Chickens' growth performance and pancreas development exposed to soy cake varying in trypsin inhibitor activity, heat-degraded lysine concentration, and protein solubility in potassium hydroxide

D. Hoffmann,*[,1] S. Thurner,[†] D. Ankerst,[‡] K. Damme,[§] W. Windisch,* and D. Brugger*

**Technical University of Munich (TUM), TUM School of Life Sciences Weihenstephan, Chair of Animal Nutrition, Liesel-Beckmann-Str. 2, 85354 Freising-Weihenstephan, Germany; [†] Institute for Agricultural Engineering and Animal Husbandry, Bavarian State Research Center for Agriculture, Voettingerstr. 36, 85354 Freising-Weihenstephan, Germany; [‡] Department of Mathematics, Biostatistics, Technical University of Munich (TUM), Boltzmannstr. 3, 85748 Garching, Germany; and [§] Department for Education and Poultry Research, Bavarian State Research Center for Agriculture, Mainbernheimerstr. 101, 97318 Kitzingen, Germany*

ABSTRACT This study focused on the effect of varying trypsin inhibitor activity (**TIA**), heat-degraded lysine concentration and protein solubility in potassium hydroxide on broiler performance and pancreas weight. Two soybean breeds were subject to varying thermal, hydrothermal, pressure, and kilning processing. This resulted in a total of 34 soy cake variants, widely varying in TIA (0.25 to 23.6 mg/g), heat-degraded lysine (1.40 to 8.60 g/kg), and potassium hydroxide (65.5 to 97.6%), respectively. These soy cake variants as well as a commercial soybean meal extract were included into a common grower and finisher diet for broiler chicks at fixed amounts (grower: 35%; finisher: 25%) and tested in a 35 d fattening experiment with 1680 broiler chicks (grower phase: day 11 to 24; finisher phase day 25 to 35). TIA was the dominant factor affecting zootechnical performance and pancreas weight at slaughter (day 35), depressing liveweight at day 24 ($P < 0.006$), and day 35 (0.026), weight gain (grower: $P < 0.006$) and feed: gain ratio during grower phase ($P < 0.005$) and increasing pancreas weight ($P < 0.010$) at the time of slaughter. Negative effects of TIA were also visible in soy cake variants below recommended thresholds. This highlights the necessity of complete elimination of TIA in broiler diets as far as technically possible.

Key words: soybean, heat degraded lysine, trypsin inhibitor, broiler, growth performance

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INTRODUCTION

Globally, soybean is the most important feed protein compound in the diets of monogastric livestock (Lee et al., 2007). Nevertheless, the utilization of raw soybeans for feed is mainly limited by the presence of protease inhibitors, which interfere with intake, digestion, absorption, and metabolism of nutrients as well as with health status of the animal (Liener, 1994; Clarke and Wiseman, 2005). In this context, pancreatic hypertrophy and pancreatitis have been recognized in growing animals in response to activities of protease inhibitors (Applegarth et al., 1964; Kakade et al., 1973; Clarke and Wiseman, 2005; Clarke and Wiseman, 2007). These inhibitors and in particular trypsin inhibitors (**TI**) require heat treatment in order to become inactivated (Liener, 1994). The trypsin inhibitor activity (**TIA**) varies among different soybean breeds and ranges around 70 to > 100 mg/g (Vollmann et al., 2003). During growth of the soybean plant, TIA is further affected by environmental and genetic factors (Vollmann et al., 2003).

Different feed processing methods, especially a combination of heat and pressure, decrease TIA and thus increase the nutritional value of respective feedstuffs (Liener, 1962; Rohe et al., 2017). On the other hand, Parsons et al. (1992) reported that excessive heat treatment causes denaturation of amino acids, resulting in reduced protein quality and lower feed efficiency. In this context, lysine appears to be the amino acid that is most vulnerable to heat due to Maillard reaction (e.g., epsilon-fructose lysine) (Adrian, 1974). Therefore, indicators used to refer to protein degeneration are protein solubility in potassium hydroxide (**KOH**) or heat-degraded lysine (**HDL**). With an increase in processing, KOH decreases and HDL increases, respectively.

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[1]Corresponding author: d.hoffmann@tum.de

Current upper limits tolerable for TIA in soybean products fed to monogastric livestock are considered to be ≤ 4 mg/g for broiler chickens and ≤ 4.7 mg/g for pigs (Batterham et al., 1993; Clarke and Wiseman, 2005). However, available data on the matter are quite limited and sometimes contradictory. For example, Huisman and Tolman (2001) already recognized adverse effects in fattening performance of pigs at TIA levels of ≥0.5 mg/g. Furthermore, there has been to our knowledge only one investigation by Heger et al. (2016) on the interaction between heat degraded amino acids and TIA on animal performance. Parsons et al. (1991) found that a decrease in chickens' growth performance could be seen when KOH was below 59% and generally seems to be critical below 70% according to Araba and Dale (1990).

Therefore, the goal of this study was to assess the dose-response relationship between TIA, HDL, and KOH, respectively, and zootechnical performance and pancreas weight of broilers in high resolution under practical feeding conditions.

MATERIALS AND METHODS

Soybean Processing

Two homogenous batches of each soybean breed (breed 1: Sultana; origin: conventional farming, harvested in Bavaria, Germany; breed 2: Merlin; origin: organic farming, harvested in Romania) were subject to a total of 17 different combinations of thermal, hydrothermal, pressure, and kilning processing variants in order to vary levels of TIA, HDL, and KOH, respectively. Treatments comprised over-, under- and optimum treatment of soy cake. Table A1 gives an overview of processing parameters as published earlier in detail by Hoffmann et al. (2017). In addition to the 34 soy cake variants, 1 batch of commercial soybean meal was included into the study, thus resulting in a total of 35 differentially processed soybean products. Respective concentrations of nutrients, TIA, HDL, and KOH are presented in Table A2.

Experimental Diets

The 35 soybean products were used to compose grower and finisher feeds for boiler chicks meeting or exceeding the feeding recommendations for male Ross 308 broilers with a final weight of 1.7 to 2.4 kg (Aviagen, 2014) (Table 1). Inclusion of soybean products into grower and finisher diets accounted for 35 and 25% of final feed, respectively. In total, 70 feed batches were produced (35 dietary variants within grower feed and finisher feed, respectively). Basal dietary components without soybean products were purchased from a commercial feed mixer (Likra West, Ingolstadt, Germany) and final feeds were completed at the experimental feed mixing plant of University of Hohenheim (Germany). Concentrations of nutrients, TIA, HDL, and KOH of the final grower and finisher feeds are presented in Table A3 and A4, respectively.

Table 1. Feed composition of grower and finisher diets and analyzed nutrient contents of grower and finisher basal components.

Fed ratio	Grower diet	Finisher diet
Soy cake sample/soybean meal reference (%)	35.0	25.0
Basal components (%)	65.0	75.0
Ingredients (%)		
Soy cake sample/soybean meal reference (%)	35.0	25.0
Maize	50.7	45.4
Wheat	11.1	26.3
Monocalcium phosphate	1.17	1.35
Calcium carbonate	0.83	0.89
Premix[2]	0.63	0.50
Sodium bicarbonate	0.36	0.41
Soybean oil	0.20	0.23
Sodium chloride	0.07	
Analyzed nutrients of basal components (%)		
KOH-CP[3]	56.4	66.3
ME[4] (MJ/kg)	13.2	13.0
Crude protein	8.80	9.30
Crude ash	5.50	5.50
Crude fat	3.90	3.50
Crude fiber	1.10	1.90
Calcium	0.91	0.91
Phosphor	0.76	0.76
Threonine	0.30	0.30
Lysine	0.25	0.30
TIA[5] (mg/g)	0.20	0.20
Sodium	0.18	0.15
Methionine	0.15	0.18
Tryptophan	0.08	0.10

[1]Analyzed nutrient concentrations and TIA of soy products and fed diets are displayed in Tables A2 to 4.
[2]Content per kg base diet: 12,500 IU vit. A; 5500 IU vit. D3; 125 mg vit E (α-tocopherol acetate); 62.5 mg L-Carnitine; 50 mg Fe (iron-(II)- sulphate); 125 mg Zn (zinc sulphate); 150 mg Mn (manganese-(II) sulphate); 20 mg Cu (copper-(II)-sulphate); 1.6 mg I (calcium iodate); 0.4 mg Se (sodium selenite); propyl gallate (E310); fumaric acid (E-297); calcium formate (E238); citric acid (E330); calcium lactate (E327); orthophosphoric acid (E338); propionic acid (E-280); 20,000 BXU Endo-1.4-ß-Xylanase; 625 FTU 3-phytase.
[3]KOH-CP: amount of potassium hydroxide soluble crude protein.
[4]ME (MJ/kg): Metabolizable Energy (ME) concentration was estimated on base of feed table information (DLG, 2018).
[5]TIA: trypsin inhibitor activity.

Animals and Housing

The animal study was reviewed and approved by responsible welfare authorities of the District Government of Lower Frankonia, Federal State of Bavaria, Germany (registered case number: 55.2–2532-2–331). The animal field trials were conducted at the Department for Education and Poultry Research, Bavarian State Research Center for Agriculture (Lower Frankonia, Germany).

Two consecutive and identical experimental runs with all 35 mixtures were conducted to provide an appropriate sample size. In each experimental run, a total of 840 male broiler chicks (1680 birds in total) (Ross 308; Aviagen Group, Huntsville, AL, USA) were obtained from a commercial hatchery (Brüterei Süd, Regenstauf, Germany). The mean BW at d 0 after hatching during the first experimental run was 44.0 (SD = 2.3) g and during the second run 40.9 (SD = 1.8) g.

Birds were weighed and raised for the first 10 days in 3 big cages of 280 chicks/cage. Cages were bedded with straw pellets. All birds were fed a commercial soybean meal based starter diet containing 12.4 MJ ME/kg and 21.5% crude protein from day 0 to 10, which met or exceeded all the nutrient requirements of ROSS 308 broilers according to Aviagen (2014).

At day 11 birds were individually weighed, wing tagged, and assigned to 35 individual grower diets in a balanced block design with BW as blocking factor yielding 105 separate blocks of 8 animals each. The mean initial BW at day 11 during the first experimental run was 174.5 g (SD = 21.8) and during the second run 157.8 g (SD = 17.4). Birds were fed the assigned experimental grower diet from day 11 to 24 and the experimental finisher diet from day 25 to 35. The mean initial BW at day 25 was 645.4 g (SD = 183.3) and 614.9 g (SD = 160.4) for the first and second experimental run, respectively. Cages (1.6 m^2/cage) were bedded with straw pellets. Birds had ad libitum access to feed and water during all stages of the study. Light program and ventilation were adjusted due to recommendations for Ross broilers by Aviagen (Aviagen, 2015). From day 11 to 35 birds received additional vitamin supplementation via the drinking water supply (Bela-Multivit AD3E forte: 0.1 ml/bird/d, Vechta, Germany; Biozink soluble: 1 g/l water, Vechta, Germany). Both sets of birds were vaccinated on day 16 for Newcastle Disease (Avipro ND LASOTA, Elanco Deutschland GmbH, Germany) and Infectious Bronchitis (Nobilis IB Ma5, MSD Tiergesundheit, Germany). On day 35 all birds were killed using electrical stunning and cervical dislocation.

In summary, the study comprised 35 different feeds, each of them represented by 6 cages (3 per experimental run) with 8 birds/cage.

Parameters of Zootechnical Performance

At day 1, 10, 24, and 35, birds were individually weighed to evaluate the liveweights (**LW**) at given time points as well as changes in total weight gain (**TWG**) over time. Furthermore, health conditions were evaluated according to Knierim et al. (2016). Total feed intake (**TFI**) was recorded per cage and production phase (grower, finisher), to estimate the feed conversion ratio as feed: gain (**FCR**). At day 35, 6 of 8 birds per cage were dissected after slaughtering and the pancreas of each bird was weighed.

Chemical Analyses

All chemical analyses were carried out in duplicates. TIA, KOH-soluble crude protein (KOH-CP) and crude nutrient analyses were determined for raw soybeans, processed soy cake samples, as well as in the experimental diets according to published standard procedures (DIN, 2001; VDLUFA, 2012). The amount of reactive (= non-glycosylated) lysine in soybean products was analyzed by the homoarginine method according to Pahm et al. (2008) and used to calculate the total contents of HDL in soybean products and final feed mixtures, respectively. Amino acids (except for tryptophan and tyrosine) were analyzed by ion-exchange chromatography according to Brugger et al. (2016).

Statistical Analyses

Data of experimental run 1 and 2 were merged to one common dataset. Individual animal data were pooled within cage and the mean values per cage were considered as the statistical replicate (n = 6 per feed variant). A preceding multi-factorial ANOVA analysis (data not shown) did not indicate any significant effect of the soybean breed; therefore, we excluded this aspect from all subsequent data analysis.

Data of the 35 variants of grower and finisher diets were statistically analyzed using descriptive statistics, linear regression analysis (y = a + bx), and, if applicable, broken-line regression analysis. Since broken-line regression analysis distinguished 2 ranges of dietary TIA with and without presence of correlation to HDL, the complete dataset was split into 2 subsets, each one comprising data derived from diets containing TIA below and above the respective breakpoint. Each of this subsets ('below breakpoint', 'above breakpoint') was individually analysed using linear regression. The threshold of significance was considered to be $\alpha \leq 0.05$ for all statistical procedures.

RESULTS

Dietary Parameters

Differential feed processing yielded a finely-graded range of TIA in complete feed (grower: 0.5 to 8.7 mg/g, finisher: 0.3 to 7.2 mg/g) and, at the same time, different degrees of heat damage to lysine (grower: 0.47 to 3.4 g/kg, finisher: 0.34 to 2.43 g/kg) and KOH (grower: 60.8 to 84.9%; finisher: 59.4 to 88.4%) (Table A2).

Figure 1 shows the relationship of HDL and KOH, respectively, to TIA in complete feed. Table 2 presents the statistical measures of the regression models in addition to Figure 1. Stepwise reduction in dietary TIA until a threshold of 1.8 mg/g in grower diets and 1.4 mg/g in finisher diets did not correlate to HDL. However, below the respective thresholds, HDL significantly increased with further declining TIA (slopes: -0.81 ± 0.33, $P < 0.02$ for grower and slopes: -0.67 ± 0.34, $P = 0.05$ for finisher diets, respectively). The latter was statistically evident for the grower diets ($P = 0.02$) and showed a similar trend in finisher diets ($P = 0.05$). It was not possible to estimate a broken-line regression for the relationship between KOH and TIA in complete feed. Therefore, we analyzed it using linear regression, which expressed a significant direct relationship in grower (slope = 1.37 ± 0.36, $P = 0.0006$) and finisher diets (slope = 1.94 ± 0.50, $P = 0.0005$), respectively (Figure 1, Table 2).

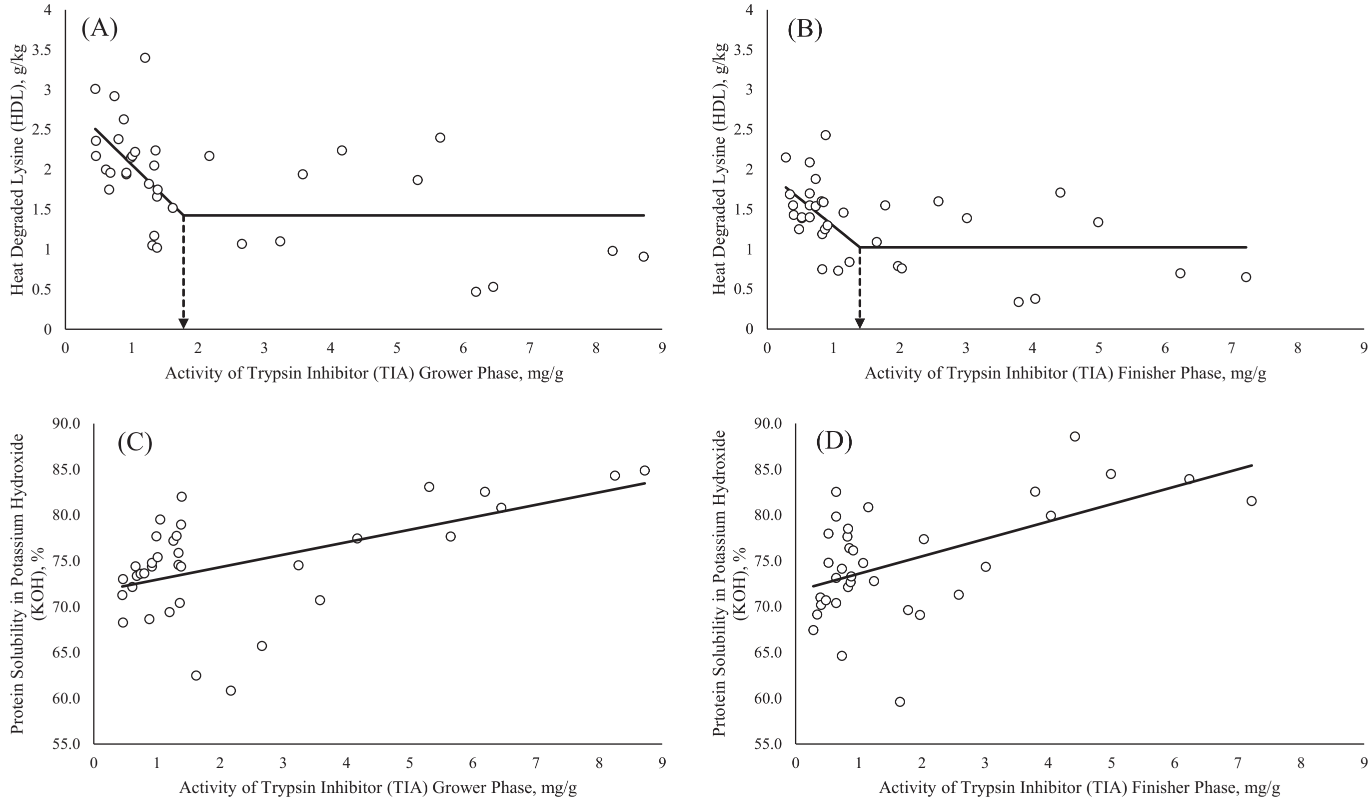

Figure 1. Broken-line regression analyses for data of grower (A) and finisher phase (B) displaying heat-degraded lysine (HDL) in relation to trypsin inhibitor (TIA) and regression analyses for data of grower (C) and finisher phase (D) displaying protein solubility in potassium hydroxide (KOH) in relation to trypsin inhibitor (TIA). A breakpoint is detected for grower feed (1.8 mg/g TIA) and finisher phase (1.4 mg/g TIA) indicating a plateau for HDL above the breakpoint. Each dot represents the dietary analysis of each of the 35 feed mixtures. Statistical data can be seen in Table 2.

Table 2. Broken-line regression analyses to examine the correlation between trypsin inhibitor activity (TIA) and heat-degraded lysine (HDL) in grower and finisher phase and regression analyses to examine the correlation between activity of TIA and protein solubility in potassium hydroxide (KOH).

	Regression models	Parameter estimates	*P* values	[1]R^2
Heat-degraded lysine (HDL), g/kg, grower Phase	$y = a + bx$ for $x \leq X_B$	a, 2.87 ± 0.17	<0.0001	0.31
	$y = Y_B$ for $x > X_B$	b, −0.81 ± 0.33	0.0200	
		X_B, 1.78 ± 0.40	<0.0001	
Heat-degraded lysine (HDL), g/kg, finisher Phase	$y = a + bx$ for $x \leq X_B$	a, 1.96 ± 0.12	<0.0001	0.29
	$y = Y_B$ for $x > X_B$	b, −0.67 ± 0.34	0.0547	
		X_B, 1.40 ± 0.41	0.0015	
Solubility in potassium hydroxide (KOH), %, grower phase	$y = a + bx$	a, 1.37 ± 71.6	<0.0001	0.30
		b, 1.37 ± 0.30	0.0006	
Solubility in potassium hydroxide (KOH), %, finisher phase	$y = a + bx$	a, 1.94 ± 71.6	<0.0001	0.31
		b, 1.94 ± 0.50	0.0005	

a: intercept; b: regression factor x and y.
[1]Coefficient of determination of the respective regression model.

Due to the potential for autocorrelation between dietary HDL and TIA below the aforementioned thresholds, all animal related data were divided into pre- and post-threshold subsets for subsequent regression analysis.

Zootechnical Performance and Pancreas Weight

All animals performed well, and no veterinary interventions were necessary during the whole trial. Overall mortality rate for the first and second experimental run was 1.4% and 1.3%, respectively.

Ranges of LW, TWG, TFI, and FCR at the end of the grower phase revealed a high degree of variation, from 383 to 798 g/bird, 213 to 633 g/bird, 550 to 759 g/bird, and 1.10 to 2.79, respectively (Table A5).

In groups fed with diets containing TIA of 1.8 mg/g and below during the grower phase, linear regression analyses revealed no significant relationship between zootechnical performance and TIA, HDL, or KOH, respectively (Table 3). In strong contrast, animals

Table 3. Regression models of live weight (LW), total weight gain (TWG), total feed intake (TFI), and feed conversion ratio (FCR) of broilers as affected by varying ratios of dietary trypsin inhibitor activity (TIA), heat-degraded lysine (HDL) concentrations, and solubility in potassium hydroxide (KOH) during grower feed.

	Trypsin inhibitor activity (TIA)	Regression models	Estimates of a	Estimates of b	$^1R^2$
Data of grower feed below breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 717 ± 42.8 (P < 0.0001)	b, − 30.4 ± 40.2 (P = 0.4581)	0.03
	Total weight gain (TWG), g/bird	y = a + bx	a, 546 ± 42.3 (P < 0.0001)	b, − 31.2 ± 39.8 (P = 0.4406)	0.03
	Total feed intake (TFI), g/bird	y = a + bx	a, 693 ± 29.9 (P < 0.0001)	b, − 12.5 ± 28.0 (P = 0.6596)	0.01
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 1.29 ± 0.09 (P < 0.0001)	b, 0.08 ± 0.09 (P = 0.3948)	0.03
Data of grower feed above breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 636 ± 38.9 (P < 0.0001)	b, − 25.2 ± 7.03 (P = 0.0059	0.59
	Total weight gain (TWG), g/bird	y = a + bx	a, 472 ± 39.8 (P < 0.0001)	b, − 25.6 ± 7.19 (P = 0.0061)	0.59
	Total feed intake (TFI), g/bird	y = a + bx	a, 674 ± 28.4 (P < 0.0001)	b, − 7.22 ± 5.13 (P = 0.1925)	0.18
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 1.25 ± 0.21 (P < 0.0002)	b, 0.14 ± 0.04 (P = 0.0049)	0.60
	Heat-degraded lysine (HDL)				
Data of grower feed below breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 599 ± 48.5 (P < 0.0001)	b, 42.5 ± 22.8 (P = 0.0750)	0.13
	Total weight gain (TWG), g/bird	y = a + bx	a, 431 ± 48.3 (P < 0.0001)	b, 40.5 ± 22.7 (P = 0.0876)	0.13
	Total feed intake (TFI), g/bird	y = a + bx	a, 643 ± 16.5 (P < 0.0001)	b, 18.0 ± 16.5 (P = 0.2866)	0.05
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 1.55 ± 0.11 (P < 0.0001)	b, -0.09 ± 0.05 (P = 0.0787)	0.14
Data of grower feed above breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 498 ± 53.0 (P < 0.0001)	b, 6.65 ± 33.6 (P = 0.8568)	0.01
	Total weight gain (TWG), g/bird	y = a + bx	a, 6.24 ± 54.0 (P < 0.0002)	b, 6.65 ± 34.22 (P = 0.8502)	0.01
	Total feed intake (TFI), g/bird	y = a + bx	a, 654 ± 26.75 (P < 0.0001)	b, -11.7 ± 17.0 (P = 0.5075)	0.05
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 2.13 ± 0.28 (P < 0.0001)	b, − 0.12 ± 0.18 (P = 0.4995)	0.05
	Solubility in Potassium Hydroxide (KOH)				
Data of grower feed below breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 439 ± 245 (P < 0.0863)	b, 3.35 ± 3.31 (P = 0.3218)	0.05
	Total weight gain (TWG), g/bird	y = a + bx	a, 304 ± 243.7 (P < 0.2260)	b, 2.86 ± 3.29 (P = 0.3948)	0.03
	Total feed intake (TFI), g/bird	y = a + bx	a, 440 ± 165 (P < 0.0142)	b, 3.25 ± 2.24 (P = 0.1603)	0.09
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 1.46 ± 0.55 (P < 0.0145)	b, − 0.01 ± 0.01 (P = 0.8703)	0.01
Data of grower feed above breakpoint (1.8 mg/g TIA)	Live weight (LW) at day 24, g/bird	y = a + bx	a, 936 ± 180 (P < 0.0006)	b, − 5.60 ± 2.34 (P = 0.0402)	0.39
	Total weight gain (TWG), g/bird	y = a + bx	a, 770 ± 185 (P < 0.0025)	b, 5.61 ± 2.41 (P = 0.0449)	0.38
	Total feed intake (TFI), g/bird	y = a + bx	a, 742 ± 113.8 (P < 0.0001)	b, 1.37 ± 1.48 (P = 0.3794)	0.09
	Feed conversion ratio (FCR), ratio	y = a + bx	a, -0.03 ± 0.98 (P <0.7765)	b, 0.03 ± 0.01 (P = 0.0475)	0.37

a: intercept; b: slope of model.
[1]Coefficient of determination of the respective regression model.

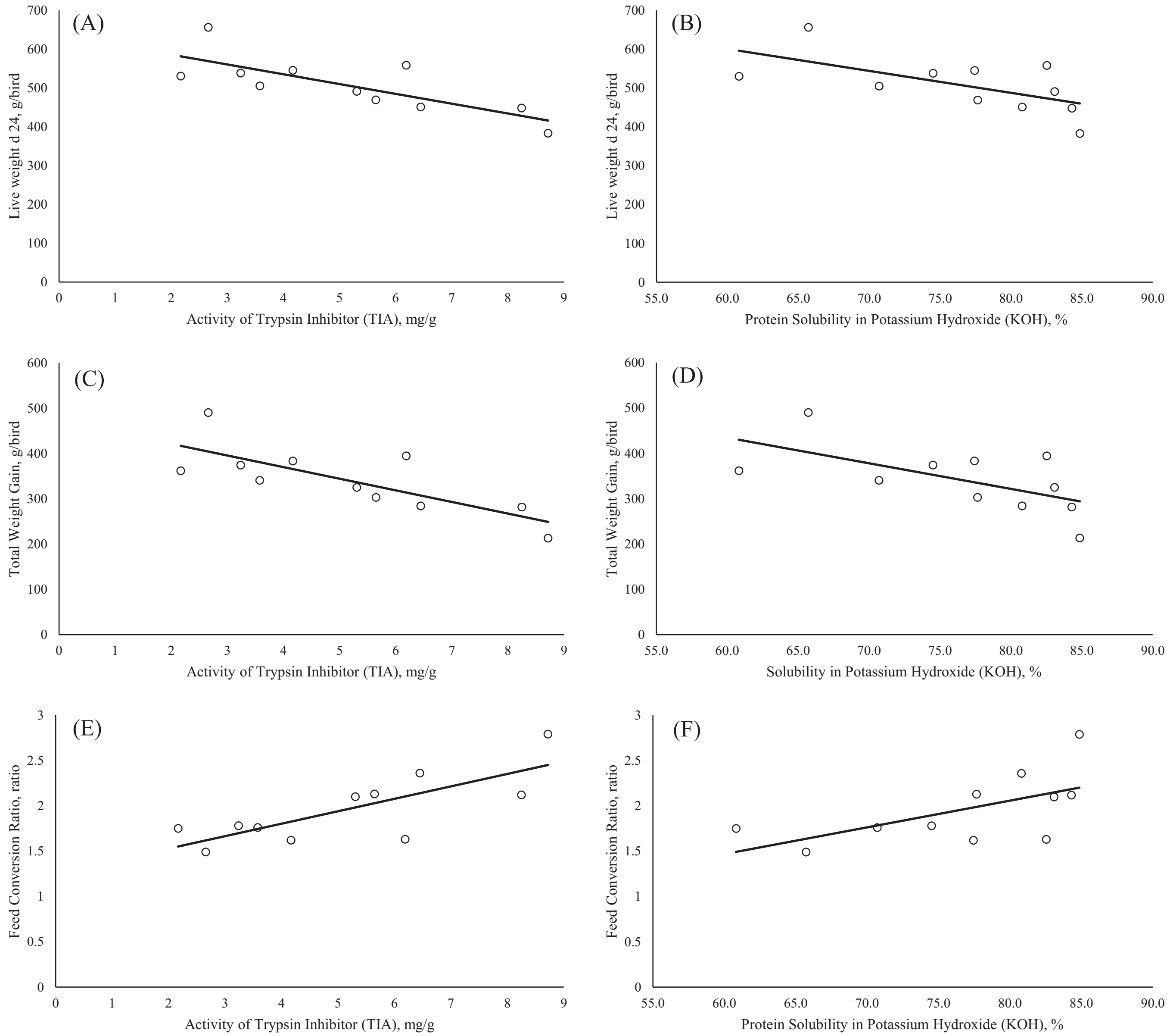

Figure 2. The effect of trypsin inhibitor activity (TIA) and protein solubility in potassium hydroxide (KOH) in processed soy cake fed to broiler chickens on live weight (LW) (A, B), total weight gain (TWG) (C, D), and feed conversion ratio (FCR) (E, F) for dietary treatments with above calculated breakpoints in the grower phase. Statistical data behind graphs can be seen in Table 3. Each dot represents the mean value of each dietary treatment (n = 11). Treatment means of LW are each calculated on base of n = 48 individual values (bird-wise). Treatment means of TWG and FCR are each calculated on base of n = 6 individual values (cage-wise).

challenged with TIA above 1.8 mg/g experienced an inverse linear response of LW (slope = −25.2 ± 7.03, $P = 0.0059$) and TWG (slope = −25.6 ± 7.19, $P < 0.0061$) (Figures 2 A, C, Table 3) and a direct response of FCR (slope = 0.14 ± 0.04, $P < 0.0049$) with decreasing TIA (Figure 2 E, Table 3), whereas TFI was not affected whatsoever (Table 3). Comparable response patterns were also evident in these groups as demonstrated in the relationship between dietary KOH and LW (slope = −5.60 ± 2.34, $P < 0.0402$) and TWG (slope = 5.61 ± 2.41, $P < 0.0449$) as well as for FCR (slope = 0.03 ± 0.01, $P < 0.0475$) (Figures 2 B, D, F, Table 3), but not for TFI. HDL did not affect zootechnical performance in grower phase within groups receiving dietary TIA >1.8 mg/g (Table 3).

At the end of the finisher phase, LW, TWG, TFI, and FCR exhibited ranges from 679 to 1317 g/bird, 290 to 590 g/bird, 699 to 1201 g/bird, and 1.98 to 3.33, respectively. Groups receiving dietary TIA 1.4 mg/g and below during finisher phase exhibited no significant changes of zootechnical parameters in response to dietary TIA, HDL, and KOH, respectively (Table 4). In contrast, above this threshold LW was negatively and significantly correlated to TIA (slope = 35.4 ± 14.6, $P < 0{,}0255$) (Figure 3 A, Table 4), whereas FCR directly responded to KOH (slope = −0.02 ± 0.01, $P < 0.014$) in a linear fashion (Figure 3 B, Table 4). All other parameters were neither significantly affected by TIA nor HDL (Table 4).

Table 4. Regression models of live weight (LW), total weight gain (TWG), total feed intake (TFI), feed conversion ratio (FCR), and pancreas weight (at day of slaughter) of broilers as affected by varying ratios of dietary trypsin inhibitor activity (TIA), heat-degraded lysine (HDL) concentrations, and solubility in potassium hydroxide (KOH) during finisher feed.

	Trypsin inhibitor activity (TIA)	Regression models	Estimates of a	Estimates of b	[1]R^2
Data of finisher feed below breakpoint (1.4 mg/g TIA)	Live weight (LW) at day 35, g/bird	y = a + bx	a, 1199 ± 72.3 ($P < 0.0001$)	b, − 33.7 ± 95.8 ($P = 0.7208$)	0.01
	Total weight gain (TWG), g/bird	y = a + bx	a, 488 ± 41.4 ($P < 0.0001$)	b, − 18.97 ± 54.8 ($P = 0.7325$)	0.01
	Total feed intake (TFI), g/bird	y = a + bx	a, 1089 ± 66.47 ($P < 0.0001$)	b, − 30.4 ± 88.0 ($P = 0.7331$)	0.01
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 2.26 ± 0.20 ($P < 0.0001$)	b, 0.20 ± 0.27 ($P = 0.4662$)	0.03
	Pancreas weight, g/bird	y = a + bx	a, 3.85 ± 0.26 ($P < 0.0001$)	b, 0.24 ± 0.35 ($P = 0.5045$)	0.02
Data of finisher feed above breakpoint (1.4 mg/g TIA)	Live weight (LW) at d35, g/bird	y = a + bx	a, 1034 ± 59.1 ($P < 0.0001$)	b, − 38.4 ± 14.6 ($P = 0.0255$)	0.41
	Total weight gain (TWG), g/bird	y = a + bx	a, 402 ± 40.8 ($P < 0.0001$)	b, − 6.74 ± 10.1 ($P = 0.5198$)	0.04
	Total feed intake (TFI), g/bird	y = a + bx	a, 949 ± 59.4 ($P < 0.0001$)	b, − 23.5 ± 14.7 ($P = 0.1409$)	0.20
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 2.45 ± 0.13 ($P < 0.0001$)	b, − 0.03 ± 0.03 ($P = 0.3612$)	0.08
	Pancreas weight, g/bird	y = a + bx	a, 3.27 ± 0.41 ($P < 0.0001$)	b, 0.33 ± 0.10 ($P = 0.0090$)	0.51
	Heat-degraded lysine (HDL)				
Data of finisher feed below breakpoint (1.4 mg/g TIA)	Live weight (LW) at d35, g/bird	y = a + bx	a, 1038 ± 85.6 ($P < 0.0001$)	b, 91.6 ± 55.6 ($P = 0.1145$)	0.11
	Total weight gain (TWG), g/bird	y = a + bx	a, 408 ± 45.0 ($P < 0.0001$)	b, 45.2 ± 32.3 ($P = 0.1770$)	0.09
	Total feed intake (TFI), g/bird	y = a + bx	a, 950 ± 79.2 ($P < 0.0001$)	b, 79.4 ± 51.5 ($P = 0.1377$)	0.10
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 2.74 ± 0.25 ($P < 0.0001$)	b, − 0.23 ± 0.16 ($P = 0.1654$)	0.09
	Pancreas weight, g/bird	y = a + bx	a, 3.80 ± 0.33 ($P < 0.0001$)	b, 0.14 ± 0.21 ($P = 0.5067$)	0.02
Data of finisher feed above breakpoint (1.4 mg/g TIA)	Live weight (LW) at d35, g/bird	y = a + bx	a, 922 ± 79.9 ($P < 0.0001$)	b, − 26.8 ± 71.1 ($P = 0.7140$)	0.01
	Total weight gain (TWG), g/bird	y = a + bx	a, 416 ± 41.7 ($P < 0.0001$)	b, − 37.1 ± 37.1 ($P = 0.3407$)	0.09
	Total feed intake (TFI), g/bird	y = a + bx	a, 919 ± 67.1 ($P < 0.0001$)	b, − 54.1 ± 59.7 ($P = 0.3860$)	0.08
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 2.20 ± 0.14 ($P < 0.0001$)	b, 0.14 ± 0.12 ($P = 0.2842$)	0.11
	Pancreas weight, g/bird	y = a + bx	a, 5.46 ± 0.50 ($P < 0.0001$)	b, − 0.98 ± 0.45 ($P = 0.0525$)	0.33
	Solubility in Potassium Hydroxide (KOH)				
Data of finisher feed below breakpoint (1.4 mg/g TIA)	Live weight (LW) at d35, g/bird	y = a + bx	a, 806 ± 401 ($P < 0.0573$)	b, 4.98 ± 5.41 ($P = 0.3676$)	0.04
	Total weight gain (TWG), g/bird	y = a + bx	a, 73.3 ± 217 ($P < 0.7382$)	b, 5.43 ± 2.92 ($P = 0.0773$)	0.14
	Total feed intake (TFI), g/bird	y = a + bx	a, 896 ± 374 ($P < 0.0258$)	b, 2.32 ± 5.05 ($P = 0.6507$)	0.01
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 4.52 ± 1.06 ($P < 0.0004$)	b, − 0.03 ± 0.01 ($P = 0.0584$)	0.16
	Pancreas weight, g/bird	y = a + bx	a, 1.23 ± 1.35 ($P < 0.3745$)	b, 0.04 ± 0.02 ($P = 0.0511$)	0.17
Data of finisher feed above breakpoint (1.4 mg/g TIA)	Live weight (LW) at d35, g/bird	y = a + bx	a, 1062 ± 318 ($P < 0.0075$)	b, − 2.17 ± 4.11 ($P = 0.6091$)	0.03
	Total weight gain (TWG), g/bird	y = a + bx	a, 208 ± 167 ($P < 0.2400$)	b, 2.20 ± 2.16 ($P = 0.3309$)	0.09
	Total feed intake (TFI), g/bird	y = a + bx	a, 906 ± 279 ($P < 0.0088$)	b, − 0.55 ± 3.61 ($P = 0.8831$)	0.01
	Feed conversion ratio (FCR), ratio	y = a + bx	a, 3.59 ± 0.42 ($P < 0.0001$)	b, − 0.02 ± 0.01 ($P = 0.0140$)	0.47
	Pancreas weight, g/bird	y = a + bx	a, − 1.83 ± 1.41 ($P < 0.2237$)	b, 0.08 ± 0.02 ($P = 0.0012$)	0.67

a: intercept; b: slope of model.
[1]Coefficient of determination of the respective regression model.

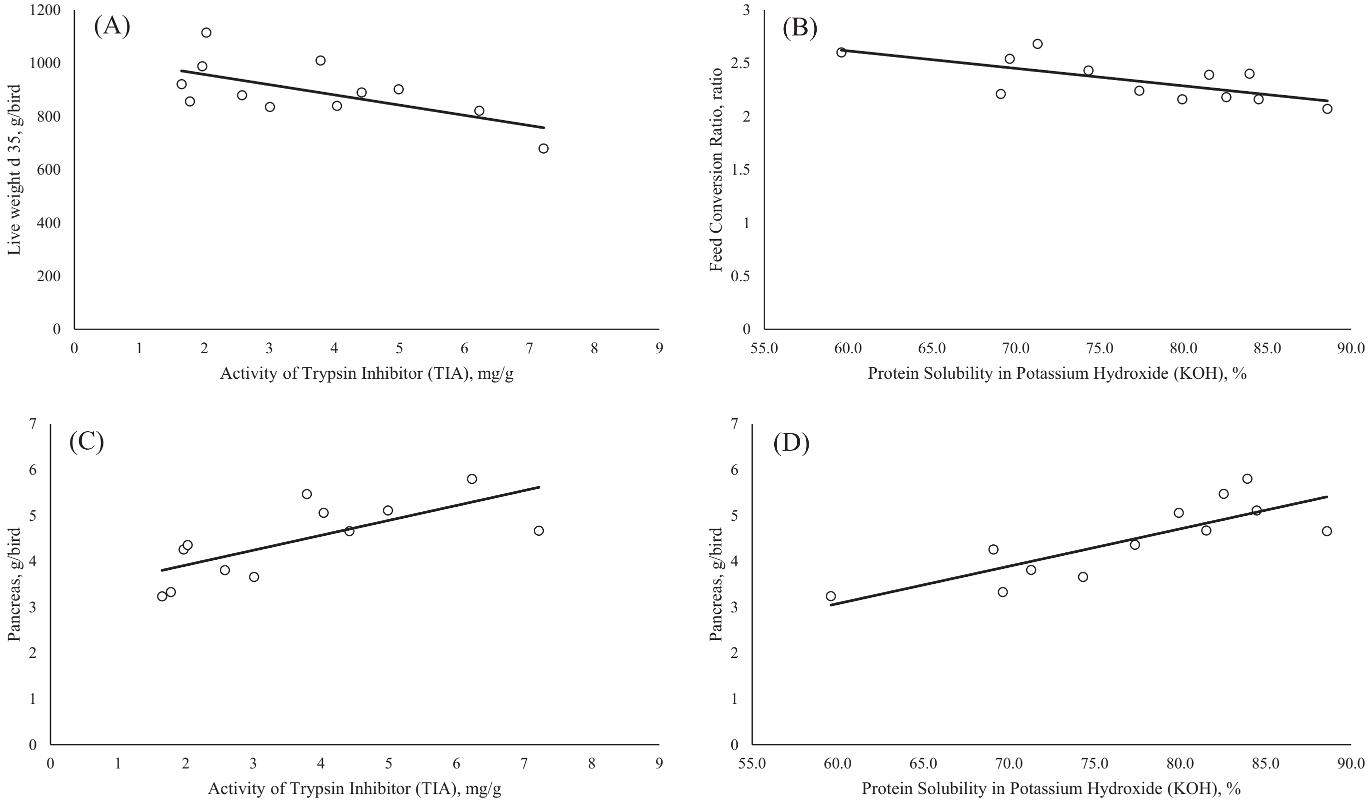

Figure 3. The effect of trypsin inhibitor activity (TIA) and protein solubility in potassium hydroxide (KOH) in processed soy cake fed to broiler chickens on live weight (LW) (A), feed conversion ratio (FCR) (B), and pancreas weight (C, D) for dietary treatments with above calculated breakpoint in finisher phase. Statistical data behind graphs can be seen in Table 4. Each dot represents the mean value of each dietary treatment (n = 12). Treatment means of LW are each calculated on base of n = 48 individual values (bird-wise). Treatment means of FCR are each calculated on base of n = 6 individual values (cage-wise).

Pancreas weight ranged from 3.24 to 5.80 g/bird at the time point of slaughtering. Below the TIA threshold of 1.4 mg/g in finisher feed, regression analyses did not reveal significant relationships between pancreas weight and dietary TIA, HDL, or KOH, respectively (Table 4). In groups fed diets with TIA above this threshold, pancreas weight responded in a straight linear fashion to TIA (slope = 0.33 ± 0.10, $P < 0.009$) and KOH (slope = 0.08 ± 0.02, $P < 0.0012$) (Figure 3 C, D, Table 4) but not HDL (Table 4).

Because intercepts and slopes derived from separate linear regression analyses of the 2 subsets of data (below and above TIA threshold, Figure 1) were quite similar, we performed a linear regression analyses over the entire dietary range. We analyzed the reaction of FCR during the grower phase in relation to dietary TIA over all treatment groups (Figure 4, Table 5) because FCR exhibited the strongest correlation to TIA ($R^2 = 0.77$) and exhibited a straight linear increase of FCR with increasing TIA (slope = 0.14 ± 0.01, $P < 0.0001$). Implementation of a broken-line model was not possible for this dataset.

DISCUSSION

This study investigated the response of zootechnical performance and pancreas weight of broiler chicks to varying dietary TIA, HDL, and KOH, as applied by different processing techniques of soybean cake. Consequently, we were able to generate a wide range of these parameters in the final grower and finisher feed mixtures. This applied particularly to low TIA levels. For example, a recent feeding study with broiler chicks conducted by Heger et al. (2016) reached TIA minimum levels of 1.01 mg/g and 0.88 mg/g in final grower and finisher feeds, respectively. Currently, the upper limits tolerable for TIA in full-fat soybeans are considered to be below 4 mg/g when used for broiler chickens (Clarke and Wiseman, 2005; Clarke and Wiseman, 2007). By increasing the range of TIA well below and above the practical threshold of TIA in broiler diets, this study allowed greater conclusions to be drawn about the birds' response.

High processing intensities depressed TIA to very low values but also elevated HDL. This increase occurred below comparably small TIA values of 1.8 mg/g (grower feed) and 1.4 mg/g (finisher feed), respectively. HDL reflects the degree of lysine degradation through Maillard reactions and hence is an accepted parameter of overprocessing that indicates a decreasing availability of amino acids in soybean products (Faldet et al., 1992; Fontaine et al., 2007). Indeed, responses of zootechnical performance and pancreas weight as observed in the present study at low TIA levels might have been

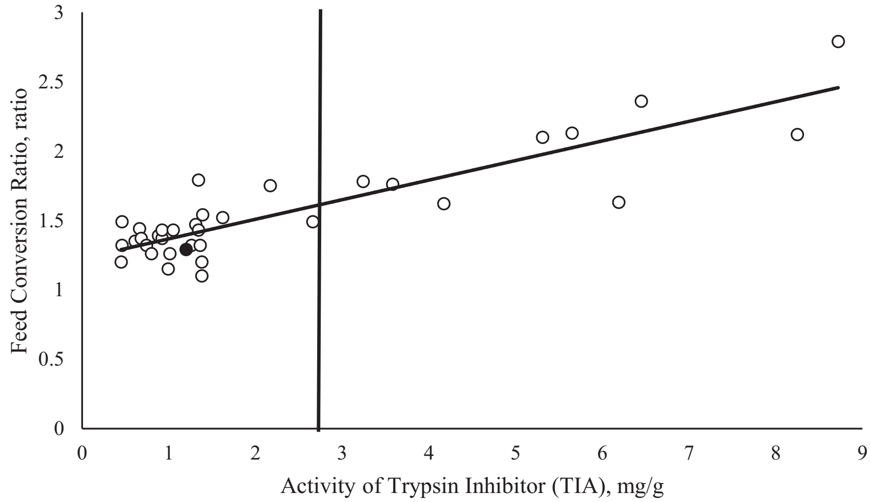

Figure 4. The effect of trypsin inhibitor activity (TIA) in processed soy cake fed to broiler chickens on feed conversion ratio (FCR) for all dietary treatments in grower phase. Statistical data provided in Table 5. Each dot represents the mean value of each dietary treatment (n = 35). Treatment means of FCR are each calculated on base of n = 6 individual values (cage-wise). Black dot represents commercial soybean meal. Black vertical line shows adapted TIA threshold for soy cake in fed diet (2.6 mg/g). Threshold in fed ratio was calculated using the accepted threshold of 4 mg/g for full fat soybeans (as published by Clarke and Wiseman 2005), estimating 15% oil content and 35% of soy cake in grower feed.

Table 5. Regression model of feed conversion ratio (FCR) of broilers as affected by varying ratios of dietary trypsin inhibitor activity (TIA) during grower phase.

	Regression models	Estimates of a	Estimates of b	[1]R^2
Feed conversion ratio (FCR), ratio	y = a + bx	a, 1.22 ± 0.04 ($P < 0.0001$)	b, 0.14 ± 0.01 ($P < 0.0001$)	0.77

a: intercept; b: slope of model.
[1]Coefficient of determination of the respective regression model.

affected at least theoretically by additive effects of both TIA and HDL. For this reason, the statistical evaluations were performed separately for subsets of data below and above the respective TIA thresholds. Nevertheless, it must be kept in mind that rising HDL may affect performance only under the condition of inducing a limitation in essential amino acids, namely lysine. Since our experimental diets were composed according to recommendations, it may be assumed that limiting amino acids were provided with some safety margins that compensated for rising HDL. Therefore, it may be concluded that TIA had the most significant impact on zootechnical performance compared to HDL under the present experimental conditions.

Another indicator for protein degradation through feed processing is decreasing protein solubility in KOH. According to Araba and Dale (1990), KOH levels below 70% indicate over-processing. Indeed, our study reached dietary KOH levels of 60%. Again, diet formulations of our experimental feeds may be assumed to provide some safety margins in protein supply compensating potentially depressing effects of KOH on performance. Furthermore, since dietary KOH was correlated to TIA (r = 0.55 and 0.56 for grower and finisher, respectively), some effects statistically attributable to KOH might have descended from corresponding changes in TIA.

There is plenty of evidence that processing of soybeans improves their feed value (Osborne and Meldel, 1917; Liener, 1962; Van Der Poel et al., 1990; Heger et al., 2016; Rohe et al., 2017), thereby increasing the utilization of feed within the gastrointestinal tract through a decrease in antinutritive substances (Liener, 1962; Rohe et al., 2017). In the present study, untreated soybeans caused the lowest growth performance in the grower and finisher phases and a stepwise reduction of dietary TIA improved zootechnical performance and reduced pancreatic weight accordingly, which is in line previous results (e.g., Heger et al., 2016). Lower pancreas weights are considered to reflect relief from pancreatic hypertrophy induced specifically by dietary presence of TI (Applegarth et al., 1964; Kakade et al., 1973; Han and Parsons, 1991; Leeson and Atteh, 1996; Clarke and Wiseman, 2007). However, KOH was also inversely correlated with pancreas weight and associated with a reduction in FCR. This somewhat contradicts the current stage of knowledge of reduced KOH (below ∼ 70%) and its adverse effects on animal performance (Araba and Dale, 1990). Given the aforementioned significant interaction between dietary TIA and KOH, we conclude this observation reflects an indirect TIA effect due to autocorrelation with dietary KOH. In summary, zootechnical performance and pancreas weights were mainly affected by dietary TIA rather than HDL or KOH during the present study.

This assumption is further supported by the fact that intercepts and slopes derived from separate linear regression analysis of the 2 subsets of data (below and above TIA threshold as depicted in Figure 1) were quite similar. Consequently, it seems to be justified to perform linear regression analysis over the entire range of dietary TIA. As an example, we analyzed the reaction of FCR during the grower phase in relation to dietary TIA over all treatment groups. We chose this parameter because it exhibited the strongest correlation to TIA ($R^2 = 0.77$) and thus would be the best parameter of our dataset to derive a critical threshold of dietary TIA. As demonstrated, FCR responded directly in a linear fashion to dietary TIA without any indication of a plateau that would point towards a certain threshold of upper tolerable activities within complete feed. Compared to the threshold of TIA commonly used in practice (4 mg/g in full-fat soybeans (Clarke and Wiseman, 2005; Clarke and Wiseman, 2007), corresponding to around 2.6 mg/g assuming the inclusion levels used in the present study), further reductions in dietary TIA continued to improve FCR (the lowest FCR was evident in a group with TIA = 1.38 mg/g, HDL = 1.66 g/kg, KOH = 74.4%).

In conclusion, feed processing jointly modifies TIA, HDL, and KOH, but TIA was revealed to be the most important parameter negatively affecting zootechnical performance and pancreas weight. Since effectiveness of TIA was demonstrated over the whole range of dietary activities, including very minute concentrations below currently applied thresholds, it is recommended to completely eliminate dietary TIA under practical dietary conditions as far as technically possible with generous safety margins in terms of essential amino acid supply.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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