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ORIGINAL ARTICLE

Volatile fatty acid profile for grass hay or alfalfa hay fed to alpacas (*Vicugna pacos*)

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Summary

The purpose of this study was to determine the diurnal composition and concentration of volatile fatty acids (VFA) and to determine VFA composition and concentration differences between stomach compartment 1 (C1) and caecum of alpacas fed grass and alfalfa hay. The study was divided into two experiments. In Experiment 1 (EXP 1), 10 male alpacas (3+ years old, 65 kg BW) were divided into two groups, housed in drylot pens, provided *ad libitum* water and fed alfalfa (AH) or grass hay (GH) for 30 days. The alpacas were slaughtered and the digestive tract collected, divided into sub-tract sections, weighed and digesta sampled for pH, dry matter (DM) and NDF. Volatile fatty acid composition and concentration were determined on C1 and caecal material. Four adult male (3+ years old, 60 kg BW), C1 fistulated alpacas were housed in metabolism crates and divided into two forage groups for Experiment 2 (EXP 2). Alpacas were fed the forages as in EXP 1. Diurnal C1 VFA samples were drawn at 1, 3, 6, 9, 12, 18 and 24 h post-feeding. There were no differences between forages for tract weight, C1 and caecum digesta DM or NDF. Differences were noted ($p < 0.05$) for pH between forages and sub-tract site. Volatile fatty acids concentrations were different ($p < 0.05$) for forage and site, and total VFA was higher for AH than GH (110.6 and 79.1 mM) and C1 than caecum (40.7 and 27.6 mM). Proportion of VFA was significant ($p < 0.05$) for forage and site, C1 acetate highest for GH (84.8 vs. 74.0 mM) and caecum acetate 83.7 and 76.2 mM for GH and AH respectively. These data demonstrate the level of VFA produced in C1 and the caecum of alpacas and the diurnal VFA patterns. Composition of VFA is similar to other ruminant species.

Keywords alpaca, volatile fatty acid, compartment 1, caecum, diurnal

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Introduction

South American camelids have a unique digestive physiology that has not been extensively researched. Alpacas are classified as pseudo-ruminants; they have three stomachs instead of the typical four in ruminant animals (cattle and sheep). Alpacas also have a glandular saccule organ attached to the first stomach that secretes bicarbonate and phosphate-buffering agents to maintain the rumen pH to near neutral (Eckerlin and Stevens, 1973). Robinson et al. (2013) showed that compartment 1 (C1) pH of alpacas fed various forages and grains would decrease post-feeding then return to pre-feeding levels. Values ranged from slightly above neutral (~7.2) to slightly below (~6.5). Rumen pH is associated with the level of organic acids, mainly volatile fatty acids (VFA), produced (Brossard et al., 2003). The three main VFAs associated with rumen fermentation are acetate, propionate and butyrate. Of these, acetate and butyrate are incorporated into the Krebs' cycle as acetyl-CoA and propionate is shuttled to the gluconeogenic pathway in the liver for glucose synthesis. Van Saun (2006) makes the point that propionate is produced from the fermentation of sugars and starch and that because alpaca diets are most often low in sugars and starch, propionate may not be the major precursor for gluconeogenesis.

Diet composition has an effect on the VFA ratios, where hay and grain diets result in differing ratios between the three VFAs (Dijkstra, 1994). Substrate availability can affect subsequent rumen pH. In cattle and sheep, VFAs have been shown to produce 80% of the metabolizable energy absorbed from the

rumen and an additional 9–17% absorbed from caecal and colic fermentation (Siciliano-Jones and Murphy, 1989; Bergman, 1990). Understanding the production and site concentrations is the first step in understanding the utilization of VFAs in alpacas. The objectives of this study were to determine the effects of forage variations on VFA production, to determine VFA concentration differences between C1 and the caecum and to characterize the diurnal VFA concentration patterns in alpacas fed alfalfa hay or grass hay.

Material and methods

Animals and treatments

Two experiments were conducted following animal use and care guidelines (FASS, 2010) and under the approval of The Camelid Center Animal Use Committee. Alpacas in Experiment 1 (EXP 1) were housed in drylot paddocks for the duration of the study. Water and a commercial free-choice salt and mineral supplement were available *ad libitum*. The animals were fed once daily *ad libitum*, where the previous days orts were measured, discarded and fresh feed made available. Averaged daily dry matter intake was determined by dividing the total forage consumed (delivered minus orts) by the total number of days ($n = 30$) and alpacas ($n = 5$) in the pen.

Alpacas in Experiment 2 (EXP 2) were housed in individual metabolism crates. Water was available *ad libitum*, and the alpacas were fed *ad libitum* once daily. In addition, the alpacas had access to a commercial free-choice salt and mineral supplement – a supplement they have had access to for several months prior to this experiment. Orts were removed daily, and for the week prior to the sample collection, feed intake was determined by collecting daily orts, drying at 60 °C and subtracting dry matter (DM) orts from DM fed. The DM intake for the week of sampling for each alpaca was used to determine statistical differences as outlined below.

The alfalfa hay (AH; *Medicago sativa*) and grass hay (GH; tall fescue, *Festuca arundinacea*) forage used in both experiments were from the same source and were fed from the same stored material. Each forage was ground through a 25-mm screen, mixed in a feed mixer to provide homogeneity throughout the two experiments. Grab samples of the forages were collected throughout the experiments, and the composite samples analysed by a commercial forage laboratory (Dairy One Forage Lab, Ithaca, NY, USA) using NIR and wet chemistry methods. The feed compositions are provided in Table 1.

Table 1 Chemical composition* of forages expressed on a dry matter basis

	Alfalfa	Grass
Dry matter, %	91.1	90.8
Crude protein, %	18.2	17.0
NDF, %	49.7	59.7
ADF, %	35.9	34.0
Lignin, %	7.0	4.4
Fat, %	2.0	3.4
Ash, %	8.7	10.0
Non-fibre carbohydrate†, %	26.0	15.4
Non-structural carbohydrate†, %	12.9	12.9
Starch, %	2.7	1.8
Calcium, %	1.30	0.49
Phosphorus, %	0.20	0.35
Potassium, %	1.32	2.73
Sodium, %	0.12	0.04

*Chemical composition was determined by Dairy One Forage Lab (Ithaca, NY, USA) using NIR (minerals were determined by wet chemistry methods) from two composite grab samplings.
†Fractions determined by calculations. NFC = 100–CP–NDF–Fat–Ash–Bound protein; NSC = Starch + water-soluble.

Experiment 1

Ten adult male alpacas (3+ years old, 65 ± 4 kg BW) were randomly assigned to one of two diets, AH or GH. Because the alpacas were group-fed for this experiment, no statistics were determined. After being acclimated to the diet for 30 days, the alpacas were slaughtered by a commercial slaughtering facility 2 h post-feeding. The digestive tract was isolated and removed as quickly as possible after slaughter and a total weight taken. The tract was then divided into the stomachs (compartment 1–3, C1–3), duodenum, jejunum, ileum, caecum and large intestine with ligatures. Digesta from the C1 and caecum were evacuated, weighed, and subsamples were taken for VFA analysis. Each sub-tract section was weighed, and digesta samples from each section were taken and measured for pH, DM and neutral detergent fibre (NDF). Immediately upon collection, digesta pH was determined using a pH meter (#209, Hanna Instruments, Smithfield, RI, USA). Samples were dried at 60 °C for 48 h or until a constant weight was obtained to determine dry matter. Duplicate digesta sample aliquotes were collected from the C1 and caecum sites and placed in vials, centrifuged at 2000 *g* for 20 min. A 1-ml aliquot of the supernatant was mixed with 200 ul of formic acid/metaphosphoric acid (1:2 ratio) and frozen for future analysis (Filípek and Dvořák, 2009). The samples were analysed using gas chromatography for VFA composition and concentration. A

mean of the duplicates was used for statistical analysis.

Experiment 2

This experiment is to provide preliminary data for future research. Four adult male alpacas (3+ years old, 60 ± 3 kg BW) fitted with C1 fistula were also divided into two groups: one group fed AH and the other GH. The alpacas were acclimated to the forage diets for 30 days prior to initiating sampling protocol as outlined. Compartment 1 samples were drawn at 1, 3, 6, 9, 12, 18 and 24 h post-feeding. The sampling occurred once for each alpaca. Samples consisted of drawing 20 ml of C1 fluid through the fistula from the anterior-caudal, and both from lateral-caudal and the posterior-caudal regions of C1 using a rumen sampler tube. The three samples were then pooled into one sample for measurements. The pH was measured on each sample immediately after collected. A 1-ml aliquot was prepared for VFA analysis as outlined above.

Sample analysis

Neutral detergent fibre was determined on duplicate samples using the Ankom fibre analyzer (A200; Ankom Technologies, Macedona, NY, USA). Volatile fatty acids were determined on a 6890 GC System gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). An Agilant Innowax capillary column was used, 30 m × 250 μm × 0.25 μm.

Statistical analysis

Experiment 1

Statistical analysis of digestive tract, digesta weights and pH was made using a linear model with forage as fixed effect. Forage, site and the interaction were fixed effects for the analysis of the VFA data. The SAS (SAS, Inst., Cary, NC, USA) PROC GLM was used for all calculations. Level of significance was set at $p \leq 0.05$. Least-squares means for forage and site were determined using unadjusted *t* tests.

Experiment 2

Dry matter intake data were analysed using a linear mixed model with forage, day and the forage × day as the main effects. The diurnal VFA and pH data were analysed using a linear mixed model with forage, time, and forage × time interaction as main effects with time treated as a repeated measure (Littell et al., 1998). The SAS (SAS, Inst.) procedure MIXED was used for all calculations, and a probability of $p < 0.05$ was considered significantly different. Least-squares means for levels of the forage × time factors were calculated and compared using unadjusted *t* tests.

Results

Averaged DM intake over the acclimation period for EXP 1 was 1450 and 1375 g/day for AH and GH forages respectively. Feed intake for EXP 2 was not different, and mean intake was 1422 and 1351 g/day (±91 SEM) for AH and GH respectively.

Experiment 1

The results of the data collected from EXP 1 are presented in Table 2. There were no differences for whole digestive tract weights between the two forage groups. This trend continued on for the weights of the subtract sections. Dry matter and NDF mass of the digesta obtained from the C1 and caecum were not different between the forages.

The pH (Table 2) increased from 6.59 and 6.86, for AH and GH, respectively, as the digesta passed through the tract until it reached the jejunum (7.68 and 7.63 for AH and GH). The pH along the remainder of the tract was lower for both AH and GH at the caecum and large intestine ($p < 0.05$) than the jejunum

Table 2 Gastrointestinal tract parameters of alpacas* fed alfalfa or grass hay determined 2 h post-feeding

	Alfalfa	Grass	SEM
Whole tract†, g	15 847	17 107	1083
Foregut sub-tract†, g	11 863	13 687	929
Small intestine sub-tract†, g	1707	1570	86
Caecum sub-tract†, g	736	569	118
Large intestine sub-tract†, g	1183	1544	243
Compartment 1 digesta, g‡	1215	1382	90
Caecum digesta, g‡	102	40	85
Compartment 1 digesta NDF, g‡	694	792	46
Caecum digesta NDF, g‡	48	22	49
pH			
Compartment 1	6.59[a]	6.86[a]	0.12
Duodenum	6.28[b]	6.73[a]	0.15
Jejunum	7.68[c]	7.63[c]	0.11
Ileum	7.84[c]	7.93[c]	0.10
Caecum	7.28[e]	7.52[d]	0.06
Large intestine	7.39[de]	7.47[de]	0.05

[abcde]Row or column means with differing superscript are different at $p < 0.05$. Variable means without superscripts are not different.
*n = 5 male alpacas (3+ years old, 65 ± 4 kg BW) for each forage.
†Includes tract tissue and digesta content.
‡Dry matter digesta content.

and ileum, but were not as low as the C1 and the duodenum. Duodenal and caecal pH were different ($p < 0.05$) between the two forage treatments, but the other sites showed no differences between forages.

Volatile fatty acid concentrations (Table 3) were different between forages ($p < 0.05$) and site ($p < 0.05$). Acetate concentration was greatest for both forages and sites, followed by propionate and butyrate. Total VFA concentration is 170% greater in the C1 versus the caecum. Acetate, propionate and butyrate are 130, 295 and 581% greater in the C1 versus the caecum ($p < 0.05$). Although the concentration was greater between forage and sites, the VFA proportions were only different between the C1 and caecum ($p < 0.01$). Total VFA mass was determined based on the fluid fraction of the site digest and concentration of VFA for the site. Differences ($p < 0.05$) were noted between sites, but not between forages.

Experiment 2

Compartment 1 pH (Table 4) ranged between 6.9 and 7.2 for both forage treatments and was not different for time or forage. Total VFA concentrations (Table 4) showed a difference ($p < 0.05$) between the 12 h (82.9 mM) and the 24 h (53 mM) for AH. No time differences were noted for GH, but a numerical peak was noted at 18 h (85.5 mM). Acetate, propionate and butyrate proportions were not different across time or between forage treatments. The Ac/Pr ratio was different ($p < 0.05$) for AH between the 18 h time and all the other times. There were no Ac/Pr ratio differences for GH.

Table 3 Volatile fatty acid profile of the compartment 1 (C1) and caecum from alpacas* fed alfalfa or grass hay determined 2 h post-feeding

	Forage				
	Alfalfa		Grass		
	C1	Caecum	C1	Caecum	SEM
Concentration, mM					
Acetate	81.9^{c}	34.6^{a}	60.2^{b}	23.0^{a}	5.6
Propionate	17.8^{c}	4.5^{a}	12.3^{b}	3.9^{a}	1.4
Butyrate	10.9^{b}	1.6^{a}	6.6^{b}	0.7^{a}	0.8
Total VFA	110.6^{c}	40.7^{a}	79.1^{b}	27.6^{a}	7.2
Ac/Pr†	4.8^{a}	8.2^{b}	5.0^{ab}	6.0^{ab}	0.9
Proportion, %					
Acetate	74.0^{a}	76.2^{a}	84.8^{b}	83.7^{b}	1.1
Propionate	16.1^{b}	15.6^{b}	11.4^{a}	14.1^{ab}	1.1
Butyrate	9.9^{d}	8.2^{c}	3.8^{b}	2.2^{a}	0.01
Total VFA mass, g					
Acetate	41.3^{b}	17.5^{a}	40.7^{b}	14.6^{a}	4.6
Propionate	10.0^{b}	2.5^{a}	9.3^{b}	2.8^{a}	1.1
Butyrate	7.3^{b}	1.1^{a}	5.8^{b}	0.5^{a}	0.7

VFA, volatile fatty acids.

^{abcd}Row means with differing superscripts are different at $p < 0.05$.

*n = 5 male alpacas (3+ years old, 65 ± 4 kg BW) for each forage.

†Ac/Pr, acetate/propionate ratio.

Table 4 Diurnal changes in volatile fatty acid profile of compartment 1 (C1) in alpacas* fed alfalfa or grass hay

	Sample Time (h)						
	1	3	6	12	18	24	SEM
pH							
Alfalfa	7.22	7.15	7.05	7.0	7.23	7.23	0.09
Grass	7.02	7.13	7.07	6.95	6.93	7.01	
Total volatile fatty acids, mM							
Alfalfa	56.2^{ab}	61.5^{ab}	64.6^{ab}	82.9^{bc}	64.4^{ab}	53.0^{a}	7.78
Grass	63.6^{abc}	67.8^{abc}	69.9^{abc}	76.8^{bc}	85.5^{c}	64.2^{abc}	
Acetate, %							
Alfalfa	77.3	77.6	78.0	77.6	80.1	77.1	1.23
Grass	75.4	75.4	75.7	76.8	76.8	76.1	
Propionate, %							
Alfalfa	16.0	15.8	15.9	15.5	12.8	15.5	1.14
Grass	17.5	17.3	16.9	15.8	15.9	16.6	
Butyrate, %							
Alfalfa	6.7	6.6	6.2	6.9	7.4	7.4	0.43
Grass	7.1	7.3	7.4	7.5	7.4	7.3	
Ac/Pr†							
Alfalfa	4.9^{a}	4.9^{a}	4.9^{a}	5.0^{ab}	6.9^{b}	5.0^{ab}	0.63
Grass	4.3^{a}	4.4^{a}	4.5^{a}	4.9^{a}	4.8^{a}	4.6^{a}	

^{abc}Row and column means with differing superscripts are different at $p < 0.05$. Variable means without superscripts are not different.

*n = 2 male alpacas (3+ years old, 60 ± 3 kg BW) for each forage. Means were determined from a sample pool of three sampling sites within C1.

†Ac/Pr, acetate/propionate ratio.

Discussion

In ruminants, microbial fermentation occurs in the rumen and in the caecum (Elsden et al., 1946). Bergman (1990) summarized the production and absorption of VFA from the rumen stating that all species, ruminant and non-ruminant, produce and absorb VFA from the lower digestive tract gaining some additional energy benefit. Engelhardt and Sallman (1972) showed that VFAs were absorbed in large quantities from the C1 and C2 in guanaco, especially associated with the glandular saccule region. They also showed that in this region, VFA absorption occurred more rapidly. Engelhardt et al. (1979) found in llamas that absorption of water, sodium and VFAs also occurred in the C3. Volatile fatty acid concentrations are affected by rumen volume, dilution rates and absorption rate, and these three factors account for species

differences between camelids and ruminants (Elsden et al., 1946; Abbas et al., 1995).

The purpose of these experiments was to determine the differences in VFA concentration and composition in alpacas fed alfalfa and grass hays and to determine the differences in VFA profile between the C1 and caecum. Our intent was to characterize aspects of digesta passing through the alpaca digestive tract, while not affecting the integrity of the C1 and caecum VFA samples. The alpacas in EXP 1 were slaughtered 2 h post-feeding. The VFA samples collected from C1 digesta were from the fluid phase after the C1 content was evacuated. Thus, the sample location within C1 cannot be specified, only to say it came from the fluid phase. Caecal VFA samples were collected from the evacuated caecal digesta after it was mixed, so it is representative of the entire caecal content. Vallenas and Stevens (1971) expressed concern over these two factors when comparing their data with those of others (Williams, 1963) who could not specify where samples were collected from or the timing associated with intake. We attempted to stay consistent in both experiments as samples were collected to decrease the variability between time post-feeding and content of collections.

Total VFA concentrations in the C1 and caecum of our alpacas were 111 and 41 mM for AH and 79 and 28 mM for GH with caecal VFA accounting for 26% of total C1-caecal VFA concentrations. Faichney (1969) demonstrated that approximately 5.3% of digestible energy requirement in sheep, fed grass cubes, was met by caecal VFA production. Ulyatt et al. (1975) concluded that total VFA production by the caecum accounted for 8.6–16.8% in sheep. In an earlier study, Faichney (1968) showed differences in total VFA concentration between the rumen and caecum of sheep where the concentration for the rumen rose from 29 to 130 mM then back to 31 mM over a 24-h period. DeGregorio et al. (1982) showed total caecal concentrations of 46.3 mM for sheep fed an alfalfa/orchard grass hay with a pH of 7.2 in relation to 7.8 in the ileum. A similar pattern was evident in our alpacas, although more pronounced in alpacas fed AH. DeGregorio et al. (1982) showed a proportion of 77:16:6 for acetate:propionate:butyrate for caecal VFA. Under the conditions of our study, the caecal proportions were 76:16:8 for AH and 84:14:2 for GH for acetate: propionate:butyrate respectively. The A:P ratio was 8.2 and 6.0 for AH and GH. Caecal total VFA did not show a distinct rise-and-fall pattern as the rumen and ranged from 47 to 96 mM over the same 24-h period (Faichney, 1968). The concentrations of total VFA between the rumen and caecum in sheep and our C1 to caecum in alpacas are similar. Caecal contribution appears higher in our alpacas, leading us to believe that alpacas do obtain VFA energy from caecal fermentation and absorption as ruminants, but further research is needed to determine whether this is the case.

The VFA profile of the alpacas in EXP 1 was different according to the type of forage fed to the alpacas. There was a shift towards acetate at the expense of both propionate and butyrate for GH C1 and butyrate for GH caecum. There was no difference in the VFA profile for EXP 2. This may be due to the sampling, where for EXP 1, the entire C1 content was mixed and the sample taken from the mixture. For EXP 2, the samples were drawn from the same site, not including VFA production in the mat material. Ramos et al. (2009) compared alfalfa- and grass hay-based diets switched from a 70:30 forage:concentrate ratio to 30:70 ratio in sheep. The concentrate was a combination of rapidly fermenting and slow-fermenting grains. They concluded that forage type did not affect differences between fermentation profile, but the abrupt increase in fermentable substrate did change acetate and butyrate concentrations. The more highly fermentable fraction of alfalfa may account for the shift in EXP 1.

Total VFA, measured over the 24-h period, increased from 56 to 83 mM and 64 to 86 mM for AH and GH respectively. In Faichney's sheep study (1968), total VFAs peaked at around 2 h, whereas in our alpacas, the peak occurred much later at approximately 12–18 h. Abouheif et al. (2010) showed with once-a-day feeding of a high concentrate diet in lambs resulted in total VFA concentrations peaking around 10 h post-feeding. Our alpacas were fed *ad libitum* once daily, so they did not consume their feed within the first 2 h as Faichney's sheep did. This may account for the extended peak time, but other factors may be responsible, including rapid absorption of VFAs (Rubsamen and Engelhardt, 1978; Engelhardt and Sallman, 1972) and slower passage of solid-phase material (Clemens and Stevens, 1980). Diurnal VFA proportions between the two forages did not change across time. Again, alpacas in our study were fed *ad libitum*, so rapid consumption did not occur. Consumption by the alpacas was more evenly spaced throughout a 24-h period. The ratios of 77:16:7 for AH and 75:18:7 for GH, acetate:propionate:butyrate, are similar to those found in the literature (Rumsey et al., 1970; Cantalapiedra-Hijar et al., 2009; Estrada et al., 2010) for forage-only or high forage diets.

It cannot be disputed that VFAs are an important component of the energy needs of the alpaca.

The ratios of the three main VFAs, measured from the liquid phase, are similar to those of other ruminant species and show similar increases in diurnal patterns. The extent to which each of these contribute to the energy needs (i.e. propionate to gluconeogenesis) will require further research.

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