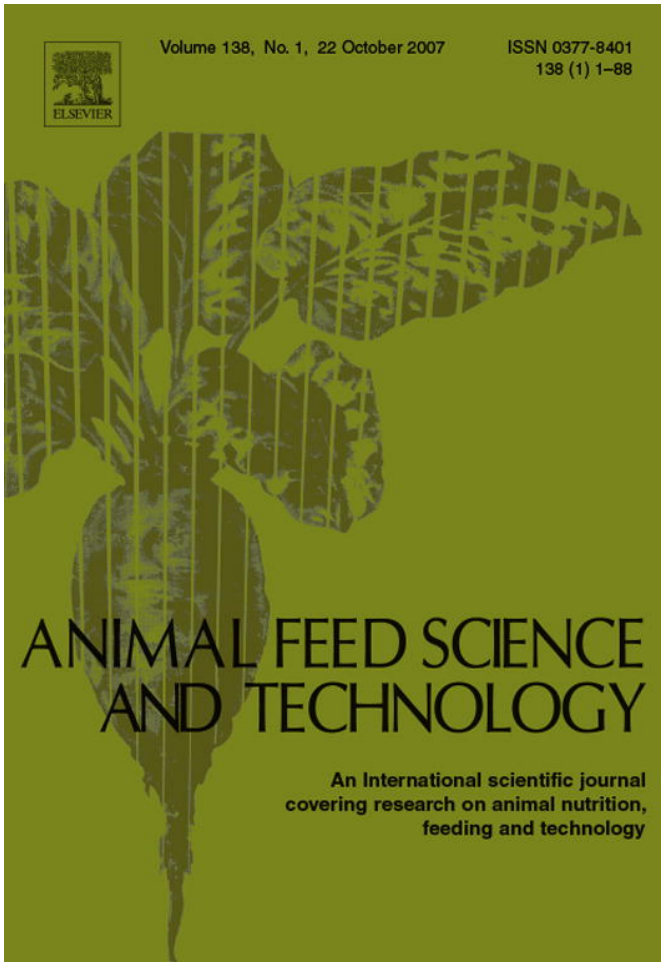

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Dietary low-phytate mutant-M 955 barley grain alters phytate degradation and mineral digestion in sheep fed high-grain diets

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Abstract

Greater production demands for ruminants require increased dietary inclusion of high-energy feeds. Grains and oil seeds are most commonly used to enhance diet energy density. However, use of such feeds proportionally increases the amount of dietary phytate phosphorus (P), which the ruminant may not be able to fully utilise. Our objectives for this study were to determine the extent of phytate degradation and mineral digestion in wethers fed high-grain diets consisting of either a non-mutant or low-phytate mutant barley grain. In two separate experiments, mature Columbia wethers ($n = 7$) fitted with rumen and duodenal cannulas and Columbia × Polypay wether lambs ($n = 8$) were individually fed one of two finishing diets formulated with either non-mutant Harrington (HARR) variety or low-phytate mutant-M 955 (M955) barley grains. Total-P intake was similar (P=0.46–0.70) between the M955 and HARR treatments for mature (5756 and 5550 mg/day, respectively) and lamb (5207 and 4894 mg/day, respectively) wethers. Dietary water-soluble P was 3.6 times greater in M955 *versus*

Abbreviations: ARS, Agricultural Research Service; Ca, calcium; Fe, iron; HARR, Harrington variety barley grain; M955, mutant-M 955 variety barley grain; Mg, magnesium; P, phosphorus; USDA, United States Department of Agriculture; Zn, zinc

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HARR diets and phytate P was 11 times greater in HARR *versus* M955 treatment diets. Apparent total-P digestion was similar between M955 and HARR treatments (P=0.52–0.69). More monoester P was identified in the duodenal chyme of mature wethers fed HARR treatment diet, presumably due to incomplete hydrolysis of phytate P in the rumen. Feeding M955, compared to HARR, treatment diet resulted in greater (P<0.05) apparent partial-tract digestion of calcium (Ca) and total-tract digestion of iron (Fe), magnesium (Mg), and zinc in mature wethers and apparent total-tract digestion of Mg and Fe and retention of Ca, Fe, and Mg in wether lambs. These results indicate that phytate in diets formulated with Harrington variety barley grain may not be fully digested in the rumen. Subsequent passage of partially digested phytate from the rumen may interfere with mineral digestion in wethers fed high-grain diets.

Keywords: Phytate; M 955 mutant; Barley; Digestion; Mineral; Sheep; 31P-NMR

1. Introduction

Grains and oil seeds are high in phosphorus (P), of which the majority exists as phytate (Nelson et al., 1968). Unlike non-ruminant animals, ruminants can uniquely derive their daily-P need from phytate via rumen microbial phytase (Morse et al., 1992; Yanke et al., 1998; Guyton et al., 2003). The extent of phytate-P hydrolysis in ruminants varies considerably and may be incomplete resulting in undegraded phytate entering the small intestine. Dietary feed type (Konishi et al., 1999), intrinsic and endogenous phytase (Bravo et al., 2002; Kincaid et al., 2005), feed processing (Park et al., 2000), and decreasing forage:concentrate ratio (Bravo et al., 2003) may alter phytate-P hydrolysis in ruminants. Phytate can bind and inhibit digestive enzymes (Bedford, 1996) or complex with proteins (Gifford and Clydesdale, 1990) and micronutrients (Maenz et al., 1999), impairing digestion. Specifically, phytate alters digestion of calcium (Ca), iron (Fe), magnesium (Mg), and zinc (Zn) in non-ruminant animals (Sugiura et al., 1999; Veum et al., 2002; Hurrell, 2003).

Awareness of P excretion into the environment (Sharpley et al., 1994) has brought about an intensive search for, and subsequent identification of, mutant low-phytate cereal grains (*e.g.*, Raboy et al., 2000). These mutant grains contain similar amounts of total P as the non-mutant varieties, but have substantially less P existing as phytate. For example, the low-phytate mutant-M 955 barley grain has been reported to contain <0.2 mg phytate P/g, representing >90% reduction in grain phytate compared to the non-mutant Harrington variety, which contained >2.0 mg phytate P/g (Dorsch et al., 2003). Non-ruminant performance and P utilisation increased when low-phytate mutant grains were fed in place of non-mutant types (Veum et al., 2002; Jang et al., 2003). Furthermore, whether through inclusion of low-phytate mutants (Hambidge et al., 2005) or diet dephytinisation treatment (Fredlund et al., 2003), the reduction of dietary phytate enhanced absorption of minerals, such as Ca and Zn, in non-ruminant animals.

Considering that undegraded phytate may appear in the chyme of ruminant animals consuming high-grain diets (Konishi et al., 1999; Park et al., 2000; Bravo et al., 2003; Kincaid et al., 2005), inclusion of mutant low-phytate grains may be a strategy for reduc-

ing total dietary phytate, while maintaining recommended daily P need. Our objectives for this study were to determine the extent of phytate degradation through the digestive tract using phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) and to assess apparent P, Ca, Fe, Mg, and Zn absorption in wethers fed high-grain diets containing either the low-phytate mutant-M 955 or the non-mutant Harrington variety barley grain.

2. Materials and methods

2.1. Animal care and use

An Animal Care and Use Committee (USDA, ARS, U.S. Sheep Experiment Station, Dubois, ID) reviewed all procedures (protocol #304) described herein and approved the surgical placement of the cannulas and experimental use of the animals.

2.2. Experiment 1

2.2.1. Animals and treatment assignment

Mature Columbia wethers ($n = 8$; body weight mean ± standard deviation = 84.1 ± 4.2 kg), fitted with ruminal and duodenal cannulas, were used to determine the effects of two phytate barley-grain types on apparent partial- (rumen-complex) and total-tract P, Ca, Fe, Mg, and Zn digestion, and chyme- and faecal-P composition. Wethers were housed in an indoor climate- and light-controlled facility and placed in individual pens (160 cm × 240 cm) with feed and automatic-water receptacles. Before treatment assignment, wethers were fed an adaptation diet (Table 1) for 14 days. Subsequently, one of two finishing-diet treatments (Table 1) was randomly assigned to each wether: Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley-grain diets. Barley grains (whole berry), grown at the University of Idaho Agricultural Research and Extension Centre (Tetonia, ID), were incorporated into the diet as a total mixed ration. Mutant-M 955 barley grain had less total starch (618 g/kg *versus* 641 g/kg, respectively; starch availability determined according to Xiong et al. (1990) with modifications of Brown et al. (1998)) and greater N (Kjeldahl; 22 g/kg *versus* 19 g/kg, respectively) than the Harrington variety. As such, purified corn starch and urea were used to balance the starch and N contents of the treatment diets. Treatments were fed in two equal portions, twice daily (07:00 and 19:00 h), for 25 days at a targeted intake of 18 g/kg body weight (dry matter basis) to accommodate maintenance requirements of mature wethers (NRC, 1985). Weekly subsamples of treatment diets were collected and combined over the duration of the study to generate a composite sample that was stored at −20 °C. Immediately before sample collection (described below), one wether assigned to the M955 treatment was removed from the experiment due to continual displacement of the rumen cannula. Therefore, for all analyses in Period 1, there were $n = 3$ and 4 for M955 and HARR treatments, respectively. Because of the M955 experimental unit loss, we selected four wethers at the completion of sample collection (Period 1), having functional cannulas, and repeated the treatment protocol (Period 2). The four wethers were fed the original

Table 1
Experiment 1 and 2 feedstuff composition (dry matter basis) of the adaptation diet and feedstuff and nutrient composition the treatment diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Item	Adaptation diet[a]	Treatment diets	
		M955	HARR
Feedstuffs (g/kg)			
Barley grain (whole berry)	650	829	838
Alfalfa hay (2 cm chop)	278	90	99
CSB[b]	50	35	35
Corn starch	–	24	–
Urea	3	–	6
Trace mineral salt[c]	10	10	10
Limestone[d]	9	12	12
Nutrients			
Organic matter (g/kg)		950	943
N (g/kg)		22	22
Total P (mg/kg)		3441	3234
Water-soluble P (mg/kg)		2157	584
Ca (mg/kg)		7692	6195
Fe (mg/kg)		210	150
Mg (mg/kg)		1202	625
Zn (mg/kg)		30	19

[a] Harrington variety barley grain was used during the adaptation period (14 days).
[b] Condensed separator byproduct from sugarbeet molasses.
[c] Redmond natural trace mineral (NTM) salt, Redmond, UT. Composition (g/kg; dry matter basis): NaCl = 950; Ca = 5.5; Cu = 0.007; I = 0.02; Fe = 0.7; Mg = 0.9; Mn = 0.007; P=0.5; K = 1.2; S = 1.3.
[d] Thermocal Mines of Idaho, Dubois, ID. Composition (g/kg; dry matter basis): Ca = 380; Fe = 1.2; Mg = 4.3.

adaptation diet (Table 1) for 14 days and treatment and sampling protocols were repeated (Period 2). During Period 2, a minimal amount of chyme was collected from one wether assigned to the M955 treatment due to limited duodenal flow. Therefore, for partial-tract analyses from Period 2, there were $n = 1$ and 2, and for total-tract digestibility, and digesta-P composition analyses, there were $n = 2$ and 2 for M955 and HARR treatments, respectively.

2.2.2. Sample collection

Beginning on day 18 of treatment and throughout the remainder of the trial, wethers received a twice-daily intrarumen bolus of 2.5 g Cr_2O_3. Daily diet refusals were collected, weighed, subsampled (200 g/kg of wet weight), and stored. On day 25, immediately before feeding (hour 0), samples were obtained from the rumen, duodenum, and rectum of each wether, and repeated at 1.5 h intervals for 10.5 h. Duodenal (25 mL chyme) and rectal (10 g faeces) samples were lyophilised, ground (2 mm), and stored (−20 °C). Rumen samples were placed in a loose-mesh cotton cloth and pressed. The resultant fluid (15 mL) was centrifuged (27,000 × g, 10 min) and the supernatant was stored at −20 °C. For analysis, an equal subsample was obtained from each time-point sample and pooled across the sampling period to form duodenal, faecal, and rumen–fluid sample composites.

2.3. Experiment 2

2.3.1. Animals and treatment assignment

Growing Columbia × Polypay wether lambs ($n = 8$; body weight mean ± standard deviation = 53.5 ± 5.6 kg) were used to determine the effects of two phytate barley-grain types on P, Ca, Fe, and Mg balance and digestion, and faecal-P composition. Wethers were housed, fed an adaptation diet (Table 1), and received treatment assignment as described in Experiment 1. Treatments were fed (two equal portions twice daily at 07:00 and 19:00 h) for 15 days at a targeted intake of 30 g/kg body weight (dry matter basis) to accommodate rapid growth potential (NRC, 1985). Weekly subsamples of treatment diets were collected and combined over the duration of each study to generate a composite sample that was stored (−20 °C).

2.3.2. Sample collection

On days 10–15, wether lambs were placed in individual metabolism stations (43 cm × 137 cm) with feed and automatic-water receptacles. Daily diet refusals, faeces, and urine (acidified with 6N HCl to achieve a pH ∼ 2.0) were collected and weighed, and daily subsamples (200 g/kg wet weight) were pooled over the collection period and stored (−20 °C). Faecal composites were lyophilised and ground (2 mm) before analysis.

2.4. Sample analyses and calculations

Diet, faeces (Experiments 1 and 2), chyme (Experiment 1), and urine (Experiment 2) composites were analysed for P, Ca, Fe, Mg, and Zn (Zn analysis limited to Experiment 1) and water-soluble P (excluding urine) using inductively coupled plasma-optical emission spectroscopy (ICP-OES) as previously described (Turner and Leytem, 2004). For total P, Ca, Fe, Mg, and Zn, diet, faeces, and chyme were ground (0.5 mm) and digested (microwave-assisted) in duplicate (1 g dry matter or 1 mL of urine), in concentrated HNO_3 and H_2O_2 (300 g/kg), and subsequent digests were analysed. For water-soluble P, diet, faeces, and chyme were ground (0.5 mm) in triplicate (2 g) and transferred to a conical tube with 40 mL of deionised water. Contents were agitated for 1 h at 20 °C, centrifuged (10,000 × g for 30 min), and supernatant was removed and analysed. All samples, excluding urine, were analysed for organic matter (ashing procedure; AOAC, 1990) and acid detergent fibre (ADF; procedures of Van Soest et al. (1991) modified for use in an Ankom 200 fibre analyser; Ankom Technology, Fairport, NY). Diet, faeces and chyme composites from Experiment 1 were analysed for Cr (Fenton and Fenton, 1979). Rumen fluid (Experiment 1) total P, Ca, Fe, Mg, and Zn were determined using inductively coupled plasma-mass spectrometry (ICP-MS) after acid digestion (Utah Veterinary Diagnostic Laboratory, Logan, UT). Briefly, samples were digested in heated HNO_3 and diluted with ultrapure water to a final nitric acid concentration of 0.8 M (similar matrix of standards). The mineral contents of the diluted digests were calculated from a series of known standard preparations (Spex Certiprep, Metuchen, NJ).

^{31}P NMR spectroscopy (Turner, 2004) was used to determine P composition in the duodenal and faecal samples. Briefly, P was extracted in triplicate by shaking 2.00 ± 0.01 g of dried material with 40 mL of a solution containing 0.5 M NaOH and 0.05 M EDTA for 4 h

at 20 °C. Extracts were centrifuged at 10,000 × *g* for 30 min and aliquots analysed for total P by ICP–OES. The remaining solutions from the triplicate extracts were combined, frozen rapidly at −80 °C, lyophilised, and ground to a fine powder. Freeze-dried extracts were re-dissolved in 0.1 mL of D_2O (for signal lock) and 0.9 mL of a solution containing 1 M NaOH and 0.1 M EDTA, and then transferred to a 5 mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer (Rheinstetten, Germany) operating at 202.456 MHz for ^{31}P. A 5 μs pulse (45°), a delay time of 5.0 s, an acquisition time of 0.8 s, and broadband proton decoupling was used for all samples. The number of scans varied between 9000 and 14,000, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in parts per million (ppm) relative to 8.7 M H_3PO_4 and assigned to individual P compounds or functional groups based on literature reports (Turner et al., 2003). Signal areas were calculated from integration and P concentrations calculated by multiplying the proportion of total spectral area assigned to a specific signal by the total P concentration (g P/kg dry sample) in the original extract. This NMR procedure detects concentrations of P compounds of approximately 0.1 mg P/kg dry matter (Turner, 2004).

2.5. Statistical analysis

2.5.1. Experiment 1

Daily digesta flow into the duodenum and faeces excreted were estimated from the Cr_2O_3 marker concentration of the chyme and faeces composites as follows: digesta excreted (g/day) = (marker dosed (g/day)/marker in digesta (g/g)). Organic matter and nutrient intake, excretion, and apparent digestion coefficients were analysed using the mixed models procedure of SAS (2003). The model included treatment and period as fixed effects and the period × treatment interaction was considered a random effect. A treatment effect was significant when the probability of a greater *F*-test statistic was <0.05.

2.5.2. Experiment 2

Nutrient intake, excretion, digestion (apparent), and retention were analysed as a complete randomised design using the mixed models procedure of SAS (2003). The model included treatment as the fixed effect. A treatment effect was significant when the probability of a greater *F*-test statistic was <0.05.

3. Results

3.1. Effect of low-phytate barley on phosphorus composition of the diets, chyme, and faeces

The P composition of the two treatment diets (Experiments 1 and 2) varied greatly (Table 2). Inorganic phosphate was 0.71 g/g of total P (signal at approximately 6.1 ppm) in the diet formulated with low-phytate mutant-M 955 barley grain and was 0.20 g/g of total P in the diet formulated with the Harrington variety. Phytate P was 0.04 and 0.56 g/g of total P in the diets containing the mutant-M 955 and Harrington variety barley grains, respectively.

Table 2
Phosphorus distribution among various P compounds, determined by solution ^{31}P NMR spectroscopy, in treatment diets, chyme, and faeces from Experiments 1 and 2 where wethers were fed diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains (g P/kg (dry matter basis))

Treatments	Total NaOH–EDTA P[a]	Phosphate[b]	Phosphate monoesters[b,c]	Phosphate diesters[b]	Pyrophosphate[b]	Phytic acid[b]	Phosphonates[b]
Experiments 1 and 2: Diets (combined)							
M955	2.47 (0.70)	1.74 (0.71)	0.72 (0.29)	ND	0.017 (<0.01)	0.11 (0.04)	ND
HARR	2.16 (0.70)	0.42 (0.20)	1.73 (0.80)	ND	0.003 (<0.01)	1.21 (0.56)	ND
S.E.	0.11	0.08	0.04	–	0.003	0.06	–
P-value	0.19	0.01	<0.01	–	0.08	0.01	–
Experiment 1: Duodenal chyme							
M955	15.08 (0.99)	12.32 (0.80)	2.11 (0.14)	0.36 (0.02)	0.15 (<0.01)	0.11 (<0.01)	0.06 (<0.01)
HARR	14.48 (0.94)	10.54 (0.73)	3.16 (0.22)	0.40 (0.03)	0.17 (0.01)	0.04 (<0.01)	0.05 (<0.01)
S.E.	1.91	1.67	0.29	0.06	0.03	0.08	0.04
P-value	0.82	0.45	0.03	0.63	0.56	0.52	0.94
Experiment 1: Faeces							
M955	15.14 (0.97)	13.92 (0.92)	0.81 (0.05)	0.11 (<0.01)	0.29 (0.02)	ND	ND
HARR	15.62 (0.93)	13.78 (0.88)	1.23 (0.08)	0.17 (0.01)	0.35 (0.02)	0.18 (0.01)	0.05 (<0.01)
S.E.	1.61	1.63	0.18	0.03	0.07	–	–
P-value	0.83	0.95	0.11	0.22	0.50	–	–
Experiment 2: Faeces							
M955	12.89 (0.97)	10.41 (0.81)	1.72 (0.14)	0.24 (0.02)	0.34 (0.03)	0.14 (0.01)	0.03 (<0.01)
HARR	11.98 (0.95)	9.06 (0.76)	1.52 (0.17)	0.37 (0.03)	0.39 (0.03)	0.56 (0.05)	0.07 (<0.01)
S.E.	1.31	1.48	0.32	0.11	0.04	0.20	0.03
P-value	0.64	0.54	0.67	0.42	0.42	0.19	0.34

[a] Values are total P extracted with NaOH–EDTA; values in parenthesis are the proportions (g/g) of the total P determined using inductively coupled plasma-optical emission spectroscopy following microwave-assisted digestion in concentrated HNO_3 and H_2O_2.
[b] Values in parenthesis are the proportions (g/g) of the NaOH–EDTA extracted P.
[c] Phosphate monoesters includes all monoester forms of P including phytic acid and the lower inositol esters of phytic acid. ND = not detected. S.E. = standard error of the mean.

In Experiment 1, irrespective of grain source, the majority of P in the chyme was inorganic phosphate (Table 2). The only difference in chyme-P concentrations between treatments was for the phosphate monoesters (includes phytate P and all lower inositol phosphate esters). The concentration of phosphate monoesters in HARR treatment was greater than M955 treatment (3.16 g/kg *versus* 2.11 g/kg DM, respectively; P<0.05). The proportion of P present as phosphate monoesters was greater in HARR than in M955 treatment (0.22 g/g *versus* 0.14 g/g of total P, respectively; P<0.05); inversely, the amount of P present as inorganic P was less in HARR than in M955 treatment (0.73 g/g *versus* 0.80 g/g of total P; P<0.05). The remaining P fractions were similar between treatments. Barley grain variety did not influence P composition of faecal samples and, as with the chyme, the majority of P in the faeces was inorganic phosphate regardless of treatment (Table 2). A small amount of phytate P was detected in the faeces from HARR treatment wethers, while none was found in faeces from M955 treated wethers. Phosphate diesters and pyrophosphate were also detected in small amounts in all faeces, with a minimal amount of phosphonates detected in the faeces from HARR treatment wethers.

In Experiment 2, treatment did not affect P composition of faeces (Table 2). The majority of P excreted from the animals was in the form of inorganic phosphate, which comprised 0.81 and 0.76 g/g of total P excreted from M955 and HARR treated wethers, respectively. The remaining P was in the form of phosphate monoesters (including phytate), phosphate diesters, pyrophosphate and phosphonates.

3.2. *Effect of low-phytate barley on phosphorus digestion*

In Experiment 1 (Table 3), mature wethers consumed, digested (apparent partial- and total-tract), and excreted similar (P=0.55–0.97) amounts of total P regardless of the barley-grain type consumed. As would be expected, wethers fed mutant-M 955 barley grain consumed more (numerically; P=0.09) soluble P than those fed the Harrington variety (3620 mg/day *versus* 1007 mg/day, respectively). Despite this, the amount of soluble P reaching the duodenum and excreted in faeces was similar (P=0.43 and 0.99) between treatments. This resulted in HARR treatment wethers having a negative partial-tract digestion (apparent) coefficient ($-5.15 < 0$; P=0.02).

The wether lamb responses to treatment in Experiment 2 (Table 4) was similar to the mature wethers, whereas total P consumed, digested, and retained did not differ (P=0.28–0.46) between treatments. Proportional to intake, soluble P excreted was greater (P=0.02) in HARR than M955 treatment wethers; a similar tendency (P=0.07) was observed for mature wethers (Table 3). Because of higher (1.4–1.5 times) dietary intake to accommodate growth, Experiment 2 wether lambs consumed more total P per unit of body weight than the Experiment 1 mature wethers. However, this seemed to have no bearing on total- and soluble-P apparent total-tract digestion (absorption) response to the barley grain variety.

3.3. *Effect of low-phytate barley on mineral digestion and metabolism*

In Experiment 1 (Table 5), apparent total-tract digestion (absorption) of Fe, Mg, and Zn was greater (P<0.01) in wethers fed diets formulated with the low-phytate mutant-M 955

Table 3
Phosphorus digestion in mature wethers consuming diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Item	Treatments[a]		P-value[b]
	M955[c]	HARR[c]	
Total P			
Intake (mg/day)	5756 ± 385	5550 ± 351	0.70
Reaching the duodenum (mg/day)	8691 ± 1001	7765 ± 970	0.58
Excreted in faeces (mg/day)	4532 ± 539	4506 ± 492	0.97
Partial-tract digestibility[d,e]	−0.53 ± 0.13	−0.43 ± 0.11	0.55
Total-tract digestibility[d]	0.22 ± 0.08	0.18 ± 0.07	0.69
Soluble P			
Intake (mg/day)	3620 ± 287	1007 ± 272	0.09
Reaching the duodenum (mg/day)	7520 ± 1055	6057 ± 1013	0.43
Excreted in faeces (mg/day)	232 ± 86	230 ± 81	0.99
Partial-tract digestibility[d,e]	−1.11 ± 0.44)	−5.15 ± 0.38	0.02
Total-tract digestibility[d]	0.94 ± 0.06	0.76 ± 0.06	0.07

[a] For intake and faecal variables, $n = 5$ and 6 for M955 and HARR, respectively. For duodenal variables, $n = 4$ and 6 for M955 and HARR, respectively.
[b] *F*-statistic probability.
[c] Least squares means (±standard error of the mean) are presented.
[d] Apparent digestibility coefficients (g/g) do not account for endogenous nutrient contribution.
[e] Partial-tract digestibility based on samples obtained from the duodenum.

Table 4
Phosphorus digestion in wether lambs consuming diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Item	Treatments[a,b]		S.E.	P-value[c]
	M955	HARR		
Total phosphorus				
Intake (mg/day)	5207	4894	282	0.46
Excreted in faeces (mg/day)	4711	4012	419	0.28
Excreted in urine (mg/day)	56	179	91	0.38
Digestibility[d]	0.09	0.18	0.07	0.38
Balance (mg/day)	440	704	275	0.52
Soluble phosphorus				
Intake (mg/day)	3361	927	123	<0.01
Excreted in faeces (mg/day)	1054	929	201	0.68
Digestibility[d]	0.69	0.03	0.14	0.02

[a] $n = 4$ per treatment.
[b] Least squares means and standard error (S.E.) of the means are presented.
[c] *F*-statistic probability.
[d] Apparent digestibility coefficients (g/g) do not account for endogenous nutrient contribution.

as opposed to the normal-phytate Harrington variety barley grain. Apparent partial-tract (rumen-complex) digestion of Ca and Mg was greater (P<0.05) in M955 treatment wethers (Table 5). Barley grain variety did not influence apparent total-tract digestion of Ca (P=0.15). Likewise, in Experiment 2 (Table 6), apparent digestion of Mg and Fe was greater (P<0.01) in wether lambs fed the mutant-M 955 compared to the Harrington variety barley grain diet, and a similar tendency (P=0.07) was observed for Ca digestion. As a result, retention of Mg, Fe, and Ca was greater (P<0.04) in M955 than HARR treatment wether lambs (Table 6). Introduced earlier, Experiment 2 wether lambs were fed, in proportion to body weight, more diet on a daily basis than Experiment 1 wethers to accommodate a greater growth potential.

Table 5
Mineral digestion in mature wethers consuming diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Item	Treatments[a]		P-value[b]
	M955[c]	HARR[c]	
Calcium			
Intake (mg/day)	13068 ± 1891	10668 ± 1870	0.35
Reaching the duodenum (mg/day)	5213 ± 804	7099 ± 656	0.11
Excreted in faeces (mg/day)	10292 ± 1876	11832 ± 1792	0.46
Partial-tract digestibility[d,e]	0.56 ± 0.11	0.31 ± 0.09	0.05
Total-tract digestibility[d]	0.20 ± 0.09	−0.10 ± 0.08	0.15
Iron			
Intake (mg/day)	352 ± 22	257 ± 20	0.01
Reaching the duodenum (mg/day)	150 ± 53	229 ± 43	0.28
Excreted in faeces (mg/day)	238 ± 21	275 ± 19	0.22
Partial-tract digestibility[d,e]	0.56 ± 0.20	0.11 ± 0.16	0.12
Total-tract digestibility[d]	0.33 ± 0.04	−0.07 ± 0.03	<0.01
Magnesium			
Intake (mg/day)	2014 ± 135	1061 ± 125	<0.01
Reaching the duodenum (mg/day)	706 ± 60	815 ± 49	0.19
Excreted in faeces (mg/day)	289 ± 50	374 ± 46	0.24
Partial-tract digestibility[d,e]	0.63 ± 0.06	0.24 ± 0.06	<0.01
Total-tract digestibility[d]	0.86 ± 0.04	0.64 ± 0.03	<0.01
Zinc			
Intake (mg/day)	51.1 ± 9.4	32.0 ± 9.3	<0.01
Reaching the duodenum (mg/day)	70.6 ± 17.3	95.0 ± 14.1	0.31
Excreted in faeces (mg/day)	103 ± 19	112 ± 18	0.67
Partial-tract digestibility[d,e]	−0.59 ± 0.61	−2.33 ± 0.53	0.17
Total-tract digestibility[d]	−1.06 ± 0.40	−2.81 ± 0.37	<0.01

[a] For intake and faecal variables, $n=5$ and 6 for M955 and HARR, respectively. For duodenal variables, $n=4$ and 6 for M955 and HARR, respectively.
[b] *F*-statistic probability.
[c] Least squares means (±standard error of the mean) are presented.
[d] Apparent digestibility coefficients (g/g) do not account for endogenous nutrient contribution.
[e] Partial-tract digestibility based on samples obtained from the duodenum.

Table 6
Mineral digestion in wether lambs consuming diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Item	Treatments[a,b]		S.E.	P-value[c]
	M955	HARR		
Calcium				
Intake (mg/day)	12903	10380	641	0.03
Excreted in faeces (mg/day)	8385	7981	591	0.64
Excreted in urine (mg/day)	158	61	27	0.04
Digestibility[d]	0.35	0.23	0.04	0.07
Balance (mg/day)	4360	2337	523	0.04
Iron				
Intake (mg/day)	305	224	14	<0.01
Excreted in faeces (mg/day)	191	185	13	0.76
Excreted in urine (mg/day)	0.87	0.68	0.31	0.68
Digestibility[d]	0.37	0.18	0.04	<0.01
Balance (mg/day)	114	39	11	<0.01
Magnesium				
Intake (mg/day)	1818	989	76	<0.01
Excreted in faeces (mg/day)	1585	1522	134	0.75
Excreted in urine (mg/day)	947	769	67	0.11
Digestibility[d]	0.13	−0.54	0.08	<0.01
Balance (mg/day)	−715	−1301	149	0.03

[a] $n = 4$ per treatment.
[b] Least squares means and standard error (S.E.) of the means are presented.
[c] F-statistic probability.
[d] Apparent digestibility coefficients (g/g) do not account for endogenous nutrient contribution.

As with P, daily Mg, Fe, and Ca intakes for wether lambs were 1.4–1.5 times greater per unit of body weight than mature wethers. Regardless, digestibility of these minerals was consistently greater in wethers when the low-phytate mutant-M 955 compared to the normal-phytate Harrington variety barley grain was included in the diet. In Experiment 1, rumen

Table 7
Mineral concentration (mM) of rumen fluid supernatant (27,000 × g, 10 min) from mature wethers consuming diets formulated with either Harrington (HARR) variety or mutant-M 955 (M955; Dorsch et al., 2003) barley grains

Mineral	Treatments[a]		P-value[b]
	M955[c]	HARR[c]	
Phosphorus	43.2 ± 3.3	33.5 ± 3.1	0.07
Calcium	17.1 ± 2.4	19.0 ± 2.2	0.57
Iron	0.22 ± 0.03	0.22 ± 0.03	0.98
Magnesium	5.8 ± 0.5	5.3 ± 0.5	0.52
Zinc	0.16 ± 0.02	0.15 ± 0.02	0.77

[a] $n = 5$ and 6 for M955 and HARR, respectively.
[b] F-statistic probability.
[c] Least squares means (±standard error of the means) are presented.

fluid (supernatant) P tended to be greater (P=0.07) in M955 than HARR treated wethers, but Ca, Fe, Mg, and Zn concentrations were similar (P=0.52–0.98) between treatments (Table 7).

Post-experiment analysis of Experiments 1 and 2 treatment diets, revealed that mature wethers (Table 5) and wether lambs (Table 6) fed the mutant-M 955 treatment diets consumed more (P<0.03) daily Mg, Fe, and Zn, and Mg, Fe, and Ca, respectively, than those fed the HARR treatment. This was due to greater concentration of these minerals in the M955 diet than in the HARR (Table 1). The mutant-M 955 barley grain had greater (mg/kg DM) Ca (733 *versus* 561), Fe (48.3 *versus* 39.5), Mg (1427 *versus* 1185), and Zn (29.4 *versus* 22.6) than the Harrington variety. However, based on the level of grain inclusion (>810 g/kg DM; Table 1), these differences in the grain do not account for the proportionally greater Fe, Mg, and Zn in the M955 *versus* HARR treatment diets. It seems that the differences are partly due to the limestone and trace-mineral salt supplements added to the diets.

4. Discussion

Dietary replacement of non-mutant Harrington variety with the low-phytate mutant-M 955 barley grain had no effect on total P intake, excretion, or apparent total-tract P digestion in wethers. This indicates that, irrespective of the dietary phytate content, the rumen microbial phytase readily hydrolysed phytate P. In addition to microbial phytase, high intrinsic phytase activity, associated with the barley grain (Yingran et al., 2005), may increase the extent of ruminal phytate P hydrolysis. As such, intrinsic or exogenous phytase may ameliorate measurable differences in total-tract P digestibility (Kincaid et al., 2005). Substantially lower water-soluble P was available in Harrington barley based diets. Regardless, similar amounts of soluble P arrived in the duodenum of the HARR and M955 treatment wethers. Again, this seems to indicate extensive preduodenum microbial and(or) intrinsic phytase activity. The low soluble P intake in conjunction with a high amount of ruminal-P solubilisation, resulted in HARR treatment wethers having a negative partial-tract digestion (apparent) coefficient (−5.15). This implies a much greater release of soluble P from the rumen-complex than consumed and that phytate P was readily hydrolysed in the rumen. Likewise, additional dietary phytate did not affect total P apparent digestibility in lactating dairy cows (Guyton et al., 2003), and rumen microbes, *in vitro*, readily hydrolysed the phytate P added in the form of common feedstuffs (Morse et al., 1992). There is evidence suggesting incomplete hydrolysis of dietary phytate in ruminants due to feedstuff type (Konishi et al., 1999), intrinsic feed phytase (Kincaid et al., 2005), feed processing (Park et al., 2000), addition of exogenous phytase (Bravo et al., 2002; Kincaid et al., 2005), and decreasing forage:concentrate ratio (Bravo et al., 2003). Taken together, it seems that the use of total-P apparent-digestion estimates to indirectly assess the extent of phytate digestion in ruminants may not be appropriate due to lack of specificity and(or) sensitivity.

Using ^{31}P NMR characterisation, a greater concentration of monoester phosphates were found in the duodenal chyme of mature wethers fed the HARR barley treatment, despite similar amounts of soluble P appearing in the duodenum of M955 and HARR treatment wethers. The monoester phosphate fraction would include phytate and the breakdown products of

phytate (lower inositol phosphate esters). Based on this evidence, phytate in the HARR treatment was not completely hydrolysed in the rumen resulting in a small portion of the phytate and the lower inositol esters of phytate to appear in the duodenum. In support, approximately 30% of phytate in rapeseed meal was not degraded in the rumen of sheep (Konishi et al., 1999; Park et al., 1999) and 22% of phytate in rapeseed meal reached the duodenum of sheep (Park et al., 2000).

Dietary inclusion of the low-phytate mutant-M 955 barley grain in place of the Harrington variety resulted in greater apparent digestion (absorption) of Mg, Fe, and Zn in mature wethers and Mg, Fe, and Ca in wether lambs. Indications of altered mineral digestion were observed in the foregut and became readily pronounced as the digesta passed from the rumen-complex and through the small and large intestines of the mature wethers (Table 5). Ultimately, use of the Harrington variety grain in diets decreased retention of Mg, Fe, and Ca in wether lambs. Several plausible explanations for this alteration of mineral metabolism are available in the literature.

First, the formation of mineral–phytate and mineral–inositol ester complexes in the rumen and small intestine could result in reduced mineral absorption. Formation of such complexes in the rumen may, in turn, alter the extent of phytate hydrolysis. For example, mineral–phytate complexes reduced microbial–phytase hydrolysis of phytate (Maenz et al., 1999) with the ranked potency of these inhibitors being $Zn^{2+} \gg Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ (pH 7). Furthermore, ternary complexes of phytate, Ca^{2+}, and protein may be formed (Cheryan, 1980). Such complexes seem responsible for phytate induced alteration of Mg (Bohn et al., 2004), Ca, Fe, and Zn absorption (Hurrell, 2003) in humans. Regardless of whether mineral absorption was inhibited or phytate hydrolysis was impaired in HARR treatment wethers, identification of undegraded phytate in the duodenal chyme correspond with the lower partial- and total-tract apparent mineral digestion coefficients.

Second, absorption of P, Ca, and Mg across the ruminal wall is enhanced depending on the concentration of these minerals, alone and in proportion to each other, in the rumen liquid phase. Magnesium and Ca, to a lesser extent, are absorbed across the ruminal wall (Sklan and Hurwitz, 1985; Joblin and Lee, 1990). Calcium absorption is positively correlated with Ca concentration at the ruminal mucosal surface (Schroder et al., 1997; Höller et al., 1988). The lower presence of phytate in the rumen of wethers fed the mutant-M 955 grain may have resulted in greater, compared to wethers fed the Harrington variety, concentration of absorbable Ca at the mucosal surface. In support, the apparent Ca partial-tract digestion was greater in mature wethers fed the mutant-M 955 grain. Inorganic P concentration of rumen fluid may also play an important role in Ca and Mg absorption. As inorganic P increased, Ca (Beardswoth et al., 1989; Dua and Care, 1999) and Mg (Dua and Care, 1999) absorption increased in the rumen of sheep. Furthermore, as the P:Ca ratio was increased, net flux of Ca across the rumen wall shifted from secretion to absorption (Höller et al., 1988). This information together with the known high soluble-P and low-phytate-P intake of M955 treatment wethers suggests a ruminal environment more suitable for greater Ca and Mg transfer across the ruminal wall than would occur in HARR treatment wethers. Phosphorus concentration of rumen fluid supernatant from M955 treatment mature wethers was slightly greater than fluid HARR treatment wethers.

5. Conclusion

Dietary inclusion of the mutant-M 955 low-phytate barley grain, in place of the Harrington variety, in high-grain diets fed to wethers decreased the amount of undegraded phytate passing into the duodenum and seemed to increase mineral absorption and retention. Based on this evidence, dietary substitution with mutant low-phytate grains may reduce mineral wastage in sheep fattening and finishing operations.

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