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The effect of green tea waste extract on ruminal degradability and intestinal digestibility of barley grain

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Abstract: This study was conducted to investigate the effects of different levels of green tea waste extract (GE) on ruminal degradability and intestinal digestibility of dry matter (DM), crude protein (CP), and starch of barley grain. Therefore, barley grain was treated with 0 as control, and 5%, 10%, 15%, and 20% of GE. The potential of DM degradability ($a + b$) of barley grain treated with 15% and 20% of GE was significantly lower compared to other treatments (Linear [L] $P < 0.05$). The lowest ($a + b$) fraction of CP degradability was found in the barley grain treated with 20% GE (L $P < 0.05$). The ($a + b$) fraction and effective degradability of starch in barley grain treated with 15% and 20% of GE were significantly lower than those in other treatments (L and quadratic [Q] $P < 0.05$). The highest intestinal DM, CP, and starch digestibility were associated with barley grain treated with 10% GE (L and Q $P < 0.05$). Based on recent results, processing barley grain with GE leads to a decrease of DM, CP, and starch degradability. The treatment of 10% GE improved intestinal digestibility of the aforementioned nutrients; therefore, it can be used to protect barley grain against ruminal degradability.

Key words: Cereals, feed value, dry matter, protein, starch, plant extract

1. Introduction

In many parts of the world, barley grain (BG) is one of the main components of the concentrated elements of ruminant rations (1). There is less protein content in BG than in oil seed meal; however, since it is a significant portion of the concentrate used in feeding ruminants, it supplies a large amount of protein in the diet. Degradability of BG protein is high in the rumen, and only a small amount of this protein passes through the rumen to the small intestine (2). It is essential to protect proteins in productive animals whose protein requirement is not met only through the synthesis of microbial proteins. Therefore, there is a need to reduce protein degradation in these animals (3,4). Accordingly, many different processing methods, such as heating (5) and the use of formaldehyde (6), have been applied to protect proteins from ruminal degradation and to allow proteins to bypass the rumen to other digestive tract sectors. These processing methods may reduce intestine bioavailability of some amino acids like lysine, cysteine, tyrosine, and leucine (7,5). Moreover, an increasing demand for natural and healthy food has led to the development of organic livestock farming, which is eco-friendly, has healthy breeding conditions, high animal welfare standards, and avoids using common growth promoters, animal wastes, and other chemical supplements in the diets of livestock. Using formaldehyde or other chemicals to protect proteins from ruminal degradation is not permitted in organic farming. Hence, it seems necessary to search for an alternative way to protect proteins in order to enhance their use and efficiency of the animals' digestion. Accordingly, condensed tannins are often used in the diet to protect proteins (8).

The dry matter of BG consists of approximately 50% to 60% starch; 80% to 90% of this starch is degradable in the rumen. Hence, only a small amount of this starch enters the small intestine, which increases the risk of producing ruminal acidosis in livestock in whose diet a large amount of BG has been used. Increasing the amount of nondegradable BG starch might be useful for high-producing livestock (9). Cereal starch can be considered a suitable source of glucose if an appropriate processing method is available which reduces ruminal degradability and increases intestinal digestion. Starch, in the form of protected granules joined to proteins, is capable of establishing covalent bonds with aldehyde compounds due to their free amino acid groups; thus, processing

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cereals with aldehyde compounds may protect starch from ruminal degradation (10).

Tea waste is a potential feed for ruminants; studies have shown the presence of some secondary plant metabolites like tannins and saponins in this waste (11,12). Green tea waste is produced during the process of dried green tea production in tea-drying factories. The amounts of DM, organic matter, CP, ether extract, natural detergent fiber, acid detergent fiber, acid detergent lignin, calcium, phosphorus, total phenols, total tannins, and saponins of green tea waste are 94.8, 94.4, 16.3, 1.0, 42.1, 32.9, 8.6, 0.35, 0.21, 16.2, 12.2, and 14.6 (percentages in dry matter), respectively (13).

Tannins are plant polyphenol compounds that have the property of linking macromolecules, usually to proteins, but also to starch and structural polysaccharides. Recent studies have shown that using tannin leads to a reduction in ruminal degradability of plant proteins and enhances intestinal bioavailability of amino acids in ruminants (14,15). Published reports indicate that using high levels of condensed tannin reduces ruminal degradability of fast-fermenting carbohydrates and hemicelluloses, but increases intestinal digestion (16).

Further research on tea waste has focused on the waste produced by beverage companies manufacturing various tea drinks (spent tea leaves); there is little information about the waste produced by tea-drying factories. Green tea waste is rich in considerable amounts of tannin. There is no available information on the effects of green tea waste produced by tea-drying factories on the ruminal degradability of BG. Therefore, this study was conducted to assess the effect of green tea waste extract (GE) on ruminal degradability and intestinal digestion of DM, CP, and starch of BG.

2. Materials and methods

This study was conducted at the Animal Science Research Institute of Iran at Karaj, Alborz. Analysis of chemical components was performed in the Animal and Poultry Nutrition Laboratory, and ruminal degradability experiments were conducted in the Nutrition and Physiology Laboratory.

2.1. Preparation of green tea waste extract and processing barley grain with the extract

Green tea waste was provided by tea-drying factories and dried at 40 °C for 72 h; the waste was then ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) using a 1-mm mesh. A solution was first prepared that included, in each 100 mL, 10 mL of methanol (Merck Co., Darmstadt, Germany), 10 mL of ethanol (Merck Co.), and 80 mL of distilled water; thereafter, the waste was soaked in the aforementioned solution at a ratio of 1:8, kept at 39 °C for an hour, and then filtered using Whatman filter paper Grade 40 (Whatman, Buckinghamshire, UK) (17).

The GE was converted to powder form after evaporation at 50 °C in order to determine components and to apply in processing BG. To treat BG with green tea waste extract, amounts of 0, 5, 10, 15, and 20 g of this extract were dissolved in distilled water and then sprayed on experimental samples (BGs). The treated samples were dried at 35–40 °C and milled with a 2-mm sieve mill to undergo a rumen degradation test (17).

2.2. Ruminal degradability experiment

The ruminal degradability was conducted using 2 rumen-fistulated Talyshi steers (350 ± 4.5). The steers were fed with a base diet containing alfalfa hay, wheat straw, and concentrate at the level of maintenance requirements, according to NRC (18) recommendations. To adjust the rumen's condition, the diet was offered 2× daily, at 08:00 and 18:00. Water and rock salt were readily available for the livestock.

To determine degradability, 5 g of each feed sample (BG treated with 0%, 5%, 10%, 15%, and 20% GE) were placed in separate nylon bags and suspended in the rumen using 30-cm–long plastic tubes. Bags containing the feed samples were kept in warm water at 39 °C to absorb enough moisture before being placed in the rumen; the bags were then set in the rumen through the rumen fistula and the lids were kept closed until the end of incubation (for each steer, there were 2 replicates of each treatment each time). Bags were removed from the rumen at definite time points of 0, 2, 4, 6, 8, 12, 24, and 48 h. Bags were transferred to cold water immediately to stop microorganism activity. The bags were then washed using a washing machine (SE1000, Sepehr Electric Co., Tehran, Iran) for 30 min and dried for 48 h in an oven (T6060, Heraeus Co., Hanau, Germany) at 55 °C. The weight differences of dried samples before and after incubation represented ruminal DM degradability at that time (19). After determination of DM degradability, ruminal CP and starch degradability were measured as the differences in CP and starch before and after incubation. DM, CP, and starch degradability of experimental samples were measured according to the Ørskov and McDonald (20) model using Fitcurve software as follows:

$P = a + b\ (1 - e^{-ct})$.

In the equation above, P = degradability of nutrient in time t; a = rapidly degradable fraction that disappears quickly; b = slowly degradable fraction; c = degradability ratio of fraction b (%/h); t = ruminal fermentation time; e = logarithm to the base of the mathematical constant e (e = 2.7182).

2.3. Intestinal digestibility

In order to determine intestinal DM, CP, and starch digestion, an amount of 5 g of each treatment was placed

in the rumen of each bull through the fistula for 12 h using plastic bags. The bags were then removed from the rumen, washed using a washing machine (SE1000; Sepehr Electric Co., Tehran, Iran) for 30 min, and dried in an oven (T6060; Heraeus Co., Hanau, Germany) at 55 °C for 48 h. Residues from the bags were pooled by animal and by sample to determine DM, CP, and starch. Approximately 0.5 g of each sample was placed inside special filter bags (Ankom F57; ANKOM Technology, Macedon, NY, USA); 3 empty filter bags were considered blank. Thirty filter bags were set in a container including 2 L of prewarmed HCl (Merck Co.) 1M with 1.9 pH plus 1 g per liter pepsin (P-7000, Sigma, St. Louis, MO, USA) on a shaker (CSL-NOR; Cleaver Scientific, Rugby, Warwickshire, UK) at 39 °C with adjusted shaking speed for an hour. The bags were later transferred to 2 L of prewarmed pancreatin solution (KH_2PO_4 [Merck Co.], 0.5 M, pH 7.75, 50 ppm thymol [Merck Co.], and 3g/L of pancreatin [P-7545, Sigma]) and were set on the shaker at 39 °C with adjusted shaking speed for 24 h. Eventually, the bags were dried in the oven at 55 °C for 48 h, and the amounts of DM, CP, and starch were measured. The weight differences between samples that had gone through ruminal incubation before and after these processes were considered intestinal DM, CP, and starch digestion of BG (21,22).

2.4. Chemical compounds measurement

Total extractable phenolic compounds, total tannin, condensed tannin, and hydrolyzable tannin of GE were measured based on Hagerman's (23) method. Total saponin of GE was measured based on Yosioka et al.'s (24) method. DM, ash, and CP of BG samples were measured according to AOAC (25), and starch was measured based on Clegg's (26) method.

2.5. Statistical analysis

A completely randomized design was used in this investigation. Treatments were set as follows: untreated BG as control, and BG treated with 5%, 10%, 15%, and 20% of GE, labeled as 0GEBG, 5GEBG, 10GEBG, 15GEBG, and 20GEBG, respectively. The polynomial contrasts statement of the SAS program was used to obtain linear and quadratic effects of GE levels on ruminal degradability and intestinal digestibility of DM, CP, and starch of BG. Four replicates were used in the ruminal degradation experiment. Intestinal digestibility tests were conducted with 6 replicates for each treatment. The statistical model of experimental design was as follows:

$Y_{ij} = \mu + T_{ij} + e_{ij}$.

In the above, Y_{ij} = observed parameter; μ = overall mean; T_{ij} = effect of treatment; and e_{ij} = trial error.

Data were analyzed using SAS 9.1 (27) and GLM procedure. Duncan test at $P < 0.05$ was used to compare means.

3. Results

3.1. Ruminal degradability

Secondary metabolites of green tea waste extract are presented in Table 1. The amount of DM, ash, CP, and starch of experimental treatments is shown in Table 2. No significant difference was found in case of DM degradability between 15GEBG and 20GEBG after 48 h of incubation; however, in both treatments, DM degradability was considerably lower than in other treatments (L $P < 0.05$). The 0GEBG treatment possessed the highest ruminal DM degradability (L $P < 0.05$, Table 3). The rapidly degradable fraction (a) of DM in the 20GEBG treatment did not show any significant difference compared to 15GEBG; but in both treatments, fraction (a) of DM was significantly lower than in other treatments (L $P < 0.05$). The highest fraction (a) of DM was that for 0GEBG; there was no significant difference compared to the 15GEBG group (Table 4). No significant differences were found among all treatments in the slowly degradable fraction (b) of barley grain DM degradability. Potential of DM degradability (a + b) of 15GEBG and 20GEBG did not show any significant difference, but in both treatments, this fraction was significantly lower than in other treatments (L $P < 0.05$, Table 4). Constant rate (c) of DM degradability did not show any noticeable difference among treatments. Effective degradability in the 15GEBG and 20GEBG treatments showed no statistical difference in all outflow rates, but compared to other treatments, both represented a lower amount (L $P < 0.05$). The 0GEBG treatment possessed the highest effective degradability in all outflow rates (L $P < 0.05$, Table 4).

Table 5 shows the effect of different levels of GE on ruminal CP degradability of treatments at different times of rumen incubation. After 48 h of rumen incubation, the 20GEBG group showed the least CP degradability (L and Q $P < 0.05$); but no significant difference was detected as compared to 15GEBG. The 0GEBG treatment had the highest amount of CP ruminal degradability among

Table 1. Secondary metabolites of green tea waste extract (% DM).

Metabolites	Contents*
Total phenolics	12.5 ± 0.60
Total tannin	9.5 ± 0.51
Condensed tannin	6.7 ± 0.53
Hydrolyzable tannin	2.8 ± 0.79
Total saponins	8.4 ± 0.40

* Each parameter was calculated with 3 replicates.

Table 2. The contents of dry matter, crude protein, ash, and starch (% DM) of barley grain (BG) treated with different levels of green tea waste extract (GE).

Treatments	DM*	CP	Ash	Starch
0GEBG	90.2 ± 0.78	12.2 ±0.27	2.7 ± 0.60	54.1 ± 0.33
5GEBG	89.7 ± 0.88	12.2 ± 0.30	2.9 ± 0.45	54.0 ± 1.11
10GEBG	90.0 ± 0.71	12.2 ± 0.76	2.9 ± 0.41	54.7 ± 0.61
15GEBG	89.8 ± 0.29	12.1 ± 0.63	2.6 ± 0.77	54.2 ± 0.99
20GEBG	89.7 ± 0.53	11.9 ± 0.72	2.8 ± 0.37	53.7 ± 0.47

* Standard deviation within treatments; for all nutrients n = 3.

Table 3. The ruminal dry matter degradability (%) of barley grain (BG) treated with different levels of green tea waste extract (GE) at different hours of incubation.

Treatments	Hours of incubation							
	0	2	4	6	8	12	24	48
0GEBG	27.7^{a}	34.3^{a}	40.7^{a}	49.3^{a}	63.2^{a}	70.7^{a}	80.8^{a}	90.9^{a}
5GEBG	26.4^{a}	32.4^{a}	39.0^{a}	46.2^{b}	60.1^{b}	69.2^{a}	79.5^{a}	87.6^{b}
10GEBG	23.8^{b}	28.2^{b}	34.7^{b}	44.4^{b}	57.0^{c}	65.3^{b}	74.5^{b}	83.6^{c}
15GEBG	20.7^{c}	25.5^{c}	31.2^{c}	39.9^{c}	54.6^{d}	62.0^{c}	71.8^{c}	79.8^{d}
20GEBG	20.4^{c}	25.5^{c}	31.3^{c}	39.8^{c}	54.8^{d}	61.4^{c}	72.0^{c}	79.0^{d}
SEM*	0.96	0.94	1.08	1.09	0.83	1.03	1.01	1.12
P value L¶	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P value Q‡	0.21	0.52	0.30	0.37	0.60	0.23	0.34	0.72

*, Standard error of the mean; L¶, linear; Q‡, quadratic; $^{a–d}$ means in the same column not sharing the same superscript are significantly different (P < 0.05).

Table 4. The ruminal degradability parameters and intestinal digestibility of dry matter of barley grain (BG) treated with different levels of green tea waste extract (GE).

Treatments	Degradability parameters*							
	a	b	(a + b)	c	ED_2	ED_5	ED_8	DMD
0GEBG	25.2^{a}	66.3	91.4^{a}	0.087	79.1^{a}	67.3^{a}	59.8^{a}	15.1^{b}
5GEBG	23.4^{a}	65.5	88.9^{a}	0.087	76.7^{b}	65.0^{b}	57.6^{b}	15.5^{b}
10GEBG	20.3^{b}	64.1	84.4^{b}	0.088	72.6^{c}	61.3^{c}	54.1^{c}	19.3^{a}
15GEBG	17.1^{c}	63.8	80.9^{c}	0.088	69.3^{d}	58.0^{d}	50.8^{d}	9.2^{c}
20GEBG	16.9^{c}	63.4	80.3^{c}	0.090	68.9^{d}	57.7^{d}	50.6^{d}	8.5^{c}
SEM¶	0.93	1.52	1.36	0.004	0.83	0.53	0.47	1.01
P value L‡	0.0001	0.09	0.0001	0.70	0.0001	0.0001	0.0001	0.0002
P value Q‡	0.28	0.83	0.61	0.93	0.41	0.21	0.13	0.0001

*Parameters: a, rapidly degradable fraction (%); b, slowly degradable fraction (%); (a + b), potential degradability (%); c, degradation rate of the fraction b (h – 1); ED, effective degradability for passage rates 2%, 5%, and 8%/h; DMD, dry matter intestinal digestibility (%); ¶, standard error of the mean; ‡L, linear; ‡Q, quadratic; $^{a–d}$ means in the same column not sharing the same superscript are significantly different (P < 0.05).

all treatments (L and Q P < 0.05). The fraction (a) of CP degradability in 0GEBG was notably higher than in other treatments (L and Q P < 0.05). The lowest fraction (a) of CP degradability was associated with the 15GEBG and 20GEBG treatments (L and Q P < 0.05, Table 6). The lowest fraction (b) of CP degradability was related to 20GEBG (L P < 0.05), but no significant difference was found between 15GEBG and 20GEBG in this fraction. The 0GEBG treatment had the highest fraction (b) of CP degradability among treatments (L P < 0.05). However, this amount was not significantly different in 0GEBG as compared to 5GEBG (Table 6). No significant difference was found between the 0GEBG and 5GEBG groups for the potential of CP degradability (a + b), while these treatments had the highest (a + b) fraction compared to other treatments (L P < 0.05). The lowest (a + b) fraction of CP degradability was for 20GEBG (L P < 0.05). The constant rate (c) of CP degradability in the 0GEBG and 5GEBG groups was considerably higher than in other treatments (L P < 0.05), but no significant difference was found with regard to this fraction in the 2 treatments (Table 6). Effective protein degradability of 0GEBG in 2% and 5% outflow rates was

Table 5. The ruminal crude protein degradability (%) of barley grain (BG) treated with different levels of green tea waste extract (GE) at different hours of incubation.

Treatments	Hours of incubation							
	0	2	4	6	8	12	24	48
0GEBG	9.1[a]	13.1[a]	19.1[a]	25.3[a]	35.3[a]	44.7[a]	54.2[a]	71.4[a]
5GEBG	9.9[a]	12.9[a]	19.5[a]	24.4[a]	33.6[b]	44.6[a]	53.4[a]	68.8[b]
10GEBG	7.7[b]	9.7[b]	15.4[b]	20.3[b]	28.2[c]	37.5[b]	46.4[b]	62.3[c]
15GEBG	5.0[c]	7.5[c]	10.1[c]	15.7[c]	23.7[d]	32.0[c]	40.5[c]	55.0[d]
20GEBG	4.9[c]	7.5[c]	10.9[c]	13.6[d]	23.6[d]	32.1[c]	39.9[c]	53.4[d]
SEM*	0.52	0.36	0.64	0.61	0.44	0.67	0.61	1.10
P value L¶	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P value Q‡	0.0004	0.001	0.0005	0.0008	0.0005	0.0001	0.0001	0.01

*, Standard error of the mean; L¶, linear; Q‡, quadratic; [a–d] means in the same column not sharing the same superscript are significantly different (P < 0.05).

Table 6. The ruminal degradability parameters and intestinal digestibility of crude protein of barley grain (BG) treated with different levels of green tea waste extract (GE).

Treatments	Degradability parameters*							
	a	b	(a+b)	c	ED_2	ED_5	ED_8	CPD
0GEBG	7.6[a]	66.7[a]	74.3[a]	0.058[a]	57.3[a]	43.6[a]	35.8[a]	53.4[b]
5GEBG	7.7[a]	64.2[ab]	71.8[a]	0.059[a]	55.6[b]	42.5[b]	35.0[a]	52.9[b]
10GEBG	5.7[b]	61.1[b]	66.7[b]	0.052[b]	49.7[c]	36.7[c]	29.7[b]	59.4[a]
15GEBG	3.1[c]	56.7[c]	59.9[c]	0.050[b]	43.6[d]	31.5[d]	25.0[c]	49.4[c]
20GEBG	2.8[c]	54.8[c]	57.6[c]	0.052[b]	42.3[d]	30.7[d]	24.4[c]	49.2[c]
SEM¶	0.49	1.72	1.66	0.002	0.78	0.42	0.35	1.27
P value L‡	0.0001	0.0001	0.0001	0.0004	0.0001	0.0001	0.0001	0.0001
P value Q‡	0.002	0.46	0.08	0.39	0.001	0.0001	0.0001	0.0001

*Parameters: a, rapidly degradable fraction (%); b, slowly degradable fraction (%); (a + b), potential degradability (%); c, degradation rate of the fraction b (h – 1); ED, effective degradability for passage rates 2%, 5%, and 8%/h; CPD, crude protein intestinal digestibility (%); ¶, standard error of the mean; L‡, linear; Q‡, quadratic; [a–d] means in the same column not sharing the same superscript are significantly different (P < 0.05).

significantly higher than in other experimental treatments (L and Q P < 0.05). With regard to these 2 outflow rates, 20GEBG had the lowest effective degradability of CP (L and Q P < 0.05), but there was no significant difference from 15GEBG (Table 6). For the 8% outflow rate, no significant difference was found between the 0GEBG and 5GEBG treatments for the effective degradability of CP; the amount of this component in the 2 treatments was significantly higher than in other treatments (L and Q P < 0.05, Table 6). Effective CP degradability of the 15GEBG and 20GEBG treatments had no significant difference for the 8% outflow rate, but results were significantly lower (L and Q P < 0.05, Table 6) compared to other treatments.

Table 7 shows ruminal starch degradability of the treatments at different times of incubation. After 48 h of rumen incubation, ruminal starch degradability of the 15GEBG and 20GEBG treatments had no significant difference, but results were significantly lower than in other treatments (L and Q P < 0.05). For fraction (a) of starch degradability, no significant difference was found between the 0GEBG and 5GEBG treatments, but the amount of this fraction in the 2 mentioned treatments was significantly higher than in other treatments (L P < 0.05). Among experimental treatments, 20GEBG had the lowest fraction (a) of starch degradability (L P < 0.05, Table 8). For fraction (b) of starch degradability, the 0GEBG and 5GEBG treatments were not significantly different, but the amount of this fraction in the 2 treatments was significantly higher than in other treatments (L and Q P < 0.05). The 15GEBG and 20GEBG treatments had no significant difference with regard to fraction (b) of starch degradability, but the amount of this fraction in the 2 treatments was significantly lower than in other experimental treatments (L and Q P < 0.05, Table 8). With regard to the potential of starch degradability (a + b), the 0GEBG and 15GEBG treatments had no significant difference, but the amount of this fraction in the 2 treatments was significantly higher than in other treatments (L and Q P < 0.05). The fraction of starch degradability was not statistically different between 15GEBG and 20GEBG, but its value in those 2 treatments was significantly lower than in the other treatments (L and Q P < 0.05, Table 8). The highest constant rate of starch degradability (c) was related to 0GEBG (L P < 0.05, Table 8). The 15GEBG and 20GEBG treatments had the lowest (L and Q P < 0.05) effective degradability of starch in all outflow rates. The 0GEBG and 5GEBG groups had the highest effective degradability of starch (L and Q P < 0.05, Table 8).

3.2. Intestine digestibility

Among experimental treatments, 10GEBG had the highest DM intestinal digestibility (L and Q P < 0.05). With regard to this, no significant difference was found between the 0GEBG and 5GEBG groups. The lowest intestinal digestibility of DM was associated with the 15GEBG and 20GEBG groups (L and Q P < 0.05, Table 4).

The intestinal CP digestibility of the different treatments is shown in Table 6. The 10GEBG group had the highest intestinal digestibility of CP (L and Q P < 0.05), while 15GEBG and 20GEBG had the lowest intestinal digestibility of CP (L and Q P < 0.05). No significant difference was found between 0GEBG and 5GEBG with regard to CP intestinal digestibility.

The lowest intestinal digestibility of starch was found with 15GEBG and 20GEBG (L and Q P < 0.05). The 10GEBG group had the highest intestinal digestibility of starch among all treatments (L and Q P < 0.05). No

Table 7. The ruminal starch degradability (%) of barley grain (BG) treated with different levels of green tea waste extract (GE) at different hours of incubation.

Treatments	Hours of incubation							
	0	2	4	6	8	12	24	48
0GEBG	28.8^{a}	32.8^{a}	39.8^{a}	45.9^{a}	52.9^{a}	62.8^{a}	78.8^{a}	90.8^{a}
5GEBG	28.8^{a}	30.1^{a}	38.1^{a}	45.1^{a}	50.1^{b}	60.9^{a}	78.1^{a}	89.0^{a}
10GEBG	24.6^{b}	27.4^{b}	31.5^{b}	36.5^{b}	40.7^{c}	52.7^{b}	67.8^{b}	79.7^{b}
15GEBG	21.1^{c}	23.1^{c}	26.0^{c}	31.5^{c}	35.7^{d}	45.8^{c}	59.9^{c}	70.9^{c}
20GEBG	20.1^{c}	23.9^{c}	25.8^{c}	30.8^{c}	34.8^{d}	45.8^{c}	61.5^{c}	71.0^{c}
SEM*	1.21	1.16	1.21	1.15	1.24	1.24	1.34	1.27
P value L¶	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P value Q‡	0.05	0.16	0.01	0.02	0.24	0.01	0.001	0.001

*, Standard error of the mean; L¶, linear; Q‡, quadratic; $^{a–d}$, means in the same column not sharing the same superscript are significantly different (P < 0.05).

Table 8. The ruminal degradability parameters and intestinal digestibility of starch of barley grain (BG) treated with different levels of green tea waste extract (GE).

Treatments	Degradability parameters*							
	a	b	(a + b)	c	ED_2	ED_5	ED_8	StarchD
0GEBG	26.7[a]	68.5[a]	95.2[a]	0.058[a]	77.1[a]	63.8[a]	55.9[a]	32.1[b]
5GEBG	26.0[a]	68.3[a]	94.4[a]	0.056[b]	76.6[a]	62.3[a]	54.4[a]	33.2[b]
10GEBG	22.0[b]	65.3[b]	87.3[b]	0.047[c]	67.9[b]	53.7[b]	46.3[b]	39.9[a]
15GEBG	18.3[c]	60.0[c]	78.3[c]	0.045[c]	60.1[c]	47.1[c]	40.3[c]	28.1[c]
20GEBG	18.1[c]	60.8[c]	79.0[c]	0.046[c]	60.5[c]	47.3[c]	40.4[c]	28.1[c]
SEM¶	1.34	0.54	1.37	0.002	1.25	1.24	1.22	1.21
P value L‡	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
P value Q‡	0.08	0.0001	0.0008	0.66	0.002	0.01	0.01	0.0001

* Parameters: a, rapidly degradable fraction (%); b, slowly degradable fraction (%); (a + b), potential degradability (%); c, degradation rate of the fraction b (h – 1); ED, effective degradability for passage rates 2%, 5%, and 8%/h; StarchD, starch intestinal digestibility (%); ¶, standard error of the mean; L‡, linear; Q‡, quadratic; [a–d], means in the same column not sharing the same superscript are significantly different ($P < 0.05$).

difference was found between 0GEBG and 5GEBG with regard to intestinal digestibility of starch (Table 8).

4. Discussion

4.1. Ruminal degradability

The results of the present study indicate that GE reduced the ruminal degradability of DM, CP, and starch of BG. Consistent with our results, Martinez et al. (28) reported that using tannic acid reduced ruminal DM, CP, and starch degradability of BG; however, they did not observe any significant effect on the ruminal degradability of BG starch. In accordance with the results of this research, Waldo (29) reported that using tannins reduced the ruminal degradability of starch.

Decreasing the ruminal degradability of proteins, peptides, and deamination of amino acids can lead to reducing ruminal CP degradability of treated BG through GE (30). Reduction of protein degradation may be due to the formation of tannin that binds with the BG protein, reducing its solubility and protecting protein from rapid degradation in the rumen (17). Phenolic compounds and the action of aldehydes in protecting starch against ruminal degradation are somewhat similar (28). Cereal starch is available in the form of capsulated granules that can bind proteins with aldehyde compounds. Therefore, processing cereals with aldehydes can protect starch from ruminal degradation (10). Hence, the phenolic compounds of GE can reduce ruminal degradation of the BG starch through the same mechanism that includes passing it into the small intestine.

4.2. Intestinal digestibility

The highest intestinal DM, protein, and starch digestibility associated with 10GEBG may be related to the effect of tannins on the intestinal digestibility of CP. Indeed, Frutos et al. (31) reported that intestinal digestibility of CP of soybean meal was reduced through treatment with high levels (25 g/kg) of tannins. However, Alipour and Rouzbehan (32) observed that the use of tannin extracts of grape pomace increased the intestinal digestibility of CP of soybean meal. Tannins make noncovalent hydrogen bonds with other molecules, which are reversible in the abomasum and small intestine pH. Thus, these phenolic compounds facilitate passing of proteins from the rumen, and bioavailability in the small intestine increases after breaking down tannin–protein bonds (28). By reducing the rumen degradation of CP by tannins, more protein passes through the rumen; this can be effective in increasing intestinal digestibility of CP, as shown in this study (32). Due to the effect of tannins on the reduction of rumen DM and disappearance of crude protein in BG, it is possible to increase the flow of starch from the rumen to the small intestine; this has an impact on the intestinal digestion of the starch (28).

In conclusion, based on the results of this study, processing BG with GE reduced the ruminal degradability of DM, CP, and starch. Among various treatments, 10% GE improved the intestinal digestion of mentioned nutrients; therefore, it can be used to protect barley grain against ruminal degradability.

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