

The Economic and Nutritional-Physiological Aspects of Dairy Cows Are Influenced by the Different Sources of Dietary Starch

Research Article

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ABSTRACT

This study utilized a Latin square experimental design to assess the effects of different nonstructural carbohydrate sources on milk production, nutrient digestibility, nitrogen and energy utilization efficiency, and plasma metabolites in 32 multiparous dairy cows. The cows, with an average of 130 ± 13 days-in-milk, produced milk at a rate of 45 ± 2.4 kg, and had a live weight of 656 ± 27 kg, were fed diets formulated based on National Research Council (NRC) recommendations. Our results indicated that cows fed a corn-based diet exhibited a higher milk fat percentage compared to those fed other diets. Additionally, diets containing wheat, barley, or corn improved milk production efficiency, as measured by the ratio of milk production to dry matter intake and net energy of milk to net energy intake, compared to diets containing potatoes. Furthermore, cows on wheat or corn diets showed enhanced digestibility of dry matter, organic matter, crude protein, and crude fat compared to those on barley or potato diets. Notably, a diet based on potatoes resulted in significantly increased triglyceride levels in plasma. These findings underscore the impact of carbohydrate source on milk yield, nutrient utilization, and metabolic profile, highlighting the importance of selecting appropriate nonstructural carbohydrates for optimizing dairy cow performance.

KEY WORDS dairy cow, efficiency, milk production, nutrient digestibility, starch.

INTRODUCTION

To enhance the milk production of dairy cows through genetic modification, it is necessary to increase the intake of energy and protein-rich concentrates, which provide essential nutrients (Cabrita *et al.* 2009). One commonly employed approach to enhance milk yield in high-producing dairy cows involves creating a diet with an increased level of non-fiber carbohydrates (NFC) (Gao and Oba, 2016). The diet of dairy cows consists of carbohydrates, including fibrous (NDF) and NFC components, which make up around 65-75% of their overall ration. Non-fibrous carbohydrates may account for approximately 30-45% of the dry matter (DM) present in their dietary intake (Hall *et al.*

2010). Dairy producers initially introduced root crops like potatoes into the feeding regimen of dairy cattle, but later replaced them with grains and corn silage due to their high cost (Eriksson *et al.* 2004). Generally, dairy rations are predominantly composed of corn and wheat as the primary sources of carbohydrates owing to their cost-effectiveness and rich digestible energy content.

The presence of starch plays a critical role in energy production in cereal grains and potatoes. Nevertheless, the quantity of starch varies across these food sources (Gómez *et al.* 2016). Starch digestibility is influenced by a variety of factors, both intrinsic and extrinsic. In their paper, Shipandeni *et al.* (2023) conducted a review on the subject and discussed the various factors that contribute to starch

digestibility. The factors can be classified into internal factors, which consist of attributes like particle size, grain/endosperm type, and processing methods, and external factors, which encompass elements such as dietary fiber content and animal-related variables like starch consumption (Tian and Sun, 2020).

Altering the levels of starch concentration and fermentability in the dietary intake can have a notable effect on the productivity of dairy cows (Gómez *et al.* 2016; Tian and Sun, 2020; Shipandeni *et al.* 2023). This phenomenon encompasses the influence of energy intake, partitioning, and protein absorption. The research conducted by Allen (2000) and Allen *et al.* (2009) has provided evidence of the influence of these modifications on the performance of dairy cows. Additionally, the fermentability of starch in the rumen has significant implications for both starch utilization and milk production. The studies carried out by Bradford and Allen (2007) and Allen *et al.* (2009) have shed light on the correlation between rumen starch fermentability and milk production. While raw potatoes cannot serve as a readily fermentable energy source like grain starch, they can potentially mitigate fluctuations in energy supply throughout the day and address rumen issues caused by graminoid starch in the diet (Hall, 2004). The animal's nutrient intake is significantly impacted by the speed of fermentation and the quantity of dietary carbohydrates, particularly starch, in the stomach (Tester *et al.* 2006). A meta-analysis by Moharrery *et al.* (2014) illustrated how rumen starch digestibility was affected by both the intake and source of starch. Conversely, the digestibility of starch in the small intestine was primarily influenced by the origin of starch, while the digestibility in the hindgut was solely dependent on the escape of pre-compartment starch (Mosavi *et al.* 2012; Gómez *et al.* 2016). Ruminal breakdown rates of different feed components, such as starch, are commonly assessed using the *in situ* technique, in which feed samples are placed in nylon bags and incubated in a ruminant with a cannula.

Nevertheless, the increasing emphasis on exploring non-animal substitutes in scientific studies highlights the necessity of establishing reliable *in vitro* approaches for assessing nutrient degradability (Ghoorchi *et al.* 2013). In a research conducted by Hindle *et al.* (2005), the breakdown of starch from different sources was examined both *in vitro* and *in vivo*, highlighting the distinction between *in vivo* starch breakdown and predictions from an *in vitro* gas production trial where donor animals were not familiar with diets including the same starch origin. The researchers suggested that *in vitro* gas production might provide a more accurate representation of potato starch fermentation *in vivo* if donor animals were adjusted to a diet with this starch source.

A greater concentration of non-structural carbohydrates in the diet may have the potential to improve the utilization of ammonia nitrogen for the production of microbial protein (MP) in the rumen (Zhou *et al.* 2022). This rise in energy availability within the rumen could lead to enhanced milk yield in dairy cows by supplying a larger quantity of metabolizable nutrients (Gozho and Mutsvangwa, 2008).

The presence of starch in dairy rations can have a profound effect on the quantity and quality of milk produced. This is attributed to the diverse pathways involved in the digestion and fermentation of different types of starch, resulting in varying end products. To enhance milk production in dairy cows, increasing the intake of non-fiber carbohydrates (NFC) has been a common strategy. However, the impact of different sources of NFC on dairy cow performance, including milk yield, nutrient digestibility, and metabolic profiles, remains underexplored. This study aims to evaluate how varying sources of non-structural carbohydrates—barley, potato, wheat, and corn—affect milk production, nutrient utilization, and plasma metabolites in Holstein dairy cows, with a focus on optimizing dietary strategies for improved dairy performance.

MATERIALS AND METHODS

The research utilized a Latin square design, encompassing thirty-two multiparous lactating Holstein cows. The study adhered to the guidelines established by the Yasouj University Council on Animal Welfare. The cows chosen for the research had an average of 130 ± 13 (Mean \pm SEM) days-in-milk, an average daily yield of 45 ± 2.4 kg/day, and an average live weight of 656 ± 27 kg. The SabzBavaran-e-NouAndish Dairy Facility in Qir-o-Karzin, south of Iran, provided individual tie-stalls for all experimental cows. These tie-stalls allowed the cows to have free access to water. Throughout each testing phase, comprising a 14-day acclimation period succeeded by seven days of sample collection and data documentation, the cattle were accommodated in separate enclosures and granted unrestricted water intake. The animals in the experiment were fed basal diets formulated to meet or exceed the necessary levels of crude protein (CP), net energy for lactation (NE_L), minerals, and vitamins for a 660 kg cow producing 45 kg of milk with 3.5% fat and 3.0% true protein levels following the NRC (2001) guidelines. The rations were provided as total mixed ration (TMR) at 8:00 a.m. and 4:00 p.m., with milking sessions at 4:00 a.m., 12:00 p.m., and 6:00 p.m. Four different types of starch sources were utilized in the experiment, namely wheat grain, barley grain, corn grain, and potato in the form of cut pieces. The detailed information regarding the ingredients and chemical compositions of each ration can be found in Table 1.

Table 1 Ingredient and nutrient compositions of the experimental diets fed to dairy cows as TMR

Ingredient, % of DM	Experimental diet			
	Potato chopping	Wheat grain	Corn grain	Barley grain
Alfalfa hay	25.00	25.00	24.00	24.00
Corn silage	24.00	24.00	24.00	24.00
Potato chopping	24.20	-	-	-
Wheat grain	-	25.1	-	-
Corn grain	-	-	27.3	-
Barley grain	-	-	-	29.50
Soybean meal	10.00	9.00	9.00	9.00
Cottonseed meal	1.70	1.30	1.10	1.50
Fish meal	1.50	1.50	1.50	1.50
Corn gluten meal	1.50	1.50	1.50	1.50
Meat meal	2.50	2.50	2.50	2.50
Heated-soybean whole seed ¹	2.50	2.50	1.50	0.50
Wheat bran	2.90	3.40	3.40	2.20
Sugar beet pulp	2.50	2.50	2.50	2.10
Vitamin and mineral premix ²	1.00	1.00	1.00	1.00
Limestone	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20
Nutrient composition of experimental diets fed to dairy cows				
DM, % as-fed	54.50	54.30	55.15	54.90
Organic matter (OM)	92.50	91.75	92.13	92.51
Neutral detergent fiber (NDF)	31.17	31.50	31.60	31.09
Forage NDF	19.80	19.20	19.20	19.10
Acid detergent fiber (ADF)	18.20	18.30	18.30	18.40
CP	16.50	17.05	16.60	16.90
Ether extract	3.72	3.81	3.65	3.61
Total fatty acids	3.52	3.62	3.81	3.64
Starch	31.50	31.60	31.70	31.30
Ethanol-soluble carbohydrates	4.52	4.29	4.14	4.31
NFC ³	40.80	40.20	40.50	40.20
Net energy for lactation (NE _L) ⁴ , Mcal/kg of DM	1.67	1.69	1.70	1.68

¹ The composition of the substance includes 91.2% dry matter (DM), 45.3% crude protein (CP), 18.8% crude fat, and 34.0 trypsin inhibitor units/mg of trypsin inhibitor.

² The vitamin-mineral premix, on a DM basis, contained the following per kilogram: vitamin A: 50000 IU; vitamin D3: 10000 IU; vitamin E: 0.10 g; Calcium: 196 g; Phosphorus: 96.0; Sodium: 71.0; Magnesium: 19.0 g; Iron: 3.00 g; Copper: 0.30 g; Manganese: 2.0; Zinc: 3.0 g; Cobalt: 0.10 g; Iodine: 0.10 g and Selenium: 0.001.

Wheat bran and ground corn were utilized as carriers at a ratio of 400 g/kg and 210 g/kg, respectively.

³ Nonfiber carbohydrates (NFC)= 100 – (NDF+CP+ether extract+ash).

⁴ Calculated based on the chemical composition of the dietary ingredients and adjusted for 24 kg of dry matter intake (DMI) (NRC, 2001).

Sampling and chemical analysis of the samples

Each animal's daily intake was documented regularly. Additionally, in the final week of each phase, the experimental diets and remaining feed were gathered and stored at -20 °C. After each phase, the diet and leftover samples were mixed equally and dehydrated in an oven at 60 °C for 48 hours. The dried samples were then pulverized using a mill (Peppink 100AN, Overijssel, The Netherlands) with a 1 mm sieve. The samples were then analyzed for their dry matter (DM), organic matter (OM), crude fat, crude protein (CP) as mentioned by Aioanei and Pop (2013), and for neutral detergent fiber (NDF; as per Van Soest *et al.* 1991 after pretreatment with amylase), acid detergent fiber (ADF), acid detergent lignin (ADL; according to Van Soest, 1973), and starch content.

On the last seven consecutive days of each experimental period, individual fecal samples were collected and stored at a temperature of -20 °C. At the conclusion of each period, the fecal samples were combined in equal proportions, dried for a period of 72 hours at a temperature of 55 °C, and ground using a mill equipped with a 1 mm sieve. The fecal samples were analyzed for DM, OM, CP, ether extract, ADF, and NDF. Digestibility was assessed through Acid Insoluble Ash analysis (Van Keulen and Young, 1977).

During each experimental period, milk production was documented twice within the last seven days. Specifically, milk samples were obtained and preserved in plastic containers with potassium dichromate at a temperature of 4 °C on the 16th and 17th days of each period. At the conclusion of each period, the milk samples were combined in propor-

tion and subjected to analysis using a milkoscan (Milko Scan 133B Foss Electric, Denmark) to determine the percentages and yield of protein, fat, lactose, and solid nonfat (SNF).

Rumen fluid samples were obtained from individual animals during the last two days of each experimental periods using a stomach tube connected to a vacuum pump, four hours after the morning feeding. To prevent contamination from saliva, the first 100 mL of ruminal fluid was discarded. The subsequent 100 mL was utilized for pH, VFA, and NH₃-N analysis. The rumen pH was promptly measured using a portable pH meter (Metrohm 744). The rumen fluid samples were then filtered through four layers of cheesecloth and prepared for NH₃-N and VFA analysis. For NH₃-N analysis, 5 mL of rumen fluid was acidified with 5 mL of 0.2 N HCl. For VFA analysis, 5 mL of rumen fluid was mixed with 1 mL of 250 g/L meta-phosphoric acid. All samples were stored at a temperature of -20 °C until further analysis. The ruminal NH₃-N was analyzed using the Kjeldahl method (method 984.13; AOAC, 2005) on a Kjeltac Auto 1030 Analyzer (Tecator), while VFA concentrations were determined by GC using a Chrompack instrument (model CP-9002; Chrompack) equipped with a 50-m (0.32 mm ID) fused-silica column (CP-Wax Chrompack Capillary Column, Varian). The carrier gas was helium, while the internal standard utilized was crotonic acid (trans-2-butenoic acid). On the 19th day of each experimental period, prior to the morning feeding, blood samples were collected from the cows' jugular veins in heparin-coated tubes. These samples were promptly transported to the laboratory while kept on ice. To separate the plasma, the blood samples were subjected to centrifugation at 2500 rpm at 4 °C for a duration of 20 minutes. The resulting plasma samples were then stored at -20 °C until further biochemical analysis. The content of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, and glucose was determined colorimetrically using commercial kits, following the manufacturer's recommended procedures.

A flowchart of the experimental design and methodology is provided in Figure 1 to facilitate understanding of the study's procedure and timeline.

Statistical analysis and experimental design

The normal distribution of data was evaluated using the UNIVARIATE procedure in SAS (2004). To account for the effects of different dietary treatments while controlling for variation, a Latin square design was employed. This design helps manage the potential variability due to time or period effects, with each animal receiving each dietary treatment in a balanced manner across different periods.

Data concerning dry matter intake, milk production, milk composition, total-tract nutrient digestibility, dry matter (DM) and nitrogen content of the feces, nitrogen and energy efficiencies for milk production, and plasma metabolites were analyzed using the MIXED procedure in SAS. The analysis included the fixed effects of dietary treatment, period, and their interactions, while accounting for animal-specific random effects to handle repeated measures and individual variability.

Model diagnostics were conducted to ensure the validity of the assumptions. Normality of residuals was assessed through residual plots and statistical tests provided by the UNIVARIATE procedure. Homogeneity of variance was evaluated using Levene's test and variance component analysis.

Outliers were identified by examining standardized residuals, with values exceeding ± 2.5 standard deviations being flagged for further review. These outliers were carefully evaluated, and any necessary adjustments were made to ensure the robustness of the statistical analysis.

Post-hoc comparisons among means were performed using the Duncan's multiple range test to determine significant differences between treatment groups. The significance level was set at $P < 0.05$ for all statistical tests and data were presented as mean \pm SEM.

RESULTS AND DISCUSSION

Table 2 presents the findings on the impact of experimental diets on feed intake, milk production, and composition. The source of dietary starch did not have a significant effect on dry matter intake ($P < 0.05$). Similar to previous studies, it was observed that diets containing corn or wheat, wheat, barley, corn, or oat, and wheat or potato did not affect DM intake in lactating cows. Nevertheless, Silveira *et al.* (2007) discovered that cattle consuming a diet containing corn exhibited greater dry matter intake in contrast to those consuming a diet containing barley.

Cabrita *et al.* (2006) proposed that diets characterized by a greater rate of starch degradation in the rumen could potentially decrease the intake of dry matter due to regulatory mechanisms. These mechanisms include a reduction in ruminal pH and an increase in the production of volatile fatty acids. The varying impacts of different starch sources on dry matter intake observed in these studies could be linked to the starch levels present in the experimental diets and the size of the forage particles. Experimental diets with elevated levels of forage NDF tend to enhance chewing activity, stimulate saliva production, and maintain optimal rumen pH, thereby offsetting any variations in the fermentability of the diets within the rumen.

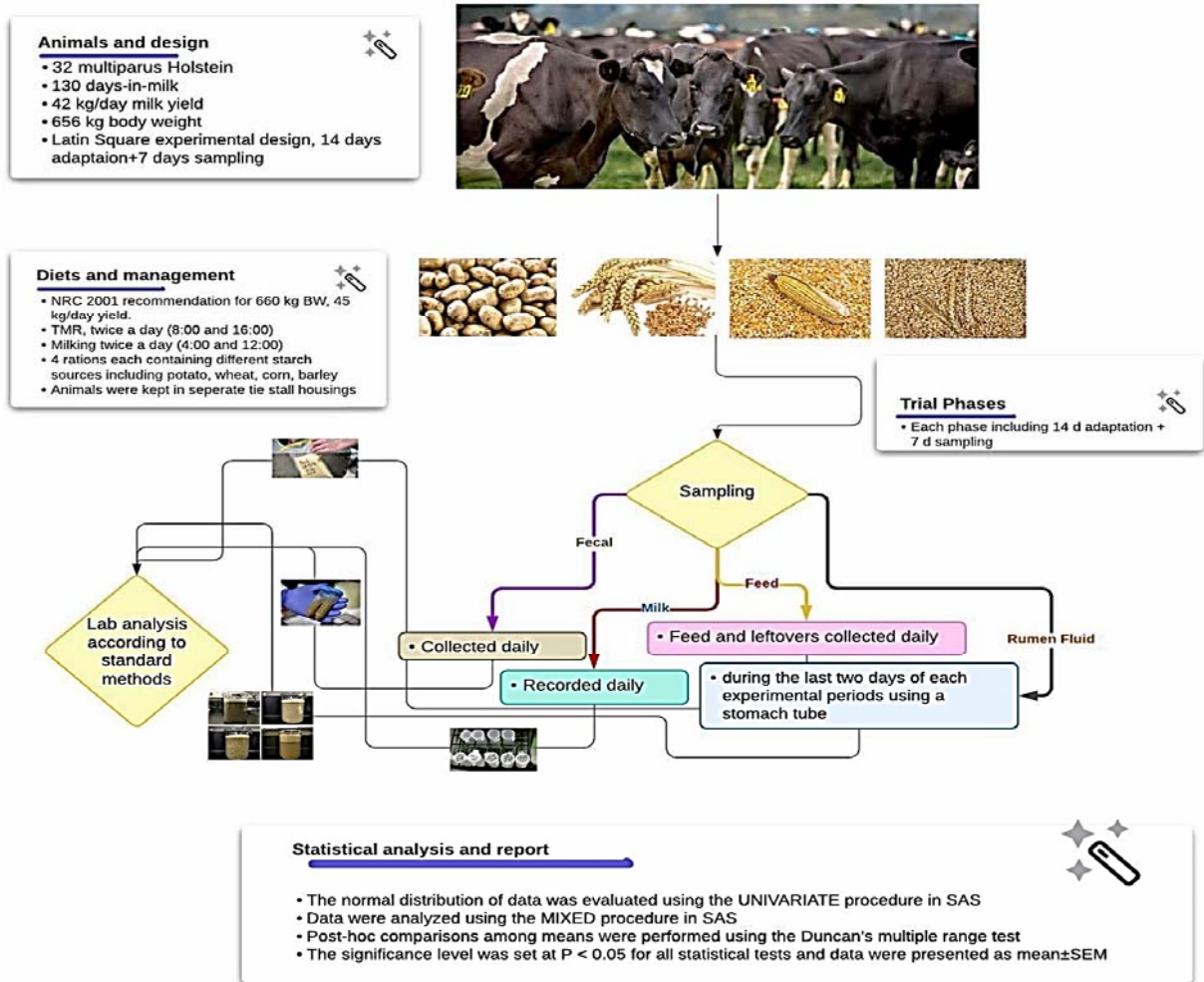


Figure 1 This flowchart provides an overview of the animals and study design, dietary management, trial phases, sampling procedure, and lab analysis methods employed to assess the impact of different starch sources on feed digestibility, milk production, and rumen fluid metabolites. Statistical analysis approaches are also detailed

Table 2 Effect of feeding different starch sources on DM intake, performance, and milk composition of dairy cows

Item	Experimental diet				SEM	P-value	
	Potato chopping	Wheat grain	Corn grain	Barley grain			
DIM (kg/day)	27.43	26.37	26.24	26.52	1.04	0.77	
Milk yield (kg/day)	43.07 ^c	46.67 ^a	44.27 ^b	45.63 ^b	0.96	0.04	
4%FCM ¹ (kg/day)	38.38 ^b	41.02 ^a	40.36 ^a	40.00 ^{ab}	0.71	<0.01	
ECM ² (kg/day)	42.14 ^b	44.92 ^a	44.25 ^a	43.91 ^{ab}	1.29	<0.01	
4%FCM/DIM	1.40	1.56	1.54	1.51	0.26	0.61	
ECM/DIM	1.54	1.70	1.68	1.66	0.31	0.59	
Milk fat	(%)	3.26 ^{ab}	3.28 ^{ab}	3.40 ^a	3.17 ^b	0.17	<0.01
	(kg/day)	1.41 ^b	1.49 ^{ab}	1.51 ^a	1.45 ^{ab}	0.04	0.03
Milk protein	(%)	3.10	3.09	3.21	3.13	0.23	0.81
	(kg/day)	1.36 ^b	1.44 ^a	1.42 ^a	1.42 ^a	0.05	0.04
Milk lactose	(%)	4.28	4.31	4.37	4.36	0.31	0.64
	(kg/day)	1.61 ^c	2.01 ^a	1.84 ^b	1.98 ^{ab}	0.16	0.02
SNF ³	(%)	8.16 ^b	8.22 ^b	8.26 ^b	8.76 ^a	0.13	0.03
	(kg/day)	3.51 ^c	3.84 ^a	3.46 ^b	3.99 ^a	0.09	<0.01

¹ 4% fat-corrected milk (FCM) yield= (0.4×kg of average milk yield) + (15×kg of fat, Xi, 2016).

² Energy-corrected milk (ECM) yield= (kg of milk×0.3246) + (kg of milk fat×12.96) + (kg of milk protein×7.04), as described by Orth (1992).

³ The solids-not-fat portion of the milk.

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

SEM: standard error of the means.

In the context of milk production, cows that were provided with a wheat-based diet showed increased milk yield as opposed to cows that were given alternative diets ($P < 0.05$). Cows fed rations consisting of barley, corn, and potato exhibited a decrease in milk production by 1.04, 2.40, and 3.60 kg on average, respectively, when compared to cows that were fed a wheat-based diet (Table 2). There is some indication to suggest that the deceleration of ruminal starch breakdown can enhance milk production in lactating cows (Deckardt *et al.* 2013). However, several other studies have failed to observe any noteworthy impact of the type of starch utilized on the production of milk (Khorasani *et al.* 2001; Gozho and Mutsvangwa, 2008). The impact of various cereal grains on milk production in lactating cows is contingent upon several factors, including the quantity of grain in the diet, the basal diet, the physical processing and composition of the cereal grains, and the level of feed intake. Although our research did not uncover any notable impact on DM intake resulting from the experimental diets, the rise in milk yield seen in cows consuming a wheat-based diet may be linked to the improved DM digestibility.

In our investigation, the inclusion of a diet predominantly composed of corn resulted in an increased fat content in milk when compared to other dietary options ($P < 0.05$). This result is consistent with earlier research conducted by Gozho and Mutsvangwa (2008) which found a higher milk fat content in cows that were fed a ration containing corn starch compared to those fed barley or wheat. However, some studies have suggested that the source of dietary starch may not have a significant impact on milk fat percentage (Weiss, 2019). Chen *et al.* (2020) found no significant effect on the milk fat percentage of their subjects when comparing low and medium concentrations of potato or wheat starch. Nevertheless, the fat percentage of cows' milk did increase when high concentrations of potatoes were included in the diet (Mosavi *et al.* 2012). The difference in milk fat content may be explained by differences in fermentation characteristic in rumen and the site and degree of digestion of these starch sources (Mosavi *et al.* 2012). Corn starch exhibits a slower degradation rate in the rumen when contrasted with wheat starch or barley starch. This delayed breakdown can potentially enhance the availability of essential precursors, such as acetate and butyrate, necessary for milk fat biosynthesis in the mammary glands. Worth noting that the rumen is commonly recognized as the primary location for starch digestion. In most cases, ruminal digestion accounts for 75 to 80% of the total intake, with around 35 to 60% of the starch entering the small intestine being broken down (Gómez *et al.* 2016). Feeding diets that contain concentrated feed has been found to have several effects on the rumen. These include lowering the pH, increasing the concentrations of volatile fatty acids (VFAs)

and osmolality, and causing metabolic disorders. Additionally, when ruminants are fed diets high in starch, it significantly enhances the activity of bacteria in the rumen that consume and produce lactic acid. These bacteria are not affected by the lower pH and therefore take advantage of the increased availability of substrate. On the other hand, feeding a significant amount of roughages can limit feed consumption, energy efficiency, and the synthesis of MP in ruminants (Giuberti *et al.* 2014). As a result, increasing the quantity of starch in the diet is considered a promising strategy to reduce methanogenesis per unit of dry matter intake by shifting ruminal fermentation towards propionogenesis, which acts as an alternative hydrogen sink to methanogenesis (Giuberti *et al.* 2014; Hassan *et al.* 2020).

A decrease in rumen pH caused by feeding disrupts the biohydrogenation pathway of C18 unsaturated fatty acids, resulting in an increase in trans fatty acid production. Many of these trans fatty acids inhibit milk fat biosynthesis in mammary tissue (Yi *et al.* 2023). Nevertheless, there exists a significant disparity in the *in vitro* fermentation kinetics among various sources of starch (Hatew *et al.* 2015).

The milk protein percentage of cows was not affected by the dietary starch sources, as indicated by the lack of significance ($P > 0.05$). However, diets containing wheat, barley, or corn resulted in increased milk protein yield compared to diets containing potatoes ($P < 0.05$). These findings are consistent with previous studies, suggesting that the milk protein percentage of cows remains unaffected by the type of dietary starch sources. According to Gozho and Mutsvangwa (2008), cows that were fed a diet with corn showed a higher milk protein percentage than those fed a diet with oats. Nevertheless, there was no notable difference in the milk protein percentage of cows fed diets containing corn, wheat, or barley (Gozho and Mutsvangwa, 2008).

Table 3 presents the effects of experimental diets on the efficiency of using nitrogen and energy for milk production. Cattle that were provided with feed composed of wheat, barley, or corn demonstrated increased milk production efficiency, as evidenced by the ratio of milk production to dry matter intake and the ratio of net energy of milk to net energy intake, in contrast to cattle that were given feed containing potatoes ($P < 0.05$). Silveira *et al.* (2007) revealed that cows that were provided with a barley-based diet exhibited greater net energy efficiency for milk production in comparison to cows that were given a corn-based diet. However, it should be noted that milk production efficiency, measured by the ratio of milk production to dry matter intake, is influenced by the diet.

The effectiveness of energy utilization following post-ruminal digestion of starch is greater when it is assimilated as glucose in comparison to when it undergoes fermentation

in the rumen, leading to conversion to propionate and subsequently to glucose in hepatocytes (Reynolds, 2006). As carbohydrate concentrations increased, there was a notable decrease in ruminal starch degradation. However, the overall digestion of starch in the entire digestive tract was not adversely affected. This suggests that the starch was broken down in both the small and large intestine, compensating for the reduced degradation in the rumen (Deckardt *et al.* 2013). These effects include ruminal acidosis, diminished fiber fermentation, and decreased efficiency in MP synthesis (Gómez *et al.* 2016). Additionally, moving the location of carbohydrate digestion and absorption from the rumen to the small intestine can offer significant energetic benefits. This phenomenon occurs because glucose, which undergoes digestion and absorption in the small intestine, can be utilized more effectively in a restricted amount, leading to a greater generation of net ATP when compared to the fermentation of glucose into short-chain fatty acids in the rumen (Trotta *et al.* 2022).

It is well established that increased ruminal starch digestion leads to increased milk production (Neubauer *et al.* 2020). The diminished milk production efficiency witnessed in cows that are fed with potatoes can be ascribed to the diminished digestibility of DM in the digestive tract and the increased excretion of DM in the feces of these animals. This aligns with the conclusions drawn by Moharrery *et al.* (2014), indicating that the digestibility of starch in the total digestive tract is primarily influenced by the source of starch. The intake of nitrogen, as shown in Table 3, was not influenced by the source of starch in the diet ($P > 0.05$). This lack of effect may be attributed to the fact that dietary starch sources do not have an impact on dry matter intake and the formulation of diets based on identical proteins. In cows fed barley or wheat, the utilization of dietary nitrogen was moderate, while it was lower in cows fed potato and higher in cows fed corn. The decreased use of dietary nitrogen in cows fed a high potato starch diet may be due to the lower digestibility of CP in the digestive tract and the higher nitrogen excretion in their feces (Hristov *et al.* 2019; Neubauer *et al.* 2020; Zhou *et al.* 2022). Our investigation indicates that the inferior efficiency of dietary nitrogen in cows fed a potato-starch ration is probably linked to a decrease in MP synthesis and an elevation in ammonia concentration within their rumen.

Surber and Bowman (1998) found that barley starch had higher rumen and post-ruminal digestibility compared to corn starch, resulting in increased energy production and improved feed conversion ratio. They also observed a 17% higher microbial nitrogen synthesis in barley-fed heifers compared to those fed corn (Surber and Bowman, 1998). Legume starch exhibited reduced digestibility in both the rumen and small intestine in comparison to starch derived

from barley and wheat grains, suggesting that the enzymatic activity of bacterial and pancreatic amylase is constrained by similar constraints (Larsen *et al.* 2009). Theurer *et al.* (1999) proposed that there exists a notable reduction in the overall consumption of starch or degradable starch in the rumen, resulting in a decline in the flow of microbial nitrogen in dairy cows.

Table 4 presents the effects of experimental diets on total-tract nutrient digestibility, DM excretion, and fecal nitrogen. The DM, OM, CP, and crude fat digestibility through the entire tract of cows fed diets containing wheat or corn was higher compared to diets containing barley or potato ($P < 0.05$). The total-tract digestibility of NDF and ADF was also higher in cows fed diets with corn, barley, or wheat compared to diets with potato. However, in some systems, there is a negative correlation between ruminal starch and NDF digestion (Volden and Larsen, 2011).

In a study conducted by Silveira *et al.* (2007), it was determined that a corn diet had a positive impact on the digestibility of DM, OM, and crude fat in dairy cows, in comparison to a barley diet. However, the digestibilities of CP and NDF were not influenced by the corn diet. Gozho and Mutsvangwa (2008) conducted a separate study which analyzed the impact of different dietary starch sources. Their findings indicated that the digestibility of DM, OM, and NDF did not vary based on the starch source. Interestingly, cows that were given an oat-based diet showed increased protein digestibility. The current investigation revealed that cows consuming diets containing barley or potato exhibited reduced CP digestibility in comparison to diets containing wheat or corn. This phenomenon may be linked to the slower breakdown of starch in rumen and the higher quantity of undigested starch that reaches the end of the intestine. While starch digested in the small intestine is more energetically efficient than fermentation in the rumen and hindgut (Neubauer *et al.* 2020), increased levels of starch reaching the end of the intestine could potentially enhance MP synthesis.

Nevertheless, due to the absence of enzymatic digestion of MP at the end of the intestine (Zhou *et al.* 2022), the synthesis of microbial organic matter from starch fermentation in the hindgut is lost in feces (Hristov *et al.* 2019), leading to decreased total-tract CP digestibility. Additionally, the disparity in CP digestibility among the experimental diets may also be influenced by variations in grain protein digestibility (Zhou *et al.* 2022).

The analysis of plasma metabolite concentration was conducted in connection with the experimental diets, and the findings can be found in Table 5. It was determined in the research that the plasma levels of glucose, total cholesterol, HDL cholesterol, and LDL cholesterol were not affected by the various dietary starch sources ($P > 0.05$).

Table 3 Effect of feeding different starch sources on nitrogen and energy efficiency in dairy cows

Item	Experimental diet				SEM	P-value
	Potato chopping	Wheat grain	Corn grain	Barley grain		
Milk yield efficiency ¹	1.57 ^c	1.77 ^a	1.69 ^b	1.72 ^{ab}	0.8	<0.01
NE efficiency ²	0.68 ^b	0.75 ^a	0.76 ^a	0.74 ^a	0.01	<0.01
Milk nitrogen (g/day)	217.60 ^b	230.41 ^a	227.29 ^a	227.36 ^a	1.16	0.03
Nitrogen intake (g/day)	719.13 ^{ab}	724.83 ^a	696.93 ^b	717.61 ^{ab}	3.12	0.01
Nitrogen efficiency ³ (%)	30.30 ^c	31.71 ^b	32.62 ^a	31.66 ^b	0.16	<0.01

¹ Milk yield efficiency= kg of daily milk ÷ kg of daily DM intake

² Net energy efficiency= milk NE_L (Mcal/day) ÷ energy intake (Mcal/day)

³ Nitrogen efficiency for milk production= milk nitrogen content (g/day) ÷ daily nitrogen intake (g/day)

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 4 Effect of feeding different starch sources on total-tract nutrient digestibility, DM and nitrogen excretion in dairy cows

Item	Experimental diet				SEM	P-value
	Potato chopping	Wheat grain	Corn grain	Barley grain		
DM (%)	43.18 ^c	62.61 ^a	57.86 ^a	48.36 ^b	1.31	0.02
OM (%)	49.48 ^c	62.84 ^a	55.42 ^a	53.44 ^b	0.87	0.01
CP (%)	52.16 ^b	69.89 ^a	64.48 ^a	56.33 ^b	1.22	<0.01
Ether extract (%)	82.43 ^b	86.57 ^a	87.74 ^a	82.91 ^b	0.77	<0.01
NDF (%)	31.37 ^c	42.94 ^a	44.86 ^{ab}	39.79 ^b	1.62	0.03
ADF (%)	31.03 ^b	40.27 ^a	34.47 ^{ab}	35.40 ^{ab}	1.98	0.02
Feces DM (kg/day)	9.97 ^a	7.48 ^d	6.78 ^{cd}	8.93 ^b	0.19	<0.01
Feces nitrogen (g/day)	289.96 ^a	217.37 ^b	213.86 ^b	227.35 ^a	10.57	<0.01

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber and ADF: acid detergent fiber.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 5 Effect of feeding different starch sources on plasma metabolites of dairy cows

Item (mg/dL)	Experimental diet				SEM	P-value
	Potato chopping	Wheat grain	Corn grain	Barley grain		
Glucose	52.77	50.84	53.18	52.59	2.78	0.62
Triglycerides	13.98 ^a	12.74 ^b	13.15 ^{ab}	12.66 ^b	0.22	0.04
Total cholesterol	200.92	217.68	207.84	203.01	10.16	0.78
HDL-cholesterol	115.99	108.15	123.42	107.14	7.39	0.59
LDL-cholesterol	82.27	83.93	82.94	80.09	6.98	0.61

HDL: high-density lipoprotein and LDL: low-density lipoprotein.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 6 Effect of feeding different starch sources on ruminal fermentation characteristics in dairy cows

Item	Experimental diet				SEM	P-value
	Potato chopping	Wheat grain	Corn grain	Barley grain		
Ruminal pH	6.09	6.04	6.02	5.89	0.07	0.49
Total VFA, mM	103.85	103.11	101.45	104.98	1.18	0.21
Acetate (mol/100 mol)	54.13	50.38	52.36	53.06	1.66	0.43
Propionate ¹ (mol/100 mol)	19.39	20.25	20.04	19.71	1.79	0.19
Butyrate (mol/100 mol)	14.97	15.19	14.93	14.90	1.09	0.44
Valerate (mol/100 mol)	1.07	0.91	0.98	0.86	0.33	0.37
Isovalerate ² (mol/100 mol)	1.85	2.10	1.95	1.72	0.39	0.21
Acetate:propionate	2.81	2.47	2.63	2.72	0.89	0.48
Ruminal NH ₃ -N, mg/dL	15.08	13.74	15.11	14.08	1.09	0.54

¹ The sum of propionate and isobutyrate.

² The sum of isovalerate and 2-methylbutyrate.

SEM: standard error of the means.

However, the animals that were given the potato-based diet demonstrated a significant increase in triglyceride levels in comparison to the other dietary interventions ($P < 0.05$). These findings support previous research that has shown that dietary starch sources do not have an impact on plasma glucose concentration (Mosavi *et al.* 2012).

It is worth noting that while the energy utilization of fermentation is less efficient than enzymatic hydrolysis of carbohydrates, a faster rate of starch digestion can lead to a rapid but temporary increase in postprandial blood glucose levels. This could potentially be more beneficial for fat deposition. On the other hand, slowly digestible starches can gradually increase glucose concentration and prolong the release of insulin into the blood, which may be more favorable for lean deposition (Yu *et al.* 2020).

The investigated diets did not have any impact on the measured ruminal fermentation characteristics items, as indicated in Table 6. In a recent study conducted by Malekhhahi *et al.* (2021), it was found that the super-conditioned corn diet had a significant impact on the molar proportion of ruminal acetate and the acetate-to-propionate ratio. Compared to the ground and steam-flaked corn diets, the super-conditioned corn diet resulted in a decrease in the molar proportion of ruminal acetate and the acetate-to-propionate ratio, while increasing the ruminal propionate concentration. The researchers concluded that the greater gelatinization of starch in the super-conditioned corn, as opposed to the ground corn, was consistent with the observed changes in ruminal proportions of acetate and propionate. The mentioned study's finding aligns with previous studies conducted by Ekinici and Broderick (1997) and Dann *et al.* (1999), which also reported a decrease in the ruminal acetate-to-propionate ratio and an increase in propionate concentration in cows fed rapidly degradable starch.

In summary, our results demonstrate that wheat-based diets significantly increased milk yield, while corn-based diets led to higher milk fat content and improved total-tract nutrient digestibility. However, potato-based diets resulted in reduced milk production efficiency and digestibility compared to other starch sources. Additionally, the different starch sources had no significant impact on plasma glucose levels or ruminal fermentation characteristics.

While this study provides valuable insights into the impact of different dietary starch sources on feed intake, milk production, and nutrient digestibility, several limitations should be acknowledged to fully understand the implications of our findings.

The experimental conditions, including housing, management practices, and climatic factors, could have influenced the outcomes of this study. Variability in environmental conditions, such as temperature and humidity, can

affect feed intake, rumen fermentation, and overall animal performance. For instance, high ambient temperatures may reduce feed intake and alter nutrient utilization, which could potentially confound the effects observed with different starch sources. Future studies should consider including environmental controls or accounting for these factors in the analysis to better isolate the effects of dietary treatments.

The specific breed of cows used in this study may also impact the generalizability of the results. Different breeds have varying metabolic rates, feed conversion efficiencies, and responses to dietary starch sources. The Holstein breed, used in this study, is known for its high milk production but may have different nutritional requirements and responses compared to other breeds. As such, the observed effects might not be directly applicable to other breeds or types of dairy cattle. Including a broader range of breeds in future research could provide more comprehensive insights and enhance the applicability of the findings to different dairy systems.

The levels of starch and the size of forage particles in the experimental diets could also influence the results. Variations in starch concentration and particle size might interact with dietary treatments in ways not fully captured in this study. For example, higher levels of forage NDF can impact chewing activity and rumen function, potentially affecting the digestibility and utilization of starches. Investigating these interactions in more detail could offer a better understanding of how different dietary factors contribute to the observed effects.

The duration of the experimental periods may also be a limitation. Short-term studies may not fully capture the long-term impacts of dietary treatments on animal performance and health. Extending the duration of experiments could provide additional insights into the sustainability and consistency of the effects observed.

Finally, the precision and accuracy of measurements, such as those for milk composition and plasma metabolites, can influence the reliability of the findings. Ensuring high precision in these measurements and considering potential sources of error is crucial for drawing robust conclusions. In conclusion, while our study provides important data on the effects of dietary starch sources on dairy cow performance, recognizing and addressing these limitations will be essential for interpreting the results accurately and applying them to broader contexts. Future research should aim to address these limitations to further elucidate the complex interactions between dietary factors, environmental conditions, and genetic variables in dairy cattle nutrition.

Our results regarding dry matter intake are consistent with findings from Khorasani *et al.* (2001) and Gozho and Mutsvangwa (2008). However, the observed increase in

milk yield with wheat-based diets contrasts with Deckardt *et al.* (2013), indicating potential differences in diet formulations and experimental conditions. The increase in milk fat with corn-based diets aligns with Gozho and Mutsvangwa (2008) but differs from Weiss (2019) and Chen *et al.* (2020), suggesting that starch degradation rates may affect milk composition in complex ways.

CONCLUSION

In conclusion, the results of this study provide strong evidence that the inclusion of wheat, barley, corn, and potato in the diets of dairy cows did not have a significant impact on their dry matter intake. However, cows fed the wheat ration showed higher milk production and milk production efficiency. Although the milk fat percentage was higher in cows fed the corn diet, the source of starch did not affect the protein percentage. Feeding a diet containing corn was found to improve the efficiency of nitrogen intake. Based on these findings, it can be concluded that feeding cereal grains, particularly wheat, can have positive effects on nutrient digestibility, nitrogen intake efficiency, and milk production in dairy cows. However, in certain economic conditions, raw potatoes can still be used as a starch source to provide energy for dairy cows.

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