



THE EFFECTS OF MALIC ACID AND ITS SALTS ON RUMEN FERMENTATION, LACTATION AND FATTENING PERFORMANCE OF CATTLE

DJELOVANJE JABUČNE KISELINE I NJEZINIH SOLI NA FERMENTACIJU U BURAGU, LAKTACIJU I TOV GOVEDA

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SUMMARY

Enzymes, buffers, yeast culture, aromatic plants and organic acids are alternative feed additives added to ruminant diets in recent years. These additives are not a warranty for high production or productivity. But they can be important to improve rumen fermentation, animals' health, performance, reproduction and the quality of animal products. Organic acids could be beneficial due to the antimicrobial effect on rumen fermentation. The most common organic acids are the carboxylic acids. Dicarboxylic acids such as malic acid naturally are found in forages at different levels. They are sometimes found in their sodium, potassium, or calcium salts, or double salts. Malic acid or its salts reduce lactic acid concentration in rumen by stimulating lactate utilization by the *Selenomonas ruminantium*. It is also possible that supplementing ruminant diets with malic acid or its salts are effective in reducing the drop in ruminal pH just after feeding. Moreover, it is known that the dietary inclusion of malic acid decreases protozoa numbers and methane emissions. Some studies report that malic acid improves animals' performance and rumen ecology, yet other studies claim no effects on these criteria. The contrastive results might be due to the differences in animals, forage type, forage maturity, forage:concentrate ratio, quantity of malic acid and the chemical form added in the diets. Therefore, more in vivo research is required so as to have a final conclusion.

Key words: Malic acid, ruminant, performance, rumen fermentation

INTRODUCTION

Organic acids are organic compounds with acidic properties. Carboxylic acids, acidity of which is associated with their carboxyl group are the most common organic acids. The usage of organic acids as feed additive started to become more popular in the early 2000s due to the ban of antibiotics within the European Union. An organic acid, like malate, could be an alternative feed additive since there is no chance of developing antibiotic resistance or having harmful residues in animal products (Martin, 1998; Castillo et al., 2004). Organic acids are present in the preservative feed additive group in the list

of EU legislation, and their use is permitted in all the livestock animals. Alongside the formic and propionic acids, the most commonly used acids are citric and lactic as well as sorbic, malic, acetic and fumaric acid. Fumarate and malate are found in biological tissues as compounds of the krebs cycle (Nelson and Cox, 2000). At the same time, they can act as an antimicrobial agent. In contrast to the antimicrobial compounds, it seems to induce rather than inhibit some definitive ruminal bacterial populations (Nisbet and Martin, 1991).

Aspartate, fumarate, and malate can stimulate the growth of *Selenomonas ruminantium* (Martin, 1998). *Selenomonas ruminantium* uses lactate as

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energy and a carbon source. Malic acid induces propionate and (or) succinate production by bacterium, thereby reducing the availability of hydrogen to methanogenic bacteria (Castillo et al., 2004). Lactate intake by *Selenomonas ruminantium* is more effective in the presence of malate; thus, the latest study has emphasized the function of malate in the prevention of a reduced ruminal pH (Nisbet and Martin, 1990). Montano et al. (1999) reported that ruminal DL-lactate concentration was closely associated ($R^2 = 0.70$) with ruminal pH. The effects of malate on in vivo ruminal fermentation and ruminant performance are limited and conflicting. On the other hand, in vitro studies have shown positive effects of malic acid and malate salts on ruminal fermentation by increasing ruminal pH and propionate production (Carro and Ranilla, 2003; Gómez et al., 2005; Newbold et al., 2005; Tejido et al., 2005; Wang et al. 2009). The contrastive results might be attributed to the differences in animals, dietary factors (e.g., forage:concentrate ratio, forage type) (Castillo et al., 2004), dose of malate and chemical form in which malate was fed (Wang et al., 2009; Carrasco et al., 2012). Callaway et al. (1997) showed that the malic acid quantity of forage varied with forage type, variety, maturity, and processing. Another effect of malate is on methane emission by ruminants. Methane yield in the rumen represents a loss of energy for the ruminants together with enteric methane by host animals that contribute to the global warming. Carro and Ranilla (2003) found that the malate supplementation on different substrates (corn, barley, wheat and sorghum) increased ($p < 0.05$) the carbon dioxide (CO_2) production and decreased the production of methane except on corn.

This review discusses the effects of malic acid and its salts on methane emission, rumen fermentation, lactation and fattening performance of ruminants.

THE EFFECTS OF MALIC ACID AND SALTS ON RUMEN FERMENTATION

Malic acid has stimulated DL-lactic acid utilization in vitro, increasing pH, total volatile fatty acid (VFA) production, and propionate volume (Nisbet and Martin, 1991; 1993; 1994; Martin and Streeter, 1995). Callaway and Martin (1996) evaluated that the addition of 8 and 12 mM L-aspartate, fuma-

rate, and DL-malate to cracked corn fermentations increased pH ($p < 0.05$), tended to increase total gas production and CO_2 volume, and reduced the acetate:propionate ratio ($p < 0.05$). In this research, L-aspartate, fumarate, and DL-malate tended to decrease methane percentages and hydrogen concentration was not changed. At the end of the study DL-malate addition at 8 and 12 mM levels decreased ($p < 0.05$) lactate accumulation. In fact, disodium malate can be effective as a buffer not only due to the properties of this organic acid, but due to the supply of the sodium, which may aid in raising the pH (Castillo et al, 2004). Crespo et al. (2002) and Carro and Ranilla (2003) carried out a study to find the effects of 4, 7 and 10 mM of disodium-calcium malate on the ruminal fermentation with corn, barley, wheat, and sorghum. For all feedstocks, the rumen pH increased as malate concentration increased. In the study by Sniffen et al. (2006), addition of 100 g malic acid supplementation increased digestibility of neutral detergent fiber and acid detergent fiber (ADF) ($p < 0.05$) but did not affect the pH, total VFAs, propionic acid, butyric acid, isobutyric acid, acetic acid:propionic acid ratio. Microbial nitrogen (N) production and availability of organic matter (OM) increased ($p < 0.05$) with supplemental malic acid. Nisbet et al. (2009) did a study to determine what effects malate and fumarate may have on intestinal pathogen populations such as *Escherichia coli* and *S. enterica Typhimurium*. Pure cultures of *E. coli* and *S. enterica Typhimurium* were grown with malate and fumarate added up to 20 mM. Malate and fumarate did not inhibit ($p > 0.1$) the growth rate or final populations of these microorganisms. The final pH and the total VFA production were increased ($p < 0.05$), and the ratio of acetic to propionic acid was decreased ($p < 0.05$) by dicarboxylic acid addition.

Malic acid could reduce methane emission without negative impairing fermentation in vitro (Li et al., 2012). Mohammed et al. (2004) reported that L-malate supplementation in diets resulted in an increase ($p < 0.05$) in total gas production, proportion of propionic acid and total VFA. Ammonia-N concentration, acetic acid, hydrogen and methane production were reduced ($p < 0.05$). Researchers concluded that L-malate could be used as supplements to reduce methane production as well as to enhance rumen fermentation and animal performance. Malic acid may reduce methane emission without nega-

tive effect on ruminant performance but additional nutritional and management practices could be beneficial to reduce methane production.

MALIC ACID AND MALATE SALTS IN THE DAIRY DIETS

Although *in vitro* studies have shown positive effects of malic acid on ruminal fermentation *in vivo* studies to evaluate the effects of malic acid on lactating cow performance are limited. It is uncertain what the optimum dose of malic acid or malate salts are for dairy cows. In the study of Alferez (1978), cows fed 105 g of malic acid had higher milk yield, fat-corrected milk yield, and fat yield than cows fed 0 or 70 g malic acid. In his study, feeding malic acid above this level did not improve feed efficiency or cows' productivity. Stallcup (1979) fed Holstein cows 0, 28, or 70 g malic acid per day, and found same results. Cows fed 70 g of malic acid had higher milk yield than cows fed unsupplemented control diet. In an other trial, cows fed hay and silage based diet with 100 g supplemental malic acid had higher milk fat content and solids corrected milk than cows fed the unsupplemented diet (Stallcup, 1979). Devant and Bach (2004) found that cows fed a diet containing 84 g supplemental malate compared to cows fed a control diet had increased milk production but the same ruminal pH in the early lactation period. In a mid-lactation dairy cow study, researchers claimed malic acid addition (50 g) increased milk yield with minimum effect on milk chemical composition. In another research, malic acid supplementation did not affect percentage of fat, protein, and lactose in milk (Sniffen et al., 2006). On the contrary, Kung et al. (1982) found that malate treatment did not affect ruminal pH and VFA concentrations, milk production and composition in mid-lactation dairy cows. In 2007, Devant and his colleagues carried out a study with early lactation cows. At the end of the research, milk production, milk fat and protein percentages were not affected by malate addition. Rumen pH did not reduce despite cows being supplemented with malic acid salt took more concentrate feed (Devant et al., 2007). Khampa et al. (2006) fed dairy steers a high level of cassava chip with sodium DL-malate supplementation up to 27 g/hd/d. At the end of their study, apparent digestibility of DM, OM, crude protein (CP) and NDF was not significantly different between treatments and ADF digestibility in all the

malate treatments tended to be higher ($p > 0.05$). They found higher and more stable pH values than on the control treatment and malate could result in a decrease ($p < 0.05$) of lactate production and an increase in the production of CO_2 ($p < 0.05$). Mean total VFAs and propionate concentrations in the rumen increased with increasing levels of malate in the diet. The reduction in pH associated with increased concentrate feeding was associated with increased VFA concentrations. They concluded that the use of high level of cassava chip with supplementation of sodium DL-malate at 18 g/hd/d could be beneficial for rumen ecology and performance in ruminants. In early lactation research, Wang et al. (2009) evaluated the effects of malic acid on performance, blood metabolites and energy balance of Holstein dairy cows. Cows were fed control, supplemental 70, 140 and 210 g malic acid per day. At the end of the study, malic acid supplementation did not affect feed consumption and milk chemical composition but milk production increased ($p = 0.04$). Malic acid increased plasma glucose and serum insulin concentrations ($p < 0.01$) and decreased non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA) concentration and urine ketones ($p < 0.01$) of lactation cows. Researchers concluded that malic acid supplementation in the dairy cow diets, energy availability and nutrient digestibility may have been improved.

MALIC ACID AND MALATE SALTS IN THE FATTENING DIETS

Intensive fattening systems require high concentrate diets to assure animals' fast growth. Cereals such as barley, wheat and corn, are commonly used in intensive fattening diets. Nevertheless, feeding ruminants 80–95 % concentrate diets can result in ruminal acidosis (Krehbiel et al., 1995), decreased feed intake, impaired nutrient absorption and deteriorated animal performance (Owens et al., 1998). Acidosis also prejudices several secondary disorders such as laminitis, parakeratosis, ruminitis, and polienccephalomalacia (Underwood, 1992; Krehbiel et al., 1995; Nagaraja et al., 1998; Owens et al., 1998; Bagley, 2001; Hernandez-Bermudez, 2002). For this reason, it is very important to optimize rumen pH in high concentrate feeding fattening animals. Very low levels of malic acid supplementation (200 mg/d) did not influence rumi-

nal VFA concentrations in steers fed a 79 % high-moisture corn-based finishing diet (Kung et al., 1982). Montano et al. (1999) found that malic acid (80 g/animal/day) in high concentrate finishing diets facilitated a higher ruminal pH without harmful effects on microbial growth or ruminal nutrients (starch, fiber or protein) digestion. Malic acid in Holstein male calves (90 to 120 d old) improved average daily gain and feed efficiency (Sanson and Stallcup, 1984) and feedlot steers (Streeter et al., 1994; Liu et al., 2009). Contrary to that, Hackett et al. (1995) evaluated that, malic acid supplementation (70 g/d) did not affect the birth weight and weight gain of the calves or the body weight and condition score of the cows. In another study, it was concluded that the DL-malate supplemented finishing diets for cattle did not change fattening performance (Streeter et al., 1994; Hill et al., 1997; Martin et al., 1999; Carrasco et al. 2012). However, Carrasco et al. (2012) found that malate added to steers' diet resulted in higher ($p < 0.05$) ammonia-N concentrations than control and malic acid groups. Martin et al. (1999) found that DL-malate were effective in decreasing the drop in ruminal pH (1 to 2 h) just after feeding ($p < 0.01$). Thus, DL-malate in finishing diets could be effective in reducing acidosis. Moreover, average daily gain was increased by the DL-malate treatment, during the dietary adaptation period ($p = 0.09$). Malates' most important problem is its high cost (Martin, 1998; Carro and Ranilla, 2002; Castillo et al., 2004). Therefore, it may be more economical to add DL-malate to the diets of cattle when they arrive at the feedlot rather than adding it throughout the finishing period (Martin et al., 1999). In a beef cattle study, increasing dietary malic acid led to reduction in the dry matter intake (DMI) ($p < 0.001$) and daily methane production ($p < 0.001$). Compared with the control diet, the highest concentration of malic acid decreased daily methane emissions by 16 %, but DMI was also reduced ($p < 0.001$) which could have potentially negative effects on animal performance (Foley et al., 2009). In a fattening trial, Liu et al (2009) suggested the optimum malic acid usage was 15.6 g malic acid per kg DM in Simmental steers. In their study, the digestibility of DM, OM, NDF and ADF in the total tract also increased by increasing malic acid, and no differences in terms of CP and ether extract digestibility were found.

CONCLUSION

Malic acid and its salts supplementation could be effective to high-producing lactation cows or feedlot cattle fed high concentrate diets by increasing ruminal pH, propionic acid production, and decreasing lactic acid accumulation. The dietary factors, such as forage to concentrate ratio and forage type, are important in determinant effects of malic acid/salts addition because malic acid content in the ruminants' diet varies. It is difficult to decide the optimum dose in terms of different production conditions based on the available information. Thus, more in vivo research is required to evaluate the effects of the malic acid and malate supplementation on ruminant performance of the cows fed different diets.

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SAŽETAK

Zadnjih su godina enzimi, kulture kvasaca, aromatične biljke i organske kiseline alternativni dodaci hrani preživača. Ti dodaci nisu jamstvo za visoku proizvodnju ili proizvodnost, ali mogu biti važni za poboljšanje fermentacije u buragu, zdravlje životinja, proizvodne pokazatelje, reprodukciju i kakvoću životinjskih proizvoda. Organske kiseline mogu biti korisne zahvaljujući antimikrobnom djelovanju na fermentaciju u buragu. Najčešće organske kiseline su karboksilne kiseline. Dikarboksilne kiseline kao što je jabučna kiselina prirodno se nalaze u krmivima u različitim razinama. Katkada se nalaze u natrijevim, kalijevim ili kalcijevim solima ili dvostrukim solima. Jabučna kiselina ili njezine soli smanjuju koncentraciju mliječne kiseline u buragu stimulirajući korištenje laktata pomoću *Selenomonas ruminantium*. Isto tako je moguće da je dodavanje jabučne kiseline ili njezinih soli u hranu preživača djelotvorno u smanjenju pada pH neposredno nakon hranjenja. Osim toga, poznato je da uključenje jabučne kiseline u hranu smanjuje broj protozoa i emisije metana. Prema nekim radovima jabučna kiselina poboljšava performanse životinja i ekologiju buraga, dok prema drugim radovima nema djelovanja prema tim kriterijima. Oprečni rezultati mogu se pripisati razlikama u životinjama, vrsti krmiva, dozrelosti krmiva, omjeru krmivo:koncentrat, količini jabučne kiseline i kemijskom sastavu jabučne kiseline dodane u hranu. Stoga je potrebno dalje *in vivo* istraživanje kako bi se došlo do konačnih zaključaka.

Ključne riječi: jabučna kiselina, preživač, performanse, fermentacija u buragu