International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Organic Acids as Rumen Modifiers

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Abstract: ***Organic acids have been used as rumen modifiers in ruminants for more than three decades to reduce bacterial and fungal growth in feedstuffs and maintain their storage life. Organic acids in the rumen help to prevent the fall in ruminal pH and reducing methanogenesis. Thus, in the rumen, these acids can stimulate ruminal growth of prominent bacteria and consequently change favorably ruminal fermentation, improving ruminant performance. Acids used as feed additives are predominantly compounds that naturally occur in cell metabolism, thus they are natural products with minimum level of toxicity. Performance and health promoting effects have been demonstrated for a number of organic acids, including citric, fumaric, formic, and lactic acid and their salts. The acids showed significant effects on feed digestion and absorption and on stabilization of rumen microflora which have been demonstrated in a number of studies. The object of this paper is to review the different studies with organic acids and discuss their impact on ruminants.***

Keywords: Organic acid, Rumen modifier, Microbes, Ruminants, Fermentation

1. Introduction

The knowledge about various nutrients in the animal feed and better predicting what they will consume is essential to minimize losses from nutrient waste. The purpose of maintaining a healthy rumen will sustain a relatively constant pattern of nutrient supply to the animal which in turn should optimize efficiency of nutrient use. Currently a number of tools are available to modify rumen function in a favorable manner. Over the past few years, several studies aimed at evaluating alternative means to manipulate the gastrointestinal microflora in livestock production. In feed legislation they are registered as preservatives, but when used as feed additive in optimum levels shows positive effects on animal health and performance (Castillo *et al.*, 2004). They are enlisted as feed additives authorized by the European legislation in use for all ruminant species, because they do not leave harmful residues in animal origin products. These additives are considered to be important to promote rumen fermentation, and consequently improves animal health, performance and quality of the animal products. Organic acids in the rumen aid in preventing the drop of ruminal pH and reducing methanogenesis (Castillo *et al.*, 2004). Thus, in the rumen, these acids can stimulate ruminal growth of prominent bacteria and consequently change favorably ruminal fermentation, improving ruminant performance (Martin, 1998). Acids used as feed additives are predominantly compounds that naturally occur in cell metabolism, thus they are natural products with minimum level of toxicity (Kirchgessner and Roth, 1988). Performance and health promoting effects have been demonstrated for a number of organic acids, including citric, fumaric, formic, and lactic acid and their salts. The acids showed significant effects on feed digestion and absorption and on stabilization of rumen microflora which have been demonstrated in a number of studies. Organic acids could be beneficial due to their antimicrobial effect on rumen fermentation. They are sometimes found in their salts like sodium, potassium, or calcium salts, etc. Malic acid or its salts reduce lactic acid concentration in rumen by stimulating lactate utilization by the *Selenomonas ruminantium*. Supplementing ruminant diets with malic acid or its salts are found to be effective in reducing the drop in ruminal pH just after feeding. Moreover, it is known that the dietary inclusion of malic acid decreases protozoa population and methane emission. Some studies also reported that malic acid improves animal's health, performance and rumen ecosystem, yet other studies claim no effects on these criteria. These variations in results might be due to the differences in animals, forage type, forage maturity, roughage: concentrate ratio, quantity and chemical state of malic acid added in the diets. Hence, more *in vivo* research is required to give a concrete final conclusion.

Organic acids can stimulate the growth of the most prominent ruminal bacterium, *Selenomonas ruminantium*, can favorably alter the mixed ruminal microorganism fermentation, and improve the performance of feedlot steers (Nisbet and Martin, 1990; Callaway and Martin, 1996; Martin et al., 1999). Therefore, the aim of this review is to bring forth the different studies with organic acids and discuss their impact on dairy cattle.

2. Organic Acids

The most common organic acids are the carboxylic acids, commonly found in biological tissues. Dicarboxylic acids such as malic acid are found naturally in forages at different levels. Dicarboxylic acids like aspartate, fumarate and especially malate stimulate lactate utilization in the rumen, moderating pH. In a research based on high quality concentrate diets, malate has been found to increase total VFA production, decrease the acetate: propionate ratio and reduce methanogenesis. Results were less clear with low quality diets. Aspartate, malate and fumarate have been evaluated as feed additives because of their ability to decrease methanogenesis by 'sinking' hydrogen (H_2) during their conversion to propionate (Newbold and Rode, 2006). This theory has also been analyzed in several studies (Moss and Newbold, 2002; Wallace et al., 2007). The increased H_2 removal could also stimulate cellulolytic bacteria and increase cellulose digestion (Newbold and Wallace, 2006).

However, such acids have had inconsistent effects on animal health and performance (Martin et al., 1999). Although these organic acids have been categorized under GRAS (Generally Recognized As Safe) status, problems with palatability (Moss and Newbold, 2002), decreased ruminal pH (Asanuma et al., 1999), inconsistent responses, and high costs (Newbold and Rode, 2006), have limited their acceptance.

2.1 pka Value of Organic Acids

The effects of organic acids in the reduction of pH and their antimicrobial activity vary considerably depending on their dissociation level. The amount of dissociation depends on the pH value of the environment, and is described by the specific pK (dissociation constant) value for each acid, which defines the pH at a 50% dissociation rate. A lower the pK value, denotes the acid to be strong, which relates to its ability to drop the pH of the rumen environment. Acids used as feed additives have pK values between 3 and 5, and are categorized under intermediate strength. The physico-chemical properties of different organic acids are depicted in table 1. At a pK value of around 3, fumaric acid, citric acid and lactic acid are found to be stronger than acetic, propionic and formic acid. Water solubility is categorized as a good or very good for most acids and salts, except for fumaric acid, Ca-lactate and Ca-formate. The nutritive value differs considerably between acids, and is found to be the highest in propionic acid and lowest in formic acid. For easy handling, solid forms are preferred to liquid forms as the later are tend to be corrosive.

Table 1: Physico-chemical properties of different organic acids

Organic acids	Physical form	Solubility in water	Molecular weight (gram)	pKa value	Gross energy (Kilojoule/gram)
Acetic acid	liquid	very good	60.1	4.75	14.8
Propionic acid	liquid	very good	74.1	4.87	20.8
Butyric acid	liquid	very good	88.1	4.82	24.9
Formic acid	liquid	very good	48.0	3.75	5.8
Lactic acid	liquid	good	90.1	3.08	15.1
Citric acid	solid	good	210.1	3.14	10.3
Fumaric acid	solid	poor	116.1	3.03	11.5
Ca-formate	solid	poor	130.1	-	3.9
Na-formate	solid	very good	68.0	-	3.9
Ca-propionate	solid	good	16.6	-	16.6
Ca-lactate	solid	poor	10.2	-	10.2

2.2 Effect on Rumen

Among the different rumen modulators aspartate, fumarate and malate can stimulate the growth of *Selenomonas ruminantium* (Martin, 1998). *S. ruminantium* bacteria use lactate as a source of energy Khampa, S., and Wanapat, M. (2007). Malic acid induces propionate and succinate by this bacteria thereby preventing the availability of hydrogen (H_2) to methanogenic bacteria (Castillo et al., 2004). In the presence of malate, *S. ruminantium* effectively utilizes lactate. Nisbet and Martin (1990) observed significant effects of malate in inhibition of a reduced ruminal pH. Organic acids have been suggested to act as an electron sink for *S. ruminantium* (Nisbet and Martin, 1990;; Newbold, et al., 2005). Treatment of mixed ruminal microorganism fermentations with DLmalate yielded responses similar to those of ionophores (i.e., increased propionate, decreased methane, decreased lactate), suggesting that organic acids have an effect on electron flow (Martin et al., 1999). Ionophore effects closely associated with electron redistribution (decreased lactate, increased propionate) were enhanced by organic acid treatment (Callaway and Martin, 1996). Therefore, by providing an electron sink in the form of organic acids, the effects of monensin are enhanced in some cases. All of the studies reviewed up to now have dealt with examining the effects of organic acids on ruminal microorganism fermentation. Experiments have also been conducted to determine the effects of cellobiose and monensin on the *in vitro* fermentation of all three organic acids by mixed ruminal bacteria (Callaway and Martin, 1997). Cellobiose addition to organic acid fermentations increased the rate of organic acid utilization by the mixed bacterial population. Total VFA concentrations were increased by cellobiose addition to all fermentations. A lag period (< 4 h) occurred in monensin treated fermentations before organic acids were utilized; however, total VFA were enhanced and the acetate: propionate ratio was dropped by addition of monensin. When cellobiose and monensin were added together, propionate release and organic acid utilization were increased. Disappearance rates of organic acids and concentrations of total VFA were found to be highest, and the acetate: propionate ratio was the lowest, in incubations treated with cellobiose plus monensin. Therefore, the beneficial effects (i.e., increased total VFA and propionate concentrations) of organic acids on fermentation by mixed ruminal bacteria are apparently enhanced by the addition of cellobiose and monensin.

2.3 Effect on GIT

A two fold effect of organic acids is found in the GIT (gastro-intestinal tract). They decrease gastric and small intestine pH. The acid dissociation in the bacterial cell and accumulation of the anionic salts inhibit microbial growth. Dose response studies reveal a substantial pH reduction in feed with the most effective acid concentrations, while their salts have low impacts on the pH value (Roth and Kirchgessner, 1989). Apart from acid concentration, pH reduction in feed also depends on pK value and buffering capacity of the feed ingredients. Animal protein, extensively used in animal feed, has a more than 10 fold higher buffering capacity compared to cereal grains (Kirchgessner and Roth, 1988). There is also a wide difference in buffering capacity of mineral sources; MgO and $CaCO_3$ buffer acids to a greater extent than mineral complexed with organic salts. Inadequate drop in gastric pH inhibits pepsin activity and impairs digestion of protein. A pH below 4 is required for effective proteolytic activity, and is enhanced at lower pH values. Organic acids showed positive effects on protein hydrolysis have been mentioned in various published trials (Mroz *et al.*, 2000). Similarly at higher pH, duodenal secretion of pancreatic enzymes is decreased thus affecting the overall digestion. Dietary inclusion with organic acids leads to decreased duodenal pH, increased N retention and improved nutrient digestibility (Overland *et al.*, 2000; Kluge *et al.*, 2004). In growing animals, formic acid supplementation resulted in improved fat digestibility (Partanen *et al.*, 2001) and better nutrient retention leads to higher feed conversion rate and daily weight gain (Eidelsburger *et al.*, 2000; Hasselmann, *et al.*, 2003; Ettle *et al.*, 2004).). The inclusion of various acids, salts and their combinations showed significant impact on feed intake, daily weight gain, feed conversion rate and incidence of diarrhoea (Freitag *et al.*, 1998).

2.4 Effect on Microbes

Organic acids and their salts inhibit growth of microbes in gastrointestinal tract through pH reduction and cation-anion interaction in the microbial cell. A reduction in the growth rates of many microbes like, *Escherchia coli*, *Salmonella* species, *Clostridium* species etc. is observed below pH 5, while acid tolerant microbes remain unharmed. Due to high acidity and low pH a barrier against microbes is created ascending from the ileum and large intestine. This reduction in pH alters cell integrity and enzyme activity, thus inhibiting intra-luminal microbial growth of pathogens. Several studies have reported a reduction in bacterial cell count in the stomach (Kluge *et al*., 2004) and the duodenum (Hebeler *et al*., 2000). However *Lactobacillus* species were not affected (Hellweg *et al.*, 2006). Low concentrations of organic acids are specific for each acid (Strauss and Hayler, 2001), for example, gram positive bacteria are sensitive to long chain acids whereas gram negative bacteria are only sensitive to acids with less than eight carbon atoms (Partanen, 2001). Formic acid was found to be more effective when compared to propionic or lactic acids against the majority of bacteria (Strauss and Hayler, 2001). The overall importance of anions in for rumen performance can be demonstrated by comparison of acids and salts in different feeding trials (Kirchgessner and Roth, 1988) with formic acid and its sodium salts, where same amounts of anions were added to both groups. Daily weight gain and feed conversion ratio, were around 50% in the salt group compared with those receiving formic acid. The reason might be due to the anionic effect, whereas the higher impacts were due to the pH reduction. In contrast, reduction of gut microbe cell counts might be due to the anionic effect. An experimental study suggests that the *E. coli* and *Enterococcus* species counts can be reduced to similar levels with either formic acid or its calcium salts (Kirchgessner and Roth (1988). The data analyzed, revealed the effectiveness of anions in the regulation of gut microbe composition and the performance promoting effects of organic salts. Any change in the gut microflora causes change in the intestinal environment, resulting in reduced lactic acid and ammonia concentrations in stomach and small intestine (Hebeler *et al.*, 2000). An overall reduction in gut micro flora decreases the microbial metabolic needs and increases the absorption rate of nutrients, especially amino acids (Overland *et al*., 2000), leading to improved daily weight gain and better feed efficiency (Hasselmann *et al*., 2003; Ettle *et al*., 2004). However, sufficient amounts of anions are indispensable, as acids with low molecular weight seem to have more significant activities than those with higher molecular weights (Eidelsburger, 1997). In a recent study, feed supplementation with potassium diformate significantly enhanced dry matter content in the caecum (Hellweg *et al.*, 2006). The frequency of diarrhoea can also be reduced by inhibition of pathogens like *E. coli* to adhere to the intestinal wall. After binding to specific receptors on the gut wall most microbes can only proliferate, affect epithelial function or produce toxins. These results might be more closely related to anions instead of undissolved acids (Diebold and Eidelsburger, 2006).

2.5 Effect on Energy Metabolism

Most organic acids contribute a considerable amount of energy (table 1). Organic acids are mostly absorbed through the intestinal mucosa by passive diffusion. The short chain acids can be used for ATP generation in the citric acid cycle, for example, 1 mole (M) of fumaric acid produces 18M ATP. If ATP synthesis is compared to the energy content of 1340 kJ/M fumaric acid, it becomes evident that 74.3 kJ is required per mole of ATP, approximately the same amount of energy that is required in ATP production from glucose. Hence fumaric acid can be compared with glucose in energetic terms. It is same in case of citric acid, whereas propionic and acetic acid need 15% and 18% more energy for 1 mole of ATP production (Kirchgessner and Roth, 1988). As the gross energy of organic acids show complete involvement during metabolism, it should be taken into account in energy calculation of feed rations. For example, propionic acid contains five times more energy than wheat (Diebold and Eidelsburger, 2006). Initial research characterized the effects of aspartate, fumarate, and malate on lactate utilization by the predominant ruminal bacterium, *Selenomonas ruminantium*. Based on this pure-culture research, progress has been made in demonstrating that some of these organic acids, especially malate, alter mixed ruminal microorganism fermentation in a manner that is equivalent to that of ionophores. Malate was also found to be effective in reducing the drop in ruminal pH normally seen one to two hours after feeding a high-grain diet and hence improved cattle performance. Therefore, the finishing diets or high-producing dairy cattle diets supplemented with malate might be effective in reducing subclinical acidosis.

2.6 Effect on Milk Yield

In the tropics, due to constant exposure of dairy cattle to high temperature causes thermal stress. Thermal stress includes high body temperature, reduction in feed intake, alterations in endocrine profiles consequently leading to decreased milk production (Prasad *et al*., 2012). Hence, thermal stress is considered as a main factor which causes a drop in milk production during summer season (Prasad *et al*., 2012). Milk production can decrease between 10 to 35% during prolonged thermal stress period (St-Pierre *et al.*, 2003). Collier *et al*. (2008) reported a sudden drop in milk yield due to decrease in energy metabolism. The digestible energy utilized in dairy cows was reported to be 60% at 21°C but it decreased to 40% after 7 days exposure to 32°C. Due to heat stress, a negative correlation was found between production and temperature humidity index (Linville and Pardue, 1992). Data on milk yield of the dairy cows supplemented with different levels of organic acids solution containing 50g of lactic acid, 80g citric acid, 90g phosphoric acid, and 10g of copper sulphate per litre of distilled water (Ali *et al.,* 2013) are summarized in table 2. A significant ($P<0.05$) increase was observed in the milk yield with the use of the organic acids solution supplemented in the drinking water during high heat conditions. The higher level (1.5ml) of organic acids solution results in higher milk production. The increase in the milk yield might be due to an increase in microbial efficiency and microbial nitrogen production, and minimum methane production the rumen (Newbold *et al*., 2005; Sniffen *et al*. 2006; Khampa and Wanapat, 2007).

Table 2: Effect of supplementation of different levels of organic acids on feed intake and milk yield in dairy cows

Organic acids (ml/L of H_2O)	Feed intake (kg/day)	Milk yield (kg/day)
0	$31.3^b \pm 9.94$	$10.4^c \pm 0.99$
0.5	$35.4^a \pm 2.71$	$11.8^b \pm 0.98$
1	$36.0^a \pm 2.38$	$12.5^b \pm 0.81$
1.5	$37.3^a \pm 2.84$	$13.4^a \pm 0.30$

Values bearing different superscripts in a row differ significantly ($P<0.05$)

3. Conclusion

Organic acids and their salts can be used as rumen modifiers to improve animal health and performance. They potentially provide an alternative to the currently used antimicrobials. They stimulate, instead of inhibiting specific rumen microbes. The initial studies on the effects of dicarboxylic acids on lactate utilization by *S. ruminantium*, further led to studies on mixed culture fermentations, and limited *in vivo* studies. When compared, both *in vivo* and *in vitro* research works that have been conducted on other feed additives, little research has been made with organic acids. Hence, a detail understanding on rumen fermentation by judicious use of organic acids is needed if nutritionists and microbiologists successfully manipulate ruminal microorganism in the future experimental studies.

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