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Effect of Triticale Dried Distillers Grains with Solubles on Ruminal Bacterial Populations as Revealed by Real Time Polymerase Chain Reaction

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ABSTRACT : Real time PCR was used in this study to determine the effect of triticale dried distillers grains with solubles (TDDGS) as a replacement for grain or barley silage in finishing diets on the presence of six classical ruminal bacterial species (*Succinivibrio dextrinosolvens*, *Selenomonas ruminantium*, *Streptococcus bovis*, *Megasphaera elsdenii*, *Prevotella ruminicola* and *Fibrobacter succinogenes*) within the rumen contents of feedlot cattle. This study was divided into a step-wise adaptation experiment (112 days) that examined the effects of adaptation to diets containing increasing levels of TDDGS up to 30% (n = 4), a short-term experiment comparing animals (n = 16) fed control, 20%, 25% or 30% TDDGS diets over 28 days, and a rapid transition experiment (56 days) where animals (n = 4) were rapidly switched from a diet containing 30% TDDGS to a barley-based diet with no TDDGS. It was found that feeding TDDGS as replacement for barley grain (control vs. 20% TDDGS) decreased 16S rRNA copy numbers of starch-fermenting *S. ruminantium* and *S. bovis* ($p<0.001$ and $p = 0.04$, respectively), but did not alter 16S rRNA copy numbers of the other rumen bacteria. Furthermore, feeding TDDGS as a replacement barley silage (20% vs. 25% and 30% TDDGS) increased 16S rRNA copy numbers of *S. ruminantium*, *M. elsdenii* and *F. succinogenes* ($p<0.001$; $p = 0.03$ and $p<0.001$, respectively), but decreased ($p<0.001$) the 16S rRNA copy number of *P. ruminicola*. Upon removal of 30% TDDGS and return to the control diet, 16S rRNA copy numbers of *S. ruminantium*, *M. elsdenii* and *F. succinogenes* decreased ($p = 0.01$; $p = 0.03$ and $p = 0.01$, respectively), but *S. dextrinosolvens* and *S. bovis* increased ($p = 0.04$ and $p = 0.009$, respectively). The results suggest that replacement of TDDGS for grain reduces 16S rRNA copy numbers of starch-fermenting bacteria, whereas substitution for barley silage increases 16S rRNA copy numbers of bacteria involved in fibre digestion and the metabolism of lactic acid. This outcome supports the contention that the fibre in TDDGS is highly fermentable. (**Key Words :** Cattle, Real Time PCR, Rumen Microorganisms, Triticale Dried Distillers Grains with Solubles)

INTRODUCTION

The rumen possesses a complex symbiotic microbiota that ferments feedstuffs to a mixture of VFA that are the major energy source for ruminants. When cattle are fed high-grain diets, the ruminal pH often falls to levels below 5.8 (Dunlop, 1972; Nocek, 1997; Owens et al., 1998) resulting in a reductions in the conversion of fibre to VFA and in microbial protein synthesis (Strobel and Russell, 1986). If the pH reduction is severe (i.e., <5.2) acids can accumulate in the rumen, and clinical ruminal acidosis may occur (Dawson and Allison, 1988). Dried distillers grains with solubles (DDGS) have been used in diets both as an energy and protein concentrate (Schingoethe et al., 1999; Liu et al., 2000; Anderson et al., 2006). Previous work has suggested that including corn or wheat DDGS at 20% of the diet (DM basis) optimizes growth performance in feedlot cattle (Buckner et al., 2007; Gibb et al., 2008). Substitution of DDGS for grain in feedlot diets replaces starch with more slowly fermentable fibre, thereby decreasing the rate of acid production in the rumen and possibly the occurrence of sub-acute ruminal acidosis (SARA). Furthermore, DDGS commonly contains 320-460 g/kg neutral detergent fibre (NDF) on a DM basis (Al-Suwaiegh et al., 2002; Anderson et al., 2006) with reported values as high as 750 g/kg

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(Ojowi et al., 1997). This high NDF level may also enable DDGS to replace a portion of the forage component of the diet while still maintaining proper rumen function.

Bacterial changes associated with ruminal acidosis primarily include shifts in the populations of starch- and soluble sugar-fermenting bacteria and lactic acid-utilizing bacteria (Nagaraja and Titgemeyer, 2007). *Streptococcus bovis* has been considered as key etiologic agent in the development of clinical acidosis as it is capable of rapid growth on starch-based substrates and produces lactic acid as a primary fermentation end-product (Mackie and Gilchrist, 1979; Russell and Hino, 1985; McAllister et al., 1990). Lactic acid-utilizing bacteria such as *Megasphaera elsdenii*, and *Selenomonas ruminantium* play a central role in preventing the accumulation of ruminal lactic acid in grain-adapted cattle (Counotte et al., 1981).

Triticale (genus *Triticosecale*) is a highly adaptive, high-yielding cereal grain grown in western Canada that was developed by crossing wheat (*Triticum aestivum* or *Triticum turgidum*) with rye (*Secale cereale*). Triticale grain contains approximate 65% starch (Chapman et al., 2005) making it a potential carbohydrate source for bioethanol production. However, information regarding the impact of triticale DDGS (TDDGS) on populations of rumen bacteria involved in starch, fibre and lactic acid metabolism is lacking. For this purpose, the impact of TDDGS on 16S rRNA copy numbers of six rumen bacteria; *Succinivibrio dextrinosolvens*, *S. ruminantium*, *S. bovis*, *M. elsdenii*, *Prevotella ruminicola* and *F. succinogenes* was examined using real-time PCR. It was hypothesized that replacement of grain with TDDGS would reduce 16S rRNA copy numbers of starch-fermenting and lactic acid-utilizing bacteria and increase 16S rRNA copy numbers of fibre-fermenting bacteria. Further replacement of the forage with TDDGS was hypothesized to increase 16S rRNA copy numbers associated with both fibre-fermenting and lactic acid-utilizing bacteria.

MATERIALS AND METHODS

Reference strains

Reference strains obtained from the Lethbridge Research Centre culture collection used in this study are listed in Table 2. Reference strains of *Succinivibrio dextrinosolvens* 22b, *Megasphaera elsdenii* T81 and *Prevotella ruminicola* 23 were kindly provided by Dr. Paul Weimer at US Dairy Forage Research Centre. Bacteria were cultured for 18 h in medium under anaerobic conditions. Cells were isolated by centrifugation at 10,000×g for 10 min at 4°C. The supernatant was discarded and cell pellets were processed for DNA extraction.

Animal diets

Ruminally cannulated continental crossbred beef steers weighing 457±36 kg were randomly allocated to 8 pens. TDDGS was included in diets as a replacement for a portion

Table 1. Ingredients and nutrient composition of the four diets containing varying percentage of triticale dried distillers grains with solubles (TDDGS)

	TDDGS[1] (%)			
	Control	20	25	30
Ingredients, % dry matter (DM)				
Dry-rolled barley grain[2]	85	65	65	65
Barley silage[3]	10	10	5	0
Mineral and vitamin mix[4]	5	5	5	5
Nutrient composition, % DM				
DM	78.5	78.6	83.4	90.6
Protein	13.4	17.2	17.7	19.7
Neutral detergent fibre (NDF)	23.6	25.8	23.2	22.2
Acid detergent fibre (ADF)	8.0	10.2	8.7	7.9
peNDF[5]	2.02	2.29	1.42	0.83
Starch	51.4	41.5	41.8	44.0
Oil[6]	1.9	2.4	2.5	2.6
Calcium	0.78	0.78	0.71	0.87
Phosphorus	0.41	0.52	0.55	0.58
Ca:P	1.91	1.51	1.30	1.49

[1] Composition of TDDGS: 36.7% CP, 32.6% NDF, 5.9% starch (DM basis), and 11.4% ADIN (% of N).
[2] Composition of barley grain: 10.8% CP, 20.1% NDF, and 6.6% ADF (DM basis) according to NRC (1985).
[3] Composition of barley silage 12.5% CP, 46.1% NDF, 33.2% ADF, and 11.1% starch (DM basis).
[4] Supplement contained 562.6 g/kg ground barley, 100 g/kg canola meal, 25 g/kg molasses, 30 g/kg white salt, 10 g/kg feedlot mineral, 20 g/kg urea, 0.5 g/kg flavour, 0.66 g/kg Vitamin E, 2.32 g/kg rumensin (25 mg/kg) and 250 g/kg calcium carbonate.
[5] peNDF: physical effectiveness of neutral detergent fibre.
[6] Calculated from ingredient composition.

of barley grain, and subsequent as a substitute for barley silage. Steers were randomly assigned to one of following four diets (Table 1): i) 0% TDDGS diet (control): containing total mixed ration (TMR) of dry-rolled barley grain (85%), barley silage (10%), ii) 20% TDDGS diet: containing dry-rolled barley grain (65%), TDDGS (20%), barley silage (10%), iii) 25% TDDGS diet: containing dry-rolled barley grain (65%), TDDGS (25%), barley silage (5%), and iv) 30% TDDGS diet: containing dry-rolled barley grain (65%), TDDGS (30%). All four diets contained supplements (5% vitamins and minerals). Diets were formulated to meet or exceed the minimum nutrient requirements for beef cattle as recommended by National Research Council (2000). Total mixed rations were prepared daily and animals were offered *ad libitum* access to feed and water. The two replicates fed the same dietary treatments were spatially separated during the entire study. All animals were cared for according to the guidelines set out by the Canadian Council on Animal Care (1993).

Experimental design

The study was divided into three experimental periods. Period 1 was composed of 112-days were 4 steers were adapted in a step-wise manner to examine the effects of substituting 20% TDDGS for barley grain with a further increase in the level of TDDGS in the diet to 25 and 30% being substituted for barley silage (Table 1). This substitution resulted in a decline in starch and peNDF content of the diet. Rumen samples were collected at day 0, 28, 56, 84 and 112. Period 2 examined the effect of different diets over a period of 28 days. In the short-term study steers (n = 16) were divided into four groups (n = 4 per treatment) and assigned to control, 20, 25 or 30% TDDGS diets. Rumen samples were collected on day 0 and 28. Period 3 consisted of a 56-day rapid transition study where steers (n = 4) were initially fed a 30% TDDGS and then rapidly shifted to the control diet on day 28. This portion of the study examined the degree to which 16S rRNA copy number for each of the bacterial species of interest returned to those initially measured during the control period. For this portion of the study, rumen samples were collected at day 0, 28 and 56.

Sample collection

Rumen contents were sampled via fistula from each animal before the morning feeding on each sampling day and collected from the reticulum, ventral and dorsal sacs, and the feed mat (250 ml/site). Fluid and solid contents were combined, thoroughly mixed and placed directly into a flask on crushed ice. Samples were immediately transported to the laboratory for DNA extraction.

DNA extraction and PCR

Total DNA was extracted from rumen contents according to Mosoni et al. (2007). In brief, a representative sample (solid and liquid phases mix) was homogenized for 10 min using a Stomacher (Seward Ltd., Worthing, West Sussex, UK). Total DNA was extracted from 0.5 g homogenized rumen contents using the Power Soil® DNA Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Upon extraction, total DNA was quantified using Nanodrop 1000® (Nanodrop, Thermo Fisher Scientific, Wilmington, DE, USA) and 5 μl was resolved on a 0.7% (wt/vol) agarose gel. A control PCR was performed on all extracted DNA to rule out presence of inhibitors by amplifying 16S rRNA using forward primer 5' TCC TAC GGG AGG CAG CAG T 3' and reverse primer 5' GGA CTA CCA GGG TAT CTA ATC CTG TT 3' (Nadkarni et al., 2002) using the conditions described by Sharma et al. (2004).

Real time quantification

The average 16S rRNA copy number for *S. dextrinosolvens*, *S. ruminantium*, *S. bovis*, *M. elsdenii*, *P. ruminicola* and *F. succinogenes* was estimated by real time PCR (qPCR) using Brilliant SYBR® Green QPCR master mix (Stratagene, La Jolla, CA, USA). Each reaction mix (12.5 μl) contained 20 ng of DNA template and 500 nM of each primer. For *F. succinogenes*, *S. ruminantium* and *S. bovis*, PCR conditions according to Tajima et al. (2001) were used. For *S. dextrinosolvens*, *M. elsdenii*, *P. ruminicola*, PCR conditions as described by Stevenson and Weimer (2007) were used. Details of all primer sequences are provided in Table 2.

Purified genomic DNA from each of the specific rumen bacteria was used as control. Amplified fragments were cloned into pDrive® (Qiagen, Mississauga, ON, Canada) and sequenced. Confirmed plasmid containing the bacterial fragment of interest for each organism was used as standard in qPCR. Ten-fold serial dilutions were made in Tri-EDTA prior to qPCR. In total, six real-time PCR standards were prepared. The respective 16S rRNA gene for each organism was quantified using standard curves obtained with known concentrations of the plasmid DNA. All PCR reactions were performed in triplicate and normalized using an internal ROX reference dye. QPCR amplification was performed using the Stratagene Mx3005p® system (Stratagene). The 16S rRNA copy number/gram DM of rumen contents was calculated from three independent qPCR reactions according to Mosoni et al. (2007).

Statistical analysis

Copy numbers of each organism were analyzed as a completely randomized design using the PROC MIXED procedure (SAS, 2005) with diet in the model as a fixed effect. Day was treated as a repeated measures effect to account for potential correlations and different variances among the days for the 112-d step-wise adaptation (Period

Table 2. PCR primers and reference strains used to amplify target rumen bacteria in this study

Target bacteria	Primers (5′→ 3′)	Reference strains	Reference
Fibrobacter succinogenes	F:GGTATGGGATGAGCTTGC R:GCCTGCCCCTGAACTATC	*Fibrobacter succinogenes* subsp. *succinogenes* S85	Tajima et al., 2001
Succinivibrio dextrinosolvens	F:CGTCAGCTCGTGTCGTGAGA R:CCCGCTGGCAACAAAGG	*Succinivibrio dextrinosolvens* 22b	Stevenson and Weimer, 2007
Selenominas ruminantium	F:TGCTAATACCGAATGTTG R:TCCTGCACTCAAGAAAGA	*Selenomonas ruminantium* subsp. *lactilytica* (ATCC 19205)	Tajima et al., 2001
Streptococcus bovis	F:CTAATACCGCATAACAGCAT R:AGAAACTTCCTATC TCTAGG	*Streptococcus bovis* 45 S1	Tajima et al., 2001
Megasphaera elsdenii	F:AGATGGGACAACAGCTGGA R:CGAAAGCTCCGAAGAGCCT	*Megasphaera elsdenii* T81	Stevenson and Weimer, 2007
Prevotella ruminicola	F:GAAAGTCGGATTAATGCTCTATGTTG R:CATCCTATAGCGGTAAACCTTTGG	*Prevotella ruminicola* 23	Stevenson and Weimer, 2007

1) and 56-d rapid transition study (Period 3). Various variance-covariance matrices were fitted and the one with the lowest AIC value was used for the final analysis. The UNIVARIATE procedure was used to check the residuals for normality and for potential outliers.

RESULTS AND DISCUSSION

Total DNA extracted using the Power Soil® DNA Kit was of high quality and yield allowing for successful real time PCR. Each real time PCR yielded acceptable slopes and R^2 values. The trends observed in this study can be understood in context of two aspects. Firstly, control vs. 20% TDDGS diet supplementation where TDDGS was substituted for barley grain, reducing the starch content and increasing the fibre content of the diet. Secondly, where barley silage was replaced by TDDGS which resulted in an increased concentrate:forage ratio from 90:10, 95:5 to 100:0, for the 20%, 25% and 30% TDDGS diets, respectively.

Succinivibrio dextrinosolvens, Selenomonas ruminantium, Streptococcus bovis

In the step-wise adaptation study, comparable 16S rRNA copy numbers of *S. dextrinosolvens* were detected

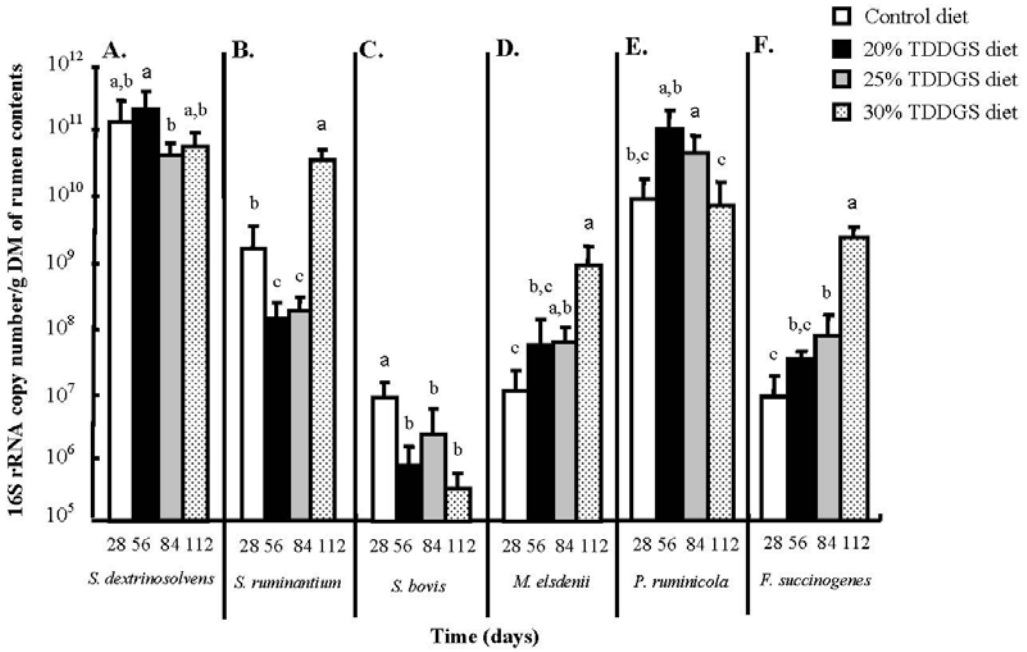

Figure 1. Average copy number of 16S rRNA from A) *S. dextrinosolvens*, B) *S. ruminantium*, C) *S. bovis*, D) *M. elsdenii*, E) *P. ruminicola* and F) *F. succinogenes* quantified using qPCR from rumen contents of animals in the four groups (n = 4 animals per group). Animals were fed either control, 20%, 25% or 30% triticale dried distillers grains with solubles (TDDGS) diet over 112-d step-wise adaptation study (Period 1). Significant differences between diets (control, 20%, 25% and 30% TDDGS) ($p<0.05$) are noted by different lower case letters within each organism.

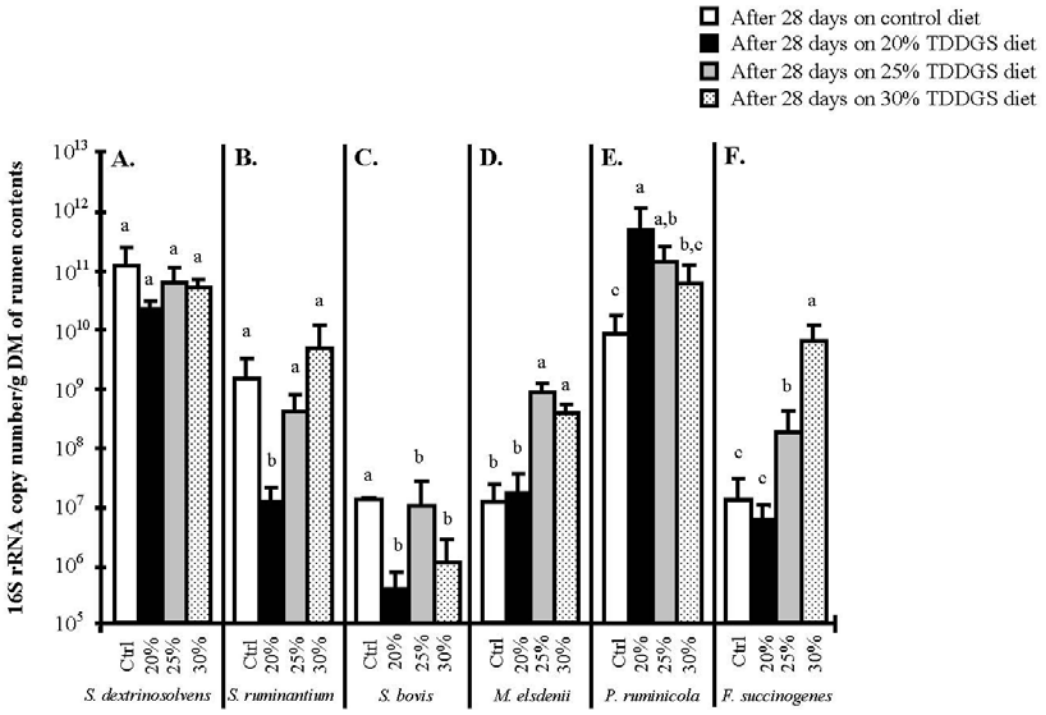

Figure 2. Average copy number of 16S rRNA from A) *S. dextrinosolvens*, B) *S. ruminantium*, C) *S. bovis*, D) *M. elsdenii*, E) *P. ruminicola* and F) *F. succinogenes* quantified using qPCR from rumen contents from animals fed control, 20%, 25% and 30% triticale dried distillers grains with solubles (TDDGS) diets for 28 days (Period 2). A sample from one steer on the control diet was not obtained on day 28. For all other dietary treatments, four steers were sampled. Significant differences between diets (control, 20%, 25% and 30% TDDGS) ($p<0.05$) are noted by different lower case letters within each organism.

from steers on all four diets. However, the highest *S. dextrinosolvens* 16S rRNA copy number was recorded from steers fed 20% TDDGS which was higher ($p<0.001$) than from steers fed 25% TDDGS (Figure 1A). In the short term study, no difference in 16S rRNA copy number of *S. dextrinosolvens* was observed among diets (Figure 2A). In the rapid transition study, we found the transition from the 30% TDDGS diet to the control diet increased *S. dextrinosolvens* 16S rRNA copy numbers (Figure 3A). These results suggest that TDDGS inclusion (either substitute for grain or forage) had negligible effects on the population of *S. dextrinosolvens*. Similarly, Stevenson and Weimer (2007) reported that *S. dextrinosolvens* was unlikely to make a significant contribution to starch digestion in cattle fed high-grain diets. In addition, others have shown that 16S rRNA copy number associated with *S. dextrinosolvens* declined when cows were transitioned from a hay to a grain diet (Tajima et al., 2001).

S. dextrinosolvens is known to possess amylolytic activities and to be a potent utilizer of pectin and dextrins (Stewart et al., 1997; Russell and Rychlik, 2001). Among starch- and soluble sugar-fermenting bacteria we monitored, *S. ruminantium* and *S. bovis* exhibit the highest growth rate and amylolytic activities (Cotta, 1988, 1992; McAllister, 1990). Therefore, the comparatively constant 16S rRNA copy numbers of *S.dextrinosolvens* suggest that this bacterium did not undergo major shifts in population as a result of changes in starch or fibre content of the diet.

During the step-wise adaptation study, the 16S rRNA copy number of the major ruminal starch-fermenting bacterium, *S. bovis* was observed to decrease ($p<0.001$) as steers were fed increasing levels of TDDGS (Figure 1C), a result that was also observable in the short term study (Figure 2C). Similarly, the 16S rRNA copy number of *S. ruminantium* decreased ($p<0.001$) as TDDGS was substituted for barley grain, but as TDDGS was substituted for barley silage the 16S rRNA copy number of this lactate-utilizer increased ($p<0.001$, Figure 1B and 2B). Such a response suggests that substitution of the highly fermentable fibre in TDDGS for the less fermentable fibre in barley silage may have increased the amount of lactate available for metabolism by *S. ruminantium*. This possibility is supported by the fact that transition from the 30% TDDGS decreased ($p = 0.01$) 16S rRNA copy number associated with *S. ruminantium* while increasing ($p = 0.009$) the 16S rRNA copy number associated with *S. bovis* (Figure 3).

In the current study, the lack of an increase in 16S rRNA copy number associated with *S. bovis* suggests that this bacterium did not proliferate with increasing levels of

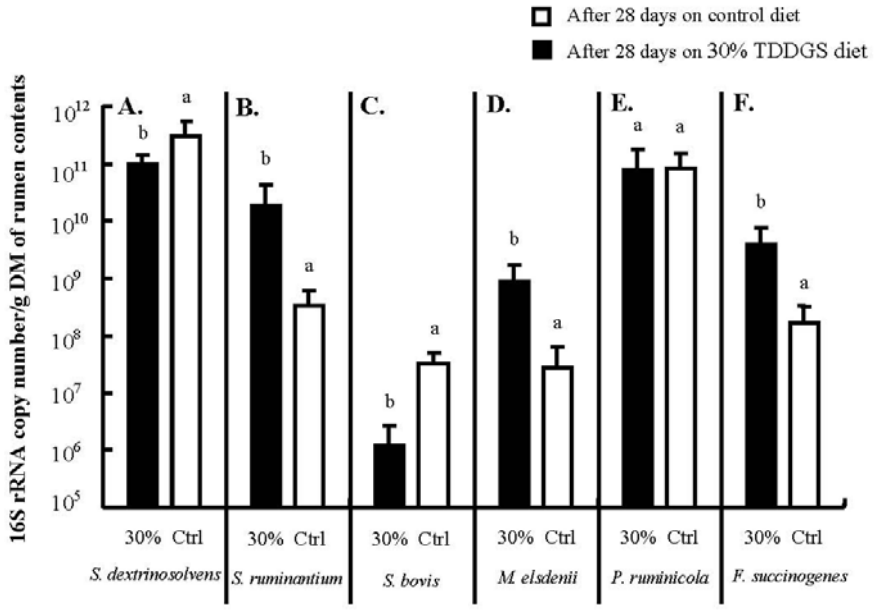

Figure 3. Average copy numbers of 16S rRNA from A) *S. dextrinosolvens*, B) *S. ruminantium*, C) *S. bovis*, D) *M. elsdenii*, E) *P. ruminicola* and F) *F. succinogenes* quantified using qPCR from rumen contents from animals (n = 4) fed 30% triticale dried distillers grains with solubles (TDDGS) diet for 28 days followed by control diet for an additional 28 days (Period 3). Significant differences between control and 30% TDDGS diet ($p<0.05$) are noted by different lower case letters within each organism.

TDDGS in the diet, even though there is evidence that this feeding practice lowers ruminal pH and increases the incidence of liver abscesses in feedlot cattle (Wierenga et al., 2010). This observation is consistent with Weimer et al. (2008) where they observed that *S. bovis* accounted for a comparably low proportion of total bacteria even in cattle fed high-starch diets. In this context it is important to note that none of the steers used in the current study exhibited symptoms of clinical acidosis.

Similarly, Tajima et al. (2001) reported that the 16S rRNA copy number of *S. bovis* decreased when cows were adapted from a hay to a grain diet. Wells et al. (1997) also observed that the numbers of *S. bovis* declined 10,000-fold and were similar to those in forage-fed cattle when cattle were adapted to a high-grain diet. In addition, even in the occurrence of acute acidosis, the explosive growth of *S. bovis* appears to be of a transient nature, as lactobacilli rapidly out-compete it at low rumen pH (Nagaraja and Titgemeyer, 2007). Bacteriocins may also contribute to conferring lactobacilli with a competitive advantage over *S. bovis* (Wells et al., 1997).

Megasphaera elsdenii

In the step-wise adaptation study, 16S rRNA copy number of *M. elsdenii* was found to increase ($p = 0.03$) with increasing TDDGS in the diet, but did not differ between the control and 20% TDDGS diet (Figure 1D). A similar pattern was observed in the short term study where 16S rRNA copy number of *M. elsdenii* increased with increasing TDDGS in the diet, but did not differ between the 20% TDDGS and control diet (Figure 2D). Conversely, switching steers from the 30% TDDGS diet to the control diet resulted in a decline ($p = 0.03$) in 16S rRNA copy numbers of *M. elsdenii* (Figure 3D). These results suggest that substitution of more fermentable fibre in TDDGS for less fermentable fibre in barley silages increased 16S rRNA copy numbers associated with *M. elsdenii* in the rumen. This response may also reflect an increase in the availability of lactic acid for metabolism by *M. elsdenii* as a result of this change in dietary composition. Others have reported that the abundance of *M. elsdenii* increases in cattle adapted to high grain diets, likely as result of increased lactate production (Krause et al., 2003). Previous research reported that 16S rRNA from *M. elsdenii* was undetectable when cattle were fed hay but was detected 2 days after the introduction of grain into the diet, with levels increasing for up to 12 days (Klieve et al., 2003).

Prevotella ruminicola

In the step-wise adaptation study, 16S rRNA copy number of *P. ruminicola* was highest in the 20% TDDGS diet and lowest ($p<0.001$) in steers fed 30% TDDGS (Figure 1E). The same trend was observed in the short term study (Figure 2E), but 16S rRNA copy number of *P. ruminicola* in the 20% TDDGS was higher than in the control diet. No difference in 16S rRNA copy number of *P. ruminicola* was found when cattle were transferred from the 30% TDDGS to the control diet (Figure 3E). Previous

research reported that *P. ruminicola* was the predominant species among 13 rumen bacteria measured in cows fed hay and that its predominance declined when the cows were shifted to a grain diet (Tajima et al., 2001).

P. ruminicola is also known to possess carboxymethyl-cellulase and xylanase activities, but it appears that the dietary manipulations employed in this study had little influence on this bacterium as one form of fibre was swapped for another as TDDGS replaced barley silage. *P. ruminicola* is also known to play an important role in peptide metabolism (Stewart et al., 1997), but this capacity appeared to have little effect on 16S rRNA copy numbers even though the protein content of the diet dramatically increased as barley silage was replaced by TDDGS in the diet.

Fibrobacter succinogenes

The 16S rRNA copy number associated with *F. succinogenes* increased ($p<0.001$) as TDDGS was substituted for barley silage (Figure 1F). However, this difference was not significant when 20% TTDGS was substituted for barley grain. Similar results were found in the short term study, where increasing TDDGS also tended to increase the 16S rRNA copy number associated with *F. succinogenes* (Figure 2F). Conversely, transition from the 30% TDDGS diet to the control diet resulted in a decline ($p = 0.01$) in *F. succinogenes* 16S rRNA copy number in rumen contents (Figure 3F). Taken together, these observations support the fact that *F. succinogenes* played an increasing role in fermentation in the rumen as the concentration of fermentable fibre in the rumen increased. This is consistent with the general understanding that *F. succinogenes* plays an important role in the fermentation of high forage diets (Stewart et al., 1997; Tajima et al., 2001). Although not assessed in the current experiment, it is equally possible that the 16S rRNA copy number of two other important cellulolytic bacteria in the rumen, *Ruminococcus albus* and *Ruminococcus flavefaciens* also increased as TDDGS was substituted for barley silage.

Although quantification of total and relative abundance of each bacterium was not undertaken in the current experiment, changes in the copy number of the 16S rRNA gene generally reflect changes in the predominance of the target bacterium within a microbial community. Substitution of TDDGS for barley silage increased the 16S rRNA copy numbers associated with the cellulolytic bacterium, *F. succinogenes* and lactate-utilizers such as *S. ruminantium* and *M. elsdenii*. These changes suggest that significant quantities of lactate may still arise from TDDGS, in spite of its high fibre content. The fact that the 16S rRNA copy number associated with *F. succinogenes* increased in spite of TDDGS being associated with a significant decline in ruminal pH (Wierenga et al., 2010) raises the need to revisit the role of fibrolytic bacteria in the fermentation of fibre in DDGS even when rumen pH remains below 5.5 for a significant portion of the day.

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