Organic Acid and Plant Botanical Supplementation in Heat-Stressed Holstein Calves

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Introduction

Dietary organic acid and plant botanical (OA/PB) supplementation represents a promising strategy to support and reduce antibiotic usage in livestock production systems. These natural compounds have unique antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, which when combined, have potential to improve gastrointestinal health by controlling bacterial pathogen growth and enhancing barrier function. Organic acid and plant botanical feeding is a common practice on swine and poultry farms; however, these additives have received minimal attention for growing and lactating ruminants. This conference proceeding aims to review the fundamental concepts related to OA/PB feeding in domestic animals considering post-absorptive metabolism and intestinal health. The composition and properties of organic acids (i.e., citric and sorbic acids) and plant botanicals (i.e., thymol and vanillin) are summarized. The effects of OA/PB supplementation on feed intake and growth performance during heat stress conditions is discussed. A recent comprehensive study at Cornell University that investigated two levels of OA/PB supplementation in weaned and heat stressed Holstein calves and its effects on growth performance is presented in support.

Definitions and functions of organic acid and plant botanicals

Citric acid: A weak organic acid and intermediary metabolite of the citric acid cycle within mitochondria. It possesses antimicrobial properties. Its mode of action is proposed to involve the reduction in bacterial intracellular pH causing damage to enzymatic activity, protein, DNA, and extracellular membranes.

Sorbic acid: A short-chain unsaturated fatty acid that exerts antimicrobial and antifungal actions by inhibiting the microbial enzymatic apparatus and uncoupling the cell's nutrient transport system.

Thymol: A natural monoterpenoid phenol that has antioxidant properties, and promotes bactericidal activity and membrane permeabilizing actions towards pathogens such as *Salmonella enterica*.

Vanillin: A phenolic aldehyde widely used to increase palatability. The compound has anti-microbial activity but also anti-inflammatory and antioxidant potential.

Dietary organic acid and plant botanical supplementation: Lessons learned from swine and poultry production

The concept of feeding acidifiers such as citric and sorbic acids has been commonplace in swine and poultry production (Sofos et al., 1985; Roth and Kirchgessner, 1998; Partanen and Mroz, 1999). These compounds have also been utilized in the food industry for their protective effects against bacteria, fungus and mold (MacDonald and Reitmeier, 2017). As a feed additive, there is an extensive body of literature demonstrating their important role in maintaining gut health in livestock species. Their benefits and applications involve improving nutrient digestibility, enhancing immune function, exerting antimicrobial effects against pathogenic bacteria, and increasing growth performance (Pearlin et al., 2020). In swine production, an important feature of organic acid supplementation is the acidification of the digestive tract, especially for suckling animals. It has been shown that diet acidification for weaned piglets with 1% citric acid caused a reduction in stomach pH from 4.6 to 3.5 (Sciopioni et al., 1978). This pH control enables piglets to maintain an optimal pH for enzymatic action in the stomach and therefore improve protein digestion (Cranwell et al., 1976). The lowering of stomach pH may also restrict the growth of pH-sensitive pathogenic bacteria like *Escherichia coli (E. coli)*, *Salmonella* spp. and *Clostridium perfrigens*. The undissociated form of acidifiers can penetrate the bacterial cell, which possesses a neutral pH, and dissociate causing a reduction in intracellular pH, while inhibiting enzymatic reactions and nutrient transport (Mroz et al., 2006).

The *in vivo* effects of dietary OA/PB supplementation (25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride matrix) on intestinal integrity and inflammation of weaned pigs was also recently investigated (Grilli et al., 2015b). Dietary OA/PB supplementation promoted greater average daily gain and body weights of study pigs. The investigation also involved the collection of ileal and jejunal tissue samples post-weaning for Ussing chamber analysis of transepithelial electrical resistance, intermittent short-circuit current, and dextran flux. Results indicated that pigs fed OA/PB at 5 g/kg of body weight tended to have reduced intermittent short-circuit current in the ileum, which suggests improved intestinal barrier. These findings were supported by increased trans-epithelial resistance in Caco-2 cells grown in the presence of OA/PB (0.2 or 1 g/L; Grilli et al., 2015b). The authors were also able to demonstrate that feeding OA/PB downregulated the ileal gene expression of inflammatory cytokines including interleukin-12 and transforming growth factor-β) in pigs. This may mean that dietary OA/PB may ensure the integrity of the intestinal barrier by minimize inflammation. In a different study, Bonetti et al. (2020) demonstrated that sorbic acid and thymol reduce the growth of *E. coli* that express K88, the etiological agent of post-weaning diarrhea in pigs. Thymol also reduced the expression levels of *E. coli* K88 virulence genes. Lastly, Emami et al. (2017) supplemented *E. coli* K88-challenged broilers with three different organic acid mixtures. Organic acid therapy improved growth performance, ileal morphology, and primary and secondary immune responses, and increased cecal lactobacilli and reduced cecal *E. coli*.

In growing broiler chickens, Hassan et al. (2010) tested a 0.06% and 0.1% dietary supplementation level of two mixtures of organic acids (blends of fumaric acid, calcium format, calcium propionate, potassium sorbate, or citric acid, calcium formate, butyrate, calcium lactate, essential oils and flavoring compounds). Independent of mixture, birds supplemented with organic acids had increased body weight gain and feed conversion ratio compared to an unsupplemented control. Both organic acid mixtures also decreased intestinal counts for *E. coli* and *Salmonella* spp. Furthermore, Smulikowska et al. (2009) demonstrated that dietary organic acid supplementation with a blend of fumaric acid, calcium formate, calcium propionate and potassium sorbite at 1 g/kg of pelleted diet increased villi height, crypt depth, and the width of the tunica muscularis of broiler chicks. These histological outcomes are postulated to improve intestinal functionality by enhancing nutrient absorption to support growth.

Heat stress in Holstein dairy calves: Dietary organic acid and plant botanical supplementation to improve resilience

One of the greatest challenges facing dairy production in the United States is heat stress. It is estimated that ~$2.3 billion in economic losses are associated with decreased performance in heat-stressed gestating and lactating cows (St-Pierre et al., 2003; Ferreira et al., 2016). Growing dairy cattle also experience impaired growth performance that contributes to these economic losses. The physiological response to heat stress is characterized by decreased feed intake, increased sweating and respiration rates, and increased body temperature (Collier et al., 1982). These changes contribute to increases in maintenance energy costs that can range from 25 to 30% (Fox and Tylutki, 1998). Another important hallmark of heat adaptation in mammals is the redirection of blood supply from the visceral organs towards the body periphery (Hall et al., 1999). This causes ATP depletion, acidosis, altered ion pump activity, and oxidative stress in the intestinal epithelium (Hall et al., 1999, 2001). The insult provokes paracellular permeability and tight junction opening (Lambert, 2009), which may promote intestinal permeability and leakage of bacteria and their endotoxin into the circulation to stimulate local and systemic immune responses (Ghosh et al., 2020).

It is important to consider that growing animals experiencing heat stress not only possess an increased maintenance energy requirement but also experience decreased feed intake (Nonaka et al., 2008; O'Brien et al., 2010; Yazdi et al., 2016). In growing cattle, it appears that the reduction in feed intake accounts for the deficit in growth that occurs during heat exposure. O'Brien et al. (2010) determined that heat-stressed Holstein bull calves experienced a 12% reduction in feed intake during heat exposure (29.4 to 40°C for a period of 9 d), which completely accounted for the decrease in average daily gain. In a similar manner, Yazdi et al. (2016) demonstrated that lowered dry matter intake during heat exposure was the driver of lowered body weights in Holstein bull calves. However, it is important to note that in these previous studies, carcass composition was not evaluated, the effects of extended heat stress were not tested, and heifer calves were not studied. Moreover, the direct effects of heat stress on physiology and metabolism within the context of growth still deserves consideration in dairy calves. Although some of the post-absorptive metabolism changes in terms of increased circulating insulin levels seem to be fairly similar between growing and lactating cattle (Rhoads et al., 2009; O'Brien et al., 2010), our understanding of the mechanisms and implications of heat-induced intestinal permeability in growing animals is still undeveloped. In addition, dietary therapies that enhance heat stress resilience in dairy calves need to be considered.

The ability of dietary citric and sorbic acids, thymol, and vanillin to enhance growth in heat-stressed calves has scientific merit. Using an *in vitro* approach, Grilli et al. (2015a) evaluated the effects dietary OA/PB supplementation on the growth of foodborne pathogens such as *E. coli* and *Salmonella typhimurium* using pure bacterial cultures as well as mixed ruminal microorganism fermentations. Several concentrations (i.e., 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0% vol/vol) of water-solubilized OA/PB were tested. The results demonstrated that 2% OA/PB inclusion (relative to total culture volume) reduced pathogen growth rates. Pathogen populations were also reduced by OA/PB. These findings suggest that dietary organic acid and plant botanical supplementation may be a means to reduce potentially harmful populations of pathogenic bacteria found in the digestive tract of young dairy cattle experience heat stress. However, the effects of dietary OA/PB on growth in young calves was not yet defined.

Therefore, our lab completed a study which evaluated the effects of dietary OA/PB supplementation on growth performance in Holstein calves challenged by heat stress. In a completely randomized design, 62 bull and heifer calves were assigned to one of five groups (n = 11-14/group): thermoneutral conditions (TN-Con), HS conditions (HS-Con), thermoneutral conditions pair-fed to HS-Con (TN-PF), HS with low-dose microencapsulated OA/PB (75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride for rumen protection; AviPlus R; Vetagro, Italy; HS-Low), or HS with high-dose microencapsulated OA/PB (150 mg/kg of body weight; AviPlus R; HS-High). Supplements were delivered as a twice daily bolus via the esophagus wk 1 through 13 of life; all calves received boluses equivalent for triglyceride. Post weaning, calves (62 ± 2 d; 91 ± 10.9 kg) remained in thermoneutral conditions (temperature-humidity index [THI]: 60 to 69) for a 7-d covariate period. Thereafter, calves remained in TN conditions or were moved to HS conditions (THI: 75 to 83) for 19 d. Clinical assessments and body weight were recorded, and blood was routinely sampled. Organs from HS-Con and TN-Con calves were harvested at trial completion. Statistical analyses were carried out using the mixed model procedure of SAS (v9.4, SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of body weight at birth, treatment, time, and their interactions as well as the random effect of calf.

Housing post-weaned Holstein calves in moderate heat stress conditions for 19 d markedly increased rectal (39.9, 39.9, 40.0 vs. 38.9 and 38.7°C; $P < 0.01$) and skin (38.7, 38.7, 38.8 vs. 32.8 and 31.8°C; $P < 0.01$) temperatures, as well as respiration rates (104, 104, 101 vs. 64 and 58; $P = 0.05$) of calves grouped in heat stress conditions (HS-Con, HS-Low, and HS-High, respectively) compared to calves housed in thermoneutrality (TN-Con and TN-PF, respectively). Exposure to high ambient temperatures significantly decreased dry matter intake of heat-stressed calves ($P < 0.01$). Calves in the HS-Con group consumed approximately 18% less feed than calves that were assigned to the TN-

Con group (*P* = 0.02). In accordance with our experimental design, TN-PF had similar dry matter intake as compared to HS-Con (*P* = 0.99). Although dry matter intake was comparable for the heat stress and pair-fed groups (HS-Con, HS-High and TN-PF), a low level of OA/PB supplementation presented an intermediate response and was similar to the observed intake of TN-Con (*P* = 0.20). Body weight was not modified by treatment; however, HS-Con and HS-Low had approximately 35% lower average daily gain, relative to TN-Con (*P* < 0.01). However, it is important to highlight that a high level of OA/PB supplementation (HS-High) during heat stress conditioning caused an intermediate response in average daily gain, which was similar to TN-Con (*P* = 0.16). Heat-stressed calves had lower small intestine (2.74 vs. 3.05 kg; *P* ≤ 0.15) and liver weights (2.74 vs. 3.11 kg; *P* < 0.05), and greater kidney weights (686 vs. 589 g; *P* < 0.10) when compared to calves maintained in thermoneutrality. We conclude that reductions in dry matter intake account for losses in growth during heat stress and dietary OA/PB supplementation enhances heat stress resilience in calves.

Summary

Dietary organic acid and plant botanical supplementation is common practice in swine and poultry production, and science now suggests that we consider the practice in young dairy cattle. The justification is the consistent ability of OA/PB feeding to enhance growth, intestinal functionality, and reduce gastrointestinal bacterial pathogens. This said, microencapsulation of OA/PB to avoid rumen degradation of these compounds is likely needed in dairy cattle to elicit benefits in the lower gut. Our findings in Holstein calves are early evidence that dietary microencapsulated OA/PB feeding is a means to partially restore feed intake and average daily gain post-weaning when challenged by heat exposure. On-going investigations are examining whether dietary OA/PB influences the gastrointestinal bacteria profile in relation to changes growth performance.

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