Research Article

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Differences in muscle histidine-containing dipeptides in broilers

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Abstract

BACKGROUND: Poultry meat has high levels of histidine-containing dipeptides (HCD) and consumption of meat rich in HCD may elicit certain health benefits. The aim of this work was to compare the HCD content (anserine and carnosine) in the breast and thigh muscles of two broiler strains differing in growth rate, feeding regime, and age at slaughter. A 3 (production system) × 2 (sex) × 2 (age at slaughter) full factorial arrangement was applied with fast-growing Ross 308 chicks fed *ad libitum* (ROSS-AL), slow-growing Sasso T451 chicks fed *ad libitum* (SASSO-AL), and Ross 308 chicks given limited feeding (ROSS-LIM). At the age of 40 and 62 days, eight birds per production system × sex combination were randomly selected for sampling of the breast and thigh muscle. Muscle HCD content was determined by high-performance liquid chromatography (HPLC).

RESULTS: Across treatments, levels of anserine were 2.5- and 1.9-fold higher than carnosine in breast and thigh muscle respectively ($P < 0.001$), and levels of anserine and carnosine were 2.2- and 2.8-fold higher respectively in breast versus thigh muscle ($P < 0.001$). In breast muscle, SASSO-AL had higher levels of HCD than ROSS-AL and ROSS-LIM ($P < 0.001$). Considering different market meat types, breast muscle of 62-day-old SASSO-AL birds had more than threefold higher content of HCD compared to thigh muscle of 40-day-old ROSS-AL birds ($P < 0.001$).

CONCLUSION: Large differences in muscle HCD content were found, varying according to type of muscle and broiler.

Keywords: chicken; muscle; age; carnosine; anserine

INTRODUCTION

Poultry meat consumption and production have been increasing in recent decades and it is forecast to rise further and become the most commonly consumed meat worldwide. Poultry meat is a source of high-quality animal protein.[1] It is also a good source of histidine-containing dipeptides (HCD).[2] Histidine-containing dipeptides are a group of bioactive peptides that include carnosine, anserine, and balenine (also known as ophidine). Carnosine is composed of β-alanine and L-histidine. Anserine and balenine are the methylated analogs, better defined as β-alanyl-N-π-methyl-histidine and β-alanyl-N-τ-methyl-histidine respectively.[3] Histidine-containing dipeptides are synthesized in muscle and are widely abundant in mammals and other vertebrates, although their distribution differs considerably across species and tissues.[4] Carnosine is the predominant HCD in beef and pork, whereas anserine is the major HCD in poultry meat and salmonid fishes.[5] Compared to other farm animal species, poultry has the highest total HCD content, ranked as follows: turkey > chicken > horse > pig > rabbit > beef.[6]

It can be hypothesized that consumption of meat rich in HCD may elicit certain health benefits. Histidine-containing dipeptides display several physiological roles including intracellular buffering in skeletal muscle and free radical quenching.[7] Histidine-containing dipeptides also show a direct reactive carbonyl species quenching mechanism, preventing the formation of advanced lipoxidation end products and advanced glycoxidation end products.[3,8] An improvement in recovery from mental fatigue in subjects with heavy workloads after consumption of chicken meat rich in both carnosine and anserine has also been reported.[9] The benefits of using HCD supplements in humans have been particularly emphasized in the elderly because carnosine content and skeletal muscle mass decrease with age.[3,5]

As the intake of HCD through the consumption of poultry meat may be relevant for human health, it is important to understand the determinants of the muscle HCD content in broilers from commercial production systems. In this study two broiler strains, Ross 308 and Sasso T451, differing in growth potential and raised on different feeding regimes and until two slaughter ages, were compared for the HCD content in their breast and thigh muscles. Ross 308 is a fast-growing hybrid broiler that reaches the commercial slaughter weight of approximately 2.5 kg at 40 days of age. It is a feed-efficient broiler with a good meat yield, which is used in

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intensive production systems. Sasso T451 is a slow-growing broiler that reaches a similar slaughter weight at 65 to 75 days of age. It is the typical broiler used in organic production and more extensive production systems, e.g. free-range Label Rouge productions in France. Differences in muscle fiber type and metabolic properties, and sensory and technological meat properties between these broiler strains have long been documented[10] but the effects of differences in growth rate on muscle HCD content are not well established. Developments in genetics and feeding may also have changed certain muscle properties. We hypothesized that broilers genetically selected for slower growth may accumulate more HCD because the amino acid demand for protein synthesis in tissues is lower, potentially allocating residual dietary amino acids to the synthesis of non-proteinogenic compounds. Different feeding regimes were installed and birds were slaughtered at different ages to allow the effects of growth rate and age to be separated.

MATERIALS AND METHODS

Birds and experimental design

The experiment was conducted at the Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, and met the guidelines approved by the institutional animal care and use committee. The experiment followed a 3 (production system) × 2 (sex) × 2 (slaughter age) full factorial arrangement. Three production systems were installed as a combination of broiler strain and feeding level: Ross 308 birds fed *ad libitum* (ROSS-AL), Sasso T451 birds fed *ad libitum* (SASSO-AL), and Ross 308 birds given limited feed (ROSS-LIM). The ROSS-LIM group was restricted to the same daily feed intake as the SASSO-AL birds. For this purpose, a small group of SASSO chicks (10 males and 11 females) was kept separately in two pens starting 1 week before the start of the main experiment. The feed intake was measured daily, and the same amount of feed, adjusted on a weekly basis, was given to the ROSS-LIM birds.

Male and female chicks were raised in separate pens with 25 birds per pen for the ROSS groups and 42 birds per pen for the SASSO groups, and two replicate pens per sex × production system combination (368 chicks in total). The larger number of SASSO versus ROSS birds per pen only lasted until day 40 of the experiment, when 17 birds per pen were removed for another experiment. Considering the slower growth of SASSO birds, the pen density was never critical. Water was available *ad libitum*. No medications were added to the feed or the water. All treatments received the same diets – a starter feed for the first 2 weeks, a grower-1 feed in weeks 3 and 4, a grower-2 feed in weeks 5 and 6, and a finisher feed from week 6 onward. All feeds were manufactured at ILVO, Melle. The composition of the diets is given in Table 1.

Slaughtering and sampling of the birds from all treatments was done at 40 and 62 days of age. These dates were chosen because ROSS-AL and SASSO-AL reached approximately the commercial slaughter weight of 2.5 kg at 40 and 62 days of age respectively. Four birds from each pen were chosen at random on each of the slaughter days (eight birds per production system × sex combination). After 5 hours of feed withdrawal, birds were individually weighed, killed by cervical dislocation followed by exsanguination and eviscerated. Breast and thigh muscles of both sides of the carcass were collected, vacuum packed and frozen at −20 °C.

Performance data were recorded at pen level at 40 and 62 days of age.

Carnosine and anserine analysis

The carnosine and anserine content was determined using a modification of the method described by Kobe *et al.*[11] Minced samples (1 g) from both breast and thigh muscles were homogenized with 25 mL of 0.01 mol L^{-1} phosphate buffer (pH 7.4) using the Ultra turrax (type 18/10, Janke und Kunkel, KG, Germany). After homogenization, 1.0 mL Ultra gradient HPLC grade acetonitrile (Filter Service, Eupen, Belgium) was added to 2.0 mL of this mixture. The samples were kept overnight at 4 °C, and subsequently centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was filtered using a cellulose syringe filter (filter chromafil RC-20/25, pore size of 0.20 µm and Ø 25 mm) and 20 µL of this solution was injected into a high-performance liquid chromatography (HPLC) column equipped with an Agilent 1200 series quaternary pump, degasser, auto-sampler, and column oven. An amino-propyl EC250/4.6 Nucleosil 120 – 7 NH2 column was used (Machery-Nagel, Düren, Germany ref. 720058.46). A diode array detector was used at 210 nm (Agilent). The concentration was determined by comparing standard solutions of both anserine (Flamma, Chignolo, Italy) and carnosine (Sigma-Aldrich, Overijse, Belgium), with known concentrations between 0.02 and 0.10 mg mL^{-1}. Separation was carried out at a flow rate of 1 mL min^{-1} in an isocratic way. The mobile phase was composed of acetonitrile/200 mM phosphate buffer (pH 4.5) in a 60/40 ratio. The data are expressed on a wet muscle weight basis.

Table 1. Ingredient and nutrient composition of the diets (g kg^{-1} as fed)

	Starter	Grower 1	Grower 2	Finisher
Ingredient composition				
Wheat	520.0	545.1	582.0	607.5
Corn	100.0	75.0	50.0	50.0
Soybean meal 48	213.0	202.0	182.5	170.4
Full fat soybeans	100.0	100.0	100.0	80.0
Animal fat	23.5	41.0	52.5	61.4
Vitamins and mineral mix	10.0	10.0	10.0	10.0
$CaCO_3$	6.0	5.0	3.9	3.1
Di-Ca-phosphate	15.4	12.3	10.3	9.5
NaCl	2.2	2.3	2.4	2.4
Na-bicarbonate	1.5	1.4	1.3	1.2
L-Lysine HCl	2.95	2.49	2.21	2.02
DL-methionine	2.55	2.32	2.14	1.91
L-threonine	0.83	0.53	0.33	0.21
Ronozyme WX	0.20	0.20	0.20	0.20
Ronozyme NP	0.20	0.20	0.20	0.20
Clinacox	0.20	0.20	0.20	0.00
Estimated nutrient composition				
Crude protein	210	205	200	190
Digestible lysine	11.00	10.25	10.00	9.75
Available P	4.0	3.5	3.0	2.5
Metabolizable energy, MJ kg^{-1}	11.90	12.10	12.25	12.40

Statistical analysis

Data were analyzed with the GLM procedure in SAS Enterprise Guide (Version 7.15, SAS Institute Inc., Cary, NC, USA). Data for the muscle HCD contents were analyzed per muscle separately. The model included the fixed effects of production system (strain and feeding regime combination, i.e. ROSS-AL, ROSS-LIM, and SASSO-AL), age (40 or 62 days old), sex (males and females), and their interaction terms age × production system, age × sex, production system × sex and age × production system × sex.

Table 2. Mean values for broiler performance data, according to age at slaughter, production system, and sex

Age (A)	Production system (P)	Sex (S)	BW (g)	ADFI (g)	ADG (g)	FCR
40 days	ROSS-AL	Female	2405[a]	98.9[a]	59.0[a]	1.68[a]
		Male	2857[b]	111.8[b]	70.3[b]	1.59[a,b]
	ROSS-LIM	Female	1333[c]	57.4[c]	32.2[c]	1.78[c,a]
		Male	1629[d]	70.6[d]	39.6[d]	1.78[c,a]
	SASSO-AL	Female	1324[c]	60.9[c]	32.1[c]	1.90[d]
		Male	1463[e]	68.0[d]	35.5[e]	1.91[d]
RMSE			22.2	0.93	0.57	0.027
P-value						
Production system			<0.001	<0.001	<0.001	<0.001
Sex			<0.001	<0.001	<0.001	0.164
Production system × sex			<0.001	0.006	<0.001	0.077
62 days	ROSS-AL	Female	4298[a]	140.3[a]	68.6[a]	2.05[a]
		Male	5580[b]	158.5[b]	89.3[b]	1.78[b]
	ROSS-LIM	Female	2815[c]	84.5[c]	44.7[c]	1.89[b]
		Male	3244[d]	97.4[d]	51.6[d]	1.89[b]
	SASSO-AL	Female	2313[e]	86.4[c]	36.7[e]	2.36[c]
		Male	2719[c]	98.1[d]	43.2[c]	2.27[c]
RMSE			74.8	1.92	1.20	0.031
P-value						
Production system			<0.001	<0.001	<0.001	<0.001
Sex			<0.001	<0.001	<0.001	<0.001
Production system × Sex			<0.001	0.113	<0.001	0.002

Values are means based on two pens.
Mean values, within a column, with different superscripts, are significantly different at $P < 0.05$.
ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio; RMSE, root mean square error; ROSS-AL, Ross 308 fed *ad libitum*; ROSS-LIM, Ross 308 fed limited; SASSO-AL, Sasso T451 fed *ad libitum*.

Performance data were analyzed per slaughter age separately with a model including the fixed effects of production system, sex and their interaction term. Least square means were separated statistically with Tukey's HSD method and the significance level was set at $P < 0.05$.

RESULTS

Body weight and performance data

As expected, at 40 days of age and across sexes, ROSS-LIM (1.73 ± 0.4 kg) and SASSO-AL (1.36 ± 0.1 kg) had a lower body weight (BW) than ROSS-AL (2.54 ± 0.1 kg) ($P < 0.001$), with ROSS-LIM being heavier than SASSO-AL ($P < 0.05$) (Table 2). Similarly, BW at 62 days of age decreased in the order ROSS-AL > ROSS-LIM > SASSO-AL across sexes (4.79 ± 0.8, 3.11 ± 0.5 and 2.52 ± 0.4 kg respectively) ($P < 0.001$). Across production systems and ages, males were significantly heavier than females, as expected ($P < 0.001$). Across sexes, the commercial market-type birds ROSS-AL at 40 days and SASSO-AL at 62 days did not differ in BW and reached the intended BW at slaughter (mean 2.54 versus 2.52 kg).

At both 40 days and 62 days of age, and across sexes, ROSS-AL had a higher average daily feed intake (ADFI) and average daily gain (ADG) than ROSS-LIM and SASSO-AL ($P < 0.001$). At both ages and across sexes, ROSS-LIM and SASSO-AL consumed the same amount of feed as intended. However, at both ages, and across sexes, ROSS-LIM had a higher ADG than SASSO-AL ($P < 0.01$). At 40 days of age, and across sexes, the feed conversion ratio (FCR) increased in the order ROSS-AL < ROSS-LIM < SASSO-AL ($P < 0.001$). At 62 days of age and across sexes, ROSS-AL and ROSS-LIM had a lower FCR than SASSO-AL ($P < 0.001$). Within each production system and age, males had a higher ADFI and ADG than females ($P < 0.01$). At 40 days, there was no effect of sex on FCR. At 62 days, male ROSS-AL broilers had a lower FCR than females ($P < 0.001$), whereas there was no effect of sex for ROSS-LIM and SASSO-AL.

Effect of muscle and age on HCD content

The mean HCD content of breast and thigh muscles across treatments is displayed in Fig. 1. Anserine is the main HCD with 2.5- and 1.9-fold higher contents across treatments for breast and thigh muscles respectively compared to carnosine. Across treatments, breast muscle contained levels of anserine and carnosine that were 2.2- and 2.8-fold higher, respectively, than thigh muscle ($P < 0.001$). Slaughter age had a contrasting effect in the two muscles. In breast, a lower anserine content was found at 62 days than at 40 days (30.8 ± 6.1 and 33.1 ± 6.0 mmol kg^{-1} respectively, $P < 0.05$), whereas there was no age effect for carnosine (overall mean value 12.8 ± 7.4 mmol kg^{-1}, $P > 0.05$). In thigh muscle, the contents of both anserine ($P < 0.001$) and carnosine ($P < 0.05$) were higher at 62 days compared to 40 days (anserine: 13.3 ± 1.9 and 9.3 ± 1.4 mmol kg^{-1} respectively; carnosine: 6.1 ± 1.5 and 5.5 ± 1.4 mmol kg^{-1}, respectively).

Effect of production system on HCD content

The effect of production system on the muscle HCD content is illustrated in Fig. 2. Across sexes and slaughter ages, the breast anserine content increased ($P < 0.05$) in the order ROSS-AL < ROSS-LIM < SASSO-AL (26.1 ± 4.5, 33.3 ± 3.6 and

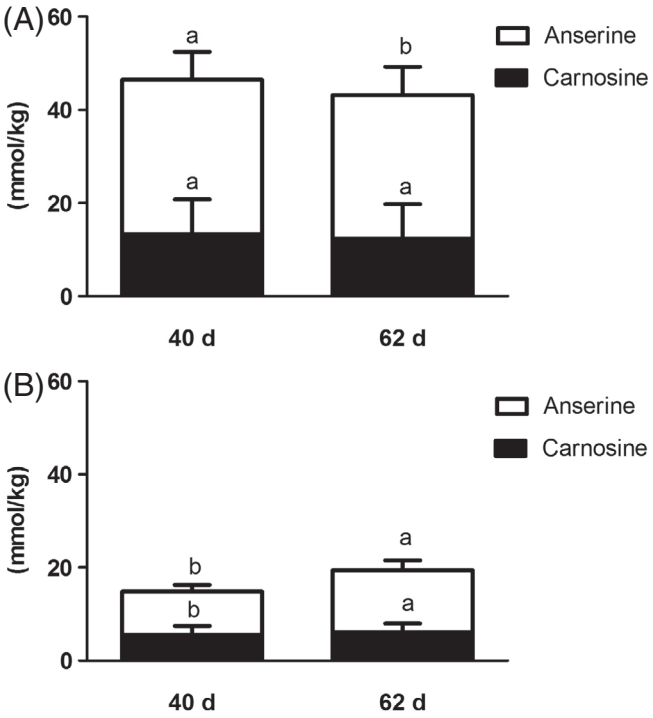

Figure 1. Effect of age at slaughter (40 days or 62 days) on the HCD content (mmol kg^{-1}) of breast (A) and thigh (B) muscles in broilers (across production systems and sexes). Values are means based on 47 and 48 birds for the 40 day and 62 day age group respectively. Means with different superscripts are significantly different ($P < 0.05$). Error bars represent standard deviations.

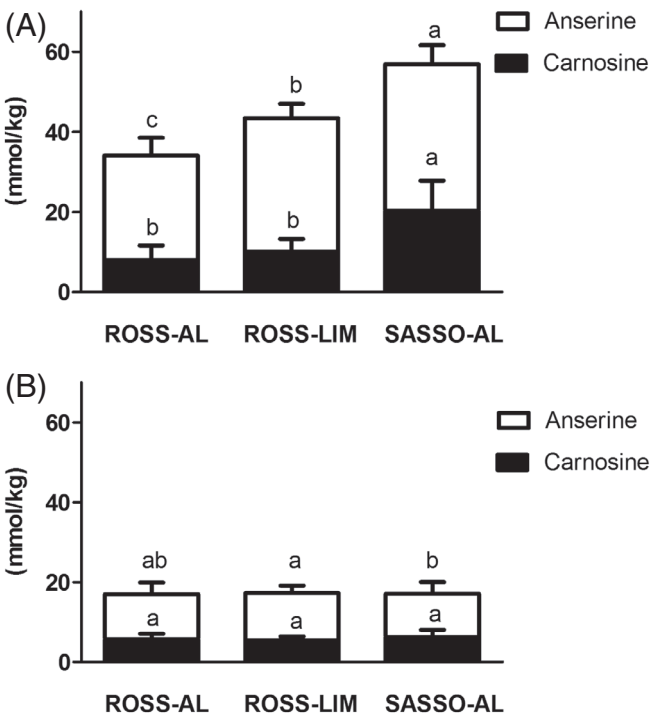

Figure 2. Effect of production system (ROSS-AL, ROSS-LIM and SASSO-AL) on the HCD content (mmol kg^{-1}) in breast (A) and thigh (B) muscles of broilers (across age groups and sexes). Values are means based on 32 birds for ROSS-AL and SASSO-AL, and 31 birds for ROSS-LIM. Means with different superscripts are significantly different ($P < 0.05$). Error bars represent standard deviations.

36.6 ± 4.7 mmol kg^{-1}, respectively). The breast carnosine content was higher ($P < 0.05$) for SASSO-AL than the two ROSS production systems (20.3 ± 7.5, 8.0 ± 3.6 and 10.1 ± 3.2 mmol kg^{-1} for SASSO-AL, ROSS-AL, and ROSS-LIM, respectively). In thigh, the production system effect was less marked. There was only a small but significant difference ($P < 0.05$) between ROSS-LIM and SASSO-AL for the anserine content (11.8 ± 1.8 and 10.8 ± 3.0 mmol kg^{-1} respectively), with an intermediate value for ROSS-AL (11.3 ± 2.9 mmol kg^{-1}).

Effect of sex and interaction effects on HCD content

Table 3 gives the mean values for the HCD content in breast and thigh muscles for the individual production system × age × sex treatments. The effects of production system and age were mentioned above. In breast, carnosine and total HCD content was higher in females than males (carnosine: 14.1 ± 8.3 and 11.6 ± 6.2 mmol kg^{-1} respectively; HCD: 46.4 ± 11.0 and 43.3 ± 11.8 mmol kg^{-1} respectively) ($P < 0.05$) – however, with a significant age × sex interaction effect for total HCD ($P < 0.05$). Females had higher anserine and total HCD values at 62 days but not at 40 days. In thigh, sex had no effect on the HCD content, and there was also no age × sex effect ($P > 0.05$). The production system × age and production system × sex interaction effect was not significant ($P > 0.05$), except for a small but significant ($P < 0.05$) production system × age interaction on the anserine content in thigh muscles.

Market meat comparison for HCD content

ROSS-AL birds slaughtered at 40 days correspond to the typical fast-growing broilers from intensive production systems, whereas SASSO-AL birds slaughtered at 62 days correspond to slow-growing chickens in alternative systems, both yielding birds of approximately 2.5 kg BW at slaughter. The HCD content in these market meats is displayed in Fig. 3. In both breast and thigh muscles, 62-day SASSO-AL had higher content of anserine ($P < 0.001$) and carnosine ($P < 0.001$ in breast and $P < 0.05$ in thigh) compared to 40-day-old ROSS-AL. The HCD content in breast muscle of SASSO-AL 62-day-old birds was more than threefold higher than thigh muscle of ROSS-AL 40-day-old birds ($P < 0.001$).

DISCUSSION

This study explored several factors that might contribute to variation in the muscle HCD content of market broilers. It is well established that anserine is the predominant HCD in broiler skeletal muscles;[5] this was confirmed in the present study. The large differences in HCD content between breast and thigh muscles in the present study are also in line with the literature, and they can be explained by differences in fiber type composition and metabolism.[5,12,13] The *pectoralis* muscle constitutes about half of the breast meat in chicken and it is almost exclusively composed of type IIb or white fibers.[14] In contrast, the *biceps femoris* muscle in thigh meat is composed of type I, IIa, and IIb fibers.[15] Type IIb fibers are fast-fatiguing and are used for brief bursts of activity. They have a more glycolytic metabolism and a stronger tendency to create an acidic cell environment, hence requiring larger amounts of dipeptides as physico-chemical buffer against protons.[16,17] This may explain why HCD synthesis during growth and development is less prioritized in thigh compared to breast muscles.

Less is known about how production systems contribute to variability in muscle HCD contents. A noteworthy difference in breast

Table 3. Mean values for HCD content (mmol kg^{-1}) in breast and thigh muscles according to age, production system, and sex

Age (A)	Production system (P)	Sex (S)	BW (kg)	Breast			Thigh		
				Anserine	Carnosine	Total HCD	Anserine	Carnosine	Total HCD
40 days	ROSS-AL	Male	2.59	27.5	8.60	36.1	9.00	5.44	14.4
		Female	2.49	26.3	7.70	34.0	8.99	5.30	14.3
	ROSS-LIM	Male	1.98	35.4	10.6	46.1	10.2	5.46	15.6
		Female	1.48	35.0	11.3	46.3	10.6	5.16	15.8
	SASSO-AL	Male	1.43	38.6	19.5	58.1	8.32	5.48	13.8
		Female	1.29	36.1	22.3	58.4	8.62	6.40	15.0
62 days	ROSS-AL	Male	5.35	22.2	5.28	27.5	13.4	5.58	19.0
		Female	4.24	28.3	10.6	38.9	13.9	6.54	20.4
	ROSS-LIM	Male	3.43	30.8	8.33	39.2	12.8	5.58	18.4
		Female	2.79	32.0	10.2	42.2	13.6	5.96	19.6
	SASSO-AL	Male	2.80	35.4	17.3	52.7	13.1	6.06	19.2
		Female	2.24	36.3	22.3	58.6	13.0	7.08	20.1
RMSE			0.288	4.04	5.07	5.89	1.65	1.40	1.96
P-value									
Age			<0.001	0.006	0.275	0.005	<0.001	0.044	<0.001
Production system			<0.001	<0.001	<0.001	<0.001	0.057	0.113	0.805
Sex			<0.001	0.417	0.022	0.012	0.393	0.099	0.059
Age × Production system			<0.001	0.426	0.836	0.406	0.045	0.931	0.061
Age × Sex			<0.001	0.015	0.127	0.003	0.846	0.287	0.356
Production system × Sex			0.174	0.294	0.575	0.585	0.788	0.412	0.897
Age × Production system × Sex			0.012	0.375	0.590	0.188	0.833	0.771	0.602

Values are means based on eight birds (except $n = 7$ for the female ROSS-LIM group slaughtered at 40 days).
RMSE, root mean square error; ROSS-AL, Ross 308 fed *ad libitum*; ROSS-LIM, Ross 308 fed limited; SASSO-AL, Sasso T451 fed *ad libitum*.

anserine and carnosine content and in thigh anserine content was observed in the present study between the slow-growing SASSO strain and the fast-growing ROSS strain. The large difference in growth rate between these two strains is accompanied by a series of metabolic differences,[18] but the size of the difference in muscle HCD content seems to be larger than for other metabolites or characteristics. It is well known that selection for fast growth and high meat yield promotes transformation of type I (red) to type II (white) muscle fibers, and is accompanied by a larger muscle fiber diameter.[10] Muscle fiber characteristics were not assessed in the present study but, according to the literature, the slow-growing SASSO strain is expected to be richer in type I muscle fibers.[19] In this context, we found somewhat surprisingly higher contents of anserine and carnosine in the breast of the slow-growing SASSO broilers. We also found an effect of age on the HCD levels in thigh but not in breast muscles. Regardless of the production system, more dipeptides accumulated in thigh muscles of 62-day-old broilers compared to 40-day-old broilers. This suggests that HCDs play a more prominent physiological role in breast compared to thigh muscles from early life.

It is unclear from the literature how selection for fast growth and efficiency in modern broilers in recent decades may have altered basic metabolic processes, e.g. the synthesis of non-proteinogenic dipeptides. Selection for fast growth and high feed efficiency has been very successful and has resulted in large decreases in the number of days and the amount of feed required to reach market slaughter weight.[20] Effects of this selection on important body composition traits (e.g. breast yield, body fatness) are more variable; however, it has in general resulted in leaner animals with higher breast yield, which also depends on the weight of these traits in the composite breeding goals. According to the review by Tallentire *et al.*,[20] there is some indication that higher protein deposition has been achieved through selection for efficient growth by lowering protein breakdown; however, net protein turnover seems not to be affected. In contrast, Dransfield and Sosnicki[18] showed that protein turnover is higher in muscles of slow-growing birds. Differences in protein turnover and in efficiency of feed amino acid utilization between the broiler types in the present study may have resulted in differences in the pool of free L-histidine available for synthesis of HCD. This needs to be further explored. Our findings seem to suggest that, in fast-growing broilers, the available L-histidine is directed with priority towards muscle protein synthesis, leaving less of this amino acid available for the synthesis of HCD. Conversely, in slow-growing chickens, the synthesis of anserine and carnosine may have been favored by a higher availability of L-histidine. It should also be realized that the different broiler types received the same diet, which was formulated according to requirements for fast-growing broilers. This diet may have provided a surplus of L-histidine in the slow-growing chickens, which have a lower protein deposition rate. The inhibitory effect of selection for fast growth on muscle HCD deposition is further supported in the present study by the higher content of anserine in the breast muscle of the group of ROSS birds whose feed was limited compared to the group fed *ad libitum*. In conclusion, the effects of growth rate and genetic constitution on the synthesis and deposition of HCD, in interaction with the dietary supply of precursors, warrant further investigation.

According to this reasoning, free L-histidine would be the rate-limiting precursor for HCD synthesis in broilers, in contrast to horses[21] and humans,[22–24] where β-alanine has been clearly identified as the rate-limiting precursor. Attempts have been made in the past to increase broiler muscle carnosine

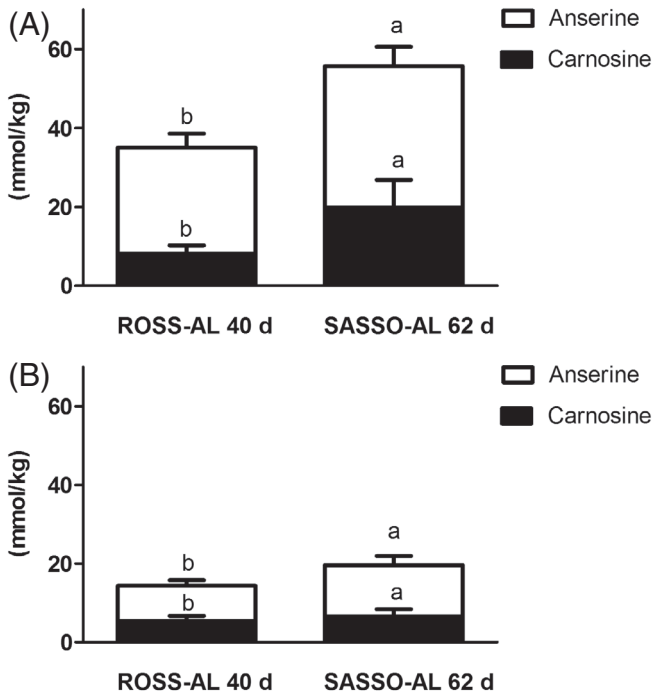

Figure 3. Differences in the HCD content (mmol kg^{-1}) of breast (A) and thigh (B) muscles of broilers slaughtered at a BW of approximately 2.5 kg from typical commercial production systems SASSO-AL at 62 days of age and ROSS-AL at 40 days of age. Values are means based on 16 birds. Means with different superscripts are significantly different ($P < 0.05$). Error bars represent standard deviations.

and anserine concentrations through either L-histidine[25–28] or β-alanine supplementation in the diet.[26,29–33] Feeding broilers a diet low in L-histidine (at 67% of the requirements) resulted in considerably lower anserine levels in muscle, and carnosine was no longer detected.[28] Supplementation with 1 g histidine per kg feed resulted in a 64% increase in carnosine and a 10% increase in anserine in broiler muscles.[25] Dietary supplementation with 3 g histidine per kg feed increased the carnosine content in broiler breast by 18%,[27] whereas supplementation with 5 g histidine per kg feed induced an increase in muscle carnosine content of 26%.[26] Divergent results are instead available in the literature on the effect of β-alanine supplementation on the deposition of HCD in muscles. Five days' supplementation with 22 mmol kg^{-1} β-alanine were effective in increasing the carnosine concentration by 61% in the *pectoralis superficialis* muscle of newly hatched chickens.[29] More recently, an increase in carnosine concentration in the *pectoralis superficialis* muscle after various doses of β-alanine supplementation in different broiler's growing stages has been reported.[31,34] In contrast, older broilers, supplemented for 4 weeks with different β-alanine concentrations, did not show any increase in carnosine or anserine level in muscles.[30]

A number of differences seem to exist in muscles between female and male broilers, with males exhibiting higher muscle fiber number than female ones.[35] Three to five times wider pectoralis muscle fibers have been found in fast-growing male chickens compared to slow-growing chickens.[18] Surprisingly, we found a higher content of carnosine and total HCD in breast from female birds compared to males. A higher content of carnosine in female chickens compared to males in both breast and thigh has been reported before.[5,13] The physiological basis for this sex effect in growing chickens is unclear. The effect of sex on skeletal muscle carnosine content also seems to differ among animal species. In mice, the muscle carnosine content in male rats is higher than in female rats and a positive correlation between carnosine content and testosterone has been reported.[36] Likewise, human males also display higher muscle carnosine concentrations than females.[37] However, it should be kept in mind that prepubertal broilers, as used in the present study, cannot simply be compared with sexually mature animals from other species.

Based on the results of the present study, it seems that HCDs are considerably more abundant in meat from extensive and organic broiler production systems (exemplified by the Sasso T451 strain here) compared to the more widespread intensive broiler production systems, which are based on fast-growing genetic lines (exemplified here by the Ross 308 strain). Within the market meat types, a threefold higher HCD content was found in the breast muscle of SASSO-AL birds compared to the thigh muscle of ROSS-AL birds. The effect on the human supply of HCD and the concomitant possible physiological effects following the consumption of broiler meat from different production systems varying in HCD content warrants further investigation.

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