

Rumen Protozoal Community Structures Are Not Altered In Lactating Dairy Cows Offered Alternative Forage Crops During Short-Term Grazing Experiments

Abstract #16394

Laura M. Cersosimo¹, Rinske Tacoma¹, Sabrina Greenwood¹, Kelsey Juntwait², Andre F. Brito², Jana Kraft¹

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The objective of this study was to compare the rumen protozoal community structures and volatile fatty acids (VFA) in cows grazing pasture strip-tilled with alternative forage crops (AFC) or traditional grass-legume pasture mix.

Abstract

The study consisted of two 21-d experiments, spring (SPR) and summer (SUM). Sixteen lactating Jersey cows (SPR: 85 ± 46 DIM; SUM: 143 ± 58 DIM) were split into two groups with eight cows assigned to the AFC (AFC (+)) and eight cows assigned to traditional mixed grasses-legumes (AFC (-)). Pasture comprised 40% of the diet (DM basis), while a TMR comprised 60%. The SUM AFC (+) group had a lower ($P=0.02$) isobutyrate proportion (0.80% of total VFA) than the SUM AFC (-) group (0.98% of total VFA). Abundance of protozoal taxa did not differ between groups in either experiment.

Introduction

- The use of AFC (e.g., small grains and legumes) is one potential solution to overcome periods of low pasture mass.
- Rumen protozoa are anaerobic ciliates that ferment feedstuffs in concert with bacteria and fungi.

Methods

- Sixteen Jersey dairy cows (8 cows grazing AFC, AFC(+), 8 cows grazing control mixed grasses/legumes, AFC (-)) were co-housed at the University of New Hampshire Organic Research Farm.

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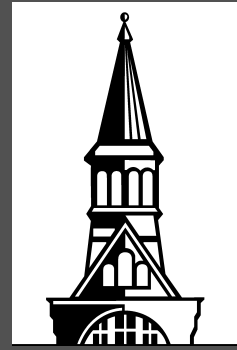
[Table 2.](#) Composition of rumen VFA from AFC (-) and AFC (+) fed lactating Jersey cows during SPR and SUM experiments.

Conclusions

- Abundances of major VFA and protozoal taxa were not altered by the addition of AFC.
- Protozoal taxa correlated with animal performance.
- As AFC matured within a 21d period, future studies may characterize the rumen protozoa after AFC regrowth or during each week of the 21d period.

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The study consisted of two, 21-d experiments, spring (SPR) and summer (SUM). Sixteen lactating Jersey cows (SPR: 85 ± 46 DIM; SUM: 143 ± 58 DIM) were split into two groups with eight cows assigned to the AFC (AFC (+)) and eight cows assigned to traditional mixed grasses-legumes (AFC (-)). Pasture comprised 40% of the diet (DM basis), while a TMR comprised 60%. SPR AFC (2.4% of total DM) included barley, hairy vetch, rye, triticale, and wheat, and SUM AFC (10.0% of total DM) included buckwheat, oats, and chickling vetch. Milk samples were collected for four consecutive milkings (d 19-21). Individual whole rumen digesta samples (500 mL) were collected on d 20 and 21 of each experiment. Ruminal VFA samples were analyzed by gas-liquid chromatography. Microbial DNA was extracted and the V3-V4 regions of the protozoal 18S rRNA gene were amplified via PCR. The program MOTHUR was used to perform bioinformatics analyses. A completely randomized block design with PROC MIXED compared the LSM between groups and the PROC CORR model in SAS (v.9.4) performed Pearson correlations between rumen protozoal genera, animal performance, and VFA. Yields of milk, milk fat, and milk protein (kg/d) in SPR were: AFC (-), 22.5; 1.08; 0.80 and AFC (+), 23.4; 1.15; 0.85 and in SUM: AFC (-), 17.3; 0.74; 0.59 and AFC (+), 18.9; 0.92; 0.70, respectively. Total VFA (mM), and molar proportions of acetate (70.8%), propionate (16.0%), and butyrate (1.93%) did not differ in either experiment. The SUM AFC (+) group had a lower ($P=0.02$) isobutyrate proportion (0.80%) than the SUM AFC (-) group (0.98%). Abundance of protozoal taxa did not differ between groups in either experiment. The protozoal genera *Eudiplodinium* (CON: 43.0%; TRT: 49.3%) and *Entodinium* (AFC (-): 48.1%; AFC (+): 37.1%) were most abundant in SPR and SUM, respectively. The protozoal genus *Diplodinium* (SPR: 6.48%; SUM: 3.28%) was positively correlated ($P<0.001$) with milk production ($r=0.69$), milk fat ($r=0.64$), and protein yields ($r=0.61$), and ruminal propionate ($r=0.38$; $P=0.04$). The protozoal genus *Entodinium* was negatively correlated with milk yield ($r=-0.48$; $P<0.01$). In conclusion, the rumen protozoal community structures and predominant VFA were not altered in AFC-fed cows, yet, the genus *Diplodinium* was positively correlated to animal performance and VFA.

Introduction

Methods

Figure 1 AFC

Figure 2
Protozoal Taxa

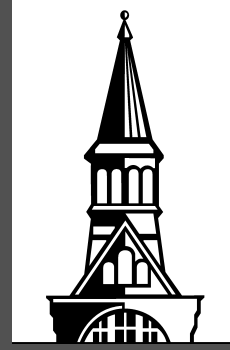
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Introduction

- In the Northeastern United States, early spring and mid-late summer are periods of decreased pasture mass.
- The use of alternative forage crops (AFC; e.g., small grains and legumes) is one potential solution to overcome this challenge.
- Rumen protozoa are anaerobic ciliates that ferment feedstuffs into VFA in concert with bacteria and fungi.
- VFA, such as acetate, butyrate, and propionate are major energy sources used for milk production.
- *Diplodinium* spp. ingest starch granules, plant fragments, and prey on bacteria and other protozoa.
- Holotrich protozoa from the genera *Isotricha*, attach to the plant cell surface, whereas entodiniomorphs, *Epidinium caudatum* and *Eudiplodinium maggii*, use their oral cavities to attach to plant fibers¹.

Why evaluate the rumen protozoal community structure?

- Previous research has been conducted to investigate if the abundances of certain protozoal taxa are altered by consumption of AFC.
- Jami *et al.*² and Bainbridge *et al.*³ evaluated the relationship between rumen bacterial taxa and animal performance (milk protein, fat, and milk production), but no relationship has been described between rumen protozoal taxa and animal performance.

¹Orpin CG. The role of ciliate protozoa and fungi in the rumen digestion of plant cell walls. *Anim Feed Sci Technol* 1984;10:121–143., ²Jami E, White BA, Mizrahi I. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PLoS One* 2014;9:e85423., ³Bainbridge M, Cersosimo L, Wright A-DG, Kraft J. Rumen bacterial communities shift across a lactation in Holstein, Jersey, and Holstein x Jersey dairy cows and correlate to rumen function, bacterial fatty acid composition, and production parameters. *FEMS Microb Ecol* 2016. 92: 1-14.

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Figure 1 AFC
offered

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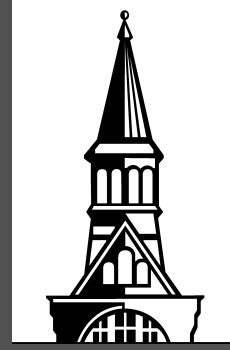
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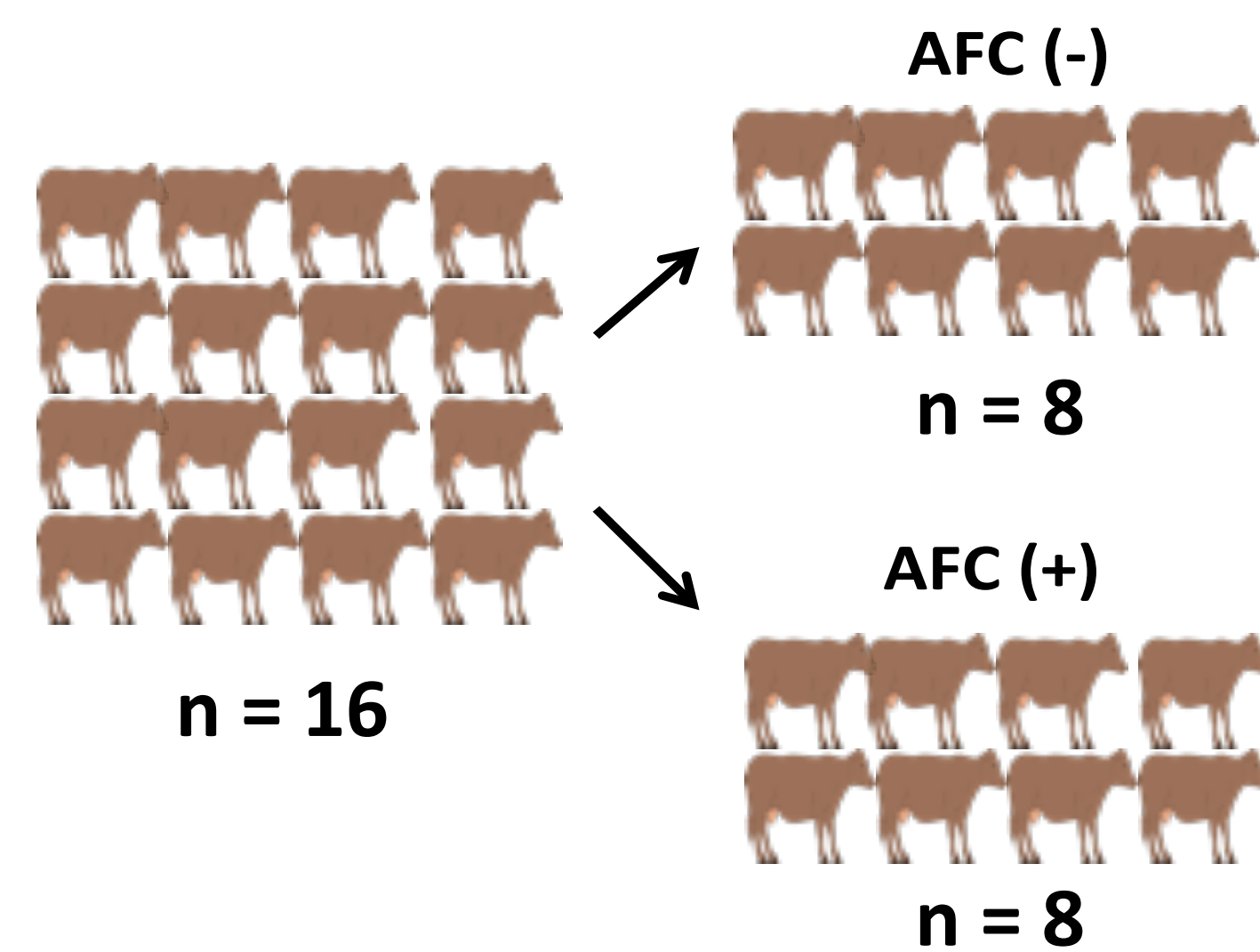


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Experimental Design



- In both experiments, cows were blocked by days in milk (DIM), milk production, and parity.
- A completely randomized block design with PROC MIXED compared the LSM between groups.
- Pearson correlations between rumen protozoal genera, animal performance, and VFA were performed using the PROC CORR model in SAS (v. 9.4).

Sample Collection



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



- Rumen Digesta**
- Collected d20-d21
 - 500 mL
 - Esophageal intubation



- Milk**
- d19-d21
 - 4 consecutive milkings
 - Preserved in 2-bromo-2-nitropropan-1,3-diol and pooled by cow

Sample Analyses



- Forage**
- Nutrient composition was analyzed by Cumberland Valley
 - Pasture DMI (kg/d) was estimated using chromium oxide as a biomarker



- Rumen Digesta**
- V3-V4 hypervariable region of the 18S rRNA gene was amplified (316F,758R)
 - MiSeq v.3 sequencing platform
 - Bioinformatics with MOTHUR
 - VFA were analyzed via gas-liquid chromatography



- Milk**
- Milk was analyzed for milk solids (fat and protein) by the Dairy Herd Improvement Association
 - Feed efficiency (kg milk produced/kg DMI/d)

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Figure 2 Protozoal Taxa

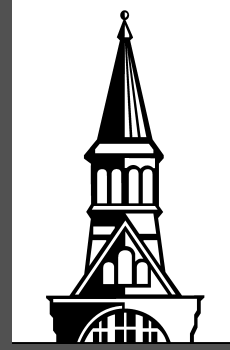
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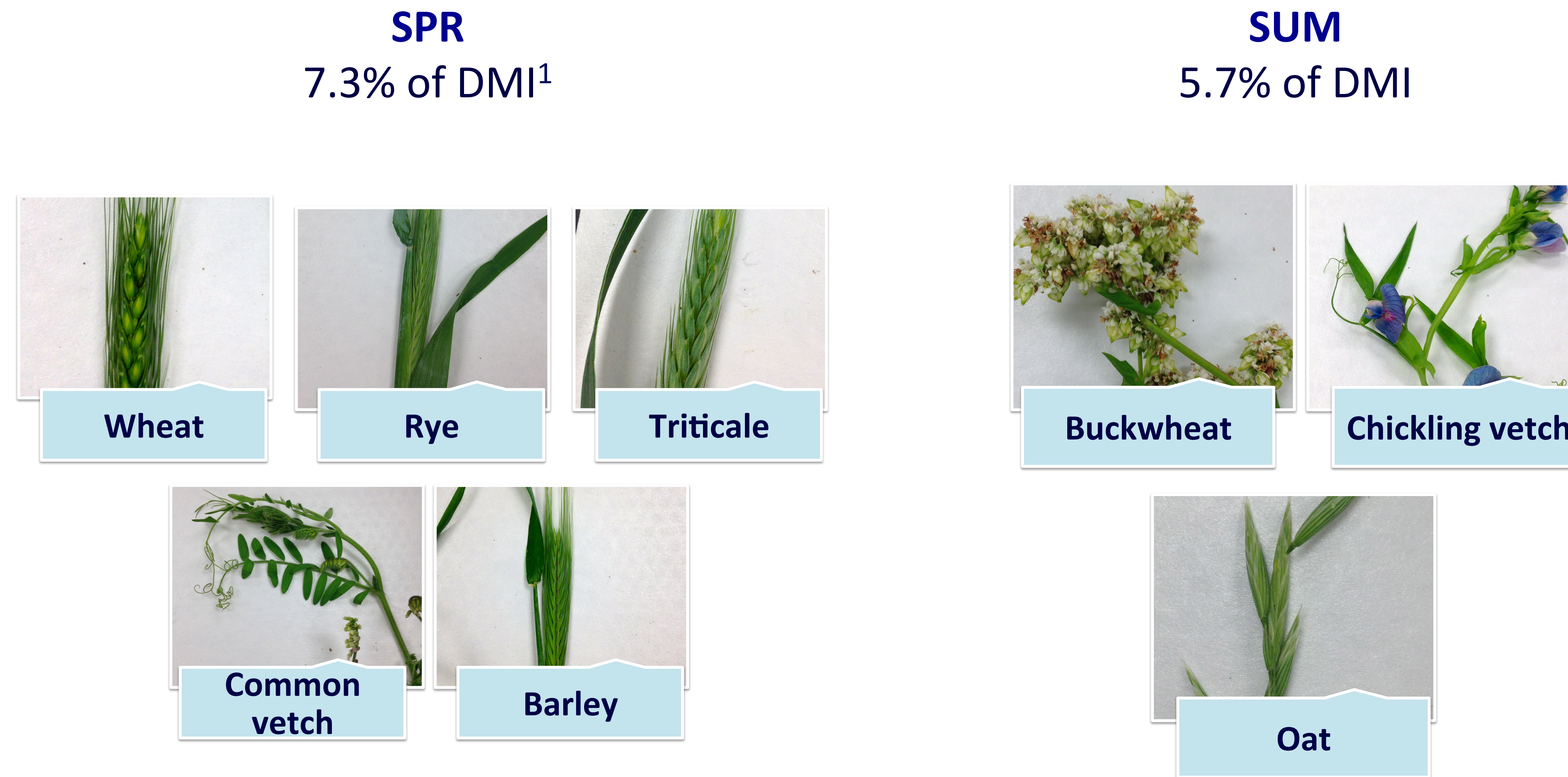


Figure 1: Alternative forage crops offered at each period.

¹DMI, dry matter intake

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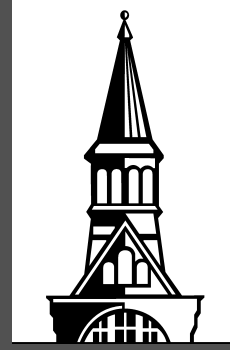
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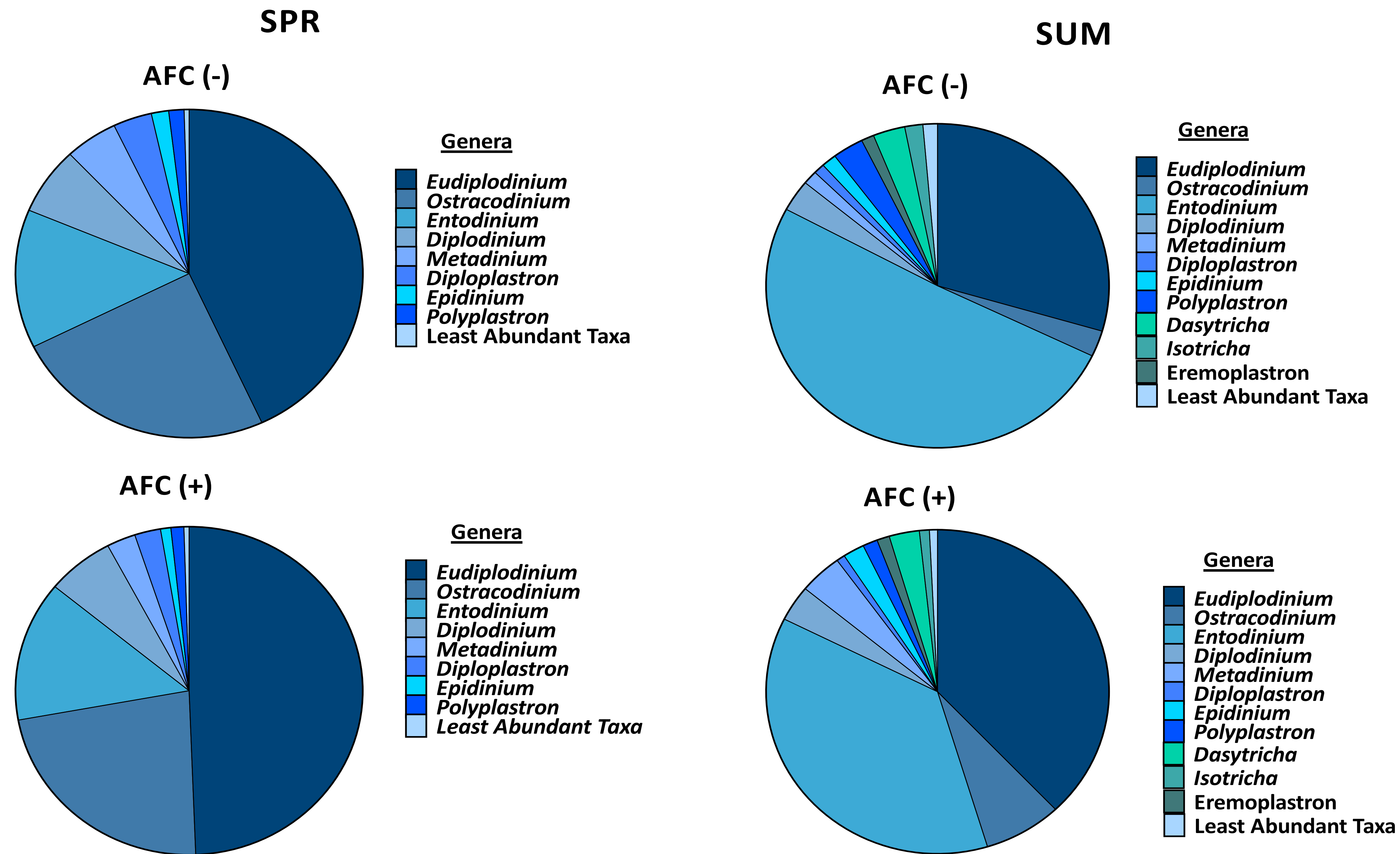


Figure 2: Percent abundance of 18S rRNA gene sequence reads classified into rumen protozoal taxa during the SPR and SUM experimental periods in lactating Jersey cows.

- Abundance of protozoal taxa did not differ between AFC (-) and AFC (+) groups during SPR and SUM experiments.
- The genus *Entodinium* was more abundant in the SUM period.

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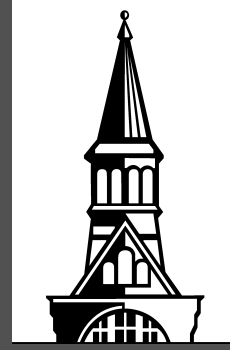
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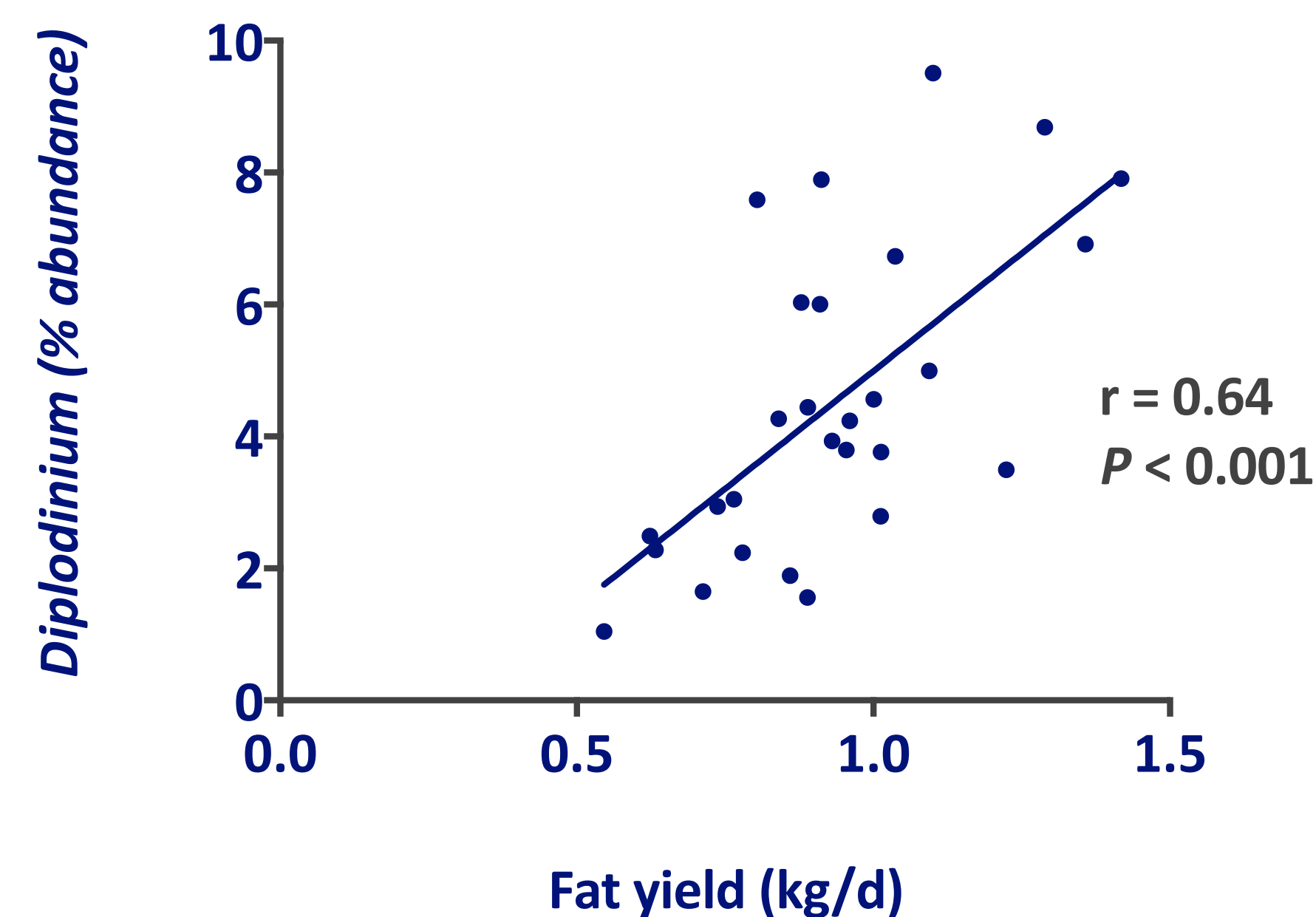
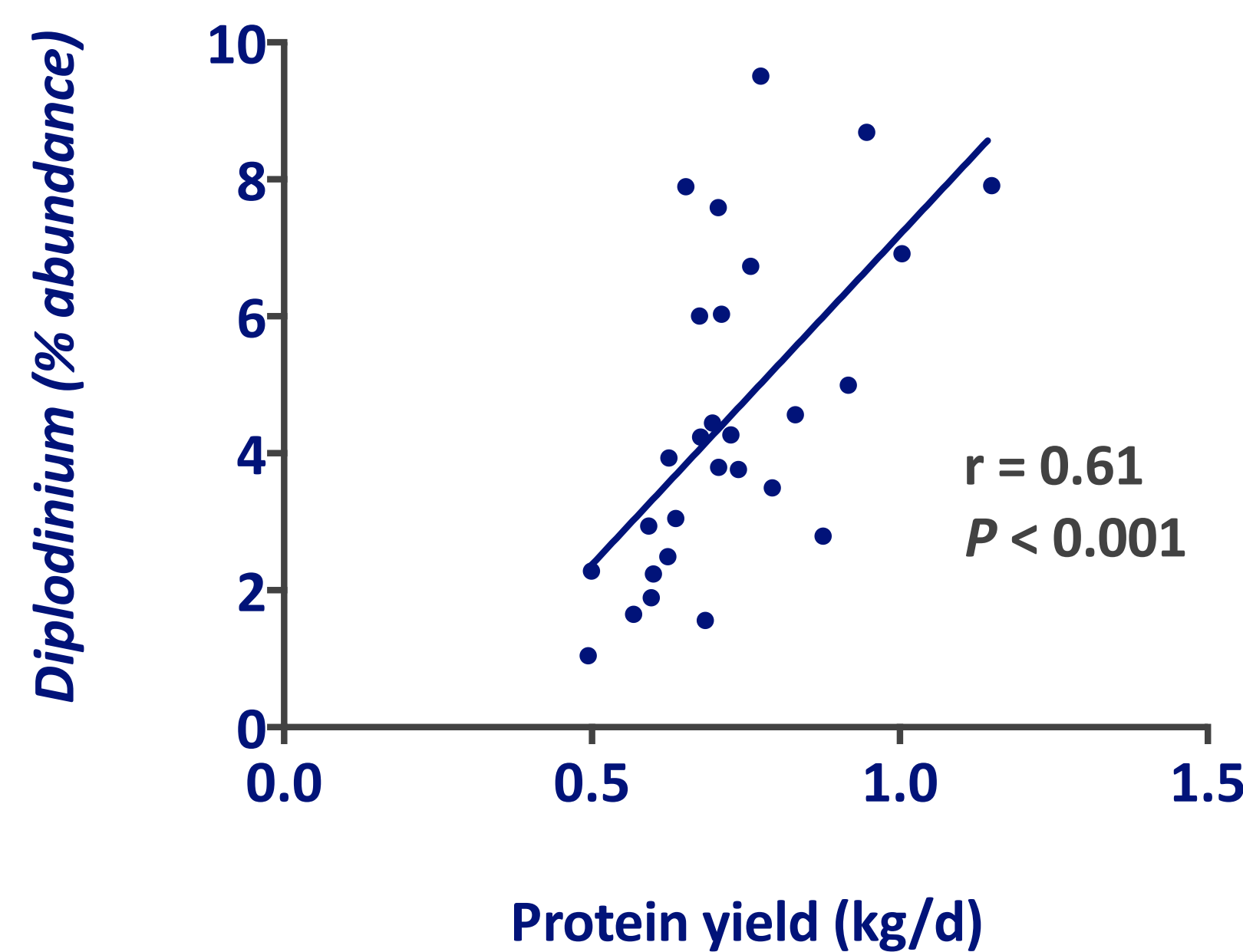
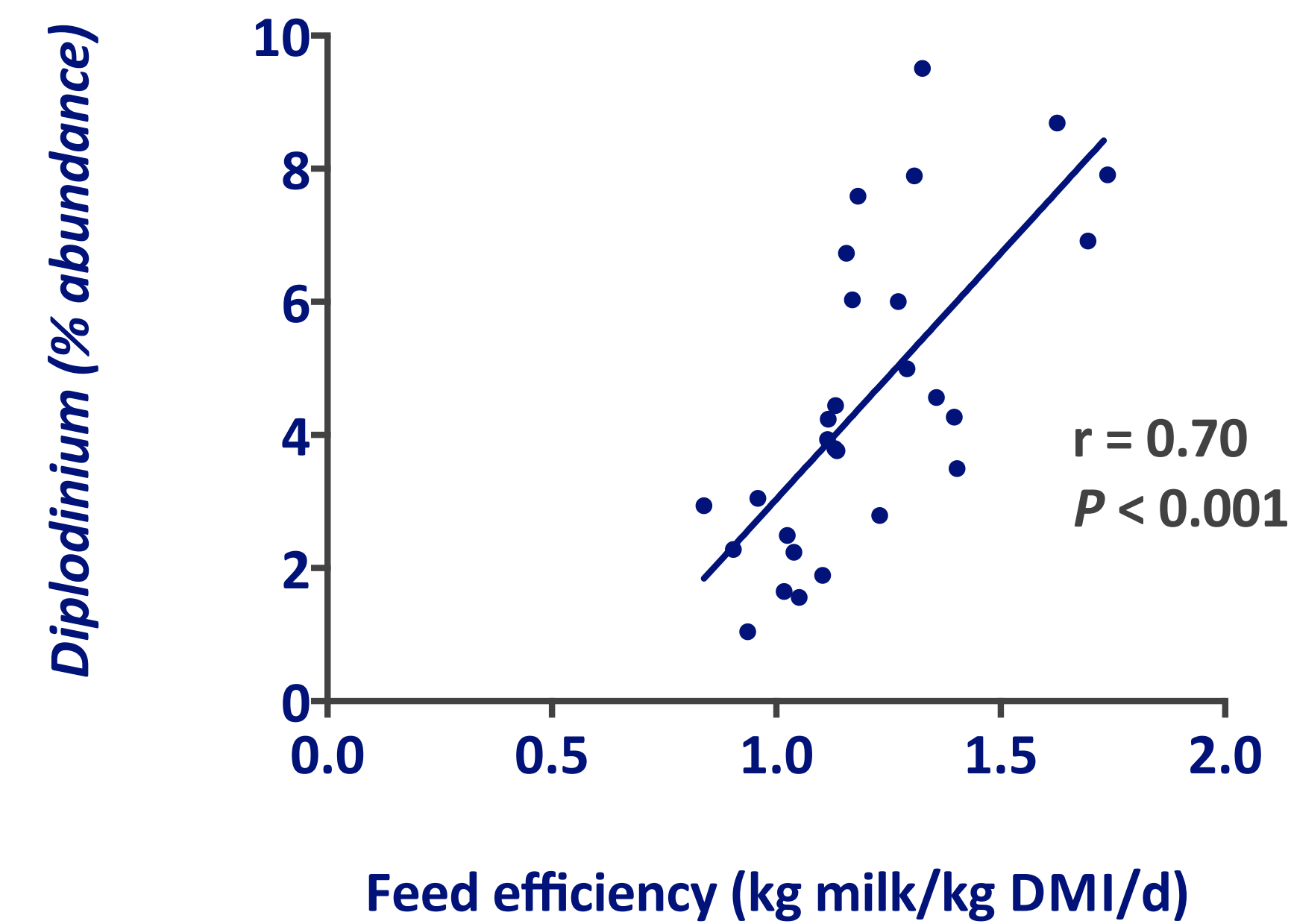
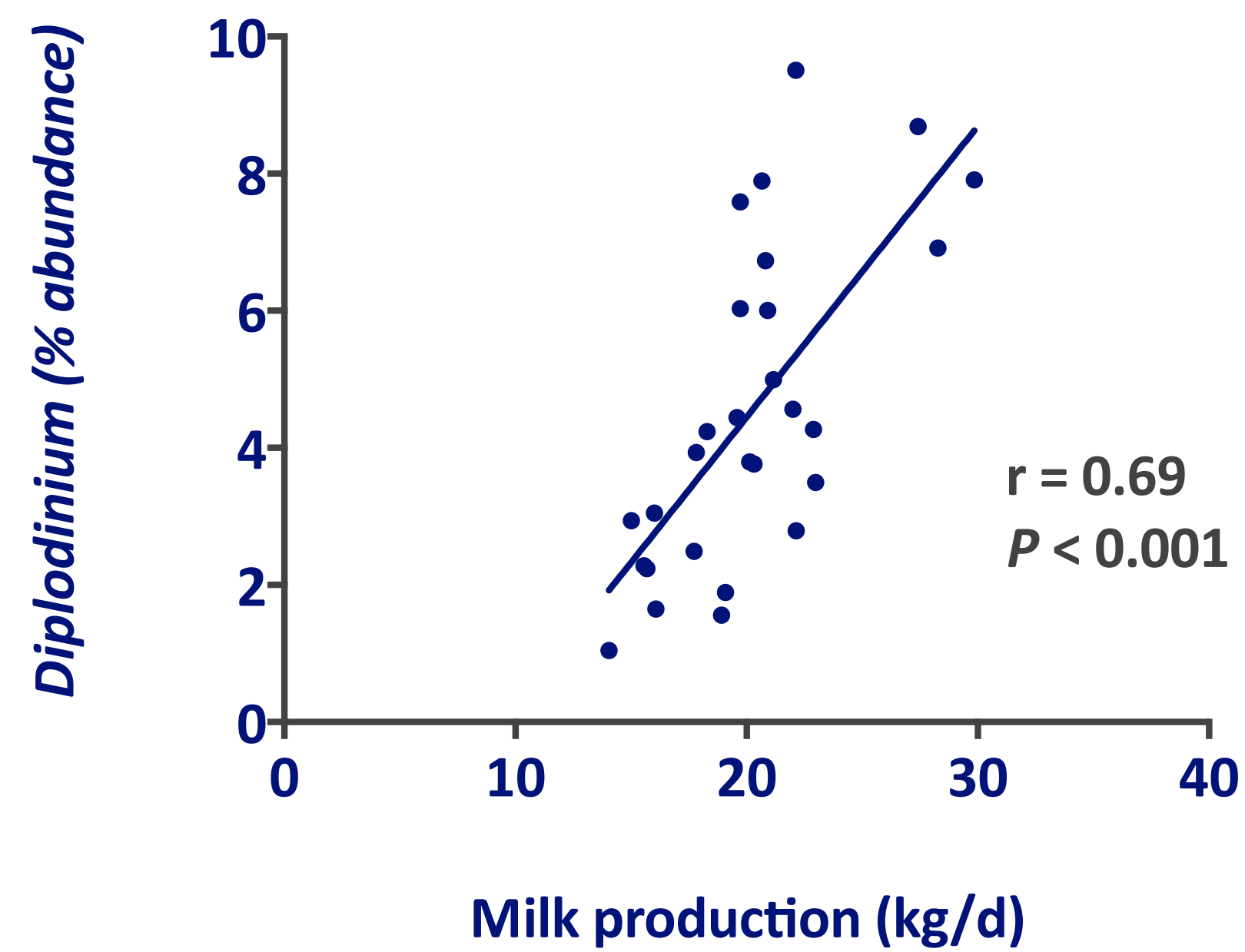


Figure 3: Pearson correlations between the protozoal genus *Diplodinium* and animal performance.

- *Diplodinium* correlated with milk fat and protein yields, feed efficiency, and milk production ($P < 0.001$).
- *Entodinium* was negatively correlated with milk production ($r = -0.48$, $P < 0.01$).

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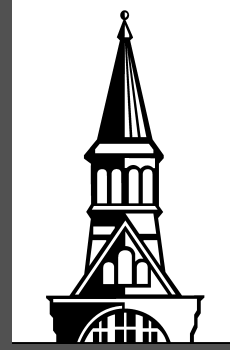
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Table 1: Nutrient composition (%DM) of the diets provided to lactating Jersey cows during the SPR and SUM experiments.

	DMI (kg/d)	CP ^a	ADF ^b	NDF ^c	lignin	ESC ^d	starch
SPR							
TMR ^e	10.7 ± 0.1	16.8	21.9	29.4	3.1	4.2	26.8
AFC (-) ^f mixed grasses	5.9 ± 0.4	13.2	35.2	58.5	4.7	7.9	3.2
AFC (-) legumes	1.4 ± 0.1	-	-	-	-	-	-
AFC (-) broadleaf weeds	1.1 ± 0.1	-	-	-	-	-	-
AFC (+) ^g mixed grasses	5.0 ± 0.4	11.0	35.8	59.2	4.8	8.4	3.2
AFC (+) legumes	1.2 ± 0.1	-	-	-	-	-	-
AFC (+) broadleaf weeds	0.7 ± 0.1	13.7	27.3	40.4	6.5	10.2	5.0
AFC (+) AFC small grains ^h	1.1 ± 0.3	8.5	42.9	67.6	5.7	7.3	2.6
SUM							
TMR	11.3 ± 0.4	15.2	19.9	32.8	3.1	10.0	23.8
AFC (-) mixed grasses	5.9 ± 0.6	14.7	34.7	56.0	4.7	9.2	2.2
AFC (-) legumes	0.9 ± 0.3	20.7	23.0	30.1	5.1	12.3	4.0
AFC (-) weeds/dead material	1.7 ± 0.2	15.0	31.7	48.9	7.1	7.9	3.1
AFC (+) mixed grasses	5.4 ± 0.4	15.3	33.5	55.3	4.8	9.4	2.7
AFC (+) legumes	1.2 ± 0.2	20.6	22.5	29.3	5.2	12.5	3.7
AFC (+) weeds/dead material	1.0 ± 0.1	16.2	29.5	44.2	6.4	9.0	2.3
AFC (+) oats	0.1 ± 0.0	11.1	33.4	51.5	4.3	11.2	3.9
AFC (+) AFC broadleaf ⁱ	1.1 ± 0.1	11.3	31.5	35.2	4.8	18.6	8.1

^a CP= crude protein, ^b ADF= acid detergent fiber, ^c NDF= neutral detergent fiber, ^d ESC= ethanol soluble carbohydrates, ^eTMR = total mixed ration (60:40 forage:concentrate), ^fAFC (-) = control diet, no annual forage crops, ^gAFC (+) = treatment, annual forage crops provided, ^halternative forage crop small grains included barley, rye, triticale, and wheat, ⁱAFC broadleaf = chickling vetch and buckwheat.

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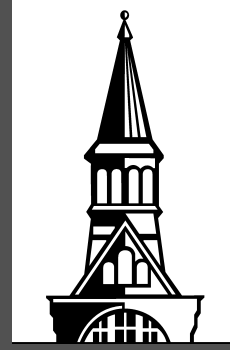
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Table 2: Composition of rumen VFA from AFC (-) and AFC (+) fed lactating Jersey cows during SPR and SUM experiments.

VFA (% of total)	SPR			SUM		
	AFC (-)	AFC (+)	SE	AFC (-)	AFC (+)	SE
Acetate	70.20	69.56	0.54	71.21	72.04	0.65
Propionate	16.21	16.49	0.35	15.62	15.57	0.36
Butyrate	11.10	11.29	0.30	10.96	10.43	0.23
Isobutyrate	0.99	0.96	0.05	0.98	0.80	0.03
Valerate	0.92	1.06	0.10	0.67	0.63	0.10
Isovalerate	0.57	0.66	0.86	0.56	0.54	0.12
Total VFA (mM)	70.38	78.20	4.27	71.91	78.20	5.19

- Percentages of acetate, propionate, and butyrate did not vary in SPR or SUM periods.
- Cows consuming AFC had less ruminal isobutyrate than those offered AFC (-) pasture ($P = 0.02$).

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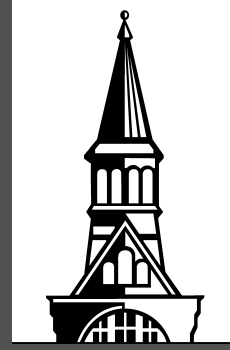
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Conclusions

- Concentration of major VFA and abundances of protozoal taxa were not altered by the addition of AFC.
- Protozoal taxa correlated with animal performance.
- Potential explanations for why differences in major VFA and protozoal taxa were not observed include: i) low AFC inclusion in the diet, ii) intra- and inter-animal variations, and iii) short length of experimental periods.
- Because AFC matured within a 21d period, future studies may characterize the rumen protozoa after AFC regrowth and/or during each week of the 21d period.

Funding Support

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