

## Original Research Article

### **Evaluation of partial replacement of barley grain with heated potato processing by-products in the diet of dairy cows using an *in vitro* gas production technique**

#### **ABSTRACT**

The effects of replacing barley grain with heated potato slices and French fries waste in an iso-energetic total mixed rations of dairy cow on cumulative gas production and *in vitro* DM degradability was evaluated using *in vitro* techniques. Seven experimental dairy cow total mixed ration (TMR) were formulated based on replacing barley grain with heated potato slices (HPS) or French fries waste (FFW) including: **Control**: a diet containing 16% (DM basis) barley grain as one of the main sources of non-forage carbohydrates; **5 HPS**: a diet containing 11% barley grain and 5% HPS; **10 HPS**: a diet containing 6% barley grain and 10% HPS; **15 HPS**: a diet containing 1% barley grain and 15% HPS; **5 FFW**: a diet containing 11% barley grain and 5% FFW; **10 FFW**: a diet containing 6% barley grain and 10% FFW; **15 FFW**: a diet containing 1% barley grain and 15% FFW. Two *in vitro* gas production approaches were conducted separately to measure the production of gas volumes and dry matter (DM) degradability at 2, 4, 6, 12, 24, 36, and 48 hours of incubation. After 48 h of incubation, the gas production volume varied between 174 and 197 ml/g DM, with the highest value observed with control and the lowest value with FFW treatments ( $P < 0.001$ ). Also, the rate of gas production for the insoluble fraction varied from 0.031 to 0.054 ml/h and was higher for HPS treatments than for control and FFW treatments ( $P < 0.001$ ). After 48 h of incubation, DM degradability was significantly lower for control than other treatments. DM effective degradability, with a passage rate of  $0.05 \text{ h}^{-1}$ , was found to be higher for FFW and HPS compared with the control. Moreover, the rate of the B fraction degradation was significantly lower for the control ( $0.14 \% \text{ h}^{-1}$ ) than for treatments supplemented with potato processing by-products. Totally, the results of the current study showed that heated potato processing by-products can be replaced with barley grain by 15% in the diet of dairy cows without negative effects on ruminal fermentation.

**Keywords:** *in vitro* gas production, potato waste, energy source, dairy cow.

#### **INTRODUCTION**

The data shows that potato (*Solanum tuberosum*) is the fourth non-cereal carbohydrate source used in human nutrition globally [1]. Based on the published data, the total production area of the Iranian potato sector is estimated at 160,000 – 170,000 hectares. Total annual production of about 5 – 5.5 million tons [2]. The potato processing industry in Iran is growing and about 13% of the total volume of potatoes production is processed (about 700,000 to 800,000 tons per year). French fries and chips are the most important segments in the processing industry [2]. In addition to this, potatoes are processed for flakes, mash potatoes and salads as well. The co-products from processing for producing frozen food products are quantitatively the most important ones. Shortly, in the French fries production factories, potatoes are first peeled by steaming (the co-product is called steam peel), then peeled potatoes are cut into slices, which are heated at 80–90 °C for 5 minutes to reduce the oil absorption during the frying process. Next, the heated slices are passed through an electronic optic, where malformed slices are removed from the production line (heated potato slice; HPS), and after that, the slices are fried in oil at 170 °C for 40 seconds. Then the fried slices pass through a freezing tunnel, after which the malformed fried slices are removed before packaging by an electronic optic (French fries waste; FFW).

The feeding values of potato co-products are well documented, and they are classified as high-energy, low-fiber feed sources with low DM content [3, 4, 5]. Most of the studies on using potato co-products have focused on feeding potato peels, screen solids, bits and pieces, and belt solids [3, 4, 5, 6], and data related to heated potato by-products (like fried or cooked potato co-products) are limited. It has been shown that heat treatments increase the rate and extent of starch degradation in the rumen [7, 8]. So, it is expected that the degradability of heated potato co-products will be different from their raw and unheated shapes.

On the other side, the *in vitro* gas production technique, as a promising technique, has been used to evaluate the possibility of using food industry residuals and by-products as animal feed sources. It has been shown that there is a good correlation between results from *in vitro* and *in vivo* experiments [9]. The objective of the current study was to evaluate the effects of replacing barley grain with heated potato slices (HPS) and French fries wastes (FFW) in a total mixed ration (TMR) on cumulative gas production and *in vitro* DM degradability.

## **MATERIAL AND METHODS**

### **Preparation of an Experimental Diets**

The heated potato slices (HPS) and French fries waste (FFW) used in the current study were provided by Talachin Agro-industrial Company in Qazvin, Iran. Seven iso-energetic TMR diets

for lactating dairy cows (700 kg BW, producing 40 kg milk/day, and 90 days in milk) were formulated using NASEM Dairy-8 R 2022.10.08 software [10] (Table 1) as **Control**: a diet containing 16% (DM basis) barley grain as one of the main sources of non-forage carbohydrates; **5 HPS**: a diet containing 11% barley grain and 5% HPS; **10 HPS**: a diet containing 6% barley grain and 10% HPS; **15 HPS**: a diet containing 1% barley grain and 15% HPS; **5 FFW**: a diet containing 11% barley grain and 5% FFW; **10 FFW**: a diet containing 6% barley grain and 10% FFW; **15 FFW**: a diet containing 1% barley grain and 15% FFW. Prior to TMR preparation, all feed ingredients were subjected to a drying process at a temperature of 60°C for 72 hours. After drying, they were milled through a sieve with a mesh size of 1 mm. Also, DM (method ID 934.01), crude protein (CP, method ID 984.13), ether extract (method ID 920.30), and ash (method ID 942.05) were determined according to AOAC [11]. Starch content was determined according to Hall method [12]. A Fibertec<sup>TM</sup> (Foss, Denmark) fiber analyzer was used to determine the amounts of acid detergent fiber (ADF) and neutral detergent fiber (NDF) using reagents that were reported by Van Soest et al. [13] and added heat-stable amylase and sodium sulfite. The analyses of fatty acids (FA) profile of the HPS, FFW, and barely were performed using gas chromatography [14]. Table 2 shows the chemical composition of FFW and HPS.

#### ***In vitro* cumulative gas production and kinetics**

The rumen fluid was collected from two infertile ruminally-cannulated Holstein cows (average BW 700 kg) that were fed a diet consisting of 60% forages and 40% concentrates, twice daily from 07:00 to 15:00. Rumen fluid was mixed with the pre-heated (39 °C) buffer mixture, in a 1:2 ratio (rumen fluid: buffer). The *in vitro* gas production technique was conducted using Menke and Steingass technique [15], where, approximately 500 mg of experimental samples were weighed (based on DM) in triplicate into 120 ml serum vials. A total of 45 ml of prepared solution (rumen-fluid and buffer mixture) was added to each bottle. The glass vials were then incubated in incubated-shaker at 39 °C and gas production was recorded after 2, 4, 6, 12, 24, 36, and 48 h using a water displacement apparatus. For the *in vitro* study, four replications of each experimental total mixed ration (TMR) were used, and two runs were conducted to ensure the reliability of the findings. Gas production kinetics fractions were estimated using the NLIN procedure of SAS [16] according to the non-linear exponential model of Ørskov and McDonald [17]:

$$G = a + b (1 - \exp^{-ct})$$

where, G represents the gas generated at a given time (t), a represents the gas production (ml/g DM) from the immediately soluble fraction, b represents the gas production (ml/g DM) from

the insoluble fraction,  $a + b$  represents the potential gas production (ml/g DM),  $c$  represents the gas production rate constant (ml/h) for the insoluble fraction, and  $t$  represents the incubation time (h).

### ***In vitro* dry matter degradability**

To evaluate the effect of partial replacement of barley grain with heated potato slices (HPS) or French fries waste (FFW), a modified *in vitro* technique was employed. This involved weighing 500 mg of the prepared total mixed ration (TMR) into nylon bags measuring 2.5×3 and with a pore size of 45µm. The bags were then heat-sealed and placed into 120 ml serum vials [18]. The vials were incubated at 39 °C before the injection of a combination consisting of 45 ml of rumen fluid (15 ml) and buffer (30 ml) in a ratio of 1:2 into each vial.

In this part of the experiment, it was tried to record the amount of gas production and DM degradation of the experimental treatments simultaneously. In this way, for each incubation time (2, 4, 6, 12, 24, 36, and 48 h), 4 vials (replicates) were allocated to each of the experimental treatments, and 4 vials were assigned to the blank as well. Each run contains 224 vials (including 7 experimental treatments plus blank, 7 incubation times, and 4 replications). It should be mentioned that current *in-vitro* gas production is done over two consecutive runs on two different days. There were four different times the vials were incubated: 2, 4, 6, 12, 24, 36, and 48 hours. At each time, the bags inside were taken out, rinsed, and washed with phosphate buffer (pH 7.4) to make sure that any microbial contamination caused by rumen microbes was removed [18]. The *in vitro* DM degradability (IVDMD) for each time point was calculated as the weight of substrate incubated initially minus the weight of residue at the related time point. Also, the partitioning factor for 12 and 24 hours of incubation was found by dividing the mg of truly degraded DM (TDM) by the ml of gas that was made in the lab at those times. We then used the NLIN procedure in SAS [16] to figure out the kinetic parameters of *in vitro* DM degradability (IVDMD) by fitting the data to the model of Ørskov and McDonald [17]:

$$D = a + b (1 - \exp^{-kdt})$$

Where  $D$ : degradability at the time ( $t$ ),  $a$ : intercept,  $b$ : potentially degradable fraction, and  $kd$ : rate of degradation of the  $b$  fraction.

Also, the effective degradability of DM (ED) was calculated assuming a fractional passage rate ( $kp$ ) of 2, 5, and 8%/h using the following equation:

$$ED = a + b \times kd / (kd + kp)$$

Where:  $ED$ : effective degradation at ruminal passage rates;  $a$ : the immediately degradable fraction;  $b$ : the potentially degradable fraction;  $kp$ : fractional passage rate; and  $kd$ : the fractional degradation rate.

## Statistical analysis

The SAS Inst., Inc., Cary, NC, USA, PROC MIXED (version 9.1) was used to analyze the data. Treatments were regarded as fixed effects, and subsequent runs were taken to be random effects. The analysis employed the following model:  $Y_{ij} = \mu + T_i + R_j + e_{ij}$ . For this model,  $Y_{ij}$  is the observation of the dependent variable  $ij$ ,  $\mu$  is the overall mean of  $Y$ ,  $T_i$  is the effect of treatment ( $i = 7$ ),  $R_j$  is the fixed effect of incubation run as replications ( $j = 2$ ), and  $e_{ij}$  is the random residual error that is linked to observation  $ij$ . The treatments' means were compared using the probability of difference (PDIFF) option of the least squares means statement.  $P < 0.05$  differences were regarded as statistically significant differences.

## RESULTS AND DISCUSSION

The chemical analysis of the wastes related to the heated potato slices (HPS), French fries waste (FFW), and barley grain is presented in Table 2. The DM contents for HPS and FFW were 22.5 and 37.1%, respectively. Among the currently studied co-products, French fries had the second-highest DM percentage. During the processing of French fries at a higher temperature of approximately 170 °C, the tissue of the potato absorbed oil, and the moisture content was evaporated, which was replaced by oil [19]. Therefore, this leads to increased DM content as compared to heated potato slices.

The starch levels in barley grain and HPS were higher compared to FFW, with values of 57.2% and 54.7% of DM, respectively, while FFW had a starch content of 38.3%. However, due to the frying process, FFW had a higher fatty acid (FA) content of 19.80% compared to the others.

**Table 1. Ingredient and chemical composition (% of DM) of the treatments used in *in vitro* gas production technique**

Diet ingredients	Control	Heated potatoes slices (HPS)			French fries wastes (FFW)		
		5%	10%	15%	5%	10%	15%
Alfalfa hay	11.00	11.00	11.00	11.00	12.07	12.07	12.07
Corn silage	27.00	27.00	27.00	27.00	22.01	22.01	22.01
Wheat straw	2.00	2.00	2.00	2.00	1.20	1.20	1.20
Barley grain (grounded)	16.00	11.00	6.00	1.00	15.40	10.40	5.40
Heated potato slices (HPS)	0.00	5.00	10.00	15.00	0.00	0.00	0.00
French fried wastes (FFW)	0.00	0.00	0.00	0.00	5.00	10.00	15.00
Corn grain (grounded)	20.00	20.00	20.00	20.00	14.58	14.58	14.58
Soybean meal	8.80	8.80	8.80	8.80	15.71	15.71	15.71
Cotton seed meal	5.00	5.00	5.00	5.00	2.50	2.50	2.50
Corn Gluten meal	4.00	4.00	4.00	4.00	0.87	0.87	0.87
Wheat bran	3.30	3.30	3.30	3.30	1.61	1.61	1.61
Toxin binder	0.25	0.25	0.25	0.25	0.16	0.16	0.16

Sodium bicarbonate	0.60	0.60	0.60	0.60	0.86	0.86	0.86
Salt	0.40	0.40	0.40	0.40	0.27	0.27	0.27
Vit/Min premix <sup>1</sup>	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Limestone	0.80	0.80	0.80	0.80	0.43	0.43	0.43
Magnesium oxide	0.20	0.20	0.20	0.20	0.21	0.21	0.21
Chemical composition %							
Crude protein	17.20	17.03	16.87	16.74	16.95	16.71	16.48
RDP <sup>2</sup> , % of CP	62.2	61.90	61.30	68.58	61.86	61.53	61.19
RUP <sup>3</sup> , % of CP	37.8	38.10	38.70	38.70	38.14	38.47	38.81
Starch	31.29	31.12	30.96	30.79	30.30	29.32	28.33
Non- fiber carbohydrate	41.70	41.99	42.28	42.58	41.26	40.83	40.39
Neutral detergent fiber	30.06	29.98	29.89	29.81	29.89	29.71	29.54
Acid detergent fiber	17.00	16.82	16.69	16.55	16.92	16.89	16.85
Crude ash	7.97	8.00	8.03	8.10	7.93	7.89	7.85
Ether extract	3.09	3.01	2.93	2.58	3.98	4.86	5.75
NEI <sup>3</sup> , Mcal/kg DM	1.63	1.63	1.64	1.64	1.65	1.67	1.69

<sup>1</sup> Provided (kg of DM): 500,000 IU of vitamin A, 200,000 IU of vitamin D3, 1000 mg of vitamin E, 500 mg of antioxidants, 190 g of Ca, 80 g of P, 21 g of Mg, 3000 mg of Fe, 60 g of Na, 300 mg of Cu, 2000 mg of Mn, 3000 mg of Zn, 100 mg of Co, 100 mg of I, and 30 mg of Se.

<sup>2</sup> Rumen degradable protein

<sup>3</sup> Rumen undegradable protein

<sup>4</sup> Net energy for lactation were estimated using the NASEM 2021 equation [10].

DM content for HPS and FFW was 22.57 and 37.18 percent, respectively. The higher DM for FFW in comparison with HPS is related to the replacement of the water with fatty acids (FA) during the frying process of a potato slice in oil at 170 °C for a couple of seconds. It is noteworthy that absorbing FA into FFW raised its energy content from 2.03 to 2.31 Mcal/kg. The starch contents of barley grain and HPS (57.2 and 54.7% of DM, respectively) were higher than FFW (38.3 %). Also, NDF concentrations for barley, HPS, and FFW were 18.92, 17.20, and 15.41%, respectively. The non-fiber carbohydrate (NFC) content in potato co-products and barley grain varies from highest to lowest in the following order: barley grain, HPS, and FFW. Most of the potato by-products have less starch content compared to barley grain. Frying, a heat-based process, can dehydrate food, reducing moisture and increasing carbohydrates and lipids, potentially leading to a decrease in protein content, as per previous studies [20].

**Table 2. Chemical composition of barley grain, heated potato particles and French fries wastes used in current study**

Items (% of DM)	Barley grain	HPS	FFW
-----------------	--------------	-----	-----

DM	88.50	22.57	37.18
Crude Protein	11.30	9.70	8.15
Starch	57.21	54.70	38.30
NDF	18.92	17.20	15.41
ADF	7.20	4.50	6.50
NFC	63.50	69.42	54.85
Ash	2.65	3.19	1.79
FA	2.11	0.49	19.80
NEI <sup>1</sup> (Mcal/kg DM)	1.94	2.03	2.31

<sup>1</sup> were calculated based on the NASEM 2021 equation. DM: dry matter; crude protein; NDF: neutral detergent fiber; NFC: non-fiber carbohydrates; FA: fatty acids; NEI: net energy for lactation. HPS: heated potato slices; FFW: French fries waste.

Replacing the barley grain with HPS and FFW in batch culture affected *in vitro* gas production at different times of incubation, as shown in Table 3. The gas production volume within the first two hours of incubation for the control (ml/g DM) was higher than that of other treatments, followed by treatments containing 5 and 10% HPS. The result showed that adding HPS to diets by 10 percent did not have a significant effect on gas production; however, higher inclusion of HPS (i.e., 15%) decreased gas production significantly. There are several reasons why the lowest HPS level (15%) led to less gas production. These include the amorphous nature of potato starch granules and the fact that rumen bacteria are not used to dealing with these structures [21].

Inversely, the inclusion of French fries waste in the diet decreased gas production not only within the initial hours of incubation but also during the latter hours. The lowest gas production after 48 hours of incubation was related to the treatment containing 15% of FFW. A decrease in gas production companions through the inclusion of FFW could be related to FFW's oil content. Menke and Steingass [15] and Cone and van Gelder [22] reported less gas production for fat in comparison to carbohydrates (2 vs. 370 ml gas/g substrate, respectively). On the other side, the lower volume of gas production for treatments that contain 10 and 15% French fries waste could be attributed to the negative effects of unsaturated fatty acids on rumen bacterial fermentation. Yang et al. [23] using the *in vitro* gas production technique, showed that an increasing proportion of unsaturated fatty acids in the fermentation substrate reduces CH<sub>4</sub> and CO<sub>2</sub> emissions, affecting rumen fermentation and the microbial community. Also, other studies mentioned that the inhibition of unsaturated fatty acids on rumen fermentation may be due to FA adhering to the feed surface and the toxic effects of FA on rumen bacteria [24, 25].

The gas production from the immediately soluble fraction (a) and the rate and extent of gas production from the insoluble fraction (b) are shown in Table 3. The highest amount of gas production for a soluble fraction (a) belonged to 15% HPS, and its value was significantly higher than control (7.24 vs. 4.95 ml). Also, Melesse et al. [26] found that heat treatment of potatoes caused the gelatinization of potato starch, which led to more solubility. However, the replacement of barley grain with FFW at all studied levels decreased gas production from the immediately soluble fraction. Similarly, the gas production from the insoluble fraction (b) was highest for the 15% HPS and the lowest for the 15% FFW. Also, the rate of gas production for the insoluble fraction (ml/h) varied from 0.031 to 0.054 ml/h and was the highest for diets containing 5% HPS.

The in-vitro gas production method, on the other hand, is appealing to ruminant nutritionists since it can quickly assess the volume of gas production over time. However, the measurement of gases only implies the measurement of gases that are nutritionally wasteful and environmentally dangerous (CO<sub>2</sub> and CH<sub>4</sub>). There is a widespread misconception that the rate and extent of gas generation are similar to the rate and extent of feed degradation. This misconception has been found in the majority of previous investigations [27]. It has been shown that measuring the amount of DM truly degraded along with gas production for each time point gives more information regarding the partitioning of the degraded substrate between waste gases and volatile fatty acids on one side and microbial mass production on the other.

**Table 3. Effects of replacing barley grain with heated potato slices and French fries on *in vitro* cumulative gas production (ml/g DM) and its kinetics during *in vitro* batch culture incubation**

Time (hours)	Heated potatoes slices (HPS) %				French fries wastes (FFW) %			SEM	P-value
	Control	5	10	15	5	10	15		
2	10.1 <sup>a</sup>	10.3 <sup>a</sup>	7.55 <sup>bc</sup>	6.50 <sup>cd</sup>	8.60 <sup>ab</sup>	7.25 <sup>bc</sup>	5.12 <sup>d</sup>	1.3642	0.03
4	23.70 <sup>a</sup>	21.85 <sup>a</sup>	13.02 <sup>cd</sup>	10.22 <sup>d</sup>	23.05 <sup>a</sup>	18.05 <sup>b</sup>	14.00 <sup>c</sup>	2.49193	0.01
6	55.85 <sup>a</sup>	37.45 <sup>b</sup>	31.62 <sup>c</sup>	28.95 <sup>c</sup>	38.35 <sup>b</sup>	39.35 <sup>b</sup>	28.85 <sup>c</sup>	2.99386	0.03
12	96.90 <sup>a</sup>	84.75 <sup>c</sup>	77.27 <sup>d</sup>	89.97 <sup>b</sup>	67.95 <sup>e</sup>	68.75 <sup>e</sup>	61.30 <sup>f</sup>	3.30371	0.01
24	138 <sup>a</sup>	126.65 <sup>b</sup>	122.10 <sup>c</sup>	89.97 <sup>e</sup>	108.95 <sup>c</sup>	108.85 <sup>c</sup>	103.63 <sup>d</sup>	2.92578	0.01
36	175.50 <sup>a</sup>	172.25 <sup>ab</sup>	169.23 <sup>bc</sup>	164.10 <sup>c</sup>	158.70 <sup>d</sup>	153.45 <sup>d</sup>	148.00 <sup>e</sup>	3.69186	0.01
48	197.25 <sup>a</sup>	196.25 <sup>a</sup>	195.75 <sup>a</sup>	185.98 <sup>b</sup>	181.50 <sup>bc</sup>	177.15 <sup>cd</sup>	174.23 <sup>d</sup>	3.81984	0.04
a	-4.95 <sup>b</sup>	-4.87 <sup>b</sup>	-4.66 <sup>b</sup>	-7.24 <sup>a</sup>	-3.82 <sup>bc</sup>	-1.21 <sup>d</sup>	-2.94 <sup>c</sup>	1.049	0.03

b	197.60 <sup>cd</sup>	203.10 <sup>bc</sup>	209.73 <sup>ab</sup>	217.43 <sup>a</sup>	211.63 <sup>a</sup>	198.05 <sup>cd</sup>	191.90 <sup>d</sup>	5.419	0.01
a+b	192.65 <sup>cd</sup>	198.23 <sup>bc</sup>	205.07 <sup>ab</sup>	210.19 <sup>a</sup>	207.81 <sup>a</sup>	196.84 <sup>c</sup>	188.96 <sup>d</sup>	5.177	0.01
k	0.032 <sup>d</sup>	0.054 <sup>a</sup>	0.042 <sup>b</sup>	0.037 <sup>c</sup>	0.031 <sup>d</sup>	0.034 <sup>cd</sup>	0.037 <sup>c</sup>	0.002	0.02

<sup>1</sup> Control: a diet containing 16% (DM basis) barley grain as the main source of non-forage carbohydrate; 5 HPS: a diet containing 11% barley grain and 5% HPS; 3) 10 HPS: a diet containing 6% barley grain and 10% HPS 4) 15 HPS: a diet containing 1% barley grain and 15% HPS; 5) 5 FFW: a diet containing 11% barley grain and 5% FFW; 6) 10 FFW: a diet containing 6% barley grain and 10% FFW; 7) 15 FFW: a diet containing 1% barley grain and 15% FFW, SEM; standard error of the mean; HPS: heated potato slices; FFW: French fries waste.

a: the amount of gas produced from the fraction that is soluble quickly (mL); b: the amount of gas produced from the insoluble fraction (mL); a+b represents the potential gas production (mL); k represents the gas production rate constant for the insoluble fraction in milliliters per hour (ml/h); and t is the incubation time in hours (h).

<sup>a-f</sup> Means within a row with different superscripts differ significantly ( $P < 0.05$ ).

The results related to the DM degradability of the experimental treatments are given in Table 4. DM degradability after 2, 4, and 6 hours of incubation for the control was significantly lower than other treatments. The highest amount of DM degradability for treatments containing HPS was the highest, and it was intermediate for the FFW treatments.

The previous studies reported a slower ruminal degradation rate for raw potato starch than barley and wheat [28, 29]. Different types of grains and cooking methods degrade at different rates in the rumen. In general, the ruminal degradation rate was the fastest (32% h), then oats (29% h), barley (29% h), potatoes (5% h), corn (2% h), and sorghum [30, 31]. The slower ruminal degradation rate of raw potato starch is attributed to the protein properties and starch nature of raw potatoes. According to Alvani et al. [32], the amount of branching in amylopectin in potato starch is lower than that in cereals. Additionally, Hernández-Medina et al. [33] demonstrated that it undergoes gelatinization at relatively low temperatures, ranging from 64.4 to 69.9 °C, in comparison to other types of starches, and Vasanthan and Bhatta [34] noted that it exhibits a high degree of expandability. Also, in their study, Sarian et al. [35] evaluated the enzymatic degradation of granular potato starch by *Microbacterium aurum* strain B8.A. They found that the hydrolysis of potato starch granules occurred at a slower rate compared to wheat and tapioca starch. The variation in degradation rate can be attributed to variations in the structure and/or surface area of the starch granules.

Previous studies showed that heat treatments increase the rate and extent of starch degradation in the rumen [7, 8]. Sveinbjörnsson et al. [8] demonstrated that cooking increased the *in vitro* starch degradation rate of isolated potato starch from 0.038 to 0.197/h. In another study,

Sveinbjörnsson et al. [8] showed that the heat treatment of pure potato starch, peas, barley, and maize increased the proportion of starch degradation after 8 hours of *in vitro* incubation. Specifically, the degradation rate for pure potato starch increased from 0.155 to 0.870 /h, peas from 0.491 to 0.815, barley from 0.686 to 0.913, and maize from 0.351 to 0.498. This indicates that the greatest increase in starch degradation was observed in pure potato starch, with 0.87 being degraded when it was in the cooked form, compared to only 0.155 in the raw form. It seems that the heat processing of potato co-products increases starch's ruminal degradation rate.

It should be mentioned that part of the slower DM degradability for barley in comparison to potato by-products could be related to higher NDF content with a slower degradability rate. After 48 hours of incubation, DM degradability for the barley treatment was significantly lower than for other treatments. Also, for the mentioned time point, 48 h, the inclusion of FFW at the 15% level caused a decrease in degradability compared to the lower levels (i.e., 5 and 10% of FFW), which could be attributed to the higher oil content, which leads to impaired ruminal microorganism activity. Previous studies have shown that high levels of unsaturated fatty acids can reduce microbial activity in the rumen due to toxicity and physical inhibition, leading to a decrease in the extent of degradation [36, 37, 38].

It has been shown that the partitioning of fermented nutrients between microbial biomass and waste gas (CO<sub>2</sub> and CH<sub>4</sub>) production plays an important role in ranking the feeds. For the *in vitro* gas production method, the partitioning factor (PF) is a measure of how well microbial biomass is produced. It is calculated by dividing the amount of truly degradable substrate (mg) by the amount of net gas production (ml) [27]. After 12 hours of incubation, the PF for 10% and 15% FFW was the highest (8.09 and 7.57), for 5% FFW, 5% HPS and 10% HPS were the lowest, and for the control (barley grain), 15% HPS was intermediate. As aforementioned, animal feeds with high PF indicate that a greater amount of degradable materials were incorporated into the microbial mass in batch culture. Also, the PF value after 24 hours of incubation for treatments containing FFW was higher than HPS and control (barley grain) treatments. The current results showed that replacing HPS and FFW with barley by 15% didn't affect ruminal fermentation negatively.

The average value for the soluble fraction (a) varied between 2.52% and 4.32%, with the highest value observed with 10% HPS, an intermediate value with the control, and the lowest value with 5% HPS ( $P < 0.001$ ). On the other hand, the average value for the potentially degradable fraction (b) ranged from 53.90% to 66.04%, with the lowest value observed with the control and the highest value with 5% HPS ( $P < 0.001$ ). The DM's effective degradability,

with a passage rate of 0.05 h<sup>-1</sup>, was found to be higher for FFW and HPS compared to the control (Table 4). Also, the Kd of the b fraction was significantly lower for the control (0.14 % h<sup>-1</sup>) than for treatments supplemented with potato processing by-products.

It has been shown that rate of raw potato starch degradability is significantly lower than that of normal cereal grain, which is used in dairy cow diets (i.e., corn, barley, and wheat grain). Monteils et al. [39] reported that the degradability rates of potato peel and wheat starches were 5% and 34% h<sup>-1</sup>. However, heating potato starch, like what happened in the current study, increases the rate of potato starch degradability to an extent similar to that of barley starch and may be higher [8, 29]. The higher rate of starch degradability for heated potato by-products attributed to two process. Firstly, the starch gelatinization which occurred by heating process and consequently solubilization of amylose starch [40, 41]. Secondly, denaturation of proteins which cover potato starch granules [42].

**Table 4. Effects of replacing barley grain with different levels of heated potato slices and French fries on partitioning factors, DM degradability and it's kinetics during *in vitro* batch culture incubation**

Time (hours)	Heated potatoes slices (HPS) %				French fries wastes (FFW) %			SEM	P-value
	Control	5	10	15	5	10	15		
2	19.87 <sup>d</sup>	24.25 <sup>abc</sup>	24.04 <sup>abc</sup>	25.88 <sup>a</sup>	23.57 <sup>bc</sup>	24.77 <sup>ab</sup>	22.14 <sup>c</sup>	1.541	0.01
4	28.55 <sup>e</sup>	34.80 <sup>c</sup>	37.16 <sup>b</sup>	39.26 <sup>a</sup>	32.27 <sup>d</sup>	35.80 <sup>bc</sup>	32.33 <sup>d</sup>	1.359	0.01
6	35.46 <sup>c</sup>	46.77 <sup>a</sup>	43.76 <sup>a</sup>	46.66 <sup>a</sup>	44.91 <sup>a</sup>	42.28 <sup>ab</sup>	40.38 <sup>bc</sup>	1.536	0.03
12	42.19 <sup>d</sup>	54.04 <sup>ab</sup>	49.03 <sup>c</sup>	52.26 <sup>b</sup>	52.32 <sup>b</sup>	54.93 <sup>a</sup>	51.99 <sup>b</sup>	3.243	0.01
24	51.12 <sup>c</sup>	63.09 <sup>a</sup>	60.52 <sup>b</sup>	59.47 <sup>b</sup>	60.22 <sup>b</sup>	61.42 <sup>ab</sup>	59.17 <sup>b</sup>	1.611	0.03
36	58.86 <sup>c</sup>	69.77 <sup>a</sup>	69.14 <sup>a</sup>	68.68 <sup>a</sup>	69.02 <sup>a</sup>	68.18 <sup>a</sup>	64.79 <sup>b</sup>	1.600	0.04
48	60.80 <sup>c</sup>	73.55 <sup>a</sup>	74.05 <sup>a</sup>	74.16 <sup>a</sup>	74.13 <sup>a</sup>	74.42 <sup>a</sup>	70.37 <sup>b</sup>	1.717	0.04
a	3.50 <sup>ab</sup>	2.52 <sup>b</sup>	4.32 <sup>a</sup>	2.91 <sup>b</sup>	3.17 <sup>ab</sup>	3.21 <sup>ab</sup>	2.64 <sup>b</sup>	0.411	0.02
b	53.90 <sup>d</sup>	66.04 <sup>a</sup>	63.39 <sup>bc</sup>	63.60 <sup>bc</sup>	64.96 <sup>ab</sup>	64.95 <sup>ab</sup>	62.48 <sup>c</sup>	1.120	0.01
a+b	0.14 <sup>c</sup>	0.17 <sup>b</sup>	0.15 <sup>bc</sup>	0.19 <sup>a</sup>	0.15 <sup>bc</sup>	0.16 <sup>bc</sup>	0.15 <sup>bc</sup>	0.014	0.04
kd	57.40 <sup>d</sup>	68.57 <sup>a</sup>	67.71 <sup>ab</sup>	66.51 <sup>bc</sup>	68.13 <sup>ab</sup>	68.16 <sup>ab</sup>	65.1 <sup>c</sup>	1.215	0.02
ED 0.02	50.46 <sup>d</sup>	61.45 <sup>a</sup>	60.18 <sup>b</sup>	60.35 <sup>b</sup>	60.41 <sup>ab</sup>	60.84 <sup>ab</sup>	57.82 <sup>c</sup>	0.715	0.01
ED 0.05	42.91 <sup>d</sup>	53.26 <sup>a</sup>	51.74 <sup>b</sup>	53.07 <sup>a</sup>	51.76 <sup>b</sup>	52.51 <sup>ab</sup>	49.60 <sup>c</sup>	0.577	0.02
ED 0.08	37.48 <sup>e</sup>	47.06 <sup>ab</sup>	45.52 <sup>c</sup>	47.44 <sup>a</sup>	45.38 <sup>c</sup>	46.29 <sup>bc</sup>	43.52 <sup>d</sup>	0.658	0.01
PF 12h	6.90 <sup>ab</sup>	5.58 <sup>cd</sup>	5.79 <sup>c</sup>	6.77 <sup>bc</sup>	5.83 <sup>cd</sup>	8.09 <sup>a</sup>	7.57 <sup>ab</sup>	0.371	0.03
PF 24h	4.94 <sup>bc</sup>	4.57 <sup>c</sup>	4.78 <sup>c</sup>	4.87 <sup>c</sup>	6.70 <sup>a</sup>	5.64 <sup>b</sup>	5.44 <sup>b</sup>	0.178	0.04

<sup>1</sup>Control: a diet containing 16% (DM basis) barley grain as the main source of non-forage carbohydrate; 5 HPS: a diet containing 11% barley grain and 5% HPS; 3) 10 HPS: a diet containing 6% barley grain and 10% HPS 4) 15 HPS: a diet containing 1% barley grain and 15% HPS; 5) 5 FFW: a diet containing 11% barley grain and 5% FFW; 6) 10 FFW: a diet containing 6% barley grain and 10% FFW; 7) 15 FFW: a diet containing 1% barley grain and 15% FFW, SEM; standard error of the mean; HPS: heated potato slices; FFW: French fries waste.

a: the immediately degradable fraction; b: the potentially degradable fraction; k: the fractional degradation rate; a+b: the total degradable fraction; Ed 0.02, Ed 0.05, and Ed 0.08: effective degradation at ruminal passage rates of 2%, 5%, and 8% per h.

PF 12h: Partitioning factor after 12 h of incubation; PF 24h: Partitioning factor after 24 h of incubation

<sup>a-f</sup> Means within a row with different superscripts differ significantly ( $P < 0.05$ ).

## CONCLUSION

The previous studies indicated raw potato starch as a slow-degradable source of carbohydrate in ruminant nutrition; however, the current study findings revealed that heating increases the rate of potato starch degradability to an extent similar to barley starch and may be higher. In general, heated potato processing by-products can be replaced with barley grain by 15% in the diet of dairy cows without negative effects on ruminal fermentation.

## REFERENCES

1. Pathak, P. D., S. A. Mandavgane, N. M. Puranik, S. J. Jambhulkar, and B. D Kulkarni. (2017). Valorization of potato peel: a biorefinery approach. *Crit. Rev. Biotechnol.* 38: 218–230. <https://doi.org/10.1080/07388551.2017.1331337>.
2. Schripsema, A., and Meijer, B. (2017). Food losses in the Iranian potato sector: Identification mission (No. 1750). Wageningen Food & Biobased Research. <https://library.wur.nl/WebQuery/wurpubs/523027>
3. Nelson, M.L. (2010). Utilization and Application of Wet Potato Processing Co-Products for Finishing Cattle. *J. Anim. Sci.* 88:133- 142. <https://doi.org/10.2527/jas.2009-2502>.
4. Nelson, M.L., J.R. Busboom, J.D. Cronrath, L. Falen and A. Blankenbak (2000). Effects of graded levels of potato by-products in barley- and corn-based beef feedlot diets. I. Feedlot performance, carcass traits, meat composition, and appearance. *J. Anim. Sci.*78: 1829-1836. <https://doi.org/10.2527/2000.7871829x>.
5. Charmley, E., D. Nelson and F. Zvomuya (2006) Nutrient cycling in the vegetable processing industry: Utilization of potato by-products. *Can. J. Soil Sci.* 86(4): 621-629. <https://doi.org/10.4141/S05-118>

6. Nelson, M.L., D.J. Marks, J.R. Busboom, J.D. Cronrath and L. Falen (2004). Effects of supplemental fat on growth performance and quality of beef from steers fed barley-potato finishing diets. 1. Feedlot performance, carcass traits, appearance, water-binding, retail storage, and palatability attributes. *J. Anim. Sci.* 82:3600–3610. <https://doi.org/10.2527/2004.82123600x>.
7. Offner, A., A. Bach, and D. Sauvant (2003). Quantitative review of in situ starch degradation in the rumen. *Feed Sci. Technol.* 106: 81-93. [https://doi.org/10.1016/S0377-8401\(03\)00038-5](https://doi.org/10.1016/S0377-8401(03)00038-5).
8. Sveinbjörnsson, J., M. Murphy and P. Udén (2007). In vitro evaluation of starch degradation from feeds with or without various heat treatments. *Anim. Feed Sci. Technol.* 132(3-4):171-185. <https://doi.org/10.1016/j.anifeedsci.2006.03.018>
9. Huhtanen, P., Seppala, A., Ots, M., Ahvenjarvi, S., & Rinne, M. (2008). In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. *Journal of Animal Science*, 86(3), 651-659. <https://doi.org/10.2527/jas.2007-0246>
10. National Academies of Science, Engineering, and Medicine. 2021. Nutrient Requirements of Dairy Cattle, 8th rev. ed. National Acad Press, Washington DC.
11. AOAC (2007). Official Methods of Analysis. 18th Edition, Association of Official Analytical chemists, Gaithersburg.
12. Hall, M. B. (2009). Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. *J. AOAC Int.*, 92, 42-49. <https://doi.org/10.1093/jaoac/92.1.42>
13. Van Soest, P. V., J. B. Robertson and B. A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74(10):3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
14. Oyebade, A., L. Lifshitz, H. Lehrer, S. Jacoby, Y. Portnick, and U. Moallem. 2020. Saturated fat supplemented in the form of triglycerides decreased digestibility and reduced performance of dairy cows as compared to calcium salt of fatty acids. *Animal* 14:973–982. <https://doi.org/10.1017/S1751731119002465>
15. Menke, K.H. and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *A. nim. Res. Develop.*, 28: 7-55.
16. SAS. (2003). User's Guide Version 9.1.3. SAS Institute, Inc., Cary, NC.

17. Ørskov, E.R. and I.M. McDonald (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92: 499-503. <https://doi.org/10.1017/S0021859600063048>
18. Parnian-Khajehdizaj, F., Taghizadeh, A., Hosseinkhani, A., and Mesgaran, M. D. (2018). Evaluation of dietary supplementation of B vitamins and HMBI on fermentation kinetics, ruminal or post-ruminal diet digestibility using modified in vitro techniques. *Journal of BioScience and Biotechnology*, 7(2-3), 125-133. <https://editorial.uni-plovdiv.bg/index.php/JBB/article/view/200>
19. Millin, T. M., I. G. Medina-Meza, B. C. Walters, K. C. Huber, B. A. Rasco and G. M. Ganjyal (2016). Frying oil temperature: impact on physical and structural properties of French fries during the par and finish frying processes. *Food Bioproc. Tech.* 9:2080-2091. <https://doi.org/10.1007/s11947-016-1790-2>
20. Romano, A., V. D'Amelia, V. Gallo, S. Palomba, D. Carputo, and P. Masi (2018). Relationships between composition, microstructure and cooking performances of six potato varieties. *Food Res. Int.* 114:10-19. <https://doi.org/10.1016/j.foodres.2018.07.033>
21. Schneider, P. L., M. R. Stokes, L. S. Bull, and C. K. Walker (1985). Evaluation of potato meal as a feedstuff for lactating dairy cows. *J. Dairy Sci.* 68: 1738-1743. [https://doi.org/10.3168/jds.S0022-0302\(85\)81020-1](https://doi.org/10.3168/jds.S0022-0302(85)81020-1).
22. Cone, J. W., & van Gelder, A. H. (1999). Influence of protein fermentation on gas production profiles. *Animal Feed Science and Technology*, 76(3-4), 251-264. [https://doi.org/10.1016/S0377-8401\(98\)00222-3](https://doi.org/10.1016/S0377-8401(98)00222-3)
23. Yang, Z., Liu, S., Xie, T., Wang, Q., Wang, Z., Yang, H. & Wang, W. (2022). Effect of Unsaturated Fatty Acid Ratio In Vitro on Rumen Fermentation, Methane Concentration, and Microbial Profile. *Fermentation*, 8(10), 540. <https://doi.org/10.3390/fermentation8100540>
24. Brask, M.; Lund, P.; Weisbjerg, M.R.; Hellwing, A.L.F.; Poulsen, M.; Larsen, M.K.; Hvelplund, T. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. *J. Dairy Sci.* 2013, 96, 2356–2365. <https://doi.org/10.3168/jds.2011-5239>
25. Sun, X. G., Wang, Y., Xie, T., Yang, Z. T., Wang, J. D., Zheng, Y. H. & Li, S. L. (2021). Effects of high-forage diets containing raw flaxseeds or soybean on in vitro ruminal fermentation, gas emission, and microbial profile. *Microorganisms*, 9(11), 2304. <https://doi.org/10.3390/microorganisms9112304>

26. Melesse, A., H. Steingass, M. Schollenberger and M. Rodehutschord (2018). Component composition, in vitro gas and methane production profiles of fruit by-products and leaves of root crops. *J. Agric. Sci.* 156(7): 949-958. <https://doi.org/10.1017/S0021859618000928>
27. Makkar, H.P. (2004). Recent advances in the in vitro gas method for evaluation of nutritional quality of feed resources. *Assessing quality and safety of animal feeds*. FAO Animal Production and Health Series 160:55-88. [http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/570\\_EN-04.htm](http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/570_EN-04.htm)
28. Cone, J. W., W. Cliné-Theil, A. Malestein and A. T. van't Klooster (1989). Degradation of starch by incubation with rumen fluid. A comparison of different starch sources. *J. Sci. Food Agric.* 49(2):173-183. <https://doi.org/10.1002/jsfa.2740490206>
29. Eriksson, T., and Murphy, M. (2004). Ruminal digestion of leguminous forage, potatoes and fodder beets in batch culture: I. Fermentation pattern. *Animal feed science and technology*, 111(1-4), 73-88. <https://doi.org/10.1016/j.anifeedsci.2003.05.001>
30. Callison, S. L., Firkins, J. L., Eastridge, M. L., & Hull, B. L. (2001). Site of nutrient digestion by dairy cows fed corn of different particle sizes or steam-rolled. *Journal of Dairy Science*, 84(6), 1458-1467. [https://doi.org/10.3168/jds.S0022-0302\(01\)70179-8](https://doi.org/10.3168/jds.S0022-0302(01)70179-8)
31. Mosavi, G. H. R., Fatahnia, F., Mehrabi, A. A., Mirzaei Alamouti, H. R., & Darmani Kohi, H. (2012). Effect of dietary starch source on milk production and composition of lactating Holstein cows. *South African Journal of Animal Science*, 42(3), 201-209. <https://hdl.handle.net/10520/EJC124544>
32. Alvani, K., Qi, X., Tester, R. F., & Snape, C. E. (2011). Physico-chemical properties of potato starches. *Food Chemistry*, 125(3), 958-965. <https://doi.org/10.1016/j.foodchem.2010.09.088>
33. Hernández-Medina, M., Torruco-Uco, J. G., Chel-Guerrero, L., & Betancur-Ancona, D. (2008). Caracterización fisicoquímica de almidones de tubérculos cultivados en Yucatán, México. *Food Science and Technology*, 28, 718-726. <https://doi.org/10.1590/S0101-20612008000300031>
34. Vasanthan, T., & Bhatta, R. S. (1996). Physicochemical properties of small-and large-granule starches of waxy, regular, and high-amylose barleys. *Cereal chemistry (USA)*, 73(2). <https://www.cerealsgrains.org/publications/cc/backissues/1996/Documents/CC1996a33.html>
35. Sarian, F. D., van der Kaaij, R. M., Kralj, S., Wijbenga, D. J., Binnema, D. J., van der Maarel, M. J., & Dijkhuizen, L. (2012). Enzymatic degradation of granular potato

- starch by *Microbacterium aurum* strain B8. *A. Applied microbiology and biotechnology*, 93, 645-654. <https://doi.org/10.1007/s00253-011-3436-7>
36. Sepelev, I. and R. Galoburda (2015). Industrial potato peel waste application in food production: a review. *Res Rural Dev.* 1:130-136. [https://ilufb.llu.lv/conference/Research-for-Rural-Development/2015/LatviaResearchRuralDevel21st\\_volume1-130-136.pdf](https://ilufb.llu.lv/conference/Research-for-Rural-Development/2015/LatviaResearchRuralDevel21st_volume1-130-136.pdf)
37. Ahmad, F. and S. T. Khan (2019). Potential industrial use of compounds from by-products of fruits and vegetables. Health and safety aspects of food processing technologies, 273-307. [https://link.springer.com/chapter/10.1007/978-3-030-24903-8\\_10](https://link.springer.com/chapter/10.1007/978-3-030-24903-8_10)
38. Sampaio, S. L., S. A. Petropoulos, A. Alexopoulos, S. A. Heleno, C. Santos-Buelga, L. Barros and I. C. Ferreira (2020). Potato peels as sources of functional compounds for the food industry: A review. *Trends Food Sci. Technol.* 103:118-129. <https://doi.org/10.1016/j.tifs.2020.07.015>
39. Monteils, V., S. Jurjanz, G. Blanchart and F. Laurent (2002). Kinetics of ruminal degradation of wheat and potato starches in total mixed rations. *J. Anim. Sci.* 80(1): 235-241. <https://doi.org/10.1093/ansci/80.1.235>
40. Gidley, M. J. (1992). Structural order in starch granules and its loss during gelatinisation. In G. O. Phillips, P. A. Williams, & G. J. Wedlock (Eds.), *Gums and stabilisers for the food industry* (pp. 87–92). Oxford: IRL Press.
41. Bogracheva, T. Y., Meares, C., and Hedley, C. L. (2006). The effect of heating on the thermodynamic characteristics of potato starch. *Carbohydrate polymers*, 63(3), 323-330. <https://doi.org/10.1016/j.carbpol.2005.08.065>
42. Prates, L. L., Lei, Y., Refat, B., Zhang, W., & Yu, P. (2018). Effects of heat processing methods on protein subfractions and protein degradation kinetics in dairy cattle in relation to protein molecular structure of barley grain using advanced molecular spectroscopy. *Journal of Cereal Science*, 80, 212-220. <https://doi.org/10.1016/j.jcs.2018.01.008>