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Effects of ammonia-treated maize on growth performance of beef cattle

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ABSTRACT

High-moisture cereal grains are often treated with ammonia to prevent spoilage and for perceived animal performance advantages, but there are few reports on the effects of ammonia treatment of cereal on cattle performance. This study was carried out on 101 Charolais cattle to quantify the effects of feeding ammonia-treated maize on the performance of intensively fattened beef cattle. Animals were assigned to 16 pens in two sheds and all animals in each pen were fed on the same diet for 176 d. The cereal in the diets was either ammonia-treated maize (ATM) or untreated maize diet (UTM), formulated to be approximately isoenergetic and isonitrogenous. Cattle fed ATM had a lower feed conversion ratio (FCR) (6.14 ± 0.39 vs 6.49 ± 0.41 kg DM/kg BW; $p = 0.011$) and tended to have a higher average daily liveweight gain (ADG) (1.60 ± 0.10 vs 1.54 ± 0.10 kg/d; $p = 0.060$). The pH of the ruminal fluid at slaughter was higher ($p < 0.001$) in animals that were fed the ATM diet (6.35 ± 0.69) compared to those fed the UTM diet (5.46 ± 0.33). No differences were found in the concentrations of NH_3 or total VFA in ruminal fluid between the two groups. The molar proportion of butyric acid from all VFAs was lower in animals fed ATM than UTM (14.69 ± 3.55% and 18.04 ± 2.97% respectively; $p = 0.012$), and the molar proportion of propionic acid was higher in the ATM than the UTM groups (22.14 ± 1.54% and 19.54 ± 1.72% respectively; $p < 0.001$). The ratio of acetic acid to propionic acid was significantly lower in animals fed ATM compared to those fed UTM (ATM vs UTM: 2.64 ± 0.26 vs 2.97 ± 0.29; $p = 0.0038$). Faecal starch was lower in cattle fed ATM than untreated maize (UTM) (14.73 ± 5.65 vs 18.14 ± 4.27% DM; $p < 0.001$). The pH of faeces in the ATM group was higher than that in the UTM group (4.75 ± 0.19 vs 4.55 ± 0.19; $p < 0.001$). In conclusion, ammonia treatment of maize resulted in increased ADG and decreased FCR, with reduced faecal starch concentration, suggesting possible gains in efficiency of starch utilisation.

1. Introduction

Feed quality for livestock can be adversely affected by pests and microorganisms during storage and effective feed preservation is

Abbreviations: ATM, diet contains ammonia-treated maize; UTM, diet contains untreated maize; FCR, feed conversion ratio; ADG, average daily liveweight gain; DM, dry matter; DMI, dry matter intake; BW, bodyweight; VFA, volatile fatty acid; CP, crude protein; NPN, non-protein nitrogen.

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beneficial to animal health. Many approaches have been taken to create an environment that is not conducive to the survival and reproduction of microorganisms and pests: controlling moisture and temperature, creating an anaerobic environment by adding deoxidiser or filling containers with carbon dioxide (CO_2) or nitrogen (N_2) (Raeker, 1990; Hashem et al., 2012; Navarro, 2012). Additives such as microbial inoculants, chemicals and enzymes, are also commonly used to both preserve and enhance the digestibility and nutritive value of rations for livestock (Muck et al., 2018). Cereal grains contribute a large proportion of the diets for fattening cattle in Europe, and are often harvested with a high moisture content in northern and western Europe, necessitating preservation (Olsson et al., 2002). High starch content of the cereals also increases the risk of reticuloruminal acidosis (Owens et al., 1998). Whereas acidifying treatments are effective preservatives (Campling, 1991) but do not address acidosis, alkalization treatments of cereal grains have the potential to address both spoilage and acidosis (Humer and Zebeli, 2017).

Ammonia treatment has been applied to low quality roughages (Oji et al., 1977) and whole grains (Han et al., 1978; Horton, 1978; Laksesvela, 1981; Kraiem et al., 1991) to increase digestibility, to prevent mould growth and to inhibit bacterial proliferation (Bothast et al., 1972; Kabak et al., 2006). Ammonia treatment increases non-protein nitrogen (NPN) (Oji et al., 1977; Horton, 1978; Herrera-Saldana et al., 1982; Males and Gaskins, 1982; Kraiem et al., 1991), which contributes to improved reticuloruminal microbial activity and might therefore improve animal performance (Spanghero et al., 2017; Belanche et al., 2021). Improvements in feed intake and feed efficiency in steers (Mathison et al., 1989) and increases in milk yield and milk protein in dairy cows were noted when ammonia-treated high-moisture barley was fed (Robinson and Kennelly, 1989). Laksesvela (1981) reported an increase in dry matter intake (DMI) of adult female sheep fed ammonia-treated barley in addition to a higher lambing percentage than those fed untreated barley. Due to the alkalinising nature of ammonia, it is also expected to decrease the rate of proton accumulation in the rumen like other alkali treatments (Humer and Zebeli, 2017), thereby reducing the risk of reticuloruminal acidosis.

Most previous studies that demonstrated beneficial effects of ammonia treatment on performance of beef cattle used direct insufflation of bagged cereal grains with anhydrous ammonia gas (Laksesvela, 1981; Phillip et al., 1985; Mathison et al., 1989; Goonewardene et al., 1998). We recently conducted a study with only limited replication on enzymic catalysis of urea to ammonia in barley on a commercial beef farm in Scotland; it showed a significant improvement in feed conversion ratio and a tendency to increased average daily liveweight gain (ADG; Jonsson et al., 2018). In the present study, we aimed to establish whether a commercially available method of cereal grain preservation using enzymic catalysis of urea to ammonia in on-farm commodity bays would deliver similar performance benefits to those previously documented using direct insufflation with anhydrous ammonia gas. The beef cattle in the fattening system were fed on a maize-based diet with or without the ammonia treatment. The primary aim was to characterise the performance effects of enzyme-catalysed ammonia-treatment of grain in beef cattle. We hypothesized that cattle fed the ammonia-treated diet would have higher ADG and lower FCR than cattle fed the untreated diets. Secondary aims of the study were intended to suggest possible explanations for any observed differences in performance of animals on the diets and made use of opportunistically collected samples. They included the characterisation of ruminal volatile fatty acid (VFA) profiles and analysis of faeces.

2. Materials and methods

This study was conducted on a commercial beef fattening unit near Milan, in northern Italy. Experiments were carried out in accordance with the European Communities Council Directive (2010/63/EU), transposed by the Italian Ministry of Health (DL 26, 4 March 2014).

Table 1
Ingredients and average nutritional values of the diets during the experimental period.

	Untreated Maize (UTM)	Ammonia-treated Maize (ATM)
Ingredient as fed (kg/head/d)		
Maize Silage	9.0	9.0
Maize meal	5.2	0
Maxammon[a] maize meal	0	6.5
Brewers' grains	3.0	3.0
Straw	1.0	1.0
Rape cake	2.0	1.0
Minerals and vitamins mix	0.2	0.2
Total as fed (kg/head/d)	20.40	20.70
Total as DM (kg/head/d)	11.00	11.00
ME[b] (MJ/kg)	12.05	12.05
Nutrient (% of DM)		
CP	13.78	13.79
Fat	4.11	4.06
NDF	31.37	29.73
Starch	42.23	47.96
Ca	0.70	0.69
P	0.40	0.37

a Maxammon (Harbro Limited, Turriff, Scotland): 15 kg urea and 5 kg Maxammon per ton of maize.
b ME: metabolizable energy; CP: crude protein; NDF: neutral detergent fibre.

2.1. Animals and treatments

One hundred and three Charolais cattle (475 ± 39 days old, 454 ± 37 kg) were used in the trial. All animals were treated on arrival against endo- and ectoparasites using a broad-spectrum endectocide and were vaccinated against bovine herpesvirus-1, parainfluenza-3, bovine respiratory syncytial virus and bovine viral diarrhoea virus. After ranking by weight, animals were randomly allocated by initial coin toss and subsequent alternation to 16 pens (5–7 animals/pen, 8 pens/group) in two sheds on 19/03/2018. All animals in each pen were fed on the same treated diet for 176 d.

Table 1 shows the composition of the two diets, which were intended to be approximately isoenergetic and isonitrogenous, and the average energy and nutrient composition during the experimental period. All components were analysed by near infrared spectrophotometry (NIR) performed by Harbro Limited (Turriff, Scotland) using a FossNIRSystems 5000 + machine while TMRs were analysed weekly, using a portable NIR instrument (Polispec – ITPhotonics, Italy). In total, 51 cattle were fed ATM and 52 cattle were fed UTM. Maize silage and straw were produced on farm, and other ingredients included in the diet were sourced locally. Ground maize was treated with Maxammon according to the instructions provided by Harbro Limited: 15 kg urea and 5 kg Maxammon per ton of grain, with the addition of 30 l water/t if the moisture content was 14–15% and an additional 10 l water/t for each additional percentage point below 14%. The cattle had ad libitum access to hay for 7 d after arrival with progressively increasing portion of TMR (from 50% to 100% from D-2 to D-7). After the initial transition period, cattle had ad libitum access to TMR provided every morning at 07:00 h. Cattle always had free access to fresh water.

2.2. Health status and growth performance

Animals were inspected daily for lameness, bloat, and bovine respiratory disease (BRD) and weighed on D-1, D-60, D-116 and D-176 (last day) of the trial. The feed intake of each pen was calculated once a week by weighing the feed offered and the residue in the feed-trough 24 h post-feeding and then corrected for the diet dry matter, evaluated each time using a portable NIR instrument (Polispec, IT Photonics, Italy). The ADG of each animal was obtained by dividing the difference between the liveweight on D-176 and D-1 of the trial by 175 (days on feed). The FCR of each pen was calculated by dividing the average daily feed dry matter intake of each pen by the ADG of animals in that same pen.

2.3. Ruminal fermentation observations

Twenty-eight animals (14 animals per group, with at least 1 animal from each pen) were convenience-selected for post-mortem sampling in the abattoir. Within 30–40 min after slaughter, ruminal fluid (200 mL per animal) was collected from the dorsal sac of the rumen for the determination of pH, NH_3, total and proportional volatile fatty acids (lactic acid, propionic acid, butyric acid and acetic acid). The pH was measured immediately on collection using a portable pH-meter (HI 5522, HANNA Instruments, Woonsocket, USA). The concentration of NH_3 was measured using a modified colorimetric method developed by Broderick and Kang (1980). The VFA concentrations were analysed using high-performance liquid chromatography (Shimatzu, Kyoto, Japan).

2.4. Faecal consistency, undigested fraction and chemical characteristics

Faeces were collected from 28 animals (14 animals were convenience-sampled per group and avoiding animals under medical treatment) on D-60, D-116 and D-176. Faecal consistency was scored from 1 to 5, 1 being very compact, semi-solid and 5 being very liquid, using a method based on criteria discussed by Hall (2002). Faecal fractional size profile was estimated by sieving faeces with a 3-plate sieve, with sieve dimensions of 4.76 mm, 3.17 mm and 1.55 mm. The pH of the faeces was measured using a portable pH-meter (HI 5522, HANNA Instruments, Woonsocket, USA). Faecal measurements were carried out according to the AOAC guidelines (1990): faecal moisture content (method 934.01); concentrations of crude protein (CP) (method 920.87); lipids (method 920.85) and starch (method 996.11). The concentrations of faecal ADF and NDF were measured using the method described by Van Soest et al. (1991).

2.5. Statistical analysis

Data were analysed in R version 4.0.3 (R Core Team, 2020) For the dependent variables, the effects of treatments and other potential factors (shed, pen or sampling day) were tested by fitting a linear mixed-effects model (LMM) using the 'lme4′ package (Bates et al., 2015), or a generalized linear model (GLM). Pen is the experimental unit, except for the post-mortem ruminal samples, when it was not possible to link the animal back to the pen of origin. The distributional assumptions of LMMs and GLMs were checked visually by plotting residuals against fitted values. Variables such as ruminal NH_3 concentration and faecal starch concentration required natural-log-transformation to achieve normally distributed residuals. Significance was defined at $p < 0.05$ and a trend was defined at $0.05 \leq p < 0.10$. The cattle were allocated to two sheds; four pens of cattle in each shed were fed one of two diets (ATM or UTM).

The ADG and FCR were analysed using LMM:

$$Y_{ijnk} = \mu + T_i + S_j + (T \times S)_{ij} + P_n + e_{ijnk};$$

Where Y_{ijnk} is the dependent, continuous variable; μ is the overall mean; T_i is the fixed effect of the diet (i = ATM and UTM); S_j is the fixed effect of the shed (j = 1 and 2); $(T \times S)_{ij}$ is the interaction between the diet and the shed; P_n is the normally distributed random effect of the pen (n = 1–16) and e_{ijnk} is the normally distributed residual error. The interaction between the diet and the shed was removed when $p > 0.10$, and the resulting model was fitted again to evaluate the effects.

For the faecal data collected over time, a GLM was applied:

$$Y_{imk} = \mu + T_i + D_m + (T \times D)_{im} + e_{imk}$$

Where Y_{imk} is the dependent, continuous variable; μ is the overall mean; T_i is the fixed effect of the diet (i = ATM and UTM); D_m is the fixed effect of the sampling day (m = D-60, D-116 and D-176); $(T \times D)_{im}$ is the interaction between the diet and the sampling day and e_{imk} is the normally distributed residual error. Analysis of deviance for the fit of the GLM (*F*-test in 'anova' function) was performed to compare the model with the interaction term with the model with main effects only – the interaction term was removed if $p > 0.10$.

The model for analysing ruminal content was:

$$Y_{ik} = \mu + T_i + e_{ik},$$

where Y_{ik} is the dependent variable; μ is the overall mean; T_i is the fixed effect of the diet (i = ATM and UTM), e_{ik} is the residual error.

3. Results

Health and performance: During the study, two cattle that were fed UTM were slaughtered before the due date – one due to severe BRD and one due to severe bloat. Twenty-three of fifty-two cattle in the UTM group and 21/51 cattle in the ATM group were diagnosed with BRD. There were eight lame cattle in the UTM group and six in the ATM group; seven cattle in the UTM group and three in the ATM group had bloat that was responsive to treatment or recovered with no treatment. Animal performance data are shown in Table 2. Although initial and final liveweights differed among pens, there were no differences between the two treatments in these variables. The FCR of animals fed ATM was lower than those fed the UTM diet (6.14 ± 0.39 vs 6.49 ± 0.41 kg DM/kg BW; $p = 0.011$; Fig. 1).

Ruminal fluid characteristics at slaughter: The pH of the ruminal fluid at slaughter was higher ($p = 0.0011$) in animals that were fed the ATM diet (6.35 ± 0.69) compared to those fed the UTM diet (5.46 ± 0.33). No differences were found in the concentrations of NH_3 or total VFA in ruminal fluid between the two groups. The proportion of butyric acid was 14.69 ± 3.55% of the total VFAs in animals fed the ATM diet, while it was 18.04 ± 2.97% of the total VFAs in the UTM fed group ($p = 0.012$). The proportion of propionic acid was 22.14 ± 1.54% of the total VFA in the ATM group but only 19.54 ± 1.72% VFA in the UTM group (p < 0.001). The ratio of acetic acid to propionic acid was lower in animals fed ATM compared to those fed UTM (ATM vs UTM: 2.64 ± 0.26 vs 2.97 ± 0.29; $p = 0.0038$). There was no difference in the concentration of lactic acid between groups, nor were there any differences in the proportion of acetic acid within the total VFA concentration (Table 2).

Faecal characteristics: No differences were found in the faecal consistency between groups. The UTM and ATM groups (42 faecal samples per group) had 28 and 27 faecal samples respectively that were scored 4 or 5. The remaining samples in both groups were scored 3. No differences were found in the results of 3-level sieving between groups (Table 3). However, faecal composition differed between the two groups and differed over time (Table 3, Fig. 2). The pH of faeces in the ATM group was higher than that in the UTM group (4.75 ± 0.19 vs 4.55 ± 0.19; $p < 0.001$). The faecal starch concentration in the ATM group was lower than that in the UTM group (14.73 ± 5.65 vs 18.14 ± 4.27% DM; $p < 0.001$). All the faecal composition variables changed significantly over time ($p < 0.001$) and interactions between the diet and sampling day affected pH ($p < 0.001$), NDF proportion ($p = 0.022$) and starch concentration ($p = 0.0097$) in faeces (Fig. 2, Table 3). The proportion of CP in faecal DM tended to be higher in the ATM group when

Table 2
Effects of feeding the ammonia-treated maize on growth performance and ruminal content at slaughter of beef cattle.

	Untreated Maize (UTM)	Ammonia-treated Maize (ATM)	*p* - value (diet)	*p* - value (shed)
Growth performance[a]	n = 50 cattle	n = 51 cattle		
Initial liveweight (kg)	458 ± 38	450 ± 36	0.41	0.02
Final liveweight (kg)	727 ± 24	729 ± 25	0.93	0.0089
ADG (kg/d)	1.54 ± 0.10	1.60 ± 0.10	0.060	0.10
Feed efficiency	n = 8 pens	n = 8 pens		
DMI (kg/d)	10.18 ± 0.48	9.82 ± 0.42	0.18	0.22
FCR (kg DM/kg BW)	6.49 ± 0.41	6.14 ± 0.39	0.011	0.19
Ruminal content	n = 14 cattle	n = 14 cattle	***p* - value (diet)**[b]	
pH	5.46 ± 0.33	6.35 ± 0.69	< 0.001	
NH_3 (mmol/L)	19.22 ± 8.82	21.28 ± 10.84	0.88	
VFA (mmol/L)	167.84 ± 26.77	154.32 ± 31.70	0.23	
Lactic acid (% VFA)	1.39 ± 1.19	0.78 ± 0.44	0.19	
Acetic acid (% VFA)	57.67 ± 2.12	58.20 ± 2.53	0.55	
Butyric acid (% VFA)	18.04 ± 2.97	14.69 ± 3.55	0.012	
Propionic acid (% VFA)	19.54 ± 1.72	22.14 ± 1.54	< 0.001	
Acetic acid: Propionic acid	2.97 ± 0.29	2.64 ± 0.26	0.0038	

[a] Two animals in the UTM group were removed from the analysis because of the death. Continuous variables are presented by mean ± SD. The *p* - values were obtained by fitting the variable with the LMM with the diet and the shed as fixed effects, the pen as random effects.

[b] The *p* - values were obtained by fitting the dependent variables with the GLM with the diet as a fixed effect.

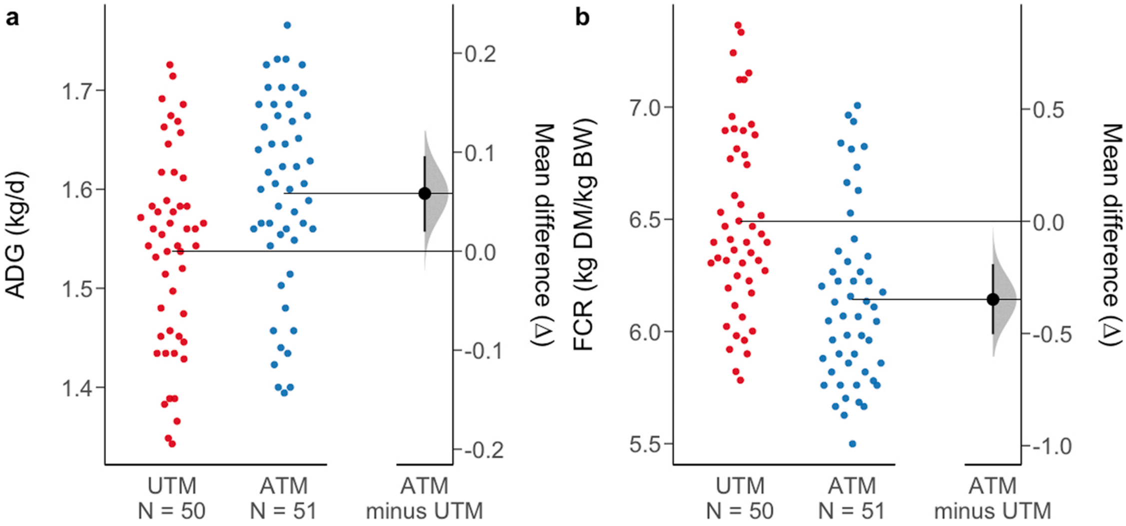

Fig. 1. (a) The average daily liveweight gain (ADG) and (b) feed conversion ratio (FCR) of cattle fed the diet containing ammonia-treated maize (ATM) and the diet containing untreated maize (UTM). Gardner-Altman plots show the individual mean values for each animal on the left, the mean difference of the ATM mean from the UTM mean (dark horizontal line), and the distribution of the deviations of the ATM observations from the UTM mean (shaded in grey).

Table 3
Effects of feeding the ammonia-treated maize on faecal consistency and composition of beef cattle.

	Diet†		Day			*p* - value¶ (diet)	*p* - value (day)	*p* - value (diet x day)
	UTM (n = 42)	ATM (n = 42)	D-60 (n = 28)	D-116 (n = 28)	D-176 (n = 28)			
Physical evaluation‡								
Sieve§ I (%)	3.07 ± 1.22	3.00 ± 1.43	3.07 ± 1.33	3.14 ± 1.53	2.89 ± 1.10	0.81	0.77	–
Sieve II (%)	16.43 ± 3.90	16.62 ± 3.13	16.61 ± 4.24	16.68 ± 2.89	16.29 ± 3.40	0.81	0.91	–
Sieve III (%)	80.50 ± 4.30	80.38 ± 3.58	80.32 ± 4.38	80.18 ± 3.78	80.82 ± 3.72	0.92	0.81	–
Chemical evaluation‡								
pH	4.55 ± 0.19	4.75 ± 0.19	4.67 ± 0.16	4.58 ± 0.27	4.69 ± 0.18	< 0.001	0.0094	< 0.001
DM (%)	19.96 ± 1.44	20.14 ± 1.38	21.24 ± 1.20	19.47 ± 1.22	19.32 ± 0.97	0.48	< 0.001	–
CP (% of DM)	15.20 ± 1.29	15.57 ± 1.27	14.32 ± 1.02	16.56 ± 0.63	15.28 ± 1.00	0.059	< 0.001	–
Fat (% of DM)	4.83 ± 0.92	4.90 ± 0.78	4.08 ± 0.46	4.81 ± 0.59	5.70 ± 0.55	0.55	< 0.001	–
NDF (% of DM)	49.91 ± 3.77	53.04 ± 5.45	55.19 ± 3.18	52.57 ± 3.70	46.67 ± 3.32	< 0.001	< 0.001	0.022
ADF (% of DM)	28.38 ± 3.01	29.17 ± 3.13	30.94 ± 2.56	29.47 ± 1.23	25.92 ± 2.69	0.11	< 0.001	–
Starch (% of DM)	18.14 ± 4.27	14.73 ± 5.65	14.30 ± 2.56	12.56 ± 3.05	22.45 ± 3.40	< 0.001	< 0.001	0.0097

† UTM: untreated maize; ATM: ammonia-treated maize.
‡ Continuous variables are presented by mean ± SD.
§ Dimensions of sieve I to III are 4.76 mm, 3.17 mm and 1.55 mm, respectively.
¶ The *p* - values were obtained by fitting the variable with the GLM with the diet, the sampling day and their interaction as fixed effects.

compared to the UTM group (15.57 ± 1.27 vs 15.20 ± 1.29% DM; $p = 0.059$). No significant differences in faecal DM proportion, or proportions of fat and ADF in faecal DM caused by the ammonia treatment were found.

4. Discussion

Ammonia treatment of livestock feed is not new. Early investigations of ammonia made use of anhydrous ammonia gas and demonstrated effective preservation (Bothast et al., 1972) and some enhanced animal performance (Robinson and Kennelly, 1989). However, until recently, relatively little use has been made of ammonia treatment, probably because of the logistic challenges associated with treating large volumes of feed. Several systems have recently been developed commercially, in which ammoniation is achieved by mixing cereal grain with urea and a source of enzyme to catalyse the conversion of urea to ammonia. Feed can be treated readily on-farm using mixer wagons, deposited in a commodity-bay, and covered with a plastic sheeting for 7–10 days, during which ammonia gas percolates through the cereal grains and is absorbed. In the present study, the diets were broadly equivalent in energy and protein. The study was carried out on a commercial farm and was intended to contrast the effect of the feeding system rather than any independent, direct effect of the ammonia treatment for two main reasons. Firstly, the study was conducted on a commercial farming unit to maximise the relevance of the results to cattle producers. This implies considerable constraints on the types of rations to

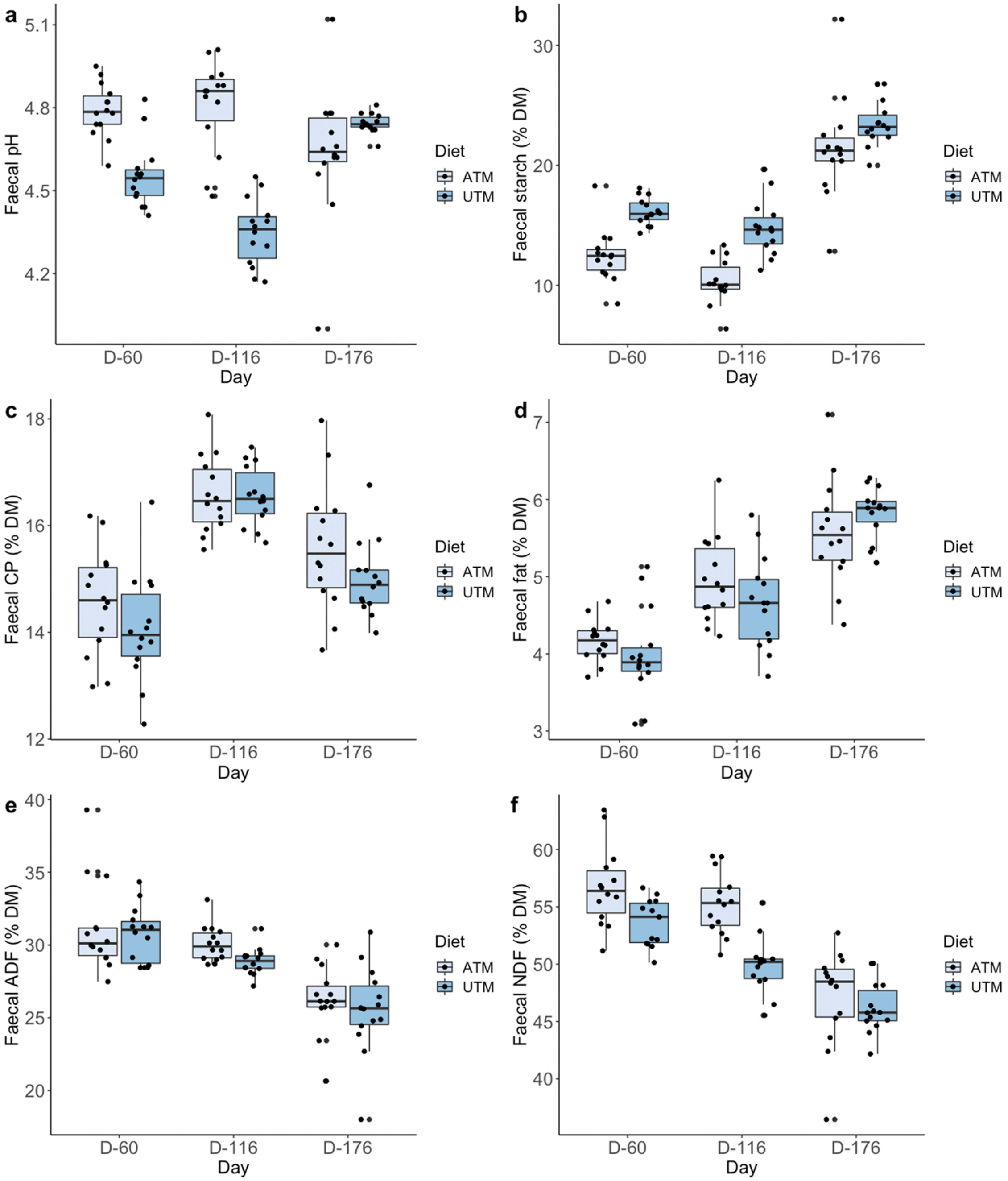

Fig. 2. The effect of feeding the ammonia-treated maize (ATM) on faecal pH (a), and faecal proportions of starch (b), CP (c), fat (d), ADF (e), and NDF (f). Sampling day significantly affected all of the variables ($p = 0.0094$ for pH and $p < 0.001$ for all other variables), and the main effect of treatment was significant for pH, NDF and starch ($p < 0.001$) and CP tended to be higher in ATM animals ($p = 0.059$). Significant interactions of sampling day by treatment were seen for pH, NDF and starch ($p < 0.001$, $p = 0.022$ and $p = 0.0097$, respectively).

be fed and an expectation that each of the alternative treatments be used in a way that maximises the potential productivity of animals given each treatment. Nested within this reason, the ammonia treatment has been marketed to farmers partly on the basis that its presumed buffering effect should enable higher rates of starch to be fed than when using other preservatives (anecdotal information provided to NJ and RJ by Scottish farmers and feed suppliers). Secondly, the ammonia treatment provides an additional source of NPN, which must be balanced somehow, using a comparison that is commercially plausible. A treatment with urea in the absence of enzymes to catalyse its conversion to ammonia would not be feasible in this study because it is not an accepted method of preservation and because some (possibly most) of the urea would be expected to be converted to ammonia as a result of endogenous cereal enzymes

(Patra and Aschenbach, 2018), reducing the validity of the control treatment.

Consistent with our hypothesis, our previous study (Jonsson et al., 2018), and with earlier studies in which ammoniation was achieved through direct gaseous insufflation (Phillip et al., 1985; Goonewardene et al., 1998), the FCR estimates for cattle which were fed the ammonia-treated diets were lower than those fed the untreated diet, and the ADG of cattle which were fed the ATM diet tended to be higher than those fed the UTM diet. Hence, there was an observed FCR benefit of 6.9% (0.45/6.49 kg/kg, $p = 0.011$) – broadly consistent with the DMI being 3.5% less in the animals on the ATM diet (0.36/10.18 kg/d, $p = 0.18$) and the ADG being 3.8% higher (0.06/1.60 kg/d, $p = 0.06$). It is possible that the performance benefits seen with ammonia treatment arise from improved digestibility of the cereal grains, as well as increased nutrient utilisation attributed to the increased microbial growth arising from nitrogen. The improved digestibility of whole DM (Ørskov et al., 1982), organic matter (OM) (Laksesvela, 1981) or crude fibre (Low and Kellaway, 1983; Mandell et al., 1988) of whole moist barley due to the ammonia treatment have been reported, while Robinson and Kennelly (1989) and a recent study which used Maxammon (Belanche et al., 2021) did not detect an effect on digestibility of OM. However, higher *in sacco* N degradability and a tendency to higher total tract apparent N digestibility of barley were noted by Belanche et al. (2021), and Low and Kellaway (1983) reported a significant increase in apparent N digestibility of whole wheat grain due to the ammonia treatment. Ammonia treatment has been shown to increase the production of urinary purine derivatives and microbial DNA, suggesting higher microbial protein synthesis (Belanche et al., 2021). It might be expected that this effect would be similar if feed was ammonia-treated or if it was simply supplemented with urea, because urea in the rumen is converted to ammonia, which is the key nitrogen substrate for microbial growth (Patra and Aschenbach, 2018). However, cereal that has been insufflated with ammonia gas (either directly or by pre-feeding treatment with urea and enzyme) has the ammonia incorporated within the cereal grain (Nikulina et al., 2018). In in vitro studies, ammonia in ruminal fluid builds more slowly after ingestion of ammonia-treated cereal than cereal to which an equivalent amount of urea had been added (Nikulina et all, 2018). It is possible that this slower release of ammonia might provide more stable conditions in the rumen for microbial protein synthesis, as suggested by higher urinary purine derivatives and microbial DNA in sheep on ammonia-treated diets (Belanche et al., 2021).

Higher ruminal microbial activity would be expected to result in more rapid and complete utilisation of available starch within the rumen. In the present study, cattle fed the ammonia-treated grain had lower faecal starch concentrations, which was consistent with either increased digestibility of the grain or increased utilisation (Zinn et al., 2007; Fredin et al., 2014). Alkalization of cereal grain has been proposed to reduce the risk of rumen fermentation disorders (Ørskov, 1979; Robinson and Kennelly, 1988, 1989; Huntington et al., 2006; Humer and Zebeli, 2017). The observations from our study were broadly consistent with this: fewer animals fed on the ammonia-treated ration developed bloat, although bloat incidence was low overall and not tested statistically (8 cases in total fed on UTM versus 3 cases on ATM). Faecal and ruminal pH were also higher in animals on the ATM diet. This is consistent with the findings of Belanche et al. (2021) in sheep.

Volatile fatty acids are often used for evaluation of the effect of differing diets and dietary treatments on ruminal fermentation (Hall et al., 2015). There was no difference in ruminal total VFA concentration between groups in the present study. A higher proportion of ruminal propionic acid and lower butyrate proportion were noted in cattle that were fed on the ammonia-treated maize than those fed on the untreated diet. Consistent with this result, Laksesvela (1981) reported increased propionic acid in the ruminal fluid of cattle that were fed ammonia-treated barley. Inconsistency among studies in the results of VFA analysis may arise in part from the timing of sampling in relation to the dietary change and to the time of the last meal. Cattle on a higher rate of starch supplementation are likely to adapt by developing a higher rate of VFA clearance, which in turn would result in lower concentrations of VFAs if sampling was carried out post-mortem and several hours after the last substantial meal (Jonsson et al., 2019). In the present study, our main interest in examining VFA was to quantify lactate concentration, which might be considered a relatively more stable indicator of a predisposition to chronic acidosis, but lactate concentrations were similar in the two treatments.

Changes in the composition of single faecal samples in cattle should be interpreted cautiously due to substantial diurnal variation in their relative compositions (Jancewicz et al., 2016). In our study, faecal variation over time-points was pronounced for all chemical variables but not for physical or structural variables. The most notable effects were the treatment × sampling day interactions for pH and proportions of starch and NDF. Faecal pH was higher in animals on ATM at the first two time points (D-60 and D-116) but by the third time point (D-176) they were similar in both groups, likely due to progressive adaptation of all animals to the high starch diet. The lower faecal starch concentrations measured in the ATM group at the first two time-points was consistent with our previous study of beef cattle fed on barley treated with ammonia or with propionate (Jonsson et al., 2018). The reason for the increase seen in faecal starch concentration in animals on both treatments by the third time-point and the equivalence in the two groups requires further investigation. Matthé et al. (2003) demonstrated that faecal starch absorption was maximal at 94.5% when cows were given a 21-d adaptation period. Although the composition of the diet was fixed in our study after the initial 7-d adaptation period, the absolute feed intake of animals increased from about 6.5 kg/head/d to about 12 kg/head/d as the animals grew, so it is possible that the high faecal starch proportions seen by the end of the feeding period might result from saturation of absorptive capacity in both groups. This might also involve an allometric scaling relationship between body-mass (BM) and absorptive capacity, in which gut fill scales to $BM^{1.0}$ but the rate of metabolic and absorptive processes scales to $BM^{0.75}$ (for example, see Müller et al., 2013). Faecal NDF was slightly higher in ATM animals at the first two time points but decreased in both groups over time and were similar by the last time-point. The significant reduction in NDF and ADF in faecal DM in both treatment groups over time suggests progressive adaptation to the diets with improved fibre degradation. The weak effect of diet on NDF and absence of an effect on ADF in faeces is consistent with the variability in results of other studies. Several studies reported no changes in apparent total tract digestibility (aTTD) of NDF and ADF in ruminants resulting from the ammonia treatment of cereal grains (Mathison et al., 1989, 2021)., whereas Mowat et al. (Mowat et al., 1981) and Low and Kellaway (1983) reported improved aTTD of ADF and NDF in steers with ammonia treatment of maize and wheat respectively.

5. Conclusions

The enzyme-catalysed ammonia treatment of grain showed similar effects to those previously reported in studies using direct insufflation with anhydrous ammonia, increasing the growth performance of cattle in a beef fattening system. It increased the ADG of cattle and decreased FCR. This method of processing cereal grains has the potential to increase nutrient utilization on commercial cattle farms.

Author contributions

Huang: data curation, formal analysis, visualisation, writing original draft; Jones: data curation, formal analysis, investigation, methodology, writing -review and editing; Compiani & Grossi: investigation, data curation, methodology, formal analysis, project administration, resources, writing – review and editing; Johnson: supervision, formal analysis, visualisation, validation, writing – review and editing; Eckersall: supervision, writing – review and editing; Rossi: conceptualisation, investigation, data curation, methodology, project administration, resources, writing – review and editing; Jonsson: conceptualisation, supervision, investigation, data curation, methodology, project administration, resources, writing, review and editing.

CRediT authorship contribution statement

Y. Huang: Investigation, Data curation, Formal analysis. **R. Jones:** Data curation, Formal analysis. **R. Compiani:** Conceptualisation, Project administration, Resources, Investigation, Data curation, Formal analysis. **S. Grossi:** Conceptualisation, Project administration, Resources, Investigation, Investigation. **P.C.D. Johnson:** Supervision, Writing – original draft, Writing – review & editing. **P.D. Eckersall:** Supervision. **C.A. Sgoifo Rossi:** Conceptualisation, Project administration, Resources, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **N.N. Jonsson:** Conceptualisation , Project administration, Resources, Supervision, Conceptualisation. All authors, led by YH.

Declaration of Competing Interest

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References

Association of official analytical chemists - AOAC, 1990. Official methods of analysis, Arlington, VA.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67.
Belanche, A., Martín-García, I., Jiménez, E., Jonsson, N.N., Yañez-Ruiz, D.R., 2021. A novel ammoniation treatment of barley as a strategy to optimize rumen pH, feed degradability and microbial protein synthesis in sheep. J. Sci. Food Agric. 101, 5541–5549.
Bothast, R.J., Lancaster, E.B., Hesseltine, C.W., 1972. Ammonia kills molds in corn. J. Dairy Sci. 56, 241–245.
Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63, 64–75.
Campling, R.C., 1991. Processing cereal grains for cattle - a review. Livest. Prod. Sci. 28, 223–234.
Fredin, S.M., Ferraretto, L.F., Akins, M.S., Hoffman, P.C., Shaver, R.D., 2014. Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows. J. Dairy Sci. 97, 1862–1871.
Goonewardene, L.A., Spicer, H.M., Engstrom, D.F., ZoBell, D.R., Yaremcio, B.J., 1998. A study on feeding ammoniated and processed barley to feedlot steers. Anim. Feed Sci. Technol. 74, 135–142.
Hall, M.B., 2002. Characteristics of manure: What do they mean? In Proceedings of the Tri-State Dairy Nutrition Conference.
Hall, M.B., Nennich, T.D., Doane, P.H., Brink, G.E., 2015. Total volatile fatty acid concentrations are unreliable estimators of treatment effects on ruminal fermentation in vivo. J. Dairy Sci. 98, 3988–3999.
Han, Y.W., Yu, P.L., Smith, S.K., 1978. Alkali treatment and fermentation of straw for animal feed. Biotechnol. Bioeng. 20, 1015–1026.
Hashem, M.Y., Risha, E.-S.M., El-Sherif, S.I., Ahmed, S.S., 2012. The effect of modified atmospheres, an alternative to methyl bromide, on the susceptibility of immature stages of angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). J. Stored Prod. Res. 50, 57–61.
Herrera-Saldana, R., Church, D.C., Kellems, R.O., 1982. The effect of ammoniation treatment of intake and nutritive value of wheat straw. J. Anim. Sci. 54, 603–608.
Horton, G.M.J., 1978. The intake and digestibility of ammoniated cereal straws by cattle. Can. J. Anim. Sci. 58, 471–478.
Humer, E., Zebeli, Q., 2017. Grains in ruminant feeding and potentials to enhance their nutritive and health value by chemical processing. Anim. Feed Sci. Technol. 226, 133–151.
Huntington, G.B., Harmon, D.L., Kristensen, N.B., Hanson, K.C., Spears, J.W., 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. Anim. Feed Sci. Technol. 130, 225–241.
Jancewicz, L.J., Penner, G.B., Swift, M.L., McKinnon, J.J., Waldner, C.L., McAllister, T.A., 2016. Characterization of the variation in the daily excretion of faecal constituents and digestibility predictions in beef cattle fed feedlot diets using near-infrared spectroscopy. Can. J. Anim. Sci. 96, 532–549.
Jonsson, N.N., Wolff, G., MacKenzie, D. Effect of Maxammon grain treatment on performance of finishing beef cattle. Proceedings of the 9th Nordic Feed Science Conference, Uppsala, Sweden, 12–13 June 2018, Edited by: Uden P, Eriksson T, Sporndly R, Rustas BO, Liljeholm M. Pages 163–165.
Jonsson, N.N., Ferguson, H.J., Koh-Tan, H.H.C., McCartney, C.A., Cernat, R.C., Strachan, E.M., Thomson, W., Snelling, T.J., Harvey, C.D., Andonovic, I., Michie, C., Wallace, R.J., 2019. Postmortem observations on rumen wall histology and gene expression and ruminal and caecal content of beef cattle fattened on barley-based rations. Animal 14, 1447–1460.

Kabak, B., Dobson, A.D., Var, I., 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit. Rev. Food Sci. Nutr. 46, 593–619.
Kraiem, K., Abdouli, H., Goodrich, R.D., 1991. Comparison of the effects of urea and ammonia treatments of wheat straw on intake, digestibility and performance of sheep. Livest. Prod. Sci. 29, 311–321.
Laksesvela, B., 1981. A note on the use of whole, moist barley treated with ammonia as a feed supplement for sheep. Anim. Prod. 32, 231–233.
Low, S.G., Kellaway, R.C., 1983. The utilization of ammonia-treated whole wheat grain by young steers. Anim. Prod. 37, 113–118.
Males, J.R., Gaskins, C.T., 1982. Growth, nitrogen retention, dry matter digestibility and ruminal characteristics associated with ammoniated wheat straw diets. J. Anim. Sci. 55, 505–515.
Mandell, I.B., Nicholson, H.H., Christison, G.I., 1988. The effects of barley processing on nutrient digestion within the gastrointestinal tract of beef cattle fed mixed diets. Can. J. Anim. Sci. 68, 191–198.
Mathison, G.W., Engstrom, D.F., Kennelly, J.J., Roth, L., Beck, B.E., 1989. Efficiacy of anhydrous ammonia and sulfur dioxide as prservatives for high moisture grain and their effect on the nutritive values of barley for growing-finishing cattle. Can. J. Anim. Sci. 69, 1007–1020.
Matthé, A., Lebzien, P., Hric, I., Flachowsky, G., 2003. Influence of prolonged adaptation periods on starch degradation in the digestive tract of dairy cows. Anim. Feed Sci. Technol. 103, 15–27.
Mowat, D.N., McCaughey, P., Macleod, G.K., 1981. Ammonia or urea treatment of whole high moisture shelled corn. Can. J. Anim. Sci. 61, 703–711.
Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung Jr., L., 2018. Silage review: recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980–4000.
Müller, D.W.H., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J., Clauss, M., 2013. Assessing the Jarman–Bell Principle: Scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. Comp. Biochem Physiol. Part A: Mol. Integr. Physiol. 164, 129–140.
Navarro, S., 2012. The use of modified and controlled atmospheres for the disinfestation of stored products. J. Pest Sci. 85, 301–322.
Nikulina, A., Sarnataro, C., Fabro, C., Mason, F., Spanghero, M., 2018. In vitro ammonia release of urea-treated high moisture barley and maize grain. J. Anim. Feed Sci. 27, 173–178, 2018.
Oji, U.I., Mowat, D.N., Winch, J.E., 1977. Alkali treatments of corn stover to increase nutritive value. J. Anim. Sci. 44, 798–802.
Olsson, J., Borjesson, T., Lundstedt, T., Schnurer, J., 2002. Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Int. J. Food Microbiol 72, 203–214.
Ørskov, E.R., 1979. Recent information on processing of grain for ruminants. Livest. Prod. Sci. 6, 335–347.
Ørskov, E.R., Reid, G.W., Holland, S.M., Tait, C.A.G., Lee, N.H., 1982. The feeding value for ruminants of straw and whole crop barley and oats treated with anhydrous or aqueous ammonia or urea. Anim. Feed Sci. Technol. 8, 247–257.
Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1998. Acidosis in cattle, a review. J. Anim. Sci. 76, 275–286.
Patra, A.K., Aschenbach, J.R., 2018. Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: a review. J. Adv. Res 13, 39–50.
Phillip, L.E., Garino, H.J., Alli, I., Baker, B.E., 1985. Effect of anhydrous ammonia on amino acid preservation and feeding value of high-moisture ear corn for growing steers. Can. J. Anim. Sci. 65, 411–417.
R Core Team, 2020. R: A language and environment for statistical computing, Vienna, Austria.
Raeker, M.O., 1990. Preservation of high moisture corn by propionate treatment. Iowa State University.
Robinson, P.H., Kennelly, J.J., 1988. Influence of ammonia of high moisture barley on its in situ rumen degradation and influence on rumen fermentation in dairy cows. Can. J. Anim. Sci. 68, 839–851.
Robinson, P.H., Kennelly, J.J., 1989. Influence of ammoniation of high-moisture barley on digestibility, kinetics of rumen ingesta turnover, and milk production in dairy cows. Can. J. Anim. Sci. 69, 195–203.
Soest, P.J.V., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Spanghero, M., Mason, F., Zanfi, C., Nikulina, A., 2017. Effect of diets differing in protein concentration (low vs medium) and nitrogen source (urea vs soybean meal) on in vitro rumen fermentation and on performance of finishing Italian Simmental bulls. Livest. Sci. 196, 14–21.
Zinn, R.A., Barreras, A., Corona, L., Owens, F.N., Ware, R.A., 2007. Starch digestion by feedlot cattle: predictions from analysis of feed and fecal starch and nitrogen. J. Anim. Sci. 85, 1727–1730.