In Vitro Determination of Wheat Dry Matter Solubility and Protein Digestibility

Rodica Căpriță, Adrian Căpriță, Iuliana Crețescu, Valeria Nicu

Banat’s University of Agricultural Sciences and Veterinary Medicine from Timisoara – 300645, Calea Aradului 119, Romania

Abstract
The objectives of the present study were to determine the in vitro dry matter (DM) solubility and protein digestibility (PD) of wheat grains. Two experiments were conducted. In experiment 1, samples were incubated for different time periods with pepsin, simulating gastric digestion, and in experiment 2, samples were digested following an in vitro two-step procedure, simulating gastric and small intestine digestion. DM solubility in gastric digestion showed an increase with the incubation time. DM solubility of wheat ranged in experiment 1 from 0.1532 g/g at 30 minutes digestion time, to 0.1714 g/g at 120 minutes digestion time. Samples showed higher DM solubility and PD after small intestine incubation than after gastric incubation. DM solubility increased with 16.67% and PD increased with 24.87% when gastric digestion was followed by 240 minutes intestinal digestion.

Keywords: dry matter solubility, in vitro digestion, protein digestibility, wheat

1. Introduction
When predicting the nutritional quality of feeds, information on the digestibility of the various nutrients is of utmost importance. Digestibility is a measure of the availability of nutrients. The biological availability of nutrients is of great importance in formulating a balanced ration to attain maximum productivity in animals.

Direct determination of energy values of feeds by in vivo trials is expensive and time-consuming; it also requires animal facilities and relatively large amounts of experimental diets. Therefore, much effort has been devoted to the development of in vitro procedures.

Only that portion which is soluble or is rendered soluble by hydrolysis or some other chemical or physical change can be taken up into the circulation and assist in supplying the animal body with material for building and repair of tissue or supply the energy necessary for body functions. In addition, measures of digestibility are somewhat easier to obtain than measures of intake, and thus considerable effort has been made by animal nutritionists to develop effective means of determining digestibility [1]. 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 [2].

The specificities of the enzymes determine which bonds are hydrolyzed. However, the hydrolysis of a particular bond depends on the access of the enzyme to the substrate. Therefore, the degradation of other bonds by enzymes with other specificities will influence the total degradation. For that reason it is recommended that in vitro incubations include the same enzymes as those occurring in the digestive tract [3].

The in vitro techniques intend to simulate the digestion process, using either an inoculum prepared from pig digestive contents [4] or enzymatic preparations [5]. Many in vitro methods are based on consecutive incubations with pepsin and pancreatin, suggesting that pancreatin contains all the...
necessary enzymes for solubilizing the potentially digestible nutrients [6,7,8].

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 digestion such as endogenous secretions, absorption, and transit [10,11].

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

2. Materials and methods

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 DM solubility 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 (0.1 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 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:

\[
\text{DM solubility (g/g)} = \frac{(G_0 - G_1)}{G_0},
\]

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:

\[
\text{PD (g%)} = \frac{(\text{CP wheat} - \text{CP undigested})}{(\text{CP wheat})} \times 100.
\]

3. Results and discussion

DM solubility 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. DM solubility of wheat ranged in experiment 1 from 0.1532 g/g at 30 minutes digestion time, to 0.1714 g/g at 120 minutes digestion time.

DM solubility in experiment 2 was 0.1832 g/g at 120 minutes gastric digestion time. After small intestinal digestion (240 minutes) samples showed higher DM solubility and PD compared to those achieved after gastric digestion. The results of the pepsin-pancreatin digestion, given in Table 2, show that 54.87% of the total protein was solubilized.
Table 1. DM solubility of wheat in gastric digestion

<table>
<thead>
<tr>
<th>Gastric digestion time (min)</th>
<th>DM solubility (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.1532</td>
</tr>
<tr>
<td>60</td>
<td>0.1604</td>
</tr>
<tr>
<td>90</td>
<td>0.1644</td>
</tr>
<tr>
<td>120</td>
<td>0.1714</td>
</tr>
</tbody>
</table>

Table 2. DM solubility and PD of wheat in gastric and intestinal digestion

<table>
<thead>
<tr>
<th>Intestinal digestion time (min)</th>
<th>DM solubility (g/g)</th>
<th>PD (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.1832</td>
<td>43.94</td>
</tr>
<tr>
<td>240</td>
<td>0.2083</td>
<td>54.87</td>
</tr>
</tbody>
</table>

Figure 1. Dynamics of DM solubility in gastric digestion of wheat

DM solubility increased with 16.67%, and PD increased with 24.87% 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.

Figure 2. Increase of DM solubility and PD in intestinal digestion of wheat

4. Conclusions

DM solubility in gastric digestion showed an increase with the incubation time. After small intestinal digestion (240 minutes) samples showed higher DM solubility and PD compared to those achieved after gastric digestion.

Acknowledgements

This work was supported by CNCSIS–UEFISCSU, project number 1054/2009 PNII – IDEI code 894/2008.

References

