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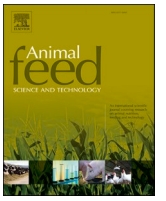

Short communication

Effect of acidification or fermentation of barley grain using Limosilactobacillus reuteri or Weissella cibaria on inositol phosphate hydrolysis in vitro

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ABSTRACT

Fermentation of cereal grains may hydrolyse *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (**InsP$_6$**) and increase concentration of lower order inositol phosphates (**InsP**) in vitro, thereby increasing nutrient digestibility in diets of swine and poultry. Effects of chemical acidification or fermentation with the lactic acid-producing bacteria *Limosilactobacillus reuteri* or *Weisella cibaria* of high β-glucan hull-less barley grain on pH, organic acid concentration, InsP$_6$ hydrolysis and lower order InsP formation were assessed in vitro. In 3 batches, ground barley grain was treated in four forms balanced for water content: 1) unfermented barley (**Control**); 2) chemically acidified barley with lactic acid and acetic acid (0.019 L/kg barley grain at a ratio of 4:1 (volume/volume)) (**ACD**); 3) barley fermented with *L. reuteri* TMW 1.656 (Fermented with *L. reuteri*); and 4) barley fermented with *W. cibaria* 10 M FUA3120 (Fermented with *W. cibaria*). For 24 h in an incubator, ACD and Fermented with *L. reuteri* were incubated at 37 °C and Fermented with *W. cibaria* at 30 °C. Barley inositol phosphate$_6$-phosphorus (**InsP$_6$-P**) was 68%, 64% and 41% lower, respectively, for ACD, Fermented with *L. reuteri* or Fermented with *W. cibaria* than Control. Barley grain pH after 24 h, concentration of InsP$_6$-P, inositol phosphate$_5$-phosphorus (**InsP$_5$-P**), inositol phosphate$_4$-phosphorus (**InsP$_4$-P**) and sum inositol phosphate$_{6-2}$-phosphorus (**InsP$_{6-2}$-P**) were lower ($P < 0.05$) for ACD and Fermented barley grain than Control and Fermented with *L. reuteri* than *W. cibaria*. Acetate, lactate, inositol phosphate$_3$-phosphorus (**InsP$_3$-P**) and inositol phosphate$_2$-phosphorus (**InsP$_2$-P**) concentration was greater ($P < 0.05$) for ACD and Fermented barley grain than Control. Lactate and InsP$_2$-P concentration were greater ($P < 0.01$) for Fermented with *L. reuteri* than *W. cibaria*. Total P release, cumulative P release of InsP$_6$-P, InsP$_5$-P, InsP$_4$-P and InsP$_3$-P were greater ($P < 0.01$) for Fermented with *L. reuteri* than *W. cibaria*. In conclusion, lower pH in ACD and Fermentation indicated that acidification is important for InsP$_6$ hydrolysis via intrinsic phytase activation in barley grain in vitro. Between strains,

Abbreviations: AA, amino acid; ACD, chemically-acidified barley; ADF, acid detergent fibre; CDC, Crop Development Centre; CFU, colony-forming unit; CP, crude protein; DM, dry matter; GE, gross energy; HPLC, high-performance liquid chromatography; InsP, inositol phosphate; InsP$_2$, inositol phosphate$_2$; InsP$_3$, inositol phosphate$_3$; InsP$_4$, inositol phosphate$_4$; InsP$_5$, inositol phosphate$_5$; InsP$_6$, *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); InsP-P, inositol phosphate- phosphorus; InsP$_2$-P, inositol phosphate$_2$- phosphorus; InsP$_3$-P, inositol phosphate$_3$- phosphorus; InsP$_4$-P, inositol phosphate$_4$- phosphorus; InsP$_5$-P, inositol phosphate$_5$- phosphorus; InsP$_6$-P, inositol phosphate$_6$- phosphorus; sum InsP$_{6-2}$-P, inositol phosphate$_{6-2}$- phosphorus; MRS, De Man, Rogosa and Sharpe; NDF, neutral detergent fibre; TDF, total dietary fibre; trt, treatment.

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fermentation with *L. reuteri* maximised acidification, lactate concentration and degradation of $InsP_6$-P, $InsP_5$-P, $InsP_4$-P in barley grain.

1. Introduction

Barley grain remains an important feed grain especially in Northern hemispheres (AAFC, 2023; FAOSTAT, 2023). Barley grain contains 4.30 g total P/kg dry matter (**DM**) (Rodehutscord et al., 2016); however, a major part of P (about 75%) is stored as inositol phosphate$_6$-phosphorus (**$InsP_6$-P**)) and its salt phytate (Stewart et al., 1988). In monogastric animals, hydrolysis of $InsP_6$ remains incomplete as the small intestine lacks sufficient endogenous phytase (Selle and Ravindran, 2008). *Myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (**$InsP_6$**) acts as antinutritive factor, because it reduces the absorption of protein and minerals (Woyengo and Nyachoti, 2013).

Sourdough fermentation reduces pH promoting $InsP_6$ hydrolysis by activating intrinsic phytase of barley grain (Leenhardt et al., 2005) and by increasing $InsP_6$ solubility (Grynspan and Cheryan, 1983). As a result, $InsP_6$ is hydrolysed to lower order inositol phosphate (**InsP**) forms inositol phosphate$_4$ (**$InsP_4$**) to inositol phosphate$_2$ (**$InsP_2$**) prior to feeding that can be almost entirely hydrolysed by pigs by the end of the gastro-intestinal tract (Rosenfelder-Kuon et al., 2020). However, effects of fermentation of barley grain using different lactic acid-producing bacteria on InsP hydrolysis have rarely been reported. Previously, fermentation of highly fermentable and high β-glucan hull-less barley grain with *Limosilactobacillus reuteri* decreased $InsP_6$ by 54% and thereby increased apparent total tract digestibility of DM, crude protein (**CP**) and gross energy (**GE**) (Heyer et al., 2021).

Barley grain β-glucan content ranges from 44 to 103 g/kg of DM (Fouhse et al., 2017). The hypotheses of the present study were that chemical acidification or fermentation of high β-glucan barley grain using lactic acid-producing bacteria would hydrolyse $InsP_6$

Table 1
Analysed nutrient content of highly fermentable and high β-glucan hull-less barley grain.

Item, g/kg dry matter	Hull-less barley[a]
Gross energy (MJ/kg)	18.9
Starch	543.6
Crude protein (N × 6.25)[b]	143.0
Ether extract	13.2
Total dietary fibre	166.5
Soluble fibre	0.20
Insoluble fibre	156.3
Neutral detergent fibre	161.3
Acid detergent fibre	24.2
Crude fibre	15.7
β-glucan[c]	85.4
Ash	21.1
Calcium	0.36
Total phosphorus	4.09
Inositol phosphates[d,e]	
$InsP_6$	7.58
$InsP_6$-P	2.12
$InsP_5$-P	0.45
$InsP_4$-P	1.43
$InsP_3$-P	0.33
$InsP_2$-P	0.11

[a] Fibar; Crop Development Centre, Saskatoon, SK, Canada.

[b] Indispensable amino acids (g/kg dry matter): arginine, 6.6; histidine, 3.0; isoleucine, 5.2; leucine, 9.7; methionine, 2.1; phenylalanine, 7.4; threonine, 4.5; tryptophan, 1.4; valine, 7.1; dispensable amino acids (g/kg dry matter): alanine, 5.5; aspartic acid, 7.9; cystine, 3.1; glutamic acid, 35.3; glycine, 5.4; proline, 15.6; serine, 5.3; tyrosine, 3.9; total amino acids, 136.6; chemically-available lysine, 4.9.

[c] β-glucan content was analysed in CDC Fibar barley grain of a different harvest year (Fouhse et al., 2017).

[d] Inositol phosphates were measured in Control barley grain. Control was not incubated.

[e] $InsP_6$, *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_2$-P, inositol phosphate$_2$-phosphorus; $InsP_3$-P, inositol phosphate$_3$-phosphorus; $InsP_4$-P, inositol phosphate$_4$-phosphorus; $InsP_5$-P, inositol phosphate$_5$-phosphorus; $InsP_6$-P, inositol phosphate$_6$-phosphorus; P, phosphorus.

thereby increasing lower order InsP in vitro and that using *L. reuteri* during fermentation would degrade $InsP_6$ further than *W. cibaria* because *L. reuteri* produces more lactate and acetate. To account for the pH-dependent activation of InsP hydrolysis by intrinsic phytase, a chemically acidified control was used. The objective was to compare the content of different InsP forms of barley grain that was chemically acidified or fermented using *L. reuteri* or *W. cibaria*.

2. Materials and methods

A highly fermentable and high β-glucan hull-less barley grain cultivar (Fibar; Crop Development Centre [**CDC**], Saskatoon, SK, Canada) was test ingredient (Fouhse et al., 2017; Table 1). Barley grain was ground using a 1-mm screen in a centrifugal mill (model ZM200; Retsch, Haan, Germany). Ground barley grain was treated as follows balanced for water content in 3 batches with each fermentation starting with a different preculture: 1) unfermented barley (**Control**); 2) chemically acidified barley (**ACD**); 3) barley fermented with *L. reuteri* TMW1.656 (**Fermented with *L. reuteri***); 4) barley fermented with *W. cibaria* 10 M FUA3120 (**Fermented with *W. cibaria***). The Control barley was mixed with an equal weight of sterile deionized water prior to sampling. The ACD barley was prepared by adding sterile deionized water, lactic acid (800 g/kg; MilliporeSigma Canada Co., Oakville, ON, Canada) and glacial acetic acid (MilliporeSigma Canada Co., Oakville, ON, Canada) in 4:1 ratio (volume/volume; 0.019 L/kg barley grain) (Le et al., 2016). The ACD was incubated for 24 h at 37 °C. For the fermented barley grain, the sourdough isolates *L. reuteri* TMW1.656 or *W. cibaria* 10 M FUA3120 were used. These strains were grown on modified De Man, Rogosa and Sharpe (**MRS**) agar (Meroth et a, 2003), subcultured in mMRS broth and incubated anaerobically at 37 °C for *L. reuteri* and 30 °C for *W. cibaria* (Yang et al., 2015). Cells were harvested by centrifugation and resuspended in an equal volume of sterile tap water. The barley fermentation was started by mixing ground barley with an equal weight of culture of *L. reuteri* or *W. cibaria* in tap water to achieve an initial cell count of about 10^8 colony-forming units (**CFU**)/g. The mixture was incubated for 24 h at 37 °C for *L. reuteri* and 30 °C for *W. cibaria*. Microbial cell counts and pH were measured as described in Heyer et al. (2021). Observation of a uniform colony morphology matching the strain that was used as inoculum was used to verify the identity of the fermentation microbes with the inoculum.

Barley grain was analysed for moisture (method 930.15; AOAC, 2006), CP (method 990.03; N × 6.25; AOAC, 2006), GE using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany), ash (method 942.05; AOAC, 2006), ether extract (method 920.39 A; AOAC, 2006), starch (assay kit STA-20; Sigma, St. Louis, MO, USA), crude fibre (method 978.10; AOAC, 2006), acid detergent fibre (**ADF**) inclusive of residual ash (method 973.18; AOAC, 2006), neutral detergent fibre (**NDF**) assayed without heat stable amylase and expressed inclusive of residual ash (Holst, 1973), total dietary fibre (**TDF**), soluble and insoluble dietary fibre (method 991.43; AOAC, 2006), Ca and P (method 985.01; AOAC, 2006). The amino acid (**AA**) content in barley grain was analysed by high-performance liquid chromatography (**HPLC**) (method 982.30E; AOAC, 2006) and chemically available lysine was determined by spectrophotometry (method 975.44; AOAC, 2006). Concentration of acetate and lactate was analysed by HPLC (Zhang and Gänzle, 2010). The content of $InsP_6$, inositol $phosphate_5$ (**$InsP_5$**), $InsP_4$, inositol $phosphate_3$ (**$InsP_3$**) and $InsP_2$ was determined according to Newkirk and Classen (1998) by HPLC on an Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) (Heyer et al., 2022).

The inositol $phosphate_{6–2}$-P (**$InsP_{6–2}$-P**) was calculated as the sum of all detected inositol phosphate-P (**InsP-P**) ($InsP_6$-P to inositol $phosphate_2$-P (**$InsP_2$-P**)) in the sample. Total P release (g/kg DM) for the three treatments (**trt**) ACD, Fermented *L. reuteri* and *W. cibaria* was calculated by subtracting the sum of $InsP_6$-P – $InsP_2$-P (g/kg DM) for each trt from Control. The cumulative degraded $InsP_x$ (μmol/g DM) for trt considering the degradation of all greater InsP ($InsP_{>x}$) was calculated using the following equations:

$InsP_{6\ degraded} = InsP_{6\ Control} - InsP_{6\ trt}$.

$InsP_{5\ degraded} = InsP_{6\ degraded} + [InsP_{5\ Control} - InsP_{5\ trt}]$.

$InsP_{4\ degraded} = InsP_{5\ degraded} + [InsP_{4\ Control} - InsP_{4\ trt}]$.

$InsP_{3\ degraded} = InsP_{4\ degraded} + [InsP_{3\ Control} - InsP_{3\ trt}]$.

$InsP_{2\ degraded} = InsP_{3\ degraded} + [InsP_{2\ Control} - InsP_{2\ trt}]$.

The P release of degraded $InsP_x$ (g/kg DM) was calculated by multiplying by the molecular weight of P (31.0 g P/mol): P released (1 phosphate group) $InsP_x = InsP_{x\ degraded} \times 31.0$.

Data were analysed using the MIXED procedure of SAS (2016) with 3 incubation observations per treatment. Normality and homogeneity of variance of the residuals for each variable were confirmed prior to the ANOVA. Treatment was the fixed effect and batch

Table 2
The pH and acetate and lactate concentration of acidified or fermented hull-less barley grain[a,b].

Item	Control	ACD	Fermented		SEM	P-value[c]		
			L. reuteri	*W. cibaria*		Acid	Fermentation	Bacteria
pH after 24 h incubation[d]	5.89	3.90	3.76	4.13	0.05	<0.001	<0.001	<0.001
Acetate, mMol/kg fresh matter	0.00	9.95	29.5	21.9	4.04	0.049	<0.001	0.112
Lactate, mMol/kg fresh matter	0.00	73.1	103.7	47.5	6.59	<0.001	<0.001	<0.001

[a] Control barley; ACD, chemically-acidified barley; Fermented *L. reuteri*, fermented barley with *Limosilactobacillus* (*L.*) *reuteri*; Fermented *W. cibaria*, fermented barley with *Weissella* (*W.*) *cibaria*.

[b] Least squares means based on 3 observations per treatment.

[c] Acid = acid addition (Control vs. ACD); Fermentation = Control vs. Fermented *L. reuteri* and Fermented *W. ciberia*; Bacteria = Fermented *L. reuteri* vs. Fermented *W. cibaria*.

[d] Control was not incubated and the pH was measured shortly after mixing with deionized sterile water. The pH before incubation for ACD, Fermented *L. reuteri* and Fermented *W. cibaria* was 3.93, 5.67 and 5.33.

was the random effect in the model. Single-degree of freedom contrasts were used to test effect of acidification (Control vs. ACD), fermentation (Control vs. Fermented *L. reuteri* and *W. ciberia*) and bacteria strain (Fermented *L. reuteri* vs. *W. cibaria*) on pH, organic acid and InsP (Littell et al., 2006). Data are presented as least squares means with SEM. To test the hypotheses, $P < 0.05$ was considered significant and $0.05 \leq P < 0.10$ was considered a trend.

3. Results

Total P content of barley grain was 4.09 g/kg DM of which 52% were bound as $InsP_6$-P (Table 1). The pH after 24 h in vitro incubation of barley grain was lower ($P < 0.001$) for ACD or Fermented barley grain than Control, and for Fermented *L. reuteri* compared to Fermented *W. cibaria* (Table 2). Both fermentation cultures are heterofermentative lactobacilli and produce lactate, acetate, and ethanol from hexoses. Acetate and lactate concentration of barley grain was greater ($P < 0.05$) for ACD and Fermented barley grain than Control and lactate concentration was greater ($P < 0.001$) for Fermented *L. reuteri* than Fermented *W. cibaria*. The concentration of $InsP_6$-P, inositol phosphate$_5$-P (**$InsP_5$-P**), inositol phosphate$_4$-P (**$InsP_4$-P**) and the sum of $InsP_6$-P to $InsP_2$-P was lower ($P < 0.05$) for ACD or Fermented barley grain than Control and for Fermented *L. reuteri* than Fermented *W. cibaria* (Table 3). The inositol phosphate$_3$-P (**$InsP_3$-P**) and $InsP_2$-P concentration was greater ($P < 0.001$) for ACD or Fermented barley grain than Control and $InsP_2$-P concentration was greater ($P < 0.01$) for Fermented *L. reuteri* than Fermented *W. cibaria*. Total and cumulative P release for $InsP_6$-P, $InsP_5$-P, $InsP_4$-P and $InsP_3$-P was greater ($P < 0.01$) for Fermented *L. reuteri* than Fermented *W. cibaria*.

4. Discussion

Hull-less barley grain contained 4.09 g P/kg DM, similar than reported previously (Rodehutscord et al., 2016). The intrinsic phytase activity of barley grain was not measured and differs among barley cultivars; however, a CDC Fibar barley grain of a different harvest year contained 1120 U intrinsic phytase activity/kg DM, similar than reported previously (490 to 1100 U/kg DM, Steiner et al., 2007; Rodehutscord et al., 2016).

Cereal processing strategies to degrade $InsP_6$ into lower forms of InsP prior to feeding are warranted to increase digestibility of plant P in cereal-based diets for pigs, because pigs lack endogenous phytase activity within the gastro-intestinal tract (Golovan et al., 2001). Sourdough fermentation activates intrinsic enzymes (proteases, amylases, pentosanases, phytases) stimulating degradation of fibre and antinutritional factors including phytate thereby increasing nutrient availability (Leenhardt et al., 2005; Gänzle et al., 2014). Barley phytases are acidic phytases with an optimum pH of 5.0 to 6.0 (Greiner et al., 2000). Acidification including barley sourdough will solubilise phytate salts while maintaining phytase activity and thus support stepwise conversion of $InsP_6$ into the water soluble $InsP_4$, $InsP_3$ and $InsP_2$ (Gänzle et al., 2014).

Lactic acid-producing bacteria evolved to form stable associations with insects and vertebrate hosts are often used to ferment food (Li and Gänzle, 2020). Both *L. reuteri* and *W. cibaria* are heterofermentative lactic acid bacteria. *Limosilactobacillus reuteri* is a host-adapted gut symbiont that has formed stable associations with rodents, birds, herbivores, and pigs but the ecology of *W. cibaria*

Table 3
Inositol phosphate concentration and inositol phosphate-P release of acidified or fermented hull-less barley grain[a,b].

Item, g/kg dry matter	Control	ACD	Fermented		SEM	P-value[c]		
			L. reuteri	*W. cibaria*		Acid	Fermentation	Bacteria
$InsP_6$-P	2.12	0.68	0.77	1.26	0.05	<0.001	<0.001	<0.001
$InsP_5$-P	0.45	0.17	0.18	0.49	0.04	<0.001	0.018	<0.001
$InsP_4$-P	1.43	0.31	0.46	0.96	0.06	<0.001	<0.001	<0.001
$InsP_3$-P	0.33	0.63	0.62	0.61	0.04	<0.001	<0.001	0.928
$InsP_2$-P	0.11	1.18	0.81	0.52	0.08	<0.001	<0.001	0.009
Sum $InsP_6$-P – $InsP_2$-P	4.45	2.98	2.84	3.84	0.16	<0.001	<0.001	<0.001
Total P release[d]	-	1.47	1.61	0.61	0.21	-	-	0.009
Cumulative P release[e]								
$InsP_6$-P	-	0.24	0.23	0.14	0.01	-	-	<0.001
$InsP_5$-P	-	0.30	0.28	0.14	0.01	-	-	<0.001
$InsP_4$-P	-	0.57	0.52	0.25	0.03	-	-	<0.001
$InsP_3$-P	-	0.47	0.42	0.16	0.04	-	-	0.002
$InsP_2$-P	-	-0.07	0.07	-0.04	0.07	-	-	0.184

InsP2-P, inositol phosphate2- phosphorus; InsP3-P, inositol phosphate3- phosphorus; InsP4-P, inositol phosphate4- phosphorus; InsP5-P, inositol phosphate5- phosphorus; InsP6-P, inositol phosphate6- phosphorus; sum InsP6-2-P, inositol phosphate6-2- phosphorus; P, phosphorus.

[a]Control barley; ACD, chemically-acidified barley; Fermented *L. reuteri*, fermented barley with *Limosilactobacillus* (*L.*) *reuteri*; Fermented *W. cibaria*, fermented barley with *Weissella* (*W.*) *cibaria*.

[b]Least squares means based on 3 observations per treatment.

[c]Acid = acid addition (Control vs. ACD); Fermentation = Control vs. Fermented *L. reuteri* and Fermented *W. ciberia*; Bacteria = Fermented *L. reuteri* vs. Fermented *W. cibaria*.

[d]Total P release (g/kg DM) for ACD, Fermented *L. reuteri* and *W. cibaria* (**trt**) was calculated by subtracting the sum of $InsP_6$-P – $InsP_2$-P (g/kg DM) for trt from Control.

[e]Cumulative P release of degraded $InsP_x$ (g/kg DM) considering the degradation of all greater InsP ($InsP_{>x}$) was calculated by multiplying by the molecular weight of P (31.0 g P/mol): P released (1 phosphate group hydrolysed) $InsP_x = InsP_{x\ degraded} \times 31.0$.

remains unknown. Schwab et al. (2008) conducted an in vitro study to assess effects of *L. reuteri* LTH5448 and *W. cibaria* 10 M on pH, lactate and acetate formation during fermentation (24 h at 37°C) using sorghum flour. After incubation, cell counts of *W. cibaria* were about 10-fold lower than for *L. reuteri*. In addition, lactate and acetate formation were numerically lower for *W. cibaria* than for *L. reuteri* resulting in a numerically greater pH for *W. cibaria* (pH 4.0 vs. 3.8), which matches the results of the present study. During cereal fermentations with *L. reuteri*, carbohydrates, non-starch polysaccharides, proteins and phenolic compounds are degraded by microbial activity or by the activity of intrinsic cereal enzymes (Gänzle, 2014; Gaur and Gänzle, 2023). Cereal fermentation using *L. reuteri* decreased NDF, CP and ADF content (Le et al., 2016; Heyer et al., 2021). Considering the close structural relationship between dietary fibre and $InsP_6$ in the aleurone layer, cereal fermentation might open up the fibre matrix increasing access for enzymatic degradation and increase nutrient digestibility in pigs (Newkirk and Classen, 1998; Blaabjerg et al., 2011).

Plant phytase and $InsP_6$ in barley grain are primarily located in the aleurone layer (Raboy, 2003). The ACD, Fermented with *L. reuteri* and *W. cibaria* reduced $InsP_6$-P concentration in barley grain by 68%, 64%, and 41%, respectively, and increased total P release by 1.47, 1.61, 0.61 g/kg DM, respectively, compared with Control, thus minimising the antinutritional effect of $InsP_6$ to reduce nutrient digestibility in vivo (Schlemmer et al., 2001). Plant phytase efficacy and solubility of phytate is greatest with pH below 4 (Grynspan and Cheryan, 1983). The present study confirmed the decisive influence of the pH. Of note, the final pH of ACD was numerically greater than Fermented with *L. reuteri*; however, the pH of ACD was low throughout the 24 h fermentation. Instead, the pH of Fermented with *L. reuteri* was only gradually reduced from pH ~ 5.5 to less than 4.0. In ACD, Fermented with *L. reuteri* and Fermented with *W. cibaria*, the $InsP_4$-P content decreased numerically by 1.1, 1.0 and 0.5 g/kg DM, respectively, compared to Control while the $InsP_2$-P content increased numerically in the opposite direction by 1.1, 0.7, and 0.4 g/kg DM, respectively. Acidification with 5% lactic acid lowered the pH to 2.4 and reduced $InsP_6$-P in barley grain by converting $InsP_6$ into lower order InsP forms (Metzler-Zebeli et al., 2014). Degradation of $InsP_6$ into $InsP_4$ is the limiting step for plant P utilisation for the exogenous phytase used in the study by Rosenfelder-Kuon et al. (2020). In the present study, ACD and Fermented barley grain increased $InsP_3$-P concentration in barley grain by up to 48% and thus is promising to maximise InsP degradation prior to feeding; however, an end-product inhibition of the phytase by released P might have prevented a complete hydrolysis of InsP molecule (Żyła et al., 1995).

5. Conclusion

Lower pH in chemically acidified and fermented barley indicated that acidification is important for $InsP_6$ hydrolysis via intrinsic phytase activation in barley grain in vitro. Between strains, fermentation with *L. reuteri* maximised acidification, lactate concentration and degradation of inositol $phosphate_6$, inositol $phosphate_5$ and inositol $phosphate_4$ in barley grain. In weanling piglets, *L. reuteri* TMW1.656 showed probiotic properties indicating that cereal fermentation is a potential processing technique to improve gut health in swine production.

CRediT authorship contribution statement

Zijlstra Ruurd: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Dorper A.:** Data curation, Formal analysis. **Heyer C.M.E.:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Validation, Writing – original draft, Writing – review & editing. **Ganzle M.G.:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Sommerfeld V.:** Data curation, Formal analysis, Methodology.

Declaration of Competing Interest

On behalf of all authors, I mention that no financial or other contractual agreements exist that might cause conflicts of interest or be perceived as causing conflicts of interest. Also, financial arrangements between any of the authors and any company whose product is mentioned prominently in the submitted manuscript do not exist.

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