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Prediction of apparent, standardized, and true ileal digestible total and reactive lysine contents in heat-damaged soybean meal samples¹

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ABSTRACT: Forty-two individually housed entire male pigs weighing 37.5 ± 0.15 kg (mean \pm SEM) were used in a randomized block design having 7 dietary treatments ($n = 6$). The dietary treatments were 5 semisynthetic diets containing 350 g/kg of soybean (*Glycine max*) meal (SBM) with variable heat treatments (0, 7, 14, 21, and 28 min autoclaved at 135°C), a protein free (N-free) diet, and an enzymatically hydrolyzed casein (EHC) diet. Heat treatment linearly decreased ($P < 0.001$) total Lys content from 27.5 to 19.2 g/

kg and reactive Lys content from 23.4 to 11.7 g/kg. Apparent, standardized, and true ileal digestible total and reactive Lys contents linearly decreased ($P < 0.001$) with increasing severity of heat treatment and were accurately predictable ($P < 0.001$) from total and reactive Lys content in heat-damaged SBM. These data indicate that excessive heat processing of SBM reduced both the content ($P < 0.001$) and digestibility ($P < 0.001$) of total and reactive Lys in SBM.

Key words: ileal digestibility, pig, reactive lysine

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INTRODUCTION

Variation in available AA content of protein sources hinders precise formulation and hence efficiency of AA use in pig diets. Soybean meal (SBM) is a common protein ingredient in pig diets worldwide with a superior AA profile compared with other plant protein sources. However, during heat processing or prolonged storage of feedstuffs, the ϵ -amino group of Lys can react with reducing sugars and form biologically unavailable Lys derivatives (unreactive Lys). This unreactive Lys decreases ileal digestibility and is unavailable to animals even after absorption in the small intestine (van Barneveld et al. 1995). However, some unreactive Lys can revert to Lys with acid hydrolysis in conventional AA analysis, thereby causing inaccurate determinations of biologically available Lys (Rutherford and Moughan, 1997). Only Lys with a free ϵ -NH₂ group is reactive Lys and considered biologically available Lys for protein deposition (Rutherford and Moughan, 2007). This research was conducted to establish linear relations between Lys content (total and reactive) and the apparent, standardized, and true ileal digestible total

and reactive Lys content in autoclaved SBM with an *in vivo* ileal digestibility study.

MATERIALS AND METHODS

Forty-two entire male pigs (37.5 ± 0.15 kg; mean \pm SEM) were allocated to 7 dietary treatments in a randomized block design ($n = 6$). Pigs were blocked and stratified within block based on initial BW. The treatments were 5 SBM-based semisynthetic diets containing 350 g/kg SBM with variable heat treatments (0, 7, 14, 21, and 28 min autoclaved at 135 °C), a N-free diet, and an enzymatically hydrolyzed casein (EHC) diet. A synthetic diet was used as a basal formulation (Stein et al., 2007) and either SBM (350 g/kg) or EHC (120 g/kg) was the sole protein source in the diet except for the N-free diet. The SBM was autoclaved for 0, 7, 14, 21, and 28 min at 135°C. The autoclaved SBM were ground through a hammer mill fitted with a 4-mm screen before mixing. As a digestibility marker, 3 g/kg TiO₂ was included in diets.

Pigs were individually housed and fed the experimental diet at 2.5 times maintenance energy requirement [$2.5 \times (0.458 \text{ MJ} \times \text{BW}^{0.75})/\text{diet DE}$] for 5 d to eliminate any influence of feed intake on digestibility. On day 6, pigs were euthanized 9 h \pm 20 min after start

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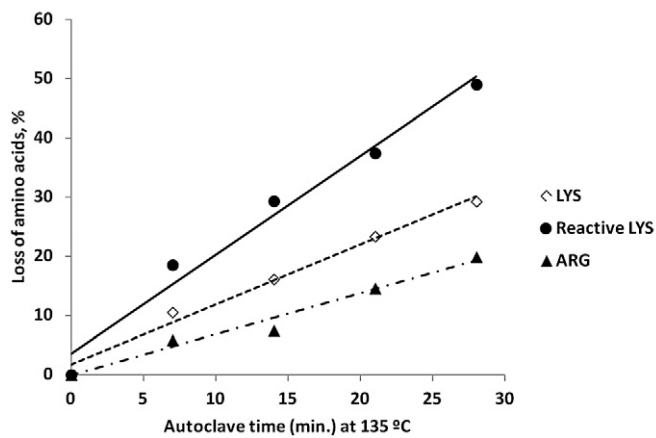


Figure 1. Loss of Arg, total Lys, and reactive Lys (%) in autoclaved soybean meal samples. The contents of Arg, Lys, and reactive Lys in soybean meal sample prior to autoclaving (0 min.) were 34.1, 27.5, and 23.4 g/kg as-is basis.

of feeding. Ileal samples were collected and TiO_2 was measured using established methods (Kim et al., 2009). Ileal samples collected from the pigs fed the EHC diet were ultrafiltered to remove any protein molecules of dietary origin that were smaller than molecular weight 10,000 daltons using the method described by Hodgkinson et al. (2000). Guanidination was carried out by a modified method of Maga (1981) and Fontaine et al. (2007). The AA content was measured as described by Kim et al. (2009) using a reverse phase HPLC (Hewlett Packard 1100 HPLC with Diode array detector). Apparent, standardized, and true ileal digestibility were calculated as described by Stein et al. (2007) using the endogenous AA flows (EAAF) determined from the pigs fed the N-free and EHC diets, respectively. Digestibility data were analyzed by 1-way ANOVA using a polynomial comparison. Block was used as a random factor and SBM was used as a fixed factor in the analysis. Individual pig was the experimental unit in the model. Linear regression analysis was conducted to establish prediction equations for apparent, standardized, and true ileal digestible total

and reactive Lys contents in the SBM samples.

RESULTS AND DISCUSSION

The autoclaved SBM samples were analyzed for AA composition and AA susceptible to heat treatment: Lys, reactive Lys, and Arg (Figure 1). The content of other AA was not changed after heat processing. The slope of linear reduction due to heat treatment was greater for reactive Lys than total Lys (Figure 1). Thus, the gap between total Lys and reactive Lys (i.e., reverted Lys) increased as severity of heat damage increased. Consequently, loss of AA after 28 min heat treatment was 20, 30, and 50% for Arg, total Lys, and reactive Lys, respectively.

Basal and specific EAAF were determined by feeding a protein-free diet (N-free) and an EHC diet with subsequent ultrafiltration, and our results were within reported ranges (Hodgkinson et al., 2000; Gonzalez-Vega et al., 2011). The EAAF determined using the EHC method is positively influenced by the proportion of casein used in the diet (Hodgkinson et al., 2000). The amount of protein in the EHC diet (120 g/kg) were lower than that in SBM test diets (approximately 160 g/kg) and therefore the EAAF determined with the EHC method may have slightly underestimated the actual EAAF in pigs fed the SBM test diets. Nevertheless, the EAAF determined using the N-free and EHC methods were used to estimate standardized and true ileal digestibility of AA, respectively.

Apparent, standardized, and true ileal digestibility of Lys and reactive Lys values in heat-treated SBM (Table 1) are similar to published values (Gonzalez-Vega et al., 2011). Heat treatment linearly decreased ($P < 0.001$) ileal digestibility of total and reactive Lys. Heat treatment reduced content and digestibility of total and reactive Lys and their apparent, standardized, and true ileal digestibility in SBM (Figure 1; Table 1).

Based on the determined in vivo ileal digestible Lys contents, linear regression equations were developed to

Table 1. Apparent, standardized, and true ileal digestibility (%) of Lys and reactive Lys in autoclaved soybean meals¹

Item	Soybean meal autoclave time at 135°C, min					Pooled SE	P-value Linear
	0	7	14	21	28		
Apparent ileal digestibility							
Lys	89.8	85.3	82.9	80.5	75.6	1.53	0.001
Reactive Lys	94.3	91.3	88.9	85.6	82.7	1.16	0.001
Standardized ileal digestibility ²							
Lys	93.0	88.4	86.2	84.0	79.7	1.53	0.001
Reactive Lys	96.5	93.7	91.6	88.9	86.7	1.16	0.001
True ileal digestibility ³							
Lys	94.7	90.1	88.0	85.9	81.8	1.53	0.001
Reactive Lys	97.8	95.2	93.3	90.9	89.1	1.16	0.001

¹Total and reactive Lys in autoclaved soybean meals for 0, 7, 14, 21, and 28 min at 135°C were 27.5, 24.7, 22.4, 20.6, and 19.2 g/kg and 23.4, 19.2, 16.1, 14.4, and 11.7 g/kg as-is basis, respectively.

²Adjusted using endogenous AA flow determined under N-free method: 289 mg/kg DMI Lys and 193 mg/kg DMI reactive Lys.

³Adjusted using endogenous AA flow determined under the enzymatically hydrolyzed casein ultrafiltration method: 443 mg/kg DMI Lys and 312 mg/kg DMI reactive Lys.

Table 2. Linear regression equations to predict of apparent ileal digestible (AID), standardized ileal digestible (SID) and true ileal digestible (TID) Lys and reactive Lys contents from total and reactive Lys contents, respectively, in soybean meal

Item	Intercept	Slope	RSD ¹	R ²	P-value
Prediction from total Lys					
AID total Lys, g/kg as-fed	-8.37	1.2002	0.810	0.95	0.001
SID total Lys, g/kg as-fed	-7.88	1.2126	0.810	0.95	0.001
TID total Lys, g/kg as-fed	-7.62	1.2192	0.811	0.95	0.001
Prediction from reactive Lys					
AID reactive Lys, g/kg as-fed	-2.841	1.0626	0.392	0.99	0.001
SID reactive Lys, g/kg as-fed	-2.440	1.0666	0.393	0.99	0.001
TID reactive Lys, g/kg as-fed	-2.193	1.0691	0.393	0.99	0.001

¹RSD = residual standard deviation.

predict amounts of apparent, standardized, and true ileal digestible total and reactive Lys from total and reactive Lys contents in SBM, respectively (Table 2). The linear response of total Lys content to heat treatment is similar to another study, in which SBM was autoclaved for 0, 15, and 30 min. at 125°C (Gonzalez-Vega et al., 2011). However, the slope of the regression was steeper in our study because we included values for SBM autoclaved at the higher temperature of 135°C for a similar duration.

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