Using NIR to measure reactive lysine - the potential implications for the animal feed industry

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Lysine

Lysine is an essential amino acid for poultry and swine, meaning it cannot be produced via transamination and must be supplied to the animal through dietary intake. Lysine is usually a limiting amino acid if not supplemented in synthetic form, and due to its high concentration in tissue deposition, formulation of diets for amino acid requirements often relies upon ratios between lysine and all other amino acids. The total lysine that has been measured in the raw material or finished feed is not a measure of the bioavailability to the animal, this only comes with measuring the performance response to lysine in animal trials. Apparent ileal digestibility gives a measurement of the apparent disappearance of lysine in the small intestine. Standardised ileal digestibility, which accounts for basal endogenous losses, is widely accepted as the most advanced method for quantification of the dietary-origin amino acids digested and absorbed in the small intestine. Synthetic lysine is often added to diets, based on price, to supply animal requirements.

The Maillard Reaction

Reducing sugars are able to bind to free amino groups found on amino acids, especially the ε-amino group found on lysine. This is known as the Maillard reaction and results in the production of modified lysine, such as Amadori and Maillard products, including furosine and carboxymethyl-lysine (see Figure 1). The Maillard reaction is an irreversible reaction which makes the lysine unavailable for digestion. Reactive lysine, the lysine that has not undergone the Maillard reaction and is metabolisable, can be described as unmodified lysine which possesses a free side chain amino group and can be either free or protein bound. In the past, reactive lysine has been called a number of names including chemically reactive lysine, chemically available lysine, available lysine and total available lysine. Measuring total lysine in feeds involves the acid hydrolysis of the proteins present; during hydrolysis some of the damaged lysine can be released and analysed as lysine. However, as discussed above, this lysine is not available to be metabolised by the animal. To identify the lysine that can be both digested and metabolised by the animal, ileal digestible reactive lysine must be determined.

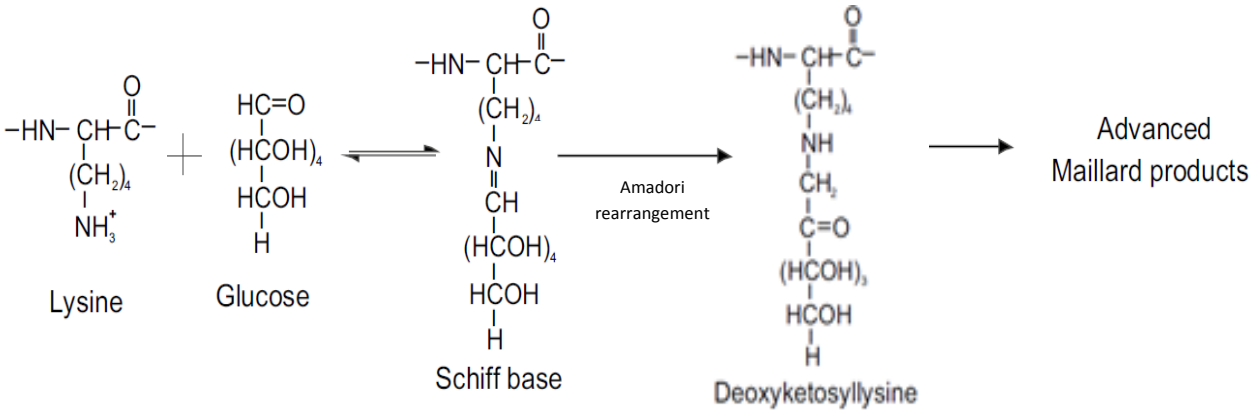

Figure 1. Maillard reaction of protein bound lysine with glucose. Adapted from Rutherford, 2015.

The effect of processing on lysine

Protein rich feedstuffs are often subjected to heat processing before inclusion in monogastric diets. During heat processing, cooking and long term storage at ambient temperature, the ε-amino group of lysine can react with other compounds including reducing sugars, fats and their oxidation products, polyphenols, vitamins and other amino acids. Processing is associated with increased levels of the Maillard products, furosine and carboxymethyl-lysine, as well as increased levels of lysinoalanine, a cross-linked amino acid that occurs due to heat treatment in soybean meal and canola meal. As such, measuring the content of reactive lysine as a % of total lysine of a raw material can act as a measure of heat damage during processing.

Attempts have been made to understand the effects of heat treatment on the reactive lysine using autoclaving. In a study by Kim and Mullan (2012), the effect of autoclaving on the reactive lysine content of soybean meal was investigated. A soybean meal sample was subject to increasing autoclaving time (0-30 minutes with increments of 5 minutes, 135°C). The results showed a strong correlation between autoclaving time and reactive lysine content (see Figure 2).

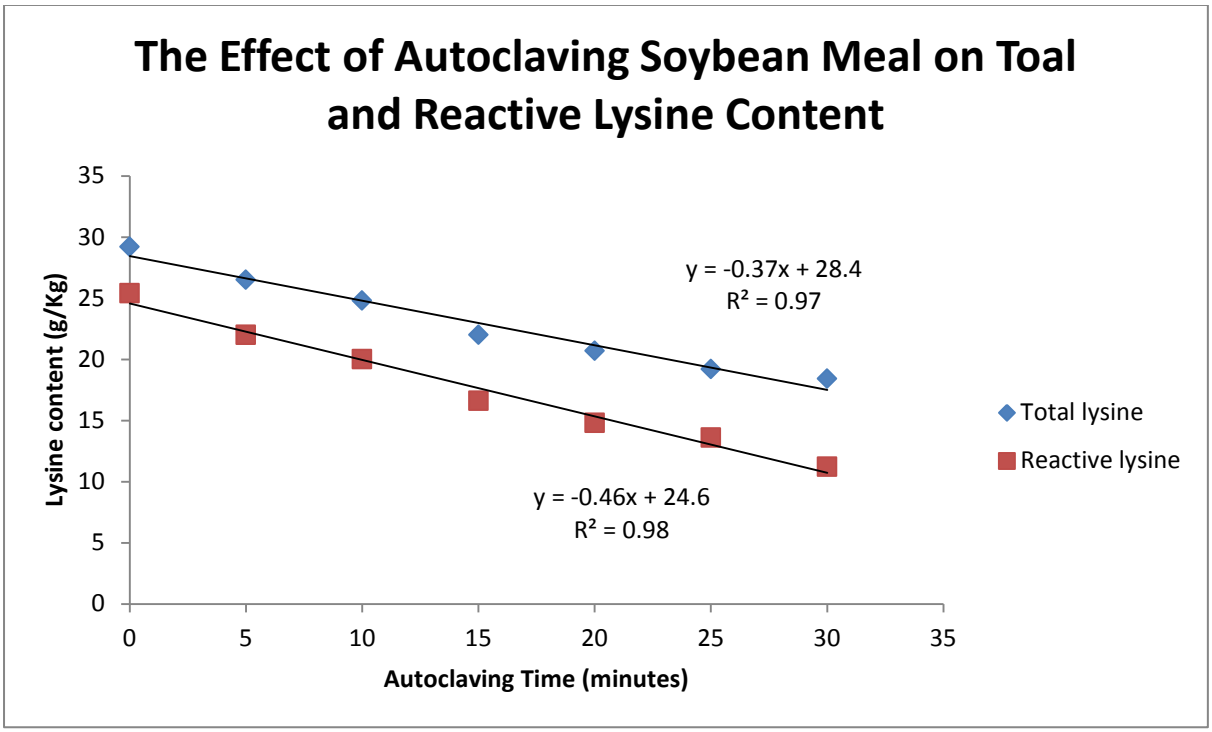

Figure 2. Correlation between autoclaving time and total and reactive lysine content of a soybean meal sample.

Digestible reactive lysine

Whilst reactive lysine is a measure of heat damage, standardised ileal digestible (SID) reactive lysine is a measure of the proportion of the lysine that will be bioavailable to the animal. The same study by Kim and Millen (2012) found that with autoclaving treatment the content of SID reactive lysine is also reduced (see Figure 3).

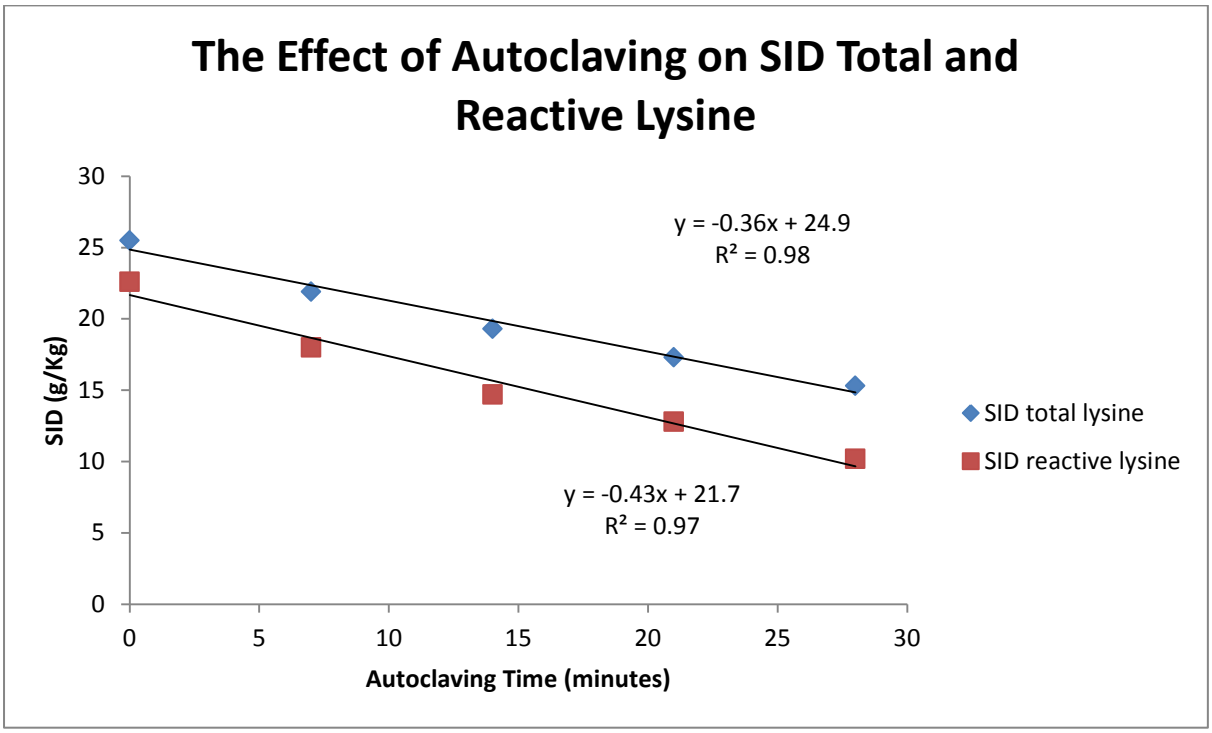

Figure 3. The effect of increasing autoclaving time on SID total and reactive lysine contents of soybean meal.

Determining reactive lysine and SID reactive lysine

Reactive lysine can be determined by a number of wet chemistry methods including fluorodinitrobenzene (FDNB), guanidination, dye-binding, trinitrobenzenesulphonic acid, sodium brorohydride and furosine methods. The most common methods used are the guanidination, furosine and FDNB methods. Measurements of digestible reactive lysine are obtained through taking ileal samples from pigs fed diets containing a marker. Standardised ileal digestibility of reactive lysine is calculated taking into account endogenous and feed associated ileal reactive lysine flows. NIR calibrations can be developed for reactive lysine content of raw materials using any of the above methods as a reference and NIR calibrations for SID reactive lysine can be developed using *in vivo* animal work.

When evaluating the amount of reactive lysine in feedstuffs as a measure of heat damage, it is important to consider reactive lysine as a proportion of total lysine (analysable modified plus unmodified lysine). Total lysine must also be considered when measuring reactive lysine because, if the total lysine is low, then it would be expected that the reactive lysine would also be low.

Nutritional value of reactive lysine

Recent work at Wageningen University looked at the effect of further heat processing (toasting at 95°C for 30 min) on the composition and nutritive value of soyabean meal and canola meal. This further processing reduced the analysed contents of both total and reactive lysine in both feedstuffs (Table 1).

Table 1. The influence of further processing (95°C steam treatment in the presence of lignosulfonate for 30 min) on the SBM and RSM content of total lysine and reactive lysine.

	Standard SBM (A)	**Further processed SBM (B)**	**□(A-B)**	**Standard CM (C)**	**Further processed CM (D)**	**□(C-D)**
Total lysine (%)	3.4	2.4	1.0	2.1	1.6	0.5
Reactive lysine (%)	3.2	1.9	1.3	1.9	1.2	0.7

(Hulshof et al, 2016)

When semi-purified diets with these feedstuffs as the only protein source were fed to growing pigs, further heat processing was shown to decrease animal performance (see Table 2). When diets including processed SBM and RSM were supplemented with crystalline AA to meet 90% of the SID lysine requirements, the performance depression was counter-acted (see Table 2).

Table 2. Performance in pigs fed from 15.6 to 41.3 kg LW with semi-purified diets based on standard or further heat processed (95°C, 30 min) soyabean (SBM) or canola meal (CM).

	SBM	**pSBM**	**pSBM + AA**	**CM**	**pCM**	**pCM + AA**
ADFI (g/d)	1138^{a}	$1130^{a,b}$	1115^{b}	1117^{b}	$124^{a,b}$	1139^{a}
ADG (g/d)	637^{a}	432^{c}	612^{a}	542^{b}	449^{c}	555^{b}
G:F (g/g)	0.56^{a}	0.38^{c}	0.55^{a}	0.49^{b}	0.40^{c}	0.49^{b}
Days to slaughter	39^{d}	57^{a}	39^{d}	44^{c}	52^{b}	45^{c}

Soybean meal (SBM), processed SBM (pSBM) and pSBM with the addition of crystalline AA to standardised ileal digestible AA levels in the SBM (pSBM + AA)
Rapeseed meal (CM), processed RSM (pCM) and pCM with the addition of crystalline AA to standardised ileal digestible AA levels in the SBM (pCM + AA)
$^{a-d}P<0.001$ with significant effects of protein source, diet type and protein source * diet type in all cases.
(Hulshof et al, 2016)

Global NIR predicted data from 2016

Soybean Meal

Using an NIR calibration, the reactive lysine content (as % of total lysine) was analysed in global soybean meal samples. The majority of global soybean meal samples surveyed had a reactive lysine percentage of above 72.5%. However, as can be seen in Figure 4, there were also some samples that had as low as 65% reactive lysine.

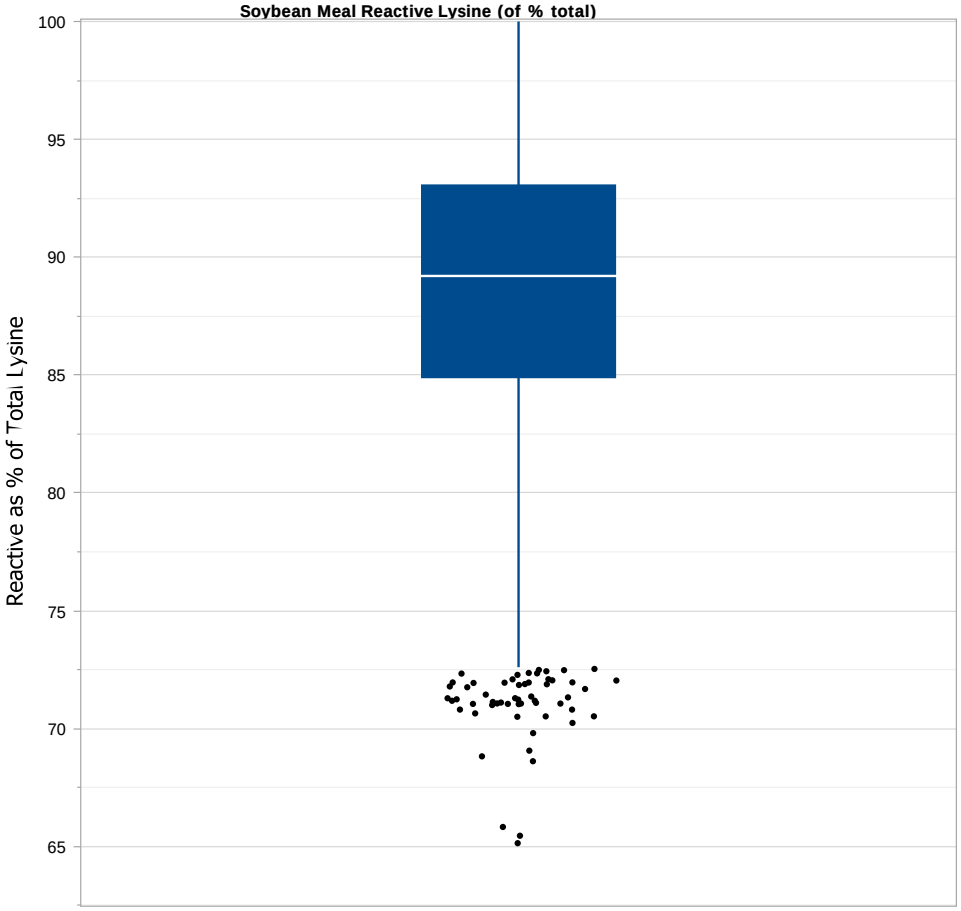

Figure 4: Distribution of reactive (% total lysine) for global soybean meal samples as predicted by NIR (n=8400).

Canola Meal

The reactive lysine content of global rapeseed meal samples (as % of total lysine) was also analysed using an NIR calibration. The majority of canola meal samples had a reactive as % total lysine value of above 81% with some outliers higher and some lower than this (see Figure 5).

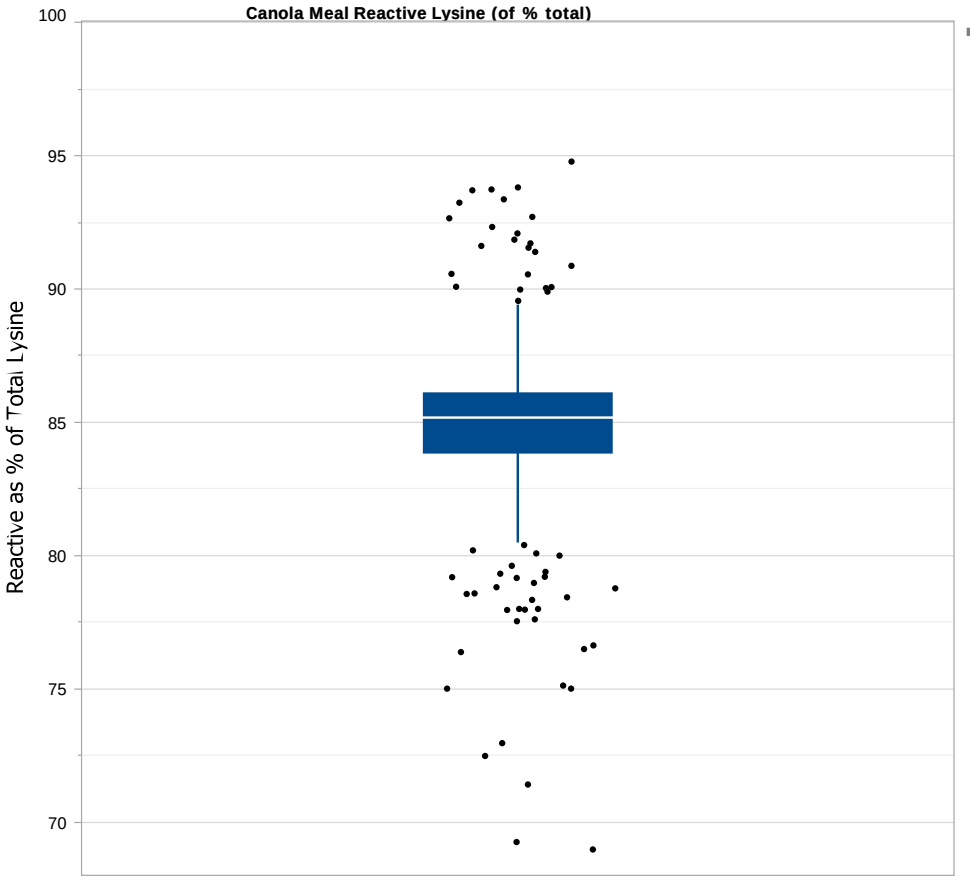

Figure 5: Distribution of reactive (% of total lysine) for global canola meal samples as predicted by NIR (n=800).

Summary

Reactive lysine, the portion of lysine that is chemically intact following heat treatment and SID reactive lysine, a measure of the lysine that will be bioavailable to the animal are both vulnerable to heat processing. As such, it is interesting to analyse the reactive lysine and SID reactive lysine content of oilseed meals. NIR calibrations for reactive lysine and SID reactive lysine offer the ability to analyse larger numbers of samples due to economic savings on analysis costs.

Until such time that it is possible to formulate diets to reactive lysine requirements, there are still significant benefits of understanding the reactive lysine content of protein sources. Published research has shown the potential implications on animal performance of feeding diets containing oil seed meals with reduced reactive lysine content, including negative effects on average daily gain, gain to feed ratio and days to slaughter.

The reactive lysine content of global soybean meal and canola meal samples has the potential to be variable due to differences in processing and indeed is variable in global samples. NIR allows for the rapid and cost effective analysis of reactive lysine content and gives the opportunity to better understand the variation found in processed oilseed meals.