Milk production, rumen function, and digestion in dairy cows fed diets differing in predominant forage and concentrate type

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Abstract

The objective was to determine the effect of dietary ratio of neutral detergent fibre (aNDFom) to starch within diets differing in grass to maize silage ratio on rumen function, diet digestion, serum haptoglobin, and production of lactating dairy cows. Four isonitrogenous diets were formulated with a forage to concentrate ratio of 50:50, with the forage proportion containing either a high or low ratio of grass silage to maize silage (82:18 [GS] or 18:82 [MS] on a dry matter [DM] basis, respectively) and the concentrates containing either a high (F) or low (S) aNDFom to starch ratio, giving 4 dietary ratios of aNDFom to starch. Diets were fed to 4 early lactation Holstein dairy cows in a 4 × 4 Latin square design with 28-d periods. Feed intake, eating behaviour, milk production and composition, total tract digestion, nitrogen (N) excretion, aNDFom passage rate and *in-situ* degradation, rumen pH, and serum haptoglobin were measured during the last week of each period. Cows fed the MS diets consumed 1.34 kg/d more DM (P = 0.047) and 2.38 kg/d more starch (P = 0.001) compared to GS diets and produced 2.46 kg/d more milk (P = 0.038). Milk fat concentration was higher (+2.88 g/kg) for cows fed GS diets compared to MS diets (P = 0.007), while cows fed S concentrates had a higher milk fat concentration (+1.8 g/kg) irrespective of forage source (P = 0.033). Digestibility of aNDFom was higher (+0.106 kg/kg) for GS diets than for MS diets (P = 0.004). Similarly, aNDFom digestibility was higher (+0.057 kg/kg) for F concentrates (P = 0.031). Rumen and total-tract particle retention times were higher (+11.9 and +9.1 h, respectively) for cows fed GS diets (P = 0.009 and P = 0.037, respectively). Milk N yield/N intake was higher for the MS diets versus GS diets (P = 0.045), due to a greater (+130 g/d) milk protein yield (p = 0.015). Cows fed the MS diets spent 187 min/d more with rumen pH below 5.8 compared to GS diets (P = 0.006). Serum haptoglobin concentration, a purported marker of gut inflammation, was 5.3 ng/ml higher for

cows fed S concentrates versus F concentrates (P = 0.023). In conclusion, changes in concentrate aNDFom:starch ratio had little effect on DM intake, milk yield and composition, rumen function, and eating behaviour compared to effects of silage source (MS vs GS), where replacing a portion of diet GS with MS increased feed intake, milk yield, rumen passage rate, and N digestion, but also reduced fibre digestion and milk fat concentration. These observations suggest a greater effect of forage type on lactation performance than concentrate type per se under the conditions of the current study.

*Key word*s**:** starch, effective fibre, nitrogen excretion, rumen function.

Abbreviations: ADFom, acid detergent fibre; aNDFom, neutral detergent fibre; BCS, body condition score; BW, body weight; DM, dry matter; F, diets with high aNDFom concentrates; GS, grass silage; GS-F, high grass silage diet with high aNDFom concentrates; GS-S, high grass silage diet with high starch concentrates; MS, maize silage; MS-F, high maize silage diet with high aNDFom concentrates; MS-S, high maize silage diet with high starch concentrates; S, diets with high starch concentrates; VFA, volatile fatty acids; R-MRT; rumen mean retention time; N, nitrogen; SARA, subacute rumen acidosis.

1. Introduction

The average milk yield of dairy cows continues to increase worldwide, leading to increased energy and protein requirements (Eastridge, 2006; March et al., 2014). To meet these higher nutritional requirements, large amounts of cereal grains and other concentrate feeds are often included in dairy cow rations, supplying high quantities of readily degradable starch which may lead to negative effects on rumen metabolism, such as subacute rumen acidosis (SARA; Kleen et al., 2003; Plaizier et al., 2008). In the UK dietary starch concentrations are generally lower than those encountered in North America (Eastridge, 2006), but the higher inclusion of wheat and barley that are rapidly degraded in the rumen (Offner et al. 2003; Endres and Espejo, 2010), increases the risk of SARA at lower diet starch concentrations than when maize grain is fed (Tayyab et al., 2018). Additionally, grass silage, which is often wet and acidic, is the main forage fed on many dairy farms in the UK (March et al., 2014; Tayyab et al., 2018) and may also increase the risk of SARA. The incidence of SARA can result in inflammation of the gut wall that disrupts the epithelium of the reticulo-rumen by altering the tight junctions of the epithelial lining (Steele et al., 2011; Zebeli and Metzler-Zebeli, 2012). Increases in endothelial permeability allows ruminal endotoxins to enter into the blood circulation that can trigger the release of acute phase proteins such as haptoglobin as an innate immune response (Ametaj et al., 2010; Plaizier et al., 2012).

The dietary inclusion of sufficient fibre can help to ensure optimum rumen function by maintaining an appropriate rumen pH, increasing particle retention time and improving overall diet digestibility in dairy cows (Zebeli et al., 2012). The dietary proportion of fibre and starch can also alter the rate of production and proportion of ruminal VFA in the rumen, which can impact on animal performance and milk quality (Zebeli et al., 2010). The composition of rumen-

fermentable carbohydrates and physically effective neutral detergent fiber (peNDF), and their interaction should therefore be considered when formulating diets (Allen, 1997; Armentano and Pereira, 1997; Mertens, 1997), and the aNDFom to starch ratio has been proposed as a key indicator to evaluate the effect of carbohydrate composition on nutrient digestibility and milk production (Beckman and Weiss, 2005).

Our previous study reported that feeding a short compared to a longer particle length grass silage had little effect on the reticulo-rumen pH in dairy cows, but altered intake and milk performance when fed alone or in combination with maize silage (Tayyab et al., 2019). However, the effects of different dietary aNDFom to starch levels in diets based on a short chop grass silage or grass/maize silage mixtures on rumen metabolism and performance are unclear. It was hypothesized that diets containing a high level of starch relative to aNDFom would reduce rumen pH and fibre digestion, while those containing a higher concentration of aNDFom would decrease rumen passage rate and DMI. Therefore, the objective was to determine the effects of the dietary ratios of aNDFom to starch and grass to maize silage on rumen function and passage kinetics, eating behaviour, serum haptoglobin concentration, and milk yield and composition of dairy cows.

2. Materials and methods

2.1. Forages and diets

A first cut perennial ryegrass silage (*Lolium perenne*) was mown and harvested using a self-propelled precision forage harvester and ensiled in a concrete-walled clamp with an additive containing lactic acid producing bacteria (Axphast Gold, Biotal, Worcestershire, UK) at two litres/tonne. Maize silage (*Zea mays*) was harvested and ensiled in a concrete-walled clamp without additive. The mean geometric particle size (X_m) of the maize silage and ryegrass silage

were 10.2 and 23.6 mm, respectively (measured as described by Tayyab et al., 2018). Four TMR diets with a forage:concentrate ratio of 50:50 (DM basis) were formulated to have two ratios of GS to MS; either 82:18 (GS) or 18:82 (MS) on a DM basis, respectively. Silage clamp core samples of the GS and MS used analyzed by infrared spectroscopy (Trouw Nutrition, Ashbourne, UK) for diet formulation had the following predicted composition, respectively: 643 and 737 g digestible OM/kg DM (D value); 10.3 and 11.75 MJ ME/kg DM; pH 3.8 and 4.2; 29 and 57 g NH3N/kg totalN); and 102 and 37 g/kg DM lactic acid. Concentrates for the diets were formulated with either a high (F) or low (S) aNDFom:starch ratio, primarily by substitution of soyhulls as a primary aNDFom source with cracked wheat and maize as starch sources (Table 1). The two GS to MS and concentrate aNDFom:starch ratios were used in a 2 × 2 factorial arrangement resulting in 4 diets consisting of high GS with a high aNDFom concentration (82:18 G:M, 414 g/kg aNDFom and 90 g/kg starch; GS-F), high GS with a high starch concentration (82:18 G:M, 309 g/kg aNDFom and 220 g/kg starch; GS-S), high MS with a high aNDFom concentration (18:82 G:M, 345 g/kg aNDFom and 214 g/kg starch; MS-F), and high MS with a high starch concentration (18:82 G:M, 258 g/kg aNDFom and 319 g/kg starch; MS-S) on a DM basis (Table 1). Diets were formulated to contain a similar crude protein (CP) concentration (170 g/kg DM) and provide similar amounts of metabolizable protein sufficient to meet predicted requirements (Thomas, 2004). The formulated diet aNDFom to starch ratio was highest in GS-F at 4.6 and lowest for MS-S at 0.8.

2.2. Animals, feeding and experimental routine

Four early lactation (61 ± 0.2 [SD] DIM) Holstein dairy cows (in their 2nd parity and producing 44.2 kg milk/d [± 0.1 SD]) fitted with a rumen cannula (#1C, Bar Diamond, PO Box 60, 29575 Bar Diamond Lane, Parma, Idaho, USA) at the end of their previous lactation were

initially assigned randomly to one of the 4 dietary treatments within a 4 × 4 Latin square design, balanced for carryover effects, with 4 periods each of 28-d duration. The experiment was conducted under the authority of the UK Animals (Scientific Procedures) Act (1986; amended 2013). The first week of each period was used for incremental change to the new treatment diet, week 2 for adaptation to the diet, with weeks 3 and 4 designated as sampling weeks. Diets were prepared daily using a Calan Data Ranger (American Calan, New Hampshire, USA). During the first two weeks of each period, cows were housed in a cubicle yard with individual feeding through Calan gates (American Calan, New Hampshire, USA). Cows were fed 4 times/d (0500, 1000, 1600 and 2200 h) throughout the experiment, and refusals were removed daily at 0930 h. Whilst in the cubicle yard cows were milked twice daily at 0600 and 1600 h in a 50-stall rotary parlour (Dairy Master, Worcestershire, UK). At the start of week 3, cows were moved to individual metabolism stalls and followed a similar feeding and milking routine using facilities described previously (Thomson et al., 2017). One cow was removed from the study in period 2 due a health problem unrelated to the study and replaced with another cow of similar yield and parity for measurements in period 3 and 4 that did not require a rumen fistula. Data from the cow that became ill was not used.

2.3. Intake and milk yield and composition

Measurements of DMI, milk yield and milk composition were taken over the last 6-d of each period. Fresh feed was offered daily for ad libitum intake with 10% refusals. Daily TMR and forage samples were composited for the final week of each period and stored at -20°C for subsequent analysis. Forage samples were collected daily to determine DM concentration and to allow the adjustment of the fresh weight inclusion of the diet components. Consecutive milk samples were collected for the last 6-d of each period and analysed for fat, protein, casein,

lactose, urea, and milk FA as described previously by Thomson et al. (2017). The body weight of cows was recorded at the start of the study and at end of each period. Fresh water was available continuously.

2.4. Rumen degradability and passage kinetics

On d-15 of each period, the *in situ* dacron bag method was used to estimate the degradability of GS aNDFom (GS-aNDFom; Åkerlind et al., 2011). Duplicate samples of GS (5 ± 0.13 g DM) were incubated in the rumen of each cow for 0, 2, 4, 8, 16, 24, 48 and 96 h intervals as described previously by Tayyab et al. (2016). Particle passage kinetics was estimated using chromium-mordanted GS aNDFom (Cr-aNDFom) according to Udén et al. (1980). The Cr-aNDFom was inserted directly in the rumen via the cannula (or fed to the intact cow by top-dressing the diet at 0800 h) on d-21 of each period. Faeces was collected at -1 (to measure the background concentration of the marker), 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 40, 44, 48, 52, 56, 64, 72, 80, 88, 96, 108, 120, 132 and 144 h to estimate particle passage kinetics (Hammond et al., 2014).

2.5. *Eating and rumination behaviour*

Continuous recordings of the eating and ruminating behaviour of each cow were made for a 4-d period commencing on d-15 of each period using jaw movement recorders (Rutter et al., 1997). Recordings commenced daily at 1000 h and continued for 23.5 h; data were downloaded daily during the remaining 30 min period. Jaw movement recording was analysed with proprietary software (Rutter, 2000) to identify periods of eating and ruminating.

2.5. Particle size determination and sorting activity

Offered diets and refusals were sampled for particle size determination for 5-d during the final week of each period and stored at -20°C for subsequent analysis. Samples were defrosted at room temperature for 6 h, pooled across each treatment diet and period and assessed in triplicate

using a modified Penn State Particle Separator (Tayyab et al., 2018) to determine particle size distribution (DM basis). The Penn State Particle Separator contained sieves with holes that measured 33, 19, 8 and 4 mm diameter, and a bottom pan. The X_m of the diets and forages was calculated using the method described by ASABE (2007). The physical effectiveness factor (pef) was determined as the DM proportion of particles longer than 4 or 8 mm (Lammer et al., 1996; Thomson et al., 2017). The physically effective fibre concentration (peNDF) was calculated by multiplying the aNDFom concentration of the diet by its pef (Mertens, 1997). Sorting activity was calculated as the actual intake of each fraction expressed as a percentage of the predicted intake of each fraction, where a sorting value of < 100% indicated selective refusals, > 100% preferential consumption, and 100% no sorting (Leonardi and Armentano, 2003).

2.6. Diet digestion and nitrogen excretion

During the last 5-d of each period, a total collection of faeces and urine was performed by using a harness and chute fitted on each cow (Thomson et al., 2017). Faeces were collected via the chute into a tray that was emptied at regular intervals into a large bucket. Urine was collected via a collection cup glued over the vulva of the cow and tube that emptied into a 25 L container containing 1200 mL of 10N sulphuric acid to maintain urine pH < 2.0. The urine collection container was agitated several times during the day to ensure mixing of the acid and urine. Sub-samples of the mixed 24 h collections were bulked as a proportion of the daily excretion to account for daily differences in excreta weight (5% for faeces, 1.25% for urine) and stored in a sealed container at 4℃ until the end of sampling week. At the end of each sampling week the bulked sample was mixed and subsamples stored at -20℃ for subsequent analysis. Water intake was also recorded for 6-d during the final week of each period.

2.7. Rumen pH, ammonia, and volatile fatty acids and blood sampling

On day 22 of each period spot samples of rumen liquor were taken prior to feeding and then at 0.5, 1.5, 3 and 6 h post feeding for the subsequent determination of pH, VFA and ammonia concentration as described by Thomson et al. (2017). Approximately 80 ml of rumen fluid was collected into a beaker by inserting a fixed probe through the seal of the rumen cannula bung to a fixed depth in the ventral sac of the rumen. Following the measurement of pH a subsample for ammonia analysis was acidified (pH < 2) and then acidified and unacidified samples for VFA analysis were immediately frozen and stored at -20℃ until analyzed (Thomson et al., 2017). An indwelling pH probe (Sentix 41–3 probe, WTW Trifthof, Weilheim, Upper Bavaria) was also used to monitor rumen pH in the ventral sac for a 3-d period commencing at 1000 h on day 22 (Thomson et al., 2017). The pH probe was calibrated in standard solution of pH 4 and 7 prior to insertion and data was recorded at 15 min intervals. Blood samples were collected from all cows by coccygeal venepuncture on the 26th day of each sampling week at 0930 and 1530 h and held at room temperature for 3 h prior to centrifuging at 3000 g for 10 min and the serum separated and stored at -20°C prior to subsequent analysis for haptoglobin concentration.

2.8. Chemical Analysis

The diet samples were analyzed for DM concentration (AOAC, 2012; 988.05) and then milled through a 1 mm screen hammer mill (Crompton Control Series 2000, Wakefield West Yorkshire UK). The ash (942.05), ether extract (920.39) and CP (988.05) content was measured as described by AOAC (2012). Faecal samples were oven dried at 60°C for 72 h followed by subsequent determination of CP and ash concentration as described for feed samples and urinary N concentration was determined using the macro Kjeldahl method (Thomson et al., 2017). The aNDFom (using sodium sulphite and heat-stable α-amylase; Sigma, Gillingham, UK) and ADFom concentrations of mixed diets, forages, and faeces were measured according to the

237 procedure described by Mertens (2002) and expressed exclusive of residual ash. The starch
238 concentration of the MS and mixed diets was determined using the method described by
239 McCleary et al. (1997). Milk samples were analysed for fat, CP, casein, lactose, urea, and fatty
240 acid (FA) concentrations using mid-infrared spectroscopy on a Combi Foss machine (National
241 Milk Laboratories, Wiltshire, UK). Serum samples were analysed for haptoglobin (HP) using an
242 ELISA assay (Abcam, Cambridge, UK; intra-assay CV 9.1%). All spectrophotometric
243 measurements were undertaken using a BioTeck microplate reader (BioTeck Instruments Ltd,
244 Potton, UK) at 450 nm absorbance. Rumen VFA concentrations were determined using a gas
245 chromatograph (3400, Varian Inc., Crawley, UK) using the methods described by Aikman et al.
246 (2011), which included use of a 4% Carbowax 20M column (Supelchem, Sawbridgeworth, UK),
247 pivalic acid (2.5 mg/mL) as an internal standard, an oven temperature gradient between 180 and
248 200°C, and injector and detector temperatures of 220°C., Rumen ammonia concentrations were
249 determined by a colorimetric procedure (Sutton et al., 2003). Faecal chromium concentration
250 was analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, NexION® 2000,
251 PerkinElmer, Seer Green, UK) as described by Cope et al. (2009), with an intra-assay CV of
252 6.6%.

253 *2.9. Statistical Analysis*

254 Fat corrected (40 g/kg) milk yield was calculated as described previously (Gaines, 1928).
255 Rumen degradability profiles were fitted assuming an exponential degradation curve including a
256 lag time using SigmaPlot (Systat Software Inc., Berkshire, UK) according to the procedure
257 described by Ørskov and McDonald (1979). Effective rumen degradability (ED) of aNDFom
258 was determined at rumen fractional passage rate of 5 or 8%/h (including lag time) (Åkerlind et

al., 2011). Rumen retention time was calculated according to the procedure described by Dhanoa et al. (1985).

Data was analysed as a Latin square design using mixed models procedures of GenStat 17.1 (VSN International Ltd., Oxford, UK), with main effects of forage type (MS or GS), concentrate type (aNDFom:starch ratio), and their interaction using the following model:

$$Y = \mu + Fi + Cj + F\times Cij + Pj + Ak + €ijk,$$

Where Y is the observation, μ the overall mean, Fi is the forage type effect, Cj is the concentrate type effect, C×Fij is the interaction between F and C, Pj the fixed effect of period, Ak the random animal effect and €ijk the residual error. Data for manual and logger rumen pH, VFA and acute phase protein were analysed as repeated measurements. Results are presented as means ± SED, with a significance level of < 0.05 and a tendency at < 0.10.

3. Results

3.1. Diet composition

As intended, the forage aNDFom and diet aNDFom concentrations of the GS diets were numerically higher compared to the MS diets (Table 2), whilst starch concentration was numerically higher for MS diets. Similarly, within silage type differences in concentrate formulations were reflected by numerical differences in aNDFom and starch concentrations. Samples of GS and MS taken over the course of sampling periods for the current study contained (respectively, DM basis) 524 and 363 g/kg aNDFom, 306 and 178 g/kg aADF, 130 and 80 g/kg crude protein. The GS diets had a higher ($P = 0.001$) proportion of DM retained on the > 33 and 19 – 33 mm screens, while the MS diets had a greater ($P = 0.01$) proportion of particles retained on the 4 – 8 and 9 – 19 mm screens. Concentrate type also influenced diet particle size distribution, with the F diets (GS-F and MS-F) having a higher ($P = 0.001$) proportion of DM

retained on the 4 – 8 mm screen and a lower (P = 0.04) proportion retained on the < 4 mm screen compared to the S diets (GS-S and MS-S). The X_m of the GS diets was higher (P = 0.01) than the MS diets (7.55 and 5.96 mm, respectively). Both forage (P = 0.003) and concentrate type (P = 0.001) had an effect on the pef concentration (peNDF>4), with the GS-F diet having the highest (25.1%) and MS-S diet the lowest (15.2%) concentration.

3.2. Intake and milk yield and composition

Cows fed the MS diets consumed 1.34 kg/d more (P = 0.047) DM compared to the GS diets (Table 3). Similarly, milk yield was 2.46 kg/d greater (P = 0.038) for cows fed MS compared to GS diets. Milk fat concentration was 2.88 g/kg higher (P = 0.007) in cows fed GS diets compared to the MS diets, while cows fed the S concentrates had higher fat concentration (1.8 g/kg; P = 0.033) compared to the F concentrates. Milk crude protein (P = 0.007) and casein (P = 0.004) concentrations and milk protein yield (P = 0.015) were higher for cows fed the MS diets. Milk fat to protein ratio (F:P) was higher (P = 0.002) for cows fed the GS diets compared to the MS diets. The concentrations of total saturated fatty acids (SFA; P = 0.009), total unsaturated fatty acids (P = 0.034), C16:0 (P = 0.002) and C18:0 (P = 0.010) were higher in milk from cows fed GS compared to MS diets. The S diets resulted in 0.147 g/100g FA higher total milk SFA concentration compared to the F diets (P = 0.008), due mainly to a higher C16:0 concentration (P = 0.002).

3.3. Diet digestibility and grass silage fibre degradation and passage kinetics

Digestibility of OM was higher (P = 0.044) and there was a tendency (P = 0.056) for a higher DM digestibility for the S vs F diets (Table 4). Cows fed the MS diets excreted more faecal DM (P = 0.005) and OM (P = 0.004) compared to cows fed the GS diets, due to greater diet intake. In contrast, cows fed the S diets excreted less faecal DM and OM (P = 0.006) due to higher DM

and OM digestibility. The aNDFom and ADFom intakes were higher (P = 0.001) in cows fed the F diets, and there was a tendency (P = 0.062) for a higher aNDFom intake, and a higher ADFom intake (P = 0.013) for cows fed the GS diets compared to the MS diets. In contrast, cows fed the MS diets consumed 2 times more starch than cows fed the GS diets (P = 0.001) and cows fed S concentrates consumed on average 2.58 kg more starch daily than when they were fed the F concentrates (P = 0.001). Cows fed the GS diets also had higher (P < 0.004) aNDFom and ADFom total digestion and digestibility compared to the MS diets. Similarly, cows fed the F diets had higher (P = 0.031) aNDFom and ADFom total digestion and digestibility than when fed the S diets.

There was no effect of either silage or concentrate type on the overall *in situ* degradation kinetics of GS aNDFom, although the initial rate of disappearance was greater for the GS diets compared to the MS diets (Table 5). In contrast, the Cr-aNDFom escaped the rumen at a faster rate (P = 0.004) when cows were fed the MS compared to the GS diets, but concentrate type had no effect on Cr-aNDFom passage rate (P = 0.329). Similarly, rumen mean retention time and total-tract retention time was higher (P = 0.009 and P = 0.037, respectively) in cows when receiving the GS compared to the MS diets.

3.4. Nitrogen digestion and excretion

There was a tendency (P = 0.092) for a higher N intake for cows fed the MS compared to the GS diets, due to the higher DMI for the MS diets (Table 6). Faecal N output was higher (P = 0.023) in cows fed the GS diets, such that N digestibility was higher (P = 0.003) in cows fed the MSdiets. For urine N excretion an interaction was found between forage and concentrate type (P = 0.035), where the high S concentrate decreased urinary-N output when cows were fed the GS diets, but had no effect when the MS diets were fed. Milk N output increased (P = 0.015) when

cows were fed the MS compared to the GS diets, while there was no effect of concentrate type. Milk N output as a % of N intake was also higher (P = 0.045) in cows when fed the MS compared to the GS diets.

3.5. Rumen pH, ammonia, volatile fatty acids and serum haptoglobin

There was no effect of forage or concentrate type on mean, minimum or maximum rumen pH measured continuously (Table 7). However, cows fed the MS diets spent 187 min/d more (P = 0.006) with a rumen pH below 5.8. In contrast, cows fed the GS diets spent a longer time at a rumen pH of 6.2-6.5 (P = 0.010). There was a tendency (P = 0.071) for a longer time spent at rumen pH of 6.5-6.8 in cows fed the S diets compared to the F diets. Rumen fluid pH of individual samples in cows were similar to the rumen pH values measured by indwelling pH probe (Supplementary Figure S1). Rumen ammonia concentrations increased post feeding at 1000 h and reached a peak at 1130 h, with cows fed the MS diets having a 31.1 mg/L higher (P = 0.003) ammonia concentration compared to cows fed the GS diets (Figure 1). The F diets increased (+ 20 mM; P = 0.012) rumen acetate concentration in cows compared to the S diets (Table 7). The concentration of propionate was 9 mM higher (P = 0.001) in cows fed the MS compared to the GS diets (Table 7). Similarly, the acetate to propionate ratio was higher in cows fed the GS diets (+ 0.79; P = 0.001) or the F diets (+ 0.24; P = 0.001) compared to the MS diets or S diets, respectively (Table 7). There was an interaction between forage and concentrate type for both iso-valerate and caproate (P = 0.038 and 0.032, respectively), where their concentrations increased when the F concentrate was fed with GS, but concentrate type had little effect when MS diets were fed. The blood serum concentration of HP was 5.3 ng/ml higher in cows fed the S diets compared to the F diets (P = 0.023; Figure 2). There was no effect of time, forage type or their interaction on HP concentration.

351 *3.6. Eating behaviour and sorting activity*

352 There was no difference in eating time expressed as total (min/d), min/kg DMI, min/kg
353 aNDFom intake, and min/% peNDF between the dietary treatments (Table 8). Total rumination
354 time tended ($P = 0.060|$) to be higher in cows fed the F diets compared to the S diets. Cows fed
355 the GS diets had a 2.2 min/kg DMI longer ($P = 0.019$) rumination time compared to the MS
356 diets. When rumination time was calculated per kg aNDFom intake or per % peNDF, cows fed
357 the S diets had a longer ($P = 0.005$) rumination time compared to those fed F diets. There was no
358 main effect of forage or concentrate type ($P > 0.05$) on sorting activity of the different dietary
359 fractions.

360 **4. Discussion**

361 *4.1. Forage and diet composition*

362 Increasing starch concentrations in concentrates fed was achieved primarily by replacing
363 soyhulls with wheat and maize starch, more than doubling the starch to aNDFom ratio for both
364 GS and MS diets, and reducing the total aNDFom concentrations of the MS diet to values well
365 below recommended concentrations in the UK (Thomas, 2004) and USA (NRC, 2001). The
366 current study is part of a larger project where the particle size and peNDF of forages and diets
367 fed on the UK dairy herds were characterised (Tayyab et al., 2018, 2019). The particle size of the
368 grass silage used in the current study was within the shortest 2% of the mean values fed on UK
369 dairy herds reported in Tayyab et al. (2018). However, the particle size of the maize silage used
370 in the current study was similar to the mean values fed on UK dairy herds (Tayyab et al., 2018)
371 but higher than that fed ($X_m = 9.01$ mm) on North American herds (Maulfair et al., 2010).

372 *4.2. Milk production*

373 Cows had higher DMI when fed the MS diets compared to the GS diets, a finding in
374 agreement with Hart et al. (2015) and Tayyab et al. (2019) where DMI was increased when a
375 proportion of the GS in the diet was replaced by MS. This may partly be due to the longer
376 particle X_m for the GS diets compared to the MS diets that increased rumen retention time
377 (Table 5) and likely increased rumen fill and limited DMI (Zebeli et al., 2012; Nasrollahi et al.,
378 2015). The higher DMI in cows when fed the MS diets resulted in a higher milk yield compared
379 to the GS diets. Feeding dairy cows with diets containing a high fibre concentration is usually
380 associated with a higher milk fat concentration (Mertens, 1997). However, milk composition is
381 less responsive to dietary particle size in early to mid-lactation cows because of their negative
382 energy balance and mobilisation of body fat reserves resulting in an increase in fatty acids
383 available for milk fat synthesis (Zebeli et al., 2006). Contrary to previous findings, in the current
384 study, feeding cows a higher starch concentrate increased milk fat concentration compared to the
385 higher aNDFom concentrates. The reasons for this increase in milk fat concentration are unclear
386 as rumen acetate:propionate ratio was decreased when the S concentrates were fed. However,
387 feeding the higher starch concentrate may have increased glucose supply to the mammary gland
388 and there is evidence of a positive effect of glucose on milk fatty acid synthesis (Osorio et al.,
389 2016). Milk fat yield was not affected, and the increased milk fat concentration may in part be
390 due to a numerical decrease in milk yield when the S concentrate diets were fed. Cows fed the S
391 diets did have a higher rumination time relative to %peNDF$_4$ or %peNDF$_8$ and the relatively
392 rapid rumen degradation rate of soyhulls (Ipharraguerre and Clark, 2003) may also be factors.
393 Additionally, feeding excessive dietary peNDF (> 14-18%) has not been reported to increase the
394 milk fat concentration (Zebeli et al., 2012).

395 *4.3. Diet digestibility, nitrogen excretion, and rumen fibre degradation and passage kinetics*

396 The digestibility of DM and OM were not affected by forage type, however the S diets had
397 higher digestibility coefficients. Higher starch concentration in concentrates fed may have
398 provided a greater energy supply to rumen microbes to degrade and digest the diet compared to
399 the high aNDFom diets, as there was a trend for higher DM and OM digestibilities in cows when
400 fed high starch diets in the study by Caton and Dhuyvetter (1997). The more likely reason for the
401 increase in OM digestibility is that the starch that replaced aNDFom in the high starch
402 concentrate is more digestible compared to aNDFom (NRC, 2001). The digestibility of aNDFom
403 was depressed in cows fed the S diets, a finding in agreement with Ipharraguerre and Clark
404 (2003) who reported a lower total-tract aNDFom digestibility when starch replaced soyhulls in
405 the diet of dairy cows. Replacing a fibrous component of the diet with starch typically reduces
406 the total-tract digestibility of fibre (aNDFom or ADFom) in cows (Valadares et al., 2000). In
407 contrast, the digestibility of aNDFom and ADFom were both greater for GS compared to MS
408 diets, which may in part reflect the increased rumen retention time for GS aNDFom, more time
409 spent ruminating per kg DMI and fNDFom intake, and the greater amount of time rumen pH was
410 below 5.8 for MS diets. These are all factors that although associated with lower total DMI
411 would contribute to increased aNDFom and ADFom digestibility.

412 Nitrogen digestibility, milk N output and milk-N % of total N intake were higher in cows fed
413 the MS diets, as reported previously (O'Mara et al., 1998; Sinclair et al., 2015; Tayyab et al.,
414 2019). This was likely due to the higher starch and metabolizable energy concentration of the
415 MS diets, alongside the resulting increase in DMI. The values for milk N output and milk-N as a
416 % of total N intake were somewhat higher than reported in previous studies (Nevens et al., 2006;
417 Powell et al., 2010; Reynolds et al., 2014; Moorby et al., 2016), reflecting the higher milk
418 protein yield of cows used in the present study. The amount of intake N not recovered as milk,

faeces, and urine, which includes milk retained in the body and any volatile losses of N during sample handling and analysis, is similar to other studies reported in the literature (Sphangero and Kowalski, 2021) and not affected by treatment (data no shown).

In a previous study by Tafaj et al. (2001), a shorter particle size diet resulted in a higher passage rate through the gastrointestinal tract of dairy cows compared to a longer particle size. Rumen passage rate is influenced by various factors including diet composition, and especially diet starch and fibre concentration (Tafaj et al., 2007). However, in the current study, concentrate type did not affect the passage rate of grass-NDF, but the GS diets resulted in a higher R-MRT compared to the MS diets. The high R-MRT could explain a lower DMI in cows fed the GS diets due to a negative effect of rumen fill on intake (Zebeli et al., 2007). Previous studies have found no relationship between forage particle size and digesta passage rate through the rumen (Beauchemin and Yang, 2005; Tafaj et al., 2007). This lack of an effect of particle size on passage rate may be due to particle size reduction by chewing and mastication that may potentially increase the rate of finer particles escaping from the rumen (Beauchemin and Yang, 2005).

4.4. Rumen pH, VFA, and ammonia and serum haptoglobin

Rumen pH primarily depends on dietary composition (e.g. forage source, amount of concentrates, fermentability of concentrates and amount of fibre in the diet) and subsequent rate of saliva production and VFA absorption across the rumen epithelium (Zebeli et al., 2012; Nasrollahi et al., 2016). On a low forage diet (<50 % forage), rumen pH has been shown to decrease with decreasing particle size, but there was no effect when the forage proportion was high (Nasrollahi et al., 2016). To avoid SARA, Zebeli et al. (2012) suggested a high forage to concentrate ratio (56:44 DM basis) in the diet, but in the current study forages composed 50%

(DM basis) of the diet and were fed along with a high starch concentrate (MS diet) that was formulated to induce SARA. The starch concentration of MS-S diet was well above recommended levels in the UK and would be expected to induce SARA (Tayyab et al., 2019). Tafaj et al. (2007) reported a strong positive association (R^2 = 0.41) between aNDFom concentration and rumen pH, but in the current study feeding the S diets did not significantly affect mean rumen pH. This may be explained by the inclusion of maize meal as a starch source that is more resistant to rumen degradation compared to wheat-based starch (Moharrery et al., 2014) and the use of soyhulls in the F concentrates. Sub-acute ruminal acidosis has been defined as cows spending 5-6 h/d (300-360 min/d) under a rumen pH of 5.8 (Zebeli et al., 2008). In the current study, no cow experienced SARA according to this criteria, however, when cows were fed the MS diets they spent an average of 269 min/d under pH 5.8 compared to when fed the GS diets where they spent 82 min/d, irrespective of concentrate type (Table 7). Feeding a high starch diet (320 g/kg DM) to dairy cows has been reported to decrease the acetate concentration and increase the propionate concentration in the rumen compared to when fed a low starch diet (Oba and Allen, 2003), which is in agreement with the current findings. The higher acetate to propionate ratio in the current study was also in agreement with Beckman and Weiss (2005), where a high NDF:Starch diet (1.27) increased the acetate:propionate ratio in the rumen by 0.35 compared to a low NDF:Starch (0.74) diet. The higher ammonia concentration in cows fed the MS diets was likely due to a higher proportion of soybean meal and rapeseed meal and lack of rumen-protected soybean meal (Sopralin) compared to the GS diets. The serum concentration of HP in the current study was higher in cows fed the S diets compared to when they received the F diets, a finding in agreement with Khafipour et al. (2009) where cows fed high grain diets had increased serum HP concentrations (+475.6 μg/ml) compared to those fed a high NDF diet with

a low starch concentration. Serum HP concentration was lower in the current study compared to concentrations reported by Khafipour et al. (2009), which may be due to the higher starch concentration (33.4% starch) lower forage concentration (400 g/kg DM) of the diet fed and the occurrence of SARA in the study of Khafipour et al. (2009).

4.5. Feeding behaviour and sorting activity

The lack of an effect of forage or concentrate type on eating time in the current study could be due to the comparatively low X_m (< 8 mm) and peNDF>8 concentration (< 20%) of the diets fed. Feeding a longer dietary particle size diet generally results in an increase in eating and rumination time in dairy cows (Beauchemin and Yang, 2005; Tafaj et al., 2007). For example, increasing forage particle size in the diet from 6.7 to 10 mm resulted in an increase in eating time (+19 min/d) and ruminating time (+ 28 min/d) (Nasrollahi et al., 2016). The GF diet had the highest aNDFom concentration at 399 g/kg DM, but 38% of the aNDFom concentration was contributed by soyhulls that are a highly degradable source of fibre in the rumen and may not be as effective as forage aNDFom in promoting rumination (Ipharraguerre and Clark, 2003). Feeding the S diets s in the current study increased rumination time per kg aNDFom intake or per unit peNDF compared to the F diets. Sorting activity is often associated with an excessive consumption of starch rich concentrates in the diet and a lower fibre intake, which can decrease rumen pH and induce SARA (Leonardi and Armentano, 2003). Particle size of the diets in the present study was relatively short compared to the average particle size (19.5 mm) of dairy rations in the UK (Tayyab et al., 2018). Based on particle size distributions of the diets and refusals there was little sorting measured across all diets, which may be attributed to the individual and frequent feed provision in the current study.

5. Conclusions

In general, there were very few interactions observed between forage type and concentrate starch concentration, which may in part reflect the limited number of experimental observations obtained for some variables. Feeding diets higher in MS increased DMI, milk yield, rumen passage rate, nitrogen digestibility and nitrogen efficiency, but decreased milk fat concentration, aNDFom digestibility, rumen pH, rumen acetate to propionate ratio, and rumination time in dairy cows compared to feeding diets higher in grass silage. Concentrate type (aNDFom:starch ratio) had little effect on DMI, milk production, or grass silage aNDFom degradability or rumen passage rate, despite effects on rumen pH and aNDFom digestion. Feeding dietary starch levels well in excess of that currently recommended in the UK (150 to 200 g/kg DM) through added ground maize and wheat grains did not induce SARA, despite the short particle size of the GS fed. In the present study, forage type had a greater impact on digestion and production than concentrate aNDFom and starch concentrations, confirming the benefits of replacing grass silage with maize silage for feeding intake and milk yield.

Conflict of interest

The authors of the above manuscript have no conflicts of interest to declare.

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Table 1

Dietary formulation (kg/kg DM) and predicted composition (g/kg DM) of experimental diets.

	Treatment[1]			
Ingredients	GS-F	GS-S	MS-F	MS-S
Grass silage	410	410	090	090
Maize silage	90	90	410	410
Cracked wheat	56	170	80	140
Maize meal	-	72	-	090
Soyhulls	212	30	150	-
Soybean meal	52	40	120	120
Sopralin[2]	80	88	-	-
Rapeseed meal	50	50	100	100
Molasses	20	20	20	20
Limestone	5	5	5	5
Salt	5	5	5	5
Hi-mag mineral[3]	10	10	10	10
Megalac[4]	10	10	10	10
Predicted composition[5]				
ME (MJ/kg DM)	11.6	11.9	12.1	12.4
MPE[6]	113	114	116	118
MPN[7]	127	127	122	122
aNDFom	414	309	345	258
Starch	90	220	214	319
aNDFom:starch[8]	4.6	1.4	1.6	0.8

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S).

[2] Soybean meal treated to reduce rumen degradation (Trouw Nutrition, Belfast, UK).

[3] Mineral/vitamins premix supplied calcium (230 g/kg), sodium (95 g/kg), magnesium (40 g/kg), selenium (30 mg/kg), phosphorous (20 g/kg), zinc (5.2 g/kg), manganese (2.2 g/kg), copper (1.2 g/kg), and vitamin A (400,000 IU/kg), vitamin D (80,000 IU/kg), and vitamin E (2,000 IU/kg).

[4] A calcium salts of fatty acids (Volac, Royston, UK).

[5] Forumlated using Feed into Milk by Thomas (2004), diets were formulated for 37 kg/d milk[6]MPE, metabolizable protein-rumen energy limited.

[7] MPN, metabolizable protein-rumen nitrogen limited

[8] aNDFom to starch ratio.

Table 2
Measured chemical composition (g/kg DM) and particle size distribution in experimental diets.

	Treatments[1]					P value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
DM, g/kg	450	444	455	449				
OM	912	916	927	931				
CP	175	173	174	173				
Ether extract	20	25	24	22				
aNDFom[3]	399	295	347	266				
ADFom	253	168	208	144				
Forage aNDFom	248	248	196	196				
Starch	117	236	215	323				
aNDFom:Starch	3.44	1.26	1.70	0.84				
faNDFom:Starch	2.13	1.05	0.94	0.61				
Particle size distribution								
>33 mm	6.39	5.94	0.39	0.43	0.810	0.001	0.940	0.432
19-33 mm	21.66	21.78	13.01	13.78	1.625	0.001	0.898	0.819
8-19 mm	20.40	21.06	29.82	30.96	1.010	0.001	0.150	0.474
4-8 mm	14.51	9.64	16.01	11.72	0.401	0.002	0.001	0.225
<4 mm	37.04	41.57	40.78	43.10	1.718	0.078	0.039	0.384
$X_{m,}$ mm[4]	7.40	7.69	6.08	5.85	0.549	0.010	0.947	0.542
SD_{xm5}	3.15	3.16	2.71	2.79	0.061	0.001	0.371	0.395
$pef_{>4}$, %[6]	62.96	58.43	59.11	56.90	1.718	0.078	0.039	0.384
$pef_{>8}$, %	48.45	48.79	43.31	45.17	1.791	0.018	0.423	0.572
$peNDF_{>4}$, %[7]	25.07	17.27	20.46	15.16	0.851	0.003	0.001	0.094
$peNDF_{>8}$, %	19.28	14.43	14.95	12.04	0.767	0.002	0.001	0.133

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S).
[2] F = forage source, C = concentrate source, F × C = interaction between F and C
[3] faNDFom = forage aNDFom.
[4] Xm = geometric mean particle size.
[5] SDxm = SD of X_m.
[6] pef = physical effectiveness factor.
[7] peNDF = physically effective fibre.

Table 3
Production performance of cows fed diets differing in forage type and aNDFom:starch ratios.

	Treatments[1]					*P* value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
DMI, kg/d	23.1	23.1	24.9	24.1	0.67	0.047	0.436	0.450
Milk yield, kg/d	40.9	40.6	44.5	41.9	1.15	0.038	0.161	0.239
4% FCM, kg/d[3]	40.7	41.4	40.7	40.4	0.99	0.531	0.753	0.504
Feed efficiency[4]	1.76	1.76	1.79	1.75	0.027	0.259	0.665	0.352
Fat, g/kg	39.7	41.2	36.5	38.7	0.79	0.007	0.033	0.584
Fat, kg/d	1.63	1.66	1.63	1.62	0.04	0.531	0.753	0.504
Protein[5], g/kg	30.3	30.8	31.5	32.0	0.34	0.007	0.107	0.837
Protein[5], kg/d	1.23	1.24	1.40	1.34	0.046	0.015	0.476	0.308
F:P ratio[6]	1.32	1.33	1.16	1.22	0.026	0.002	0.092	0.303
Lactose, g/kg	46.9	46.9	46.8	46.8	0.36	0.796	0.920	0.935
Lactose, kg/d	1.92	1.91	2.08	1.96	0.044	0.023	0.098	0.165
Casein, g/kg	2.41	2.46	2.52	2.55	0.025	0.004	0.073	0.701
Urea, mg/kg	240	240	243	242	26.0	0.913	0.958	0.976
BW, kg[7]	664	669	667	671	5.13	0.537	0.260	0.819
Water intake, kg/d	95.5	83.0	86.5	82.5	5.47	0.287	0.100	0.337
Milk FA, g/100 milk[8]								
∑MUFA	0.93	0.93	0.87	0.90	0.029	0.087	0.366	0.424
∑PUFA	0.15	0.14	0.15	0.15	0.006	0.214	0.794	0.329
∑SFA	2.69	2.82	2.47	2.63	0.058	0.008	0.023	0.820
∑UFA	1.09	1.09	1.00	1.05	0.031	0.034	0.352	0.358
C16:0	1.15	1.23	1.03	1.12	0.022	0.002	0.006	0.793
C18:0	0.35	0.35	0.31	0.32	0.011	0.010	0.498	0.633
C18:1	0.80	0.81	0.75	0.78	0.031	0.146	0.403	0.548
n	4	3	3	4				

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S). Measurements averaged over the last 6 days of each period.

[2] F = forage source, C = concentrate source, F × C = interaction between F and C.

[3] FCM = fat corrected milk.

[4] Feed efficiency = kg milk/ kg DMI.

[5] Crude protein.

[6] F:P = Fat to protein ratio.

[7], BW = final body weight.

[8] FA = fatty acids, ∑ = total sum.

Table 4
Intake and digestion of diet components in cows fed diets differing in forage type and aNDFom:starch ratios.

	Treatments[1]					P-value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
DM, kg/d[3]								
Intake	22.97	22.80	24.87	23.68	0.908	0.096	0.350	0.471
Faecal output	6.24	5.69	6.99	6.21	0.160	0.005	0.004	0.368
Digestion	16.73	17.12	17.88	17.47	0.863	0.285	0.987	0.552
Digestibility, kg/kg	0.728	0.750	0.719	0.737	0.0108	0.226	0.056	0.764
OM, kg/d[4]								
Intake	20.94	20.93	23.05	22.05	0.866	0.058	0.455	0.467
Faecal output	5.42	4.88	6.14	5.46	0.159	0.004	0.006	0.565
Digestion	15.52	16.05	16.91	16.59	0.818	0.172	0.867	0.507
Digestibility, kg/kg	0.740	0.767	0.734	0.752	0.0107	0.222	0.044	0.614
Starch intake, kg/d	2.68	5.66	5.46	7.63	0.426	0.001	0.001	0.248
aNDFom, kg/d								
Intake	9.14	6.84	8.65	6.31	0.281	0.062	0.001	0.927
Faecal output	3.07	2.65	3.79	3.09	0.068	0.001	0.001	0.044
Digestion	6.07	4.19	4.86	3.22	0.174	0.003	0.001	0.529
Digestibility, kg/kg	0.663	0.607	0.558	0.501	0.0246	0.004	0.031	1.000
ADFom, kg/d								
Intake	5.80	3.82	5.16	3.42	0.174	0.013	0.001	0.389
Faecal output	2.08	1.71	2.43	1.87	0.048	0.002	0.001	0.049
Digestion	3.72	2.11	2.72	1.55	0.098	0.001	0.001	0.096
Digestibility, kg/kg	0.641	0.544	0.523	0.444	0.0255	0.004	0.008	0.632
n	4	3	3	4				

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S). Measurements made over the last 5 days of each period.

[2] F = forage source, C = concentrate source, F × C = interaction between F and C.

[3] DM = dry matter.

[4] OM = organic matter..

Table 5
In situ rumen degradation (% DM disappearance over time) and passage kinetics of grass silage aNDFom in cows fed diets differing in forage type and aNDFom:starch ratios.

	Treatments[1]					P value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
Degradation curve parameters[3]								
a, %	10.4	9.5	9.1	9.1	0.66	0.156	0.357	0.377
b, %	81.2	87.1	82.6	81.5	4.59	0.564	0.521	0.362
c, h	0.038	0.026	0.031	0.034	0.0051	0.823	0.297	0.130
lag time, h	2.84	3.76	3.41	3.45	0.543	0.763	0.303	0.332
ED5, %	37.6	31.6	32.4	33.6	2.55	0.429	0.281	0.141
Rumen passage kinetics, h[4]								
k1, /h	0.0252	0.0263	0.0344	0.0370	0.00236	0.004	0.329	0.642
k2, /h	0.1212	0.1175	0.1216	0.1167	0.01196	0.978	0.637	0.947
Tp	39.58	39.25	38.92	40.52	2.721	0.883	0.757	0.642
TT	18.23	17.74	19.58	19.75	1.902	0.280	0.912	0.819
R-MRT	41.3	36.4	27.2	28.2	3.30	0.009	0.444	0.280
TT-MRT	67.8	62.8	55.2	57.1	4.20	0.037	0.632	0.310
cT	203.3	188.4	165.6	171.3	12.60	0.037	0.632	0.310
n	4	3	3	4				

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S).
[2] F = forage source, C = concentrate source, F × C = interaction between F and C.
[3] a = soluble fraction, b = potentially degradable fraction, c = rate of degradation, ED5 = effective degradability at 5%/h passage rate.
[4] k1 = emptying rate of rumen, k2 = emptying rate of intestines, Tp = time to peak marker flow, TT = transit time, R-MRT = rumen mean retention time, TT-MRT = total-tract mean retention time, cT = clearance time.

Table 6
Nitrogen intake and excretion in cows fed diets differing in forage type and aNDFom:starch ratios.

N, g/d	Treatments[1]					P-value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
Intake	643	630	691	656	23.7	0.092	0.229	0.546
Faecal output	225	217	211	191	7.8	0.023	0.063	0.317
Digested	418	413	480	465	20.2	0.016	0.535	0.757
Digestibility, g/g	0.650	0.656	0.695	0.709	0.0109	0.003	0.276	0.620
Faecal-N of intake N, %	35.0	34.4	30.5	29.1	1.09	0.003	0.276	0.620
Urine	162	112	151	167	15.1	0.109	0.178	0.035
Urine-N of manure N, %	41.7	34.1	41.4	46.6	2.85	0.039	0.589	0.034
Urine-N of intake N, %	25.3	17.7	21.5	25.5	3.12	0.406	0.464	0.058
Milk N	197	199	224	214	7.4	0.015	0.476	0.308
Milk-N of intake N, %	30.6	31.6	32.5	32.9	0.77	0.045	0.257	0.634
n	4	3	3	4				

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S).

[2] F = forage source, C = concentrate source, F × C = interaction between F and C.

Table 7
Rumen pH and rumen volatile fatty acid concentration (mM) of cows fed diets differing in forage type and aNDFom:starch ratios.

Parameter	Treatments[1]					P value[2]		
	GS-F	GS-S	MS-F	MS-S	SED	F	C	F × C
Mean pH	6.19	6.20	6.08	6.11	0.055	0.087	0.607	0.796
Min pH	5.72	5.84	5.71	5.69	0.112	0.380	0.552	0.461
Max pH	6.47	6.58	6.59	6.61	0.151	0.561	0.574	0.692
T <5.5 pH[3]	20	71	35	16	43.6	0.560	0.337	0.642
T <5.8 pH	60	103	262	275	37.8	0.006	0.373	0.603
T 5.8-6.0 pH	134	193	283	285	52.9	0.049	0.478	0.497
T 6.0-6.2 pH	486	278	420	224	53.0	0.208	0.013	0.877
T 6.2-6.5 pH	661	541	345	404	55.9	0.010	0.493	0.110
T 6.5-6.8 pH	69	227	79	179	53.0	0.712	0.071	0.585
T >6.8 pH	4	20	27	33	14.7	0.185	0.370	0.670
Acetate	139.4	108.4	115.9	107.8	22.03	0.110	0.012	0.130
Propionate	39.6	34.8	44.8	47.6	6.80	0.001	0.677	0.104
A:P ratio[b]	3.46	3.26	2.72	2.43	0.171	0.001	0.001	0.432
Butyrate	29.0	24.9	26.0	24.9	4.35	0.304	0.079	0.307
Iso-Butyrate	1.2	1.1	1.0	1.2	0.18	0.898	0.770	0.014
Valerate	3.3	2.8	3.3	3.3	0.53	0.142	0.113	0.179
Iso-valerate	2.8	2.1	2.4	2.3	0.41	0.516	0.028	0.038
Caproate	2.4	1.7	1.6	1.4	0.36	0.001	0.001	0.032
n	3	3	3	3				

[1] Diets formulated to contain a high grass:maize silage ratio with a high aNDFom concentration (GS-F), a high grass:maize silage ratio with high starch concentration (GS-S), a low grass:maize silage ratio with high aNDFom concentration (MS-F), and a low grass:maize silage ratio with high starch concentration (MS-S).
[2] F = forage source, C = concentrate source, F × C = interaction between F and C.
[3] Time (min/d) spent under different pH levels during a day.
[4] Acetate:propionate ratio

Table 8
Eating behaviour in cows when fed diets containing a high grass:maize silage ratio with a high aNDFom concentration (GS-F), high grass:maize silage ratio with a high starch concentration (GS-S), low grass:maize silage ratio with a high aNDFom concentration (MS-F) or a low grass:maize silage ratio with a high starch concentration (MS-S)

Parameter	Treatments				SED	P value		
	GS-F	GS-S	MS-F	MS-S		F	C	F × C
Eating								
min/d	313	294	285	253	40.0	0.285	0.419	0.821
min/kg DMI	13.4	12.6	11.7	10.5	1.66	0.175	0.423	0.863
min/kg aNDFomI	33.8	41.8	34.1	39.0	4.57	0.713	0.115	0.663
min/kg faNDFomI	55.2	55.9	61.2	52.7	6.51	0.767	0.438	0.361
min/% $peNDF_{>4}$	12.5	16.3	14.1	16.9	1.75	0.422	0.057	0.680
min/% $peNDF_{>8}$	16.2	19.7	19.2	21.3	2.15	0.204	0.136	0.660
Ruminating								
min/d	561	515	522	500	18.6	0.108	0.060	0.395
min/kg DMI	24.1	22.2	21.5	20.7	0.75	0.019	0.061	0.329
min/kg aNDFomI	60.4	75.3	61.3	77.3	3.97	0.623	0.005	0.858
min/kg faNDFomI	97.8	96.0	112.9	104.6	5.19	0.023	0.228	0.422
min/% $peNDF_{>4}$	22.4	29.5	25.4	33.4	2.10	0.079	0.007	0.772
min/% $peNDF_{>8}$	29.1	35.5	34.8	42.1	2.84	0.038	0.027	0.835
n	4	3	3	4				

F = forage source, C = concentrate source, F × C = interaction between F and C, aNDFomI = aNDFom intake, faNDFomI = forage aNFDom intake

Fig. 1. Rumen ammonia concentrations in cows when fed diets containing a high grass:maize silage ratio with a high aNDFom concentration (GS-F;--×--), high grass:maize silage ratio with a high starch concentration (GS-S;--●--), low grass:maize silage ratio with a high aNDFom concentration (MS-F;--×--) or a low grass:maize silage ratio with a high starch concentration (MS-S;--●--) (SED = 1.93, Time effect P <0.001, F effect P = 0.003, C effect P = 0.51, F × C effect P = 0.63).

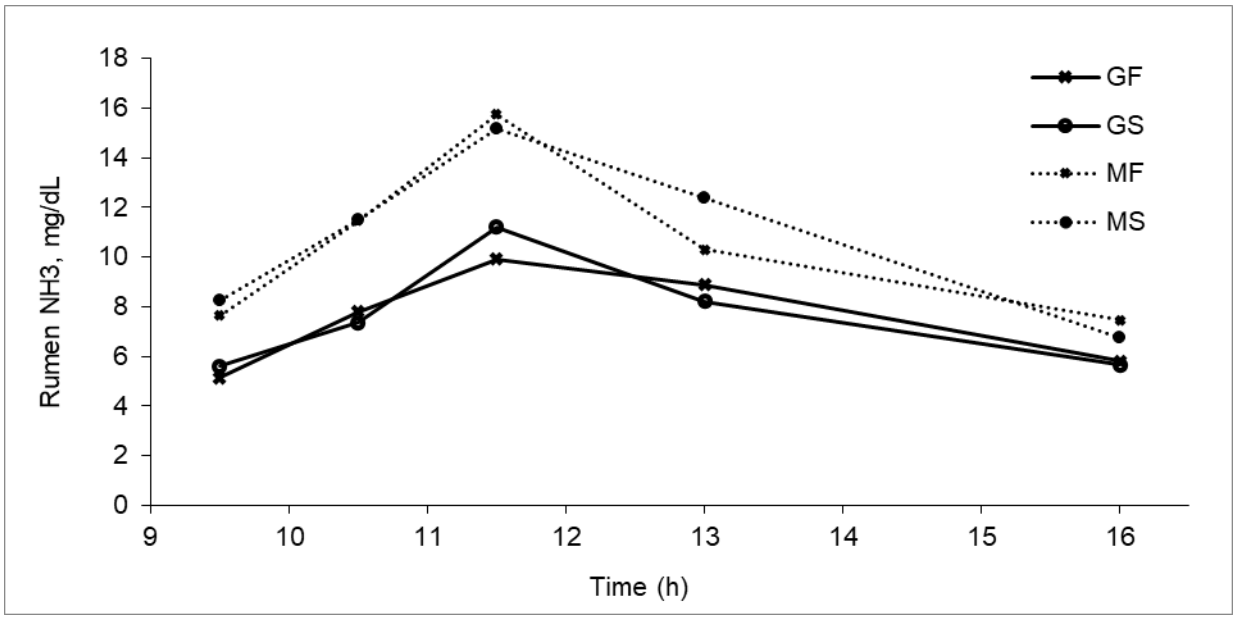

Fig. 2. Concentration of serum haptoglobin (HP) in cows when fed diets containing a high grass:maize silage ratio with a high aNDFom concentration (GS-F; --×--), high grass:maize silage ratio with a high starch concentration (GS-S; --●--), low grass:maize silage ratio with a high aNDFom concentration (MS-F; --×--) or a low grass:maize silage ratio with a high starch concentration (MS-S; --●--)
(SED= 4.04; F effect $P = 0.86$, C effect $P = 0.023$, F × C effect $P = 0.26$).