ESTIMATION OF *IN VITRO* DRY MATTER SOLUBILITY AND PROTEIN DIGESTIBILITY OF BARLEY GRAINS

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Abstract

The biological availability of nutrients is of great importance in formulating a balanced ration to attain maximum productivity in animals. In vitro digestibility techniques are useful to provide a quick, inexpensive, and precise prediction of in vivo amino acid and protein digestibility for humans and animals. The objectives of the present study were to determine the in vitro dry matter solubility (DMS) and protein digestibility (PD) of barley grains. Two experiments were conducted: experiment 1 simulated gastric digestion and experiment 2 simulated gastric and small intestinal digestion. DMS in gastric digestion showed an increase with the incubation time. DMS of barley grains ranged in experiment 1 from 0.1067 g/g at 30 minutes digestion time, to 0.1475 g/g at 120 minutes digestion time. Samples showed higher DMS and PD after pepsin-pancreatin digestion than after pepsin digestion. DMS increased with 19.22% and PD increased with 16.18% when pepsin digestion was followed by 240 minutes intestinal digestion.

Key words: dry matter solubility, *in vitro* digestion, protein digestibility, barley

INTRODUCTION

Information on the digestibility of nutrients is of great importance when predicting the nutritional quality of feeds [5]. Digestibility is a measure of the biological availability of nutrients and it is important in formulating a balanced ration in order to obtain maximum productivity in animals. Only that part which is soluble or which becomes soluble by hydrolysis or other chemical or physical changes can be taken up into the circulation and used by the animal as building materials or energy source.

Determination of energy values of feeds by *in vivo* trials is expensive and time-consuming, and requires animal facilities and relatively large amounts of experimental diets.

Measurement of *in vitro* dry matter digestibility (DMD) and protein digestibility (PD) have been used extensively to analyze feeds because of a high degree of correlation to *in vivo* digestibility [7]. *In vitro* protein digestibility assays must be accurate, rapid, cheap, simple, robust, adaptable and relevant to the processes of digestion, absorption, and metabolism.

The *in vitro* techniques simulate the digestion process, using either an inoculum prepared from pig digestive contents [6], or enzymatic preparations [2, 3]. The hydrolysis of a particular bond depends on the specificities of the enzymes and on the access of the enzyme to the substrate. Therefore, it is recommended that *in vitro* incubations include the same enzymes as those occurring in the digestive tract [1].

In vitro methods used to assess feed ingredients, as potential components of monogastric diets, are based on consecutive incubations with pepsin and pancreatin, suggesting that pancreatin contains all the necessary enzymes for solubilizing the potentially digestible nutrients [4, 10, 11].

The two-step pepsin-pancreatin system simulates the digestion in the stomach and the small intestine, and appears to be an effective system to predict organic matter digestibility in pigs [9], although it doesn't take into account some aspects of *in vivo*

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digestion, such as endogenous secretions, absorption, and transit [8, 12].

The objectives of the present study were to determine the *in vitro* dry matter solubility (DMS) and protein digestibility of barley grains.

MATERIAL AND METHOD

Two experiments were conducted. Experiment 1 (the first step), simulating the digestion in the stomach, was an enzymatic hydrolysis with a pepsin solution at pH 2.0 and 37°C, in the presence of chloramphenicol.

In experiment 2 (two-step simulation), first step was followed by hydrolysis with the multi-enzyme pancreatin (mixture of protease, amylase and lipase, from porcine pancreas), at pH 6.8 and 37°C, for 4 h.

The *in vitro* DMS and PD were calculated from the difference between concentrations in the sample and the indigested residue.

For the gastric digestion, a sample of 1 g air-dried material, ground to pass a 0.5 mm screen, was weighed with an accuracy of 0.1 mg into a 15 mL plastic centrifuge tube. To each sample were added 4 mL of phosphate buffer (0.l M, pH 6.0), 0.2 mL HCl 2M, and 1 mL freshly prepared 4% pepsin solution (P7012 Sigma-Aldrich; ≥ 2,500 units/mg of protein, from porcine gastric mucosa). In order to prevent bacterial growth, 0.5 mL of a chloramphenicol solution (0.5 g chloramphenicol, Sigma C-0378, per 100 ml ethanol) was added.

The tubes closed with stoppers were placed in a shaking water bath (LabTech LSB-015S) at 37°C, r = 120 rpm. The gastric digestion was monitored at different incubation times: 30, 60, 90, and 120 minutes. Samples were then centrifuged at 5000g for 10 minutes with a Hettich 320R centrifuge. Residue was dried for 16 h at 100°C and analyzed for DM solubility and crude protein content (CP).

For the gastric and intestinal digestion, 4 g air-dried material, ground to pass a 0.5 mm screen, was weighed with an accuracy of 0.1 mg into a 50 mL plastic centrifuge tube. After 120 minutes gastric digestion of the sample, 2 ml phosphate buffer (0.2 M, pH 6.8), 2 ml of 0.6 N NaOH (to adjust pH to 6.8), and 2 ml 2% pancreatin (Sigma P7545) were added to the mixture.

The tubes were incubated in the water bath with shaking at 120 rpm, at 37°C for 240 minutes. Samples were then centrifuged at 5000g for 10 minutes. Residue was dried for 16 h at 100°C and analyzed for DM solubility and CP content.

All samples for *in vitro* analysis were done in duplicate.

Dry matter solubility was calculated as the loss in weight due to digestion, calculated with the formula:

$DMS(g/g) = (G_0 - G_1)/(G_0)$,

where G_0 = DM before digestion and

G_1 = DM of residue after digestion.

Crude protein (CP) was determined by the macro-Kjeldahl technique (%N× 6.25) [12].

PD was calculated with the formula:

PD (g%) = (CP barley - CP undigested)/(CP barley) x100.

RESULTS AND DISCUSSIONS

DMS in gastric digestion showed an increase with the incubation time. Protein is hydrolyzed and solubilized mostly during gastric digestion.

Solubility values of dry matter after gastric digestion are presented in Table 1 and Figure 1. DMS of barley ranged in experiment 1 from 0.1067 g/g at 30 minutes digestion time, to 0.1475 g/g at 120 minutes digestion time.

DMS in experiment 2 was 0.1571 g/g at 120 minutes gastric digestion time.

After small intestinal digestion (240 minutes) samples showed higher DMS and PD compared to those achieved after gastric digestion. The results of the pepsin-pancreatin digestion, given in Table 2, show that 49.12% of the total protein was solubilized.

DMS increased with 19.22%, and PD increased with 16.18% when gastric digestion was followed by 240 minutes intestinal digestion (Figure 2). This is due to higher solubility of proteins and non-starch polysaccharides at higher pH values.

Table 1 DMS of barley in gastric digestion

Gastric digestion time (min)	DMS (g/g)
30	0.1067
60	0.1157
90	0.1392
120	0.1475

Table 2 DMS and PD of barley in gastric and intestinal digestion

Intestinal digestion time (min)	DMS (g/g)	PD (g%)
0	0.1571	42.28
240	0.1873	49.12

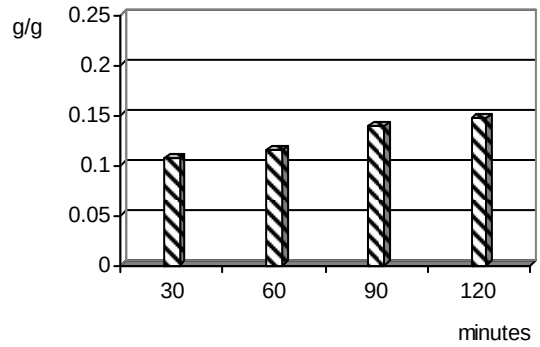

Fig. 1 Dynamics of DMS in gastric digestion of barley

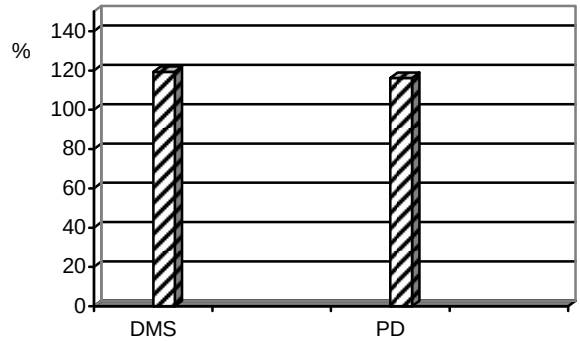

Fig. 2 Increase of DMS and PD in intestinal digestion of barley

CONCLUSIONS

DMS in gastric digestion showed an increase with the incubation time.

After small intestinal digestion (240 minutes) samples showed higher DMS and PD compared to those achieved after gastric digestion.

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