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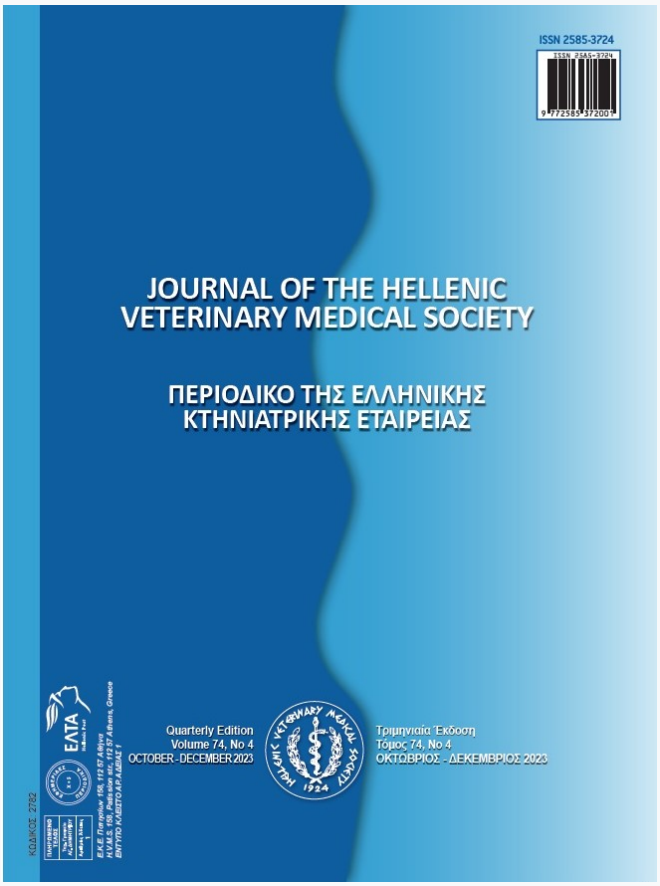

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Review article
Ανασκόπηση

Ruminal Acidosis
Part II: Diagnosis, Prevention, and Treatment

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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

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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 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 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 thecolor, 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 consistof 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 CO_2^- content (TCO_2) and bicarbonate and anincreased anion gap (Sabes et al., 2017; Snyder and Credille, 2017). The standard base excess (SBE) values are between 14 and16 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 50-150mmol/l (Nagaraja and Titgemeyer, 2007; Henning et al., 2010). On the other hand, the lactic acid concentration is increased by less than 1mM 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 from7.8 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 withSARA (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).

Ahypertonic saline solution (HSS) (7.2%, dose of 4ml/kg over a period of 10 minutes) should use to compensate fora 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% $NaHCO_3$ solution is recommended at 5L over 30 minutes for a 450kg/animal, followed by 1.3% $NaHCO_3$ infusion at a volume of 150ml/kg of body weight (BW) over 6 to 12hours. Another solution of $NaHCO_3$ (8.4%) is used nowadays for a shorter treatment at a rate of 5ml/kg BW over a time of 10 to 20 minutes. $NaHCO_3$ 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 (Pereiera 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 between5.7 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 $5x10^9$ 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 *Cinnamonum cassia* (cinnamon) present antimicrobial ability, pH buffering and anti-inflammatory response (Jaramillo-Lopez et al., 2017; Oetzel, 2017;Puvača, 2022). Particularly, a mixtureof 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 antimicrobialse.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 bya 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.

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