Dynamics of methanogenesis, ruminal fermentation, and alfalfa degradation during adaptation to monensin supplementation in goats

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ABSTRACT

This study aimed to examine the temporal (hourly within a day and daily over the long term) effects of monensin on CH₄ emissions, ruminal fermentation, and in situ alfalfa degradation in dairy goats during dietary monensin supplementation by controlling the confounding effects of feed intake and ambient temperature. Six ruminally cannulated dairy goats were used, and they were housed in environmental chambers and fed a restricted amount of ration throughout the experiment. The experiment included a baseline period of 20 d followed by a treatment period of 55 d with 32 mg of monensin/d. During the whole experiment, CH₄ production was measured every 5 d, whereas fermentation characteristics and in situ alfalfa degradation were analyzed every 10 d. The CH₄-depressing effect of monensin was time dependent on the duration of treatment, highly effective at d 5 but thereafter decreased gradually until d 55 even though CH₄-suppressing effect still remained significant. The decreasing effects of monensin on ruminal acetate proportion and acetate to propionate ratio also faded over days of treatment, and the acetate proportion returned up to the pre-supplementation level on d 50. Monensin supplementation elevated ruminal propionate proportion and decreased the effective ruminal degradability of alfalfa NDF, but both measurements tended to recover over time. The postprandial increase rate of hourly CH₄ emissions was reduced, whereas that of propionate proportion was enhanced by monensin supplementation. However, the postprandial responses to monensin in CH₄ emission rates, ruminal VFA profiles, and in situ degradation kinetics declined with both hours after feeding and days of treatment. Our results suggest that the CH₄-suppressing effect of monensin supplementation in goats was attributed to reductions in both ruminal feed

degradation and acetate to propionate ratio, but those reductions faded with time, hours after feeding, and days of treatment.

Key words: goat, methane emission, monensin, ruminal fermentation and degradation

INTRODUCTION

Ruminant production is confronted with massive challenges in animal health and productivity. Ruminal acidosis is commonly observed in ruminants when they are fed grain-based diets to increase production (Russell and Rychlik, 2001). Negative energy balance and subclinical ketosis are inherent to ruminants during the transition period and early lactation (Drackley, 1999). Additionally, ruminal CH₄ emission contributes to global greenhouse gas emissions and represents an energy loss for the host animal (Johnson and Johnson, 1995; Hristov et al., 2013). As demonstrated by Shabat et al. (2016), inefficient cows produced less propionate but more CH₄ in the rumen than their efficient peers. Monensin, as an ionophore, has been used in ruminant production to alleviate the above problems and improve animal's energy efficiency (McGuffey et al., 2001; Duffield et al., 2008a, 2008b, 2012; Patra et al., 2017).

However, the persistence of monensin effects remains controversial, especially its CH₄-depressing effects (Sauer et al., 1998; Guan, 2006; Odongo et al., 2007; Appuhamy et al., 2013). For example, Guan (2006) reported that the CH₄-depressing effect of monensin was short lived in cattle, whereas it was long lived in the study of Odongo et al. (2007). The animal's feed intake may interfere with the evaluation of monensin effects as discrepancies in its CH₄-suppressing effects were found between measurements per day and per unit feed intake (Odongo et al., 2007). Additionally, it is well documented that ambient temperature can also influence CH₄ production (Salles et al., 2008; Bernier et al., 2012) by affecting animal's DMI and nutrient degradation (Kennedy and Milligan, 1978; von Keyserlingk and Mathison, 1993). However, the ambient temperature fluctuates in long-term studies, such as in

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the study of Guan (2006) where ambient temperature varied between -28.6 to 6.7°C over 16 wk.

Furthermore, to our knowledge, the effects of monensin on postprandial variations of CH_4 emissions, ruminal VFA profiles, and in situ degradation kinetics over days of treatment have never been studied, limiting our understanding of the temporal responses of the ruminal ecosystem to monensin. The objective of the present study was to investigate how monensin affects CH_4 production, ruminal fermentation, and in situ degradation of alfalfa hay during a long-term (55 d) treatment in dairy goats by controlling the confounding factors of feed intake and ambient temperature. The postprandial temporal effects of monensin on the above measurements were also examined hourly following feeding monensin.

MATERIALS AND METHODS

All experimental procedures were approved by the Northwest A&F University Animal Care and Use Committee.

Animals, Diets, and Experimental Design

Six nonlactating Xinong Saanen dairy goats, approximately 4 yr of age, with similar BW (54 ± 2.4 kg), body condition (health), and feed intake behavior (good appetite) were used in this study. The goats were fitted with permanent ruminal cannulas and had never been exposed to monensin before the experiment. A TMR (Table 1) was formulated to meet the nutrient requirements for maintenance (NY/T 816–2004; Ministry of Agriculture of China, 2004). The goats were fed the same amount of TMR equivalent to 330 g of alfalfa hay, 281 g of corn silage, and 408 g of concentrate twice daily in 2 equal meals at 0800 and 1800 h. The goats had free access to drinking water. The amounts of feeds refused, if any, were recorded daily.

The goats were first allowed to adapt to the environmental chambers and the TMR diet for 2 mo. Then, feeding experiment lasted for 75 d, including a baseline period (without monensin) for 20 d and a treatment period of 55 d (d 1–55), during which 16 mg of monensin (20% monensin in the Rumensin premix, Elanco Animal Health, Greenfield, IN) was top dressed onto a small amount of corn silage that was offered to each goat before each feeding.

Five indoor environmental chambers ($7.4 \text{ m} \times 4.2 \text{ m} \times 2.7 \text{ m}$) were used in this study, with 2 chambers serving as adaptation chambers to allow the goats to adapt to the chamber environments and 3 chambers as gas measurement chambers. The goats were randomly

divided equally into 2 groups, and the 3 goats of each group were housed in 1 of the 2 adaptation chambers. The 3 goats were separated by placing each in a metabolic cage ($1.5 \text{ m} \times 1.0 \text{ m} \times 1.5 \text{ m}$). The adaptation chambers were kept open to the indoor atmosphere to ensure adequate air exchange. The feeding experiment started in a staggered manner for the 2 groups of goats with a 3-d interval so that gas emissions from each group of the 3 goats could be measured individually using the 3 gas measurement chambers. The temperature inside the chambers was maintained between 6 and 14°C , and the goats were subjected to a diurnal cycle of 14 h of light and 10 h of darkness throughout the experiment. The goats were housed in the adaptation chambers and were moved to the gas measurement chambers only when their gas emissions were measured.

Measuring Methane Emissions and DMI

During both the baseline and the monensin treatment periods, CH_4 and CO_2 emissions from each goat were measured every 5 d using the approach of Goopy et al. (2011) with minor modifications. By this approach, gas emission from each goat was measured by determining the gas accumulation inside each airtight chamber with a fixed volume over a period of time. The system-alarm concentration of CO_2 was set at 2,000 ppm, much lower than the threshold level of 10,000 ppm for animals (Weissman et al., 1984; Hellwing et al., 2012). The internal volume of each chamber was 83.9 m^3 , which is about 137 times larger than that (0.613 m^3) used by Goopy et al. (2011), allowing for an extended duration of gas measurement in the closed chambers.

Table 1. Ingredients and chemical composition of the experimental diet

Item	% (DM)
Ingredient	
Corn silage	26.8
Alfalfa hay	33.3
Crushed corn	28.0
Soybean meal	10.8
Vitamin-mineral premix ¹	0.1
CaHPO_4	0.3
CaCO_3	0.3
Salt	0.4
Chemical composition	
DM	49.3
OM	93.8
CP	14.7
NDF	33.9
ADF	23.9

¹Vitamin-mineral premix (per kg): 600 mg of Mn, 950 mg of Zn, 430 mg of Fe, 650 mg of Cu, 30 mg of Se, 45 mg of I, 20 mg of Co, 450 mg of nicotinic acid, 800 mg of vitamin E, 45,000 IU of vitamin D, and 120,000 IU of vitamin A.

Briefly, a total of 24 h of gas emissions from each goat was measured in 2 consecutive days over 3 separate periods: 0000 to 0800 h and 1800 to 2400 h on the first day and then 0800 to 1800 h on the second day. At the end of each period, the chamber doors were kept open to allow fresh air in, and the chamber was cleaned before the next period. During the gas measurement periods, the air inside each chamber was mixed for 30 s every 10 min by 4 draft fans, and continuously and constantly pumped at a rate of 2 L/min to the CH₄ analyzer (model 55i, Thermo Fisher Scientific Inc., Waltham, MA) and CO₂ analyzer (BAIF-Maihak Analytical Instrument Co. Ltd., Beijing, China). The concentrations of CH₄ and CO₂ in the 3 chambers were analyzed sequentially, 5 min for each chamber every 15 min (4 times per h for each chamber).

The hourly and daily CH₄ emissions were calculated as follows:

$$\text{CH}_4 \text{ emission (L/h or L/d)} = \Sigma [(C_i - C_{i-1}) \times V_c + V_f \times (C_i - C_0)] / 1,000,$$

where $(C_i - C_{i-1})$ = the difference of CH₄ concentration (ppm) in every 15 min; C_0 = the initial CH₄ concentration (ppm) of each period; V_c = the chamber volume (83.9 m³); and V_f = the gas volume pumped from each chamber over each 15-min measurement (0.03 m³).

Feed and individual ort samples were collected on the days of gas measurement, dried at 55°C for 72 h, and then ground through a 1-mm screen. Those samples were analyzed to determine the content of DM (method 934.01, AOAC International, 2005), ash (method 942.05, AOAC International, 2005), and CP (method 954.01, AOAC International, 2005). The NDF and ADF contents were determined according to the method of Van Soest et al. (1991) using sodium sulfite and a heat-stable α -amylase (Ankom A200I fiber analyzer, Ankom Technology, Macedon, NY).

Collection and Analysis of Ruminal Fluid

During the baseline and the monensin treatment periods, rumen samples of each goat were collected from the anterior ventral sac of the rumen at 0, 1, 2, 4, 6, and 8 h after the morning feeding, and the samples were collected every 10 d on the day after every other gas measurement. Ruminal pH was measured immediately after sampling using a pH meter. Then, the ruminal samples were strained through 4 layers of cheesecloth. A subsample of 5 mL of ruminal fluid was mixed with 1 mL of 25% metaphosphoric acid and stored at -40°C until it was analyzed for VFA.

Ruminal VFA concentrations were analyzed by GC (Agilent Technologies 7820A GC system, Santa Clara, CA) using a 30 m $\times$ 0.25 mm $\times$ 0.33 μ m fused silica column (AE-FFAP, Atech Technologies Co. Ltd., Shanghai, China) after removing the solid particle and protein according to the procedures of Li et al. (2014).

In Situ Alfalfa Hay Degradation

In situ alfalfa hay degradation was determined using nylon bags as recommended by NRC (2001) every 10 d in the baseline period and the first 20 d of the monensin treatment period, and then every 15 d. Briefly, alfalfa hay was milled through a 2-mm screen. About 2.5 g (DM) of alfalfa hay were each placed in individual nylon bags (10 $\times$ 8 cm) with a pore size of 50 μ m, resulting in a ratio of the sample weight to the bag surface area of 15 mg/cm². The bags were placed inside the goat rumen through the cannula before the morning feeding. Two nylon bags were each retrieved after 2, 4, 8, 16, 24, 48, and 72 h of incubation. The retrieved nylon bags were rinsed in tap water and then dried at 55°C for 72 h. The alfalfa residues in each bags were analyzed for DM and NDF contents. Degradation parameters and effective ruminal degradability (ERD) were calculated using the nonlinear regression procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC) according to the following model (Ørskov and McDonald, 1979):

$$Y_t = a + b \times (1 - e^{-kt}),$$

in which Y_t = disappearance proportion at time t , a = rapidly degradable fraction; b = slowly degradable fraction; k = constant rate of degradation of fraction b ; and t = time of incubation (h).

$$\text{ERD} = a + b \times k / (k + kp),$$

in which a , b , and k are the degradation parameters as described above and kp is passage rate of roughage (0.04 h⁻¹, Offner et al., 2003).

Statistical Analysis

The measurements during the baseline period were averaged for each goat as the pre-supplementation values. The CH₄ emissions, ruminal fermentation parameters, and in situ degradation were analyzed as a 1-way repeated measures ANOVA using the PROC MIXED program in SAS with animal as a random effect. Days of the whole experimental period and days of the monensin treatment period were treated as repeated fac-

tors to identify statistically significant fluctuations over time.

Hourly CH₄ emissions during the first 11 h [from preprandial 1 h (the 0 h data) to the postprandial 10 h] were calculated for each goat by averaging that of the morning and afternoon feeding. And the 2 measurements of postprandial hourly CH₄ emissions in every 10 d were averaged to match the timelines of other measurements. The postprandial hourly variations in CH₄ emissions and ruminal VFA profiles over days of the entire experimental period were analyzed using 2-way ANOVA for repeated measures using the PROC MIXED program with day and hour as repeated factors and animal as a random effect; interaction of day × hour was also examined.

Student *t*-test for paired samples was performed to test differences among days of the experimental period and differences between the baseline and the monensin treatment periods. Statistical significance was declared at $P < 0.05$, whereas a trend was declared at $0.05 \leq P < 0.10$.

RESULTS

Effects of Monensin on DMI and Methane Production

On average, greater than 97% of the diets offered daily to each goat was consumed, and no difference was observed in DMI between the baseline and the monensin treatment periods ($P = 0.328$) or over days of the experiment ($P = 0.782$; Table 2). The monensin supplementation decreased the CH₄ emissions ($P = 0.001$) in goats expressed either as liters per day or as liters per day per kilogram of DMI. However, the CH₄-depressing effect decreased gradually over days of the monensin treatment ($P < 0.001$). Compared with the baseline period, the daily CH₄ production was decreased effectively (by 20.3 and 18.4% based on L/d and L/kg DMI, respectively) at d 5, but the CH₄-depressing effect decreased gradually during the monensin treatment period, and the CH₄ production was only decreased by 5.3 and 4.5%, when expressed as liters per day and liters per kilogram of DMI, respectively, at d 55.

The postprandial temporal variations of CH₄ emission rates (L/h) after feeding monensin are presented in Figure 1. Hourly CH₄ emissions were significantly affected by days of the experiment ($P < 0.001$) and hours after feeding ($P < 0.001$), and an interaction between day and hour was also observed ($P < 0.001$). Hourly CH₄ emissions peaked at the first postprandial hour during the baseline period, whereas during the monensin treatment period, it peaked at the fourth postprandial hour during d 5 to 10, and thereafter, it peaked sooner during d 15 to 50, at the second postprandial hour.

Table 2. Methane emissions from goats during the baseline and the monensin treatment (32 mg/d) periods

Item	Baseline period ¹	Days of monensin treatment										<i>P</i> -value ³				
		5	10	15	20	25	30	35	40	45	50	55	SED ²	M	T _W	T _M
CH ₄ (L/d)	24.29	19.36	20.77	21.08	21.40	20.90	21.91	21.65	22.19	22.67	22.69	23.00	0.355	0.001	<0.001	<0.001
Pairwise comparison ⁴	a	e	d	cd	bcd	d	bcd	bcd	bcd	bc	bc	b				
Reduction ⁵ (%)	0	20.3	14.5	13.2	11.9	14.0	9.8	10.9	8.6	6.7	6.6	5.3				
DMI (kg/d)	1.02	0.99	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.01	0.006	0.328	0.782	0.953
CH ₄ (L/kg of DMI)	23.92	19.51	20.85	21.12	21.47	21.06	21.91	21.55	22.29	22.61	22.63	22.85	0.326	0.001	<0.001	<0.001
Pairwise comparison ⁴	a	e	d	cd	bcd	d	bcd	bcd	bcd	bc	bc	b				
Reduction ⁵ (%)	0	18.4	12.8	11.7	10.3	12.0	8.4	9.9	6.8	5.5	5.4	4.5				

¹Average of 3 measurements of methane emissions from each goat.

²SED = standard error of the difference.

³M = monensin effect based on comparison between baseline period and treatment period; T_W = temporal fluctuations during the whole experimental period (baseline and treatment periods); T_M = temporal fluctuations during the monensin treatment only.

⁴Different letters within the same row indicate a significant ($P < 0.05$) difference in daily methane production.

⁵Averaged reduction in methane production relative to that of the baseline period.

Moreover, the postprandial hourly CH₄ emissions were also reduced by monensin, but the magnitude of the reduction declined with hours after feeding and with days of treatment.

Effects of Monensin on Ruminal Fermentation Characteristics

The effects of monensin supplementation on rumen fermentation parameters during the baseline and the monensin treatment periods are summarized in Table 3. The ruminal pH tended to decrease over the course

of the entire experiment ($P = 0.059$), but it did not differ between the baseline and the monensin treatment periods ($P = 0.115$) or over the course of the monensin treatment period ($P = 0.841$). The monensin supplementation changed the VFA profiles in a time-dependent manner except those of butyrate and valerate. Ruminal total VFA concentration was reduced ($P = 0.005$) by monensin, and the magnitude of the decrease fluctuated with days of treatment ($P < 0.001$). Ruminal acetate molar proportion ($P = 0.033$) and the ratio of acetate to propionate (**A:P**, $P = 0.003$) were lowered by monensin, but both measurements increased

day\hour	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h
Baseline	a	a	a	a	a	a	a	a	a	a	a
d 5-10	bc	c	d	d	b	b	d	c	b	b	b
d 15-20	bc	c	c	cd	b	ab	bc	c	b	ab	b
d 25-30	c	c	bc	c	b	b	c	bc	ab	ab	b
d 35-40	b	c	b	abc	ab	ab	c	ab	b	ab	ab
d 45-50	ab	b	ab	bc	ab	ab	b	ab	ab	ab	ab

Figure 1. Postprandial temporal variations of CH₄ emission rates (L/h) in the goats. The hourly CH₄ emissions from preprandial 1 h (0 h) to postprandial 10 h were calculated for each goat by averaging the CH₄ production of the morning and afternoon feeding. Error bars represent SEM (n = 6). Different lowercase letters (a–d) within the same column in the table indicate significant difference ($P < 0.05$). All the statistical significances of day, hour, and their interaction were less than 0.001. Color version available online.

Table 3. Ruminal fermentation parameters of the goats during the baseline period and the monensin supplementation (32 mg/d) period

Item	Baseline period ¹	Days of monensin treatment					P-value ³			
		10	20	30	40	50	SED ²	M	T _W	T _M
pH	6.58	6.37	6.41	6.41	6.41	6.42	0.091	0.115	0.059	0.841
Total VFA (mM)	57.63 ^{ab}	51.42 ^b	63.71 ^a	45.95 ^c	41.19 ^c	56.34 ^{ab}	1.262	0.005	<0.001	<0.001
Individual VFA molar proportion (%)										
Acetate	70.63 ^a	64.61 ^{cd}	64.48 ^d	65.80 ^{bc}	66.18 ^{bc}	67.75 ^{ab}	1.657	0.033	<0.001	0.012
Propionate	15.34 ^c	21.21 ^a	21.18 ^a	21.00 ^{ab}	20.73 ^{ab}	19.61 ^b	0.769	0.001	<0.001	0.091
Butyrate	10.70	9.64	10.06	9.27	9.06	9.02	1.096	0.291	0.350	0.475
Valerate	0.95	1.18	1.13	1.11	1.13	1.02	0.085	0.114	0.109	0.367
Acetate:propionate	4.66 ^a	3.05 ^c	3.07 ^c	3.14 ^{bc}	3.20 ^{bc}	3.50 ^b	0.278	0.003	<0.001	0.028

^{a-d}Values with different superscripts within the same row differ significantly ($P < 0.05$).¹Average of 2 measurements for each goat.²SED = standard error of the difference.³M = monensin effects based on comparison between the baseline and the monensin treatment periods; T_w = temporal fluctuations during the whole experimental period (baseline and treatment periods); T_M = temporal fluctuations during the monensin treatment only.

gradually with days of treatment ($P < 0.05$), and the acetate proportion was restored up to the pre-supplementation level on d 50. The increasing effect ($P = 0.001$) of monensin on ruminal propionate proportion tended to decline over days of treatment ($P = 0.091$).

The postprandial responses to monensin in ruminal acetate molar proportion declined with both hours after feeding ($P = 0.003$) and days of treatment ($P < 0.001$), and an day $\times$ hour interaction ($P = 0.001$) was also observed (Figure 2A). Compared with that of the baseline period, acetate molar proportion was lowered by monensin from 0 to 4 h after morning feeding, but thereafter it recovered to the baseline level at sixth and eighth postprandial hour on d 10 and 20 (Figure 2A). The acetate inhibition also gradually diminished with days of treatment, and by d 50, acetate molar proportion recovered to the baseline value at all the sampling time points. The propionate-promoting effect of monensin also decreased with both hours after feeding ($P < 0.001$) and days of treatment ($P < 0.001$), and no difference in propionate molar proportion was seen between the eighth postprandial hour on d 50 and the baseline period (Figure 2B).

Effect of Monensin on In Situ Alfalfa Hay Degradation

The in situ degradation kinetic parameters and ERD of alfalfa hay in goats are presented in Table 4. The ERD of alfalfa hay DM and NDF ($P < 0.05$) were reduced by monensin, and the ERD of alfalfa hay NDF tended to increase over days of monensin treatment ($P = 0.079$). The degradations of rapidly degradable fractions of alfalfa hay DM and NDF were decreased ($P < 0.05$) by monensin but in a time-dependent manner ($P < 0.01$), as they declined from per supplementation to d 10 and thereafter increased gradually up to the baseline value on d 50. However, the degradation of slowly degradable fraction of NDF tended to be higher during the monensin treatment period than in baseline period ($P = 0.093$).

DISCUSSION

Relationships Among Ambient Temperature, DMI, and Rumen Fermentation

In the current study, fluctuations in DMI during the entire experiment period were minimized by controlling the amount of TMR offered and maintaining a relatively stable temperature (6 to 14°C) in the chambers. Temperature is one factor that can affect DMI, with low temperature increasing DMI but decreasing feed degradation (Kennedy and Milligan, 1978; von Keyserlingk

A

B

Figure 2. Postprandial variations of molar proportions of ruminal acetate (A) and propionate (B) in the goats. Error bars represent SEM (n = 6). The *P*-value showed the effects of day, hour, and their interaction determined using 2-way ANOVA analysis for repeated measures. Different lowercase letters within the same column in the table indicate a significant difference ($P < 0.05$). Color version available online.

and Mathison, 1993; Salles et al., 2008) by increasing passage rate and thus shortening the mean retention time (Kennedy and Milligan, 1978; Kadzere et al., 2002). Mathematically, DMI can influence the production of end products in rumen fermentation, and this is demonstrated by CH₄ emissions being proportional to DMI (Boadi and Wittenberg, 2002; Grainger et al., 2007). Therefore, temperature can also affect rumen fermentation and CH₄ emissions indirectly (Salles et al., 2008; Bernier et al., 2012). Although several studies have shown that monensin has no effect on DMI (Odongo et al., 2007; Grainger et al., 2008; Waghorn et al., 2008), 2 meta-analyses (Duffield et al., 2008a; Apuhamy et al., 2013) revealed a 2.3 to 2.6% decrease in DMI in dairy cows fed monensin. Odongo et al. (2007) observed a 6.5% reduction in CH₄ emissions (g/d) by cows fed 24 mg of monensin/kg of DM, and the CH₄ depression was sustained for 6 mo; however, when expressed as liters of CH₄ per kilogram of NDF intake, the monensin treatment had no effect. Discrepancies in CH₄-depressing effects of monensin were also reported between CH₄ production per day and per unit of feed intake in the same study (Guan, 2006; da Fonseca et al., 2016). Apparently, controlling confounding factors, such as temperature and DMI, is necessary to assess the effect of monensin and to advance the mechanistic understanding of monensin on rumen fermentation and CH₄ emissions. By controlling these 2 confounding factors, we showed a consistent CH₄-depressing effect when CH₄ emissions were expressed as liters per day or as liters per kilogram of DMI.

Effects of Monensin on Daily Methane Production During Adaptation to Monensin

Monensin still exhibited significant inhibition of CH₄ production at the end of the monensin treatment (d 55), but its long-term efficacy is probably small as its CH₄-depressing efficacy continued to fade over time, even during the latter days of the treatment. Johnson and Johnson (1995) and Guan (2006) showed that the CH₄-depressing effects of monensin were short lived in cattle and lasted for less than 30 d. The longer efficacy observed in the present study is probably attributable to the experimental design of repeated measures and the high sensitivity of the Student *t*-test for paired samples (self-comparison) because one-way ANOVA test of the CH₄ data showed no difference in CH₄ production between the baseline period and the latter days (d 35 to 55) of the monensin treatment (data not shown). These results corroborate the development of resistance to monensin by ruminal microbes (Johnson and Johnson, 1995). Sauer et al. (1998) found that the ruminal microbiota in cows could become adapted to monensin after the first treatment and did not respond to repeated monensin supplementation. Guan (2006) observed a reduction of ruminal protozoal populations in cow receiving monensin, but that reduction appeared to be a short-term effect, with a total recovery after 4 to 6 wk of the treatment. Several studies also documented resistance of gram-positive bacteria to monensin (Newbold et al., 1993; Ravva et al., 2013; Kim et al., 2014b). No alteration in the abundance or diversity of metha-

Table 4. In situ alfalfa hay degradation in the rumen of the goats during the baseline period and the monensin supplementation (32 mg/d) period

Item	Baseline period ¹	Days of monensin treatment				SED ²	<i>P</i> -value ³		
		10	20	30	50		M	T _W	T _M
DM degradation ⁴									
<i>a</i> (%)	20.90 ^a	14.15 ^d	16.14 ^c	16.59 ^{bcd}	19.88 ^{ab}	1.216	0.026	<0.001	0.002
<i>b</i> (%)	31.79 ^c	37.31 ^{ab}	37.05 ^{abc}	37.97 ^a	35.00 ^{bc}	2.497	0.113	0.035	0.170
<i>k</i> (%/h)	8.34	8.18	7.69	7.00	6.54	0.404	0.071	0.138	0.256
<i>a</i> + <i>b</i> (%)	52.69	51.45	53.19	54.57	54.89	1.541	0.616	0.248	0.143
ERD (%)	42.25 ^a	39.17 ^b	40.44 ^{ab}	40.69 ^{ab}	41.13 ^{ab}	0.449	0.014	0.009	0.112
NDF degradation ⁴									
<i>a</i> (%)	10.34 ^a	2.73 ^c	5.95 ^b	5.50 ^b	7.79 ^{ab}	1.044	0.010	<0.001	0.001
<i>b</i> (%)	36.07 ^b	42.21 ^a	39.90 ^{ab}	41.71 ^{ab}	40.53 ^{ab}	2.285	0.093	0.043	0.413
<i>k</i> (%/h)	6.50	5.99	5.75	5.60	5.36	0.446	0.137	0.506	0.831
<i>a</i> + <i>b</i> (%)	46.41	44.94	45.86	47.20	48.32	1.295	0.903	0.264	0.162
ERD (%)	32.54 ^a	27.93 ^b	29.43 ^b	29.66 ^b	30.34 ^b	0.472	0.002	0.001	0.079

^{a-d}Values with different superscripts within the same row differ significantly (*P* < 0.05).

¹Average of 2 measurements for each goat.

²SED = standard error of the differences.

³M = monensin effects based on comparison between the baseline and the monensin treatment periods; T_W = temporal fluctuations during the whole experimental period (baseline and treatment periods); T_M = temporal fluctuations during the monensin treatment only.

⁴*a* = rapidly degradable fraction; *b* = slowly degradable fraction; *k* = constant rate of degradation of fraction *b*; *a*+*b* = potential extent of degradation; effective ruminal degradability (ERD) = $a + b \times k / (k + kp)$, where *kp* is the passage rate of roughage (0.04 h⁻¹, Offner et al., 2003).

nogens was observed upon monensin supplementation (Chen and Wolin, 1979; Hook et al., 2009; Scharen et al., 2017), suggesting that methanogens are resistant to monensin. Therefore, the CH₄-depressing effects of monensin are most likely attributed to its ability to lower the availability of methanogenic substrates, especially H₂, rather than to direct inhibition to methanogens (McGuffey et al., 2001). Based on the pathways of carbohydrate fermentation, the H₂ available for methanogenesis depends primarily on the amount of dietary carbohydrate fermented and the ruminal VFA profiles. Given the same DMI between the baseline and the monensin treatment periods in the current study, our results suggest that monensin inhibits CH₄ production by lowering the rate of ruminal dietary degradation and shifting the ruminal VFA files to a lower A:P (Russell, 1998; Ungerfeld, 2015).

Effects of Monensin on Ruminal VFA Profiles During Adaptation to Monensin

Acetate production during rumen fermentation is accompanied by H₂ production, whereas propionate production is accompanied with H₂ consumption (Hegarty 1999; Janssen, 2010). Consistent with the findings of other studies, monensin decreased the ruminal A:P (Brown and Hogue, 1985; Tomkins et al., 2015; Wang et al., 2015) but had no effect on the proportion of butyrate or valerate (Gehman et al., 2008; Tomkins et al., 2015; Vendramini et al., 2015). These shifts in VFA profiles could be driven by inhibition of gram-positive bacteria and protozoa (Russell, 1987; Russell and Houlihan, 2003; Kim et al., 2014a), both of which produce acetate and H₂ as fermentation end products (McGuffey et al., 2001; Tedeschi et al., 2003). The shift from acetate to propionate reduces the H₂ available for methanogenesis (Russell, 1998; Ungerfeld, 2015), explaining, at least partially, the monensin inhibition to CH₄ production seen in this study.

The time-dependent effect of monensin on ruminal propionate proportion ($P = 0.091$) was weaker than that on CH₄ production ($P < 0.001$) during the monensin treatment. The relatively persistent shifts in VFA profiles by monensin are in general accordance with previous observations (Rogers et al., 1997; Guan, 2006). Guan (2006) reported that the monensin-induced reduction of ruminal A:P was maintained for at least 12 weeks, whereas that of CH₄ emissions was maintained up to 4 wk. The increased propionate production by monensin cannot only decrease H₂ availability for methanogenesis but also increase the precursor for gluconeogenesis in animals, which would improve animal's energy efficiency (Shabat et al., 2016). A previous meta-analysis by Duffield et al. (2012) revealed that

monensin supplementation improved feed efficiency in cattle by 1.3%. Because monensin can provide a persistent energetic advantage by enhancing propionate production, we agree with the assertion of Tedeschi et al. (2003) that monensin is effective to reduce CH₄ emissions from ruminants, despite potential microbial adaptation, because it can decrease the CH₄ output per kilogram of milk or meat.

The effects of monensin supplementation on total VFA concentration have been variable among previous studies (Salles et al., 2008; Khorrami et al., 2015; Lemos et al., 2016), which may be attributed to the duration of monensin treatment. In this long-term study, temporal variations in total VFA concentration were seen over the course of the monensin treatment ($P < 0.001$), which is in agreement with the results of Rogers et al. (1997). Although monensin reduced the total VFA concentration, no difference in ruminal pH was observed, which is consistent with previous observations (Fairfield et al., 2007; Khorrami et al., 2015; Tomkins et al., 2015; Mirzaei-Alamouti et al., 2016). The monensin-induced decrease in total VFA concentration might be probably too small to change the ruminal pH.

Effects of Monensin on ERD of Alfalfa Hay During Adaptation to Monensin

Consistent with previous studies (Reveneau et al., 2012; Montano et al., 2015), monensin reduced ruminal digestion of OM and fiber. Such adverse effects of monensin on ruminal fiber degradation have been attributed to its inhibition of cellulolytic bacteria and protozoa in the rumen (Khorrami et al., 2015; Wang et al., 2015). As confirmed in the present study, the reduced DM digestibility corresponded with the decreased ruminal VFA concentration and CH₄ production (Jentsch et al., 2007). However, monensin did not lower apparent total-tract digestibility in numerous previous studies (Gehman et al., 2008; Salles et al., 2008; Reveneau et al., 2012; Khorrami et al., 2015), probably because the reduced ruminal digestibility was compensated by increased postruminal digestion (Haimoud et al., 1995; Reveneau et al., 2012).

So far no studies in the literature have evaluated the dynamics of in situ feed degradation over days of the monensin treatment. In the present study, we showed that the inhibition of ERD of alfalfa hay by monensin became weak over the course of the monensin treatment period. The time-dependent patterns in ERD of alfalfa NDF mirror that of ruminal A:P, corroborating the theory of Nozière et al. (2011) that NDF digestibility positively correlates with acetate proportion but negatively correlates with propionate proportion. It should be noted that Benchaar et al. (2006) reported

no effect of monensin on ERD of grass silage DM and NDF in dairy cows using a Latin square design. The lack of response in the aforementioned study could be due to the pre-exposure of the animals to monensin, which would lower the responses of ruminal microbiota to repeated monensin supplementation (Sauer et al., 1998).

Postprandial Responses of Methane Emissions, VFA Profiles, and In Situ Alfalfa Hay Degradation to Monensin

This study is the first to show that the postprandial responses to monensin in CH₄ emission rates, ruminal VFA profiles, and in situ degradation kinetics declined with both hours after feeding and days of treatment. Consistent with previous studies (Rooke et al., 2014), the hourly CH₄ emissions peaked shortly after feeding during the baseline period. Monensin both delayed CH₄ production from peaking and reduced the CH₄ emission rates after feeding. The degradation of the rapidly degradable fraction of alfalfa NDF was decreased, whereas that of slowly degradable fraction tended to be increased by monensin, suggesting the inhibitory effect of monensin on alfalfa NDF degradation weakened with hours after feeding. Therefore, the CH₄-depressing effects of monensin dwindled with time, hours after feeding, and days of treatment, likely because of its stimulatory effect on ruminal propionate proportion and its inhibitory effect on alfalfa NDF degradation weakened with time. The fading of postprandial responses to monensin with hours after feeding was probably due to the passage of monensin from the rumen, whereas the weakening of postprandial responses to monensin with days of treatment probably reflects the development of resistance to monensin by ruminal microbes. Further studies are needed to investigate the adaptive changes of ruminal microbes upon long-term exposure to monensin.

CONCLUSIONS

Using dairy goats as a model, we showed that monensin decreased CH₄ production, ruminal A:P, and fiber digestibility without the confounding effects of DMI and temperature. However, all these effects faded, though at varying rates, during long-term monensin supplementation, suggesting adaptation by developing resistance to monensin by ruminal microbes. The effect of monensin on postprandial CH₄ emission rates, ruminal VFA profiles, and in situ degradation kinetics also dwindled with hours after feeding. Despite the adaptation and the small magnitude of CH₄ inhibition, monensin can still help mitigate CH₄ emissions from

ruminants because it can increase propionate production relatively persistently and then decrease the CH₄ output per kilogram of milk or meat.

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