing literature and providing a basis for future studies and interventions.

UNIVERSITY OF THESSALY



SCHOOL OF HEALTH SCIENCES

FACULTY OF VETERINARY MEDICINE

CLINICAL VETERINARY MEDICINE DEPARTMENT

ADVANCING THE UNDERSTANDING OF RUMINAL ACIDOSIS.

Doctoral Thesis by

Nikolaos M. Voulgarakis

Summary

This PhD dissertation comprehensively examines Subacute Ruminal Acidosis (SARA), a major nutritional disorder in ruminants that significantly impacts animal health, welfare, and farm economics. SARA is particularly prevalent in intensive livestock farming, leading to complications such as liver abscesses, milk fat depression, reduced milk yield, and early culling. The dissertation is structured into three chapters: a literature review and two research investigations.

The literature review focuses on the definition, types, epidemiology, economic impacts, welfare implications, diagnosis, prevention, and treatment of ruminal acidosis.

The first research investigation explores the use of computerized rumen colorimetry as a novel diagnostic tool for SARA. Diagnosing SARA is challenging due to the need to evaluate multiple parameters, such as milk fat/protein ratio, ruminal lactate, and hemogram, instead of relying on a single definitive symptom or diagnostic method. This study evaluated the effectiveness of computerized rumen colorimetry in detecting SARA in beef cattle. Over one year, 75 cattle aged 8–10 months from five farms were periodically monitored for rumen pH prior to slaughter. Samples of rumen wall and rumen content were obtained at slaughter for analysis. The study found that the darkening of the rumen epithelium's color was attributed to SARA incidence and was caused by the effect of rumen pH on the keratinized layer of the epithelium. Furthermore, an increase in the thickness of ruminal epithelium layers, particularly non-keratinized and total epithelium, was observed in animals with a history of SARA. It is possible that lower rumen pH increases the rate of replacement of the keratinized epithelium, with the non-keratinized epithelium overgrowing to compensate. In conclusion, computerized rumen colorimetry shows promise as a reliable method for managing SARA in bovine farms by monitoring the condition in the slaughterhouse. Further research is needed to evaluate its effectiveness in detecting SARA in live animals.

The second research investigation addresses SARA in Greek dairy sheep. This study investigates the prevalence and impact of SARA in Greek dairy sheep farming and explores the use of computerized rumen colorimetry as a management and diagnostic tool for SARA in dairy sheep farms. Forty-two dairy sheep flocks were monitored regularly for rumen pH, milk composition, and milk yield over an approximately 9-month milking period. Computerized Rumen Mucosal Colorimetry was applied to 280 culled, aged ewes from these farms, with rumen wall samples taken for histological examination. SARA cases were identified in 19 farms (45%, n=42). Farms with SARA cases had lower milk fat levels, although milk yield was higher in these farms, while milk protein levels did not differ based on the SARA status of the farms.

114

In culled ewes, rumen color was significantly associated with the farm's SARA status. Additionally, ewes from farms with more SARA cases exhibited increased thickness in the ruminal epithelium layers, particularly in the non-keratinized and total epithelium layers. In conclusion, SARA is a common disorder in the Greek dairy sheep industry, lowering the fat content of milk. The study suggests that computerized rumen epithelium colorimetry in aged culled ewes shows promise as a diagnostic tool for SARA in dairy sheep farms.

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ



ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΤΜΗΜΑ ΚΤΗΝΙΑΤΡΙΚΗΣ

ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ

Συμβολή της Δυσπεπτικής Οξέωσης των Μηρυκαστικών

Διδακτορική Διατριβή του

Νικόλαος Μ. Βουλγαράκης

Περίληψη

Η διατριβή εξετάζει την Υποκλινική Δυσπεπτική Οξέωση [Subacute Ruminal Acidosis (SARA)] των μηρυκαστικών. Η SARA επηρεάζει σημαντικά την υγεία, την ευζωία και τις αποδόσεις των ζώων και είναι ιδιαίτερα διαδεδομένη στην εντατική κτηνοτροφία, προκαλώντας επιπλοκές όπως αποστήματα στο ήπαρ, μείωση της λιποπεριεκτικότητας του γάλακτος, μείωση της γαλακτοπαραγωγής και πρόωρη αξιοποίηση των ζώων. Η διατριβή περιλαμβάνει τρία κεφάλαια: μία βιβλιογραφική ανασκόπηση και δύο ερευνητικές μελέτες.

Η βιβλιογραφική ανασκόπηση εστιάζει στον ορισμό, τους τύπους, την επιδημιολογία, τις οικονομικές επιπτώσεις, τις συνέπειες για την ευζωία, τη διάγνωση, την πρόληψη και τη θεραπεία της δυσπεπτικής οξέωσης.

Η πρώτη ερευνητική μελέτη εξετάζει τη χρήση της ψηφιακής χρωματομετρίας του βλεννογόνου της μεγάλης κοιλίας, ως ένα νέο διαγνωστικό εργαλείο για τη διάγνωση της SARA. Η μελέτη αξιολόγησε την αποτελεσματικότητα της ψηφιακής χρωματομετρίας της μεγάλης κοιλίας σε παχυνόμενα βοοειδή. Για ένα χρόνο, 75 βοοειδή ηλικίας 8-10 μηνών από πέντε εκτροφές παρακολουθούνταν περιοδικά με μετρήσεις του pH της μεγάλης κοιλίας. Κατά τη σφαγή των ζώων, δείγματα του τοιχώματος της μεγάλης κοιλίας ελήφθησαν για ιστολογικές και χρωματομετρικές εξετάσεις. Η έρευνα διαπίστωσε ότι η SARA προκαλούσε σκούρο χρωματισμό στο επιθήλιο της μεγάλης κοιλίας. Ο σκούρος χρωματισμός αφορούσε το κερατινοποιημένο στρώμα του επιθηλίου και αποδόθηκε στην επίδραση του όξινου pH στην επιφάνεια του επιθηλίου. Επιπλέον, παρατηρήθηκε αύξηση στο πάχος των στρωμάτων του επιθηλίου της κοιλίας, ιδιαίτερα του μη κερατινοποιημένου και του συνολικού επιθηλίου, σε ζώα με ιστορικό SARA. Είναι πιθανό ότι το χαμηλότερο pH της μεγάλης κοιλίας να αυξάνει τον ρυθμό αντικατάστασης του κερατινοποιημένου επιθηλίου, με αποτέλεσμα το μη κερατινοποιημένο επιθήλιο να πολλαπλασιάζεται για να αντισταθμίσει τη συχνότερη ανάγκη παραγωγής κερατινοποιημένων στρωμάτων. Συμπερασματικά, η ψηφιακή χρωματομετρία του βλεννογόνου της μεγάλης κοιλίας φαίνεται να είναι μια αξιόπιστη μέθοδος για τη διαχείριση της SARA σε εκτροφές βοοειδών, μέσω εκτίμησης της εικόνας των ζώων στο σφαγείο. Απαιτείται περαιτέρω έρευνα για την αξιολόγηση της αποτελεσματικότητάς της μεθόδου στην ανίχνευση της SARA στα ζώντα ζώα.

Η δεύτερη ερευνητική μελέτη αφορά τη διερεύνηση της SARA σε εκτροφές γαλακτοπαραγωγικών προβάτων στη χώρα μας. Αυτή η μελέτη διερευνά τη συχνότητα της SARA στις εκτροφές αυτές και εξετάζει τη χρήση της ψηφιακής χρωματομετρίας του βλεννογόνου της μεγάλης κοιλίας ως εργαλείο διαχείρισης της SARA. Σαράντα δύο εκτροφές γαλακτοπαραγωγικών προβάτων συμπεριλήφθηκαν στην έρευνα όπου παρακολουθούνταν τακτικά το pH της μεγάλης κοιλίας των ζώων, η γαλακτοπαραγωγή και η σύνθεση του παραγόμενου γάλακτος για το σύνολο της αρμεκτικής περιόδου, διάρκειας περίπου 9 μηνών. Επίσης, λήφθηκε βλεννογόνο της μεγάλης κοιλίας από 280 προβατίνες των παραπάνω εκτροφών, οι οποίες αντικαταστάθηκαν λόγω ηλικίας και αξιοποιήθηκαν στο σφαγείο. Στα δείγματα μεγάλης κοιλίας εφαρμόστηκε ψηφιακή χρωματομετρία και επιπλέον έγιναν ιστολογικές εξετάσεις. Περιπτώσεις SARA εντοπίστηκαν σε 19 εκτροφές (45%, n=42). Οι εκτροφές με SARA είχαν χαμηλότερα επίπεδα λιποπεριεκτικότητας γάλακτος, αν και η γαλακτοπαραγωγή ήταν υψηλότερη σε αυτές τις εκτροφές, ενώ τα επίπεδα πρωτεΐνης στο γάλα δεν διέφεραν βάσει της συχνότητας SARA. Στις σφαζόμενες προβατίνες, το χρώμα του βλεννογόνου της μεγάλης κοιλίας επηρεάζονταν σημαντικά από τη συχνότητα SARA στις εκτροφές προέλευσης. Επιπλέον, οι προβατίνες από εκτροφές με υψηλότερο ποσοστό SARA παρουσίαζαν αύξηση στο πάχος των στρωμάτων του επιθηλίου της μεγάλης κοιλίας, ιδιαίτερα του μη κερατινοποιημένου και του συνολικού επιθηλίου. Συμπερασματικά, η SARA είναι μια συχνή κατάσταση στην ελληνική γαλακτοπαραγωγική προβατοτροφία και προκαλεί μείωση της λιποπεριεκτικότητας του γάλακτος. Ακόμη, προτείνεται ότι η ψηφιακή χρωματομετρία του επιθηλίου της μεγάλης κοιλίας σε ηλικιωμένες προβατίνες που αντικαθίστανται μπορεί να αξιοποιηθεί στη διαχείριση της SARA στις εκτροφές γαλακτοπαραγωγικών προβάτων.

ANNEX I

Nikolaos Voulgarakis' CV

I. Personal information:

- Full Name: Nikolaos Voulgarakis
- Father's name: Moschos
- Nationality: Greek
- Date of Birth:
- Place of Birth: Chania
- Residency: Karditsa Greece
- Email:

Αφαίρεση προσωπικών δεδομένων
(Υπηρεσία Βιβλιοθήκης & Πληροφόρησης
Πανεπιστημίου Θεσσαλίας)

II. Education, Academic Degrees Professional Titles

- **Education**

Sept. 2013 – Sept. 2014 & Sept. 2013 – Sept 2019: University education:

Department of Nursing, School of Medicine of the National and Kapodistrian University of Athens, Athens (Sept. 2013-Sept. 2014).

Department of Veterinary Medicine, University of Thessaly, Karditsa (Sept. 2014- Sept. 2019)

Nov. 2019 - Nov. 2020 & Dec. 2020 – Until now: Postgraduate education: Department of Veterinary Science University of Thessaly, Karditsa.

Postgraduate student at the Department of Veterinary Medicine, University of Thessaly. Subject of study: “Clinical, Laboratory Postgraduate education at the Department of Clinical Veterinary Medicine of Farm Animals (Nov. 2019 – Nov. 2020).

PhD candidate in Department of Clinical Veterinary Medicine of Farm Animals, University of Thessaly. Supervisor: Professor G. Christodouloupoulos. Subject: Contribution to the study of Ruminant Acidosis in ruminant. Under support of my doctoral thesis (Dec 2020 – July 2024)

- **Qualifications**

Degree in Veterinary Medicine (November 07, 2019), grade: Very Good (7.11 / 10).

Clinical and Laboratory Postgraduate Education (December 2020), grade: Excellent.

- **Professional titles**

License Of Veterinary Practitioner from the Geotechnical Chamber of Greece.

- **Foreign Languages**

English (LOWER B2).

III. CAREER DEVELOPMENT

June 2017 – September 2019

Internship in Farm Animal Veterinary Office of Veterinary Chatzidakis Eleftherios, Chania Crete

March 2020 - September 2020, May 2021 - September 2021

Academic Fellow at Department of Veterinary Medicine of Thessaly Subject of lectures: teaching courses to undergraduate students (Farm Animal Medicine).

January 2022 – October 2022

Academic Fellow at Department of Veterinary Medicine of Thessaly: *Subject of employment: teaching courses, laboratories and management of clinical cases (emergencies) in companion animals and farm animals.*

27 February 2023 – 3 March 2023

Veterinary Visitor in the Department of Animal Medicine, Production and Health — MAPS of the University of Padova to participate in a short internship program: *Subject of employment: a) followed the education and training program in farm animal medicine (ruminants, pigs), under the supervision of Prof. Mattia Cecchinato, Prof. Rudi Cassini, Prof. Matteo Giancesella and Prof. Michele Drigo and b) visited several bovine, goat and swine farms, where in*

collaboration with Prof. Mateo Giancesella, Prof. Rudi Cassini and Prof. Michele Drigo he participated in activities related to field trials

.March 2023 – June 2023

Academic Fellow at Department of Veterinary Medicine of Thessaly: Subject of employment: teaching courses, laboratories and management of clinical cases (emergencies) in companion animals and farm animals.

IV. TEACHING EXPERIENCE

- *Department of Veterinary Medicine. University of Thessaly*

March 2020 - September 2020, May 2021 - September 2021, January 2022 – July 2022

Auxiliary participation in academic and clinical practice of undergraduate students of 6th, 8th and 10th semester of the Department of Veterinary Medicine of the University of Thessaly, in sheep, goat and cow farms.

V. PUBLICATIONS

Original publications and presentation in congress from this thesis

- 1. “Ruminal Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease.”**

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Angelidou-Tsifida M, Papatsiros V, Athanasiou L, Christodoulopoulos G. Journal of the Hellenic Veterinary Medical Society, 2023;74(3):5883–5891. <https://doi.org/10.12681/jhvms.31237>

- 2. “Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment.”**

Voulgarakis N, Athanasiou L, Psalla D, Gougoulis D, Papatsiros V, Christodoulopoulos G. Journal of the Hellenic Veterinary Medical Society, 2024;74(4),6329–6336. <https://doi.org/10.12681/jhvms.31438>

3. “Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle?”

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Katsoulos PD, Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodoulopoulos G. Veterinary Research Communications. 2024; 48(1):475-484. <https://doi.org/10.1007/s11259-023-10231-w>

4. “Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management”

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, , Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodoulopoulos G. Preprints 2024; 2024061041. <https://doi.org/10.20944/preprints202406.1041.v1>

5. “Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management”

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, , Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodoulopoulos G. Animals 14(14), 2061. <https://doi.org/10.3390/ani14142061>

6. “Proposal of an easy clinical field test for the diagnosis of Subacute Ruminal Acidosis in ruminants.”

Voulgarakis N, Gougoulis D, Athanasiou LV, Psalla D, Papatsiros V, Christodoulopoulos G. Proceedings of 15th Hellenic Veterinary Conference (Athens, Greece, 4-6 November 2022): Oral presentation no 45 (OP 45), page 70.

List of other original publications

1. Effects of Injectable Administration of Dexamethasone Alone or in Combination with Vitamin E/Se in Newborn Low Birth Weight Piglets.

Papakonstantinou GI, Gougoulis DA, **Voulgarakis N**, Maragkakis G, Galamatis D, Athanasiou LV, Papatsiros VG. Vet Sci. 2023; 10(2): 135. <https://doi.org/10.3390/vetsci10020135>

2. Outbreak of Acute Clinical Mastitis in Primigravidae Ewes in the Immediately Pre-Partum Period.

Gougoulis D, Athanasiou LV, Vasileiou NG, **Voulgarakis N**, Dimoveli K, Mavrogianni V. Ruminants. 2023; 3(2):133-139. <https://doi.org/10.3390/ruminants3020012>

3. Effects of a Natural Polyphenolic Product from Olive Mill Wastewater on Oxidative Stress and Post-Weaning Diarrhea in Piglets.

Papakonstantinou GI, Meletis E, Petrotos K, Kostoulas P, Tsekouras N, Kantere MC, **Voulgarakis N**, Gougoulis D, Filippopoulos L, Christodoulopoulos G, Athanasiou LV, Papatsiros VG. Agriculture. 2023; 13(7):1356. <https://doi.org/10.3390/agriculture13071356>

4. Effects of a Multi-Component Mycotoxin-Detoxifying Agent on Oxidative Stress, Health and Performance of Sows.

Papatsiros VG, Eliopoulos C, **Voulgarakis N**, Arapoglou D, Riahi I, Sadurní M, Papakonstantinou GI. Toxins (Basel). 2023; 15(9) :580. <https://doi.org/10.3390/toxins15090580>

5. Effects of a Curcumin/Silymarin/Yeast-Based Mycotoxin Detoxifier on Redox Status and Growth Performance of Weaned Piglets under Field Conditions.

Papatsiros VG, Papakonstantinou GI, **Voulgarakis N**, Eliopoulos C, Marouda C, Meletis E, Valasi I, Kostoulas P, Arapoglou D, Riahi I, Christodoulopoulos G, Psalla D. Toxins (Basel). 2024; 16(4): 168. <https://doi.org/10.3390/toxins16040168>

6. Effects of a Phytogetic Feed Additive on Redox Status, Blood Haematology, and Piglet Mortality in Primiparous Sows.

Papatsiros VG, Papakonstantinou GI, Katsogiannou E, Gougoulis DA, **Voulgarakis N**, Petrotos K, Braimaki S, Galamatis DA, El-Sayed A, Athanasiou LV. Stresses. 2024; 4(2):293-307. <https://doi.org/10.3390/stresses4020018>

7. Precision Livestock Farming Technology: Applications and Challenges of Animal Welfare and Climate Change.

Papakonstantinou GI, **Voulgarakis N**, Terzidou G, Fotos L, Giamouri E, Papatsiros VG. Agriculture. 2024; 14(4):620. <https://doi.org/10.3390/agriculture14040620>

List of other conference oral presentations and poster presentations

1. Effect of centrifugation on selected dipstick test results in ovine urine.

Athanasίου LV, Katsogiannou EG, Pazarakioti AT, Kokkinaki KCG, Kantere MC, **Voulgarakis NM**, Dedousi A, Katsoulos PG. Proceedings of European Society of Veterinary Clinical Pathology (ESVCP)/ European College of Veterinary Clinical Pathology (ECVCP) 24th Annual Meeting 2022 (Belgrade, Serbia, 5-8 October): Poster presentation no 25 (P25), page 162.

2. Effects of a multi-component mycotoxin detoxifier on the antioxidant status and the performance of sows. Proceedings of The World Mycotoxin

Sadumi M, Riahi I, Eliopoulos C, **Voulgarakis N**, Arapoglou D, Papakonstantinou GI, Papatsiros VG. Proceedings of The World Mycotoxin Forum 2023 (Antwerp, Belgium, 9-11 October): Poster presentation no 67 (P67), page 131-132.

3. Evaluating Ocular Manifestations of Bluetongue Disease in Sheep.

Katsoulos PD, Gougoulis DA, Katsogiannou EG, Doukas D, Tsokana CN, **Voulgarakis N**, Athanasίου LV. Proceedings of the XXI International Society for Animal Clinical Pathology Congress 2024 (Heraklion, Greece, 14-18 May): Oral presentation no 3.80 (OP 3.80), page 124.

4. Impact of polyphenolic phytogenic feed additive on redox status, hematological parameters of primiparous sows, and piglet mortality.

Papatsiros VG, Papakonstantinou GI, Katsogiannou E, **Voulgarakis N**, Petrotos K, Braimaki S, Galamatis DA. Gougoulis DA. Proceedings of the XXI International Society for Animal Clinical Pathology Congress 2024 (Heraklion, Greece, 14-18 May): Oral presentation no 3.81 (OP 3.81), page 125.

6. In vivo effects of an anti-mycotoxins product in weaned pigs challenged by fumonisins: Insights into the animal performance and antioxidant status.

Riahi I, Della Badia A, Codina R, Eliopoulos C, **Voulgarakis N**, Papakonstantinou G, Papatsiros V. Proceedings of 45th Mycotoxin Workshop 2024 (Vienna, Austria, 2-5 June): Poster presentation no. 172 (P 172).

ANNEX II

Publications from PhD dissertation

1. “Ruminal Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease.”

Published

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Angelidou-Tsifida M, Papatsiros V, Athanasiou L, Christodouloupoulos G. Ruminal Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease. J Hellenic Vet Med Soc, 2023;74(3):5883–5891. <https://doi.org/10.12681/jhvms.31237>

Ruminal Acidosis

Part I: Clinical manifestations, epidemiology, and impact of the disease

N. Voulgarakis^{1*}, D. Gougoulis¹, D. Psalla², G. Papakonstantinou¹,
M. Angelidou-Tsifida², V. Papatsiros¹, L.V. Athanasiou¹, G. Christodouloupoulos¹

¹Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

²Laboratory of Pathology, School of Veterinary Medicine, Aristotle University, Thessaloniki, Macedonia, Greece

ABSTRACT: Ruminal acidosis is presented as the most considerable nutritional disorder of ruminants with severe impacts on animal health, animal welfare and considerable economics losses in small ruminant and bovine herds. The disease can be distinguished as acute (ARA) and subacute ruminal acidosis (SARA). Subacute ruminal acidosis constitutes the main nutritional disorder in intensive ruminant farming with several complications, such as liver abscesses, milk fat depression, reduced milk yield, and early culling. This paper constitutes the first part of a thorough review of ruminal acidosis. The first part focuses on the definition, types of ruminal acidosis, epidemiology, economic impacts and welfare implications.

Keywords: ruminal acidosis; acute ruminal acidosis; subacute ruminal acidosis; milk fat; milk production; animal welfare

Corresponding Author:

Nikolaos Voulgarakis, Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, P.O. Box 199, GR-43100, Karditsa, Greece
E-mail address: nvoulgarakis@uth.gr

Date of initial submission: 31-08-2022
Date of acceptance: 22-02-2023

INTRODUCTION

Ruminal acidosis has been introduced in the literature since 1959 with several names, such as rumen overload, toxic indigestion, grain engorgement, grain overload, grain poisoning, and acute indigestion (Krotkova et al., 1959; Dehkordi and Dehkordi, 2011). It is classified into two basic types, acute and subacute (also called latent or subclinical) based on the degree of ruminal pH reduction in the rumen and the clinical manifestations (Aschenbach et al., 2011; Reis et al., 2014). Acute Ruminal Acidosis (ARA) is usually observed in feedlots with a significant morbidity and mortality rate compared to subacute ruminal acidosis (SARA) which is frequently recorded in dairy farms and compromises farm profitability due to treatment costs, reduced herd productive and early culling (Kleen et al., 2003; Krause and Oetzel, 2006; Snyder and Credille, 2017).

Ruminal acidosis is considered to be one of the most frequent metabolic disorders especially for SARA, many authors have highlighted the considerable economic losses mainly due to reduced milk production and low body condition (Krause and Oetzel, 2006; Chaudhry et al., 2018). From an animal welfare perspective, SARA is considered a source of further complications in animal health due to consequent pathological situations such as abdominal pain and depression (Oetzel, 2003; Wang et al., 2019).

The aim of this part (I) of the review is to present a comprehensive aspect of the etiology, pathophysiology, and clinical presentation of ruminant acidosis in cattle and small ruminants.

DEFINITION

Ruminant acidosis is a ruminant nutritional disorder that emerge due to the consumption of feed with high carbohydrate and low digestive fiber content (Mahmood et al., 2013; Snyder and Credille, 2017). The pathophysiological definition of ruminal acidosis is the rapid reduction of ruminal pH due to extensive accumulation of small chain fat acids (SCFAs) (Kawas et al., 2007; Bramley et al., 2008).

TYPES OF RUMINAL ACIDOSIS

As mentioned, two main types of ruminal acidosis, ARA and SARA, have been suggested by the majority of the researchers.

In bovine medicine, physiological rumen pH varies between 6 and 7 for animals on a roughage diet

(Gruenberg and Constable, 2009). In small ruminants, normal ruminal pH ranges between 5.8 and- 6.8 (Jasmin et al., 2011; Wang et al., 2019). A range of ruminal pH at 5.0-5.6 is suggestive for SARA. Ruminal pH below 5.0, approaching 4.5 or lower, characterizes the ARA (Krause and Oetzel, 2006). In small ruminants, rumen pH decreases below 5.2 in cases of ARA and between 5.2 and 5.8 in cases of SARA (Church, 1991; Commun et al., 2009).

Acute Ruminal Acidosis (ARA)

Acute Ruminal Acidosis called clinical ruminal acidosis or acute ruminal lactic acidosis (ARLA). Ruminal pH value lower than 5 is suggested as the main diagnostic feature for ARA (Commun et al., 2009; Kleen and Cannizzo, 2012). In bovine, ARA is characterized by a large accumulation of lactic acid (50-150 mM) (Blanch et al., 2009). In small ruminants during ARA, concentrations of lactic acid varying around 34 mM and total Volatile Fatty Acids (VFAs) around 94 mM (Wiryawan and Brooker, 1995).

In addition, several changes evoke in microbial populations, such as the decreased population of Gram-negative bacteria and lactic acid-utilizers, the increased population of Gram-positive bacteria (*Streptococcus bovis*, *Lactobacillus* spp.), the increased population of lactic acid-producers and the absence or reduction of ciliated protozoa. Furthermore, other microbial toxic products are present in cases of ARA such as ethanol, amines, and endotoxins (Wiryawan and Brooker, 1995; Nagaraja and Titgemeyer, 2007).

During ARA, blood profile is also altered with blood pH recorded below 7.35 (normal range 7.35-7.45), lactic acid increased (especially the D-lactic acid), bicarbonate decreased <20 mEq/L and packed cell volume (PCV) increased >40% (Nagaraja and Titgemeyer, 2007).

It has been well established that ARA causes damage to the epithelial barrier integrity in the rumenoreticulum and/or abomasum, which allows toxins (Lipopolysaccharide- LPS), biogenic amines and bacteria, such as *Fusobacterium necrophorum*, to infiltrate across ruminal epithelium (Tadepalli et al., 2009; Minuti et al., 2014).

Epidemiology

The acute form is considered rarer and mainly accidental compared to the subacute form (Vasconcelos and Galyean, 2008; Giger-Reverdin, 2018). The in-

cidence of ARA varies between 0.3-3% during lactation, with a considerably higher rate during the 1st-month post-calving (Krause and Oetzel, 2006; Lean et al., 2007).

Acute ruminal acidosis emerges after the significant consumption of rapidly fermentable carbohydrates such as starch, corn, wheat and barley in ruminants unadapted to a high-concentrate diet. The administration of wheat flour at a dose of 50 g/kg of body weight (BW) in sheep and a dose of 80-100 g/kg BW in goats can cause ARA (Aslan et al., 1995; Lettat et al., 2010). Similarly, ruminal acidosis can be provoked due to the unintended gross consumption of sorghum flour amounts especially for goats at a dose of 50 g/kg BW (Nour et al., 1998). Further, ARA is observed in the early feeding period when newly feedlot cattle are introduced to a primarily concentrate-based ration and stepped up too rapidly (Krause and Oetzel, 2006).

Feeding practices with a pasture of low neutral detergent fiber (NDF) and high non-structural carbohydrate (NSC) may induce acidosis (Wales et al., 2001). Diets that are deprived of forage (<10% of total Dry Matter Intake, DMI) significantly increase the risk of ruminal acidosis (Enemark et al., 2002). Ruminal acidosis may be invoked from the ingestion of small particle size (diameter less than 1.78mm) (Yansari et al., 2004; White et al., 2014). ARA is more frequent when animals are grouped than alone due to the psychological competition for overconsuming (Smith, 2009).

A great risk of acidosis for sheep is recorded when animals gain access to cropping regions especially after harvest. This risk is attributed to the availability of large amounts of grain, either remaining after harvest or split during the handling, in combination with the lack of any adaption stage. Similar issues are provoked by the rapid introduction of sheep to crops, particularly brassicas such as rapes, kales or turnips, that are highly digestible (Lean et al., 2007).

Clinical signs

Acute ruminal acidosis emerges as one of the most significant causes of increased morbidity and mortality in ruminant production systems (Plaizier et al., 2017).

The affected ruminants are presented with tachypnea, shallow respiration, diarrhea, profuse salivation, nasal discharge, abdominal respiration, and bilateral abdominal dictation. Behaviour alterations are man-

ifested by anorexia, weakness, depression, lethargy, staggering, recumbency, flank watching, and lameness (Meyer and Bryant, 2017; Pupin et al., 2017). Clinical and laboratory testing reveal pyrexia in early stage and hypothermia in later stages, tachycardia, dehydration, congestion of mucous membranes and vessels of sclera, pneumonia, metabolic acidosis, hypocalcaemia, severe toxemia, acidified urine, and ruminal atony (Snyder and Credille, 2017; Reis et al., 2018).

Moreover, central nervous system symptoms may be presented such as ataxia, blindness, head pressing, opisthotonos, altered gait, teeth grinding, incoordination, tremors, seizures, prostration, and coma. The recumbent ruminants are perceived with their heads tucked in their flanks and similar presentation of parturient paresis (Meyer and Bryant, 2017; Sabes et al., 2017). Ruminants with a peracute form of acidosis emerge dead with few or no obvious clinical signs (Snyder and Credille, 2017).

In bovine medicine, profuse, watery foul-smelling or fetid diarrhea can be presented with a gray color and undigested grains or the presence of blood due to the increased passage and decreased digestibility (Meyer and Bryant, 2017; Snyder and Credille, 2017). In small ruminant medicine, diarrhea could be watery with yellowish color and acidic odor or greenish or soupy or just watery feces (Minuti et al., 2014; Pupin et al., 2017).

Pathoanatomical lesions

The hallmark of macroscopic examination of ruminant epithelium in ARA is easy detachment of ruminal papillae accompanied by patchy areas of sloughed papillae and multifocal erosion or ulceration (Ismail et al., 2010). In ARA and repeated ruminal acidosis episodes parakeratosis is also observed. Parakeratosis is the thickening of the *stratum corneum* and emerges with the dark color of ruminal mucosa (Enemark, 2008; Lopez-Campos et al., 2010). The ruminal papillae mucosa appears brown, friable, and easily detached. Parakeratosis often results in rumenitis due to impaired epithelial barrier caused by the decreased ruminal pH (Steele et al., 2009). Rumen content with undigested grain and sour smell and profuse amounts of fluid in the whole gastrointestinal tract are common findings in bovine with ARA (Meyer and Bryant, 2017). Nodules on papillae in both dorsal and ventral ruminal sacs, hyperaemia of papillae in *atrium ruminis*, epithelial necrosis, dyskeratosis, and acute super-

ficial ulcers have been referred in ARA cases, as well (Aslan et al., 1995; Fanning et al., 2018). On the other hand, Odongo et al., (2006) reported a reduced number of papillae on the ventral sac of the rumen during ruminal acidosis in lambs.

Ruminal fluid during ARA (intra-ruminal lactate concentration >30 mg/100 ml or 3.3 mmol/L) presents several aspects such as milky/greenish-brown or gray or yellow to orange color, sticky/sour or putrid odor and frothy consistency with contained pods and fragments. Laboratory analysis reveals watery or thin viscosity, no or fast flotation/sedimentation, pH 5.2-3.8, methylene blue reduction (MBR) test >5 min, reduced glucose fermentation test, absence or reduced protozoal population and dominance of gram-positive bacteria (Snyder and Credille, 2017; Ribeiro et al., 2020).

At the microscopical level, the most prominent lesions seen in rumen epithelium after ARA and repeated ruminal acidosis episodes are increased apoptosis of ruminal epithelium and parakeratosis (Steele et al., 2009; Minuti et al., 2014). Attenuation of the cell defense system due to acidosis is suspected to be the mechanisms that render epithelial cells more vulnerable to cellular damage and apoptosis (Steele et al., 2009; Hollmann et al., 2013). Ruminal parakeratosis is characterized by the accumulation of keratinized, nucleated squamous epithelial cells. Moreover, the cellular adhesion in the *stratum corneum* and *stratum spinosum* is weakened after ARA episodes. Large gaps among *stratum corneum* and *stratum granulosum* lead to microbial colonization of rumen epithelium and consequent infiltration of inflammatory cells (Steele et al., 2009). In small ruminants, parakeratosis in the rumen, omasum and reticulum is referred to as the main lesion with mild to severe detachment of the mucosa from the submucosa, ballooning degeneration of mucosal cells with cellular debris, swollen and vacuolated epithelia with intraepithelial pustules, necrosis, and presence of neutrophils (Bacha et al., 2017; Pupin et al., 2017).

Additionally, LPS, endotoxins, and histamine release evoke endotoxic shock, cardiovascular collapse, rumen ulceration, mycotic or bacterial rumenitis, renal failure, liver abscess, laminitis, enterotoxaemia, systemic acidemia, and thromboembolic respiratory disease (Snyder and Credille, 2017; Ribeiro et al., 2020). Lungs are characterized by congestion with severe diffuse edema (Lira et al., 2013). Liver failure, muscular weakness, and further rhabdomyolysis are

aggravated due to recumbency during ARA (Constable, 2010; Fartashvand and Haji-Sadeghi, 2018).

Economic impacts

ARA emerges as a financial significant loss in the beef production industry, where the estimated treatment cost is 10 dollars/animal (Snyder and Credille, 2017). In Australia, the financial losses are estimated at 9 million dollars/year in beef feedlots (Shu et al., 2000).

A survey over six years about mortality and morbidity in feedlot cattle presents that SARA and ARA emerge with 3-7% morbidity and 14-36% mortality (Edwards, 1996). Rahman et al., (2014) reported morbidity rates at 10-50% in goats with ARA. The fatality rates are 90% for untreated animals and 30-40% for treated animals (Oliveira et al., 2009).

Subacute Ruminal Acidosis (SARA)

It is also called Subclinical Ruminal Acidosis or Latent Acidosis. The consumption of huge amounts of rapidly fermentable carbohydrates combined with inadequate fiber induce SARA, which is characterized by a low ruminal pH (5.0-5.5), an increase in VFA (150-225 mM) and a fluctuating lactate acid (10-40 mM) (Calsamiglia et al., 2012; Li et al., 2014). In the subacute form of acidosis, the clinical signs are not obvious. Several microbial changes are observed during the incident of SARA such as an increase of *Lactobacillus* spp., lactic acid-producing bacteria and lactic-acid utilizers, and the absence (or) reduction of ciliated protozoa (Nagaraja and Titgemeyer, 2007).

For definition of SARA in bovine medicine several cut-off values have been suggested based on the duration of decreased pH, such as 6.0, 5.8, 5.6, and 5.5, with the majority to agree with values below 5.6 for a period of 3h/day (Petzold et al., 2014; Bilal et al., 2016).

Several authors demonstrated as the main characteristic of subacute ruminal acidosis, in small ruminants, short periods with a pH range between 5.0-5.6 (Shen et al., 2019b). In caprine medicine, a range of 5.5 to 6.0 is used as rumen pH cut-off in SARA definition for at least 3 hours (Jiang et al., 2014; Taghipoor et al., 2020).

Furthermore, SARA is based on the presence of butyrate or propionate or both acids. The butyric form of SARA is defined as a consequence of increased corn consumption (1.2% of BW) and characterized

by a ruminal pH among 5.5-5.8, an increased butyrate percentage (from 13 to 22% of total VFA), the total value of VFA at 107 mM and a reduction of lactate concentration (<5 mM) (Doreau et al., 2001; Lettat et al., 2012). According to Lettat et al., (2010), the propionic form of SARA in small ruminants is caused by increased consumption of beet pulp (1.2% of BW) and is characterized by a mean ruminal pH among 5.8-6.2, a propionate percentage at 35%, a total VFA concentration at 110 mM and lactate concentration less than 10 mM. However, in bovine, a propionic form of SARA has as its characteristics, a mean pH of 5.67, a total VFA concentration at 114 mM, and a lactate concentration of less than 3 mM (Khafipour et al., 2009).

Epidemiology

SARA emerges commonly in intensive livestock production systems (Lopez-Campos et al., 2010; Gao and Oba, 2014). Episodes of low ruminal pH and consequent SARA in lactating cows are recorded among calving and 5 months post-calving (Kleen et al., 2013). However, the beef feedlot cattle could be exposed anytime to SARA, from the onset of their gathering into the feedlots until culling time, with pH <5.5 and prevalence of 14 to 42% (Nagaraja and Lechtenberg, 2007).

In dairy cattle, the occurrence of SARA is estimated at 11-26% with 11-20% in early and 18-26% in mid-lactation (Kitkas et al., 2013; Atkinson, 2014; Stefanska et al., 2016). A higher SARA frequency was recorded at a farm with history of high SARA prevalence in early- and mid-lactation [48.2%, 53.8% and 65.3% at 30, 90 and 150 days in milk (DIM), respectively] (Kitkas et al., 2019). The early lactation period is at high risk due to the diminished size and reduced absorption ability of rumen epithelium/papillae, fewer adapted rumen microflora and the rapid introduction to high energy-dense diets (DeVries et al., 2009). During mid-lactation, SARA emerges due to management errors such as automatic feeding misadjustment, wrong calculation of components in the preparation of total mixed ration (TMR), inconsistency in feeding time- schedule, and insufficient feed bunk space per cow (Kleen et al., 2003, Kitkas et al., 2013).

In sheep farming, similar findings were presented by Lira et al., (2013) in an extended survey in north-eastern Brazil, where ruminal acidosis emerged at a frequency of 13.9% in goats and 12.4% in sheep. A cross-sectional survey of 19 dairy sheep farms in Ar-

gentina reported that 42.1% of flocks challenged presented ruminal acidosis (Suarez and Busetti, 2009).

Primiparous cows present a higher frequency of SARA (29% than 19% of multiparous cows) and occurrence earlier in lactation. The above emerged because primiparous cows are not yet capable of managing their feed intake during the consumption of a high-energy diet and are not able to have access to feed bunk for small and frequent meals due to socially domination (Oetzel, 2017; Kitkas et al., 2013).

Clinical signs

The clinical signs of SARA emerge after a delay of onset time or disguise by other common diseases, such as lameness and considerable deterioration of body condition (Colman et al., 2015).

The affected by SARA ruminants are usually presented with intermittent diarrhea, poor body condition and reduced or variable feed intake, and rumination. Behaviour alterations comprise mild transient anorexia, alternating mood, depression, and weakness. Clinical and laboratory examination present signs of dehydration, rumen stasis, abdominal pain, feces alterations, laminitis, metabolic disorders, lower fecal and urinary pH, reduced milk yield, and reduced fat and protein concentration in milk (Snyder and Credille, 2017; Shen et al., 2019a). In bovine, continuous tail swishing, cud drooping during rumination, and increased colic incidence are presented as further clinical signs of SARA (Abdela, 2016; Meyer and Bryant, 2017).

Feces may be presented brighter and of yellowish color with the sweet-sour smell. The consistency may be loose and frothy with fibrin casts, undigested particles (1-2 cm) and whole cereal grains. Significant body fecal soiling, especially in the hindquarters has been reported in the case of SARA (Meyer and Bryant, 2017; Oetzel, 2017).

Furthermore, SARA impact on bovine reproduction consists of infertility, abortions, stillbirths, premature births and being prone to environmental mastitis (Abdela, 2016; Snyder and Credille, 2017).

Pathoanatomical lesions

Macroscopic examination of the rumen wall in SARA reveals grey to dark brown mucosa and is considered a typical sign of parakeratosis and/or hyperkeratosis (Ploger et al., 2012). The major impacts of SARA are parakeratosis, thickening and detachment

of epithelium and rumenitis (Steele et al., 2009). While parakeratosis is considered a persistent finding, the thickness of the total epithelium, *stratum corneum*, *stratum granulosum* and *stratum spinosum* may appear debilitated during SARA cases as well (Steele et al., 2011; Zhang et al., 2020). The pathogenetic mechanisms responsible for these lesions are similar to those pertaining to ARA.

Furthermore, necropsy besides rumen lesions may reveal liver abscesses, subcutaneous abscesses, pulmonary bacterial emboli, peritonitis, or inflammation in organs such as kidneys (pyelonephritis), lungs (pneumonia), heart valves (endocarditis) and joints (arthritis) (Kleen et al., 2003; Abdela, 2016).

Economic impacts

According to literature cost estimate of SARA in the USA dairy industry, based on milk production losses, reduction efficiency of milk production, premature and high culling rate, rises up to 1.12 USD/day/cow. Economic losses of SARA are estimated up to 500 million - 1 billion dollars/year in total USA dairy industry (Enemark, 2008; McCann et al., 2016).

For the beef USA industry, Stock, (2000) reports a financial loss of 9.40 dollars/feedlot steer due to acidosis impact on the reduction of final cattle weight. Furthermore, the reduced DMI due to SARA leads to the decreased growth of beef calves with losses of 10-13 dollars/animals (Stock and Britton, 1996).

Effect of SARA on milk production

Administration of the HC diet (High Concentrate diet; concentrate 60%) may increase milk quality and milk yield in a short duration of time, but also may reduce rumen pH and increase the risk of SARA in dairy ruminants (Abaker et al., 2017; Shen et al., 2019a). SARA reduces milk production and milk fat concentration but has no effect on protein and energy levels (Chang et al., 2015; Xu et al., 2015). On the other hand, short-term SARA has no effect on milk fat or protein concentration (Li et al., 2012; Tian et al., 2017).

A ratio of milk fat: milk protein lower than 1 in animals feed with an HC diet, could be a useful diagnostic sign of SARA in ruminants (Enemark et al., 2002; Giger-Reverdin et al., 2014). According to Cook et al., (2005) in bovine medicine, the incidence of SARA should be suspected when 10% of cows have individual milk fat percentages of 2.5 or less.

Also, Danscher et al., (2015) reported a reduction of milk fat content at 4.14% during SARA.

The estimated reduction in milk production, milk fat and true protein is 2.7-3 kg/cow/day, from 37 to 34 g/kg (0.3% points) and 29 to 28 g/kg (0.12%), respectively (Xu et al., 2016). However, the financial losses of reduced milk fat and milk protein are estimated at 400-475 dollars/cow without taking into account the early culling and veterinary expenses (Abdela, 2016).

The increase in milk Somatic Cell Count (SCC) is reported as a sign of SARA due to reduced milk production (higher cell concentration) and due to the greater inflammatory status in cases of HC diets (Shen et al., 2019a; Antanaitis et al., 2020).

WELFARE IMPLICATIONS

Several authors reported that ruminants affected with acidosis will be distinguished due to their feeding behavior, which is dependent on daily DMI, cumulative intake patterns, fractional intake rate, sorting behavior, and chewing behavior (Calsamiglia et al., 2012; Giger-Reverdin, 2018).

During SARA a higher rate of saturation or an aversion to diet can be mainly presented by reduced concentrated feed intake and increased roughage intake (Nocek, 1997; Giger-Reverdin et al., 2014). The "off-feed" periods during acidosis decrease and alter feeding intake patterns (Calsamiglia et al., 2012).

Ruminants present different patterns in dealing with ruminal acidosis based on chewing behavior for buffering rumen pH, which explains why several animals suffer acidosis more than others in a herd with the same diet. During SARA, the chewing/kg Dry Matter (DM) duration and saliva production is decreased (Giger-Reverdin et al., 2014). It is noteworthy that goats can browse rather than graze, which allows them to choose their feed in a feedstuff variety during SARA (Desnoyers et al., 2008).

Animals with acidosis prefer to nibble or eat small amounts of food. This can be explained as an effort to increase the daily intake rate and further reduce the movement of high rapidly fermentable carbohydrates amount into the rumen during acidosis (Giger-Reverdin, 2012). The total time of eating, chewing, and ruminating is decreased in goats with HC diets, due to the reduction of NDF (Maekawa et al., 2002; Desnoyers et al., 2008).

The episodes of ruminal acidosis may be manifest-

ed by a slightly total effect on cow behavior such as standing time, lying time, or feeding time (DeVries et al., 2009). Brzozowska et al., (2013) report that SARA causes discomfort which stimulates the cow to increase feeding time due to an increase in both meal frequency and duration. Lying time and rumination during SARA is decreased (by 1h/day) and standing time is increased. Furthermore, high acidosis risk (HR) cows sort their ratio to a greater level than low acidosis risk (LR) cows (DeVries et al., 2009).

Goats with SARA demonstrate a position curled up as a sign of sickness or pain mainly in the afternoon, especially after feeding hours (Desnoyers et al., 2008). On the other hand, sheep are more aggressive and less responsive, and they are devoting less time to recumbency and more time to standing with the head up posture and ears pricked up during SARA (Commun et al., 2012).

Water or salt consumption may be higher during the acidosis challenge. Salt supplementation in water causes 1.4 times more drinking water during acidosis (Commun et al., 2012).

CONCLUSION

It is generally accepted that ruminal acidosis and especially subacute ruminal acidosis emerges as one

of the main restraining factors in livestock productivity. In ruminal research, topics concerning the definition and clinical manifestation are adequately investigated and presented.

On the other hand, crucial topics such as epidemiology, productivity implications, and financial consequences are still under research, especially in the small ruminant herd with still vital space in research and justification.

The necessity to define the actual prevalence and incidence of SARA in small ruminant herds is unquestionable. Methods and techniques applied in cow husbandry will provide useful information for understanding and addressing these metabolic processes in small ruminants. Additionally, the investigation of resource-based and management-based measures will provide beneficial information on the economic impacts and welfare status of dairy herds.

Undoubtedly, every sign of illness in a dairy herd could potential be an indication of acidosis and therefore as a scientific community, we should be vigilant and prepared for perceiving and assessing these metabolic issues.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

REFERENCES

- Abaker JA, Xu TL, Jin D, Chang GJ, Zhang K, Shen XZ (2017) Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J Dairy Sci* 100:666–678.
- Abdela N (2016) Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. *Achiev Life Sci* 10:2:187-196.
- Antanaitis R, Juozaitienė V, Malašauskienė D, Televičius M (2020) Inline Reticulorumen pH as an Indicator of Cows Reproduction and Health Status. *Sensors (Basel)* 20(4):1022.
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2011) Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89:1092-1107.
- Aslan V, Thamsborg SM, Jorgensen RJ, Bassel A (1995) Induced Acute Ruminal Acidosis in Goats Treated with Yeast (*Saccharomyces cerevisiae*) and Bicarbonate. *Acta Vet Scand* 36:65-77.
- Atkinson O (2014) Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract* 22(1):1-9.
- Bacha FB, Pupin RC, Leal PV, Carvalho NM, Franco GL, Ítavo CCBF, Riet-Correa F, Lemos RAA (2017) Experimental poisoning by *Enterobacterium contortilis* in sheep. *Pesqui Vet Bras* 37(1):23-30.
- Bilal MS, Abaker JA, Aabdin ZU, Xu T, Dai H, Zhang K, Liu X, Shen X (2016) Lipopolysaccharide derived from the digestive tract triggers an inflammatory response in the uterus of mid-lactating dairy cows during SARA. *BMC Vet Res* 12(1):284.
- Blanch M, Calsamiglia S, DiLorenzo N, DiCostanzo A, Muetzel S, Wallace RJ (2009) Physiological changes in rumen fermentation during acidosis induction and its control using a multivalent polyclonal antibody preparation in heifers. *J Anim Sci* 87:1722-1730.
- Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND (2008) The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. *J Dairy Sci* 91:308-321.
- Brzozowska AM, Sloniewski K, Oprzadek J, Sobiech P, Kowalski ZM (2013) Why are dairy cows not able to cope with the subacute ruminal acidosis?. *Pol J Vet Sci* 16:4:813-821.
- Calsamiglia S, Blanch M, Ferret A, Moya D (2012) "Is subacute ruminal acidosis a pH related problem? Causes and tools for its control". *Anim Feed Sci Technol* 172(1-2):42-50.
- Chang G, Zhuang S, Seyfert HM, Zhang K, Xu T, Jin D, Guo J, Shen X (2015) Hepatic TLR4 signaling is activated by LPS from digestive tract during SARA, and epigenetic mechanisms contribute to enforced TLR4 expression. *Oncotarget* 6:36.
- Chaudhry AS, Khan MA, Khan MS, Ashraf K, Saleem MH, Khan NU, Usman T, Ahmad I (2018) Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J Anim Plant Sci* 28(1):80-84:1018-7081.
- Church DC (1991) *Livestock Feeds and Feeding*, third ed Prentice-Hall Inc USA 349 pp.
- Colman E, Waegeman W, De Baets B, Fievez V (2015) Prediction of sub-acute ruminal acidosis based on milk fatty acids: a comparison of linear discriminant and support vector machine approaches for model development. *Comput Electron Agric* 111:179-85.
- Commun L, Mialon MM, Martin C, Baumont R, Veissier I (2009) Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J Anim Sci* 87:3372-3379.
- Commun L, Silberberg M, Mialon MM, Martin C, Veissier I (2012) Behavioural adaptations of sheep to repeated acidosis challenges and effect of yeast supplementation. *Anim* 6:2011-2022.
- Constable PD (2010) Grain overload in ruminants. *The Merck Veterinary Manual* 10th ed (Khan C, Ed) Merck & Co Inc Whitehouse USA pp:200-203.
- Cook NB, Oetzel GR, Nordlund KV (2005) Clinical forum-subacute ruminal acidosis-a problem in UK dairy herds?. *UK Vet* 10:40-46.
- Dansch AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC (2015) Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Vet Scand* 57(1):39.
- Dehkordi AJ, Dehkordi ZK (2011) Occurrence of metabolic alkalosis in rumen lactic acidosis: a review article. *Comp Clin Pathol* 20(1):1-3.
- Desnoyers M, Duvaux-Ponter C, Rigalma K, Roussel S, Martin O, Giger-Reverdin S (2008) Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. *Anim* 2:12:1802-1808.
- DeVries TJ, Beauchemin KA, Dohme F, Schwartzkopf-Genswein KS (2009) Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feeding, ruminating, and lying behaviour. *J Dairy Sci* 92:5067-5078.
- Doreau M, Ollier A, Michalet-Doreau B (2001) An atypical case of ruminal fermentations leading to ketosis in early lactating cows. *Rev Med Vet (Toulouse)* 152:301-306.
- Edwards A (1996) Respiratory diseases of feedlot cattle in central USA. *Bovine Practitioner* 0:30:5-7.
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet J* 176:1:32-43.
- Enemark JMD, Jørgensen RJ, Enemark P (2002) Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Vet Ir Zootech* 20(42):16-29.
- Fanning JP, Hynd PI, Cockcroft PD (2018) The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. *Small Rumin Res* 162:57-62.
- Fartashvand M, Haji-Sadeghi Y (2018) Evaluation of serum cardiac biomarkers in sheep with acute lactic acidosis. *J Hell Vet Medical Soc* 68(2):219-224.
- Gao X, Oba M (2014) Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet. *J Dairy Sci* 97(5):3006-16.
- Giger-Reverdin S (2018) Recent advances in the understanding of sub-acute ruminal acidosis (SARA) in goats, with focus on the link to feeding behaviour. *Small Rumin Res* 163:24-28.
- Giger-Reverdin S, Lebarbier E, Duvaux-Ponter C, Desnoyers M (2012) A new segmentation-clustering method to analyse feeding behaviour of ruminants from within-day cumulative intake patterns. *Comput Electron Agric* 83:109-116.
- Giger-Reverdin S, Rigalma K, Desnoyers M, Sauvant D, Duvaux-Ponter C (2014) Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *J Dairy Sci* 97:4367-4378.
- Gruenberg W, Constable P (2009) Function and Dysfunction of the Ruminant Forestomach. in *Current Veterinary Therapy Elsevier Inc* pp. 12-19.
- Hollmann M, Miller I, Hummel K, Sabitzer S, Metzler-Zebeli BU, Razazi-Fazeli E, Zebeli Q (2013) Downregulation of Cellular Protective Factors of Rumen Epithelium in Goats Fed High Energy Diet. *PLoS ONE* 8(12):e81602.
- Ismail M, Mahmoud A, Nasr M, Badr Y (2010) Clinical and laboratory studies on experimentally induced acute ruminal lactic acidosis in male goats. *Alex J Vet Sci* 31:53-62.
- Jasmin BH, Boston RC, Modesto RB, Schaer TP (2011) Perioperative ruminal pH changes in domestic sheep (*Ovis aries*) housed in a biomedical research setting. *J Am Assoc Lab Anim Sci* 50(1):27-32.
- Jiang XY, Ni YD, Zhang SK, Zhan YS, Shen XZ (2014) Identification of Differentially Expressed Proteins in Liver in Response to Sub-acute Ruminal Acidosis (SARA) Induced by High-concentrate Diet. *Asian-Australas J Anim Sci* 27:8:1181-1188.
- Kawas JR, Garcia-Castillo R, Fimbres-Durazo H, Garza-Cazares F, Hernandez-Vidal JFG, Olivares-Saenz E, Lu CD (2007) Effects of sodium bicarbonate and yeast on nutrient intake, digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. *Small Rumin Res* 67:149-156.
- Khafipour E, Krause DO, Plaizier JC (2009) Alfalfa pellet-induced sub-

- acute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. *J Dairy Sci* 92:1712-1724.
- Kitkas GC, Valergakis GE, Karatzias H, Panousis N (2013) Subacute ruminal acidosis: prevalence and risk factors in Greek dairy herds. *Iran J. Vet. Res.* 14:183-189.
- Kitkas GC, Valergakis GE, Kritsepi-Konstantinou M, Gelasakis A, Arsenos G, Kalaitzakis E, Panousis (2019) Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation. *J Hell Vet Med* 70:1551-1560.
- Kleen JL, Cannizzo C (2012) Incidence, prevalence and impact of SARA in dairy herds. *Anim Feed Sci Technol* 172:4-8.
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): A review. *J Vet Med A Physiol Pathol Clin Med* 50:406-414.
- Kleen JL, Uppgang L, Rehage J (2013) Prevalence and consequences of subacute ruminal acidosis in German dairy herds. *Acta Vet Scand* 55:48.
- Krause KM, Oetzel GR (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim Feed Sci Technol* 126:215-236.
- Krotkova AP (1959) Effect of sugar beet on acidosis in the ruminant. *Zhivotnovodstvo* 7:63-64.
- Lean IJ, Annison F, Bramley E, Browning G, Cusack P, Farquharson B, Little S, Nandapi D (2007) Ruminal Acidosis- aetiopathogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR) A V Association ed Blackwell Publishing Asia Pty Ltd Carlton Vic Australia pp. 1-60.
- Lettat A, Nozière P, M Silberberg, Morgavi P, Berger C, Martin C (2010) Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. *J Anim Sci* 88:3041-3046.
- Lettat A, Nozière P, Silberberg M, Morgavi DP, Berger C, Martin C (2012) Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. *BMC Microbiol* 12:142.
- Li F, Cao Y, Liu N, Yang X, Yao J, Yan D (2014) Subacute ruminal acidosis challenge changed in situ degradability of feedstuffs in dairy goats. *J Dairy Sci* 97:5101-5109.
- Li S, Gozho G, Gakhar N, Khafipour E, Krause DO, Plaizier JC (2012) Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can J Anim Sci* 92:353-364.
- Lira MAA, Simões SVD, Riet-Correa F, Pessoa CMR, Dantas AFM, Miranda Neto EG (2013) Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui Vet Bras* 33(2):193-198.
- Lopez-Campos O', Bodas R, Prieto N, Giraldez FJ, Perez V, Andres S (2010) Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim* 4:6:958-964.
- Maekawa M, Beauchemin KA, Christensen DA (2002) Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *J Dairy Sci* 85(5):1165-75.
- Mahmood AK, Khan MS, Khan MA, Kahn MA, Bilal M, Farooq U (2013) Lactic Acidosis in Goats: Prevalence, Intra-ruminal and Haematological investigations. *J Anim Plant Sci* 23(6):1527-1531.
- McCann JC, Luan S, Cardoso FC, Derakhshani H, Khafipour E, Looor JJ (2016) Induction of Subacute Ruminal Acidosis Affects the Ruminal Microbiome and Epithelium. *Front Microbiol* 7:701.
- Meyer NF, Bryant TC (2017) Diagnosis and Management of Rumen Acidosis and Bloat in Feedlots. *Vet Clin North Am Food Anim Pract* 33(3):481-498.
- Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Jahan N, Bani P (2014) Experimental acute rumen acidosis in sheep: Consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. *J Anim Sci* published online July 1.
- Nagaraja TG, Lechtenberg KF (2007) Acidosis in feedlot cattle. *Vet Clin Food Anim Pract* 23:333-350.
- Nagaraja TG, Tittgemeyer EC (2007) Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci* 90(E. Suppl.):E17-E38.
- Nocek JE (1997) Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005-1028.
- Nour MMS, Abusamra MT, Hago BED (1998) Experimentally induced lactic acidosis in Nubian goats: Clinical, biochemical and pathological investigations. *Small Rumin Res* 31:7±17.
- Odongo NE, Alzahal O, Lindenger MI, Duffield TF, Valdes EV, Terrell SP, McBride BW (2006) Effects of mild heat stress and grain challenge on acid-base balance and rumen tissue histology in lambs. *J Anim Sci* 84:447-455.
- Oetzel GR (2003) Subacute ruminal acidosis in dairy cattle. *Advances in Dairy Cattle* 15:307-317.
- Oetzel GR (2017) Diagnosis and Management of Subacute Ruminal Acidosis in Dairy Herds. *Vet Clin North Am Food Anim Pract* 33:3:463-480.
- Oliveira DM, Medeiros JMA, Assis ACO, Neves AB, Galiza GJN, Simões SVD, Dantas AFM, Riet-Correa F (2009) Acidose láctica ruminal aguda em caprinos. *Ciênc Anim Bras* 117-122.
- Petzold M, Meyer U, Spilke J, Danicke S (2014) Using rumen probes to examine effects of conjugated linoleic acids and dietary concentrate proportion on rumen pH and rumen temperature of periparturient dairy cows. *J Anim Physiol Anim Nutr* 98:785-796.
- Plaizier JC, Li S, Tun HM, Khafipour E (2017) Nutritional models of experimentally-induced subacute ruminal acidosis (SARA) differ in their impact on rumen and hindgut bacterial communities in dairy cows. *Front Microbiol* 7:2128.
- Ploger S, Stumpff F, Penner GB, Schulzke JD, Gabel G, Martens H (2012) Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann N Y Acad Sci* 1258: 52-59.
- Pupin RC, Leal PV, Lima SC, Melo GKA, Pott A, Araújo MA, Barros CSL, Lemos RAA (2017) *Enterolobium contortisiliquum* is a cause of acute ruminal acidosis in sheep. *Toxicon* 126:90-95.
- Rahman MdM, Islam MS, Adam GO, Alam MdR, You MJ (2014) Prevalence of ruminal lactic acidosis and clinical assessments of four therapeutics in goats of Bangladesh. *J Vet Clin* 31:0-6.
- Reis LF, Araújo CASC, Sousa RS, Minervino AHH, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto Júnior RA, Mori CS, Ortolani EL (2018) Prevention of acute ruminal lactic acidosis in sheep by probiotic or monensin supplementation: clinical aspects. *Semin Ciênc Agrar* 39:4:1575-1584.
- Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Ortolani EL (2014) Comparison of Rumen Fluid pH by Continuous Telemetry System and Bench pH Meter in Sheep with Different Ranges of Ruminal pH. *Hindawi Publishing Corporation Scientific World Journal Volume, Article ID 195782*, 4 pages.
- Ribeiro ACS, Conceição AI, Soares GSL, Correia FR, Oliveira-Filho JP, Soaresd PS, Mendonça CL, Afonso JAB (2020) Hemogasometry, cardiac biomarkers and blood metabolites in goats with experimentally induced acute ruminal lactic acidosis. *Small Rumin Res* 191:106187.
- Sabes AF, Girardi AM, Fagliari JJ, Oliveira JA, Marques LC (2017) Serum proteinogram in sheep with acute ruminal lactic acidosis. *Int J Vet Sci Med* 5:1:35-40.
- Shen Y, Zhao F, Yu L, Yang W, Wang M, Wang H (2019a) Starch sources and concentration in diet of dairy goats affected ruminal pH and fermentation, and inflammatory response. *Anim Prod Sci* 59:9:1640-1647.
- Shen YZ, Ding LY, Chen LM, Xu JH, Zhao R, Yang WZ, Wang HR, Wang MZ (2019b) Feeding corn grain steeped in citric acid modulates rumen fermentation and inflammatory responses in dairy goats. *Anim* 13:2:301-308.
- Shu Q, Gill HS, Leng RA, Rowe JB (2000) Immunization with *Streptococcus bovis* vaccine administered by different routes against lactic acidosis in sheep. *Vet J* 159:262-269.
- Smith BP (2009) Large animal internal medicine: diseases of the alimentary system. In: Murray MJ, Guard C editors. *Large animal internal medicine* 4th ed New York (NY): Mosby.
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33(3):451-461.
- Steele MA, AlZahal O, Hook SE, Croom J, McBride BW (2009) Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet Scand* 51:39.

- Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K, McBride BW (2011) Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am J Physiol Regul Integr Comp Physiol* 300:6:1515-1523.
- Stefańska B, Nowak W, Komisarek J, Taciak M, Barszcz M, Skomiał J (2016) Prevalence and consequence of subacute ruminal acidosis in polish dairy herds. *J Anim Physiol Anim Nutr* 101(4):694-702.
- Stock R (2000) Acidosis in cattle: an overview. In: *Proceedings of the 33rd Annual Convention of the American Association of Bovine Practitioners*, Rapid City USA pp. 30-37.
- Stock R, Britton RL (1996) Acidosis. *NebGuide*, University of Nebraska-Lincoln. October 1995 <<http://www.ianr.unl.edu/pubs/animaldisease/g1047.htm>> (assessed 27.11.99).
- Suárez VH, Buseti MR (2009) Health management practices and disease prevalence in dairy sheep systems in Argentina. *Pesqui Vet Bras* 29(11):931-937.
- Tadepalli S, Narayanan SK, Stewart GC, Chengappa MM, Nagaraja TG (2009) *Fusobacterium necrophorum*: A ruminal bacterium that invades liver to cause abscesses in cattle. *Anaerobe* 15:36-43.
- Taghipoor M, Delattre M, Giger-Reverdin S (2020) A novel modelling approach to quantify the response of dairy goats to a high-concentrate diet. *Sci Rep* 10:20376.
- Tian P, Luo Y, Li X, Tian J, Tao D, Hua C, Geng Y, Ni Y, Zhao R (2017) Negative effects of long-term feeding of high-grain diets to lactating goats on milk fat production and composition by regulating gene expression and DNA methylation in the mammary gland. *J Anim Sci Biotechnol* 8:74.
- Vasconcelos JT, Galyean ML (2008) ASAS centennial paper: contributions in the *Journal of Animal Science* to understanding cattle metabolic and digestive disorders. *J Anim Sci* 86:1711-1721.
- Wales WJ, Williams YJ, Doyle PT (2001) Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial ryegrass pastures. *Aust J Exp Agric* 41:465-71.
- Wang K, Zheng M, Ren A, Zhou C, Yan Q, Tan Z, Zhang P, Yi K (2019) Effects of High Rice Diet on Growth Performance, Nutrients Apparent Digestibility, Nitrogen Metabolism, Blood Parameters and Rumen Fermentation in Growing Goats. *Kafkas Univ Vet Fak Derg* 25(6):749-755.
- White BH, Clayton EH, Friend MA (2014) The effect of mechanical processing of wheat straw on intake and rumen parameters following grain feeding in sheep. *Anim Prod Sci* 54:1455-1459.
- Wiryawan KG, Brooker JD (1995) Probiotic control of lactate accumulation in acutely grain-fed sheep. *Aust J Agric Res* 46:1555-68.
- Xu C, Shen T, Yang W, Yu H, Gao S, Huang B (2016) The effect of subacute ruminal acidosis of dairy cows on productivity, digestibility and greenhouse gas emission. *J Agric Sci* 8:92.
- Xu T, Tao H, Chang G, Zhang K, Xu L, Shen X (2015) Lipopolysaccharide derived from the rumen down-regulates stearoyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet Res* 11:52.
- Yansari AT, Valizadeh R, Naserian A, Christensen DA, Yu P, Shahroodi FE (2004) Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. *J Dairy Sci* 87:3912-3924.
- Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, Wang HR (2020) Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 103:1931-1943.

2. “Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment.”

Published

Voulgarakis N, Athanasiou L, Psalla D, Gougoulis D, Papatsiros V, Christodouloupoulos G.

Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment. J Hellenic Vet Med Soc, 2024;74(4),6329–6336. <https://doi.org/10.12681/jhvms.31438>

Journal of the Hellenic Veterinary Medical Society

Vol 74, No 4 (2023)



Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment

N Voulgarakis, LV Athanasiou, D Psalla, D Gougoulis, V Papatsiros, G Christodouloupoulos

doi: [10.12681/jhvms.31438](https://doi.org/10.12681/jhvms.31438)

Copyright © 2024, N Voulgarakis, LV Athanasiou, D Psalla, D Gougoulis, V Papatsiros, G Christodouloupoulos



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

Voulgarakis, N., Athanasiou, L., Psalla, D., Gougoulis, D., Papatsiros, V., & Christodouloupoulos, G. (2024). Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment. *Journal of the Hellenic Veterinary Medical Society*, 74(4), 6329–6336. <https://doi.org/10.12681/jhvms.31438>

Ruminal Acidosis *Part II: Diagnosis, Prevention, and Treatment*

N. Voulgarakis^{1*}, L.V. Athanasiou¹, D. Psalla², D. Gougoulis¹, V. Papatsiros¹,
G. Christodouloupoulos³

¹*Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece*

²*Laboratory of Pathology, School of Veterinary Medicine, Aristotle University, Thessaloniki, Macedonia, Greece*

³*Department of Animal Science, Agricultural University of Athens, Athens, Greece*

ABSTRACT: Ruminal acidosis is presented as the most significant nutritional disorder of ruminants with severe impact on animal health, welfare and considerable economics losses. This pathological condition presented in two forms acute ruminal acidosis (ARA) and subacute ruminal acidosis (SARA). The present manuscript constitutes the second part of ruminal acidosis review focusing on diagnosis, prevention, and treatment. Diagnosis of SARA presents several difficulties due to the non-clinically patent symptoms. Prevention and treatment are based on the amelioration of nutrition including the use of dietary supplements, and antimicrobial administration as well as the improvement of management practices.

Keywords: ruminal acidosis; acute ruminal acidosis; subacute ruminal acidosis; diagnosis; prevention; treatment

Corresponding Author:

Nikolaos Voulgarakis, Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, P.O. Box 199, GR-43100, Karditsa, Greece
E-mail address: E-mail address: nvoulgarakis@uth.gr

Date of initial submission: 17-09-2022
Date of acceptance: 09-01-2023

INTRODUCTION

Acidosis is defined as a reduction in the alkali (base excess) in body fluids relative to the acid (hydrogen ion) content (Dehkordi and Dehkordi, 2011). Ruminal acidosis is categorized into two major types subacute ruminal acidosis (SARA) and acute ruminal acidosis (ARA) based on ruminal pH range and clinical manifestations (Reis et al., 2014; Oetzel, 2017).

Several diagnostic examinations are applied not only for ruminal acidosis evaluation but also for prognosis and preventative interventions (Snyder and Credille, 2017). Rumination activity, biochemical profiles, blood-gas analysis, milk analysis, feces, and urinalysis provide crucial data for SARA management. Ruminal acidosis prevention is performed at the herd level with interventions in nutrition preparation and management and further to ancillary dietary or not supplementations such as buffers, probiotics, yeast, plant-derived extracts with antimicrobial properties, antibiotics, vaccination, and rumen fluid transformation (Oetzel, 2017).

The aim of this part (II) of the review is to present the current diagnostic methods, management interventions, and treatment protocols of acidosis in bovines and small ruminants reported over the last 30 years (1990-2020).

DIAGNOSTIC APPROACH

Sampling protocol and rumen pH values

ARA diagnosis is considered straightforward when the rumen pH is between 4.0 and 5 for a period of 6 to 24 hours after induction of acidosis (Kirbas et al., 2014; Reis et al., 2018).

On the contrary several but non unanimously accepted protocols have been recommended for diagnosis of SARA. In cattle twelve (12) samples collected by rumenocentesis is considered to be an optimum sample number for SARA evaluation. The samples should be collected within 2-4h after concentrate meal supplementation in herds or within 5-8h in grass only or total mixed ration (TMR) fed herds. The above-mentioned protocol should be performed at 45-150 days in mid-lactation or among 2 and 180 lactation days. There are two reported cut-off points, pH ≤ 5.5 (positive) and pH > 5.8 (negative) for SARA diagnosis. Several authors report that if at least 3/12 samples have ruminal pH ≤ 5.5 then herd is considered SARA positive. In any other situation, (prevalence of low ruminal pH between 16.7-33.3%) additional sam-

pling or another diagnostic index of SARA is recommended (Kleen et al., 2003; Oetzel, 2017).

In small ruminants, several authors proposed slightly different limits for SARA diagnosis with rumen pH between 5.2 to 5.8 for a duration of 3h/d (Ma et al., 2018; Wang et al., 2020).

Sampling of rumen content and pH measurement

The standard diagnostic method is rumen fluid extraction via rumenocentesis and measurement of pH with portable pH meter (Morgante et al., 2009; Chaudhry et al., 2018). The most important disadvantages of rumenocentesis are the limited volume of rumen fluid collected and the estimation of ruminal pH alone without any other data such as the color, the odor, and the microflora populations (Mialon et al., 2012). Moreover, abscess or haematoma formation or septic peritonitis are among the reported complications of the procedure (Aceto et al., 2000; Penner et al., 2009).

Oral stomach tube has also been used to collect ruminal fluid samples for pH determination. However, is not reliable, as pH varies along the ruminal areas reached by the tube, saliva contamination (alkaline pH values), sampling in the fiber mat of the rumen and time of sampling in relation to feeding (Lean et al., 2007; Lee et al., 2019).

In ruminants, rumen cannula is proposed for collecting reliable samples of ruminal fluid, but it is only used in research (Nocek, 1997). However, welfare issues have been raised regarding this practice and there are a number of practical constraints, such as frequently cleaning, repeated removal and replacement of cannula cover and monitor rumen cannula sites, restricting their utilize in field situations (Lean et al., 2007; Tajik and Nazifi, 2011).

Bench pH-meter is the most commonly used technique for evaluation of rumen pH (Sato et al., 2012; Zhang et al., 2020). Despite being more practical, the test paper strips for pH evaluation do not provide reliable results due to the color of ruminal fluid which may conflict with the interpretation of the color change on the strip and also the large fluctuations of pH (Kirbas et al., 2014; Oetzel, 2017).

It is worth noted that collection of rumen samples with different techniques in same animal show different pH values (Duffield et al., 2004; Al Zahal et al., 2007; Bramley et al., 2008). Comparing pH values

in samples collected by rumenocentesis vs stomach tube, several authors report a mean difference in rumen pH from 0.28 to 1.1 with the lower values detected by rumenocentesis (Nordlund et al., 1995; Garrett et al., 1999; Enemark et al., 2004). Also, samples by rumenocentesis present 0.28 units lower ruminal pH than ruminal cannula (Garrett et al., 1999).

Telemetric systems

In recent years, telemetric systems are used for continuously measurements of rumen pH and temperature. Telemetric systems consist of a submersible electrode, a data logger, a transfer cable and specific software. The electrodes are mainly placed in the ventral sac of the rumen. The continuous telemetry measurement system evaluates rumen fluid pH every 5 minutes and has a sensitivity of 0.01 pH units. Telemetry units present several limitations such as rapid fluctuation in pH values, bulky size, rumen fistula insertion of pH sensor and necessity of retrieval for calibration and battery replacement (Oetzel, 2017; Reis et al., 2018).

Electronic devices that are called 'smart pills' or 'smart boluses' belong also to the category of telemetric systems. Such devices are administered once, are completely maintenance-free, and measure direct, insightful values, including rumen pH, with the highest accuracy inside your cows, in the reticulum. Specific readout devices with integrated Internet connection read out the data from the 'smart boluses'. SARA is included in the diagnostic possibilities of such devices (Kilic, 2011).

Ancillary diagnostic tests

In addition to measuring the rumen pH, many other tests have been proposed to diagnose SARA and predict the onset of ARA, and some have been successful even without having yet been widely applied in practice.

The detection of rumination activity via automated sensors can be used as a possible SARA indicator in cattle (Oetzel, 2017). Cattle that are ill feed less consequently ruminate less, thus; the estimation of ruminating time could be used as a health index. A range of methods using microphones, accelerometers and laser interferometry have been evaluated to derive this information (Hamilton et al., 2019).

The fat percentage in milk is also considered as evidence of absence of SARA in a herd, but the case of

low value could not be used as an index of SARA due to more causative agents of milk fat reduction. Milk fat values must be evaluated frequently (at least once a week) for better evaluation of SARA status (Enemark, 2008). Acidosis should be further investigated if more than 10% of cows in a herd test have a higher milk protein than milk fat or a rapid fall in milk fat of 0.3-0.5% in a week or a sudden fall in milk protein of >0.3% in a week occurs (Kleen et al., 2003; Lean et al., 2007).

Rumen temperature can be used as a new diagnostic tool for the evaluation of ruminal acidosis. Particularly ruminal temperature can be increased above 39.2°C and reach up to 41°C with ruminal pH values among 5.6 and 5.0. During ARA, ruminal temperature can reach maximum value of 40.5°C with pH value of 4.2 (AlZahal et al., 2008; Reis et al., 2018).

Blood pH is also used in the diagnosis of acidosis. Low blood pH (7.1-7.44) is reported in all forms of acidosis (Li et al., 2012; Chaudhry et al., 2018). The affected animals with ARA or SARA present a reduction of plasma pH, base excess, total CO₂⁻ content (TCO₂) and bicarbonate and an increased anion gap (Sabes et al., 2017; Snyder and Credille, 2017). The standard base excess (SBE) values are between 14 and 16 mmol/l in case of acidosis induction (Aslan et al., 1995).

Ruminal lactic acid can also be used as another acidosis index. The normal ruminal lactic acid values are between 0-5 mmol/l, whereas during ARA are increased at 50-150 mmol/l (Nagaraja and Titgemeyer, 2007; Henning et al., 2010). On the other hand, the lactic acid concentration is increased by less than 1 mM during SARA (Sun et al., 2018).

In addition, urine pH measurement has been used in the diagnosis of ruminal acidosis. In ruminants, decreased urine pH values of 5.3-6.7 have been reported during ARA (Patra et al., 1993; Sabes et al., 2017). Urine pH during SARA ranges from 7.8 to 8.3 (Li et al., 2012; Danscher et al., 2015). Moreover, when the net acid-base excretion (NABE) is <83 mmol/l, the inorganic phosphate concentration is >5.7 mmol/l and the urine pH is <7.8, the cow should be considered as subacute acidotic (Furll, 2014).

Stool pH <5 is indicative of ARA, while when it falls below 6.0 it is compatible with SARA (Li et al., 2012; Minuti et al., 2014; Danscher et al., 2015; Chaudhry et al., 2018). Investigation of acidosis is

recommended if more than 15% of a herd is scouring. In farm routine, the stool scoring of at least 25 fresh manure samples in the field using the 1-5 scoring system is also recommended and taking action if more than 5/25 samples are score 2 or less (Lean et al., 2007).

Furthermore, values of acute phase proteins (APPs) have been proposed as indicators of ruminal acidosis by many researchers (Gonzalez et al., 2010; Zebeli et al., 2012; Nikvand et al., 2020).

TREATMENT

In ARA cases, treatment strategy should aim to adjust ruminal pH through prevention of lactic acid accumulation, correction of electrolyte losses, adequate circulating blood volumes and normal gastrointestinal motility (Radostits et al., 1994). Recumbent ruminants are usually of poor prognosis. However, severely affected ruminants should be euthanized for welfare reasons (Meyer and Bryant, 2017).

Hypertonic saline solution (HSS) (7.2%, dose of 4ml/kg over a period of 10 minutes) should use to compensate for a considerable volume deficit. However, the results of the above solution are of short duration and should be followed by oral or intravenous fluids infusion (5-10L/liter of HS infusion) (Snyder and Credille, 2017). For clinical acidosis, 5% NaHCO₃ solution is recommended at 5L over 30 minutes for a 450kg/animal, followed by 1.3% NaHCO₃ infusion at a volume of 150ml/kg of body weight (BW) over 6 to 12 hours. Another solution of NaHCO₃ (8.4%) is used nowadays for a shorter treatment at a rate of 5ml/kg BW over a time of 10 to 20 minutes. NaHCO₃ administration should be followed by isotonic intravenous or oral fluid (Snyder and Credille, 2017).

The therapeutic protocol for repairing rumen microflora requires the removal of acidic rumen content, the infusion of rumen buffers and the transformation of rumen fluid. The transformation of acidic rumen fluid can be achieved by either rumenotomy or rumen lavage. Rumen lavage is applied by repeated flushing of the rumen with warm water via a large-bore stomach tube in mild acidosis cases (Snyder and Credille, 2017).

Rumenotomy or rumen lavage should be followed by infusion of rumen fluid from a donor with a normal diet. Regarding the optimum amount of rumen fluid for the infusion, several authors propose the 3-10 L for cattle heavier than 400 kg of BW and 2-4 L for

smaller ones (Snyder and Credille, 2017).

The ruminal fluid transfaunation (RFT) is considered as a routine procedure in ruminant production systems (DePeters et al., 2014). Healthy RFT provides rapid recovery of rumen fluid color, odor, and consistency, unaffected fermentative activity of rumen microflora and rapid return of protozoa population (Pereira et al., 2018). RFT relieves the damage of rumen epithelium morphology and function due to an increase of total Volatile Fatty Acids (VFA), acetate, propionate, and butyrate concentrations, reduction of lactate and Lipopolysaccharide (LPS) concentrations and thus the restoration of rumen epithelial integrity (Liu et al., 2019).

PREVENTION

Nutrition

An adequate intake of neutral detergent fiber (NDF) and acid detergent fibre (ADF) is necessary for keeping ruminal pH within the normal interval (Lean et al., 2007). A minimum of 25% NDF in dry matter of a total diet for dairy cattle (Li et al., 2014). Likewise, in small ruminant, a proportion 30% of NDF is proposed (Lean et al., 2007; Jaramillo-Lopez et al., 2017).

Management

A considerable management issue is the bunk space for easily feeding of all animals without excessive competition. The social issues among cows, such as domination, could be vanished if the bunk space is 0.38-0.46m/cow (Lean et al., 2007; Snyder and Credille, 2017).

For the prevention of SARA, a further nutritional management strategy could include meal size and frequency. The frequency of meals fluctuates between 8 and 12 meals/day, but there is a significant animal-to-animal variation as well as day-to-day variation in meal frequency and size (Meyer and Bryant, 2017).

Dietary Buffers

The dietary buffers cannot eliminate ruminal acidosis but they can minimize or prevent it (Enemark, 2008). The oral administration of buffers is used in affected animals, which are not treated with rumenotomy or lavage (Snyder and Credille, 2017).

a) Sodium Bicarbonate (SB)

Sodium bicarbonate (SB) addition in feedstuffs

increases rumen pH and palatability and prevents rumen epithelium from damage by the proliferation of the acid-tolerant lactobacilli during lactic acidosis episodes (Kleen et al., 2003; Dai et al., 2017). In dairy cattle rations, SB, at a dose of 150g/day, increases dry matter intake (DMI), milk production and milk fat percentage (Enemark, 2008). In bovine diets based on corn silage, a dose of 200-300g SB/animal/day is proposed (Lean et al., 2007).

b) Sodium Butyrate (NaB)

Sodium butyrate (NaB) is used as an anti-inflammatory agent to prevent SARA (Bilal et al., 2017). NaB supplement administration evokes the reduction of LPS and pro-inflammatory cytokine concentrations (Aabdin et al., 2018). Administration of 10 g NaB with or without 10 g SB in a high concentrate (HC) diet keeps steady ruminal pH and prevents LPS accumulation (Li et al., 2018; Ma et al., 2018).

c) Various buffers

Magnesium hydroxide is recommended at a dose of 1g/kg of BW which is diluted in a volume of water adequate for entering throughout the rumen (Snyder and Credille, 2017).

Magnesium oxide raises rumen pH, milk production and milk fat percentage at a dose of 0.4-0.8% dry matter (DM) on corn silage and pasture-based diets. The rumen pH is increased after the administration of 0.5% magnesium oxide and 1.5% SB to both low and high-forage diets. A dose of 30-45g magnesium oxide /animal/day is proposed for acidosis prevention (Lean et al., 2007).

In addition, the administration of limestone at 1-2% in diet can keep steady rumen pH between 5.7 and 6.0 and ameliorate DMI in fattening lambs and cattle (Jaramillo-Lopez et al., 2017).

Probiotics (Direct-Fed Microbials DFM) and yeast

Several direct-fed-microbials (DFM) are used as feed additives for acidosis prevention, such as *Enterococcus faecium*, *Lactobacillus plantarum*, and yeast *Saccharomyces cerevisiae*, via intraruminal administration in the concentration of 10^5 cfu/ml. In lactating cows, 5g *Saccharomyces cerevisiae*/day (10^{10} cfu) can improve the average ruminal pH after one week of administration (Bach et al., 2007). In addition, the combination of *Enterococcus faecium* and yeast at a dose of 5×10^9 cfu/day has been proven to increase the ruminal digestion of forage DM and DM consump-

tion in early lactation cows (Enemark, 2008).

Probiotic yeasts have a symbiotic relationship with lactic acid-consuming bacteria [*Selenomonas ruminantium* (*Sel ruminantium*), *Megasphaera elsdenii* (*Me elsdenii*)] (Kleen et al., 2003; Henning et al., 2010). Moreover, the combination of *Sel ruminantium* subsp *lactilytica* and *Me elsdenii* could prevent lactic acidosis due to lactate fermentation by *Me elsdenii* and to glucose-lactate fermentation by *Sel ruminantium* subsp *lactilytica*. The above combination contributes to the growth stimulation of other rumen bacteria and the consequent reduction of *Streptococcus bovis* (*Str bovis*) domination (Wiryawan and Brooker, 1995).

Flavonoids, Essential Oils, and Antioxidants

Alternative strategies are been used for acidosis prevention, such as plants with antimicrobial properties and plants with similar results on ruminal fermentation as in-feed antibiotics (Durmic et al., 2012). β -sitosterol (BSS), one phytosterol (plantsterol), has been proven to relieve inflammatory response and control ruminal fermentation after high-grain diets consumption (Xia et al., 2020). Additional, Naringin, a type of citrus flavonoid with proven antioxidant properties, reduces the discomfort that might be associated with damage to the ruminal wall in cases of subclinical acidosis (Lopez-Campos et al., 2010).

Flavonoids and essential oils are defined as plant-derived compounds (Puvača et al., 2022). Flavonoids ameliorate fermentation and increase the population of lactate-consuming bacteria in the rumen. Essential oils from *Allium sativa* (garlic), *Origanum vulgare* (oregano), *Capsicum annum* (pepper), *Yucca schidigera* (mojave yucca), and *Cinnamomum cassia* (cinnamon) present antimicrobial ability, pH buffering and anti-inflammatory response (Jaramillo-Lopez et al., 2017; Oetzel, 2017; Puvača, 2022). Particularly, a mixture of cinnamaldehyde (0.18 g/d) and eugenol (0.09g/day) could reform the rumen microbial fermentation in beef heifers-fed high-grain diets (Jaramillo-Lopez et al., 2017).

Antimicrobials

Several antimicrobials have approved for the control of lactate-producing bacteria and liver abscess in feedlot cattle, as inhibitors of Gram-positive bacteria (Nurmeiliasari et al., 2017). The main antimicrobial-categories that have been used are ionophores (lasalocid, monensin) and non-ionophores (virginiamycin,

tylosin) (Kleen et al., 2003).

a) Ionophore Antibiom

In bovine, monensin ameliorates the ruminal pH due to the inhibition of most lactate-producing bacteria in favor of lactate-consuming bacteria (Oetzel, 2017). Monensin is added at 250-300mg/animal/day in dairy cattle for at least two days before the carbohydrate challenge for effective control of acidosis (Lean et al., 2007).

In small ruminants, monensin is used in high doses (22-33mg/kg of total DMI) for ruminal pH control (Meissner et al., 2010; Lammie et al., 2016). However, Miranda Neto et al., (2011) reported that monensin usage (33mg/kg DM) do not help to prevention of ARA in goats.

Lasalocid (0.66-1,32mg/kg BW) is also used as a feed additive in the worldwide cattle industry. This antibiotic provides a higher rumen pH and lowers L- and D- lactate concentrations in dairy cattle (Lean et al., 2007; Meyer and Bryant, 2017).

b) Non-Ionophore Antimicrobials

In small ruminants, virginiamycin (VM) administration (30-80mg/animal per day) reduces the proliferation of lactic acid-producing bacteria during the fermentation of grain (Jassim et al., 2003; Hutton et al., 2010). Virginiamycin, could be added to the diet of bovines at 20ppm or could be combined with ionophore antimicrobials e.g., monensin 20ppm and VM 20ppm (Lean et al., 2007).

Administration of tylosin (150mg/animal/day) combined with monensin or other ionophores antibiotics presented similar beneficial results with virginiamycin (Lean et al., 2007).

The usage of antibiotics can potentially provoke

negative effects, such as depressed ruminal fermentation and antimicrobial resistance and public health concerns due to antimicrobial residues while production costs are also increased (Wiryawan and Brooker, 1995). Due to this concerns limitations and bans of antibiotics usage have been applied by several countries (Lammie et al., 2016).

Vaccines

Immunization with intramuscular injection of a live *Str. bovis* vaccine (*Str. bovis* strain SB-5) followed by a booster dose at 28 days has been reported to reduce the risk of lactic acidosis (Enemark, 2008; Jaramillo-Lopez et al., 2017).

CONCLUSION

Ruminal acidosis emerges as the most important nutritional disorder in ruminant intensive farming. Especially SARA causes significant economic losses due to milk fat depression and liver abscesses. The diagnosis of SARA in practice remains questionable. Prevention plays a key role in controlling the epidemiologic occurrence and the consequent financial losses of SARA. Prevention should be based mainly in equilibration of providing diet with or without additions of dietary additives such as probiotics, buffers and minerals. Antibiotics should be considered as the ultimate solution with great concern on antimicrobial resistance issues.

Veterinarians should try to comprehend the mode of feed assimilation, energy accumulation, and pH regulation in ruminants. It is the only path to achieving desirable productivity goals with an appreciation of animal needs and welfare.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Aabdin ZU, Bilal MS, Dai H, Abaker JA, Liu X, Benazir S, Yan J, Shen X (2018) NOD1/NF- κ B signaling pathway inhibited by sodium butyrate in the mammary gland of lactating goats during sub-acute ruminal acidosis. *Microb Pathog* 122:58-62.
- Aceto H, Simeone AJ, Fergusson JD (2000) Effect of rumenocentesis on health and productivity in dairy cows. *J Anim Sci* 78:(1)(Abstr. 162).
- AlZahal O, Kebreab E, France J, Froetschel M, McBride BW (2008) Ruminal temperature may aid in the detection of subacute ruminal acidosis. *J Dairy Sci* 91:(1):202-7.
- Alzahal O, Kebreab E, France J, McBride B (2007) A Mathematical Approach to Predicting Biological Values from Ruminal pH Measurements. *J Dairy Sci* 90:3777-85.
- Aslan V, Thamsborg SM, Jorgensen RJ, Bassel A (1995) Induced Acute Ruminal Acidosis in Goats Treated with Yeast (*Saccharomyces cerevisiae*) and Bicarbonate. *Acta Vet Scand* 36:65-77.
- Bach A, Dinare's M, Devant M, Carre X (2007) Associations between lameness and production, feeding, and milking attendance of Holstein cows milked with a robotic milking system. *J. Dairy Res* 74:52-57.
- Bilal MS, Abaker JA, Aabdin ZU, Waheed U, Xu T, Dai H, Junfei G, Shen X (2017) High grain diet triggers inflammation in the goat uterus: A comprehensive regulation diet modulates the immune response. *Int J Agric Biol* 19:34-40.
- Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND (2008) The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. *J Dairy Sci* 91:308-321.
- Chaudhry AS, Khan MA, Khan MS, Ashraf K, Saleem MH, Khan NU, Usman T, Ahmad I (2018) Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J Anim Plant Sci* 28:(1):80-84:1018-7081.
- Dai H, Liu X, Yan J, Aabdin ZU, Bilal MS, Shen X (2017) Sodium butyrate ameliorates high-concentrate diet-induced inflammation in the rumen epithelium of dairy goats. *J Agric Food Chem* 65:596-604.
- Dansch AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC (2015) Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Vet Scand* 57:(1):39.
- Dehkordi AJ, Dehkordi ZK (2011) Occurrence of metabolic alkalosis in rumen lactic acidosis: a review article. *Comp Clin Pathol* 20:(1):1-3.
- DePeters EJ, George LW (2014) Rumen transfaunation. *Immunol Lett* 162:69-76.
- Duffield T, Plaizier J, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J, McBride B (2004) Comparison of Techniques for Measurement of Rumen pH in Lactating Dairy Cows. *J Dairy Sci* 87:59-66.
- Durmie Z, Hutton P, Murray K, Vercoe PE (2012) Inclusion of selected levels of Australian native plant *Eremophila glabra* in fermentation substrate can influence events leading to rumen lactic acidosis in vitro and in vivo carbohydrate-challenged systems. *Anim Feed Sci Technol* 178:57-66.
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet J* 176:1:32-43.
- Enemark JMD, Jorgensen RJ, Kristensen NB (2004) An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. *Vet Res Commun* 28(8):687-709.
- Fürll M (2014) Möglichkeiten zur praktischen Diagnostik von Pansenfermentationsstörungen bei Kühen. *Tierärztl Umsch* 69:315-322.
- Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR (1999) Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. *J Dairy Sci* 82:1170-1178.
- Gonzalez FHD, Ruiperez FH, Sanchez JM, Souza JC, Martinez-Subiela S, Ceron JJ (2010) Haptoglobin and serum amyloid A in subacute ruminal acidosis. *Rev Med Vet Zoot* 57:159-167.
- Hamilton AW, Davison C, Tachtatzis C, Andonovic I, Michie C, Ferguson HJ, Somerville L, Jonsson NN (2019) Identification of the Rumination in Cattle Using Support Vector Machines with Motion-Sensitive Bolus Sensors. *Sensors* 19(5):1165. <https://doi.org/10.3390/s19051165>.
- Henning PH, Horn CH, Steyn DG, Meissner HH, Hagg FM (2010) The potential of *Megasphaera elsdenii* isolates to control ruminal acidosis. *Anim Feed Sci Technol* 157:13-19.
- Hutton PG, Durmie Z, Vercoe PE (2010) Investigating *Eremophila glabra* as a bioactive agent for preventing lactic acidosis in sheep. *Anim Prod Sci* 50:449-453.
- Jaramillo-Lopez E, Itzá-Ortiz M, Peraza-Mercado G, Chávez J (2017) Ruminal acidosis: Strategies for its control. *Austral J Vet Sci* 49:139-148.
- Jassim ARAM, Gordon GLR, Rowe JB (2003) The effect of basal diet on lactate-producing bacteria and the susceptibility of sheep to lactic acidosis. *Anim* 77:459-469.
- Kilic U (2011) Use of Wireless Rumen Sensors in Ruminant Nutrition Research. *Asian J Anim Sci* 5:46-55. DOI: 10.3923/ajas.2011.46.55.
- Kirbas A, Baydar E, Kandemir FM, Dorman E, Kizil O, Yildirim BA (2014) Evaluation of serum cardiac troponin I concentration in sheep with acute ruminal lactic acidosis. *Vet Arh* 84:(4):355-364.
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): A review. *J Vet Med A Physiol Pathol Clin Med* 50:406-414.
- Lammie SL, Hughes JM (2016) Antimicrobial resistance, food safety, and one health: The need for convergence. *Annu Rev Food Sci Technol* 7:287-312.
- Lean IJ, Anison F, Bramley E, Browning G, Cusack P, Farquharson B, Little S, Nandapi D (2007) Ruminal Acidosis- aetiopathogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR) pp 1-60.
- Lee M, Jeong S, Seo J, Seo S (2019) Changes in the ruminal fermentation and bacterial community structure by a sudden change to a high-concentrate diet in Korean domestic ruminants. *Asian-Australas J Anim Sci* 32:1:92-102.
- Li F, Yang XJ, Cao YC, Li X, Yao JH, Li Z, Sun FF (2014) Effects of dietary effective fiber to rumen degradable starch ratios on the risk of sub-acute ruminal acidosis and rumen content fatty acids composition in dairy goat. *Anim Feed Sci Technol* 189.
- Li L, He ML, Wang K, Zhang YS (2018) Buffering Agent via Insulin-Mediated Activation of PI3K/AKT Signaling Pathway to Regulate Lipid Metabolism in Lactating Goats. *Physiol Res* 67:753-764.
- Li S, Gozho G, Gakhar N, Khafipour E, Krause DO, Plaizier JC (2012) Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can J Anim Sci* 92:353-364.
- Liu J, Li H, Zhu W, Mao S (2019) Dynamic changes in rumen fermentation and bacterial community following rumen fluid transplantation in a sheep model of rumen acidosis: implications for rumen health in ruminants. *FASEB J* 33:8453-8467.
- Lopez-Campos O, Bodas R, Prieto N, Giraldez FJ, Perez V, Andres S (2010) Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim* 4:6:958-964.
- Ma N, Abaker JA, Bilal MS, Dai H, Shen X (2018) Sodium butyrate improves antioxidant stability in sub-acute ruminal acidosis in dairy goats. *BMC Vet Res* 14:275.
- Meissner HH, Henning PH, Horn CH, Leeuw KJ, Hagg FM, Fouché G (2010) Ruminal acidosis: A review with detailed reference to the controlling agent *Megasphaera elsdenii* NCIMB 41125. *S Afr J Anim Sci* 40:(2).
- Meyer NF, Bryant TC (2017) Diagnosis and Management of Rumen Acidosis and Bloat in Feedlots. *Vet Clin North Am Food Anim Pract* 33:(3):481-498.
- Mialon MM, Deiss V, Andanson S, Anglard F, Doreau M, Veissier I (2012) An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anesthesia. *Vet J* 194:55-59.
- Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Jahan N, Bani P (2014) Experimental acute rumen acidosis in sheep: Conse-

- quences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. *J Anim Sci* published online July 1.
- Miranda Neto EG, Silva STG, Mendonça CL, Drummond ARF, Afonso JAB (2011) Aspectos clínicos e a bioquímica ruminal de caprinos submetidos à acidose láctica experimental e suplementados ou não com monensina sódica. *Pesqui Vet Bras* 31:416-424.
- Morgante M, Giancesella M, Casella S, Ravarotto L, Stelletta C, Giudice E (2009) Blood gas analyses, ruminal and blood pH, urine and faecal pH in dairy cows during subacute ruminal acidosis. *Comp Clin Path* 18:229-232.
- Nagaraja TG, Titgemeyer EC (2007) Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci* 90(E. Suppl.):E17-E38.
- Nikvand AA, Nouri M, Razi Jalali M, Teimory Azadbakht MR (2020) Effects of activated charcoal and zeolite on serum lipopolysaccharides and some inflammatory biomarkers levels in experimentally induced subacute ruminal acidosis in lambs. *Turk J Vet Anim Sci* 44:830-837.
- Nocek JE (1997) Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005-1028.
- Nordlund KV, Garrett EF, Oetzel GR (1995) Herd based rumenocentesis: a clinical approach to the diagnosis of subacute rumen acidosis. *Compendium Contin Educ Pract Vet* 17:8:48-56.
- Nurmeiliasari N, Priyanto R, Astuti DA, Salundik, Takahashi J (2017) Utilization of Rumen Mechanical Stimulator as Pseudo Fiber in Ruminant to Minimize Metabolic Problem. *Wartazoa* 27:2:067-080.
- Oetzel GR (2017) Diagnosis and Management of Subacute Ruminal Acidosis in Dairy Herds. *Vet Clin North Am Food Anim Pract* 33:3:463-480.
- Patra RC, Lal SB, Swarup D (1993) Physicochemical alterations in blood, cerebrospinal fluid and urine in experimental lactic acidosis in sheep. *Res Vet Sci* 54:217-220.
- Penner GB, Aschenbach JR, Gäbel G, Rackwitz R, Oba M (2009) Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to sub-acute ruminal acidosis in sheep. *J Nutr* 139:1714-1720.
- Pereira PFV, Romão FTNAM, Penzetti EM, Sanches JFZ, Curti JM, Flai-ban KKMC, Lisboa JAN (2018) Value of transfaunation for the treatment of induced ruminal lactic acidosis in goats and sheep. *Pesqui Vet Bras* 38:(4):670-678.
- Puvača N (2022) Bioactive Compounds in Dietary Spices and Medicinal Plants. *JATEM* 5:704-711.
- Puvača N, Tufarelli V, Giannenas I (2022) "Essential Oils in Broiler Chicken Production, Immunity and Meat Quality: Review of *Thymus vulgaris*, *Origanum vulgare*, and *Rosmarinus officinalis*" *Agriculture* 12(6):874.
- Radostits OM, Blood DC, Gay CC (1994) *Veterinary Medicine* 8th ed., Bailliere Tindall, London.
- Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Ortolani EL (2014) Comparison of Rumen Fluid pH by Continuous Telemetry System and Bench pH Meter in Sheep with Different Ranges of Ruminal pH. *Hindawi Publishing Corporation Scientific World Journal Volume*, Article ID 195782, 4 pages.
- Reis LF, Sousa RS, Oliveira FLC, Rodrigues FAML, Araújo CASC, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Minervino AHH, Ortolani EL (2018) Comparative assessment of probiotics and monensin in the prophylaxis of acute ruminal lactic acidosis in sheep. *BMC Vet Res* 14:9.
- Sabes AF, Girardi AM, Fagliari JJ, Oliveira JA, Marques LC (2017) Serum proteinogram in sheep with acute ruminal lactic acidosis. *Int J Vet Sci Med* 5:1:35-40.
- Sato S, Ikeda A, Tsuchiya Y, Ikuta K, Murayama I, Kanehira M, Okada K, Mizuguchi H (2012) Diagnosis of subacute ruminal acidosis (SARA) by continuous reticular pH measurements in cows. *Vet Res Commun* 36:201-205.
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33(3):451-461.
- Sun YY, Cheng M, Xu M, Song LW, Gao M, Hu HL (2018) The effects of subacute ruminal acidosis on rumen epithelium barrier function in dairy goats. *Small Rumin Res* 169:1-7.
- Tajik J, Nazifi S (2011) Serum Concentrations of Lipids and Lipoproteins and Their Correlations together and with Thyroid Hormones in Iranian Water Buffalo (*Bubalus bubalis*). *Asian J Anim Sci* 5:196-201.
- Wang Y, Liu J, Huang J, Chang G, Roy AC, Gao Q, Cheng X, Shen X (2020) Sodium butyrate attenuated iE-DAP induced inflammatory response in the mammary glands of dairy goats fed high-concentrate diet. *J Sci Food Agric* 101(3):1218-1227.
- Wiryawan KG, Brooker JD (1995) Probiotic control of lactate accumulation in acutely grain-fed sheep. *Aust J Agric Res* 46:1555-68.
- Xia G, Sun J, Fan Y, Zhao F, Ahmed G, Jin Y, Zhang Y, Wang H (2020) β -Sitosterol Attenuates High Grain Diet-Induced Inflammatory Stress and Modifies Rumen Fermentation and Microbiota in Sheep. *Anim* 10:171.
- Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W (2012) Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. *J Dairy Sci* 95:1041-1056.
- Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, Wang HR (2020) Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 103:1931-1943.

3. “Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle?”

Published

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Katsoulos PD, Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodouloupoulos G. Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle? Vet. Res. Commun.. 2024; 48(1):475-484. [https://doi.org/10.1007/s11259-023-10231-](https://doi.org/10.1007/s11259-023-10231-w)

[w](https://doi.org/10.1007/s11259-023-10231-w)



Can computerized rumen mucosal colorimetry serve as an effective field test for managing subacute ruminal acidosis in feedlot cattle?

Nikolaos Voulgarakis¹ · Dimitrios Gougoulis¹ · Dimitra Psalla² · Georgios Papakonstantinou¹ · Panagiotis-Dimitrios Katsoulos³ · Konstantinos Katsoulis⁴ · Mariana Angelidou-Tsifida² · Labrini Athanasiou¹ · Vasilios Papatsiros¹ · Georgios Christodouloupoulos⁵

Received: 12 August 2023 / Accepted: 4 October 2023
© The Author(s) 2023

Abstract

Subacute ruminal acidosis (SARA) is one of the major nutritional disorders in the dairy and beef industries, leading to significant financial losses. Diagnosing SARA is challenging due to the need to evaluate multiple parameters, such as milk fat/protein ratio, ruminal lactate, and hemogram, instead of relying on a single definitive symptom or diagnostic method. This study aimed to evaluate the effectiveness of computerized rumen colorimetry in detecting SARA in beef cattle. Over one year, 75 cattle aged 8–10 months from five farms were periodically monitored for rumen pH prior to slaughter. Samples of rumen wall and rumen content were obtained at slaughter for analysis. The study found a positive correlation coefficient between rumen pH and color components, particularly for Red (0.853) and color lightness (L) (0.862). The darkening of the rumen epithelium's color was attributed to the effect of rumen pH on the keratinized layer of the epithelium. Furthermore, an increase in the thickness of ruminal epithelium layers, particularly non-keratinized and total epithelium, was observed in animals with a history of SARA. It is possible that the lower rumen pH increases the rate of replacement of the keratinized epithelium, and the non-keratinized epithelium overgrows to compensate for the need to produce keratinized layers. In conclusion, computerized rumen colorimetry shows promise as a reliable method for managing SARA in bovine farms by monitoring the condition in the slaughterhouse. Further research is needed to evaluate its effectiveness in detecting SARA in live animals.

Keywords Subacute ruminal acidosis · Colorimetry · Rumen epithelium · Beef

Introduction

High-concentrate diets find common usage in both dairy and beef industries to enhance production efficiency. Nonetheless, this practice can precipitate ruminal acidosis (RA) due to the rapid reduction of ruminal pH (Plaizier et al. 2012; Abaker et al. 2017). RA manifests as acute (ARA) or subacute (SARA) categories based on pH reduction and clinical manifestations (Aschenbach et al. 2011). Within intensive livestock farming, SARA is among the important nutritional disorders due to its potential for substantial welfare and economic repercussions. These encompass the deterioration of milk quality and quantity, diminished feed intake, weight loss, formation of liver abscesses, and elevated culling rates (Kleen et al. 2003; Krause and Oetzel 2006; Enemark 2008).

The task of diagnosing and confirming SARA proves to be intricate due to the absence of pathognomonic symptoms and precise diagnostic techniques (Nocek 1997; Tajik and

✉ Georgios Christodouloupoulos
gc@aua.gr

¹ Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece

² Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Macedonia, Greece

³ Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Macedonia, Greece

⁴ Department of Animal Husbandry and Nutrition, Faculty of Veterinary Science, School of Health Sciences, University of Thessaly, Karditsa, Greece

⁵ Department of Animal Science, Agricultural University of Athens, Athens, Greece

Nazifi 2011; Snyder and Credille 2017). Field diagnosis of SARA primarily relies on measuring rumen fluid pH, whereby a rumen pH below 5.5–5.8 indicates the presence of SARA (Krause and Oetzel 2006; Plaizier et al. 2008; Humer et al. 2018). However, this pH measurement is subject to various influencing factors, including the method of measurement, sampling location, and timing of sampling (Neubauer et al. 2017; Villot et al. 2017).

Numerous researchers have noted a connection between the gray to dark discoloration of the rumen wall epithelium during necropsies of ruminants or culling and subacute and acute rumenitis (Enemark 2008). Nonetheless, an accurate interpretation of this coloration presents a challenge. Visual interpretation can be susceptible to bias, whereas instrumental colorimetric methods lack the capability for remote analysis and are particularly sensitive to the size of the colorimeter and spectrophotometer apertures (Yancey and Kropf 2008). Offering a practical and dependable alternative, computerized Red, Green, and Blue (RGB) analysis has found application in studies within the food industry (Chmiel et al. 2012; Silva et al. 2022).

This study seeks to establish a correlation between rumen epithelial color and SARA status in feedlot cattle, as determined through long-term periodic evaluations of rumen pH. The primary goal of this study was to assess the viability of computerized rumen colorimetry as a diagnostic tool for SARA within the context of herd management for feedlot cattle.

Materials and methods

Selection of farms

A total of 21 beef farms located in Thessaly, central Greece, were preselected for the study. These farms raise a breed of beef known as the “Greek Red Breed,” an adapted variant of the Limousin breed. The final selection was based on their ration and three-year medical history, extracted from clinical case records at the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly. The 21 pre-selected beef farms were divided into 2 categories: farms with no previous history of ARA (2 farms) and farms with a history of ARA (19 farms). Among the latter, 3 subgroups were formed based on the percentage of digestive fiber in their ration: (i) 5 farms with less than 15% Neutral Detergent Fiber (NDF); (ii) 8 farms with 15–20% NDF; and (iii) 6 farms with 20–25% NDF. The study encompassed all farms with no prior history of ARA (Category I, 2 farms) and a random selection of 3 farms with a history of ARA from each of the 3 subgroups (Category II, 3 farms), using computer-generated random numbers. Table 1 presents a breakdown of the feed composition for the 5 farms included in the study. There were no modifications to the dietary composition over the course of the 12-month trial period.

Experimental design

In each of the 5 participating beef farms, 15 cattle aged 8–10 months were subject to periodic monitoring every 2 weeks for rumen pH over the course of 1 year before slaughter (from September 2021 to August 2022). Inclusion criteria encompassed a body weight range of 220–246 kg (with an

Table 1 Ingredients, Chemical Composition, and Forage-to-Concentrate Ratio (F:C) in Total Mixed Rations for Farm 1 and Farm 2 (Category I), and Farms 3, 4, and 5 (Category II)

Item	Farm 1 Diet	Farm 2 Diet	Farm 3 Diet	Farm 4 Diet	Farm 5 Diet
Ingredients (% of DM)					
Alfalfa silage	20.0	15.0		20.0	19.0
Corn Silage	15.0	10.0	20.0	5.0	11.0
Wheat straw	5.0	15.0			
Ground Corn	25.0	20.0	60.0	40.0	40.0
Ground wheat			5.0		7.5
Ground barley	15.0	18.0		20.0	10.0
Soybean meal	12.0	14.5	12.0	12.5	10.0
Wheat Bran	5.0	5.0			
Vitamins and minerals	3.0	2.5	3.0	2.5	2.5
F:C	40:60	40:60	20:80	25:75	30:70
Chemical composition					
ME (Mcal/kg DM)	0.25	0.24	0.27	0.26	0.26
CP (% of DM)	16.1	15.2	14.0	16.3	15.2
NDF (% of DM)	31.9	34.4	14.8	22.2	23.2
ADF (% of DM)	18.3	20.7	9.1	12.2	12.9

ME = Metabolizable energy, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

initial body weight of 233 ± 13 kg, $n = 75$) and an absence of prior medical history. Measurement of rumen pH involved sampling the rumen content after esophageal tubing, specifically 3 h after the morning administration of concentrated feed. The average pH of all measurements taken throughout the year (24 measurements per animal) was designated as pH_{farm} . Each animal was distinguished with distinct national registration numbers affixed to plastic ear-tags on both ears. All animals received uniform preventive medication, including vaccination and antiparasitic drugs.

The 15 monitored cattle on each farm were co-housed with an additional 4–12 cattle of the same age range (8–10 months at the onset of monitoring). All calves within these enclosures were slated for slaughter in September 2022, attaining an age of 20–22 months. During the slaughterhouse phase, samples of rumen wall and rumen fluid were procured from the monitored animals designated for regular production cycle slaughter. It is important to note that no animals were slaughtered exclusively for experimental purposes.

At the time of slaughter, all 75 monitored animals were sampled. Directly following evisceration, samples of rumen content and rumen wall were collected. The pH of rumen fluids was measured, the color of the rumen epithelium was visually assessed, and a digital gross image of the rumen wall epithelium was captured. The recorded pH measurement at this juncture was denoted as $\text{pH}_{\text{abattoir}}$.

A total of 40 abdominal wall samples (20 from Category I and 20 from Category II) were submitted for histological examination at the Pathology Laboratory, Veterinary School, Aristotle University of Thessaloniki. The 40 rumen samples submitted were sourced from the initial 20 follow-up animals from each farm category sent to the slaughterhouse.

Sample size justification

According to the literature, the range of SARA prevalence is relatively wide from 14 to 42% (Nagaraja and Lechtenberg 2007). In Greece, there is limited information about the prevalence of SARA (Kitkas et al. 2013). An average value of 22% prevalence was selected in the sample size calculation enhanced by the fact that animals were partly selected by farms with a previous history of ARA. Therefore, a sample size of 66 animals was calculated with precision 0.1 and confidence level of 95% to which 10% were added for any sample depreciation (Sergeant 2018). Based on this calculation, the 75 samples investigated in this study were considered an adequate sample size.

Esophageal tubing (Rumen scoop)

Rumen fluid sampling was conducted using the Rumen Scoop (Flora, profs-products.com, Futtermittelüberwachung Bayern Regierung von Oberbayern, Sachgebiet 56, 80,534 München) (Geishauser et al. 2012). This all-metal device featured a 2.7-meter-long hose with a head and handle on each end. The head, functioning as a fluid scoop, was a cylindrical container measuring 3 cm in diameter and 21 cm in length, with a 40 mL capacity and side openings controlled by a screw mechanism.

To collect the rumen fluid, the head was inserted through the esophagus into the rumen for 10 to 20 s, allowing the fluid to be naturally drawn into the head without applying a vacuum. Subsequently, the handle activated the closing mechanism of the openings, and the device was carefully withdrawn. The head was then unscrewed and emptied. The gathered rumen fluid was immediately analyzed for pH using a digital portable pH meter (HI-2002 Edge pH Meter, Hanna instruments).

Abattoir work protocol

Prior to slaughter, cattle were subjected to an approximate 3-hour fasting period, during which they underwent a comprehensive physical examination. They were marked with spray paint on the fleece for monitoring from slaughter to evisceration. After evisceration, each rumen was re-marked with a labeled cable before entering the gut room, where it was separated by slaughterhouse staff, following the procedure described by Jonsson et al. (2020).

The rumen fluid sample was obtained through aspiration, using a 16G 105-mm needle and a 50 mL syringe, from the eviscerated rumen. Subsequently, it was drained into a sterile glass container with a capacity of 100 mL. The pH of the rumen content was promptly measured using the same digital portable pH meter mentioned earlier (HI-2002 Edge pH Meter, Hanna instruments).

The procedure for sampling the rumen wall involved specific steps. Firstly, the rumen was completely emptied of its contents. Next, the reticulorumen was oriented left-facing, with the reticulum in the front, the large rumen behind, the dorsal ruminal sac cranially, and the ventral ruminal sac caudally, with the esophagus positioned in front and dorsally. The location for the incision was determined based on standard measurements (Fig. 1), and a 5×5 cm square of the rumen wall was extracted for sampling. Subsequently, the sample was thoroughly washed with water to remove any visible residues of the rumen content. After cleaning, the sample epithelium was visually assessed, and was graded based on its color using a three-tiered score: (a) white or pale white (score 0), (b) grey (score 1), and (c) dark grey or

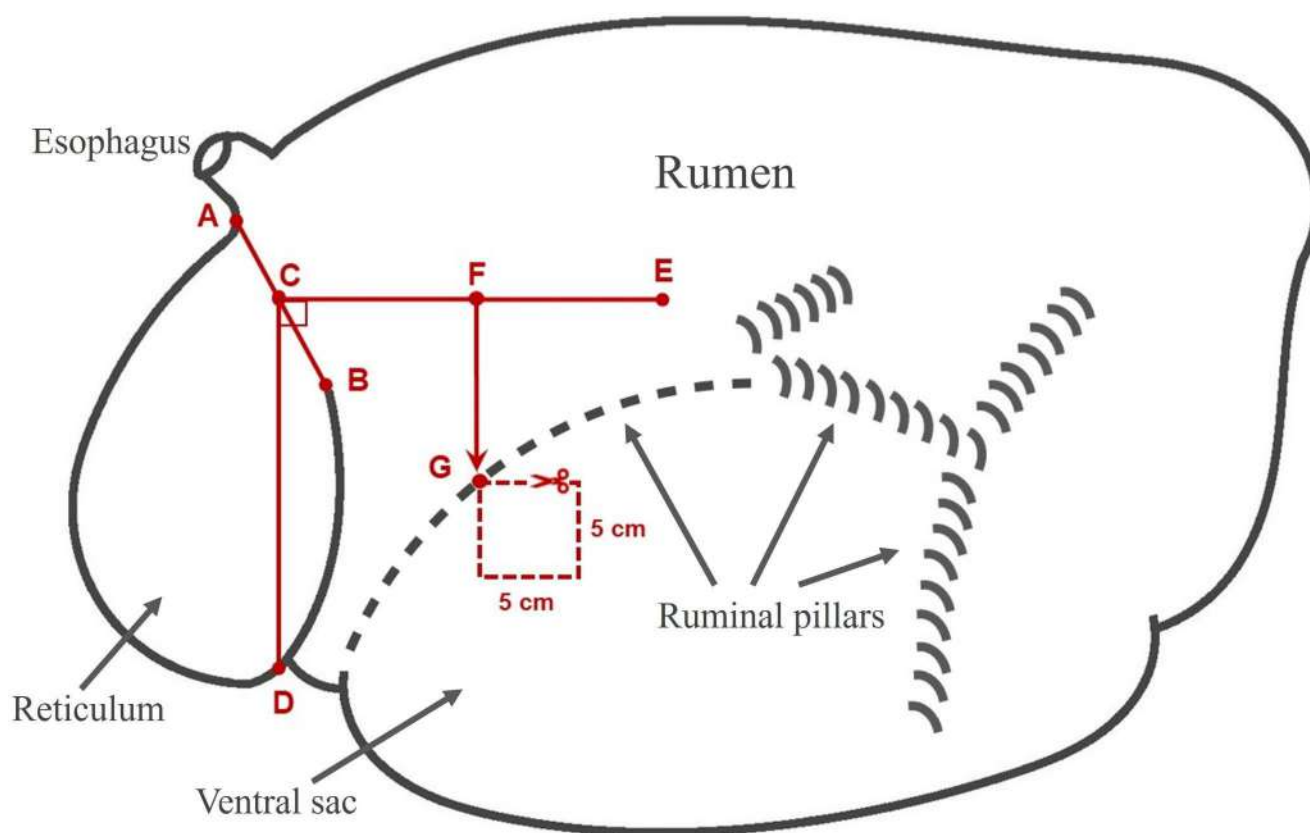


Fig. 1 The procedure for sampling the rumen wall was performed following specific standard seven steps: (i) Commence by drawing a straight line between point A, the lower point of esophageal adhesion, and point B, the dorsal-caudal reticulorumen adhesion point. (ii) Locate the midpoint of line AB and designate it as point C. (iii) Draw a vertical line crossing point C and mark point D, where the vertical line intersects the edge of the reticulum. Measure the distance CD. (iv) Find point E in a manner that ensures the distance from C to E is equal

to the distance from C to D and also ensures that angle DCE forms a right angle. (v) Identify the midpoint of line CE and label it as point F. (vi) Determine the point on the rumen pillar where a vertical line from point F intersects with the ventral pillar and mark it as point G. (vii) We now have the sampling region defined: it will be a square in the ventral ruminal sac, with the cranial side extending from the FG and point G as its upper-left corner (cranial-dorsal corner). The square should have dimensions of 5 cm x 5 cm

black (score 2) (Fig. 2). Finally, a digital gross image was obtained following the standard procedure and the biopsy sample was placed in a container with a formalin solution for histological examination.

Image acquisition and color measurement

For image acquisition, the collected samples were placed on a specific desk, and digital gross images were taken. A NIKON COOLPIX L330 digital camera with a 60-mm macro lens was used, with standard lighting conditions and settings. The images were saved in raw format.

The quantification of rumen discoloration was performed through software applications, namely RGB (RGB Color Detector 2022) and CIELab (CIE 1986). Within the RGB application, computations were made for color components encompassing Red, Green, and Blue (RGB), as well as Hue, Saturation, and Value (HSV), alongside Hue, Saturation, and Lightness (HSL). The determination of color components

L (color lightness), a (redness-greenness of the color), and b (yellow-blueness of the color) was carried out utilizing CIELab (CIE 1986). For every rumen wall sample, the average values of these color components were computed from 9 distinct spot measurements, serving to facilitate subsequent statistical analysis. It is important to highlight that while the L, a, and b values are technically denoted as L^* , a^* , and b^* respectively, for the sake of simplicity, this text omits the asterisks.

To distinguish between Category I and Category II, the color difference Delta E (ΔE) was calculated using the average values of L, a, and b color components. The following equation was used:

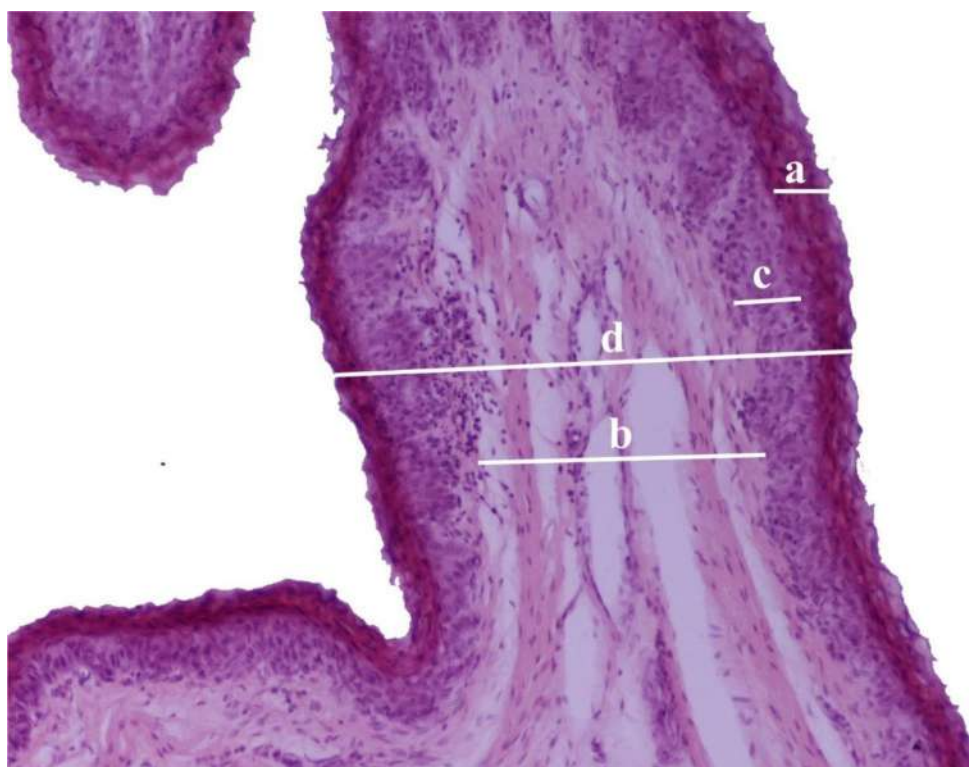
$$\Delta E = \sqrt{(L_I - L_{II})^2 + (a_I - a_{II})^2 + (b_I - b_{II})^2}$$

Where ΔE = color difference; L_I , a_I , b_I = mean values of the color components determined for Category I, and L_{II} , a_{II} , b_{II} = mean values of the color components determined for Category II. ΔE is measured on a scale from 0 to 100, where



Fig. 2 A three-tiered scale for visually assessing the rumen epithelium: (a) white/pale white (score 0); (b) grey (score 1); (c) dark grey or black (score 2)

Fig. 3 Measurements of the beef ruminal papillae: (a) Stratum corneum thickness. (b) Width of the connective tissue. (c) Thickness of the non-keratinized epithelium. (d) Total epithelium thickness. (Hematoxylin-eosin staining; magnification x10)



0 indicates less color difference, and 100 indicates complete distortion (Brainard 2003).

Histological examination

For histological examinations, the rumen wall samples were cut into 1 × 1 cm particles and fixed in 10% formalin buffer for 48 to 72 h. Afterward, they were dehydrated using alcohol solutions and routinely embedded in paraffin (Paraplast Plus®, Kendall, England). Dewaxed 3–5 µm-thick sections were stained with hematoxylin and eosin (H&E) for histopathological evaluation. From each sample, randomly

selected fields with a x10 objective were captured with a microscope-coupled camera, and ImageJ software was used to measure stratum corneum thickness, connective tissue width, non-keratinized epithelium thickness, and total epithelium thickness in each photomicrograph (Fig. 3).

Statistical analysis

Data on continuous variables were checked for normal distribution, and appropriate log transformations were applied to address skewness. A t-test was conducted to assess differences in pH values, color measurements, and rumen

Table 2 Comparison of pH_{farm}, pH_{abattoir}, and color components (mean ± SD) between two categories of farms: Category I (without SARA) and Category II (with SARA) farms

	Category I (n=30)	Category II (n=45)	p value	95% Confidence Interval of the difference
pH _{farm}	5.93 (0.12)	5.72 (0.15)	< 0.001***	0.14 to 0.28
pH _{abattoir}	5.97 (0.13)	5.74 (0.18)	< 0.001***	0.16 to 0.30
L	48.1 (11.7)	29.62 (10.3)	< 0.001***	13.3 to 23.5
a	-2.9 (9.1)	0.55 (5.1)	0.420	0.13 to 6.7
b	14.5 (13.2)	0.77 (5.1)	< 0.001***	9.36 to 18.0
Red	118.1 (31.0)	71.4 (23.0)	< 0.001***	34.1 to 59.1
Green	115.3 (29.4)	70.1 (23.5)	< 0.001***	32.9 to 57.4
Blue	89.9 (33.8)	69.5 (23.8)	0.003*	7.2 to 33.7
RGB-SUM	323.3 (85.0)	211.1 (69.0)	< 0.001***	76.6 to 147.8
HSV	20.1 (26.6)	19.9 (16.4)	0.968	9.7 to 10.1
HSL	40.1 (13.5)	25.6 (11.5)	< 0.001***	8.7 to 20.3

*Significant difference at $p \leq 0.05$ ***Significant difference at $p \leq 0.001$

L: color lightness

a: redness-greenness of the color

b: yellow-blueness of the color

HSV: Hue, Saturation and Value

HSL: Hue, Saturation, and Lightness

epithelium characteristics between the two animal categories. Correlations between pH values, color characteristics, and epithelium thickness were investigated through scatter plots and Pearson calculations (r). The mean pH status was calculated by averaging the measurements taken every 2 weeks for the continuously monitored animals. The chi-square test for independence, a non-parametric statistical procedure, was used to compare visual (categorical) data. Data analysis was performed using IBM SPSS Statistics version 29.0.0.0. (241), and statistical significance was set at either the 0.001 or 0.05 level.

Results

All animals successfully completed the study, and prior to sampling at the slaughterhouse, all animals underwent clinical examination with no evidence of acute acidosis or other clinical disease recorded.

Table 3 Comparison of the visual examination results between Category I (Farms without SARA) and Category II (Farms with SARA) [number of samples (percentage)]

	Category I (n=30)	Category II (n=45)	p value	Pearson Chi-Square
White/Pale White	27 (90.0%)	29 (64.4%)	0.044*	6.225
Grey	2 (6.7%)	10 (22.2%)		
Dark Grey/Black	1 (3.3%)	6 (13.3%)		

*Significant difference at $p \leq 0.05$

The monitored animals had a pH_{farm} range from 5.27 to 6.15, with a mean value of 5.80 (Standard Error [SE]=0.020). Animals from farms with previous cases of ARA had a pH range of 5.27 to 5.90, with a mean value of 5.72 (SE=0.023). In contrast, animals from farms without ARA cases had a pH range of 5.70 to 6.15, and their mean pH was 5.93 (SE=0.021, $p < 0.001$).

Similarly, in the abattoir, the mean pH_{abattoir} value of Category I samples was 5.97 (SE=0.023), which was statistically higher than the mean value of Category II samples (5.74, SE=0.026, $p < 0.001$) (Table 2).

Color measurements

The chi-square test for visual examination showed significant differences, with a higher percentage of grey and black discoloration in Category II compared to Category I ($p = 0.044$) (Table 3).

The average value of the L component in Category I samples was 48.1 units (SE=2.14), which was significantly ($p < 0.001$) higher than the value for Category II (29.6 units, SE=1.53); a similar trend was observed for the b component. However, there was no significant difference in the average value of the a component (Table 2).

The color difference (ΔE) between Category I and Category II rumen wall samples was calculated using the L, a, and b color components. A significant color difference ($\Delta E = \sim 13.2177$) was found between the 2 categories (Table 2).

Concerning the color measurement using the RGB Analysis system, Category I samples exhibited significantly higher mean values for Red, Green, Blue, and RGB-SUM in comparison to Category II samples. Additionally, significant differences were observed for the L value, but not for the V value (Table 2).

Ruminal epithelium histopathology and histomorphometry

Histological examinations showed no pathological findings in the rumen epithelium samples, and there were no signs

of parakeratosis. In samples with the darkest coloration, the dark color was limited to the keratinized layer.

Based on the results, Category II exhibited significantly greater thickness in 2 key measurements: Non-keratinized epithelium thickness (mean 131.5, SE = 9.94) and Total epithelium thickness (mean 278.1, SE = 12.37), in contrast to Category I, where these measurements were lower (mean 88.3, SE = 5.48 and mean 237.1, SE = 9.40, respectively). In contrast, no significant disparities were observed in stratum corneum thickness and connective tissue width (Table 4).

Linear regression analysis

Linear regression analysis between pH_{farm} and values of each color component using CIELab system showed that L had the highest correlation coefficient [$r=0.862$ ($p<0.001$)], while b had a smaller degree of correlation [$r=0.456$ ($p<0.001$)]. However, a showed a lower and non-significant degree of correlation [$r=-0.160$ ($p=0.170$)] (Table 5).

In linear regression analysis between pH_{farm} and each component from RGB color analysis, significant correlations were found between the RGB, RGB-SUM, HSV, and HSL components. Red had the highest degree of correlation [$r=0.853$ ($p<0.001$)], while Green and RGB-SUM had smaller degrees of correlation [$r=0.844$ ($p<0.001$) and $r=0.835$ ($p<0.001$), respectively]. However, HSL, Blue, and HSV had lower degrees of correlation [$r=0.753$ ($p<0.001$), $r=0.650$ ($p<0.001$), and $r=0.402$ ($p<0.001$), respectively] (Table 5).

Non-keratinized epithelium thickness had the highest correlation coefficient [$r=-0.778$ ($p<0.001$)] among the layers of ruminal epithelium, while Total epithelium thickness showed a smaller but significant degree of correlation [$r=-0.578$ ($p<0.001$)]. However, there was no significant correlation coefficient for stratum corneum thickness and connective tissue width (Table 6).

Among CIELab system components, L showed a high degree of correlation [$r=-0.637$ ($p<0.001$)], while b showed a smaller but significant correlation with non-keratinized epithelium thickness. However, a showed no significant correlation coefficient (Table 7).

Similarly, correlation analysis between non-keratinized epithelium thickness and RGB color components revealed that Red had the highest correlation coefficient [$r=-0.634$ ($p<0.001$)], while Green, RGB-SUM, HSL, and Blue had smaller correlation coefficients [$r=-0.629$ ($p<0.001$), $r=-0.605$ ($p<0.001$), $r=-0.605$ ($p<0.001$), and $r=-0.565$ ($p<0.001$), respectively]. However, HSV showed no significant correlation coefficient [$r=0.086$ ($p=0.597$)] (Table 7).

The correlation coefficient for total epithelium thickness and values of each color component using the CIELab

Table 4 Comparison of histological measurements in the layers of the ruminal epithelium (mean $\pm$ SD) among rumen samples from Category I (without SARA) and Category II (with SARA) farms

	Cat- egory I (n = 20)	Category II (n = 20)	p value	95% confi- dence interval of the difference
Stratum corneum (μm)	28.8 (10.1)	30.0 (10.7)	0.72	-5.4 to 7.8
Connective tissue (μm)	120.1 (30.2)	116.7 (32.0)	0.73	-23.3 to 16.5
Non-keratinized epithelium (μm)	88.3 (24.5)	131.5 (44.4)	0.001***	20.2 to 66.2
Total epithelium (μm)	237.1 (42.1)	278.1 (55.3)	0.012*	9.5 to 72.5

*Significant difference at $p \leq 0.05$

***Significant difference at $p \leq 0.001$

Table 5 Linear regression analysis of color components (x) and pH_{farm} (y)

Analysis System	Color component	pH_{farm} Pearson test [r (p value)]	R ²	Equation
CIELab	L	0.862 (<0.001)***	0.743	$y = -3.7e^2 + 70.19x$
	a	-0.160 (0.170)		
	b	0.456 (<0.001)***	0.208	$y = -1.67e^2 + 29.95x$
RGB	Red	0.853 (<0.001)***	0.727	$y = -9.08e^2 + 1.72e^2x$
	Green	0.844 (<0.001)***	0.713	$y = -8.76e^2 + 1.66e^2x$
	Blue	0.650 (<0.001)***	0.422	$y = -5.69e^2 + 1.12e^2x$
	RGB-SUM	0.835 (<0.001)***	0.697	$y = -2.35e^3 + 4.5e^2x$
	HSV	0.402 (<0.001)***	0.161	$y = -2.61e^2 + 48.46x$
	HSL	0.753 (<0.001)***	0.568	$y = -3.27e^2 + 61.71x$

***Significant difference at $p \leq 0.001$

L: color lightness

a: redness-greenness of the color

b: yellow-blueness of the color

HSV: Hue, Saturation and Value

HSL: Hue, Saturation, and Lightness

system revealed that L had the greatest correlation coefficient [$r=-0.474$ ($p=0.002$)], while a and b showed no significant correlation (Table 8).

Similarly, correlation analysis between total epithelium thickness and RGB color components showed that Red had a high degree of correlation [$r=-0.476$ ($p=0.002$)], while Green, RGB-SUM, HSL, and Blue had smaller correlation coefficients [$r=-0.466$ ($p=0.002$), $r=-0.467$ ($p=0.002$), $r=-0.428$ ($p=0.006$), and $r=-0.392$ ($p=0.012$),

Table 6 Linear regression analysis of histological measurements in the layers of the ruminal epithelium (x) and pH_{farm} (y)

	pH _{farm}		
	Pearson test	R ²	Equation
	[r (p value)]		
Stratum corneum (μm)	0.085 (0.604)		
Connective tissue (μm)	0.034 (0.835)		
Non-keratinized epithelium (μm)	-0.778 (<0.001)***	0.606	$y = 1.03e^3 - 1.6e^2x$
Total epithelium (μm)	-0.578 (<0.001)***	0.334	$y = 1.12e^3 - 1.51e^2x$

***Significant difference at $p \leq 0.001$ **Table 7** Linear regression analysis of color components (x) and non-keratinized epithelium thickness (y)

Analysis System	Color component	Non-keratinized epithelium (μm)	
		Pearson test	R ²
		[r, (p value)]	Equation
CIELab system	L	-0.637 (0.001)***	0.406
	a	0.248 (0.123)	
	b	-0.464 (0.003) ^b	0.215
RGB System	Red	-0.634 (<0.001)***	0.402
	Green	-0.629 (<0.001)***	0.395
	Blue	-0.461 (0.003)*	0.212
	RGB-SUM	-0.605 (<0.001)***	0.366
	HSV	0.086 (0.597)	
	HSL	-0.565 (<0.001)***	0.319

*Significant difference at $p \leq 0.05$ ***Significant difference at $p \leq 0.001$

L: color lightness

a: redness-greenness of the color

b: yellow-blueness of the color

HSV: Hue, Saturation and Value

HSL: Hue, Saturation, and Lightness

respectively]. However, HSV showed no significant correlation coefficient [$r = 0.55$ ($p = 0.738$)] (Table 8).

Discussion

SARA is defined as increased periods of rumen pH under 5.6–5.8 (Kleen et al. 2003; Krause and Oetzel 2006). The animals in Category II showed a mean ruminal pH value lower than 5.8, indicating the presence of subacute ruminal acidosis (SARA). In contrast, animals in Category I had a mean ruminal pH value above 5.8, indicating the absence of SARA.

Sampling for pH measurements using the Rumen Scoop has generated concerns about its accuracy among veterinary clinicians. Nevertheless, this device has proven its precision

Table 8 Linear regression analysis of color components (x) and total epithelium thickness (y)

Analysis System	Color component	Total epithelium (μm)	
		Pearson test	R ²
		[r, (p value)]	Equation
CIELab system	L	-0.474 (0.002)*	0.224
	a	0.137 (0.399)	
	b	-0.255 (0.112)	
RGB System	Red	-0.476 (0.002)*	0.226
	Green	-0.466 (0.002)*	0.217
	Blue	-0.392 (0.012)*	0.153
	RGB-SUM	-0.467 (0.002)*	0.218
	HSV	0.055 (0.738)	
	HSL	-0.428 (0.006)*	0.184

*Significant difference at $p \leq 0.05$

L: color lightness

a: redness-greenness of the color

b: yellow-blueness of the color

HSV: Hue, Saturation and Value

HSL: Hue, Saturation, and Lightness

in specific research studies, as exemplified by Geishauser et al. (2012), and has consistently delivered reliable results in our clinical practice. Additionally, taking into account animal welfare concerns, it's crucial to highlight that esophageal tubing stands as the sole non-invasive method for collecting rumen fluid samples. Therefore, for our current research, we have chosen this method, giving precedence to animal welfare considerations.

The mean values of color components, regardless of the evaluation method (RGB or CIELab), were significantly lower in Category II samples, indicating the presence of dark coloration in the ruminal epithelium. This finding was consistent with the results of Alhidary et al. (2016), where a correction of the ratio in lambs resulted in more light-colored epithelium. Other researchers have observed grey to dark ruminal epithelium due to parakeratosis resulting from lowered pH and increased accumulation of volatile fatty acids (VFAs) in rumen fluid (Enemark 2008; Steele et al. 2009, 2011). However, in our study, we observed an increase in non-keratinized rumen epithelium in Category II animals without parakeratosis. The darkening of the rumen epithelium's color should be attributed to the effect of rumen pH on the keratinized layer of the epithelium.

The L component in CIELab analysis showed a strong correlation with the average pH_{farm} and could potentially be used as an indicator of improper pH range in the rumen. Similarly, Chmiel et al. (2012) reported that the L component had the greatest correlation coefficient for the color and pH of meat, while the b component was determined to have the greatest correlation coefficient by Wulf et al. (1997). In our study, the b component followed with the next highest correlation coefficient ($r = 0.456$) (Table 5).

Additionally, using the RGB Analysis system, Red presented the highest degree of correlation with the average pH_{farm} and could be used as an indicator for critical pH values in the rumen (Table 5). Similarly, Chmiel et al. (2012) reported that Red presented with the highest correlation coefficient for the color and pH of meat, with V and L color components to follow. However, in our survey, the Green and RGB-SUM components presented with the next highest correlation coefficient ($r = 0.844$ and $r = 0.835$, respectively) (Table 5). Therefore, the Green and RGB-SUM components could be used as alternative parameters for the prediction of abnormal deviations in rumen pH.

The animals in Category II had increased non-keratinized epithelium thickness and total epithelium thickness compared to Category I (Table 4). The thickness of the non-keratinized epithelium showed the strongest correlation with the average pH_{farm} (Table 6). It is possible that the lower rumen pH may increase the rate of replacement of the keratinized epithelium, leading to an increase in the non-keratinized epithelium to correspond to the need of producing keratinized layers.

A correlation between the layers of the ruminal epithelium and color components has remained unexplored in existing literature. In the course of our investigation, a significant statistical correlation was established between the L component and both the non-keratinized epithelium and the total epithelium ($r = -0.637$ and $r = -0.474$, respectively). Similarly, within the RGB Analysis system, the Red component exhibited the highest degree of correlation with both the non-keratinized epithelium and the total epithelium ($r = -0.634$ and $r = -0.476$, respectively). Following closely in correlation coefficient were the Green, RGB-SUM, and L indexes (as shown in Tables 7 and 8). This noteworthy correlation can be attributed to the prolonged impact of rumen pH on both the color of the epithelium and the thickness of the non-keratinized epithelium, as the animals' diet remained constant throughout the year-long monitoring period. It is anticipated that conducting further research with a larger sample size will serve to reinforce and enhance these observed correlations.

Conclusions

This study affirms the potential utility of computerized rumen colorimetry in investigating subacute ruminal acidosis (SARA) in beef cattle. The availability of portable computing devices equipped with color analysis software offers a convenient, swift, and dependable means of assessing the chromatic variation in rumen epithelium. This assessment can be conducted during exploratory necropsy or at the abattoir, rendering it a valuable resource for monitoring the

effectiveness of management, nutritional, and preventive interventions within a beef farm setting. To fully explore the prospective application of computerized rumen colorimetry as a diagnostic instrument for live animals, further research is warranted, involving the utilization of a gastroscope.

Acknowledgements The authors express their sincere gratitude to Mathis Christodoulouopoulos for his invaluable assistance in creating the graph presented in Fig. 1.

Authors' contributions The author's contributions have as follow (authors are referred by their initials): Conceptualization, G.C.; methodology, G.C., D.A.G., D.P., V.G.P.; validation, G.C., D.A.G., D.P., V.G.P., L.V.A.; formal analysis, G.C., D.A.G., N.V., D.P., P.D.K.; investigation, N.V., G.C., D.A.G., D.P., G.I.P., K.K., M.A.T.; resources, N.V.; data curation, N.V., D.A.G., G.C., D.P., P.D.K.; writing—original draft preparation, N.V., D.A.G.; writing—review and editing, G.C., D.A.G., L.V.A., D.P., V.G.P.; visualization, G.C.; supervision, G.C., D.P., D.A.G., V.G.P.

Funding Open access funding provided by HEAL-Link Greece.

Declarations

Statement of animal ethics The research was carried out in compliance with existing animal welfare regulations (Directive 98/58/EC) and was approved by the Ethics Committee of the Faculty of Veterinary Science at the University of Thessaly, Greece (protocol number 151/16-05-2021). Throughout the experiment, no modifications were made to their diet or treatment for experimental purposes.

Conflict of Interest The authors affirm that there are no financial or personal relationships, nor any other conflicts of interest, that could potentially exert undue influence or bias the content of this paper. Funding for essential materials, integral to the first author's PhD studies, was generously provided by the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly.

Consent to participate Informed written consent was acquired from the farm proprietors.

Consent for publication Our study does not involve the use of individual personal data. The farm owners granted written consent for the publication of data pertaining to their farms in a journal article.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abaker JA, Xu TL, Jin D, Chang GJ, Zhang K, Shen XZ (2017) Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. *J Dairy Sci* 100:666–678. <https://doi.org/10.3168/jds.2016-10871>
- Alhidary I, Abdelrahman MM, Alyemni AH, Khan RU, Al-Mubarak AH, Albaadani HH (2016) Characteristics of rumen in Naemi lamb: morphological changes in response to altered feeding regimen. *Acta Histochem* 118:331–337. <https://doi.org/10.1016/j.acthis.2016.03.002>
- Aschenbach JR, Penner GB, Stumpff F, Gabel G (2011) Ruminant Nutrition Symposium: role of fermentation acid absorption in the regulation of ruminal pH. *J Anim Sci* 89:1092–1107. <https://doi.org/10.2527/jas.2010-3301>
- Brainard DH (2003) 5-Color appearance and Color Difference Specification. In: Shevell SK (ed) *The Science of Color*, 2nd edn. Elsevier, Optical Society of America, Amsterdam, Boston, pp 191–216
- Chmiel M, Slowinski M, Dasiewicz K, Florowski T (2012) Application of a computer vision system to classify beef as normal or dark, firm, and dry. *J Anim Sci* 90:4126–4130. <https://doi.org/10.2527/jas.2011-5022>
- CIE (1986) Colorimetry: official recommendations of the international commission on illumination. CIE Publ. No. 15.2. CIE Central Bureau, Vienna
- Enemark JMD (2008) The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): a review. *Vet J* 176(1):32–43. <https://doi.org/10.1016/j.tvjl.2007.12.021>
- Geishauser T, Linhart N, Neidl A, Reimann A (2012) Factors associated with ruminal pH at herd level. *J Dairy Sci* 95:4556–4567. <https://doi.org/10.3168/jds.2012-5380>
- Humer E, Aschenbach JR, Neubauer V, Kröger I, Khiaosa-ard R, Baumgartner W, Zebeli Q (2018) Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J Anim Physiol Anim Nutr* 102:380–392. <https://doi.org/10.1111/jpn.12850>
- Jonsson NN, Ferguson HJ, Koh-Tan HH, McCartney CA, Cernat RC, Strachan EM et al (2020) Postmortem observations on rumen wall histology and gene expression and ruminal and caecal content of beef cattle fattened on barley-based rations. *Animal* 14:1447–1460. <https://doi.org/10.1017/S1751731119002878>
- Kitkas GC, Valergakis G, Karatzias H, Panousis N (2013) Subacute ruminal acidosis: prevalence and risk factors in greek dairy herds. *Iran J Vet Res* 14:183–189. <https://doi.org/10.22099/IJVR.2013.1679>
- Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP (2003) Subacute ruminal acidosis (SARA): a review. *J Vet Med A Physiol Pathol Clin Med* 50:406–414. <https://doi.org/10.1046/j.1439-0442.2003.00569.x>
- Krause KM, Oetzel GR (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: a review. *Anim Feed Sci Technol* 126:215–236. <https://doi.org/10.1016/j.anifeedsci.2005.08.004>
- Nagaraja TG, Lechtenberg KF (2007) Acidosis in feedlot cattle. *Vet Clin Food Anim Pract* 23:333–350. <https://doi.org/10.1016/j.cvfa.2007.04.002>
- Neubauer V, Humer E, Kröger I, Braid T, Wagner M, Zebeli Q (2017) Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J Anim Physiol Anim Nutr* 102(1):343–349. <https://doi.org/10.1111/jpn.12675>
- Nocek JE (1997) Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005–1028. [https://doi.org/10.3168/jds.S0022-0302\(97\)76026-0](https://doi.org/10.3168/jds.S0022-0302(97)76026-0)
- Plaizier JC, Krause DO, Gozho GN, McBride BW (2008) Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Vet J* 176:21–31. <https://doi.org/10.1016/j.tvjl.2007.12.016>
- Plaizier JC, Khafipour E, Li S, Gozho GN, Krause DO (2012) Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Anim Feed Sci Technol* 172:9–21. <https://doi.org/10.1016/j.anifeedsci.2011.12.004>
- RGB Color Detector (2022) Available from: com.TheProgrammer. RGBColorDetector (Version 3.0.7). Accessed 10 Oct 2022
- Sergeant ESG (2018) Epitools Epidemiological Calculators. Ausvet. Available at: <http://epitools.ausvet.com.au>. Accessed 15 August 2021
- Silva EPE, Moraes EP, Anaya K, Silva YMO, Lopes HAP, Andrade Neto JC et al (2022) Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. *PLoS ONE* 7(2):e0263714. <https://doi.org/10.1371/journal.pone.0263714>
- Snyder E, Credille B (2017) Diagnosis and treatment of clinical rumen acidosis. *Vet Clin North Am Food Anim Pract* 33:451–461. <https://doi.org/10.1016/j.cvfa.2017.06.003>
- Steele MA, AlZahal O, Hook SE, Croom J, McBride BW (2009) Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Vet Scand* 51:39. <https://doi.org/10.1186/1751-0147-51-39>
- Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K et al (2011) Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am J Physiol Regul Integr Comp Physiol* 300:1515–1523. <https://doi.org/10.1152/ajpregu.00120.2010>
- Tajik J, Nazifi S (2011) Serum concentrations of lipids and lipoproteins and their correlations together and with thyroid hormones in Iranian Water Buffalo (*Bubalus bubalis*). *Asian J Anim Sci* 5:196–201. <https://doi.org/10.3923/ajas.2011.196.201>
- Villot C, Meunier B, Bodin J, Martin C, Silberberg M (2017) Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal* 12:481–490. <https://doi.org/10.1017/S1751731117001677>
- Wulf DM, O'Connor SF, Tatum JD, Smith GC (1997) Using objective measures of muscle color to predict beef longissimus tenderness. *J Anim Sci* 75:684–692. <https://doi.org/10.2527/1997.753684x>
- Yancey JWS, Kropf DH (2008) Instrumental reflectance values of fresh pork are dependent on aperture size. *Meat Sci* 79:734–739. <https://doi.org/10.1016/j.meatsci.2007.11.006>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

4a. “Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management”

Published

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, , Katsoulis K, Angelidou-Tsifida M, Athanasiou L, Papatsiros V, Christodouloupoulos G. Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management. Preprints 2024; 2024061041.
<https://doi.org/10.20944/preprints202406.1041.v1>

Article

Not peer-reviewed version

Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management

[Nikolaos Voulgarakis](#) , [Dimitrios Gougoulis](#) , [Dimitra Psalla](#) , [Georgios I. Papakonstantinou](#) ,
Konstantinos Katsoulis , Mariana Angelidou-Tsifida , [Labrini V. Athanasiou](#) , [Vasileios G. Papatsiros](#) ,
[Georgios Christodouloupoulos](#) *

Posted Date: 17 June 2024

doi: 10.20944/preprints202406.1041.v1

Keywords: subacute rumen acidosis; rumen mucosa colorimetry; dairy sheep; milk fat depression; epidemiology; Greece



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management

Nikolaos Voulgarakis ¹, Dimitrios A. Gougoulis ¹, Dimitra Psalla ², Georgios I. Papakonstantinou ¹, Konstantinos Katsoulis ³, Mariana Angelidou-Tsifida ², Labrini V. Athanasiou ¹, Vasileios G. Papatsiros ¹ and Georgios Christodoulopoulos ^{4,*}

¹ Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece; nvoulgarakis@vet.uth.gr (N.V.); dgoug@vet.uth.gr (D.A.G.); geopapak@vet.uth.gr (G.I.P.), lathan@vet.uth.gr (L.V.A.), vpapatsiros@vet.uth.gr (V.G.P.)

² Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Macedonia, Greece; dpsalla@vet.auth.gr (D.P.), mtangelid@vet.auth.gr (M.A.T.)

³ Department of Animal Husbandry and Nutrition, Faculty of Veterinary Science, School of Health Sciences, University of Thessaly, Karditsa, Greece; kkatsoulis@vet.uth.gr (K.K.)

⁴ Department of Animal Science, Agricultural University of Athens, Athens, Greece; gc@aia.gr (G.C.)

* Correspondence: gc@aia.gr; Tel.: +30-2105294386

Simple Summary: Subacute rumen acidosis (SARA) is a significant metabolic issue in high-yield livestock, including dairy sheep, characterized by a rumen pH below 5.8 for at least four hours daily. This condition results in economic losses due to reduced milk production and lower milk fat content. High-concentrate, low-fiber diets increase the risk of SARA by disrupting rumen fermentation. Diagnosing SARA is challenging, with rumen fluid pH measurement being the gold standard. This study investigated SARA's prevalence and impact in 42 Greek dairy sheep flocks and evaluated computerized rumen colorimetry as a diagnostic tool. Over nine months, rumen pH and milk composition were monitored, and rumen wall samples were analyzed for color changes and histological alterations. Findings revealed increased epithelium thickness and discoloration in SARA-affected sheep. The study highlights the prevalence of SARA in the modern intensive dairy sheep industry, underscores the need for balanced feeding strategies, and suggests computerized rumen colorimetry as a promising tool for SARA detection and management in dairy sheep farms.

Abstract: Subacute rumen acidosis (SARA) is a prevalent issue in intensive livestock farming, particularly associated with high-concentrate diets. This study investigates the prevalence and impact of SARA in Greek dairy sheep farming and explores the use of computerized rumen colorimetry as a management and diagnostic tool for SARA in dairy sheep farms. Forty-two dairy sheep flocks were monitored regularly for rumen pH, milk composition, and milk yield over an approximately 9-month milking period. Computerized Rumen Mucosal Colorimetry was applied to 280 culled, aged ewes from these farms, with rumen wall samples taken for histological examination. SARA cases were identified in 19 farms (45%, n=48). Farms with SARA cases had lower milk fat levels, although milk yield was higher in these farms, while milk protein levels did not differ based on the SARA status of the farms. In culled ewes, rumen color was significantly associated with the farm's SARA status. Additionally, ewes from farms with more SARA cases exhibited increased thickness in the ruminal epithelium layers, particularly in the non-keratinized and total epithelium layers. In conclusion, SARA is a common disorder in the Greek dairy sheep industry, lowering the fat content of milk. The study suggests that computerized rumen epithelium colorimetry in aged culled ewes shows promise as a diagnostic tool for SARA in dairy sheep farms.

Keywords: subacute rumen acidosis; rumen mucosa colorimetry; dairy sheep; milk fat depression; epidemiology; Greece

1. Introduction

In high-yield livestock farming, rumen acidosis (RA) is a common metabolic issue [1][2]. It occurs in two forms: acute (ARA), characterized by a rumen pH below 5.0, and sub-acute (SARA),

with a pH consistently lower than 5.8 for at least four hours per day [3–7]. SARA results in economic losses due to reduced milk production and lower milk fat content [6,8,9].

Feeding high-concentrate diets with low percentages of dietary fiber for extended periods increases the risk of subacute rumen acidosis, negatively affecting both milk production and fat concentration [10–12]. The subsequent lower rumen pH disrupts fermentation and bacterial synthesis, leading to increased production of long-chain fatty acids (LCFA), which hinders milk fat synthesis in the udder [13,14].

Several hematobiochemical parameters have been suggested as diagnostic markers for SARA in small ruminants, but none have proven entirely accurate [6,15–17]. Rumen fluid pH measurement is the gold standard, with a pH between 5.2 and 5.8 for more than four hours per day indicating SARA in intensive small ruminant livestock farming [5–7,17].

Rumen acidosis, marked by rumen wall discoloration, especially in subacute rumenitis, manifests as a gray to dark discoloration [18–21]. Objective color estimation using colorimeters and spectrophotometers, particularly through Red, Green, and Blue (RGB) analysis, has emerged as a precise method for assessing rumen epithelium health [22–25].

While extensively studied in other livestock, SARA's prevalence and impact on sheep are not well-explored [26]. This study aims to address this critical gap by evaluating the prevalence and impact of SARA in Greek sheep farming. Additionally, it aims to assess the effectiveness of computerized rumen colorimetry as a tool for managing and detecting SARA in dairy sheep farms.

2. Materials and Methods

2.1. Selection of Farms

Forty-two dairy sheep flocks were thoughtfully selected to participate in this study. These farms are situated in Thessaly, a central region of Greece. Inclusion criteria required farms to be within reach of the Department of Clinical Veterinary Medicine, Faculty of Veterinary Science, University of Thessaly, Greece. Farms also needed to have crossbreed ewes of Lacaune with the local Karagouniko breed, maintain a stable ration composition throughout the entire milking period, and be confirmed as free of paratuberculosis and progressive pneumonia, as evidenced by the Department's archives.

2.2. Experimental Design

The selected farms were monitored every two months for rumen pH throughout the entire milking period, spanning approximately 9 months. Five visits were conducted at each farm during the first 10 days of the 1st, 3rd, 5th, 7th, and 9th months post-partum.

At each visit, six ewes were randomly chosen and assessed for rumen pH, ensuring they had not been previously selected for rumen pH measurement to prioritize animal welfare. Rumen fluid sampling took place four hours after the morning administration of concentrated feed. Simultaneously, milk samples were gathered from the bulk milk tank during these visits. Additionally, data on daily milk yield was sourced from the farmers' records.

Furthermore, between July and August 2021, during the culling of aged ewes from the above 42 farms, rumen sampling was performed in slaughterhouses. Due to economic constraints, a total of 280 ewes were included in the sampling, with at least 6 ewes from each farm.

2.3. Sample size justification

According to the existing literature, the prevalence of subacute rumen acidosis (SARA) in dairy sheep is approximately 12% [27]. To ensure a desired level of accuracy with a margin of error (e) of ± 0.05 and a 95% confidence interval ($Z = 1.96$), the estimated sample size (n) was calculated using the formula $n = (Z^2 \times P \times (1-P)) / e^2$, yielding a value of 162. This study exceeded this calculated sample size requirement during both farm and slaughterhouse sampling phases.

2.4. Ethical approval

The study adhered to established animal welfare regulations (Directive 98/58/EC) and obtained approval from the Ethics Committee of the Faculty of Veterinary Science at the University of Thessaly, Greece (Approval No. 152/16-05-2021). No modifications were made to the animals' diet or treatment for experimental purposes during the entire duration of the study.

2.5. Clinical and laboratory procedures

2.5.1. Rumenocentesis and rumen pH measurement

Rumenocentesis, conducted without sedation, was employed for the collection of rumen fluid samples, following established methodologies from prior studies [28]. The procedure included an external abdominal puncture using a 16G 105-mm needle (Intralune PP, Vygon, France) in the left abdominal region, specifically targeting the ventral sac of the rumen and aspirating the fluid with a 50 mL syringe [6,29]. Subsequently, the obtained rumen fluid underwent immediate pH determination, utilizing a digital portable pH-meter (HI-2002 Edge pH Meter, Hanna instruments).

2.5.2. Milk Analyses

To maintain sample quality, they were refrigerated at 4°C during transportation to the laboratory. On the same day of collection, the milk composition, including fat, protein, lactose, and total solid content, was analyzed using an infrared analysis technique with the assistance of an FTIR interferometer—specifically, the Milkoscan FT6000 Analyzer manufactured by Foss Electric in Denmark. The annual average values for the mentioned milk components were calculated based on the five measurements in each farm, along with the monthly milk yield, following basic arithmetic procedures [30].

2.5.3. Rumen wall Sampling and image acquisition

The sampling procedure for the rumen wall followed a standardized protocol as detailed by Voulgarakis et al. [25]. The incision location was determined based on standard measurements outlined in the same study. A 5x5 cm square from a specific location on the rumen wall was excised for sampling. After excision, the sample underwent thorough washing with water to eliminate any visible remnants of rumen content. Subsequently, a digital gross image was captured once again using the standard procedure described by Voulgarakis et al. [25]. The sample was then placed in a container with a 10% formalin solution for subsequent histological examination.

2.5.4. Rumen Wall Image Color Analysis

The quantification of rumen mucosa color utilized software applications, specifically RGB Colour Detector 3.0.7 [31] and CIELab [32]. We followed a procedure and color component measurement consistent with the method detailed by Voulgarakis et al. [25].

In the RGB application, the analyzed color components included Red, Green, and Blue (RGB), Value (V), Lightness (L), Hue, Saturation, and Value (HSV), and Hue, Saturation, and Lightness (HSL). Additionally, the summary of the Red, Green, and Blue color components was computed and labeled as RGB-SUM [23]. Using the CIELab software, the L (color lightness), a (redness-greenness of the color), and b (yellow-blueness of the color) color components were assessed [32].

For each rumen wall sample, average values of these color components were calculated from nine distinct spot measurements, and the resulting average value was employed for subsequent statistical analysis. It is essential to note that, although the L, a, and b values are technically denoted as L*, a*, and b*, respectively, for simplicity, this text omits the asterisks.

2.5.5. Histological Examination

Hematoxylin-eosin staining was employed on rumen wall sample sections. A microscope-coupled camera captured a randomly selected field with a x10 objective from each sample. ImageJ software was utilized to measure the thickness of the stratum corneum, connective tissue, non-keratinized epithelium, and total epithelium in each photomicrograph (Figure 1).

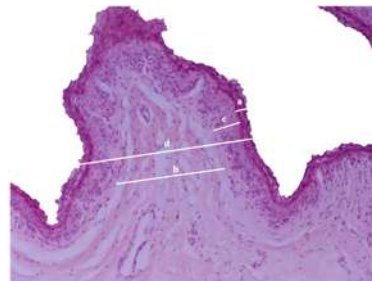


Figure 1. Measurements of the ewe rumen papillae: (a) Stratum corneum thick-ness. (b) Width of the connective tissue. (c) Thickness of the non-keratinized epithelium. (d) Total epithelium thickness. (Hematoxylin-eosin staining; magnification x10).

2.6. Statistical analysis.

The consistency of rumen pH measurements over time in each farm was examined using the Intraclass Correlation Coefficient (ICC). Continuous variables in the dataset, including pH values, milk parameters (such as yield, fat, protein, lactose, and total solids content), color measurements, and characteristics of the rumen epithelium, were checked for normal distribution. When necessary, log transformations were applied to address any observed skewness in the data. To explore potential differences in these variables among farms with low, moderate, and high SARA prevalence, an Analysis of Variance (ANOVA) was conducted. The results were presented using the mean and standard deviation (SD) as measures of central tendency and variability, respectively. The analysis was performed using IBM SPSS Statistics version 29.0.0.0 (241), with a significance level of 0.05 used to determine statistical significance.

3. Results

All animals involved in the study completed the entire monitoring period without any adverse effects on their welfare.

The 42 farms were categorized into three groups based on the number of rumen pH measurements below 5.8 out of 30 measurements conducted in each farm [$n=5$ visits ($\times$) 6 ewes each visit= 30 measurements per farm]. The categories and the number of farms in each category are as follows:

Category 1: Farms with a low prevalence of SARA, including 17 farms (41% of the total), with each farm exhibiting only 0-2 cases of pH <5.8 in the 30 measurements.

Category 2: Farms with a moderate prevalence of SARA, comprising 19 farms (45% of the total), with each farm showing 3-9 cases of pH <5.8 in the 30 measurements.

Category 3: Farms with a high prevalence of SARA, consisting of 6 farms (14% of the total), with each farm having 10-14 cases of pH <5.8 in the 30 measurements.

Throughout the farming sampling period, the mean pH values of the participating farms, with six randomly selected ewes each time, ranged from 5.73 to 5.93, yielding a mean pH of 5.83 (Standard Deviation SD=0.06). Specifically, in Category 1 farms, the pH range was between 5.87 and 5.93, with a mean pH of 5.89 (SD=0.01). In Category 2, the pH range was from 5.78 to 5.84, with a mean pH of 5.81 (SD=0.02). Lastly, in Category 3, the pH range was recorded between 5.73 and 5.76, with a mean pH of 5.74 (SD=0.01). The sample size for each category was consistent. Post hoc analysis using Bonferroni adjustment revealed significant differences among all possible pairwise comparisons between the three farm categories ($p < 0.001$) (Table 1).

In the abattoir, post hoc analysis revealed significant differences in the color components among all possible pairwise comparisons of the three farm categories, with the exception of the a* component across all farm categories and the b* component between Category 1 and Category 2 ($p < 0.001$) (Table 2).

Table 1. Comparison of pH_{farm}, and milk components (mean $\pm$ SD) between three categories of farms: Category 1 (Farms with a low Prevalence of SARA Farms), Category 2 (Farms with a moderate Prevalence of SARA Farms) and Category 3 (Farms with a high Prevalence of SARA Farms) farms.

Farm sampling period	Category 1 (n=17)	Category 2 (n=19)	Category 3 (n=6)	p-value (Between groups)
pH _{farm}	5.89 (0.01)	5.81 (0.02)	5.74 (0.01)	<0.001***
Milk Yield (Liter)	7160.3 (100.9)	7227.4 (104.8)	7399.1 (66.1)	<0.001***
Milk Fat content (%)	5.92(0.13)	5.69(0.11)	5.65(0.06)	<0.001***
Milk Protein content (%)	5.72(0.10)	5.72(0.12)	5.83(0.05)	0.064
Milk Lactose content (%)	4.71(0.09)	4.81(0.14)	4.95(0.16)	0.001*
Milk Total Solids content (%)	10.87(0.23)	11.06(0.31)	11.73(0.40)	<0.001***
Milk Fat: Milk Protein ratio	1.04(0.02)	0.99(0.02)	0.96(0.01)	<0.001***

*Significant difference at $p \leq 0.05$; ***Significant difference at $p \leq 0.001$.

Table 2. Comparison of color components (mean $\pm$ SD) between three categories of farms: Category 1 (Farms with a low Prevalence of SARA Farms), Category 2 (Farms with a moderate Prevalence of SARA Farms) and Category 3 (Farms with a high Prevalence of SARA Farms) farms.

Abattoir sampling period	Category 1 (n=111 ewes)	Category 2 (n=124 ewes)	Category 3 (n=45 ewes)	p-value (Between groups)
L*	39.93(5.24)	30.59(4.92)	21.98(6.92)	<0.001***
a*	0.66(4.03)	1.01(4.34)	2.28(4.04)	0.091
b*	9.54(5.80)	9.53(4.67)	4.49(6.92)	<0.001***
Red	100.94(14.33)	78.38(9.98)	58.41(12.34)	<0.001***
Green	93.38(12.69)	71.16(13.14)	51.60(16.13)	<0.001***
Blue	78.92(15.15)	57.53(14.52)	46.33(17.81)	<0.001***
RGB -SUM	273.23(36.99)	207.08(32.36)	156.34(43.06)	<0.001***
HSV	0.40(0.06)	0.33(0.06)	0.24(0.05)	<0.001***
HSL	35.47(4.85)	26.82(4.08)	20.44(5.57)	<0.001***

***Significant difference at $p \leq 0.001$; L*: color lightness; a*: redness-greenness of the color; b*: yellow-blueness of the color; HSV: Hue, Saturation and Value; HSL: Hue, Saturation, and Lightness.

3.1. Milk analysis

During the farming sampling period, milk yield differed across farm categories, with Category 3 having the highest average yield (mean 7399.1, SD= 66.1) and Category 1 the lowest (mean 7160.3, SD= 100.9). Post hoc analysis indicates significant differences between Category 1 and Category 3 and between Category 2 and Category 3 for milk yield ($p < 0.05$). Conversely, milk fat content varied across farm categories, with Category 1 having the highest average fat content (mean 5.92, SD= 0.13) and Category 3 the lowest (mean 5.65, SD= 0.06). Post hoc analysis indicates significant differences between Category 1 and Category 2, and Category 1 and Category 3 ($p < 0.001$). The milk protein content also showed variation, ranging from 5.46 to 5.90. Category 1 had the lowest average protein content (mean 5.72, SD= 0.10), while Category 3 had the highest (mean 5.83, SD= 0.05). Milk lactose content ranged from 4.36 to 5.14, with variations across categories. A significant difference was found between Category 1 and Category 3 ($p < 0.001$). Milk total solid content ranged from 10.54 to 12.21, with Category 3 having the highest average (mean 11.73, SD= 0.40) and Category 1 the lowest (mean

10.87, SD= 0.23). Significant differences were observed between Category 1 and Category 3, as well as between Category 2 and Category 3 ($p < 0.001$). The milk fat-to-milk protein ratio was 1.01 overall, with Category 1 having the highest ratio (1.04) and Category 3 the lowest (0.96) (Table 1).

3.2. Colour Measurement

Pairwise comparisons of mean L^* component values among all categories (1, 2, 3) revealed statistically significant differences ($p < 0.001$). Specifically, Category 1 exhibited the highest mean L^* value (mean 39.93, SD=5.24), whereas Category 3 reported the lowest mean L^* value (mean 21.98, SD=6.92). Significant differences were also observed in the mean values of the b^* component between Category 1 (mean 9.54, SD= 5.80) and Category 3 (mean 4.49, SD= 6.92), as well as between Category 2 (mean 9.53, SD= 4.67) and Category 3 (mean 4.49, SD= 6.92). However, no statistically significant differences were detected in the mean values of the a^* component across all possible comparisons (Table 2).

Regarding the color measurement using the RGB Analysis system, the mean values of Red, Green, Blue, and RGB-SUM were found to be significantly higher in Category 1 when compared to the mean values of Category 2 and Category 3 samples. Similarly, Category 2 exhibited significantly higher mean values compared to Category 3. Furthermore, significant differences were observed for the HSV value and HSL value among all possible comparisons, with Category 1 having the highest values compared to Category 2 and Category 3. Lastly, Category 2 revealed significantly higher mean values compared to Category 3 (Table 2).

3.3. Ruminal epithelium histopathology and histomorphometry

Histological analyses revealed no abnormal findings in the rumen epithelium samples, and parakeratosis was not observed. The darkest coloration was confined solely to the keratinized layer in those samples.

According to the statistical analysis, Category 3 exhibited significantly higher thickness in non-keratinized epithelium thickness (mean 166.67, SD=21.41) and total epithelium thickness (mean 356.47, SD=74.08), in contrast to Category 2 (mean 116.47, SD=28.64 and mean 294.53, SD= 61.26, respectively) and Category 1 (mean 80.13 SD=14.46 and mean 217.07, SD=51.69). Additionally, the non-keratinized epithelium thickness and total epithelium thickness were significantly higher in samples of Category 2 compared to samples of Category 1. However, no significant differences were observed in stratum corneum thickness and connective tissue width in any of the possible comparisons (Table 3).

Table 3. Comparison of histological measurements in the layers of the ruminal epithelium (mean $\pm$ SD) among rumen samples from Category 1 (Farms with a low Prevalence of SARA Farms), Category 2 (Farms with a moderate Prevalence of SARA Farms) and Category 3 (Farms with a high Prevalence of SARA Farms) farms.

	Category 1 (n=15 ewes)	Category 2 (n=15 ewes)	Category 3 (n=15 ewes)	p-value (Between groups)
Stratum corneum (μm)	33.67(12.83)	43.07(24.97)	41.67(16.25)	0.342
Connective tissue (μm)	103.27(39.35)	135.00(61.32)	148.13(63.83)	0.090
Non-keratinized epithelium (μm)	80.13(14.46)	116.47(28.64)	166.67(21.41)	<0.001***
Total epithelium (μm)	217.07(51.69)	294.53(61.26)	356.47(74.08)	<0.001***

***Significant difference at $p \leq 0.001$.

4. Discussion

This research represents the first attempt to investigate the epidemiology of subacute rumen acidosis (SARA) in dairy sheep farms in Greece. The findings provide strong evidence that SARA is a common disorder in the modern Greek dairy sheep industry. These results are not surprising given the prevalent nutritional practices in dairy sheep farming in Greece. Ewes are typically fed a concentrate mixture exceeding 1 kg per day throughout the milking period and are additionally offered cereal hay, usually *ad libitum*. The concentrate mixture is consumed rapidly, and the limited consumption of hay fails to meet the animals' daily fiber needs. Feeding more than 500 grams of cereals daily increases the risk of ruminal acidosis in sheep [33]. Furthermore, long-term diets high in grain concentration and low in dietary fiber can lead to SARA, resulting in a rumen pH between 5.0 and 5.8 for several hours daily [3–5,7].

In this study, farms with a higher number of SARA cases (Categories 2 and 3) produced higher milk yields. A higher incidence of SARA correlates with increased cereal consumption, providing higher energy intake. Energy intake is a primary factor for milk production in dairy ewes [33]. However, these farms also exhibited lower milk fat content, likely due to the high prevalence of SARA. This effect is attributed to alterations in rumen fermentation and changes in fatty acid profiles. Multiple studies, including those by Tao et al. [34], Zhang et al. [35], Li et al. [13], and Kitkas et al. [14], have consistently observed lower milk fat percentages in high-concentrate groups compared to low-concentrate groups in both dairy cows and goats. The reduction in ruminal pH associated with SARA modifies the composition of rumen bacteria and fermentation patterns, leading to increased production of specific long-chain fatty acids (LCFA). These LCFA, once absorbed, can inhibit milk fat synthesis in the udder [13,14]. Therefore, further research is needed to explore optimal feeding strategies for dairy sheep that balance high energy intake with adequate dietary fiber, such as incorporating concentrate mixtures into a total mixed ration (TMR).

This research proposes computerized measurement of rumen epithelium color as a promising method for monitoring SARA status. This technique can be easily applied during the slaughter of aged culled ewes, providing health advisors with reliable data on SARA status in the farm. Currently, rumen pH measurement is considered the gold standard for diagnosing SARA in ruminants [8,36,37]. However, pH measurement can be influenced by various factors, including the method of measurement, sampling location, and timing [38,39]. Beyond pH measurements, diagnostic indicators such as milk fat percentage and the milk fat-to-protein ratio have been proposed for detecting SARA in dairy cows. Rapid weekly drops in milk fat of 0.3–0.5% or a milk fat-to-protein ratio below 1 suggest potential SARA cases [40–44]. However, these indexes have not been established for the dairy sheep industry, partly due to ongoing genetic improvements in dairy sheep breeds. Additionally, chronic diseases such as progressive pneumonia and Johne's disease are associated with drops in milk fat [45,46].

Existing literature indicates that the prevalence of SARA in dairy sheep is approximately 12%, but this study was conducted in Brazil [27]. This study exceeded the calculated sample size requirement based on this figure to account for the lack of extensive data from Greece and other Mediterranean countries.

The mean color values, measured by RGB or CIELab systems, were significantly lower in Category 3 samples compared to Categories 1 and 2, indicating darker ruminal epithelium discoloration. Similar results were reported in feedlot cattle by Voulgarakis et al. [25]. Factors contributing to this darkening include pelleting, fiber particle size, dietary iron levels, and the buffering system. Lambs' epithelium showed lighter color after ration correction, consistent with our findings [47]. Feeding high fermentable TMR is associated with gray to dark color, leading to reduced ruminal pH and increased volatile fatty acid and lactic acid accumulation [18–21,47,48]. High iron consumption may cause dark color due to black stain formation [47,49]. In conclusion, rumen pH plays a decisive role in the darkening of the keratinized layer in the ruminal epithelium.

Our histological findings showed that the darkening of the rumen epithelium's color was due to the effect of rumen pH on the keratinized layer rather than parakeratosis. This is a notable finding as previous research attributed rumen epithelium darkening to parakeratosis [41].

Furthermore, our research observed an increase in the thickness of ruminal epithelium layers, particularly non-keratinized and total epithelium, in animals from farms with a high incidence of SARA. Steele et al. [50] observed increased total epithelial thickness and stratum corneum thickness in animals fed a high-grain diet. Bo Trabi et al. [51] found that animals fed a low-grain non-pelleted TMR concentrate had higher total epithelium and stratum corneum thickness compared to those fed high-grain non-pelleted TMR concentrate or high-grain TMR in pelleted form. Wang et al. [52] reported increased total epithelium and stratum corneum thickness in animals fed a ground corn high-grain diet compared to a whole corn high-grain diet. Our study found no significant difference in stratum corneum thickness among the categories, but Category 3 samples had a greater length of the stratum corneum compared to Category 1 samples. Garcia Diaz et al. [53] reported higher total epithelium and stratum corneum thickness, but lower connective tissue width and non-keratinized thickness, in animals fed a grain-based diet compared to those on an additive-supplemented grain-based diet. Lower rumen pH may increase the rate of replacement of keratinized epithelium, stimulating non-keratinized epithelium growth to produce keratinized layers [25].

5. Conclusions

Subacute rumen acidosis (SARA) is a prevalent condition in the modern dairy sheep industry of Greece, significantly affecting farms with higher milk yields. This study demonstrates that SARA not only impacts production metrics but also induces notable changes in the rumen epithelium, detectable through rumen colorimetry concerning the color of the epithelium. The discoloration in the rumen epithelium, observed as a darker hue, is attributed to the acidified rumen content's effect on the upper layers of the epithelium, rather than the presence of parakeratosis as previously suggested.

The use of computerized rumen colorimetry in ewes slaughtered as part of the replacement stock offers a promising management tool for assessing the SARA status of a farm. By implementing this technique, farmers and health advisors can gain reliable insights into the prevalence of SARA and make informed decisions to optimize feeding strategies and overall herd health.

Overall, the findings highlight the need for continued research and the development of comprehensive feeding practices that balance high energy intake with adequate dietary fiber. Incorporating concentrate mixtures into a total mixed ration (TMR) may provide a viable solution, mitigating the risk of SARA while supporting optimal milk production and animal well-being in dairy sheep farms.

Author Contributions: Conceptualization, G.C.; methodology, G.C., D.A.G., D.P. and V.G.P.; software, N.V., D.A.G.; validation G.C., D.A.G., D.P., V.G.P., L.V.A.; formal analysis, G.C., D.A.G., N.V., D.P.; investigation, N.V., G.C., D.A.G., D.P., G.I.P., K.K., M.A.T.; resources, N.V.; data curation, N.V., D.A.G., G.C., D.P.; writing—original draft preparation, N.V., G.C.; writing—review and editing, G.C., D.A.G., L.V.A., D.P., V.G.P.; visualization, G.C.; supervision, G.C., D.P., D.A.G., V.G.P.; project administration, G.C.; funding acquisition, G.C. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for essential materials, integral to the first author's PhD studies, was generously provided by the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly.

Institutional Review Board Statement: The trial protocol received approval by the Institutional Ethical Research Committee (University of Thessaly, approval number: 152, date of approval: 16 Mayr 2021).

Informed Consent Statement: Informed written consent was acquired from the farm proprietors.

Data Availability Statement: All data generated for this study are presented within the manuscript.

Acknowledgments: The authors wish to thank the managers of the trial farms for their support

Conflicts of Interest: The authors affirm that there are no financial or personal relationships, nor any other conflicts of interest, that could potentially exert undue influence or bias the content of this paper.

References

- Blanch, M.; Calsamiglia, S.; Di Lorenzo, N.; Di Costanzo, A.; Muetzel, S.; Wallace, R.J. Physiological changes in rumen fermentation during acidosis induction and its control using a multivalent polyclonal antibody preparation in heifers. *J. Anim. Sci.* **2009**, *87*, 1722–1730.
- Xia, G.; Sun, J.; Fan, Y.; Zhao, F.; Ahmed, G.; Jin, Y.; Zhang, Y.; Wang, H. β -Sitosterol Attenuates High Grain Diet-Induced Inflammatory Stress and Modifies Rumen Fermentation and Microbiota in Sheep. *Anim* **2020**, *10*, 171.
- Church, D.C. *Livestock Feeds and Feeding*, 3rd ed.; Publisher: Prentice-Hall Inc., USA, 1991; pp. 349.
- Commun, L.; Mialon, M.M.; Martin, C.; Baumont, R.; Veissier, I. Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J. Anim. Sci.* **2009**, *87*, 3372–3379.
- Chang, G.; Liu, X.; Ma, N.; Yan, J.; Dai, H.; Roy, A.C.; Shen, X. Dietary Addition of Sodium Butyrate Contributes to Attenuated Feeding-Induced Hepatocyte Apoptosis in Dairy Goats. *J. Agric. Food Chem.* **2018**, *66*, 9995–10002.
- Chaudhry, A.S.; Khan, M.A.; Khan, M.S.; Ashraf, K.; Saleem, M.H.; Khan, N.U.; Usman, T.; Ahmad, I. Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J. Anim. Plant Sci.* **2018**, *28*(1), 80–84; 1018–7081.
- Wang, Y.; Liu, J.; Huang, J.; Chang, G.; Roy, A.C.; Gao, Q.; Cheng, X.; Shen, X. Sodium butyrate attenuated iE-DAP induced inflammatory response in the mammary glands of dairy goats fed high-concentrate diet. *J. Sci. Food Agric.* **2020**, *101*(3), 1218–1227.
- Krause, K.M.; Oetzel, G.R. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* **2006**, *126*, 215–236.
- Voulgarakis, N.; Gougoulis, D.; Psalla, D.; Papakonstantinou, G.; Angelidou-Tsifida, M.; Papatsiros, V.; Athanasiou, L.; Christodouloupoulos, G. Ruminal Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease. *J. Hellenic Vet. Med. Soc.* **2023**, *74*(3), 5883–5891. <https://doi.org/10.12681/jhvms.31237>
- Desnoyers, M.; Duvaux-Ponter, C.; Rigalma, K.; Roussel, S.; Martin, O.; Giger-Reverdin, S. Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. *Anim.* **2008**, *2*(12), 1802–1808.
- Chang, G.; Zhuang, S.; Seyfert, H.M.; Zhang, K.; Xu, T.; Jin, D.; Guo, J.; Shen, X. Hepatic TLR4 signaling is activated by LPS from digestive tract during SARA, and epigenetic mechanisms contribute to enforced TLR4 expression. *Oncotarget* **2015**, *6*, 36.
- Xu, T.; Tao, H.; Chang, G.; Zhang, K.; Xu, L.; Shen, X. Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet. Res.* **2015**, *11*, 52.
- Li, S.; Gozho, G.N.; Gakhar, N.; Khafipour, E.; Krause, D.O.; Plaizier, J.C. Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can. J. Anim. Sci.* **2012**, *92*, 353–364.
- Kitkas, G.; Valergakis, G.; Kritsepi-Konstantinou, M.; Gelasakis, A.; Arsenos, G.; Kalaitzakis, E.; Panousis, N. Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation. *J. Hellenic Vet. Med. Soc.* **2019**, *70*, 1551–1560. [10.12681/jhvms.20861](https://doi.org/10.12681/jhvms.20861).
- Kirbas, A.; Baydar, E.; Kandemir, F.M.; Dorman, E.; Kizil, O.; Yildirim, B.A. Evaluation of serum cardiac troponin I concentration in sheep with acute ruminal lactic acidosis. *Vet. Arh.* **2014**, *84*(4), 355–364.
- Sabes, A.F.; Girardi, A.M.; Fagliari, J.J.; Oliveira, J.A.; Marques, L.C. Serum proteinogram in sheep with acute ruminal lactic acidosis. *Int. J. Vet. Sci. Med.* **2017**, *5*(1), 35–40.
- Voulgarakis, N.; Athanasiou, L.; Psalla, D.; Gougoulis, D.; Papatsiros, V.; Christodouloupoulos, G. Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment. *J. Hellenic Vet. Med. Soc.* **2024**, *74*(4), 6329–6336. <https://doi.org/10.12681/jhvms.31438>
- Enemark, J.M.D. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet. J.* **2008**, *176*(1), 32–43.
- Lopez-Campos, O.; Bodas, R.; Prieto, N.; Giraldez, F.J.; Perez, V.; Andres, S. Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Anim.* **2010**, *4*(6), 958–964.
- Andrés, S.; Jaramillo, E.; Bodas, R.; Blanco, C.; Benavides, J.; Fernández, P.; González, E.P.; Frutos, J.; Belenguera, A.; López, S.; Giraldez, F.J. Grain grinding size of cereals in complete pelleted diets for growing lambs: Effects on ruminal microbiota and fermentation. *Small Rumin. Res.* **2018**, *159*, 38–44.

21. Fanning, J.P.; Hynd, P.I.; Cockcroft, P.D. The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. *Small Rumin. Res.* **2018**, *162*, 57-62.
22. Yancey, J.W.S.; Kropf, D.H. Instrumental reflectance values of fresh pork are dependent on aperture size. *Meat Sci.* **2008**, *79*, 734-739.
23. Chmiel, M.; Slowinski, M.; Dasiewicz, K.; Florowski, T. Application of a computer vision system to classify beef as normal or dark, firm, and dry. *J Anim Sci* **2012**, *90*(11), 4126-30.
24. Silva, E.P.E.; Moraes, E.P.; Anaya, K.; Silva, Y.M.O.; Lopes, H.A.P.; Andrade Neto, J.C.; Oliveira, J.P.F.; Oliveira, J.B.; Rangel, A.H.N. Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. *PLoS One.* **2022**, *17*(2), e0263714.
25. Voulgarakis, N.; Gougoulis, D.A.; Psalla, D.; Papakonstantinou, G.I.; Katsoulos, P.D.; Katsoulis, K.; Angelidou-Tsifida, M.; Athanasiou, L.V.; Papatsiros, V.G.; Christodouloupoulos, G. Can Computerized Rumen Mucosal Colorimetry Serve as an Effective Field Test for Managing Subacute Ruminal Acidosis in Feedlot Cattle? *Vet Res Commun.* **2024**, *48*, 475-484 Oct 9. doi: 10.1007/s11259-023-10231-w.
26. Li, F.; Cao, Y.; Liu, N.; Yang, X.; Yao, J.; Yan, D. Subacute ruminal acidosis challenge changed in situ degradability of feedstuffs in dairy goats. *J Dairy Sci* **2014**, *97*, 5101-5109.
27. Lira, M.A.A.; Simões, S.V.D.; Riet-Correa, F.; Pessoa, C.M.R.; Dantas, A.F.M.; Miranda Neto, E.G. Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui Vet Bras* **2013**, *33*(2), 193-198.
28. Mialon, M.M.; Deiss, V.; Andanson, S.; Anglard, F.; Doreau, M.; Veissier, I. An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anaesthesia. *The Veterinary Journal* **2012**, *194*(1), 55-59.
29. Morgante, M.; Stelletta, C.; Berzaghi, P.; Ganesella, M.; Andrighetto, I. Subacute rumen acidosis in lactating cows: An investigation in intensive Italian dairy herds. *J Anim Physiol Anim Nutr* **2007**, *91*, 226-234.
30. Johnson, R.R.; Kubly, P.J. (2011) *Elementary Statistics* 11th (eleventh) Edition, Cengage Learning.
31. RGB Color Detector (2022) Available from: com.TheProgrammer.
32. CIE. (1986) Colourimetry: Official recommendations of the international commission on illumination. CIE Publ. No. 15.2. CIE Central Bureau, Vienna.
33. Povey, G.; Stubbings, L.; Phillips, K. Feeding the ewe A manual for consultants, vets and producers, 2nd ed.; Publisher: AHDB Agriculture and Horticulture Development Board Middlemarch Business Park Siskin Parkway East, Coventry, England, **2018**; pp. 1-64.
34. Tao, H.; Chang, G.; Xu, T.; Zhao, H.; Zhang, K.; Shen, X. Feeding a High Concentrate Diet Down-Regulates Expression of ACACA, LPL and SCD and Modifies Milk Composition in Lactating Goats. *PLoS ONE* **2015**, *10*(6), e0130525. doi:10.1371/journal.pone.0130525.
35. Zhang, H.; Peng, A.L.; Zhao, F.F.; Yu, L.H.; Wang, M.Z.; Osorio, J.S.; Wang, H.R. Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci.* **2020**, *103*(2), 1931-1943. doi: 10.3168/jds.2019-16944. Epub 2019 Dec 16. PMID: 31837780.
36. Plaizier, J.C.; Krause, D.O.; Gozho, G.N.; McBride, B.W. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet J* **2008**, *176*, 21-31.
37. Humer, E.; Aschenbach, J.R.; Neubauer, V.; Kröger, I.; Khiaosa-Ard, R.; Baumgartner, W.; Zebeli, Q. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J Anim Physiol Anim Nutr (Berl)* **2018**, *102*(2), 380-392.
38. Neubauer, V.; Humer, E.; Kröger, I.; Braid, T.; Wagner, M.; Zebeli, Q. Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J Anim Physiol Anim Nutr* **2017**, *102*(1), 343-349. https://doi.org/10.1111/jpn.12675
39. Villot, C.; Meunier, B.; Bodin, J.; Martin, C.; Silberberg, M. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal* **2017**, *12*, 481-490. https://doi.org/10.1017/S1751731117001677
40. Enemark, J.M.D.; Jørgensen, R.J.; Enemark, P. Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Vet ir Zootech* **2002**, *20*(42), 16-29.
41. Kleen, J.L.; Hooijer, G.A.; Rehage, J.; Noordhuizen, J.P. Subacute ruminal acidosis (SARA): A review. *J. Vet. Med. A Physiol Pathol Clin Med* **2003**, *50*, 406-414.
42. Cook, N.B.; Oetzel, G.R.; Nordlund, K.V. Clinical forum-subacute ruminal acidosis—a problem in UK dairy herds? *UK Vet* **2005**, *10*, 40-46.

43. Lean, I.J.; Annison, F.; Bramley, E.; Browning, G.; Cusack, P.; Farquharson, B.; Little, S.; Nandapi, D. Ruminal Acidosis- aetiopathogenesis, prevention and treatment: A review for veterinarians and nutritional professionals. Reference Advisory Group on Fermentative *Acidosis of Ruminants (RAGFAR)* **2007**, pp 1-60.
44. Giger-Reverdin, S.; Rigalma, K.; Desnoyers, M.; Sauvant, D.; Duvaux-Ponter, C. Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *J Dairy Sci* **2014**, *97*, 4367–4378.
45. Christodoulou, G. Maedi-Visna: Clinical review and short reference on the disease status in Mediterranean countries, *Small Rumin Res* **2006**, *62*(1–2), 47-53.
46. McKenna, S.L.; Keefe, G.P.; Tiwari, A.; VanLeeuwen, J.; Barkema, H.W. Johne's disease in Canada part II: disease impacts, risk factors, and control programs for dairy producers. *Can Vet J.* **2006**, *47*(11), 1089-99.
47. Alhidary, I.; Abdelrahman, M.M.; Alyemni, A.H.; Khan, R.U.; Al-Mubarak, A.H.; Albaadani, H.H. Characteristics of rumen in Naemi lamb: Morphological changes in response to altered feeding regimen. *Acta Histochem* **2016**, *118*(4), 331-7.
48. Abdelrahman, M.M.; Alhidary, I.; Alyemni, A.H.; Khan, R.U.; Bello, A.R.; Al-Saiady, M.Y.; Amran, R.A. Effect of alfalfa hay on rumen fermentation patterns and serum biochemical profile of growing Naemi lambs with ad libitum access to total mixed rations. *Pak. J. Zool.* **2005**, *49*, 15–19.
49. Hamada, T.; Maeda, S.; Kameoka, K. Effects of minerals on formation of colour in the rumen epithelium of kids. *J. Dairy Sci.* **1969**, *53*, 588–591.
50. Steele, M.A.; Greenwood, S.L.; Croom, J.; McBride, B.W. An increase in dietary non-structural carbohydrates alters the structure and metabolism of the rumen epithelium in lambs. *Can. J. Anim. Sci.* **2012**, *92*, 123-130.
51. Bo Trabi, E.; Seddik, H.E.; Xie, F.; Wang, X.; Liu, J.; Mao, S. Effect of pelleted high-grain total mixed ration on rumen morphology, epithelium-associated microbiota and gene expression of proinflammatory cytokines and tight junction proteins in Hu sheep. *Anim. Feed Sci. Technol.* **2020**, *263*, 114453.
52. Wang, L.; Qi, W.; Mao, S.; Zhu, W.; Liu, J. Effects of whole corn high-grain diet feeding on ruminal bacterial community and epithelial gene expression related to VFA absorption and metabolism in fattening lambs. *J Anim Sci.* **2022**, *100*(3), skac056.
53. Garcia Diaz, T.; Ferriani Branco, A.; Jacovaci, F.A.; Cabreira Jobim, C.; Bolson, D.C.; Pratti Daniel, J.L. Inclusion of live yeast and mannanoligosaccharides in high grain-based diets for sheep: Ruminal parameters, inflammatory response and rumen morphology. *PLoS ONE* **2018**, *13*(2), e0193313.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

4b. “Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management”

Published

Voulgarakis N, Gougoulis D, Psalla D, Papakonstantinou G, Katsoulis K, Angelidou Tsifida M, Athanasiou L, Papatsiros V, Christodouloupoulos G. Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact, and Colorimetry Management. *Animals* 2024, 14(14), 206. <https://doi.org/10.3390/ani14142061>

Article

Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact and Colorimetry Management

Nikolaos Voulgarakis ¹, Dimitrios A. Gougoulis ¹, Dimitra Psalla ², Georgios I. Papakonstantinou ¹, Konstantinos Katsoulis ³, Mariana Angelidou-Tsifida ², Labrini V. Athanasiou ¹, Vasileios G. Papatsiros ¹ and Georgios Christodouloupoulos ^{4,*}

- ¹ Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, 431 00 Karditsa, Greece; nvoulgarakis@vet.uth.gr (N.V.); dgoug@vet.uth.gr (D.A.G.); geopapak@vet.uth.gr (G.I.P.); lathan@vet.uth.gr (L.V.A.); vpapatsiros@vet.uth.gr (V.G.P.)
- ² Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece; dpsalla@vet.auth.gr (D.P.); mtangelid@vet.auth.gr (M.A.-T.)
- ³ Department of Animal Husbandry and Nutrition, Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly, 431 00 Karditsa, Greece; kkatsoulis@vet.uth.gr
- ⁴ Department of Animal Science, Agricultural University of Athens, 118 55 Athens, Greece
- * Correspondence: gc@aua.gr; Tel.: +30-2105294386

Simple Summary: Subacute rumen acidosis (SARA) is a significant clinical issue in high-yield livestock, including dairy sheep, and it is characterized by rumen pH dropping below 5.8 for at least four hours daily. This condition leads to economic losses due to reduced milk production and lower milk fat content. High-concentrate, low-fiber diets increase the risk of SARA by disrupting rumen fermentation. Diagnosing SARA is challenging, with rumen fluid pH measurement being the gold standard. This study investigated the prevalence of SARA and its impact in forty-two Greek dairy sheep flocks, as well as the potential of computerized rumen colorimetry as a diagnostic tool *post mortem*. Over nine months, rumen pH and milk composition were monitored, and rumen wall samples were analyzed for color and histological changes. The results showed increased epithelium thickness and discoloration in SARA-affected sheep. This study highlights the prevalence of SARA in the modern intensive dairy sheep industry, underscores the need for balanced feeding strategies, and suggests computerized rumen colorimetry as a promising tool for SARA detection and management in dairy sheep farms.



Citation: Voulgarakis, N.; Gougoulis, D.A.; Psalla, D.; Papakonstantinou, G.I.; Katsoulis, K.; Angelidou-Tsifida, M.; Athanasiou, L.V.; Papatsiros, V.G.; Christodouloupoulos, G. Subacute Rumen Acidosis in Greek Dairy Sheep: Prevalence, Impact and Colorimetry Management. *Animals* **2024**, *14*, 2061. <https://doi.org/10.3390/ani14142061>

Academic Editor: Umberto Bernabucci

Received: 14 June 2024
Revised: 8 July 2024
Accepted: 12 July 2024
Published: 14 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Subacute rumen acidosis (SARA) is a current issue in intensive livestock farming, and it is particularly associated with feeding high-concentrate diets. This study investigated the prevalence and impact of SARA in forty-two Greek dairy sheep flocks by recording rumen pH, milk composition, and milk yield over a period of nine months. Moreover, it explored the use of computerized rumen colorimetry as a management and diagnostic tool for SARA in dairy sheep. In culled ewes, computerized rumen mucosal colorimetry was applied, and rumen wall samples taken for histological examination. SARA cases were identified in 19 farms (45%, $n = 42$). Farms with SARA cases had lower milk fat levels, while milk yield and milk protein levels did not differ based on the SARA status of the farms. In culled ewes, rumen color was significantly associated with the flock's SARA status, and affected ewes showed increased thickness in non-keratinized and total epithelial layers. It was concluded that computerized rumen mucosal colorimetry in aged, culled ewes shows promise as an indicator, *post mortem*, of SARA present in dairy sheep flocks whose impact can be minimized by making significant changes in dietary management.

Keywords: subacute rumen acidosis; rumen mucosa colorimetry; dairy sheep; milk fat depression; epidemiology; Greece

1. Introduction

In high-yield livestock farming, rumen acidosis is a common clinical issue [1,2]. It occurs in two forms: acute, which is characterized by a rumen pH below 5.0, and subacute (subacute ruminal acidosis—SARA), which is characterized by rumen pH being lower than 5.8 for at least four hours per day [3–5]. In contrast to acute rumen acidosis, SARA does not cause acute rumenitis, and any potential mucosal degenerative changes either do not occur or are reversible. However, SARA still leads to economic losses due to reduced milk production and lower milk fat content [5,6].

Feeding high-concentrate diets with low percentages of dietary fiber for extended periods increases the risk of subacute rumen acidosis, negatively affecting both milk production and fat concentration [7–9]. The subsequent lower rumen pH disrupts fermentation and bacterial synthesis, leading to an increased production of long-chain fatty acids (LCFAs), which hinders milk fat synthesis in the udder [10,11].

Several hematobiochemical parameters have been suggested as diagnostic markers for SARA in small ruminants, but none have proven to be entirely accurate [5,12]. Rumen fluid pH measurement is the gold standard, with a pH between 5.2 and 5.8 for more than four hours per day indicating SARA in intensive small ruminant livestock farming [5,12].

Rumen acidosis, marked by rumen wall discoloration, especially in subacute rumenitis, manifests as a gray to dark discoloration [13–16]. In cattle, the objective estimation of color using spectrophotometers or colorimeters through Red, Green, and Blue (RGB) analysis has emerged as a potential method for assessing various health issues [17–19].

Sheep farming in Greece is a significant component of the country's agricultural landscape, with approximately 7.3 million animals across 83,000 holdings [20,21]. Sheep farming in Greece is distinguished by its high dairy specialization, with 93% of sheep being dedicated to milk production. The primary use of this milk is for the production of feta cheese, a Protected Designation of Origin product and the most important export of primary Greek agriculture [22]. Despite its importance, the Greek sheep population has seen a decline of over 10% since 2010. This reduction has been marked by a decrease in the number of small and medium-sized holdings, which have been increasingly replaced by fewer, larger operations housing more than 500 ewes each. These larger enterprises predominantly employ an intensive indoor production system, relying heavily on imported concentrate feeds, supplemented by locally produced hay and sometimes ensilage. The current sheep population mainly consists of crossbreeds between various indigenous sheep and foreign breeds, such as mainly Lacaune and secondary Friesian, of which sheep of Lacaune cross adapt especially well to the intensive farming methods now prevalent in Greece.

While SARA has been extensively studied in other livestock, research on this topic in dairy sheep is limited [23]. This study aims to address this gap by evaluating the prevalence and impact of SARA in Greek dairy sheep farming and by assessing the effectiveness of computerized rumen colorimetry—a tool developed by the authors in a previous publication [19]—for managing and detecting SARA in these farms.

2. Materials and Methods

2.1. Selection of Farms

Forty-two dairy sheep flocks, each consisting of 500–1000 milking ewes, were carefully selected to participate in this study. These farms are situated in Thessaly, a central region of Greece. Inclusion criteria required farms to be within reach of the Department of Clinical Veterinary Medicine, Faculty of Veterinary Science, University of Thessaly, Greece. Farms also needed to have crossbreed ewes of Lacaune with the local Karagouniko breed, maintain a stable ration composition throughout the entire milking period, and be confirmed as free of paratuberculosis and ovine progressive pneumonia, as evidenced by the department's archives. The ration composition on the 42 farms consisted of a specified amount of complementary concentrate mixture given per head and cereal hay provided *ad libitum*. Throughout the 9-month trial period, the complementary feed remained unchanged.

2.2. Experimental Design

The selected farms were monitored every two months for rumen pH throughout the entire milking period, spanning approximately nine months. Five visits were conducted at each farm during the first 10 days of the 1st, 3rd, 5th, 7th, and 9th month post-partum.

At each visit, six ewes were randomly chosen and assessed for rumen pH, ensuring they had not been previously selected for rumen pH measurement to prioritize animal welfare. Rumen fluid sampling took place four hours after the morning administration of concentrated feed. Simultaneously, milk samples were gathered from the bulk milk tank during morning visits. Additionally, data on daily milk yield were sourced from the farmers' records.

Furthermore, between July and August 2021, during the culling of aged ewes from the above 42 farms, rumen sampling was performed in slaughterhouses. Due to economic constraints, a total of 280 ewes were included in the sampling, with at least 6 ewes from each farm.

2.3. Sample Size Justification

According to the existing literature, the prevalence of subacute rumen acidosis (SARA) in dairy sheep is approximately 12% [24]. To ensure a desired level of accuracy with a margin of error (e) of ± 0.05 and a 95% confidence interval ($Z = 1.96$), the estimated sample size (n) was calculated using the formula $n = (Z^2 \times P \times (1 - P)) / e^2$, yielding a value of 162. This study exceeded this calculated sample size requirement during both farm and slaughterhouse sampling phases.

2.4. Ethical Approval

This study adhered to established animal welfare regulations (Directive 98/58/EC) and obtained approval from the Ethics Committee of the Faculty of Veterinary Science at the University of Thessaly, Greece (approval number: 152/16 May 2021). No modifications were made to the animals' diets or treatments for experimental purposes during the entire duration of this study.

2.5. Clinical and Laboratory Procedures

2.5.1. Rumenocentesis and Rumen pH Measurement

Rumenocentesis, conducted without sedation, was employed for the collection of rumen fluid samples, following established methodologies from prior studies [25]. The procedure included an external abdominal puncture using a 16 G (105 mm) needle (Intralune PP, Vygon, Écouen, France) in the left abdominal region, specifically targeting the ventral sac of the rumen and aspirating the fluid with a 50 mL syringe [5,26]. The point for rumenocentesis was identified at the intersection of two lines: one horizontal, passing through the stifle joint, and one vertical, passing through the midpoint between the base of the last rib and the hip bone. The obtained rumen fluid underwent immediate pH determination, utilizing a digital portable pH meter (HI-2002 Edge pH Meter, Hanna instruments, Leighton Buzzard, UK).

2.5.2. Milk Analyses

To maintain sample quality, milk samples were refrigerated at 4 °C during transportation to the laboratory. On the same day of collection, the milk composition, including fat, protein, lactose, and total solids' content, was analyzed using an infrared analysis technique with the assistance of an FTIR interferometer (Milkoscan FT6000 Analyzer, Foss Electric, Hilleroed, Denmark). The annual average values for the mentioned milk components were calculated based on the five measurements in each farm, along with the monthly milk yield, following basic arithmetic procedures [27].

2.5.3. Rumen Wall Sampling and Image Acquisition

The sampling procedure for the rumen wall followed a standardized protocol, as detailed by Voulgarakis et al. [19], ensuring that the same anatomical area of the rumen wall was sampled each time. This standardized protocol, developed specifically for this purpose, was based on certain measurements and was created by the Clinical Veterinary Medicine Department, Faculty of Veterinary Science, University of Thessaly. Finally, a 5 cm × 5 cm square section of the rumen wall was excised for sampling. After excision, the sample underwent thorough washing with water to eliminate any visible remnants of rumen content. For image acquisition, the collected samples were placed on a specific desk 35–37 mm between the lens and ruminal epithelium surface, and digital gross images were taken. A digital camera (NIKON COOLPIX L330, Tokyo, Japan) was used with an EF-S 60 mm macro lens (NIKKOR Wide-Angle Optical Zoom Lens 21×, Nikon Corporation, Tokyo, Japan) with standard lighting conditions [halogen lighting (4 matt bulbs, 18 W each, color temperature of 2800 K, color rendering index of 90 to 100)] and settings (manual operation mode with an f/3.5 aperture, 1/30 s shutter speed, and ISO range on 160). The images were saved in raw format [19]. The sample was then placed in a container with a 10% formalin solution for subsequent histological examination.

2.5.4. Rumen Wall Image Color Analysis

The quantification of rumen mucosa color was determined using software applications, specifically RGB Color Detector 3.0.7 [28] and CIELab 1.0 [29].

In the RGB application, the analyzed color components included Red, Green, and Blue (RGB), Value (V), Lightness (L), Hue, Saturation, and Value (HSV), and Hue, Saturation, and Lightness (HSL). Additionally, the summary of the Red, Green, and Blue color components was computed and labeled as RGB-SUM [17]. Using the CIELab software 1.0, the L (color lightness), a (redness/greenness of the color), and b (yellowness/blueness of the color) color components were assessed [29].

For processing and analyzing the image, a 1 × 1 cm area within the region of interest was randomly selected from each sample. For each rumen wall sample, average values of these color components were calculated from nine distinct spot measurements, and the resulting average value was employed for subsequent statistical analysis. It is essential to note that, although the L, a, and b values are technically denoted as L*, a*, and b*, respectively, for simplicity, this text omits the asterisks.

2.5.5. Histological Examination

For the histological examinations, the rumen wall samples were cut into 1 cm × 1 cm size particles. Tissues were fixed in 10% formalin buffer for 48 to 72 h. The processed tissues were dehydrated by being immersed in a series of alcohol solutions and embedded in paraffin sections (Paraplast Plus®, Kendall, UK). Afterwards, 4 µm thick sections were obtained via semi-automatic precision microtome (CUT 5062, SLEE medical GmbH, Nieder-Olm, Germany), dewaxed and stained with hematoxylin and eosin (H&E), for histopathological evaluation. From each sample, randomly selected fields with a ×10 objective were captured using a microscope (Nikon eclipse 50i, Tokyo, Japan)-coupled camera (Nikon, Digital sight DS-L1, Tokyo, Japan). ImageJ software 1.53 was utilized to measure the thickness of the stratum corneum, connective tissue, non-keratinized epithelium, and total epithelium in each photomicrograph (Figure 1).

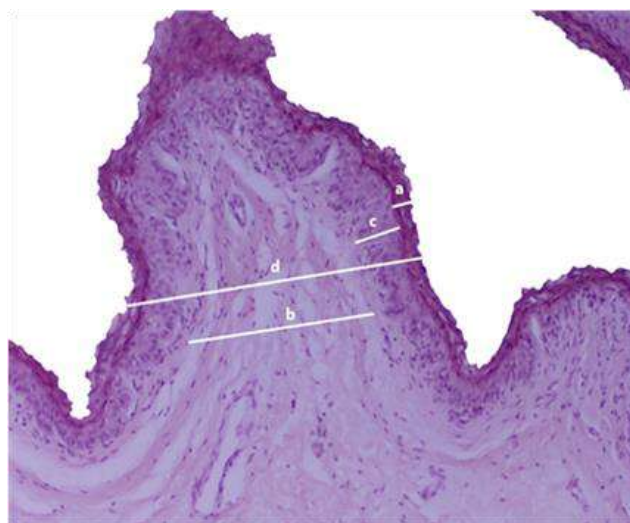


Figure 1. Measurements of the ewe rumen papillae: (a) Stratum corneum thickness. (b) Width of connective tissue. (c) Thickness of non-keratinized epithelium. (d) Total epithelium thickness. (Hematoxylin-eosin staining; magnification $\times 10$).

2.6. Statistical Analysis

The consistency of rumen pH measurements over time in each farm was examined using the intraclass correlation coefficient (ICC). Continuous variables in the dataset, including pH values, milk parameters (such as yield, fat, protein, lactose, and total solids' content), color measurements, and characteristics of the rumen epithelium, were checked for normal distribution. When necessary, log transformations were applied to address any observed skewness in the data. To explore potential differences in these variables among farms with low, moderate, and high SARA prevalence, a one-way analysis of variance (ANOVA) was conducted using IBM SPSS Statistics version 29.0.0.0 (241). The results were presented using mean and standard deviation (SD) as measures of central tendency and variability, respectively. The analysis was performed using IBM SPSS Statistics version 29.0.0.0 (241), with a significance level of 0.05 used to determine statistical significance.

3. Results

All animals involved in this study completed the entire monitoring period, without any adverse effects.

The 42 farms were categorized into three groups based on the number of rumen pH measurements below 5.8 out of 30 measurements conducted in each farm [$n = 5$ visits ($\times$) 6 ewes each visit = 30 measurements per farm]. The categories and the number of farms in each category were as follows:

- Category 1: Farms with a low prevalence of SARA, including 17 farms (41% of the total), with each farm exhibiting only 0–2 cases of $\text{pH} < 5.8$ in the 30 measurements.
- Category 2: Farms with a moderate prevalence of SARA, comprising 19 farms (45% of the total), with each farm showing 3–9 cases of $\text{pH} < 5.8$ in the 30 measurements.
- Category 3: Farms with a high prevalence of SARA, consisting of 6 farms (14% of the total), with each farm having 10–14 cases of $\text{pH} < 5.8$ in the 30 measurements.

Table 1 shows the chemical composition and feeding amount per head of concentrate mixtures used on farms. Although there are arithmetic differences between the three farm categories, these differences were not statistically significant.

Throughout the farming sampling period, the mean pH values of the participating farms, with six randomly selected ewes each time, ranged from 5.73 to 5.93, yielding a mean pH of 5.83 (standard deviation (SD) = 0.06). Specifically, in Category 1 farms, the pH range was between 5.87 and 5.93, with a mean pH of 5.89 (SD = 0.01). In Category 2, the pH range was from 5.78 to 5.84, with a mean pH of 5.81 (SD = 0.02). Lastly, in Category 3, the pH

range was recorded between 5.73 and 5.76, with a mean pH of 5.74 (SD = 0.01). The sample size for each category was consistent. Post hoc analysis using the Bonferroni correction revealed significant differences among all possible pairwise comparisons between the three farm categories ($p < 0.001$) (Table 2).

Table 1. Chemical composition and feeding amount per head (mean $\pm$ SD) of the complementary concentrate mixture used on farms.

Chemical Composition	Category 1 ⁱ (n = 17)	Category 2 ⁱⁱ (n = 19)	Category 3 ⁱⁱⁱ (n = 6)	p-Value
ME (Mcal/kg DM)	2.65 $\pm$ 0.07	2.66 $\pm$ 0.07	2.66 $\pm$ 0.08	0.96
CP (% of DM)	16.06 $\pm$ 0.97	16.05 $\pm$ 0.97	16.50 $\pm$ 1.38	0.72
NDF (% of DM)	14.47 $\pm$ 0.80	14.42 $\pm$ 0.77	14.33 $\pm$ 1.21	0.95
ADF (% of DM)	5.94 $\pm$ 0.83	6.11 $\pm$ 0.81	6.17 $\pm$ 0.75	0.77
Feeding Amount (g/day)	1,682 $\pm$ 107	1,705 $\pm$ 118	1,758 $\pm$ 150	0.43

ⁱ Farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA; ME = metabolizable energy; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; DM = dry matter.

Table 2. Comparison of rumen pH, milk yield, and milk components (mean $\pm$ SD).

Farm Sampling Period	Category 1 ⁱ (n = 17)	Category 2 ⁱⁱ (n = 19)	Category 3 ⁱⁱⁱ (n = 6)	p-Value
Rumen pH	5.89 $\pm$ 0.01	5.81 $\pm$ 0.02	5.74 $\pm$ 0.01	<0.001
Milk yield (liters/ewe/milking period)	412.0 $\pm$ 1.1	414.5 $\pm$ 2.1	415.1 $\pm$ 0.6	0.143
Milk fat content (%)	5.92 $\pm$ 0.14	5.69 $\pm$ 0.11	5.65 $\pm$ 0.06	<0.001
Milk protein content (%)	5.72 $\pm$ 0.10	5.72 $\pm$ 0.12	5.83 $\pm$ 0.05	0.064
Milk lactose content (%)	4.71 $\pm$ 0.09	4.81 $\pm$ 0.14	4.95 $\pm$ 0.16	0.001
Milk total solids' content (%)	10.87 $\pm$ 0.23	11.06 $\pm$ 0.31	11.73 $\pm$ 0.40	<0.001
Milk fat/milk protein ratio	1.04 $\pm$ 0.02	0.99 $\pm$ 0.02	0.96 $\pm$ 0.01	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA.

In the abattoir, post hoc analysis revealed significant differences in the color components among all possible pairwise comparisons of the three farm categories, with the exception of the a component across all farm categories and the b component between Category 1 and Category 2 ($p < 0.001$) (Table 3).

Table 3. Comparison of color components (mean $\pm$ SD) in rumen mucosa samples.

Color Components	Category 1 ⁱ (n = 111)	Category 2 ⁱⁱ (n = 124)	Category 3 ⁱⁱⁱ (n = 45)	p-Value
L	39.93 $\pm$ 5.24	30.59 $\pm$ 4.92	21.98 $\pm$ 6.92	<0.001
a	0.66 $\pm$ 4.03	1.01 $\pm$ 4.34	2.28 $\pm$ 4.04	0.091
b	9.54 $\pm$ 5.80	9.53 $\pm$ 4.67	4.49 $\pm$ 6.92	<0.001
Red	100.94 $\pm$ 14.33	78.38 $\pm$ 9.98	58.41 $\pm$ 12.34	<0.001
Green	93.38 $\pm$ 12.69	71.16 $\pm$ 13.14	51.60 $\pm$ 16.13	<0.001
Blue	78.92 $\pm$ 15.15	57.53 $\pm$ 14.52	46.33 $\pm$ 17.81	<0.001
RGB-SUM	273.23 $\pm$ 36.99	207.08 $\pm$ 32.36	156.34 $\pm$ 43.06	<0.001
HSV	0.40 $\pm$ 0.06	0.33 $\pm$ 0.06	0.24 $\pm$ 0.05	<0.001
HSL	35.47 $\pm$ 4.85	26.82 $\pm$ 4.08	20.44 $\pm$ 5.57	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA; L: color lightness unit ranged from 0 to 100. a: redness/greenness of the color unit ranged from -128 to 127 . b: yellowness/blueness of the color unit ranged from -128 to 127 . Red, Green, and Blue unit ranged from 0 to 255 in an 8-bit per channel representation. RGB-SUM is labeled as the summary of the Red, Green, and Blue color components; HSV: hue, saturation, and value. Value unit is a percentage (0% to 100%). HSL: hue, saturation, and lightness. Lightness unit is a percentage (0% to 100%).

3.1. Milk Analysis

Milk fat content varied across farm categories, with Category 1 having the highest average fat content and Category 3 the lowest, as shown in Table 2. Post hoc analysis indicated significant differences between Category 1 and Category 2, as well as between Category 1 and Category 3 ($p < 0.001$). As a direct consequence of these fat differences, there were also variations in the milk fat/milk protein ratio, with Category 1 having the highest ratio and Category 3 the lowest. Milk yield and milk protein did not vary between farm categories; however, small but statistically significant differences were found in the concentration of lactose and the total solids' content (Table 2).

3.2. Color Measurement

Pairwise comparisons of mean L component values among all categories (1, 2, 3) revealed statistically significant differences ($p < 0.001$). Specifically, Category 1 exhibited the highest mean L value, whereas Category 3 reported the lowest mean L value, as reported in Table 3. Significant differences were also observed in the mean values of the b component between Category 1 and Category 3, as well as between Category 2 and Category 3, as shown in Table 3. However, no statistically significant differences were detected in the mean values of the a component across all possible comparisons (Table 3).

Regarding the color measurement using the RGB analysis system, the mean values of Red, Green, Blue, and RGB-SUM were found to be significantly higher in Category 1 when compared to the mean values of Category 2 and Category 3 samples. Similarly, Category 2 exhibited significantly higher mean values compared to Category 3. Furthermore, significant differences were observed for the HSV value and HSL value among all possible comparisons, with Category 1 having the highest values compared to Category 2 and Category 3. Lastly, Category 2 revealed significantly higher mean values compared to Category 3 (Table 3).

3.3. Ruminal Epithelium Histopathology and Histomorphometry

Histological analyses revealed no abnormal findings in the rumen epithelium samples, and parakeratosis was not observed. The darkest coloration was confined solely to the keratinized layer in these samples.

According to the statistical analysis, Category 3 exhibited a significantly higher thickness in non-keratinized epithelium thickness and total epithelium thickness in contrast to Category 2 and Category 1, as shown in Table 4. Additionally, the non-keratinized epithelium thickness and total epithelium thickness were significantly higher in samples of Category 2 compared to samples of Category 1. However, no significant differences were observed in stratum corneum thickness and connective tissue width in any of the possible comparisons (Table 4).

Table 4. Comparison of histological measurements in the layers of the ruminal epithelium (mean $\pm$ SD).

Ruminal Epithelium	Category 1 ⁱ (n = 15)	Category 2 ⁱⁱ (n = 15)	Category 3 ⁱⁱⁱ (n = 15)	p-Value
Stratum corneum (μ m)	33.67 $\pm$ 12.83	43.07 $\pm$ 24.97	41.67 $\pm$ 16.25	0.342
Connective tissue (μ m)	103.27 $\pm$ 39.35	135.00 $\pm$ 61.32	148.13 $\pm$ 63.83	0.090
Non-keratinized epithelium (μ m)	80.13 $\pm$ 14.46	116.47 $\pm$ 28.64	166.67 $\pm$ 21.41	<0.001
Total epithelium (μ m)	217.07 $\pm$ 51.69	294.53 $\pm$ 61.26	356.47 $\pm$ 74.08	<0.001

Significant difference at $p \leq 0.001$; ⁱ farms with a low prevalence of SARA; ⁱⁱ farms with a moderate prevalence of SARA; ⁱⁱⁱ farms with a high prevalence of SARA.

4. Discussion

This research represents the first attempt to investigate the epidemiology of SARA in dairy sheep farms in Greece. The findings strongly indicate that SARA is a common disorder

in the modern Greek dairy sheep industry. These results are unsurprising given the typical nutritional management practices on farms. Ewes were provided with a complementary concentrate mixture that exceeded 1500 g per day throughout the milking period (see Table 1) and were also offered cereal hay *ad libitum*. Typically, ewes consume the allotted amount of the complementary concentrate mixture promptly, but the consumption of cereal hay often depends on its quality, leading to variable intake and potentially failing to meet the animals' daily fiber requirements. It is known that feeding more than 500 g of cereals daily increases the risk of ruminal acidosis in sheep; therefore, the recommendation is that if more than 500 g is fed to a ewe, it should be in the form of a total mixed ration (TMR) rather than as a complementary feed [30].

The complementary concentrate mixtures used on the farms did not show significant differences in their chemical composition or feeding amounts (see Table 1). This was expected as feeding practices and ration formulation in modern Greek sheep farming are quite homogeneous. The lack of differences in the concentrate mixtures among the investigated farms underscores the causative role of hay in the cases of SARA documented in this research.

In this study, farms with a higher number of SARA cases (Categories 2 and 3) exhibited lower milk fat content. Lower ruminal pH is attributed to alterations in rumen fermentation and changes in the composition of rumen bacteria and fermentation patterns, leading to an increased production of specific LCFAs. These LCFAs, once absorbed, can inhibit milk fat synthesis in the udder [10,11]. Multiple studies, including those by Tao et al. [31], Zhang et al. [32], Li et al. [10], and Kitkas et al. [11], have consistently observed lower milk fat percentages in high-concentrate groups compared to low-concentrate groups in both dairy cows and goats. To the best of the authors' knowledge, this research is the first to document this effect in dairy ewes.

This research proposes a computerized measurement of rumen epithelium color as a promising method for monitoring SARA status. This technique can be easily applied during the slaughter of aged, culled ewes, providing health advisors with reliable data on SARA status in the farm. Currently, rumen pH measurement is considered the gold standard for diagnosing SARA in ruminants [33,34]. However, pH measurement can be influenced by various factors, including the method of measurement, sampling location, and timing [35,36]. Beyond pH measurements, diagnostic indicators such as milk fat percentage and the milk fat/protein ratio have been proposed for detecting SARA in dairy cows. Rapid weekly decreases in milk fat content of 0.3–0.5% or a milk fat/protein ratio below 1 suggest potential SARA cases [37–40]. However, these indexes have not been established for the dairy sheep industry, partly due to the ongoing genetic improvements in dairy sheep breeds. Additionally, chronic diseases such as progressive pneumonia and Johne's disease are associated with decreases in milk fat content [41,42].

The existing literature indicates that the prevalence of SARA in dairy sheep is approximately 12%, but this study was conducted in Brazil [24]. This study exceeded the calculated sample size requirement based on this figure to account for the lack of extensive data from Greece and other Mediterranean countries.

The mean color values, measured by RGB and CIELab systems, were significantly lower in Category 3 samples compared to Categories 1 and 2, indicating darker discoloration of the ruminal epithelium. Extensive research and reviews on this topic, both in dairy and fattening lambs, have shown that feeding highly fermentable feed, which leads to reduced ruminal pH and increased accumulation of volatile fatty acids and lactic acid, is associated with a gray to dark coloration [13–16,43,44]. Similar results were reported in feedlot cattle by Voulgarakis et al. [19]. Unfortunately, as far as is known, no research data from dairy ewes have been published yet. Other factors contributing to the darkening of the ruminal mucosa include pelleting, fiber particle size, dietary iron levels, and the buffering system [43,45]. However, the generally accepted conclusion among sheep practitioners is that rumen pH plays a decisive role in the darkening of the keratinized layer in the ruminal epithelium.

Histological findings showed that the darkening of the rumen epithelium's color was due to the effect of rumen pH on the keratinized layer rather than parakeratosis. This is a notable finding as previous research attributed rumen epithelium darkening to parakeratosis [37].

Furthermore, an increase in the thickness of ruminal epithelium layers, particularly non-keratinized and total epithelium, was observed in animals from farms with a high incidence of SARA. Steele et al. [46] observed increased total epithelial thickness and stratum corneum thickness in animals fed a high-grain diet. Bo Trabi et al. [47] found that animals fed a low-grain non-pelleted TMR concentrate had higher total epithelium and stratum corneum thickness values compared to those fed a high-grain non-pelleted TMR concentrate or high-grain TMR in a pelleted form. Wang et al. [48] reported increased total epithelium and stratum corneum thickness values in animals fed a ground corn high-grain diet compared to a whole corn high-grain diet. No significant difference in stratum corneum thickness was found among the categories, but Category 3 samples had a greater stratum corneum length compared to Category 1 samples. Garcia Diaz et al. [49] reported higher total epithelium and stratum corneum thickness values but lower connective tissue width and non-keratinized thickness in animals fed a grain-based diet compared to those on an additive-supplemented grain-based diet. Lower rumen pH may increase the keratinized epithelium's rate of replacement, stimulating non-keratinized epithelium growth to produce keratinized layers [19].

5. Conclusions

In conclusion, this study sheds light on the prevalence and impact of subacute rumen acidosis (SARA) in Greek dairy sheep flocks. Our findings highlight that SARA significantly affects dairy sheep farms, manifesting in notable changes in the rumen epithelium, which can be detected through computerized rumen colorimetry. The observed discoloration is indicative of acidic effects on the upper layers of the epithelium, rather than the previously suggested presence of parakeratosis.

The use of computerized rumen colorimetry in culled ewes as a diagnostic tool shows promise for assessing SARA status in dairy sheep farms. This technology provides farmers and health advisors with reliable data on SARA prevalence, enabling informed decisions to optimize feeding strategies and enhance herd health.

Looking ahead, further research and the development of comprehensive feeding practices are crucial. Strategies such as integrating concentrate mixtures into total mixed rations (TMRs) could mitigate SARA risks while promoting optimal milk production and ensuring animal welfare in dairy sheep farms.

Author Contributions: Conceptualization, G.C.; methodology, G.C., D.A.G., D.P. and V.G.P.; software, N.V. and D.A.G.; validation G.C., D.A.G., D.P., V.G.P. and L.V.A.; formal analysis, G.C., D.A.G., N.V. and D.P.; investigation, N.V., G.C., D.A.G., D.P., G.I.P., K.K. and M.A.-T.; resources, N.V.; data curation, N.V., D.A.G., G.C. and D.P.; writing—original draft preparation, N.V. and G.C.; writing—review and editing, G.C., D.A.G., L.V.A., D.P. and V.G.P.; visualization, G.C.; supervision, G.C., D.P., D.A.G. and V.G.P.; project administration, G.C.; funding acquisition, G.C. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for essential materials, integral to the first author's PhD studies, was generously provided by the Clinical Veterinary Medicine Department, Faculty of Veterinary Medicine, University of Thessaly, through the Account of the Research Committee of the University of Thessaly (code: 2557, Scientific Supervisor: Prof. E. Saridomichelakis).

Institutional Review Board Statement: The trial protocol received approval from the Institutional Ethical Research Committee (University of Thessaly; approval number: 152; date of approval: 16 Mayr 2021).

Informed Consent Statement: Informed written consent was acquired from the farms' proprietors.

Data Availability Statement: All data generated for this study are presented within this manuscript.

Acknowledgments: The authors wish to thank the managers of the trial farms for their support.

Conflicts of Interest: The authors affirm that there are no financial or personal relationships, nor any other conflicts of interest, that could potentially exert undue influence or bias the content of this paper.

References

1. Penner, G.B.; Aschenbach, J.R.; Gäbel, G.; Rackwitz, R.; Oba, M. Epithelial Capacity for Apical Uptake of Short Chain Fatty Acids Is a Key Determinant for Intraruminal pH and the Susceptibility to Subacute Ruminal Acidosis in Sheep. *J. Nutr.* **2009**, *139*, 1714–1720. [PubMed]
2. Xia, G.; Sun, J.; Fan, Y.; Zhao, F.; Ahmed, G.; Jin, Y.; Zhang, Y.; Wang, H. β -Sitosterol Attenuates High Grain Diet-Induced Inflammatory Stress and Modifies Rumen Fermentation and Microbiota in Sheep. *Animals* **2020**, *10*, 171. [CrossRef] [PubMed]
3. Church, D.C. *Livestock Feeds and Feeding*, 3rd ed.; Prentice-Hall Inc.: Hoboken, NJ, USA, 1991; p. 349.
4. Commun, L.; Mialon, M.M.; Martin, C.; Baumont, R.; Veissier, I. Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. *J. Anim. Sci.* **2009**, *87*, 3372–3379. [CrossRef] [PubMed]
5. Chaudhry, A.S.; Khan, M.A.; Khan, M.S.; Ashraf, K.; Saleem, M.H.; Khan, N.U.; Usman, T.; Ahmad, I. Effect of different level of concentrate feeding on hematobiochemical parameters in an experimentally induced sub-acute ruminal acidosis (SARA) in sheep and its management with different concentration of antacids. *J. Anim. Plant Sci.* **2018**, *28*, 80–84.
6. Voulgarakis, N.; Gougoulis, D.; Psalla, D.; Papakonstantinou, G.; Angelidou-Tsifida, M.; Papatsiros, V.; Athanasiou, L.; Christodoulouopoulos, G. Ruminal Acidosis Part I: Clinical manifestations, epidemiology and impact of the disease. *J. Hell. Vet. Med. Soc.* **2023**, *74*, 5883–5891. [CrossRef]
7. Desnoyers, M.; Duvaux-Ponter, C.; Rigalma, K.; Roussel, S.; Martin, O.; Giger-Reverdin, S. Effect of concentrate percentage on ruminal pH and time-budget in dairy goats. *Animal* **2008**, *2*, 1802–1808. [CrossRef] [PubMed]
8. Xu, Y.; Ding, Z. Physiological, biochemical and histopathological effects of fermentative acidosis in ruminant production: A minimal review. *Span. J. Agric. Res.* **2011**, *9*, 414–422. [CrossRef]
9. Xu, T.; Tao, H.; Chang, G.; Zhang, K.; Xu, L.; Shen, X. Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Vet. Res.* **2015**, *11*, 52. [CrossRef] [PubMed]
10. Li, S.; Gozho, G.N.; Gakhar, N.; Khafipour, E.; Krause, D.O.; Plaizier, J.C. Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. *Can. J. Anim. Sci.* **2012**, *92*, 353–364. [CrossRef]
11. Kitkas, G.; Valergakis, G.; Kritsepi-Konstantinou, M.; Gelasakis, A.; Arsenos, G.; Kalaitzakis, E.; Panousis, N. Effects of ruminal pH and subacute ruminal acidosis on milk yield and composition of Holstein cows in different stages of lactation. *J. Hell. Vet. Med. Soc.* **2019**, *70*, 1551–1560. [CrossRef]
12. Voulgarakis, N.; Athanasiou, L.; Psalla, D.; Gougoulis, D.; Papatsiros, V.; Christodoulouopoulos, G. Ruminal Acidosis Part II: Diagnosis, Prevention and Treatment. *J. Hell. Vet. Med. Soc.* **2024**, *74*, 6329–6336. [CrossRef]
13. Enemark, J.M.D. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet. J.* **2008**, *176*, 32–43. [CrossRef]
14. Lopez-Campos, Ó.; Bodas, R.; Prieto, N.; Giraldez, F.J.; Perez, V.; Andres, S. Naringin dietary supplementation at 0.15% rates does not provide protection against sub-clinical acidosis and does not affect the responses of fattening lambs to road transportation. *Animal* **2010**, *4*, 958–964. [CrossRef] [PubMed]
15. Andrés, S.; Jaramillo, E.; Bodas, R.; Blanco, C.; Benavides, J.; Fernández, P.; González, E.P.; Frutos, J.; Belenguera, A.; López, S.; et al. Grain grinding size of cereals in complete pelleted diets for growing lambs: Effects on ruminal microbiota and fermentation. *Small Rumin. Res.* **2018**, *159*, 38–44. [CrossRef]
16. Fanning, J.P.; Hynd, P.I.; Cockcroft, P.D. The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. *Small Rumin. Res.* **2018**, *162*, 57–62. [CrossRef]
17. Chmiel, M.; Slowinski, M.; Dasiewicz, K.; Florowski, T. Application of a computer vision system to classify beef as normal or dark, firm, and dry. *J. Anim. Sci.* **2012**, *90*, 4126–4130. [CrossRef] [PubMed]
18. Silva, E.P.E.; Moraes, E.P.; Anaya, K.; Silva, Y.M.O.; Lopes, H.A.P.; Andrade Neto, J.C.; Oliveira, J.P.F.; Oliveira, J.B.; Rangel, A.H.N. Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. *PLoS ONE* **2022**, *17*, e0263714. [CrossRef]
19. Voulgarakis, N.; Gougoulis, D.A.; Psalla, D.; Papakonstantinou, G.I.; Katsoulos, P.D.; Katsoulis, K.; Angelidou-Tsifida, M.; Athanasiou, L.V.; Papatsiros, V.G.; Christodoulouopoulos, G. Can Computerized Rumen Mucosal Colorimetry Serve as an Effective Field Test for Managing Subacute Ruminal Acidosis in Feedlot Cattle? *Vet. Res. Commun.* **2024**, *48*, 475–484. [CrossRef] [PubMed]
20. Eurostat 2021. Available online: <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220517-2> (accessed on 7 July 2024).
21. Papanikolopoulou, V.; Vouraki, S.; Priskas, S.; Theodoridis, A.; Dimitriou, S.; Arsenos, G. Economic Performance of Dairy Sheep Farms in Less-Favoured Areas of Greece: A Comparative Analysis Based on Flock Size and Farming System. *Sustainability* **2023**, *15*, 1681. [CrossRef]

22. Pulina, G.; Milán, M.J.; Lavín, M.P.; Theodoridis, A.; Morin, E.; Capote, J.; Thomas, D.L.; Francesconi, A.H.D.; Caja, G. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *J. Dairy Sci.* **2017**, *101*, 6715–6729. [\[CrossRef\]](#)
23. Atkinson, O. Prevalence of subacute ruminal acidosis (SARA) on UK dairy farms. *Cattle Pract.* **2014**, *22*, 1–9.
24. Lira, M.A.A.; Simões, S.V.D.; Riet-Correa, F.; Pessoa, C.M.R.; Dantas, A.F.M.; Miranda Neto, E.G. Diseases of the digestive system of sheep and goats in the semiarid region of Brazil. *Pesqui. Vet. Bras.* **2013**, *33*, 193–198. [\[CrossRef\]](#)
25. Mialon, M.M.; Deiss, V.; Andanson, S.; Anglard, F.; Doreau, M.; Veissier, I. An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anaesthesia. *Vet. J.* **2012**, *194*, 55–59. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Morgante, M.; Stelletta, C.; Berzaghi, P.; Ganesella, M.; Andrighetto, I. Subacute rumen acidosis in lactating cows: An investigation in intensive Italian dairy herds. *J. Anim. Physiol. Anim. Nutr.* **2007**, *91*, 226–234. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Johnson, R.R.; Kubly, P.J. *Elementary Statistics*, 11th ed.; Cengage Learning: Boston, MA, USA, 2011.
28. RGB Color Detector. 2022. Available online: <https://www.the-programmer.com/> (accessed on 24 September 2021).
29. CIE. *Colourimetry: Official Recommendations of the International Commission on Illumination*; CIE Publ. No. 15.2; CIE Central Bureau: Vienna, Austria, 1986.
30. Povey, G.; Stubbings, L.; Phillips, K. *Feeding the Ewe A Manual for Consultants, Vets and Producers*, 2nd ed.; AHDB Agriculture and Horticulture Development Board Middlemarch Business Park Siskin Parkway East: Coventry, UK, 2018; pp. 1–64.
31. Tao, H.; Chang, G.; Xu, T.; Zhao, H.; Zhang, K.; Shen, X. Feeding a High Concentrate Diet Down-Regulates Expression of ACACA, LPL and SCD and Modifies Milk Composition in Lactating Goats. *PLoS ONE* **2015**, *10*, e0130525. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Zhang, H.; Peng, A.L.; Zhao, F.F.; Yu, L.H.; Wang, M.Z.; Osorio, J.S.; Wang, H.R. Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J. Dairy Sci.* **2020**, *103*, 1931–1943. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Plaizier, J.C.; Krause, D.O.; Gozho, G.N.; McBride, B.W. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet. J.* **2008**, *176*, 21–31. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Humer, E.; Aschenbach, J.R.; Neubauer, V.; Kröger, I.; Khiaosa-Ard, R.; Baumgartner, W.; Zebeli, Q. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. *J. Anim. Physiol. Anim. Nutr.* **2018**, *102*, 380–392. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Neubauer, V.; Humer, E.; Kröger, I.; Braid, T.; Wagner, M.; Zebeli, Q. Differences between pH of indwelling sensors and the pH of fluid and solid phase in the rumen of dairy cows fed varying concentrate levels. *J. Anim. Physiol. Anim. Nutr.* **2017**, *102*, 343–349. [\[CrossRef\]](#)
36. Villot, C.; Meunier, B.; Bodin, J.; Martin, C.; Silberberg, M. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal* **2017**, *12*, 481–490. [\[CrossRef\]](#)
37. Kleen, J.L.; Hooijer, G.A.; Rehage, J.; Noordhuizen, J.P. Subacute ruminal acidosis (SARA): A review. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **2003**, *50*, 406–414. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Cook, N.B.; Oetzel, G.R.; Nordlund, K.V. Clinical forum—subacute ruminal acidosis—a problem in UK dairy herds? *UK Vet.* **2005**, *10*, 40–46.
39. Lean, I.J.; Annison, F.; Bramley, E.; Browning, G.; Cusack, P.; Farquharson, B.; Little, S.; Nandapi, D. *Ruminal Acidosis—Aetiopathogenesis, Prevention and Treatment: A Review for Veterinarians and Nutritional Professionals*; Reference Advisory Group on Fermentative Acidosis of Ruminants (RAGFAR): Melbourne, Australia, 2007; pp. 1–60.
40. Giger-Reverdin, S.; Rigalma, K.; Desnoyers, M.; Sauvant, D.; Duvaux-Ponter, C. Effect of concentrate level on feeding behavior and rumen and blood parameters in dairy goats: Relationships between behavioral and physiological parameters and effect of between-animal variability. *J. Dairy Sci.* **2014**, *97*, 4367–4378. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Christodoulouopoulos, G. Maedi-Visna: Clinical review and short reference on the disease status in Mediterranean countries. *Small Rumin. Res.* **2006**, *62*, 47–53. [\[CrossRef\]](#)
42. McKenna, S.L.; Keefe, G.P.; Tiwari, A.; VanLeeuwen, J.; Barkema, H.W. Johne’s disease in Canada part II: Disease impacts, risk factors, and control programs for dairy producers. *Can. Vet. J.* **2006**, *47*, 1089–1099.
43. Alhidary, I.; Abdelrahman, M.M.; Alyemni, A.H.; Khan, R.U.; Al-Mubarak, A.H.; Albaadani, H.H. Characteristics of rumen in Naemi lamb: Morphological changes in response to altered feeding regimen. *Acta Histochem.* **2016**, *118*, 331–337. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Abdelrahman, M.M.; Alhidary, I.; Alyemni, A.H.; Khan, R.U.; Bello, A.R.; Al-Saiady, M.Y.; Amran, R.A. Effect of alfalfa hay on rumen fermentation patterns and serum biochemical profile of growing Naemi lambs with ad libitum access to total mixed rations. *Pak. J. Zool.* **2005**, *49*, 15–19. [\[CrossRef\]](#)
45. Hamada, T.; Maeda, S.; Kameoka, K. Effects of minerals on formation of colour in the rumen epithelium of kids. *J. Dairy Sci.* **1969**, *53*, 588–591. [\[CrossRef\]](#)
46. Steele, M.A.; Greenwood, S.L.; Croom, J.; McBride, B.W. An increase in dietary non-structural carbohydrates alters the structure and metabolism of the rumen epithelium in lambs. *Can. J. Anim. Sci.* **2012**, *92*, 123–130. [\[CrossRef\]](#)
47. Bo Trabi, E.; Seddik, H.E.; Xie, F.; Wang, X.; Liu, J.; Mao, S. Effect of pelleted high-grain total mixed ration on rumen morphology, epithelium-associated microbiota and gene expression of proinflammatory cytokines and tight junction proteins in Hu sheep. *Anim. Feed Sci. Technol.* **2020**, *263*, 114453. [\[CrossRef\]](#)

48. Wang, L.; Qi, W.; Mao, S.; Zhu, W.; Liu, J. Effects of whole corn high-grain diet feeding on ruminal bacterial community and epithelial gene expression related to VFA absorption and metabolism in fattening lambs. *J. Anim. Sci.* **2022**, *100*, skac056. [[CrossRef](#)] [[PubMed](#)]
49. Garcia Diaz, T.; Ferriani Branco, A.; Jacovaci, F.A.; Cabreira Jobim, C.; Bolson, D.C.; Pratti Daniel, J.L. Inclusion of live yeast and mannanoligosaccharides in high grain-based diets for sheep: Ruminal parameters, inflammatory response and rumen morphology. *PLoS ONE* **2018**, *13*, e0193313.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.