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# Changes in body composition in broilers by a sulfur amino acid deficiency during growth

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**ABSTRACT** In the factorial approach, amino acid (AA) requirements are determined using the AA composition of retained protein, which is assumed to be constant. However, this hypothesis may not be valid because the AA composition of body protein can be affected by the diet. The objective of this study was to quantify the changes in chemical body composition of broilers receiving diets either deficient (TSAA<sup>-</sup>) or sufficient (TSAA<sup>+</sup>) in TSAA. Diet TSAA<sup>+</sup> was formulated according to the Ross recommendation. Diet TSAA<sup>-</sup> provided 36% true digestible Met:Lys and 64% true digestible TSAA:Lys, which were, respectively, 34 and 22% lower compared with diet TSAA<sup>+</sup>. Performance and tissue weight gain between 7 and 42 d of age were not affected by the TSAA supply. In TSAA<sup>-</sup> chickens, protein gain was lower in the carcass ( $P < 0.01$ ) and tended to be lower in the empty body ( $P = 0.06$ ) and pectoralis major muscle ( $P = 0.10$ ). Compared with

TSAA<sup>+</sup> chickens, lipid gain in TSAA<sup>-</sup> chickens was 78% greater in the pectoralis muscle ( $P < 0.001$ ), 28% greater in abdominal fat ( $P < 0.05$ ), and 10% greater in the carcass ( $P = 0.10$ ). In the pectoralis muscle, there was a tendency for an increase in the redness value (a\*;  $P = 0.10$ ). The TSAA supply affected the AA composition of tissues and tissue gain, but the Met and Cys concentrations were changed only in the offal ( $P = 0.08$ ). The deficient TSAA supply resulted in an increase in the Ser concentration in the empty body, carcass, and pectoralis muscle ( $P < 0.05$ ). In contrast, it resulted in a decrease in the concentrations of Lys and Glu in the empty body, of Phe, Tyr, Gly, and Glu in the pectoralis muscle, and of Ala in the offal ( $P < 0.05$ ). This indicates that although chickens cope with a TSAA deficiency predominantly by changing the protein and lipid concentration in the body, the AA composition is also affected. This calls into question the use of a constant ideal AA profile in poultry nutrition.

**Key words:** broiler, body composition, deficiency, sulfur amino acid, tissue gain

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## INTRODUCTION

The sulfur amino acids (AA) Met and Cys are typically the first-limiting AA for protein deposition in poultry diets (NRC, 1994). As an indispensable AA, Met is also an important methyl donor and precursor for the biosynthesis of compounds such as creatine, carnitine, and polyamines (Baker et al., 1996; Kim, 2005). Cysteine is considered a semi-essential AA because it can be synthesized from Met and Ser by transsulfuration (Stipanuk, 2004).

The AA requirements in growing animals are often determined by the factorial approach considering the AA composition of retained protein, the maximum efficiency of AA utilization for growth, and the maintenance AA requirement. It is then assumed that the relative contribution of these traits to the AA requirements is constant, resulting in a constant ideal AA profile. However, there are indications that the AA composition in the body is affected by genotype (Geraert et al., 1990; Pesti et al., 1994), age (Fisher et al., 1981; Rivera-Torres et al., 2011), sex (Dozier et al., 2008; Stilborn et al., 2010), and the protein and AA content in the diet (Jeroch and Pack, 1995; Huyghebaert and Pack, 1996; Fatufe and Rodehutsord, 2005). In pigs, tissues were affected differently by a Met deficiency, suggesting that the growth response alone is insufficient to appreciate the AA requirement (Conde-Aguilera et al., 2010).

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Broiler studies aiming to assess the response to the TSAA supply mostly focus on performance, carcass yield, and the chemical composition of the whole animal or breast meat (Huyghebaert and Pack, 1996; Fatufe and Rodehutsord, 2005). However, as shown in pigs, tissues may respond differently to a deficient TSAA supply. Therefore, the objective of this study was to quantify the response of broilers to a deficient TSAA supply on performance, meat quality, and chemical composition of different tissues.

## MATERIALS AND METHODS

Animal care procedures were carried out according to current French legislation on animal experimentation, and the senior researchers are authorized by the French Ministry of Agriculture to conduct experiments on living animals at the INRA facilities in Nouzilly, France.

### *Birds, Experimental Design, and Housing*

Fifty male broiler chicks (*Gallus gallus*; Ross PM3 strain) were obtained at 1 d of age from a hatchery (Grelier, St Laurent de la Plaine, France) and were reared conventionally until 7 d of age. Thirty birds ( $181 \pm 7$  g) were then selected, 6 of which were slaughtered to estimate the initial body composition. The remaining 24 animals were divided in 2 homogenous groups of 12 birds each. Each group consisted of birds receiving a diet either deficient or sufficient in TSAA (**TSAA-** and **TSAA+**, respectively) during a 5-wk period. The 24 birds were housed individually in wire-floored cages (length 30 cm  $\times$  width 30 cm  $\times$  height 36 cm). Room temperature was gradually decreased from 29°C at 7 d of age to 22°C at 23 d of age to be maintained until the end of the experiment. Artificial lighting was provided 23 h/d during the 3 first d of age and then changed to 18 h/d until the end of the experiment. Birds were weighed after an overnight fast at 7 and 42 d of age. All birds were slaughtered at 42 d of age; 12 birds (6 birds per treatment) were used for body composition analysis, whereas the other 12 birds were used to determine meat quality and organ weights. The latter group was designated as the sampling group.

### *Diets and Feeding*

Before the start of the experiment (i.e., from d 1 to 6), chicks were offered ad libitum a starter diet with 20% CP and 1.19% Lys. Four diets, based on corn, wheat, and soybean meal, were formulated and served as a basis for the experimental diets (Table 1). These 4 diets differed in TSAA contents on the one hand and in energy, CP, and AA contents on the other hand to account for the change in nutrient requirements between wk 1 (d 7 to 14) and wk 5 (d 36 to 42). Differences in TSAA content were created by substituting DL-Met for cornstarch. Birds received a different diet each week

by proportional blending of the diets formulated for wk 1 and 5. Diets were formulated so that the nutrient composition (other than TSAA) met or exceeded the NRC (1994) recommendations. The anticipated true digestible (**TD**) Lys content decreased from 1.10 to 0.94% from wk 1 to 5, which is in accordance with the recommendations of the breeder (Aviagen, 2009). Diet TSAA- provided 36% TD Met:Lys and 64% TD TSAA:Lys, which are, respectively, 34 and 22% lower than the supply of these AA in diet TSAA+.

All diets were offered in crumble form. Because the TSAA concentration in the diet may affect voluntary feed intake, the daily food allowance was slightly below the anticipated ad libitum intake capacity. The allowance was adjusted daily avoiding feed refusals, which (if any) were weighed to calculate actual ADFI. Water was freely available.

### *Comparative Slaughter Measurements*

The change in body composition was determined by comparing the body composition of the initial group (slaughtered at 7 d of age,  $n = 6$ ) with that of birds having received the experimental diets for 5 wk (slaughtered at 42 d of age,  $n = 6$  per treatment). Animals were slaughtered by cervical dislocation and exsanguination. Before slaughter, birds were kept for 4 to 6 h in a light room without access to feed but with free access to water. After slaughter, the animal was divided into blood, feathers, carcass (without head, feet, and neck), and offal [i.e., the emptied small and large intestines, liver, kidneys, stomach, heart, lungs, diaphragm, genital tract, bladder, gizzard, spleen, and abdominal fat (leaf fat surrounding the cloaca and abdominal fat surrounding the gizzard) together with head, feet, and neck]. Each animal was weighed before and after exsanguination to estimate blood weight. Blood was collected, pooled by treatment and age to create a representative sample, and stored at  $-20^{\circ}\text{C}$ . The body was then soaked in hot wax ( $60$  to  $70^{\circ}\text{C}$ ) for 10 to 20 s and plucked. The weight before and after plucking was used to determine feather mass. Before plucking, some feathers of each bird were picked from different parts of the body (from the same location for different birds) to create a representative feather sample. Feathers samples were then pooled by treatment and age and stored at  $-20^{\circ}\text{C}$ . Frozen samples of pooled blood and feathers were freeze-dried. The pectoralis major muscle (**PM**) was separated from the carcass at slaughter, and these 2 components were analyzed separately. The PM was weighed, cut into small pieces, frozen, and freeze-dried later. Carcass samples were weighed, frozen, ground in a cutter-slicer (Hobart FR15 cutter/slicer, Marne la Vallée, France), and then freeze-dried. Offal was weighed, frozen, and freeze-dried. Samples of the freeze-dried blood, feathers, carcass (without PM), PM, and offal were independently homogenized in a cutter mill (Grindomix GM200, Restsch, Newton, PA).

**Table 1.** Ingredients and nutrient composition of the basal diets (as-fed basis)<sup>1</sup>

Item (%)	wk 1 (d 7 to 14)		wk 5 (d 36 to 42)	
	TSAA-	TSAA+	TSAA-	TSAA+
<b>Ingredient</b>				
Corn	43.35	43.35	46.26	46.26
Soybean meal	32.00	32.00	29.20	29.20
Wheat	15.00	15.00	15.00	15.00
Soybean oil	5.18	5.18	5.39	5.39
Corn starch	1.00	0.79	1.00	0.81
DL-Methionine	0.10	0.31	0.06	0.25
L-Lysine HCl	0.20	0.20	0.12	0.12
L-Threonine	0.08	0.08	0.04	0.04
Salt	0.30	0.30	0.30	0.30
Calcium carbonate	0.23	0.23	0.24	0.24
Dicalcium phosphate	2.01	2.01	1.85	1.85
Trace minerals and vitamin premix <sup>2</sup>	0.50	0.50	0.50	0.50
Monensin	0.05	0.05	0.05	0.05
<b>Analyzed composition<sup>3</sup></b>				
Protein (N × 6.25)	20.0	20.0	19.1	18.9
Crude fat	7.2	7.2	7.4	7.3
Starch	38.2	38.4	40.0	39.3
Ash	5.1	5.2	4.9	4.8
Gross energy (kcal/kg)	4,053	4,062	4,078	4,044
<b>Essential amino acid</b>				
Lys	1.18	1.19	1.05	1.01
Met	0.42	0.61	0.36	0.53
TSAA	0.74	0.95	0.68	0.85
Thr	0.81	0.81	0.74	0.73
Trp <sup>4</sup>	0.20	0.20	0.20	0.20
Ile	0.84	0.85	0.82	0.80
Leu	1.60	1.65	1.60	1.53
Val	0.92	0.92	0.89	0.89
His	0.55	0.54	0.53	0.53
Phe	0.95	0.97	0.94	0.90
Arg	1.26	1.33	1.28	1.18
Tyr	0.77	0.75	0.74	0.71
<b>Calculated composition<sup>3,4</sup></b>				
AME <sub>n</sub> (kcal/kg)	3,050	3,066	3,120	3,111
True digestible Lys	1.08	1.09	0.95	0.92
True digestible Met:Lys	37	54	36	55
True digestible TSAA:Lys	63	81	65	86

<sup>1</sup>The 4 diets differed in TSAA contents on the one hand and in energy, CP, and amino acids contents on the other hand to account for the change in nutrient requirements between wk 1 and 5. These diets were blended proportionally each week. TSAA- = diet deficient in TSAA; TSAA+ = diet sufficient in TSAA.

<sup>2</sup>Supplied per kilogram of feed: Fe, 58.2 mg; Cu, 20.05 mg; Mn, 80.85 mg; Zn, 90.1 mg; I, 2 mg; Se, 0.2 mg; vitamin A (retinyl acetate), 15,000 IU; cholecalciferol, 5,000 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 100 IU; vitamin K<sub>3</sub> (menadione), 5 mg; thiamine, 5 mg; riboflavin, 8 mg; pantothenic acid, 25 mg; niacin, 100 mg; pyridoxine, 7 mg; folic acid, 3 mg; biotin, 0.3 mg; vitamin B<sub>12</sub>, 0.02 mg; and choline, 550 mg.

<sup>3</sup>Adjusted for 87.3% DM.

<sup>4</sup>AME<sub>n</sub> and Trp values were estimated from Sauvant et al. (2004). True digestible values were obtained from the calculated digestibility of the ingredients for poultry (Sauvant et al., 2004) combined with the measured amino acid contents.

Subsamples were taken and ground with a ball mill (Vangoumil 300, Darmstadt, Germany) for AA analyses.

The 12 chickens of the sampling group were also slaughtered at 42 d of age in a fed state. The PM was removed and weighted to determine meat color and the pH 24 h after slaughter, and stored in plastic bags at 4°C. The iliotibialis and sartorius muscles, liver, abdominal fat, duodenum, jejunum, ileum, and ceca were removed and weighed.

## Chemical Analyses

All analyses were performed in duplicate. Chemical analyses were carried out in diets and samples of indi-

vidual chickens for PM, carcass without PM, and offal, and in samples pooled by treatment for feathers and blood. Analyses included DM, ash, protein (N × 6.25), crude fat, and gross energy. Samples were analyzed for DM (ISO 6496–1983) and ash contents (ISO-5984) using methods of the International Organization for Standardization (ISO methods, <http://www.iso.org>). The N content was analyzed according to the Dumas procedure [NF V18–120, 1997 (with a Rapid N cube, Elementar France, Villeurbanne, France)] of the AFNOR group (NF methods, <http://www.afnor.org/en>). The crude fat content (NF V18–117, 1997) was determined by solvent extraction with an automatic extraction system (Leco TFE 2000 Instrument, Leco, St. Joseph, MI). Energy content (ISO 9831–1998) was

**Table 2.** Performance of broilers offered a diet with a deficient (TSAA-) or sufficient (TSAA+) TSAA supply from 7 to 42 d of age<sup>1</sup>

Item	Treatment		Significance <sup>2</sup>	
	TSAA-	TSAA+	RSD	<i>P</i> -value
Initial BW (g)	180	181	7	0.90
Final BW (g)	2,590	2,641	73	0.11
ADFI (g/d)	114	114	NA	NA
ADG (g/d)	68.9	70.3	2.1	0.11
G:F (g/g)	1.66	1.62	0.06	0.10

<sup>1</sup>Values correspond to the average of 12 replicate measurements per treatment from birds used to determine the body composition and those used to determine meat quality and organ weights. No statistical analysis was performed for daily feed intake because feed was not offered ad libitum.

<sup>2</sup>RSD = residual SD; *P* = probability of a treatment effect; NA = not applicable.

measured with an adiabatic bomb calorimeter (IKA C5000, IKA, Staufen, Germany). Feed samples were also analyzed for starch content (ISO 6493-2000) using a digital polarimeter (ADP220, Bellingham, UK). The AA analyses of feed and body compartments (ISO 13903-2005) were performed with a HPLC (Alliance System, Waters, Guyancourt, France) after hydrolysis with 6 *N* HCl at 110°C for 23 h under reflux according to the Waters Pico Tag method (Cohen et al., 1989). The Met and Cys contents were obtained after performic acid oxidation before hydrolysis.

The pH in PM was measured 24 h after slaughter at a depth of 2.5 cm below the surface (pH meter model 506, Crison Instruments SA, Barcelona, Spain) equipped with a spear electrode. Meat color of meat was determined using a Miniscan spectrophotometer (HunterLab, Reston, VA) reporting in CIELAB system values of lightness (**L\***), redness (**a\***), and yellowness (**b\***). Color was measured 24 h after slaughter at 3 points in every PM (Petracci and Baeza, 2011).

### Calculations and Statistical Analyses

Empty body composition was calculated as the sum PM, carcass without PM, offal, blood, and feathers. Carcass composition was calculated from the analyzed composition of PM and carcass without PM. The initial body composition was assumed identical for all birds and was determined through the analyzed body composition of the initial group. Tissue gain and composition of gain was calculated as the difference between the measured composition of chickens slaughtered at the end of the trial and the estimated initial body composition of these birds. The Cys supply from Met catabolism was calculated from the difference between digestible Met intake and Met retention, and taking into account the difference in molar weights between Met and Cys. It was assumed that there were no material Met losses (e.g., as endogenous secretions or integuments) and that all excess Met was degraded through the transsulfuration pathway.

Statistical analyses were performed using the GLM procedure of SAS (SAS Institute Inc., 2008) by one-way

ANOVA using the individual animal as the experimental unit. Differences among means were tested using the least significant difference method, and significance was accepted at  $P \leq 0.05$ .

## RESULTS

All birds appeared to be in good health and showed normal behavior during the experiment. Some birds had occasional feed refusals that were recovered quantitatively and weighed. During the experiment, daily feed refusals ranged from 2 to 7% of the feed allowance.

### Performance and Composition of Empty BW and Empty BW Gain

No differences ( $P > 0.05$ ) in ADG, final BW, and G:F were observed in response to the TSAA supply (Table 2). Although certain probability values were close to the tendency limit, none of the performance traits differed more than 2.5%.

The body composition of broilers slaughtered at the beginning of the experiment was  $160 \pm 2$ ,  $95.2 \pm 5.0$ ,  $21.6 \pm 0.7$ , and  $723 \pm 4$  g/kg of empty BW for protein, fat, ash, and water, respectively, and  $1,851 \pm 34$  kcal/kg of empty BW for energy. The composition of the empty body at 42 d of age is shown in Table 3. Compared with birds that received diet TSAA+, birds receiving diet TSAA- tended to have a lower protein content in the empty body ( $P = 0.06$ ), whereas the water, lipid, and ash contents were not different between diets. The AA concentrations in empty body protein at the start of the experiment were as follows (in g/16 g of N): Lys,  $7.00 \pm 0.10$ ; Met,  $2.39 \pm 0.05$ ; Cys,  $1.66 \pm 0.08$ ; Thr,  $4.19 \pm 0.07$ ; Val,  $4.94 \pm 0.08$ ; Ile,  $4.21 \pm 0.06$ ; Leu,  $7.49 \pm 0.07$ ; Phe,  $4.10 \pm 0.05$ ; Tyr,  $2.48 \pm 0.10$ ; His,  $2.89 \pm 0.06$ ; Arg,  $8.03 \pm 0.22$ ; Ser,  $4.04 \pm 0.20$ ; Gly,  $7.07 \pm 0.14$ ; Ala,  $6.06 \pm 0.09$ ; Asp,  $8.17 \pm 0.14$ ; Glu,  $12.90 \pm 0.17$ ; Pro,  $6.02 \pm 0.09$ ; and total,  $93.65 \pm 1.92$ . Neither the Met nor the Cys content in empty body protein were affected by the TSAA supply ( $P = 0.83$  and  $P = 0.26$ , respectively). However, the Lys and Glu contents in body protein were lower ( $P < 0.05$ ), whereas the Ser

**Table 3.** Composition of the empty body and empty BW gain in broilers offered a diet with a deficient (TSAA-) or sufficient (TSAA+) TSAA supply from 7 to 42 d of age<sup>1</sup>

Item	Body composition				Composition of gain			
	Treatment		Significance <sup>2</sup>		Treatment		Significance <sup>2</sup>	
	TSAA-	TSAA+	RSD	P-value	TSAA-	TSAA+	RSD	P-value
Initial BW (g)	180	180	7	0.94				
Final BW (g)	2,589	2,632	100	0.48				
Empty BW and gain (g; g/d)	2,520	2,572	91	0.35	67.0	68.4	2.62	0.35
Composition (g/kg)								
N × 6.25	193	196	2	0.06	196	199	3	0.06
Lipid	138	127	11	0.12	141	130	12	0.12
Ash	26.2	27.0	1.4	0.37	26.6	27.4	1.5	0.38
Water	644	650	10	0.32	637	644	11	0.32
Energy (kcal/kg)	2,221	2,125	103	0.14	2,260	2,157	111	0.14
Composition (g/16 g of N)								
Lys	6.77	6.91	0.09	0.03	6.75	6.91	0.10	0.03
Met	2.30	2.31	0.06	0.83	2.29	2.30	0.06	0.83
Cys	1.99	1.94	0.07	0.26	2.01	1.96	0.08	0.25
Thr	4.13	4.11	0.03	0.30	4.12	4.11	0.03	0.30
Val	4.96	4.93	0.05	0.32	4.97	4.94	0.05	0.31
Ile	4.22	4.25	0.06	0.43	4.23	4.26	0.06	0.43
Leu	7.25	7.28	0.09	0.50	7.24	7.27	0.09	0.50
Phe	3.96	3.97	0.05	0.77	3.95	3.96	0.06	0.77
Tyr	3.06	3.09	0.05	0.35	3.10	3.13	0.06	0.37
His	2.99	2.98	0.09	0.93	2.99	2.99	0.10	0.92
Arg	7.62	7.56	0.20	0.60	7.60	7.54	0.22	0.61
Ser	4.39	4.19	0.11	0.01	4.42	4.20	0.12	0.01
Gly	7.28	7.31	0.19	0.81	7.30	7.33	0.20	0.82
Ala	6.00	6.00	0.08	0.97	6.00	6.00	0.08	0.97
Asp	7.88	7.96	0.11	0.26	7.87	7.96	0.12	0.25
Glu	12.62	12.82	0.15	0.04	12.61	12.82	0.16	0.04
Pro	6.36	6.29	0.11	0.32	6.38	6.31	0.11	0.31
Total	93.8	93.9	0.7	0.80	93.9	94.0	0.8	0.81

<sup>1</sup>Values correspond to the average of 6 replicate measurements per treatment from birds used to determine the body composition.

<sup>2</sup>RSD = residual SD; P = probability of a treatment effect.

content was greater ( $P < 0.01$ ) in birds receiving diet TSAA-. These differences resulted in differences in the composition of empty BW gain. In birds receiving diet TSAA-, the protein content in BW gain tended to be lower ( $P = 0.06$ ). In addition, the Lys and Glu contents in body protein gain were lower ( $P < 0.05$ ), whereas the Ser content was higher ( $P < 0.01$ ) when chickens were offered diet TSAA-.

### Tissue Composition and Tissue Gain

The TSAA content in the diet did not affect the weight gain of tissues (Table 4). Birds that received diet TSAA- had a lower protein content in carcass weight gain ( $P < 0.01$ ) and tended to have a lower protein content in PM weight gain ( $P = 0.10$ ). In these birds, the lipid content was higher in PM weight gain ( $P < 0.001$ ) and tended to be greater in carcass weight gain ( $P = 0.10$ ). The response of the lipid content was considerably greater in PM compared with the carcass. Broilers offered diet TSAA- had a slightly lower ash content in PM weight gain ( $P < 0.05$ ). Except for Met in the offal, neither the Met nor the Cys contents of tissues were affected by the TSAA supply. In the offal, the Met and Pro contents in protein gain tended to be lower when birds received diet TSAA- ( $P = 0.08$ ), and the Ala content was lower ( $P < 0.05$ ). Different tissues

responded differently to the TSAA content in the diet. The response appeared to be minimal for the carcass followed by the offal, whereas PM was the most affected tissue. For both carcass and PM, the Ser content of protein gain was greater in birds receiving diet TSAA- ( $P < 0.05$ ). In addition, the Phe, Tyr, Gly, and Glu contents in protein gain of PM were lower in these birds ( $P < 0.05$ ). The chemical composition of the carcass, PM, offal, feathers, and blood and the composition of the tissue gain is given in Supplemental Tables A to E (available online at <http://ps.fass.org/>).

### Relative Organ Weights and Meat Quality

The PM, iliotibialis, and sartorius muscles, liver, duodenum, jejunum, ileum, and ceca weights were not affected by the TSAA content in the diet (Table 5). However, the relative weight of abdominal fat was higher in birds receiving diet TSAA- ( $P = 0.04$ ). The TSAA content in the diet did not affect meat quality traits in PM, although the a\* value tended to be greater in birds that received diet TSAA- ( $P = 0.10$ ; Table 5).

### Supply and Retention of Met and Cys

Most Met was retained in the carcass (81%), whereas most of the Cys was retained in the feathers (50%;

**Table 4.** Composition of tissue weight gain in broilers offered a diet with a deficient (TSAA-) or sufficient (TSAA+) TSAA supply from 7 to 42 d of age<sup>1</sup>

Item	Tissue <sup>2</sup>																			
	Carcass				PM				Offal				Feathers				Blood			
	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+	TSAA-	TSAA+		
Tissue gain (g/d)	49.7	51.1	11.1	11.5	12.3	12.5	3.07	3.05	1.91	1.88										
% of empty BW gain	74.3	74.7	16.6	16.8	18.4	18.3	4.58	4.46	2.85	2.74										
Composition (g/kg)																				
N × 6.25	185**	191**	230†	237†	162	157	539	539	122	124										
Lipid	134†	121†	16.9***	9.1***	224	212	12.5	12.3	1.22	1.17										
Ash	28.2	29.0	11.4*	11.8*	28.8	29.8	2.94	3.30	8.33	7.68										
Water	653	659	742	742	586	600	446	446	868	867										
Energy (kcal/kg)	2,262	2,163	1,487	1,448	3,026	2,861	3,145	3,088	707	716										
Composition (g/16 g of N)																				
Lys	7.76	7.87	8.69	8.98	5.98	6.12	2.03	2.06	8.53	8.83										
Met	2.65	2.60	2.88	2.85	2.24†	2.42†	0.59	0.59	1.32	1.34										
Cys	1.08	1.10	1.19	1.20	1.20	1.29	8.25	7.92	1.85	1.84										
Thr	4.03	3.98	4.32	4.45	3.69	3.85	5.23	5.13	4.90	4.98										
Val	4.58	4.56	5.08	5.13	4.34	4.52	7.87	7.60	6.25	6.42										
Ile	4.26	4.28	4.90	4.98	3.55	3.69	5.06	4.93	3.86	4.02										
Leu	7.13	7.16	7.91	8.01	6.52	6.71	8.48	8.34	10.49	10.71										
Phe	3.74	3.80	4.06*	4.25*	3.81	3.78	5.15	4.93	6.13	6.17										
Tyr	3.12	3.15	3.61*	3.81*	3.02	3.02	3.08	3.16	3.80	3.61										
His	3.47	3.39	4.28	4.28	2.47	2.57	0.77	0.79	5.42	5.70										
Arg	7.41	7.35	6.94	6.89	8.98	8.95	7.49	7.33	6.17	6.29										
Ser	3.39**	3.16**	3.36*	3.22*	3.83	3.96	11.14	10.85	3.69	3.65										
Gly	6.61	6.65	4.34*	4.53*	11.13	11.36	7.33	7.24	3.64	3.74										
Ala	6.13	6.04	5.67	5.75	6.54*	7.06*	4.64	4.47	7.28	7.42										
Asp	8.18	8.24	9.01	9.28	7.59	7.67	6.70	6.71	8.55	8.66										
Glu	13.19	13.39	14.03*	14.78*	11.86	12.38	11.01	10.69	10.35	10.36										
Pro	5.32	5.20	3.73	3.72	7.38†	7.98†	11.65	11.65	3.92	3.93										
Total	92.0	91.9	94.0	96.1	94.1	97.3	106.5	104.2	96.2	97.7										

<sup>1</sup>Values correspond to the average of 6 replicate measurements per treatment (for feathers and blood, 1 pooled measurement per treatment) from birds used for the comparative slaughter study. No statistical analysis was carried out for feathers and blood composition of gain because there were no replicate measurements. The residual SD for each tissue is presented in the supplemental tables (available online at <http://ps.fass.org/>).

<sup>2</sup>PM = pectoralis major muscles; carcass includes PM but not head, feet, and neck; offal includes head, feet, tail, and internal organs (i.e., the emptied small and large intestine, liver, kidneys, stomach, heart, lungs, diaphragm, heart, genital tract, bladder, gizzard, and spleen).

\*Within a row and tissue component, means differ ( $P \leq 0.05$ ).

\*\*Within a row and tissue component, means differ ( $P \leq 0.01$ ).

\*\*\*Within a row and tissue component, means differ ( $P \leq 0.001$ ).

†Within a row and tissue component, means tend to differ ( $P \leq 0.10$ ).

**Table 5.** Relative organ weights and meat quality traits in broilers offered a diet with a deficient (TSAA-) or sufficient (TSAA+) TSAA supply from 7 to 42 d of age<sup>1</sup>

Item	Treatment		Significance <sup>2</sup>	
	TSAA-	TSAA+	RSD	P-value
Tissue (g/kg of BW)				
Muscle				
Pectoralis major	150	161	15	0.27
Iliotibialis	11.2	10.5	0.7	0.12
Sartorius	2.87	3.11	0.46	0.39
Liver	26.3	27.0	4.6	0.80
Abdominal fat	19.8	15.5	3.1	0.04
Intestine				
Duodenum	6.18	5.75	0.59	0.24
Jejunum	8.81	8.48	1.32	0.67
Ileum	7.92	7.60	1.14	0.64
Ceca	2.46	2.28	0.33	0.37
Meat quality trait				
pH (24 h)	5.63	5.66	0.03	0.15
Lightness (L*)	51.2	51.4	3.0	0.94
Redness (a*)	0.613	-0.152	0.741	0.10
Yellowness (b*)	13.60	13.17	1.37	0.59

<sup>1</sup>Values correspond to the average of 6 replicate measurements per treatment from birds used to determine meat quality and organ weights.

<sup>2</sup>RSD = residual SD; *P* = probability of a treatment effect.

Table 6). The distribution of Met and Cys retentions among tissues was little affected by the TSAA supply. Birds fed diet TSAA- tended to have a lower Met retention in the offal ( $P = 0.08$ ) and a slightly lower Cys retention in the carcass ( $P < 0.05$ ). In these birds, the carcass stored 37% of all retained Cys compared with 40% for TSAA+ broilers. However, neither Met nor Cys retentions in the PM and feathers were affected by the TSAA content in the diet. The

PM and feathers stored, respectively, 25 and 3% of all retained Met, and 12 and 50% of all retained Cys. As expected, the TD Met intake and the total Cys supply (by the diet and the transsulfuration pathway) were lower in birds fed diet TSAA- compared with TSAA+ ( $P = 0.001$ ). They were retained by 73 and 57%, respectively, in TSAA- animals. For TSAA+ birds, retention of Met and Cys was, respectively, 51 and 39% of the supply.

**Table 6.** Methionine and Cys supply and retention in broilers offered a diet with a deficient (TSAA-) or sufficient (TSAA+) TSAA supply from 7 to 42 d of age<sup>1</sup>

Amino acid flow (g/d) <sup>2</sup>	Treatment		Significance <sup>3</sup>	
	TSAA-	TSAA+	RSD	P-value
Met intake	0.442	0.643	0.019	0.00
True digestible Met intake	0.415	0.617	0.018	0.00
Met retention	0.302	0.315	0.018	0.23
Carcass	0.244	0.255	0.017	0.32
Pectoralis muscle	0.073	0.078	0.008	0.33
Offal	0.044	0.048	0.003	0.09
Feathers	0.010	0.010	NA	NA
Blood	0.003	0.003	NA	NA
Cys intake	0.376	0.372	0.012	0.40
True digestible Cys intake	0.330	0.326	0.011	0.40
Cys supply from Met catabolism	0.130	0.354	0.028	0.00
Cys retention	0.264	0.268	0.016	0.73
Carcass	0.100	0.108	0.006	0.05
Pectoralis muscle	0.030	0.033	0.004	0.22
Offal	0.024	0.025	0.001	0.11
Feathers	0.136	0.130	NA	NA
Blood	0.004	0.004	NA	NA

<sup>1</sup>Values correspond to the average of 6 replicate measurements per treatment from birds used to determine the body composition.

<sup>2</sup>The true digestibility was obtained from table values for feed ingredients (Sauvant et al., 2004). The Cys supply from Met catabolism was calculated from the difference between digestible Met intake and Met retention and taking into account the difference in molar weights between Met and Cys. It was assumed that there were no material Met losses (e.g., as endogenous secretions or integuments) and that all excess Met was degraded through the transsulfuration pathway.

<sup>3</sup>NA = not applicable; RSD = residual SD; *P* = probability of a treatment effect.



## DISCUSSION

In our experimental conditions, the TSAA– diet provided for 35 d did not affect performance. A deficient supply of some AA can result in a reduction in appetite (Kiraz and Sengul, 2005; Bunchasak and Keawarun, 2006), and a deficient Met supply has been reported to reduce feed intake (Picard et al., 1993; Bunchasak, 2009). The main reason to equalize feed intake between treatments in this study was to avoid a possible interfering effect of feed intake. Compared with the feed intake potential reported by the bird supplier, the imposed feed restriction required to equalize feed intake between treatments was relatively modest (approximately 4%). Moreover, the sufficient level of TSAA used in this study was calculated to meet the recommended requirement for Ross broilers, which is about 13% greater than the recommendation of the NRC (Kalinowski et al., 2003; Mehri et al., 2012). The deficient level corresponded to approximately 22% of the supply of diet TSAA+, but this level was only marginally deficient compared with the recommendation of the NRC. This may explain that the performance response to the Met deficiency was moderate.

Different mechanisms can be used by the animal to cope with a (marginally) deficient AA supply, such as a reduction in growth rate and a change in the composition of growth. In this experiment, the second mechanism was most apparent because protein gain was reduced in the whole animal, carcass, and PM, whereas lipid gain was increased. Similar responses to a TSAA deficiency have been observed by others (Jensen et al., 1989; Baker et al., 1996; Kiraz and Sengul, 2005). The marginally deficient TSAA supply we used resulted in an important increase in lipid gain in PM (78%), abdominal fat (28%), and to a lesser extent, in the carcass (10%). This difference in magnitude is also due to the relative contribution of lipids in these tissues to whole body lipid and the very low lipid content of PM (1 to 2%). The increase in lipid content can be explained because a large fraction of the AA in birds offered diet TSAA– could not be used for protein deposition. These AA will then be deaminated, and the carbon chains will be used for lipid deposition (Jensen et al., 1989; Jeroch and Pack, 1995). In addition, others reported a significant increase in fat content in breast meat (Bunchasak et al., 1997), abdominal fat (Austic, 1985), and increased lipogenesis in response to a TSAA deficiency (Baker et al., 1996).

Despite changes in body composition, little or no change in Met and Cys contents of body proteins was observed. This suggests that the bird could cope with the TSAA deficiency by reducing the protein content of gain, without changing the TSAA content in protein. This contrasts with an earlier study, where pigs responded to a strong Met deficiency by reducing both the protein content in the body and the Met content in body protein (Kalinowski et al., 2003; Conde-Aguilera

et al., 2010). In that study, the Met and Cys contents of protein gain in the longissimus dorsi muscle changed the most, whereas the composition of the intestines remained constant. Other studies also indicated that the AA composition of the intestines is relatively constant and relatively resistant to nutrient deficiencies (Ebner et al., 1994; Hamard et al., 2009). Interestingly, in the present study the offal (which includes the intestines) was the only tissue for which the Met content in protein was affected by the TSAA supply. The postdigestive efficiency of retention can be calculated from the data given in Table 6. For birds offered TSAA–, this efficiency was 73% for Met and 57% for Cys (including the supply of Cys from excess Met). Although there is no direct evidence, this large difference suggests that Met may have been more limiting than Cys in this study. In piglets, Conde-Aguilera et al. (2010) estimated the efficiency of Met utilization at 69%. The fact that that study was carried out using a more severe restriction of 43% below recommendations may indicate that there is an upper limit to the efficiency of Met utilization because these efficiencies are of similar magnitude in both species. If this would be the case, the TSAA deficiency used in the present study would have been the maximum the broilers would be able to cope with. A more severe TSAA deficiency would then force the birds to reduce protein deposition to a greater extent, change the TSAA composition of body proteins, or both.

In our conditions of a marginally deficient TSAA supply, the composition of some AA was affected, albeit to a limiting extent, in different body compartments. The concentrations of Lys and Glu in the empty body, Phe, Tyr, Gly, and Glu in PM, and Ala in the offal were reduced, and that of Ser in the empty body, carcass, and PM was increased (Tables 3 and 4). Similarly, a TSAA deficiency altered AA composition of tissues and tissue gain in piglets (e.g., His and Glu in the empty body and longissimus muscle (Conde-Aguilera et al., 2010). In chickens, Farran and Thomas (1992) reported that a Val deficiency changed the AA composition of feather proteins. There is also evidence that the AA profile of deposited whole body protein was affected by dietary Lys supply (Fatufe et al., 2004). This means that the animal has a certain ability to cope with a limiting AA supply by changing the AA composition of tissue protein. Differences in the AA composition of whole body protein could be the consequence of the changing proportions of different types of proteins in the body (Chung and Baker, 1992; Fatufe and Rodehutschord, 2005). For example, the proportion of major proteins such as collagen may depend on dietary AA provision as previously suggested (Fatufe et al., 2004; Fatufe and Rodehutschord, 2005; Conde-Aguilera et al., 2010). Using a proteomic approach, differences in muscle protein expression have been shown in response to a dietary Met deficiency, with the exclusive presence of 3 proteins expressed in PM when broilers were offered a Met-deficient diet (Corzo et al., 2006). Nevertheless,

the consequences of these modifications are unknown. More research is certainly needed to understand how an animal can change the AA composition of body protein.

The fact that the Ser content of protein gain of the empty body, carcass, and PM was increased in broilers receiving diet TSAA- (see above) is to highlight. Relationships between Ser synthesis and Met metabolism have been identified in conditions of low availability in methyl groups, with in particular a negative correlation between Ser synthesis and homocysteine remethylation to form Met (Cuskelly et al., 2001; Pillai et al., 2006). In our experimental conditions of a deficient TSAA supply (Met itself is a source of methyl groups), increased Ser synthesis may reflect an adaptation of methyl metabolism. However, there was no change in Ser concentrations due to TSAA deficiency in piglets irrespective of tissues or compartments (Conde-Aguilera et al., 2010). Whether these findings are related to metabolic differences between species remains an open issue. Serine (as Gly, from which Ser can be synthesized through a reversible reaction) is sometimes considered as semi-essential AA in poultry because the requirement for these AA may exceed the capacity of de novo synthesis (NRC, 1994; Baker, 2009). Birds also exhibit particular features with regard to Gly compared with mammals. Indeed, birds excrete uric acid as an end product of nitrogen metabolism and Gly is required for the synthesis of uric acid (Baker, 2009).

In conclusion, a marginal TSAA supply affects differently growth traits and the chemical composition of tissues and certain aspects of meat quality. This underlines the ability of broilers to cope with a dietary TSAA deficiency and calls into question the use of a constant ideal AA profile in poultry nutrition. Furthermore, it indicates the limitations of using daily gain as the only criterion to appreciate AA requirements.

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