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Reducing rumen starch fermentation of wheat with 3% NaOH does not reduce whole tract starch digestibility and increases energy utilization in wethers during heat stress

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Abstract

Previous studies have suggested that feeding slowly fermentable grains might improve heat-tolerance in ruminants, due to increased post-rumen starch digestion and a reduction in the heat increment of feeding. During heat stress gastrointestinal function may be compromised which could impact on the site and extent of digestion. To characterize the *in vitro* rumen starch and DM disappearance of corn, wheat, and 3% NaOH-treated wheat grains, grain samples were incubated during 0, 5, 8, and 24 h at 39˚C in buffered rumen fluid. Then faecal pH and whole tract apparent starch, DM, and organic matter (**OM**) digestibility were determined in wethers fed, with either corn (**CD**), wheat (**WD**) or 3% NaOH- treated wheat (**TWD**) based diets during heat stress in two experiments. In experiment 1, 22 wethers were fed either CD or WD (*n*=11 per diet) during three different stages in climate controlled rooms: stage 1 (**TNFR**), 7 days of thermoneutral conditions 18 to 21˚C and 40 to 50% relative humidity) and 1.3 times maintenance feed intake; stage 2 (**HSFR**), 7 days of heat stress (28 to 38˚C and 30 to 50% relative humidity) and feed intake as **TNFR**; and stage 3 (**HSFU**), 7 days of heat stress as in **HSFR** stage and 1.5 times maintenance feed intake. In experiment 2, 31

wethers were fed either CD (*n*=10), WD (*n*=10) or TWD (*n*=11) during three different stages with the same temperature and relative humidity regimes as experiment 1, however during TNFR and HSFR stages wethers received 1.7 times maintenance feed intake while in HSFU stage wethers received 2 times maintenance feed intake. After 24 h of incubation, untreated wheat had the fastest rate of starch and DM disappearance followed by 3% NaOH-treated wheat and corn grain (*P*<0.001). Wethers fed CD had lower apparent starch (P<0.001) and higher DM (P<0.001) and OM (P<0.001) digestibility than those fed WD, which was associated with lower faecal pH (P<0.001) and higher faecal starch content (P<0.001) than wheat diets. 3% NaOH treatment of wheat did not affect the whole tract starch digestibility of wheat being faecal pH and starch content of TWD like WD. At low feed intakes (1.3 times maintenance) there was no effect of heat stress on digestibility whereas at higher feed intakes heat stress reduced starch, OM, and DM digestibility. However, starch digestibility of TWD was similar to WD and metabolizable energy intake, DM and OM digestibility were higher. It is concluded that 3% NaOH treatment of wheat could reduce the rumen starch disappearance (*in vitro* condition) without reducing the whole tract starch digestibility (tested *in vivo*) of wheat in heat-stressed wethers. The study demonstrated the treated wheat with NaOH can be a strategy to increase OM digestibility and energy retention of wethers under heat stress.

Implications

The results of this study supports the theory that the feeding of slowly fermentable grains increases the proportion of starch that by-passes rumen fermentation and is digested in the small intestine, thus reducing the heat increment of feeding. Therefore, NaOH treatment of wheat grain is a feasible alternative to reduce rumen starch fermentability of wheat and to increase the metabolizable energy intake and utilization in ruminants under heat stress conditions.

Introduction

Cereal grains are often fed to ruminants to counteract the reduction in DM intake that can occur during periods of high ambient temperatures (Baumgard et al., 2014). While all cereal grains are relatively high in starch concentrations (Rodehutscord et al., 2016), the efficiency of starch utilization in ruminants is influenced by a combination of factors including grain processing, extent of starch fermentation in the rumen and digestion in the small intestine, type and amount of VFA produced in the rumen and in the hindgut and whole tract digestibility (Rowe et al., 1999). It is suggested that the efficiency of starch utilization can be maximized if starch by-passes the rumen, because of reductions in heat from fermentation and metabolic heat losses (Owens et al., 1986; Reynolds et al., 2001), which can be beneficial during heat stress (HS) conditions (Russell, 2007).

Feed digestibility decreases with increasing feed intake (Leaver et al. 1969) and while heat stress (HS) reduces feed intake (Chauhan et al. 2014; Mahjoubi et al. 2015) in sheep. There have been varying reports on the effect of heat stress on digestibility suggesting that the effects of elevated temperature on digestibility were uncertain and with the changes in feed intake (Bhattacharya and Uwayjan, 1975; Hyder et al., 2017). For example, it is possible that some of the direct effects of HS on digestibility may be masked by a reduction in feed intake.

Previous studies have demonstrated the lower *in vitro* rumen fermentability of corn and 3% NaOH-treated wheat grains compared to wheat grains and that feeding diets based on corn and 3% NaOH-treated wheat to sheep resulted in improved heat-tolerance, expressed as reduced respiration rates and rectal and skin temperature compared with untreated wheat-based diets (Gonzalez-Rivas et al., 2016; Gonzalez-Rivas et al., 2017). The most plausible explanation for this improved heat-tolerance is the difference in the proportion of post-rumen starch digestion between grains leading to a reduction of the heat increment of feeding. High ambient temperatures also divert blood flow from the gastrointestinal and visceral tissues to

the periphery to assist in heat dissipation from the skin (Bell et al., 1986; Wong and Hollowed, 2016). Consequently, the gut can become hypoxic resulting in ischemic damage and possible reductions in digestive capacity (Cronje, 2005). Therefore, this study aimed to characterize the *in vitro* starch and DM disappearance of corn, wheat, and 3% NaOH- treated wheat and to determine the whole tract apparent DM, organic matter (**OM**), and starch digestibility of grain-based diets in lambs feeding at different intake levels under heat stress conditions.

Material and methods

Animal ethics approval was provided by The University of Melbourne, Faculty of Veterinary and Agricultural Sciences Animal Ethics Committee (AEC 1413253.2 and AEC 1513585.1).

In vitro experiments

Starch and DM disappearance were determined from grain samples during the *in vitro* incubation of 84 replicates of 1 g (dry weight) of 1-mm ground corn (72.4% starch), Australian standard white (**ASW**) wheat (65.8% starch), and 3% NaOH–treated ASW wheat (65.6% starch) grains as described in (Gonzalez-Rivas et al., 2017). Briefly, grain replicates were weighed into 250-mL serum flasks in 4 *in vitro* fermentation runs at 39˚C using buffered rumen fluid in an automated gas production system (Ankom RF; Ankom Technology Corp, Macedon, NY) with 7 replicates per incubation time (5, 8, and 24 h) per run.

Six zero-hour (T_0) flask containing 1 g of grain sample and 100 ml of Kansas State pH 6.8 buffer solution (Marten and Barnes, 1980) followed by 15 min of incubation at 39˚C (Szasz et al., 2005) were included per run. T_0 starch and DM disappearance were used to calculate the readily digestible fraction that disappears from the media before microbial fermentation (Ørskov et al., 1980). In addition, four backgrounds flasks containing only buffered rumen fluid were included in each run to correct for residual DM.

Fermentation was terminated at 5, 8, and 24 h of incubation by placing the flasks in an ice bath. Then, the supernatant was discarded and the residues were recovered using a filter mesh (54 T DP Screen mesh, 114 µ mesh opening; Unirich, GJS Machinery Pty Ltd, Revesby, NSW, Australia) and an electric vacuum pump (Air Admiral Vacuum/ Pressure station; Barnant Company, Barrington, IL, USA) following the procedure described by Navarro-Villa et al. (2011).

Residues were then dried at 60°C for 48 h to maintain starch stability. After drying, residues were weighed and subtracted from the background residue weight to calculate DM digestibility. Dry residues were ground using a mortar and pestle to pass a 0.5 mm sieve for starch determination using a kit (K-TSTA; Megazyme International Ireland, Wicklow, Ireland) containing thermostable α- amylase and amyloglucosidade (McCleary et al., 1997) as per manufacturer's instructions. The OM content was measured by combustion of the dry residue in a furnace chamber at 650ºC for four hours (AOAC, 1990).

In vitro starch (**IVSD**) and DM disappearance (**IVDMD**) for each sample were calculated as the amount of starch and DM that disappeared during the *in vitro* incubation and were fitted to the non-linear kinetic model (Ørskov and McDonald, 1979; Szasz et al., 2005) $p\ (\%) = a + b\ (1- e^{[-ct]})$ where p is the starch or DM that disappeared at incubation time $t\ (h)$; a is the soluble and rapidly degradable fraction (%); b is the potentially degradable fraction (%); c is the fractional rate at which b disappeared per hour. The effective DM and starch degradability (**ED**) were determined as per (Szasz et al., 2005; Anele et al., 2015); $ED = a + bc/(c+k)'$ where, a, b, and c are the constants obtained from the non-linear model, and k is the fractional outflow rate, assuming a medium passage rate of rumen solids outflow (5.0% h^{-1}).

In vivo experiments

Animals and experimental design

Faecal starch and pH concentration, metabolizable energy (**ME**), DM, OM and starch intake, and the whole tract apparent digestibility were determined in two *in vivo* experiments. Details on animals and experimental design have been reported elsewhere (Gonzalez-Rivas et al., 2016; Gonzalez-Rivas et al., 2017). Briefly, in experiment 1 (Gonzalez-Rivas et al., 2016), 22 wethers (41.2±2.4 kg) housed individually in metabolic crates in two climate-controlled rooms, were fed either crushed corn grain plus forage (**CD**; 39% starch) or crushed wheat grain plus forage (**WD**; 37% starch), *n*=11 per diet, according to the feeding schedule presented in Table 1. The daily ration of feed was split into 3 equal meals fed at 0900, 1300, and 1700 h. The amount of feed offered on a daily basis as DM was calculated before each period based on body weight (BW) using the following equation: feed (kg DM/d) = Level x $W^{0.75}$ × 450/1,000/ ME, in which Level is the multiplier of maintenance being applied, $W^{0.75}$ is the metabolic BW, 450 is the maintenance energy requirement for sheep (kJ/kg $W^{0.75}$), and ME is the ME content of the diet (Liu and McMeniman, 2006).

The experiment had 3 stages with differences in heat load and feed intake. Stage 1 (**TNFR**; Thermoneutral feed restriction) consisted 7 days of thermoneutral conditions (18 to 21°C and 40 to 50% relative humidity with a 12-h light and 12-h dark cycle) and 1.3 times maintenance feed intake (M) ; Stage 2 (**HSFR**; Heat stress feed restriction), 7 days of heat stress (28 to 38°C and 30 to 50% relative humidity with a 12-h light and 12-h dark cycle) and feed intake as in TNFR; and stage 3 (**HSFU**; Heat stress feed unrestricted), 7 days of heat stress as in TNFR and 1.5 M.

In experiment 2, (Gonzalez-Rivas et al., 2017), 31 wethers (46.3±2.8 kg), housed in two climate-controlled rooms, were fed either crushed corn grain plus forage (**CD**, 42.7% starch, *n*=10), crushed wheat grain plus forage (**WD**, 39.4% starch, *n*=10) or 3% NaOH-treated

whole wheat plus forage (**TWD**, 39.3% starch, n = 11) according to the feeding schedule presented in Table 1 during three different stages with the same temperature and relative humidity regimes as experiment 1, except that during TNFR and HSFR stages wethers received 1.7 M while in HSFU stage wethers received 2 M. Grains for both studies were crushed through a roller mill to a particle size of 2 to 5 mm and for Experiment 2 whole wheat was treated with 3% NaOH according to the technique described by De Campeneere et al. (2006). Daily feed intake as DM was determined in both experiments by weighing the refusals before the morning feeding (0830 h) and water consumption was measured daily at 0900 and 1700 h.

The nutrient content of the ingredients and diets from both experiments were determined by wet chemistry or near infrared spectrophotometry by an external commercial laboratory (Symbio Laboratories, Brooklyn, Victoria, Australia).

Faecal samples (~250 g per animal) were collected from the metabolic crates in the morning before feeding (0830 h) on days 3, 4, and 5 of each stage in both experiments which allows for a minimum of approximately twice the mean passage rate of concentrate diets in sheep (Bartocci et al. 1997) before faecal collection began in each period.

Faecal pH was measured using a pH meter (HI 5221; Hanna Instruments Australia, Keysborough, Victoria, Australia) immediately after 50 g of fresh faeces were mixed with 100 mL reverse osmosis (RO) water. Faecal samples were composited by animal at the end of each stage and kept frozen (-20°C) until analysis. Faecal samples were then thawed, weighed and dried at 60°C in open aluminium tins for 48 h until stable weight. After drying, DM content was calculated, and subsamples were ground through a 0.5 mm sieve using a cyclone grinder

for starch determination. Faecal starch concentrations were determined as described previously for fermentation residues.

Whole tract apparent starch, DM and OM digestibility were determined using the acid insoluble ash technique (AIA; Van Keulen and Young (1977). Briefly, samples of each diet and composited faeces were ground through a 1 mm sieve using a cyclone grinder. Then, 3 g of faeces and 5 g of diet (as DM) were weighed into pre-weighed crucibles and ashed in a muffle furnace (Tetlow Kilns & Furnaces Pty Lt, Notting Hill, Victoria, Australia) set at 600°C during four hours. Ash content was then calculated to obtain OM% ; *Ash(%) = (sample ash weight/ sample DM weight) × 100,* and OM (%) = 100 – Ash (%).

Each ashed sample was then transferred into pre-weighed 50-mL Falcon tubes. To each tube, 10 mL of 4 N HCl was added and vortexed at maximum speed. Then, samples were placed into a water bath (20 L Analogue water bath-WB20; Ratek Instruments Pty Ltd, Victoria, Australia) set at 100°C for 30 min. After 30 min, samples were cooled and centrifuged for 10 min at 3,000 × g in a refrigerated centrifuge set at 4°C (Kubota 5700; Kubota Corporation, Tokyo, Japan). The supernatant was removed with a transfer pipette and 20 ml of hot RO water was added to each sample and vortexed. Then, the samples were centrifuged for 10 min and rinsed again. This washing and centrifuging process was done twice. The supernatant was removed using a transfer pipette and tubes containing the samples were placed in a drying oven set at 100°C for 24 h until a stable weight was obtained. The AIA content was determined using the known weight of the tubes and initial sample ash weights: *AIA (%)= (Wf-We)/Ws × 100* where *Wf* is the weight of tube with ash, *We* is the weight of the empty tube and *Ws i*s the weight of sample as DM (Van Keulen and Young, 1977).

The apparent digestibility coefficients for DM, OM and starch were calculated according to Zhong et al. (2008) and Bergero et al. (2009) respectively; *DM digestibility (%) = (1- A/B) × 100* where *A* and *B* are the AIA (%) in feed and faeces, respectively. *OM and Starch digestibility (%) = 100 × [1-(A/B) × (NB/NA)]* where *NA* and *NB* are the nutrients (either OM or starch%) in the feed and faeces, respectively.

Statistical Analysis

In vitro starch and DM disappearance parameters were analysed using the restricted maximum likelihood analysis (REML) in GenStat V16 (GenStat release 16 VSN International Ltd., Hemel Hempstead, UK). Data were analysed using grain type (corn, wheat, and 3% NaOH wheat) as a fixed factor and run (1 to 4) as a random factor. Faecal pH and starch percentage, ME, starch, DM and OM intake, and digestibility coefficients were analysed using RELM using diet (WD, CD, or TWD) and stage (TNFR, HSFR, and HSFU) as fixed factors and wethers as random factors. Correlation between faecal starch percentage and pH was analysed using Pearson correlation.

Differences between estimated means were determined using the Bonferroni test. The Wald test was used to test significance and significance was declared if $P \leq 0.05$. Results are reported as means and pooled SED.

Results

In vitro starch and dry matter disappearance

At T_0, the IVDMD was significantly higher ($P<0.001$) for untreated wheat followed by corn and 3% NaOH-treated wheat and this was reflected in the estimation of the rapidly degradable fraction (*a*) of DM for each grain (Table 2). At 5, 8, and 24 h wheat had the highest ($P<0.001$) IVDMD followed by 3% NaOH-treated wheat and corn (Table 2). Consequently, after 24 h the

IVDMD was 93.1% for untreated wheat compared to 80.3 and 87.3% for corn and 3% NaOH-treated wheat, respectively (Table 2, P<0.001). From the disappearance kinetics, the calculated rate of DM disappearance (c) for corn was only 31% that of untreated wheat with 3% NaOH-treated wheat being intermediate (Table 2, Fig 1a, P<0.001). However, there were no differences (P>0.05) between the grains in the effective degradability (ED) and potentially degradable fraction (b) of DM.

At T_0, untreated wheat had the highest (P<0.001) IVSD followed by corn and 3% NaOH-treated wheat and this was reflected in the estimation of a of starch for each grain (Table 2) . At 5, 8, and 24 h wheat had the highest (P<0.001) IVSD followed by 3% NaOH-treated wheat and untreated corn (Table 2). Consequently, after 24 h the IVSD was 98.2% for untreated wheat compared to 92.1 and 96.1% for corn and 3% NaOH-treated wheat, respectively (Table 2, P<0.001). the calculated rate of c for starch in corn was only 61% that of untreated wheat with 3% NaOH-treated wheat being intermediate (Table 2, Fig 1b, P<0.001).. Untreated wheat and corn had similar b of starch, both of which were lower than 3% NaOH- treated wheat (P<0.001). There were no differences between the grains in ED (Table 2, Fig. 1b).

Calculated Metabolizable energy, starch, DM and OM intake

In experiment 1, while there were no differences in calculated ME and OM intake between the diets, the different experimental stages had a significant influence on the ME, DM, OM, and starch intakes (P<0.001) (Table 3). As designed, there was no difference in ME, DM, OM, and starch intakes in wethers between the TNFR and HSFR periods whereas there was a substantial increases (ca. +16%, P<0.001) in ME, DM, OM, and starch intakes during HSFU when ME offered was increased from 1.3 M to 1.5 M. However, there were Stage x Diet interactions for ME (P=0.010), DM (P=0.021), OM (P=0.012) and starch (P<0.001) intakes such

that the various intakes increased to a greater extent between HSFR and HSFU in those wethers consuming the CD diet than the WD diet, albeit the differences were small.

In experiment 2, wethers fed WD had slightly lower calculated ME intake than those fed CD and TWD (14.4 vs 14.3 and 14.2 MJ/day for CD, TWD, and WD respectively; P=0.014) and ME intake was 13% higher (P<0.001) during HSFU than during TNFR and HSFR (Table 4). However, there was a Diet x Stage interaction (P=0.041) such that the difference between the diets was largely due to a 5.5% lower ME intake in those wethers fed the WD diet than fed the CD or TWD diets during the HSFU phase (Table 4). Wethers fed the CD had lower DM intake than those fed TWD with WD intermediate (1178 vs 1224 and 1197 g/day for CD, TWD, and WD respectively; P<0.001), in part because of the slightly different calculated ME contents of the diets which impacted the DM offered. Although DM intake was 13% higher (P<0.001) during HSFU than during TNFR and HSFR (Table 4), there was a Diet x Stage interaction (P=0.044) such that the TWD wethers consumed more offered DM than those fed the WD and CD diets during HS, particularly during the HSFU phase. There was no significant effect of diet on OM intake (1139 vs 1161 and 1171 g/day for CD, TWD, and WD respectively; P=0.010) and OM intake was 13% higher (P<0.001) during HSFU than during TNFR and HSFR (Table 4). However, there tended to be a Diet x Stage interaction (P=0.058) such that the difference between the diets was largely due to a slightly lower OM intake in those wethers fed the CD diet than fed the CD or TWD diets during HS (Table 4). Wethers fed CD had higher starch intake than those fed both TWD and WD (503 vs 480 and 472 g/day for CD, TWD, and WD respectively; P<0.001), in part because of the slightly different starch contents of the diets which impacted the starch offered. Although, starch intake was 13% higher (P<0.001) during HSFU than during TNFR and HSFR (Table 4), there was a Diet x Stage interaction

(*P*=0.036) such that the increase in starch intake between HSFR and HSFU was lower in the wethers consuming the WD diet than the other groups (Table 4).

Starch, DM and OM apparent digestibility coefficients and faecal characteristics

In experiment 1, the apparent DM digestibility of the CD was much higher than the WD (80.9 vs 72.9% for CD and WD respectively; *P*<0.001) and tended to be lower during HSFU than the other periods (76.9 vs 78.2 and 75.6% for TNFR, HSFR and HSFU respectively; *P*=0.055) (Table 3). The apparent OM digestibility of the CD was much higher than the WD (82.7 vs 75.6% for CD and WD respectively; *P*<0.001) and tended to be lower during HSFU than HSFR (79.0 vs 80.3 and 78.1% for TNFR, HSFR and HSFU respectively; *P*=0.079) (Table 3). The apparent starch digestibility of the CD was lower than the WD (97.6 vs 99.4% for CD and WD respectively; *P*<0.001) and tended to be lower during HSFU than both TNFU and HSFR (98.8 vs 98.8 and 98.0% for TNFR, HSFR and HSFU respectively; *P*=0.016) (Table 3). As a consequence, faecal starch was higher in wethers consuming CD compared to WD (4.66 vs 0.83% for CD and WD respectively; *P*<0.001) and was higher during HSFU than other stages (2.45 vs 2.39 and 3.40% for TNFR, HSFR and HSFU respectively; *P*=0.041). This was particularly so for those wethers consuming the CD diet as indicated by a trend for a Diet x Stage interaction (*P*=0.091). Faecal pH was lower in wethers consuming CD compared to WD (7.36 vs 7.87 for CD and WD respectively; *P*<0.001) but was not affected by stage of study (7.56 vs 7.82 and 7.47 for TNFR, HSFR and HSFU respectively; *P*=0.12). There was a negative correlation between faecal starch percentage and pH (r = - 0.544; *P*<0.001) (data not shown).

In experiment 2, the apparent DM digestibility of the CD and TWD were much higher than the WD (83.5 vs 85.3 and 75.9% for CD, TWD and WD respectively; *P*<0.001). Apparent DM digestibility was decreased by HS particularly at the higher level of feed intake (85.8 vs 82.6 and 76.3% for TNFR, HSFR and HSFU respectively; *P*<0.001) and this was particularly so

for the WD diet as indicated by the strong Diet x Stage interaction (*P*=0.004) (Table 4). The apparent OM digestibility of the CD and TWD were much higher than the WD (84.0 vs 85.9 and 77.1% for CD, TWD and WD respectively; *P*<0.001). Apparent OM digestibility was decreased by HS particularly at the higher level of feed intake (86.5 vs 83.3 and 77.3% for TNFR, HSFR and HSFU respectively; *P*<0.001) and this was particularly so for the WD diet as indicated by the strong Diet x Stage interaction (*P*=0.005) (Table 4). The apparent starch digestibility of the CD was lower than both the TWD and WD (97.7 vs 99.5 and 99.5% for CD, TWD and WD respectively; *P*=0.007). Apparent OM digestibility was decreased by HS particularly at the higher level of feed intake (99.2 vs 98.9 and 98.6% for TNFR, HSFR and HSFU respectively; *P*<0.001) (Table 4). As a consequence, faecal starch concentration was higher in wethers consuming CD compared to both TWD and WD (5.24 vs 1.41 and 0.83% for CD, TWD and WD respectively; *P*<0.001) although it was not affected by stage of study (2.48 vs 2.39 and 2.61% for TNFR, HSFR and HSFU respectively; *P*=0.77) (Table 4). Faecal pH was higher in wethers consuming TWD than WD with CD being even lower still (6.23 vs 7.10 and 6.78 for CD, TWD and WD respectively; *P*<0.001) but was not affected by stage of study (6.63 vs 6.73 and 6.75 for TNFR, HSFR and HSFU respectively; *P*=0.48) (Table 4). There was a negative correlation between faecal starch percentage and pH (r = - 0.502; *P*<0.001) (data not shown).

Discussion

A major finding of this study were that 3% NaOH treatment of wheat reduced the *in vitro* rumen starch degradability of wheat grain but did not affect the apparent whole tract starch digestibility of wheat diets *in vivo*. Another important finding was that heat stress reduced starch, OM and DM digestibility in wheat-based diets at high feed intakes (2 M) but not at lower feed intakes (<1.7 M). For corn-based diets the effects of heat stress were observed at

intakes of 1.7 M and above. Furthermore, it was demonstrated that by reducing the rumen starch fermentation of wheat grain with 3% NaOH the starch digestibility of wheat diet was unaffected, and the ME intake and DM and OM digestibility were improved in wethers during heat stress. Therefore, these results suggest that treating wheat with NaOH reduces rumen starch fermentability of wheat, thus reducing heat produced during digestion (Gonzalez et al. 2017) and potentially increasing energy utilization and intake in ruminants during heat stress conditions.

Similar results were observed *in vitro* and *in situ* by others (Ørskov, 1976; Berger et al., 1981; Moran, 1986). Data obtained in this study agree with those of Nocek and Tamminga (1991) and Rowe and Pethick (1994) who reported greater rumen starch digestibility for barley and wheat than for corn. In this study, a reduction in the *in vitro* rumen starch disappearance of wheat was observed with 3% NaOH treatment. A slow rate of grain fermentation and starch disappearance is associated with a large amount of starch by-passing the rumen (Hindle et al., 2005), and reductions of heat from fermentation and metabolic heat losses which may result in up to 40% increased efficiency in energy utilization (Owens et al., 1986; Reynolds et al., 2001), and improving heat tolerance in small ruminants (Gonzalez-Rivas et al., 2016; Gonzalez-Rivas et al., 2017)

Dry matter kinetic parameters of wheat and corn agreed with the values reported by McAllister et al. (1990) where wheat showed higher potential degradable fraction (*b*) than corn (68.6 and 67.1%, respectively) and a faster fractional rate of disappearance (*c*) (0.121 vs 0.017 h^{-1} for wheat than for corn; respectively). The rapid starch degradability of untreated wheat in this experiment was mainly due to a large rapidly degradable fraction at T_0 (*a*=31.4%), a small potential degradable fraction (*b*=67.3%) and a faster constant rate of degradation (*c*=0.39 h^{-1}) compared with corn and 3% NaOH treated wheat.

The variations in ME, starch, DM, and OM digestibility observed in the wethers could be due to the slower ruminal fermentation of starch in TWD compared to WD. Additionally, factors like the detrimental effects of heat stress, the starch content of the diet, the experimental design can also influence the ME, starch, DM, and OM intake and digestibility (Gonzalez-Rivas et al., 2017). The experiments presented herein demonstrate the lower DM and OM digestibility of diets containing rapidly fermentable grains (wheat) compared to those containing slowly fermentable grains (corn and 3% NaOH treated wheat).

Previous studies have shown that lactating dairy cows fed large amounts of crushed wheat in mixed rations had a linear reduction of NDF and ADF digestibility (Leddin et al., 2009, 2010). *In vitro* and *in vivo* studies also demonstrated that cellulose digestion could be significantly inhibited by slight declines in rumen pH due to its negative effect on cellulolytic bacteria activity (Russell and Wilson, 1996). Thus, feeding strategies aimed to raise rumen pH by chemical treatments of grains offer a feasible alternative to improve dietary fibre utilization (Leddin et al., 2010). The higher DM digestibility of diets containing 3% NaOH treated wheat compared to those containing untreated wheat grains could be due to optimal rumen pH for fibre digestion, and to the effect of NaOH on the whole grain. Sodium hydroxide produces partial hydrolysis of the hemicellulose of the whole grain and gelatinization of the outer starch granules, slowing the attack of rumen bacteria and digestive enzymes in an alkaline environment (Ørskov and Greenhalgh, 1977; Anderson et al., 1981; Berger et al., 1981) improving the digestibility of fibre (Ørskov and Greenhalgh, 1977; Anderson et al., 1981; McNiven et al., 1995) and increasing the proportion of the starch digested post-rumen (Schmidt et al., 2006).

Compared to the starch granules in the wheat, the corn starch granules have greater amylose content and the amylose is strongly bound to proteins that limit the bacterial

attachment and enzymatic attack reducing rumen fermentation (Rooney and Pflugfelder, 1986; Rowe et al., 1999) explaining the lower apparent *in vitro* starch disappearance and whole tract starch digestibility of the corn-based diet. Rowe and Pethick (1994) reported total tract starch digestibility of corn and wheat of 93 and 98% respectively for ruminants although there are differences between sheep and cattle in their ability to ferment and digest starch. For example, Rowe et al. (1999) collated data from a number of studies and summarise that total apparent digestibility of starch from maize were 93 and 100% for cattle and sheep respectively, with considerably less maize starch fermented in the rumen of cattle (76 vs 86% of intake). Total apparent starch digestibility in steers was 84 and 96% for cracked corn and wheat respectively (Philippeau et al., 1999) which is qualitatively similar to the collation by Rowe et al. (1999) for cattle (93 vs 98% for cracked corn and wheat).

Chemical treatment of wheat with NaOH reduces the *in vitro* and in situ rumen degradation of starch (De Campeneere et al., 2006; Hetta et al., 2013), increases rumen pH (Ørskov and Greenhalgh, 1977; Anderson et al., 1981) and the amount of starch that reaches the small intestine (Homolka et al., 2001; Schmidt et al., 2006). It has been suggested that the slow rate of fermentation of 3% NaOH-treated wheat might improve the efficiency of nutrient utilization by allowing the starch to be absorbed in the small intestine (Mayne and Doherty, 1996). However, some authors have suggested that the ruminant small intestine has a limited ability to digest starch and some starch is fermented in the hindgut and the lactic acid produced has negative consequences for animal health and productivity (Matthé et al., 2001). However, sheep can consume up to 12 g/kg of corn starch without negatively impacting apparent total tract starch digestibility (Catro-Perez et al. 2013), and the maximum starch intake in the present study was around this amount.

The higher concentration of starch in the faeces confirms lower starch digestibility of cracked corn compared to milling wheat (Zinn et al. 2007) and was associated with low faecal pH in wethers fed CD. These data agreed with previous studies reporting low faecal pH associated with a reduction in intestinal starch digestion (Wheeler and Noller, 1977), increasing starch fermentation in the hindgut (Reynolds et al., 2001). This reduction in starch digestibility is associated with reduced activity of pancreatic alpha-amylase in the small intestine due to suboptimal pH (pH < 6.9), suggesting that dietary modifications which lead to higher intestinal pH could increase the utilization of dietary starch (Wheeler and Noller, 1977). Nevertheless, Anderson et al. (1981) demonstrated that the alkali treatment of corn increased the rumen and faecal pH and DM digestibility in dairy cows. These results are comparable to our results, demonstrating that despite a slower fermentation of TWD in the rumen, the intestinal pH obtained with the alkali treatment facilitated the efficient absorption of starch in the small intestine to an equal level as WD and improved DM and OM digestibility of wheat diets.

While some authors indicate that heat stress reduces feed intake, and the rate of digesta passage with a subsequent increase in DM and OM digestibility (Bernabucci et al., 1999; Dixon et al., 1999), others report negative or no effects of high environmental temperatures on rumen function and diet digestibility in ruminants (Mathers et al., 1989; Bernabucci et al., 2009). In this study, it was demonstrated that heat stress reduced starch, DM and OM digestibility in wheat-based diets at high feed intakes (2 M) but not at lower feed intakes (<1.7 M) whereas for corn-based diets the effects of heat stress were observed at lower intakes as well. However, during heat stress wethers fed TWD maintained similar nutrient digestibility as during thermoneutral conditions (TNFR vs HSFR periods) suggesting that TWD might improve nutrient utilization during heat stress compared to WD and CD. At

higher feed intakes there was a reduction in nutrient digestibility during heat stress, but this is likely, at least in part, due to the increase in feed intake between HSFR and HSFU.

It has been reported that animals fed NaOH treated grains had increased water intake (Ørskov and Greenhalgh, 1977), and this can be associated with a greater rate of passage and lower digestibility (Bernabucci et al., 1999). As expected, an increase in water intake was observed due to heat stress in this study, however, there were no differences in water intake between wethers fed CD and TWD and wethers fed CD and TWD and wethers in both groups had greater water intake than wethers fed WD (Gonzalez-Rivas et al., 2017), and whole tract DM and OM digestibility values were larger for wethers fed CD and TWD than those fed WD. Thus, water intake was unlikely to be associated with a reduction of DM digestibility.

There is some resistance to using NaOH treated wheat because of its corrosive properties and the safety concerns about handling NaOH on farm. Nevertheless, there is a commercial application of NaOH treated wheat in the Western Australian dairy industry where there is limited access to slowly fermenting grains such as corn or barley (McDonnell et al. 2017).

In conclusion, this study confirmed the lower *in vitro* starch disappearance of corn and 3% NaOH treated wheat and the lower in vivo starch digestibility of corn-based diets compared to wheat-based diets. It also demonstrated that the treatment of whole wheat with 3% NaOH did not affect the whole tract starch digestibility of wheat-based diets. Furthermore, it was also demonstrated that heat stress reduced starch, OM and DM digestibility in wheat-based diets at high feed intakes (2 M) but not at lower feed intakes (<1.7 M) whereas for corn-based diets the effects of heat stress were observed at lower intakes. Reducing rumen starch fermentation of wheat with 3% NaOH didn't impact starch digestibility of wheat whereas DM and OM digestibility of a wheat-based diet were improved. Thus, NaOH

treatment of wheat grain is a feasible alternative to reduce rumen starch fermentability of wheat and to increase the nutrient utilization in ruminants under heat stress conditions.

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Table 1. Composition of experimental diets fed to wethers on a dry matter basis[1]

Item	Experimental Diet Experiment 1		Experimental Diet Experiment 2		
	Wheat (WD)	Corn (CD)	Wheat (WD)	Corn (CD)	3% NaOH-wheat (TWD)
Ingredient, % DM basis					
Crushed corn grain	0	48	0	50	0
Crushed wheat grain	48	0	50	0	0
Whole 3% NaOH-treated wheat	0	0	0	0	50
Oaten chaff	24	24	25	25	25
Lucerne chaff	24	24	25	25	25
Ram Balancer supplement [2]	4	4	0	0	0
Chemical composition, DM basis					
OM, %[3]	95.0	95.9	96.6	96.7	94.9
DM, %	87.6	87.1	90.1	89.2	88.8
Estimated ME, (MJ/kg DM)	13.3	13.4	11.9	12.2	11.7
CP, %	25.0	23.9	11.6	10.1	11.8
ADF, %	18.0	17.8	15.9	16.7	16.1
NDF, %	29.9	30.8	28.3	26.7	26.4
AIA, %[3]	0.86	0.60	0.56	0.36	0.37
Total starch, %	37.2	39.1	39.4	42.7	39.3

[1] Near-infrared spectroscopy (NIRS) and wet chemistry analysis performed by an external laboratory.

[2] Ram Balancer Supplement (Lienert Australia, SA, Australia) included 12.5% Ca, 0.8% P, and 30% CP and provided 8,000 IU vitamin A, 1,600 IU vitamin D3, 50 IU vitamin E, 5 mg vitamin B1, 1 mg Co, 0.5 mg Y, 30 mg Fe, 420 mg Mg, 150 mg Se, 128 Mg S, 40 mg Zn, 33 mg Lasalocid, and 20 mg antioxidant per kilogram of mixed ration,.

[3] Values obtained by the acid insoluble ash (AIA) technique.

Experiment 1 (Gonzalez-Rivas et al., 2016); Experiment 2 (Gonzalez-Rivas et al., 2017)

Table 2. *In vitro* Dry matter (IVDMD) and starch (IVSD) disappearance parameters of corn, untreated wheat, and 3% NaOH treated wheat grain incubated in buffered rumen fluid

Item	Grain			SED	*P*-value[1]
	Corn	Wheat	3% NaOH Wheat		
IVDMD					
Incubation time, h					
0	20.0[a]	26.4[b]	15.9[c]	1.3	<0.001
5	46.7[a]	81.0[b]	74.1[c]	2.2	<0.001
8	57.7[a]	89.9[b]	78.4[c]	2.2	<0.001
24	80.3[a]	93.1[b]	87.3[c]	2.2	<0.001
Disappearance kinetics[2]					
a, %	20.0[a]	26.2[b]	16.0[c]	0.97	<0.001
b, %	69.2	67.3	70.2	3.49	0.70
c, h^{-1}	0.11[a]	0.36[b]	0.29[c]	0.031	<0.001
ED [3], %	94.2	98.5	90.8	3.99	0.19
Model fit, R	0.998	0.995	0.995		
IVSD					
Incubation time, h					
0	24.5[a]	31.5[b]	14.6[c]	1.3	<0.001
5	70.3[a]	87.9[b]	84.1[c]	2.6	<0.001
8	83.4[a]	96.2[b]	87.0[c]	2.6	<0.001
24	92.1[a]	98.2[b]	96.1[c]	2.6	<0.001
Disappearance kinetics[2]					

a, %	24.3[a]	31.4[b]	14.6[c]	1.14	<0.001
b, %	68.7[a]	67.3[a]	80.5[b]	1.84	<0.001
c, h^{-1}	0.24[a]	0.39[b]	0.32[c]	0.034	<0.001
ED [3], %	97.9	100	99.6	1.98	0.18
Model fit, R	0.998	0.995	0.993		

[1]Probability associated with REML

[2]Parameters from fitting the IVDMD values of 7 replicates per grain per incubation times to the Non-Lineal exponential model $p = a + b \{1- ^{exp}[-ct]\}$ where p is the starch or DM that disappeared at incubation time t (h); a is the soluble and rapidly degradable fraction (%); b is the potentially degradable fraction (%); c is the fractional rate at which b disappeared per hour.

[3]Effective DM degradability calculated using the equation $ED= a +bc/(c+k)$, where k is the fractional outflow rate assumed 5.0 % h^{-1} (Ørskov and McDonald, 1979)

[abc] Superscripts within a row indicate significant differences.

Table 3. Metabolizable energy, starch, dry matter and organic matter intake, apparent digestibility coefficients and faecal pH and starch percentage of wethers fed corn (CD) and wheat (WD) based diets and subjected to three different stages of heat load during experiment 1[1]. Means are predicted means pooled within stage

Experiment 1										
Diet (D)	Corn (CD)			Wheat (WD)				Significance		
Stage (S)	TNFR	HSFR	HSFU	TNFR	HSFR	HSFU	SED[2]	D	S	D × S
ME intake (MJ/d)	10.1^{b}	9.86^{b}	11.9^{a}	10.3^{b}	10.3^{b}	11.8^{a}	0.19	0.12	<0.001	0.010
DM intake (g/d)	755^{b}	736^{b}	891^{a}	771^{b}	771^{b}	887^{a}	14.2	0.019	<0.001	0.021
DM digestibility (%)	81.2^{a}	81.8^{a}	79.6^{a}	72.6^{bc}	74.6^{b}	71.6^{c}	1.32	<0.001	0.055	0.78
OM intake (g/d)	724^{b}	732^{b}	851^{a}	732^{b}	733^{b}	843^{a}	13.6	0.79	<0.001	0.012
OM digestibility (%)	82.9^{a}	83.6^{a}	81.5^{a}	75.1^{b}	77.0^{b}	74.6^{b}	1.26	<0.001	0.079	0.80
Starch intake (g/d)	295^{b}	298^{b}	348^{a}	287^{b}	287^{b}	330^{a}	5.4	<0.001	<0.001	<0.001
Starch digestibility (%)	98.0^{b}	98.2^{b}	96.7^{c}	99.5^{a}	99.4^{a}	99.3^{a}	0.44	<0.001	0.016	0.11
Faecal starch (%)	4.24^{ab}	3.83^{b}	5.91^{a}	0.65^{c}	0.95^{c}	0.88^{c}	0.833	<0.001	0.041	0.091
Faecal pH	7.31^{cd}	7.45^{bc}	7.33^{cd}	7.81^{ab}	8.18^{a}	7.61^{bc}	0.201	<0.001	0.12	0.35

[1] TNFR consists of thermoneutral and 1.3 × maintenance feed intake, HSFR stage consists of heat stress and feed intake as TNFR , and HSFU stage consists of heat stress and 1.5 × maintenance feed intake

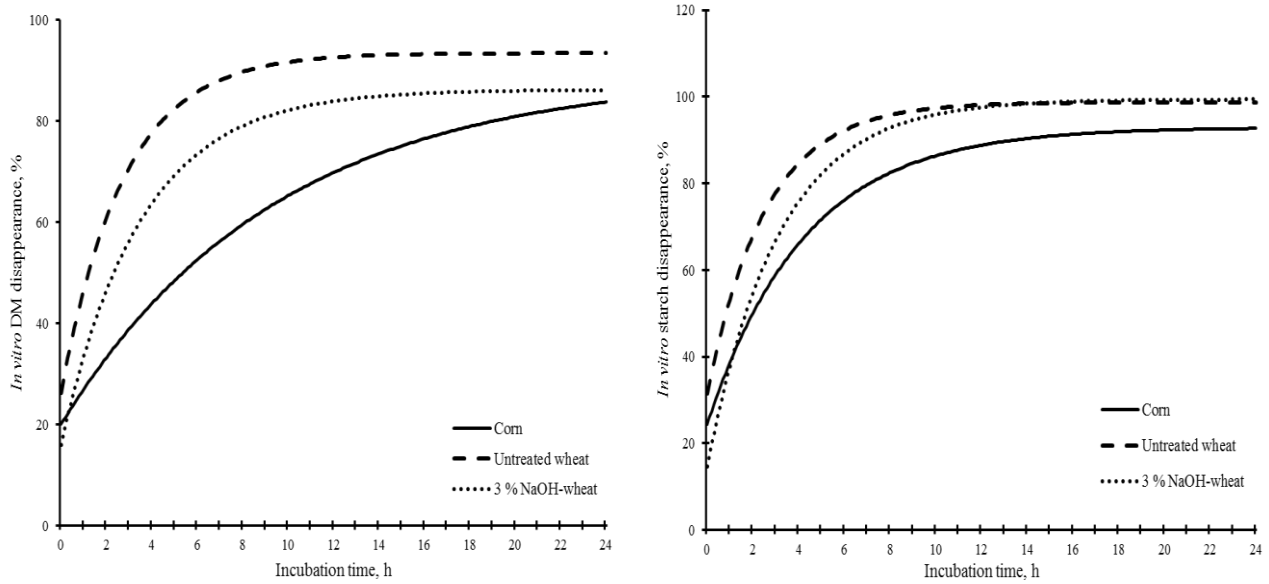
[2] Standard error of the difference for the diet × stage interaction.

Table 4. Metabolizable energy, starch, dry matter and organic matter intake, apparent digestibility coefficients and faecal pH and starch percentage of wethers fed corn (CD), wheat (WD) and 3% NaOH-treated wheat (TWD) based diets and subjected to three different stages of heat load during experiment 2[1]. Means are predicted means pooled within stage

Experiment 2													
Diet (D)	Corn (CD)			3% NaOH-Wheat (TWD)			Wheat (WD)				Significance		
Stage (S)	TNFR	HSFR	HSFU	TNFR	HSFR	HSFU	TNFR	HSFR	HSFU	SED[3]	D	S	D × S
ME intake (MJ/d)	13.6^{cd}	13.7^{cd}	15.8^{a}	13.2^{e}	13.9^{c}	15.9^{a}	13.5^{de}	13.9^{c}	15.4^{b}	0.26	0.014	<0.001	0.041
DM intake (g/d)	1,115^{e}	1,122^{d}	1,298^{b}	1,125^{d}	1,191^{c}	1,357^{a}	1,133^{d}	1,167^{cd}	1,292^{b}	22.9	<0.001	<0.001	0.044
DM digestibility (%)	87.4^{a}	82.6^{b}	80.6^{bc}	88.2^{a}	87.1^{a}	80.5^{bc}	81.8^{bc}	78.2^{c}	67.8^{d}	1.87	<0.001	<0.001	0.004
OM intake (g/d)	1,078^{c}	1,084^{c}	1,255^{a}	1,067^{c}	1,130^{b}	1,287^{a}	1,095^{b}	1,130^{b}	1,287^{a}	22.1	0.10	0.001	0.058
OM digestibility (%)	88.0^{a}	82.8^{b}	81.3^{b}	88.8^{a}	87.7^{a}	81.2^{b}	82.8^{b}	79.3^{b}	69.3^{c}	1.82	<0.001	<0.001	0.005
Starch intake (g/d)	476^{d}	479^{d}	554^{a}	441^{e}	467^{d}	532^{b}	446^{e}	460de	509^{c}	9.3	<0.001	<0.001	0.036
Starch digestibility (%)	98.3^{bc}	97.7^{cd}	97.2^{d}	99.6^{a}	99.5^{a}	99.3^{a}	99.7^{a}	99.5^{a}	99.2^{ab}	0.48	0.007	<0.001	0.12
Faecal starch (%)	5.33^{a}	5.00^{a}	5.39^{a}	1.41^{b}	1.39^{b}	1.43^{b}	0.69^{b}	0.79^{b}	1.00^{b}	1.053	<0.001	0.77	0.97
Faecal pH	6.28^{cd}	6.16^{d}	6.26^{cd}	6.96^{ab}	7.31^{a}	7.04^{ab}	6.66^{bc}	6.73^{bc}	6.95^{ab}	0.236	<0.001	0.48	0.27

[1] TNFR consists of thermoneutral and 1.7 × maintenance feed intake, HSFR consists of heat stress and feed intake as TNFR, and HSFU consists of heat stress and 2 × maintenance feed intake

[2] Standard error of the difference for the diet × stage interaction.



12

13 **Figure 1.** *In vitro* DM (a) and Starch (b) disappearance curves of incubation of ground corn,
14 untreated wheat, and 3% NaOH- treated wheat grains. IVDMD and IVSD curves are
15 representative of 24 h incubation at 39°C in buffered rumen fluid. Curves are the result of the
16 application of the non-lineal exponential model to the estimated means of IVDM and IVSD
17 values of 7 replicates per grain per incubation time after curve fitting $p= a + b \{1- ^{exp\ [-ct]}\}$;
18 $P<0.001$.

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