

Rumen Undegradable Protein (RUP) and Its Intestinal Digestibility after Steam Flaking of Cereal Grains

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ABSTRACT

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While it is known that heat treatment of cereal grains generally improves the nutritional value for ruminants, simultaneous information on rumen degradability and intestinal digestibility of the rumen by-pass is scarce, especially for non-starch constituents. The effect of steam flaking at 90°C for 30 min on protein quality of maize, wheat, and barley was studied. In addition to proximal chemical analyses, protein rumen degradability was determined *in vitro* and intestinal digestibility of rumen undegraded protein was determined using the mobile bag method. No significant effects of steam flaking on chemical composition of cereal grains (crude protein, acid detergent fibre, neutral detergent fibre, and starch) were observed. The protein fractions that are relevant to rumen degradability were significantly influenced by the steam flaking: the non-protein nitrogen fraction (A) was reduced ($P < 0.05$) by 43–93% for all three cereal grains, whereas the fraction that represents true soluble protein (B1) was reduced ($P < 0.05$) for wheat grains (by 77%) and barley (by 93%). Although the difference was not significant, a decrease of 41% for B1 fraction was also observed for maize. On the other hand, steam flaking markedly increased buffer insoluble but neutral detergent soluble protein fraction (B2) by 15–25% for all three cereal grains, whereas effects on B3 fraction were not significant. Steam flaking was also associated with an increase of the rumen undegradable protein fraction (C) in the case of barley, from 6.6 to 11.1% of total N ($P < 0.05$) and wheat, from 5.2 to 8.4% of the total N ($P < 0.05$). Overall, the changes of the protein fractions led to a considerable increase of the rumen undegradable protein (RUP) for all steam-flaked cereal grains ($P < 0.05$). Steam flaking also increased ($P < 0.05$) intestinal digestibility of RUP of the cereal grains (by 3.6–34.8%), leading to a more efficient protein use in ruminants.

Keywords: heat treatment; protein fractions; protein degradability

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Cereals are used as energy, but also as protein source for ruminants. Although the content is low, the protein from these grains can constitute a significant part of the overall dietary protein supply. Better nutrient utilization by ruminants can be obtained by applying various processing methods. For example, heat treatment of cereal grains may improve their feeding value for ruminants by influencing the ruminal degradation of starch and protein and their post-ruminal digestion (Prestlokken 1999; Razzaghi et al. 2016).

Steam flaking involves moderate to high temperatures, uptake of moisture, and physical disruption of the starch kernel by mechanical forces. The primary purpose of flaking is to change the physical form of the grain and to make it more digestible. Main focus of the previous trials aiming to assess effects of the heat treatment on cereals was starch digestion and utilization (Plascencia et al. 2011). However, the heat treatment also influences the properties of non-starch organic matter, such as proteins (Corona et al. 2005; May et al. 2009).

Proteins from cereal grains such as wheat, barley, and oat are considered highly degradable in the rumen (Ceresnakova et al. 2000; Kopceková et al. 2008). Fiems et al. (1990) showed that grains processing, such as steam flaking, decreased ruminal degradability of crude protein. Heat treatment of plant proteins often reduces the amount of soluble and degradable protein in the rumen and increases the amount of rumen undegraded protein (RUP). This RUP might have a high intestinal digestibility if not heat-damaged (Dakowski et al. 1996). Therefore, in the case of ruminants, the heat treatment of feeds is a matter of optimization: while lowering ruminal degradability is desirable, lowering intestinal digestibility is to be avoided.

Modern systems for protein evaluation in ruminants are evolving toward a better prediction of the rumen undegradable protein and its intestinal digestibility. Moreover, some of them, such as the Cornell Net Carbohydrate and Protein System – CNCPS (Fox et al. 2004), take into account the dietary protein fractions in a more detailed manner, which is relevant from the viewpoint of the potential effects of heat treatments, such as steam flaking, on protein quality. However, the results in this direction are still scarce, preventing an appropriate prediction of the real protein values of the processed cereal grains.

The objective of this experiment was to evaluate the effects of steam flaking on protein quality in

maize, wheat, and barley, upon their effects on the various fractions of the dietary proteins. The evaluation focused on the differences in solubility and degradability of proteins as well as on the intestinal digestibility of RUP.

MATERIAL AND METHODS

The experimental procedures were reviewed and approved by the Animal Care Committee of the National Agricultural and Food Centre, Research Institute for Animal Production Nitra and by the Research & Development Ethical Committee of INCDBNA, Balotesti, Romania.

Sample description and preparation. Wheat, maize, and barley grains were steam-flaked or left untreated (control). Steam flaking was done at 90°C, for 30 min, and the resulting flake density was 440–460 g/l. Ten samples were collected and analysed for each experimental group.

All 60 samples (3 × 10 for untreated and 3 × 10 for steam-flaked grains) were ground in a hammer mill, using a 3-mm screen for *in vitro* digestibility and *in situ* incubations and a 1-mm screen for chemical analyses and solubility assessment.

Chemical analyses and *in vitro* digestibility. Nitrogen (Kjeldahl method) and dry matter were analysed according to European Commission Regulation (EC) No 152/2009, whereas neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin were determined by van Soest procedures (Lutonska and Pichl 1983). Starch content was determined by the enzymatic method according to Salomonsson et al. (1984). Nitrogen fractionation was based on solubility in borate-phosphate buffer and in detergent solutions (Licitra et al. 1996), whereas tungstic acid (TA) was used for non-protein nitrogen (NPN) determination instead of trichloroacetic acid. *In vitro* organic matter digestibility was estimated both by the Tilley-Terry method (Tilley and Terry 1963) and the pepsin-cellulase method (Aufrère et al. 2007).

Rumen degradability. Rumen degradability of the studied feeds was assessed according to the NRC system (NRC 2001), where five fractions of crude protein (CP) – A, B1, B2, B3, and C – are identified using borate-phosphate buffer as well as acid detergent and neutral detergent solutions.

The rumen degradable protein (RDP) value was calculated according to the following formula:

$$\text{RDP} = A + B_1 \left[\frac{\text{kd}_{B_1}}{\text{kd}_{B_1} + \text{kp}} \right] + B_2 \left[\frac{\text{kd}_{B_2}}{\text{kd}_{B_2} + \text{kp}} \right] + B_3 \left[\frac{\text{kd}_{B_3}}{\text{kd}_{B_3} + \text{kp}} \right]$$

where:

A = percentage of CP that is soluble in borate-phosphate buffer but not precipitated with a protein denaturant, tungstic acid

C = percentage of total CP recovered with ADF (i.e. ADIN)

B₁ = percentage of CP soluble in borate-phosphate buffer and precipitated with TA

B₃ = difference between the portions of total CP recovered in NDF (i.e. NDIN) and ADF (i.e. fraction C)

B₂ = the remaining CP, calculated as total CP minus the sum of fractions A, B₁, B₃, and C

kd_{B1}, kd_{B2}, kd_{B3} = rates of rumen degradation of fractions B₁, B₂, and B₃, respectively

kp = rate of passage from the rumen

Degradation rates of individual N fractions and passage rate were used from CNCPS (NRC 2001). The applied degradation rates (per h) were: for wheat, kd_{B1} = 0.30, kd_{B2} = 0.16, kd_{B3} = 0.05; for maize, kd_{B1} = 0.1, kd_{B2} = 0.10, kd_{B3} = 0.06; for barley, kd_{B1} = 0.30, kd_{B2} = 0.14, kd_{B3} = 0.05; kp = 0.08% per h. The undegraded protein passing to the small intestine was calculated as the difference up to 100: RUP = 100 – RDP.

Intestinal digestibility of the rumen by-pass.

Intestinal digestibility of RUP was determined by the mobile bag technique (Hvelplund et al. 1992), using cows with T-cannula installed in the distal duodenum. Three rumen fistulated cows were used for 16-hour rumen incubations. The cows were fed a typical diet consisting of maize silage (17 kg), alfalfa hay (4.5 kg), and a concentrate mixture based on cereal grains (43% wheat, 43% barley, and 14% protein-vitamin-mineral premix). The diet was

calculated for maintenance nutritive requirements and was fed twice daily, in equal meals.

Incubation residues were lyophilised, then weighed (about 0.3–0.5 g) and introduced into small bags (3 × 5 cm, pore size 42 µm; Uhelon).

The bags were incubated for 1 h at 37°C in pepsin/0.1M HCl solution. Pepsin with 700 U/g activity (Merck, Germany) was used. Thereafter bags were inserted into the duodenum and, after recovery from the faeces, washed in tap water, dried, and analysed for nitrogen content. The bags were collected within 20 h after insertion into the duodenum.

Statistical analysis. The effect of steam flaking versus non-treating was assessed using the Student's *t*-test; significance was declared at *P* < 0.05.

RESULTS AND DISCUSSION

Chemical composition and in vitro digestibility. The chemical composition of untreated or steam-flaked wheat, maize, and barley grains is presented in Table 1. Although no consistent and significant effect of the steam flaking on the content of nutrients was detected, a tendency for higher starch content (significant in the case of maize) and lower NDF content was noticed. Such differences in the starch content between untreated and heat-treated cereal grains were also observed by Tothi et al. (2008). Also, in other studies, heat treatment increased the solubility of fibres (and decreased the NDF), but the observed effects might be analytical rather than nutritional (Vranjes and Wenk 1995). In contrast to our results, where no consistent and significant effect on CP was observed, a higher CP content of the

Table 1. Chemical composition of untreated and treated cereal grains (means ± SEM)

Studied feeds	<i>n</i>	Content of nutrients (g/kg DM)			
		CP	starch	NDF	ADF
Wheat, untreated	10	135.0 ± 4.77	681.2 ± 4.31	120.1 ± 5.35	39.0 ± 5.89
Wheat, steam-flaked	10	128.9 ± 1.33	689.7 ± 2.27	120.2 ± 9.67	45.2 ± 1.54
Maize, untreated	10	91.9 ± 1.74	727.0 ± 0.40 ^a	108.6 ± 6.28	30.4 ± 3.04
Maize, steam-flaked	10	89.2 ± 1.04	780.0 ± 7.36 ^b	93.8 ± 11.66	28.7 ± 0.90
Barley, untreated	10	120.9 ± 0.48	597.1 ± 2.40	173.9 ± 3.62	58.2 ± 1.97
Barley, steam-flaked	10	120.6 ± 1.02	589.8 ± 2.30	168.4 ± 0.05	63.9 ± 4.19

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre

^{a,b}means within grain type with different superscript in the same column are significantly different (*P* < 0.05)

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Table 2. *In vitro* organic matter digestibility of the studied feeds

Studied feeds	Tilley-Terry digestibility	Pepsin-cellulase digestibility
Wheat, untreated	92.14 ± 0.80	95.94 ± 0.11
Wheat, steam-flaked	90.46 ± 1.01	94.83 ± 0.24
Maize, untreated	92.19 ± 0.81	93.42 ± 0.98
Maize, steam-flaked	91.86 ± 0.73	96.75 ± 0.21
Barley, untreated	85.91 ± 1.20	86.04 ± 0.03
Barley, steam-flaked	84.01 ± 0.43	89.44 ± 0.53

steam-flaked maize compared with untreated maize was reported by Swift (1997). On the other hand, Berger (2006) found a lower content of CP in steam-flaked maize than in the whole grain. Also, Kelzer et al. (2010) found a slightly lower protein content in distillers grains exposed to high temperature. However, overall, such differences in the chemical composition are not likely to induce noticeable changes in protein value of the steam-flaked cereal grains.

Organic matter digestibility, assessed *in vitro* both by the Tilley-Terry and the pepsin-cellulase methods (Table 2) showed no significant differences between treated and untreated cereal grains. As the two methods are estimators of the total tract digestibility, the lack of difference between treated and untreated cereal grains suggested that steam flaking did not render the protein indigestible. Other authors (Corona et al. 2005; Kelzer et al. 2010) reported either positive or negative effects of the heat treatment on the total tract digestibility of cereals; however the magnitude of these effects was rather small, which is consistent with the results of the current study.

Rumen degradability. In the current study, the steam flaking treatment of cereal grains influenced rumen protein degradability, assessed by the CNPS. Steam flaking significantly reduced the proportion of protein soluble in borate buffer (A + B1 protein fractions), as shown in Table 3. The largest decrease was observed for barley (from 26.2 to 2.0%) followed by wheat (from 28.4 to 10.1%). While other authors (Lykos and Varga 1995) found similar decrease in the case of maize steam flaking (from 22.3 to 1.7%), in our study the extent of decrease is less pronounced, although still noticeable (from 15.8 to 4.0%). This is consistent with the fact that maize is generally less degradable in the rumen, presumably due to a lower proportion of soluble proteins.

This reduction of protein solubility is important from the nutritional point of view as it contributes to the reduction of protein degradability in rumen, which in most feeding situations is considered an advantage.

While the effects of steam flaking on the soluble fractions of cereal grains nitrogen are easy to determine and often reported, the effects on various protein fractions are scarcely reported.

Almost all CNCPS protein fractions were significantly influenced by the heat treatment for all three cereals (Table 3). The fraction, representing the NPN, decreased significantly ($P < 0.05$) in all steam-flaked grains, whereas the fraction representing the true soluble protein (B1) was reduced by approximately 77% in steam-flaked wheat ($P < 0.05$) and by up to 93% in steam-flaked barley ($P < 0.05$), compared to the untreated samples.

Steam flaking does not influence B3 fraction; however, B3 does not constitute a high proportion of cereal grains' protein except for maize.

Table 3. Protein fractions (means ± SEM) according to Cornell Net Carbohydrate and Protein System (CNCPS) in untreated and in steam-flaked cereal grains

Studied feeds	n	Protein fractions (% of total CP)				
		A	B ₁	B ₂	B ₃	C
Wheat, untreated	10	11.8 ± 2.16 ^a	16.6 ± 2.46 ^a	58.8 ± 1.03 ^a	8.9 ± 1.54	5.2 ± 0.13 ^a
Wheat, steam-flaked	10	6.2 ± 0.22 ^b	3.9 ± 1.03 ^b	74.8 ± 1.97 ^b	7.7 ± 1.43	8.4 ± 0.07 ^b
Maize, untreated	10	10.2 ± 3.02 ^a	5.6 ± 3.01	67.2 ± 3.32 ^a	7.8 ± 2.92	9.9 ± 2.17
Maize, steam-flaked	10	0.7 ± 0.36 ^b	3.3 ± 0.28	79.1 ± 0.02 ^b	7.3 ± 0.89	11.0 ± 0.38
Barley, untreated	10	13.2 ± 0.88 ^a	13.0 ± 0.05 ^a	57.0 ± 0.21 ^a	9.1 ± 0.48	6.6 ± 0.46 ^a
Barley, steam-flaked	10	1.1 ± 0.05 ^b	0.9 ± 0.09 ^b	75.9 ± 0.42 ^b	8.9 ± 1.31	11.1 ± 0.69 ^b

CP = crude protein

^{a,b}means within grain type with different superscript in the same column are significantly different ($P < 0.05$)

Also, fraction B3 is slowly degraded in the rumen because it is associated with the cell wall; within this context its variation (e.g. caused by heat treatment) does not noticeably influence the overall nitrogen degradability in the rumen.

In the present study, steam flaking influenced C fraction differently, depending on the cereal species: it increased significantly in wheat (from 5.2 to 8.4 g/100 g CP) and barley (from 6.6 to 11.1 g/100 g CP), while the influence was not significant for maize (Table 3). The C fraction is usually associated with lignin, tannins, and protein complexes of Maillard products and is generally considered both undegradable in the rumen and indigestible in the small intestine (Lanzas et al. 2007), therefore its increase is likely to be associated with a higher loss of faecal N.

The B2 fraction also increased significantly after steam flaking, by 27.2% in the case of wheat, 17.7% in the case of maize, and 33.1% in the case of barley (Table 3). The B2 fraction represents the insoluble but degradable protein and forms the largest fraction in both untreated and treated cereals (NRC 2001); in the current study being much higher than the sum of all other fractions (A + B1 + B3 + C).

Overall, the easily degradable protein fractions (A + B1) were reduced by steam flaking, whereas the slowly degradable fractions (B2 + B3) were increased. It is important to underline that fraction B2 represents a major proportion of dietary proteins, which together with microbial and endogenous protein contributes to metabolizable protein supply.

Steam flaking decreased the RDP and increased the RUP for all three cereal grains, in various

extents (Table 4). Reduction of rumen protein degradability following heat treatment (pelleting or expanding; temperatures varying from 80 to 130°C) was also observed by Ljokjel et al. 2003 for barley and wheat, but not for maize. However, other authors (Lykos and Varga 1995; Kelzer et al. 2010) reported depressing effects of the heat treatments on the rumen degradability of maize proteins, which support the effects found in this study.

Intestinal digestibility of the rumen protein by-pass. The same reactions that are responsible for decreasing the rumen degradability of protein may determine the decrease of the intestinal digestibility of the rumen by-pass (Prestlokken 1999). Therefore, the challenge is to lower the rumen degradability without depressing the intestinal digestibility of rumen escape protein, too, which is a matter of optimisation of the processing conditions.

Over time, studies focusing on protein-rich feeds (e.g. rapeseed meal, Fava beans, etc.) retrieved identification of optimal processing conditions that ensures both reduced rumen degradability and increased digestibility of the rumen escape protein (Karlsson et al. 2012). As most of the previous studies addressing steam flaking of cereal grains focused on starch digestion, the information referring to the fate of proteins is rather scarce.

Results of the studies on protein-rich feeds suggested that, as long as the heat treatment is not excessive, the intestinal digestibility is not affected (Schroeder et al. 1996). In the present study, the cereal grains were subjected to a mild heat treatment, 90°C for 30 min, which is unlikely to compromise the intestinal digestibility. This is consistent with the fate of the protein fractions in

Table 4. Partition between rumen degradable (RDP) and undegradable protein (RUP) and intestinal digestibility of RUP for treated and untreated cereal grains (% of CP)

Studied feeds	RDP	RUP	ID	DP
Wheat, untreated	67.5 ± 1.12 ^a	32.5 ± 1.12 ^a	81.2 ± 3.82 ^a	26
Wheat, steam-flaked	59.9 ± 1.30 ^b	40.1 ± 1.30 ^b	96.8 ± 0.05 ^b	39
Maize, untreated	54.6 ± 0.64 ^a	45.4 ± 0.64 ^a	93.4 ± 1.01 ^a	42
Maize, steam-flaked	32.4 ± 0.83 ^b	67.6 ± 0.83 ^b	96.8 ± 0.28 ^b	65
Barley, untreated	63.2 ± 0.86 ^a	36.8 ± 0.86 ^a	71.2 ± 0.62 ^a	26
Barley, steam-flaked	50.8 ± 0.37 ^b	49.2 ± 0.37 ^b	96.0 ± 0.14 ^b	47

CP = crude protein, ID = intestinal digestibility of the rumen undegraded protein, DP = proportion of the RUP digestible in the intestine (RUP × ID/100)

^{a,b}means within grain type with different superscript in the same column are significantly different ($P < 0.05$)

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the rumen: the reduction of rumen degradability is achieved at the expense of the rapidly soluble fractions. Although C fraction (presumably indigestible in the intestine) increased, the increase of B2 fraction (digestible in the intestine) is even larger. Therefore, the structure of the rumen escape protein (undegraded B2 + B3 fractions vs C fraction) changed and makes it more digestible.

In the present study, the intestinal digestibility, measured using the mobile nylon bags method, increased from 71.2% (untreated barley), 81.2% (untreated wheat), and 93.4% (untreated maize) to 96.0–96.8% (Table 4). The authors of the present study are not aware of similar effects reported in the literature; however, previous findings indirectly support these results. Gonzalez et al. (2003) reported that heat treatment of maize led to an increase of the effective intestinal digestibility from 82.6 to 91.6%, an increase three times higher than that obtained for maize in the current study. Li et al. (2010) varied the processing conditions during corn distilling and found that, even if the RUP of the distillers grains varies with the processing conditions (e.g. temperature), the intestinal digestibility of the rumen protein bypass is rather constant (unaffected by the temperature). Safaei and Yang (2017) claimed that the shift of the site of protein digestion (from rumen to intestine), that is well documented in the case of heat-treated protein-rich feeds, also occurs in the case of steam-flaked cereal grains. However, it has to be noted that in the current study the flakes density was 0.44–0.46 kg/l, i.e. significantly higher than the flakes density usually encountered in the previous reports and above the values considered as optimal (0.32–0.39 kg/l) (Plascencia and Zinn 1996).

CONCLUSION

Steam flaking of the wheat, maize, and barley grains induced noticeable changes of the protein fractions that are relevant for protein degradability in rumen. Of these, fraction B2 increased by 17–33%, at the expense of A and B1 fractions, whose sum decreased by 65–92%. This means much less soluble protein and more insoluble, yet rumen degradable protein. Overall, these changes induced a decrease of the RDP/RUP ratio, which is favourable in most feeding situations.

Moderate heat treatment (steam flaking at 90°C for 30 min) also led to an increased digestibility of the rumen escape protein with 4–34%, depending on the cereal species. This also contributed to a higher protein value of the steam-flaked cereal grains.

As most ruminant diets have the disadvantage that the dietary protein is too degradable, the decrease of the protein degradability of the cereal grains contributes to a better overall protein efficiency. Furthermore, a higher intestinal digestibility of the rumen escape protein added even more to their real protein value. This is more important in the feeding situations where the cereal grains represent an important part of the ruminant diets.

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