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Effect of Propionibacteria Supplementation to Sheep Diets on Rumen Fermentation, Nutrients Digestibility and Blood Metabolites

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ABSTRACT

Background: This study was carried out to evaluate the effect of supplementing ruminant ration with propionibacterium P169 by using 3X3 Latin square Rahmani rams on feed intake, nutrients digestibility, rumen fermentation, blood plasma metabolites. **Methods:** Rams were divided into three groups with three successive experimental periods. Each period consisted of 21 days. Animals were fed on: rations consisting of 70% Concentrate Feed Mixture (CFM) and 30% roughage on dry matter basis as control; control ration plus 0.2 g (6×10^9 CFU head^{-1} day^{-1}, low dose) (*Propionibacterium freudenreichii* strain P169) (P169 L) and control ration plus 0.4 g (1.2×10^{10}CFU, high dose) head^{-1} day^{-1} (P169 H). **Results:** Results showed that dry matter intake, nutrients digestibility, Nitrogen balance and nutritive value were not differed ($p>0.05$) between treatments and the control. Rumen propionate increased ($p<0.05$) while, isobutyrate and acetate/propionate ratio decreased ($p<0.05$) with group treated low-dose P169. No difference ($p>0.05$) in rumen pH, ammonia concentration, Total Volatile Fatty Acids (TVFAs) and VFAs (acetate and butyrate) between treatments. Blood plasma parameters were not affected by treatments ($p>0.05$). **Conclusion:** It could be concluded that supplementing rations with propionibacterium might have a slightly effect on rumen propionate proportion.

Key words: Propionibacteria, nutrients digestibility, blood parameters

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INTRODUCTION

Propionibacteria 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[1,2], thus directly feeding propionibacteria may be a natural way to increase hepatic glucose production and positively influence metabolism[3]. The need for glucose and other energy-providing substrates increases dramatically as a dairy cow transitions from gestation to lactation. If these requirements are not met, cow health and milk production can be compromised[4]. Ruminally derived propionate is the major precursor for gluconeogenesis in early-lactation dairy cows[5], theoretical efficiency of propionate as a source of energy in the form of Adenosine triphosphate (ATP) is 109% compared with glucose[6]. The efficiency of utilization for maintenance of propionic acid is 0.86 vs. 0.59 for acetate and 0.76 for butyrate[6], thus, increasing ruminal synthesis of propionate may increase glucose supply, reduce ketosis and provide increased substrate for lactose synthesis.

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Supplemental direct-fed microbials such as propionibacteria may increase the molar proportion of ruminal propionate and reduce rumen acidosis and increase hepatic gluconeogenesis and increase weight gain when fed to ruminants[7,8,9,10]. However, Raeth-Knight *et al.*[11] did not observe any differences in ruminal pH or Volatile Fatty Acids (VFA) concentrations, or nutrient digestibility of mid-lactation cows fed diets supplemented with *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*.

Inclusion of propionibacteria 169 (P169) in the diet increased concentrations of glucose and insulin in plasma but did not influence reproduction of lactating dairy cows[10,12].

The aim of the current study is evaluating the effect of supplementing ruminant ration with propionibacterium on rams' performance (feed intake, nutrients digestibility, rumen fermentation, blood plasma metabolites).

MATERIALS AND METHODS

This study was carried out at the Agricultural Experiments Station, Faculty of Agriculture, Cairo University, in collaboration with Dairy Science

Table 1: Chemical composition of dietary ingredients (%DM) for digestion trial

Items	Diet ingredients		
	CFM	Berseem hay	Rice straw
DM	90.14	86.24	89.34
OM	89.73	88.42	85.37
Ash	10.72	11.58	14.63
CP	11.48	13.04	03.96
EE	03.40	03.10	02.95
NFE	62.37	47.56	47.45
CF	12.48	31.85	35.01
NDF	19.83	52.10	64.22
ADF	05.85	38.15	45.22

CFM: Concentrate feed mixture, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NFE: Nitrogen free extract, CF: Crude fiber, NDF: Neural detergent fiber, ADF: Acid detergent fiber

Department, National Research Centre, Dokki, Giza, during the period from November 1, 2011 to January 2, 2012.

Three mature male Rahmani Rams were randomly assigned to dietary treatments low dose, high dose of propionibacteria 169 (P169); control in a 3×3 Latin square design with 21 days periods. Periods consisted of 14 days for adaptation, followed by a 7 days collection period during which voluntary feed intake was measured and total collection of feces and urine were done. Average body weight 42 kg and aged 1.2-1.5 years. All animals were kept in individual pens.

The *propionibacteria freudenreichii* strain 169 (Dairy ProP169®) is a dried material of viable bacteria prepared by Bio-Vet Inc. USA., contains a live bacterial count: 30 Billion (3×10^{10}) Colony Forming Units (CFU) g^{-1}). The additive was kept frozen at -20°C as recommended until feeding. Live bacterial numbers of product was enumerated with specie specificity for genus *P. freudenreichii* before feeding. Direct Fed Microbial (DFM) package was analyzed for total counts of *Propionibacteria* according to Vedamuthu[13].

All rams were subject into feed intake regime which was formulated to meet nutrient requirements according to NRC[14]. Rams were fed on 20% berseem hay (*Trifolium alexandrinum*), 10% rice straw and 70% concentrate mixture consisted of 50% yellow corn, 20% soybean meal, 25% wheat bran, 2% vitamins and minerals mixture, 2% limestone and 1% common salt. Experimental rations were offered for each ram at 9.00 am. Water was available in plastic buckets at all times. The bacterial additives were top-dressed once daily at the time of feeding. Chemical analysis of dietary ingredients is present in Table 1. Supplementary doses were calculated according to the manufacturer's instructions for the first (low-dose) as 0.2 g (6×10^{9}CFU/Dairy ProP169®) and the second level (high-dose) was 0.4 g (1.2×10^{10}CFU).

Sampling procedures

Feces and urine collections: Representative samples of the voided feces and excreted urine were taken daily just after collection. Urine samples, 50 mL were stored in tight covered bottles containing sulfuric acid 1:1 for each animal and taking into consideration the real urine amount to capture NH_3-N and refrigerated at 4°C for N-determination. Fecal samples were weighed and dried at 60°C for 24 h. The dried samples of feces and feeds were ground to pass through 1 mm sieve and stored for chemical analysis. Residues of feeds if any were also recorded. Dry matter and nutrients digestibility were determined by Acid Insoluble Ash (AIA) method using 2 N HCl procedure[15].

Rumen fluid samples: Rumen fluid samples were collected from each ram at end of collection period. The samples were taken just before morning feeding, 3 and 6 h post feeding. Rumen fluid samples were collected through rubber stomach tube at the end of digestibility trial for each period. Samples of rumen fluid were strained through two layers of cheesecloth and its pH was immediately recorded after collection with Beckman pH meters. Strained Rumen Fluid (SRL) samples were acidified with 0.1 N hydrochloric acid and concentrated orthophosphoric acid and stored by freezing for determination of Total Volatile Fatty Acids (TVFA's).

Blood sampling: Blood samples were collected at the final day of the treatment period at 4 h after morning concentrate feeding at 8:00 am from the jugular vein. Blood samples were directly collected into clean dried glass culture tubes containing EDTA as anti-coagulant and centrifuged at 4000 rpm for 20 min; blood plasma was then transferred into a clean dried glass vial and kept frozen for the latter analysis.

Chemical analysis: Proximate analyses of these samples were determined of feeds and feces were analyzed according to AOAC[16]. Crude Fiber (CF), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were analyzed by Ankom[200] Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest *et al.*[17].

Plasma glucose, triglycerides and cholesterol (Stanbio Laboratory, Boerne, TX). β-Hydroxybutyrric acid, β-HBA (BEN Biochemical Enterprise, BEN S.r.l., Milan, Italy). Plasma total lipids (Biodiagnostic, Cairo, Egypt). The biochemical analyses of the selected parameters were spectrophotometrically measured (T80 UV/VIS Spectrometer, PG Instruments Ltd., UK) according to the standard protocols of the suppliers (brochures).

The pH value of rumen fluid samples was determined using pH meter. Twenty five milliliters of rumen fluid was acidified with 1 mL of 50% sulfuric acid, vortexed and freezed until analyzed for ammonia using the Kjeldahl procedure[16]. The concentration of total VFA's was determined in rumen fluid by the steam distillation method[18] using Mrkham micro distillation apparatus. Individual volatile fatty acids were analyzed by HPLC[19].

Statistical analysis: Data of body weight, dry matter intake, nutrients digestibility, rumen fermentations and blood plasma metabolites were analyzed as a Latin square. Data were analyzed using the PROC GLM procedures of SAS[20]. The differences among means were separated according to Duncan's New Multiple Range Test[21].

RESULTS AND DISCUSSION

Body weight, DMI, nutrients digestibility and nutritive value: Average body weight of Rahmni rams were increased ($p<0.05$) with low dose and high dose P169 than sheep fed control diet as shown in Table 2. Dry matter intake was similar with all experimental diets. Nutrients digestibility coefficients for DM, OM, CP, CF, EE, NFE, NDF and ADF showed insignificant ($p<0.05$) differences between treatments. This result was agreed in Ebeid[22].

The results were similar between all treatments for nutritive value of Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) are presented in Table 2.

Nitrogen balance: Effect of feeding propionibacteria (P169) on nitrogen balance is shown in Table 3. Data of nitrogen balance was not ($p<0.05$) different between all dietary groups' treatment and control diet. N-intake value was equal among dietary treatment of P169 and control diet, however N feces slightly higher in high dose P169 supplement than low dose P169 and control diet. N urine was lower ($p>0.05$) among dietary treatments than control diet. Results of N-excreted were reflected digested N insignificant differences ($p>0.05$) among dietary group of treated in P169 supplementation and sheep fed on control diet.

Results related to N-balance showed slightly higher insignificant ($p>0.05$) with low dose and high dose P169 than control diet, also data indicated insignificant difference ($p>0.05$) (27.28, 26.65 and 25.32) for low dose, high dose P169 and control diet, respectively.

Nitrogen balance ratio get the same trend observed in the results obtained by Lehloenya *et al.*[23] who reported that there was no effect of feeding P169 on N intake, duodenal N flow, or N fecal.

Table 2: Effect of propionibacteria (P169) supplementation on body weight, DMI, nutrients digestibility and nutritive value of the experimental rations for Rahmni sheep

	Propionibacteria (P169)			
Items	Control	P169 (L)	P169(H)	±SE
Average b.wt. (kg)	41.73	42.03	41.83	0.70
DMI (g h^{-1} d^{-1})	856.83	857.03	857.03	14.62
Nutrients digestibility (%)				
DM	62.82	61.10	61.43	0.51
OM	67.74	68.40	67.65	0.27
CP	61.07	61.47	60.32	0.40
CF	54.33	54.23	51.92	0.95
EE	74.70	74.46	72.06	0.77
NFE	78.23	77.95	78.29	0.47
NDF	52.20	53.43	50.88	0.85
ADF	52.62	50.85	50.18	1.46
Nutritive value (%)				
TDN	63.25	63.84	63.07	0.22
DCP	11.00	11.00	11.00	0.01

DMI: Dry matter intake, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NFE: Nitrogen free extract, CF: Crude fiber, NDF: Neural detergent fiber, ADF: Acid detergent fiber, TDN: Total digestible nutrients, DCP: Digestible crude protein

Table 3: Effect of supplementation propionibacteria (P169) on nitrogen balance

	Propionibacteria (P169)			
Item	Control	P169 (L)	P169 (H)	±SE
Nitrogen intake (g day^{-1})	15.13	15.13	15.13	1.27
Nitrogen in feces (g day^{-1})	5.64	05.63	05.76	0.15
Nitrogen in urine (g day^{-1})	5.62	05.35	05.31	0.26
Digested nitrogen (g day^{-1})	9.50	09.78	09.82	1.16
Nitrogen balance (day)	3.87	04.15	04.06	1.17
Nitrogen balance % of nitrogen intake	25.32	27.28	26.65	6.00

Table 4: Effect of supplementation propionibacteria (P169) on rumen parameters

	Propionibacteria (P169)			
Item	Control	P169 (L)	P169 (H)	±SE
pH	06.60	06.58	06.55	0.08
NH3-N (mg dL^{-1})	11.62	11.25	12.26	0.87
TVFAs	12.55	11.80	12.04	0.66
Individual volatile fatty acids (%)				
Acetate	47.93	47.69	48.65	0.41
Propionate	26.97^{ab}	28.89^{a}	26.24^{b}	0.58
Iso-butyrate	01.82	01.27	01.83	0.38
Butyrate	23.28	22.14	23.26	0.47
Acetate/propionate	01.78^{a}	01.66^{b}	01.87^{a}	0.04

Means in the same row with different letters are significantly different ($p<0.05$). NH_3-N: Ammonia nitrogen, TVFs: Total volatile fatty acids

Rumen fermentation: Table 4 is shown pH values, ammonia nitrogen (NH_3-N), Total Volatile Fatty Acids (TVFAs) concentrations and VFAs proportions in rumen fluid.

Ruminal pH was similar ($p>0.05$) among treatments. It was noticed that animals fed on P169 recorded the lowest values of pH at all sampling times versus those fed on control diet. Similar results were obtained by Ghorbani *et al.*[9], Raeth-Knight *et al.*[11], Lehloenya *et al.*[23], Thompson[24] and Yang *et al.*[25].

Ammonia-N (NH3-N) is one of the most important end products of protein breakdown in the rumen. Results in Table 4 indicated that *Propionibacteria* insignificantly ($p>0.05$) affected on rumen ammonia concentrations. However, NH3-N concentrations recorded the highest values with rams fed high-dose P169 compared with these fed on low-dose P169 and control groups at 3, 6 h after feeding.

The mean value of NH3-N followed the same trend. This may be due to digestion N was higher with group fed on high dose than low dose and control. Similar trend of NH3-N was reported by Ghorbani *et al.*[9], Raeth-Knight *et al.*[11], Lehloenya *et al.*[23] and Yang *et al.*[25]. The beneficial effect of high level of ammonia-N might be in experiment due to increasing the amount of substrate available for microbial protein synthesis in the rumen[26].

Total TVFAs concentrations and individual VFAs: Effect of feeding propionibacteria P169 on total VFA concentrations and proportions of individual VFA in the rumen are shown in Table 4. Total VFA concentration did not differ ($p>0.05$) among the low dose, high dose and control treatments. In general, increases in VFA were observed with control treatment versus supplement P169 post feeding, but no significant ($p>0.05$) changes occurred between 0, 3 and 6 h post feeding between all treatments. Ruminal acetate was not affected by supplementation P169, however, ruminal acetate trended to insignificantly ($p>0.05$) increase with animals fed high dose P169 at 3 and 6h post feeding versus low dose and control treatments. Also, the mean values for ruminal acetate increased with high dose P169 treatment than other treatments.

Ruminal propionate recorded the highest value with low dose P169 ($p<0.05$) treatment versus control and high dose P169 treatments for all times (0, 3 and 6 h), this result affected on mean values between treatments. But, there were no differences among high dose P169 and control diet.

Dietary treatments did not affect ($p>0.05$) the proportions of isobutyrate, or butyrate, however, low dose P169 recorded lower values with them compared with other treatments result of increased in ruminal propionate. While, the acetate:propionate ratio tended ($p<0.05$) to be lower for animals fed low dose P169 compared with control and high dose treatments, respectively. Also, this result is related by ruminal propionate values, because there is negative correlation between propionate and acetate propionate ratio.

These results was more accepted with reported by Lehloenya *et al.*[23] who concluded there were decreased molar proportion of acetate, increased molar proportion of propionate (by 9.7%) and tended to decrease acetate: Propionate ratio compared with control steers in feeding P169 and suggested that feeding P169 alters Ruminal metabolism toward increased propionate without affecting feed intake or ruminal kinetics. The same result was observed by Weiss and Akay[4,27]. In the same context, *P. acidipropionici* supplementation appeared altered with ruminal metabolism toward less acetate and more propionate. Butyrate concentration decreased as the dose of *P. acidipropionici* increased. They suggested that *P. acidipropionici* did effectively reduce butyrate concentration in the rumen[8].

Table 5: Effect of propionibacteria (P169) supplementation on plasma blood metabolites

	Propionibacteria (P169)			
Items	Control	P169 (L)	P169 (H)	±SE
Glucose (mg dL^{-1})	66.67	65.33	70.67	0.34
Cholesterol (mg dL^{-1})	185.20	198.40	182.20	7.10
β-hydroxy butyric acid (mmol L^{-1})	0.40	00.30	00.34	0.05
Total lipid (g L^{-1})	2.26	02.28	01.83	0.20
Triglyceride (g dL^{-1})	2.73	03.86	02.73	0.43

In contrast, *propionibacteria* P15 has no significant difference between treatments for acetate, propionate, butyrate or total VFA concentration, or acetate/propionate ratio was reported except for caproic acid which was slightly higher for the control than the DFM supplementation[11,24,25].

Blood metabolites: Data related to blood metabolites are presented in Table 5. The average values of plasma glucose were 66.67, 65.33 and 70.67 mg dL^{-1} for control, P169 (L) and P169 (H), respectively with insignificant ($p>0.05$) differences among rams fed on dietary group treatment compared with control diet.

Plasma cholesterol, total lipid and triglyceride concentrations were not ($p>0.05$) differ among dietary groups treatments and control diet, however the trend was slightly ($p>0.05$) higher with low dose P169 treatment than control and high dose P169 diet. On the other hand, plasma β-HBA concentration was lower ($p>0.05$) among dietary group treatment of P169 and sheep fed control diet without any significant differences. This result was agreed with Lehloenya *et al.*[22].

CONCLUSION

Results of the current study showed that supplementing rations with Dairy Prop P169 might have a slightly effect on rumen propionate proportion. And this topic needs more studies to complain to clarify the reasons and justifications for not getting the expected results relate to performance.

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