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Review Article
Propionibacteria in Ruminant's Diets: An Overview

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Abstract

Pionibacteria are natural inhabitants of the rumen that make up 1.4% of ruminal microflora and produce propionate, a major precursor for glucose production by hepatic gluconeogenesis. Several mechanisms have been suggested for the mode of action of direct-fed bacteria in ruminants include stimulation of desirable microbial growth in the rumen, alteration of ruminal fermentation pattern and end product formation, increasing postruminal nutrient flow, increasing nutrient digestibility and alleviation of stress through enhanced immune response. Propionibacteria have the ability to convert lactic acid and glucose to acetic and propionic acids, reduce the risk of acidosis and increase weight gain and milk production of treated animals. On the other hand, enteric CH_4 is the single largest contributing source of greenhouse gases production which causes global warming crisis. Propionibacteria also act to alter the biohydrogenation of polyunsaturated fatty acids in the rumen and increasing the generation of health-promoting fatty acids such as Conjugated Linoleic Acid (CLA). The impact of feeding of propionibacteria on the performance of the ruminant animals has been evaluated but results were inconsistent, this may be attributed to many of factors involved the used bacterial strain and its viability, bacterial inclusion level in the diet, diet composition and frequency of feeding, animal status including age, breed, health and physiological condition. In this review the focus will be on surveying impact of feeding propionibacteria on the productive performance of the ruminants including the effects on nutrients digestibility, rumen activity, blood parameters, milk yield and milk composition.

Key words: Propionibacteria, ruminants, nutrients digestibility, rumen activity, blood parameters and milk production

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INTRODUCTION

The global concerns with the antibiotics (e.x. ionophores) utilization as animal growth-promoter encourage the nutritionists to search for alternative safer feed supplements[1]. In this milieu, utilization of beneficial bacteria in ruminant diets is appearing to be a promising mean for improving feed nutrients utilization and enhance overall of animal's productivity[2-4]. There are many bacterial strains have being used as bacterial feed additives but Lactobacilli, Bifidobacteria and Propionibacterium are the most common direct fed bacteria.

Propionibacteria are found naturally in the rumen and make up 1.4% of total ruminal microflora[3]. Propionibacteria play a vital role in production of glucose (gluconeogenesis), spares glucogenic amino acids and inhibition of hepatic lipid oxidation[5]. Therefore, they have been used for improving energy metabolism for dairy animals' especially in transition period from gestation to lactation[6]. Theoretically, efficiency of propionate as a source of energy in the form of ATP represents 109% compared with glucose[7]. In addition, the efficiency of propionic acid utilization for the maintenance is 0.86 versus 0.59 for acetate and 0.76 for butyrate[7], thus, increasing ruminal synthesis of propionate may increase glucose supply and increase milk and lactose production[8,9], weight gain and feed efficiency[10,11] and decrease the incidence of acidosis and ketosis[12,13].

On the other hand, Propionibacteria as a feed additive may play an important role in reductions of methane (CH_4) production by increase competition for hydrogen through producing more propionate in the rumen[14,15]. Moreover, many of propionibacteria species have antimicrobial activity[16-18]. Therefore, there inclusion in ruminant diets may reduce CH_4 production through their antimicrobial activity and redirect fermentation toward propionate formation.

The impact of feeding of propionibacteria on the ruminant performance and methane production has been evaluated but the results were inconsistent. This may be attributed to many of factors involved the bacterial strain and its viability, bacterial inclusion level in the diet, diet composition and frequency of feeding, animal status including age, breed, health and physiological condition[3,4]. In this review the focus will be on surveying impact of propionibacteria inclusion in ruminant's diets on the animal's productive performance and methane production.

PROPIONIBACTERIA MORPHOLOGY AND ADVANTAGES

Organisms of the genus Propionibacterium are classified as a Gram-positive, slow growing, non-spore forming, non-motile, usually pleomorphic bacteria ranging in size from 0.3 to 1.3 µm in diameter and 1-10 µm in length. Fermentation products from glucose include combinations of propionic and acetic acids and frequently lesser amounts of isovaleric, formic, succinic or lactic acids and carbon dioxide[19], *Propionibacterium* spp. are acid intolerant, anaerobic to aerotolerant organisms which grow best at 30-37°C at a pH near[20] 7.0. *Propionibacteria* spp. including (*P. freudenreichii*, *P. jensenii*, *P. acidipropionici* and *P. thoenii*) are extensively used by the dairy-food industry as starter cultures for production of Swiss-type cheeses[21], commercial production of vitamin[22] B_{12}, production of antimicrobial agents such as propionic acid, propionins (antiviral peptides) and bacteriocins which used as preservatives in the food industry[23]. *Propionibacterium* strains are also employed as inoculants for silage production[24].

Propionibacteria mode of action: Direct fed Propionibacteria have been used in the ruminant's nutrition for over 20 years, primarily to improve growth performance, feed conversion and milk production efficiency[25]. They are administered to animal's diets in the form of an encapsulated bolus or mixed with the feed. Propionibacteria in the rumen act to convert substrate to propionic acid, this leads to increase propionic acid concentration in the rumen and subsequently in the blood. The increased levels of propionate in the blood lead to increased concentrations of key enzymes in the gluconeogenesis pathway such as pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver. These enzymes are involved in the gluconeogenesis pathway that converts propionate into glucose. This leads to an increase in available glucose, which may be used by the mammary gland. Within the mammary gland, alveoli secretory epithelial cells increase lactose in the lumen of the alveoli. Lactose is an osmotic regulator of milk production and causes an increase of water in the lumen and thus, an increase in milk production[26-28]. In addition, propionibacteria serve as an alternate means of hydrogen disposal to ruminal methanogenesis[25,29-31]. Its well known that, enteric methane (CH_4) is a normal product of ruminal fermentation and represents a mechanism to remove H^+ and avoid the accumulation of reduced electron carriers during fermentation[31]. However, enteric CH_4 is the single largest contributing source of greenhouse gases which lead to global warming crisis. Hence, substantial efforts are now being directed toward developing strategies to mitigate enteric CH_4 emissions. Increasing ruminal synthesis of propionate at the expense of acetate favors reduced CH_4 emissions, as propionate is a net H^+ sink in the fermentation process[32].

Recently, the *in vitro* work of Alazzeh *et al.*[29] identified the ability of *P. freudereichii strain T54* to reduce CH_4 production, beside their ability to alter the biohydrogenation of polyunsaturated fatty acids[33,34] and increasing the generation of health-promoting fatty acids such as conjugated linoleic acid (CLA). It has also been reported that several species of Propionibacteria exert antimicrobial activity and produce antimicrobial peptides that may contribute to a reduction[16-18] in CH_4.

Propionibacteria impact on feed intake and feed efficiency: Concerning with effect of Propionibacteria addition to ruminants diets and their impact on feed intake and feed efficiency, Swinney-Floyd *et al.*[10] stated that *Propionibacterium freudenreichii* (P-63) did not affect the dry matter intake (DMI) and feed efficiency in the newly weaned calves. Also, Rust *et al.*[35] reported that the combination of *Propionibacterium freudenreichii* (PF24) with three different levels of *Lactobacillus acidophilus* strains did not affect the dry matter intake in finishing cattle but the final live weight was considerably higher for all treated groups compared to the control. Galyean *et al.*[36] found that adding live cultures of *Lactobacillus acidophilus* strain 45 and (or) strain 51 plus *Propionibacterium freudenreichii* (PF-24) for growing finishing steers slightly increased daily dry matter intake by 2.4% above the control. Moreover, Huck *et al.*[37] found that heifers fed *L. acidophilus* for 28 day followed by *P. freudenreichii* showed greater gain (5.0%) and improved feed efficiency (5.1%) compared with controls. In addition, Allen[38] added the *Lactobacillus acidophilus* and *Propionibacterium freudeneichii* into the diets of finishing cattle and found that feed conversion improved by 2.4% with insignificant decreased in feed intake. But, there was a trend for feed intake to be numerically reduced by the addition of the microbial preparation. Also, Francisco *et al.*[39] reported that cows fed supplemental *Propionibacteria* (17/head) showed improved energy balance but lower daily dry matter intake at the first week of lactation, while Ghorbani *et al.*[13] found that dry matter intake was not affected by ruminally cannulated steers supplemented with *Propionibacterium* (P15) or P15 in combination with *Enterococcus faecium* EF212. However, Kim and Rust[40] found that the addition of *Propionibacteria acidipropionici* strain (DH42) at rate of 10^9 CFU/head/day to cattle fed a high concentrate diet decreased dry matter intake and average daily gain, while McPeake *et al.*[41] reported that treated steers with various combinations and concentrations of *Lactobacillus acidophilus* strains (45 and 51) and *Propionibacterium freudenreichii* (PF24) had a greater final live weight, average daily gain and dry matter intake than untreated steers. In other studies, Elam *et al.*[42] found no significant differences in dry matter intake. In contrast, Daivis[43] found that the treated heifers with mixture of *Propionibacteria* strains P169 and yeast at level of 5 g/head increased daily feed intake from 9.32 kg to 10.09 (kg/day) without significant differences in feed efficiency. Also, Raeth-Knight *et al.*[44] found that feeding dairy cattle combination of *L. acidophilus* (LA747), *L. acidophilus* strains (LA45) and *P. freudenreichii* (PF24) had no effect on DMI.

Lehloenya *et al.*[11] found that treated steers with *Propionibacterium* strain (169) strain insignificantly increased the intakes of organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF). Also, De Ondarza and Seymour[45] reported that supplementation of commercial dairy herd with *Propionibacteria freudenreichii* (P169) at level of 6×10^{10} CFU/day/cow increased the dry matter intake under summer heat and humidity condition. On the other hand, Vasconcelos *et al.*[46] found that feeding of feedlot cattle (British and British x Continental steers) with diet supplemented with 1×10^7 CFU (Low), 1×10^8 CFU (Medium) or 1×10^9 CFU (High) of *Propionibacterium freudenreichii* (strain NP 24) did not affect the final body weight or dry matter intake. While, Weiss *et al.*[47] reported that dairy cows fed *Propionibacterium* strain P169 at level of 6×1011CFU/day had lower DMI. However, Boyd[48] stated that feeding live bacterial inoculant (Bovamine®) at level of 4×10^9 CFU/h/day of a combination of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24 to mid lactating Holstein cows during hot weather lead to lower dry matter intake. In contrast, Thompson[49] reported that supplementation of Holstein cows with 10^9 CFU/g of *Lactobacillus acidophilus* and 10^9 CFU/g *Propionibacterium freudenreichii* had no effect on dry matter intake or nutrients digestibility. Also, West and Bernard[50] found no effect on dry matter intake by feeding lactating Holstein cows on bacterial inoculants (*Propionibacterium freudenreichii strain* NP24, *Lactobacillus acidophilus* strain NP51 and *L. acidophilus* strain NP45. In addition, Azzaz *et al.*[3] showed significant ($p<0.05$) increase of all nutrients digestibility coefficients by buffaloes fed rations supplemented with yeast culture+ *Propionibacterium* (P169) compared with those fed the control ration. Also, the nutritive values of the experimental rations expressed as Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) take the same trend of nutrients digestibility coefficients.

Propionibacteria impact on ruminal fermentation: The rumen is a dynamic and continuous-culture type fermentation container with a highly complex and competitive microbial ecosystem within, yet the rumen microbial ecosystem represents and facilitates a classic symbiotic association between the microbes and the host animal. Propionibacteria represent a good example for relation between the microbes and the host animal. In this concern, Kim *et al.*[51] fed *Propionibacterium acidipropionici*, strain DH42 at levels 0, 10^7, 10^8, 10^9 and 10^{10} CFU to steers fed high concentrate diet. They concluded that *P. acidipropionici* may alter ruminal metabolism toward less production of acetate and butyrate but more propionate with no effect on rumen pH, lactate or branched-chain fatty acids. In contrast, Akay and Dado[52] reported that *Propionibacterium* P5 increased the *in vitro* total VFA's, propionate, acetate, butyrate and valerate at all experimental inclusion levels (10^3, 10^6 and 10^9 CFU mL^{-1}). In this concern, Ghorbani *et al.*[13] found that steers received diet supplemented with *Propionibacterium* P15 (P15) at level of 1×10^9 CFU g^{-1} did not show any change in ruminal pH, total VFA, propionate, iso-butyrate and iso-valerate concentrations or the acetate:propionate ratio. Also, Yang *et al.*[53] found no significant effect on the *in vitro* pH, acetate, propionate, butyrate or total VFA's concentration or acetate/propionate ratio when *Propionibacterium* P15 at level of 1×10^9 CFU g^{-1} was tested. Moreover, Stein *et al.*[8] reported that Holstein cows supplemented with Propionibacteria strain 169 at level of 6×10^{11} CFU/day for 30 week postpartum showed reduction in ruminal pH and greater ruminal propionate production which leads to decrease ruminal acetate/propionate ratio. However, the molar percentage of ruminal acetate and butyrate were not affected by the treatment. In addition, Raeth-Knight *et al.*[44] stated that Holstein cows treated with *L. acidophilus* and *P. freudenreichii* did not show any change on their ruminal total VFA's and ammonia concentrations. While, Lehloenya *et al.*[11] found that feeding *Propionibacterium* strain P169 and yeast culture (XPY) increased molar proportion of propionate (by 9.7%) but decreased molar proportion of acetate and acetate: propionate ratio compared to control steers. They suggested that feeding P169 alters ruminal metabolism toward increased propionate without affecting feed intake or ruminal kinetics. Also, Weiss *et al.*[47] reported that dairy cows fed the *Propionibacterium* strain at a rate of 6×10^{11} CFU/day had lower concentrations of acetate but higher concentrations of propionate and butyrate than control. On the other hand, Thompson[49] reported that supplementation of Holstein cows with 10^9 CFU g^{-1} of *Lactobacillus acidophilus* and 10^9 CFU g^{-1} *Propionibacterium freudenreichii* had no effect on rumen kinetics, pH, acetate, propionate, butyrate and acetate/propionate ratio.

Propionibacteria impact on animal's blood metabolites: In ruminants, Francisco *et al.*[39] found that feeding dairy cows on propionibacteria 169 at level of 6×10^{10} CFU/cow during the first 12 week postpartum did not influence concentrations of glucose and cholesterol in cow's blood plasma. Similarly, Ghorbani *et al.*[13] found that steers received diet supplemented with *Propionibacterium* P15 at level of 1×10^9 CFU g^{-1} did not show any change in blood glucose. Also, Lehloenya *et al.*[54] stated that blood plasma glucose concentrations of cows received supplemented diets with *Propionibacterium* strain P169 were not changed compared to control cows. Moreover, Daivis[43] found no significant effect of Propionibacteria strains P169, P5 and yeast supplementation on plasma glucose and insulin concentrations of Angus$\times$ Hereford heifer's blood. In addition, Aleman *et al.*[9] studied the effect of feeding primiparous Holstein cows at two levels of Propionibacteria (high dose, 6×10^{11} CFU/head/day and low dose, 6×10^{10} CFU/head/day) on metabolic indicators during lactation. They found that plasma glucose levels reach 67.9 mg dL^{-1} in low-dose P169 treated cows, which represent 6-9% greater plasma glucose than high-dose P169 treated and control cows, respectively. In contrast, Weiss *et al.*[47] found that plasma concentrations of glucose and β-hydroxybutyrate (BHB) of dairy cows were not affected by P169 treatment. Similarly, Boyd[48] found no significant effect on concentration of serum glucose by feeding mid lactating Holstein cows on a combination of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24) at level of 4×10^9 CFU/head/day. Also, Thompson[49] reported that supplementation of Holstein cows with 2×10^9 CFU/day of *Lactobacillus acidophilus* and 2×10^9 CFU/day of *Propionibacterium freudenreichii* had no effect on the blood metabolites: glucose and β-hydroxybutyrate. In addition, West and Bernard[50] reported that serum glucose content was not altered by similar treatment. In addition, Azzaz *et al.*[3] showed no significant differences among buffaloes fed rations supplemented with yeast culture+*Propionibacterium* (P169) and buffaloes fed the control rations in the overall means of plasma glucose, ALT, AST, total lipids, total protein, albumin, globulin concentration and albumin/globulin ratio.

***Propionibacteria* impact on animal's milk yield and milk composition:** In lactating ruminants, Francisco *et al.*[39] found that feeding dairy cows on propionibacteria 169 at level of

6×10^{10} CFU/cow during the first 12 week postpartum did not influence daily milk yield or 4% fat corrected milk production. In addition, Stein *et al.*[8] found limited positive responses in milk yield to Propionibacteria supplementation for multiparous cows in early lactation. Similarly, Raeth-Knight *et al.*[44] found that supplementing mid lactating dairy cows with *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* had no effect on milk yield or milk components. In contrast, Lehloenya *et al.*[54] reported that daily milk and 4% FCM production for cows fed propionibacteria strain P169 (6×10^{11} CFU/head/day)+ 56 g/head of yeast were 9-16% greater than the control during mid lactation (9-30 weeks). Also, milk protein and SNF percentages and yields increased in treatments compared to control. In addition, De Ondarza and Seymour[45] stated that inclusion of propionibacteria in the diet increased ($p<0.05$) milk production, especially in early lactation and in older cows. However, the production of 3.5% fat-corrected milk and milk protein percentage were not affected by P169 supplementation. Moreover, Weiss *et al.*[47] found that cows fed the *Propionibacterium* strain P169 2 weeks before calving to 119 postpartum at rate of 6×10^{11} CFU/cow/day had comparable milk yield and composition as the control cows. Concentrations and yields of milk fat, milk protein, yield of energy corrected milk were greater ($p<0.05$) during the first week of lactation. Additionally, Boyd[48] found no significant effect on milk yield, energy corrected milk and milk fat percentage by feeding mid lactating Holstein cows with combination of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24) at level of 4×10^{9} CFU/head/day. Similarly, Thompson[49] found that supplementation of dairy cows with *Propionibacterium freudenreichii* had no significant effects on milk production, milk components or milk fatty acids profile compared to control cows. While, West and Bernard[50] found that supplementation of Holstein cows with 1×10^{9} CFU/day of *Lactobacillus acidophilus* and 2×10^{9} CFU/day of *Propionibacterium freudenreichii* increased significantly their yields of milk fat, FCM and energy-corrected milk than cows of control. Also, efficiency of milk production (defined as energy-corrected milk yield per unit of DMI) was greater for cows fed bacterial inoculants compared with control cows. However, the effects of treatment on milk fat percentage or milk protein yield or percentage were not significant. In addition, Azzaz *et al.*[3] showed that milk yield and 4% fat corrected milk (FCM) yield were significantly higher for yeast culture+*Propionibacterium* (P169) treated buffaloes compared to control. Also, the percentages and yields of milk fat, protein, lactose, Total Solids (TS) and Solid Not Fat (SNF) take the same trend of milk productivity.

CONCLUSION

It could be concluded that, propionibacteria as feed supplements can play a vital role in enhancement of ruminant's productive performance through:

- Improving energy metabolism for dairy animals' especially in transition period from gestation to lactation as Propionibacteria have important role in production of glucose (gluconeogenesis), spares glucogenic amino acids and inhibition of hepatic lipid oxidation
- Increasing ruminal synthesis of propionate which led to increase glucose supply to mammary gland and consequently increase milk and lactose production
- Increasing weight gain and feed efficiency
- Decrease the incidence of the metabolic disorders like acidosis and ketosis
- Mitigate enteric CH_4 emissions and consequently reduce the production of single largest contributing source of greenhouse gases which lead to global warming crisis

SIGNIFICANCE STATEMENT

This study discover the possibility of using propionibacteria as feed supplements for enhancement of ruminant's productive performance and environment protection through mitigate farm animal's CH_4 emissions. This study will help the ruminant animal's breeders to: (1) Use propionibacteria as alternative for harmful antibiotics (e.x. ionophores) in their animal's diets and (2) Reduce their animals feeding cost to become at the minimum and maximizing their profits.

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