Allometric growth of protein, amino acids, fat and minerals in slow- and fast-growing young chickens

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ABSTRACT: Allometric growth of body constituents and apparent efficiency of amino acid and nitrogen retention were examined in slow-growing laying-type cockerels (SG) and in fast-growing male broiler hybrids (FG) during the growth period from hatch to Day 22. The respective allometric coefficients for water, protein (N × 6.25), ash and fat in relation to body weight were 0.971, 1.080, 1.096 and 1.284 for SG chickens and 0.977, 1.099, 0.993, and 1.198 for FG chickens. The respective allometric coefficients describing the relationships of water, fat and ash weight with protein weight were 0.894, 1.014, and 1.186 for SG chickens and 0.893, 0.910, and 1.097 for FG chickens. High allometric coefficients for ash in both genotypes likely indicate the rapid growth of skeletal tissues which requires adequate mineral nutrition during this period of growth. The deposition of ash relative to protein was significantly higher ($P < 0.05$) in SG chickens thus suggesting that the relative growth of ash may be affected by genotype. Allometric coefficients relating amino acids to body protein were less than unity in most cases which indicates that an increasing amount of non-protein N is deposited in the body with advancing age. Except for cysteine, the apparent efficiency of amino acid retention was lower in SG as compared to FG chickens. The high retention efficiency of cysteine in SG genotype was likely associated with the conversion of surplus methionine to cysteine, required for feather protein synthesis in laying-type birds at an early age.

Keywords: chickens; age; growth rate; chemical allometry; amino acid retention

Changes in the proportion of various body components of a chicken body as a consequence of growth were studied more than eighty years ago (Mitchell et al., 1926). Since then many experiments have been carried out to obtain data enabling to predict growth, body composition and nutrient requirements of chickens of various genotypes under various environmental conditions (Hurwitz et al., 1978; Hruby, 1994; Gous et al., 1999). It is now generally accepted that the first step in predicting growth is the prediction of protein deposition rate, from which the growth of other body components (i.e. water, fat, and ash) can be calculated by allometric equations (Emmans, 1981). It is also assumed that the amino acid composition of body protein is independent of genotype or environmental factors (Emmans, 1989; Hruby, 1994). However, there is experimental evidence suggesting that ash content need not be directly related to protein content (Eits et al., 2002) and that the amino acid pattern of body protein may be affected by both genotype and nutrition (Fatufe et al., 2004). The aim of the present

experiment was to study relative growth rates of body components in two contrasting genotypes of chickens during the first 22 days of postembryonal life. The amino acid composition of whole body protein (N × 6.25) and their efficiency of retention were also studied.

MATERIAL AND METHODS

Animals and procedures

The animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno. Chickens of two contrasting genotypes were used: slow-growing male chickens of the hybrid combination Isa Brown (SG; 95 birds) and fast-growing Ross 308 cockerels (FG; 52 birds). The chickens were kept in balance cages in an air-conditioned room. The initial environmental temperature was 35°C and decreased daily by 0.7°C. Permanent artificial lighting was used. Growth of body components and nitrogen and amino acid retention were investigated within subsequent two-day balance periods. During the whole experiment, all chickens were fed on a non-pelleted starter diet (Table 1) containing 225 g crude protein (i.e. 36 g N) and 12.06 MJ nitrogen-corrected metabolisable energy per kg. The diet was formulated to meet or exceed amino acid requirements for male broilers (Zelenka et al., 2007). Feed was supplied *ad libitum* and its consumption was recorded. The body weight of chickens was recorded at the end of each balance period.

In two-day intervals from hatch to the age of 22 days, samples of chickens were selected from each group so that their body weight was approximately the same as the mean body weight of the group and the total weight of the sample was at least 300 g. The selected chickens were euthanatized and the content of the digestive tract was removed. The chickens were then autoclaved for 6 h at 130°C and 270 kPa pressure, freeze-dried, finely ground and stored for subsequent analysis.

Table 1. Composition of the diet[1]

Ingredient	(g/kg)
Maize	510.0
Wheat	120.5
Soyabean meal	260.0
Meat-and-bone meal	60.0
Fishmeal	30.0
Dicalcium phosphate	7.0
Ground limestone	5.0
Sodium chloride	1.5
DL-Methionine	2.0
Premix of feed additives[2]	4.0

[1]The diet contained (g/kg diet): crude protein 225; crude fat 37; crude fibre 33; lysine 13.5; methionine 5.1; cysteine 4.8; calcium 11.1; total phosphorus 9.2; available phosphorus 5.1 and 12.06 MJ nitrogen-corrected metabolisable energy per kg

[2]The premix supplied(mg/kg diet): Cu 9.6; Zn 19.2; Fe 35.2; Mn 64; Co 0.096; Se 0.128; I 0.72; retinyl acetate 4.13; cholecalciferol 0.06; DL-α-tocopherol acetate 32; menadione 0.8; thiamine 2.4; riboflavin 4.8; pyridoxine 4; cyanocobalamine 0.0272; biotin 0.112; niacinamid 24; folic acid 1.12; pantothenic acid 8.8; L-lysine.HCl 1152; sodium monensinate 80

Chemical analyses

The diet and samples of carcasses were analysed for moisture, nitrogen, crude fat, and crude ash. The whole-body amino acid composition was determined in lipid-extracted samples by ion-exchange chromatography using an AAA 400 amino acid analyser (INGOS Prague, Czech Republic). The samples were hydrolysed with hydrochloric acid (c = 6 mol HCl per l) for 23 h. To determine cysteine and methionine, separate samples were oxidised to form acid-stable cysteic acid and methionine sulphone, respectively. The oxidized samples were subsequently hydrolysed as described above. All analyses were performed using the methods specified by Commission Regulation (EC) (2009). Tryptophan content was analysed following alkaline hydrolysis with LiOH (c = 4.2 mol per l) (Kráčmar and Liška, 2002). No corrections for a possible destruction of amino acids in the course of hydrolysis were used.

Calculations and statistical analysis

For the expression of the accelerating growth phase of chickens, the exponential function suggested by Brody (1945) was used:

$$W = Ae^{kt}$$

where:
W = body weight at time t
A = extrapolation of the weight for time 0
e = base of natural logarithms
k = rate of growth
t = time from hatching (in days)

Weights of water, dry matter, protein, fat, ash, and amino acids were calculated by multiplying their concentrations in the whole body by live weights of chickens. Protein content was calculated as N × 6.25. Amino acids were also expressed as a percentage of protein. Allometric relationships were calculated using the power function of Brody (1945):

$$Y = aX^b$$

where:
Y = content of the body component in g
X = live weight or protein weight of chicken in g
a = extrapolation of Y for $X = 1$
b = allometric coefficient, the ratio of percentage change in Y to the corresponding percentage change in X

Apparent efficiency of nitrogen/amino acid retention was calculated as the proportion of nitrogen/amino acid consumed which was retained in the body. The significance of differences between the data for the two genotypes was evaluated by a paired t-test. The statistical analyses were performed using the Statgraphic Plus package (version 3.1, Statistical Graphic Corp., Rockville, MD, USA).

RESULTS

Both types of chicken hybrids were raised under identical environmental and dietary conditions. Their growth during the experiment is shown in Figure 1. During the first two days of postembryonal life, body weight of slow-growing chickens decreased while that of broilers increased starting on the second day. The coefficients k in exponential equations were 0.098 and 0.137 for SG and FG chickens, respectively. At the end of the experiment, the body weights of SG and FG chickens were 258 and 782 g, respectively. Growth of body protein was expressed by the equations:

$Y = 5.114e^{0.1061t}$ ($r = 0.992$) for SG genotype

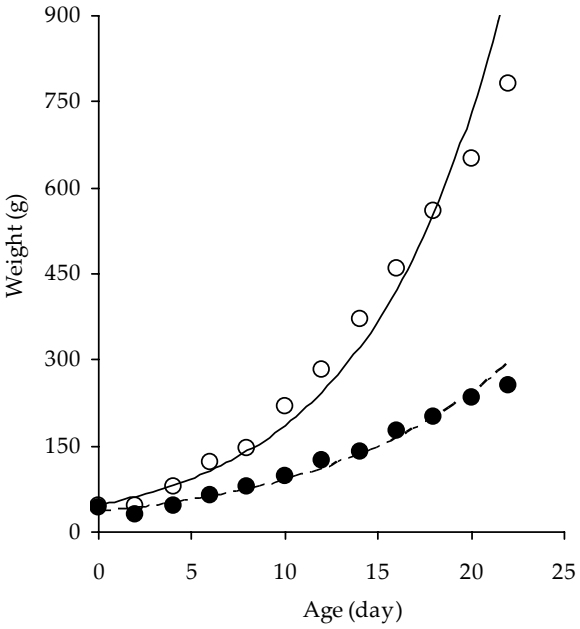

Figure 1. Growth curves of slow-growing (SG) and fast-growing (FG) genotypes of chickens. Plotted from equations: $Y = 35.14e^{0.098t}$; $r = 0.979$ (SG, closed circles) and $Y = 47.27e^{0.137t}$; $r = 0.963$ (FG, open circles)

$Y = 6.226e^{0.1504t}$ ($r = 0.969$) for FG genotype

The predicted protein weights on Day 22 were 52.8 and 170.3 g for the SG and FG genotype, respectively.

Mean concentrations of potentially limiting amino acids as well as of total amino acids in the whole-body protein of chickens are presented in Table 2.

Table 2. Mean amino acid composition of whole body protein of chickens (g/16 g N)

Amino acid	Genotype		Pooled SEM
	SG	FG	
Arginine	6.78[a]	6.83[a]	0.08
Cysteine	2.39[a]	1.77[b]	0.09
Lysine	5.78[a]	5.64[a]	0.05
Methionine	2.35[a]	2.22[a]	0.06
Threonine	3.75[a]	4.24[b]	0.05
Tryptophan	0.97[a]	0.94[a]	0.02
Total amino acids	91.12[a]	91.08[a]	0.79

SG – slow-growing chickens, FG – fast-growing chickens
[a,b]means within a row with different superscript differ ($P < 0.001$)

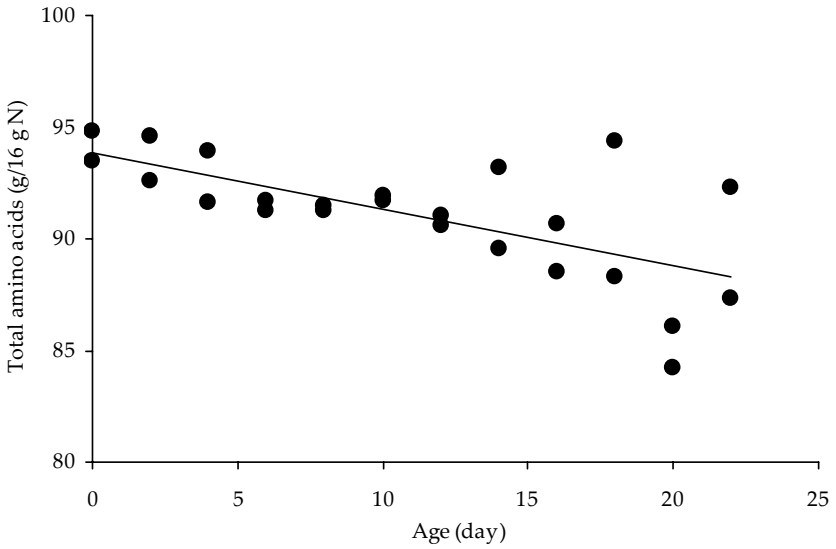

Figure 2. Relationship between the total amino acid concentration in body protein (Y) and age of chickens (X). Plotted from the equation: $Y = 93.89 - 0.253X$ ($r = 0.668$)

While the concentration of total amino acids was similar in both genotypes, cysteine was significantly higher and threonine was significantly lower in SG than in FG genotype. The amino acid pattern of body protein changed during growth; their concentration gradually decreased in most cases. Since no significant effect of the genotype was found, the data on total amino acids per 16 g N from both genotypes were combined and regressed on age (Figure 2).

Parameter estimates for the allometric relationships of water, protein, fat and ash weight with live

Table 3. Allometric coefficients (b) and indexes of correlation (I_{YX}) for allometric relations between body components and live weight or protein weight in two chicken genotypes

Y	Genotype	Allometric function $Y = aX^b$		
		b	I_{YX}	F-value
Relations to live body weight (g)				
Water	SG	0.9710[a]	0.999	9 046**
	FG	0.9771[a]	0.999	43 741**
Protein	SG	1.0804[a]	0.993	738**
	FG	1.0994[a]	0.998	2 013**
Fat	SG	1.0964[a]	0.931	65**
	FG	0.9929[a]	0.960	118**
Ash	SG	1.2841[a]	0.998	3 062**
	FG	1.1978[a]	0.999	8 059**
Relations to protein weight (g)				
Water	SG	0.8941[a]	0.994	829**
	FG	0.8933[a]	0.997	1 459**
Fat	SG	1.0141[a]	0.969	154**
	FG	0.9097[a]	0.988	397**
Ash	SG	1.1863[a]	0.997	1 759**
	FG	1.0974[b]	0.999	3 639**

SG – slow-growing chickens; FG – fast-growing chickens

X – live body weight or protein weight (g); Y – analyte weight (g)

[a,b]significant difference between genotypes ($P < 0.05$)

**significance of I_{YX} $P < 0.01$

weight are summarized in Table 3. As indicated by the allometric coefficients, the proportion of water weight in chicken bodies decreased and the deposition of minerals was much higher than the rate of growth. These differences were more pronounced in SG than in FG chickens. Except for fat weight, all allometric coefficients were significantly different from unity ($P < 0.05$). However, the comparison of allometric coefficients for the same body component showed no significant differences between the two genotypes.

Allometric coefficients describing the relationships of water, fat, and ash weight with protein weight (Table 3) indicated that the deposition of water in both genotypes was considerably slower than that of protein while the rate of mineral deposition was higher. Allometric coefficients for fat were not significantly different from unity. In SG and FG chickens, the deposition of ash was 9.74% and 18.63% higher than the deposition of protein, the difference being significant ($P < 0.05$). There was no significant difference in the other allometric coefficients between the two genotypes.

Table 4 summarizes allometric coefficients relating amino acids to body protein (N × 6.25). Coefficients for most amino acids were lower than 1.0, the exceptions were tryptophan and alanine in SG group and histidine, methionine, tryptophan, alanine and tyrosine in FG group. On average, the values found in SG chickens were slightly lower

Table 4. Allometric coefficients (b) and indexes of correlation (I_{YX}) for allometric relations between amino acids and protein in two chicken genotypes

Amino acid	Genotype					
	SG			FG		
	b	I_{YX}	F-value	b	I_{YX}	F-value
Arginine	0.985[a]	0.998	2 592**	0.997[a]	0.996	1 294**
Histidine	0.954[a]	0.994	885**	1.011[b]	0.999	5 225**
Isoleucine	0.968[a]	0.998	2 045**	0.995[b]	0.998	2 034**
Leucine	0.949[a]	0.997	1 725**	0.972[a]	0.998	2 449**
Lysine	0.986[a]	0.998	1 999**	0.992[a]	0.993	668**
Methionine	0.933[a]	0.996	1 338**	1.007[b]	0.997	1 571**
Phenylalanine	0.985[a]	0.995	1 073**	0.987[a]	0.998	2 635**
Threonine	0.973[a]	0.997	1 830**	0.973[a]	0.998	2 262**
Tryptophan	1.091[a]	0.997	1 686**	1.045[a]	0.999	3 641**
Valine	0.976[a]	0.998	2 427**	0.958[a]	0.982	264**
Alanine	1.042[a]	0.998	3 078**	1.020[b]	0.998	2 787**
Aspartic acid	0.970[a]	0.964	132**	0.983[a]	0.990	518**
Cysteine	0.909[a]	0.982	270**	0.885[a]	0.983	285**
Glutamic acid	0.978[a]	0.999	4 411**	0.937[a]	0.928	62**
Glycine	0.969[a]	0.998	3 080**	0.983[a]	0.998	2 298**
Proline	0.969[a]	0.997	1 505**	0.972[a]	0.998	2 538**
Serine	0.917[a]	0.994	826**	0.990[b]	0.998	2 700**
Tyrosine	0.945[a]	0.997	1 683**	1.003[b]	0.998	2 465**
Total amino acids	0.970[a]	0.998	2 838**	0.982[a]	0.998	2 653**

SG – slow-growing chickens; FG – fast-growing chickens

[a,b]Significant difference between genotypes ($P < 0.05$)

**Significance of I_{YX} $P < 0.01$

Table 5. Mean values of apparent efficiency of amino acid and nitrogen retention in chickens (%)

Amino acid	Genotype		Pooled SEM
	SG	FG	
Arginine	59.4[a]	70.2[a]	8.9
Cysteine	52.5[a]	43.7[a]	8.5
Lysine	49.6[a]	56.5[a]	6.5
Methionine	52.1[a]	57.7[a]	7.5
Methionine + Cysteine	52.3[a]	50.8[a]	6.7
Threonine	45.0[a]	60.1[b]	7.1
Tryptophan	58.8[a]	64.2[a]	10.0
Total amino acids	44.3[a]	51.7[a]	6.2
Total N	44.9[a]	53.3[a]	5.6

SG – slow growing chickens; FG –fast growing chickens
[a,b]Significant difference between genotype ($P < 0.05$)

than in FG chickens. In both genotypes, the lowest value was found in cysteine.

Data on the apparent efficiency of amino acid and nitrogen retention are presented in Table 5. In general, FG chickens retained amino acids more efficiently than SG birds, the only exception being cysteine, the efficiency of which was higher in SG genotype. As a result, the utilization of total sulphur amino acids for protein accretion was also higher in this genotype. The efficiency of total N retention followed the same trend as in amino acids, the mean values being 44.9% and 53.3% for SG and FG genotype, respectively. Except for threonine, there was no statistical significance between both genotypes as evaluated by the paired *t*-test.

DISCUSSION

As expected, both growth rate and protein deposition were substantially higher in broiler chickens than in laying-type birds. At the end of the experiment, the FG chickens were about three times heavier than SG chickens and a similar difference between the genotypes was observed in protein weight. As shown by Plavnik and Hurwitz (1983) and Shires et al. (1987), this difference remained nearly unchanged also at 10 weeks of age.

The present study showed that the amino acid composition of whole body protein of broilers and laying-type chickens was similar. Only the concentration of cysteine was much higher in SG chickens, the difference being about 35%. This may be attributed to a greater proportion of feathers in the laying-type birds in the first weeks of life. As compared to feather-free whole body protein, feather protein is characterized by a high concentration of cysteine (Nitsan et al., 1981; Stilborn et al., 1997). Due to the smaller body size of SG compared to FG genotype, feather protein contributes more to the whole body in laying-type chickens than in broilers. Similar results were reported by Fatufe et al. (2004), who found a significantly higher cysteine concentration in the whole body protein gain of the layer genotype than in broilers. However, in contrast to the present results, Fatufe et al. (2004) observed significant differences between the genotypes in the majority of the other amino acids and concluded that the amino acid profile of the deposited protein was genotype-dependent. In their study of the amino acid composition of protein gain from 8 to 21 days of age, the aforementioned authors found significantly higher levels of lysine, methionine, alanine, aspartic acid, glutamic acid and glycine in broilers than in laying-type chickens. While the increased deposition of lysine may be explained by its higher concentration in muscle protein (Mahan and Shields, 1998), the role of non-essential amino acids is less clear. It is interesting to note that, unlike the present study, Fatufe et al. (2004) reported no significant effect of genotype on threonine concentration. We were not able to find any explanation of this discrepancy.

The mean concentration of total amino acids in body protein (91.1 g/16 g N) agrees well with the value of 91.0 reported by Kyriazakis and Emmans (1993), who analyzed the amino acid composition of whole body protein in pigs. However, as shown in Figure 2, the concentration of total amino acids gradually decreased with advancing age, thus suggesting that an increased proportion of non-amino acid nitrogenous compounds such as creatine, nucleotides, purines, pyrimidines or amines might be retained in the body. In contrast, the results of Mahan and Shields (1998) demonstrated that the relative concentration of amino acids in the whole body protein of pigs slightly increased with increasing body weight. Whether or not this represents a real difference between the species remains unclear.

As indicated by the allometric coefficients, the proportion of water in the body decreased while that of protein and ash increased with the increasing age of birds. Similar results were reported also by

Kwakkel et al. (1997) and Gous et al. (1999). In SG chickens, the growth of fat was relatively faster than body weight gain while the opposite was true for FG chickens. Conversely, Fatufe et al. (2004) found that the fat content in gained body weight from 8 to 21 days was lower in laying-type males than in broilers. Nevertheless, the allometric coefficients for fat estimated in the present study were not significantly different from unity, which might be due to the young age of chickens. As shown in experiments with laying-type pullets by Kwakkel et al. (1997), fat was proportionally related to the growth of the fat-free body up to about 60 days of age; thereafter, its relative deposition rate more than doubled. The remarkably high allometric coefficients for ash relative to body weight in both genotypes likely indicate the rapid growth of skeletal tissues, since the concentration of minerals in bones is higher than that in non-skeletal body components. Consequently, adequate mineral nutrition during this period of growth is of particular importance.

When the relative growth of body components is expressed as a function of body weight, the results may be biased by the varying fat deposition rate, which is closely related to the level of feeding (Kwakkel et al., 1997). Therefore, in most models predicting growth or defining nutrient requirements, the starting point is usually the determination of protein gain (Gous et al., 1999) from which the deposition rates of other body components may be predicted using allometric equations (Emmans, 1981). In order to eliminate the possible confounding effect of fat, this approach was also applied in the present study and the allometric coefficients were calculated relative to protein. The results showed that both dry matter and fat developed proportionally to body protein (*b* value close to 1.0). Allometric coefficients for water were significantly lower than unity whereas those for ash were much higher. The decreasing deposition rate of water relative to body weight during growth is usually ascribed to the increasing proportion of body fat. The present results as well as those by Gous et al. (1999) suggest that the reduction of water weight relative to protein weight might be due to changes in the relative proportion of tissues with different water to protein ratio such as muscle protein vs. collagen (Ashgar et al., 1986).

In the growth simulation models, it is commonly assumed that ash weight is a simple function of protein weight, being independent of other factors, e.g. nutrition or genotype (Emmans, 1981; Black et al., 1986) and that the allometric exponent for ash is close to unity (Emmans and Kyriazakis, 1997). This assumption was questioned by Eits et al. (2002), who demonstrated that the relationship between ash and protein in broiler chickens was strongly affected by the dietary protein to energy ratio. The aforementioned authors also postulated that, due to a lower proportion of bones, the relative growth of ash in broilers is slower than that in laying-type chickens. The results of the present experiment support this hypothesis as the allometric coefficient for ash in FG chickens was significantly lower than in SG chickens. On the other hand, both values found in our study were significantly higher than 1.0 while Eits et al. (2002) reported for broilers the value of 0.998. Allometric exponents for ash close to 1.0 were also found in White Leghorn pullets (Kwakkel et al., 1997) and in pigs (Kyriazakis and Emmans, 1992). In contrast, Sakomura et al. (2005), who studied allometric relationships of body ash with body protein in Ross broilers reported the values of 1.080 and 1.085 for males and females, respectively. The reason for this discrepancy is not clear. For the prediction of body growth, possible errors in estimating the relative growth of ash are only of minor importance due to a small proportion of ash in the body (Emmans and Kyriazakis, 1997). However, correct information on the growth rate of ash is important for the factorial estimation of mineral requirements.

Although the allometric coefficients relating amino acids to body protein were lower in SG chickens than in FG birds, the differences were significant only in a few cases and the coefficients for total amino acids were almost the same. The present experiment thus failed to give any convincing evidence on the effect of genotype on the amino acid composition of deposited protein. Nevertheless, the values below 1.0 indicate that an increasing amount of non-protein N may be deposited in the body with advancing age. The changes in total amino acid concentration in body protein (Figure 2) support this hypothesis. The low allometric coefficient for cysteine in both genotypes suggested that the deposition rate of feather protein was slower than that of whole body protein. In contrast, the comparison of Gompertz rate parameters for feather weight and whole body protein weight reported by Hancock et al. (1995) and Gous et al. (1999) showed that the relative growth rate of feathers was greater than that of body protein.

The present study showed that, except for cysteine and total sulphur amino acids, the apparent efficiency of amino acid retention was lower (though non-significantly) in laying-type chickens than in broilers. For both total amino acids and total N, the difference between the genotypes was approximately 15%. Conversely, most models predicting protein accretion implicitly assume that the efficiency with which ideal protein is utilized is constant across genotypes (Emmans and Kyriazakis, 1997; Sandberg et al., 2005). The main reason for this apparent inconsistency is that the experimental diet fed in the present study to both genotypes contained amino acids at levels sufficient to meet the requirements for broilers. Since the protein deposition potential of SG chickens was substantially lower than that of broilers, the excessive part of amino acids was deaminated and their nitrogen excreted. As a consequence, the retention efficiency of amino acids and of total N was reduced. Nevertheless, there are experiments demonstrating genotype-dependent differences in amino acid utilization even under conditions of their suboptimal intake. Thus Fatufe et al. (2004) found out that the marginal efficiency of lysine utilization was much lower in the laying genotype than in broilers, although it was estimated under a comparable degree of lysine deficiency. These authors suggested that the better utilization of amino acids in broilers was a consequence of the long-term selection for muscle growth. In the case of essential amino acids, the greatest differences between SG and FG chickens were observed in threonine and cysteine. There is no obvious explanation for threonine, as the concentration of this amino acid in empty body protein, inner organs or feathers is similar. The high apparent efficiency of cysteine utilization in SG chickens is likely associated with feather growth. As mentioned above, feather protein is rich in cysteine (7.0 g/16 g N) compared to the rest of the body (1.1 g/16 g N – Emmans, 1989). The data on the utilization of methionine clearly showed that in SG chickens methionine was partly converted to cysteine, thus meeting the higher demand for this amino acid.

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