Influence of reducing starch in the diets with similar protein and energy contents on lactation performance, ruminal fermentation, digestibility, behaviour and blood metabolites in primiparous and multiparous dairy cows

B. Akhlaghi1 | E. Ghasemi1 | M. Alikhani1 | A. Ghaedi1 | S. M. Nasrollahi2 | M. H. Ghaffari3

1 Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
2 Horizon Ideologists Co., Isfahan, Iran
3 Physiology Unit, Institute of Animal Science, University of Bonn, Bonn, Germany

Correspondence
B. Akhlaghi, Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran. Email: behzad.akhlaghi67@yahoo.com

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Abstract

**Background:** It is not clearly known whether parity can affect the outcomes of starch reduction in the diet of lactating dairy cows.

**Introduction:** A 2 x 2 factorial study was conducted to evaluate the effects of reducing starch in the diets with similar protein and energy contents on lactation performance, ruminal fermentation, nutrient digestibility, behaviour and blood metabolites in primiparous (PP) and multiparous (MP) dairy cows.

**Methods:** Twenty PP cows (DIM = 37 ± 10; 40 ± 5 kg/day of milk; mean ± SD) and 20 MP cows (DIM = 37 ± 9; 48 ± 5 kg/day of milk) were used in present study. Treatments were a factorial arrangement of two levels of starch (high vs. low) and two parity categories (PP vs. MP): (1) high-starch diet (29.2% ± 0.70) and PP cows (HS-PP); (2) low-starch diet (22.3% ± 0.52) and PP cows (LS-PP); (3) high-starch diet and MP cows (HS-MP) and (4) low-starch diet and MP cows (LS-MP). All diets were formulated to be similar in crude protein (16.1 % of dry matter) and NEL (1.60 Mcal/kg of dry matter) contents. The amount of metabolise protein was 2688 g/day in high-starch diet and 2728 g/day in low-starch diet. The experiment was conducted over two consecutive periods and included 4 weeks for adaptation and 3 weeks for data collection.

**Results:** Dry matter intake and the yield of milk true protein and lactose increased but milk fat; protein ratio and nutrient digestibility decreased for cows fed the HS diets compared with the LS diets. The ruminal proportion of propionate was greater but acetate, the acetate to propionate ratio and sorting against long particles (19 and 8 mm) were lower for cows fed the HS diets than the LS diets. Multiparous cows had a greater nutrient intake and milk yield, longer rumination meal length, greater BW, but lower plasma total antioxidant capacity, non-esterified fatty acids, faeces pH compared with PP cows. An interaction between parity and the dietary level of starch was
detected on feed efficiency measured as FCM yield/DMI in the way that only within PP cows low-starch diet was more efficient than HS diets. We found another interaction effect of parity × starch on back fat thickens (BFT) change in the way that only within PP cows BFT change was greater for HS compared with LS diet.

**Conclusion:** Overall, regardless of the benefit derived from feeding a reduced-starch diet by partially replacing grains with sugar beet pulp in the diets on nutrient digestibility, a reduced-starch diet may be used more efficiently in PP than in MP cows but at expense of body reserves (i.e. BFT) loses.

**KEYWORDS**
dairy cow, lactation performance, parity, starch

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1 | INTRODUCTION

Increase in grain prices has renewed the interest in feeding low-starch diets to high-producing cows (>40 kg/day). Moreover, the price of and demand for cereal grains (e.g. for human-edible products and ethanol production) have increased in the last two decades (2000–2020). This encouraged the dairy nutritionists to reconsider the current feeding regimen and to formulate lower-starch diets (Krause & Oetzel, 2006). Feeding high-starch diets (28–32% of the total ration DM) to dairy cows may decrease the ruminal pH and increase the risk of subacute ruminal acidosis (Khafipour et al., 2009). Replacing cereal grain with high-quality forages, high-sugar feeds or by-product feeds are strategies to reduce the dietary starch content while maintaining the high yield potential in lactating cows (Münnich et al., 2018; Naderi et al., 2016; Nemati et al., 2020).

Several studies have examined the effect of decreasing dietary starch content through replacing grain with non-forage fibre sources (NFFS) on lactation performance (MacRae & Armstrong, 1969; Mertens & Loften, 1980; Nemati et al., 2020), ruminal fermentation and total-tract nutrient digestibility (Ferraretto et al., 2011; Nemati et al., 2020), and milk fatty acid composition (Akins et al., 2014; Ranathunga et al., 2010) of dairy cows. A recent study showed that replacing corn with molasses sugar beet pulp (BP) improved the rumen and hindgut conditions and fibre digestibility by promoting the physiological pH and bacterial diversity (Petri et al., 2019). Commonly, dietary starch content recommendations for lactating cows ranged between 23% and 30% of DM (Grant, 2005), 24% and 26% of DM (Staples, 2007) and greater than 24% of DM (Shaver, 2008). However, the effectiveness of this approach depends on several factors such as starch fermentability, available feed alternatives and animal status (e.g. parity and stage of lactation).

Periparturient dairy cows are highly variable in their ability to cope with the shift to energy-rich diets after calving (Penner et al., 2009; Bannink et al., 2012). Primiparous (PP) cows are generally more susceptible to low ruminal pH, higher ruminal volatile fatty acids (VFA) concentration and developing ruminal acidosis after calving compared with multiparous (MP) cows possibly due to differences in feeding patterns and variability in nutrient intake (Krause and Oetzel, 2006; Stauder et al., 2020). Besides, this is because PP cows have not been exposed to high-starch diets after calving (Enemark et al., 2004) and may differ in their feeding patterns, ruminal fermentation and metabolic characteristics compared with MP cows (Penner et al., 2007; Nasrollahi et al., 2017; Stauder et al., 2020). Therefore, low-starch diets may be suited better for the conditions of PP versus MP cows.

To our knowledge, there is a paucity of data to reveal how changes in the dietary starch content affect the lactation performance in PP compared with MP. We hypothesised that decreasing dietary starch content by partially replacing grains (corn and barley) with BP in the diets with similar protein and energy contents would not impair the intake, lactation performance or behaviour of dairy cows in early lactation, but we expected better performance response in PP cows compared with MP cows. This study aims at investigating the combined effects of a reduced-starch diet with parity (PP vs. MP) on lactation performance, ruminal fermentation, nutrient digestibility, blood metabolites and behaviour of dairy cows.

2 | MATERIALS AND METHODS

2.1 | Experimental design, cow management and treatments

The experiment was conducted at the Dairy Research Facilities of the Lavark Research Station from Isfahan University of Technology (Iran). Guidelines for the care and use of animals were approved by the Iranian Council of Animal Care (1995), as well as an advisory committee of the Isfahan University of Technology approved all experimental procedures.

Twenty PP cows (DIM = 37 ± 10; 40 ± 5 kg/day milk; parity = 2.2 ± 0.44; mean ± SD) and 20 MP cows (DIM = 37 ± 9; 48 ± 5 kg/day milk) were used for the present study. Treatments were a factorial arrangement of two starch levels (high vs. low) and two parity categories (PP vs. MP): (1) high-starch diet (29.2% ± 0.70% of DM, mean ± SD) and PP cows (HS-PP); (2) low-starch diet (22.3% ± 0.52% of DM, mean ± SD) and MP cows (LS-PP).
TABLE 1 Ingredients and chemical composition of the experimental diets

| Item                                      | Diet                  | High starch | Low starch |
|-------------------------------------------|-----------------------|-------------|------------|
| Ingredient composition, % of DM           |                       |             |            |
| Alfalfa hay                               | 12.6                  | 12.6        |            |
| Corn silage                               | 21.6                  | 21.5        |            |
| Wheat straw                               | 1.47                  | 1.47        |            |
| Beet pulp                                 | 1.82                  | 11.9        |            |
| Corn grain, ground                        | 17.9                  | 12.6        |            |
| Barley grain, ground                      | 17.9                  | 12.6        |            |
| Soybean meal                              | 11.6                  | 11.7        |            |
| Canola meal                               | 3.68                  | 3.66        |            |
| Whole soybean seeds, extruded             | 3.37                  | 3.36        |            |
| Cottonseed – high lint                    | 2.21                  | 2.20        |            |
| Wheat bran                                | 1.07                  | 1.06        |            |
| Energy booster                           | 0.99                  | 1.78        |            |
| Buffer                                    | 1.00                  | 1.00        |            |
| Calcium carbonate                         | 0.68                  | 0.48        |            |
| Salt                                      | 0.32                  | 0.32        |            |
| Magnesium carbonate                       | 0.28                  | 0.27        |            |
| Sodium bentonite                          | 0.40                  | 0.40        |            |
| Vitamin premix                            | 0.48                  | 0.47        |            |
| Mineral premix                            | 0.40                  | 0.39        |            |

Chemical composition, % of DM, unless otherwise stated (SD in parentheses)

| Item                                      | Diet                  | High starch | Low starch |
|-------------------------------------------|-----------------------|-------------|------------|
| Dry matter, % as fed                      | 46.0 ± 0.82           | 46.5 ± 1.05 |            |
| Organic matter                            | 90.8 ± 0.29           | 91.1 ± 0.33 |            |
| Crude protein                             | 16.1 ± 0.26           | 16.1 ± 0.52 |            |
| Ether-extract                              | 4.40 ± 0.20           | 4.80 ± 0.64 |            |
| Ash                                       | 9.22 ± 0.29           | 8.95 ± 0.33 |            |
| Forage neutral detergent fibre            | 17.4 ± 0.2            | 18.8 ± 0.3  |            |
| neutral detergent fibre                   | 32.9 ± 3.87           | 35 ± 3.51   |            |
| Non-fibre carbohydrate                    | 40.0 ± 1.48           | 38.0 ± 2.46 |            |
| Starch                                    | 29.2 ± 0.70           | 22.3 ± 0.52 |            |
| Water-soluble carbohydrate                | 3.66 ± 0.45           | 5.30 ± 0.48 |            |
| NEL,4 Mcal/kg of DM                       | 1.60                  | 1.60        |            |
| % DM retained on screens7                 |                       |             |            |
| 19 mm                                     | 6.47                  | 6.10        |            |
| 8 mm                                      | 28.4                  | 27.6        |            |
| 1.18 mm                                   | 41.7                  | 40.8        |            |
| Pan                                       | 23.3                  | 25.3        |            |
| peNDF > 8, % of DM                        | 34.8                  | 33.7        |            |
| peNDF > 1.18, % of DM                     | 76.6                  | 74.6        |            |
| (Continues)                               |                       |             |            |
However, before that the PP cows (as pregnant heifers) received a growing diet containing ~80% forage, and MP cows received a lactating diet (contained ~40% forage) followed by a far-off diet (contained ~80% forage for 6 weeks).

2.2 Intake, digestibility and analyses

Cows were fed individually twice daily at 0930 and 1730 h for ad libitum intake with a target refusal of 10% of DM/day. Each morning before feeding, the leftover was weighed and recorded. During the 3 sampling weeks, representative samples for BP (pooled within the period), as well as TMR (pooled by diet within the period) and residue (pooled by cow) were collected once a day. For the measurement of apparent total tract digestibility, the manure produced by cows was sampled during the last 4 days of each sampling period at 1100, 2000, 0500, 1400, 0800, 1700 and 0200 h and stored at −20°C until analysis. Dry matter of composited samples of BP, TMR and refusal was determined by drying at 60°C in a forced-air oven for 48 h, then adjusted to 100°C according to AOAC International (2002; method 925.40). Manure samples were thawed and dried in a forced-air oven at 60°C for 72 h. Before chemical analysis, dried samples were ground to pass through a 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Samples were analysed for crude protein (CP, Kjeltec 1030 Auto Analyzer; Tecator, Höganäs, Sweden; AOAC, 2002, ID 955.04), ether extract (AOAC, 2002, ID 920.39), water-soluble carbohydrate (Dubois et al., 1956), ash (AOAC, 2002; ID 942.05), starch (Zhu et al., 2016) and neutral detergent fibre (NDF) using heat-stable α-amylase (100 μl/0.5 g of sample, Van Soest et al., 1991). Apparent total tract digestibility of nutrients was determined using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977).

2.3 Rumen sampling and analysis

About 3 ml of rumen fluid samples were taken from the ventral sac 4 h after the morning feeding on the last day of the period (day = 49) via rumenocentesis technique (Nordlund and Garrett, 1994). Ruminal pH was determined using a portable digital pH meter (HI 8318; Hanna Instruments, Cluj- Napoca, Romania), then 4 ml of ruminal fluid was acidified with 1 ml meta-phosphoric acid 25% and the fluid samples were stored at −18°C until analysis for volatile fatty acids (VFA). VFAs were measured using the gas chromatography method (Chrompack, model CP-9002; Chrompack International BV, Middelburg, the Netherlands). Ruminal fluids were prepared with 50-m (0.32 mm i.d.) silica-fused column (CP-Wax Chrompack Capillary Column; Varian Inc., Palo Alto, CA), then nitrogen gas was entered as oven initial, as well as a carrier. Crotonic acid was used as an internal standard. Final temperatures were 55°C and 195°C, respectively. Detector and injector temperatures were set at 250°C.

2.4 Milk yield and components

Milk yield was recorded and milk samples were collected each day from 3 consecutive milkings (0100, 0900 and 1700 h) during the 3 weeks of sampling. Milk samples were pooled to the corresponding milk yield and kept with preservative potassium dichromate at 4°C before analysis for fat, protein and lactose using an infrared analyser (MilkoScan 134 BN; Foss Electric, Hillerod, Denmark; AOAC International, 2002; method 972.16). The milk urea nitrogen content was determined by enzymatic assay (Wilson et al., 1998). Fat corrected milk (4%FCM) yield was calculated as [(0.432 × kg of milk) + (16.23 × kg of milk fat)] (NRC, 2001).

2.5 Bodyweight and body condition

Bodyweights were measured after the morning milking of the beginning and the end day (day 1 and day 49) of the experimental period and the change in BW was calculated. Simultaneously, the BCS of cows was recorded using a 5-point scale with 0.25 intervals, where 1 = emaciated and 5 = obese (Ferguson et al., 1994). The backfat thickness (BFT) in the sacral region was measured by a veterinarian using ultrasound technique (Portable B-mode ultrasound generator; SonoVet 600V; BCF Technology Ltd., West Lothian, UK) with a linear transducer and frequency between 5.0 and 6.5 MHz (Kargar et al., 2013). Energy partitioning was calculated according to the equations recommended by Boerman et al. (2015).

2.6 Blood sampling and analyses

Blood samples were taken from the coccygeal vein of all cows approximately 4 h after feeding on the last day of the sampling period (day = 49). Blood samples were drawn into evacuated tubes with an anticoagulant of EDTA (1.95 mg/ml). Plasma was separated by centrifugation at 3000 × g for 20 min at 4°C and three aliquots of separated plasma were stored at −20°C before blood analysis. Complete blood count and blood gas analyses were accomplished by using an auto haematology analyser (Mindray, BC-5100) and Blood Gas and Electrolyte Analyzer (PTI CCA-TS), respectively, following the manufacturer’s instructions.

The plasma samples were analysed for cholesterol, glucose, albumin, total triacylglycerol, total protein, blood urea N (BUN; Pars Azmoon Co., Tehran, Iran), alanine transaminase (ALT, Pars Azmoon Co., Tehran, Iran) and aspartate transaminase (AST), using standard commercial kits and an automatic analyser (Alycon 300i, Dual voltage instrument; Abbott Laboratories Ltd., Chicago, IL). The analyser was calibrated with the control sera N and P (TrueLab NR and TrueLab PR, respectively; Pars Azmoon Co., Tehran, Iran) and aspartate transaminase (True-Cal UR, Pars Azmoon Co., Tehran, Iran) to ensure acceptable assay performance. Blood Globulin concentration was calculated as [(total protein – albumin)]. Plasma concentrations of non-esterified fatty acid
(NEFA) were determined by an enzymatic method (Randox Lab. Ltd., Ardmore, UK) using the same autoanalyzer. The intra- and inter-assay CVs for NEFA were 6.65% and 7.80%. Plasma malondialdehyde (MDA) was determined based on a colour complex formed from the reaction of MDA with 2-thiobarbituric acid (2-TBA) in an acidic environment as described by Chen et al. (2013). Total antioxidant capacity (TAC) was measured using a commercial kit (Randox Lab. Ltd., Ardmore, UK). Biorex Fars kit was used to measuring haptoglobin, while the ELISA kit with the Bioassay Technology laboratory (BT LAB, Shanghai 200090 China) was used for serum amyloid A (SAA) determination. All measurements were performed at the desired wavelengths using the ELISA Reader System (DANA-3200 ELISA READER).

### 2.7 Particle size distribution and behaviour

To determine the physical characteristics of the diets, the frozen samples were thawed and representative subsamples prepared for determination of particle size distributions via Penn State Particle Separator (PSPS; NASCO) equipped with three sieves (19.0, 8.0 and 1.18 mm). After separation of particle into four fractions of long (>19 mm), medium-size between 8 and 19 mm, short between 1.18 and 8 mm and eventually fine (<1.18 mm), the DM of each separated fraction was determined by oven drying at 65°C for 72 h. In this experiment, physical effectiveness factor [pef; the cumulative proportion of DM of particles retained on 2 sieves (Lammers et al., 1996) and 3 sieves (Kononoff et al., 2003) of the PSPS] was designated as pef > 8 and pef > 1.18, respectively. The particle distribution of the diets is summarised in Table 1. The physically effective NDF of either 2 (peNDF > 8) or 3 sieves (peNDF > 1.18) was calculated by multiplying the fraction of pef > 8 and pef > 1.18, respectively. To determine the sorting index of feed (SI), the ratio of actual intake of each particle fraction (>19 mm, 8–19 mm, 1.18–8 mm and pan) was expressed to the expected intake of that fraction (Leonardi and Armen-tano, 2003). The predicted intake of each fraction was calculated as the product of the intake of the total diet multiplied by the fraction in the offered TMR in percentage. A sorting index of 100 indicates no sorting, while indexes >100 and <100 imply sorting for particles and sorting against particles, respectively.

Chewing activity for each cow was monitored visually on day 48 of the collection period over a 24-h period. Chewing data include the duration of eating, ruminating and total chewing time, the number of ruminating bolus, and the chews per bolus and finally chews per minute. Estimation of time spent for ruminating and eating per kg of DM carried out based on data of neutral detergent fibre, forage NDF, peNDF > 8 and peNDF > 1.18 intakes, as well as average intake within the experimental period.

The data on eating and ruminating activities were registered at every 5-min with a 5-min interval between observations (Beauchemin et al., 2003; Krause et al., 2003). A period of ruminating by the cows was based on a 10-min interval including at least 5-min ruminating registered after 5 min without ruminating (Kargar et al., 2010). A meal was defined as at least one observation of eating activity occurring after at least 20 min without eating activity (Wangsness et al., 1976), while meal size (kg of DM/meal) was calculated as DMI divided by meal frequency (Crossley et al., 2018). The total chewing time was calculated based on the time spent for ruminating and eating, while the number of chews per bolus (chewing rate) during each ruminating period for each cow were counted for the first 10 boluses for a ruminating period were recorded and averaged to obtain a bolus chewing number for that rumination event (Kargar et al., 2013).

### 2.8 Statistical analyses

Data were analysed as a completely randomised, block (period) design with covariate using the MIXED procedure of SAS (version 8.1, SAS Institute Inc., Cary, NC). The model included the fixed effects of period, starch, parity, starch × parity, time (week) and starch × parity × time and the random effect of cow within starch × parity. The corresponding value of the dependent variable from the covariate period was considered as covariate (when available). When the time of treatment was included as a repeated measure, five covariance structures were tested (compound symmetry, compound symmetry with heterogeneous variance, autoregressive order 1, autoregressive order 1 with heterogeneous variance and antedependence 1) to select the structure with the lowest Akaike information criterion. For the variables without repeated measures during the study, time and starch × parity × time were removed from the model. The threshold of significance was set at \( p \leq 0.05 \); trends were declared at \( 0.05 < p \leq 0.10 \).

### 3 RESULTS

#### 3.1 Diet characteristics

The nutrient composition and physical characteristics of the diets are presented in Table 1. Crude protein and net energy contents were similar across diets, but high-starch (HS) diets had lower NDF (32.9% vs. 35% of DM), but greater non-fibrous carbohydrates (NFC, 40% vs. 38% of DM) and starch (29.2% vs. 22.3% of DM) contents compared with LS diets.

#### 3.2 Nutrient intake and feeding behaviour

Data on nutrient intake, sorting index, and meal patterns are presented in Table 2. Dry matter intake (kg/day or % of BW) and the intakes of NEL, OM, CP, starch and NFC \((p < 0.01)\) were greater for HS-fed cows compared to those fed the LS diets. Intakes of peNDF > 1.18 \((p = 0.06)\) and peNDF > 8 \((p = 0.09)\) tended to be higher for HS diets than LS diets. Intakes of NDF and EE were similar for all treatments. Cows fed HS diets sorted against long particles (19 and 8 mm; \(p = 0.03\)) of the diets.

Multiparous cows had higher intakes of DM, OM, CP, NEL, NDF, starch, NFC, EE, peNDF > 8 and peNDF > 1.18 \((p < 0.01)\) compared with PP cows. The dry matter intake for bodyweight per cent was
similar between MP and PP cows. Parity did not affect the sorting index either.

Data on chewing activity are presented in Table 3. Meal and rumination patterns were not affected by the level of dietary starch. Dietary treatments did not affect total chewing, eating and ruminating time. The number of eating bouts per day tended \((p = 0.09)\) to be lesser in PP cows than in MP cows. Primiparous cows had a greater energy partitioning (as a percentage of energy intake) for maintenance \((p < 0.01)\) compared with PP cows, but milk component concentrations were not affected by diets. Cows fed LS diets had greater FCM yield/DMI than cows fed HS diets \((p < 0.01)\). The average BW, BCS and the feed efficiency measured as milk yield/DMI and energy partitioning (as a percentage of energy intake) did not differ across dietary treatments.

Multiparous cows produced more fat, protein, lactose, milk and FCM \((p < 0.01)\) compared with PP cows, but milk component concentrations were similar for both groups. Multiparous cows had greater BW, BCS and the feed efficiency measured as milk yield/DMI and energy partitioning (as a percentage of energy intake) for maintenance \((p = 0.01)\). Compared with MP cows, primiparous cows had a greater energy partitioning (as a percentage of energy intake) for maintenance \((p = 0.01)\).

An interaction between parity and the dietary level of starch was detected on feed efficiency measured as milk yield/DMI \((p = 0.04)\) that only within PP cows LS diets were more efficient than HS diets. We found an interaction effect of parity \(\times\) starch on BFT change \((p = 0.05)\) that only within PP cows BFT change was greater for HS than LS diets.

### 3.3 Lactation performance

Data on lactation performance, body measurements and energy partitioning are presented in Table 4. Reducing the level of dietary starch from 29.2% to 22.3% did not affect yields of milk, 3.5% FCM and fat. The yield of milk true protein and lactose increased \((p = 0.04)\) for HS-fed cows compared to those fed the LS diets. Also, feeding LS diets tended \((p = 0.08)\) to decrease milk protein percentage compared with HS diets. Cows fed LS diets had greater concentration of milk \(\beta\)-hydroxybutyrate \((\text{BHB}, p < 0.01)\), milk urea nitrogen \((p = 0.02)\) and fat: protein ratio \((p = 0.05)\) than those fed HS diets. Lactose, SNF and fat percentage were not affected by diets. Cows fed LS diets had greater FCM yield/DMI than cows fed HS diets \((p < 0.01)\). The average BW, BCS and the feed efficiency measured as milk yield/DMI and energy partitioning (as a percentage of energy intake) did not differ across dietary treatments.

Multiparous cows produced more fat, protein, lactose, milk and FCM \((p < 0.01)\) compared with PP cows, but milk component concentrations were similar for both groups. Multiparous cows had greater BW compared with PP cows \((p < 0.01)\). Compared with MP cows, primiparous cows had a greater energy partitioning (as a percentage of energy intake) for maintenance \((p = 0.01)\).

### 3.4 Ruminal fermentation

Data on ruminal fermentation parameters are presented in Table 5. The ruminal pH and total VFA concentration in the rumen were not affected by the diets. The proportion of propionate \((p < 0.01)\) was greater but

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**TABLE 2** Nutrient intake, sorting index and meal patterns of primiparous and multiparous cows fed high-starch (29.2% ± 0.70; means ± SD) vs. low-starch (22.3% ± 0.52; means ± SD) diets

| Item               | Primiparous | Multiparous | SEM | Parity | Starch | Parity \(\times\) Starch |
|--------------------|-------------|-------------|-----|--------|--------|--------------------------|
| Nutrient intake    |             |             |     |        |        |                          |
| DM, kg/day         | 23.0        | 21.8        |     |        |        |                          |
| DM % BW            | 4.33        | 3.97        |     |        |        |                          |
| NEL intake, Mcal/day | 38.1     | 30.1        |     |        |        |                          |
| OM, kg/day         | 21.6        | 19.6        |     |        |        |                          |
| CP, kg/day         | 3.83        | 3.48        |     |        |        |                          |
| NDF, kg/day        | 7.83        | 7.54        |     |        |        |                          |
| Starch, kg/day     | 6.95        | 4.80        |     |        |        |                          |
| NFC, kg/day        | 9.52        | 8.19        |     |        |        |                          |
| EE, kg/day         | 1.04        | 1.03        |     |        |        |                          |
| peNDF > 8, kg/day  | 3.35        | 3.10        |     |        |        |                          |
| peNDF > 1.18, kg/day | 7.37   | 6.80        |     |        |        |                          |

**Sorting index, %**

| 19 mm  | 96.5 | 78.4 | 86.5 | 72.2 | 6.98 | 0.24 | 0.03 | 0.76 |
| 8 mm   | 99.0 | 93.8 | 98.3 | 94.5 | 1.99 | 0.99 | 0.03 | 0.73 |
| 1.18 mm| 101  | 102  | 101  | 102  | 0.65 | 0.93 | 0.09 | 0.73 |
| Pan    | 101  | 106  | 102  | 106  | 0.87 | 0.70 | <0.01 | 0.43 |

1 Contrasts for parity (Par), starch level (St) and interaction (Par \(\times\) St).

2 peNDF > 8 and peNDF > 1.18 = calculated by multiplying pef (DM retained on 19- and 8-mm sieves) and pef > 1.18 (DM retained on 19-, 8- and 1.18-mm sieves) by the NDF content of the diet (DM basis), respectively.

3 Sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi & Armentano, 2003).
acetate (p = 0.02) and the acetate to propionate ratio (p < 0.01) were lower for HS-fed cows compared to those fed the LS diets. The molar proportion of butyrate tended (p = 0.07) to be lower for HS-fed cows compared to those fed the LS diets. No differences were observed among the diets for the proportion of valerate, isovalerate and isobutyrate. The digestibilities of DM, OM and NDF were lower for HS-fed cows compared to those fed the LS diets (p < 0.01) but the digestibility of starch was not influenced by dietary treatments. No differences were detected in faeces pH and faeces score across treatments.

The molar proportion of butyrate tended to be lower in MP compared with PP cows (p = 0.07), the molar proportion of total VFA, acetate, propionate, isobutyrate, isovalerate, valerate and the acetate: propionate ratio was not affected by parity. Multiparous cows had lower faeces pH compared with PP cows (6.21 vs. 6.38; p < 0.01).

### 3.5 Blood parameters

The data of blood parameters are presented in Table 6. The blood concentration of AST was greater for HS-fed cows compared to those fed the LS diets (p < 0.01). The concentration of blood gas and complete blood count in plasma were not affected by the level of dietary starch.

| Table 3 Lactation performance, body measurements and energy partitioning of primiparous and multiparous cows fed high-starch (29.2% ± 0.70; means ± SD) vs. low-starch (22.3% ± 0.52; means ± SD) diets |
|---|---|---|---|---|---|---|
| Item | Primiparous | Multiparous | p Value<sup>1</sup> |
| | High starch | Low starch | High starch | Low starch | SEM | Parity | Starch | Parity × starch |
| Yield, kg/day | | | | | | | | |
| Milk | 40.4 | 41.5 | 53.2 | 48.7 | 1.76 | <0.01 | 0.31 | 0.11 |
| 3.5% FCM<sup>2</sup> | 36.6 | 38.4 | 48.6 | 46.0 | 1.58 | <0.01 | 0.82 | 0.18 |
| Fat | 1.15 | 1.24 | 1.51 | 1.48 | 0.05 | <0.01 | 0.62 | 0.35 |
| Protein | 1.22 | 1.18 | 1.58 | 1.41 | 0.05 | <0.01 | 0.04 | 0.19 |
| Lactose | 1.98 | 1.91 | 2.54 | 2.28 | 0.07 | <0.01 | 0.04 | 0.18 |
| Composition, % | | | | | | | | |
| Fat | 2.84 | 3.03 | 2.94 | 3.10 | 0.12 | 0.48 | 0.15 | 0.91 |
| Protein | 2.96 | 2.90 | 2.93 | 2.92 | 0.02 | 0.97 | 0.08 | 0.23 |
| Lactose | 4.80 | 4.68 | 4.74 | 4.73 | 0.04 | 0.88 | 0.17 | 0.24 |
| SNF<sup>3</sup> | 7.44 | 7.32 | 7.38 | 7.40 | 0.04 | 0.93 | 0.13 | 0.18 |
| Fat: protein | 0.95 | 1.04 | 0.99 | 1.06 | 0.03 | 0.61 | 0.05 | 0.91 |
| Milk urea nitrogen, mg/dl | 11.7 | 12.4 | 11.7 | 13.0 | 0.44 | 0.56 | 0.02 | 0.49 |
| β-hydroxybutyrate, mmol/L | 0.05 | 0.07 | 0.05 | 0.07 | <0.01 | 0.29 | <0.01 | 0.90 |
| MY/DMI | 1.75 | 1.89 | 1.87 | 1.71 | 0.05 | 0.61 | 0.40 | 0.08 |
| 3.5% FCM/DMI | 1.56a | 1.75b | 1.68 | 1.71 | 0.04 | 0.37 | <0.01 | 0.04 |
| Body measurements | | | | | | | | |
| BW, kg<sup>4</sup> | 549 | 544 | 658 | 638 | 16.4 | <0.01 | 0.44 | 0.63 |
| BCS<sup>5</sup> | 2.86 | 2.79 | 2.85 | 2.79 | 0.48 | 0.19 | 0.62 | 0.16 |
| BFT, mm | 26.8 | 25.9 | 25.5 | 25.9 | 0.06 | 0.54 | 0.54 | 0.14 |
| BW change, kg/day | 0.11 | 0.01 | 0.20 | 0.05 | 0.11 | 0.60 | 0.28 | 0.86 |
| BCS change | 0 | 0.07 | 0.06 | 0.03 | 0.06 | 0.75 | 0.87 | 0.21 |
| BFT change, mm<sup>6</sup> | 0.90a | -0.90b | -1.20b | -0.70a | 0.59 | 0.12 | 0.28 | 0.05 |
| Energy partitioning, % of intake | | | | | | | | |
| Maintenance | 25.9 | 26.5 | 23.1 | 25.1 | 0.84 | 0.01 | 0.13 | 0.42 |
| Milk | 71.9 | 73.4 | 73.7 | 74.5 | 1.62 | 0.38 | 0.48 | 0.82 |
| Body tissue gain | 2.21 | 0.06 | 3.11 | 0.40 | 2.08 | 0.76 | 0.25 | 0.89 |

<sup>1</sup>Contrasts for parity (Par), starch level (St) and interaction (Par × St).

<sup>2</sup>FCM yield = 0.432 × milk yield + 16.23 × fat yield (Council, 2001).

<sup>3</sup>SNF = solid non-fat.

<sup>4</sup>BW = over a 7-week period from week 1 of adaptation to week 6 of sampling.

<sup>5</sup>BCS = body condition score was determined using a five-scale method where 1 = emaciated and 5 = obese (Ferguson et al., 1994).

<sup>6</sup>BFT = backfat thickness was measured using the ultrasonographic method (Schröder & Staufenbiel, 2006).
TABLE 4  Chewing activities of primiparous and multiparous cows fed high-starch (29.2% ± 0.70; means ± SD) vs. low-starch (22.3% ± 0.52; means ± SD) diets

| Item                          | Primiparous | Multiparous | p Value¹  |
|-------------------------------|-------------|-------------|-----------|
|                              | High starch | Low starch  | High starch | Low starch | SEM | Parity | Starch | Parity × starch |
| Eating time                   |             |             |            |            |     |        |        |                  |
| Min/day                       | 362         | 330         | 384        | 369        | 19.4 | 0.12   | 0.23   | 0.64             |
| Min/kg of DMI                 | 15.7        | 15.2        | 13.8       | 14.0       | 1.07 | 0.15   | 0.87   | 0.73             |
| Min/kg of NDF intake          | 38.3        | 36.6        | 34.0       | 33.8       | 2.61 | 0.18   | 0.70   | 0.78             |
| Min/kg of peNDF² > 8         | 111         | 111         | 99.8       | 103        | 8.79 | 0.26   | 0.81   | 0.87             |
| Min/kg of peNDF² > 1.18      | 49.9        | 49.7        | 44.7       | 45.9       | 3.71 | 0.23   | 0.89   | 0.86             |
| Ruminating time               |             |             |            |            |     |        |        |                  |
| Min/day                       | 484         | 498         | 509        | 507        | 21.6 | 0.42   | 0.78   | 0.71             |
| Min/kg of DMI                 | 20.7        | 22.9        | 18.3       | 19.2       | 1.28 | 0.02   | 0.23   | 0.62             |
| Min/kg of NDF intake          | 50.7        | 55.1        | 45.2       | 46.0       | 3.17 | 0.02   | 0.42   | 0.58             |
| Min/kg of peNDF > 8          | 147         | 167         | 132        | 138        | 11.2 | 0.05   | 0.26   | 0.55             |
| Min/kg of peNDF > 1.18       | 66.4        | 74.5        | 59.5       | 62.0       | 4.65 | 0.04   | 0.25   | 0.55             |
| Total chewing time            |             |             |            |            |     |        |        |                  |
| Min/day                       | 846         | 828         | 893        | 877        | 30.0 | 0.11   | 0.56   | 0.97             |
| Min/kg of DMI                 | 36.4        | 38.1        | 32.1       | 33.3       | 2.03 | 0.03   | 0.49   | 0.89             |
| Min/kg of NDF intake          | 89.1        | 91.7        | 79.3       | 79.8       | 5.04 | 0.03   | 0.75   | 0.83             |
| Min/kg of peNDF > 8          | 259         | 279         | 232        | 241        | 18.1 | 0.08   | 0.42   | 0.76             |
| Min/kg of peNDF > 1.18       | 116         | 124         | 104        | 107        | 7.49 | 0.06   | 0.44   | 0.77             |
| Meals                         |             |             |            |            |     |        |        |                  |
| No. of bouts/day              | 10          | 9.50        | 10.8       | 10.2       | 0.44 | 0.09   | 0.22   | 0.91             |
| Length, min/meal              | 37.1        | 34.8        | 35.5       | 36.4       | 1.98 | 0.99   | 0.71   | 0.43             |
| Eating rate, g of DM/min      | 68.9        | 69.3        | 76.8       | 74.1       | 5.58 | 0.26   | 0.84   | 0.78             |
| Meal size, kg of DM           | 2.50        | 2.34        | 2.64       | 2.66       | 0.14 | 0.13   | 0.64   | 0.53             |
| Rumination                    |             |             |            |            |     |        |        |                  |
| No. of bouts/day              | 12.4a       | 14.0a       | 12.80b     | 11.5c      | 0.64 | 0.11   | 0.81   | 0.03             |
| Bout length, min/meal         | 39.3        | 36.1        | 40.7       | 45.4       | 2.53 | 0.04   | 0.76   | 0.12             |

²Within parity, means of starch levels with different superscripts differ (p ≤ 0.05).
²Contrasts for parity (Par), starch level (St) and interaction (Par × St).
²peNDF = the physically effective NDF of 2 (peNDF > 8) and 3 sieves (peNDF > 1.18), respectively.

Multiparous cows had a lower level of plasma TAC (p < 0.01), NEFA (p = 0.02) and BE (p = 0.02) compared with PP cows and there was a trend (p < 0.10) for a greater concentration of BP and ALT in MP compared with PP cows. Multiparous cows had lower plasma red blood cells compared with PP cows (p = 0.02).

4 | DISCUSSION

The objective of the present study was to compare the effects of feeding a reduced-starch diet on lactation performance, ruminal fermentation, digestibility, behaviour and blood metabolites between PP and MP dairy cows. As mentioned, this hypothesis was constructed based on previous observations on a greater sensitivity of PP cows to the high-starch diets at both levels of digestion (i.e. rumen) and metabolism (Penner et al., 2007; Nasrollahi et al., 2017; Stauder et al., 2020).

4.1 | Nutrient intake, sorting index and feeding behaviour

In the current study, lower dietary starch content decreased DMI, which was not expected because increasing starch in early lactation may decrease intake due to excess fermentable fuels in the liver (HOT theory) (Allen et al., 2009). In addition, beet pulp is a non-effective NDF source and would not be expected to decrease DMI (NRC 2001). However, in our experiment, feed intake decreased, possibly due to a high level of administration in place of grains. Indeed, substituting a
TABLE 5  Ruminal fermentation and nutrient digestibility of primiparous and multiparous cows fed high-starch (29.2% ± 0.70; means ± SD) vs. low-starch (22.3% ± 0.52; means ± SD) diets

| Item                      | Primiparous |                | Multiparous |                | p Value1 |          |          |          |          |          |          |
|---------------------------|-------------|----------------|-------------|----------------|----------|----------|----------|----------|----------|----------|----------|
|                           | High starch | Low starch     | High starch | Low starch     | SEM      | Parity   | Starch   | Parity × | SEM      | Parity   | Starch   | Parity × |
| Ruminal fermentation      |             |                |             |                |          |          |          |          |          |          |          |          |
| pH                        | 5.98        | 6.14           | 6.08        | 6.07           | 0.20     | 0.28     | 0.69     | 0.67     |          |          |          |          |
| Total VFA, mM             | 100         | 108            | 108         | 101            | 8.43     | 0.89     | 0.96     | 0.40     |          |          |          |          |
| Acetate                   | 60.6        | 63.7           | 60.5        | 63.7           | 1.29     | 0.99     | 0.02     | 0.95     |          |          |          |          |
| Propionate                | 25.3        | 21.6           | 26.1        | 22.6           | 1.16     | 0.44     | <0.01    | 0.91     |          |          |          |          |
| Butyrate                  | 10.0        | 11.0           | 9.60        | 10.1           | 0.40     | 0.07     | 0.07     | 0.49     |          |          |          |          |
| Isobutyrate               | 0.66        | 0.56           | 0.58        | 0.53           | 0.04     | 0.24     | 0.12     | 0.66     |          |          |          |          |
| Isovalerate               | 1.81        | 1.67           | 1.55        | 1.62           | 0.14     | 0.31     | 0.82     | 0.49     |          |          |          |          |
| Valerate                  | 1.54        | 1.38           | 1.55        | 1.36           | 0.12     | 0.95     | 0.18     | 0.92     |          |          |          |          |
| Acetate: propionate       | 2.56        | 3.04           | 2.37        | 2.90           | 0.17     | 0.36     | <0.01    | 0.88     |          |          |          |          |
| Nutrient digestibility    |             |                |             |                |          |          |          |          |          |          |          |          |
| Dry matter                | 69.7        | 74.4           | 68.1        | 74.2           | 1.17     | 0.45     | <0.01    | 0.56     |          |          |          |          |
| Organic matter            | 72.1        | 76.4           | 70.5        | 76.3           | 1.12     | 0.45     | <0.01    | 0.52     |          |          |          |          |
| NDF                       | 53.6        | 63.6           | 50.6        | 62.2           | 2.69     | 0.42     | <0.01    | 0.76     |          |          |          |          |
| Starch                    | 95.2        | 94.8           | 94.4        | 95.0           | 0.42     | 0.48     | 0.82     | 0.21     |          |          |          |          |
| Faeces pH                 | 6.36        | 6.40           | 6.20        | 6.23           | 0.05     | <0.01    | 0.52     | 0.85     |          |          |          |          |
| Faeces score              | 2.91        | 2.96           | 2.88        | 2.88           | 0.07     | 0.45     | 0.75     | 0.75     |          |          |          |          |

1 Contrasts for parity (Par), starch level (St) and interaction (Par × St).

high-fibre by-product for grains may have resulted in the limitation of intake by physical filling effects of the diet (Forbes, 1995) and this effect is more pronounced when subject animals are early lactation cows (Allen et al., 2009) like the present study. The LS diets contained more fat which could also partially cause a lower intake. Some previous studies have observed decreased DMI (Voelker and Allen, 2003a; Alamouti et al., 2009) when the starch content of the diets decreased but others found no effects on DMI (Fanchone et al., 2013; Dann et al., 2014; Alamouti et al., 2014) or increased DMI of dairy cows (Hall and Chase, 2014; Poorkasegaran and Yansari, 2014). This discrepancy among studies suggests that reduced dietary starch content alone does not reduce feed intake and this could be due to differences in nutrient composition of the diets.

Decreasing the starch content in the diets resulted in changes in the sorting index but did not affect the eating or rumination activities of dairy cows in the current study. When the dietary starch content was reduced from 29.2% to 22.3%, we observed a reduction in the extent of sorting against long particles (19.0 mm) and medium particles (8.0 mm) but not for very fine particles (pan). There is also more fibre in the LS, making it more difficult to sort of grain/starch. In the current study, the nutrient intake was higher in MP than in PP cows; however, the DMI expressed as a percentage of BW was not affected by parity, which indicates the role of body size on the greater intake of the MP cows (Maekawa et al., 2002). Beauchemin and Rode (1994) also showed that the PP cows had approximately 5 kg/day less DMI compared with MP. In this study, the meal size (kg of DM/meal), meal length (min/meal) and eating rate (kg of DM/min) were similar between PP and MP cows, which is consistent with the literature (Naderi et al., 2019). Similarly, Beauchemin et al. (2002) reported that meal interval did not differ between parity in mid-lactation, but PP cows consumed less feed per meal than MP cows. In the present study, MP cows had a longer interval between ruminating bouts than PP cows, which might be associated with more efficient rumination activity in older cows than younger cows.

4.2 | Lactation performance

The dietary starch content in this study was manipulated by lowering the dry ground corn and barley inclusion (percentage of DM) from 17.95 (HS) to 12.62 (LS) and increasing the inclusion of BP from 1.82 (HS) to 11.96 (LS). In spite of similar energy and protein contents, the LS diets on average contained 6.9 percentage of DM-less starch (from 22.3 to 29.2%) and 2.1 percentage of DM units more NDF than HS diets in the current study. The decreasing dietary starch content did not influence the milk yields and 3.5% FCM because of similar milk fat content and percentage. The literature regarding the effects of different dietary starch content on the milk yield of cows is inconclusive. For example, some studies observed no differences in the yields of milk or milk fat when comparing the HS diet with the LS diets (Alamouti et al., 2014; Boguhn et al., 2010; Akins et al., 2014). In contrast, other studies found lower milk fat yield and percentage...
TABLE 6  Blood metabolites of primiparous and multiparous cows fed high-starch (29.2% ± 0.70; means ± SD) vs. low-starch (22.3% ± 0.52; means ± SD) diets

| Item                          | Primiparous | Multiparous | p Value1 |
|------------------------------|-------------|-------------|----------|
|                              | High starch | Low starch  | High starch | Low starch  | SEM | Parity | Starch | Parity × starch |
| Blood biochemical parameters |             |             |           |           |     |        |        |                |
| Glucose, mg/dl               | 71.1        | 69.5        | 71.5      | 68.4      | 2.14 | 0.85   | 0.28   | 0.73            |
| Triglyceride, mg/dl          | 11.1        | 12.0        | 9.27      | 8.48      | 1.86 | 0.15   | 0.98   | 0.65            |
| AST, U/L                     | 41.4        | 24.4        | 38.8      | 24.7      | 5.21 | 0.81   | <0.01  | 0.78            |
| ALT, U/L                     | 39.6        | 40.6        | 36.9      | 37.1      | 1.78 | 0.08   | 0.75   | 0.83            |
| TAC, mmol/L                  | 0.57        | 0.59        | 0.52      | 0.45      | 0.02 | <0.01  | 0.39   | 0.17            |
| Albumin, g/dl                | 3.88        | 3.73        | 3.84      | 3.74      | 0.08 | 0.77   | 0.15   | 0.77            |
| Globulin, g/dl               | 2.81        | 2.98        | 2.13      | 3.01      | 0.13 | 0.20   | 0.85   | 0.28            |
| Total protein, g/dl          | 6.70        | 6.72        | 6.97      | 6.75      | 0.15 | 0.34   | 0.52   | 0.44            |
| NEFA, mmol/L                 | 0.37b       | 0.50a       | 0.34      | 0.34      | 0.03 | 0.02   | 0.09   | 0.09            |
| Malondialdehyde, nmol/ml     | 1.67        | 1.55        | 2.06      | 1.64      | 0.19 | 0.24   | 0.17   | 0.46            |
| BUN, mg/dl                   | 16.6        | 16.4        | 17.2      | 15.5      | 1.12 | 0.88   | 0.41   | 0.50            |
| Haptoglobin, g/l             | 0.66        | 0.66        | 0.72      | 0.64      | 0.06 | 0.80   | 0.58   | 0.56            |
| SAA, yg/ml                   | 393         | 387         | 414       | 355       | 36.4 | 0.88   | 0.37   | 0.48            |
| Complete blood count (10^9/L)|             |             |           |           |     |        |        |                |
| Platelets                    | 300         | 307         | 288.      | 250       | 36.9 | 0.35   | 0.69   | 0.54            |
| Red blood cells              | 6.49        | 6.83        | 6.30a     | 5.82b     | 0.24 | 0.02   | 0.77   | 0.10            |
| White blood cells            | 8.89        | 11.4        | 10.5      | 10.1      | 1.03 | 0.87   | 0.29   | 0.16            |
| Blood gas                    |             |             |           |           |     |        |        |                |
| pH                           | 7.37        | 7.34        | 7.35      | 7.40      | 0.02 | 0.41   | 0.53   | 0.08            |
| Blood pressure, mmHg         | 620         | 620         | 620       | 620       | 0.21 | 0.07   | 0.35   | 0.35            |
| pO2, mmHg                    | 160         | 137         | 144       | 151       | 10.6 | 0.98   | 0.46   | 0.17            |
| pCO2, mmHg                   | 49.7        | 54.1        | 53.7      | 47.4      | 3.91 | 0.73   | 0.81   | 0.19            |
| BE, mmol/l                   | 1.58        | 1.40        | 2.47      | 4.37      | 0.79 | 0.02   | 0.30   | 0.21            |
| SBE, mmol/l                  | 2.46        | 2.78        | 3.14      | 4.00      | 0.98 | 0.35   | 0.55   | 0.78            |
| O2SAT, %                     | 98.9        | 98.3        | 98.5      | 98.8      | 0.22 | 0.72   | 0.43   | 0.09            |
| O2-CT, %                     | 20.3        | 20.1        | 20.2      | 20.3      | 0.07 | 0.80   | 0.45   | 0.14            |
| P50, mm/Hg                   | 27.8        | 28.6        | 28.4a     | 26.9b     | 0.65 | 0.41   | 0.57   | 0.09            |

a,bWithin parity, means of starch levels with different superscripts differ (p ≤ 0.05).
1Contrasts for parity (Par), starch level (St) and interaction (Par × St).
AST = aspartate aminotransferase; ALP = alkaline phosphatase; TAC = total antioxidant capacity; NEFA = non-esterified fatty acids; BUN = blood urea nitrogen; SAA = serum amyloid A; pO2 = partial pressure of oxygen; pCO2 = partial pressure of carbon dioxide; BE = base excess; SBE = standard base excess; O2SAT = oxygen saturation.

when cows were fed a diet containing LS compared with HS content (Poorkasegaran and Yansari, 2014; Shahmoradi et al., 2015). The main differences between the studies are probably due to differences in the amount of starch in the control and treatment groups, stage of lactation, fermentability of the starch sources and the level of effective/forage NDF in the diet. It should be noted that in the present study, FCM yield was numerically greater (2.6 kg/day) in MP and lower (1.8 kg/day) in PP cows when comparing HS versus LS diets, although this was not statistically significant. Therefore, future studies are warranted to test the results of the present study with a larger number of animals.

In the current study, cows fed the LS diets had less milk protein and lactose content compared with those fed the HS. The observation is in line with the results of Dias et al. (2018), who reported that the milk protein (percentage and yield) decreased for cows fed the LS diet compared to those fed the HS diets (23% vs. 29% of the diet DM) which were also consistent with the finding of Poorkasegaran and Yansari (2014) and Shahmoradi et al. (2015). The higher percentage and yield of milk protein for the HS diets compared with the LS diets is probably due to the higher feed intake, CP intake and greater ruminal microbial protein production as well as the greater ruminal percentage of propionate (Oba and Allen, 2003; N R C , 2001). In the current study, the milk...
fat-to-protein ratio was higher in the LS diets compared with the HS diets, which might indicate a lower risk of sub-acute ruminal acidosis in cows fed the LS diets (NRC, 2001; Enemark et al., 2008).

In the current study, an increase in the concentrations of milk urea nitrogen was observed in cows fed the LS diets, although the difference (1 mg/dl) was small and likely of little biological significance. The higher milk urea nitrogen concentrations in the cows fed LS diets are likely related to a lower intake of starch that compromises microbial protein synthesis or due to a lower ruminal ammonia utilisation for microbial protein (Oba and Allen, 2003; Hristov et al., 2005).

4.3 | Ruminal fermentation and nutrients digestibilities

Cows fed the LS diets had a lower molar proportion of ruminal propionate but higher acetate and butyrate (tendency) compared with those fed the HS diets, with no effect on total VFA and ruminal pH, which is in line with previous studies (Voelker and Allen, 2003b; Zhao et al., 2013). Many studies found that reduced starch increased the acetate percentage and decreased propionate (Mojtahedi and Mesgaran, 2011; Zhao et al., 2013). In the current study, the molar proportion of propionate in total VFA decreased from 25.7% to 22.1% as starch intake was reduced from 7.75 to 5.34 kg/day, although the starch digestibility did not affect by decreased dietary starch content from 29.2% to 22.3%.

Decreasing the dietary starch content from 29.2% to 22.3% increased the digestibility of DM, OM and NDF in the current study. In line with our results, Sánchez-Duarte et al. (2019) showed that the apparent total tract digestibility of DM, OM and starch were greater in calves fed the LS diets (21% of DM) than the HS diets (27% of DM). Higher total tract digestibilities of NDF and starch for moderate- to low NFC diets were possibly due to the positive associative effects of fibre on ruminal fermentation (Batajoo and Shaver, 1994).

4.4 | Blood metabolites

Decreasing the dietary starch content did not influence the majority of blood parameters (except for AST) in the current study. According to the results, dietary treatment did not alter plasma urea nitrogen in this study, suggesting that despite greater nitrogen (N) intake due to increasing milk protein yield (removing protein by milk), a similar amount of nitrogen was available for ureagenesis. The biochemical parameters of AST and ALT in plasma are used to evaluate liver function (Ghouri et al., 2010). Interestingly, the HS cows showed higher concentrations of plasma AST compared with the LS cows, which is likely a sign of liver damage. Recently, this greater level of AST is associated with low rumen pH and possibly acidosis susceptibility of dairy cows (Nasrollahi et al., 2019). Putting together this finding and lower milk fat to protein ratio in cows fed HS versus LS diets, it could be postulated that cows fed the HS diet were at the risk of ruminal acidosis and to test this hypothesis, continuous measurement of ruminal pH is recommended for future studies.

In the current study, the PP cows had greater NEFA concentration than MP cows. In two previous studies where the animals mainly used pasture diets, it was reported that the PP cows had a higher pronounced metabolic disequilibrium than the MP cows which was found by greater concentrations of NEFA (Cavestany et al., 2005; Meikle et al., 2004). Bernabucci et al. (2005) reported a positive association between oxidative status and NEFA as indicators for lipo-mobilisation (Bernabucci et al., 2005). Higher NEFA concentration in the PP cows compared to the MP cows indicates a greater negative balance in this group of cows (Drackley et al., 2003). The PP cows had greater plasma concentrations of ALT and TAC compared to the MP cows, which agrees with Nasrollahi et al. (2017). The NEFA concentration reflects the mobilisation of adipose tissue to supply energy. It seems that first-lactation heifers that have not been exposed to high-concentrate diets until after calving (Penner et al., 2007) are not enough accustomed to manage and clear the metabolites loaded in the blood (Nasrollahi, 2017). Thus, first-lactation heifers with poorly regulated metabolism would be observed among the PP cows, whereas such cows are more likely culled in later lactations (Oetzel, 2007; Nasrollahi et al. 2017).

4.5 | Feed efficiency

One of the interesting findings of the present study was improving feed efficacy expressed as FCM yield/DMI by switching from HS to LS diets that happened only in PP cows and not in MP cows. Part of the reasoning for this observation could be explained by losing body reserves and mobilisation of fatty acids since the switching from HS to LS diets caused a lowering BFT and increasing blood concentration of NEFA in PP cows. The extent of improvement in feed efficiency in PP cow fed the LS diets than those fed the HS diets were much bigger than the amount of body losses and therefore the improvement of feed efficiency might be related to other factors that are not known. Before calving, PP cows as heifers are used to be fed with a high-fibre (80–100% forage) and nutrient-diluted diets and after calving, it suddenly changed to a high-starch diet. Previous studies indicated a clear adverse effect of such high-starch feeding on dropping rumen pH of PP cows detected by continuous measuring of reticular pH (Stauder et al., 2020). Moreover, at the level of metabolism, as previously explained, there are some problems for PP cows to regulate the overload of metabolites and the HS diet with a high rate of digestion and absorption of nutrients may exacerbate this condition. As result, the HS diet at both levels of digestion and metabolism is prone to make stress on PP cows, and therefore using LS diets contained non-forage fibre sources can help this animal to manage the first lactation better and production efficiency would be improved.

5 | CONCLUSIONS

Feeding a reduced-starch diet by partially replacing grains with BP in the diets with similar energy and protein contents resulted in lower DM intake, protein yield and blood concentration of AST but similar
milk production and greater digestibility and ratio of milk fat to protein than the HS diets. Multiparous cows had greater nutrient intake and milk production but lower plasma total antioxidant capacity compared with PP cows. In PP cows and not in MP cows, feed efficiency, measured as FCM yield/DMI, was greater on LS compared with HS diet, but it was associated with more BFT losses. Overall, the results showed reduced-starch diets may improve digestion, and metabolism of dairy cows, and the reduced-starch diet may be used more efficiently in PP than in MP cows but at the expense of the body reserves loss.

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ETHICS STATEMENT
The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines pages, have been adhered to. No ethical approval was required as this is a review article with no original research data.

CONFLICT OF INTEREST
The authors declare that there are not any conflicts of interest.

ANIMAL WELFARES STATEMENT
Guidelines for the care and use of animals were approved by the Iranian Council of Animal Care (1995), as well as an advisory committee of the Isfahan University of Technology approved all experimental procedures (IUT2018/22). The experiment was completely non-invasive. The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

AUTHOR CONTRIBUTIONS
Behzad Akhlaghi: Conceptualization; Formal analysis; Methodology. Ebrahim Ghasemi: Conceptualization; Funding acquisition; Investigation; Supervision; Writing original draft; Writing review & editing. Masoud Alikhani: Investigation; Supervision. Ali Ghaedi: Methodology. Morteza Hosseini Ghaffari: Writing original draft; Writing review & editing.

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Data sharing not applicable to this article as no datasets were generated or analyzed for this review.

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ORCID
B. Akhlaghi https://orcid.org/0000-0001-8282-0667
S. M. Nasrollahi https://orcid.org/0000-0002-5348-1106

REFERENCES
Akins, M. S., Perfield, K. L., Green, H. B., Bertics, S. J., & Shaver, R. D. (2014). Effect of monensin in lactating dairy cow diets at 2 starch concentrations. Journal of Dairy Science. Journal of Dairy Science, 97, 917–929. https://doi.org/10.3168/jds.2013-6756.
Alamouti, A., Alikhani, M., Ghorbani, G., & Zebeli, Q. (2009). Effects of inclusion of neutral detergent soluble fibre sources in diets varying in forage particle size on feed intake, digestive processes, and performance of mid-lactation Holstein cows. Animal Feed Science and Technology, 154, 9–23. https://doi.org/10.1016/j.anifeedsci.2009.07.002.
Alamouti, A., Alikhani, M., Ghorbani, G., Teimouri-Yansari, A., & Bagheri, M. (2014). Response of early lactation Holstein cows to partial replacement of neutral detergent soluble fibre for starch in diets varying in forage particle size. Livestock Science, 60, 60–68. https://doi.org/10.1016/j.livsci.2013.12.009.
Allen, M., Bradford, B., & Oba, M. (2009). Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of Dairy Science, 87, 3317–3334. https://doi.org/10.2527/jas.2009-1779.
American National Standards Institute. (1995). Method of determining and expressing fineness of feed material by sieving. Page 461 in ASAE Standards 1995 ASAE St. Joseph, MI.
AOAC International. (2002). Official methods of analysis (17th edn.). Gaithersburg, MD: AOAC Int.
Bannink, A., Gerrits, W., France, J., & Dijkstra, J. (2012). Variation in rumen fermentation and the rumen wall during the transition period in dairy cows. Animal Feed Science and Technology, 172, 80–94. https://doi.org/10.1016/j.anifeedsci.2011.12.010.
Batajoo, K. K., & Shaver, R. D. (1994). Impact of nonfiber carbohydrates on intake, digestion, and milk production by dairy cows. Journal of Dairy Science, 77, 1580–1588. https://doi.org/10.3168/jds.S0022-0302(94)77100-9.
Beauchemin, K. A., Maekawa, M., & Christensen, D. A. (2002). Effect of diet and parity on meal patterns of dairy cows. Canadian Journal of Animal Science, 82, 215–223. https://doi.org/10.4141/A01-080.
Beauchemin, K. A., Yang, W. Z., & Rode, L. M. (2003). Effects of particle size of alfalfa-based dairy cow diets on chewing activity, rumen fermentation, and milk production. Journal of Dairy Science, 86, 630–643. https://doi.org/10.3168/jds.S0022-0302(03)73641-8.
Beauchemin, K., & Rode, L. (1994). Compressed baled alfalfa hay for primiparous and multiparous dairy cows. Journal of Dairy Science, 77, 1003–1012. https://doi.org/10.3168/jds.S0022-0302(94)77036-3.
Bernabucci, U., Ronchi, B., Lacetera, N., & Nardone, A. (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. Journal of Dairy Science, 88, 2017–2026. https://doi.org/10.3168/jds.S0022-0302(05)72878-2.
Boguhn, J., Kluth, H., Bulang, M., Engelhard, T., & Rodehutschild, M. (2010). Effects of pressed beet pulp silage inclusion in maize-based rations on performance of high-yielding dairy cows and parameters of rumen fermentation. Animal, 4, 30–39. https://doi.org/10.1017/S1751731109990735.
Boerma, J. P., Potts, S. B., VandeHaar, M. J., & Lock, A. L. (2015). Effects of partly replacing dietary starch with fiber and fat on milkproduction and energy partitioning. Journal of Dairy Science, 98, 7264–7276. https://doi.org/10.3168/jds.2015-9467.
Cavestany, D., Blanc, J., Kulsar, M., Uriarte, G., Chilibraste, P., Meikle, A., Fehl, H., Ferraris, A., & Kral, E. (2005). Studies of the transition cow under a pasture-based milk production system: Metabolic profiles.
Drackley, J. K., Cicela T & LaCount, D. (2003). Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. Journal of Dairy Science, 86, 1306–1314. https://doi.org/10.3168/jds.S0022-0302(03)73714-X.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350–356. https://doi.org/10.1021/ac60111a017.

Enemark, J. M. D. (2008). The monitoring, prevention and treatment of subacute ruminal acidosis (SARA): A review. The Veterinary Journal, 176, 32–43. https://doi.org/10.1016/j.tvjl.2007.12.021.

Enemark, J. M. D., Jørgensen, R. J., & Kristensen, N. B. (2004). An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. Veterinary Research Communications, 28, 687–709.

Fanchone, A., Noziere, P., Portelli, J., Duriot, B., Largeau, V., & Doreau, M. (2013). Effects of nitrogen underfeeding and energy source on nitrogen ruminal metabolism, digestion, and nitrogen partitioning in dairy cows. Animal Feed Science and Technology, 191(2):895–906. https://doi.org/10.1016/j.anifeedsci.2012.05.007.

Ferguson, J. D., Galligan, D. T., & Thomsen, N. (1994). Principal descriptors of body condition score in Holstein cows. Journal of Dairy Science, 77, 2695–2703. https://doi.org/10.3168/jds.S0022-0302(94)77212-X.

Ferraretto, L. F., Shaver, R. D., Espineira, M., Gencoglu, H. & Bertics, S. J. (2011). Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. Journal of Dairy Science, 94, 1490–1499. https://doi.org/10.3168/jds.2010-3736.

Forbes, J. (1995). Voluntary food intake and diet selection in farm animals. Wallingford, Oxford: CAB International.

Fox, D., Tylutki, T., Czymmek, K., Rasmussen, C., & Durbal, V. (2000). Development and application of the Cornell University nutrient management planning system. In Proceedings of the Cornell Nutrition Conference for Feed Manufacturers, Rochester, NY (pp. 167–179). Ithaca, NY: Cornell University.

Ghouri, N., Preiss, D., & Sattar, N. (2010). Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: A narrative review and clinical perspective of prospective data. Hepatology, 52, 1156–1161. https://doi.org/10.1002/hep.23789.

Grant, R. (2005). Optimizing starch concentrations in dairy rations. In Proceedings of the Tri-State Dairy Nutrition Conference, Fort Wayne, IN (pp. 73–79). Columbus: The Ohio State University.

Hall, M., & Chase, L. (2014). Responses of late-lactation cows to forage substitutes in low-forage diets supplemented with by-products. Journal of Dairy Science, 97, 3042–3052. https://doi.org/10.3168/jds.2013-5759.

Hristov, A. N., Ropp, J., Grandeen, K., Abedi, S., Etter, R., Melgar, A., & Foley, A. (2005). Effect of carbohydrate source on ammonia utilization in dairy cows. Journal of Animal Science, 83, 408–421. https://doi.org/10.2527/2005.832408x.

Iranian Council of Animal Care. (1995). Guide to the care and use of experimental animals (Vol. 1). Isfahan, Iran: Isfahan University of Technology.

Kargar, S., Ghorbani, G., Khovash, M., Alikhani, M., & Yang, W. (2010). Effects of dietary fat supplements and forage: Concentrate ratio on feed intake, feeding, and chewing behavior of Holstein dairy cows. Journal of Dairy Science, 93, 4297–4301. https://doi.org/10.3168/jds.2010-3168.

Krause, K. M., & Oetzel, G. R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Animal Feed Science and Technology, 126, 215–236. https://doi.org/10.1016/j.anifeedsci.2005.08.004.

Krause, K., Combs, D., & Beauchemin, K. (2003). Effects of increasing levels of refined cornstarch in the diet of dairy cows on performance and ruminal pH. Journal of Dairy Science, 86, 1341–1353. https://doi.org/10.3168/jds.S0022-0302(03)73719-9.

Krause, K. M., & Oetzel, G. R. (2006). Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Animal Feed Science and Technology, 126, 215–236. https://doi.org/10.1016/j.anifeedsci.2005.08.004.

Krause, K., Combs, D., & Beauchemin, K. (2003). Effects of increasing levels of refined cornstarch in the diet of dairy cows on performance and ruminal pH. Journal of Dairy Science, 86, 1341–1353. https://doi.org/10.3168/jds.S0022-0302(03)73719-9.

Lammers, B., Buckmaster, D., & Heinrichs, A. J. (1996). A simple method for the analysis of particle sizes of forage and total mixed rations. Journal of Dairy Science, 79(5), 922–928. https://doi.org/10.3168/jds.S0022-0302(96)76442-1.

Leonardi, C., & Armentano, L. E. (2003). Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. Journal of Dairy Science, 86, 557–564. https://doi.org/10.3168/jds.S0022-0302(03)73634-0.

Maekawa, M., Beauchemin, K., & Christensen, D. (2002). Chewing activity, saliva production, and ruminal pH of primiparous and multiparous dairy cows. Journal of Dairy Science, 85, 1176–1182. https://doi.org/10.3168/jds.S0022-0302(02)74180-5.

Mertens, D., & Loften, J. (1980). The effect of starch on forage fiber digestion in cattle animals. Journal of Animal Science, 59(2):895–906. https://doi.org/10.2527/2005.832408x.
Nasrollahi, S., Ghorbani, G., Zali, A., & Kahyani, A. (2017). Feeding behaviors, metabolism, and performance of primiparous and multiparous dairy cows fed high-concentrate diets. *Livestock Science*, 198, 115–119. https://doi.org/10.1016/j.livsci.2017.02.017

Nasrollahi, S., Zali, A., Ghorbani, G., Khami, M., Maktabii, H., & Beauchemin, K. (2019). Effects of increasing diet fermentability on intake, digestion, rumen fermentation, blood metabolites and milk production of heat-stressed dairy cows. *Animal*, 13, 2527–2535. https://doi.org/10.1017/S1751731119001113

Nemati, M., Hashemzadeh, F., Ghorbani, G., Ghasemi, E., Khorvash, M., Ghafvari, M., & Nasrollahi, S. (2020). Effects of substitution of beet pulp for barley or corn in the diet of high-producing dairy cows on feeding behavior, performance, and ruminal fermentation. *Journal of Dairy Science*, 103, 8829–8840. https://doi.org/10.3168/jds.2020-18308.

Nordlund, K. V., & Garrett, E. F. (1994). Rumenocentesis: A technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. *Compendium on Continuing Education for the Practicing Veterinarian*, 28, 109–109.

NRC. (2001). *Nutrient requirements of dairy cattle* (7th rev. edn.). Washington, DC: The National Academies Press.

Oba, M., & Allen, M. (2003). Effects of diet fermentability on efficiency of microbial nitrogen production in dairy cows. *Journal of Dairy Science*, 86, 195–207. https://doi.org/10.3168/jds.S0022-0302(03)73600-5.

Oetzel, G. (2007). Subacute ruminal acidosis in dairy herds: Physiology, pathophysiology, milk fat responses, and nutritional management. In 40th Annual Conference (pp. 89–119). American Association of Bovine Practitioners.

Penner, G. B., Beauchemin, K. A., & Mutsvangwa, T. (2007). Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. *Journal of Dairy Science*, 90, 365–375. https://doi.org/10.3168/jds.S0022-0302(07)72638-3.

Penner, G. B., Taniguchi, M., Guan, L. L., Beauchemin, K. A., & Oba, M. (2009). Effect of dietary forage to concentrate ratio on volatile fatty acid absorption and the expression of genes related to volatile fatty acid absorption and metabolism in ruminal tissue. *Journal of Dairy Science*, 92, 2767–2781. https://doi.org/10.3168/jds.2008-1716.

Petri, R. M., Munnoch, M., Zebedi, Q., & Klevenhusen, F. (2019). Graded replacement of corn grain with molassed sugar beet pulp modulates the fecal microbial community and hindgut fermentation profile in dairy cows. *Journal of Dairy Science*, 102, 5019–5030. https://doi.org/10.3168/jds.2018-15704.

Poorkasegaran, S., & Yansari, A. T. (2014). Effects of different sources of carbohydrates on intake, digestibility, chewing, and performance of Holstein dairy cows. *Journal of animal science and biotechnology*, 5(1), 6.

Ranathunga, S. D., Kalscheur, K. F., Hippen, A. R., & Schingoethe, D. J. (2010). Replacement of starch from corn with nonforage fiber from distillers' grains and soyhulls in diets of dairy cows. *Journal of Dairy Science*, 93, 1086–1097. https://doi.org/10.3168/jds.2009-2323.

Sanchez-Duarte, J. J., Kalscheur, K. F., Casper, D. P., & Garcia, A. D. (2019). Performance of dairy cows fed diets formulated at 2 starch concentrations with either canola meal or soybean meal as the protein supplement. *Journal of Dairy Science*, 102, 7970–7979. https://doi.org/10.3168/jds.2018-15760.

Schröder, U. J., & Staufenbiel, R. (2006). Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *Journal of Dairy Science*, 89, 1–14. https://doi.org/10.3168/jds.S0022-0302(06)72064-1.

Shahmoradi, A., Alikhani, M., Riasi, A., Ghorbani, G., & Ghaffari, M. (2015). Effects of partial replacement of barley grain with beet pulp on performance, ruminal fermentation, and plasma concentration of metabolites in transition dairy cows. *Journal of Animal Physiology and Animal Nutrition, 100, 178–188. https://doi.org/10.1111/jpn.12205.

Shaver, R. D. (2008). Coping with high corn prices: Low starch diets and lactation performance by dairy cows. In Proceedings of the 6th Mid-Atlantic Nutrition Conference, MD. Accessed June 8, pp. 128–133. https://ansc-old.seed.umd.edu/sites/ansc.umd.edu/files/images/uploaded/Proceedings%20of%20the%206th%20Mid-Atlantic%20Nutrition%20Conference%20-%202008.pdf.

Stauder, A., Humer, E., Neubauer, V., Reisinger, N., Kaltenegger, A., & Zebeli, Q. (2020). Distinct responses in feed sorting, chewing behavior, and ruminal acidosis risk between primiparous and multiparous Simmental cows fed diets differing in forage and starch levels. *Journal of Dairy Science*, 103, 8467–8481. https://doi.org/10.3168/jds.2019-17760.

Staples, C. R. (2007). Feeding dairy cows when corn prices are high. In *Proceedings of the 44th Florida Dairy Production Conference*, Gainesville, FL (pp. 7–22). Gainesville: University of Florida Extension.

Van Keulen, J., & Young, B. A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, 44, 282–287. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Van Soest, P. R., Robertson, J., & Lewis, B. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597. https://doi.org/10.3168/jds.2002-0302(91)78551-2.

Voelker, J., & Allen, M. (2003a). Pelleted beet pulp substituted for high-moisture corn: 1. Effects on feed intake, chewing behavior, and milk production of dairy cows. *Journal of Dairy Science*, 86, 3542–3552. https://doi.org/10.3168/jds.2002-0302(03)73959-9.

Voelker, J., & Allen, M. (2003b). Pelleted beet pulp substituted for high-moisture corn: 2. Effects on digestion and ruminal digestion kinetics in dairy cows. *Journal of Dairy Science*, 86, 3535–3561. https://doi.org/10.3168/jds.2002-0302(03)73960-5.

Wangness, P. J., Chase, L., Petersen, A., Hartsock, T., Kellmell, D., & Baumgardt, B. (1976). System for monitoring feeding behavior of sheep. *Animal Feed Science and Technology*, 42, 1544–1549. https://doi.org/10.2527/jsas.1976.4261544x.

Wilson, R. C., Overton, T. R., & Clark, J. H. (1998). Effects of Yucca shidigera extract and soluble protein on performance of cows and concentrations of urea nitrogen in plasma and milk. *Journal of Dairy Science*, 81, 1022–1027. https://doi.org/10.3168/jds.S0022-0302(97)75664-4.

Xiao, X., Liu, C., Liu, Y., Li, C., & Yao, J. (2013). Effects of replacing dietary starch with neutral detergent–soluble fibre on ruminal fermentation, microbial synthesis and populations of ruminal cellulosytic bacteria using the rumen simulation technique (RUSITEC). *Journal of Animal Physiology and Animal Nutrition*, 97, 1161–1169. https://doi.org/10.1111/jpn.12025.

Zhu, L., Jones, C., Guo, Q., Lewis, L., Stark, C. R., & Alavi, S. (2016). An evaluation of total starch and starch gelatinization methodologies in pelleted animal feed. *Journal of Animal Science*, 94, 1501–1507. https://doi.org/10.2527/jas.2015-9822.