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The inclusion of low quantities of lipids in the diet of ruminants fed low quality forages has little effect on rumen function

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Research highlights

- An overview of the changes in fatty acids profile in the rumen fluid of cattle receiving a tropical grass hay, of longer retention time, was studied;
- The inclusion of low quantities of lipids in the diet had little, if any, effect on rumen function. However, soybean oil did increase the fractional outflow rate of liquid from the rumen and this may be of benefit to animals grazing low quality pastures;
- Differences in fatty acids profile of oils were only partially translated into the fatty acids profile in the rumen fluid, without great changes in the proportion of CLA isomers observed;
- None of the oils utilised in this experiment would affect fat synthesis and as such it is unlikely that the small quantities of lipids present in protein supplements fed to cattle in northern Australia would have any detrimental effect on fat synthesis when grazing low quality tropical pastures in the dry season.

Abstract

Biohydrogenation within rumen fluid (RF) proceeds to varying degrees depending on retention time (RT) and type of basal diet, especially the profile of fatty acid (FA) being hydrogenised. The objective of this study was to examine the FA profile and the RT of liquid in the rumen of steers fed a low crude protein (CP) tropical grass (*Chloris gayana* hay, 38g CP, 17g crude lipid and 752g neutral detergent fiber (aNDFom)/kg dry matter (DM)) supplemented with various lipids. Five rumen cannulated *Bos indicus* cross, five-year-old steers (799 ± 15kg live weight (LW)) were allocated to a 5x5 Latin square design. The treatments were control, hay only, or the addition of 30g/kg hay DM of lipid sources: Coconut (high lauric acid), cottonseed and soybean (high linoleic acid) or fish oil (high long chain FA (LCFA)). Retention time

decreased with addition of soybean oil (14h) but no differences between other treatments (mean 17h). Coconut oil increased lauric and myristic acids in RF. There were no changes in total saturated FA (TSFA) in RF, with exception of a lower concentration for fish oil treatment. Addition of fish oil also decreased the concentration in RF of stearic and linolenic acid, but no differences to coconut and cottonseed treatments for linolenic acid. Fish oil also resulted in higher LCFA, linoleic and total unsaturated FA (TUFA), but no differences to soybean oil for the latter two acids. The conjugated linoleic acid (CLA) was only different in RF between cottonseed and fish oil treatments. Differences in FA profile of oils were only partially translated into the FA profile in RF of steers fed a tropical hay, without great changes in the proportion of CLA isomers observed.

Abbreviations: RT, retention time; FA, fatty acid; RF, rumen fluid; CP, crude protein; aNDFom, neutral detergent fibre; ADFom, acid detergent fibre; DM, dry matter; LW, live weight, LCFA, long chain FA; TSFA, total saturated FA; TUFA, total unsaturated FA; CLA, conjugated linoleic acid; TOBCFA, total odd branched-chain FA; MCP, microbial crude protein.

Keywords: Oil; rumen fluid; fatty acid; biohydrogenation; CLA isomers

1. Introduction

Fatty acid (FA) profile in the rumen fluid (RF) or adipose tissue of ruminant is influenced by the addition of oils in the diet, such as sunflower oil (Bessa et al., 2007), safflower oil (O'Kelly and Spiers, 1991), fish oil (Kim et al., 2008) and linseed oil (Shingfield et al., 2011). The common objectives in most studies using addition of FA in the diet, is to decrease saturated and trans FAs and increase conjugated linoleic acid (CLA)s, 18:3n-3, 20:5n-3, and 22:6n-3. These changes in FAs are related to human-health benefits (Ha et al., 1987; Ochoa et al., 2004),

changes in the microbial population (Boeckaert et al., 2006), influences on animal metabolism (Bauman et al., 2011) or to enhancement of the overall performance of offspring, when fed to dams during pregnancy and lactation (Pickard et al., 2008; Or-Rashid et al., 2010). There are known differences between the FA profile of meat of grass fed animals and animals fed silage or concentrate-based diets (Poulson et al., 2004; Noci et al., 2005), but there is little information regarding the FA profile of cattle fed tropical grasses. Lipid profile and concentration in the diet are two factors that can influence the profile of fat in muscle and milk. Some isomers resulting from the biohydrogenation process are known to have effects on fat synthesis, and the longer RT of fluid in the rumen of animals fed tropical grasses may affect the extent of biohydrogenation and hence FA profile compared to other basal diets. One hypothesis in this study was that a higher polyunsaturated FA content of the supplement (highest being fish oil) was associated with a higher total unsaturated fatty acids (TUFA) of RF. A second hypothesis was that supplementation with lipids varying in FA profile resulted in different concentrations of CLA isomers, reflecting the FA profile of the supplement and the extent of biohydrogenation. The aim of this experiment was to determine differences in FA in response to the lipid supplement sources in cattle consuming a tropical grass hay.

2. Materials and methods

The experiment was conducted at the Center of Advanced Animal Science (CAAS). The procedures were conducted in accordance with the guidelines of the Australian code of practice for the care and use of animals for scientific purposes and were reviewed and approved by the University of Queensland animal ethics committee.

2.1. Animals, experimental design and treatments

Five Brahman-cross rumen cannulated steers (799 ± 15 kg LW, 5 years of age) at the commencement of the experiment were randomly allocated to one of five individual pens. Steers remained in their pens throughout the experimental period. Prior to the start of the experiment the steers were offered *ad libitum* Rhodes grass (*Chloris gayana*) hay containing 876 g organic matter (OM), 38 g crude protein (CP), 752 g ash-free neutral detergent fiber (aNDFom), 440 g ash-free acid detergent fiber (ADFom) and 17 g crude lipids per kg of dry matter (DM). The experiment consisted of a seven day adaptation period followed by an experimental period of five runs, each of which consisted of a feeding period of 18 days followed by a three day collection period (total 21 days).

The experiment consisted of a 5x5 Latin square design, consisting of five replicates (steers) and five treatments (lipids). Steers were allocated to one of the following treatments:

1. Control (Rhodes grass (RG) hay only); 2. Coconut oil plus RG; 3. Cottonseed oil plus RG; 4. Fish oil plus RG and 5. Soybean oil plus RG.

2.2. Feeding

Steers were offered RG at a total daily allocation of 9 g DM/kg LW in approximately equal amounts at 0700 and 1600 h each day. This level of intake was the average of the daily *ad libitum* intake in the 7 day adaptive feeding period. Lipids were offered at 30 g/kg hay intake on

a DM basis, following the dose used by Shingfield et al. (2003), as total daily allocation split in half doses at 0700 and 1600 h each day and were administered via the cannula. No mineral or vitamin supplements were added. Hay residues of each steer were collected weekly.

2.3. Sampling procedures and measurements

2.3.1. Liveweight

Steers were weighed, unfasted, prior to feeding on the first day of each run.

2.3.2. Retention time of chromium ethylene diaminetetraacetic acid (Cr-EDTA) in the rumen

On day 19 of each run (day 1 of the RF collection period), a single dose of Cr-EDTA (approximately 65 mL/100 kg LW; 2.8 mg Cr/mL) was administered to three sites in the rumen (i.e. cranial, ventral and caudal sacs) of all steers via the cannula immediately prior to the morning feeding. Rumen fluid samples (approximately 300 mL) were collected prior to dosing and feeding (0 h) and then 4, 8, 12, 16, 24, 28, 32 and 48 h after dosing (and feed offered) with the use of a sampling probe.

2.3.3. Analytical procedures for feeds and rumen physical-chemical parameters

Duplicate sub-samples of weekly hay offered and residues were oven dried to a constant weight at 60^{o}C. Weekly samples were bulked within runs and ground through a 1 mm screen (RetschZM 200; Haan, Germany) and stored for chemical analysis. Residual moisture content of all samples was determined by drying samples at 105^{o}C for 24 h. Organic matter content of samples was determined after incineration at 550^{o}C for 8 h in a muffle furnace (Modutemp Pty. Ltd.; Perth, WA, Australia). Nitrogen content of the hays offered for each run was determined by the Kjeldahl method using a N analyser (Kjeltec, 8400 FOSS; Hillerod, North Zealand, Denmark), according to Thiex et al. (2002). A conversion factor of 6.25 was used to convert the total N to CP. Ash-free neutral detergent fibre was assayed with a heat stable amylase (aNDFom)

(Mertens, 2002), and acid detergent fibre expressed exclusive of residual ash (ADFom) according to Van Soest et al. (1991).

Total crude lipid content of samples was determined using an adaptation of the low-toxicity solvent method (Hara and Radin, 1978). Approximately 5 g of sample was mixed with 50 mL Chloroform:Methanol (2:1) and incubated at room temperature overnight. The sample was then filtered into a 100 mL volumetric cylinder through Whatman paper (12.5 cm, N^{o}. 1) and rinsed with Chloroform:Methanol (2:1) solution (between 10 to 20 mL). A 0.88% NaCl solution, 20% of the filtered sample volume, was added to the sample which was then shaken well and incubated at room temperature for 2 h, resulting in the separation of two distinct phases. The volume of the lower phase chloroform extract was recorded, and the upper phase was discarded. The chloroform extract (15 mL) was then transferred to a pre-weighed vial and then evaporated to a constant weight at room temperature and crude lipid weight determined.

Crude lipids (%) = (lipids in 15 mL/10) x (total volume/sample weight) x 100

Rumen fluid samples were stored on ice for approximately 15 min before RF pH was measured and sub-samples of RF stored for the following analysis: Cr concentration (10 mL rumen fluid); FA profile (10 mL rumen fluid), rumen NH_3N (8 mL RF + 1 mL 1 M H_2SO_4) and rumen volatile FA (VFA) (3 mL RF (1 mL at 0, 8 and 16 h) + 1 mL 20% metaphosphoric acid + internal standard (*i.e.* 4 methyl n-valeric acid)). All samples were stored at -20^{o}C until analysis. Samples collected at 0, 4, 8, 12, 16, 24, 28, 32 and 48 h were analysed for Cr concentration. Samples collected at 0, 4, 8, 12 and 16 h were analysed for FA profile. Samples collected at 0, 8 and 16 were analysed for NH_3N concentration. Chromium concentration was determined in RF samples diluted 1 in 10 with distilled water, centrifuged at 4000 g and set at 4° C for 5 min and then aspirated directly into the ICP (inductively coupled plasma spectrometer; Optima 7300 DV,

PerkinElmer; Waltham, MA, USA). To overcome matrix effects, standards were prepared for samples in each run by diluting known amounts of Cr with 0 h rumen fluid after bulking from all animals (*i.e.* each run had a separate standard curve); this bulked fluid was also diluted 1 in 10 with distilled water and centrifuged at 4000 g for 5 min prior to use. In order to calculate RT, fluid outflow rate (FOR) and rumen volume, the change in concentration of CrEDTA with time was measured. The slope (k) of Ln [Cr] against time is the fractional outflow rate (FOR, /h) and 1/k is the retention time (RT, h) of the marker in the rumen. The rumen volume, or pool size, was calculated by dividing the total amount of Chromium injected at time zero by the predicted concentration at time zero by extrapolating the regression of Ln [Cr] to time zero (Binnerts et al., 1968). In this approach the limitations due to non-steady state conditions are recognised. Fatty acids were analysed using modifications of the method of Kramer et al. (1997). The RF and feed samples (0.05-0.1 g) were weighed into 5 ml tubes with 20 uL internal standard and 2.0 ml of 0.5 N NaOH in methanol and vortexed. The tubes were heated for 15min at 50°C on heat block, then cooled at ambient temperature. Two ml of 2% H_2SO_4 in methanol was added, and the tubes heated for 1hr at 50°C, then cooled at ambient temperature and vortexed. Both heptane and deionised water were added at 1ml each, and the tubes centrifuged at 1500 g for 5 min. The upper heptane layer was transferred to a 2.0 ml micro-centrifuge tube, and 0.1 g of anhydrous sodium sulphate added to remove any residual water. Activated charcoal (0.01 g) was added, and the tubes stood for 1hr at ambient temperature, then centrifuged at 13000 rpm for 20 min and the upper heptane layer again transferred to a 0.2 ml vial and stored at -20°C until analysis. The GC operation used initial temperature of oven of 85°C, held for 4 min, ramp rate of 13°C/min to 175°C, held for 27 min, ramp rate of 4°C/min to 215°C, held for 35 minutes. The inlet split

injection ratio was 1:15 with the flame ionisation detector set at 250°C, and helium gas pressure on column and linear velocity at 16.7cm/sec.

The NH_3N concentration in RF was determined by distillation using a Büchi 321 distillation unit (Flawil, St. Gallen, Switzerland). Sodium tetraborate was added to excess to buffer the sample at around pH 9.5 and decrease hydrolysis of non-ammonia compounds, ammonia was distilled from the mixture using steam. Boric acid captured the ammonia gas, forming an ammonium-borate complex. Ammonia concentration was calculated after titration against a 0.01 M HCl solution of known molarity using a TIM 840 Titration workstation manager (Radiometer Analytical SAS, Villeubanne, Cedex, France). The concentration of VFAs present in RF were determined by gas liquid chromatography (GC17, Shimadzu; Kyoto, Honshu, Japan) using a polar capillary column (ZB-FFAP, Phenomenex; Lane Cove, NSW, Australia) based on the methods of Cottyn and Boucque (1968), Ottenstein and Bartley (1971) and Playne (1985).

2.4. Statistical analysis

The experiment was a 5 (steers) x 5 (runs) Latin square design, with the five treatments randomly allocated to ensure balance with both animal and run. Analyses were carried out using the General Linear Model (GLM) procedure in the SAS statistical system (SAS® version 9.2©, 2008). The Least Significant Difference test (LSD) test was used for multiple comparisons, adopting a 5% level to assess statistical significance in all cases.

3. Results

3.1 Rumen Parameters

Rumen NH_3N concentration was very low (less than 10 mg/L) for all steers (Table 1). Steers supplemented with cottonseed oil had lower rumen NH_3N concentration than control steers and steers supplemented with fish oil ($P<0.05$), but not significantly different to steers supplemented

with soybean or coconut oil (P>0.05). Total VFA concentration was lower for steers supplemented with coconut oil compared with all other steers (P<0.05). Steers supplemented with fish oil had a lower molar proportion of acetic acid and a higher molar proportion of propionic acid (P<0.05) than the other steers, but no different to control steers (P>0.05), with no differences in the molar proportion of butyric acid or branched-chain FA apparent between treatments (P>0.05). Retention time of Cr-EDTA was shorter in the rumen of steers provided with soybean oil (P<0.05), with no difference between the other lipids and control, resulting in an increase in fractional outflow rate of approximately 20% in steers administered with soybean oil compared with control steers (P<0.05). Steers supplemented with cottonseed, fish and soybean oils had lower rumen volume (P<0.05) than control and coconut oil supplemented steers.

Insert Table 1 here

3.2. Fatty acids profile of rumen fluid

The FA profile of the hay and different lipid sources used in this experiment are presented in Table 2. Total odd and branched-chain fatty acid (TOBCFA) concentrations were low for all feeds with the highest concentration present in fish oil (*i.e.* 0.74% of total FA). Soybean oil had the highest concentration of TUFA and TC18s, and the lowest concentration of TSFA (P<0.05). Coconut oil had the highest concentration of TSFAs, C12:0 (lauric acid) and C14:0 (mystiric acid) and the lowest concentration of TUFA, TC18s and C16:0 (palmitic acid) (P<0.05) when compared with the other oils and hay. Rhodes grass hay had a higher concentration of palmitic acid and C18:3n-3 and a lower concentration of C18:1*c*9 than any of the lipid sources. All feeds had a very low or undetectable concentration of C15:0anteiso.

Insert Table 2 here

Palmitic acid was present in the feeds and RFof steers at the highest concentration, regardless of treatment, followed by C18:2n-6 for cottonseed, soybean and fish oils, C14:0 for coconut oil and C18:3n-3 for hay in the FA profile of feeds and C18:2n-6 was the second highest in RF of all treatments (Table 3). The TOBCFA concentration did not differ ($P>0.05$) between the cottonseed, fish and soybean treatments. In addition, there was no difference ($P>0.05$) between the control, cottonseed, soybean and coconut treated diets for TOBCFA. However, the fish oil treatment was less than ($P<0.05$) the control and coconut oil treatments for TOBCFA. The saturated fatty acid concentration did not differ ($P>0.05$) between the fish and soybean treatments. However, it was significantly lower for steers supplied with fish oil in comparison with the other treatments ($P<0.05$), whilst there was no difference in comparison with the concentration of TSFA ($P>0.05$) in the RF of steers supplied with soybean oil. Steers supplied with fish oil had a higher concentration of TUFA in the RF than control steers or steers supplied with coconut and cottonseed oils ($P<0.05$). Steers supplied with coconut oil had a higher concentration of C12:0, C14:0 in the RF compared to all other treatments ($P<0.05$). Linolenic acid (C18:3n-3) was higher in the RF of steers supplied with soybean oil, compared to the other oils ($P<0.05$), but was not significantly different ($P>0.05$) to the concentration in the RF of control steers.

Insert Table 3 here

4. Discussion

The present experiment evaluated the effect of vegetable and fish oils on the rumen FA profile of steers fed a low CP tropical forage. The vegetable oils used in the current study are likely to be present in small quantities in protein supplements (copra meal, soybean meal and cottonseed meal) commonly used by the cattle industry in northern Australia. Fish oil was used as it

contains FAs which are present in some algae species and as such may reflect the FA profile in the RF of cattle supplemented with certain algae spp. It was not possible to source and use oil from algae species in the quantities required and the fish oil approach was used to supply FAs of interest that would be supplied by certain marine algae species.

The hay used in this study averaged 38 g CP/kg DM and this low CP concentration is typical of forages grazed by cattle during the dry season in northern Australia (Hennessy, 1980; McLean et al., 1983), which typically results in rumen NH_3N concentration lower than that required for maximal microbial crude protein (MCP) production. The lipid sources used in the present experiment were supplied at 30 g/kg DM. A preliminary feeding experiment indicated that roughage (maize silage) intake was reduced when soybean oil was supplied at 50 g/kg DM, which is below the maximal level of 94 g lipids/kg DM reported by Kucuk et al. (2004), where soybean oil was utilized without marked influence in fibre digestion. While the amount of lipids supplied in the current experiment are found in use by other researchers (Kitessa et al., 2001; Shingfield et al., 2011), and are lower than elsewhere (Bauman et al., 2011), the low amounts of lipids supplied more closely reflect the situation in extensive grazing scenarios where the source of lipids is most likely to come from small quantities remaining in protein supplements after various lipid extraction techniques have occurred, with varying levels of efficiency.

Fatty acid profile in the RF and adipose tissue of ruminants is influenced by the addition of lipids to the diet (Bessa et al., 2007; Shingfield et al., 2011), or the addition of supplements high in lipids (*e.g.* algae; Or-Rashid et al., 2008; Boeckaert et al., 2008), or the inclusion of whole cottonseed and soybean to the diet (Mohamed et al., 1988). It is known that the FA profile in RF of dairy cows grazing a high quality ryegrass (*Lolium perenne* cv. Bealey) and white clover (*Trifolium repens*) pasture can vary considerably over the grazing cycle (Sun and Gibbs, 2012),

but there is little information available about a tropical grass. The basal diet in the current experiment was hay and it had a slightly higher linolenic content in comparison to linoleic, but the RF of control animals had more linoleic, indicative of biohydrogenation within the rumen. The addition of oils in this experiment resulted in a range of minor changes in the FA profile in the RF of steers but no great changes in the proportion of CLA isomers were observed, and therefore effects on fat metabolism would not be expected. This may be a consequence of the long RT of the fluid phase within the rumen and the extent of biohydrogenation. There is a close relationship between RT of fluid and particles in the rumen (Poppi et al., 1981) and so the long RT of CrEDTA is indicative of the RT of both phases with FA able to move between both phases.

The TOBCFA concentration was higher in the RF than in the hay and oils used in the present experiment, which is in agreement with TOBCFA being almost exclusively microbial in origin (Kim et al., 2005; Vlaeminck et al., 2006). The TOBFCA concentration was lower in the RF of steers supplied with fish oil compared to control steers, but not different to that in the rumen of steers supplied with cottonseed and soybean oils. The lower TOBCFA concentration in the rumen of steers supplied with fish oil could be due to differences in the microbial community, which have been reported to occur in the rumen of cattle fed fish oil (Kim et al., 2008).

Despite the higher concentration of TSFA and lower concentration of TC18s in coconut oil compared to the other oils and Rhodes grass hay used in this experiment, these differences were not observed in the RF of steers supplied with coconut oil. The increased levels of palmitic acid C16:0 observed in the RF of all treatments and possibly longer chains C18s could have resulted from an extensive biohydrogenation of unsaturated isomers, but also by synthesis of FAs. Emmanuel (1974) reported protozoal elongation of shorter chain FAs, which mainly resulted from elongation of butyric acid into palmitic acid, but the biosynthesis of other FAs, which

included stearic acid, was also reported. Palmitic acid was the most abundant FA in the RF of all steers, consistent with our findings, with no significant differences between treatments. The FA profile of the RF was very high in C16:0 and C18:0 was quite low in comparison to other studies with temperate grasses (Scollan et al., 2001) or using finishing diets (Sackmann et al., 2003). This reflects the high C16:0 in the tropical grass hay sample that was used as the basal diet, as found in O'Kelly and Reich, (1976). The higher concentration of TSFA in the RF of steers supplied with coconut oil in the current experiment is due to an increase in the concentration of lauric and mystiric acids in the rumen of these steers, reflecting their higher concentration in the coconut oil itself. Coconut oil, rich in both lauric and mystiric acids, has been reported to have positive effects when added in the diet of ruminants by suppressing methane production (Machmüller et al., 2002; Soliva et al., 2004), but the evaluation of these FAs individually had no effects on methane reduction, indicating that it was an additive effect or the combination of both acids which resulted in the antimethanogenic effects (Hristov et al., 2011).

The concentration of TUFA and TC18s were highest in the rumen of steers supplied with fish and soybean oil. Fish oil had a higher concentration of C22:6, unsaturated C18s and LCFA (C20 or longer) and the lowest concentration of C18:2n-6. Steers supplied with fish oil had higher concentrations of C16:1c7, C18:1c11, C20:1c11, C20:4 and C22:1c13 and lower concentration of C18:0 and these results are in agreement with other work using fish oil (Kim et al., 2008; Shingfield et al., 2011). Some marine algae are rich sources of specific long chain FAs. For example, docosahexaenoic acid (DHA), C22:6n-3, contributes as much as 39% of total FA in *Schizochytrium* sp. (Jiang et al., 2004; Hauvermale et al., 2006), or 68.9% of total FA in *Crypthecodinium cohnii* (Pickard et al., 2008). While all other feeds had relatively low or undetectable concentrations of docosahexanoic acid (DHA), fish oil had a significantly higher

concentration (*i.e.* 7.68% of total FA). Fish oil is a rich source of DHA. However, the concentration for this specific fatty acid found in the literature can vary substantially. Shingfield et al. (2011) reported concentrations of DHA of 3%, whilst the fish oil utilized by Ulven et al. (2011) had concentration of DHA of 24% of total FAs. Nevertheless, despite the high concentration of DHA observed in the fish oil utilized in this study, the effects on RF concentrations of C22:6 concentration were non-existent, with levels undetectable in the RF of steers in the current experiment. Kitessa et al. (2001) reported transfers of DHA into milk of goats ranging from 3.5 to 7.6%. However, the authors utilized a high quality basal diet (*i.e.* chaffed lucerne hay-oat grain: 60/40 w/w), and utilized two sources of tuna oil, a protected and an unprotected one. It remains unknown if feeding marine algae with high concentrations of C22:6PUFA to ruminants fed low protein, high fibre diets will alter the RF profile of these animals, and subsequent tissue accretion of these longer chain FA.

Total unsaturated FA concentration was higher in the RF of steers supplied with fish oil and cottonseed oil, compared to steers supplied with coconut oil. In the past decade, research groups have focused their studies on the effects of CLA isomers, most importantly the CLA*t*10*c*12, on lipid metabolism in the mammary gland and subcutaneous and intramuscular fat (Bauman et al., 2011). The extent of formation of this specific isomer CLA*t*10*c*12 within the rumen of steers in the current experiment could not be accurately measured with the method utilised. However, the concentration of CLA*c*9 *t*11, which is the main CLA isomer, was very low and did not differ significantly between any of the treatments studied. Using the numerically higher concentration of CLA*c*9*t*11 found in the RF of steers supplied with cottonseed oil (*i.e.* 0.4% of total FA), a total lipid concentration of RF of approximately 0.2%/L (Patton et al., 1970), considering that one half of lipids would be represented by FA (Doreau and Ferlay, 1994), the rumen volume of

steers supplied with cottonseed oil (*i.e.* 70.6 L) and the value of FOR (*i.e.* 5.7%/h) over 24 h, the total production of CLA*c*9*t*11 would be approximately 0.39 mg/d. While CLA*c*9*t*11 is present at a higher concentration compared to other isomers (Bauman et al., 2008) it would still be found in the RF of steers in the present experiment at much lower concentrations than the amount of CLA*t*10*c*12 required to influence fat synthesis in the mammary gland (*i.e.* 25g/d) (Bauman et al., 2008). Therefore, no effects on fat synthesis in any tissue levels could be expected from any of the treatments studied here.

Under extensive grazing scenarios the inclusion of oils in the diets would be impractical and most likely the addition of these oils would be through small quantities remaining in processed protein supplements that are commonly used. A simple calculation using the oil concentration and the intake of cottonseed meal supplement of steers in Costa et al. (2016), indicated that a 200kg weaner consuming approximately 1.3kg of cottonseed meal supplement a day (1.2 kg DM), would end up consuming 54 g of lipids. Considering around 50% of the lipids is represented by FA (Doreau and Ferlay, 1994) and the TUFA concentration in FA of cottonseed oil is approximately 32%, it could be estimated that there is a total consumption of less than 9g of TUFA through cottonseed meal intake. This example clearly demonstrates how little quantities of these or any other individual FAs are ingested through protein supplements and how little effect they would be likely to have under current commercial conditions. Feeding whole seeds, *e.g.* whole cottonseed or soybean seeds or algae species high in lipid content would potentially have a much greater effect.

The provision of cottonseed oil to steers decreased rumen NH_3N concentration compared to control steers. High levels of lipids can affect fermentation and protein degradation (Jenkins, 1993) which may result in decreased NH_3N concentration. Biologically this is of little

significance given the low rumen NH_3N concentration in response to all treatments and in control animals within this experiment. Similarly, while steers supplied with fish oil had a lower molar proportion of acetic acid and a higher molar proportion of propionic acid in the rumen compared to the other steers, which has been reported previously (Chalupa et al., 1984), it is unlikely that the changes would be of biological significance. The shorter RT of a liquid marker in the rumen of steers supplemented with soybean was unexpected, since the main concern with the use of oils is related to detrimental effects on fibre digestion (Jenkins, 1993). The shorter RT could result in beneficial implications, such as an increase in intake and MCP production of steers grazing low CP and high aNDFom diets. Shifts in microbial population have been reported with the use of soybean oil resulting in an inhibitory effect on methane production and an increase in MCP production (Mao et al., 2010).

In summary, specific differences in FA profile resulted from the use of oils in this experiment related to FA profile of the oils used. However, none of the oils utilised in this trial would cause changes in CLA concentration which could result in effects on fat synthesis. Increases in long chain PUFAs could be expected from the fat of ruminants fed with fish oil. In this experiment, the longer RT observed for the tropical grass, with a resultant longer time for biohydrogenation of the fish oil resulted in similar intermediates as the ones reported for cattle (Shingfield et al. 2011) and goats (Kitessa et al., 2001) fed fish oil with higher quality diets.

5. Conclusions

The inclusion of low quantities of lipids in the diets of ruminants grazing low quality pastures will have little, if any, effect on rumen function. However, soybean oil did increase the fractional outflow rate of liquid from the rumen and this may be of benefit to animals grazing low quality pastures. While there were large differences in the FA profile of the oils used in the

present experiment, this did not translate into major differences in the FA profile in the rumen of steers that were supplied with the oils when fed a low quality tropical forage.

None of the oils utilised in this experiment would affect fat synthesis and as such it is unlikely that the small quantities of lipids present in protein supplements fed to cattle in northern Australia would have any detrimental effect on fat synthesis when grazing low quality tropical pastures in the dry season. It is not known if these responses would be different for higher quality, wet season tropical forages where higher rumen NH_3N and shorter RT of material in the rumen are likely to exist.

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Table 1. Rumen NH_3N and volatile fatty acid (VFA) concentration, fractional outflow rate (FOR), retention time (RT) of liquid phase in the rumen and rumen volume of steers fed Rhodes grass hay and provided with coconut, cottonseed, soybean and fish oils

Parameter	Control	Coconut oil	Cottonseed oil	Fish oil	Soybean oil	SEM
NH_3N (mg N/L)	8.0^{b}	5.8^{ab}	3.4^{a}	8.9^{b}	7.0^{ab}	5.6
Total VFA (mM)	79.8^{b}	69.4^{a}	78.6^{b}	78.3^{b}	76.7^{b}	5.0
Acetic (% total VFAs)	76.1^{ab}	77.6^{b}	76.6^{b}	74.3^{a}	76.4^{b}	1.4
Propionic (% total VFAs)	14.9^{b}	13.5^{a}	14.4^{ab}	17.4^{c}	14.6^{ab}	1.1
Butyric (% total VFAs)	7.3	7.5	7.3	6.6	7.3	0.7
Isobutyric (% total VFAs)	0.57	0.61	0.61	0.58	0.63	0.08
Valeric (% total VFAs)	0.54	0.51	0.55	0.56	0. 60	0.11
Isovaleric (% total VFAs)	0.47	0.46	0.47	0.48	0.47	0.05
RT (h)	17.0^{b}	17.1^{b}	18.1^{b}	16.9^{b}	14.4^{a}	1.6
FOR (%/h)	6.0^{a}	5.9^{a}	5.7^{a}	6.1^{a}	7.2^{b}	0.7
Rumen volume (L)	77.0^{b}	80.4^{b}	70.6^{a}	72.4^{a}	73.4^{a}	3.6

Values are means with standard error of the mean (SEM). Different alphabetical superscripts across the rows indicate significant difference between treatments ($P<0.05$).

Table 2. Fatty acids of Rhodes grass hay (Hay), coconut oil (coconut), cottonseed oil (cottonseed), fish oil (fish) and soybean oil (soybean) fed to steers[1]

FA	Hay	Coconut	Cottonseed	Fish	Soybean	SEM
	% of total FA[2]					
C12:0	1.29[a]	47.28[b]	0.02[a]	0.08[a]	0.01[a]	1.21
C14:0	1.42[b]	16.32[d]	0.48[a]	3.50[c]	0.08[a]	0.45
C15:0iso	0.02[a]	0.00[a]	0.00[a]	0.17[b]	0.00[a]	0.02
C15:0anteiso	0.10[b]	0.00[a]	0.00[a]	0.06[ab]	0.00[a]	0.03
C15:0	0.64[c]	0.02[a]	0.01[a]	0.45[b]	0.01[a]	0.03
C15:1	0.07[b]	0.00[a]	0.00[a]	0.11[c]	0.00[a]	0.02
C16:0	30.48[e]	8.52[a]	18.00[d]	13.50[c]	11.03[b]	0.60
C17:0iso	0.07[b]	0.00[a]	0.00[a]	0.41[c]	0.01[a]	0.04
C17:0anteiso	0.05[a]	0.00[a]	0.00[a]	0.10[b]	0.00[a]	0.03
C18:0	3.94[d]	2.61[b]	2.41[a]	3.56[c]	3.87[d]	0.13
C18:1*t*11	0.00	0.00	0.00	0.00	0.00	0.00
C18:1*c*9	4.40[a]	7.00[b]	22.12[d]	26.64[e]	19.54[c]	0.40
C18:1*c*11	0.51[b]	0.17[a]	0.74[c]	3.84[e]	1.18[d]	0.07
C18:2n-6	14.80[c]	5.04[b]	53.26[d]	1.30[a]	54.92[d]	1.44
C18:3n-3	22.30[c]	0.32[a]	0.39[a]	0.48[a]	7.56[b]	1.03
CLA*c*9*t*11	0.00	0.02	0.00	0.03	0.00	0.02
C19:0	0.21[b]	0.00[a]	0.01[a]	0.00[a]	0.00[a]	0.03
C20:0	3.00[b]	0.07[a]	0.26[a]	0.23[a]	0.36[a]	0.60
C20:1*c*9	0.00[a]	0.01[a]	0.01[a]	0.60[b]	0.02[a]	0.02
C20:1*c*11	0.16[b]	0.06[a]	0.10[ab]	8.43[c]	0.18[b]	0.05
C20:3	0.00[a]	0.06[ab]	0.00[a]	0.10[b]	0.00[a]	0.04
C20:4	0.00[a]	0.01[a]	0.00[a]	0.82[b]	0.00[a]	0.01
C22:0	2.19[c]	0.05[a]	0.18[a]	0.19[a]	0.45[b]	0.09
C22:1*c*11	0.00[a]	0.01[a]	0.00[a]	4.04[b]	0.01[a]	0.03
C22:1*c*13	0.10[a]	0.00[a]	0.00[a]	1.19[b]	0.01[a]	0.06
C22:2*c*13*c*16	0.81[b]	0.00[a]	0.00[a]	0.02[a]	0.00[a]	0.03
C22:6	0.00[a]	0.01[a]	0.00[a]	7.68[b]	0.01[a]	0.08
C23:0	0.17[d]	0.00[a]	0.02[b]	0.02[b]	0.04[c]	0.01
C24:0	2.58[b]	0.06[a]	0.10[a]	0.06[a]	0.15[a]	0.11
C24:1*c*15	0.00[a]	0.00[a]	0.00[a]	1.25[b]	0.01[a]	0.01
C26:0	1.43[b]	0.01[a]	0.02[a]	0.00[a]	0.01[a]	0.08
TOBCFA[3,4]	0.24[b]	0.02[a]	0.00[a]	0.74[c]	0.01[a]	0.10
TSFA[3,4]	48.69[c]	86.86[d]	21.56[b]	23.06[b]	16.07[a]	2.25
TUFA[3,4]	45.53[b]	12.82[a]	77.68[d]	69.97[c]	83.61[e]	2.34
TC18s[3,4]	46.33[c]	15.20[a]	79.39[d]	37.06[b]	87.16[e]	2.31

[1]Samples of feed offered were collected in duplicate for each run, but arithmetic average values are presented here. [2]Identifiable and quantifiable fatty acids presented only. Values expressed as % of total FA and some are not presented in this Table.[3]TOBCFA = total odd branched-chain fatty acids; TSFA = total saturated fatty acids; TUFA = total unsaturated fatty acids; TC18s = total fatty acids containing 18 carbon chains.[4]Total of individual fatty acids listed in the Table plus other identifiable fatty acids; Values are means with standard error of the mean (SEM).

Different alphabetical superscripts across the rows indicate significant difference between treatments ($P<0.05$).

Table 3. Fatty acids in the rumen fluid of steers fed Rhodes grass hay only (control), coconut oil (coconut), cottonseed oil (cottonseed), fish oil (fish) and soybean oil (soybean)[1]

FA	Control	Coconut	Cottonseed	Fish	Soybean	SEM
	% of total FA[2]					
C12:0	1.88[a]	8.45[b]	1.48[a]	0.94[a]	1.91[a]	1.03
C12:1	0.90[b]	0.92[b]	0.85[b]	0.38[a]	0.70[ab]	0.28
C13:1	1.68	1.73	1.53	1.02	1.35	0.41
C14:0	5.86[b]	14.24[c]	5.46[ab]	3.92[a]	4.66[ab]	1.34
C15:0iso	4.48[b]	6.30[c]	4.15[ab]	2.72[a]	3.32[ab]	1.13
C15:0anteiso	7.02	6.21	6.28	4.33	5.01	1.67
C15:0	4.44[c]	1.61[a]	3.29[b]	1.70[a]	2.83[b]	0.69
C15:1	2.55	2.16	2.05	1.56	1.58	0.62
C16:0	28.20	26.13	30.05	23.74	24.57	3.41
C16:1*c*7	0.57[a]	0.65[a]	0.58[a]	2.37[b]	0.48[a]	0.48
C17:0iso	1.66[b]	0.88[a]	1.23[ab]	0.93[a]	0.84[a]	0.38
C17:0anteiso	2.26[c]	1.28[ab]	1.78[bc]	1.14[a]	1.49[ab]	0.45
C17:0	1.73[c]	0.60[a]	1.31[bc]	0.94[ab]	1.26[b]	0.32
C18:0	8.21[b]	6.70[b]	8.19[b]	3.96[a]	8.26[b]	1.25
C18:1*t*11	0.66[a]	0.48[a]	1.86[b]	0.69[a]	1.69[b]	0.58
C18:1*c*9	6.33[a]	4.28[a]	7.60[ab]	12.11[b]	9.30[ab]	3.66
C18:1*c*11	1.20[a]	1.23[a]	1.64[a]	2.15[b]	1.61[a]	0.34
C18:2n-6	9.38	4.49	10.15	19.94	18.26	9.90
C18:3n-3	2.54[bc]	1.54[a]	1.79[ab]	1.30[a]	3.02[c]	0.60
CLA*c*9*t*11	0.14	0.17	0.40	0.08	0.26	0.19
C19:0	0.14[b]	0.00[a]	0.04[ab]	0.07[ab]	0.04[ab]	0.10
C20:0	0.96[b]	0.78[ab]	0.71[ab]	0.51[a]	0.66[a]	0.22
C20:1*c*11	0.05[a]	0.29[a]	0.18[a]	1.49[b]	0.26[a]	0.48
C20:4	0.00[a]	0.08[ab]	0.00[a]	0.41[b]	0.04[a]	0.26
C22:1*c*13	0.00[a]	0.05[a]	0.00[a]	0.21[b]	0.02[a]	0.10
C24:0	0.55[b]	0.46[ab]	0.42[ab]	0.34[a]	0.41[ab]	0.14
C24:1*c*15	0.05	0.00	0.10	0.10	0.11	0.11
C26:0	0.00	0.05	0.00	0.00	0.00	0.05
TOBCFA[3,4]	16.10[b]	15.14[b]	14.01[ab]	9.23[a]	11.24[ab]	3.63
TSFA[3,4]	66.14[b]	64.18[b]	62.51[b]	43.74[a]	53.91[ab]	9.73
TUFA[3,4]	27.62[ab]	20.53[a]	31.55[ab]	47.76[c]	40.77[bc]	11.07
TC18s[3,4]	28.92[ab]	19.42[a]	32.87[ab]	40.60[b]	43.19[b]	11.98

[1]Bulk samples of rumen fluid collected at five different times over a 16 h period

[2]Identifiable and quantifiable fatty acids presented only. Values expressed as % of total FA and some are not presented in this Table.

[3]TOBCFA = total odd branched-chain fatty acids; TSFA = total saturated fatty acids; TUFA = total unsaturated fatty acids; TC18s = total fatty acids containing 18 carbon chains.

[4]Total of individual fatty acids listed in the Table plus other identifiable fatty acids.

Values are means with standard error of the mean (SEM). Different alphabetical superscripts across the rows indicate significant difference between treatments ($P<0.05$).