

# 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

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## Funding information

The Isfahan University of Technology and Kimia-Roshd Sepahan Co

## 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 × 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

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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  $\times$  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) losses.

#### KEYWORDS

dairy cow, lactation performance, parity, starch

## 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 & Lofton, 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 =  $37 \pm 10$ ;  $40 \pm 5$  kg/day milk; parity =  $2.2 \pm 0.44$ ; mean  $\pm$  SD) and 20 MP cows (DIM =  $37 \pm 9$ ;  $48 \pm 5$  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 ( $29.2\% \pm 0.70\%$  of DM, mean  $\pm$  SD) and PP cows (HS-PP); (2) low-starch diet ( $22.3\% \pm 0.52\%$  of DM, mean  $\pm$  SD)

**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 <sup>1</sup>	0.99	1.78
Buffer <sup>2</sup>	1.00	1.00
Calcium carbonate	0.68	0.48
Salt	0.32	0.32
Magnesium oxide	0.28	0.27
Sodium bentonite	0.40	0.40
Vitamin premix <sup>3</sup>	0.48	0.47
Mineral premix <sup>4</sup>	0.40	0.39
Chemical composition, % of DM, unless otherwise stated (SD in parentheses) <sup>3</sup>		
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 <sup>5</sup>	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, <sup>6</sup> Mcal/kg of DM	1.60	1.60
% DM retained on screens <sup>7</sup>		
19 mm	6.47	6.10
8 mm	28.4	27.6
1.18 mm	41.7	40.8
Pan	23.3	25.3
pef > 8	34.8	33.7
pef > 1.18	76.6	74.6
peNDF > 8, % of DM	14.2	14.2
peNDF > 1.18, % of DM	31.3	31.3

(Continues)

**TABLE 1** (Continued)

Item	Diet	
	High starch	Low starch
MPS, <sup>8</sup> mm	4.15	3.87
SDPS, <sup>9</sup> mm	2.96	3.02

<sup>1</sup>Fractionated refined palm oil containing (% of total FA) 16:0 (74.4%), 18:0 (4.88%), cis-9 18:1 (15.1%), 18:2n-6 (2.82%), and 18:3n-3 (2.80%) (0.11; RumiFat R100, Ecolex, Selangor, Malaysia).

<sup>2</sup>Kimia buff, Kimia-Roshd Sepahan Co. Iran.

<sup>3</sup>Containing (DM basis) 1,300,000 IU/kg of vitamin A, 360,000 IU/kg of vitamin D3 and 12,000 IU/kg of vitamin E.

<sup>4</sup>Containing (DM basis) 10 g/kg of manganese (manganese sulphate), 16 g/kg of zinc (zinc sulphate), 4 g/kg of copper (copper sulphate), 0.15 g/kg of iodine (potassium iodate), 0.12 g/kg of cobalt (cobalt sulphate), 0.8 g/kg of iron (iron sulphate) and 0.08 mg/kg of selenium (sodium selenite).

<sup>5</sup>Non-fibrous carbohydrate = 100 - (CP + NDF + ether extract + ash) (NRC, 2001).

<sup>6</sup>Net energy for lactation was calculated according to NRC (2001).

<sup>7</sup>Particle length variables were measured using the Penn State Particle Separator (The Pennsylvania State University, University Park (Kononoff et al., 2003). pef > 8 and pef > 1.18 = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; peNDF > 8 and peNDF > 1.18 = physically effective NDF determined as NDF content of TMR multiplied by pef > 8 and pef > 1.18, respectively.

<sup>8</sup>MPS = geometric mean particle size, calculated according to the method of the American Society of Agricultural Engineers (ASAE, 1995; method S424.1).

<sup>9</sup>SDPS = geometric SD of particle size, calculated according to the method of the American Society of Agricultural Engineers (ASAE, 1995; method S424.1).

and PP cows (LS-PP); (3) high-starch diet and MP cows (parity = 2.2 ± 0.44, mean ± SD) (HS-MP) and (4) low-starch diet and MP cows (parity = 2.2 ± 0.44, mean ± SD) (LS-MP). Milk yield (kg/day ± SD), DIM (days ± SD) and BW (kg ± SD) were 40 ± 5 versus 48 ± 5, 37 ± 10 versus 37 ± 9 and 543 ± 40 versus 640 ± 49 for PP versus MP, respectively. Within parity, the assignment of cows to the two diets was random. Cows were housed individually in box stalls (4 × 4 m) that were equipped with concrete feed bunk and automatic water troughs. Clean wood shavings and sand were used as bedding and refreshed daily. Diets were formulated as total mixed ration (TMR) using the Cornell Net Carbohydrate and Protein System (version 5.0; Fox et al., 2000) to meet or exceed predicted nutrient requirements for a lactating dairy cow with 643 kg weight and milk yield of 45 kg/day containing 3% true protein and 3.2% fat (Table 1). Due to the limitation in stall numbers, cows were enrolled in the study during 2 consecutive 49-day periods with 5 cows per treatment in each period. Each period consisted of 4 weeks of adaptation to feed and 3 weeks of sampling.

The first calving occurred at an average age of 24.6 months ± 1.7 in PP cows and 24.0 months ± 1.9 in MP cows. PP cows were sired by 5 different sires, whereas MP cows were sired by 12 and the sires of PP cows differed from the sires of MP cows. From 3 weeks before parturition until the start of the experiment, nutritional conditions were the same for PP and MP cows [a close-up diet (contained ~50% forage) for 3 weeks, followed by a lactation diet (contained ~40% forage)].

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, 2300, 0800, 1700 and 0200 h and stored at -20°C until analysis. Dry matter of composited samples of BP, TMR and refusals were 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, Kjeltex 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  $\alpha$ -amylase (100  $\mu$ l/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, Hillerød, 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  $\times$  kg of milk) + (16.23  $\times$  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  $\times$  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-5150) 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 a calibrator solution (TrueCal 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 autoanalyser. 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 Armantano, 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 rumination registered after 5 min without rumination (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 (Wangness 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 rumination period for each cow were counted for the first 10 boluses for a rumination 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



**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	<i>p</i> Value <sup>1</sup>		
	High starch	Low starch	High starch	Low starch		Parity	Starch	Parity × Starch
Nutrient intake								
DM, kg/day	23.0	21.8	29.3	26.5	0.68	<0.01	<0.01	0.28
DM % BW	4.33	3.97	4.41	4.14	<0.01	0.19	<0.01	0.82
NEL intake, Mcal/day	38.1	30.1	46.8	36.9	1.15	<0.01	<0.01	0.40
OM, kg/day	21.6	19.6	26.5	24.0	0.68	<0.01	<0.01	0.68
CP, kg/day	3.83	3.48	4.71	4.24	0.12	<0.01	<0.01	0.67
NDF, kg/day	7.83	7.54	9.62	9.23	0.25	<0.01	0.18	0.83
Starch, kg/day	6.95	4.80	8.54	5.88	0.20	<0.01	<0.01	0.21
NFC, kg/day	9.52	8.19	11.7	10.0	0.30	<0.01	<0.01	0.55
EE, kg/day	1.04	1.03	1.28	1.26	0.03	<0.01	0.62	0.88
peNDF > 8, kg/day <sup>2</sup>	3.35	3.10	4.15	3.77	0.18	<0.01	0.09	0.74
peNDF > 1.18, kg/day <sup>2</sup>	7.37	6.80	9.13	8.3	0.35	<0.01	0.06	0.71
Sorting index, % <sup>3</sup>								
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

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

<sup>2</sup>peNDF > 8 and peNDF > 1.18 = calculated by multiplying pef > 8 (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.

<sup>3</sup>Sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi & Armentano, 2003).

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 greater ruminating time as minutes per kg of DMI ( $p = 0.02$ ), per kg of NDF intake ( $p = 0.02$ ), per kg of peNDF > 8 intake ( $p = 0.05$ ) and per kg of peNDF > 1.18 intake ( $p = 0.04$ ) and longer rumination meal length than PP cows ( $p = 0.04$ ) compared with MP cows. Primiparous cows also had longer chewing time per kg of DMI ( $p = 0.03$ ), per kg of NDF intake ( $p = 0.03$ ), per kg of peNDF > 8 intake ( $p = 0.08$ ) and per kg of peNDF > 1.18 intake ( $p = 0.06$ ) compared with MP cows.

### 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 (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 ( $p = 0.01$ ).

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 × starch on BFT change ( $p = 0.05$ ) that only within PP cows BFT change was greater for HS than LS diets.

### 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

**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		SEM	p Value <sup>1</sup>		
	High starch	Low starch	High starch	Low starch		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	1.82	0.05	0.61	0.40	0.08
3.5% FCM/DMI	1.56 <sup>a</sup>	1.75 <sup>b</sup>	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	0.66	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>	0.90 <sup>a</sup>	-0.90 <sup>b</sup>	-1.20 <sup>b</sup>	-0.70 <sup>a</sup>	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>a,b</sup>Within parity, means of starch levels with different superscripts differ ( $p \leq 0.05$ ).

<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).

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 4** Chewing activities of primiparous and multiparous cows fed high-starch ( $29.2\% \pm 0.70$ ; means  $\pm$  SD) vs. low-starch ( $22.3\% \pm 0.52$ ; means  $\pm$  SD) diets

Item	Primiparous		Multiparous		SEM	p Value <sup>1</sup>		
	High starch	Low starch	High starch	Low starch		Parity	Starch	Parity $\times$ 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 peNDF <sup>2</sup> > 8	111	111	99.8	103	8.79	0.26	0.81	0.87
Min/kg of peNDF <sup>2</sup> > 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	12.40 <sup>a</sup>	14.0 <sup>a</sup>	12.80 <sup>a</sup>	11.5 <sup>b</sup>	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

<sup>a,b</sup>Within parity, means of starch levels with different superscripts differ ( $p \leq 0.05$ ).

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

<sup>2</sup>peNDF = 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 ( $29.2\% \pm 0.70$ ; means  $\pm$  SD) vs. low-starch ( $22.3\% \pm 0.52$ ; means  $\pm$  SD) diets

Item	Primiparous		Multiparous		SEM	<i>p</i> Value <sup>1</sup>		
	High starch	Low starch	High starch	Low starch		Parity	Starch	Parity $\times$ starch
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

<sup>1</sup>Contrasts for parity (Par), starch level (St) and interaction (Par  $\times$  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 ( $29.2\% \pm 0.70$ ; means  $\pm$  SD) vs. low-starch ( $22.3\% \pm 0.52$ ; means  $\pm$  SD) diets

Item	Primiparous		Multiparous		SEM	$p$ Value <sup>1</sup>		
	High starch	Low starch	High starch	Low starch		Parity	Starch	Parity $\times$ 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	0.37 <sup>b</sup>	0.50 <sup>a</sup>	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	6.30 <sup>a</sup>	5.82 <sup>b</sup>	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
pO <sub>2</sub> , mmHg	160	137	144	151	10.6	0.98	0.46	0.17
pCO <sub>2</sub> , 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
O <sub>2</sub> SAT, %	98.9	98.3	98.5	98.8	0.22	0.72	0.43	0.09
O <sub>2</sub> -CT, %	20.3	20.1	20.2	20.3	0.07	0.80	0.45	0.14
P50, mm/Hg	27.8	28.6	28.4 <sup>a</sup>	26.9 <sup>b</sup>	0.65	0.41	0.57	0.09

<sup>a,b</sup>Within parity, means of starch levels with different superscripts differ ( $p \leq 0.05$ ).

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

AST = aspartate aminotransferase; ALP = alkaline phosphatase; TAC = total antioxidant capacity; NEFA = non-esterified fatty acids; BUN = blood urea nitrogen; SAA = serum amyloid A; pO<sub>2</sub> = partial pressure of oxygen; pCO<sub>2</sub> = partial pressure of carbon dioxide; BE = base excess; SBE = standard base excess; O<sub>2</sub>SAT = 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; NRC, 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 and 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 (Ghoury 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.

#### ACKNOWLEDGEMENTS

The Isfahan University of Technology and Kimia-Roshd Sepahan Co. are acknowledged for funding this study. The authors express their appreciation to A. Ghaderi (farm staff at Research Center of Dairy Cow, Faculty of Agriculture, Isfahan University of Technology) for cooperation.

#### 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

#### DATA AVAILABILITY STATEMENT

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

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.722>

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**How to cite this article:** Akhlaghi, B., Ghasemi, E., Alikhani, M., Ghaedi, A., Nasrollahi, S. M., & Ghaffari, M. H. (2022). Influence of reducing starch in the diets with similar protein and energy contents on lactation performance, ruminal fermentation, digestibility, behavior, and blood metabolites in primiparous and multiparous dairy cows. *Veterinary Medicine and Science*, 8, 808–821. <https://doi.org/10.1002/vms3.722>