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Age and Gender Dependent Amino Acid Concentrations in the Feather, Feather-Free and Whole Empty Body Protein of Fast Growing Meat-Type Chickens

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Abstract

Two consecutive growth experiments with meat-type chickens (Ross 308) were conducted in order to quantify the age-dependent amino acid (AA) content in the whole body protein of male and female birds based on experimental data of the feather and feather-free body protein fractions. Birds were reared under uniform housing and feeding conditions (floor pens, 15 pens per gender, 5 birds per pen) during the starter (day 1 to 22) and grower period (day 22 to 36). Both the starter and grower diet based on corn, wheat, soybean meal, soybean protein concentrate and feed amino acids was formulated to ensure an equal feed protein quality close to the ideal amino acid ratio by adjusting a constant mixture of the feed proteins. At start of the experiment and further on weekly up to the end of the 5th week, 15 birds per gender (each 3 pens of 5 birds) were selected and fasted for 24 h, to emptying of gastro-intestinal tract, respectively. Subsequently, birds were euthanized and the feathers were manually removed. Nitrogen (N) and AA content were determined both in the feather and feather-free body fraction. The concentration of individual AAs in both of body protein fraction is varying considerably. Explicitly higher Cys, Ser and Pro but importantly lower Met, Lys and His concentrations were found in the feather protein. Furthermore, significant differences ($p < 0.001$) for nearly all AAs of the studied body protein fractions and the whole empty body protein dependent on age of birds were observed. Especially high deviations were obtained during the first week of age and at the end of the experiment. According to this observed variation of AA concentrations must be concluded that the body AA composition of meat-type chickens during growth is not constant. The detected gender-specific differences for several AAs in the feather and body protein of male and female birds were rather low and with very low variation.

Keywords

Growing Chickens, Feather Amino Acids, Body Amino Acids, Age, Gender

1. Introduction

Body composition data of modern fast growing meat-type broiler chickens provide important information for further optimization of nutrient supply. However, the database for amino acid (AA) content of whole body and body fractions like feathers of current genotypes is scarce. Furthermore, different assumptions exist regarding the age and gender dependent body AA composition. Several studies supposed a rather uniform AA content of the whole body protein of broiler chickens during growth [1] [2] [3] [4] [5]. In consequence, constant AA contents in feather and feather-free body protein were applied as reference data for assessing AA requirement data [6] [7]. However, this assumption is invalid according to influencing factors like genotype [8] [9] [10], gender [11] [12] and age [11] [13]-[18]. Specifically, an age-dependent change of the whole body protein AA composition can be expected due to different allometric growth rates of individual body tissues and organs [7] [19] [20] [21]. Additionally, the dietary protein and AA supply can also impact on body AA composition [9] [10] [15] [22]-[27]. An increased accuracy in estimations of the adequate dietary AA supply could be achieved if substantial body protein fractions are initially investigated separately.

Therefore, in addition to our experimental data including both the age and gender dependent proportions of the feather and feather-free body fraction including their protein partitioning [28] [29], the present study aimed to provide detailed results about the AA composition of the whole body protein based on weekly analyses of feather and feather-free body fractions from male and female meat-type chickens during the entire growing period up to market weight.

2. Materials and Methods

Both growth experiments utilized a total of 180 growing broiler chickens (male to female birds = 1:1) were conducted at the facilities of the Division Animal Nutrition Physiology, Department of Animal Sciences at Georg-August-University Göttingen and were approved by the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

2.1. Animals and Housing

Freshly hatched meat-type chickens (ROSS 308) were obtained from a commercial hatchery and placed together in a floor pen on wood shavings. At next day (d1) averaged weighed birds were randomly allotted to 15 pens per gender (each 5 birds per pen) and kept under uniform management and feeding conditions (starter period: 1 to 22 d; grower period: 22 to 36 d). Further details were already reported elsewhere [28].

2.2. Diets and Feeding

Both starter and grower diets [28] were based on corn, wheat and soybean meal, soybean protein concentrate and crystalline feed AAs as main ingredients. Diet formulation aimed to meet current recommendations and was adjusted close to the ideal dietary AA ratios [30]. A constant mixture of the protein sources yielded equal feed protein quality during both of the feeding periods.

2.3. Collection and Sampling

At start of the experiment and weekly up to the end of the 5th week, 15 male and 15 female chickens (3 pens per week and gender) were placed separately with free access to drinking water. Selected birds were fasted for 24 hours for emptying the digestive tract to yield an "empty body mass". According to animal welfare regulations, these birds were subsequently euthanized by CO_2 inhalation. Each sample of quantitatively collected feathers and of feather-free empty body was separately stored in plastic bags at −20˚C for further processing as previously described in detail [28].

2.4. Chemical Analyses

Feed ingredients, diets, feathers and feather-free empty bodies were analysed in duplicates according to the German standard procedures [31]. N analyses were conducted by DUMAS-method (LECO® TruMac, LECO Instrument GmbH, Kirchheim, Germany) and crude protein (CP) was calculated by factor as N × 6.25 [31] [32]. AA composition of the feed protein sources, feathers and feather-free bodies was analysed by ion-exchange chromatography (Biochrom® 30, Biochrom Ltd. Cambridge, England) following acid hydrolysis without and with application of an oxidation step for quantitative determination of sulphur containing AAs [32]. Tryptophan (Trp) was quantified by ion-exchange chromatography (LC 300, Biotronik, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) following hydrolysis with lithium hydroxide based on the modified method 4.11.2 according to [31].

2.5. Statistical Analyses

Statistical analyses run with SPSS software package (Version 23.0 for Windows; IBM SPSS Statistics Inc., Chicago, IL, USA). Two-way analysis of variance (ANOVA) was performed to compare means of variables depending both on age and gender of broiler chickens as main effects, inclusive their interactions. Verification of variance homogeneity was evaluated by Levene-test. Significant differences ($p \leq 0.05$) were identified by Games-Howell test and Tukey post-hoc test, respectively.

3. Results

The age and gender dependent data for development of feather and feather-free body mass were already reported [28] and further applied for the current calculations. Table 1 summarizes N and AA contents in the feather protein depending on age and gender, respectively. Generally, high N contents significantly influenced by age of birds ($p < 0.001$) were observed in the dry matter (DM) of feathers. Accordingly, the feather N content was significantly reduced from day old to 15d old chickens, but with further increasing of age the N concentrations in the feather N tends to increase again. However, no gender-specific effect on feather N content was found and no interaction occurred between age and gender.

Feathers contain rather high quantities of the dispensable AAs glutamic acid (Glu), serine (Ser) and proline (Pro). Among indispensable AAs, specifically high concentrations of leucine (Leu), arginine (Arg) and cysteine (Cys) are located in the feather protein fraction. Otherwise, feathers contain a very low quantity of methionine (Met). For all AAs age-dependent effects ($p < 0.001$) were identified in feathers of male and female birds, except for glycine (Gly). The contents of lysine (Lys), Met, Cys and Glu initially increased up to respective near to the end of the starter period and afterwards declined up to slaughter weight. In contrast,

Table 1. Content of N (% of dry matter) and amino acids (g/16 g N) in feathers of broiler chickens depending on age and gender.[1]

Item	Age (days)						Gender		Mean	SEM	Significance (p)[3]		
	1	8	15	22	29	36[2]	Male	Female			A	G	A × G
N	16.21^{a}	15.94^{ab}	15.40^{c}	15.58^{bc}	15.75^{abc}	15.86^{abc}	15.76	15.71	15.74	0.05	0.001	0.909	0.385
Lys	1.49^{c}	2.09^{b}	2.43^{a}	2.36^{a}	2.13^{b}	2.05^{b}	2.11	2.22	2.17	0.05	0.000	0.000	0.000
Met	0.38^{e}	0.52^{c}	0.60^{a}	0.56^{b}	0.50^{cd}	0.47^{d}	0.51	0.54	0.52	0.01	0.000	0.000	0.000
Cys	6.07^{c}	6.45^{bc}	6.74^{ab}	7.14^{a}	7.22^{a}	6.87^{ab}	6.79	6.88	6.83	0.08	0.000	0.416	0.003
Thr	4.05^{d}	4.20^{c}	4.44^{b}	4.66^{a}	4.70^{a}	4.60^{a}	4.44	4.54	4.49	0.04	0.000	0.000	0.000
Trp[4]	1.01^{a}	0.96^{ab}	0.86^{b}	0.73^{c}	0.64^{cd}	0.61^{cd}	0.78			0.04	0.000		
Arg	7.38^{a}	7.00^{b}	6.61^{c}	6.72^{c}	6.81^{bc}	6.70^{c}	6.83	6.78	6.81	0.04	0.000	0.430	0.335
His	1.82^{a}	1.44^{b}	1.02^{c}	0.82^{d}	0.69^{e}	0.64^{e}	1.02	0.93	0.98	0.06	0.000	0.000	0.000
Ile	4.02^{c}	4.06^{c}	4.14^{c}	4.38^{b}	4.55^{a}	4.53^{ab}	4.26	4.36	4.31	0.04	0.000	0.012	0.207
Leu	7.26^{b}	7.31^{b}	7.37^{b}	7.60^{a}	7.67^{a}	7.70^{a}	7.49	7.53	7.51	0.04	0.000	0.395	0.044
Phe	5.22^{a}	4.79^{b}	4.51^{c}	4.58^{c}	4.60^{c}	4.60^{c}	4.64	4.66	4.65	0.03	0.000	0.522	0.085
Tyr	4.27^{a}	3.85^{b}	3.42^{c}	3.20^{d}	2.97^{e}	2.84^{e}	3.38	3.25	3.32	0.08	0.000	0.001	0.006
Val	5.17^{c}	5.41^{bc}	5.45^{b}	5.92^{a}	6.04^{a}	5.96^{a}	5.70	5.74	5.72	0.06	0.000	0.586	0.022
Ala	3.21^{d}	3.66^{c}	4.04^{b}	4.24^{a}	4.24^{a}	4.19^{a}	3.94	4.10	4.02	0.06	0.000	0.000	0.000
Asp	6.93^{a}	6.92^{a}	6.90^{a}	6.90^{a}	6.75^{ab}	6.62^{b}	6.81	6.84	6.83	0.03	0.000	0.118	0.000
Glu	10.46^{d}	11.22^{c}	11.79^{ab}	12.02^{a}	12.03^{a}	11.69^{b}	11.59	11.75	11.67	0.09	0.000	0.004	0.000
Gly	6.98	7.02	6.77	6.91	7.00	6.90	6.98	6.87	6.92	0.05	0.619	0.238	0.164
Pro	9.03^{d}	9.38^{cd}	9.72^{bc}	10.26^{ab}	10.53^{a}	10.27^{ab}	9.86	10.08	9.97	0.11	0.000	0.225	0.013
Ser	10.61^{b}	10.05^{c}	9.86^{c}	10.52^{b}	11.06^{a}	11.06^{a}	10.45	10.58	10.52	0.09	0.000	0.149	0.088

N = nitrogen, Lys = lysine, Met = methionine, Cys = cysteine, Thr = threonine, Trp = tryptophan, Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Phe = phenylalanine, Tyr = tyrosine, Val = valine, Ala = alanine, Asp = aspartic acid, Glu = glutamic acid, Gly = glycine, Pro = proline, Ser = serine, SEM = standard error of mean, A = age, G = gender, A × G = interaction between age and gender. [1]At 1d of age only two pooled feather samples (one per gender due to the low feather yield per bird), for remaining age periods n = 6 pooled samples of each five birds (3 per gender) and n = 16 pooled samples of each five birds per gender. [2]Final body mass of male birds was determined at 35d of age. [3]Significance level (p) of the two-way ANOVA. [4]Due to technical reasons tryptophan was not determined in feathers of female birds. [abc]Means of age-dependent data with different superscript letters within lines are significantly different ($p < 0.05$).

threonine (Thr), isoleucine (Ile), Leu, valine (Val), alanine (Ala), Pro and Ser increased, but Trp, Arg, histidine (His), phenylalanine (Phe), tyrosine (Tyr) and aspartic acid (Asp) contents in the feather protein declined with increasing age of the birds. In addition, some gender-specific effects on AA composition of feathers were observed, but can hardly be explained.

The N content in the feather-free body DM of birds (**Table 2**) declined continuously with increasing of age ($p < 0.001$). Male birds contained significantly more N in the feather free body than female counterparts. As related to the AA composition of the feather protein, higher contents of Glu, Lys, His and Ala were present in the feather-free body protein. In contrast, lower quantities of Cys, Phe and Val were observed. Individual body AA composition was also age-dependent ($p < 0.001$). However, no significant age-dependent difference ($p > 0.05$) was observed for Ala and Asp in the feather-free body. Mostly, superior AA concentrations were found in the body protein of day-old chickens, but subsequently decreasing. In contrast, a progression in increase with increasing of age was obtained for Lys, Met, His, Ile and Glu. However, between 15 d to 36 d only trends were observed. Additionally, gender-specific effects ($p < 0.05$) on AA content of feather-free body protein were observed, except for Cys. Also for most of AAs significant interaction between age and gender could be detected.

A summary of the observed effects is illustrated in **Figure 1**. The columns are presented as means of AA concentrations in the feather protein as related to the

Table 2. Content of N (% of dry matter) and amino acids (g/16 g N) in the feather-free empty body of broiler chickens depending on age and gender.[1]

Item	Age (days)						Gender		Mean	SEM	Significance (p)[3]		
	1	8	15	22	29	36[2]	Male	Female			A	G	A × G
N	11.33^{a}	10.41^{b}	10.14^{bc}	10.07^{bcd}	9.78^{cd}	9.63^{d}	10.58	9.87	10.23	0.12	0.000	0.000	0.000
Lys	6.51^{d}	6.75^{c}	6.83^{bc}	6.83^{bc}	7.03^{ab}	7.06^{a}	6.72	6.95	6.84	0.04	0.000	0.000	0.167
Met	1.92^{c}	1.92^{c}	1.94^{c}	1.94^{bc}	2.00^{ab}	2.01^{a}	1.91	2.00	1.96	0.01	0.000	0.000	0.025
Cys	1.07^{a}	1.00^{b}	0.95^{bc}	0.94^{c}	0.92^{c}	0.94^{c}	0.97	0.97	0.97	0.01	0.000	0.798	0.027
Thr	4.04^{a}	3.89^{b}	3.84^{b}	3.84^{b}	3.88^{b}	3.81^{b}	3.80	3.96	3.88	0.02	0.000	0.000	0.004
Trp[4]	1.43^{a}	1.33^{b}	1.26^{c}	1.23^{cd}	1.28^{bc}	1.19^{d}	1.29			0.02	0.000		
Arg	6.53^{a}	6.25^{b}	6.06^{bc}	6.04^{c}	6.11^{bc}	6.09^{bc}	6.10	6.26	6.18	0.04	0.000	0.000	0.007
His	2.17^{e}	2.34^{d}	2.50^{c}	2.56^{bc}	2.65^{a}	2.62^{ab}	2.43	2.52	2.48	0.03	0.000	0.000	0.008
Ile	3.61^{c}	3.72^{ab}	3.67^{bc}	3.71^{abc}	3.80^{a}	3.80^{a}	3.64	3.79	3.72	0.02	0.000	0.000	0.031
Leu	7.04^{a}	6.84^{b}	6.78^{b}	6.79^{b}	6.89^{ab}	6.83^{b}	6.76	6.97	6.86	0.03	0.001	0.000	0.013
Phe	3.85^{a}	3.70^{b}	3.60^{b}	3.61^{b}	3.64^{b}	3.62^{b}	3.61	3.73	3.67	0.02	0.000	0.000	0.024
Tyr	3.17^{a}	2.95^{b}	2.87^{b}	2.86^{b}	2.91^{b}	2.88^{b}	2.90	2.98	2.94	0.02	0.000	0.000	0.316
Val	4.06^{a}	3.92^{b}	3.78^{c}	3.83^{bc}	3.84^{bc}	3.79^{c}	3.96	3.78	3.87	0.02	0.000	0.000	0.005
Ala	6.00	5.84	5.91	5.93	5.98	5.93	5.89	5.97	5.93	0.02	0.103	0.032	0.003
Asp	8.31	8.22	8.22	8.32	8.43	8.32	8.12	8.49	8.30	0.04	0.077	0.000	0.003
Glu	14.52	14.49	14.48	14.55	14.88	14.85	14.09	15.17	14.63	0.10	0.016	0.000	0.262
Gly	7.74^{a}	7.13^{b}	7.07^{b}	6.97^{bc}	6.91^{bc}	6.76^{c}	7.19	7.01	7.10	0.06	0.000	0.003	0.012
Pro	5.49^{a}	5.02^{b}	4.94^{bc}	4.91^{bc}	4.71^{c}	4.82^{bc}	5.07	4.89	4.98	0.05	0.000	0.002	0.007
Ser	4.40^{a}	3.92^{b}	3.80^{bc}	3.76^{cd}	3.75^{cd}	3.66^{d}	3.84	3.93	3.88	0.04	0.000	0.002	0.001

Item (Abbreviations see **Table 1**), SEM = standard error of mean, A = age, G = gender, A × G = interaction between age and gender. [1]Per age period: n = 6 pooled samples (3 per gender) of each five birds; per gender: n = 18 pooled samples of each five birds. [2]Final body mass of male birds was determined at 35 d of age. [3]Significance level (p) of the two-way ANOVA. [4]Due to technical reasons tryptophan was not determined in the body of female birds. [abc]Means of age-dependent data with different superscript letters within lines are significantly different ($p < 0.05$).

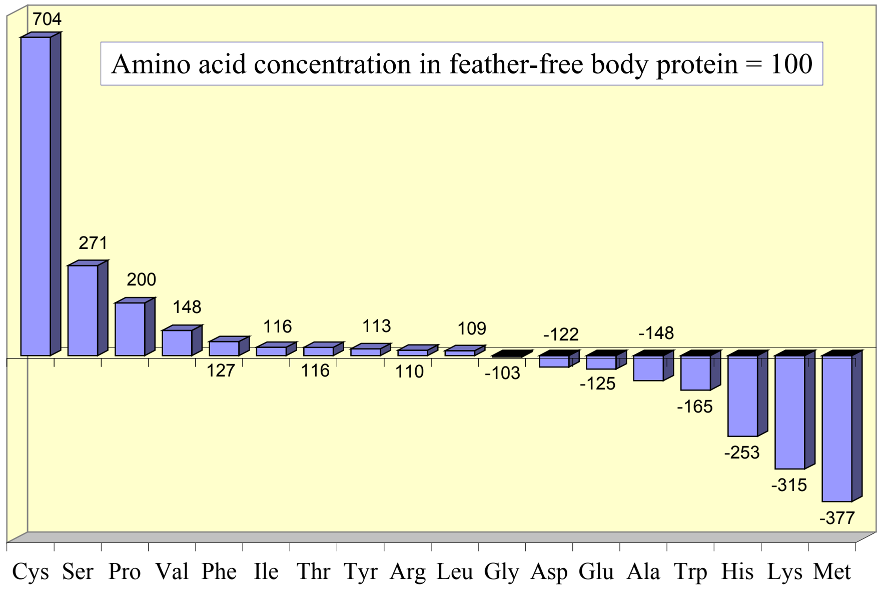

Figure 1. Relative deviation of the individual AA content in the protein of feather and feather-free body (Abbreviations see **Table 1**).

corresponding data in the feather-free body protein as reference. Accordingly, the mean Cys content in the feather protein fraction exceeded the Cys concentration in the feather-free body protein for more than seven times. In addition, markedly higher Ser, Pro and Val contents were obtained in feathers. In contrast, the feather protein contains approximately 3 to 4 times lower Lys and Met as well as 1.5 to 2.5 lower Ala, Trp and His than the feather-free body protein.

The N and AA composition of the whole empty body (**Table 3**) is quite similar to the feather-free body fraction due to the high protein partitioning of this fraction to the whole body protein [29]. If AA concentrations of both fractions (**Table 2** vs. **Table 3**) are compared directly, a large divergence for Cys and Ser is obvious, but explained by their very high concentrations in the feather protein (**Table 1**, **Figure 1**). Generally, day-old and in parts 8d old birds indicate significantly higher or lower AA concentrations ($p < 0.05$) than found in the body of older birds. No significant or extremely low differences were observed between remaining age periods (≥8 d). As compared with male birds, the body of female birds contains significantly lower N but higher AA concentrations, except for Val, Gly and Pro. For most of body AA concentrations, significant interactions between age and gender were identified.

Table 4 summarizes the partitioning of individual AAs of the feather fraction expressed as percentage of the whole empty body AA content. As expected, a very high individual variation between AAs was observed, also depending on age ($p < 0.001$). Due to the high DM and protein content in feathers of 1 d old chickens [28] [29], the highest relative proportion of individual feather AAs was found in one day old birds, the lowest ($p < 0.05$) at 8 d or 15 d of age. Subsequently, the AA partition in the feather fraction increased continuously, except for His.

Table 3. Content of N (% of dry matter) and amino acids (g/16 g N) in the whole empty body of broiler chickens depending on age and gender.[1]

Item	Age (days)						Gender		Mean	SEM	Significance (p)[3]		
	1	8	15	22	29	36[2]	Male	Female			A	G	A × G
N	11.83^{a}	10.67^{b}	10.37^{bc}	10.36^{bc}	10.12^{c}	10.06^{c}	10.89	10.24	10.57	0.12	0.000	0.000	0.066
Lys	5.81^{b}	6.43^{a}	6.54^{a}	6.48^{a}	6.58^{a}	6.51^{a}	6.31	6.48	6.39	0.05	0.000	0.000	0.034
Met	1.70^{b}	1.83^{a}	1.85^{a}	1.83^{a}	1.86^{a}	1.84^{a}	1.78	1.86	1.82	0.01	0.000	0.000	0.002
Cys	1.77^{a}	1.38^{de}	1.33^{e}	1.42^{cd}	1.49^{c}	1.58^{b}	1.46	1.53	1.49	0.03	0.000	0.000	0.000
Thr	4.04^{a}	3.91^{b}	3.88^{b}	3.90^{b}	3.95^{ab}	3.89^{b}	3.85	4.01	3.93	0.02	0.001	0,000	0.002
Trp[4]	1.14^{bc}	1.31^{a}	1.24^{ab}	1.06^{c}	1.23^{ab}	1.14^{bc}	1.19			0.02	0.001		
Arg	6.65^{a}	6.30^{b}	6.09^{c}	6.09^{c}	6.18^{bc}	6.15^{bc}	6.17	6.32	6.24	0.04	0.000	0.000	0.005
His	2.12^{d}	2.28^{c}	2.40^{b}	2.43^{ab}	2.47^{a}	2.41^{b}	2.33	2.38	2.35	0.02	0.000	0.000	0.001
Ile	3.67^{b}	3.74^{b}	3.70^{b}	3.76^{b}	3.87^{a}	3.88^{a}	3.70	3.84	3.77	0.02	0.000	0.000	0.028
Leu	7.08^{a}	6.88^{b}	6.82^{b}	6.85^{b}	6.96^{ab}	6.92^{b}	6.82	7.02	6.92	0.03	0.000	0.000	0.007
Phe	4.04^{a}	3.77^{b}	3.66^{c}	3.69^{bc}	3.73^{bc}	3.73^{bc}	3.71	3.83	3.77	0.03	0.000	0.000	0.015
Tyr	3.32^{a}	3.02^{b}	2.90^{c}	2.89^{c}	2.91^{c}	2.87^{c}	2.95	3.02	2.98	0.03	0.000	0.000	0.320
Val	4.22^{a}	4.02^{b}	3.90^{c}	3.99^{bc}	4.04^{b}	4.03^{b}	4.10	3.97	4.03	0.02	0.000	0.000	0.017
Ala	5.61^{b}	5.69^{ab}	5.79^{a}	5.79^{a}	5.82^{a}	5.74^{ab}	5.71	5.77	5.74	0.02	0.006	0.043	0.000
Asp	8.12	8.13	8.13	8.20	8.28	8.13	8.00	8.33	8.17	0.04	0.158	0,000	0.000
Glu	13.96^{b}	14.26^{ab}	14.29^{ab}	14.35^{ab}	14.61^{a}	14.50^{a}	13.84	14.81	14.33	0.10	0.001	0.000	0.072
Gly	7.64^{a}	7.13^{b}	7.04^{bc}	6.97^{bc}	6.92^{bc}	6.78^{c}	7.17	6.99	7.08	0.06	0.000	0.002	0.009
Pro	5.99^{a}	5.32^{b}	5.25^{b}	5.33^{b}	5.24^{b}	5.42^{b}	5.47	5.38	5.42	0.05	0.000	0.101	0.003
Ser	5.27^{a}	4.34^{cd}	4.21^{e}	4.29^{de}	4.41^{bc}	4.47^{b}	4.42	4.58	4.50	0.06	0.000	0.000	0.000

Item (Abbreviations see Table 1), SEM = standard error of mean, A = age, G = gender, A × G = interaction between age and gender. [1]Per age period: n = 6 pooled samples (3 per gender) of each five birds; per gender: n = 18 pooled samples of each five birds. [2]Final body mass of male birds was determined at 35 d of age. [3]Significance level (p) of the two-way ANOVA. [4]Due to technical reasons tryptophan was not determined in the body fractions of female birds. [abc]Means of age-dependent data with different superscript letters within lines are significantly different ($p < 0.05$).

Table 4. Percentage of feather amino acids as related to the whole empty body amino acid content of broiler chickens depending on age and gender.[1]

AA	Age (days)						Gender		Mean	SEM	Significance (p)[3]		
	1	8	15	22	29	36[2]	Male	Female			A	G	A × G
Lys	3.56^{a}	2.24^{c}	2.45^{bc}	2.84^{b}	2.93^{b}	3.43^{a}	2.65	3.17	2.91	0.10	0.000	0.000	0.008
Met	3.08^{a}	1.96^{d}	2.13^{cd}	2.41^{bc}	2.41^{bc}	2.80^{ab}	2.25	2.68	2.46	0.09	0.000	0.000	0.006
Cys	47.78^{a}	32.19^{c}	33.19^{c}	39.28^{b}	43.56^{ab}	47.05^{a}	38.43	42.58	40.51	1.21	0.000	0.000	0.002
Thr	13.99^{a}	7.40^{b}	7.57^{c}	9.35^{b}	10.74^{b}	12.85^{a}	9.74	10.89	10.31	0.48	0.000	0.001	0.000
Trp[4]	13.62^{a}	4.98^{b}	3.70^{b}	5.00^{b}	4.09^{b}	5.05^{b}	6.07			0.84	0.000		
Arg	15.54^{a}	7.66^{d}	7.13^{d}	8.65^{cd}	9.97^{c}	11.83^{b}	9.67	10.58	10.13	0.54	0.000	0.007	0.000
His	11.96^{a}	4.35^{b}	2.75^{c}	2.64^{c}	2.53^{c}	2.89^{c}	4.62	4.42	4.52	0.58	0.000	0.376	0.002
Ile	15.35^{a}	7.47^{d}	7.36^{d}	9.13^{c}	10.61^{c}	12.72^{b}	9.91	10.97	10.44	0.55	0.000	0.002	0.000
Leu	14.36^{a}	7.32^{de}	7.11^{e}	8.69^{cd}	9.95^{c}	12.09^{b}	9.44	10.40	9.92	0.50	0.000	0.002	0.000
Phe	18.05^{a}	8.74^{d}	8.08^{d}	9.74^{cd}	11.16^{c}	13.46^{b}	11.00	12.07	11.54	0.63	0.000	0.007	0.000
Tyr	17.95^{a}	8.80^{c}	7.70^{c}	8.67^{c}	9.21^{bc}	10.76^{b}	10.22	10.81	10.51	0.63	0.000	0.098	0.000
Val	17.12^{a}	9.27^{c}	9.27^{c}	11.64^{b}	13.51^{b}	16.22^{a}	11.75	13.93	12.84	0.62	0.000	0.000	0.000
Ala	7.99^{a}	4.43^{c}	4.63^{c}	5.74^{b}	6.59^{b}	7.95^{a}	5.72	6.72	6.22	0.29	0.000	0.000	0.000
Asp	11.93^{a}	5.87^{d}	5.58^{d}	6.58^{cd}	7.36^{c}	8.86^{b}	7.36	8.03	7.70	0.41	0.000	0.009	0.000
Glu	10.50^{a}	5.42^{d}	5.41^{d}	6.56^{cd}	7.42^{c}	8.75^{b}	7.08	7.61	7.34	0.35	0.000	0.022	0.000
Gly	12.78^{a}	6.79^{d}	6.30^{d}	7.79^{cd}	9.16^{c}	11.17^{b}	8.38	9.62	9.00	0.46	0.000	0.000	0.000
Pro	21.03^{a}	12.15^{d}	12.18^{d}	15.11^{c}	18.23^{b}	20.72^{a}	15.16	17.98	16.57	0.75	0.000	0.000	0.000
Ser	28.10^{a}	15.93^{d}	15.48^{d}	19.20^{c}	22.66^{b}	26.88^{a}	20.24	22.51	21.37	0.94	0.000	0.000	0.000

AA = amino acid (Abbreviations see Table 1), SEM = standard error of mean, A = age, G = gender, A × G = interaction between age and gender. [1]Per age period: n = 6 pooled samples (3 per gender) of each five birds; per gender: n = 18 pooled samples of each five birds. [2]Final body mass of male birds was determined at 35 d of age. [3]Significance level (p) of the two-way ANOVA. [4]Due to technical reasons tryptophan was not determined in the body fractions of female birds. [abc]Means of age-dependent data with different superscript letters within lines are significantly different ($p < 0.05$).

At 36 d of age, approximately 50 percent of feather AAs proportions came near to the initial level as observed in one day old chicken. With the exception of His and Tyr, also significant gender-specific effects of AA partitioning in the feather fraction were detected. In line with **Figure 1**, the highest relative proportions of feather AAs were demonstrated for Cys, Ser and Pro, the lowest proportions for Met, Lys and His.

4. Discussion

Our actual results have demonstrated that individual AA concentration in the feather and feather-free body protein of modern meat-type chicken is highly variable. This observation is in line with previous studies directly comparing the AA content of both body fractions [6] [7] [11] [13] [16] [18] [33] [34]. As summarized in **Figure 1**, important differences were observed for Cys, Ser and Pro with significantly higher and for Met, Lys and His with significantly lower content in the feather protein as compared to the feather-free body protein fraction. Likewise, considerable differences were found as the relative AA concentrations of both protein fractions were compared to the ideal dietary AA ratio with Lys as reference AA [35].

It is well-known that Cys is present in extremely high concentration in keratins [36]. In detail, this was reported for proteins from hair [37] [38] [39] [40], feathers [6] [7] [13] [14] [16] [18] [27] [33] [37] [40]-[45] and wool [37] [40]. In contrast, the remaining body protein fractions, predominantly the muscle and organ tissues of birds are rich in Lys [5] [6] [7] [11] [13] [15] [18] [44] [45] [46] [47]. Therefore, both the variation and displacement of the mostly important fractions like feather and carcass protein dependent on genotype, gender, age and nutrient supply may impact on the AA composition of the whole body protein of birds.

Nitsan *et al.* [33] reported that the AA content of feathers is quite consistent across most domesticated species. Stilborn *et al.* [16] found no significant effect on the feather AA content from two distinct broiler strain crosses and concluded later on that the carcass AA content, when expressed as percentage of protein, was also relatively constant despite genetic improvements [11]. The significantly higher Cys content in the accreted body protein of a laying bird as compared to a fast growing meat-type chicken [10] is not in contrast to these observations, but indicates a higher feather protein proportion [28]. Otherwise, no significant gender effect on feather AAs in growing chickens was reported [14] [16]. We observed marginal and negligible gender-specific effects in the feather protein (**Table 1**). According to Stilborn *et al.* [11], this conclusion also fits for all AAs in the feather-free body protein (**Table 2**). Higher AA contents were analysed in the feather-free body protein of female chickens, but corresponding with lower body protein as compared to their male counterparts [29]. Some small differences were abundant during the latter growth period reflecting different maturation rates between male and female birds [20].

Depending on age, the AA composition of the feather, feather-free body and whole body protein was assumed to be rather constant [1] [2] [3] [33]. However, according to our observations there are references for age-dependent effects on the content of individual AA both in the body and several body protein fractions of growing birds. In line with several reports [13] [14] [16] [18] [41] an age-dependent effect exists on individual feather AAs, indicating elevated Cys, Thr, Ile, Leu and Val but declined His, Trp and Tyr concentrations in the feather protein with increasing of age. Simultaneously, the content of nearly all dispensable AAs in feathers were also elevated with increasing age of the birds. In contrast, different trends were observed for Lys, Met, Arg and Phe. Additionally, it has to be pointed out that the feather AA data from freshly hatched day old chicken differ from other age periods. When these early data up to 1 week of age were excluded from data analysis, no or only marginal age-dependent differences for most of the individual feather AAs were observed. However, according to earlier reports [28] [29] the contribution of feathers to the whole body protein changes with age, mainly during early rapid growth.

But also feather structure and composition are age-dependent and individual morphological parts of fowl feathers show differences in AA composition [40] [48] [49] [50] [51]. In addition, also effects of diets deficient in branched chain AAs and methionine on AA content of the feather protein were reported [27] [43]. In case of deficient AA supply, feather protein synthesis is of superior priority [27] [52].

The observed variation of the AA content in the feather-free body protein was lower as compared to the feather protein. Apart from elevated Lys and His, but declined Cys, Trp, Val, Gly, Pro and Ser contents, the carcass AAs were rather constant when the data up to the end of the 1st week were excluded. This statement agrees with several chicken and turkey studies [11] [13] [18] [44] [45]. Age and diet related changes of feather-free body AA composition could result from displacements between the muscle, bone and connective tissue proportions.

Looking on AA composition of the whole body protein (carcass plus feathers) of chickens, more data are available. Earlier investigations [1] [2] [3] indicate age-independent rather constant AA proportions. However, more recent reports also indicate some divergences for several AAs during first days of the growth period [3] [15] [45] or near to the market weight of meat-type chickens [3] [11]. These observations are in good agreement with our results (**Table 3**). Almost constant AA composition data are observed when both the 1st week and last week (d 36) data are excluded. Generally, the differences between d 8 and d 36 were negligible, except for Ile and Trp with advancing age. In contrast, superior content of Cys was found in day old chickens which declined within two weeks. Afterwards, an elevated Cys content was observed with increasing age. The age-dependent variation between individual AAs is explained by the increasing ratio of feather protein to whole body protein [3] [15] [24] [29] [53] [54] due to changes in feathering rate and resulting AA partitioning within body protein (**Table 4**). Altered proportions of different tissue proteins during growth [6] [10]

[15] [20] [25] [33] [55] are additional factors. Muscle tissue is rich in Lys, bone and connective tissue is rich in Gly and Pro but low in Cys and Trp [40] [46] [56]. In consequence, elevated Lys content in the whole body protein indicates an elevated contribution of muscle tissue to the whole body [17]. Accordingly, lower contribution of bone and connective tissue results in lower contents of Gly, Pro and Trp in the featherless body protein with increasing of age.

In addition, both dietary energy and protein supply may contribute to modify whole body AA composition of broiler chickens, possibly by influencing the proportions of individual body tissues [15] [54]. Such observations are also reported dependent on dietary supply of Lys [9] [10] [22], Met or Met + Cys [25] [26] [27] [57], Thr [24], Trp [5] and Val [23] [43].

Finally has to be stated that published AA data of body protein fractions vary considerably, also attributed to different methodical procedures during sample preparation and chemical analysis [14] [46] [47] [58] [59]. Exemplarily, substantial Cys losses can be expected during autoclaving process of carcasses [47] [60]. In consequence, lower Cys contents were observed in feather meal following hydrothermal and pressure processing as compared to untreated feather protein [61] [62] [63]. Extended Cys degradation was also reported by commercial processing of feathers [39] [40] [64] [65] [66]. The current study dried and milled the feathers before analyses, but autoclaved the feather-free body samples [28]. This factor of pre-treatment needs attention when body AA composition data are compared.

Accordingly, literature data on AA content in the body of birds are diverse and the reference basis is not always clearly defined. Some data include the feather fraction whereas other data are related to the feather-free whole body. Sometimes there is no indication whether the so-called "carcass" fraction includes head, legs or internal organs. Furthermore, the exclusion of gastro-intestinal content in the analysis samples is a further factor limiting the comparability of reported data.

Despite this fact, **Table 5** summarizes the AA composition of the feather, feather-free body and whole body protein as derived from the literature. Statistical analysis excluded single data which exceeded or remained below 30% of the calculated average for the individual AA. Regardless of the discussed factors of influence on individual AA content in body protein fractions, the averaged values fit our mean values as presented in **Tables 1-3** very well. Minor divergences were within the range of the relatively high Min/Max variation (**Table 5**). Systematic differences were only found for Val and Glu, but are difficult to explain.

5. Conclusions

In fast growing meat-type chickens, the AA composition of the feather protein is not constant during the growth period up to market weight. However, minor gender dependent effects were observed for most of the AAs. According to different tissue growth and partitioning of the feather and remaining body protein

Table 5. Amino acid content (g/16 gN) in body fractions of growing chickens and observed variation from literature data[1].

AA	Feather protein			Feather-free protein			Whole empty body protein		
	n	Mean	Min/Max	n	Mean	Min/Max	n	Mean	Min/Max
Lys	12	**2.0**	1.6/2.5	6	**7.1**	6.5/7.5	16	**6.4**	5.7/7.5
Met	9	**0.6**	0.5/0.7	3	**2.2**	1.8/2.5	13	**1.9**	1.6/2.3
Cys	11	**8.1**	7.0/9.5	3	**1.1**	1.0/1.3	11	**1.6**	1.1/2.0
Thr	15	**4.5**	3.6/5.4	7	**4.0**	3.2/4.5	11	**4.0**	3.4/4.5
Trp	5	**0.8**	0.5/1.2	4	**1.0**	0.8/1.5	3	**1.0**	0.8/1.1
Arg	14	**6.6**	5.4/7.4	6	**7.0**	6.6/7.9	11	**6.8**	5.8/7.7
His	11	**0.7**	0.6/0.9	6	**2.4**	2.0/3.0	10	**2.4**	1.8/3.2
Ile	12	**4.6**	3.7/5.4	5	**4.0**	3.9/4.2	11	**4.0**	3.3/4.7
Leu	13	**7.8**	6.4/9.1	5	**7.3**	6.6/7.7	11	**7.1**	6.6/8.0
Phe	14	**4.6**	3.5/5.5	6	**4.2**	3.9/5.0	11	**4.1**	3.5/4.7
Tyr	13	**2.7**	2.2/3.4	6	**2.8**	2.4/3.2	9	**2.9**	2.5/3.1
Val	13	**7.4**	6.3/8.9	6	**5.4**	4.8/6.7	12	**4.9**	3.8/5.8
Ala	9	**4.0**	3.4/4.4	4	**6.4**	5.5/6.9	9	**6.0**	5.4/6.6
Asp	11	**6.7**	5.6/7.9	4	**8.7**	7.1/9.4	9	**8.4**	7.8/9.2
Glu	11	**9.4**	7.9/11.3	4	**14.0**	12.0/15.0	10	**12.9**	11.8/13.9
Gly	11	**6.8**	5.7/9.1	4	**8.0**	7.5/8.3	8	**7.9**	6.6/9.2
Pro	9	**9.2**	7.5/10.9	2	**6.1**	5.6/6.7	9	**6.3**	5.1/7.3
Ser	9	**11.0**	8.9/11.6	3	**4.5**	4.5/4.6	9	**4.5**	3.6/4.9
Ref.	[6] [7] [13] [14] [16] [21] [27] [40] [41] [43] [44] [45] [61] [62] [63]			[6] [7] [11] [13] [21] [44] [45]			[1] [2] [3] [4] [5] [9] [10] [15] [23] [24] [25] [26] [27] [57] [67]		

AA = amino acid (Abbreviations see Table 1), n = Number of included literature values, Min/Max = range of averaged literature data (lowest/highest value), Ref. = references for AA data of the presented protein fraction. [1]Only results between 1 d and 42 d of age were included.

fractions, the AA content in the feather-free body protein is varied considerably and responds on age-dependent empty body AA composition. These observations justify a separate evaluation of both fractions in factorial AA requirement studies because such important AAs like Lys and SAAs are involved and impacts on the derived ideal dietary AA ratio cannot be excluded.

The described procedure to derive AA body composition based on AAs in feathers and feather-free empty body yields reliable data for modelling of AA deposition in meat-type chickens providing an important precondition for valid evaluation of AA requirements based on factorial approaches.

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