Determining available lysine in processed feedstuffs

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Introduction

Many protein sources, commonly used in feedstuffs for pig production, undergo some form of processing. During processing, proteins can be exposed to heat, both wet and dry, pressure and alkali. When feedstuffs are subjected to these kinds of conditions certain amino acids can react with other compounds present in the food resulting in nutritionally unavailable compounds. Lysine is particularly susceptible to this type of modification and can react with compounds, especially reducing sugars, that may be present in a feedstuff to form Maillard compounds (Hurrell and Carpenter, 1981). Maillard compounds are not acid stable and during conventional amino acid analysis a proportion of these Maillard compounds revert back to lysine which leads to an overestimation (as much as 100%) of the amount of lysine present in the feedstuff. The formation of these Maillard compounds causes serious problems when attempting to determine the available lysine content of a processed feedstuff.

Several methods have been developed to determine available lysine including animal growth assays, reactive lysine chemical methods (reactive lysine being the lysine that has remained unmodified during processing) and digestibility assays. Growth assays determine the growth rates of pigs fed increasing levels of lysine, these growth rates are then compared to those of animals fed a test feedstuff and from this comparison the available lysine content of the feedstuff can be estimated. These assays are laborious and time consuming, are highly variable and the results are difficult to interpret. Reactive lysine assays (e.g. FDNB reactive lysine), which are chemical assays that measure the unmodified lysine content of a feedstuff, do provide an accurate estimate of the potentially available lysine in a feedstuff assuming that all the available lysine was digested and absorbed. However, for many protein sources not all the reactive lysine is digested and absorbed from the small intestine of the animal and therefore reactive lysine assays are not always reliable methods for determining available lysine (Moughan et al., 1996). Ileal digestibility assays measure the lysine content in the feedstuff and also the undigested lysine at the end of the small intestine (ileal digesta) of an animal fed that feedstuff. From the difference between dietary lysine and undigested lysine in the digesta the proportion of lysine that has been digested and absorbed can be calculated. The ileal digestibility assay does accurately determine lysine digestibility for unprocessed proteins as well as the digestibility of most amino acids in
processed feedstuffs but, since this method uses amino acid analysis to determine lysine content in diets and digesta, it does not accurately determine lysine digestibility when applied to processed feedstuffs. Essentially, therefore, there is currently no reliable method for measuring available lysine in processed protein sources.

**The true ileal digestible reactive lysine assay**

The true ileal digestible reactive lysine assay (available lysine assay) has been developed by Moughan and Rutherfurd (1996) and has been thoroughly reviewed (Rutherfurd and Moughan, 2007). The assay combines the guanidination method and a true ileal digestibility assay. The guanidination method (a method for determining reactive lysine) involves the conversion of reactive lysine to the stable compound homoarginine which can then be determined by conventional amino acid analysis. Essentially, the test feedstuff is fed to a group of animals and the digesta are collected from these animals. The reactive lysine content of both the diet and digesta are determined using the guanidination method and the digestibility of reactive lysine is then calculated. This digestibility estimate is corrected to a true digestibility estimate by correcting for the endogenous lysine that is secreted into the pigs small intestine in the form of mucus, digestive enzymes and sloughed gut cells. Correction for endogenous loss is made using the enzyme hydrolysed casein technique (Butts et al., 1991; Moughan et al., 1990). The reactive lysine content of the original feedstuff is also determined and multiplied by the reactive lysine digestibility value resulting in an estimate of the true ileal digestible reactive lysine content of the feedstuff. The digestible reactive lysine content is by definition the available lysine content and as such can be used in the formulation of pig diets.

A comparison of digestible reactive lysine (new assay) with conventional digestible total lysine in a variably heated skim milk powder has been reported by Rutherfurd and Moughan (1997) and is shown in Table 1. For all heat treatments, digestible total lysine overestimated the digestible reactive lysine (available lysine) content. The overestimation ranged from 12% after only 1 min heating to a very large 50% after 10 min heating clearly demonstrating firstly, the inaccuracy of the traditional method in determining available lysine in heated proteins, and secondly, the sensitivity of the new assay in detecting differences in available lysine in proteins that have undergone relatively minor heating.

The assay has also been applied to a range of commercially available processed protein sources (Rutherfurd et al., 1997a). For some protein sources under certain processing conditions the digestible total lysine was similar to the digestible reactive lysine, for example, a blood meal sample (85.9 g kg\(^{-1}\) compared to 85.1 g kg\(^{-1}\)). However, for a number of processed protein sources the digestible total lysine was significantly different from the digestible reactive lysine
(available lysine) content, for example, cottonseed meal (12.9 g kg\(^{-1}\) compared to 10.3 g kg\(^{-1}\)), heated lactose/casein (45.2 g kg\(^{-1}\) compared to 50.5 g kg\(^{-1}\)) and wheat meal (3.2 g kg\(^{-1}\) compared to 2.9 g kg\(^{-1}\)). These results highlight the inadequacy of the traditional digestibility assay and the value of the new assay for determining digestible reactive lysine (available lysine) in processed feedstuffs.

Table 1. Comparison of the digestible total lysine (conventional digestibility assay) and digestible reactive lysine content (available lysine)(g kg\(^{-1}\) sample) in a variably heated skim milk powder. From Rutherfurd and Moughan (1997).

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Digestible total lysine</th>
<th>Digestible reactive lysine</th>
<th>Overall SE</th>
<th>Significance</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.8</td>
<td>38.1</td>
<td>0.09</td>
<td>***</td>
<td>3.4</td>
</tr>
<tr>
<td>1</td>
<td>31.6</td>
<td>28.0</td>
<td>0.53</td>
<td>***</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>19.8</td>
<td>16.6</td>
<td>0.25</td>
<td>***</td>
<td>15.8</td>
</tr>
<tr>
<td>5</td>
<td>13.7</td>
<td>11.0</td>
<td>0.62</td>
<td>*</td>
<td>19.8</td>
</tr>
<tr>
<td>10</td>
<td>11.2</td>
<td>5.7</td>
<td>0.73</td>
<td>***</td>
<td>49.5</td>
</tr>
</tbody>
</table>

1Skim milk powder was autoclaved at 121°C for 1 to 10 minutes.

Feedstuffs that have undergone processing clearly contain different levels of available lysine than has been originally thought. This new assay not only highlights a weakness in the evaluation of foods, i.e. the inaccurate determination of available lysine in processed protein sources, but also allows for a more accurate assessment of available lysine and consequently more accurate formulation and description of feedstuffs.

The accuracy of the digestible reactive lysine assay has been evaluated using a detailed validation study with growing pigs (Rutherfurd et al., 1997b). The results from this study clearly demonstrated that the assay accurately predicts the uptake of available lysine in heated proteins and that conversely the traditional ileal lysine digestibility assay is inaccurate.

Summary

The reaction between O-methylisourea and lysine (guanidination reaction) can be used to determine structurally unaltered lysine residues in heat-processed feedstuffs. Further, it can be uniquely used to determine structurally unaltered lysine residues in the digesta of animals fed these heat-processed feedstuffs. Therefore, if the guanidination reaction is used in conjunction with a true ileal amino acid digestibility assay the digestibility of reactive lysine can be determined.

References


