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## **Heat damage, Maillard reactions, and measurement of reactive lysine in feed ingredients and diets**

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### **Introduction**

Feed cost represents 70% of the total cost of poultry and pork production (Patience et al., 2015); therefore, a number of processing techniques have been developed to maximize utilization of nutrients in feed ingredients and diets for optimum animal growth performance. Oilseed meals are commonly exposed to varying degrees of heat to remove solvents used during oil extraction, increase nutrient digestibility, improve storage life, and to reduce anti-nutritional factors (Liener, 1994; Rehman and Shah, 2005; Goebel and Stein, 2011). Heat is also applied in the production of distillers dried grains with solubles (**DDGS**) from dry grind ethanol facilities during the liquefaction and drying stages (Bothast and Schlicher, 2005). Thus, heat treatment of many processed feed ingredients is a necessity. However, excessive heat results in reduced nutrient digestibility due to formation of Maillard reaction products (González-Vega et al., 2011; Pahm et al., 2008). Maillard reaction involves the reaction of reducing sugars with the terminal/ε-amino group of amino acids (**AA**; particularly Lys), and products of the Maillard reaction can render AA in diets and/or feed ingredients unavailable (Finot and Magnenat, 1981). Factors affecting the rate of formation of Maillard reaction products include temperature, pH, substrate, and water activity, and each of these factors may affect the kinetics of the reactions in specific ways. Caramelization, which involves the oxidation of sugars and is another form of heat damage may also take place (Pahm et al., 2008). Formation of enzymatically resistant bonds between ε-amino groups of Lys and carboxyl groups of Glu and Asp can also occur, and these indigestible peptides can reduce protein digestibility and block AA transport across the intestinal wall (Dworschák, 1980). Other consequences of heat damage include conversion of some of the

L-forms of AA into D-forms (racemization) and formation of lysinoalanine (Hirano et al., 1973; Meade et al., 2005).

The objective of this contribution is to review the current understanding about heat damage, stages and products of Maillard reaction, and measurement of reactive Lys in diets and feed ingredients. Effects of heat damage in feed ingredients and diets on nutrient digestibility and growth performance of pigs will also be discussed.

### **Stages of the Maillard Reactions**

The Maillard reaction is named after Louis Maillard who first described formation of brown pigments due to the reaction of a reducing sugar with AA in the presence of heat. Later, it was documented that the Maillard reaction consists of a series of reactions where initially, a carbonyl group of a reducing sugar condenses with an amino group of an AA (Hodge, 1953). The Maillard reaction consists of seven different types of reactions, and due to the complexity of these reactions, the Maillard reaction is commonly divided into 3 main stages (Hodge, 1953): initial stage (colorless), intermediate stage (colorless or yellow), and final stage (highly colored).

#### ***Initial Stage***

The initial stage of the Maillard reaction consists of a sugar-amine condensation, and products of this reaction are further rearranged and converted to Amadori compounds (Gerrard, 2002). The terminal amino group of AA with an amino group in the side chain is most susceptible to the reaction. As a consequence, Lys is the most susceptible of all AA due to the presence of its  $\epsilon$ -amino group in the side chain, which may condense with reducing sugars. After condensation, the formed glycosylamine is dehydrated to yield a Schiff's base, which then undergoes rearrangement to form Amadori compounds (Zhang et al., 2009).

#### ***Intermediate Stage***

The intermediate stage of the Maillard reaction involves the dehydration, fragmentation, and degradation of Amadori compounds (Nursten, 2005). At this stage, visible browning that can be detected by spectrophotometric measurements has started (Hodge, 1953). Products of sugar dehydration include furfurals and reductones whereas some of the products from sugar

fragmentation include carbonyl and hydroxycarbonyl compounds (Nursten, 2005). Compounds formed from sugar fragmentation can also react with  $\alpha$ -AA to form aldehydes via Strecker degradation, and the formed aldehydes and pyrazine provides aroma in heated feed ingredients and/or diets (Zhang et al., 2009).

### ***Final Stage***

The final stage of the Maillard reaction involves aldol condensation and aldehyde-amine polymerization, which leads to the formation of high molecular weight heterogeneous polymers known as melanoidins (Hodge, 1953; Nursten, 2005). The chemical structure of melanoidins has not been fully elucidated, but is believed to be anionic, brown-colored, and contain approximately 3 to 4% nitrogen (Langner and Rzeski, 2014).

### **Measurement of reactive lysine in feed ingredients and diets**

The concentration of digestible Lys in heated feed ingredients and diets may be overestimated due to the presence of unreactive Lys that can be analyzed as part of the total Lys (Finot and Magnenat, 1981). Therefore, to obtain a more accurate value for digestible Lys in heated feeds, ileal digestibility of reactive Lys (rather than total Lys) must be determined. Conventional methods of AA analysis cannot separate the unreactive Lys and reactive Lys from the total Lys, and several methods to quantify the concentration of reactive Lys in heat-damaged proteins have been developed (Pahm et al., 2009). Some of the most common methods used to determine the concentration of reactive Lys in heat-damaged proteins are the homoarginine procedure, the furosine procedure, and the fluorodinitrobenzene procedure (Couch and Thomas, 1976; Moughan, 2003).

### ***Homoarginine procedure***

Reactive Lys may be measured using the homoarginine procedure that transforms the unbound Lys to homoarginine through a guanidination reaction (Pahm et al., 2008). This procedure has been effective in measuring reactive Lys in feedstuffs that have undergone moderate or severe heating. In this procedure, the unreactive Lys is transformed to homoarginine through a guanidination reaction using O-methylisourea before the protein sample is acid-hydrolyzed (Kimmel, 1967). Because the reactive Lys is converted to homoarginine, the

analyzed Lys in the chromatogram only represents the unreactive Lys. The analyzed concentration of homoarginine can then be mathematically converted to Lys on a molar basis to estimate the concentration of reactive Lys in feed ingredients and diets (Pahm et al., 2009).

### ***Furosine procedure***

The furosine procedure is used to calculate the concentration of reactive Lys on the basis of the concentration of furosine in heated proteins (Pahm et al., 2008). Furosine is a basic AA that is produced from Amadori compounds during the acid hydrolysis step of AA analysis. Hydrolysis of Amadori compounds yields 32% furosine, 40% regenerated Lys, and 28% pyridosine (Bujard et al., 1978). Therefore, measuring the concentration of furosine using high-performance liquid chromatography allows for the calculation of regenerated Lys (i.e., unreactive Lys) in feedstuffs. Therefore, the calculated amount of unreactive Lys based on the furosine concentration can be subtracted from the analyzed total Lys to calculate the concentration of reactive Lys (Pahm et al., 2009; Kim et al., 2012).

### ***Fluorodinitrobenzene procedure***

Reactive Lys is converted to dinitrophenyl or trinitrophenyl-Lys by reacting a feed sample with fluorodinitrobenzene (Rutherford and Gilani, 2009). Because dinitrophenyl or trinitrophenyl-Lys is a colored compound, this can be quantified using a spectrophotometer (Mehta and Deeth, 2016). Therefore, the Lys that remained colorless after reacting with fluorodinitrobenzene is considered the unreactive Lys of a protein sample. However, fluorodinitrobenzene may react with carbohydrates during acid hydrolysis, and this may deteriorate the color of fluorodinitrobenzene-reactive Lys (Mehta and Deeth, 2016).

### **Effects of heat damage in feed ingredients**

Heating improves the nutritional values of soybeans and soybean meal (**SBM**) because it inactivates the trypsin inhibitors and other anti-nutritional factors that may be present in raw soybeans (González-Vega et al., 2011; Oliveira et al., 2020b). In commercial production of SBM, heat treatment is applied during de-solventizing and during the drying-cooling process and the temperature may reach 100 to 110 °C in de-solventizing process and may be up 150 °C in the drying-cooling process. However, autoclaving of SBM at 150 °C for 3 min reduced standardized

ileal digestibility (**SID**) of AA by growing pigs, whereas autoclaving at 110 °C for 30 min did not reduce SID of AA compared with non-autoclaved SBM. Autoclaving at 150 °C for 18 min increased the reduction in SID of AA compared with autoclaving for 3 min (Oliveira et al., 2020b).

The negative effects of heating has also been reported in 00-rapessed meal (**RSM**) that was autoclaved at 100 °C or 150 °C, which resulted in decreased SID of AA and the SID of Lys was reduced more than that of other AA. The greater reduction in the SID of Lys, Arg, and sulfur AA compared with other AA reported by Oliveira et al. (2020a,b) confirms that Lys is the AA that is most susceptible to Maillard reactions because the  $\epsilon$ -amino group in the sidechain can react directly with reducing sugars under moist and heat conditions (Eklund et al., 2015).

Formation of Maillard reaction products during heating depends on the type of heat that is applied, the length of heating, and the feed ingredient that is being heated. Heating of SBM by oven drying at 125 °C for 30 min did not affect the SID of AA when compared with non-heated SBM, but digestibility of AA in SBM that was heated by autoclaving at 125 °C for 30 min was less than in SBM that was oven dried for 30 min (González-Vega et al., 2011). However, if corn DDGS is subjected to oven drying at 50, 75, or 100 °C, the concentration of total Lys, reactive Lys, and digestible Lys is reduced (Pahm et al., 2008).

Autoclaving is associated with pressure, moisture, and high temperature, whereas oven drying is only a thermal treatment. Amino acids are less stable at greater pressure, and the rate of reaction between the amino group of AA and glucose increases if the humidity is increased. The formation of Maillard reaction products depends on water activity, temperature, pH, time of heating, and the type and availability of the reactants (Ramírez-Jiménez et al., 2001; Fontaine et al., 2007; González-Vega et al., 2011). Based on the concentration of Lys, autoclaving corn DDGS for 10 min was sufficient to cause heat damage, but increasing time of autoclaving to 20 or 30 min did not result in further decreases in Lys concentration, which indicates that the extent of the reduction in the concentration and digestibility of Lys and other AA varies among feed ingredients that are being heated (Almeida et al., 2013).

When feed ingredients are heat damaged, the concentration of Lys is reduced whereas the concentration of crude protein (**CP**) remains constant (Table 1; Pahm et al., 2008; González-

Vega et al., 2011; Kim et al., 2012; Almeida et al., 2013). Therefore, the degree of heat damage in a feed ingredient may be estimated by calculating the Lys:CP ratio (Stein et al., 2009; Cozannet et al., 2010). In SBM that has not been heat damaged, the Lys:CP ratio is greater than 6.0% (González-Vega et al., 2011; Oliveira et al., 2020b). However, the Lys:CP ratio can be reduced to less than 4.0% as the severity of heating increases, which results in a reduction in SID of Lys (Oliveira et al., 2020b). Likewise, the Lys to CP ratio in non-heat damaged corn DDGS, 00-rapeseed meal, canola meal, sunflower meal and cottonseed meal is 3.0, 3.7, 5.2, 3.6, and 4.1, respectively and ratios below these values indicate heat damage (Almeida et al., 2013, 2014a,b). Thus, each protein source has a unique Lys to CP ratio if not heat damaged and values below this unique ratio indicates heat damage.

Heat damage of feed ingredients is also associated with an increase in analyzed acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**) because some of the melanoidins formed in the Maillard reaction are analyzed as fiber. When sunflower meal and cottonseed meal were subjected to increasing duration of autoclaving at 130 °C, analyzed ADF and NDF increased and SID of AA decreased (Almeida et al., 2014a). However, a decrease in insoluble dietary fiber and increase in soluble dietary fiber in SBM if autoclaved at 110 °C or 150 °C, indicate that autoclaving solubilized some insoluble dietary fiber (Oliveira et al., 2020).

Overheating may also reduce digestible energy and metabolizable energy of feed ingredient. Digestible energy and metabolizable energy in soybean meal and 00-rapeseed meal were reduced by approximately 400 kcal/kg if ingredients were autoclaved at 150 °C for 18 min compared with non-autoclaved ingredients (Table 2; Oliveira et al., 2020a,b). The reduction in digestible energy and metabolizable energy may be a result of sugars being bound to Lys and other AA during the Maillard reaction, and therefore, these sugars are not available for absorption. The activity of amylase and amyloglucosidase may also be reduced if Maillard reaction products enters the small intestine (Chung et al., 2012). Dietary fat may also be affected by overheating, if diets are heat damaged during extrusion processing, some dietary fat gets oxidized or depolymerized during heating, which will reduce the energy value of fat (Kerr et al., 2015).

Weanling pigs fed diets containing heat damaged SBM or heat damaged corn DDGS, had decreased average daily feed intake, average daily gain, gain to feed ratio, and subsequently

reduced final body weight, compared with pigs fed diets containing non-heat damaged SBM or non-heat damaged corn DDGS (Almeida et al., 2014c). Therefore, if heat damaged feed ingredients are used in diet formulation assuming the same concentration and digestibility of lysine and other amino acids as in non-heat damaged feed ingredients, diets that are deficient in digestible amino acids are formulated. If a heat damaged feed ingredient is included in diets for pigs it is, therefore, necessary to add extra energy and extra AA to the diets to avoid the negative impacts on pig growth performance.

### Summary

The Maillard reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars and results in a decrease in the digestibility of Lys and other AA. Excess heat during processing of feedstuffs may reduce not only the concentration, but also the digestibility of most AA and energy. Further research is needed to determine optimal processing conditions (e.g., temperature and humidity) of feedstuffs to avoid heat damage and subsequently increase nutrient digestibility and pig growth performance.

### Literature Cited

- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. *J. Anim. Sci. Biotechnol.* 4:44-44. doi 10.1186/2049-1891-4-44
- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2014a. Digestibility by growing pigs of amino acids in heat-damaged sunflower meal and cottonseed meal. *J. Anim. Sci.* 92:585-593. doi 10.2527/jas.2013-6769
- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2014b. Effects of heat treatment on the apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. *Anim. Feed Sci. Technol.* 187:44-52. doi 10.1016/j.anifeedsci.2013.09.009
- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2014c. Effects of balancing crystalline amino acids in diets containing heat-damaged soybean meal or distillers dried grains with solubles fed to weanling pigs. *Animal* 8:1594-1602. doi 10.1017/S175173111400144X
- Bothast, R. J., and M. A. Schlicher. 2005. Biotechnological processes for conversion of corn into ethanol. *Appl. Microbiol. Biotechnol.* 67:19-25. doi 10.1007/s00253-004-1819-8



- Bujard, É., P. A. Finot, R. Madelaine, A. Lê van Kiet, R. Deutch, and A. Isely. 1978. Mesure de la disponibilité et du blocage de la lysine dans les laits industriels. *Ann. Nutr. Alim.* 32:291-305.
- Couch, J. R., and M. C. Thomas. 1976. A comparison of chemical methods for the determination of available lysine in various proteins. *J. Agric. Food Chem.* 24:943-946. doi 10.1021/jf60207a033
- Cozannet, P., Y. Primot, C. Gady, J. P. Métayer, P. Callu, M. Lessire, F. Skiba, and J. Noblet. 2010. Ileal digestibility of amino acids in wheat distillers dried grains with solubles for pigs. *Anim. Feed Sci. Technol.* 158:177-186. doi 10.1016/j.anifeedsci.2010.04.009
- Chung, S. Y., S. H. Han, S. W. Lee, and C. Rhee. 2012. Effect of Maillard reaction products prepared from glucose–glycine model systems on starch digestibility. *Starch - Stärke* 64:657-664. doi <https://doi.org/10.1002/star.201100176>
- Dworschák, E. 1980. Nonenzyme browning and its effect on protein nutrition. *Crit. Rev. Food Sci. Nutr.* 13:1-40. doi 10.1080/10408398009527292
- Eklund, M., N. Sauer, F. Schöne, U. Messerschmidt, P. Rosenfelder, J. K. Htoo, and R. Mosenthin. 2015. Effect of processing of rapeseed under defined conditions in a pilot plant on chemical composition and standardized ileal amino acid digestibility in rapeseed meal for pigs. *J. Anim. Sci.* 93:2813-2825. doi 10.2527/jas.2014-8210
- Finot, P., and E. Magnenat. 1981. Metabolic transit of early and advanced Maillard products. *Prog. Food Nutr. Sci.* 5:193-207.
- Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. *J. Agric. Food Chem.* 55:10737-10743. doi 10.1021/jf071747c
- Gerrard, J. A. 2002. New aspects of an ageing chemistry - recent developments concerning the Maillard reaction. *Aust. J. Chem.* 55:299-310. doi 10.1071/CH02076
- Goebel, K. P., and H. Stein. 2011. Ileal digestibility of amino acids in conventional and low-kunitz soybean products fed to weanling pigs. *Asian-Australas J. Anim. Sci.* 24. doi 10.5713/ajas.2011.90583
- González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. *J. Anim. Sci.* 89:3617-3625. doi 10.2527/jas.2010-3465
- Hirano, F., H. Kato, and M. Fujimaki. 1973. Racemization of amino acid residues in proteins during roasting. *Agric. Biol. Chem.* 37:191-192. doi 10.1080/00021369.1973.10860651
- Hodge, J. E. 1953. Dehydrated foods, chemistry of browning reactions in model systems. *J. Agric. Food Chem.* 1:928-943. doi 10.1021/jf60015a004

- Kerr, B. J., T. A. Kellner, and G. C. Shurson. 2015. Characteristics of lipids and their feeding value in swine diets. *J. Anim. Sci. Biotechnol.* 6:30. doi:10.1186/s40104-015-0028-x
- Kim, B. G., D. Y. Kil, Y. Zhang, and H. H. Stein. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles fed to pigs. *J. Anim. Sci.* 90:3798-3808. doi 10.2527/jas.2011-4692
- Kimmel, J. R. 1967. Guanidination of proteins. Pages 584-589 in *Methods in Enzymology* Academic Press.
- Langner, E., and W. Rzeski. 2014. Biological properties of melanoidins: A review. *Int. J. Food Prop.* 17:344-353. doi 10.1080/10942912.2011.631253
- Liener, I. E. 1994. Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr.* 34:31-67. doi 10.1080/10408399409527649
- Meade, S. J., E. A. Reid, and J. A. Gerrard. 2005. The impact of processing on the nutritional quality of food proteins. *J. AOAC. Int.* 88:904-922.
- Mehta, B. M., and H. C. Deeth. 2016. Blocked lysine in dairy products: Formation, occurrence, analysis, and nutritional implications. *Compr. Rev. Food Sci. Food Saf.* 15:206-218. doi 10.1111/1541-4337.12178
- Moughan, P. J. 2003. Amino acid availability: Aspects of chemical analysis and bioassay methodology. *Nutr. Res. Rev.* 16:127-141. doi 10.1079/NRR200365
- Nursten, H. 2005. *The Maillard Reaction. Chemistry, Biochemistry, and Implications.* Royal Society of Chemistry, Cambridge, UK.
- Oliveira, M. S. F., M. K. Wiltafsky-Martin, and H. H. Stein. 2020a. Excessive heating of 00-rapeseed meal reduces not only amino acid digestibility but also metabolizable energy when fed to growing pigs. *J. Anim. Sci.* 98. doi 10.1093/jas/skaa219
- Oliveira, M. S. F., M. K. Wiltafsky, S. A. Lee, W. B. Kwon, and H. H. Stein. 2020b. Concentrations of digestible and metabolizable energy and amino acid digestibility by growing pigs may be reduced by autoclaving soybean meal. *Anim. Feed Sci. Technol.* 269:114621. doi <https://doi.org/10.1016/j.anifeedsci.2020.114621>
- Pahm, A. A., C. Pedersen, and H. H. Stein. 2008. Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs. *J. Agric. Food Chem.* 56:9441-9446. doi 10.1021/jf801618g
- Pahm, A. A., C. Pedersen, and H. H. Stein. 2009. Standardized ileal digestibility of reactive lysine in distillers dried grains with solubles fed to growing pigs. *J. Agric. Food Chem.* 57:535-539. doi 10.1021/jf802047d

- Patience, J. F., M. C. Rossoni-Serão, and N. A. Gutiérrez. 2015. A review of feed efficiency in swine: Biology and application. *J. Anim. Sci. Biotechnol.* 6. doi 10.1186/s40104-015-0031-2
- Ramírez-Jiménez, A., B. García-Villanova, and E. Guerra-Hernández. 2001. Effect of toasting time on the browning of sliced bread. *J. Sci. Food Agric.* 81:513-518. doi 10.1002/jsfa.840
- Rehman, Z., and W. H. Shah. 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem.* 91:327-331. doi 10.1016/j.foodchem.2004.06.019
- Rutherford, S. M., and G. S. Gilani. 2009. Amino acid analysis. *Curr. Protoc. Protein Sci.* 58:11.19.11-11.19.37. doi 10.1002/0471140864.ps1109s58
- Stein, H. H., S. P. Connot, and C. Pedersen. 2009. Energy and nutrient digestibility in four sources of distillers dried grains with solubles produced from corn grown within a narrow geographical area and fed to growing pigs. *Asian-Australas J. Anim. Sci.* 22:1016-1025. doi 10.5713/ajas.2009.80484
- Zhang, Q., J. M. Ames, R. D. Smith, J. W. Baynes, and T. O. Metz. 2009. A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: Probing the pathogenesis of chronic disease. *J. Proteome Res.* 8:754-769. doi 10.1021/pr800858h

**Table 1.** Concentrations of crude protein (CP) and Lys in feed ingredients heat damaged.

| Feed ingredient               | Non-heat damaged |        |                           | Heat damaged |        |                           |
|-------------------------------|------------------|--------|---------------------------|--------------|--------|---------------------------|
|                               | CP, %            | Lys, % | Lys:CP ratio <sup>1</sup> | CP, %        | Lys, % | Lys:CP ratio <sup>1</sup> |
| Soybean meal <sup>2,3</sup>   | 47.0             | 2.9    | 6.1                       | 47.0         | 2.3    | 4.8                       |
| Corn DDGS <sup>3,4</sup>      | 27.0             | 0.82   | 3.0                       | 27.0         | 0.61   | 2.3                       |
| 00-rapeseed meal <sup>5</sup> | 35.0             | 2.0    | 5.7                       | 36.0         | 1.2    | 3.3                       |
| Canola meal <sup>6</sup>      | 36.8             | 1.9    | 5.2                       | 36.9         | 1.4    | 3.7                       |
| Sunflower meal <sup>7</sup>   | 37.0             | 1.3    | 3.6                       | 36.4         | 1.0    | 2.8                       |
| Cottonseed meal <sup>7</sup>  | 41.8             | 1.7    | 4.1                       | 43.8         | 1.7    | 3.8                       |

<sup>1</sup>Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009)

<sup>2</sup>Adapted from Oliveira et al. (2020b).

<sup>3</sup>Adapted from Fontaine et al. (2007).

<sup>4</sup>Adapted from Almeida et al. (2013).

<sup>5</sup>Adapted from Oliveira et al. (2020a).

<sup>6</sup>Adapted from Almeida et al. (2014b).

<sup>7</sup>Adapted from Almeida et al. (2014a).

**Table 2.** Concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) in feed ingredients heat damaged.

| Feed ingredient               | Non-heat damaged |       |             | Heat damaged |       |       |
|-------------------------------|------------------|-------|-------------|--------------|-------|-------|
|                               | DE               | ME    | ME:DE ratio | DE           | ME    | ME:DE |
| Soybean meal <sup>1</sup>     | 3,990            | 3,665 | 0.92        | 3,046        | 2,562 | 0.85  |
| 00-rapeseed meal <sup>2</sup> | 3,161            | 2,873 | 0.91        | 2,737        | 2,458 | 0.88  |

<sup>1</sup>Adapted from Oliveira et al. (2020b).

<sup>2</sup>Adapted from Oliveira et al. (2020a).