# Intake, nutrient digestibility, rumen parameters, growth rate, carcase characteristics and cannabinoid residues of sheep fed pelleted rations containing hemp (*Cannabis sativa* L.) stubble

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**ABSTRACT:** The feeding value and impact of hemp stubble in the diet of ruminants is unknown. Fifteen Merino castrated male sheep were maintained in individual pens and fed one of three pelletized experimental inclusion diets, as a 0% (Control), 28% (Hemp 1), and 56% (Hemp 2) pellet that delivered a diet meeting the nutrient requirements of the animals. Inclusion of hemp stubble had no effect ($P > 0.05$) on either DM intake, live weight gain or the feed to gain ratio but positively impacted ($P < 0.05$) on nutrient digestibility. Hemp stubble inclusion increased the concentration (but not molar proportions) of acetic and butyric acids and increased both the concentrations and molar proportions of iso-butyric, isovaleric, hexanoic and heptanoic acids, possibly due to increased protein digestibility and/or changes in the composition of rumen cellulolytic bacteria. Tetrahydrocannabinolic acid (THCA) was the only cannabinoid found in plasma in the sheep fed the hemp-containing diets, and this was found at very low concentrations (<16 µg/L). The psychoactive cannabinoid delta-9-tetrahydrocannabinol ($\Delta^9$-THC) was not detected in any plasma samples. THCA was detected in the liver of two sheep fed the Hemp 1 pellets and two sheep fed the Hemp 2 pellets. Cannabidiol (CBD) was detected in the liver of one sheep fed the Hemp 2 pellets (but no liver THCA was detected in this sheep). $\Delta^9$-THC was detected in both the kidney fat and subcutaneous fat of all sheep fed hemp stubble, with the concentrations being higher ($P < 0.05$) in the sheep fed the Hemp 1 pellets. THCA was also detected in the subcutaneous fat of one of the sheep fed the Hemp 1 pellets. Four of the five sheep fed the Hemp 1 pellet and one of the five sheep fed Hemp 2 pellet had detectable levels of $\Delta^9$-THC in the meat (loin). No other cannabinoids were detected in the meat. Current food standards regulations in Australia prohibit presence of any cannabinoid residues in commercial meat products; thus, determination of a withholding period is required to enable the safe feeding of hemp-stubble to sheep. Further research is also required to gain a greater understanding of the rumen metabolism of cannabinoids.

**Key words:** hemp, novel forage, summer crops, tetrahydrocannabinol



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## INTRODUCTION

Hemp, the low delta-9-tetrahydrocannabinol ($\Delta^9$-THC) variety of *Cannabis sativa*, is a high yielding multi-purpose crop and the stem (stubble) has the potential to be grazed or incorporated into pelletized diets for ruminants after the seed crop is harvested. Very little; however, is known about the nutritional quality, palatability and effects of feeding hemp stubble on ruminant animal performance. Hemp is characterized by a relatively low concentration of THC (Cherney and Small, 2016); however, it may contain higher concentrations of cannabidiol (CBD) (Marks *et al.*, 2009). In plants, THC and CBD are derived from their acidic precursors $\Delta^9$- tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), with the latter the predominant cannabinoid in fiber-type hemps (Andre et al., 2016). Despite the stem containing only trace amounts of THC (Cappelletto et al., 2001), it may still have the potential to affect animals that are consuming it as well as humans consuming the animals (meat) and their products (milk). Following assimilation via the blood, THC is rapidly absorbed into the fat tissues, brain and muscle resulting in a rapid decrease in plasma concentrations (Sharma et al., 2012). It is unknown how much THC and its precursors transfer to animal tissues when hemp stubble is consumed. Therefore, the major aim of the current study was to determine the effect of hemp stubble on apparent nutrient digestibility, rumen parameters and THC residues in meat and fat of sheep fed diets containing varying proportions of hemp stubble.

## MATERIALS AND METHODS

The animal house-based study was conducted at the New South Wales Department of Primary Industry Animal Nutrition Unit at Wagga Wagga (35°2′S, 147°19′E), NSW. The use and care of animals was approved by Charles Sturt University's Animal Care and Ethics Committee (Protocol number: A20016) and was compliant with the Animal Research Act 1985 (as amended) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Analytical chemistry was conducted at Charles Stuart University and at the NATA certified laboratories of ChemCentre in Western Australia.

### *Experimental Animals and Housing*

Fifteen Merino castrated male sheep (54.09 ± 6.09 kg) of approximately 12 mo of age were used. The animals were sourced from the same property to minimize potential differences in the composition of their rumen microbial populations and to minimize any differences that might occur due to environmental adaptation.

The sheep were maintained indoors, at varying times, in either individual pens (1.2 × 2.4 m) or metabolism cages (1.0 m × 1.5 m). At all times the sheep had access to fresh, clean water.

### *Experimental Design*

The experimental period comprised a total of 56 d including a 14 d dietary and housing adaptation period and a 7 d digestibility study (during which time the animals were housed in metabolism cages). A randomized complete block design with three diets and five replicates per treatment was used. Prior to the commencement of the experiment, the sheep were weighed using electronic scales (Prattley Industries Ltd; Ruddweigh 700) that were calibrated with a known weight. The sheep were stratified and allocated to diets based on live weight (LW). Once allocated to their respective diet, the sheep were randomized to the individual pens.

### *Experiment Diets*

The experimental diets were pelleted rations that included hemp stubble at 0% (Control), 28% (Hemp 1), and 56% (Hemp 2) of the total diet dry matter (DM), with oaten chaff as the substitute forage. Due to processing issues, 56% was the maximum possible inclusion level of the hemp stubble and this then set the 56% inclusion level of cereal straw in the control diet. The diets were formulated to be a complete feed for sheep of this age and were manufactured by a commercial feed mill. It was intended that the three pelleted rations would be isocalorific and isonitrogenous; however, as indicated in Table 1, there were variations between the three diets. The cannabinoids content of the pelleted rations is also presented in Table 1. The sheep were fed ad libitum twice daily at 0900 h and 1600 h.

### *Animal Measurements*

Unfasted LW of wethers was measured (before the morning feed at approximately 0800 h) on d 0, 16, 22, 30, 37, 44, 51, and 56 using electronic scales that were calibrated at each weighing with a known weight.

The amounts of feed offered, and feed refusals (orts) were recorded daily to enable determination of

**Table 1.** Nutritional parameters and cannabinoids profile of the experimental diets (on DM basis).

| Parameter | Control 0% hemp stubble | Hemp 1 28% hemp stubble | Hemp 2 56% hemp stubble |
|---|---|---|---|
| Dry matter, g/kg as fed | 94.7 | 92.6 | 92.2 |
| Metabolisable energy[a], MJ/kg DM | 9.9 | 9.1 | 9.6 |
| Dry matter digestibility,% | 67.3 | 62.7 | 65.3 |
| Crude protein, g/100 g DM | 8.7 | 10.3 | 10.6 |
| Organic matter, g/100 g DM | 93.3 | 94.8 | 95.8 |
| Neutral detergent fiber g/100 g DM | 40.8 | 45.0 | 40.86 |
| Acid detergent fiber, g/100 g DM | 24.5 | 31.0 | 28.0 |
| Lignin, g/100 g DM | 2.7 | 4.3 | 3.6 |
| Polyphenolics, mg/kg DM | 3520 | 2960 | 2400 |
| Cannabichromene (CBC), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabidiol (CBD), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabidiolic acid (CBDA), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabidivarin (CBDV), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabigerol (CBG), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabigerolic acid (CBGA), % w/w | <0.001 | <0.001 | <0.001 |
| Cannabino1 (CBN), % w/w | <0.001 | <0.001 | <0.001 |
| $\Delta^8$-Tetrahydrocannabinol ($\Delta^8$-THC), % w/w | <0.001 | <0.001 | <0.001 |
| $\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC), % w/w | <0.001 | 0.001 | <0.001 |
| Tetrahydrocannabinolic acid (THCA), % w/w | <0.001 | 0.001 | 0.002 |
| Tetrahydrocannabivarin (THCV), % w/w | <0.001 | <0.001 | <0.001 |
| Total cannabidiol (CBD), % w/w | <0.001 | <0.001 | <0.001 |
| Total tetrahydrocannabinol (THC) (%) | <0.001 | 0.002 | 0.002 |

[a]Metabolisable energy calculated using the formula: ME = 0.164 × (%DMD + %Fat) − 1.6 (Oddy et al., 1983).

Dry matter digestibility calculated using the formula: DMD = 83.58 − (NDF × 0.824) + (protein × 0.42) (Oddy et al., 1983).

"<" signifies a result is less than the limit of quantitation for the method.

Total THC calculated based upon THC + 0.877 × THCA.

Total CBD calculated based upon CBD + 0.877 × CBDA.

feed intake (on a DM basis). Average feed intake was aligned to the weighing of the animals and was thus determined for d 1–15, 16–21, 22–29, 30–36, 37–43, 44–50, and 51–56. Feed efficiency (feed:gain ratio) was determined by dividing the total amount of feed consumed (kg DM) by the total LW gain (kg).

### *Orts and Feces*

During the digestibility study and prior to feeding, the orts (from the previous day) were collected, the weight recorded and then retained for subsequent analysis of DM and chemical composition. The total daily amount of feces voided by each animal was recorded. Representative sub samples (15%) of feces were taken each day and stored at −18 °C for subsequent chemical analysis.

### *Ruminal Fluid*

At the end of the collection period and upon return to their individual pens, a ruminal fluid sample was collected from each animal approximately 3 h after their morning feeding using a stomach tube. A small length (less than 15 cm) of slightly wider tubing (to prevent the sheep chewing on the actual stomach tube) was inserted (and held) into the sheep's mouth and then the stomach tube (2 mm of wall thickness and 6 mm of internal diameter) was fed through and down the esophagus. Before commencing the procedure, the length of tube required to be passed was measured to ensure that the tube would rest in the rumen. A brass sampling "bolus" with fine holes and a smooth surface was attached to the end of the tube to allow filter collection of the ruminal fluid.

Each sample was assessed for saliva contamination, and if contaminated the sample was discarded and another taken. If the second sample was also contaminated, the sample was kept, but the value was not included in the analysis for ruminal fluid pH (Packer et al., 2011). The pH of the sample was assessed immediately after collection, after which subsamples were taken and stored at −18 °C until analysis of volatile fatty acid (VFA) concentrations and molar proportions, and ammonia concentrations.

### *Blood Collection*

On the last day of the feeding trial (d 56), and prior to dispatch to the abattoir for slaughter, a

blood sample was collected from the jugular vein of each sheep using a heparinized vacutainer and an 18-gauge needle. The whole blood samples were then centrifuged at 3,000 rpm for 10 min at 4 °C and the plasma was decanted and stored at −20 °C until analysis of cannabinoids content.

### *Sample Preparation*

The orts collected from each sheep during the digestibility study were bulked and a subsample (at least 500 g) was freeze-dried and the moisture content determined. The dry feed was then milled in a Christy and Norris cross beater mill to pass a 2 mm screen and used for subsequent determination of as analyzed DM content and chemical composition [ash, crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF)].

The frozen fecal samples were thawed at room temperature, and then the samples for each sheep were thoroughly mixed to create a composite sample. The composite samples were then dried in a fan-forced oven at 100 °C to constant weight to determine DM content and the dried sample was subsequently used for determination of ash, CP, NDF, and ADF contents.

### *Slaughter and Carcase Sampling*

The sheep were removed from their pens and weighed the morning of d 56 before being transported (at 1100 h) approximately 80 km to a local, commercial abattoir. The animals had ad libitum access to water prior to slaughter. The sheep were slaughtered the following morning (0600 h) in a single run and in accordance with the Australian standard practice. Electrical stimulation along the chain was used post-slaughter. Ear tag identification was matched with corresponding abattoir body number to maintain traceability throughout the chiller.

Before entering the chiller, the hot carcase weight (HCW) was recorded. AusMeat assessment of carcases included lean, fat, and bone percentage. The liver, kidney and kidney fat were collected from the offal table (at the abattoir), chilled overnight (28 h at 4 °C) transported under refrigeration (1 h) and then weighed, before storing at −18 °C until analysis of cannabinoids contents.

### *Meat Samples*

After chilling overnight (28 h) the carcases were transported from the abattoir to the Meat Laboratory at Charles Sturt University and aged in the chiller (set at 3 °C) for 7 d. The carcases were split and from the left side a sample of subcutaneous fat was removed at the rump, placed in a seal-top container, and stored at −18 °C. The *Longissimus lumborum* muscle (striploin, AUS-MEAT product identification number 4862) was boned out from the left side of each carcase. Briefly, the striploin was denuded of all fat, sinew and epimysium and starting from the anterior end, the first 2.5 cm slice was retained for future volatiles analysis, the next 2.5 cm slice was used for cannabinoids analysis and the next (third) 2.5 cm slice was also retained. Each slice was individually vacuum sealed, labelled and stored at −18 °C until analysis. The second slice from the striploin and the subcutaneous fat sample for each carcase were used for analysis of cannabinoids content.

### *Analytical Procedures*

Feed moisture content was determined as the percent weight loss incurred by freeze-drying the feed material. Samples were milled and allowed to equilibrate with atmospheric moisture prior to analysis. All samples utilized were taken from this equilibrated, homogeneous representative sample suitable for laboratory analysis.

The analytical moisture, DM (AOAC 2004-5; M934.01) and ash contents (AOAC 2004−5; M942.05) of ground samples were determined. The N content of feed, orts and feces (0.2 g ground samples) was determined using the Dumas combustion method (AOAC 2000, method 990.03) and the CP content of the samples was calculated by multiplying the N content by the factor 6.25 (Sosulski and Imafidon, 1990). The NDF and ADF contents of the feed, orts and feces were determined according to the procedures described by Van Soest and Goering (1970) and Van Soest et al. (1991). For quality control and assurance, a reagent blank sample, a control material and two replicate samples were prepared precisely the same way and placed in with each run.

After centrifuging the thawed ruminal fluid sample at 3,000 × *g* for 10 min, 100 µL of the supernatant was transferred in to a 1.5 mL Eppendorf tube containing 900 µL VFA reagents (1% formic acid−1% orthophosphoric acid, containing 184 ppm 4-methylvaleric internal standard). This was then centrifuged for 4 min at 10,000 rpm and 1 mL of supernatant was transferred to a gas chromatograph (GC) vial. Volatile fatty acid concentrations were determined using a Shimadzu GC17A

gas chromatograph with an autosampler and autoinjector. The method used a wide bore capillary column (SGE BP21 column; 12 × 0.53 mm internal diameter (ID) and 0.5 µm film thickness, SGE International, Ringwood, VIC, Australia, P/N 054473) with retention gap kit (including a 2 × 0.53 mm ID guard column, P/N SGE RGK2). For GC analysis, the carrier gas was helium with a total flow rate of 48.0 mL/min, a split ratio of 5:1 and a column flow of 7.84 mL/min. The inlet temperature was 155 °C, inlet pressure was 19 kPa and injection volume was 1 µL. The oven temperature was set at 80 °C for 2 min, increasing 6 °C/min to 122 °C, 12 °C/min to 144 °C, then increasing 40 °C/min up to 220 °C, which was then maintained to give a total run time of approximately 20 min. The flame ionization detector temperature was set at 200 °C, with the following gas flow rates: hydrogen at 40 mL/min, instrument air at 500 mL/min and nitrogen make-up gas at 30 mL/min (Packer et al., 2011). Sample VFA peaks were identified by comparing their retention times with those of a standard mixture of genuine VFA (Sigma Aldrich) and quantified using Agilent OpenLab (ChemStation A01.03) and Microsoft Excel using 4-methylvaleric acid as the internal standard.

Ruminal fluid ammonia ($NH_3$-N) concentrations were determined using a Flow Injection Analyzer Spectroscopy (Lachat 8000 series FIA). This method was adapted from Lachat (2012), using ammonia chloride as the standard.

Cannabinoids in plant material were determined using liquid chromatography mass spectrometry (LC-MS/MS). Samples were freeze dried and milled prior to analysis. An aliquot of sample was extracted in methanol with deuterated internal standards and analyzed using a Waters Xevo TQMS LC−MS/MS and quantified against certified reference standards (Cerilliant, USA). The limit of quantification for all determined cannabinoids was 10 mg/kg (w/w). Cannabinoids and associated metabolites in biological matrices were determined using LC−MS/MS. Tissue and organ samples were freeze dried prior to extraction; plasma and urine samples were analyzed as received. An aliquot of sample, spiked with deuterated internal standards, was extracted in methanol prior to matrix clean-up using solid phase extraction (SPE). Samples were analyzed using an Agilent 6470A LC-MS/MS and quantified against certified reference standards (Cerilliant, USA). Limits of detection and quantification are detailed in Table 2.

### *Calculations*

The apparent digestibility of a feed or individual nutrient is most accurately defined as the proportion that is not excreted in feces, and therefore assumed to be absorbed by the animal. The amount of each constituent (DM, OM, CP, NDF, ADF) was determined for the feed offered, orts and feces. By subtracting the amount of each constituent in the feed refusals from that in the offered feed, nutrient intakes were calculated. The digestibility of the nutrients (DM, OM, CP NDF, and ADF) were then determined, expressed on a DM basis, using the following formula (AFIA 2006):

$$\text{Digestibility}\,(\,\%\,) = [(\text{dietary intake} - \text{faecal output})\,/\text{dietary intake}] \times 100$$

Molar proportions of individual VFA were calculated from total and individual VFA concentrations. The ratios of acetic acid to propionic acid (Ac:Pr) and the propionic acid to acetic acid plus two times butyric acid (Pr:Ac + 2 × Bu) were calculated on the basis of concentrations as well as molar proportions. The ratios were included in the experimental analysis as they help explain the energy partitioning of ATP between the individual VFA; for example, one mole of butyric acid is formed from 1 mol of hexose while 2 mol of propionic or acetic acids are formed from 1 mol of hexose (Dijkstra et al., 2012).

**Table 2.** Cannabinoids and metabolites determined using LC−MS/MS

| | Organs and tissues | | Blood | |
|---|---|---|---|---|
| Compound | LoD (µg/kg DM) | LoQ (µg/kg DM) | LoD (µg/L) | LoQ (µg/L) |
| $\Delta^9$-THC | <15 | 50 | <2 | 5 |
| THCA | <30 | 100 | <2 | 5 |
| CBD | <15 | 50 | <2 | 5 |
| CBDA | <15 | 50 | <2 | 5 |
| 11-nor-9-carboxy-$\Delta^9$-Tetrahydrocannabinol | <15 | 50 | <2 | 5 |
| 11-Hydroxy-$\Delta^9$-Tetrahydrocannabinol | <15 | 50 | <2 | 5 |
| 11-nor-9-carboxy-$\Delta^9$-Tetrahydrocannabinol glucuronide | <15 | 50 | <2 | 5 |

### *Statistical Analysis*

In preliminary analyses, the model assumptions were tested for normal distribution of the residuals. The model assumption of constant variance of the residuals was also tested.

Data that was collected from the same animal over time (feed intake and LW) were analyzed as Repeated Measures Analysis, using the Mixed Model procedure in the SAS statistical program (SAS Institute Inc., 1997). The analysis included the fixed effects of "dietary treatment" and "day" and the interaction between the fixed effects with the "pen" as a random effect. The most appropriate covariance structure was chosen with reference to the Bayesian Information Criterion (BIC, Littell et al., 2000). For the analysis of liveweight, initial liveweight (pre-treatment d 0) was used as a covariate. Data collected from animals at a single point in time (nutrient digestibility, ruminal fluid, blood plasma and carcass data) were analyzed using the Mixed Model procedure in SAS and included the fixed effect of 'dietary treatment'. All results were reported as least squares means ± SE of the least squares means, and differences were significant when $P < 0.05$.

A blood sample from one sheep fed the Hemp 2 pellets could not be collected due to difficulties experienced with venipuncture.

## RESULTS

### *Intake*

Inclusion of hemp stubble in the pelleted diets had no effect ($P > 0.05$) on DM intake, with the average (±SEM) daily DM intake (post the 14 d adaptation period) being 1283 ± 87.5, 1357 ± 88.1, and 1345 ± 101.8 g/d for Control, Hemp 1 and Hemp 2, respectively.

### *Liveweight Gain and Feed to Gain Ratio*

As shown in Table 3, at the conclusion of the feeding trial there were no significant differences ($P > 0.05$) in the LW or average daily gain (ADG) between the treatment groups, although at various time points throughout the feeding period the LW of the sheep fed the hemp containing diets were significantly higher ($P < 0.001$) than for those fed the control (0% hemp) diet.

The feed to gain ratio after 56 d on feed (including the 14 d adaptation period) was not significantly ($P > 0.05$) different when hemp stubble was included in the diet compared with the control diet.

### *Apparent Nutrient Digestibilities*

There was one outlier (one sheep fed the control diet) which was not included in the analysis, because of their highly variable intake over the period of the digestibility study. The effects of diet on nutrient digestibility are shown in Table 4. The apparent digestibilities of DM and OM were significantly higher ($P = 0.0026$ and $P = 0.0030$, respectively) in those sheep fed the Hemp 2 diet compared with the Control and Hemp 1. Crude protein apparent digestibility was significantly higher ($P = 0.0147$) for Hemp 2 than the Control diet, although Hemp 1 did not differ ($P > 0.05$) from Hemp 2 or the

**Table 3.** Predicted mean (±SE) liveweight (LW), average daily LW change and feed:gain ratio of Merino sheep fed pelleted diets containing varying proportions of hemp stubble

| | Dietary treatment | | | *P* value | | |
|---|---|---|---|---|---|---|
| Parameter | Control (0% hempstubble) | Hemp 1 (28% hempstubble) | Hemp 2 (56% hemp stubble) | Treatment | Day | T × D[b] |
| Liveweight, kg | | | | 0.0334 | <0.001 | 0.0431 |
| Day 16 | 54.8 (± 0.85) | 55.3 (± 0.85) | 56.05 (± 0.85) | | | |
| Day 22 | 55.4 (± 0.85) | 57.3 (± 0.85) | 56.2 (± 0.85) | | | |
| Day 30 | 56.4 (± 0.85)[a] | 59.0 (± 0.85)[b] | 58.3 (± 0.85)[ab] | | | |
| Day 37 | 56.6 (± 0.85)[a] | 58.9 (± 0.85)[b] | 59.3 (± 0.85)[b] | | | |
| Day 44 | 57.4 (± 0.85)[a] | 59.41 (± 0.85)[ab] | 60.85 (± 0.85)[b] | | | |
| Day 51 | 57.6 (± 0.85)[a] | 60.2 (± 0.85)[b] | 60.8 (± 0.85)[b] | | | |
| Day 56 | 58.2 (± 0.85) | 59.7 (± 0.85) | 60.55 (± 0.85) | | | |
| Average daily LW change (g/d)[a] | 75.6 (± 17.59) | 100.4 (17.59) | 115.4 (17.59) | 0.273 | | |
| Feed:gain ratio | 15.7 (± 1.59) | 13.1 (± 1.59) | 11.5 (± 1.593) | 0.211 | | |

Different superscripts within rows for each parameter indicates that there is a significant difference ($P \leq 0.05$).

[a]Average daily liveweight change (g/d) was calculated based on the change in LW over the total feeding period (including 14 d adaptation period).

[b]Level of significance for the interaction between dietary treatment (T) and day (D).

Control. The apparent digestibilities of both NDF and ADF were significantly lower ($P = 0.0052$ and $P = 0.0027$, respectively) for the Control diet compared to Hemp 1 and Hemp 2, with no differences ($P > 0.05$) between Hemp 1 and Hemp 2.

### *Ruminal pH and Ammonia Concentrations*

Inclusion of hemp in the pelleted rations had no effect ($P > 0.05$) on either rumen pH or ruminal ammonia-N concentrations (Table 5); although, concentrations tended to be higher ($P = 0.0558$) for the sheep fed the pellets containing hemp stubble (Hemp 1 and Hemp 2).

### *Volatile Fatty Acids*

There were significant differences between diets in the concentrations of acetic ($P = 0.0377$), butyric ($P = 0.0366$), iso-butyric ($P < 0.0001$), valeric ($P < 0.0001$), iso-valeric ($P < 0.0001$), hexanoic ($P = 0.0022$) and heptanoic acids ($P = 0.0197$) but not propionic acid or total VFA ($P > 0.05$) (Table 5). The concentrations of acetic acid and butyric acid were significantly higher in sheep fed Hemp 1 compared to the Control but not compared to Hemp 2. The concentrations of iso-butyric, valeric, isovaleric, hexanoic and heptanoic concentrations were significantly lower ($P < 0.0001$) for the Control compared with Hemp 1 and Hemp 2, which did not differ ($P > 0.05$) to each other. There was no significant difference ($P > 0.05$) in the ratio Pr:Ac 2 x Bu (based on concentrations) between the diets.

The inclusion of hemp in the pelleted diets resulted in significant ($P < 0.05$) increases in the molar proportions of iso-butyric, valeric, isovaleric, hexanoic and heptanoic acids but not those of acetic acid, propionic acid and butyric acid ($P >$

**Table 4.** Apparent nutrient digestibility in Merino sheep fed pelleted diets containing varying levels of hemp stubble

| | Diet | | | |
|---|---|---|---|---|
| **Apparent digestibility, %** | Control, 0% hemp stubble | Hemp 1, 28% hemp stubble | Hemp 2, 56% hemp stubble | *P* value |
| Dry matter | 62.9 (± 1.40)[b] | 65.4 (± 1.25)[b] | 71.4 (± 1.25)[a] | 0.0026 |
| Organic matter | 64.3 (± 1.41)[b] | 67.4 (± 1.25)[b] | 72.9 (± 1.25)[a] | 0.0030 |
| Crude protein | 69.7 (± 1.79)[b] | 73.0 (± 1.60)[ab] | 77.1 (± 1.96)[a] | 0.0147 |
| NDF | 30.9 (± 3.62)[b] | 43.9 (± 3.23)[a] | 48.6 (± 3.85)[a] | 0.0052 |
| ADF | 19.3 (± 3.66)[b] | 41.3 (± 3.28)[a] | 37.9 (± 3.28)[a] | 0.0027 |

Values are least squares means ± standard errors of the least squares means.

NDF: neutral detergent fiber; ADF: acid detergent fiber.

Different superscripts within rows for each parameter indicates that there is a significant difference ($P \leq 0.05$).

**Table 5.** Mean ruminal pH, ammonia-N (mg/L), total volatile fatty acid (VFA) concentrations mmol/L), and individual VFA molar proportions (%) in Merino sheep fed pelleted diets containing varying levels of (industrial) hemp stubble

| | Diet | | | |
|---|---|---|---|---|
| Parameter | Control (0% hemp stubble) | Hemp 1 (28% hemp stubble) | Hemp 2 (56% hemp stubble) | *P* value |
| Rumen pH | 6.87 (± 0.112) | 6.89 (± 0.105) | 6.84 (± 0.123) | 0.9446 |
| Ammonia-N | 154 (± 34.4) | 270 (± 34.4) | 270 (± 34.4) | 0.0558 |
| Total VFA | 69.10 (± 3.158) | 79.89 (± 3.158) | 76.28 (± 3.158) | 0.0651 |
| Acetic acid | 63.15 (± 1.194) | 62.39 (± 1.143) | 63.85 (± 1.278) | 0.6310 |
| Propionic acid | 24.19 (± 1.135) | 22.03 (± 1.135) | 20.69 (± 1.135) | 0.1087 |
| Butyric acid | 11.62 (± 0.566) | 13.20 (± 0.566) | 12.84 (± 0.566) | 0.1352 |
| Iso-butyric acid | 0.30 (± 0.028)[b] | 0.57 (± 0.028)[a] | 0.65 (± 0.028)[a] | <0.0001 |
| Valeric acid | 0.62 (± 0.081)[b] | 0.97 (± 0.074)[a] | 1.12 (± 0.093)[a] | <0.0001 |
| Iso-valeric acid | 0.51 (± 0.095)[b] | 0.97 (± 0.085)[a] | 1.20 (± 0.110)[a] | <0.0001 |
| Hexanoic acid | 0.07 (± 0.034)[b] | 0.22 (± 0.034)[a] | 0.24 (± 0.034)[a] | 0.0025 |
| Heptanoic acid | 0.001 (± 0.0035)[b] | 0.014 (± 0.0035)[a] | 0.011 (± 0.0035)[a] | 0.0247 |
| Pr:Ac 2 × Bu[a] | 0.28 (± 0.017) | 0.25 (± 0.017) | 0.24 (± 0.017) | 0.1233 |
| Acetic:propionic | 2.74 (± 0.263) | 2.90 (± 0.251) | 3.38 (± 0.282) | 0.1524 |

Values are least squares means ± standard errors of the least squares means.

[a]Pr:Ac 2 × Bu = ratio of propionic acid to acetic acid + 2 × butyric acid.

Different superscripts within rows for each parameter indicates that there is a significant difference ($P \leq 0.05$).

0.05; Table 5). In addition, there were no significant differences ($P > 0.05$) between the diets in the ratios (based on molar proportions) of acetic: propionic and Pr:Ac 2 × Bu

### *Carcass Traits*

The inclusion of varying proportions of hemp stubble ($P < 0.05$) had no effect on any of the carcase parameters assessed (Table 6). Although liver weight was numerically higher when sheep were offered either the Hemp 1 or Hemp 2 diets compared with the Control, the difference was not statistically significant ($P = 0.163$).

### *Plasma Cannabinoid Concentrations*

As expected, there were no detectable cannabinoids (< 5 µg/L) in the plasma of sheep fed the control diet. The only cannabinoid detectable in the plasma of sheep fed hemp stubble was tetrahydrocannabinolic acid (THCA); no $\Delta^9$-THC was detectable (< 5 µg/L) in the plasma of these sheep. THCA concentrations did not differ ($P > 0.05$) when sheep were fed either the Hemp 1 (10.9 ± 0.57 µg/L; range 9.2−12.2 µg/L, $n = 5$) or Hemp 2 pellets (10.1 ± 2.04 µg/L; range 5.4−15.3 µg/L, $n = 4$). Of the plasma samples from the sheep fed Hemp 2 pellets, one was below the limits of quantification (<5 µg/L).

### *Tissue Cannabinoid Residues*

As expected, there were no detectable cannabinoids (<50 µg/kg DM) in the liver, kidney fat, subcutaneous fat, or meat (loin sample) of the sheep fed the Control diet. THCA was detected in the livers of two sheep fed the Hemp 1 (208 and 381 µg/kg DM) and two sheep fed Hemp 2 (163 and 201 µg/kg DM) pellets. CBD (188 µg/kg DM) was detected in the liver of one sheep fed the Hemp 2 pellets, but no liver THCA was detected in this sheep.

For all sheep fed hemp stubble, both the kidney fat and the subcutaneous fat contained $\Delta^9$-THC. The kidney fat $\Delta^9$-THC concentration was higher ($P < 0.05$) in sheep fed the Hemp 1 pellets (161.6 ± 33.08 µg/kg DM; range 63−229 µg/kg DM) compared with those fed the Hemp 2 pellets (71.6 ± 5.55 µg/kg DM; range 55−91 µg/kg DM). Similarly, $\Delta^9$-THC concentration of the subcutaneous fat was higher ($P < 0.05$) in the sheep fed the Hemp 1 pellets (224 ± 20.00 µg/kg DM; range 184−290 µg/kg DM) compared with those fed the Hemp 2 pellets (98.3 ± 8.11 µg/kg DM; range 76−120 µg/kg DM). THCA (220 µg/kg DM) was also detected in the subcutaneous fat of one of the sheep fed the Hemp 1 pellets, giving a total THC concentration [THC + (0.877 × THCA)] of 387 µg/kg DM in the subcutaneous fat for this particular sheep.

The meat (loin) of four of the five sheep fed the Hemp 1 pellet contained $\Delta^9$-THC at detectable levels (63 ± 3.71 µg/kg DM; range 52−72 µg/kg DM). Only one of the sheep fed the Hemp 2 pellet had detectable levels of $\Delta^9$-THC in their meat (71 µg/kg DM). On a fresh weight basis (which is relevant when considering potential intake by humans), the average total THC content of the loin samples was <30 µg/kg.

## DISCUSSION

No previously published data is available from *in vivo* studies on the feeding value of hemp stubble for sheep or any other ruminant species.

### *Intake*

Inclusion of hemp stubble in the pelleted diets had no effects (positive or negative) on DM intake.

**Table 6.** Predicted mean (± SE) carcase traits of Merino sheep fed pelleted diets containing varying proportions of hemp stubble

| | Dietary treatment | | | |
|---|---|---|---|---|
| Parameter | Control (0% hemp stubble) | Hemp 1 (28% hemp stubble) | Hemp 2 (56% hemp stubble) | *P* value |
| HCW[a], kg | 26.97 (± 0.65) | 27.37 (± 0.58) | 27.55 (± 0.58) | 0.800 |
| Fat score | 4.00 (± 0.40) | 4.00 (± 0.36) | 4.00 ± (0.36) | 0.685 |
| Dressing, % | 46.10 (± 1.32) | 46.26 (± 1.18) | 45.81 (± 1.18) | 0.963 |
| Lean, % | 52.22 (± 2.11) | 52.13 (± 1.89) | 49.88 (± 1.89) | 0.637 |
| Fat, % | 32.30 (± 2.97) | 32.35 (± 2.66) | 35.51 (± 2.66) | 0.637 |
| Bone, % | 15.55 (± 0.86) | 15.52 (± 0.77) | 14.60 (± 0.77) | 0.637 |
| Liver, g | 610.88 (± 54.23) | 752.46 (± 48.51) | 735.60 (± 48.51) | 0.163 |
| Kidney, g | 118.70 (± 10.48) | 127.70 (± 9.37) | 118.58 (± 9.37) | 0.747 |
| Kidney fat, g | 991.13 (± 300.78) | 985.58 (± 269.03) | 1149.80 (± 269.03) | 0.891 |

[a]Hot carcase weight.

However, there was considerable variation in the data (CV = 17.4% within treatment) that precludes an accurate assessment statistically. However, the lack of any adverse effects on DM intake indicates that hemp stubble can successfully replace cereal straw(s) in pelleted diets for sheep.

### *Liveweight Gain and Feed to Gain Ratio*

Liveweight gain is affected by the nutritional content of the diet and the intake of the animal. Up until day 51, the feeding of the hemp-containing diets had a positive impact on the LW gain of the sheep; however, by day 56 there was no difference in the LW of the sheep regardless of which diet was fed. The lack of any adverse effects on LW gain further supports that hemp stubble can successfully replace cereal straw(s) in pelleted diets for sheep.

### *Apparent Nutrient Digestibilities*

As the level of inclusion of hemp stubble increased in the pelleted rations, so too did the apparent digestibility of both DM and OM. These results suggest that DM and OM digestibility of hemp stubble is greater than that of oaten straw. In comparison to the estimated DM digestibility (Table 1), the in vivo DM digestibility of the Control diet was lower (67.3% vs. 62.86%) while that of Hemp 1 (62.7% vs. 65.40%) and Hemp 2 (65.3% vs. 72.66%) diets was higher.

These results also indicate that the presence of any secondary metabolites in hemp stubble had no adverse effects on digestibility. However, the reason(s) for these increases in DM and OM digestibility is not clear. The Control diet had a higher content of polyphenolic compounds than the hemp-containing pellets (Table 1). Polyphenolic compounds such as tannins, decrease digestibility by binding to digestive enzymes and dietary proteins (Robbins et al., 1987), which may explain the decreased digestibility of the Control diet. Flavonoids, as found in hemp (Teh and Birch, 2013; Pellati et al., 2018; Siano et al., 2019), can decrease DM digestibility (Ehsan et al., 2013), which was not the case in the current study. Lignin concentration is negatively correlated with digestibility (Guo et al., 2001; Jung and Lamb, 2006). The lignin content increased with the inclusion of hemp stubble in the pelleted diets (Table 1) and was not negatively correlated with digestibility. Differences in the NDF digestibility of the roughage component also affect digestibility of a diet. The NDF digestibility of oaten straw is generally greater than 20% (Kafilzadeh et al., 2012) while that of hemp stalk is 12.7% (Kleinhenz et al., 2020a), indicating that hemp stalk should be less digestible than oat straw. However, the NDF digestibility of the hemp containing pellets was significantly higher than that of the control. Further investigation of the digestibility of hemp stubble is required to better explain the improvements in apparent DM, OM, NDF, and ADF digestibilities in response to the inclusion of hemp stubble in the pelleted diets.

Full substitution of oat straw (Control) with hemp stubble (Hemp 2) resulted in a significant increase in CP digestibility (69.67% vs. 77.07). Although the rations had been formulated to be isonitrogenous, ultimately the Control was 8.7% CP, Hemp 1 was 10.3% CP while Hemp 2 was 10.6% CP. Diets with elevated CP content usually have greater apparent CP digestibility (Colmenero and Broderick, 2006), partially due to dilution of metabolic fecal N and partially due to increased intake of more digestible feeds (Broderick, 2003), although the latter was not the case in this study. Thus, the differences in CP digestibility were more likely due to differences in the CP content of the rations rather than the inclusion of the hemp stubble.

The results of this study indicate that not only does the inclusion of hemp stubble in pelleted rations have no adverse effects on intake, it has positive effects on digestibility.

### *Rumen pH*

The rumen pH is one of the most important determinants of ruminant performance since it impacts the fermentation pattern in the rumen. Inclusion of hemp stubble in the pelleted ration had no effect ($P > 0.05$) on rumen pH, with the average pH for all rations >6.8. A pH in the rumen of 6.5−7 is common in ruminants fed roughage-based diets (van Houtert, 1993). Roughage based diets typically have a high NDF content (>40% for all three diets used in this study) which increases chewing, salivation, and rumination, leading to an increased rumen pH when compared to low dietary NDF (Mertens, 1997).

Due to ethical and welfare concerns of stomach tubing animals, only one ruminal fluid sample was collected at around 3−4 h post-feeding. For a greater understanding of the effects of feeding hemp stubble on rumen pH, ideally ruminal fluid samples should be collected pre- and post-feeding to assess diurnal variations.

### *Ruminal Ammonia Concentrations*

Ruminal ammonia concentrations tended to be higher ($P = 0.0558$) for the hemp-containing rations, which was expected given the higher CP content of these two diets compared to the Control.

Ammonia-N is crucial for microbial protein synthesis. The minimum threshold for microbial protein synthesis is 50−80 mg/L (Satter and Slyter, 1974), with concentrations above 200 mg/L required for maximizing cellulolysis (Krebs and Leng, 1984), feed intake and digestibility (Yusuf et al., 2013). All the rations met the minimum threshold level. When N is not limiting microbial growth, the amount of microbial protein synthesized is directly proportional to the amount of substrate fermented (Mehrez et al., 1977). Thus, the improvements in digestibility with inclusion of hemp stubble in the ration may have affected microbial protein synthesis and warrants further research.

### *Volatile Fatty Acids*

Volatile fatty acids are the metabolic end products of microbial fermentation of OM in the rumen and act as major sources of energy for ruminants. The concentration and molar proportions of VFA are largely impacted by the composition of the diet, particularly the nature of carbohydrates and their degradation rate, as well as the composition of the rumen microbial population, time of feeding, rumen pH and substrate interactions (Dijkstra, 1994; Nozière et al., 2010).

For all diets, total VFA concentrations were within the normal range expected for relatively high, roughage-based diets, and as expected for these types of diets, acetic acid was the major VFA produced (France and Dijsktra, 2005). Although there were no significant differences ($P > 0.05$), total VFA concentrations tended ($P = 0.06$) to be higher for diets containing hemp stubble, which was likely a reflection of the higher OM digestibility as well as increased concentrations of some individual VFA.

Only the roughage component effectively varied between the experimental diets and thus it was expected that the similar non-structural carbohydrates (i.e., starch) contents of the three diets would result in minimal variations in both the concentrations and molar proportions of propionic acid (Sutton et al., 2003; Lettat et al., 2010), as was the case.

Whilst the inclusion of hemp stubble in the pelleted diets had no significant effects on the molar proportions of the major VFA (acetic, propionic and butyric acids), it resulted in significant increases ($P < 0.05$) in both the concentrations and molar proportions of valeric, iso-butyric, iso-valeric, hexanoic and heptanoic acids. These VFA are either required by, or stimulate growth of, many rumen organisms and are required for cellulose digestion (Muller, 1987). The concentrations of iso-butyric, iso-valeric acid and valeric acid reflect the extent of protein degradation in the rumen (Zahedifar et al., 2002), with the increase in the molar proportions of these acids supported by increased CP digestibility. The increased molar proportions of valeric acid may have also been related to the flavonoids contained in hemp. Zhan et al. (2017) reported increased valeric acid concentrations with increasing levels of dietary (alfalfa) flavonoids.

Isobutyric and isovaleric acids are synthesized by rumen microorganisms via an oxidative deamination and an oxidative decarboxylation of the branched-chain amino acids, valine and leucine, respectively (Andries et al., 1987). These VFA are required by cellulolytic bacteria and supplementation of these VFA in vitro has increased microbial protein synthesis (Cummins and Papas, 1985) and DM digestion (Gorosito et al., 1985; Mir et al., 1986). Dewhurst et al. (2003) suggested the increase in molar proportions of iso-butyric, iso-valeric, and valeric acids (with diets based on alfalfa silage and white clover silage) reflected the greater excess ruminal degradable protein, which was not the case in this study as excess ruminal degradable protein would be indicated by significantly higher ruminal ammonia concentrations. The more likely explanation for the increased molar proportions of these VFA is due to increased populations of ruminal cellulolytic bacteria (e.g., *Ruminococcus. albus, R. flavefaciens, Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*), cellulolytic enzyme activity (caboxymethyl-cellulase, cellobiase, xylanase, pectinase), and digestive enzyme activity in the small intestine (Liu et al., 2018).

Various anaerobic bacteria have been reported as hexanoic acid producers including *Megasphaera elsdenii* (Weimer and Moen, 2013), *Megasphaera hexanoica* (Jeon et al., 2017) and *Eubacterium pyruvativorans* (Wallace et al., 2004). *Megasphaera hexanoica* has also been associated with heptanoic acid production (Jeon et al., 2017). Further research is warranted to determine if the feeding of hemp stubble favors proliferation of these species, particularly *M. elsdenii* as it has been promoted as a potentially useful probiotic species to prevent lactate accumulation in diets containing readily fermentable carbohydrates (Klieve et al., 2003).

A greater understanding of how hemp stubble affects the composition of the rumen microbial populations would greatly assist in explaining the increases in the concentrations and molar proportions of the minor VFA, and thus warrants further research.

### *Carcass Traits*

The inclusion of hemp stubble in the rations had no adverse effects on any of the carcass attributes assessed. Although not statistically significant, there was a tendency for increased liver weights in response to feeding hemp stubble. The hepatotoxicity of CBD has been confirmed in several mouse model studies (Ewing et al., 2019). Rosenkrantz et al. (1981) found sub-chronic, oral administration of CBD (ranging from 30 to 300 mg/kg) increased liver weights of rhesus monkeys. Purohit et al. (2010) also noted an increase in fatty liver as a result of THC and CBD abuse in humans. Further research on the effects of feeding hemp stubble on liver weights and the potential of reversing any such effects (Issa et al., 2019) by using a withholding period prior to slaughter is warranted.

### *Plasma Cannabinoid Concentration*

THCA was the only cannabinoid found at concentrations above the limits of quantification in the plasma of sheep fed the hemp-containing diets. The psychoactive cannabinoid $\Delta^9$-THC was not detected in any plasma samples. Kleinhenz et al. (2020b) found a greater diversity of (detectable) cannabinoids in the plasma including CBDA, THCA-A, cannabidivarinic acid, and cannabichromenic acid of cattle orally dosed (5.4 mg of CBDA/kg BW). Given that the CBDA concentrations of the rations fed to the sheep were below the limits of quantification (<10 mg/kg DM), it is unclear if there may be differences in plasma cannabinoids between ruminant species fed hemp, and warrants further research.

A more intensive sampling protocol for plasma cannabinoid concentrations than used in this study could shed more light on metabolism and excretion given there is variation in the half-life (T½) of cannabinoids. For example, CBDA had a mean apparent $T_{½}$ of 14.1 h in cattle (Kleinhenz et al., 2020b). In addition, knowledge of the impact of the rumen on the fate of oral cannabinoids, and whether there are differences between ruminant species is needed to better understand the fate of cannabinoids from the long-term intake of hemp stubble in ruminant animals.

### *Tissue Cannabinoid Residues*

The current study is the first to provide data on the deposition of cannabinoids in ruminant organs (liver) and tissues. THCA was at detectable levels in the liver of only two of the sheep fed Hemp 1 pellets and two of the sheep fed the Hemp 2 pellets. CBD was detected in the liver of one sheep fed the Hemp 2 pellets.

Cannabinoids were detected in the fat and some of the meat samples; however, the concentrations were very low. Putting this in to perspective of potential dietary intake by humans, an average portion size of meat is generally considered to be around 250 g (fresh weight) and given the average total THC content of the loin samples on a fresh weight basis was <30 µg/kg this would equate to <7.5 µg total THC/serving size.

In Australia, current regulation states zero tolerance for THC in foods of animal origin until Food Standards Australia and New Zealand (FSANZ) sets a safe or 'maximum level'. Due to a lack of testing and available data, FSANZ have not yet set this maximum level. Therefore, there is currently no tolerance for any amount of THC residue in these foods. Thus, until the maximum level of THC in foods of animal origin have been set, hemp stubble is not suitable as a feed for ruminants as cannabinoid residues were detectable in both the subcutaneous and kidney fat as well as meat. Further research is needed to determine the required withholding period to eliminate THC residues from the meat and subcutaneous fat. The tissue residues of $\Delta^9$-THC; however, were very low and may warrant changes to the feed regulations as an alternative option to dealing with residues. Alternatively, data from this study could be used to assist in the establishment (by FSANZ) of the maximum level of THC in meat.

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