

## Evaluating nutrient degradation and degradation kinetics of some starch-rich cereal and legume grains in sheep consuming a starch-rich diet

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### ABSTRACT

This study aimed to determine the *in-situ* nutrient degradation and degradation kinetics of some starch-rich cereal and legume grains in sheep consuming diet containing high (32%) level of starch. Three cannulated Akkaraman sheep, 5-6 years old, were utilized in the study. Sheep with rumen cannula were fed with diets containing 32% starch 10 days before and throughout the trial. The study used barley, and corn as starch-rich cereal grains, Hungarian vetch, and forage pea grains as pulse grains to determine nutrient degradations. The feed samples used in the *in-situ* study were incubated for 0, 2, 4, 8, 12, 24 and 48 hours in the rumen for 3 sheep as 6 replicates for each incubation time. Dry matter (DM), organic matter (OM), crude protein (CP) and starch degradation rate of feeds were calculated. The water-soluble fraction, the potentially degradable fraction, and the non-degradable nutrient fraction were also calculated. Degradation kinetics of nutrients were determined. By-pass CP contents of feedstuffs were calculated using 12 h incubation values. Except for starch, all degradation values for all incubation times, degradation rates, and nutrient fractions for all nutrients were significantly different among feedstuffs ( $P < 0.05$ ). It can be concluded from this study that DM, OM and CP degradations of corn and forage peas and Hungarian vetch (HV) were higher after 48 hours of incubation, while starch degradations of barley were higher than corn. From the point of view of acidosis, it was concluded that the degradation rates of corn and HV were significantly slower than barley and forage pea and that these feedstuffs can be preferred at higher rates, especially in high starch rations, which may reduce the risk of acidosis.

**Keywords:** *In-situ*, starch-rich grain, degradation, starch, crude protein, by-pass protein

### INTRODUCTION

The main nutrients found in animal feedstuffs are carbohydrates, fats, and proteins. These components are added to the diet in varying amounts depending on the age, physiological status, and breeding purpose of the animals. However, carbohydrates and their essential components, cellulose and starch, are crucial for the nutrition of all animals. They hold particular significance for ruminant animals, serving primarily as a primary energy source (NRC, 2001).

Starch consists of two distinct chemical substances known as amylose and amylopectin, the latter of which constitutes 70 to 80% of the total. Both amylose and amylopectin are stored by plants in the form of crystalline granules, comprising chains of glucose molecules with alpha ( $\alpha$ ) bonds. Following digestion, the final product is glucose. Starch can be digested by microorganisms found in the rumen of ruminant animals, but there are large variations in the rate of fermentation or digestion

depending on processing, storage method or plant source. Small grains such as wheat, barley and oats tend to ferment faster than coarser grain feeds such as corn or sorghum (Palangi and Macit, 2019). The starch fermentation rate may also increase with increasing starch content in feeds (Allen and Ying, 2021). The starch degradation rate of feeds is associated with the risk of acidosis in ruminants and may affect the maximum amount that can be given to animals. Therefore, the determination of the rumen carbohydrates and protein degradability gives very important information to define the nutritional value of feeds (Iomelli et al., 2022). The measurements of the rumen degradation rate of feeds, especially of starch and crude protein (CP), have become very common practice to evaluate the nutritional value of feedstuffs for ruminant animals (White and Ashes 1999; Hristov et al., 2019). However, numerous factors can influence the rumen degradability of feeds (Mohamed and Chaudhry, 2008). These include animal species (Cutrignelli et al., 2007), dietary forage/concentrate ratio (Zicarelli et al., 2008), use of supplementation (Campanile et al., 2008), and cultivars (Calabro et al., 2009). The most significant factors are the type and quantity of feed, the physical fitness of the feeds to be tested, the technological treatments they have undergone, the porosity of the bags, and the quantity of sample incubated per unit of the surface of the bags (Salazar-Cubillas and Dickhoefer, 2021). These factors were also identified as being of importance in 2009 (Musco et al., 2017).

This study aimed to determine the *in-situ* nutrient degradation and degradation kinetics of some starch-rich cereal and legume grains that contain different types of starch in sheep consuming diet containing high (32%) level of starch.

## MATERIAL AND METHODS

This study was deemed appropriate by the meeting of the Kırıkkale University Local Ethic Committee dated 06 November 2019 and the decision numbered 2019-12/55.

### Animal material

Three cannulated Akkaraman sheep, 5-6 years old, were used in the study. Sheep with rumen cannula were fed diets containing 32% starch 10 days before and throughout the trial (Table 1 and Table 2).

**Table 1.** Dry matter percentages of the feedstuffs fed to the animals in the *in-situ* study

Feedstuffs	DM, %
Corn Silage	19.15
Alfalfa hay	6.02
Wheat straw	6.17
Barley	39.86
Rice	4.87
Sunflower meal	10.02
Soybean meal	12.56
Limestone	1.35

**Table 2.** Nutrient content of feedstuffs used in the *in-situ* experiment

DM, %	Barley	Corn	FP	HV
DM	92.16	92.23	93.43	93.91
OM	97.09	97.47	97.41	97.66
CP	10.89	7.68	25.09	28.40
NDF	28.07	11.75	13.22	18.97
ADF	9.63	2.78	10.09	12.73
CF	5.69	2.66	6.63	6.18
EE	1.50	4.28	1.54	1.01
Starch	55.25	75.77	51.02	47.88
ADIN-N	1.98	0.86	0.94	0.96

DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, CF: Crude Fiber, EE: Ether Extract, ADIN-N: Acid Detergent Insoluble Nitrogen, FP: Forage peas, HV: Hungarian vetch.

The diet fed to sheep included alfalfa hay, sunflower seed meal, soybean meal (as protein sources), barley, rice (as energy sources), corn silage and wheat straw (as forage sources). After the feed materials were supplied, the nutrient contents were determined and then the diet was calculated to contain 32% starch. In the experiment, barley, corn as starch-rich cereal grains and Hungarian vetch and forage pea grains as pulse grains were used to determine nutrient degradations.

#### ***In-vivo studies***

The feeds to be incubated in the rumen were ground to a pass-through 2 mm screen after drying. All feed samples used in the study were placed in 3 sheep as 6 replicates for all incubation hours. 3-4 grams of samples were weighed into each of the dacron bags with an average pore size of 45  $\mu$ . Then, the mouths of the bags were tightly closed with rubber bands to prevent the feed samples from spilling out. These bags were placed in nylon nets, 20  $\times$  40 cm in size, with pores of 0.3 cm in diameter, with weights in order to descend to the ventral part of the rumen and left in the rumen of the sheep for given incubation times.

#### ***In-vitro studies***

The feed samples used in the *in-situ* study were incubated in the rumen for 0, 2, 4, 8, 12, 24 and 48 hours. After each incubation period, the bags were removed from the rumen and washed under tap water until running water was clear (approximately 5-10 min.). Then, after keeping the bags on the counter in order to eliminate the water in the bag for about an hour, it was dried in an oven at 65  $^{\circ}$ C for 24 hours (Çetinkaya 1992). After drying the bags, they were taken out of the oven and kept in a desiccator for a while and then weighed and their weights were recorded.

All of the samples were first oven-dried to determine dry matter content (DM). Then, all of the samples were divided into two parts, one part for chemical composition and one part for *in-situ* degradation study. Samples used to determine chemical composition were ground through

a 1-mm screen and then analyzed for ash, organic matter (OM), ether extract (EE), crude protein (CP), acid insoluble nitrogen (ADIN-N) according to AOAC (1990), starch (STN EN ISO 10520, 2002), crude fiber (CF) (Crampton and Maynard, 1938), neutral detergent fiber (NDF) (Van Soest and Robertson, 1979), and acid detergent fiber (ADF) (Goering and Van Soest, 1970). The nutrient degradation rate of feeds was calculated as follows:

$$\text{Nutrient degradability} = a + b(1 - e^{-ct})$$

(Iommelli et al., 2022).

Based on nutrient losses resulting from different incubation times in the rumen, nutrients are divided into three fractions: 1. the water-soluble fraction, 2. the potentially degradable fraction, and 3. the non-degradable fraction. By-pass protein percentages were determined using the modified version of the method (Deniz et al., 2004), defined by Mullahey et al. (1992). By-pass protein percentages were calculated by subtracting the acid detergent insoluble nitrogen (ADIN) from the amount of CP that was not degraded at the end of 12 hours of rumen incubation with the following formula (Mullahey et al., 1992):

$$\text{By-pass Protein Percentage, \% total protein} = (\text{Total residual N} - \text{total residual ADIN}) / (\text{Total sample-N} - \text{total sample ADIN}) \times 100.$$

#### ***Statistical analysis***

The data obtained in the experiment were analyzed using the GLM model and the degradation rate ( $k^{-1}$ ) and lag time (Lag) were calculated using a one-pool version of the discrete lag model defined by Mertens as modified by Wechsler (1981) calculated with the nonlinear regression analysis. Tukey test was applied to determine the difference between the groups. All statistical analysis was performed in the SAS (1995) statistical program.

## **RESULTS**

Nutrient content analysis of the feeds used in the study was performed and *in-situ* DM degradation values and OM degradation values were determined (Table 3 and Table 4).

**Table 3.** DM (%) degradation values of feedstuffs used in the *in-situ* experiment (mean  $\pm$  SD)

Incubation times, h	Barley	Corn	FP	HV	P-value
0	6.37 <sup>ab</sup> $\pm$ 0.25	6.78 <sup>a</sup> $\pm$ 0.58	5.34 <sup>b</sup> $\pm$ 0.37	5.36 <sup>b</sup> $\pm$ 0.35	0.045
2	15.24 <sup>c</sup> $\pm$ 0.58	35.45 <sup>a</sup> $\pm$ 0.91	15.41 <sup>c</sup> $\pm$ 0.57	32.83 <sup>b</sup> $\pm$ 0.66	0.001
4	47.96 <sup>a</sup> $\pm$ 1.73	37.35 <sup>c</sup> $\pm$ 0.85	44.14 <sup>b</sup> $\pm$ 1.28	37.11 <sup>c</sup> $\pm$ 0.95	0.001
8	59.47 <sup>a</sup> $\pm$ 1.86	49.64 <sup>b</sup> $\pm$ 1.05	52.73 <sup>b</sup> $\pm$ 0.99	58.96 <sup>a</sup> $\pm$ 0.95	0.001
12	74.67 <sup>a</sup> $\pm$ 1.12	62.91 <sup>b</sup> $\pm$ 1.22	58.85 <sup>c</sup> $\pm$ 1.10	61.50 <sup>bc</sup> $\pm$ 1.61	0.001
24	83.15 <sup>a</sup> $\pm$ 0.26	72.93 <sup>c</sup> $\pm$ 0.50	81.54 <sup>a</sup> $\pm$ 1.09	75.59 <sup>b</sup> $\pm$ 0.73	0.001
48	86.39 <sup>c</sup> $\pm$ 0.27	88.55 <sup>b</sup> $\pm$ 0.18	89.92 <sup>a</sup> $\pm$ 0.71	88.40 <sup>b</sup> $\pm$ 0.48	0.001

FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

**Table 4.** OM (%) degradation values of feedstuffs used in the *in-situ* experiment (mean  $\pm$  SD)

Incubation times, h	Barley	Corn	FP	HV	P-value
0	6.16 <sup>ab</sup> $\pm$ 0.26	6.75 <sup>a</sup> $\pm$ 0.58	5.34 <sup>b</sup> $\pm$ 0.37	5.21 <sup>b</sup> $\pm$ 0.34	0.045
2	15.41 <sup>c</sup> $\pm$ 0.58	35.13 <sup>a</sup> $\pm$ 0.92	15.40 <sup>c</sup> $\pm$ 0.57	32.61 <sup>b</sup> $\pm$ 0.70	0.001
4	47.67 <sup>a</sup> $\pm$ 1.75	37.34 <sup>b</sup> $\pm$ 0.85	43.98 <sup>a</sup> $\pm$ 1.29	36.76 <sup>b</sup> $\pm$ 0.96	0.001
8	59.27 <sup>a</sup> $\pm$ 1.89	49.94 <sup>b</sup> $\pm$ 1.04	52.54 <sup>b</sup> $\pm$ 1.00	58.67 <sup>a</sup> $\pm$ 0.97	0.001
12	74.80 <sup>a</sup> $\pm$ 1.12	62.68 <sup>b</sup> $\pm$ 1.23	58.95 <sup>b</sup> $\pm$ 1.10	61.38 <sup>b</sup> $\pm$ 1.62	0.001
24	83.20 <sup>a</sup> $\pm$ 0.27	72.75 <sup>c</sup> $\pm$ 0.50	81.43 <sup>a</sup> $\pm$ 1.10	75.52 <sup>b</sup> $\pm$ 0.73	0.001
48	86.34 <sup>c</sup> $\pm$ 0.27	88.49 <sup>b</sup> $\pm$ 0.12	89.87 <sup>a</sup> $\pm$ 0.72	88.36 <sup>b</sup> $\pm$ 0.48	0.001

FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

DM and OM degradations increased regularly in all grain feeds used; The highest degradations were obtained at 0 hours in corn and at 48<sup>th</sup> hours in forage pea grains. The DM and OM degradation values of the feedstuffs were significantly different among groups at all incubation hours ( $P < 0.05$ ).

The determination of DM fractions and degradation kinetics, as well as OM fractions and degradation kinetics, was accomplished through the implementation of an *in-situ* method. (Table 5 and Table 6). All DM and OM fractions and all of the degradation kinetic data were statistically different between the feedstuffs ( $P < 0.05$ ).

The organic matter fractions for barley, forage peas, Hungarian vetch, and corn were as follows: the water-soluble fractions were 6.16%, 5.34%, 5.21%, and 6.75%, respectively; the non-degradable fractions were 13.66%, 10.13%, 11.64%, and 11.51%, respectively; and the potentially degradable fractions were 80.19%, 84.53%, 83.14%, and 81.74%, respectively. The degradation rates were 0.15, 0.10, 0.07, and 0.06, respectively, for barley, forage peas, Hungarian vetch, and corn, while the lag times were 0.77, 1.11, 4.36, and 5.47, respectively. All of the aforementioned data were found to be statistically significant ( $P < 0.05$ ).

**Table 5.** DM (%) fractions of the feedstuffs used in the *in-situ* experiment (mean  $\pm$  SD)

Fractions, %	Barley	Corn	FP	HV	P-value
WSDM	6.37 <sup>ab</sup> $\pm$ 0.26	6.78 <sup>a</sup> $\pm$ 0.58	5.34 <sup>b</sup> $\pm$ 0.37	5.36 <sup>b</sup> $\pm$ 0.35	0.045
PDDM	80.02 <sup>c</sup> $\pm$ 0.24	81.77 <sup>b</sup> $\pm$ 0.66	84.58 <sup>a</sup> $\pm$ 0.56	83.04 <sup>ab</sup> $\pm$ 0.70	0.001
NDDM	13.61 <sup>a</sup> $\pm$ 0.27	11.45 <sup>b</sup> $\pm$ 0.12	10.08 <sup>c</sup> $\pm$ 0.71	11.60 <sup>b</sup> $\pm$ 0.48	0.001
k <sup>-1</sup> , h	0.14 <sup>a</sup> $\pm$ 0.01	0.06 <sup>c</sup> $\pm$ 0.01	0.10 <sup>b</sup> $\pm$ 0.01	0.07 <sup>c</sup> $\pm$ 0.01	0.001
Lag, h	0.76 <sup>b</sup> $\pm$ 0.24	5.42 <sup>a</sup> $\pm$ 0.52	1.11 <sup>b</sup> $\pm$ 0.32	4.45 <sup>a</sup> $\pm$ 0.74	0.001

WSDM: Water Soluble Dry Matter, PDDM: Potentially Degradable Dry Matter, NDDM: Non-Degradable Dry Matter, k<sup>-1</sup>: Degradation rate, Lag: Time required for feeds to start degrading in the rumen, FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

**Table 6.** OM (%) fractions of the feedstuffs used in the *in-situ* experiment (mean  $\pm$  SD)

Fractions, %	Barley	Corn	FP	HV	P-value
WSOM	6.16 <sup>ab</sup> $\pm$ 0.27	6.75 <sup>a</sup> $\pm$ 0.58	5.34 <sup>b</sup> $\pm$ 0.37	5.21 <sup>b</sup> $\pm$ 0.34	0.045
PDOM	80.19 <sup>c</sup> $\pm$ 0.24	81.74 <sup>bc</sup> $\pm$ 0.66	84.53 <sup>a</sup> $\pm$ 0.56	83.14 <sup>ab</sup> $\pm$ 0.66	0.001
NDOM	13.66 <sup>a</sup> $\pm$ 0.27	11.51 <sup>b</sup> $\pm$ 0.12	10.13 <sup>c</sup> $\pm$ 0.72	11.64 <sup>b</sup> $\pm$ 0.48	0.001
k <sup>-1</sup> , h	0.15 <sup>a</sup> $\pm$ 0.01	0.06 <sup>c</sup> $\pm$ 0.01	0.10 <sup>b</sup> $\pm$ 0.01	0.07 <sup>c</sup> $\pm$ 0.01	0.001
Lag, h	0.77 <sup>b</sup> $\pm$ 0.23	5.47 <sup>a</sup> $\pm$ 0.50	1.11 <sup>b</sup> $\pm$ 0.32	4.36 <sup>a</sup> $\pm$ 0.72	0.001

WSOM: Water Soluble Organic Matter, PDOM: Potentially Degradable Organic Matter, NDOM: Non-Degradable Organic Matter, k<sup>-1</sup>: Degradation rate, Lag: Time required for feeds to start degrading in the rumen, FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

*In-situ*, crude protein degradation values and CP fractions and degradation kinetics are determined (Table 7 and Table 8). In the study, while *in-situ* crude protein degradation was higher in forage peas (16.96% and 94.43%, respectively) at 0 and 48 hours, and Hungarian vetch (41.70%) at 2 hours, barley had higher degradation values at 4, 8, 12, and 24<sup>th</sup> hour (52.49%, 65.24%, 79.80% and 88.10%, respectively). After 48 hours of incubation, CP degradation values of Hungarian vetch and forage pea were significantly higher than the others ( $P < 0.05$ ). The crude protein fractions of feeds for barley, forage peas, Hungarian vetch, and corn exhibited the following percentages of water-soluble fractions: 10.24%, 16.96%,

14.17%, and 7.86%, respectively. The non-degradable fractions demonstrated the following percentages: 8.84%, 5.57%, 6.66%, and 8.04%, respectively. The potentially degradable fractions yielded the following percentages: 80.91%, 77.47%, 79.17%, and 84.10%, respectively. These findings were statistically significant ( $P < 0.05$ ). The degradation rates were determined to be 0.14, 0.09, 0.07, and 0.06, while the lag times were found to be 0.99, 1.77, 4.25, and 5.50, respectively. The bypass rates were established at 27.68%, 37.17%, 31.11%, and 38.54% for barley, forage peas, Hungarian vetch, and corn, respectively ( $P < 0.05$ ).

**Table 7.** CP (%) degradation values of feedstuffs used in the *in-situ* experiment

Incubation times, h	Barley	Corn	FP	HV	P-value
0	10.24 <sup>c</sup> ± 0.26	7.86 <sup>d</sup> ± 0.58	16.96 <sup>a</sup> ± 0.41	14.17 <sup>b</sup> ± 0.49	0.001
2	23.78 <sup>d</sup> ± 0.94	37.22 <sup>b</sup> ± 1.61	29.17 <sup>c</sup> ± 1.45	41.70 <sup>a</sup> ± 1.92	0.001
4	52.49 <sup>a</sup> ± 2.41	43.38 <sup>b</sup> ± 2.52	52.45 <sup>a</sup> ± 1.92	44.80 <sup>b</sup> ± 2.57	0.018
8	65.24 <sup>a</sup> ± 3.65	52.05 <sup>b</sup> ± 2.26	58.80 <sup>ab</sup> ± 3.29	63.26 <sup>a</sup> ± 2.22	0.022
12	79.80 <sup>a</sup> ± 1.58	66.77 <sup>bc</sup> ± 1.29	65.15 <sup>c</sup> ± 1.61	70.19 <sup>b</sup> ± 1.68	0.001
24	88.10 <sup>a</sup> ± 0.19	77.08 <sup>c</sup> ± 0.55	85.65 <sup>a</sup> ± 0.82	80.86 <sup>b</sup> ± 1.43	0.001
48	91.16 <sup>d</sup> ± 0.18	91.96 <sup>c</sup> ± 0.08	94.43 <sup>a</sup> ± 0.39	93.34 <sup>b</sup> ± 0.28	0.001

FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

**Table 8.** CP (%) fractions of the feedstuffs used in the *in-situ* experiment (mean ± SD)

Fractions, %	Barley	Corn	FP	HV	P-value
WSCP	10.24 <sup>c</sup> ± 0.26	7.86 <sup>d</sup> ± 0.58	16.96 <sup>a</sup> ± 0.41	14.17 <sup>b</sup> ± 0.49	0.001
PDCP	80.91 <sup>b</sup> ± 0.30	84.10 <sup>a</sup> ± 0.63	77.47 <sup>d</sup> ± 0.47	79.17 <sup>c</sup> ± 0.67	0.001
NDCP	8.84 <sup>a</sup> ± 0.18	8.04 <sup>b</sup> ± 0.08	5.57 <sup>d</sup> ± 0.39	6.66 <sup>c</sup> ± 0.28	0.001
k <sup>-1</sup> , h	0.14 <sup>a</sup> ± 0.01	0.06 <sup>c</sup> ± 0.01	0.09 <sup>b</sup> ± 0.01	0.07 <sup>bc</sup> ± 0.01	0.001
Lag, h	0.99 <sup>b</sup> ± 0.42	5.50 <sup>a</sup> ± 0.84	1.77 <sup>b</sup> ± 0.48	4.25 <sup>a</sup> ± 0.79	0.001
By-pass	27.68 <sup>d</sup> ± 0.55	38.54 <sup>a</sup> ± 0.80	37.17 <sup>b</sup> ± 0.85	31.11 <sup>c</sup> ± 0.73	0.001

WSCP: Water Soluble Crude Protein, PDCP: Potentially Degradable Crude Protein, NDCP: Non-Degradable Crude Protein, k<sup>-1</sup>: Degradation rate, Lag: Time required for feeds to start degrading in the rumen, FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

*In-situ*, starch degradation values of the feeds used in the study and starch fractions and degradation kinetics values were determined (Table 9 and Table 10). In the study, starch degradation rates were higher only in Hungarian vetch and corn at the 2<sup>nd</sup> hour, while it was higher in barley at 0, 4, 8, 12, 24 and 48 hours. Starch degradation values were statistically significant ( $P < 0.05$ ),

except at 0 and 4 hours ( $P$  values 0.055 and 0.077, respectively). The starch fractions were statistically significant, except for the WS fraction of starch ( $P = 0.055$ ). Degradation rates were 0.18, 0.10, 0.07, 0.05, lag times were 1.37, 0.88, 4.15 and 6.27 for barley, forage peas, Hungarian vetch and corn, respectively ( $P < 0.05$ ).

**Table 9.** Starch degradation values of feedstuffs used in the *in-situ* experiment, starch, % (mean  $\pm$  SD)

Incubation times, h	Barley	Corn	FP	HV	P-value
0	8.50 $\pm$ 0.45	6.87 $\pm$ 0.58	6.87 $\pm$ 0.36	7.38 $\pm$ 0.34	0.055
2	18.31 <sup>b</sup> $\pm$ 0.86	37.80 <sup>a</sup> $\pm$ 1.59	17.91 <sup>b</sup> $\pm$ 1.68	34.63 <sup>a</sup> $\pm$ 2.15	0.001
4	51.34 $\pm$ 2.47	42.90 $\pm$ 2.52	44.72 $\pm$ 2.23	42.78 $\pm$ 2.67	0.077
8	64.14 <sup>a</sup> $\pm$ 3.79	49.07 <sup>c</sup> $\pm$ 2.40	52.02 <sup>bc</sup> $\pm$ 3.83	59.40 <sup>ab</sup> $\pm$ 2.53	0.013
12	83.25 <sup>a</sup> $\pm$ 1.31	65.40 <sup>b</sup> $\pm$ 1.39	62.98 <sup>b</sup> $\pm$ 1.80	67.06 <sup>b</sup> $\pm$ 1.54	0.001
24	97.47 <sup>a</sup> $\pm$ 0.07	76.84 <sup>d</sup> $\pm$ 0.54	89.97 <sup>b</sup> $\pm$ 0.91	81.43 <sup>c</sup> $\pm$ 1.39	0.001
48	98.86 <sup>a</sup> $\pm$ 0.02	96.07 <sup>d</sup> $\pm$ 0.04	98.63 <sup>b</sup> $\pm$ 0.10	97.73 <sup>c</sup> $\pm$ 0.10	0.001

FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

**Table 10.** Starch fractions (%) of the feedstuffs used in the *in-situ* experiment

Fractions, %	Barley	Corn	FP	HV	P-value
WSS	8.50 $\pm$ 0.45	6.87 $\pm$ 0.58	6.87 $\pm$ 0.36	7.38 $\pm$ 0.34	0.055
PDS	90.36 <sup>b</sup> $\pm$ 0.44	89.20 <sup>b</sup> $\pm$ 0.61	91.76 <sup>a</sup> $\pm$ 0.31	90.36 <sup>b</sup> $\pm$ 0.38	0.007
NDS	1.14 <sup>d</sup> $\pm$ 0.02	3.93 <sup>a</sup> $\pm$ 0.04	1.37 <sup>c</sup> $\pm$ 0.10	2.27 <sup>b</sup> $\pm$ 0.09	0.001
k <sup>-1</sup> , h	0.18 <sup>a</sup> $\pm$ 0.01	0.05 <sup>d</sup> $\pm$ 0.01	0.10 <sup>b</sup> $\pm$ 0.01	0.07 <sup>c</sup> $\pm$ 0.01	0.001
Lag, h	1.37 <sup>a</sup> $\pm$ 0.22	6.27 <sup>c</sup> $\pm$ 0.79	0.88 <sup>a</sup> $\pm$ 0.33	4.15 <sup>b</sup> $\pm$ 0.70	0.001

WSS: Water Soluble Starch, PDS: Potentially Degradable Starch, NDS: Non-Degradable Starch, k<sup>-1</sup>: Degradation rate, Lag: Time required for feeds to start degrading in the rumen, FP: Forage pea, HV: Hungarian vetch., <sup>a, b, c</sup>: Different letters on the same line show statistical difference ( $P < 0.05$ )

## DISCUSSION

The DM degradation values obtained in this study were higher than the DM degradation values of barley at 0, 4, 8, 24 and 48 hours (22.09%, 34.15%, 40.19%, 57.42%, 66.37%, respectively) reported by Palangi and Macit (2019). It was stated in the study of Palangi and Macit that the heat treatment applied to barley reduced the degradation due to the change in the structure of carbohydrates. Krieg et al. (2017) reported 92%, 93%, and 90% DM degradation after 48-hour incubation for ryegrass, triticale, and barley, respectively, which were in agreement with the results of the current study. Gonzalez et al. (2003) reported the DM degradation of barley and corn after 48 hours of incubation as 89.9% and 91.6%, respectively. Lei et al. (2017) reported DM degradation for

Soybean meal and corn at 93.14% and 93%, which were higher than the value obtained in the current study. It is hypothesized that these discrepancies may be attributed to disparate treatments administered to the feeds, the rumen environment, the diets provided to livestock, and the species of animals utilized for incubation. Kamalak et al. (2005) reported WSKM fractions at 25.1% and 19.5%, and PDDM at 56.53 and 50.4% for soybean meal and sunflower meal, respectively. Although this difference is due to feed varieties, especially CF percentage of feeds affect this degradation. Batajoo and Shaver (1998) have reported that water-soluble and potentially degradable DM fractions were 19.2% and 89.10% for barley; 14.40% and 96.00% for corn, respectively. The reason for the

particularly high PDDM rates here was that PDDM values were obtained after 72 hours of incubation in the study of Batajoo and Shaver. Consistent with this study, they reported the degradation rates as 0.16 and 0.06 for barley and corn, respectively. Zhao et al. (2016) stated that the water-soluble and potentially degradable DM fractions, the degradation rate, and the lag time of barley were 6.6% and 79%, 0.10, and 0.42, respectively. Palangi and Macit (2019) reported the PDDM fraction, degradation rate, and lag time of barley as 73.62%, 0.06 and 0.73%, respectively. In comparison to the present study, it is hypothesized that the observed low PDDM fraction and degradation rate can be attributed to the heat treatment employed in the study conducted by Palangi and Macit.

OM degradation values ranged from 86.34 to 89.87% after 48 h incubation. OM degradation values for barley and corn were similar to those of Hassan and Karsli (2022). Tóthi (2008) reported OM degradations for barley and corn as 86.9% and 89.7%, respectively, similar to the results of the current study. Canbolat and Bayram (2007) reported that OM degradation rates of soybean, grass pea (*Lathyrus sativus* L.) and chickpea were 91.04%, 81.70% and 79.60%, respectively. It is thought that OM degradation of grass peas and chickpeas is low due to antinutritional factors and ADF-NDF levels. Evcı and Karslı (2018) have noted that OM degradation rates for red lentils, dried beans, green lentils and chickpeas were 45.86%, 83.31%, 75.28% and 85.23%, respectively after 48 h incubation. It is thought that it is different from the results of this study because red lentils, dried beans, green lentils and chickpeas in the study of Evcı and Karslı (2018), were packing plant waste and were high in CF and foreign material.

Palangi and Macit (2019) reported 48-hour CP degradation of barley as 43.21%. This low HP degradation level is thought to be due to the heat treatment applied to barley in that study. On the other hand, Gholizadeh et al. (2021) have reported that CP degradation values were 93.5%, 99.9% and 91.7% for barley, corn and sorghum, respectively. The high values in this study can be attributed to the fact that the incubation period was 72 hours, as

well as the better CP digestion by microorganisms in the rumen in diets with more balanced carbohydrate types (Görgülü et al., 2003).

Although barley had the highest degradation rate and the lowest degradation time among the feeds used in the study, it was noted that barley had the highest non-degradable protein fraction. Batajoo and Shaver (1998) have stated that WSCP and PDCP fraction values were 9.4% and 87.10% for barley, 9.60% and 87.70% for corn, whereas the rate of CP degradations was 0.097 and 0.041 for barley and corn, respectively. Compared with the PDCP fraction of Batajoo and Shaver, the value of this study was lower. It was hypothesized that the discrepancy observed between the Batajoo and Shaver studies could be attributed to several factors, including the 72-hour incubation period, the utilization of 1 mm particle size feeds, and the employment of nylon pouches with 52  $\mu$  pore diameter. Additionally, the differential microbial activity among the animal subjects in this particular study may have been influenced by variations in their dietary intake. Zhao et al. (2016) determined the WSCP and PDCP fractions of barley as 3.0% and 81.60%, degradation rate as 0.08 and lag time as 2.74, respectively. When compared to this study, the low rate of WSCP and high lag time are attributed to the use of rolled barley in the study of Zhao et al. (2016). Görgülü et al. (2003) reported WSCP percentages as 18.6%, 52.9% and 22.3%, PDCP percentages as 74.8%, 46.6% and 77.7%, and degradation rates as 0.08, 0.21 and 0.24, for soybean meal, grass pea and under-sieve lentils, respectively. The CP fractions of grass peas and lentils were lower than those of legume grains used in this study. It is thought that this difference may have been caused by the presence of protease inhibitors in these plants and the fact that lentils were under sieve.

Bypass protein percentages of barley, forage peas, Hungarian vetch and corn used in this study were 27.68%, 37.17%, 31.11% and 38.54%, respectively. Schumacher et al. (2020) reported that the bypass protein percentages of forage peas and corn were 33.6% and 38.1%, respectively. Compared to the current study,

the results for forage peas were low, while the results for corn were similar. Ensminger et al. (1990) reported bypass rates of 27% and 40% for barley and corn, respectively, which were consistent with the results of the current study. Although the CP content of corn is not high, the high bypass protein ratio and slow degradation rate are advantageous in ruminant diets. Considering protein fractions while preparing diets affects the expected yield and performance of ruminant animals (Güney and Karşlı, 2014).

Batajoo and Shaver (1998) reported starch degradation rates of 95.2%, 99%, and 96.7% for barley, corn, and soybean meal, respectively, which were similar to the results obtained in the present study. Similarly, Zhao et al. (2016) also stated the starch degradation rate of barley as 96.3%.

Starch digestion and degradation rate were the slowest in corn and the fastest in barley and feed peas among these feedstuffs. Therefore, corn gave the impression that it could be a safer feed material with less risk of acidosis among the feed sources used in the study. Similar to the results of the current study, Hassan and Karşlı (2022) also reported a very high (over 95%) rumen starch degradation for barley and corn at 48-hour incubation. Barley grain is characterized by a thick fibrous mantle, a high level of  $\beta$ -glucans, and simply arranged starch granules, which is the third most easily degradable cereal after oats and wheat (Iommelli et al., 2022). Krieg et al. (2017) have reported that the WS starch fractions for ryegrass, triticale and barley were 31%, 35% and 24%, respectively, whereas the maximum degradation value was 99% for all three feedstuffs. The water-soluble and potentially degradable fractions of starch were 26.70% and 68.50% for barley, 19.80% and 79.20% for corn, while the rate of starch degradation was 0.266 and 0.057 for barley and corn, respectively (Batajoo and Shaver, 1998). Zhao et al. (2016) also determined the fractions of water-soluble and potentially degradable starch of barley as 29.90% and 63.80%, degradation rate of 0.322 and a lag time of 1.03, respectively. The lower water-soluble fraction in the current study is thought to be due to the

difference in particle size samples and nylon bag washing times. The starch fermentation rate of barley is faster than that of corn. Ruminal acidosis usually occurs by feeding highly fermentable grains, such as barley. Therefore, utilizing the blends of cereal grains in a ruminant diet usually provides some advantages (Iommelli et al., 2022). Cerneau and Michalet-Doreau (1991) reported that pea starch has a degradation potential of 90%. The fact that total starch degradation of forage peas and Hungarian vetch was less than that of barley and corn. This may have resulted from the high amount of protein legume grains contain and the excess protein matrix around this starch to prevent starch degradation (Hall and Mertens, 2017).

It is known that *in-situ* degradation rates are generally affected by many factors such as the particle size of the feeds, the pore size of the nylon bags, the source of the feedstuffs, maturity of feedstuffs (forages), the composition of the diet consumed by the animal, the rumen environment, and the application of the method (Weakley et al., 1983; Nocek, 1985; Cerneau and Michalet-Doreau, 1991). Therefore, some of the differences between the results of the studies may also be due to these reasons.

## CONCLUSION

From this study, it was determined that the degradability of DM, OM, and CP in corn, HV and FP significantly increased after 48 hours of incubation, highlighting their higher degradation rates. CP degradation was higher in HV than in corn. There was no statistical difference between corn and HV in DM and OM degradation. On the other hand, barley exhibited a higher starch degradation rate compared to corn. The relationship between these findings and the risk of acidosis is crucial. Acidosis is a condition characterized by the excessive fermentation of carbohydrates in the rumen, leading to a drop in pH and subsequent health issues in ruminants. The study found that the degradation rates of corn and horse beans (HV) were significantly slower than those of barley and faba beans. This slower degradation rate is beneficial in high-starch diets because it results in a more gradual

release of fermentable carbohydrates, reducing the rapid production of volatile fatty acids that contribute to acidosis. Therefore, incorporating corn and horse beans at higher rates in high-starch rations can help mitigate the risk of acidosis by ensuring a slower and more controlled fermentation process. This strategic inclusion helps maintain a stable rumen environment, thereby promoting better health and performance in ruminants.

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