TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2023) 47: 255-269
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doi:10.55730/1300-0128.4293

The effect of *Saccharomyces cerevisiae* as a probiotic on the nutrient degradability of some commonly feedstuffs used in Turkey

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Received: 13.02.2023 • **Accepted/Published Online:** 01.06.2023 • **Final Version:** 13.06.2023

Abstract: The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* as a probiotic on in situ ruminal DM, OM, CP and starch degradability values in some energy and protein sources commonly used in ruminant nutrition. In this study, the in situ degradation kinetics and fractions of dry matter, organic matter, crude protein, and starch of 6 different feedstuffs (barley, wheat, corn, sunflower seed meal, cottonseed meal and soybean meal) commonly used in animal nutrition were determined. Three ruminally cannulated Bafra sheep were used in the experiment. The experiment was designed as carryover experimental design with two periods. Each period of the experiment, samples were incubated for 0, 2, 4, 6, 8, 12, 24, and 48 h in the rumen of 3 sheep in duplicate. Degradation kinetics and fractions of DM, OM, CP, and starch were calculated. During the entire incubation period, the DM, OM, CP, and starch degradabilities and fractions of the feedstuffs significantly differed ($p < 0.01$), and the feed probiotic interaction was observed for all incubation hours for the ruminal DM, OM, CP, and starch degradabilities ($p < 0.04$). While the DM, OM, and CP degradabilities, except 48-h incubation, were affected by probiotic supplementation of diet, starch degradabilities were affected by probiotic supplementation of diet at all incubation hours. While the potentially degradable starch fractions of the feedstuffs generally increased due to the use of probiotics, it was observed that the nondegradable fractions significantly decreased ($p < 0.01$). It can be concluded that *Saccharomyces cerevisiae* significantly increased the ruminal DM, OM, CP degradations within the 12-h incubation period of all feeds, except corn. It was observed that the addition of *Saccharomyces cerevisiae* into diet increased the starch degradation values in the starch-rich cereal grains and soybean meal and affected starch fractions, especially by decreasing the water-soluble and nondegradable starch fractions and increasing the potentially degradable starch fraction.

Keywords: Sheep, in situ degradation, *Saccharomyces cerevisiae*, starch

1. Introduction

Ruminant animals have a different digestive mechanism compared with monogastric animals such as pigs and poultry, in which feedstuffs are fermented to create energy precursors for the animal's use. Livestock owners can be able to care for and feed ruminant animals if they understand how the digestive system of the animal operates [1]. Knowing the digestive system and understanding the metabolism of nutrients in ruminant animals are of vital importance in terms of proper feeding of the animals. In addition, the ability to manipulate the metabolism in favor of animals also depends on a good knowledge of the system. Therefore, in order to feed these animals precisely and economically, it is necessary to know the structure of the digestive system and how it works.

The gastrointestinal tract (GIT) is home to a wide range of microbial diversity that aids in the generation of various responses in animals' nutritional health, physiology, and productivity. The gut microbiota also regulates food safety in the GIT by shedding pathogens, interacting with organisms, and competing for resources. It also inhibits the colonization of harmful and pathogenic microorganisms in the gut. Various ways have been explored to improve the microbiota of the GIT, which has an impact on animal production potential and growth efficiency [2].

Feed additives are substances that have been widely used in animal diets. Knowing their mode of action and manipulating the rumen metabolism with them for the benefit of animals is extremely important in terms of profitability and healthy products in animal husbandry. As a matter of fact, many studies are carried out in this context.

The use of prebiotics to modify the microbial of the gastrointestinal tract has become a popular and cost-effective way to improve animal health and productivity of animals. A prebiotic has benefited the host by increasing the microbial flora of its intestine. Several microorganisms have been

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approved as probiotics for using in ruminant diets to improve nutrient utilization and animal performance. In young calves, hens, and pigs, bacterial probiotics are more effective, whereas yeast/fungal probiotics are effective for adult ruminants [3,4].

Saccharomyces cerevisiae has been used as a preventer supplement for diarrhea and other digestive system issues in livestock for decades. They also provide cost-effective production gains, reduced digestive difficulties, and improved animal health. Dietary yeast culture supplementation improves feed intake, which improves ruminant development and productivity [5].

Probiotics and prebiotics have the potential to regulate the balance and activity of the gastrointestinal (GI) microbiota, making them advantageous to the host animal and useful as functional foods. The structure and activity of gut microbial communities in livestock animals have been found to be significantly influenced by a variety of factors, including dietary and management limitations [5].

Thus, the objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* as probiotics on in situ ruminal dry matter (DM), organic matter (OM), crude protein (CP), and starch degradability values in some energy (corn, barley, and wheat) and protein (cottonseed meal, sunflower meal, and soybean meal) sources commonly used in ruminant nutrition.

2. Materials and method

2.1. Materials

The feedstuffs namely barley, wheat, corn, sunflower seed meal (SFM), cottonseed meal (CSM) and soybean meal (SBM) were obtained from the feed milling factories in the region and were utilized as feed materials in the experiment. Feed materials were obtained from various feed milling factories in the Kırıkkale region. A total of 6 feedstuffs, 3 energy and 3 protein sources, were used. These feedstuffs were ground in a feed mill with sieves of 2 mm diameter. After grinding, they were placed in nylon bags (Dacron bag) with internal dimensions of 5 × 20 cm and 40–50 µ pore size for in situ experiments. Dacron bags were bought from a private chemical company. *Saccharomyces cerevisiae* (CFU/kg > 1.0×10^{10}) used in the experiment were bought from a private company.

Three Bafra sheep aged approximately 3-year-old were used in the experiment. Sheep were brought from Kırıkkale University Veterinary Faculty farm. Sheep were rested for three days after they were brought to the faculty. Afterwards, a rumen cannula was inserted in the clinics of the surgery department, and the necessary medical treatments were carried out by hospitalizing the animals in the hospitalization units until sheep were completely recovered from surgery (approximately 15 days). After the sheep recovered, the experiment was started.

During the experiment, oat and green meadow grass were used as roughage for the animals, and mixtures of sunflower meal and barley containing at least 12% HP and 2750 kcal/kg metabolic energy (ME) as concentrated feed was used. Both forage and concentrated feed were bought from the local market.

2.2. Method

Before starting the in situ trials, the animals were given an injection of Cydoctyn for internal and external parasites and Rabenzole in tablet form orally. Each animal was individually housed in 2 × 2 m cages. Each animal was fed twice a day at 08.00 and 20:00 with forage and concentrated feed. At the same time, vitamin-mineral blocks (per 3 kg of vitamin and mineral blocks contained; vitamin A 1,500,000 IU, vitamin D3 300,000 IU, vitamin E 450 mg, niacin 9.000 mg, phosphorus 12.000 mg, calcium 18.750 mg, iron 15.000 mg, zinc 6.000 mg, manganese 1.500 mg, copper 1.500 mg contains magnesium 36.000 mg, iodine 300 mg, cobalt 300 mg) were placed in feed bunkers and clean water was always available for animals throughout the experiment.

The experiment was designed as carryover experimental design with two periods. In the first period of experiment, all three sheep were fed with forage and concentrated feed without yeast (*Saccharomyces cerevisiae*) as a probiotic. In the second period, in addition to feed used in the first period, 1 g yeast (*Saccharomyces cerevisiae*) was daily put into the rumen of each sheep in the morning feeding. Each period of the experiment was consisted of 30 days, 10 days of adaptation periods and 20 days incubation of samples (sampling periods). After the feedstuffs were ground to pass 2 mm screen, samples were put into Dacron bags (approximately 3.5 g per bag) and then incubated for 0, 2, 4, 6, 8, 12, 24, and 48 h in the rumen of 3 sheep for each period [6]. After each incubation, the bags were removed from the rumen and washed under running tap water until the water was clear (about 15 min). Then the bags with residue were dried in an oven at 65 °C for 24 h [7]. The dried nylon bags were kept in the desiccator for a short time (about 45 min), then weighed and their weights were recorded to calculate dry matter degradability.

2.3. Chemical analysis

Feed samples used to determine chemical composition were first ground through a 1-mm screen. These samples and residues remained in the bags after given incubation times were analyzed for dry matter (DM) ash, crude protein (CP), according to AOAC [8], and starch [9]. Feed samples were analyzed for neutral detergent fiber (NDF) [10], and acid detergent fiber (ADF) [11]. Organic matter (OM) content of samples was then calculated.

2.4. Calculations

Nutrient degradability values of feedstuffs were calculated as follows:Nutrient degradability = $a + b\ (1 - e^{-ct})$ [12].

Microbial destruction as a result of incubation for different times in the rumen, followed by nutrient degradation during washing are divided into three fractions:

1. Water-soluble (WS) fraction, remaining nutrient content in the bag after 0 (zero) h incubation (wash loss);2. Potentially degradable fraction (PD) was expressed as 100 – (nondegradable fraction + water-soluble fraction);3. The nondegradable fraction (ND) was expressed as the nutrient fraction that remained undegraded in the bag after 48 h of incubation [9].

2.5. Statistical analysis

Data collected in the experiment were subjected to analysis of variance analysis using Statistical Package for the Social Sciences (SPSS 20.0 evaluation version for Windows, Trial Version). Effects of feed and probiotic were determined. Feed*probiotic interaction was also evaluated. The following statistical model has been applied:

$Yij = \mu + Ai + Bj + Ck + Dl + eij$,

where: Yij = observation on group i and period j,

μ = the overall mean,

Ai = the effect of group i,

Bj = the effect of period j,eij = random error.

3. Results

The nutrient contents of the feedstuffs used in the study are presented in Table 1. It is seen that the dry matter content of all feeds is above the minimum 85% DM level, which is required for the feeds to be stored safely. It is noted that the OM contents of the feedstuffs vary between 90.40% and 98.33%, and the OM levels of cereal grains are higher than the meal. As it is known, low OM levels are an expected situation in meals, since the mineral levels, especially phosphorus contents, of meal are higher than those of cereal grains. When the protein content of the feedstuffs is examined, it is seen that the CP content of the meal is quite high compared to grain feedstuffs as expected and CP contents of meal ranged from 19.57% to 44.54%, while it was in the range of 7.04–14.5% in cereal grains. It was observed that both NDF and ADF contents of the meals were higher than cereal grains, and the NDF and ADF contents of especially SFM and CSM from the meals and barley from the grains were significantly higher compared with other feedstuffs used in the experiment. When the starch contents of feedstuffs are examined, it is noted that cereal grains contain significantly more starch compared with meals. The starch contents of cereal grains were 41.87%, 49.59% and 78.04% for barley, wheat and maize, respectively, it was 6.61%, 7.16% and 18.18% for SFM, CSM and SBM, respectively.

The ruminal DM and OM degradabilities of the feedstuffs used in the experiment are given in Tables 2 and 3. During the entire incubation period, the DM and OM degradabilities of the feedstuffs significantly differed ($p < 0.01$), and the feed*probiotic interaction was observed for all incubation hours ($p < 0.04$). Although the use of probiotics significantly affected DM degradation during the first 12-h incubation period ($p < 0.01$), it was noted that the effect of probiotic on DM and OM degradation was not significant for 48 h incubation ($p > 0.05$). It was noted that the use of probiotics significantly affected all three fractions ($p < 0.01$), except the 48th hour, the feed*probiotic interaction was also observed in all 3 fractions for all incubation hours for both DM and OM fractions ($p < 0.05$; Tables 4 and 5).

It was seen that the ruminal CP degradabilities of the feedstuffs used in the experiment were significantly different at all incubation hours ($p < 0.01$; Table 6). It was determined that the in situ CP degradabilities of the feedstuffs were significantly affected by the use of probiotics, except at the 2- and 48-h incubations ($p < 0.04$). It was noted that the use of probiotics resulted in a decrease in CP degradation in some of the feedstuffs and an increase in others, in other words, there were the feed*probiotic interaction ($p < 0.01$).

Table 1. Nutrient contents of the feed materials used in the experiment, %DM.

	Barley	Wheat	Corn	Sunflower meal	Cottonseed meal	Soybean meal
DM	93.48	91.81	88.77	92.92	92.91	88.52
Ash	2.13	1.67	4.31	9.60	9.23	6.12
OM	97.87	98.33	95.69	90.40	90.77	93.88
CP	12.14	14.75	7.04	35.25	19.57	44.54
NDF	20.14	11.38	12.79	33.65	46.08	11.13
ADF	4.84	2.46	2.99	21.32	34.32	4.87
Starch	41.87	49.59	78.04	6.61	7.16	18.18

DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 2. Dry matter degradabilities of the feed materials used in the experiment, %DM.

Degradation hours	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro–	Pro +	F	P	F*P
0th h	53.69 ± 0.31	32.98 ± 1.55	30.73 ± 0.16	25.22 ± 0.79	29.83 ± 1.16	26.93 ± 0.92	14.66 ± 0.66	28.17 ± 1.16	25.64 ± 1.39	21.79 ± 1.03	31.79 ± 3.21	34.21 ± 0.81	0.01	0.01	0.01
2nd h	54.59 ± 0.19	52.63 ± 2.25	52.86 ± 1.10	60.99 ± 2.66	36.76 ± 1.63	40.96 ± 1.61	27.30 ± 1.59	40.49 ± 0.68	31.79 ± 2.55	34.50 ± 1.52	38.99 ± 2.80	42.17 ± 0.46	0.01	0.01	0.01
4th h	56.66 ± 0.56	70.61 ± 4.65	55.09 ± 0.61	72.69 ± 2.15	42.14 ± 2.24	53.89 ± 1.62	45.17 ± 0.58	43.92 ± 0.90	34.7 ± 1.20	36.41 ± 1.94	43.26 ± 1.14	44.14 ± 1.37	0.01	0.01	0.01
8th h	58.66 ± 0.76	76.62 ± 2.55	66.93 ± 2.77	80.32 ± 2.13	59.76 ± 3.28	56.60 ± 2.08	49.02 ± 0.92	52.94 ± 1.46	36.06 ± 3.09	39.70 ± 1.48	45.74 ± 2.58	49.11 ± 1.10	0.01	0.01	0.01
12th h	64.02 ± 3.52	80.16 ± 2.43	86.63 ± 0.92	89.94 ± 0.86	69.4 ± 1.51	63.99 ± 1.43	52.12 ± 1.55	57.88 ± 2.27	42.00 ± 1.11	46.53 ± 0.37	53.45 ± 2.54	58.64 ± 4.01	0.01	0.01	0.01
24th h	79.18 ± 5.22	85.70 ± 2.17	88.86 ± 0.66	89.75 ± 2.02	82.72 ± 1.83	70.70 ± 2.72	61.18 ± 0.38	69.91 ± 2.97	44.30 ± 1.39	50.82 ± 1.75	65.57 ± 2.30	71.26 ± 2.79	0.01	0.07	0.01
48th h	84.17 ± 3.11	90.20 ± 0.45	92.04 ± 0.54	94.01 ± 0.43	89.91 ± 0.76	88.82 ± 0.33	74.71 ± 2.01	77.92 ± 0.32	57.51 ± 2.39	54.78 ± 1.13	95.51 ± 1.13	93.18 ± 2.29	0.01	0.34	0.04

F = effects of feedstuffs, P = effects of probiotic, F*P= feedstuffs*probiotic interaction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 3. Organic matter degradabilities of the feed materials used in the experiment, %OM.

Degradation hours	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	F	P	F*P
0th h	53.41 ± 0.31	32.88 ± 1.55	30.67 ± 0.16	25.05 ± 0.79	29.23 ± 1.16	25.48 ± 0.93	13.16 ± 0.67	27.16 ± 1.18	23.70 ± 1.43	21.32 ± 1.04	30.55 ± 3.27	32.61 ± 0.83	0.01	0.01	0.01
2nd h	54.23 ± 0.20	52.86 ± 3.23	52.71 ± 1.10	61.32 ± 2.63	35.39 ± 1.66	41.29 ± 1.59	24.97 ± 1.65	39.43 ± 0.69	28.75 ± 2.66	32.00 ± 1.58	36.11 ± 2.93	39.74 ± 0.48	0.01	0.01	0.01
4th h	56.63 ± 0.56	70.54 ± 4.46	54.96 ± 0.61	72.60 ± 2.16	40.83 ± 2.30	53.28 ± 1.64	44.45 ± 0.56	43.00 ± 0.92	33.11 ± 1.23	34.19 ± 1.23	40.46 ± 1.19	41.60 ± 1.44	0.01	0.01	0.01
8th h	58.91 ± 0.76	76.75 ± 2.54	66.74 ± 2.78	80.32 ± 2.13	59.9 ± 3.31	55.97 ± 2.11	44.29 ± 0.96	50.93 ± 1.53	34.43 ± 3.25	36.47 ± 1.56	43.16 ± 2.71	46.77 ± 1.15	0.01	0.01	0.01
12th h	64.01 ± 3.52	80.42 ± 2.40	86.69 ± 0.92	89.93 ± 0.86	69.80 ± 1.49	63.52 ± 1.46	50.01 ± 1.61	55.82 ± 2.38	38.43 ± 1.48	43.05 ± 0.39	51.35 ± 2.66	56.67 ± 4.21	0.01	0.01	0.01
24th h	79.33 ± 5.19	86.07 ± 2.11	88.88 ± 0.65	89.83 ± 2.01	82.64 ± 1.84	69.85 ± 2.80	58.61 ± 0.41	68.37 ± 3.12	40.60 ± 1.39	48.48 ± 1.83	63.81 ± 2.42	70.16 ± 2.90	0.01	0.04	0.01
48th h	84.70 ± 3.00	90.60 ± 0.43	92.21 ± 0.53	94.09 ± 0.43	90.69 ± 0.69	88.46 ± 0.34	73.04 ± 2.14	76.76 ± 0.33	55.15 ± 2.53	52.61 ± 1.19	95.33 ± 1.17	93.14 ± 2.31	0.01	0.41	0.03

F = effects of feedstuffs, P = effects of probiotic, F*P = feedstuffs*probiotic interaction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 4. Dry matter fractions of the feed materials used in the experiment, %DM.

	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
Fractions	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	F	P	F*P
WS	53.69 ± 0.31	32.98 ± 1.55	30.73 ± 0.16	25.22 ± 0.79	29.83 ± 1.16	26.93 ± 0.92	14.66 ± 0.66	28.17 ± 1.16	25.64 ± 1.39	21.79 ± 1.03	31.79 ± 3.21	34.21 ± 0.81	0.01	0.01	0.01
PD	30.47 ± 2.89	57.22 ± 1.33	61.31 ± 0.66	68.79 ± 0.99	60.07 ± 1.48	61.89 ± 0.89	60.05 ± 2.16	49.75 ± 1.00	31.87 ± 1.95	32.99 ± 1.59	63.76 ± 3.20	58.99 ± 2.08	0.01	0.01	0.01
ND	15.83 ± 3.11	9.79 ± 0.45	7.96 ± 0.54	5.99 ± 0.43	10.08 ± 0.76	11.17 ± 0.33	25.29 ± 2.01	22.08 ± 0.32	42.48 ± 2.39	45.22 ± 1.13	4.49 ± 1.13	6.80 ± 2.89	0.01	0.34	0.04

WS = water-soluble fraction, PD = potentially degradable fraction, ND = nondegradable fraction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 5. Organic matter fractions of the feed materials used in the experiment, %OM.

	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
Fractions	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro -	Pro +	Pro –	Pro +	F	P	F*P
WS	53.41 ± 0.31	32.88 ± 1.55	30.67 ± 0.16	25.05 ± 0.79	29.23 ± 1.16	25.48 ± 0.93	13.16 ± 0.67	27.16 ± 1.18	23.70 ± 1.43	21.32 ± 1.04	30.55 ± 3.27	32.61 ± 0.83	0.01	0.01	0.01
PD	31.29 ± 2.78	57.73 ± 1.34	61.54 ± 0.64	69.05 ± 1.00	61.46 ± 1.45	62.98 ± 0.91	59.87 ± 2.29	49.60 ± 1.01	31.44 ± 2.05	31.29 ± 1.64	64.78 ± 3.26	60.52 ± 2.10	0.01	0.01	0.01
ND	15.29 ± 3.00	9.39 ± 0.43	7.78 ± 0.53	5.90 ± 0.43	9.30 ± 0.70	11.54 ± 0.34	26.96 ± 2.14	23.24 ± 0.33	44.85 ± 2.53	47.39 ± 1.19	4.66 ± 1.18	6.86 ± 2.31	0.01	0.41	0.03

WS = water-soluble fraction, PD = potentially degradable fraction, ND = nondegradable fraction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 6. Crude protein degradabilities of the feed materials used in the experiment, %CP.

Degradation hours	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	F	P	F*P
0th h	53.39 ± 0.31	29.71 ± 1.62	32.87 ± 0.16	28.60 ± 0.76	40.71 ± 0.97	31.85 ± 0.85	16.67 ± 0.67	28.92 ± 1.15	28.75 ± 1.34	20.04 ± .05	23.02 ± 3.63	29.18 ± 0.87	0.01	0.01	0.01
2nd h	52.03 ± 0.21	38.76 ± 4.20	33.48 ± 1.55	38.46 ± 4.20	39.07 ± 1.57	30.47 ± 1.89	26.71 ± 1.61	41.20 ± 0.67	32.68 ± 2.44	38.33 ± 1.43	25.83 ± 3.41	32.87 ± 0.53	0.01	0.31	0.01
4th h	55.87 ± 0.64	60.83 ± 6.20	50.22 ± 0.68	62.98 ± 2.91	44.94 ± 2.14	48.49 ± 1.81	49.77 ± 0.50	49.13 ± 0.82	34.38 ± 1.24	39.92 ± 1.13	34.85 ± 1.31	36.72 ± 1.56	0.01	0.01	0.01
8th h	56.35 ± 0.35	70.00 ± 3.27	68.69 ± 2.62	77.47 ± 2.44	64.55 ± 2.73	49.56 ± 2.42	53.43 ± 0.84	58.69 ± 1.28	37.52 ± 3.02	44.35 ± 1.61	35.99 ± 3.15	38.54 ± 1.33	0.01	0.04	0.01
12th h	63.21 ± 3.00	79.31 ± 2.53	87.29 ± 0.88	91.23 ± 0.75	66.03 ± 1.77	58.66 ± 1.65	55.31 ± 1.44	68.46 ± 1.80	40.57 ± 1.13	50.42 ± 0.34	41.20 ± 3.21	50.90 ± 4.76	0.01	0.01	0.01
24th h	81.60 ± 4.63	88.63 ± 1.73	90.31 ± 0.57	90.93 ± 1.79	80.49 ± 2.07	68.52 ± 3.84	69.66 ± 0.30	83.87 ± 1.59	47.45 ± 1.31	54.77 ± 1.61	56.51 ± 2.90	68.16 ± 3.09	0.01	0.04	0.01
48th h	85.48 ± 2.85	93.88 ± 0.28	94.52 ± 0.37	96.57 ± 0.26	88.73 ± 0.84	84.17 ± 0.46	90.63 ± 0.75	94.80 ± 0.18	67.99 ± 1.81	58.24 ± 1.05	95.04 ± 1.25	94.19 ± 1.96	0.01	0.91	0.01

F = effects of feedstuffs, P = effects of probiotic, F*P = feedstuffs*probiotic interaction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

The CP fractions of the feedstuffs used in the experiment are presented in Table 7. There were also statistically significant differences between the three CP fractions of the feedstuffs ($p < 0.01$). The WS fractions of the feedstuffs were between 16.67% and 53.39% ($p < 0.01$). While PD fractions ranged from 32.09% to 73.96%, ND fractions ranged from 4.96% to 41.75% ($p < 0.01$). It was determined that the use of probiotics significantly affected all three fractions ($p < 0.01$), and this effect differed between the feedstuffs. While the use of probiotics increased the PD fraction in cereal grains, it decreased in the meal, the feed*probiotic interaction was observed in all 3 fractions for all incubation hours except the 48th hour ($p < 0.01$).

It was observed that there were statistically significant differences between the in situ starch degradation values of the feedstuffs used at all incubation hours (Table 8; $p < 0.01$). Similarly, it was noted that the use of probiotics had a significant effect on starch degradation at all incubation hours ($p < 0.01$). It was determined that the effect of probiotics was different between the feeds, it means, there was a feed*probiotic interaction for all incubation hours ($p < 0.01$).

Starch fractions of the feedstuffs used in the experiment are shown in Table 9. Based on Table 9, all of three starch fractions were significantly different between the feedstuffs ($p < 0.01$) and were significantly affected by the addition of probiotics to the ration ($p < 0.01$). While the PD starch fractions of the feedstuffs generally increased due to the use of probiotics, it was observed that the ND fractions significantly decreased ($p < 001$). ND fractions were 4.67%, 1.21%, 2.77%, 0.73%, 4.55%, 1.96%, 16.99%, 3.72%, 17.64%, 6.51%, 1.20%, and 0.37% for barley, wheat, corn, sunflower meal, cottonseed meal and soybean meal with and without probiotic, respectively.

4. Discussion

The DM degradation values of the feeds during the entire incubation period were significantly different, but the use of probiotics just significantly affected DM degradation during the first 12-h incubation period it seemed that the use of probiotic increased the rate of degradation but not the extent of DM degradation. The results of the study were found to be higher than the DM degradations at 0, 4, 8, 24, and 48 h (22.09%, 34.15%, 40.19%, 57.42%, 66.37%, respectively) of Palangi and Macit [13] for the barley that was heat-treated. Heat treatment reduced DM degradation. The DM degradability values reported by González et al. [14] were higher than the value obtained in the present study. Lei et al. [15] reported DM degradations for SBM and maize as 93.14% and 93%. It is thought that the differences may be caused by treatments such as the heat treatment applied to the feeds, the rumen environment, and the animal breed (cow-goat) used in the studies.

The DM fractions of the feeds were significantly different (except for the nondegradable fraction with probiotics) and feed*probiotic interaction was observed in all of these fractions Kamalak et al. [16] reported the rates of WSDM as 19.5% and 25.1%, and PDDM as 50.4% and 56.53% for SFM and SBM, respectively, which were lower compared to the results of the current study. Although this difference is due to the feed varieties, especially the crude fiber level of SFM affects this degradation. In the study of Batajoo and Shaver [17], PDDM values were 89.10% for barley and 96.00% for corn. These differences may have resulted from the fact that the data were obtained after a 72-h incubation period in Batajoo and Shaver's study [17].

Evci [18] reported 48-h OM degradation values for barley, fodder peas, Hungarian vetch and corn as 86.34%, 89.87%, 88.36% and 88.49%, respectively. The SBM in the study was found to be lower than the 48-h incubation results of corn and barley (95.33%, 90.69% and 84.70%, respectively). The reason for this decrease was attributed to the use of animals with acidosis in the study of Evci [18]. Canbolat and Bayram [19] reported the OM degradation rates of soybean, plum, and chickpea as 91.04%, 81.70%, and 79.60%, respectively. SFM, CSM, and SBM in this study were found to be high, excluding SBM, according to the 48-h incubation results (73.04%, 55.15%, 95.33%, respectively). Gao et al. [20] reported OM degradation as 64.6% after 64 h of incubation for cotton seed meal, which was lower than the results of this study. It is thought that the OM degradation of the meal is low due to antinutritional factors and ADF-NDF levels. Similar to the results of the study, Tóthi et al. [21] reported OM degradations for barley and maize as 86.9% and 89.7%, respectively.

Gao et al. [20] reported the rates of WSOM as 27.7% and 26.7% for SFM and CSM, respectively, and 48.7% and 59.2% for PDOM. According to Gao's report, SFM and CSM WSOM values in the study (13.16% and 23.70%, respectively) were found to be low, SFM PDOM values (59.87%) high, and CSM PDOM values (31.44%) low. The reason for these low results was associated with the high ADF-NDF content of the meals.

The CP degradation values after 48 h ruminal incubations were 90.63%, 67.99%, and 95.05% for SFM, CSM and SBM without probiotics, respectively. The CP degradation values observed for soybean meal in this study were similar to those of Weakley et al. [22], Deniz and Tuncer [23], but higher than the values reported by Deniz et al. [6], Gençoğlu et al. [24]. The 48-h CP degradation values of CSM and SFM were similar to that of Deniz et al. [6], but lower than the values reported in the literature for CSM [23]. It has been stated that these differences may have resulted from both the extraction method and the cellulose content of the meals [6]. On the other hand,

Table 7. Crude protein fractions of the feed materials used in the experiment, %CP.

	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
Fractions	Pro -	Pro +	Pro -	Pro +	Pro -	Pro +	Pro -	Pro +	Pro -	Pro +	Pro -	Pro +	F	P	F*P
WS	53.39 ± 0.31	29.71 ± 1.62	32.87 ± 0.16	28.60 ± 0.76	40.71 ± 0.97	31.85 ± 0.85	16.67 ± 0.67	28.92 ± 1.15	28.75 ± 1.34	20.04 ± .05	23.02 ± 3.63	29.18 ± 0.87	0.01	0.01	0.01
PD	32.09 ± 2.63	64.17 ± 1.47	61.65 ± 0.49	67.98 ± 0.86	48.02 ± 1.39	52.33 ± 0.87	73.96 ± 1.02	65.87 ± 1.11	39.23 ± 1.50	38.0 ± 1.55	72.01 ± 3.61	65.01 ± 1.78	0.01	0.01	0.01
ND	14.51 ± 2.85	6.12 ± 0.28	5.48 ± 0.37	3.42 ± 0.25	11.27 ± 0.84	15.82 ± 0.46	9.36 ± 0.75	5.20 ± 0.17	32.01 ± 1.80	41.75 ± 1.05	4.96 ± 1.25	5.81 ± 1.96	0.01	0.91	0.01

WS = water-soluble fraction, PD = potentially degradable fraction, ND = nondegradable fraction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 8. Starch degradabilities of the feed materials used in the experiment, %Starch.

Degradation hours	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	F	P	F*P
0th h	64.69 ± 0.23	37.35 ± 1.45	57.00 ± 0.10	31.34 ± 0.73	34.05 ± 1.09	28.59 ± 0.89	34.07 ± 0.66	29.60 ± 0.98	68.06 ± 0.60	21.76 ± 1.03	34.53 ± 3.09	39.99 ± 0.74	0.01	0.01	0.01
2nd h	77.65 ± 0.10	56.88 ± 2.96	71.15 ± 0.67	71.35 ± 1.96	42.93 ± 1.47	47.46 ± 1.43	51.16 ± 1.07	30.03 ± 0.80	80.44 ± 0.73	45.46 ± 1.02	53.99 ± 1.65	59.69 ± 0.32	0.01	0.01	0.01
4th h	79.66 ± 0.26	76.89 ± 3.66	76.18 ± 0.32	82.73 ± 1.36	53.38 ± 1.80	72.84 ± 0.95	57.16 ± 0.37	35.05 ± 1.06	81.29 ± 0.34	46.28 ± 1.27	63.43 ± 0.79	58.03 ± 1.03	0.01	0.01	0.01
8th h	80.81 ± 0.34	94.26 ± .0.63	85.52 ± 1.21	89.56 ± 1.13	68.73 ± 2.55	77.16 ± 1.09	63.21 ± 0.77	52.61 ± 1.47	81.66 ± 0.89	48.51 ± 0.37	64.81 ± 1.67	91.80 ± 0.18	0.01	0.01	0.01
12th h	84.07 ± 1.56	95.61 ± 0.53	92.36 ± 0.53	95.90 ± 0.35	80.84 ± 1.77	82.85 ± 0.68	75.6 ± 0.78	71.72 ± 1.52	80.14 ± 1.63	53.17 ± 1.09	72.53 ± 3.50	95.55 ± 0.42	0.01	0.01	0.01
24th h	92.87 ± 1.79	97.18 ± 0.43	93.64 ± 0.37	98.12 ± 0.37	91.77 ± 0.87	89.72 ± 0.95	80.91 ± 0.38	89.88 ± 1.00	80.62 ± 1.77	71.86 ± 100	94.45 ± 0.37	96.91 ± 0.30	0.01	0.01	0.01
48th h	95.33 ± 0.92	98.79 ± 0.12	97.23 ± 0.19	99.27 ± 0.06	95.45 ± 0.34	98.04 ± 0.06	83.01 ± 1.35	96.28 ± 0.05	82.35 ± 0.76	93.49 ± 0.19	98.79 ± 0.30	99.63 ± 0.12	0.01	0.01	0.01

F = Effects of feedstuffs, P = Effects of probiotic, F*P = Feedstuffs*probiotic interaction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

Table 9. Starch fractions of the feed materials used in the experiment, %Starch.

Fractions	Barley		Wheat		Corn		Sunflower meal		Cottonseed meal		Soybean meal		P values		
	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	Pro –	Pro +	F	P	F*P
WS	64.69 ± 0.23	37.35 ± 1.45	57.00 ± 0.10	31.34 ± 0.73	34.05 ± 1.09	28.59 ± 0.89	34.07 ± 0.66	29.60 ± 0.98	68.06 ± 0.60	21.76 ± 1.03	34.53 ± 3.09	39.99 ± 0.74	0.01	0.01	0.01
PD	30.64 ± 0.76	61.44 ± 1.41	39.62 ± 0.26	67.93 ± 0.86	61.39 ± 1.19	69.44 ± 0.88	48.93 ± 1.55	56.67 ± 0.95	14.29 ± 1.13	71.73 ± 1.06	64.26 ± 3.04	59.64 ± 0.70	0.01	0.01	0.01
ND	4.67 ± 0.92	1.21 ± 0.05	2.77 ± 0.19	0.73 ± 0.05	4.55 ± 0.34	1.96 ± 0.06	16.99 ± 1.35	3.72 ± 0.05	17.64 ± 0.76	6.51 ± 0.16	1.20 ± 0.08	0.37 ± 0.12	0.01	0.01	0.01

WS = water-soluble fraction, PD = potentially degradable fraction, ND = nondegradable fraction, Pro – = sheep are not receiving yeast (*Saccharomyces cerevisiae*) as a probiotic, Pro + = sheep are receiving yeast (*Saccharomyces cerevisiae*) as a probiotic.

in grains, the highest CP degradation was observed in wheat, while the CP degradation values of corn and barley were close to each other after 48 h of incubation. The CP degradation values of corn and wheat obtained in this study were higher than the values reported by Gençoğlu et al. [24]. The 48-h degradation order of corn, barley and wheat appears to be in agreement with Herrera Saldana, Huber, and Swingle [25].

When the CP fractions of the samples were examined, the washing loss of CP from sunflower meal was lower than that of CSM and SBM, while the percentages of nondegradable CP in the rumen after 48 h of CSM was quite high compared to SFM and SBM. The percentages of potentially degradable CP in the rumen were lower in cottonseed meal than in sunflower meal and soybean meal. On the other hand, in cereal grains, while water-soluble and nondegradable CP fractions of barley and corn were higher, and potentially degradable CP ratios were lower than that of wheat. It was seen that the percentages of water-soluble CP found in this study for corn and wheat were higher, whereas the percentages of water-soluble CP for cottonseed meal and sunflower meal were lower than the values reported in NRC [26]. A higher water-soluble fractions obtained in this study may have resulted from the washing time and method. Similar to the results of Deniz et al. [6], cotton seed meal had the lowest potentially degradable CP and the highest nondegradable CP fractions. After 48 h of incubation, nondegradable CP fractions of the meals were similar to the values reported in the literature [23]. Nondegradable CP fractions of cereal grains, after 48 h of incubation seemed to be in agreement with the values reported by Herrera-Saldana et al. [25]. The addition of probiotics to the diet caused a significant increase in PD fraction, while decreasing WS and ND fractions in cereal grains. In the meal, probiotics caused an increase in WS fraction and a decrease in PD fraction of sunflower meal and soybean meal. In the literature, there are studies reporting that the addition of probiotics to the diet increases CP digestion [27]. Similar to the current study, İnal et al. [28] reported that while live yeast culture supplementation increased the soluble fraction (a) of barley, soluble fraction of dried distilled grain soluble (DDGS) decreased with live yeast culture supplementation. This confirms the feed*probiotic interaction seen in the current study.

In particular, starch-rich cereals had very high starch degradation values of 95.33%–97.23% after 48 h of incubation. The 48-h starch degradation values of the meals were also high in the range of 82.35%–98.79%. There are several in vitro [25], in situ [24, 29] and in vivo studies [30], which indicates that starch digestibility of different grains are different. Similar to the present study, Herrera-Saldana et al. [25] also expressed very high 48-h starch degradation values for barley, wheat and corn. The 48-h starch degradation values reported for barley, wheat and maize by Herrera-Saldana et al. [25], for barley by Krieg et al. [29] were higher than those of the current study. The starch degradation values obtained in the present study were similar to the values reported for wheat and corn by Gençoğlu et al. [24]. Similarly, it was emphasized that while the ruminal starch degradation values of wheat and barley were over 98% after 12 h of incubation, this value was below 66% for maize. There is an interaction between the starch particles in the feed and the protein matrix. Highly soluble albumins and globulins are high in lysin but low in proline and glutamic acid, whereas poorly soluble prolamins are high in proline and glutamic acid but low in lysin. It was showed that the concentrations of proline and glutamic acid were negatively correlated with the starch degradation of maize grains in the rumen [29]. In the present study, water-soluble starch fractions of cereal grains in sheep consuming probiotic-free rations were considerably higher than the values reported by Herrera-Saldana et al. [25], İnal et al. [28], Gençoğlu et al. [24], Krieg et al. [29]. However, in all these studies, the lowest value was seen in maize. Besides variation of the incubation procedure (e.g., the usage of smaller pore sizes by Benninghoff et al. [31]), the differences between studies might be related to the differences in grain varieties used. In contrast to the OM, CP degradation values of the feedstuffs, the addition of probiotics to the diet caused an increase in 48-h starch degradation values of all feedstuffs at varying rates. Probiotics supplementation also resulted in significant increases in PD starch fraction, significant reductions in water-soluble starch and nondegradable starch fractions of starch rich cereals and soybean meal. This was perceived as a sign that the effect of *S. cerevisiae* on starch degradation was more effective, indicating the potential for animals to greatly increase their utilization of the starch found in these feeds. Probiotics usage may increase the better use of the energy, which would be released by the degradation of starch in the rumen over a longer period of time, by the microorganisms in the rumen, and can positively affect the microbial protein synthesis [32]. The slow degradation of starch also makes the rumen pH more stable. Indeed, it has been reported that the addition of live yeast improved ruminal pH and cellulolytic bacteria [33]. The use of yeast in ruminant diets trigger a modulation in the microbial population in the rumen and improve feeding efficiency in dairy cows [34]. Indeed, Zhu et al. [35] have noted a significant increase in the number of cellulolytic bacteria and a reduction in lactate producing species in response to live yeast supplementation. Supplementation of diet with yeast culture has resulted in improved nutrient digestibility and higher feeding efficiency in sheep fed a diet with 80% concentrate [36].

5. Conclusion

It can be concluded that *Saccharomyces cerevisiae*, which was used as a probiotic in the experiment, significantly increased the ruminal DM, OM, CP degradations within the 12-h incubation period of all feeds, except corn. It was observed that the addition of *Saccharomyces cerevisiae* into diet increased the starch degradation values in the starch-rich cereal grains and soybean meal and affected starch fractions, especially by decreasing the water-soluble and nondegradable starch fractions and increasing the potentially degradable starch fraction. This has shown that adding *Saccharomyces cerevisiae* to the diet may allow the animal to benefit from these feeds better by utilizing the nutrients in the feed more effectively in the rumen.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The paper is prepared from the MS thesis of the first author.

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