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## Effects of infusing milk precursors into the artery on rumen fermentation in lactating cows



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### ABSTRACT

This experiment was conducted to investigate the effects of infusing milk precursors into the external pudic artery on rumen fermentation in lactating dairy cows. Eight multiparous Holstein cows were randomly assigned to Group A (experimental group) and Group B (control group) with 4 cows each. A 2 × 4 complex factor crossover design was used. Cows in Group A were fed corn straw as the only roughage, and cows in Group B were fed mixed roughage. The experiment was divided into two periods. In the first period, cows in Group A, received treatments: 1) a basal infusate as a control (CSC); 2) a milk fat precursor infusion including C16:0, C18:0, C18:1c9, C18:2c6, C18:3n3, acetic acid (CSF); 3) a milk protein precursor infusion including 16 amino acids (CSA); 4) the mixed infusion of milk fat and protein precursors (CSFA). And meanwhile, cows in Group B were infused the basal infusate as a control group. In the second period, the cows in both Groups A and B were crossed over, which cows in Group A were named as Group B and the cows originally in Group B were in Group A. The experimental results showed that cows in experimental group had higher ruminal pH compared with the control, and ruminal pH in CSC, CSF, CSA were significantly higher than those in their respective control group ( $P < 0.05$ ). The concentration of ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) was significantly higher in CSA and CSFA compared with Group B ( $P < 0.05$ ). We also observed that the infusion of mixed amino acids significantly increased the bacterial protein (BCP) content in rumen ( $P < 0.05$ ) and influenced the rumen acetic acid concentration as well as the acetic to propionic ratio ( $P < 0.05$ ). Milk fat precursors infusion significantly affected butyric acid concentration ( $P < 0.05$ ). In addition, the content of lipopolysaccharide (LPS) in CSA was significantly higher than that in the control group ( $P < 0.05$ ). It is concluded that the milk precursors infused into external pudic artery caused feedback effects on ruminal fermentation under the corn straw roughage conditions. The milk protein precursor increased the ruminal pH, the contents of BCP and acetic acid, which adjust rumen fermentation and improve milk performance.

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### 1. Introduction

Currently, most small-scale dairy farms in China use “corn straw plus concentrate” as a ration. The result is low milk production, poor milk quality and prevalence of disease, resulting in low production efficiency. Rumen fermentation is associated with lactation performance as most of the milk precursors are derived from rumen fermentation products. Some studies proposed that supplementation with amino acids (AA) improved the yield, growth rate and synthesis efficiency of ruminal microbes in dairy cows, when the animals received sufficient carbohydrates. Meantime, fatty acids (FA) are also important for regulating rumen fermentation. For instance, the presence of polyunsaturated fatty acids

(PUFA) in the rumen is known to inhibit ruminal microbial activity and fermentation (Yang et al., 2009) and decrease fiber digestion. Fisher (1972) infused different quantities of methionine, histidine or lysine intravenously into lactating cows' vein, which changed feed intake and milk yield at different levels. Volatile fatty acids (VFA) and caseinate infused into the duodenum had little effect on milk composition, but increased milk yield and protein (Hurtaud et al., 1993). In contrast, histidine infused into the abomasum did not affect dry matter intake of grass silage, rumen fermentation or diet digestibility (Vanhatalo et al., 1999). Free long-chain FA infused into the abomasum of lactating dairy cows affected feed intake, reduced production and changed the composition of milk, nutrient digestibility, and metabolites in blood (Drackley et al., 1992). However, hemoperfusion with milk precursors is relatively infrequent in the research of lactating cow nutrition. The aim of this research was to further study the effects of rumen fermentation in lactating cows with external pudic artery infusion of milk composition precursors, and to provide the basis for the relationship between breast intake and rumen fermentation.

## 2. Materials and methods

### 2.1. Experiment design

A 2 × 4 crossover design was used and eight cows were randomly divided into Groups A and B with 4 cows in each group. The experiment was divided into 2 periods. The first period consisted of a 14-d preliminary period followed by a 3-d adaptation period and a 9-d infusion period. The second period included a 7-d layoff period, a 3-d adaptation period and a 9-d infusion period. In the first period, cows of Group A, as the experimental group, received treatments: 1) a basal infusate as a control (CSC); 2) milk fat precursor infusion (CSF); 3) milk protein precursor infusion (CSA); 4) the mixed infusion of milk fat and protein precursors (CSFA). And meanwhile, cows of Group B were infused the basal infusate as the control group. In the second period, the cows of Groups A and B were crossed-over. The order of infusion was the same as the first period. The experimental design is given in Table 1.

### 2.2. Experimental animals and feeding management

The experiment was conducted at Bing Zhou Hai Dairy Company in Hohhot in September 2013. Eight multiparous Chinese Holstein cows in mid-lactation with an average milk yield of 25 ± 3 kg and similar body weight were used. The cows were fed total mixed ration (TMR) twice daily at 06:00 and 18:00 and free drinking. Cows were milked at the time of ingestion and milk production was recorded at each milking. The intra-arterial

**Table 1**  
Experimental design and grouping.

Item	Animal group	Forage	Preliminary period (14 d)	Adaptation period (3 d)	Infusion period		
					3 d	3 d	3 d
The first period							
Experimental group	A	CS	—	C	CSF	CSA	CSFA
Control group	B	MF	—	C	C	C	C
The second period							
Experimental group	B	CS	—	C	CSF	CSA	CSFA
Control group	A	MF	—	C	C	C	C

CS = corn straw; MF = mixed forage; C = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

cannula was fitted on external pudic artery at 1 wk before infusion period.

### 2.3. Experimental diet

The diets of experimental and control groups were corn straw (CS) and mixed forage (MF), respectively. The diet of corn straw had single corn straw and concentrate whereas the diet of the mixed forage was constituted with alfalfa, Chinese wildrye, whole corn silage and concentrate. The dietary concentrate to roughage ratio was 45:55. The composition and nutrient levels of diets are given in Table 2.

### 2.4. Infusate preparation

The dosage of FA and AA infusate was the FA and AA intake of control group minus the intake of experimental group. Each cow received 2 L of infusate over 6 h daily after morning feeding.

Emulsions were prepared according to the methods of Stamey Lanier et al. (2013). The infusate of AA as well as the mixed infusate of milk fat and protein precursors were prepared using the method of Shi (2012). Soy lecithin was used as a sham infusate for the control group in the adaptation period and infusion period.

Soy lecithin was added to saline solution as a carrier and was heated to approximately 70°C. Different FA were then added to the solution and stirred homogeneously by an immersion blender (model EUROSTAR 60, IKA, Germany). Subsequently, the coarse emulsions were homogenized into stable emulsions with a homogenizer (model SRH 60–70, Shanghai Samro Homogenizer Co. Ltd., Shanghai, China) 3 times at 17.2 MPa at the first stage and 3.5 MPa at the second stage. The emulsions were autoclaved for 10 min at 121°C and finally the solution was sealed for storage.

Different AA was added to the distilled water heated to 90°C and the mixture was blended with soy lecithin solution. The mixture was then passed twice through the homogenizer at the same stage. The methods of high pressure sterilization and storage are the same as before.

**Table 2**  
Composition and nutrient levels of diets (DM basis).

Item	Experimental group	Control group
Ingredient, % of DM		
Chinese wildrye		3.70
Whole corn silage		26.70
Imported alfalfa		23.40
Corn straw	53.80	
Corn	24.60	24.60
Soybean meal	14.80	14.80
Whole cottonseed	5.10	5.10
CaHPO <sub>4</sub>	0.60	0.60
NaCl	0.50	0.50
Dairy premix <sup>1</sup>	0.60	0.60
Total	100.00	100.00
Nutrient levels, %		
CP	13.61	18.14
EE	2.84	3.97
NDF	44.30	32.30
ADF	29.10	21.30
Starch	15.32	21.50
NEL, MJ/kg	1.04	1.57

CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; NEL = net energy for lactating.

<sup>1</sup> The premix provided per kg of diets: VA > 700,000 IU, VD<sub>3</sub> > 120,000 IU, VE > 2,100 mg, Fe 1,750 mg, Cu 1,600 mg, Zn 10,000 mg, Mn 3,500 mg, Se 42 mg, I 84 mg, Co 42 mg.

The mixed infusate had half volumes of milk fat and protein precursors following the same methods.

The infusate was continuously pumped into the external pudic artery by using peristaltic pumps (model BT100-1L, Longer Pump Co. Ltd., Baoding, China). The actual amounts of infusion were recorded each day. The placement of the infusion line was checked daily before infusion to ensure that there was no leakage.

The composition of infusate is given in Table 3.

### 2.5. Sampling and pretreatment

Rumen fluid samples were collected using the oral cavity collector at 1200 post-feeding on the last day of each infusion period from each cow. Rumen fluid was transferred into the centrifuge tube together with the enzyme for detecting lipopolysaccharide (LPS) in a germ-free environment. Rumen fluid was strained through 4 layers of Dacron mesh and pH of the filtrate was determined by a portable pH temperature meter (model P4-036, Yueqing Dacang Electronics Co. Ltd.). Ten milliliters of filtrate were centrifuged at  $12,000 \times g$  and  $4^\circ\text{C}$  for 10 min. A 0.5 mL supernatant was added into 9.5 mL of 0.2 N hydrochloric acid (HCl) for  $\text{NH}_3\text{-N}$  and another 4 mL supernatant were added to 1 mL of metaphosphoric acid (25%) for VFA measurement. The rest of the supernatant was stored at  $-20^\circ\text{C}$  for determining bacterial protein (BCP).

### 2.6. Laboratory analyses

Rumen pH of the filtrate was determined using a portable pH temperature meter. Ammonia nitrogen was measured using theindophenol blue colorimetric method as described by Wang (2011). Bacterial protein was determined using the Coomassie brilliant blue method (Cone et al., 1997). The VFA contents of rumen fluid samples were quantified using a gas chromatographic method (Filipek and Rudolf, 2009). Then Limulus reagent dynamic development process used to determine the LPS and pretreatment employed the method described by Gozho et al. (2005).

### 2.7. Statistical analysis

Data were analyzed using PROC MIXED of SAS 9.1(SAS Institute Inc., Cary, NC). Results are reported as least square means  $\pm$  standard errors of the mean. Significance was declared at  $P < 0.05$ .

**Table 3**  
The composition of infusate.

Carrier	Content, g/L	Fatty acids infusate	Content, %	Amino acids infusate	Content, g/d·cow
Soy lecithin	6	C16:0	6.50	Asp	0.46
		C18:0	3.34	Gly	11.09
		C18:1c9	7.74	Ser	8.94
		C18:2c6	46.44	Ala	17.52
		C18:3n3	5.57	Cys	2.12
		Acetic acid	30.21	Val	27.06
		Met	1.97	Thr	18.50
		Lys	9.49	His	5.22
		Arg	5.57	Tyr	3.68
		Glu	16.97	Ile	8.42
		Leu	9.64	Phe	4.73

## 3. Results

### 3.1. The effects of infusion of different milk composition precursors on rumen fermentation

The pH value was found to be in the normal range (Table 4). The pH in CSC, CSF and CSA of experimental group were significantly different compared with controls ( $P < 0.05$ ).

As shown in Table 5, the concentrations of  $\text{NH}_3\text{-N}$  in CSF and CSFA of experimental group were significantly higher than those of the controls ( $P < 0.05$ ). But the concentration of  $\text{NH}_3\text{-N}$  in CSA of experimental group was lower than that in its control group.

It was showed in Table 6 that the concentration of BCP in CSA of experimental group was the highest and was significantly higher than that in the control group ( $P < 0.05$ ). The ruminal BCP content in CSF of experimental group was higher than that in the control group.

### 3.2. The effects of infusion of different milk composition precursors on ruminal VFA

The concentration of acetic acid was significantly higher than that in the control group when cows were infused with a milk protein precursor ( $P < 0.05$ ) and tended to be higher when they were infused with a milk fat precursor (Table 7).

Table 8 shows that the concentration of propionic acid was lower than that in controls except for CSC, which tended to have decreased CSA compared with the control group.

The variation tendency of the concentration of butyric acid was the same as propionic acid. But the concentration of butyric acid in CSF of experimental group was significantly lower than that in control group ( $P < 0.05$ ) (Table 9).

From Table 10 it can be seen that the concentration of VFA in CSA of experimental group tended to be higher than that in the control group, and other groups had no significant differences.

The acetic acid to propionic acid ratio was significant higher than that in the control group when cows were infused with AA ( $P < 0.05$ ) (Table 11).

### 3.3. The effects of infusion of different milk composition precursors on ruminal LPS

The LPS concentration in CSA of the experimental group was significantly higher than that of the control group ( $P < 0.05$ ) (Table 12).

## 4. Discussion

### 4.1. The effects of infusion of different milk composition precursors on rumen pH

The ruminal pH depends upon the  $\text{HN}_3\text{-N}$  and total VFA concentration resulting from rumen fermentation (Wang et al., 2013).

**Table 4**  
Effects of infusion of milk precursors into external pudic artery on rumen pH in lactating dairy cows.

Treatments	Experimental group	Control group	SEM	P-value
CSC	6.81	6.45	0.078	0.01
CSF	6.67	6.39	0.078	0.01
CSA	6.99	6.72	0.075	0.02
CSFA	6.90	6.81	0.09	0.47

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 5**Effects of infusion of milk precursors into external pudic artery on rumen  $\text{NH}_3\text{-N}$  concentration in lactating dairy cows (mg/dL).

Treatments	Experimental group	Control group	SEM	P-value
CSC	9.22	8.32	1.11	0.48
CSF	10.46	7.81	1.10	0.03
CSA	6.74	8.37	1.13	0.21
CSFA	8.78	5.26	1.20	0.02

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 6**

Effects of infusion of milk precursors into external pudic artery on rumen bacterial protein (BCP) concentration in lactating dairy cows (mg/dL).

Treatments	Experimental group	Control group	SEM	P-value
CSC	23.77	19.98	3.40	0.49
CSF	23.46	13.66	3.20	0.07
CSA	33.06	20.99	3.40	0.04
CSFA	22.89	21.92	3.60	0.88

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 7**

Effects of infusion of milk precursors into external pudic artery on rumen acetic acid concentration in lactating dairy cows (%).

Treatments	Experimental group	Control group	SEM	P-value
CSC	67.00	67.65	0.45	0.53
CSF	67.22	65.94	0.45	0.12
CSA	68.60	65.39	0.50	0.01
CSFA	67.85	67.62	0.55	0.85

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 8**

Effects of infusion of milk precursors into external pudic artery on rumen propionic acid concentration in lactating dairy cows (%).

Treatments	Experimental group	Control group	SEM	P-value
CSC	19.51	18.93	0.39	0.31
CSF	18.60	18.64	0.39	0.95
CSA	18.25	19.43	0.43	0.06
CSFA	18.02	18.09	0.45	0.91

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 9**

Effects of infusion of milk precursors into external pudic artery on rumen butyric acid concentration in lactating dairy cows (%).

Treatments	Experimental group	Control group	SEM	P-value
CSC	9.93	9.87	0.56	0.94
CSF	10.22	11.81	0.56	0.04
CSA	9.66	11.09	0.61	0.09
CSFA	10.27	10.46	0.65	0.83

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

Though the pH was affected by infusion, these data were still within the normal range of 6 to 7, indicating that rumen fermentation was normal. Shen et al. (2012) considered that the pH of rumen fluid collected directly from the mouth of a cow was higher than the value in the rumen itself.

**Table 10**

Effects of infusion of milk precursors into external pudic artery on rumen total volatile fatty acid concentration in lactating dairy cows (%).

Treatments	Experimental group	Control group	SEM	P-value
CSC	96.42	96.41	0.23	0.99
CSF	96.04	96.39	0.23	0.28
CSA	96.51	95.50	0.25	0.08
CSFA	96.13	96.17	0.25	0.90

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 11**

Effects of infusion of milk precursors into external pudic artery on rumen acetic to propionic ratio in lactating dairy cows.

Treatments	Experimental group	Control group	SEM	P-value
CSC	3.44	3.58	0.09	0.31
CSF	3.62	3.54	0.09	0.59
CSA	3.76	3.39	0.10	0.01
CSFA	3.76	3.74	0.10	0.90

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

**Table 12**

Effects of infusion of milk precursors into external pudic artery on rumen lipopolysaccharide (LPS) in lactating dairy cows (EU/mL).

Treatments	Experimental group	Control group	SEM	P-value
CSC	19,291	14,688	2,530	0.19
CSF	21,547	16,841	2,525	0.18
CSA	19,127	10,212	2,610	0.02
CSFA	19,964	13,758	2,930	0.14

SEM = standard errors of the mean; CSC = carrier infusion; CSF = milk fat precursor infusion; CSA = milk protein precursor infusion; CSFA = the mixed infusion of milk fat and protein precursors.

The dietary concentrate to roughage ratio was 46 :54. Volatile fatty acid production per unit of mass decreased correspondingly because of a lower ratio of concentrate. At the same time, corn straw has a high content of crude fiber, thus it may have made cows to chew and ruminate longer and secrete more saliva than usual. The phosphate and bicarbonate in the saliva could neutralize acidic material to increase pH value. The pH value changed slightly compared with other infusion treatments, which did not affect pH through infusion. Vanhatalo et al. (1999) found the ruminal pH was higher than that in the control group without a significant difference with abomasal infusion of histidine and methionine. However, pH value throughout the gut fluctuates depending on the nutrient level and composition of diets.

#### 4.2. The effects of infusion of different milk composition precursors on rumen $\text{NH}_3\text{-N}$

The concentration of  $\text{NH}_3\text{-N}$  in the ruminal liquid is associated with dietary protein degradation in rumen, which is then utilized by the rumen microbes. In the present study, corn straw had a low content of nonprotein nitrogen and soluble protein. In addition, the degradation rate is low, contributing less to ruminal ammonia concentrations.

Ivan et al. (2001) suggested that oilseeds increase the efficiency of dietary protein utilization, and release  $\text{NH}_3\text{-N}$  into the rumen which is in an undulation state (Atasoglu et al., 1998). Indeed, in the current study, diets supplemented with FA yielded a high concentration of  $\text{NH}_3\text{-N}$ . However, high levels of unsaturated fatty acids in



the rumen led to adverse effects on ruminal fermentation, and hence total tract fiber digestion (Doreau and Chilliard, 1997). This resulted in NH<sub>3</sub> accumulation due to decreased efficiency of protein synthesis.

In the current study, the concentration of NH<sub>3</sub>-N was the lowest with cows infused with AA presumably because there was sufficient nitrogen for urea synthesis. Therefore, more ammonia enters into the rumen with saliva, which is enough to satisfy the synthesis demand of protein and improve the activity of microbes to produce BCP, which was present at the highest concentration in CSA group. Another possibility is the absorption of ammonia from deaminated AA and the nitrogen lost from urinary purines could be counterbalanced by utilization and recycling of urea and other nitrogen compounds transported from blood to the rumen (Reynolds and Kristensen, 2008). The ruminant digestive system can adjust dietary nitrogen intake based on the amount of recycled nitrogen to the rumen and replace dietary nitrogen with recycled nitrogen (Kristensen et al., 2010). Wickersham et al. (2008) reported that cattle decreased their dietary nitrogen intake by changing the proportion of endogenous urea recycled into the gut when compared with the proportion excreted in urine.

#### 4.3. The effects of infusion of different milk composition precursors on rumen BCP concentration

The available energy and appropriate protein content in the rumen are beneficial to the growth and reproduction of rumen microbes, which capture and use nitrogen to produce microbial protein. The concentration of ammonia nitrogen is the major nitrogen source for BCP synthesis.

Lipid inhibits the activity of microorganisms (Wanapat et al., 2011). Demeyer and Henderickx (1967) found C<sub>18</sub> fatty acid, especially PUFA, is toxic to ciliates that are believed to be more sensitive than bacteria. High levels of fat infused into blood may decrease microbial activity, resulting in decreased synthesis of BCP. However, Pantoja et al. (1994) reported that hydrogenation of unsaturated fatty acids could protect microbes. The BCP yield increased with adding FA into the rumen and with increased unsaturation of fat (Denman and McSweeney, 2006).

The concentration of BCP is the highest after infusing milk protein precursors in our study. The highest TVFA concentration with infusing mixed AA provided more energy and carbon-skeleton. The energy and carbon-skeleton were beneficial for large scale synthesis of BCP. Amino acids were needed by most microbe growth. When there are plenty of degradable carbohydrates in the rumen, supplying AA could increase microbial growth and synthesis ratio. Kajikawa et al. (2002) thought that mixed AA had better domino effect to improve microbial growth. Socha et al. (2005) reported that 25% of the effective nitrogen content was from AA, thus the growth of microbes was at the best level. Methionine and methionine hydroxy analogue (MHA) could accelerate microbial growth and reproduction, and therefore improve synthesis BCP.

#### 4.4. The effects of infusion of different milk composition precursors on rumen VFA concentration

Volatile fatty acids are important products of carbohydrate fermentation by the rumen (Wang et al., 2013). Volatile fatty acids are produced in the rumen account for about two-thirds of carbon flow into body metabolism, which supplies 70% to 80% of the energy to ruminants (Van Houtert, 1996). This is also vital for the synthesis of milk fat and protein.

In the current study, FA infusion had no effect on the content of VFA. This agrees with the findings of Polviset et al. (2014) who reported that the molar proportions of acetic, propionic and butyric

acid were not affected by the source of fat. A similar result was reported by Dayani et al. (2007) and Wongnen et al. (2009). On the contrary, VFA production was decreased by long-chain fatty acids which were unsaturated and contained less than 18 carbon atoms; however, stearic acid did not affect VFA production or acetate to propionate ratio *in vitro* (Chalupa et al., 1984).

Rumen bacteria require an adequate supply of AA from rumen fermentation to achieve optimal growth. The growth of bacteria that can degrade structural carbohydrates rely on HN<sub>3</sub> whereas that of organisms can degrade nonstructural carbohydrates depend on available AA. Argyle and Baldwin (1989) reported the amount of AA has a linear relationship with the growth of bacteria, but linear relationship was reduced with the increasing AA quantity. Branched chain-AA modulate protein metabolism and isoacids, the degradation products of branched chain-AA, influence the growth of cellulolytic bacteria and improve the digestion of fiber. The results showed that the concentration of TVFA was significantly higher than that in the control group after infusing AA, which illustrated that the activity of cellulolytic bacteria enhanced obviously. Different proportions of AA affect the yield of VFA, may be due to the antagonism and anoxaction effect of AA on the degradation of microbes, or due to microbes have different mechanisms of absorption and utilization to each AA. Thus, there were different ratios of acid production.

#### 4.5. The effects of infusion of different milk composition precursors on rumen LPS yield

Endotoxin is a kind of LPS and trace protein complexes on the outer membrane of cell walls of gram-negative bacteria. Lipopolysaccharide has a broad range of biological activity when released after bacterial death or disassembly (Wang, 2006).

The LPS yield did not exceed 30,000 EU/mL in the infusion groups as well as the control group according to some studies (Khafipour et al., 2008; Zebeli and Ameta, 2009; Li et al., 2011; Zhou et al., 2014). In the current study, the amounts of LPS in each infusion treatment were higher than that in the control group. The LPS content in CSA group was significantly higher than that in the control group, but there was no difference among infusion treatments. The death of gram-negative bacteria leads to the release of LPS. When cows have good metabolic balance and enough energy supply, they have enhanced rumen digestion performance, which hastens microbial life cycle (Tajima et al., 2001). This may, in turn, increases the rate of LPS entering the intestinal tract without accumulating in the rumen, because of faster chyme circulation. Amino acids play essential nutritional roles in rumen microbial metabolism and hence level of AA in the rumen has a linear relationship with the growth of bacteria (Argyle and Baldwin, 1989).

## 5. Conclusion

Infusing milk precursors into external pudic artery of cows fed diets with corn straw as roughage has feedback effects on rumen fermentation. Infusing with AA – the milk protein precursor, increased pH, elevated the concentrations of BCP, and lifted the content of acetic acid, and regulated rumen fermentation. The end result was improved milk production in lactating dairy cows.

## References

- Argyle JL, Baldwin RL. Effects of amino acids and peptides on rumen microbial growth yields. *J Dairy Sci* 1989;72:2017–27.
- Atasoglu C, Valdes C, Walker ND, Newbold CJ, Wallace RJ. De novo synthesis of amino acids by the ruminal bacteria *Prevotella bryantii* B14, *Selenomonas ruminantium* HD4, and *Streptococcus bovis* ES1. *Appl Environ Microbiol* 1998;64:2836–43.

- Chalupa W, Rickabaugh B, Kronfeld DS, Sklan D. Rumen fermentation in vitro as influenced by long chain fatty acids. *J Dairy Sci* 1984;67:1439–44.
- Cone JW, Gelder AHV, Driehuis F. Description of gas production profiles with a three-phasic model. *Animal Feed Sci And Technol* 1997;66:31–45.
- Dayani O, Ghorbani GR, Alikhani M, Rahmani HR, Mir PS. Effects of dietary whole cottonseed and crude protein level on rumen protozoa population and fermentation parameters. *Small Rumin Res* 2007;69:36–45.
- Demeyer DI, Henderickx HK. The effect of C18 unsaturated fatty acids of methane production in vitro by mixed rumen bacteria. *Biochem Biophys Acta* 1967;137:484–97.
- Denman SE, McSweeney CS. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiol Ecol* 2006;58:572–82.
- Doreau M, Chilliard Y. Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. *Rep Nutr Dev* 1997;37:113–24.
- Drackley JK, Klusmeyer TH, Trusk AM, Clark JH. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. *J Dairy Sci* 1992;75:1517–26.
- Filipek Jaroslav, Rudolf Dvořák. Determination of the volatile fatty acid content in the rumen liquid: comparison of Gas chromatography and capillary isotachopheresis. *Acta Vet Brno* 2009;78:627–33.
- Fisher LJ. Response of lactating cows to the intravenous infusion of amino acids. *J Anim Sci* 1972;52:377–84.
- Gozho GN, Plaizier JC, Krause DO, Kennedy AD, Wittenberg KM. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J Dairy Sci* 2005;88:1399–403.
- Hurtaud C, Rulquin H, Verit R. Effect of infused volatile fatty acids and caseinate on milk composition and coagulation in dairy cows. *J Dairy Sci* 1993;76:3011–20.
- Ivan M, Mir PS, Koenig KM, Rode LM, Neill L, Entz T, et al. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. *Small Rumin Res* 2001;41:215–27.
- Kajikawa H, Mitsumori M, Ohmomo S. Stimulatory and inhibitory effects of protein amino acids on growth rate and efficiency of mixed ruminal bacteria. *J Dairy Sci* 2002;85:2015–22.
- Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J Dairy Sci* 2008;92:1060–70.
- Kristensen NB, Storm AC, Larsen M. Effect of dietary nitrogen content and intravenous urea infusion on ruminal and portal-drained visceral extraction of arterial urea in lactating Holstein cows. *J Dairy Sci* 2010;93:2670–83.
- Lanier JS, Suagee JK, Becvar O, Corl BA. Mammary uptake of fatty acids supplied by intravenous triacylglycerol infusion to lactating dairy cows. *Lipids* 2013;48:469–79.
- Li S, Khafipour E, Krause DO, Kroeger A, Rodriguez-Lecompte JC, Gozho GN, et al. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J Dairy Sci* 2011;95:294–303.
- Pantoja J, Firkins JL, Eastridge ML, Hull BL. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. *J Dairy Sci* 1994;77:2341–56.
- Polviset W, Wachiraprakorn C, Yuangklang C. Effect of fat sources on digestibility and rumen fermentation in crossbred Thai native x Brahman bulls. *Indian J Anim Res* 2014;48:14–20.
- Reynolds CK, Kristensen NB. Nitrogen recycling through the gut and the nitrogen economy of ruminants—An asynchronous symbiosis. *J Anim Sci* 2008;86:E293–305.
- Shen JS, Chai Z, Song LJ, Liu JX, Wu YM. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. *J Dairy Sci* 2012;95:5978–84.
- Shi XL. Production process optimization of compound amino acid injection (18AA-II) (not PVC). Shan Dong University; 2012.
- Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab CG, et al. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen protected methionine and lysine. *J Dairy Sci* 2005;88:1113–26.
- Tajima K, Aminov RI, Nagamine T, Matsui H, Nakamura M, Benno Y. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl Environ Microbiol* 2001;67:2766–74.
- Van Houtert Martin. Challenging the retinal for altering VFA ratios in growing ruminates. *Feed Mix* 1996;41:514–25.
- Vanhatalo A, Huhtanen P, Toivonen V, Varvikko T. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *J Dairy Sci* 1999;82:2674–85.
- Wanapat M, Mapato C, Pilajun R, Toburan W. Effects of vegetable oil supplementation on feed intake, rumen fermentation, growth performance, and carcass characteristic of growing swamp buffaloes. *Live Sci* 2011;135:32–7.
- Wang JC. The role of endoxemia concentration on the pathogenesis of severe hepatitis. *Parasitoses Infect Dis* 2006;4:40–1.
- Wang JQ. Ruminant nutrition research methods. 2011.
- Wang RL, Liang JG, Lu L, Zhang LY. Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. *Biol Trace Elem Res* 2013;152:16–24.
- Wickersham TA, Titgemeyer EC, Cochran RC, Wickersham EE, Gnad DP. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *J Anim Sci* 2008;86:3079–88.
- Wongnen C, Wachiraprakorn C, Patipan C, Panpong D, Kongweha K, Namsaen N, et al. Effects of fermented total mixed ration and cracked cottonseed on milk yield and milk composition in dairy cows. *Asian-Aust. J Anim Sci* 2009;12:1625–32.
- Yang SL, Bu DP, Wang JQ, Hu ZY, Li D, Wei HY, et al. Soybean oil and linseed oil supplementation affect profiles of ruminal microorganisms in dairy cows. *Animal* 2009;3:1562–9.
- Zebeli Q, Ameta BN. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. *J Dairy Sci* 2009;92:3800–9.
- Zhou J, Dong GZ, Ao CJ. Feeding a high-concentrate corn straw diet increased the release of endotoxin in the rumen and pro-inflammatory cytokines in the mammary gland of dairy cows. *BMC Veterinary Res* 2014;10:172.