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Running head: METHANE AFFECTED BY FAT AND FIBRE DIGESTIBILITY QUALITY

Methane emissions, feed intake and total tract digestibility in lambs fed diets differing in fat content and fibre digestibility

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Abstract: This study determined enteric methane (CH_4) emissions, intake, and apparent total tract digestibility of diets varying in fibre digestibility and fat content. A Latin square design with 2 levels of fat (2.0, 6.0 % DM; low and high) and 2 diets varying in fibre digestibility (low LFbD or high HFbD) was used. Higher DMI was observed (P<0.01) for LFbD versus HFbD diets (2.56 *vs.* 2.14 kg.d^{-1}, respectively), with no effect of fat. Fibre, DM, and OM digestibility were higher (P<0.01) for HFbD than LFbD diets. Increasing fat did not affect intake or digestibility of DM or dietary constituents, but there was a fibre digestibility *x* fat content interaction (P<0.01) for fat digestibility. There was also a fat content *x* fibre digestibility interaction (P<0.05) for CH_4 (g.kg^{-1} DMI, OMI, NDFI, and % GEI), with emissions being higher when fat was added to the HFbD than the LFbD diet. The CH_4 emissions per kg of NDF digested were higher (P < 0.01) for the HFbD than the LFbD diet. Methane emissions were increased by the HFbD diet, but inclusion of fat had a differential impact on CH_4 emissions as a proportion of DMI or NDF intake in diets differing in fibre digestibility.

Key words: fibre digestibility, fat, feed quality, methane, sheep

Introduction

It is well established that livestock are significant contributors to global agricultural greenhouse gas emissions. Methane (CH_4) is of particular relevance, with 35 to 40% of global anthropogenic CH_4 arising from fermentation of feed in the digestive tracts of ruminants such as cattle and sheep (Gerber et al. 2013). Strategies for reducing enteric methane emission have been assessed at length, with much focus on dietary manipulation as a means of abatement. One promising strategy for CH_4 mitigation is the supplementation of ruminant diets with additional fats from plant or animal sources. However, the industry adoption of fats as an approach to the

mitigation of enteric CH_4 emission is influenced by their costs and potential to negatively impact feed intake and diet digestibility (Hristov et al. 2013). In a review of 27 studies assessing fat supplementation, Grainger and Beauchemin (2011) concluded that with inclusion of up to 0.08 fat in the diet, a 10 $g.kg^{-1}$ increase in fat resulted in a 1 $g.kg^{-1}$ DMI and 2.6 $g.kg^{-1}$ DMI decrease in CH_4 emissions in cattle and sheep, respectively. However, CH_4 response is affected by a number of factors including level of supplementation, fat source, fatty acid profile as well as basal diet composition (Hristov et al. 2013). Increasing dietary fat content may be achieved through variety of mechanisms including the use of oilseed products from common commodity crops in Canada. For example, the addition of canola oil to beef cattle diets was reported to reduce enteric CH_4 emissions (Beauchemin and McGinn 2006). However, in the study of Cosgrove et al. (2008), no reduction in CH_4 was observed following infusions of linseed/sunflower oil blends up to 0.05 of DMI in sheep.

Additionally, varying the nutritive quality of ruminant feeds has also been shown to influence the amount of enteric CH_4 produced (Warner et al. 2017). In particular, variations in diet digestibility affect the level of feed intake, rate of passage and rumen fermentation, factors that have important implications for CH_4 production.

To date, there is a lack of data assessing the effects of varying dietary fat content and nutritive quality simultaneously. Thus, the objective of this study was to assess CH_4 emissions, feed intake, and diet digestibility in ram lambs offered diets of low or high fibre digestibility with varying fat content.

Materials and Methods

This study was conducted in accordance with the guidelines established by Canadian Council of Animal Care (2009) and was approved by the Lethbridge (Alberta) Research and Development Centre Animal Care Committee.

Animals, Experimental Design and Diets

The study was conducted as a Latin Square design with a factorial arrangement of treatments using four experimental diets (Table 1) consisting of 1) low fibre digestibility, low fat (LFbD-LF), 2) low fibre digestibility, high fat (LFbD-HF), 3) high fibre digestibility, low fat (HFbD-LF), and 4) high fibre digestibility, high fat (HFbD -HF). Dietary NDF content of the diets ranged from 308 to 320 g.kg^{-1} DM, with differences in fibre digestibility achieved through inclusion of either oat hulls or beet pulp. On average, diets contained 21.5 (LF) and 60.0 (HF) g fat.kg^{-1} DM. The principal protein source consisted of either solvent-extracted canola meal (24 g fat.kg^{-1} DM) in LF diets and pressed canola meal (114 g fat.kg^{-1} DM) in HF diets. Both canola meals were derived from the same commercial source of *Brassic napus* L. The use of pressed canola meal assisted in the formulation of isonitrogenous and isoenergetic diets with canola oil used to achieve the desired fat concentration in the high fat diets.

Twelve Canadian Arcott ram lambs, initial BW 64.3 (SD ± 3.27 kg), and approximately 8 m of age, were assigned to diets. Each experimental period consisted of 17 d adaptation followed by 4 d of simultaneous measurement of CH_4, feed intake, and digestibility. During adaptation, lambs were housed individually in pens (2.82 m × 1.02 m) fitted with automatic water dispensers and fed once daily at 1000 h with *ad libitum* access to feed. During CH_4 and diet digestibility measurements, feed intake was restricted to 0.90 of *ad libitum* based on the previous 5 d intake. Diets were pelleted (2 cm × 0.64 cm) and samples of each feed were obtained 3 times weekly

during adaptation, and daily during the measurement periods. Feed samples were stored at -20ºC for subsequent processing and analysis.

Enteric CH_4 measurements

Production of enteric CH_4 was measured using 4 climate-controlled, open circuit chambers according to the procedures of Beauchemin and McGinn (2006). Three lambs were assigned to each chamber and accommodated individually in metabolism crates with *ad libitum* access to clean, fresh water. Each chamber measured 4.4 m wide × 3.7 m deep × 3.9 m tall (63.5 m^3 volume; model C1330; Conviron Inc., Winnipeg, MB, Canada). The processes involved in air circulation in the chambers and subsequent gas sampling as described by Avila-Stagno et al. (2013). Emissions from each chamber were calibrated by releasing a known amount of CH_4 prior to, and following completion of the 4 experimental periods, and calculating the mass balance of incoming and outgoing CH_4. Concentrations of CH_4 in the intake and exhaust air ducts were monitored using a CH_4 analyzer (model Ultramat 5E; Siemens Inc., Karlsruhe, Germany). The CH_4 analyzer was calibrated daily using primary standard CH_4 and N_2 as reference gases. The difference between the incoming and outgoing mass of CH_4 was used to calculate the amount of each gas generated in each chamber. Emissions of CH_4 per kg of DMI were then calculated by dividing this value by the total DMI for the 3 lambs in the corresponding chamber.

Diet Digestibility

Apparent total tract digestibility was determined by collecting the total output of faeces and urine from each lamb over 4 d, whilst the lambs were housed in metabolism crates within chambers. Each lamb was fitted with a harness allowing faeces to be collected into a plastic bag. Urine was collected in jugs below the slatted floor of the metabolism crate and preserved by

acidification with 4 N H_2SO_4 (targeted final pH < 2). Total output of faeces and urine were collected every 24 h, with each thoroughly mixed upon collection. An aliquot of the daily urine was diluted with distilled water at a ratio of 1:5 and stored at -20ºC until analyzed for total nitrogen. A sub-sample of faeces was oven-dried (55ºC) to a constant weight and a representative composite sample was obtained by pooling the dried daily faeces based on their respective DM content. The apparent digestibility of nutrients were computed from nutrient concentration in the faeces and the nutrient intake (Digestibility, % = (1 - (nutrient in faeces/ nutrient intake)) *100). Nitrogen retention was calculated as the difference between total N intake and total N excretion (Urinary N + Fecal N).

Chemical Analysis

Dry matter of the pelleted diets and faeces was determined by drying for 48 h at 55ºC in a forced air oven, with samples subsequently ground through a 1-mm screen (standard model 4 Wiley mill; Arthur H. Thomas, Philadelphia, PA) for chemical analysis. Analytical DM content of the substrates was determined by drying at 135°C for 2 h (method 930.15; Association of Official Analytical Chemists 2005), followed by hot weighing. The OM content was calculated as the difference between 100 and ash content (method 942.05; AOAC 2005). The NDF and ADF contents were determined according to Van Soest et al. (1991) with heat-stable amylase and sodium sulfite used in the NDF procedure. The crude fat contents were determined using ether extraction (method 2003.05, Association of Official Analytical Chemists 2006); Extraction Unit E-816 HE, BŰCHI Labortechnik AG, Flawil, Switzerland). The 1-mm ground substrates (dried pelleted diets and faeces) were reground using a ball grinder (Mixer Mill MM2000; Retsch, Haan, Germany) for determination of N content. For urinary N, 150 µL of diluted acidified urine was oven dried for 24 h. Nitrogen in pelleted diets, faeces, and urine was measured by flash

combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instrumentals, Milan, Italy) and CP calculated as N × 6.25. Gross energy in pelleted diets and fecal samples was determined using a Parr Adiabatic calorimeter (Parr Instrument Company, Moline, IL).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS software, version 9.1. (SAS Inst. Inc., Cary, NC, USA). The full linear model for intake, digestibility, and CH_4 included the fixed effects of dietary treatment (modeled as the factorial effects of fibre digestibility, added fat, fibre digestibility *x* added fat), and the random effects of period (n=4) and chamber. Lamb nested within treatment was considered a random effect with day of sampling within each period treated as a repeated measure. The individual animal (n = 12) was the experimental unit for intake and nutrient digestibility because these data were obtained from individual lambs with separate access to feed. For CH_4 emissions, the model did not include the random effect of lamb as the respiration chamber, (n = 4) representing data for 3 lambs was the experimental unit. Data are presented as least square means (± SEM), with differences declared significant at $P < 0.05$ using the PDIFF statement.

Results

Feed Intake and Apparent Total Tract Digestibility

Increasing the dietary fat content did not affect intake of DM ($P = 0.82$) and its constituents (Table 2). On average, intake of DM was lower ($P < 0.01$) with HFbD as compared to LFbD diets (2.14 *vs*. 2.56 $kg.d^{-1}$). Intake of NDF (0.64 *vs.* 0.84 $kg.d^{-1}$), ADF (0.43 *vs.* 0.51 $kg.d^{-1}$) and starch were lower ($P < 0.01$) for HFbD compared to LFbD diets. A significant fat content *x* fibre

digestibility interaction (P = 0.04) was observed for fat intake with it being higher for diet with high fat content and LFbD than for HFbD diets.

Apparent total tract digestibility of the DM, OM, CP, NDF, ADF, and GE constituents was not affected by dietary fat content (P = 0.13); however the high fat diet showed higher digestibility of starch (P = 0.01) whereas HFbD decreased the digestibility of starch (P < 0.01). However, in all cases starch digestibility exceeded 98%. The HFbD diet also exhibited increased (P < 0.01) digestibility of DM and its constituents, excepting CP. A significant fat content *x* fibre digestibility interaction (P = 0.01) was observed for fat digestibility with it being lower for diets with low fat content and for HFbD than LFbD diets.

Enteric CH_4 Emissions

A significant fibre digestibility by fat content interaction was observed for CH_4 emissions per kg of DMI (P = 0.03), OMI (P = 0.03), NDFI (P < 0.01), and %GEI (P = 0.03) with fat increasing emissions with the HFbD and decreasing it in the LFbD diets (Table 3). Differing fibre digestibility or fat content did not change the amount of CH_4 emitted per kg of DM or OM digested, but emissions per kg NDF digested were higher (P < 0.01) for LFbD than HFbD diets.

Nitrogen Excretion and Retention

Total fecal (kg DM.d^{-1}) output was not affected by dietary fat content, but less urine was excreted (3.1 *vs*. 2.8 L.d^{-1}; P = 0.05) in lambs fed high fat diets (Table 4). Fecal output was decreased for HFbD compared with LFbD (0.58 *vs.* 1.00 kg DM.d^{-1}; P < 0.01), with no effect on urine production.

Nitrogen excretion (urinary or fecal) was not affected by fat content of the diet (P = 0.66), but total N excretion was higher (P < 0.01) for the LFbD *vs*. HFbD diets (68.5 *vs* 56.4 g.d^{-1},

respectively). Lambs offered HFbD diets exhibited lower excretion of both urinary N ($P < 0.01$) and fecal N ($P = 0.02$) as compared to those offered LFbD diets. Neither fat content or fibre digestibility had an impact on urinary or fecal N excretion as a percentage of total N excreted. A significant fat content by fibre digestibility interaction was observed ($P = 0.01$) for N retention where high fat increased N retention with HFbD, but reduced it with the LFbD diet.

Discussion

Diets and Feed Composition

Canola is widely grown in Canada and the meal remaining after oil extraction is routinely used as a feed for livestock. To achieve the range in fat content with levels of canola meal in the diets used in the current study, solvent extracted canola meal, low in residual oil, was used for the low fat diets. For the high fat diets, pressed canola meal was utilized as it contains considerably more residual oil than solvent extracted canola meal (Hristov et al. 2011a). This approach resulted in a 3-fold difference in fat content of our low and high fat diets (22 *vs.* 60 $g.kg^{-1}$ DM, respectively).

Although the effect of dietary fat content on enteric CH_4 emissions has been a focal point of many studies (Grainger and Beauchemin (2011), the interaction between dietary fat and fibre digestibility as it pertains to CH_4 emissions is not well documented. Thus, to provide diets of contrasting nutritive value, oat hulls and beet pulp were chosen as the basal dietary ingredients. As oat hulls contain a higher proportion of NDF than beet pulp (494 *vs.* 378 $g.kg^{-1}$ DM, respectively), the amount of other ingredients were altered to obtain similar NDF contents across diets.

Feed Intake and Apparent Diet Digestibility

In this study, improving the nutritive quality of the offered diets reduced DMI, which is consistent with previous reports for ruminants (Decruyenaere et al. 2009; Hristov et al. 2005a). Feed related factors affecting DMI such as DM content and particle size were thought to play a minor role in the observed responses given that the DM content of the feeds was similar and all feeds were pelleted. Pelleting reduces the influence of particle size and its subsequent effects on rumen fill, passage rates and DMI, making it more likely that the nutrient content of the diets accounted for the observed differences in intake. Yearsley et al. (2001) suggested that on a 'requirement theory' basis, ruminants eat to maximise production potential under several constraints including diet quality. As a consequence, ruminants offered highly fibrous, poorly digestible feeds consume a larger amount of feed to meet their requirements (Jung and Allen 1995), a response consistent with our study. Sheep fed the oat hull-based diets exhibited, on average a 16% higher DMI than those fed beet-pulp based diets. However, it is recognised that although the fibre content of beet pulp is high, this fibre is less lignified and more digestible than the fibre in oat hulls (Allen 2000). Consequently, apparent diet nutrient digestibility (except CP) was higher for the beet pulp-based diet (HFbD diet) than the oat hull-based diets (LFbD diet). Others have observed similar differences in digestibility in sheep offered beet pulp vs cottonseed hull diets (Torrent et al. 1994).

In contrast to improving diet quality, increasing the fat content of the diet did not affect total tract DM, OM, CP or ADF digestion. It has been suggested that high fat diets can depress fibre digestion, but this suppression depends on many factors including the unsaturated to saturated fatty acid ratio, rate of oil release and the composition of the basal diet (Marín et al. 2010). In most cases, negative effects of added fat on total tract digestibility do not occur until fat content in the diet exceeds 60 to 70 $g.kg^{-1}$ DM (Grainger and Beauchemin 2011). The highest

level fed in the current study was 61 g.kg^{-1} DM and with the LFbD, NDF digestibility was actually numerically higher with the high fat diet. Others have also reported tendencies for increased total tract fibre digestion when fish oil (Doreau and Chilliard 1997) was included in ruminant diets. Although the mechanisms responsible for this response are uncertain, it is possible that an increase in hind gut digestion of fibre may have occurred as a result of fat supplementation of the LFC diet. Sutton et al. (1983) showed that supplementation with fat increased post-ruminal digestion and compensated for the depression of ruminal digestion in wethers. With HFbD diets, NDF digestibility was unaffected by fat content, in agreement with Loor et al. (2002) where including canola oil (3.3% of DM) in an alfalfa/corn-based diet did not alter fibre digestibility in Holstein cows.

Enteric CH_4 Emissions

Methane emissions per kg of DMI tended to be at the lower range of values (15.9 to 30.4 g.kg^{-1} DMI) reported for lambs of comparable age and body type fed forage diets (Clark et al. 2003; Molano and Clark 2008; Waghorn et al. 2002). The lower emissions may be partially explained by differences in the form in which the diets were offered. It has been reported that grinding or pelleting of forages diets can reduce enteric CH_4 emissions by 20 to 40% at high intakes (Johnson and Johnson 1995). The reduction in CH_4 emissions in pelleted diets may be explained by the higher rate of feed passage, subsequently reducing the duration of exposure of the feed to ruminal digestion.

When CH_4 was expressed relative to NDF intake, emissions were higher for the HFbD than the LFbD diets. Highly digestible fibre diets result in increased acetic acid formation as compared to propionic acid. The formation of acetic acid results in the release of H^+, which may then be utilized by methanogenic bacteria to produce CH_4. Conversely, propionic acid involves the net

utilization of protons and competes with methanogenesis for H^+ (Hegarty 1999). Additionally, the increase in CH_4 emissions as a proportion of GEI for the higher quality diets was also in agreement with Blaxter and Clapperton (1965), when diets of increasing digestibility were offered at maintenance. The differences in CH_4 production ($g.kg^{-1}$ DMI) for the low and high quality diets would likely have been greater had DMI been similar for both groups, as CH_4 losses adjusted for DMI decrease with increasing intake (Johnson and Johnson 1995).

Addition of high fat to the HFbD diet tended to increase CH_4 emissions per kg of DMI, OMI, and NDFI, whereas addition of this same level of fat to the LFbD diet did not alter these parameters. Alterations in CH_4 emissions as a result of fat supplementation may depend on the quality of the forage in the diet. Cosgrove et al. (2008) offered sheep a pyrennial ryegrass with intraruminal infusion of a blend of linseed and sunflower oils (3:1), and also failed to measure a reduction in CH_4 emissions per unit of DMI. In a comprehensive analysis of the effects of lipid supplementation of ruminant diets on CH_4 emissions, Grainger and Beauchemin (2011) reported that fat supplementation persistently reduced CH_4 emissions in ruminants both on a DMI and GEI basis. However, the extent to which CH_4 emissions are reduced by fat supplementation depends on several factors including fat source, fatty acid profile, type of basal diet, and form in which the fat is administered (Beauchemin et al. 2008). One of the principal modes of action by which fat reduces CH_4 emissions is through depressing fibre digestion in the rumen (Mathison 1997). However, in the current study, the addition of fat did not exert negative effects on the total tract digestion of fibre. Emissions of CH_4 are also affected by rumen pH and although fat supplementation may be expected to reduce pH as a result of suppressing ruminal protozoa populations, a number of studies in sheep have reported increases in pH following supplementation with sunflower seeds and oil (Ivan et al. 2003; Ivan et al. 2004).

When expressed relative to kg of DM or OM digested, neither fibre digestibility nor fat content exerted any effects on CH_4 emissions. However, on a NDF digested basis, emissions were reduced with increasing fibre digestibility. Thus, it may be concluded that on per unit of fibre digested basis, improving forage quality may serve as an efficient means of mitigating CH_4 emissions in sheep per unit of livestock product.

Starch levels were substantially higher in the LFbD than the HFbD diets, even though similar levels of barley grain was present in all diets. We speculate that this may be due to the presence of residual starch in the oat hulls that were used to formulate the LFbD diet. As a result the starch intake of the lambs fed the LFbD diets was more than twice that of those fed the HFbD diets. Likely due to pelleting, starch digestibility was extremely high across all diets. Methane production can be decreased by shifting hydrogen flow to alternative electron acceptors like propionate. Inclusion of more dietary starch can reduce CH_4 emissions as it increases propionate production (Hatew et al. 2015), possibly accounting for the lower CH_4 emissions as %GEI for the LFbD vs the HFbD diets.

Although sheep performance was not measured in this study, improvements in diet quality would be expected to improve liveweight and carcass gains (Pearson and Ison 1997). Increased animal productivity, through increased energy density is a projected outcome of fat supplementation. Ivan et al. (2004) offered forage and concentrate diets supplemented with sunflower seeds to sheep, and observed contrasting results with regard to animal performance. Liveweight gains were reduced when sunflower seed was fed with forages, but improvements in liveweight gains and feed conversion efficiency occurred when it was included in a concentrate diet. Such improvements in performance may have significant implications for CH_4 emissions efficiency, defined as the amount of CH_4 emitted per unit of animal product. , as the emissions per unit of animal product are reduced.

Nitrogen Excretion

In addition to CH_4, excretion of N in faeces and urine of ruminants is a significant environmental concern leading to pollutants including ammonia, N_2O, N oxides, and nitrate. Thus, reduced protein feeding and improved efficiency of utilization is necessary to limit soil, ground water and air contamination. Improving the quality of the diet through the replacement of oat hulls with beet pulp reduced gross N excretion, mainly as a result of the lower DMI of this higher quality diet. It has been reported (Hristov et al. 2005b) that the provision of a highly fermentable diet can result in a shift in N from urine to faeces. From an environmental point of view this is desirable, as urinary N excretion is considered to have a greater negative environmental effect than fecal N. Fecal N is less volatile than urinary N as it bound chemically within proteins and other compounds and thus is less readily released into the atmosphere (Hristov et al. 2011b). In the current study, no reduction in the proportion of N lost as urine was observed between low and high quality diets, likely due to a lack of difference in total tract digestibility of CP. Although diets had similar CP digestibilities (67.2-70.0%), N-retention was higher for the high fat – HFbD diet. This suggest that despite having similar CP digestibilities, the increased availability of digestible energy with the high fat – HFbD diet enabled the available N to be used more efficiently for growth.

Increasing the fat content of the diet did not affect total N excretion or its portioning between urine and faeces. This is in agreement with Machmüller and Kreuzer (1999), where coconut oil was added to supply 7% of dietary DM. Again, this result may be surprising as it is reported that fat may have inhibit rumen protozoa. Rumen protozoa are associated with increased recycling of microbial N in the rumen (Jouany 1996) and reduced AA supply to the intestine (Veira et al. 1984). These effects contribute to the inefficient utilization of N, and consequently

the elimination of protozoa from the rumen can lead to improvements in N metabolism (Ivan et al. 2004). However, as evidenced by the absence of a reduction in fibre digestion and CH_4 emissions, it is hypothesized that protozoal numbers were not notably reduced with fat supplementation in the current study.

Conclusion

In conclusion, when expressed relative to intake, improving diet quality increased CH_4 emissions. However, consideration of feed form and the effects of processing would need to be considered to extrapolate these study results to grazed or unprocessed forages. Improvements in diet quality may also be expected to improve animal performance, thus increased diet quality is an effective means of reducing emissions per unit of animal product. The impact of increasing dietary fat on CH_4 emissions kg^{-1} of DMI, may depend on the fibre digestibility of the diet with it potentially increasing emission with high NDF digestibility diets. Consequently, the well documented ability of fat supplementation to reduce CH_4 emissions may not be as readily applicable to forage diets as compared to high concentrate diets.

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Table 1. Ingredient and chemical composition of pelleted diets varying in fibre digestibility and fat content

	Low Fibre Digestibility		High Fibre Digestibility	
Item	Low Fat	High Fat	Low Fat	High Fat
Ingredient composition, $g.kg^{-1}$ DM				
Beet pulp (dehydrated)	150	150	500	500
Alfalfa meal (dehydrated)	50	50	50	50
Oat hulls	400	400	50	50
Canola oil	---	24.9	8.5	31.0
Canola meal (cold-pressed[1])	---	170	---	196
Canola meal (solvent extracted[2])	150	---	175	---
Barley grain (dry-rolled)	165	120	136	93
Molasses	50	50	50	50
Mineral & vitamin mix[3]	35.0	35.2	30.5	30.2
Chemical composition (mean ± SD), $g.kg^{-1}$ DM (unless otherwise stated)				
DM, $g.kg^{-1}$	950 ± 4.7	952 ± 4.0	951 ± 3.5	953 ± 2.6
OM, $g.kg^{-1}$	918 ± 2.9	920 ± 2.7	914 ± 3.4	914 ± 3.2
Ash	78 ± 2.8	76 ± 2.5	82 ± 3.2	82 ± 3.0
Crude fat (ether extract)	21 ± 2.4	61 ± 7.3	22 ± 2.4	58 ± 2.6
NDF	344 ± 8.9	341 ± 8.6	315 ± 5.2	308 ± 7.0
ADF	213 ± 7.4	206 ± 7.8	210 ± 6.1	204 ± 5.9
Crude protein	170 ± 6.5	168 ± 6.1	171 ± 4.6	168 ± 5.8
Starch	209 ± 15.4	204 ± 4.6	111 ± 13.7	92 ± 9.0
GE, $MJ.kg^{-1}$ DM	18.0 ± 0.15	18.9 ± 0.18	17.8 ± 0.14	18.5 ± 0.14

[a]Cold-pressed canola meal; fat content = 114 $g.kg^{-1}$ DM.
[b]Solvent extracted canola meal; fat content = 23.8 $g.kg^{-1}$DM.
[c]Containing 92.6% NaCl; 4.97% Dynamate; 0.9% $ZnSO_4$; 0.83% $MnSO_4$; 0.13% $CuSO_4$; 0.1% ethylenediamine dihydroiodide, 80% preparation; 0.005%; $CoSO_4$; 0.4% canola oil (as carrier of $CoSO_4$); and 0.0014% Na_2SeO_3. vitamin A (10 000 000 IU/kg); vitamin D (1 000 000 IU/kg); and vitamin E (10 000 IU/kg). No ionophores were included in the diet.

Table 2. Feed intake and apparent total tract digestibility in ram lambs offered pelleted diets differing in fibre digestibility and fat content[a]

	Low Fibre Digestibility		High Fibre Digestibility			*P* value		
Item	Low Fat	High Fat	Low Fat	High Fat	SEM[b]	FbD[c]	Fat	FbD × Fat
Intake, kg DM.d^{-1}								
DM, kg.d^{-1}	2.60	2.52	2.08	2.19	0.11	< 0.01	0.82	0.19
OM	2.27	2.20	1.86	1.91	0.09	<0.01	0.98	0.26
CP	0.42	0.40	0.35	0.35	0.02	<0.01	0.61	0.51
NDF	0.85	0.82	0.63	0.64	0.03	<0.01	0.76	0.31
ADF	0.53	0.49	0.42	0.43	0.25	<0.01	0.24	0.06
Starch	0.51	0.48	0.22	0.19	0.01	<0.01	0.16	0.96
Fat, g.d^{-1}	5.24*c*	14.09*a*	4.86*c*	11.99*b*	1.00	0.01	<0.01	0.04
GE, Mcal.d^{-1}	10.64	10.81	8.66	9.24	0.53	<0.01	0.12	0.34
Digestibility, %								
DM	60.6	60.6	72.7	73.1	1.15	< 0.01	0.82	0.80
OM	62.6	62.9	75.8	76.4	1.09	< 0.01	0.61	0.79
CP	70.0	67.5	67.2	69.5	1.53	0.77	0.94	0.11
NDF	22.5	27.0	55.9	57. 6	1.79	< 0.01	0.13	0.44
ADF	21.0	17.5	47.8	48.9	2.07	< 0.01	0.53	0.24
Starch	99.4	100.0	98.7	99.1	0.02	< 0.01	0.01	0.55
Fat	78.1*b*	87.8*a*	72.0*c*	87.9*a*	1.21	0.01	< 0.01	0.01
GE	59.9	60.4	72.2	73.3	1.22	< 0.01	0.39	0.73

NOTE: Within a row, means without a common superscript differ ($P < 0.05$).

[a]Low fibre digestibility; 400 g oat hulls.kg^{-1} diet DM, High fibre digestibility; 500 g beet pulp.kg^{-1} diet DM. Low fat = 21.5 g.kg^{-1} DM, High fat = 60.0 g.kg^{-1} DM.

[b]Standart error of mean presented as an average within variables.

[c]FbD = fibre digestibility.

Table 3. Methane (CH_4) emissions from ram lambs offered pelleted diets differing in fibre digestibility and fat content[a]

	Low Fibre Digestibility		High Fibre Digestibility			*P* value		
Item	Low Fat	High Fat	Low Fat	High Fat	SEM[b]	FbD[c]	Fat	FbD × Fat
CH_4, g.kg^{-1} DMI	12.1*c*	11.9*c*	14.4*b*	15.7*a*	0.66	< 0.01	0.09	0.03
CH_4, g.kg^{-1} OMI	13.2*c*	12.9*c*	15.8*b*	17.2*a*	0.74	< 0.01	0.09	0.03
CH_4, g.kg^{-1} NDFI	35.5*c*	34.8*c*	45.7*b*	50.9*a*	2.07	< 0.01	<0.01	< 0.01
CH_4, % GEI	2.8*b*	2.6*b*	3.4*a*	3.6*a*	0.16	< 0.01	0.99	0.03
CH_4, g.kg^{-1} DM digested	19.9	20.0	19.8	21.5	0.91	0.27	0.15	0.17
CH_4, g.kg^{-1} OM digested	21.1	21.0	20.8	22.5	0.94	0.32	0.20	0.17
CH_4, g.kg^{-1} NDF digested	167.2	134.7	81.2	88.9	10.59	< 0.01	0.29	0.11

NOTE: Within a row, means without a common superscript differ ($P < 0.05$).

[a]Low fibre digestibility; 400 g oat hulls.kg^{-1} diet DM, High fibre digestibility; 500 g beet pulp.kg^{-1} diet DM. Low fat = 21.5 g.kg^{-1} DM, High fat = 60.0 g.kg^{-1} DM.

[b]Standart error of mean presented as an average within variables.

[c]FbD = fibre digestibility.

Table 4. Nitrogen excretion and retention in ram lambs offered pelleted diets differing in fibre digestibility and fat content[a]

	Low Fibre Digestibility		High Fibre Digestibility			*P* value		
Item	Low Fat	High Fat	Low Fat	High Fat	SEM[b]	FbD[c]	Fat	FbD × Fat
Total outputs								
Faeces, kg DM.d^{-1}	1.03	0.97	0.57	0.59	0.04	< 0.01	0.52	0.29
Urine, L.d^{-1}	3.05	2.88	3.10	2.70	0.35	0.14	0.05	0.72
Excretion of N								
Total N, g.d^{-1}	68.0	69.0	58.3	54.4	3.13	< 0.01	0.66	0.38
Urinary N, g.d^{-1}	47.9	48.1	39.9	37.3	2.11	< 0.01	0.54	0.47
Urinary N, % of total excretion	70.2	69.9	68.1	68.1	1.56	0.10	0.87	0.88
Fecal N, g.d^{-1}	20.1	21.0	18.5	17.0	1.61	0.02	0.92	0.42
Fecal N, % of total excretion	29.8	30.1	31.9	31.9	1.37	0.10	0.87	0.88
Retained N, g.d^{-1}	-1.25*ab*	-4.40*a*	-2.27*ab*	1.70*b*	1.69	0.03	0.93	0.01

NOTE: Within a row, means without a common superscript differ ($P < 0.05$).

[a]Low fibre digestibility; 400 g oat hulls.kg^{-1} diet DM, High fibre digestibility; 500 g beet pulp.kg^{-1} diet DM. Low fat = 21.5 g.kg^{-1} DM, High fat = 60.0 g.kg^{-1} DM.

[b]Standart error of mean presented as an average within variables.

[c]FbD = fibre digestibility.