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QUOATS – Harnessing new technologies for sustainable oat production and utilisation

Developing oats for sustainable livestock agriculture

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AHDB Agriculture & Horticulture DEVELOPMENT BOARD

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Introduction

Livestock production is a significant source of UK greenhouse gas emissions (GHG) including methane (CH_4) and nitrous oxide (N_2O) and of ammonia (NH_3). To put in context, around 18% of UK GHG emissions are related to food production and consumption and livestock production is a significant source of these. In 2005, over 35% of the UK emissions of methane came from agriculture, with 749.5 kt (approximately 80%), from enteric sources (mainly from ruminants) (Climate Change, the UK programme 2006) and 119.5 kt from waste (mainly manures and slurries) (Defra report AC0206). Poultry producers are also faced with meeting environmental legislation. A major concern is to comply with IPPC requirements for poultry emissions (ammonia) which are strongly correlated with the amount of nitrogen excreted by the birds, which is dependent upon how closely the amino acids in the protein of the diet fit the bird's requirements.

The amino acid profile and high oil content make oats a valuable livestock feed, with high metabolizable energy (Cuddeford, 1995). High oil naked oats (with up to 16% oil) have been developed and molecular markers associated with oil content. There is good evidence that high oil content can reduce methane emissions from ruminants. Preliminary studies at IBERS, using an *in vitro* system, showed that high oil oats decrease methane production by 35.4% compared to wheat without reducing digestibility (Cowan et al., 2008). Although oil can be added as a supplement in the feed ration, a strategy that included high oil oats within a feed ration provides a realistic and practical approach to reducing methane emissions. As oats grow well in the west and fit well into grassland rotations more oats could be grown "on-farm" with the added benefit of reducing the CO_2 emissions associated with transporting grain and providing a sustainable solution to reducing GHG.

While naked oats are more suited to poultry, ruminants can use fibre as an energy source. Therefore husked oats are more appropriate for feeding to ruminants. A key factor influencing the feeding value of oats for ruminants is the digestibility of the husk and in particular the lignin content. Development of a high oil low lignin husked oat will have a quantifiable benefit to the UK. Based on 2005 UK data, and wheat comprising 25% of the diet, high oil/low lignin oats could reduce UK methane emissions from enteric fermentation from 749.5 kt to 702.4 kt, roughly 6%. It will also remove the limitations derived from the lower yield of naked oats. Although qualitative tests provide a relatively quick indication of 'high-lignin' or of 'low-lignin', quantitative phenotypic evaluation of lignin is difficult which makes this trait an excellent candidate for molecular marker based breeding. At present commercial oat varieties with combinations of high oil and low lignin are not available.

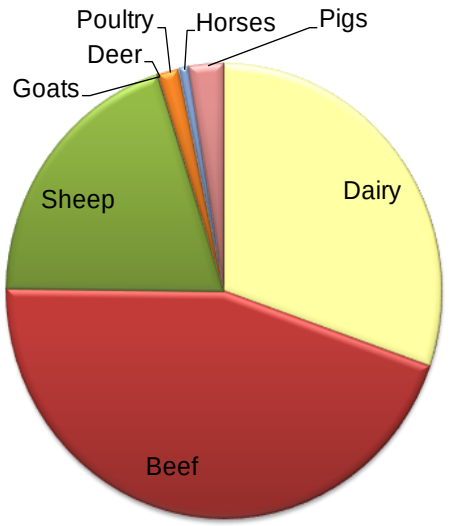

Figure 1 Proportions of methane emissions from UK livestock (2008 data)

Ruminant animals are major source of methane in UK agriculture (Fig 1), losing an average of about 6% of their gross energy intake as methane. It is well known that dietary

manipulation can modify the output of methane from the gut and from manures produced by livestock, one method of which is to increase the fat content of the animals' diet (Fig 2).

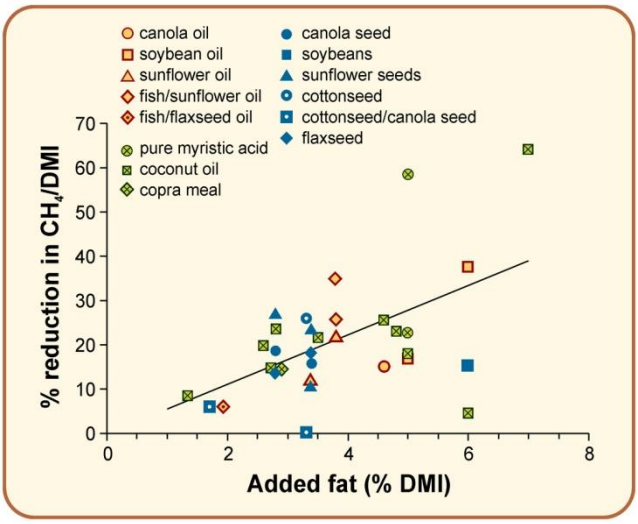

Figure 2 Effect of added fat in ruminant diets on reduction of methane emissions

Workplan

3.1 To test that markers for low lignin and high oil can be developed which can be use together in the precision breeding of HiQ oats,

3.2 To test that the HiQ oats will significantly reduce emissions, *in vitro* analyses will be carried out using gas production techniques.

3.3 To test that there are no physiological or bioenergetic barriers to combining high oil and low lignin husks in feed rations and that simple effects demonstrated *in vitro* are also manifested *in vivo*,

3.4 To test the utility of HiQ oats as straights and compound feed ingredients (and to ensure no major disadvantages that would prevent major uptake as a feed ingredient)

3.5 To test that incorporation of oats into feed rations will reduce the ecological footprint of livestock systems

Methods

Fatty acid analyses of oat grains

Fatty acid concentrations of oat samples were determined from approximately 1 g of freeze-dried material using heneicosanoic acid methyl ester (C21:0) as an internal standard (Sigma-Aldrich Co, St Louis, MO) and a one-step extraction-transesterification procedure (Sukhija and Palmquist, 1988). Fatty acid methyl esters (FAME) were separated and quantified using a gas chromatograph (CP-3800; Varian Inc., Walnut Creek, CA) equipped with a flame ionization detector, automatic injector, split injection port and a 100-m fused silica capillary column (i.d., 0.25 mm) coated with 0.2-µm film of cyanopropyl polysiloxane (CP-Sil 88; Varian Inc) using hydrogen as the fuel and helium as the carrier gas. The total FAME profile in a 1-µL sample at a split ratio of 1:30 was determined using a temperature gradient programme described by Lee et al. (2005). Peaks were identified by comparison of retention times with authentic FAME standards (ME61; Larodan fine chemicals, Malmo, Sweden; S37; Supelco, Poole, Dorset, UK).

Fatty acid data were presented as relative proportions, i.e. as a proportion of total fatty acid (FA) in the sample. All data were analysed by principal components analysis. The oat varieties were grouped into the following categories: winter naked (WN), winter low-lignin husked (WH) and spring low-lignin husked (SH), for subsequent analysis by analysis of variance.

Gas production from a range of oat lines

The gas production technique was carried out using a semi-automated method as outlined by Theodorou et al. (1994) and Davies et al. (2000). Briefly, in triplicate, approximately 1 g of sample material was incubated at 39°C in bottles with rumen fluid, and the volume and composition of gas produced over the course of 3 to 4 days was recorded (Figure 3).Gas production was measured for each of the oat lines in triplicate. The chemical composition of the oat lines used was analysed using standard laboratory methods. The relationships between methane production (both in terms of ml/g DM ml/g apparently digested DM) and a) chemical components, and b) fatty acid composition, were explored.

Figure 3 Gas analysis equipment and two incubation bottles for the gas production technique

Use of the Rumen Simulation Technique (Rusitec) to investigate promising oat lines and compare with barley.

In brief, feed samples were incubated in small fermenters primed with rumen fluid, and these were maintained for approximately 10 days. Feeds were incubated for 48 h in nylon bags, after which they were replaced with fresh feeds. Two sets of bags allowed fresh feed to be introduced to each fermenter every day by swapping out one set each day. Fermentation gases were collected and analysed, and effluent, produced as a result of the infusion of artificial saliva, was analysed for microbial protein content. Characterisation of feed sample residues after incubation allowed feed degradability to be estimated.

Experimental treatments were tested were:

1. Rolled barley
2. High oil/low lignin composite oat, cv. Racoon and SO-I in a 4:1 ratio
3. Gerald
4. Breeding line 05-46Cn14
5. Breeding line 05-44Cn18

The chemical composition of the 5 treatment feeds is provided in Table 1.

Table 1 Chemical composition of the cereals investigated in the Rusitec experiment. Values in % DM unless otherwise specified

Treatment	1	2	3	4	5
	Rolled Barley	Racoon/SO-I (4:1)	05-46Cn14	05-44Cn18	Gerald
Crude protein	10.8	8.6	11.9	10.9	11.1
Water soluble carbohydrates	3.1	3.1	3.1	2.96	2.6
Ash	2.1	2.5	2.5	2.4	2.2
Neutral detergent fibre	27.6	31.4	30.3	31.1	27.6
Acid detergent fibre	6.2	16.4	15.0	16.8	16.3
Neutral cellulase/gamanse digestibility	88.7	78.7	74.8	76.0	71.1
Starch	60.3	42.3	42.4	43.9	43.3
Total Oil	2.9	9.7	10.8	8.5	7.1
Acid detergent lignin	1.3	1.6	3.8	3.6	3.5
Metabolisable energy (MJ/kg DM)	13.1	13.5	13.2	12.8	11.7

To replicate potential animal diets, the oats were incubated as part of a diet based on grass silage. Approximately 50% of the diet DM was from a standard grass silage that was the same throughout the experiment on all treatments, and the remaining 50% diet DM comprised chopped grains (barley or oats). All feeds were incubated as fresh material (i.e. not freeze dried).

The Rusitec experiment was run for 10 days total, with 5 days for adaptation and 5 days for measurements. Two Rusitec systems were used, each with 8 incubation vessels, with 3 replicates of each treatment distributed across the two machines. Fermentation gases were collected quantitatively and analysed for methane concentrations. On the final day of the experiment, microbial protein production was estimated by labelling microbial proteins with ^{15}N.

Data collected were analysed statistically using analysis of variance.

Methane production from a range of oat varieties consumed by mature ewes

Eight mature ewes, four each of two breeds (Welsh Mountain and Welsh Mule) were fed diets comprising ryegrass silage and oats in a 1:1 ratio (on a dry matter (DM) basis) in a Latin square changeover design experiment. Feed was offered at rates designed to supply metabolisable energy requirements for maintenance (according to AFRC 1992 guidelines), i.e. at restricted rates.

<u>Diets offered</u>

The same grass silage was used throughout the experiment, and was fed with one of four oats:

A. Husked oat, cv Balado
B. Naked oat, cv Racoon
C. New breeding line oat, 14355Cn
D. 50:50 (fresh) mixture of new breeding line oat (14355Cn) and Racoon

The pre-experimental determined chemical composition of the three oats (A to C) and the mixture (D) is given in Table 2.

Table 2 Chemical composition of the three oats and calculated composition of a 1:1 mix of 14355Cn:Racoon to be fed to mature ewes (values in % of DM)

Oat	CP	OM	NDF	ADF	Oil	Total Oil	ADL
Balado	10.5	97.9	23.1	12.2	4.5	5.6	2.2
Racoon	10.5	98.2	6.1	2.4	8.5	10.2	1.1
14355Cn	10.9	97.7	19.7	9.5	3.7	4.9	1.1
1:1 mix	10.7	97.9	12.9	6.0	6.1	7.5	1.1

All experimental animals were drenched with anthelmintic prior to the experiment commencing.

The experiment consisted of four three week periods; the first two weeks of each period was used for diet adaptation, and the final six days were used for feed intake, diet DM digestibility and methane emission measurements. Diets were fed according to the schedule listed in Table 3.

Table 3 Experimental design used in the experiment

Breed	Welsh Mountain				Welsh Mule			
Chamber group	1		2		2		1	
Chamber	1	2	3	4	2	1	4	3
Sheep	1	2	3	4	5	6	7	8
Period 1	A	B	D	C	A	B	D	C
Period 2	B	C	A	D	B	C	A	D
Period 3	C	D	B	A	C	D	B	A
Period 4	D	A	C	B	D	A	C	B

The sheep were weighed two times per week at the same time of day, and feed was allocated to individual animals according to maintenance requirements.

Feeds was allocated according to AFRC (1992) energy requirement recommendations for mature barren ewes, assuming a gross energy density of 18.8 MJ/kg DM for both silage and oats, and ME densities of 11.75 MJ/kg oat DM and 10.5 MJ/kg silage DM. It is recognised that the energy densities of husked and naked oats differ, but for the purposes of this experiment it was assumed that their chemical compositions are similar.

Sheep were penned individually within the sheep unit throughout the experiment. Feeds were offered in two equal meals (at approximately 09:00 and 16:00, with feeding times recorded each day) with silage and oats being fed in separate feeders.

During the adaptation period any silage and/or oat grain refusals were weighed and the fresh weights were recorded.

At the end of each experimental period, sheep were individually housed in methane chambers and methane emissions were measured for 3 days. Feed intakes, and pre- and post-chamber measurement live weights were recorded. Faeces produced was collected, dried, and weighed, for determination of apparent whole tract digestibility of feed DM.

Data were analysed using analysis of variance, investigating the effect of dietary treatment, sheep breed, and the interaction.

Dairy cow experiment

A dairy cow experiment was carried out March to May 2014, using 9 lactating dairy cows in a 3 x 3 Latin square changeover design experiment with three 5-week periods.

Each experimental period consisted of 3 weeks for diet adaptation (including 1 week for diet change), 1 week for whole body N partitioning measurements, and 1 week for methane measurements. Feed intake and milk yields were recorded throughout, and samples for standard milk composition (fat, protein and lactose) and milk fatty acid analysis were taken at the end of each period.

Dietary treatments consisted of *ad libitum* access to ryegrass silage plus 1 of 3 concentrate treatments:

- A – including 40% wheat grain, rolled (control)
- B – including 40% oats to replace wheat (nothing else changed)
- C – wheat replaced with oats, AND other ingredients changed to give same composition as A

The concentrates were fed at a 12 kg (fresh) per cow per day split into 3 feeds, 4 kg at each milking (twice daily) and 4 kg at around midday. The oats fed as part of the concentrate portion of the diet comprised naked oat grains and oat husk (from oat cv mixed in the ratio of 3:1. For each of the treatments, 5 kg of cereal fresh matter was offered mixed with 7 kg of concentrate premix; therefore 5 kg of wheat was fed as part of diet A, and 3.75 kg oat grains plus 1.25 kg of oat husk were fed as parts of diets B and C. The cereal grains were rolled before feeding, to crack the seed coat.

The diets were formulated by Mole Valley Feed Solutions assuming a 650 kg cow yielding 40 kg of standard milk per day (Table 4).

Table 4 Diet formulations

FEEDS		A - Wheat	B - Oat 1	C - Oat 2
G. Silage Aber. 1 est.	(kg/d)	40	40	40
Wheat-rolled	(kg/d)	5	0	0
Oats Aber. 30114	(kg/d)	0	5	5
Premix Oat 2	(kg/d)	0	0	7
Premix Oat 1	(kg/d)	7	7	0
NUTRIENTS				
Fresh Weight	(kg/d)	52	52	52
Dry matter	(%)	41.9	41.7	41.6
DMI	(kg)	21.8	21.7	21.6
Forage DMI	(kg)	11.2	11.2	11.2
ME Ruminants	(MJ)	259.6	252.6	255.3
ME Adequacy	(%)	99	96	97
Energy density	(MJ/kgDM)	11.9	11.7	11.8
NDF:DM	(%)	35.2	38.6	35.4
Forage NDF:DM	(%)	25.7	25.9	25.9
Starch+sugar:DM	(%)	21.4	17.2	22.4
Crude Protein:DM	(%)	17.8	17.6	17.7
Met.Prot.Supply	(g)	2499.1	2295.7	2373.7
MP Adequacy	(%)	104	95	98

Feed intakes and milk yields were recorded throughout the experiment. Milk samples were collected for analysis of fat and protein concentrations. Nitrogen partitioning was measured over 6 days at the end of each period by collection of total outputs of faeces and urine, which together with feeds and milk were subsampled for N analysis. Methane emissions were measured for 3 days in each experimental period after measuring N partitioning.

Data were analysed using analysis of variance with orthogonal contrasts to compare between wheat and the two oat treatments, and between the two oat treatments.

Results and conclusions

Breeding of improved oat varieties for animal feed

Two approaches 1) naked oats 2) low lignin husked oats with high oil groats.


(a)


(b)


(c)

Figure 5 **a)** from left to right, husked oat spikelet with glumes, two florets in spikelet with glumes removed, primary floret with husk removed and groat visible **b)** naked oats effectively whole groats **c)** husked oats whole grain.

Naked oats have been bred at IBERS for animal feed. They have similar agronomic requirements to husked oats with a slightly higher sowing rate; however yield is lower due to lack of husk, 70-80% of the husked varieties. The target for the breeding programme has been for higher yielding lines with improved oil content. The high oil and good protein content especially the sulphur containing amino acids (table 5, fig 5) make oats an ideal animal feed. In Quoats the naked oats have been concentrated on poultry. High oil sources from IOWA state recurrent selection process have been used as parents in the crossing program to bring high oil in to UK adapted lines. NIRS calibrations developed at IBERS have been used to screen material and select for naked oats with higher oil content e.g. Mason.

Table 5 Variation in levels of crude protein, oil content and metabolisable energy of oat lines and varieties compared to feed wheat.

Selection	Crude Protein	Oil (B)	TME MJ/kg as fed
Gerald	**11.6**	**8.1**	**11.5**
Brochan	**11.0**	**7.7**	**12.4**
Hendon	**11.3**	**10.2**	**15.3**
Racoon	**14.8**	**13.6**	**16.2**
01-126Cn1	**12.2**	**12.8**	**15.8**
Mason	**13.4**	**12.7**	**15.5**
01-146Cn5	**12.6**	**13.2**	**15.8**
Zuton	**14.0**	**9.0**	**15.4**
Lennon	**13.7**	**8.9**	**15.7**
Frontier (wheat)	**12.3**	**2.5**	**13.9**

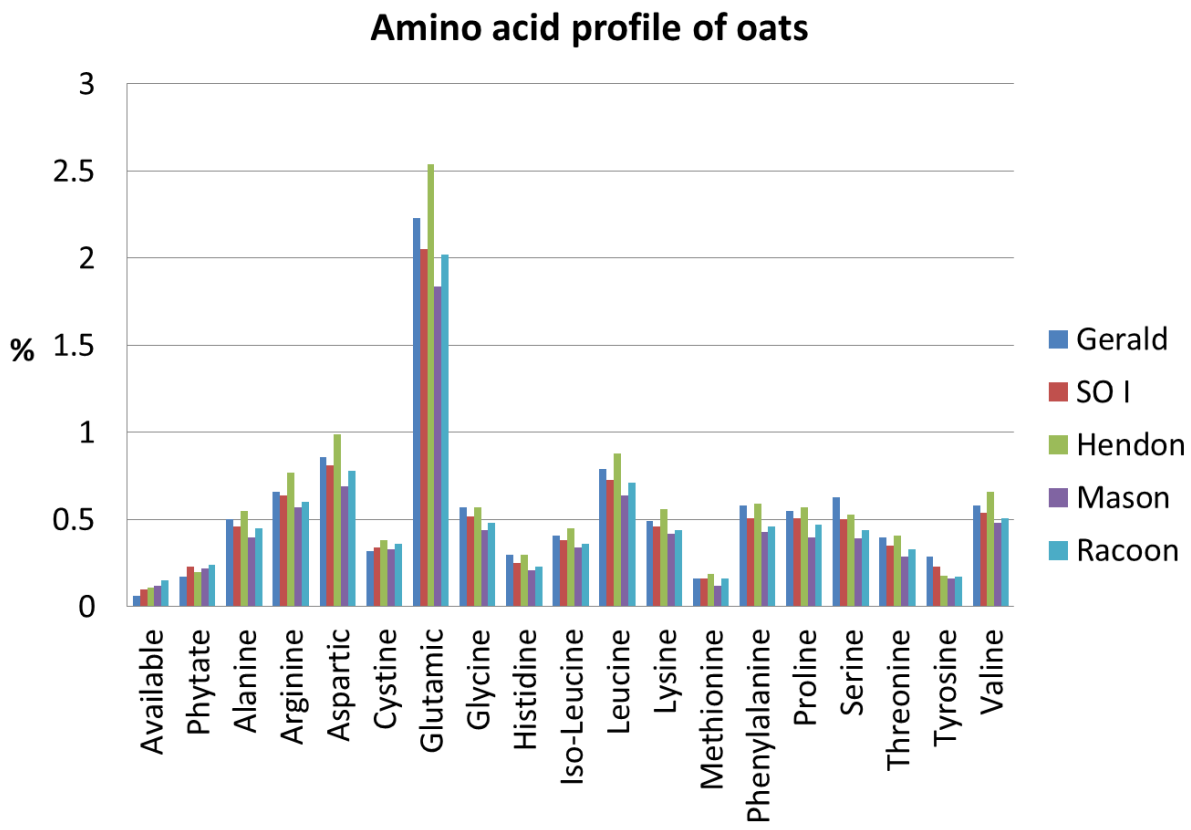

Figure 5 The range of amino acids found in various oat lines note the high levels of sulphur containing amino acids

Over the project there has been a transition to a greater emphasis on breeding of low lignin high oil husked oats.

Low lignin high oil husked oats

Sources of low lignin husk AC Assiniboia and the Australian line Mitka have been used to incorporate low lignin husk into the breeding programme. Initial laboratory studies have shown low lignin husk to be 66% more digestible than conventional husk oats. Initially lignin was tested using colorimetric pholoroglucinol staining method (Plate 1). Conventional husk stains a deep purple colour and low lignin husk does not stain. Molecular technologies within Quoats have been used for this trait and SNP markers have now been develop to screen for low lignin.

The ultimate objective is to produce an oat variety with a low lignin husk and high oil groat. This combination would make an ideal ruminant feed and overcome some of the negative comments associated with naked oats in terms of lower yield and ease of handling.

Plate 1. Effect of phloroglucinol staining on lignin content of oat husks

Fatty acid analyses of oat grains

Fatty acid analyses of 33 novel oat line grain samples, together with 6 control grain samples, found total FA content to range from 74 to 158 g/kg DM (mean 102 g/kg DM). The main FA of the grains were C16:0 (palmitic acid), C18:1 (oleic acid) and C18:2 n-6 (linoleic acid), between them accounting for an average of 97% of the total fatty acids (Fig 3). Principal components analysis of the fatty acid data (Fig 4) indicates a major proportion (about 97%) of the variation in oat grain fatty acid proportions (g/kg total fatty acid) was explained by the first principal component, which is appears to be related to the variety and/or growing conditions (spring versus winter) of the crop.

The highest total FA content was found in WN oats (Table 6) and the lowest was found in SH, although some spring lines had a reasonably high content (110.4 g/kg DM). Some individual lines within the WH group also had a total high FA content (140 g/kg DM). For all groups FA C16:0, C18:1 and C18:2 together comprised about 95% of the total FA. There was some variation in the FA proportions with the highest value of C16:0 for SH, which also had the lowest proportion of C18:1 and highest proportion of the more beneficial FAs C18:2 and C18:3. The WH group had lower proportions of C16:0 and increased proportions of the beneficial FAs C18:1, C18:2 and C18:3 than the SLLH group.

Table 6 Mean fatty acid (FA) proportions (values as % of total FA except for Total FA in g/kg DM)

	Winter naked	Winter low lignin husked	Sprint low lignin husked		
	n=4	n=16	n=18	SEM	Sig.
Total FA g/kg DM	137.6[a]	105.7[b]	90.5[c]	12.97	***
%C16:0	14.8[a]	16.8[b]	17.5[C]	0.40	***
%C16:1	0.16[a]	0.18[a]	0.22b	0.010	***
%C18	1.30[a]	1.03[b]	0.97[b]	0.100	**
%C18:1	45.5[a]	40.1[b]	35.9[c]	0.98	***
%C18:2	36.8[a]	40.4[ac]	43.6c	1.640	***
%C18:3	0.78[a]	0.92[a]	1.20[b]	0.069	***

Values in rows with different superscript letters indicate significant differences ($P < 0.05$).
Sig. = significance of treatment effect, NS = not significant, ** = $P < 0.01$, *** = $P < 0.001$.

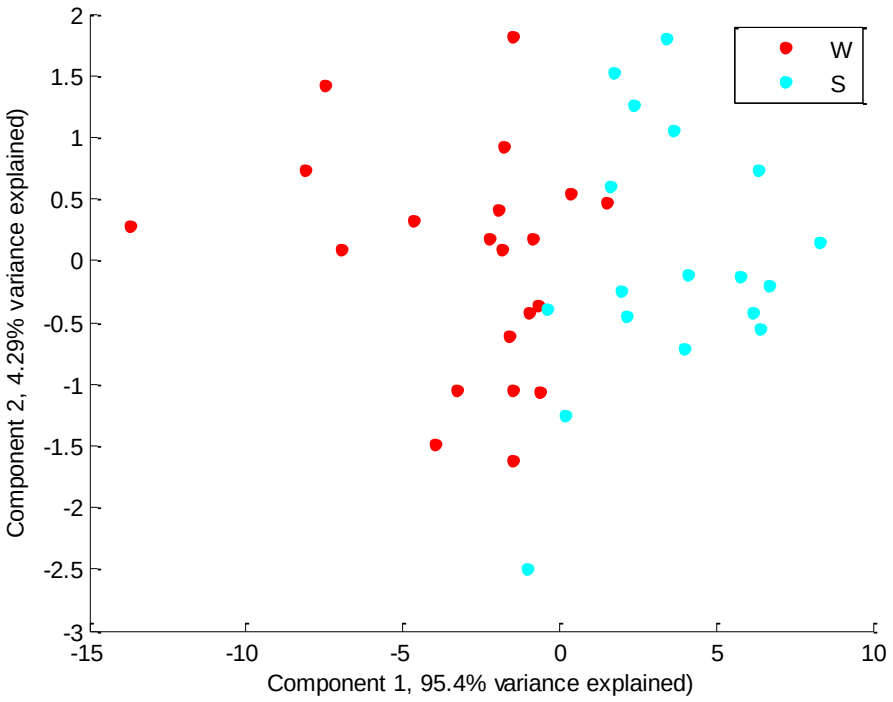

Figure 6 Principal components (1 vs 2) plot of oat grain fatty acid proportions. Red dots (W) are winter oat varieties, blue dots (S) are spring varieties.

Samples of the whole grain (including husk if present) of 4 commercial varieties of winter naked (WN) oats, together with several novel low lignin breeding lines of spring husked (SLLH; n=5) and winter husked (WLLH; n=8) oats, were analysed for standard chemical composition.

Data were analysed by ANOVA, with multiple comparisons when the effect of treatment (SN, SLLH and WLLH) was significant (P<0.05). Grain oil and CP concentrations were significantly lower in the novel husked oats than the conventional naked oat varieties (Table

7), while fibre concentrations were higher, leading to lower ME densities in the husked oats than the naked oats, as expected.

In conclusion, although the apparent feeding value of the novel husked oats was not as good as naked oats in some areas, some values of novel spring varieties in particular were similar to naked oats and show promise as ruminant feeds.

Table 7 Chemical composition of oat varieties, values in % DM unless otherwise indicated.

Oat varieties:	WN	WH	SH	SEM	Sig.
DM, %	89.4	90.9	90.9	0.49	NS
OM	97.7^{a}	97.4^{a}	97.0^{b}	0.09	***
Oil	13.8^{a}	7.5^{b}	6.3^{b}	0.74	***
CP	12.7^{a}	8.3^{b}	11.1^{a}	0.49	***
ADF	3.8^{a}	16.0^{b}	13.3^{b}	1.06	***
NDF	8.1^{a}	29.7^{b}	27.5^{b}	1.30	***
ME, MJ/kg DM	16.7^{a}	12.4^{b}	13.0^{b}	0.30	***
Starch	54.6^{a}	47.7^{b}	48.4^{b}	1.40	**
ADL	1.2^{a}	2.9^{b}	1.8a^{b}	0.49	*

Values in rows with different superscript letters indicate significant differences ($P < 0.05$).
Sig. = significance of treatment effect, NS = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Conclusions

Approximately 95% of the fatty acids in oat grains comprised palmitic acid, oleic acid and linoleic acid. The fatty acid profiles of winter and spring oats differed such that principle components analysis identified variation according to the two groups, most of which was accounted for by differences in the relative proportions of oleic and linoleic acids.

Gas production from a range of oat lines

The chemical constituents of the oat samples used in this study are presented in Table 8.

Table 8 Chemical composition (mean and range) of the oats used in the gas production analysis. Figures in g/kg DM.

	Mean	Minimum	Maximum
Crude protein	82	60	109
Organic matter	974	967	978
Neutral detergent fibre	203	54	277
Acid detergent fibre	104	26	161
Acid hydrolysis ether extract	71	39	131
Acid detergent lignin	18	7	36

Methane production varied from 28.3 to 46.1 (mean = 35.4, SD = 4.73) ml per g grain DM incubated (Figure 7), and 39.7 and 52.2 (mean = 46.3, SD = 3.68) ml per g of apparently digested DM (dDM).

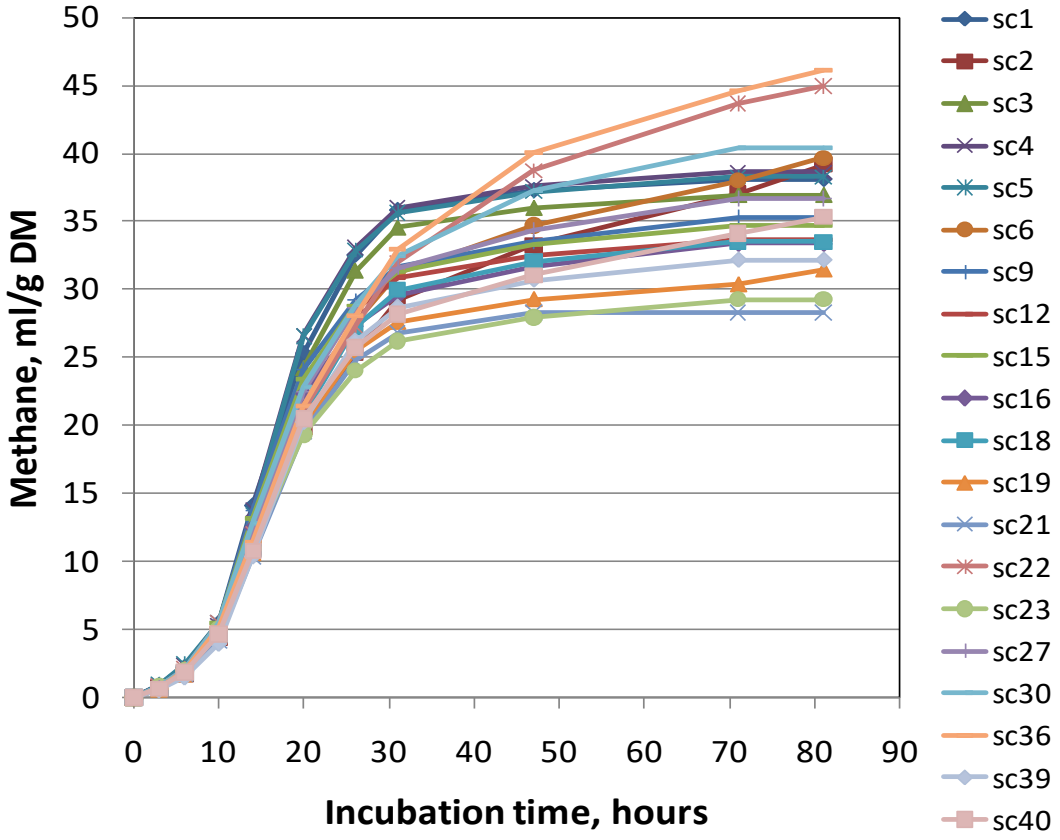

Figure 7. Cumulative methane production (ml/g DM) of the 20 oat lines over the course of approximately 80 hours.

Methane production was negatively related to grain ADL content ($r = -0.86$; $P < 0.001$) but was positively related to DM loss ($r = 0.78$; $P < 0.001$) (Figure 8). Methane production per g dDM was also negatively related to grain total fatty acid (FA) content ($r = -0.77$; $P < 0.001$). As the relative proportion (g FA/100g total FA) of some of the unsaturated FA increased, methane production per g adDM decreased (e.g. C18:1, $r = -0.76$; $P < 0.001$) (Figure 9). However, because the relative proportions of individual FA did not vary greatly among the different oat lines, the relationships with methane emissions were similar to those of total FA, particularly for those FA in greatest abundance (C16:0, C18:1, C18:2 n-6).

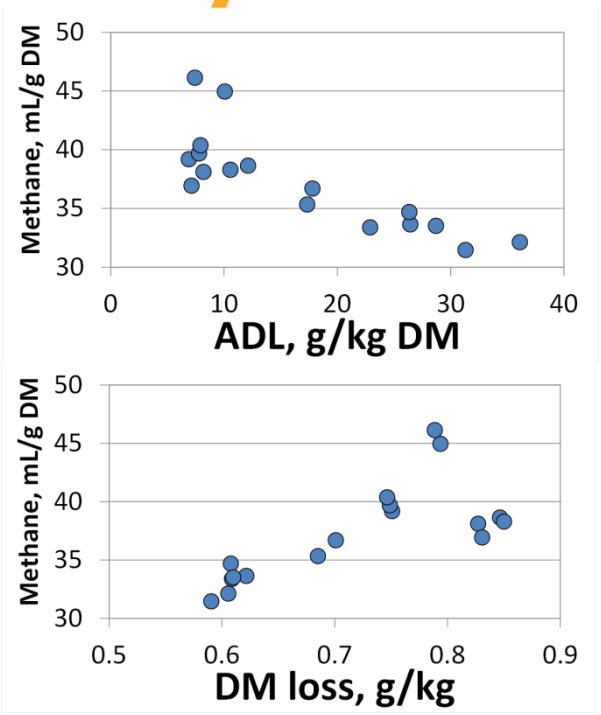

Figure 8. Methane production (g/kg DM) from oat grains in relation to ADL concentrations and total DM loss

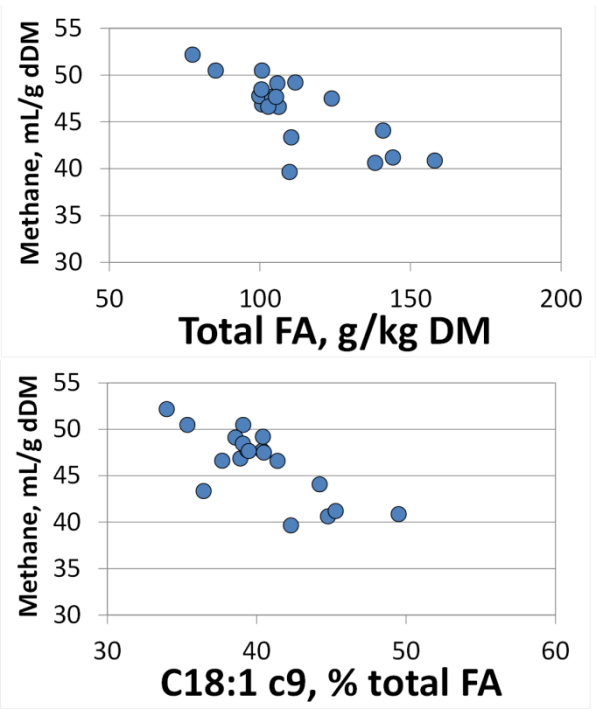

Figure 9. Methane production (g/kg dDM) from oat grains in relation to total FA concentrations and oleic acid proportion

Conclusions

There was significant variation among oats varieties/lines in the amount of methane produced, with the greatest amount of methane produced being 165% of the least. As oat lignin content increased, *in vitro* digestibility decreased and therefore methane production was reduced. As fatty acid concentrations increased, the amount of methane produced per gram of apparently digested DM decreased. Breeding husked oats for reduced lignin concentrations and increased FA concentrations to produce more digestible oat with a high oil content offers potential as a ruminant dietary ingredient that could help mitigate methane emissions.

Use of the Rumen Simulation Technique (Rusitec) to investigate promising oat lines and compare with barley.

Apparent digestibility of the 5 samples was not affected by treatment (Table 9). Similarly, methane production was not significantly affected by treatment, although microbial N production was. The greatest microbial N production was on the barley diet, the least was on the Cn18 diet, both in absolute terms (g/d) and in terms of grams produced per g of apparently digested DM.

Table 9 Mean effects of treatments on diet degradability, methane production, and microbial N production in the rusitec effluent.

	Treatment						
	Barley	Cn14	Cn18	Composite	Gerald	SED	*P*

Digestibility, g/g	0.57	0.62	0.62	0.62	0.58	0.029	0.31
Methane, ml/d	115	146	116	115	109	17.3	0.30
Methane, ml/g apparently digested	19.4	22.3	17.8	17.8	18.0	1.8	0.16
Microbial N, g/d	0.31[b]	0.26[ab]	0.22[a]	0.27[ab]	0.28[ab]	0.019	0.013
Microbial N, g/g DM apparently digested	0.053[b]	0.040[ab]	0.035[a]	0.043[ab]	0.046[ab]	0.0046	0.040

Previous results from the gas production experiment, which found an inverse relationship between oat oil content and methane production (ml per g apparently digested DM), was not replicated with the Rusitec experiment. However, there are a number of reasons why this could be. The first is that this study used diets comprising grass silage and grains, whereas the previous gas production experiment studied grains only. This may have affected the gas profile, masking the effects of the grain component of the diet.

A second possible reason is a potential change in the microbial population in the fermenter. There is limited potential for microbial population change in a batch system like the gas production system, which lasts for 2-3 days. With the much longer time course of a continuous culture system such as Rusitec, there is greater opportunity for population changes – it is well known that there are species losses in Rusitec fermenters over prolonged periods (e.g. Moumen et al, 2009). Therefore, despite being a system that allows microbial N production quantification, the methanogen population may have changed; protozoa in particular tend to be eliminated from Rusitec during the course of a study, and a relatively large proportion of methanogenic archea are associated with rumen protozoa.

Conclusions

There were no significant differences in digestibility of the 5 different cereal grain diets, although microbial N production was significantly affected. This may be related to the starch concentrations of the cereal grains, with higher yields of microbial N from those diets with higher concentrations of starch.

Methane production from a range of oat varieties consumed by mature ewes

The chemical composition of the oats offered during the experiment is listed in Table 10.

Table 10. Mean (n=8) chemical composition of the oats offered during the experiment. All values in g/kg DM.

	Balado (A)	Racoon (B)	14355Cn (C)
Crude protein	94	114	133
Organic matter	979	983	973
Water soluble carbohydrates	26	34	30
Neutral detergent fibre	315	83	375
Acid detergent fibre	139	19	168
Crude fibre	142	20	154
Starch	467	598	385
Total oil	68	130	54
Gross energy, MJ/kg DM	19.9	20.9	19.9
Acid detergent lignin	26.8	9.0	12.7

There were no treatment effects on DM intake, because sheep were fed to requirements and intake was therefore restricted (Figure 10). However, there were significant treatment effects on daily methane emissions, methane emissions per unit DM intake, per unit metabolic live weight, and as a proportion of gross energy intake (Table 11). There were no significant breed × treatment interaction effects

Table 11 Mean treatment effects on feed intake (restricted), and methane emissions (g/d, g/kg DM intake, and g/kg metabolic live weight). Means within rows with different superscripts differ significantly (P<0.05)

	Oat treatment					
	Balado	Racoon	14355Cn	Mix	SED	P
DM intake, g/d	632	639	637	639	5.23	0.510
CH_4, g/d	15.2^{a}	14.7^{a}	17.2^{b}	14.7^{a}	0.60	0.002
CH_4/DMI, g/kg	24.1^{ab}	23.0^{a}	26.9^{b}	23.9^{a}	0.96	0.003
$CH_4/LW^{0.75}$, g/kg	0.78^{a}	0.74^{a}	0.88^{b}	0.75^{a}	0.031	0.002
CH_4E/GE intake, %	7.3^{ab}	6.9^{a}	8.1^{b}	6.9^{a}	0.29	0.003

The effect of DM intake on methane emissions is clearly seen in Fig 11, with clear groups of the two breeds being evident. Variation within each breed group cluster is due to difference between the dietary treatments.

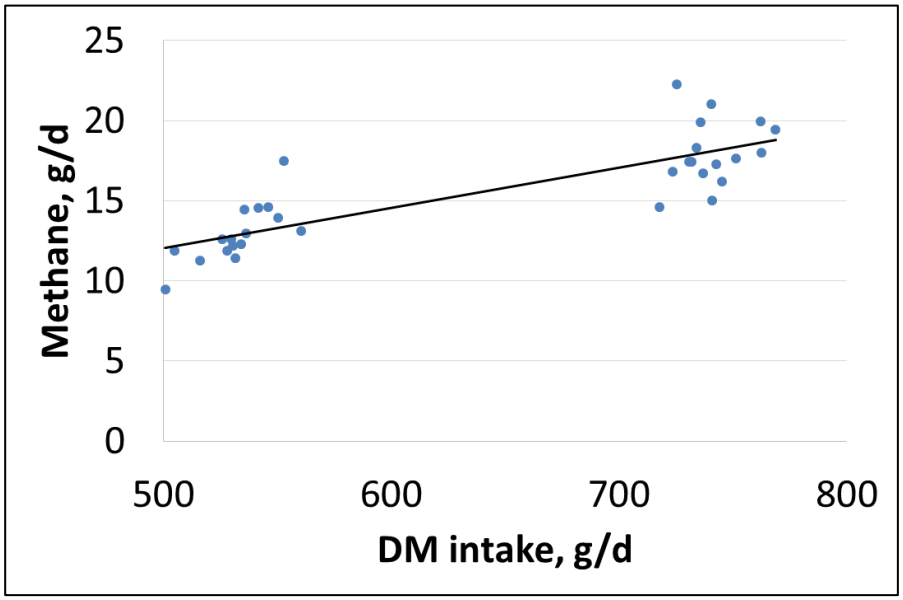

Figure 10 Effect of live weight on DM intake, which was allocated according to estimated metabolisable energy requirements. The solid line is a linear regression through all data points

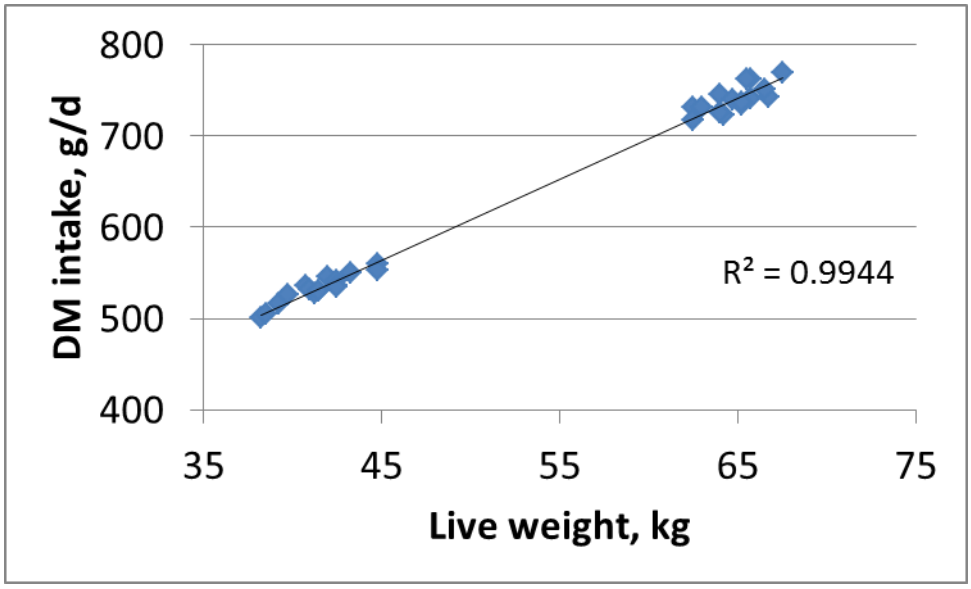

Figure 11 Effect of DM intake on methane emissions from the sheep. The solid line is the linear regression through all data (R^2 = 0.7).

Dairy cow experiment

The chemical composition of the feeds offered during the experiment is listed in Table 12.

Table 12. Mean (n=6) chemical composition of the feeds offered during the experiment. All values in g/kg DM.

	Rolled Wheat	Rolled Oats	Oat Husk	Premix A	Premix B	Conc mix A	Conc mix B	Conc mix C	Silage
Crude protein	121	142	34	311	316	248	229	229	155
Organic matter	980	977	964	893	899	919	924	929	926
WSC	38	33	7	119	104	93	82	76	82
NDF	123	93	848	221	103	177	205	250	543
ADF	38	29	405	235	62	89	109	73	339
Oil	-	-	-	-	-	-	-	-	TBD[1]
Total oil	TBD	TBD	TBD	TBD	TBD	TBD	TBD	TBD	-
Starch	655	614	48	59	208	231	241	335	-

[1] To be determined.

Treatment mean feed intakes and milk yields are presented in Table 13. There was no effect of concentrate treatment on *ad lib* silage intakes nor on milk yields. Milk fat was significantly affected by diet, with lower concentrations and yields from cows offered the Oat 2 diet. Milk protein and lactose were unaffected. Milk urea concentration tended to be lower in cows offered the Oat 1 diet. The *in vivo* whole tract organic matter digestibilities expressed as a proportion of DM intake (DOMD) did not differ significantly between treatments, which meant that the overall diet metabolisable energy density was estimated at 9.76 MJ/kg DM. This is somewhat less than the originally predicted energy density of approximately 11.8 MJ/kg DM of the formulated diet, and it likely due to differences between

assumed and actual composition of the grass silage used in the experiment. The digestibility of dietary N was significantly greater as part of the wheat-based concentrate diet compared with the oat-based diets.

Table 13. Mean effects of concentrate treatment on feed intakes, whole tract apparent digestibilities of feed, and milk yields and milk composition.

	Concentrate treatment				P	
	A - Wheat	B - Oat 1	C - Oat 2	SED	A v B+C	B v C
Silage intake, kg DM/d	10.9	10.7	10.6	0.14	0.106	0.612
Total intake, kg DM/d	21.2	21.1	21.0	0.14	0.041	0.719
DM digestibility, g/g	0.66	0.65	0.66	0.006	0.213	0.216
N digestibility, g/g	0.75	0.70	0.72	0.016	0.018	0.196
OM digestibility, g/g	0.68	0.67	0.67	0.006	0.278	0.338
Milk yields, kg/d	28.9	29.0	28.8	0.52	0.908	0.670
M yield/DMI, kg/kg	1.35	1.38	1.37	0.025	0.403	0.738
Fat, g/kg	43.2	43.6	41.4	0.49	0.325	0.002
Protein, g/kg	33.3	32.7	32.7	0.37	0.114	0.798
Lactose, g/kg	45.1	45.1	45.3	0.14	0.307	0.065
Fat yield, g/d	1497	1511	1437	17.0	0.325	0.002
Protein yield, g/d	1155	1133	1136	13.0	0.114	0.798
Lactose yield, g/d	1564	1563	1573	4.9	0.307	0.065
Urea, %	0.029	0.027	0.029	0.0010	0.202	0.031

Daily methane emissions from the dairy cows did not differ significantly between treatments (Table 14). Methane yields, i.e. g methane per kg feed DM intake, also did not differ between treatments.

There were no significant treatment effects on the outputs of N in milk, faeces and urine, nor in the apparent partitioning of dietary N to milk or urine. However, cows offered the wheat-based concentrate had significantly lower partitioning of dietary N to faeces, which reflects the increased digestibility of N on that diet.

Table 14. Mean effects of concentrate treatment on methane emissions and whole body apparent N partitioning.

	Concentrate treatment					
	A - Wheat	B - Oat 1	C - Oat 2	SED	A v B+C	B v C
Methane, g/d	371	351	370	16.9	0.490	0.293
Methane yield, g/kg DM intake	17.2	16.7	17.5	0.82	0.880	0.320
Milk N out, g/d	173	167	172	5.4	0.512	0.366
Faeces N out, g/d	171	192	177	10.4	0.160	0.186
Urine N out, g/d	265	244	259	10.5	0.153	0.183
Milk N/N In, %	25.2	25.9	26.8	0.76	0.112	0.250
Faeces N/N in, %	24.9	29.9	27.7	1.57	0.018	0.196
Urine N/N in, %	38.7	37.9	40.5	1.53	0.715	0.125

Table 15 Mean effects of concentrate treatment on proportions of major milk fatty acids.

	Concentrate treatment					
	A - Wheat	B - Oat 1	C - Oat 2	SED	A v B+C	B v C
C12:0	3.3	2.6	3.0	0.08	<0.001	<0.001
C14:0	11.3	10.1	11.0	0.16	<0.001	<0.001
C14:1 *cis*-9	1.13	0.96	1.05	0.027	<0.001	0.008
C16:0	31.0	28.5	29.7	0.53	0.003	0.045
C16:1 *cis*-9	1.51	1.42	1.44	0.073	0.225	0.735
Phytanic acid *iso*-1	0.31	0.26	0.28	0.005	<0.001	<0.001
C18:0	9.3	11.5	10.3	0.24	<0.001	<0.001
C18:1 *cis* (all isomers)	20.3	23.5	21.8	0.57	0.001	0.015
C18:1 trans (all isomers)	2.01	2.36	2.00	0.046	0.002	<0.001
C18:1 *cis*-9	19.5	22.6	20.9	0.55	<0.001	0.013
C18:2 n-6	1.53	1.51	1.56	0.049	0.889	0.389
C18:3 n-3	0.37	0.34	0.35	0.018	0.206	0.538
C20:0	0.15	0.16	0.15	0.004	0.011	0.011
Short chain FA[1]	11.7	11.0	11.6	0.16	0.006	0.004
OBCFA[2]	3.17	2.99	3.00	0.051	0.007	0.769
Long chain FA[3]	0.24	0.22	0.23	0.006	0.130	0.395

[1] < C12

[2] Odd- and branched-chain fatty acids, [3] > C20

Previous analysis of the fatty acids profiles of a range of different oat varieties and breeding lines indicated that the most significant FA in oats are palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2). These 3 FA accounted for approximately 97% of the FA in the oats analysed. In milk fat of cows offered the 3 different concentrate diets, there were significant effects of treatment on several FA, the most abundant of which are presented in Table 15.

The effects of fatty acids in the human diet and their effects on cardiovascular disease are complicated (Mensink, 2003), although it is generally accepted that a reduced consumption of saturated FA such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids and replacement with *cis* unsaturated fatty acids has potential health benefits. In this experiment, the proportions of C12:0, C14:0 and C16:0 were all reduced on the oat-based diets compared to the wheat-based diet, probably as a consequence of an increased proportion of C18:1 (both *cis* and *trans* isomers, and *trans* FA are generally considered to be detrimental to human health). However, the proportion of the *cis* isomers of C14:1 was also lower, and the proportion of C18:0 was significantly increased. There were no treatment differences in the proportions of linoleic or α-linolenic acids in total milk fat.

Conclusions

The results of the work demonstrate that oats could be used to substitute wheat in the concentrate portion of dairy cow diets without loss in productivity. No differences among treatment in the methane emissions of the cows were found, nor in the outputs of N in urine, which have the potential to influence nitrous oxide emissions. Milk fat concentrations and yields were lower from animals offered diet C, which suggests an influence of the concentrate premix fed to these animals. Finally, the fatty acid profile of the milk produced by cows offered the two oat-based diets might be considered to be generally healthier than that produced by cows when offered the wheat-based diet.

Life Cycle Assessment (LCA)

LCA of the value of oats in ruminant diets is currently being calculated using the data on feed value of oats obtained within this project. This is being conducted in parallel with an economic appraisal of the different feed rations used in these studies. This will information will be available once the analysis is complete.

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