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Effects of Different Starch Sources Used at High Levels in Cattle on Ruminal Fermentation Properties and Some Blood Parameters*

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Abstract: In this study, it was aimed to determine the effects of different starch sources on ruminal fermentation and *in situ* digestibility characteristics and some blood parameters in cows. In the study, three different total mixed rations (TMR) with similar energy, protein and starch contents were prepared and these TMR's formed the groups of the experiment. The main starch sources of the TMR's were from the barley, wheat, and corn grains, respectively. The study was carried out as two consecutive trails using 3 non-lactating Holstein female cattle with rumen cannulate within a 3 × 3 Latin square trial design. These TMRs were fed at *ad libitum* and then nutrient intakes, ruminal fermentation (pH, acetic, propionic, butyric, and lactic acids), some serum (urea, glucose, total protein, albumin, triglyceride) and blood gas parameters (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{++}, Cl^-, anion gap, lactate) were determined. Also, *in situ* dry matter and starch degradability were carried out in these animals. Nutrient intakes of cows fed different TMRs were similar ($P>0.05$), except neutral detergent fiber (NDF) intake ($P<0.05$). Both ruminal fermentation, serum and blood gas parameters did not change among treatment groups ($P>0.05$). As a result, it was determined that there were no serious changes in the ruminal fluid, serum, and blood gas parameter values of the subjects due to the content difference of the trial TMR's. On the other hand, it was determined that *in situ* dry matter (DM) and starch degradability of barley and wheat were significantly different among cereal grains, ruminal DM and starch degradability of corn followed a slower, stable, and gradual increase.
Keywords: Biochemistry, blood gas, degradability, rumen, starch

Sığırlarda Yüksek Düzeyde Kullanılan Farklı Nişasta Kaynaklarının Ruminal Fermentasyon Özellikleri ve Bazı Kan Parametreleri Üzerine Etkileri

Öz: Sunulan çalışmada, farklı nişasta kaynaklarının sığırlarda ruminal fermantasyon ve *in situ* sindirilebilirlik özellikleri ile bazı kan parametreleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Araştırmada benzer enerji, protein ve nişasta içeriklerine sahip üç farklı karma rasyon hazırlanmış ve bunlar denemenin gruplarını oluşturmuştur. Karma rasyonların ana nişasta kaynakları sırasıyla arpa, buğday ve mısır tanesi kökenlidir. Çalışma, 3 x 3 Latin kare deneme tasarımında, rumen kanüllü, laktasyonda olmayan 3 Holştayn dişi sığır kullanılarak ardışık iki deneme halinde gerçekleştirilmiştir. Bu karma rasyonlarla *ad libitum* besleme yapılmış ve ardından hayvanların besin alımları, ruminal fermantasyon (pH, asetik, propiyonik, bütirik ve laktik asitler), bazı serum (üre, glikoz, toplam protein, albümin, trigliserit), kan gazı (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{++}, Cl^-, anyon gap, laktat) parametreleri. Ayrıca nişasta kaynağı yemlerin kuru madde ve nişasta sindirilebilirliği de incelenmiştir. Farklı karma yemlerle beslenen sığırların nötral-deterjan lif (NDF) alımı ($P<0.05$) dışındaki diğer besin madde tüketimi parametreleri benzer bulunmuştur ($P>0.05$). Ruminal fermantasyon, serum ve kan gazı parametreleri deneme grupları arasında değişim göstermemiştir ($P>0.05$). Sonuç olarak deneme rasyonlarının içerik farklılığından dolayı deneklerin rumen sıvısı, serum ve kan gazı parametre değerlerinde ciddi bir değişiklik olmadığı belirlenmiştir. Diğer yandan, arpa ve buğdayın kuru madde ve nişasta sindirilebilirliğinin tahıl taneleri arasında önemli derecede farklı olduğu, mısırın rumen kuru madde ve nişasta sindirilebilirliğinin daha yavaş, istikrarlı ve kademeli bir artış gösterdiği belirlenmiştir.
Anahtar Kelimeler: Biyokimya, kan gazı, nişasta, rumen, sindirilebilirlik

Introduction

As it is known, one of the basic nutrient groups required by all living species is carbohydrates. Herbivorous and omnivorous animals basically obtain their

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carbohydrate needs from the plants they consume. Carbohydrates found in plants are classified as structural (cellulose, hemicellulose, etc.) and non-structural (starch, glucose, etc. sugars) carbohydrates. Starch has a special importance in ruminant nutrition because it is both the main metabolic energy source and one of the main activators of the ruminal fermentation mechanism (Giuberti et al., 2014). Starch can undergo ruminal and/or intestinal digestion much faster than structural carbohydrates (Huntington et al., 2006). In parallel with the rapid ruminal digestion of starch, an increase in the rate of ruminal microbial fermentation is observed, and subsequently the synthesis of organic fatty acids (and especially propionic acid) accelerates (Ferraretto, 2017). If the amount of starch in the diet is increased to a certain level, gradual increases are observed in performance of the animals, while the probability of encountering ruminal acidosis cases increases when these limits are exceeded (Boerman et al., 2015; Abdela, 2016). Studies have shown that the rate of starch digestion and the probability of acidosis formation related to it vary depending on the starch source (cereal grain) type and feed processing techniques. It has been determined that while the degradation rates of barley and wheat are generally close to each other and faster (29-34%/hour), and corn, rice, potato and sorghum starches are also close to each other but much slower (2-6/hour) (Monteils et al., 2002; Wang et al., 2009; Mosavi et al., 2012).

In the diets of high-producing ruminants, high starch-containing feedstuffs must be included to the diet to meet the animal's energy needs. However, over 28% starch in dairy cow diet causes a decrease in milk fat and the risk of subclinical acidosis. One way to prevent such problems, especially in dairy cows, is to add feed sources high in starch to the diet with low ruminal starch degradation.

When the chemical structure of starch is examined, it is seen that it basically consists of two different glucose polymers called amylose and amylopectin, and it is a molecule with a granular structure in the part called endosperm of the cereal seeds (Allen and Piantoni, 2014). However, it has been determined that starch molecules are not in a standard and stable structure and have some physical and chemical structure differences, depending on the source (plant type) from which they are obtained. Therefore, the differences in the endosperm structure of the seeds according to the plant type, the difference in starch amylose/amylopectin ratio, the granule structure size, and the processing of feed by various physical-chemical methods etc. also significantly change the ruminal/intestinal digestibility values of starch structures (Gomez et al., 2016, Qi and Tester, 2016). The granule size ranges from less than 1 µm to more than 100 µm, depending on the plant species from which the starch is obtained (Fuentes et al., 2019), this value is in the range of 1-20 µm in corn starch, while it is in the range of 1-110 µm in potato starch, and accordingly, the ruminal digestibility of potato starch is more difficult than corn starch (Monteils et al., 2002; Singh et al., 2016). It has been determined that wheat, barley, and oat starches can be digested more easily than corn due to the difference in seed endosperm structure, and that digestibility increases by processing the feeds such as grinding, gelatinization and conservation (Allen and Piantoni, 2014).

Corn, wheat, and barley grains are the most commonly used feed materials in ruminant diets. When the chemical compositions of corn, wheat and barley grains added to the diets were examined, it was found that they contained an average of 76.0%, 70.3% and 64.3% starch, respectively. It has been determined that the ruminal total digestibility values of these starches in dairy cows can vary between 72-89.9%, 88.1-88.3% and 80.7-84.6%, respectively, depending on the different feed processing techniques (Gomez et al., 2016) and feeding managements.

Today, many studies have been conducted to explain the relationships between starch and ruminal acidosis. In ruminants, it is of great importance to determine which cereal grain contains the starch type that is less and difficult to ferment, which can enable them to continue their normal digestive activities without further reducing the ruminal acidity value, and therefore delays the formation of acidity, and to prepare the appropriate diet ingredients. However, since starch is an important content of plants and an important food source for animals, it is understood that there is a need for further investigation in order to fully understand its physiological, biochemical and microbial functionality, efficiency and effects in the organism, as well as its relationship with performances and diseases. To date, studies on starch degradation have mostly been conducted in ruminants consuming a low-starch diet. In the literature, data on starch degradation in ruminants consuming a high-starch diet is limited.

In this study, it was aimed to determine the effects of different starch sources commonly used in ruminant diets on ruminal fermentation and *in situ* digestibility characteristics and some blood parameters related to acidosis in cows.

Material and Methods

The study was carried out as two consecutive trails using 3 non-lactating Holstein with rumen cannulate, aged 6 years and an average live weight of 650 kg, with the decision of the Ministry of Agriculture and Forestry, International Center for Livestock Research and Training directorate, Animal Experiments Local Ethics Committee, numbered 156/18.

In the study, nutrient contents of all feedstuffs used in the experiment were determined in the laboratory of animal nutrition. Based on the determined nutrient contents of these feedstuffs, three different feed mixtures were formulated with similar energy, protein, and starch contents. The first of these mixtures was prepared in such a way that the main starch source was from cracked barley (barley-based ration, group's named "B"), the second main starch source was from cracked wheat (wheat-based ration, group's named "W"), and the third was from cracked corn (corn-based ration, group's named "C"). These three different total mixed diets (TMR) consisted of approximately 70% concentrate and the remaining 30% roughages (consisting of equal proportions of alfalfa grass, wheat straw and corn silage). These TMRs were fed to experimental animals as two meals at 09:00 AM and 08:00 PM.

In the feeding trial, which is the first trail, three different TMRs mentioned above were given to the experimental animals in three periods within a 3×3 Latin square trial design. Animals were randomly assigned to one of three experimental group. Before the experiment, the animals were adapted to TMRs for 15 days, and in this process, the amount of concentrated feed mixes consumed by the animals were gradually increased to 70% of the total diet. At the beginning of each period, the body weights of the experimental animals were weighed. Each trial period was planned to be 18 days in total. In the first 10 days, the determination of feed consumptions and the adaptation of the experimental animals to the formulated diets, and also in the next five days the amounts of feed consumption were determined. On the 16th day, blood samples were taken from the jugular vein to determine blood gases and some blood biochemical parameters. Also, on the 16^{th} day of the experiment, approximately 50 ml of rumen fluids were taken from the rumen at 0-, 2-, 4-, 6-, 8- and 10- hours post-feeding, through rumen cannulas, and the pH of the rumen fluid was quickly determined using a portable digital pH-meter. In addition, for volatile fatty acids (VFA_s) analysis, samples were taken from these ruminal fluids in 10 ml to plastic Falcon® tubes (consisting of 9 ml of rumen fluid and 1 ml of HCl acid diluted with 50/50 distilled water) and these tubes were preserved in the cold chain and then, stored in a deep freezer at -18°C. The probability of animals experiencing ruminal acidosis during these last five days of each period was also monitored.

As a part of the second trail, on the 17^{th} and 18^{th} days of each period, incubation of feedstuffs in rumen was performed by using Dacron sacs (R510) with pore size of 40-50 µ to determine the "*in situ*" degradability rates of nutrient contents. In this Dacron bag trial, barley, wheat, and corn samples were ground to pass through a sieve with 2 mm pores. From these ground feed samples, approximately 3 g were placed in Dacron bags with 5×10 cm dimensions, and then incubated in the rumen of cannulated cows for 0-, 2-, 4-, 8-, 12-, 24- and 48- hours. After the incubation, the bags were washed with tap water until the washing water became clear (approximately 15 minutes), and then the bags were dried in the drying oven. Also, after drying, Dacron bags were weighed to determine the dry matter and starch degradability (Hassan and Karsli, 2023).

The experimental diets were formulated to be isocaloric, isonitrogenous and contained 32% starch from different starch sources, and dry matter (DM), organic matter (OM), crude protein (CP), ash contents of diets were determined according to Weende analysis methods (AOAC, 2006); neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents according to the method reported by Van Soest et al. (1991) and starch contents were analyzed according to Ewers polarimetric method (ISO, 1997). Botanical compositions of TMRs are presented in Table 1; Nutrient contents of feedstuffs used in TMRs and *in situ* digestion experiment are presented in Table 2.

The biochemical parameters in the blood samples were determined with a spectrophotometric autoanalyzer (Mindray BS-800M, Shenzhen, China), and blood gases and ions were determined using the RAPID lab® 1265 (Siemens Medical Diagnostics, Bayer, Tarrytown, NY, USA) device. Volatile fatty acids (acetic, butyric, propionic) and lactic acid contents of rumen fluid samples were determined using the Shimadzu Prominence LC 20AD HPLC (Shimadzu Corp., Kyoto, Japan) device, by modifying the method reported by Tjardes et al. (2000). Ruminal fluid samples were thawed at room temperature (22°C) before analysis, then mixed by vortexing and the supernatant was clarified by centrifugation. 1 ml of this liquid was filtered by syringe membrane filter (ISOLAB Laborgeräte GmbH, Eschau, Germany) with a pore width of 0.45 µm, specially produced for HPLC analyses. No internal standard was used. A correlation graph was created by preparing 10-20-40-60-100 -200 ppm calibration solutions of acetic, propionic, butyric, and lactic acids (Sigma Aldrich, St. Louis MO, USA) as external standards. Organic acid levels were determined using Inertsil ODS 3 HPLC analysis column (5 µm, 4.6 × 150 mm; GL Sciences Inc., Tokyo, Japan), the filtrate injection volume was 10µl, the mobile phase was 20 mM $(NH_4)H_2PO_4$ buffer solution, the flow rate was 1.5 mL/min, and the column temperature was 30°C. The amount of these four organic acids were determined in mmol/L units.

Preliminary tests were conducted to understand whether parametric test assumptions (normality and homogeneity) were met. Levene's test was performed to determine whether the group variances were homogeneous and it was found that the group variances were homogeneous. For normality, the Shapiro-Wilk test was performed. Box and Whisker

charts were examined, then data were analyzed as 3×3 Latin square method. Statistical analyzes of the data obtained in the experiment were carried out using the SPSS© 15.0 package program. The Duncan test method was used for analysis of variance (one-way ANOVA) to determine the data differences and significance values between the experimental groups and for pair wise comparisons of the means. P<0.05 was accepted as statistically significant. Average of each parameter was expressed as $\bar{x} \pm$ SEM.

Results

In the experiment, nutrient intakes of cattle consuming diets prepared with different cereal grains are shown in Table 3. The consumptions of nutrients, except NDF were similar in cows consuming diets containing different cereal grains as a source of different starch (P>0.05). NDF consumption of cows consuming a diet containing barley was found to be lower than the others (P<0.05). In general, while *in*

Table 1. Nutritional composition of TMR

	Barley Mix			Wheat Mix			Corn Mix		
Feed raw materials	**TMR* (kg)**	**%**	**% DM**	**TMR* (kg)**	**%**	**% DM**	**TMR*, (kg)**	**%**	**% DM**
Corn silage	12.5	43.3	19.15	12.5	43.40	18.95	12.5	43.33	18.93
Alfalfa	1.2	4.19	6.02	1	3.47	4.96	1.4	4.85	6.94
Wheat straw	1.2	4.19	6.17	1.4	4.86	7.13	1	3.47	5.09
Wheat bran	-	-	-	1	3.47	5.09	0.7	2.43	3.56
Barley	8	27.93	39.86	-	-	-	-	-	-
Wheat	-	-	-	7	24.30	34.59	-	-	-
Corn	-	-	-	-	-	-	6.8	23.57	33.51
Rice	1	3.49	4.87	0.85	2.95	4.10	0.8	2.77	3.85
Sunflower seed meal	2	6.98	10.02	2.3	7.99	11.40	2.8	9.71	13.87
Soybean meal	2.5	8.73	12.56	2.5	8.68	12.43	2.6	9.07	12.92
Limestone	0.25	0.87	1.35	0.25	0.87	1.34	0.25	0.87	1.34

** TMR, total mixed ration; DM, dry matter (kg).*

Table 2. Nutrient contents of feedstuffs used in TMRs and *in situ* digestion experiment (analyzed and calculated)

Diet Components	**DM**	**NE_L* (Mcal)**	**CP**	**NDF**	**ADF**	**EE**	**Ash**	**Starch**	**Ca* (g/kg)**	**P* (g/kg)**
In Both Diets and *In Situ* Experiment (%, DM)**										
Corn grain	92.14	2.14	8.53	9.35	1.92	3.85	1.33	74.08	0.3	3.2
Barley grain	92.13	2.00	12.22	20.42	6.25	2.61	3.31	56.81	0.6	3.9
Wheat grain	92.29	2.07	11.21	14.19	4.14	2.00	1.85	66.42	0.5	4.4
In Diets (%, DM)										
Corn silage	30.34	1.40	7.80	45.66	25.79	3.45	6.16	23.90	3	2.7
Alfalfa hay	92.71	0.99	17.47	44.78	32.54	2.00	10.19	3.51	11.6	2.3
Wheat straw	95.16	0.82	2.80	81.59	48.29	1.16	4.10	-	1.7	0.5
Wheat bran	95.09	1.77	14.67	55.71	17.35	4.89	5.43	13.15	1.4	11.7
Rice grain (dehulled)	91.03	2.19	9.39	20.67	6.33	1.23	0.54	87.46	0.5	2.1
Sunflower seed meal	92.60	1.17	27.84	53.11	35.06	1.15	6.39	2.64	4.5	11.2
Soybean meal	92.90	1.98	47.93	15.69	8.05	1.81	7.10	5.56	1.6	7.6
Calculated Nutrient Contents (%, DM)**										
Ration Mixes		**NE_L**	**CP**	**NDF**	**ADF**	**Starch**	**Starch Origin**	**Percentage of forage in TMR**	**Ca**	**P**
Wheat Mix Diet		1.65	16.56	33.45	17.63	32.83	69.02	31.95	0.65	0.51
Barley Mix Diet		1.64	16.72	33.08	17.31	32.57	68.57	32.25	0.66	0.44
Corn Mix Diet		1.65	16.49	31.40	17.12	34.39	71.18	31.87	0.69	0.48

** Values were calculated with the following equation: ME (Mcal/kg) = Digestible energy × 0.82; NE_L (Mcal/kg) = 0.00245 × DE - 0.12; Ca and P values were calculated according to NRC (2001) feed data tables.*

*** DM, dry matter (kg);NE_L, net energy of lactation (Mcal/day); CP, crude protein (kg); NDF, neutral detergent fiber (kg); ADF, acid detergent fiber (kg); Starch (kg).*

situ dry matter degradation was significantly higher for barley and wheat compared with corn, starch degradability values of different cereal grains were significantly different among all three grains (P<0.05; Table 4). Ruminal volatile fatty acids composition ratios and pH values were similar (P>0.05), except 4-hour post-feeding acetic acid values (Table 5). 4-hour post-feeding acetic acid value was significantly higher in cows fed barley-based diet compared with the other two groups (P<0.05). In general, serum and blood gas biochemical parameter values were similar (P>0.05) only serum urea level significantly increased during trail period (P<0.05; Table 6).

Table 3. Nutrient intakes of cattle consuming diets prepared with different cereal grain

	Treatment Groups* (x̄ ± SEM)			
Parameters**	**B**	**W**	**C**	**P-value**
DM (kg)	9.15 ±0.66	9.83 ±0.09	8.76 ±1.10	0.600
NE_L (Mcal/day)	15.00 ±1.08	16.22 ±0.48	14.45 ±1.81	0.590
CP (kg)	1.53 ±0.11	1.65 ±0.10	1.44 ±0.18	0.590
NDF (kg)	1.58 ±0.11^{b}	3.28 ± 0.08^{a}	2.78 ±0.34^{a}	0.030
ADF (kg)	1.58 ±0.11	1.73 ±0.08	1.50 ±0.19	0.260
Starch (kg)	2.97 ±0.21	3.23 ±0.06	3.01 ±0.38	0.760

* *Determined on dry matter basis; B, group were fed with Barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean.*
** *DM, dry matter;NE_L, net energy of lactation; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.*
^{a,b} Significant differences were found between values with different letters on the same line (P<0.05).

Table 4. *In situ* dry matter and starch digestibility values of different cereal grains used in the experiment

Parameters	Incubation Time (h)	Treatment Groups* (%, x̄ ± SEM)			
		B	**W**	**C**	**P-value**
Dry Matter	0	23.76 ±2.34^{a}	20.23 ±1.87^{a}	14.73 ±2.18^{b}	0.063
	2	38.85 ±1.41^{a}	40.22 ±6.93^{a}	24.22 ±1.59^{b}	0.064
	4	51.73 ±2.69^{a}	49.83 ±4.10^{a}	28.77 ±1.81^{b}	<0.001
	8	64.02 ±2.98^{a}	59.18 ±2.86^{a}	36.78 ±2.53^{b}	<0.001
	12	78.47 ±1.66^{a}	77.50 ±3.16^{a}	47.93 ±1.54^{b}	<0.001
	24	82.55 ±1.14^{a}	85.85 ±1.79^{a}	66.27 ±1.25^{b}	<0.001
	48	85.78±0.28^{b}	89.53 ±0.40^{a}	89.59 ±0.74^{a}	<0.001
Starch	0	32.54 ±2.07^{a}	25.27 ±1.75^{b}	22.43 ±1.98^{b}	0.025
	2	53.79 ±1.07^{a}	49.13 ±5.90^{a}	28.84 ±1.49^{b}	0.006
	4	64.90 ±1.95^{a}	60.18 ±3.25^{a}	33.89 ±1.68^{b}	<0.001
	8	79.52 ±1.70^{a}	68.32 ±2.15^{b}	41.79 ±2.33^{c}	<0.001
	12	95.98 ±0.31^{a}	83.07 ±2.38^{b}	54.54 ±1.34^{c}	<0.001
	24	98.33 ±0.11^{a}	93.40 ±0.84^{b}	74.38 ±0.95^{c}	<0.001
	48	99.59 ±0.01^{a}	98.63 ±0.05^{b}	97.59 ±0.17^{c}	<0.001

* *B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean. Determined on dry matter basis.*
^{a,b,c} *Significant differences were found between values with different letters on the same line (P<0.05*).

Table 5. Volatile fatty acids composition and pH values of rumen fluids obtained from cattle consuming diets prepared with different cereal grain

Time (Hour)	Parameters	Treatment Groups* (Mmol/L, x̄ ± SEM)			
		B	W	C	P-value
0th (n =9)	Acetic acid	79.21 ±8.90	65.66 ±6.77	62.85 ±4.42	0.279
	Butyric acid	5.12 ±0.78	4.24 ±0.58	3.77 ±0.09	0.303
	Propionic acid	46.64 ±10.07	34.27 ±5.97	32.06 ±5.53	0.391
	Lactic acid	12.85 ±2.02	9.51 ±1.84	9.42 ±1.81	0.400
	pH	7.00 ±0.10	7.09 ±0.09	7.04 ±0.02	0.750
2nd (n =9)	Acetic acid	65.68 ±1.62	54.92 ±3.92	60.74 ±13.05	0.651
	Butyric acid	8.32 ±1.10	6.46 ±0.53	6.73 ±1.28	0.430
	Propionic acid	38.98 ±1.88	29.15 ±5.84	35.04 ±2.70	0.272
	Lactic acid	12.14 ±0.61	10.67 ±1.32	11.93 ±1.43	0.653
	pH	6.52 ±0.06	6.32 ±0.38	6.66 ±0.05	0.404
4th (n =9)	Acetic acid	96.60 ±3.33[a]	69.16 ±0.42[b]	69.48 ±11.49[b]	0.049
	Butyric acid	9.09 ±0.91	6.68 ±0.62	6.21 ±1.45	0.196
	Propionic acid	60.45 ±5.31	38.16 ±2.92	43.50 ±11.60	0.174
	Lactic acid	15.34 ±0.42	10.87 ±0.32	11.69 ±2.68	0.184
	pH	6.10 ±0.14	6.30 ±0.16	6.48 ±0.11	0.207
6th (n =9)	Acetic acid	77.28 ±5.24	59.64 ±11.52	76.23 ±13.34	0.466
	Butyric acid	6.37 ±0.31	5.11 ±1.16	5.42 ±0.70	0.547
	Propionic acid	44.20 ±1.10	30.46 ±8.22	44.29 ±13.97	0.524
	Lactic acid	12.02 ±1.04	8.79 ±2.09	12.42 ±3.35	0.529
	pH	6.34 ±0.07	6.39 ±0.06	6.54 ±0.10	0.291
8th (n =9)	Acetic acid	74.21 ±9.30	63.00 ±9.78	82.64 ±13.44	0.491
	Butyric acid	6.10 ±0.12	5.01 ±0.94	5.80 ±0.79	0.569
	Propionic acid	39.24 ±7.04	30.32 ±4.95	46.59 ±14.50	0.533
	Lactic acid	12.02 ±1.70	9.92 ±1.56	12.91 ±3.05	0.582
	pH	6.76 ±0.07	6.71 ±0.05	6.72 ±0.20	0.969
10th (n =9)	Acetic acid	83.38 ±15.00	58.12 ±4.69	81.08 ±15.52	0.364
	Butyric acid	5.77 ±0.39	4.23 ±0.81	5.54 ±1.08	0.407
	Propionic acid	49.76 ±10.74	29.49 ±5.35	44.09 ±13.97	0.432
	Lactic acid	14.42 ±3.54	8.86 ±1.83	12.09 ±3.62	0.489
	pH	6.84 ±0.13	6.86 ±0.07	6.85 ±0.14	0.993

** B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean; n, number of samples.*

[a,b] Significant differences were found between values with different letters on the same line (P<0.05).

Table 6. Blood and blood gases biochemical parameters values

Parameters	Sampling Time (Hour)	Pre-trial Values	Treatment Groups* (x̄ ± SEM)			P-value
			B (n =6)	W (n =6)	C (n =6)	
Blood Biochemical Parameters						
Glucose (mg/dL)	0th	70.30 ±1.33	72.67 ±1.75	72.83 ±2.01	71.33 ±1.50	0.616
	6th	-	67.83 ±1.87	63.17 ±2.74	65.33 ±1.63	0.328
Total Protein (g/dL)	0th	7.40 ±0.19	7.57 ±0.17	7.37 ±0.20	7.36 ±0.11	0.862
	6th	-	6.88 ±0.46	6.45 ±0.68	6.88 ±0.29	0.796
Triglyceride (mg/dL)	0th	26.80 ±3.40	20.00 ±3.06	21.33 ±1.67	21.33 ±1.76	0.359
	6th	-	17.67 ±0.88	17.33 ±3.28	17.33 ±3.53	0.995
Urea (mg/dL)	0th	16.60 ±0.40[b]	29.67 ±3.18[a]	32.00 ±3.06[a]	29.67 ±4.63[a]	0.004
	6th	-	26.00 ±4.16	27.00 ±1.53	26.00 ±5.51	0.980
Albumin (g/dL)	0th	2.84 ±0.08	2.93 ±0.03	3.00 ±0.10	2.97 ±0.03	0.455
	6th	-	2.73 ±0.09	2.67 ±0.23	2.80 ±0.15	0.859
Blood Gases Biochemical Parameters						
pH	0th	7.47 ±0.01	7.45 ±0.01	7.46 ±0.01	7.41 ±0.03	0.110
	6th	-	7.44 ±0.02	7.45 ±0.01	7.44 ±0.01	0.765
pCO_2 (mmHg)	0th	37.66 ±1.08	37.83 ±1.69	38.43 ±0.62	40.73 ±4.64	0.767
	6th	-	39.00 ±2.31	40.07 ±0.97	38.60 ±0.50	0.776
pO_2 (mmHg)	0th	28.64 ±2.32	34.83 ±1.06	36.97 ±2.09	34.70 ±4.25	0.157
	6th	-	33.43 ±2.94	30.53 ±1.62	31.73 ±2.91	0.735
HCO_3^- (mmol/L)	0th	26.70 ±0.74	25.77 ±1.59	26.67 ±0.15	25.23 ±1.39	0.712
	6th	-	25.93 ±0.84	27.30 ±0.26	25.80 ±0.46	0.205
Na^+ (mmol/L)	0th	135.90 ±3.36	137.63 ±0.37	140.13 ±2.81	137.60 ±1.31	0.759
	6th	-	138.40 ±0.55	140.20 ±0.72	137.47 ±1.04	0.123
K^+ (mmol/L)	0th	3.87 ±0.08	4.06 ±0.15	3.16 ±0.42	3.75 ±0.48	0.268
	6th	-	3.51 ±0.30	3.58 ±0.22	3.53 ±0.26	0.982
Ca^{++} (mmol/L)	0th	1.10 ±0.02	1.13 ±0.04	1.04 ±0.07	1.10 ±0.06	0.572
	6th	-	1.07 ±0.07	0.97 ±0.08	1.06 ±0.06	0.589
Cl^- (mmol/L)	0th	99.80 ±2.65	100.67 ±4.33	104.33 ±0.88	99.67 ±4.37	0.748
	6th	-	106.00 ±2.52	103.33 ±1.45	105.00 ±0.58	0.569
Anion Gap (mmol/L)	0th	13.30 ±1.37	15.27 ±3.56	12.30 ±2.11	16.47 ±2.92	0.637
	6th	-	9.97 ±1.88	13.13 ±0.82	10.23 ±0.82	0.230
Lactate (mmol/L)	0th	1.31 ±0.14	0.66 ±0.10	0.81 ±0.28	1.10 ±0.45	0.276
	6th	-	0.65 ±0.14	0.93 ±0.17	0.66 ±0.15	0.421

** B, group were fed with barley starch; W, group were fed with wheat starch; C, group were fed with corn starch; SEM, standard error of mean; n, number of samples.*

[a,b] Significant differences were found between values with different letters on the same line (P<0.05).

Discussion and Conclusion

Nutrient intakes

Nutrient intakes of cows consuming different starch-based diets was similar, except NDF. Since the chemical composition of the diets prepared in the study were very similar to each other, it was not surprising that the nutrient consumption of the cattle consuming these diets was similar. It is thought that the low NDF consumption in cattle consuming a diet containing barley may be due to feed sorting in this group. Mosavi et al. (2012) reported that the nutrient intakes of dairy cows fed with diets containing barley, corn, wheat and potato-based starch did not change, which are in agreement with the results of the current study. It has been reported that responses of lactating cows to different cereal grains in terms of nutrient intakes depend on the level of dietary inclusion, the basal ration, physical processing of the cereal grains, the composition of a given batch of cereal grain, and the level of dietary intake (Khorasani et al., 2001). Since the characteristics of the diets were very similar in the present study, it was an expected result that the nutrient consumptions were similar.

Ruminal digestibility

In the presented study, when the *in situ* dry matter degradability values are examined, *in situ* DM degradation was similar in barley and wheat, but the DM degradation in both was significantly higher than that of corn from the beginning to the end of incubation times. This clearly shows that barley and wheat begin to undergo rapid digestion as soon as they enter the digestive system and reach an average of 84% within the first 24 hours, while corn follows a slower and stable digestion process within 48 hours (barley = wheat > corn).

When the *in situ* starch degradability values are examined, it was seen that the starch degradability rates of barley and wheat at the beginning are similar until the 4th hour, but after the 4th hour, it was observed that the starch degradation of all three grasses differed significantly in the periods until the end of incubation. Starch degradation was listed from fastest to slowest in barley, wheat and corn. At 12th hour of incubation, the degradation of barley starch was almost complete, while the degradation of wheat starch reached 83%, but only half of the degradation of corn starch was completed. Thus, when the time-dependent starch digestion rates were examined, it was seen that barley starch is digested faster than wheat and wheat is digested faster than corn starch, and that barley and wheat starch are almost complete within the first 24 hours. However, it was determined that the corn starch structure followed a slower and more stable digestion process within 48 hours (barley > wheat > corn).

In studies, it was reported that the ruminal fermentation or digestibility of corn starch is slower than other vegetable starches and it passes into the intestines without ruminal digestion at an average rate of 30% (18-42%). However, it is also known that the increase in the amount of starch intake with the diet increases the amount of starch that escapes from ruminal fermentation (Theurer, 1986; Mills et al., 1999). Similar results were also obtained in the presented study. Moreover, Overton et al. (1995) and Chibisa et al. (2015) also reported similar findings. In an experiment conducted by Hassan and Karsli (2023) in sheep consuming a forage-based diet, it was observed that the rumen starch degradation rates of barley, wheat and corn and the starch degradation values after 48 hours of incubation were higher than the data obtained in the current study. This shows that increasing the concentrated feed content of the diet reduces the rate and level of starch degradation in the rumen.

Ruminal fermentation

When the data obtained in the presented study were evaluated, it was determined that there was no statistically significant difference between the pH's and the acetic, butyric, propionic, lactic acids and total VFA values of the rumen fluid samples taken post-feeding. In the study, it is seen that ruminal pH values remained at neutral (7.00-7.09) levels in all groups at the beginning of the trial, only went down to the lowest value (6.10-6.48) at the 6-hour post-feeding, and then increased again and almost approached the initial values (6.84-6.86) at the 10-hour post-feeding. Ruminal pH values of cows fed corn-based diets were numerically higher compare with other two groups at 2- and 4-hours post-feeding, indicating a slower ruminal starch fermentation in this group. Consequently, it is seen that the pH values of the rumen fluids obtained from the experimental animals during the trial periods never fall below 6.00 to create sub clinical acidosis as it was expected (Oetzel, 2004; Khafipour et al., 2009; Morgante et al., 2009; Danscher et al., 2015).

In studies, it has been reported that high starch diets result in high ruminal VFA (acetic, propionic, butyric, isobutyric and valeric acids) and lactic acid levels, especially in animals with ruminal acidosis (Krause and Otzel, 2006; Plaizier et al., 2008; Zhao et al., 2018). However, based on the results obtained in the presented study, it shows that the preparation of diets with different starch structure does not cause any effect on ruminal fermentation and organic acid formation. The only difference was a significant increase in the level of acetic acid in the barley group at the 4th hour of the digestion process, which can be considered as an indication of the higher fermentability of barley starch. Although the starch levels of the diets in the current study were prepared to be at the high-

est levels of dairy cow diets, the expected risk of acidosis was eliminated because the feed consumption levels of the non-lactating dairy cattle used in the study were very low. For this reason, neither the expected decrease in rumen pH nor the increase in volatile fatty acids of the animals was observed. Only some numerical changes could be seen between groups.

Blood biochemistry

According to the data obtained on blood biochemistry, there was a significant change and increase in blood urea levels in the later stages of feeding with trial diets (independent of the content changes of all three ration mixes) compared to the beginning of the trial. It is thought that this change in urea is probably due to the transition from pre-experimental roughage-based diets to the experimental concentrated-based diets and the increase in nitrogenous microbial fermentation products.

In other blood parameters (glucose, total protein, albumin, and triglyceride), it was determined that there were no statistical differences depending on the dietary content changes. However, it is noticed that all these biochemical parameters examined show a decreasing trend in the advancing process from the first hour to the 6th hour of feeding, but it is understood that this situation is the result of the normal course of food digestion and metabolic process. Silveira et al. (2007) reported that no significant differences were observed in blood glucose levels between groups in feeding with barley and corn starch-based diets, and Cabrita et al. (2009) also reported that similar results in cows fed wheat and corn starch -based diets. Mosavi et al. (2012) found that no significant differences were observed between the groups in serum glucose, triglyceride, total cholesterol, LDL and HDL levels in cows fed barley, wheat, and corn -based diets. Moreover, Zhao et al. (2018) reported that no serious changes were observed in blood glucose levels even in cases ruminal acidosis.

Regarding the blood gas biochemical parameters (pH, pCO_2, pO_2, HCO_3^-, Na^+, K^+, Ca^{+2}, Cl^-, Anion Gap, and lactate) of the experimental animals, it was determined that there were no statistically significant differences among diets or sampling times (0- and 6-hours post-feeding). However, if the data between 0- and 6-hours post-feeding are evaluated, it is seen that normal changes occur at certain rates depending on possible metabolic processes. Morgante et al. (2009) determined that among the blood gas parameters pCO_2, pO_2, HCO_3 and blood pH values were 44.33, 39.76, 29.81, 7.42, respectively, in cattle control group with normal ruminal physiological values. However, they also reported that these values can change if there is a disorder such as ruminal acidosis these values were determined as 50.11, 36.60, 32.39, 7.41, respectively, in cattle with acidosis groups. Moreover, when the literatures are examined, it is seen that blood gas studies in cattle are quite limited. In the present study, it is thought that there is no statistically significant change were observed in rumen fermentation parameters due to low feed intake, as it does not cause any significant change or negatively effects in blood gas parameters of animals fed with diets formulated with different cereal grain-based with different starch types.

In conclusion, it was determined that feeding corn, wheat, and barley -based diets can significantly change *in situ* ruminal dry matter and starch digestibility values in animals according to starch types in these cereal grains, but it did not cause significant changes on ruminal pH and fermentation products (VFAs) and blood biochemical parameters with low feed intake. According to the results obtained, it was determined that feeding cows with corn-based diet may create a more stable ruminal digestibility.

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References

Abdela N. Sub-acute ruminal acidosis (sara) and its consequence in dairy cattle: a review of past and recent research at global prospective. Achievements Life Sci 2016; 10(2): 187-96.

Allen MS, Piantoni P. Carbohydrate nutrition: Managing energy intake and partitioning through lactation. Van Saun RJ. eds. In: Veterinary Clinics of North America: Food Animal Practice. New York: Elsevier Inc., 2014; pp. 577-97.

AOAC. Official methods of analysis of the association of official analytical chemists. Eighteenth Edition. Gaithersburg MD: Association of Official Analytical Chemists, 2006.

Boerman JP, Potts SB, VandeHaar MJ, Allen MS, Lock AL. Milk production responses to a change in dietary starch concentration vary by production level in dairy cattle. J Dairy Sci 2015; 98(7): 4698-706.

Cabrita ARJ, Vale JMP, Bessa RJB, Dewhurst RJ, Fonseca AJM. Effects of dietary starch source and buffers on milk responses and rumen fatty acid biohydrogenation in dairy cows fed maize silage-based diets. Anim Feed Sci Tech 2009; 152 (3-4): 267-77.

Chibisa GE, Gorka P, Penner GB, Berthiaume R, Mutsvangwa T. Effects of partial replacement of

dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows. J Dairy Sci 2015; 98(4): 2627-40.

Danscher AM, Li S, Andersen PH, Khafipour E, Kristensen NB, Plaizier JC. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. Acta Vet Scand 2015; 57(1): 1-14.

Ferraretto LF. Impact of starch content and digestibility in dairy cattle diets. Twenty-Eighth Annual Florida Ruminant Nutrition Symposium. February, 6-8, 2017; Florida-USA.

Fuentes C, Kang I, Lee J, Song D, Sjöö M, Choi J, Lee S, Nilsson L. Fractionation and characterization of starch granules using field-flow fractionation (FFF) and differential scanning calorimetry (DSC). Anal Bioanal Chem 2019; 411(16): 3665-74.

Giuberti G, Gallo A, Masoero F, Ferraretto LF, Hoffman PC, Shaver RD. Factors affecting starch utilization in large animal food production system: A review. Starch-Stärke 2014; 66(1-2): 72-90.

Gomez LM, Posada SL, Olivera M. Starch in ruminant diets: A review. Rev Colomb Cienc Pec 2016; 29(2): 77-90.

Hassan MAS, Karsli MA. The effect of *Saccharomyces cerevisiae* as a probiotic on the nutrient degradability of some commonly feedstuffs used in Turkey. Turk J Vet Anim Sci 2023; 47(3): 255-69.

Huntington GB, Harmon DL, Richards CJ. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J Anim Sci 2006; 84: 14-24.

ISO. International Organization for Standardization. ISO 10520:1997 Native starch. Determination of starch content. Ewers polarimetric method. https://www.iso.org/standard/18589.html; Accessed Date: 24.09.2023.

Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J Dairy Sci 2009; 92(3): 1060-70.

Khorasani GR, Okine EK, Kennelly JJ. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield and milk composition of Holstein cows. J Dairy Sci 2001; 84(12): 2760-9.

Krause MK, Otzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim Feed Sci Tech 2006; 126(3-4): 215-36.

Mills JAN, France J, Dijkstra J. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterization and ruminal starch digestion. J Anim Feed Sci 1999; 8(3): 291-340.

Monteils V, Jurjanz S, Blanchart G, Laurent F. Kinetics of ruminal degradation of wheat and potato starches in total mixed rations. J Anim Sci 2002; 80 (1): 235-41.

Morgante M, Gianesella M, Casella S, Ravarotto L, Stelletta C, Giudice E. Blood gas analyses, ruminal and blood pH, urine and faecal pH in dairy cows during subacute ruminal acidosis. Comp Clin Pathol 2009; 18(3): 229-32.

Mosavi GHR, Fatahnia F, Mehrabi AA, Mirzaei Alamouti HR, Darmani Kohi H. Effect of dietary starch source on milk production and composition of lactating Holstein cows. S Afr J Anim Sci 2012; 42(3): 201-9.

NRC (National Research Council). Nutrient Requirements of Dairy Cattle. Seventh Revised Edition. Washington DC: National Academy Press, 2001.

Oetzel GR. Monitoring and testing dairy herds for metabolic disease. Vet Clin North Am Food Anim Pract 2004; 20(3): 651-74.

Overton TR, Cameron MR, Elliott JP, Clark JH, Nelson DR. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. J Dairy Sci 1995, 78(9): 1981-98.

Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet J 2008; 176(1): 21-31.

Qi X, Tester RF. Effect of native starch granule size on susceptibility to amylase hydrolysis. Starch-Stärke 2016; 68(9-10): 807-10.

Silveira C, Oba M, Beauchemin KA, Helm J. Effect of grains differing in expected ruminal fermentability on the productivity of lactating dairy cows. J Dairy Sci 2007; 90(6): 2852-9.

Singh J, Colussi R, McCarthy OJ, Kaur L. Potato starch and its modification. Singh J, Kaur L eds. In: Advances in Potato Chemistry and Technology. London: Academic Press, 2016; pp. 195-247.

Theurer CB. Grain processing effects on starch utilization by ruminants. J Anim Sci 1986; 63(5): 1649-62.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci 1991; 74(10): 3583-97.

Wang M, Jiang J, Tan ZL, Tang SX, Sun ZH, Han XF. *In situ* ruminal crude protein and starch degradation of three classes of feedstuffs in goats. J Appl Anim Res 2009; 36(1): 23-8.

Tjardes K, Buskırk D, Allen M, Ames N, Bourquın L, Rust S. Brown midrib-3 corn silage improves digestion but not performance of growing beef steers. J Anim Sci 2000; 78(11): 2957-65.

Zhao C, Liu G, Li X, Guan Y, Wang Y, Yuan X, Sun G, Wang Z, Li X. Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. BMC Vet Res 2018; 14(1): 1-8.