

# Characterization Of Rumen Bacterial And Protozoal Fatty Acid Compositions From Lactating Jersey Cows



## Offered Alternative Forage Crops

Abstract #16880



Laura M. Cersosimo<sup>1</sup>, Rinske Tacoma<sup>1</sup>, Sabrina Greenwood<sup>1</sup>, Kelsey Juntwait<sup>2</sup>, Andre F. Brito<sup>2</sup>, Jana Kraft<sup>1</sup>

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The objective of this study was to compare the rumen bacterial and protozoal membrane fatty acid compositions from lactating Jersey cows fed pasture strip-tilled with alternative forage crops versus a traditional grass-legume pasture mix.

## Abstract

In spring (SPR) and summer (SUM), two separate, 21d experiments were conducted using 16 lactating Jersey cows. Cows were divided into control (AFC (-), n=8) and treatment (AFC (+), n=8) groups and offered (DM basis) 40% pasture as AFC or legume/mixed grasses and 60% TMR. Total protozoal and bacterial branched-chain FA, PUFA, as well as *trans* 18:1 isomers and 18:0, the products of rumen bacterial biohydrogenation, did not differ by group in either experiment. In conclusion, few differences were identified in the microbial FA compositions in cows consuming pasture with or without AFC.

## Introduction

- Alternative forage crops (AFC) include cool season grasses and legumes that may be used to overcome periods of limited pasture growth.
- Rumen microbial cell membranes consist of varying proportions of fatty acids (FA) that contribute to milk FA.

## Methods

- Sixteen Jersey dairy cows (SPR, 85 ± 46 days in milk, DIM; SUM, 143 ± 58 DIM) were co-housed at the University of New Hampshire Organic Research Farm.
- Dietary and microbial cell membrane FA were analyzed via gas-liquid chromatography.

## Dietary FA Results

[Table 1.](#) : FA composition of total mixed ration and pasture fed to lactating Jersey cows in SPR and SUM experiments.

[Table 2.](#) FA composition of dietary components offered to lactating Jersey cows in SPR and SUM experiments.

## FA Abbreviations

ALA,  $\alpha$ -linolenic acid; BCFA, branched-chain FA; CLA, conjugated linoleic acids; LA, linoleic acid; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SFA, saturated FA; VA, vaccenic acid

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## Figures

[Figure 1.](#) Diet components offered in SPR and SUM experiments.

[Figure 2.](#) Microbial cell membrane FA from Jersey cows from SPR and SUM experiments.

[Figure 3.](#) Key rumen bacterial cell membrane FA from Jersey cows offered AFC (-) or AFC (+) pasture.

[Figure 4.](#) Key rumen protozoal cell membrane FA from Jersey cows offered AFC (-) or AFC (+) pasture.

## Conclusions

- SPR and SUM AFC quickly matured, making their content of PUFA subsequently lower in comparison to the legume/mixed grasses.
- Potential explanations for the few differences in the microbial cell membrane FA compositions observed between AFC (-) and AFC (+) fed cows include: i) low inclusion of AFC in diet, ii) intra- and inter- animal variations, and iii) short experimental period length.

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### Abstract

Alternative forage crops (AFC) include cool and warm season grasses and legumes that could be used to overcome periods of limited pasture production. Rumen bacteria and protozoa cell membranes consist of varying proportions of fatty acids (FA) that contribute to milk FA. The objective of this study was to compare the rumen bacterial and protozoal membrane FA compositions from lactating Jersey cows fed pasture strip-tilled with AFC vs. a traditional grass-legume pasture mix. In spring (SPR) and summer (SUM), two separate, 21-d experiments were conducted using 16 lactating Jersey cows (SPR, 85 ± 46 DIM; SUM, 143 ± 58 DIM). Cows were divided into control (AFC (-), n=8) and treatment (AFC (+), n=8) groups, matched by parity, DIM, and milk production, and offered (DM basis) 40% pasture as AFC or traditional and 60% TMR. On a DM basis, SPR AFC (+) pasture consisted of AFC (barley, hairy vetch, triticale, rye, and wheat) representing 7.3% of total diet DM, while the SUM AFC (+) pasture consisted of AFC (buckwheat, chickling vetch, and oats), representing 5.7% of total diet DM. Individual whole ruminal digesta samples (500 mL) were collected via esophageal intubation on d 20 and 21 of each experiment. Bacterial and protozoal fractions were isolated by differential centrifugation. Microbial cell membrane FA were analyzed by gas-liquid chromatography. A completely randomized block design with PROC MIXED (SAS, v.9.4) was used to determine if least-squares means differed between AFC (-) and AFC (+) groups. Total protozoal and bacterial branched-chain FA, PUFA, as well as *trans* 18:1 isomers and 18:0, the products of rumen bacterial biohydrogenation, did not differ by group in either experiment. In the SPR, bacterial *cis*-11 16:1 (AFC (-), 0.45 g/100 g FA; AFC (+), 0.37 g/100 g FA), *cis*-12 18:1 (AFC (-), 0.68 g/100 g FA; AFC (+), 0.55 g/100 g FA), and *cis*-15 18:1 (AFC (-), 0.75 g/100 g FA; AFC (+), 0.70 g/100 g FA) were less abundant in the AFC (+) than AFC (-) group ( $P < 0.05$ ). In the SUM, bacterial 17:0 was lower in cows grazing AFC (+) pasture (0.67 g/100 g FA) than AFC (-) pasture (0.71 g/100 g FA;  $P < 0.01$ ). In the SUM, no differences in the protozoal FA compositions were observed. In conclusion, few differences were identified in the microbial FA compositions in cows consuming pasture with or without AFC.

Introduction

Methods

Figure 1 Diet

Figure 2  
Microbial FA

Figure 3  
Bacterial FA

Figure 4  
Protozoal FA

Table 1  
Diet FA

Table 2 Bot  
Comp FA

Conclusion

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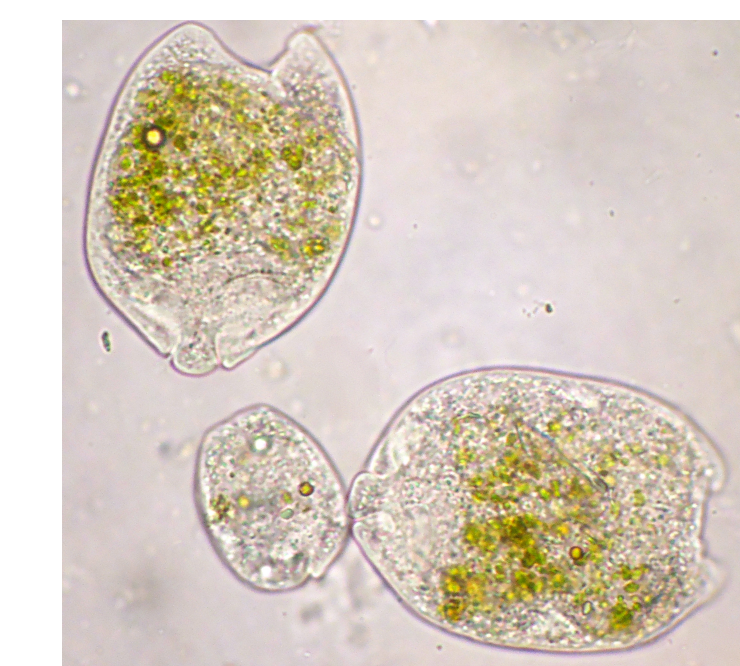
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## Introduction

- Alternative forage crops (AFC) include cool and warm season grasses and legumes that could be used to overcome periods of limited pasture production.
- Rumen bacteria biohydrogenate forage-derived PUFA into intermediates (e.g., CLA, 18:1 isomers) and the end-product, stearic acid (18:0).
- Rumen protozoa engulf chloroplasts and contain more PUFA in their cell membranes in comparison to bacteria.
- Branched-chain FA (BCFA) give microbial cell membranes fluidity.
- Rumen bacterial and protozoal cell membrane FA, such as BCFA and odd-chain FA, and 18:1 *trans* isomers are of interest because they contribute to the milk FA composition.
- Previous research demonstrated the positive impacts of BCFA- and odd-chain FA on human health<sup>1,2</sup>.



<sup>1</sup>Wongtangtharn S, Oku H, Iwasaki H, et al. Incorporation of branched-chain fatty acid into cellular lipids and caspase-independent apoptosis in human breast cancer cell line, SKBR-3. *Lipids Heal Dis* 2005;4:29.

<sup>2</sup>Jenkins B, West JA., Koulman A. A review of odd-chain fatty acid metabolism and the role of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) in health and disease. *Molecules* 2015;20:2425–44.

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### Experimental Design

- 16 Jersey dairy cows (spring, SPR, 85 ± 46 days in milk, DIM; summer, SUM, 143 ± 58 DIM) were co-housed at the University of New Hampshire Organic Research Farm.
- Cows were divided into control (AFC (-), n=8) and treatment (AFC (+), n=8) groups, matched by parity, DIM, and milk production.
- Two 21d periods were conducted in SPR (May 2015) and SUM (July 2015) when cows were grazing AFC or control pastures ([Figure 1](#)).
- A completely randomized block design with PROC MIXED compared the LSM between AFC (-) and AFC (+) groups.

### Sample Collection



- Collected d18-d21
- Total mixed ration
- Botanical Composition



- Collected d20-d21
- Esophageal intubation (500 mL collected)

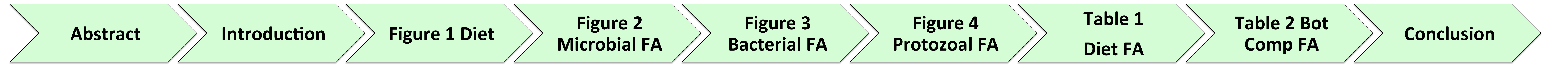
### Sample Analyses



- Dietary and microbial FA methyl esters (FAME) were made by transesterification with methanolic H<sub>2</sub>SO<sub>4</sub>
- Dietary and microbial FAME were analyzed by gas-liquid chromatography



- Methyl cellulose (1%) was added to detach microbes from feed particles
- Bacterial and protozoal cells were separated by differential centrifugation



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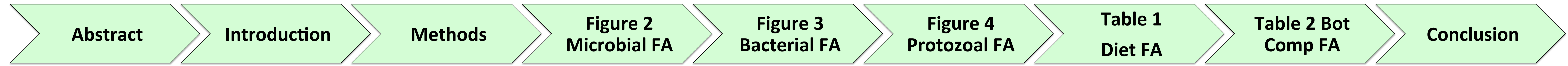
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AFC (-)		AFC (+)	
<b>Total Mixed Ration (60% of DMI)</b>	<b>Pasture (40% of DMI)</b>	<b>Total Mixed Ration (60% of DMI)</b>	<b>Pasture (40% of DMI)</b>
			AFC      CSG

- AFC (-) pasture contained a mixture of legumes/cool-season grasses (CSG)
- AFC (+) pasture was 30% strip-tilled with AFC
- SPR AFC (+) was 7.3% of total diet DMI (barley, hairy vetch, triticale, rye, wheat)
- SUM AFC (+) was 5.7% of total diet DMI (buckwheat, chickling vetch, and oats)

Figure 1: Diet components offered in SPR and SUM experiments.



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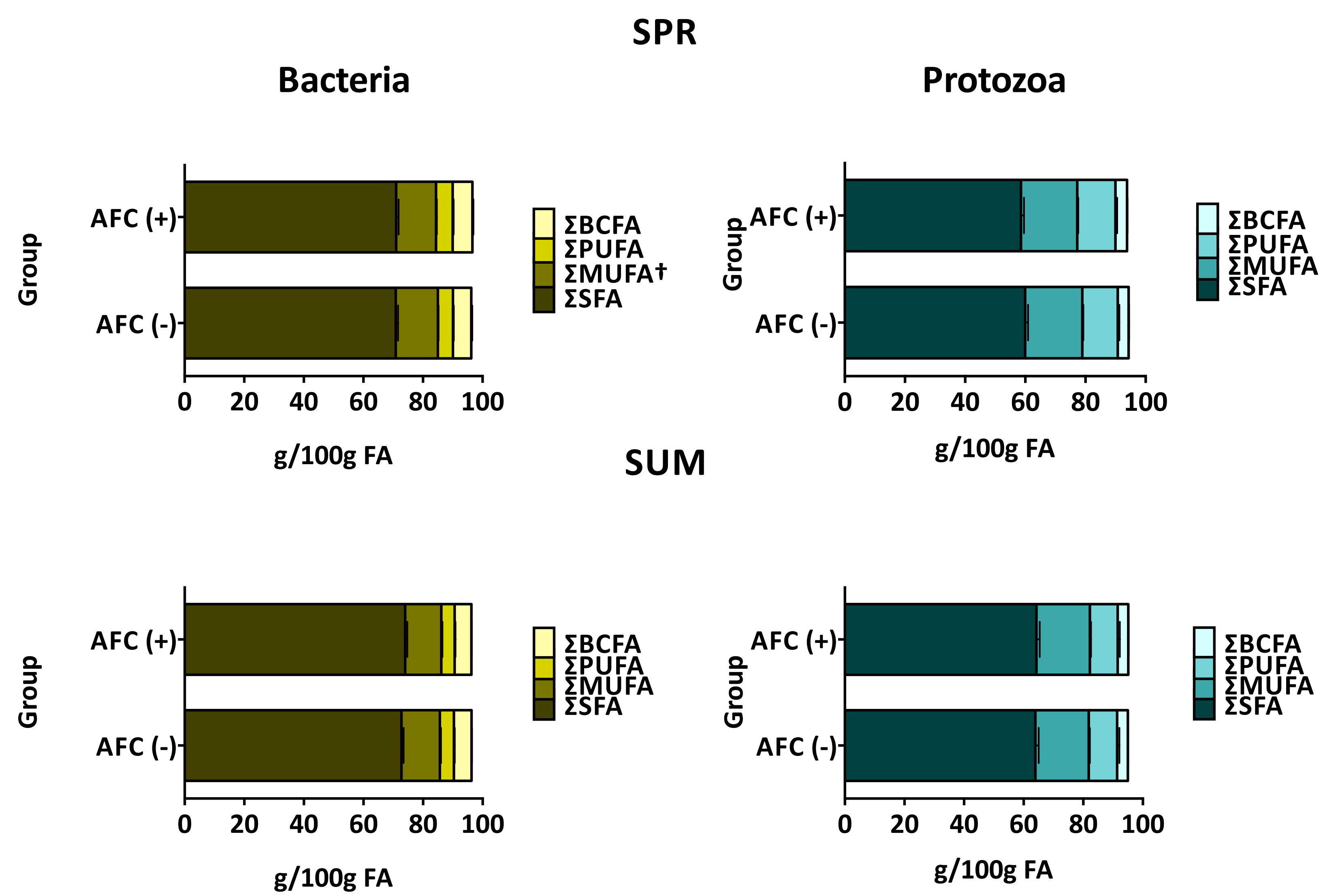
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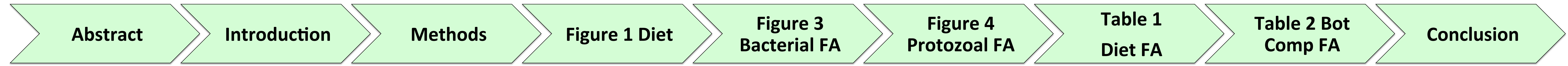
### Results

- Total protozoal and bacterial BCFA, PUFA, as well as 18:1 *trans* isomers and 18:0, the products of rumen bacterial biohydrogenation, did not differ by group in either experiment (Figures 2, 3 and 4).
- In the SPR experiment, total bacterial MUFA tended ( $P = 0.05$ ) to be less abundant in the AFC (+) than AFC (-) group (Figure 2).
- In the SUM experiment, bacterial 17:0 was lower in cows grazing AFC (+) pasture (0.67 g/100 g FA) than AFC (-) pasture (0.71 g/100 g FA;  $P < 0.01$ ).
- In the SUM experiment, no differences in the protozoal FA compositions were observed (Figure 4b).

Figure 2: Microbial cell membrane FA from Jersey cows from SPR and SUM experiments.



† 0.05 ≤ P ≤ 0.10, BCFA, branched-chain FA, MUFA, monounsaturated FA, PUFA, polyunsaturated FA, SFA, saturated FA



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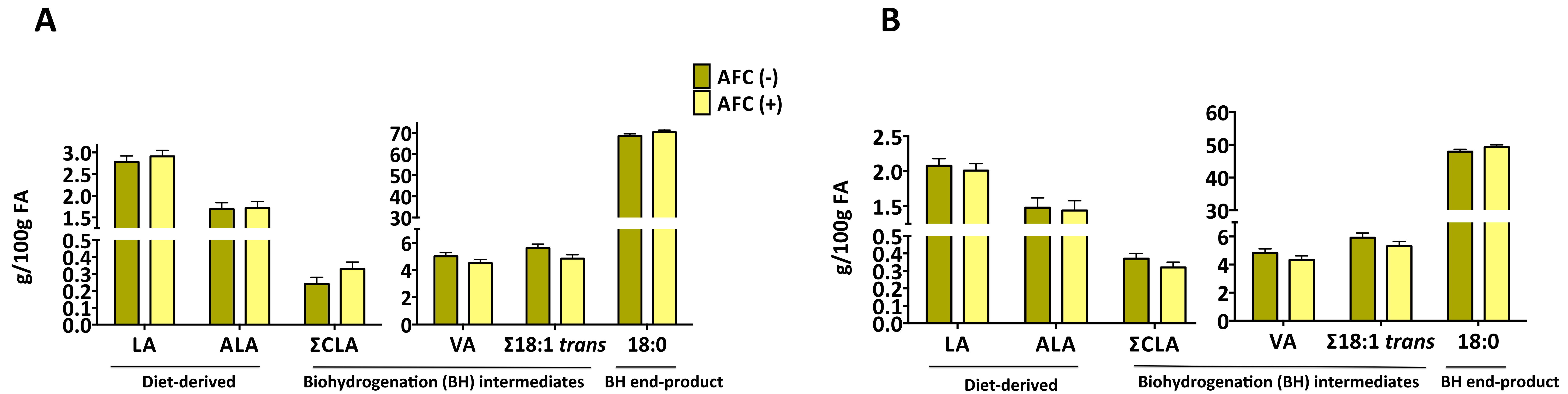
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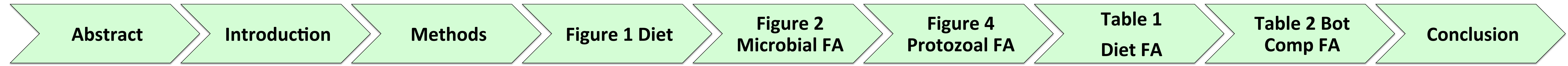
### SPR Bacteria<sup>a</sup>

### SUM Bacteria



<sup>a</sup>LA, linoleic acid; ALA, α-linolenic acid; CLA, conjugated linoleic acids; VA, vaccenic acid (18:1 *t*11); AFC, alternative forage crops

**Figure 3: Key rumen bacterial cell membrane FA from Jersey cows offered AFC (-) or AFC (+) pasture.**



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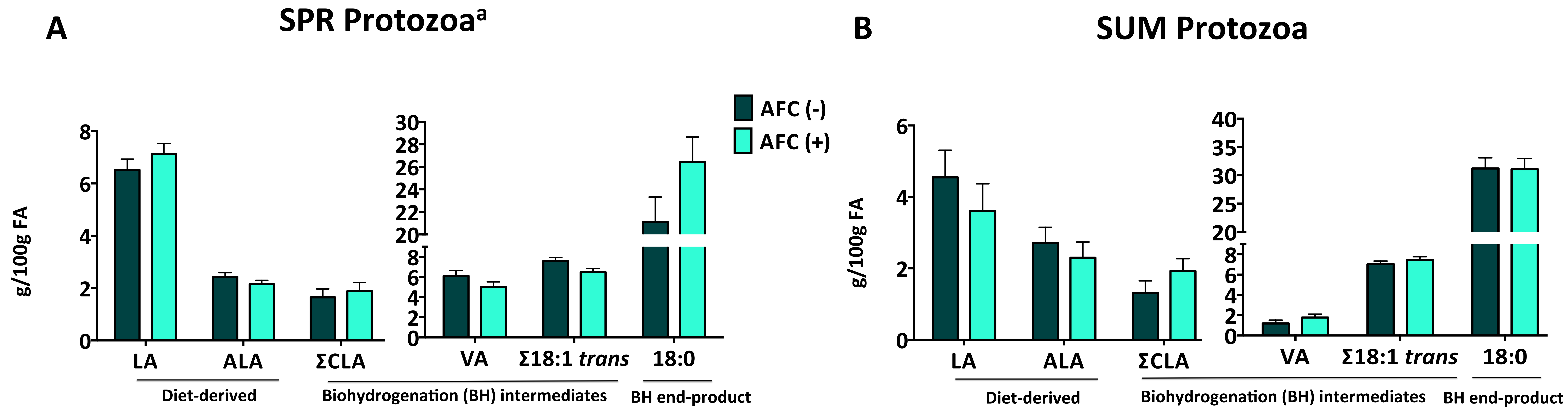
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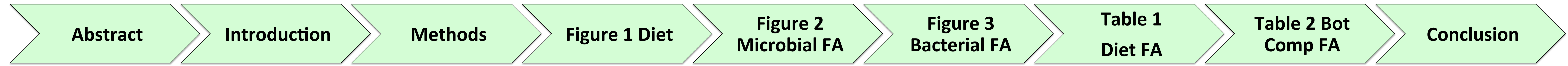
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<sup>a</sup>LA, linoleic acid; ALA, α-linolenic acid; CLA, conjugated linoleic acids; VA, vaccenic acid (18:1 *t*11); AFC, alternative forage crops

**Figure 4: Key rumen protozoal cell membrane FA from Jersey cows offered AFC (-) or AFC (+) pasture.**





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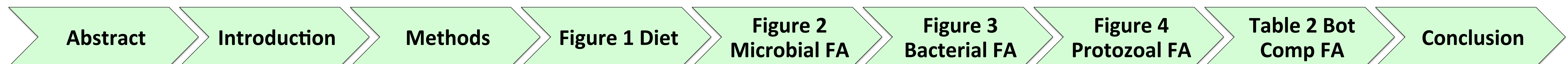
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**Table 1: FA composition of total mixed ration and pasture fed to lactating Jersey cows in the SPR and SUM experiments.**

Fatty acid group (g/100g FA)	SPR			SUM		
	TMR <sup>a</sup>	AFC <sup>b</sup> (-)	AFC (+)	TMR	AFC (-)	AFC (+)
$\Sigma$ SFA <sup>c</sup>	25.0	22.8	25.7	20.0	28.2	27.3
$\Sigma$ MUFA <sup>d</sup>	21.9	11.8	6.5	17.6	4.76	4.9
$\Sigma$ PUFA <sup>e</sup>	60.6	65.2	67.7	62.4	67.4	67.8
$\Sigma$ n-3 FA <sup>f</sup>	28.7	30.1	46.2	26.8	44.4	45.7
$\Sigma$ n-6 FA <sup>g</sup>	31.9	35.2	21.5	35.6	22.6	22.0

<sup>a</sup> TMR= total mixed ration (60% of total diet), <sup>b</sup> AFC= alternative forage crops, SPR (rye, wheat, barley, triticale, hairy vetch), SUM (oats, buckwheat, chickling vetch), <sup>c</sup>  $\Sigma$ SFA= sum of saturated fatty acids (12:0-24:0), <sup>d</sup>  $\Sigma$ MUFA= sum of monounsaturated fatty acids (16:1 c9 + 18:1 c9 + 18:1 c11 + 22:1 c9), <sup>e</sup>  $\Sigma$ PUFA= sum of polyunsaturated fatty acids (18:2 c9,c12+ 18:3 c9,c12,c15 + 20:2 c11,c14 + 20:3 c5,c8,c11 + 20:3 c11,c14,c17), <sup>f</sup>  $\Sigma$ n-3= sum of 18:3 c9,c12,c15 + 20:3 c11,c14,c17, <sup>g</sup>  $\Sigma$ n-6= sum of 18:2 c9,c12 + 20:2 c11,c14 + 20:3 c5,c8,c11



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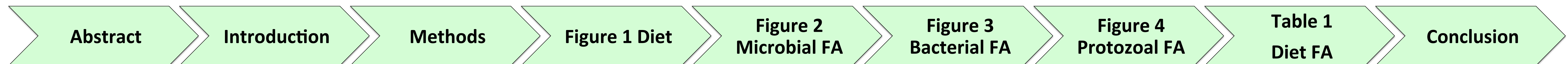
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**Table 2: FA composition of dietary components offered to lactating Jersey cows in the SPR and SUM experiments.**

FA (mg/g FA)	Total FA (%DM)	16:0	18:0	18:1 c9	LA	ALA	Σother	ΣSFA	ΣMUFA	ΣPUFA	Σn-3	Σn-6
SPR												
TMR	3.26	6.15	0.90	4.35	10.37	8.95	1.92	8.17	4.68	19.78	8.95	10.42
AFC (-) mixed grasses	2.08	4.04	0.37	1.25	4.93	8.84	1.35	5.32	1.39	14.07	8.99	5.08
AFC (+) mixed grasses	1.97	3.85	0.04	0.32	4.94	8.87	1.71	4.77	0.81	14.14	9.06	5.08
AFC (+) weeds	2.98	5.37	0.64	3.02	10.46	8.01	2.33	7.57	3.23	19.03	8.21	10.82
AFC (+) small grains	1.39	2.89	0.26	0.47	2.45	6.66	1.87	4.05	0.60	9.28	6.77	2.50
SUM												
TMR	3.19	4.96	0.64	5.21	11.26	8.34	1.46	6.37	5.60	19.88	8.55	11.33
AFC (-) mixed grasses	2.08	3.83	0.23	0.53	4.17	10.99	1.10	4.94	0.63	15.27	11.04	4.23
AFC (-) legumes	1.32	4.40	0.60	0.81	2.67	3.38	1.32	6.08	0.93	6.17	3.43	2.74
AFC (-) weeds	1.81	3.85	0.31	0.75	3.09	9.17	0.96	4.71	0.84	12.59	9.38	3.20
AFC (-) dead material	1.24	2.84	0.53	0.83	3.93	2.41	1.90	4.82	1.05	7.88	2.54	4.05
AFC (+) mixed grasses	2.26	4.20	0.33	0.56	4.40	11.77	1.33	5.55	0.69	16.36	11.86	4.50
AFC (+) legumes	1.48	4.28	0.65	0.96	3.17	4.46	1.28	5.82	1.39	7.89	4.59	3.30
AFC (+) weeds	2.85	5.39	0.39	1.05	5.38	15.25	1.07	6.63	1.12	20.79	15.33	5.46
AFC (+) dead material	1.44	3.26	0.58	0.89	4.29	3.23	2.11	5.43	1.05	7.88	3.42	4.46
AFC (+) oats	1.79	3.73	0.25	0.61	3.60	8.63	1.03	4.71	0.75	12.39	8.70	3.69
AFC (+) AFC broadleaf	1.50	3.08	0.27	1.01	4.09	4.65	1.90	4.15	1.18	9.68	5.11	4.57

- SPR AFC (+) small grains (barley, rye, wheat, triticale) contained lower contents of total PUFA (9.28 mg/g FA) than mixed grasses from either SPR AFC (-) (14.07 mg/g FA) or SPR AFC (+) (14.14 mg/g FA) pastures.
- SUM AFC (+) oats (1.79% total FA, 12.93 mg/g FA) and AFC broadleaf (chickling vetch and buckwheat, 1.50% of total FA, 9.68 mg/g FA) contained lower contents of total FA and PUFA, respectively than SUM AFC (+) mixed grasses (2.26% of total FA, 16.36 mg/g FA).
- SUM AFC (+) AFC broadleaf contained 2-fold more 18:1 c9 than SUM AFC (+) grasses.



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## Conclusions

- As the SPR and SUM AFC quickly matured within a 21d period, their content of PUFA was therefore lower in comparison to the legume/mixed grasses.
- Few differences were identified in the microbial FA compositions in cows consuming pasture with or without AFC.
- The study was limited by the inclusion of AFC in the diet, intra- and inter-animal variations, and experimental period length.
- Future studies need to characterize the rumen microbial cell membrane FA once the AFC are regrown and use culture-dependent techniques to identify bacterial and protozoal taxa and their cell membrane FA.

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