Asian-Aust. J. Anim. Sci.
Vol. 23, No. 2 : 197 - 204
February 2010

www.ajas.info

Effects of Replacing Lucerne (*Medicago sativa* L.) Hay with Fresh Citrus Pulp on Ruminal Fermentation and Ewe Performance

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ABSTRACT : Two studies were conducted to determine the effects of replacing 30% (% in diet DM) of lucerne (*Medicago sativa* L.) hay with citrus pulp in Merino ewe diets: i) an *in vitro* study which measured ruminal fermentation; and ii) an *in vivo* study in which twelve Merino ewes pre- and post-lambing were fed experimental diets in a cross-over design over 120 days to evaluate effects on ewe performance (i.e. DM intake, average daily gain (ADG) and wool growth). In both the *in vitro* and *in vivo* studies, the control treatment consisted of lucerne (91.3% in diet DM), lupins (8.3% in diet DM) and phosphate (0.42% in diet DM), while the citrus pulp treatment consisted of lucerne (57.7% in diet DM), lupins (9.5% in diet DM), phosphate (0.48% in diet DM) and fresh citrus pulp (32.3% in diet DM). Data were analysed using the mixed model procedure of SAS. In the *in vitro* study, gas production, total volatile fatty acid (VFA) yield, proportion of propionic acid to total VFA and *in vitro* dry matter digestibility (IVDMD) were higher ($p<0.02$) in the citrus pulp treatment compared to the control treatment. In contrast, *in vitro* ammonia production, pH and the acetate to propionate ratio were lower ($p<0.03$) for the citrus pulp treatment compared to the control treatment. In the *in vivo* study, DM intake of ewes fed the citrus pulp diet was lower than their control ewe counterparts throughout both the pre- and post-lambing periods (928.9 vs. 1,115.0 g/d pre-; 1,285.0 vs. 1,620.3 g/d post-lambing, $p<0.01$), however ADG was similar ($p = 0.12$). Wool growth parameters and lamb performance did not differ ($p>0.32$) between treatments. In summary, the *in vitro* study demonstrated that the replacement of 30% of a lucerne diet with fresh citrus pulp improved total VFA yield, increased total gas production and improved IVDMD, while decreasing the production of ammonia, acetic acid and rumen pH. In addition, the *in vivo* study demonstrated that the replacement of 30% of a lucerne diet with fresh citrus pulp pre- and post-lambing decreased intake but did not affect ewe performance in terms of ADG and wool growth. These findings, of course, would be of significant interest to sheep producers endeavouring to control cost of feed ingredients whilst maintaining productivity. (**Key Words :** Citrus Pulp, Growth Performance, Merino Ewe, Ruminal Fermentation, Wool Growth)

INTRODUCTION

The Australian Merino wool industry contributes approximately AUD$3 billion to Australia's economy each year (ABARE, 2003). With recent drought periods, farmers are under pressure to maintain flock numbers and sustain the health of their flocks. Nutrition plays an important role in safeguarding flock health, and farmers are endeavouring to minimise costs associated with supplementary feeding by searching for cheaper, novel feedstuffs.

Fresh citrus pulp is commonly used in the dairy industry as a high energy and low cost feedstuff. Citrus pulp is the residue after extraction of the juice, including the peel, pulp residue and the seeds (Martinez-Pascual and Fernandez-Carmona, 1980a). It is widely available from juicing factories, particularly in the sheep-wheat zones of Australia, and at AUD$40 per tonne it is a low cost alternative compared to grain supplements. Due to the high pectin content of fresh citrus pulp (25% on a dry matter (DM) basis), it is considered an energy supplement and is commonly fed to support growth and lactation at 100-150 g/kg DM in ruminant diets (Bampidis and Robinson, 2006). Pectin is rapidly and extensively broken down in the rumen, but unlike starch feedstuffs, yields little lactate, decreasing the risk of lactic acidosis (Strobel and Russell, 1986; Barrios-Urdaneta et al., 2003). Citrus pulp has a high total digestible nutrients value (82% DM), but compared with other energy feeds, it is low in protein (6-8% crude protein on DM basis; Arthington et al., 2002).

Since the onset of the drought, Australian Merino (*Ovis aries*) farmers have shown an increasing interest in the use of fresh citrus pulp in their sheep diets to maintain flock

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[a] Presented at the 2009 American Dairy Science Association (ADSA) Meeting, Montreal (QC, Canada), July 12-16.

Received June 3, 2009; Accepted August 21, 2009

health while remaining profitable. All previous fresh citrus pulp sheep studies have been conducted in Europe and South America and focused primarily towards the meat sheep and dairy sheep breeds (Bampidis and Robinson, 2006). To date, no studies have been conducted in wool producing breeds, particularly the Australian Merino, assessing the effects of supplementing fresh citrus pulp on wool growth and lamb rearing abilities. Therefore, an *in vitro* study was conducted to determine the effects of substituting 30% of fresh citrus pulp (in % of total DM basis) for lucerne (*Medicago sativa* L.) hay on rumen parameters, while an *in vivo* feeding trial was used to determine the effects of substituting 30% of fresh citrus pulp for lucerne hay on ewe performance (i.e. dry matter (DM) intake, average daily gain (ADG) and wool growth) in Australian Merino sheep.

MATERIAL AND METHODS

In vitro study

An *in vitro* incubation was conducted in a completely randomised design using the same diets (Table 1) as were fed to the animals in the *in vivo* feeding trial (control and 30% citrus pulp diets). The experiment was conducted for 24 h in a batch culture. Ruminal fluid was obtained from two Merino sheep (54.3±1.2 kg in body weight) fed 2,040 g DM with a 59:41 lucerne hay: barley grain ratio (15.7% crude protein (CP), 54.4% neutral detergent fibre (NDF)). Approximately a 1 kg sample of each of the ingredients in the diets (Table 1) were oven dried at 60°C and ground through a 1-mm screen Wiley mill (Micro Hammer Cutter Mills, Glen Creston Limited, London, UK) for use in the *in vitro* incubation.

Prior to incubation, 0.5 g DM of dietary substrate was weighed into 50 ml glass incubation bottles (n = 6 per treatment). On the day of incubation, the bottles containing the samples were pre-warmed to 39°C in an incubator for 60 min. The incubation was initiated by adding 20 ml of inoculum to each bottle. For the inoculum, ruminal contents were obtained 2 h post morning feeding from different sites within the rumen and pooled across the two sheep. The composite rumen sample was strained through four layers of cheesecloth into an insulated thermos and immediately transported to the laboratory. Prior to use, the strained ruminal fluid was maintained at 39°C in a water bath and the headspace continuously flushed with CO_2. The ruminal fluid was mixed with three volumes of pre-warmed buffer at 39°C and 0.5 ml cysteine-sodium sulfide solution as a reducing agent (Menke et al., 1979). All bottles were crimp sealed with rubber stoppers to avoid gas leakage and then placed in a water bath with temperature controlled (39±0.5°C) (Shaking incubator, The Mickle Engineering Co., Surrey, UK). Total gas production in the bottle headspace was measured at 0, 3, 6, 12, and 24 h, whereas pH, ammonia-N, total and individual concentrations of volatile fatty acids (VFA) and *in vitro* DM digestibility (IVDMD) were determined after 24 h of incubation. Six bottles without substrate (blanks) were also prepared for each time point (Benchaar et al., 2007).

Determination of fermentation gases : After 3, 6, 12 and

Table 1. Ingredients, dry matter (DM) contents, chemical composition and estimations from the small ruminant nutrition system (version 1.8.7) of the diets fed to ewes and used in the *in vitro* study

Ingredients (g/kg of DM)	Control	30% Fresh citrus pulp
Lucerne hay (fine chop)	912.8	576.6
Lupins (whole)	83.0	95.3
Fresh citrus pulp	0.0	323.3
Mono calcium phosphate	4.2	4.8
Chemical composition		
Dry matter (g/kg of feed)	883	664
Crude protein (g/kg of DM)	223	183
Neutral detergent fibre (g/kg of DM)	384	328
Total NFC[1] (g/kg of DM)	301	405
Crude fat (g/kg of DM)	39	39
Ash (g/kg of DM)	83	78
Small Ruminant Nutrition System estimations		
Metabolisable energy (MJ/kg DM)	9.8	10.6
Rumen pH	6.46	6.46
Rumen N balance (g/d)	14.3	2.40
Urea cost (MJ/d)	0.61	0.29
Apparent DM digestibility (%)	64.3	69.4

[1] NFC: Non-fibrous carbohydrates, calculated as (1,000-(NDF+CP+Crude fat+Ash)).

24 h of incubation, bottles were removed from the water bath for measurement of gas production using a water displacement technique (Fedorak and Hrudey, 1983) and then returned to the bath. Gas production on the blank bottles for each time point was also measured and used to calculate net gas production (ml per g DM incubated; Benchaar et al., 2007).

Determination of ammonia : After gas production was determined for the 24 h sample, the pH of the whole culture was measured using a pH meter (Model 209 pH/mV meter, Activon, Melbourne, Victoria, Australia) calibrated at 39°C. The bottles were then placed on ice to terminate the fermentation. Two sub-samples (1.2 ml) of the culture from each bottle were transferred to 2-ml micro-centrifuge tubes containing 200 μl trichloroacetic acid (TCA; 0.65; v/v) and centrifuged at 14,000×*g* for 10 min (Spectrafuse 16M, National Labnet Co., Edison, NJ) to precipitate particulate matter. The supernatant was transferred into 2-ml micro-centrifuge tubes and stored frozen at -20°C until analysed for ammonia-N by phenyl-hypochlorite reaction (Weatherburn, 1967). Blank 0 h samples were analysed for ammonia and used to calculate net ammonia-N production.

Determination of volatile fatty acids (VFA) : Two additional sub-samples (1.2 ml) of the culture in each bottle was also removed, acidified with 300 μl of metaphosphoric acid (0.20; w/v) and centrifuged in the same manner as the ammonia analysis. The supernatant was stored frozen at -20°C and later analysed for VFA concentrations by GLC (Hewlett Packard model 5890; Phenomenex, Torrance, CA) equipped with a ZB-FFAP silica 30 m×32 mm×1 μm capillary column. Blank 0 h samples were analysed for VFA and net total VFA production was calculated (Benchaar et al., 2007).

Determination of in vitro dry matter digestibility (IVDMD) : After samples were collected for ammonia and VFA analyses at the end of incubation, the contents of the incubation bottles were transferred into pre-weighed 50-ml centrifuge tubes, rinsed with distilled water and centrifuged twice at 500×*g* for 10 min at 4°C. The supernatant was discarded and the precipitate dried at 55°C for 48 h and weighed to estimate IVDMD.

***In vivo* study**

Animals and diets : A completely randomized block design was used to allocate twelve mature-age fine wool peri-parturient Merino ewes into one of two dietary treatment groups (0% control and 30% citrus pulp on DM basis) using three replicates. Each group of six animals were paired according to their individual weights and assigned to a four square meter pen for the study. The ewes were cared for in accordance with the guidelines of the University of Sydney Animal Ethics (National Health and Medical Research Council, 2004).

The experimental diets were mixed fresh daily and were formulated to meet the nutritional requirements according to the National Research Council (National Research Council, 1985) recommendations and the Small Ruminant Nutrition System (SRNS Version 1.8.7; Cannas et al., 2004). Citrus pulp was sourced from the Riverina region (New South Wales, Australia). The ingredients and chemical composition of the dietary treatments are presented in Table 1. All ewes had access to *ad libitum* feed and water at all times.

Experimental procedure : The trial was split into two periods; pre-lambing and post-lambing. The pre-lambing period ran for eight weeks. During this period, each pen consisted of two ewes with an individual floor space of approximately two square meters. Following this period, there was a one-week lambing interval followed by a post-lambing period of three weeks. During the post-lambing period, the ewes were put into individual pens of similar size to the pre-lambing period. The post-lambing period was designed as a cross-over treatment structure in which a ewe from each pair was placed on either a control or a diet for a period of three weeks (one week diet adaptation and two weeks of measurements).

Feed intake, average daily gain and lamb growth : Amounts of dietary treatments offered and refused were weighed and recorded daily to calculate the feed intake. Samples of the dietary ration and refusals were collected daily and oven dried at 60°C for seven days for DM determination.

Ewes were weighed at seven day intervals throughout the trial. Average daily gain (ADG) was determined by dividing the weight gain (final live weight - initial live weight) by the number of days during each period (64 days pre-lamb, 28 days cross over post-lamb). Lambs were weighed at birth, sexed and ear tagged. Lamb live weights were recorded on a weekly basis and ADG was calculated as above.

Wool measurements : Mid-side wool patches (10×10 cm area) were taken from the right-hand mid-side region of each individual ewe on day one of the feeding trial. On day 87 of the trial (prior to the crossover), patches were shorn with small animal clippers (size 40 blade) and samples stored in snap lock plastic bags. Samples were then transferred to paper envelopes and dried in an oven at 40°C for 48 h before being reweighed to determine the dry greasy weight.

Samples were washed six times with a hot 1% non ionic surfactant detergent solution and subsequently washed twice with ethanol in nylon filters. Samples were dried in paper envelopes for 48 h and re-weighed to determine clean fleece weight. From the above measurements, wool yield was determined as the proportion of greasy fleece weight to clean fleece weight (Langlands and Wheeler, 1968).

To determine the effects of dietary treatment on staple strength and length, a dyeband was placed on the left hand mid-side of each ewe on day one of the trial. A hair dye (Wella Colour, Fresh Black 2.0) was mixed in a small plastic container with a fine tip and applied to the skin of each animal for a length of 10 cm. On day 87 of the trial, the dyebands were clipped with small animal clippers (size 40 blade) and samples stored in snap lock plastic bags. Staple length (mm) was measured objectively with a ruler, while staple strength was measured subjectively through the 'flick' test (Gleeson et al., 1993). The staple was assumed to be sound and greater than 30 Newton per kilotex (N/ktex) if the staple did not break when flicked.

Chemical analyses

Weekly dietary rations and refusals were pooled for each pen and a chemical analysis performed on the samples in duplicate, and where the coefficient of variation was >5%, the analysis was repeated. All samples were oven dried at 60°C for ten days to determine dry matter (DM) content. The samples were then milled to a 1-mm screen using a blade mill (C and N Laboratory mill 8" blade, Crompton and Parkinson, England) prior to chemical analysis. Analytical DM was determined by drying the oven dried samples at 105°C for 24 h, followed by hot weighing (AOAC, 1990; method 930.05). The α-amylase neutral detergent fibre (aNDF) was determined, as described by Van Soest et al. (1991), using heat-stable α-amylase and sodium lauryl sulfate. Crude protein (CP = N×6.25) was quantified by thermal conductivity detection using the TC500 LECO Nitrogen determinator (St. Joseph, Michigan, USA). Ash was determined by igniting a 2 g sample of the dietary ingredients at 600°C for 2 h (AOAC, 1990; method 942.05). Determination of crude fat was through the ether extraction method (AOAC, 1990; method 920.39), while non-fibrous carbohydrates content (NFC) was calculated as:

NFC (g/kg of DM) = 1,000-(aNDF+CP+Crude fat+Ash)

Statistical analyses

Data were analysed with the mixed model procedure of SAS (SAS Inst. Inc., 2009). For the *in vitro* study, citrus pulp treatment means were compared using the least squares mean linear hypothesis test (LSMEANS/DIFF) against the control adjusted for Dunnett comparison. The univariate procedure of SAS was used to test for normal distribution of the data. For the *in vivo* study, intake means were compared using the least squares mean linear hypothesis test with treatment, week, and interactions included as fixed terms, ewes nested within treatment as a random effect, and week as a repeated measure by period (pre- and post-lambing). Repeated measures analysis with the minimum values of Akaike's Information Criterion (AIC) was used for selecting covariance structure for dry matter intake. Within period, initial and final ewe live weight (LW), ewe average daily gain, wool data and lamb birth weights and lamb average daily gains were analysed using a model similar to that described above, but excluding week as a repeated measure. The citrus pulp treatment was compared with control using the Dunnett test. Initial ewe weight was included in the model as a covariate. Unless otherwise specified, treatment effects were declared significant at $p<0.05$.

RESULTS

Dietary composition

Dry matter (DM) of lucerne hay, fresh citrus pulp, lupins and mono calcium phosphate averaged across the whole experiment 872.2, 167.1, 900.0 and 990.0 g/kg of feed, respectively. There was neither significant change in DM contents nor chemical composition over the duration of the trial for all dietary ingredients. The 30% citrus pulp diet comprised a lower DM content (664 vs. 883 g/kg), crude protein (183 vs. 223 g/kg) and NDF concentrations (328 vs. 384 g/kg) compared with the control diet. In contrast, total NFC content was higher for the 30% citrus pulp diet compared with the control diet (404 vs. 301 g/kg respectively). Crude fat and ash concentrations were similar between dietary treatments (Table 1).

Through the Small Ruminant Nutrition System estimations, diets were formulated to contain comparable amounts of energy with similar ruminal pH characteristics (pH 6.46). Rumen nitrogen balance was calculated to be lower for the 30% citrus pulp diet compared with the control diet (2.4 vs. 14.3 g/d respectively), while apparent DM digestibility was estimated to be numerically higher for the 30% citrus pulp diet compared to the control treatment diet (69.4 vs. 64.3%, respectively).

***In vitro* study**

Cumulative gas production was 11% higher for the 30% fresh citrus pulp diet compared to the control (144.3 vs. 129.3 ml/g DM; $p<0.01$). At the completion of the *in vitro* study, pH was lower ($p<0.01$) for the 30% fresh citrus pulp diet than the control (5.90 vs. 6.53, respectively). Total VFA production was 16% higher for the 30% fresh citrus pulp diet (Table 2). The proportion of acetic and butyric acids to total VFA production did not differ between treatments (64.8 vs. 65.9±0.42 mmol/100 mmol; $p>0.14$; 7.8±0.11 mmol/100 mmol, $p>0.82$ respectively). In contrast, the proportion of branched-chain VFA (BCVFA) and valeric acids to total VFA were 39 and 14% lower respectively ($p<0.02$) for the 30% fresh citrus pulp diet compared to the control, while the molar proportion of propionic acid was higher ($p<0.02$) for the 30% fresh citrus pulp diet than the control diet. In addition, the ratio of acetate to propionate was lower for the 30% fresh citrus pulp diet (3.2 vs. 2.84; p

Table 2. Effects of replacing 30% of lucerne hay (in % of total dietary dry matter) with fresh citrus pulp on *in vitro* cumulative gas production, pH, ammonia (NH_3), proportions and total volatile fatty acids (VFA) production and estimates of *in vitro* dry matter digestibility (IVDMD) over a 24-h incubation period

	Control	30% Fresh citrus pulp	SE[1]	p-value treatment
Gas (ml/g dry matter)	129.3	144.3	1.83	<0.01
pH	6.53	5.90	0.022	<0.01
NH_3 (g/L)	0.68	0.40	0.028	<0.01
Total VFA (μM/ml)	132.8	154.1	2.82	<0.01
VFA (mmol/100 mmol)				
Acetic (A)	65.9	64.8	0.42	0.14
Propionic (P)	20.6	22.9	0.41	0.02
Butyric	7.8	7.8	0.11	0.82
BCVFA[2]	3.2	2.3	0.03	<0.01
Valeric	2.4	2.1	0.05	0.02
Ratio A:P	3.20	2.84	0.080	0.03
IVDMD (%)	40.1	49.8	0.54	<0.01

[1] SE = Standard error. [2] BCVFA = Branched-chain volatile fatty acids (iso-butyric+iso-valeric).

= 0.03) compared to the control. *In vitro* dry matter digestibility was 24% higher for the 30% fresh citrus pulp diet compared to the control diet (49.8 vs. 40.1% respectively; p<0.01). Total ammonia production was also found to be lower for the 30% fresh citrus pulp diet versus the control diet (0.40 vs. 0.68 g/L respectively; p<0.01).

***In vivo* study**

During the pre-lambing period (Table 3), DM intakes (DMI) were lower for the ewes fed the 30% fresh citrus pulp diet compared to the ewes on the control diet (928.9 vs. 1,115 g/d; p<0.01). Average daily gains (ADG) did not differ between the 30% fresh citrus pulp diet and the control diet (197.0 vs. 154.5±30.5 g/d respectively, p = 0.35). Feed conversion ratios were much lower for the 30% fresh citrus pulp diet compared to the control diet (Table 3).

The post-lambing period produced similar results to those during the pre-lambing period (Table 4). Ewe DMI

Table 3. Pre-lambing dry matter intake (DMI) and average daily gain (ADG) of ewes fed a lucerne hay-based diet (control) or replacing 30% of lucerne hay with fresh citrus pulp

	Control	30% Fresh citrus pulp	SE[1]	p-value treatment
Initial live weight (kg)	37.3	33.6	1.65	0.15
Final live weight (kg)	45.8	44.4	2.28	0.69
DMI (g/d)	1115.0	928.9	14.36	<0.01
ADG (g/d)	154.5	197.0	30.50	0.35
Feed conversion[2]	7.2	4.7	N/A	N/A

[1] SE = Standard error. [2] Calculated as DMI/ADG.

Table 4. Post-lambing dry matter intake (DMI) and average daily gain (ADG) of ewes fed a lucerne hay-based diet (control) or replacing 30% of lucerne hay with fresh citrus pulp

	Control	30% Fresh citrus pulp	SE[1]	p-value treatment
Lambs:				
Initial LW (kg)	5.1	4.7	0.51	0.55
Final LW (kg)	15.8	14.8	0.75	0.35
ADG (g/d)	223.1	250.8	20.84	0.36
Ewes:				
Initial live weight LW (kg)	40.2	37.7	2.19	0.44
Final LW (kg)	41.5	38.2	1.67	0.19
DMI (g/d)	1620.3	1285.0	43.70	<0.01
ADG (g/d)	45.6	117.1	31.15	0.12
Feed conversion[2]	35.5	11.0	N/A	N/A

[1] SE = Standard error. [2] Calculated as DMI/ADG.

differed significantly between treatment groups (1,285.0 30% citrus pulp vs. 1,620.3 g/d control), while ADG did not differ between dietary treatments (p = 0.12). In addition, the lambs in the 30% fresh citrus pulp diet treatment group gained similar weight compared to their control diet counter parts (250.8 vs. 223.1 g/d respectively p = 0.36).

Greasy fleece weights and clean fleece weights were similar between treatment groups (p≥0.32). Wool yield and staple length did not differ significantly between treatment groups (Table 5). In addition, staple strength was not affected by replacing 30% lucerne hay with fresh citrus pulp into Merino ewe diets (data not shown).

DISCUSSION

In vitro study

Cumulative gas production was significantly higher for the 30% fresh citrus pulp diet indicating an increase in rumen fermentation. The 30% fresh citrus pulp diet had lower ruminal pH compared to those fed the control diet (5.90 vs. 6.53 respectively; p<0.01), which is consistent with the higher ruminal total VFA concentrations for the 30% fresh citrus pulp diet compared to the control. These results are in contrast to previous citrus pulp trials, where researchers have reported either a higher final ruminal pH (Strobel and Russell, 1986; Ben-Ghedalia et al., 1989; Barrios-Urdaneta et al., 2003), or they have found that the incorporation of citrus pulp into ruminant diets did not affect rumen pH or VFA profile (Wing, 1974; Leiva et al., 2000; Fonesca et al., 2001; Lanza et al., 2001; Villarreal et al., 2006).

The higher total VFA concentrations observed for the 30% fresh citrus pulp diet suggests that dietary fermentation in the rumen is enhanced by the addition of fresh citrus pulp. Such a change may be nutritionally beneficial, as VFA are the main sources of metabolisable energy for ruminants. In addition, the lower molar proportion of acetate to propionate in the rumen when fed the 30% fresh citrus pulp diet compared with the control diet, may suggest an improvement on the efficiency of utilisation of metabolisable energy and microbial protein synthesis. In contrast, previous studies have found that supplementing citrus pulp in the diets of ruminants increases the acetate to propionate ratio in the rumen (Martinze-Pascual and Fernandez-Carmona, 1980b; Strobel and Russell, 1986; Ben-Ghedalia, 1989).

Total ammonia production was found to be significantly lower for the 30% fresh citrus pulp diet compared with the control diet (0.40 vs. 0.68 g/L, respectively). These results are contradictory to previous studies where Ben-Ghedalia et al. (1989), Fonesca et al. (2001) and Barrios-Urdaneta et al. (2003) found that the incorporation of citrus pulp into ruminant diets did not affect ammonia production; however these authors made the diets under study iso-nitrogenous or balanced the ratio degradable protein/fermentable energy. This was not the case in the present study where the citrus pulp diet had 40 g crude protein/kg DM less than the control diet (Table 1). Since DM of citrus pulp was fermented to a higher extent than DM of lucerne hay (49.3 vs. 39.3%, IVDMD; data not shown), the results observed would be expected. The ammonia results obtained correlate to a lower rumen nitrogen balance (Table 1) as ammonia is formed through the degradation of amino acids in the rumen by rumen microorganisms.

In vitro dry matter digestibility (IVDMD) was found to be 24% higher for the 30% fresh citrus pulp diet compared to the control diet (49.8 vs. 40.1% respectively, p<0.01). This finding concurs with previous studies conducted in ruminants supplemented with citrus pulp (Ben-Ghedalia et al., 1989; Arthington et al., 2002; Barrios-Urdaneta et al., 2003; Villarreal et al., 2006). Due to the high total digestible nutrients and higher dietary NFC it is suggested that fresh citrus pulp would create favourable conditions for celluloysis in the rumen compared with feeding a high starch diet (Ben-Ghedalia et al., 1989). Bueno et al. (2002) found that when fresh citrus pulp was included in the diets of growing kids, DM digestibility was slightly higher for the citrus pulp diet compared with the control diet (74.6% vs. 72.4% respectively). Bueno et al.'s DM digestibility results were similar to those of Ben-Ghedalia et al. (1989), however they were numerically different from the current study.

In vivo study

During the pre-lambing period, ewes on the 30% fresh citrus pulp diet ate less feed than their control ewe counterparts (928.9 vs. 1,115.0 g/d, respectively; p<0.01). Despite this, ADG was similar between the two treatment

Table 5. Effects of replacing 30% lucerne hay (in % of total dietary dry matter) with fresh citrus pulp for on greasy fleece weight, clean fleece weight, wool yield and staple length over 87 days

	Control	30% Fresh citrus pulp	SE[1]	p-value treatment
Greasy fleece (g/cm^2)	0.063	0.058	0.0060	0.55
Clean fleece (g/cm^2)	0.048	0.040	0.0055	0.32
Wool yield (%)	75.3	72.9	2.04	0.42
Staple length (mm)	16.2	17.6	1.02	0.35

[1] SE = Standard error.

groups ($p = 0.35$). These findings are in contrast to previous studies where it has been found that the supplementation of citrus pulp either increased DMI (Fonesca et al., 2001; Bueno et al., 2002) or had no effect on the amount of feed consumed (Martinez-Pascual and Fernandez-Carmona, 1980b; Belibasakis and Tsirgogianni, 1996; Leiva et al., 2000; Lanza et al., 2001; Villarreal et al., 2006). However other citrus pulp feeding trials have produced similar results to the current study in that supplementing citrus pulp did not have an effect on ADG (Martinez-Pascual and Fernandez-Carmona, 1980b; Tsirgogianni, 1996; Lanza et al., 2001; Volanis et al., 2004; Volanis et al., 2006).

Similar trends were found during the post-lambing period, in that the ewes fed the 30% fresh citrus pulp diet ate less feed than their control ewe counterparts (1,285 vs. 1,620.3 g/d, respectively; $p<0.01$), while gaining similar amounts of weight ($p = 0.12$). In addition, it was found that lamb birth weights and lamb ADG were not significantly different between treatment groups. On the contrary, Castrillo et al. (2004) found that when supplementing dried citrus pulp to lactating Rasa Aragonesa ewes, their lambs grew at a slower rate than their control lamb counterparts (244 vs. 278 g/d, respectively). They attributed this decreased ADG to lower milk production and a variation in milk composition (less protein and fat produced each day).

Moisture content in fresh citrus pulp decreased DM content on the 30% citrus pulp diet compared to control diet (664 vs. 883 g/kg of feed, respectively; Table 1). Consequently, animals fed 30% citrus pulp diet had lower intakes compared to ewes fed control diets, but there was no negative impact upon ewe performance pre- or post-lambing (ADG; Tables 3 and 4). The effect of low DM percentage feed on voluntary feed intake was identified by Vérité and Journet (1970) who suggested water contents above 84% would lower feed intake. A relationship between intake and feed DM content was identified ($r^2 = 0.79$) by John and Ulyatt (1987), confirming observations of Vérité and Journet (1970).

Finally, it was found that replacing 30% of lucerne hay with fresh citrus pulp in the diets of ewes did not affect wool production (Table 5). This is the first fresh citrus pulp trial that has been conducted assessing the response of wool growth to the inclusion of fresh citrus pulp into Merino diets.

IMPLICATIONS

Replacing 30% of lucerne hay with fresh citrus pulp increased *in vitro* dry matter digestibility (IVDMD), total volatile fatty acid production and cumulative gas production but decreased pH and ammonia concentrations. These *in vitro* findings (i.e. IVDMD) were inconsistent with the *in vivo* trial results in which intakes were lower for the 30% fresh citrus pulp diet compared to the control diet, while average daily gains were similar between treatment groups. In addition, lamb growth and wool performance was not affected by the supplementation of citrus pulp to the ewes. In conclusion, replacing 30% of lucerne hay with fresh citrus pulp did not affect productivity in sheep even in spite of lower intake. These findings of course would be of significant interest to Australian farmers endeavouring to control cost whist maintaining productivity.

ACKNOWLEDGMENTS

Thanks to Australian Wool Innovation for funding this project and the University of Sydney for providing the resources and personnel to undertake this trial.

REFERENCES

ABARE (Australian Bureau of Agricultural and Resource Economics). 2003. Australian commodity statistics, Australian bureau of agricultural and resource economics, Canberra - 1301.0 - Year Book Australia.

Arthington, J. D., W. E. Kunkle and A. M. Martin. 2002. Citrus pulp for cattle. Vet. Clin. Food Anim. 18:317-326.

AOAC. 1990. Official methods of analysis. 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Bampidis, V. A. and P. H. Robinson. 2006. Citrus by-products as ruminant feeds: a review. Anim. Feed Sci. Technol. 128:175-217.

Barrios-Urdaneta, A., M. Fondevila and C. Castrillo. 2003. Effect of supplementation with different proportions of barley grain or citrus pulp on the digestive utilization of ammonia-treated straw by sheep. Anim. Sci. 76:309-317.

Belibasakis, N. G. and D. Tsirgogianni. 1996. Effects of dried citrus pulp on milk yield, milk composition and blood components of dairy cows. Anim. Feed Sci. Technol. 60:87-92.

Benchaar, C., A. V. Chaves, G. R. Fraser, Y. Wang, K. A. Beauchemin and T. A. McAllister. 2007. Effects of essential oils and their components on *in vitro* rumen microbial fermentation. Can. J. Anim. Sci. 87:413-419.

Ben-Ghedalia, D., E. Yosef, J. Miron and Y. Est. 1989. The effects of starch- and pectin-rich diets on quantitative aspects of digestion in sheep. Anim. Feed Sci. Technol. 24:289-298.

Bueno, M. S., E. Ferrari Jr., D. Bianchini, F. F. Leinz and C. F. C. Rodrigues. 2002. Effect of replacing corn with dehydrated citrus pulp in diets of growing kids. Small Rumin. Res. 46:179-185.

Cannas, A., L. O. Tedeschi, D. G. Fox, A. N. Pell and P. J. Van Soest. 2004. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. J. Anim. Sci. 82:149-169.

Castrillo, C., A. Barrios-Urdaneta, M. Fondevila, J. Balcells and J. A. Guada. 2004. Effects of substitution of barley with citrus pulp on diet digestibility and intake and production of lactating ewes offered mixed diets based on ammonia-treated barley straw. Anim. Sci. 78:129-138.

Fedorak, P. M. and S. E. Hrudey. 1983. A simple apparatus for

measuring gas production by methanogenic cultures in serum bottles. Environ. Technol. Lett. 4:425-432.

Fonesca, A. J. M., A. A. Dias-da-Silva and A. L. G. Lourenco. 2001. Effects of maize and citrus-pulp supplementation of urea-treated wheat straw on intake and productivity in female lambs. J. Anim. Sci. 73:123-136.

Gleeson, T., M. Lubulwa and S. Beare. 1993. Price premiums for staple measurement of wool. ABARE Research Report 93.4, Canberra, Australia.

John, A. and M. J. Ulyatt. 1987. Importance of dry matter content to voluntary intake of fresh grass forages. Proc. NZ Soc. Anim. Prod. 47:13-16.

Langlands, J. P. and J. L. Wheeler. 1968. The dyebanding and tattooed patch procedures for estimating wool production and obtaining samples for the measurement of fiber diameter. Aust. J. Exp. Agric. Anim. Husb. 8:265-269.

Lanza, M., A. Priolo, L. Biondi, M. Bella and H. Ben Salem. 2001. Replacement of cereal grains by orange pulp and carob pulp in faba bean-based diets fed to lambs: effects on growth performance and meat quality. Anim. Res. 50:21-30.

Leiva, E., M. B. Hall and H. H. Van Horn. 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83:2866-2875.

Martinez-Pascual, J. and J. Fernandez-Carmona. 1980a. Composition of citrus pulp. Anim. Feed Sci. Technol. 5:1-10.

Martinez-Pascual, J. and J. Fernandez-Carmona. 1980b. Citrus pulp in diets for fattening lambs. Anim. Feed Sci. Technol. 5:11-22.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. Estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas-production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93:217-222.

National Health and Medical Research Council. 2004. Australian Code of Practice for the care and use of animals for scientific purposes. Australian Government, Canberra, Australia.

National Research Council. 1985. Nutrient requirements of sheep, 6th Rev. Ed. Nat. Acad. Press, Washington, DC, USA.

SAS Institute, Inc. 2009. SAS OnlineDoc® 9.1.3. SAS Institute Incorporation: Cary, USA.

Strobel, H. J. and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941-2947.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vérité, R. and M. Journet. 1970. Influence de la teneur en ean et da la deshydratation de l'herbe sur sa valeur alimentaire pour less vaches laitieres. Ann. Zootech. 19:255-268.

Villarreal, M., R. C. Cochran, A. Rojas-Bourrillion, O. Murillo, H. Munoz and M. Poore. 2006. Effect of supplementation with pelleted citrus pulp on digestibility and intake in beef cattle fed a tropical grass-based diet (*Cynodon nlemfuensis*). Anim. Feed Sci. Technol. 125:163-173.

Volanis, M., P. Zoiopoulos and K. Tzerakis. 2004. Effects of feeding ensiled sliced oranges to lactating dairy sheep. Small Rumin. Res. 53:15-21.

Volanis, M., P. Zoiopoulos, E. Panagou and C. Tzerakis. 2006. Utilization of an ensiled citrus pulp mixture in the feeding of lactating dairy ewes. Small Rumin. Res. 64:190-195.

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971-974.

Wing, J. M. 1974. Effect of physical form and amount of citrus pulp on utilization of complete feeds for dairy cattle. J. Dairy Sci. 58:63-66.