Pigeon peas as a supplement for lactating dairy cows fed corn silage-based diets

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ABSTRACT

Holstein rumen-cannulated cows [$n = 7$; initial body weight (BW) 640.56 ± 71.43 kg] were fed a corn silage basal diet with 1 of 3 concentrates (C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas). Cows were randomly assigned to treatments in a replicated 3×3 Latin square and individually fed using Calan gates. Each experimental period was 21 d with 7 d for adaption and 14 d for sample collection. Ruminal fluid samples were taken the last day of each experimental period and analyzed for pH, ammonia, long-chain fatty acids, and volatile fatty acids (VFA). Consecutive a.m. and p.m. milk samples were taken during the last 2 wk of the 21-d period and analyzed for fat, protein, long-chain fatty acids, and somatic cell count. Dry matter intake (kg/d) was reduced during the second period and was greater for P10 diets. Milk protein was greater for cows fed P20 compared with P10. Energy-corrected milk was greater for cows fed the control diet compared with P10. Treatment had no effect on milk yield. Ruminal fluid pH decreased over sampling times; however, pH remained at or above 5.5. Diets did not affect ruminal fluid pH; however, pH was different for sampling periods. Ruminal ammonia decreased until 8 h postfeeding at which time it peaked consistent with changes in ammonia concentrations that usually peak 3 to 5 h postfeeding on diets high in plant proteins. Dietary treatments altered ruminal fluid VFA with reduced concentrations of acetate and greater concentrations of propionate for control diet, resulting in reduced acetate:propionate ratio. Isobutyrate exhibited an hour by treatment interaction, in which isobutyrate decreased until 8 h postfeeding and then tended to be greater for P10 than for other treatments. Animals fed the P10 diet had greater concentrations of ruminal isovalerate. Ruminal *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid (CLA) isomers were not af-

ected by dietary treatments. The P10 diet had greatest ruminal synthesis of *cis*-9,*trans*-11, but control cows had greatest ruminal synthesis of *trans*-10,*cis*-12. Milk CLA isomers were similar among treatments. Trends were observed for greater *cis*-9,*trans*-11 and *trans*-10,*cis*-12 for the P10 diet. Pigeon peas may be used as a protein supplement in dairy diets without affecting milk production, dry matter intake, or ruminal environment when they replace corn and soybean meal.

Key words: dairy, pigeon pea, fatty acid, milk

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.)], a drought-tolerant legume, is an important grain crop grown in tropical and subtropical regions (Nene and Sheila, 1990). Pigeon peas typically contain 9.9% moisture, 19.5% protein, 1.3% fat, 65.5% carbohydrate, 1.3% fiber, 3.8% ash, 0.16% Ca, 0.29% P, and 0.015% Fe (Duke, 1981; DM basis). Steers fed pigeon peas or pearl millet grain supplements with Tifton 85 bermudagrass hay had greater apparent digestion of CP for pigeon pea diets (Hill et al., 2006). The ADG of yearling heifers tended to be greater when they were fed pigeon peas compared with corn/soybean meal, whole cottonseed, or corn gluten feed (Corriher et al., 2007).

Pigeon peas contain proteinase inhibitors and tannins, which have been shown to reduce performance of pigs and poultry (Visitpanich et al., 1985; Tangtaweewipat and Elliot, 1989). Nevertheless, pigeon peas could replace soybean meal and constitute approximately half the intake of growing sheep without adverse effects (Cheva-Isarakul, 1992). Oil in pigeon peas contains 5.7% linolenic acid, 51.4% linoleic, 6.3% *trans*-vaccenic, and 36.6% saturated fatty acids (Duke, 1981). Despite having only approximately 2% total fat, the high concentration of linoleic acid (51.4%) suggests that pigeon peas could be an important dietary factor for increasing conjugated linoleic acid (CLA) concentrations in ruminant products.

Dietary lipids are extensively altered by ruminal bacterial metabolism, and one of the major changes is

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Table 1. Composition of the different total diets for dairy cows

Item	Diet ¹		
	C	P10	P20
Ingredient, % DM basis			
Corn silage	36.51	36.51	36.51
Alfalfa hay	7.69	7.69	7.69
Brewers grain	8.65	8.65	8.65
Experimental concentrate mixes, ² % DM basis			
Pigeon pea	0	9.99	19.98
Cottonseed	5.76	5.76	5.76
Soybean hulls	8.65	6.72	5.19
Ground corn	12.30	6.92	1.15
Soybean meal	6.72	4.03	1.34
Megalac ³	1.34	1.34	1.34
Concentrate premix ⁴	12.38	12.39	12.39

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

²Ingredients were mixed together before blending the TMR.

³Arm & Hammer, Princeton, NJ.

⁴Concentrate premix = citrus pulp, urea, Prolak, bicarbonate sodium, Dynamate, limestone, magox, salt, potassium carbonate, phosphate defluorinate, Zinpro Availa-4 (Zinpro, Eden Prairie, MN), Diamond V XP (Diamond V, Cedar Rapids, IA), Minvit2.

biohydrogenation of polyunsaturated fatty acids. Diet can markedly affect bacterial population and ruminal microbial processes; therefore, diet and nutrition may have major effects on fat content and fatty acid composition of milk in many species, including ruminants. Research indicates that changes in the ruminal environment will lead to changes in microbial activity that correspond to altered end-product formation. Because the ruminal environment is subject to change, it is possible that these changes will have an effect on CLA formation by ruminal microbial population (Martin and Jenkins, 2002).

Pigeon peas, which contain up to 60% unsaturated fatty acid, could increase the concentration of CLA in milk. Therefore, our objective was to determine the effect of pigeon pea supplementation on milk production and ruminal characteristics in dairy cows fed a TMR with 0, 10, and 20% pigeon peas.

MATERIALS AND METHODS

Cows and Sampling Methods

Seven primiparous ruminally cannulated Holstein cows (640.6 ± 71.4 kg of BW) were used in a 3×3 Latin square with 21-d periods. Cows were housed in a free-stall barn and averaged 89 ± 6.7 DIM and 40.1 ± 4.0 kg/d of milk at the beginning of the trial. Treatment diets consisted of 3 levels of pigeon peas, including 0 (control), 10% (**P10**), or 20% (**P20**) pigeon peas. Pigeon peas were incorporated into rations (Table 1) and individually fed once daily behind Calan doors (American Calan Inc., Northwood, NH). Treatment

diets were balanced for CP and fiber content, and they were isocaloric and isonitrogenous. Each cow was offered each treatment for 21 d, and ruminal fluid samples were taken on the last day of the experimental period and analyzed for pH, ammonia, VFA, and long-chain fatty acids (**LCFA**). Cows were milked twice daily at approximately 0400 and 1500 h. Consecutive a.m. and p.m. milk samples were taken from each cow the last 2 wk of the 21-d period and analyzed for fat, protein, SCC, and LCFA. Samples of diets and ingredients were taken every 14 d and frozen at -20°C for subsequent analyses. All cattle were managed under procedures approved by the University of Georgia Animal Care and Use Committee Guidelines. Diets were mixed daily and individually fed to cows at 1200 h daily, with amounts of feed offered and refused recorded daily. Individual milk yield was recorded electronically (Alpro, DeLaval, Kansas City, MO) at each milking and summed for each day. Cows were trained to use Calan gate feeders (American Calan Inc.) and then fed supplement treatments.

Chemical Analyses of Feeds and Samples

Forage and silage samples were lyophilized, ground through a Wiley mill (1-mm screen), and stored at -20°C for subsequent chemical analyses. Organic matter was measured as the weight loss following combustion for 8 h at 500°C . Neutral detergent fiber and ADF were sequentially determined using an Ankom 200 fiber extractor (Ankom Technology, Fairport, NY) according to the method of Van Soest et al. (1991). Crude protein concentration was determined by the combustion

Table 2. Mean DM chemical and fatty acid composition of the total diets for dairy cows

Item	Corn silage	Pigeon peas	Diet ¹		
			C	P10	P20
Chemical composition, % DM basis					
DM	41.2	88.4	52.4	52.3	52.3
CP	11.0	24.0	19.6	18.2	19.1
NDF	26.6	12.8	28.3	26.3	24.9
ADF	14.3	12.1	18.8	19.1	17.9
Total FA	3.62	2.5	4.9	4.8	4.6
FA composition, mg/100 mg of FA					
C14:0	0.70	0.02	2.02	5.16	2.20
C16:0	27.87	0.23	67.57	199.08	88.02
C18:0	3.85	0.043	12.51	28.71	12.03
C18:1	25.09	0.75	8.89	15.47	13.99
C18:2	39.26	0.57	46.09	33.80	40.19
C18:3	2.00	0.046	22.95	14.02	48.37
Others ²	1.23	0.019	7.01	5.30	5.70
Unidentified	0.0	0.8	30.20	22.79	24.48

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

²Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, and C22:0.

method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI). On the last day of the experimental period, ruminal fluid samples were collected at -2, 0, 2, 4, 6, 8, and 10 h postfeeding. Approximately 50 mL of ruminal fluid was collected and strained through 3 layers of cheesecloth, analyzed for pH and ammonia, and stored frozen for subsequent analyses of LCFA. A 40-mL subsample was strained through 3 layers of cheesecloth and immediately mixed with 10 mL of metaphosphoric acid (25% wt/vol). The sample was frozen for later analyses of VFA (Erwin et al., 1961). These samples were later thawed and centrifuged at $10,000 \times g$ for 10 min and the supernatant collected for VFA analysis using a Hewlett-Packard gas chromatograph (GLC; Varian 3400 Instrument Group, Hewlett-Packard Company, Avondale, PA) fitted with a nitroterephthalic acid modified polyethylene glycol megabore column (30 m $\times$ 0.53 mm i.d. with 1- μ m film; J&W Scientific, Folsom, CA). Initial oven temperature was 130°C for 7 min, and helium flow was 7 mL/min. The oven temperature was increased at the rate of 2.9°C/min over 7 min to a final

temperature of 150°C. Helium flow was increased to 9 mL/min. Airflow was 400 mL/min, and hydrogen flow was 29 mL/min. Heptanoic acid was used as an internal standard.

Total fatty acid percentage and fatty acid profile were determined for forage and supplement samples. Fatty acids were methylated following methods described by Park and Goins (1994) and separated by GLC procedures, as described by Duckett et al. (2002). For wet tissue lipid, 1 g of milk lipids was extracted. Milk lipid extracts containing approximately 2 mg of total lipids, based on the calculated percentage lipids on a wet tissue basis, were transmethylated (Park and Goins, 1994). Fatty acid methyl esters were analyzed using an HP6850 (Hewlett-Packard, San Fernando, CA) gas chromatograph equipped with an HP7673A (Hewlett-Packard) automatic sampler. Separations were accomplished using a 100-m Sp2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20- μ m film thickness) according to Duckett et al. (2002). Column oven temperature increased from 150 to 160°C at 1°C/min,

Table 3. Dairy cow DMI and milk composition when fed supplemental pigeon peas

Item	Diet ¹			SE	P <
	C	P10	P20		
DMI, kg/d	22.92	23.38	22.87	1.40	0.04
Fat, %	3.24	2.92	3.29	0.12	NS
Protein, %	2.83 ^a	2.57 ^b	2.90 ^a	0.10	0.05
ECM, %	35.02 ^a	29.41 ^b	33.37 ^a	3.67	0.02
lnSCC ²	1.72	1.60	1.38	0.10	0.07

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

²lnSCC = natural log of SCC.

Figure 1. Milk production of dairy cows fed supplemental pigeon peas by dietary treatments ($P < 0.34$). P10 = 10% pigeon peas; P20 = 20% pigeon peas. Color version available in the online PDF.

Figure 3. Ruminal fluid pH of dairy cows fed supplemental pigeon peas by dietary treatments ($P < 0.59$; SE = 0.07). C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas. ^{a,b}Means with different letters differ ($P < 0.05$). Color version available in the online PDF.

from 160 to 167°C at 0.2°C/min, from 167 to 225°C at 1.5°C/min, and then held at 225°C for 16 min. The injector and detector were maintained at 250°C, with a sample injection volume of 1 µL. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl heptacosanoic (C27:0) acid into each sample during methylation and expressed as g/100 g of tissue.

Statistical Analysis

Data were statistically analyzed as a replicated 3 × 3 Latin square using the MIXED procedure of SAS (SAS Institute, 2003) with animal as the experimental unit and dietary treatment and experimental period blocked. Ruminal pH, ammonia, and VFA data were subjected to ANOVA using PROC MIXED procedures

of SAS (SAS Institute, 2003). The model included cow, hour, period, treatment, hour × treatment, and error.

RESULTS AND DISCUSSION

Pigeon peas replaced corn and soybean meal in dairy diets at 2 levels (P10 = 10% pigeon peas and P20 = 20% pigeon peas; Tables 1 and 2). Experimental diets contained similar concentrations of CP and NDF (Table 2). The P20 diet contained slightly lower concentrations of NDF and ADF, reflecting the lower fiber and greater starch concentrations of the pigeon peas. Average concentrations of DM, CP, and NDF for experimental diets were 52.3, 18.9, and 26.5% of DM, respectively. Concentrations of NDF were adequate to maintain rumen function. Pigeon peas are known to have antinutritional factors such as proteinase inhibitors and tannins. The proteinase inhibitors are those that inhibit trypsin and chymotrypsin activities. This can lead to reduced protein availability and amino acid absorption from the small intestine (Dixon and Hosking, 1992). Tannins are bitter plant polyphenols that either

Figure 2. Ruminal fluid pH of dairy cows fed supplemental pigeon peas by treatment periods ($P < 0.0008$; SE = 0.07). ^{a,b}Means with different letters differ ($P < 0.05$). Color version available in the online PDF.

Figure 4. Effect of sampling time on ruminal fluid pH relative to time of feeding for dairy cows fed supplemental pigeon peas ($P < 0.002$; SE = 0.08). ^{a,b}Means with different letters differ ($P < 0.05$). Color version available in the online PDF.

Figure 5. Effect of sampling time on ruminal fluid ammonia concentration relative to time of feeding for dairy cows fed supplemental pigeon peas (g/L; $P < 0.002$; SE = 1.07). Color version available in the online PDF.

bind and precipitate or shrink proteins, amino acids, and alkaloids. Tannins may reduce intake by decreasing palatability and by negatively affecting digestion (Reed, 1995). Pigeon peas have been shown to reduce growth performance of pigs and poultry as a result of proteinase inhibitors and tannins (Visitpanich et al., 1985; Tangtaweewipat and Elliot, 1989). No adverse effects were seen when pigeon peas replaced soybean meal and constituted approximately half the intake of growing sheep (Cheva-Isarakul, 1992). Dry matter intake for treatment periods 1, 2, and 3 in the current study were 23.39, 22.76, and 23.20 kg/d, respectively. The DMI (kg/d) was reduced during the second period, and was greater for P10 diets ($P < 0.05$; Table 3). Increasing pigeon pea supplementation did not decrease DMI for cows.

The SCC in milk samples tended to be greater for cows on the control diet compared with the P10 diet ($P > 0.10$; Table 3). General agreement rests on the values of <100,000 cells/mL for uninfected cows, and >300,000 cells/mL for cows infected with significant pathogens (Smith, 1996). During treatment periods, SCC was never greater than 300,000 cells/mL; therefore,

the possible occurrence of a pathogen never affected experimental results. Diets do not generally affect SCC, but environment may affect SCC in dairy cows. The environment of tester animals was never altered during the experimental period. Milk protein percentage was greater for cows on P20 compared with P10 ($P < 0.05$; Table 3). Energy-corrected milk accounts for the true protein content of milk and it was calculated using the equation: ECM = $(0.3246 \times \text{kg of milk}) + (12.86 \times \text{kg of fat}) + (7.04 \times \text{kg of protein})$. Milk ECM was greater for cows when on the control diet compared with the P10 diet ($P < 0.05$; Table 3). The cows fed the control diet used more tissue energy to support milk production. Treatment had no effect on milk production ($P > 0.10$; Figure 1), but control cows tended to have greater milk production.

Figures 2, 3, and 4 show effects of treatment period, diet, and sampling time on ruminal fluid pH. Ruminal fluid pH decreased during the 12-h period of sampling ($P < 0.01$; Figure 4); however, the pH never decreased below 5.5. Treatment did not affect pH ($P > 0.10$; Figure 3). With typical diets fed to lactating dairy cows, the pH of ruminal fluid is usually between 5.5 and 6.5, whereas high forage diets support a ruminal fluid pH in the range from 6.2 to 7. Inclusion of pigeon peas in the TMR did not have an effect on ruminal pH; it remained between 5.5 and 6.5 throughout the sampling interval. Cellulose digestion is inhibited when pH decreases below 6 (Owens and Goetsch, 1988). Depressed fiber digestion has been attributed to low ruminal pH associated with feeding high levels of concentrate (Orskov and Fraser, 1975). They attributed this to the sensitivity of cellulolytic microbes to low ruminal pH. Depressed starch digestion has been attributed to an increased rate of flow through the rumen or small intestine, providing less time for digestion and absorption of starch (Zinn and Owens, 1980). With continuous feeding of high concentrate diets, animals tend to consume many meals per day and ruminal pH never increases sufficiently to

Figure 6. Effect of treatment period on ruminal fluid ammonia of dairy cows fed supplemental pigeon peas (g/L; $P < 0.85$; SE = 0.08). Color version available in the online PDF.

Figure 7. Ruminal fluid ammonia of dairy cows fed supplemental pigeon peas by dietary treatments (g/L; $P < 0.80$; SE = 0.08). P10 = 10% pigeon peas; P20 = 20% pigeon peas. Color version available in the online PDF.

Table 4. Ruminal fluid VFA (as % of total VFA) of dairy cows fed varying levels of supplemental pigeon peas

Ruminal VFA	Diet ¹			SE	P <
	C	P10	P20		
Acetate	0.60 ^b	0.61 ^a	0.61 ^a	0.004	0.03
Propionate	0.24 ^a	0.23 ^b	0.23 ^b	0.005	0.02
Isobutyrate ²	—	—	—	—	—
Butyrate	0.112	0.114	0.114	0.002	0.85
Isovalerate	0.014 ^b	0.016 ^a	0.014 ^b	0.0008	0.05
Valerate	0.021	0.021	0.020	0.0008	0.29
Total, mMol	95.84	98.50	100.18	1.26	0.57
Acetate:propionate	2.55 ^b	2.74 ^a	2.66 ^a	0.19	0.03

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

²Isobutyrate exhibited an hour by treatment interaction; see Table 5. No other hour by treatment interactions occurred.

initiate substantial cellulose digestion. The time after feeding when pH is lowest is usually between 0.5 and 4 h after a meal (Zinn and Owens, 1980). The lowest pH in the present study occurred at 8 to 10 h after the a.m. feeding (Figure 4); however, pH did decrease at 4 h postfeeding. This decrease in pH reflects the balance between rates of acid production, input of dietary buffers from saliva, and presence or release of buffers or bases from the feed.

When pH falls from 7 to between 5 and 5.5, many ruminal microbes cease growing, despite an ability to survive even greater concentrations of H^+ . Most acids, such as lactic acid, that inhibit microbes at high concentrations are more effective at high concentrations and when pH is low. If microbes are inhibited by pH, a reduced pH could ultimately decrease biohydrogenation in the rumen. Decreased rumen pH often results in bacterial population shifts and consequent changes in the pattern of fermentation end-products (Van Soest, 1994). Leat et al. (1977) provided evidence showing that changes in ruminal bacterial populations are associated with modifications in the biohydrogenation pathways

consistent with the altered *trans*-octadecenoic acid profile found in ruminal digesta and tissue lipids.

Figures 5, 6, and 7 show effects of sampling time, treatment period, and diet on ruminal fluid ammonia. Ruminal ammonia decreased linearly during the first 6 h postfeeding, but it increased at 8 h postfeeding to concentrations similar to those at 2 h before feeding (Figure 5). Ammonia concentrations usually peak about 1 to 2 h after a meal, whereas in diets high in plant proteins, high-level peaks are usually at 3 to 5 h postfeeding. Ammonia concentrations increased 2 h postfeeding and decreased from the 2 h concentration at 4 and 6 h postfeeding. At 8 h postfeeding, a second increase in ammonia concentration was observed, which probably resulted from a second meal taken by the cows after they returned from the 1500 h milking. Ammonia disappears from the rumen pool because of uptake by microbes, absorption through the rumen wall, and flushing to the omasum. Changes in any of these factors will alter the ammonia concentration in the rumen. The decrease in ammonia can be attributed to an increase in ammonia uptake by microbes or to an

Table 5. Isobutyrate (% of total VFA) $\times$ treatment interaction in ruminal fluid of dairy cows fed supplemental pigeon peas¹

Sample time	Diet ²			SE
	C	P10	P20	
-2 h	0.01	0.01	0.008	0.002
0 h ³	—	—	—	—
2 h	0.008	0.01	0.007	0.002
4 h	0.008	0.007	0.008	0.002
6 h	0.005	0.005	0.006	0.002
8 h	0.003	0.004	0.007	0.002
10 h	0.004	0.008	0.006	0.002

¹Sampling time $\times$ treatment interaction ($P < 0.01$).

²C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

³0 h = time of feeding; no samples were taken at 0 h.

Table 6. Ruminal fluid VFA (as % of total VFA) at different sampling times relative to feeding for dairy cows fed supplemental pigeon peas

Ruminal VFA	Sampling time						SE	<i>P</i> <	
	−2 h	0 h	2 h	4 h	6 h	8 h			
Acetate	0.61		0.62	0.62	0.62	0.59	0.60	0.01	0.13
Propionate	0.23 ^b		0.23 ^b	0.22 ^b	0.24 ^a	0.25 ^a	0.25 ^a	0.006	0.0008
Butyrate	0.11		0.11	0.12	0.11	0.12	0.11	0.008	0.52
Isovalerate	0.016		0.014	0.015	0.012	0.014	0.015	0.002	0.68
Valerate	0.02		0.02	0.02	0.02	0.021	0.021	0.0008	0.86
Total, mMol	97.69 ^a		113.06 ^a	82.14 ^b	91.90 ^b	106.70 ^a	97.55 ^{ab}	13.37	0.05
Acetate:propionate	2.70 ^a		2.76 ^a	2.85 ^a	2.68 ^a	2.46 ^b	2.45 ^b	0.20	0.04

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

increase in microbe numbers. An increase in microbe numbers could modify the biohydrogenation pathways within the rumen.

Volatile fatty acids are produced by specific microbial pathways and absorbed continually from the rumen. Ruminal concentrations represent the balance between rates of production and removal for each VFA, as well as their interconversions. Total ruminal VFA were affected by dietary treatments ($P < 0.05$; Table 4). Acetate was greater in ruminal fluid when cows were fed pigeon peas at both 10% and 20%. The control diet resulted in the greatest concentration of propionate, leading to a reduced acetate:propionate ratio. The control diet resulted in the lowest amount of butyrate but the greatest concentrations of valerate. The acetate:propionate ratio was greatest ($P < 0.05$; Table 4) for the P10 diet and did not fall below 2.5. An acetate:propionate ratio below 2.5 has been associated with milk fat depression in lactating dairy cows (Woodford et al., 1986), a syndrome that has been attributed to increased ruminal synthesis of 18:1 *trans*-10 (Griinari et al., 1998) and CLA *trans*-10,*cis*-12 (Baumgard et al., 2000). The P10 diet resulted in greater concentrations of ruminal isovalerate ($P < 0.05$; Table 4). Isobutyrate

exhibited a sampling time $\times$ treatment interaction ($P < 0.05$; Table 5). The control diet resulted in the lowest levels of isobutyrate, regardless of sampling time in relation to feeding.

The ruminal concentration of propionate increased with time following feeding ($P < 0.01$; Table 6). The VFA concentrations were not different by sampling time ($P > 0.10$). The acetate:propionate ratio decreased linearly from 4 h postfeeding to 10 h postfeeding ($P < 0.05$). The changes in pH and ruminal ammonia occurring 1 to 2 h postfeeding and then again at 8 h postfeeding may have resulted in similar changes in ruminal VFA synthesis.

Trans-vaccenic acid (C18:1 *trans*-11; **TVA**) was not affected by dietary treatments (Table 7; $P > 0.10$). The *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers were not different among dietary treatments ($P > 0.10$; Table 8). The increase in ruminal concentrations of *cis*-9,*trans*-11 is reflective of greater concentrations of linoleic acid found in pigeon peas (Table 2). *Trans*-vaccenic acid was not different among sampling times ($P > 0.10$; Table 9). However, ruminal concentrations of TVA increased linearly from 2 h postfeeding to 6 h and then decreased linearly. The *cis*-9,*trans*-11 increased linearly from 2 h

Table 7. Selected fatty acid composition (% of total fatty acids) of ruminal fluid of dairy cows fed varying levels of supplemental pigeon peas

Long-chain fatty acid	Diet ¹			SE	P <
	C	P10	P20		
C14	1.93	2.38	2.16	0.30	0.06
C14:1	3.02	3.46	3.62	0.40	0.31
C16	30.65	31.11	30.83	0.57	0.74
C16:1	0.41	0.43	0.54	0.07	0.08
C18:0	40.99	41.63	41.09	0.90	0.86
C18:1 <i>trans</i> -11	4.43 ^a	4.80 ^a	3.96 ^b	0.46	0.04
C18:1 <i>cis</i> -9	6.74	5.63	6.20	0.74	0.34
<i>cis</i> -9, <i>trans</i> -11	0.21	0.24	0.22	0.04	0.74
<i>trans</i> -10, <i>cis</i> -12	0.30	0.23	0.23	0.11	0.74
Total, mg/g	22.33	21.81	21.64	1.45	0.94

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

Table 8. Fatty acid composition (% of total fatty acids) in ruminal fluid of dairy cows fed supplemental pigeon peas

Long-chain fatty acid	Sampling time							SE	P <
	-2 h	0 h	2 h	4 h	6 h	8 h	10 h		
C14	2.30		2.01	1.97	2.14	2.11	2.42	0.31	0.46
C14:1	3.46		2.98	3.00	3.55	3.39	3.80	0.50	0.65
C16	29.66		31.85	31.37	31.02	30.10	31.15	0.70	0.12
C16:1	0.50		0.59	0.43	0.42	0.46	0.37	0.09	0.37
C18:0	41.40		40.89	40.82	38.84	45.04	40.43	1.25	0.03
C18:1 <i>trans</i> -11	4.56		4.52	4.52	4.52	3.95	4.31	0.50	0.78
C18:1 <i>cis</i> -9	4.43 ^b		7.93 ^a	7.46 ^a	6.46 ^a	5.23 ^b	6.63 ^a	0.90	0.04
<i>cis</i> -9, <i>trans</i> -11	0.19		0.20	0.30	0.25	0.20	0.21	0.05	0.51
<i>trans</i> -10, <i>cis</i> -12	0.20		0.22	0.20	0.42	0.26	0.22	0.13	0.66
Total, mg/g	24.63		20.04	23.62	21.45	21.67	20.17	2.01	0.56

^{a,b}Means within a row with different superscript letters differ ($P < 0.05$).

postfeeding to 6 h and then decreased linearly. The *trans*-10,*cis*-12 increased from 2 h postfeeding to 4 h and then peaked at 8 h.

Concentrations of *trans*-10,*cis*-12 CLA increase under certain dietary conditions, which may include feeding high levels of unsaturated fatty acids (Bauman and Griinari, 2001). The 2 main CLA isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12) in milk fat were not different among treatments in the current trial ($P > 0.10$; Table 9). The *trans*-10,*cis*-12 CLA isomer inhibits the activity of Δ^9 -desaturase (Lee et al., 1998; Bretillon et al., 1999), ultimately reducing endogenous synthesis of *cis*-9,*trans*-11 CLA. An increase in *trans*-10,*cis*-12 could have resulted in the decrease in *cis*-9,*trans*-11 in the P10 and P20 diets (Table 9). Under certain dietary conditions, such as high concentrate, low fiber diets, the profile of CLA can be altered so the concentration of the *trans*-10,*cis*-12 isomer increases in milk fat (Griinari et al., 1999). Diets low in roughage decreased ruminal lipolysis and biohydrogenation (Latham et al., 1972; Gerson and King, 1985). Therefore, it has been suggested that the main ruminal biohydrogenating bacteria are cellulolytic (Latham et al., 1972; Harfoot and Hazlewood, 1997). In addition, Griinari et al. (1998) demonstrated that

an altered rumen environment induced by feeding high concentrate, low fiber diets is associated with a change in the *trans*-octadecenoic acid profile of milk fat. In this situation, *trans*-10 octadecenoic acid replaced *trans*-11 C18:1 as the predominant *trans*-C18:1 isomer in milk fat. Further evidence in support of a specific bacterial *cis*-9,*trans*-10 isomerase is provided by observations that low fiber diets increase the proportion of *trans*-10,*cis*-12 CLA isomer in milk fat (Griinari et al., 1999). The diet is known to strongly influence the CLA content of milk and includes feedstuffs such as pasture, conserved forages, plant seed oils, cereal grains, marine oils and feeds, and animal fat.

Studies with lactating dairy cows have demonstrated that even in herds in which all cows were managed similarly and fed the same diet, there may still be a 3-fold variation in the milk fat content of CLA (Jiang et al., 1996; Kelly et al., 1998a,b). These results suggest that it may be possible to see a difference in LCFA if the amount of pigeon peas was increased in the diet well above 20%. The inclusion rates of pigeon peas at 10% and 20% of the diets were apparently too low to effect significant differences in LCFA in milk or in the rumen environment. Additional research is needed to

Table 9. Fatty acid composition (% of total fatty acids) in milk of dairy cows fed supplemental pigeon peas

Long-chain fatty acid	Diet ¹			SE	P <
	C	P10	P20		
C14	10.97	10.64	10.57	0.35	0.48
C14:1	0.82	0.75	0.73	0.05	0.15
C16	33.90	34.99	36.04	1.35	0.29
C16:1	1.81	2.40	1.70	0.58	0.44
C18	18.76	12.36	13.05	4.41	0.29
C18:1 <i>trans</i> -11	1.99	1.81	1.58	0.28	0.33
C18:1 <i>cis</i> -9	12.10	10.97	11.34	1.90	0.83
<i>cis</i> -9, <i>trans</i> -11	0.48	0.78	0.57	0.19	0.29
<i>trans</i> -10, <i>cis</i> -12	0.25	0.65	0.33	0.17	0.06

¹C = control; P10 = 10% pigeon peas; P20 = 20% pigeon peas.

evaluate the effect of higher levels of pigeon peas on the rumen environment and the concentration of CLA in muscle, subcutaneous fat, and milk.

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