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Effects of Diets Containing Monensin, Garlic Oil or Turmeric Powder on Ruminal and Blood Metabolite Responses of Sheep

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Abstract: The aim of the present study was to evaluate the effect of diets containing Garlic oil (GA), Turmeric powder (TU) or Monensin (MO) on ruminal pH, ammonia nitrogen concentration and various blood metabolites concentrations and blood gases in sheep. Four rumen cannulated Baloochi sheep were used as a 4×4 Latin square design with 4 periods and 28 day each. Treatments were: basal diet including 55% concentrate and 45% dry alfalfa hay (control), basal diet+GA (420 mg/sheep/day), basal diet+TU (20 g/sheep/day) and basal diet+MO (200 mg/sheep/day). Diets were fed once daily *ad libitum*. Ruminal fluid samples were collected before the feeding and every 15 min until 8 h post feeding at days 25 of the each experimental period. Blood samples were taken from jugular vein before the feeding and 2, 4 and 6 h post feeding at day 27 and before the feeding and 6 h post feeding at day 28 of each period of the experiment. Adding GA, TU or MO to the basal diet had no significant effect on mean and minimum of ruminal pH and ammonia nitrogen concentration ($p>0.05$) while maximum value of ruminal pH was significantly decreased by MO and TU ($p<0.05$). The experimental treatments did not change the plasma concentrations of glucose and urea-N ($p<0.05$). Supplementation with MO caused a significant increase in jugular blood partial pressure of O_2 and tended to raise blood percent O_2 saturation ($p<0.05$).

Key words: Garlic oil, turmeric powder, monensin, ruminal fermentation, plasma concentration, significant

INTRODUCTION

During ruminal fermentation a part of consumed energy and protein are excreted (as methane and ammonia nitrogen, respectively) without utilization by rumen microflora or host animal (Busquet *et al*., 2006). For this reason, ruminant nutritionists have suggested optimizing diet formulation and using feed additives. Supplementation diets with antibiotics growth promoters such as monensin and lasalocid diminish losses of energy and nitrogen (Ipharraguerre and Clark, 2003). However, the use of antibiotics as feed additives has been banned in many countries due to the risk of appearance of antibiotic residues in milk and meat and development of multi-drug bacteria (Russell and Houlihan, 2003). For many years, plant extracts have been used for remediation of diseases and also as food preservatives because of their antimicrobial characteristics (Davidson and Naidu, 2000). Results of previous studies indicated that extract of some plants can be appropriate alternatives for antibiotics growth promoters (Calsamiglia *et al*., 2006). Garlic oil possesses different curative properties such as, antiparasitic, antioxidant, anti-inflammatory and hypoglycemic. Garlic oil lessened the proportion of acetate and increased proportion of propionate and butyrate in some *in vitro* studies (Cardozo *et al*., 2004; Busquet *et al*., 2005a-c). Moreover, it reduced methane production under *in vitro* condition (Chiquette and Benchaar, 2005). However, there is limited information about the effects of garlic oil on rumianl fermentation using *in vivo* exprimments (Yang *et al*., 2007; Chaves *et al*., 2008).

A large number of researches have demonstrated different biological properties of turmeric including anti-inflammatory (Srimal and Dhawan, 1973), antimicrobial (Lutomski *et al*., 1974) and hypoglycemic (Arun and Nalini, 2002). To the knowledge, there is no information on the effect of turmeric on ruminal fermentation when it included in ruminant ration. The aim of this study was to assess the effects of diets containing Garlic oil (GA), Turmeric powder (TU) or Monensin (MO) on ruminal pH, ammonia nitrogen concentration (NH_3-N) and various blood plasma metabolites including glucose and urea-N concentrations and

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blood gases including partial pressure of O_2, partial pressure of CO_2 and percent O_2 saturation in sheep.

MATERIALS AND METHODS

Four rumen cannulated Baloochi sheep were used in a 4×4 Latin square design with 4 periods (each period of 28 days). Sheep were fed a basal diet (Table 1) without supplementation (control) or a basal diet supplemented with GA (420 mg/sheep per/day), TU (20 g/lamb per/day) or MO (200 mg/sheep per/day). Diets consisted of 55% concentrate and 45% dry alfalfa hay (Table 1) and were fed once daily ad libitum. The animals were assigned to individual metabolical cages (0.5×1.2×1 m) and had free access to salt and fresh water throughout the experiment. Each period included 21 days of adaptation and 7 days of sample collection of rumianl fluid and blood. Ruminal fluid samples (10 mL) were collected on day 25 before the feeding and every 15 min until 8 h post feeding. Samples of ruminal contents were strained through four layers of cheesecloth and pH was measured using a pH meter (Metrohm 744, Switzerland). A volume of 10 mL of the filtrated ruminal fluid acidified with 10 mL of HCL 0.2 N and stored for later determination of NH_3-N concentration. Ruminal NH_3-N was determined using distillation method (Kjeltec Auto 1030 Analyzer Tecator, tecator, Hoganas, Sweden).

On day 27, Blood samples were taken from jugular vein before the feeding, 2, 4 and 6 h post feeding with heparinized syringe. Samples were centrifuged (3500×g for 15 min at 4°C) and collected plasma was kept frozen at-20°C for further analysis. Plasma glucose and urea-N concentrations were determined by an auto-analyzer (Alcyon 300i Abbott, USA). In order to determine blood gases and pH, jugular blood samples were collected using heparinized syringes before and 4 h after the feeding on day 28. The syringes were chilled in an ice bath immediately and transported to the laboratory within 1 h. Blood partial pressure of gaseous O_2 dissolved in blood (pO_2), partial pressure of CO_2 (pCO_2) and percent O_2 saturation were measured by a pH/Blood Gas Analyzer (Stat Profile pHOx Plus blood analyzer, Nova Biomedical, USA). Data were applied to the mixed model of SAS (version 9.1; SAS Institute Inc., Cary, NC) with the following statistical model of:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + (AD)_{il} + \varepsilon_{ijklm}$$

Where:

Y_{ijklm} = The depndent variable
μ = The overral mean
A_i = The treatment effect
B_j = The period effect
C_k = The random effect of animal within treatments
D_l = The sampling time effect
$(AD)_{il}$ = The interaction effect of treatment and sampling time
ε_{jklm} = The residual error

The sampling time was included in the model as repeated measurement by using compound symmetry. Differences between least squares means were considered significant at $p<0.05$, using PDIFF in the LSMEANS statement.

Table 1: Ingredients and chemical composition of the basal diet fed to sheep (Percentage of DM)

Item	Percentage
Ingredients (% of DM)	
Alfalfa	45.0
Corn grain	15.0
Barley grain	19.0
Cottonseed meal	6.0
Soybean meal	4.0
Sugar beet pulp	3.0
Wheat bran	5.0
Calcium carbonate	1.0
Salt	1.0
Vitamin-mineral mix[1]	1.0
CP (g kg^{-1} DM)	155.0
NDF (g kg^{-1} DM)	289.0
ME (Mcal kg^{-1} DM)	2.8

[1]Composition of vitamin-mineral mix: Ca, 196.0 g kg^{-1}; P, 96.0 g kg^{-1}; Mg, 19.0 g kg^{-1}; Fe, 3.0 g kg^{-1}; Na, 71.0 g kg^{-1}; Cu, 0.3 g kg^{-1}; Mn, 2.0 g kg^{-1}; Zn, 3.0 g kg^{-1}; Co, 0.1 g kg^{-1}; I, 0.1 g kg^{-1}; Se, 0.01 g kg^{-1} and Vit A, 500,000 IU kg^{-1}; Vit D, 100,000 IU kg^{-1}; Vit E, 100 IU kg^{-1}

RESULTS AND DISCUSSION

Under present study conditions, mean of ruminal pH was not affected by GA, TU or MO (Table 2). Similarly, the lowest ruminal pH value was the same among the treatments (Table 2). However, the highest ruminal pH value was reduced in sheep fed TU or MO compared with those fed the control diet. These results are consistent with some studies (Chaves *et al*., 2008) where ruminal pH of sheep were not affected by the feeding rations supplemented with GA and the results obtained by Meyer *et al*. (2009) in which addition of MO or a blend of plant extracts in diet were not influenced the ruminal pH of steers. Yang *et al*. (2007) reported no significant changes in the mean, maximum and minimum of ruminal pH of dairy cows fed diets containing MO or GA. Furthermore, in some other studies MO did not alter ruminal pH of dairy cows (Ramanzin *et al*., 1997; Broderick, 2004). In contrast, a reduction in ruminal pH with a blend of plant extracts supplementation was reported by Benchaar *et al*. (2007) and Devant *et al*. (2007). The discrepancies among studies could be due to the type of diets and species used (Meyer *et al*., 2009).

Table 2: Ruminal pH and ammonia nitrogen concentration (NH_3-N) of sheep fed Basal Diet (BD) or plus Garlic oil (GA), Turmeric powder (TU) or monensin (mo)

	Treatments					
Item	BD	BD+GA	BBD+TU	BD+MO	SEM	p-value
pH						
Mean	6.34	6.11	6.22	6.28	0.12	0.32
Minimum	5.67	5.48	5.51	5.58	0.12	0.72
Maximum	7.48^{a}	7.27^{ab}	7.18^{bc}	7.01^{c}	0.11	0.04
N-NH_3 (mg dL^{-1})	18.74	22.41	17.73	18.51	2.72	0.31

$^{a-c}$Means within a row with different superscripts differ ($p<0.05$)

In this experiment, supplementing of basal diet with GA, TU or MO did not affect ($p>0.05$) concentration of ruminal NH_3-N (Table 2). This would agree with the results of Chaves *et al.* (2008) who reported that the addition of GA in the ration had no influence on NH_3-N concentration in the rumen of sheep. Yang *et al.* (2007) observed no effect of GA and Mo on the ruminal NH_3-N concentration of lactating cows. No change in NH_3-N concentration in ruminal fluid by MO was also reported by the results of previous studies (Ramanzin *et al.*, 1997; Broderick, 2004). Furthermore, in some *in vitro* studies a mixture of palnt extract had no significant effect on NH_3-N concentration (Busquet *et al.*, 2005c; Castillejos *et al.*, 2006). Results of Cardozo *et al.* (2004) indicated a reduction in NH_3-N concentration when GA was incubated in a continuous culture. Differences between results could be attributed to the ability of ruminal bacteria for adaptation to plant extract in long term studies after few days (Cardozo *et al.*, 2004; Busquet *et al.*, 2005c). Moreover, effects of plant extract could be varied according to the type and the plant originated (Cardozo *et al.*, 2006).

Data of plasma glucose and urea-N concentrations at pre and post feeding are shown in Table 3. Concentrations of glucose and urea-N in plasma were not influenced by additives treatments ($p>0.05$). These results confirmed the finding of Chaves *et al.* (2008) who reported no difference in serum glucose concentration of growing lambs fed diets supplemented with GA compared with control. Addition a specific mixture of essential oils in ration of periparturient and early lactation cows had no significant effect on plasma glucose and urea-nitrogen concentration (Tassoul and Shaver, 2009). Broderick (2004) reported that the adding of MO to the diet had no effect on blood urea-N concentration of dairy cows, although, plasma glucose tended to be significant.

In a earlier study (Brown and Hogue, 1987) plasma glucose concentration was not different among goats fed diet containing MO or control diet. Similar results were achieved in other studies (Duff *et al.*, 1994; Petersson-Wolfe *et al.*, 2007). There is no information about the effect of turmeric powder on glucose and urea-N in ruminants. Mehala and Moorthy (2008) demonstrated that supplementation turmeric in broiler chicken diets did not alter serum glucose concentration significantly. However, administration of turmeric or its active compound, curcumin, to diabetic rats diminished blood sugar (Arun and Nalini, 2002).

Table 3: Blood plasma glucose and urea-N concentration of sheep fed Basal Diet (BD) or plus Garlic oil (GA), Turmeric powder (TU) or monensin (mo)

	Treatments					
Item	BD	BD+GA	BD+TU	BD+MO	SEM	p-value
Glucose (mg dL^{-1})	70.87	69.57	64.55	70.62	4.46	0.46
Urea-N (mg dL^{-1})	15.57	16.06	14.67	17.31	1.47	0.21

Table 4: Jugular blood pH, partial pressure of O_2 (pO_2), partial pressure of CO_2 (pCO_2) and percent O_2 saturation of sheep fed fed Basal Diet (BD) or plus Garlic oil (GA), Turmeric powder (TU) or Monensin (mo)

	Treatments					
Item	BD	BD+GA	BD+TU	BD+MO	SEM	p-value
pH	7.405	7.429	7.402	7.420	0.02	0.79
pO_2 (mm Hg)	34.510^{a}	36.220^{ab}	31.400^{a}	39.360^{b}	1.52	0.01
pCO_2 (mm Hg)	39.670	39.740	39.000	38.510	1.68	0.94
O_2 saturation (%)	63.950	67.980	63.600	70.570	3.50	0.08

$^{a-c}$Means within a row with different superscripts differ ($p<0.05$)

Blood pH varies from 7.40-7.42 and did not differ among the animal fed the present experimental diets (Table 4). Diet supplemented by MO increased pO_2 in comparison with the control ($p<0.05$) while pCO_2 was not impacted by the experimental diets ($p>0.05$). In addition, percent O_2 saturation tended to increase in sheep fed MO ($p>0.10$). Blood gas analysis is a clinical tool for determining acid-base status in animal (Bouda *et al.*, 2000). According to the study of Hernandez *et al.* (2009) plant extracts could change blood acid-base status. They reported that a blend of plant extracts increased blood pH of growing bull calves fed a high grain diet. The results are consistent with the finding of Candanosa *et al.* (2008), who observed no change in blood pH and pCO_2 in sheep fed MO. Ionophers might affect acid/base balance and cellular activity through regulating ion translocation across cellular membrane and therefore may lead to a change in atmospheric and also tissue respiration (Yang *et al.*, 2003).

CONCLUSION

In this study, present supplementation of the basal diet with GA, TU or MO has sicnificant effect on maximal rumen pH evaluated during 8 h post feeding. The concentration of blood plasma metabolites are not altered by the present treatments. Addition of MO to the basal diet elevated pO_2 in the blood. Moreover there is a tendency to increase in blood percent O_2 saturation of

sheep fed basal diet plus MO. Present results indicate that both GA and TU have a potential to change the rumen and blood metabolite responses. However, there is a need to evaluate the effects under *in vivo* experimental condition using higher concentration of these additives.

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