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Harnessing new technologies for sustainable oat production and utilisation (QUOATS)

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CONTENTS

1.	INTRODUCTION	1
2.	MATERIALS AND METHODS	1
2.1.	Workpackage 1 Development of genetics and genomics technology	1
2.2.	Workpackage 2 Improved oats for human consumption	2
2.3.	Workpackage 3 Improved oats for livestock feed.....	2
2.4.	Workpackage 4 Agronomy of oats in conventional and organic systems	3
3.	RESULTS	4
3.1.	Workpackage 1 Development of underpinning molecular technologies.....	4
3.2.	Workpackage 2 Improved oats for human consumption	5
3.3.	Workpackage 3 Improved oats for animal feed.....	6
3.4.	Workpackage 4 Improved agronomy of oats – response of oats to N in conventional systems	7
4.	CONCLUSIONS AND IMPLICATIONS.....	11

1. Introduction

Oats are recognised as a valuable break crop in cereal rotations; nevertheless, there is a need to improve key traits that will increase the production and utilisation of oats whilst also mitigating climate and environmental change via reduced agricultural inputs. This project aimed to address these issues by developing and applying state-of-the-art genomic and metabolomic tools for targeted oat genetic improvement of key traits that will enhance the value of oats in human health improvement, realise the potential of oats as a high value animal feed and develop new opportunities for using oats through advanced fractionation.

This multi-disciplinary project combined modern phenotyping methodologies with the expertise of genomics researchers, oat breeders and end-users. Involvement of the various end-users of oats (food, feed and industrial uses) in the evaluation of novel oat lines was designed to facilitate the transfer of this research into oat breeding programmes and deliver oat varieties with the characteristics that industry requires and deliver environmental benefits to sustainable production systems.

2. Materials and methods

The project was organised in 4 workpackages. Workpackage 1 focused on the core underpinning molecular technologies for the identification of specific genes and markers associated with key traits. Workpackage 2 focused on the development of oats for human consumption and industrial uses and combined the application of molecular tools and phenotyping to the development of high quality milling oats. Central to the work was coordinated analysis of field trials that were carried out over the five years of the project incorporating analysis of grain quality and composition alongside mycotoxin analysis. Workpackage 3 focused on the development of oats for ruminants and monogastrics with the emphasis on breeding naked oats with high oil and husked oats that combine high oil with low lignin husk. Workpackage 4 focused on the agronomy of oats with an emphasis of the nitrogen use efficiency of oats in conventional and organic systems and the development of an oat lodging model.

2.1. Workpackage 1 Development of genetics and genomics technology

The methodologies associated in this work are not included in this summary but are summarised within the main text and are referenced within the main text.

2.2. Workpackage 2 Improved oats for human consumption

This workpackage focused on the development of improved oats for human consumption. Throughout the project a number of oat lines/varieties were developed with the objective of introducing traits that improved the agronomic performance of the varieties whilst improving the milling quality and grain composition as well as reducing mycotoxin levels.

Milling quality: Analysis of kernel content, hullability and specific weight was carried out at Aberystwyth University (IBERS) on all breeding material and on the multisite trials in parallel with analysis of grain size and shape using MARVIN, an image analysis system. This was reinforced through ring tests carried out with industry partners.

Multisite trials: Central to the workpackage was the analysis of grain samples for milling quality (kernel content and specific weight) and the development of novel phenotyping approaches including MARVIN. It also included multisite trials over four harvest years so that the effect of environment and management on the milling quality and grain composition of different oat varieties could be quantified.

Grain composition: Analysis of breeding material and from the multi-site trials was carried out at IBERS using high throughput NIRS technology combined with chemical analyses (beta-glucan, protein, oil and avenanthramides) as well as by The James Hutton Institute using a range of metabolomic analytical approaches (e.g. GC-MS).

Mycotoxin analysis: This was carried out by Harper Adams University.

2.3. Workpackage 3 Improved oats for livestock feed

This included a number of *in vitro* as well as *in vivo* studies using the range of improved germplasm developed within the project.

In vitro studies included the analysis of gas production from a range of oat lines followed by the use of the Rumen Simulation Technique (Rusitec) to investigate promising oat lines and compare with barley. This latter study included oat lines selected for high oil/low lignin husk.

In vivo studies included a sheep feeding study comparing the feed value and methane emissions from sheep fed different oat based diets:

- 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

This was followed by a dairy cow feeding study in which the feed intake and milk yields of dairy cows fed on different oat based diets were recorded. This also included milk composition (fat, protein and lactose) and milk fatty acid analysis as well as methane emissions. The oat based diets were:

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 oats fed as part of the concentrate portion of the diet comprised naked oat grains and oat husk.

2.4. Workpackage 4 Agronomy of oats in conventional and organic systems

Nitrogen use efficiency in conventional systems: Field trials were established near ADAS Rosemaund, Herefordshire, in the growing seasons 2010–11 and 2011–12. Four winter oat varieties (Balado, Gerald, Mascani and Tardis) were tested at contrasting seed rates and N regimes. Nitrogen treatments in each experiment were 0 kg N/ha and 140 kg N/ha (Fertiliser Manual Recommendation) and seed rates were 100 seeds/m² and 400 seeds/m² (375 seeds/m² in 2011). Yield and yield components, N partitioning and yield were determined at the end of each season.

Nitrogen use efficiency in organic systems: Field trials were conducted with the winter oat varieties used in 2.2.10 as well as others over four trial seasons (2009–10, 2010–11, 2011–12 and 2012–13) at Wakelyns Agroforestry, Suffolk, UK on a medium-clay loam. Mechanical weed control was applied in all trial years which included cultivations to control weeds in a false seed bed before drilling as well as comb harrowing and inter-row harrowing through the growing season when appropriate.

Development of a model of the lodging process for oats: The wind induced movement of oat plants grown individually in pots, or in groups in trays, was investigated by painting a tracking marker on part of the oat canopy and videoing the plant's movement. These experiments were used to better specify the relationship between wind speed and the force exerted by the plant on its stem and root system.

3. Results

3.1. Workpackage 1 Development of underpinning molecular technologies

This workpackage planned to use the best methods available at the time of submission to begin an oat genomics programme. The cost of new next generation sequencing (NGS) technologies dropped rapidly in the first years of QUOATS and in March 2011 the bacterial artificial chromosome (BAC) library and cDNA approaches were changed, with the agreement of the consortium and funders, to allow representative whole genome sequencing of the A genome diploid mapping family parents to be carried out instead. This has been very successful such that that we expanded the revised goals of recovering most gene space from the parents to include low coverage sequencing of a few informative recombinant inbred line (RIL) progeny. This allowed mapping of contigs to 'bin' intervals defined by regions of common parent-of-origin polymorphisms within each RIL. Using the Illumina short-read platforms we now have >40x coverage of the *A. atlantica* (wild) diploid parent, and >30x combined coverage of the *A. strigosa* (domesticated) parent and 15 RILs. The N50 of the *A. atlantica* assembly is ~12kb (i.e. the combined length of all contigs over 12kb is equal to the combined length of all contigs below 12kb) allowing the great majority of genes to be recovered intact on single contigs. Some 1.5Gb of *A. atlantica* contigs have been placed in ordered bins, each, on average, representing 6cM or 1% of the total map. Bioinformatic screens with sets of barley or brachypodium genes indicate that well over 90% of corresponding oat genes in the total assembly have now been mapped. 'Binned' contigs contain >1 million short indel or single nucleotide polymorphisms which can be converted into markers, giving a reasonable expectation that any gene of interest could be followed in the mapping population. Some NGS sequencing of other species related to the cultivated hexaploid has been initiated, allowing assignment of previously anonymous hexaploid markers to likely genomes of origin. Annotation of the assemblies has begun, which will allow systematic gene discovery and improved prediction of candidate genes from hexaploid quantitative trait loci (QTL).

Buffalo x Tardis Mapping Population: Genotyping methodologies rapidly developed during the project. Genome by sequencing (GBS) has been used on the Buffalo x Tardis population and we now have a new linkage map of over 7000 single nucleotide polymorphisms (SNP) linked to the new oat consensus map. This data also permits detailed identification of regions of homology with the A genome diploid map and with our C and D genome sequence databases. This provides much better coverage of the oat genome than previously obtained, helps elucidate the correct genome designation of each chromosome

and is currently being used to improve QTL analysis. QTL associated with milling quality traits, plant height, components of yield and nitrogen use efficiency (NUE) have also been identified. From the results, a series of QTL-NIL (near isogenic lines) lines have been produced targeting key QTL of interest.

AC Assiniboia crosses: A number of populations have been used to identify QTL associated with disease resistance and low lignin husk using the AC Assiniboia source of these traits. SNP markers closely linked to the low-lignin husk trait and to Pc 68 have been developed and tested in a wide range of advanced breeding lines, parental lines and in early generation segregating populations. These SNP markers are highly diagnostic and have been incorporated into the breeding programme. Unlike conventional phenotypic screening which must take place after the crop has been harvested, DNA can be extracted from young seedlings and the genotype rapidly ascertained.

Marker assisted selection: We are now routinely using markers in both the spring and winter oat breeding programmes for a wide range of traits including mildew resistance, crown rust resistance, low-lignin husk, dwarfing, flowering time, grain quality traits and distinctness, uniformity and stability (DUS) traits such as waxy/ non-waxy leaves. Markers are used at a number of stages in the breeding programme:

- Assessment of genetic diversity of breeding programme
- Selection of appropriate parents to use in the crossing programme
- Back-cross introgression of disease resistance alleles into a UK adapted background
- Fixing multiple disease resistance alleles at an early stage in the breeding programme
- Identification of individuals at an early stage in breeding programme containing desired allele combinations
- Checking uniformity of advanced breeding lines

3.2. Workpackage 2 Improved oats for human consumption

A significant number of crosses were made throughout the project with the specific objective of improving oat milling quality and composition which were evaluated in multisite trials by academic and industry partners.

Milling and grain quality parameters are of key importance for oat growers and the ability to produce high quality grain is an important target for the breeding programme. The development of high throughput methods for measuring grain quality has been one of the targets for this project. Over the course of the project near infrared spectroscopy (NIRS) calibrations for husk lignin content and kernel content have been developed. The further

development of NIRS calibration for groat oil, β -glucan and nitrogen content has also been conducted. This allows screening of early generation material from the breeding programme against quality targets. During this project a method for measuring and characterising grain size and shape has been developed allowing a better understanding of the grain size within a lot and how different varieties vary in grain size distribution. Industry partners participated in ring tests to validate analysis of kernel content and specific weight using standardised material.

T2 and HT2 are the main mycotoxins found on oats in the UK caused by *Fusarium langsethiae*. Analysis of varieties from IBERS yield trials, together with material from breeding nurseries, showed that UK grown spring oats had lower levels of mycotoxin than winter oats and organic oats had the lowest levels of all. A series of experiments using a mixture of spring and winter oat varieties was sown in the autumn and spring for two seasons to investigate whether there was a genetic or environmental effect on mycotoxin loading. Breeding for improved resistance has been hampered by the inability to artificially inoculate oats with *F. langsethiae*. This, combined with the lack of any physical symptoms of disease (unlike wheat where head blight *F. graminearum* give visibly bleached aborted and shrunken grains in the ear), makes normal methods of selection for improved disease resistance difficult.

3.3. Workpackage 3 Improved oats for animal feed

A major objective of the project was to breed oat varieties with the grain composition that made oats a valuable animal feed. A significant number of naked oat lines were developed as well as husked oats with low lignin husk that would increase the digestibility of oats by ruminants. Analysis of these oat lines showed that approximately 95% of the fatty acids in oat grains comprised palmitic acid, oleic acid and linoleic acid. The different fatty acid profiles of winter and spring oats was accounted for by differences in the relative proportions of oleic and linoleic acids. Gas production studies showed 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 the lignin content increased, *in vitro* digestibility decreased and methane production was reduced, highlighting the value of low lignin as a breeding target. As fatty acid concentrations increased, the amount of methane produced per gram of apparently digested DM decreased, suggesting that 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.

The second phase involved the evaluation of novel oats lines in sheep and dairy cow feeding trials. The sheep feeding trial found no significant differences in digestibility of the 5 different cereal grain diets, though there were differences between the two sheep breeds studied in terms DM intake and live weight gain. From the dairy feeding trials it was concluded that oats could be used to substitute wheat in the concentrate portion of dairy cow diets without loss in productivity (Table 1). 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. However, milk fat concentrations and yields were lower from animals offered the oat-based diet (C) which was formulated to have a similar composition to the wheat-based diet, 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.

Table 1. Mean effects of concentrate treatment on methane emissions and whole body apparent N partitioning.

	Concentrate treatment			SED	P-Value	
	A - Wheat	B - Oat 1	C - Oat 2		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

3.4. Workpackage 4 Improved agronomy of oats – response of oats to N in conventional systems

Balado had the high potential yield due to high numbers of grains per panicle compared to the other varieties tested. Lower yields for Gerald and Mascani at high N rates and seed rates were associated with lodging due to their height. This also meant that more N was taken up in the straw reducing their NUE and sometimes nitrogen uptake efficiency

(NUpE). Differences in yield from seed rate treatments were due to differences in shoot numbers, although at high seed rates there were also fewer grains per panicle.

Table 2. The yields (t/ha @ 15% mc) of four winter oats varieties grown at two seed rates and two nitrogen rates in the 2010–11 and 2011–12 field seasons near ADAS Rosemaund, Herefordshire.

Year	Variety	Seed rate (seeds/m ²)		Total N applied (kg N/ha)		Mean
		100	400	0	140	
2011	Balado	8.91	9.67	8.54	10.04	9.29
	Gerald	8.16	8.77	7.61	9.32	8.47
	Mascani	8.46	9.14	7.79	9.81	8.80
	Tardis	8.02	9.26	6.98	10.30	8.64
	Mean	8.39	9.21	7.73	9.87	8.80
2012	Balado	4.38	4.76	4.29	4.86	4.57
	Gerald	4.63	4.59	4.85	4.24	4.61
	Mascani	5.12	4.67	4.68	5.1	4.89
	Tardis	5.19	5.25	4.60	5.85	5.22
	Mean	4.84	4.83	4.60	5.08	4.83
SED						
	P-Value	(max.)			P-Value	SED (max.)
Year	<.001	0.107		Nrate*Variety	<.001	0.128
Variety	<.001	0.096		Year*Seedrate	<.001	0.126
Seedrate	<.001	0.068		Nrate*Seedrate	0.005	0.086
Nrate	<.001	0.053		Variety*Seedrate	0.065	0.135
Year*Variety	<.001	0.158		Year*Nrate*Variety	0.005	0.204
Year*Nrate	<.001	0.119		No other 3 rd or 4 th order interactions significant		

Nitrogen Response of Varieties: The N response experiment was carried out in harvest year 2014, a very high yield potential season (Figure1). Comparison of the response of 4 winter oat varieties to 6 levels on N showed a significant interaction between variety and N treatment. At the N rate closest to RB209 recommendations (150 kg N/ha), Balado gave the highest yield (8.22 t/ha), followed by Mascani (7.67 t/ha), Gerald (7.65 t/ha) and Tardis (7.55 t/ha). However, at the highest N rate (250 kg N/ha), Mascani gave the highest yield (8.99 t/ha) with Balado yielding slightly, but not significantly, less (8.89 t/ha) and Gerald giving the lowest yield (8.74 t/ha). These data indicate that N requirements for oats are higher than those recommended in RB209 and that further testing of modern oats varieties is required to optimise recommendations so that growers can maximise yield.

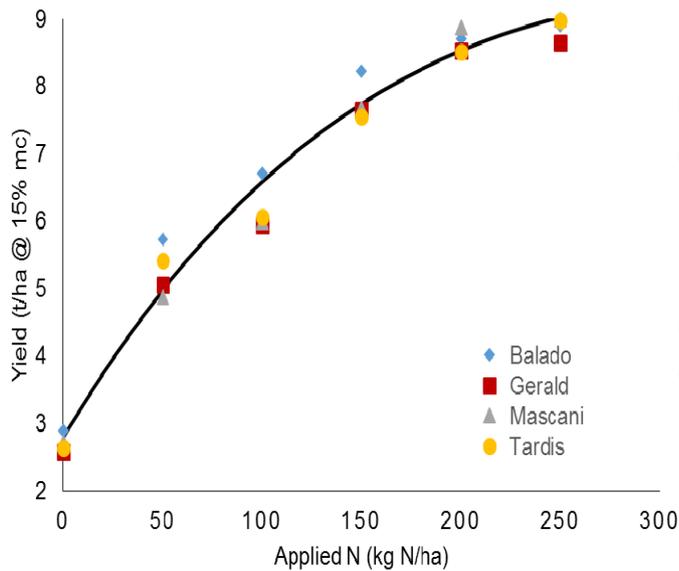


Figure 1. The yield (t/ha @ 15% mc) of the oat varieties Balado, Gerald, Mascani and Tardis at N rates of 0 to 250 kg N/ha. Line is N response curve fitted to all data (Variation Accounted For = 92.8%).

Oats in organic rotations: Competition from weeds is one of the biggest factors limiting yield in organic systems. Genotypic differences in weed tolerance were found among the varieties trialled and taller varieties tended to have a greater weed tolerance. Weed suppressive traits such as early establishment, Leaf Area Index, tillering ability and crop height were found to significantly reduce weed cover after harvest. The 60 kg/ha of available N that was supplied only increased grain yields by a small amount but greatly increased lodging. The taller varieties were much more susceptible to lodging, although stem density also seemed to contribute to lodging susceptibility. Therefore, the small yield benefit of adding fertility may well be counteracted by the increased risk of lodging, which is already of concern in organic systems where taller varieties are often used for weed competition and plant growth regulators are prohibited. Mascani had the highest average yield over the four trial years but had the smallest yield response to added fertility (Figure 2). This may highlight Mascani as a variety with low nitrogen requirements suitable for low input or organic systems.

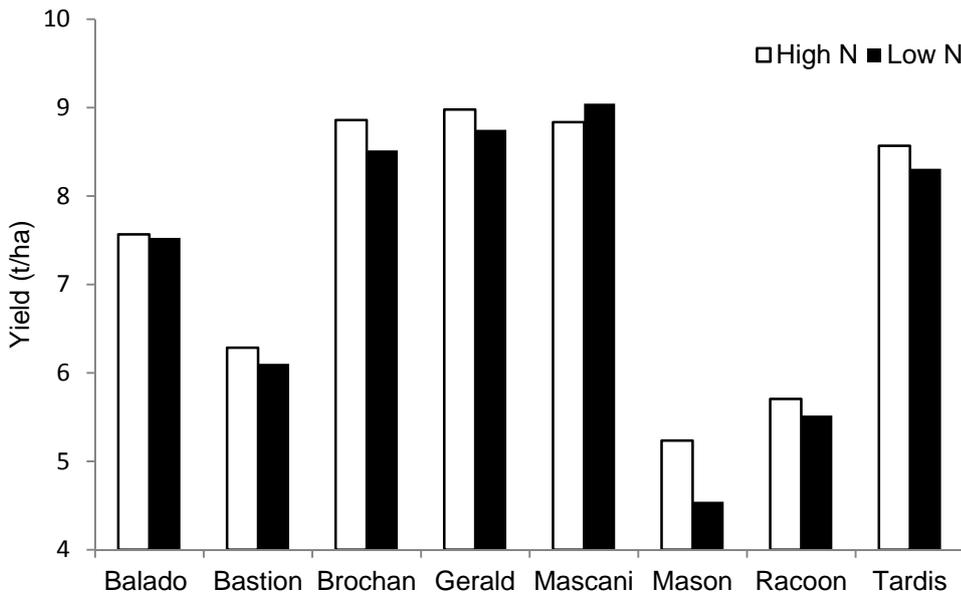


Figure 2. Mean grain yields of eight winter oat varieties under two N fertiliser levels in all four trial years

Oat performance compared to wheat and barley: Oats are generally lower yielding than wheat in conventional systems. However, yields of winter husked oat were higher and more stable than winter wheat or barley when grown under organic conditions in this study (Figure 3). The average yield of husked oats was higher than wheat and barley varieties grown at the same site for the four trial years.

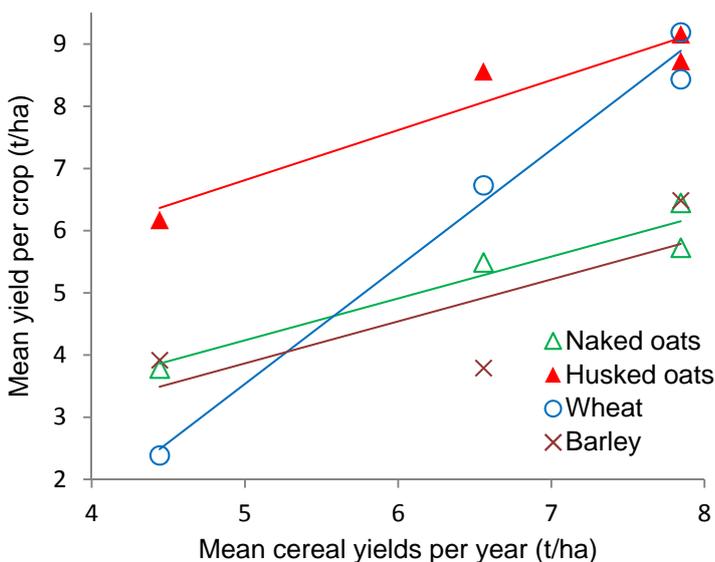


Figure 3. Joint regression analysis of mean yields of husked oats (5 varieties), naked oats (3 varieties), winter wheat (2 varieties) and winter barley (2 varieties) grown at the same site over four trial years.

Modelling the lodging of oats: This study confirmed that the plant characteristics to cause lodging and on overall lodging risk are the plant height, spread of the root plate, diameter of the stem, material strength of the stem wall and overall strength of the stem. Traditionally plant breeders have focused on improving lodging risk by shortening the plant but this

analysis shows that improving the root plate spread and stem strength would be equally effective ways of increasing lodging resistance.

4. Conclusions and implications

- This project has exploited the rapid developments in genomics technologies over the last 5 years and highlighted how this knowledge can be applied to oats and utilised within oat breeding programmes.
- It has also developed the underpinning molecular marker technology, which when allied with high throughput phenotyping, can advance oat breeding and enable the effective and efficient selection of key agronomic traits as well as grain composition traits in parallel with other high throughput approaches such as NIRS and MARVIN.
- It has shown the potential for improved milling quality and value of new approaches to the analysis of grain size and shape as means of quantifying the milling quality of varieties. Development of high-throughput methods for grain quality will help better characterise new material coming through the breeding program and improve overall oat quality aided by ring testing with commercial partners and pilot milling of individual varieties.
- Breeding husked oats for reduced lignin concentrations offers potential as a ruminant dietary ingredient that could help mitigate methane emissions.
- Molecular markers for lignin content, developed within WP1, were used to develop improved oat lines that combined high oil with a low lignin husk.
- Dairy feeding trials concluded that oats could be used to substitute wheat in the concentrate portion of dairy cow diets without loss in productivity.
- The fatty acid profile of the milk produced by cows offered the two oat-based diets which might be considered to be generally healthier than that produced by cows when offered the wheat-based diet.
- The response of oat varieties to N was studied in flowing solution culture, and in conventional as well as organic systems. Of the varieties studied in conventional systems, Balado had the high potential yield due to high numbers of grains per panicle compared to the other varieties tested. Lower yields for Gerald and Mascani at high N rates and seed rates were associated with lodging due to their height. This also meant that more N was taken up in the straw, reducing their NUE and sometimes NUPE.
- Although the response of oats to N was only carried out in 2014, it suggested that the amount of N recommended in the fertiliser manual was significantly less than was optimal. Further N response experiments are required over a range of sites, seasons and varieties in order to understand the optimum N rates of oats.
- This study reinforced the suitability of oats for organic rotations.
- Competition from weeds is one of the biggest factors limiting yield in organic systems. Genotypic differences in weed tolerance were found among the varieties trialled and taller varieties tended to have a greater weed tolerance. Mascani had the highest average yield over the four trial years but had the smallest yield response to added N fertiliser and could be regarded as a variety with low N requirements suitable for low input or organic systems.

- Winter husked oat yields were found to be higher and more stable than winter wheat or barley when grown under organic conditions. The advantage of growing oats is often greater in lower yielding environments and demonstrates the greater reliability of oats in the more marginal and variable conditions typical of organic systems.