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Determination of in situ degradation kinetics of some legume waste not used for human consumption

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Abstract: The objective of this experiment was to evaluate the chemical composition, in situ ruminal degradation kinetics, and protein fractions of subsieve dry beans (DB), chickpeas (CCP), red lentils (RL), and green lentils (GL). Four samples of those legumes were utilized as replicates and were incubated for up to 48 h in the rumen of 3 rams. RL and CCP had higher organic matter (OM) than DB and GL. Crude protein (CP) was the highest in GL ($P < 0.05$). Ether extract (EE) concentrations were higher in CCP and DB compared to those of RL and GL ($P < 0.05$). Crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) contents were the highest in RL ($P < 0.05$). The acid insoluble nitrogen (ADIN-N) content was similar in all samples evaluated. While RL had the lowest OM and CP degradability and water-soluble OM and CP concentrations, DB and CCP had the highest potentially degradable OM and CP contents at the end of the 48 h incubation period ($P < 0.05$). Escaped protein content was the highest in RL and the lowest in DB ($P < 0.05$). In conclusion, some of the pulse species that are produced locally and not suitable for human consumption can be used in the diet of ruminant animals as a source of protein based on OM and CP degradabilities and escape protein contents.

Key words: Nutrient contents, subsieve, in situ, ruminant, degradation kinetics

1. Introduction

Some of the difficulties regarding animal production in Turkey are the application of traditional production methods, lack of pasture management and feedstuff production, and imbalance between demand and production (1).

Scientists have been trying very hard to improve feed efficiency and searching for ways to minimize feed costs, which is the greatest expense in animal production. Traditionally, Turkish farmers utilize cereal residue (mainly barley and wheat) as the main sources of forage and barley and wheat grains and some common meals (sunflower and cotton seed meal) as primary sources of concentrate feed in animal production because of their low cost and availability. However, feeding animals these feedstuffs is sometimes insufficient in terms of meeting animal requirements, especially in high yielding animals. This problem can be overcome by increasing the production of feedstuffs that can be utilized as both forage and grains and by utilizing the industrial byproducts in animal feed (2).

Grain legumes are marketed after selection and packing processes for human consumption. However, grain legumes, which are below a certain standard or somehow damaged, are not used for human consumption. These grain legumes need to be efficiently used. One of the most efficient ways of utilization is to use them as animal feed. Approximately 1.2 million tons of grain legumes are produced every year and 2%–3% of this amount is eliminated as subsieve. This means that significant amount of subsieve grain legume can be used as animal feed, resulting in a source of extra income for a country's national economy (3). However, there is very limited data in the literature on the feed value of these grains when they are used in ruminant diets.

Thus, the aim of this study was to determine nutrient contents, in situ ruminal degradation kinetics, and protein fractions of subsieve dry beans (DB), chickpeas (CCP), red lentils (RD), and green lentils (GL) used as residue in the legume packing industry.

2. Materials and methods

Four samples from each pulse species (16 samples in total), collected from different regions of Turkey, were utilized in this study.

All of the samples were first oven dried in order to determine dry matter content. All of the samples were then divided into two parts, one part for chemical composition

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determination and the other part for the 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 (4), crude fiber (CF) (5), neutral detergent fiber (NDF) (6), and acid detergent fiber (ADF) (7).

In the experiment, 3 ruminally cannulated Akkaraman rams were utilized in order to determine in situ degradation kinetics. The rams were treated with Cydoctyn and Rabenzole for elimination of internal and external parasites prior to the incubation of samples. Ten days before the initiation of the experiment, a 50/50 forage/concentrate diet was offered to the rams. During the experiment, the rams were free to access clean water and vitamin-mineral blocks.

Samples used in the in situ degradation study were ground to pass through a 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 rams. For this experiment, three ruminally cannulated Akkaraman rams were used and samples were incubated as duplicates per feedstuff and incubation time. The bags were then removed from the rumen and were cleaned by running tap water for about 15 min until the water became clear. (8). The OM and CP content of residues was then also tested for determination of OM and CP degradation kinetics.

In situ degradability of OM and CP was calculated using the following equation: nutrient degradability = a + b $^{(1-e-ct)}$. Loss of DM from the bags caused by exposure of substrates to the digestive action of the rumen and the washing process that followed resulted in the partitioning of OM and CP in each of the varieties into 3 fractions: 1) soluble fractions of OM and CP (WSOM, WSCP) were determined as the differences between initial OM and CP content and the amount of OM and CP recovered at the beginning of incubation; 2) potentially degradable fractions of OM and CP (PDOM, PDCP) were determined as 100 – (nondegradable fraction and water soluble fractions of OM and CP); 3) nondegradable fractions of OM and CP (NDOM, NDCP) were determined as the difference between initial OM and CP content and the amount of OM and CP recovered after 48-h incubation of samples in the rumen.

To estimate the model of residual potentially digestible OM and CP fractions, degradation rates (OM $^{k-1}$, CP $^{k-1}$) and discrete lag times (OM lag time, CP lag time) were obtained by fitting recovery data to the one-pool version of the discrete lag model described by Mertens (9) and modified by Wechsler (10) using nonlinear regression analysis of SAS (11).

The percentages of escape protein were calculated as total residual N remaining after 12-h incubation, adjusted for the indigestible N (ADIN) using the following equations (12): bypass protein percentage: % of total protein = (total residual N – ADIN of total residue) / (total plant N – ADIN of total plant) × 100.)

All data were analyzed based on a completely randomized design using the GLM procedure of SAS (11), and means were compared with Duncan's t-test (13)

3. Results

The nutrient contents of subsieve feedstuffs (pulse species) used in the study are shown in Table 1. DM content of samples ranged from 91.19 to 92.41, and CCP had higher DM content compared to other samples ($P < 0.05$). RL and CCP had higher OM than DB and GL ($P < 0.05$). GL had the highest CP content ($P < 0.05$). EE concentrations were higher in CCP and DB compared to those of RL and GL ($P < 0.05$). RL had the highest CF, ADF, and NDF contents ($P < 0.05$). The ADIN-N content was similar in all samples evaluated ($P > 0.05$).

While OM and CP degradabilities were the lowest in RL, OM and CP degradabilities were the highest in DB and CCP at the end of the 48-h incubation (Tables 2 and 3; $P < 0.05$).

OM and CP fractions of samples are shown in Table 4. Water-soluble OM and CP contents were the lowest in RL. DB and CCP had higher PDOM and PDCP compared to GL and RL ($P < 0.05$). While DB and CCP had the lowest NDOM contents, DB had the lowest NDCP content ($P < 0.05$). Escaped protein (bypass CP) content was the highest in RL and the lowest in DB ($P < 0.05$). Rate of OM and CP degradations were similar among samples ($P > 0.05$). Lag time is defined as the necessary time for a microbe to attach substrate to initiate degradation. The lag time for CP was the lowest at RL, whereas the lag time for OM was the lowest in CCP.

4. Discussion

The nutrient contents, in situ ruminal organic matter, and crude protein degradabilities and fractions and the escape protein contents of subsieve DB, CCP, RL, and GL used as residue in pulse packing industry were evaluated in this study.

The nutrient contents of subsieve feedstuffs used in the study are shown in Table 1. DM contents of feedstuffs were similar. Dry matter contents of RL, DB, GL, and CCP were 91.19%, 1.71%, 91.73%, and 92.41%, respectively, in the experiment. Dry matter contents of RL, DB, GL, and CCP were higher than the values reported for lentils, beans, and CCP (88.00%, 89.00%, and 89.00%, respectively) by Ensminger et al. (14). All of the samples used in the experiment had over 85% DM, which is a critical level for proper storage of dry samples (15).

The OM concentrations ranged from 90.10% to 96.73% in this experiment. The OM concentrations were similar to

Table 1. Nutrient contents of subsieve feedstuffs used in the study (%).

Item	Red lentil	Dry bean	Green lentil	Chickpea	SEM
DM	91.19[b]	91.71[b]	91.73[b]	92.41[a]	0.17
Ash, % DM	3.26[c]	6.34[b]	9.89[a]	3.96[c]	0.59
OM, % DM	96.73[a]	93.65[b]	90.10[c]	96.03[a]	0.59
CP, % DM	19.02[c]	24.92[b]	27.89[a]	24.69[b]	0.71
EE, % DM	3.47[bc]	6.72[a]	2.26[c]	8.66[a]	1.29
ADIN %Total N	0.50	1.05	0.85	0.97	0.23
CF, % DM	24.03[a]	4.09[c]	10.80[b]	3.57[c]	0.52
NDF, % DM	43.28[a]	30.39[b]	29.96[b]	21.31[c]	1.04
ADF, % DM	30.99[a]	6.22[c]	10.57[b]	4.39[c]	0.59

Table 2. OM degradability values of subsieve feedstuffs used in the study (% OM).

Incubationtime (hours)	Red lentil	Dry bean	Green lentil	Chickpea	SEM
0 h	4.45[c] ± 1.45	9.26[b] ± 3.09	15.44[a] ± 5.24	9.18[b] ± 3.78	0.76
2 h	10.01[c] ± 2.72	16.63[b] ± 7.24	17.06[b] ± 3.26	22.05[a] ± 4.36	0.89
4 h	20.65[d] ± 3.95	27.54[c] ± 6.55	32.13[b] ± 4.49	40.26[a] ± 6.94	1.13
8 h	30.11[d] ± 3.53	42.45[b] ± 6.18	39.05[c] ± 2.63	50.90[a] ± 4.26	0.93
12 h	29.62[c] ± 3.20	48.51[a] ± 3.29	42.09[b] ± 2.43	47.37[a] ± 5.96	0.75
24 h	31.60[d] ± 2.51	56.71[b] ± 4.30	51.68[c] ± 5.56	64.76[a] ± 4.50	0.84
48 h	45.86[c] ± 6.94	83.31[a] ± 6.37	75.28[b] ± 3.60	85.23[a] ± 4.59	1.04

Table 3. CP degradability values of subsieve feedstuffs used in the study (% CP).

Incubation time (hours)	Red lentil	Dry bean	Green lentil	Chickpea	SEM
0 h	12.63[d] ± 1.32	18.42[c] ± 2.00	24.14[a] ± 5.35	20.81[b] ± 4.28	0.80
2 h	21.30[c] ± 1.79	33.11[ab] ± 6.15	30.80[b] ± 6.38	34.72[a] ± 4.87	0.94
4 h	27.77[c] ± 3.08	40.52[b] ± 4.16	41.16[b] ± 1.90	50.05[a] ± 3.59	0.52
8 h	44.34[d] ± 2.50	56.42[b] ± 4.25	47.72[c] ±1.95	60.66[a] ± 5.90	0.82
12 h	41.22[c] ± 2.67	61.27[a] ± 2.83	57.44[b] ± 3.46	61.01[a] ± 5.69	0.77
24 h	42.15[d] ± 5.14	68.51[b] ± 3.09	65.90[c] ± 4.20	73.00[a] ± 2.90	0.76
48 h	54.98[d] ± 3.33	89.38[a] ± 3.95	83.02[c] ± 2.72	86.92[b] ± 3.98	0.63

that of Canbolat and Bayram (16), which were observed for CCP, DB, peas, and soybeans.

Crude protein contents of RL, DB, GL, and CCP were 19.02%, 24.92%, 27.89% and 24.69%, respectively, in the experiment. The CP contents of RL were lower, but CP contents of GL were similar to the values reported by Ensminger et al. (14) and Kaya (17). The CP contents of DB in the current study were higher than those reported by Ensminger et al. (21.8%) (16) and Canbolat and Bayram (23.7%) (16); however, CP contents of CCP were between the values reported by Ensminger et al. (19.1%) (14) and Canbolat and Bayram (25.7%) (16). The differences in chemical composition among pulses were caused by variations in soil types in which pulses were grown and the varieties used (18).

Table 4. OM and CP fractions and degradation kinetics values of subsieve feedstuffs used in the study.

Item	Red lentil	Dry bean	Green lentil	Chickpea	SEM
WSOM, OM	$4.45^{c} \pm 1.00$	$9.26^{b} \pm 2.91$	$15.44^{a} \pm 4.92$	$9.17^{b} \pm 2.52$	1.66
WSCP, % CP	$12.63^{b} \pm 0.90$	$18.41^{ab} \pm 1.45$	$24.14^{a} \pm 5.35$	$20.81^{a} \pm 3.78$	1.91
PDOM, % OM	$41.41^{c} \pm 6.33$	$74.04^{a} \pm 3.55$	$59.83^{b} \pm 6.02$	$76.05^{a} \pm 2.68$	2.54
PDCP, % CP	$42.33^{c} \pm 1.77$	$70.96^{a} \pm 2.36$	$58.87^{b} \pm 5.05$	$66.10^{a} \pm 3.72$	1.97
NDOM, OM	$54.13^{a} \pm 6.24$	$16.69^{c} \pm 2.14$	$24.72^{b} \pm 1.60$	$14.76^{c} \pm 0.96$	1.80
NDCP, % CP	$45.04^{a} \pm 0.89$	$10.62^{d} \pm 1.12$	$16.98^{b} \pm 1.76$	$13.08^{c} \pm 0.48$	0.57
Bypass CP, % CP	$42.99^{a} \pm 2.61$	$27.35^{d} \pm 2.32$	$39.56^{b} \pm 4.13$	$34.70^{c} \pm 5.28$	0.79
OM k^{-1}, h	0.45 ± 0.15	0.39 ± 0.03	0.36 ± 0.02	0.41 ± 0.06	0.04
OM lag, h	$0.53^{ab} \pm 0.26$	$0.76^{ab} \pm 0.17$	$0.91^{a} \pm 0.24$	$0.48^{b} \pm 0.18$	0.12
CP k^{-1}, h	0.48 ± 0.12	0.42 ± 0.04	0.45 ± 0.08	0.50 ± 0.03	0.03
CP lag, h	$0.34^{c} \pm 0.22$	$2.08^{a} \pm 0.13$	$0.78^{b} \pm 0.47$	$0.51^{bc} \pm 0.04$	0.13

Bypass CP: bypass crude protein; OM k^{-1}: rate of degradation of organic matter; OM lag: discrete lag times of organic matter; CP k^{-1}: rate of degradation of crude protein; CP lag: discrete lag times of crude protein.

Ether extract contents of pulses varied between 2.26% and 8.66% among pulses examined in the experiment. The EE contents of pulses in the current experiment were higher than those of values (1.0%, 1.2%, and 4.1% for lentils, beans, and CCP, respectively) reported by Ensminger et al. (14). However, Ensminger et al. (14) also reported higher EE content for CCP compared to lentils and beans, which was similar to the results of the current study. These variations among pulses could be related to the growing conditions and harvesting time of pulses (19, 20).

While RL had the highest contents of CF, NDF, and ADF, CCP had the lowest CF, NDF, and ADF contents ($P < 0.05$). Even though the NDF content of DB was similar to that of GL, both CF and ADF contents of DB were significantly less than those of GL ($P < 0.05$). Crude fiber contents of RL, GL, DB, and CCP were 24.03%, 10.80%, 4.09%, and 3.57%, respectively. Ensminger et al. (14) reported that the CF contents of lentils, beans, and CCP were 3.4%, 4.1%, and 7.0%, respectively. On the other hand, Özyiğit and Bilgen (21) reported a higher CF contents for the common vetch, vetchling, and pea (20.55%, 16.22%, and 14.22%, respectively). While the CF contents of CCP, RL, and GL in the current study were higher, the CF content of DB was similar to the results reported by Ensminger et al. (14). However, the CF contents of pulses in the study by Özyiğit and Bilgen (21) were higher than the CF contents of the current study, with the exception of RL. The higher CF content of lentils was confirmed with the higher ADF and NDF contents.

The concentrations of ADIN-N were 0.50%, 1.05%, 0.85%, and 0.97% for RL, DB, GL, and CCP, respectively. These values were similar to the values observed by Goelema et al. (22) for green peas, horse beans, and beans (ranging from 0.36% to 0.99%). The lower ADIN-N contents observed in the samples, which represent indigestible and damaged N in feed, were considered as an indication of higher CP digestibilities in these feedstuffs.

Organic matter degradation values of RL, DB, GL, and CCP were 45.86%, 83.31%, 75.28%, 85.23%, respectively) after 48 h of ruminal incubation. OM degradations of peas, CCP, beans, and horse beans (91.1%, 92.1%, 91.7%, and 86.0%, respectively) reported by Arbeu and Bruno-Soares (23) were higher than the values observed in the current study. Similarly, percentages of OM degradations for both types of lentils were less than the OM degradations of soybeans, grass peas, CCP, and beans (91.04%, 81.70%, 79.60%, and 78.90%, respectively) reported by Canbolat and Bayram (16). It was thought that the low OM degradation of RL was due to its higher CF content.

Crude protein degradations of RL, DB, GL, and CCP were 54.98%, 89.38%, 83.02%, and 86.92%, respectively, after 48 h of rumen incubation in this experiment. CP degradation was 75.4% ± 7.3% in narbon vetches (15) and 74.10% ± 0.21%, 51.07% ± 0.38%, 70.47% ± 0.15%, 73.67% ± 0.33% for peas, lentils, beans, and CCP, respectively (24). The CP degradations of DB, GL, and CCP used in the study were higher than those of CP degradations of feedstuffs that have been reported in the literature. However, CP degradation of RL was similar to lentils in El-Niely's study (24) but lower than the other feedstuffs' CP degradations. It was thought that the lower CP degradation of RL could be due to protease, trypsin, and chymotrypsin inhibitors (25, 26) that RL contains.

Percentages of WSOM, PDOM, and NDOM for RL, DB, GL, and CCP were 4.45%, 9.26%, 15.44%, and 9.17%; 41.41%, 74.04%, 59.83%, and 76.05%; and 54.13%, 16.69%, 24.72%, and 14.76%, respectively. It was determined that RL had the lowest OM degradation followed by GL, DB, and CCP, respectively. There was no statistical difference among OM degradation rates. Abreu and Bruno-Soares (27) stated that the percentages of WSOM, PDOM, and NDOM for vetches and beans were 23.10% and 16.80%; 44.60% and 37.10%; 32.30% and 46.10%, respectively. They also stated that there was no statistical difference between vetches and beans in terms of degradation rates. While percentages of WSOM were lower than those of feedstuffs reported by Arbeu and Bruno-Soares (27), the percentages of PDOM of all feedstuffs in the study were only higher than that of beans reported by Arbeu and Bruno-Soares (27).

The percentage of PDOM of RL was lower than that of vetches, but the percentages of PDOM for DB, CCP, and GL were higher than that of vetches. RL had the highest percentages of NDOM among all pulse species, which was higher than those of vetches and beans reported by Arbeu and Bruno-Soares (27). The high NDOM contents of lentils might have resulted from the high CF (24.03% and 10.80%) and antinutritional materials (25,26) that they contain. WSOM and PDOM contents are also directly proportional to the amount of carbohydrate (28) and other nutrients (starch, CP, etc.) the plants contain.

While percentages of WSCP were 12.63%, 18.41%, 24.14%, and 20.8%, PDCP contents were 42.33%, 70.96%, 58.87%, and 66.10% for RL, DB, GL, and CCP, respectively. The highest NDCP content was observed in RL (45.04%) and followed by GL, CCP, and DB. The CP degradation rates were statistically similar ($P > 0.05$). Wang et al. (29) found that percentages WSCP, PDCP, and NDCP were 55.50%, 55.60%, and 72.30%; 14.10%, 22.90%, and 11.00%; 30.30%, 21.40%, and 16.70% for beans, soybeans, and peas, respectively. They stated that CP degradation rate of beans was higher than those of soybeans and peas (0.07, 0.05, and 0.05, respectively). In a study carried out by Holt and Soluski (30), NPNs that composed the majority of WSCP were 16%–40%, 25%, and 36%–46% for beans, CCP, and lentils, respectively. WSCP contents were correlated with NPN contents (30). It was thought that the high percentage of NDCP in RL could be due to protease inhibitors (25,26) and the high CF content of RL.

In this study, the highest bypass protein percentage was found in RL. It was followed by GL, CCP, and DB (42.99%, 39.56%, 34.70%, and 27.35 %, respectively). Higher bypass protein percentages for grain legumes were reported by Seifdavati and Taghizadeh (31) for vetches (66.79%), by Goelema et al. (22) for peas, and lupins and beans (52.30%, 51.20%, and 56.60%, respectively).

In conclusion, it was noted that feedstuffs (GL, CCP, and DB) used in the experiment, with the exception of RL, had a greater ruminal degradibility of both OM and CP and, moreover, had a higher escape protein contents, except in the case of DB. Therefore, these feedstuffs can be substituted with some of the common protein sources used in ruminant nutrition.

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