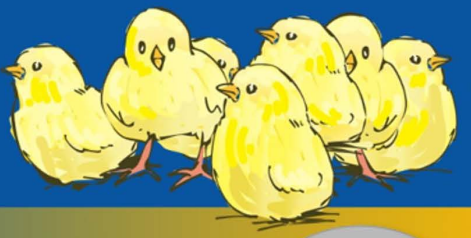

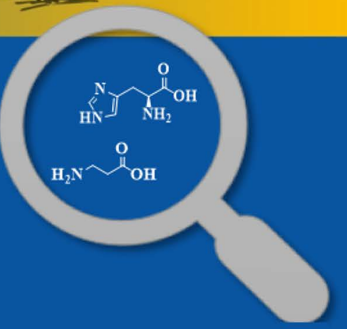

Influence of dietary concentrations of L-histidine, alone or in interaction with β-alanine, on breast meat quality and metabolic status of modern, fast-growing broiler chickens

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Dissertation

Institut für Tierwissenschaften

Influence of dietary concentrations of L-histidine, alone or in interaction with β-alanine, on breast meat quality and metabolic status of modern, fast-growing broiler chickens

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Meinen Eltern und meinem Bruder

"Imagination is more important than knowledge. Knowledge is limited. Imagination encircles the world."

Albert Einstein (1879 – 1955)

English abstract

An increasing demand for poultry meat resulted in optimization of broiler chicken's genetic towards high growth-rates and breast yield in the last decades. This breeding standard led to increased quality problems in breast meat. Various myopathies of the *Pectoralis major* muscle, such as the woody breast syndrome, the appearance of white stripping and spaghetti meat, result in high economic losses for the industry. Histidine-containing dipeptides, which are acting as natural antioxidants, pH-buffer, and metal ion chelator in skeletal muscle tissue, were found to be depleted in affected breast muscles. The aim of this thesis was to evaluate the effect of different histidine concentrations in feed on the concentration of the dipeptides carnosine and anserine, as well as the overall meat quality. Since the second precursor for the relevant dipeptides is β-alanine, the combination was also considered. Two feeding tests were carried out with commercial, fast-growing broiler breeds. In both experiments, the histidine concentration was varied in feed, based on a feed formulated for commercial use, alone or in combination with the addition of β-alanine. The first study, described in Manuscript I, focused on how the concentration of dipeptides in muscle tissue and blood serum changed, on the performance of the broilers and on the overall meat quality at two slaughter ages, with variation in histidine concentrations in the feed, without or in combination with β-alanine. Based on these results, the second study, described in Manuscript II, focused on the effect of histidine on the occurrence of different myopathies. In Manuscript III, the total metabolism was considered using an untargeted metabolomics analysis, and was performed in plasma samples from the study described in Manuscript I. The results of this work suggest that histidine is the limiting factor for the synthesis of carnosine in the skeletal muscle tissue of broilers. A higher concentration of histidine, and also the addition of β-alanine, had only marginal effects on the growth performance of the birds. The overall meat quality was only slightly affected by the concentration of histidine or the addition of β-alanine in feed, without a clear trend. However, the incidence of myopathies was dependent on the concentration of histidine. A moderate increase showed reduced incidences, whereas a higher concentration increased the incidences. The analysis of metabolic status indicated a possible deficit of folate or cobalamin at higher histidine concentration in broiler feed.

Zusammenfassung

Eine steigende Nachfrage nach Geflügelfleisch führte in den letzten Jahrzehnten zu einer Optimierung der Masthähnchengenetik hin zu hohen Wachstumsraten und Brustfleischanteil. Dieser Zuchtstandard führt vermehrt zu Qualitätsproblemen im Brustfleisch. Verschiedene Myopathien des Brustmuskels, wie das Holzbrust-Syndrom, das Auftreten von weißen Streifen und Spaghetti-artigem Fleisch, bescheren der Industrie hohe wirtschaftliche Verluste. Studien zeigten, dass Histidin-haltige Dipeptide, die als natürliche Antioxidantien, pH-Puffer und Metallionen-Chelatoren im Skelettmuskel wirken, in den betroffenen Geweben geringer konzentriert vorlagen. Ziel dieser Arbeit war es daher, die Wirkung unterschiedlicher Histidinkonzentrationen im Futter auf die Konzentration der Dipeptide Carnosin und Anserin sowie die Auswirkung auf die Fleischqualität und den metabolischen Status zu bewerten. Da der zweite Vorläufer für die relevanten Dipeptide β-Alanin ist, wurde auch die Kombination von Histidin mit β-Alanin untersucht. Es wurden zwei Fütterungsversuche mit kommerziellen, schnell wachsenden Masthähnchenrassen durchgeführt. In beiden Versuchen wurde auf der Basis eines für den kommerziell Gebrauch formulierten Futters die Histidinkonzentration variiert in Kombination ohne oder mit der Zugabe von β-Alanin. Die erste Studie, die in Manuskript I beschrieben wird, konzentrierte sich auf die Frage, wie sich die Konzentration der Dipeptide im Muskelgewebe und Blutserum verändert, auf das Wachstum der Masthähnchen, sowie auf die Gesamtfleischqualität an zwei Schlachtaltern, bei Variation der Histidinkonzentrationen im Futter, mit oder ohne β-Alanin. Basierend auf diesen Ergebnissen konzentrierte sich die zweite Studie, die in Manuskript II beschrieben wird, auf die Wirkung von Histidin auf das Auftreten der verschiedenen Myopathien. In Manuskript III wurde der Gesamtstoffwechsel mittels einer nicht-gezielten Metabolomik-Analyse von Plasmaproben betrachtet, die in der ersten Studie genommen wurden. Die Ergebnisse dieser Arbeit lassen darauf schließen, dass Histidin den limitierenden Faktor für die Synthese von Carnosin im Skeletmuskelgewebe von Masthähnchen darstellt. Eine höhere Konzentration von Histidin, und auch die Zugabe von β-Alanin, hatte nur wenig Einfluss auf die Wachstumsleistung der Tiere. Die Gesamtfleischqualität wurde durch die Konzentration von Histidin oder die Zugabe von β-Alanin im Futter nur gering beeinflusst, ohne eine klare Tendenz. Aber, die Inzidenz von Myopathien war beeinflusst von der Histidinkonzentration. Ein moderater Anstieg zeigte eine geringere Inzidenz, während eine höhere Konzentration höhere Inzidenzen aufwies. Die Analyse des metabolischen Status ergab zudem einen Hinweis auf ein mögliches Defizit von Folat oder Cobalamin bei höherer Histidinkonzentration im Futter von Masthähnchen.

Abbreviations

1MHis	1-methylhistidine
3MHis	3-methylhistidine
AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
ADP	Adenosine diphosphate
ALDH	Aldehyde dehydrogenase
AMDHD1	Imidazolonepropionase
AMEn	Apparent metabolizable energy, nitrogen-corrected
ANOVA	Analysis of variance
Ans	Anserine
AOC	Primary amine oxidase
Arg	Arginine
Asp	Aspartate
ATP	Adenosine triphosphate
BA_CON	SID His:Lys ratio of 0.44 + 0.5% supplemented β-alanine
BA_HIS1	SID His:Lys ratio of 0.54 + 0.5% supplemented β-alanine
BA_HIS2	SID His:Lys ratio of 0.64 + 0.5% supplemented β-alanine
BMM	Breast muscle myopathies
Car	Carnosine
CARNMT	Carnosine methyltransferase
CARNS	Carnosine synthase
CIE	Commission internationale de l’éclairage
CN	Carnosinases
C_{norm}	Normalized contingency coefficients
CoA	Coenzyme A
CON	SID His:Lys ratio of 0.44
CP	Crude protein
Cys	Cysteine
DAO	Diamine oxidase
DFD	Dark, firm, dry
DL	Drip loss
DNA	Deoxyribonucleic acid

DPM	Deep pectoral myopathy
DPYD	Dihydropyrimidine dehydrogenase
DPYS	Dihydropyrimidinase
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
FCR	Feed conversion ratio
Figlu	Formiminoglutamate
FTCD	Formimidoyltransferase cycloamidase
GAA	Guanidine acetic acid
GABA-T	Methylpropionate transaminase
GADL	Glutamate decarboxylase
Glu	Glutamate
Gly	Glycine
HAL	Histidine-ammonia lyase
HCD	Histidine-containing dipeptide
HDC	Histidine decarboxylase
His	Histidine
HIS1	SID His:Lys ratio of 0.54
HIS2	SID His:Lys ratio of 0.64
HIS41	SID His:Lys ratio of 0.41
HIS45	SID His:Lys ratios of 0.45
HIS49	SID His:Lys ratio of 0.49
HIS53	SID His:Lys ratio of 0.53
HIS57	SID His:Lys ratio of 0.57
HMDB	Human Metabolon Database
HNMT	Histamine-*N*-methyltransferase
HTA	Histidine transaminase
Ile	Isoleucine
Int.	Interaction
IP	Isoelectric point
iPCA	Interactive Principal Component Analysis
IU	International unit
IUPAC	International Union of Pure and Applied Chemistry
KEGG	Kyoto Encyclopedia of Genes and Genomes

L* a* b*	Color code: Lightness, redness, yellowness
Leu	Leucine
Lys	Lysine
MDA	Malondialdehyde (equivalents)
Met	Methionine
MQ	Meat quality
MS	Mass spectrometry
MSDH	Malonate semialdehyde dehydrogenase
NAD^+ / NADH	Nicotinamide adenine dinucleotide
$NADP^+$ / NADPH	Nicotinamide adenine dinucleotide phosphate
nd	Not detectable due to detection limit / by analytical method
NMR	Nuclear magnetic resonance
Oph	Ophidine
PC	Principal component
PSE	Pale, soft, exudative
RNA	Ribonucleic acid
ROS	Reactive-oxygen species
SAH	*S*-Adenosyl-homocysteine
SAM	*S*-adenosylmethionine
SEM	Standard error of the mean
SI	Sensory index
SID	Standard ileal digestible
SM	Spaghetti meat
SR	Sarcoplasmic reticulum
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TCA cycle	Tricarboxylic acid cycle
THF	Tetrahydrofolate
Thr	Threonine
Trp	Tryptophane
UHPLC	Ultra-high performance liquid chromatography
UPB	β-Ureidopropionase
UPLC	Ultra-Performance Liquid Chromatography

UROC1	Urocanate hydratase
US	United States
USDA	United States Department of Agriculture
Val	Valine
WB	Woody breast
WHC	Water-holding capacity
WLC	Water loss during cooking
WLT	Water loss during thawing
WS	White striping
α-KG	α-Ketoglutarate
βA	β-Alanine

Table of content

Introduction

1. Broiler production

The global meat demand and per capita meat consumption increased steadily during the last decades. For the food industry, poultry is one of the most important meat sources and the most consumed meat worldwide (Figure 1). In 2018, the highest poultry production was achieved in the US with 22.3 million t, followed by China (20.1 million t) and Brazil (15.5 million t), whereas the production in the European Union was 14.5 million t in the same year (Ritchie and Roser, 2017). Poultry includes all birds used commercially to produce meat, such as chickens, turkeys, ducks, and geese, but also more exotic forms like quails, pheasants, pigeons, ostriches, and emus (Mozdziak, 2019). Chicken represents the largest part of the production. For meat production, 69 billion chickens were slaughtered in 2018 worldwide, and thus chickens are the most common livestock in the world (Ritchie and Roser, 2017). The industrial production of meat-type chicken and other commercially used birds is highly organized in a complex manner, with key players specialized in different parts of the poultry business, including genetics companies, hatcheries, farms, slaughterhouses, feed producers, animal health companies and many others.

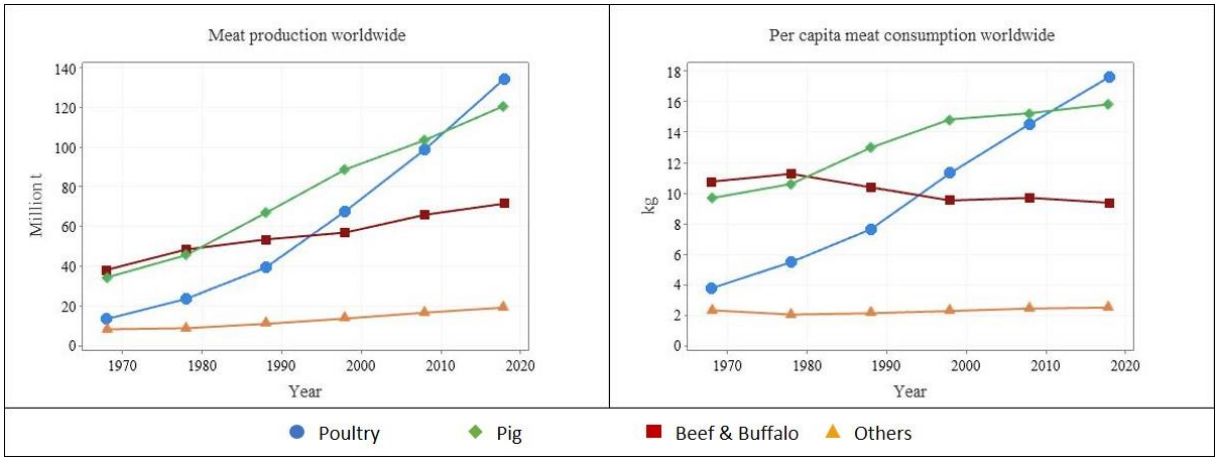

Figure 1: History of meat production by meat type and history of per capita consumption. Poultry includes all domestic meat-type birds. Others includes sheep, goat, horse, camel, and wild game meat. Per capita consumption was calculated out of the world population and the worldwide meat consumption per year. Original data given by Ritchie and Roser (2017) and Roser et al. (2013).

Modern broiler chickens have been highly selected for performance parameters such as growth rate, as a result of the increased demand and pressures on market prices. Since 1940s, different

genetic companies have focused on the genetic selection of breeds based on growth rate, i.e., to reach given body weights at lower slaughter ages. Some more recent traits were also set as breast yield, livability, and feed conversion ratio (**FCR**), as important cost factors (Havenstein et al., 2003b; Tallentire et al., 2016; Tavárez and Solis de los Santos, 2016) (Figure 2). The genetic selection was further improved by the developments in molecular genetic principles, which began in the 1950s, and the complete sequencing of the chicken genome (Burt, 2005; Tixier-Boichard et al., 2012).

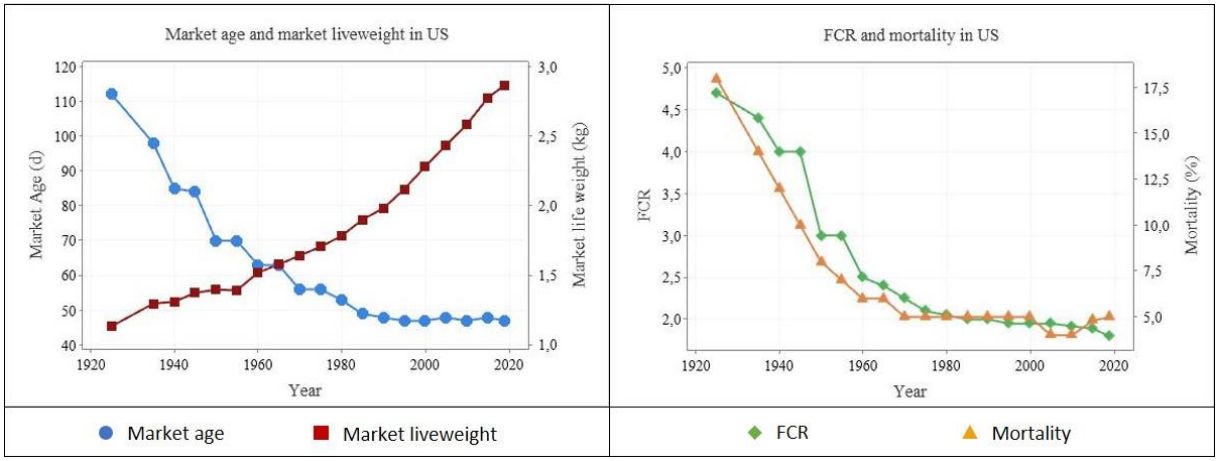

Figure 2: Development history average liveweight, market age, feed conversion ratio and mortality of broiler chickens in the US. Feed conversion ratio and mortality are based on the given slaughter ages in the graphic. FCR: Feed conversion ratio. Data from the National Chicken Council (2019). The original data were provided by the United States Department of Agriculture (USDA).

Changes of performance parameters were reported by Havenstein et al. (2003b) and Zuidhof et al. (2014) by comparing heritage breeds from 1957 with the modern broiler breed Ross 308. It was pointed out that the body weight was increased by over 460% after 56 d of age in modern fast-growing chickens. Today, in the Western world, breast fillet and processed products there from are the best marketable part of the carcass (Tavárez and Solis de los Santos, 2016). Therefore, the main difference in body weight composition was seen in breast yield, where the increased portion of the *Pectoralis major* muscle has the largest influence. Havenstein et al. (2003a) reported that the share of the *Pectoralis major* muscle of the whole carcass was 16.9% for the fast-growing and 8.4% for heritage breed at 57 d of age, whereas the proportion of the *Pectoralis minor* muscle was less changed. In contrast, the FCR decreased by 33 to 42% for the Ross 308 strain after 42 d of age. It was speculated that the energy partition between maintenance and growth changed. By modern breeds the energy used for metabolic

maintenance is reduced, thus allowing to reach these high growth rate and low FCR. Zuidhof et al. (2014) indicated a lower gut weight in proportion to total body weight and less abdominal fat in modern broiler chicken breeds as part of the explanation for lesser energy used for maintenance. In contrast to the data in Figure 2, Havenstein et al. (2003b) reported a higher mortality of 3.6% in the modern breed compared to 1.8% in the historical breed. The increased growth rate and the disproportionate breast muscle growth may explain the greater mortality in the modern breeds. This illustrates the consequences of the genetic selections in terms of performance optimization of broiler chickens. It should be mentioned that the genetic potential of the birds, especially regarding FCR, can only be reached with a good understanding of the nutritional needs of broiler chickens and an optimal feed formulation, as well as good management practices (Havenstein et al., 2003b). The excellent FCR is of particular importance for the efficient use of feed resources and thus land use, and the reduced environmental impact due to lesser nutrient losses. The importance of understanding the nutritional needs regarding feed and cost optimization is illustrated by Liebig's law of the minimum (Figure 3).

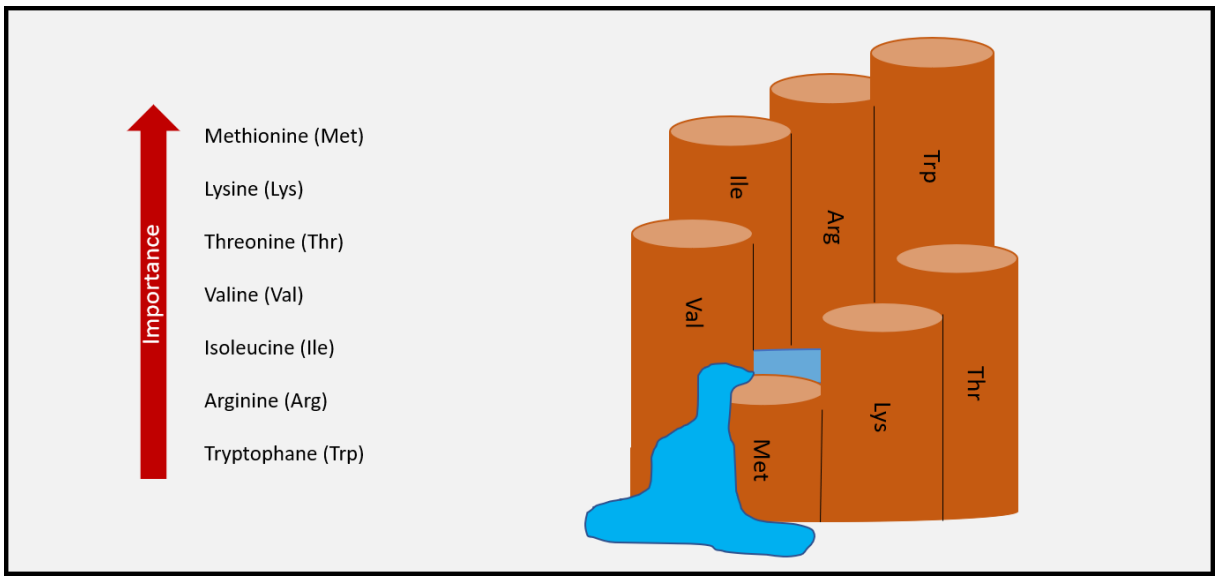

Figure 3: Illustration of the Liebig's law of the minimum for the strength of the deficiency (limited amino acids) based on a corn/soybean-based diet for broiler chickens.

Broiler meat has diverse benefits for human consumption explaining the high demand. Chicken meat is accepted by most cultures and religions (Petracci et al., 2019). Due to the high growth rate and low FCR, it is cheap and can be provided in high quantities to the market. Moreover, the low FCR and therefore optimal feed usage led to a low environmental footprint of chicken production (Tallentire et al., 2016) when compared to other livestock. Indeed, the CO_2

emissions of chicken meat (2.6 CO_2 equivalents / kg) are lower than beef and pig meat (25.0 and 4.5 CO_2 equivalents / kg, respectively) (Tondeur and Simons, 2019). For human nutrition, chicken meat shows different nutritional benefits. White chicken meat is considered healthier than red meat, because of its lower fat content, higher amounts of unsaturated fatty acids and essential polyunsaturated fatty acids, as well as low cholesterol content (Farrell, 2012; Tondeur and Simons, 2019). Moreover, for the consumer, poultry meat is tasty, with a mild natural flavor and is easy to prepare in many dishes (Tondeur and Simons, 2019).

The highly optimized poultry production system with the selection of fast-growing chicken breeds led also to different negative side effects. Tavárez and Solis de los Santos (2016) reported that the selection could have led to metabolic imbalance, which results in a decreased reproduction efficiency in breeder flocks, as well as an increase of health-related problems and meat quality abnormalities. Health issues can impact welfare and overall performance, whereas meat quality issues, like breast myopathies and stress-related metabolic changes, are negatively impacting processing parameters and buying decision of the consumer, cause economic losses. As a consequence, breeding schemes are changing due to these negative economic impacts but also due to the increasing awareness of animal welfare and environmental sustainability. Especially in Europe, welfare and quality topics push the industry towards the usage of slow-growing chicken breeds. But it has to be noted, that the high demand for poultry meat cannot be yet achieved with those breeding lines and the usage of fast-growing broilers will be still a big part of the industry.

2. The skeletal muscle

In order to understand meat quality (**MQ**) and quality abnormalities in breast meat of modern-broiler breeds, the normal properties of muscle tissue, especially the skeletal muscle tissue, need to be described. Muscles can be found in animals in the taxon *Eumetazoa* and are defined as contractile tissue which allows movement by contraction and relaxing (Burton, 2008; Spomedial, 2003). Originating from the mesoderm, muscle tissue can be found over the whole body and is classified in three categories: skeletal muscle, smooth muscle, and cardiac muscle. Due to the importance of the skeletal muscle tissue as meat for human consumption, the following part will focus on this muscle type.

The overall structure of the skeletal muscle is made of muscle fiber bundles, called muscle fascicles, and each fascicle contains numerous muscle fibers, which are the single muscle cells or myocytes. Each structural unit is arranged in rows and surrounded by connective tissue: epimysium (whole muscle), perimysium (fascicle) and endomysium (fibers). The muscle tissue is crossed by blood vessels and motoric neurons. Muscle movements are performed by contraction and relaxation of each single fiber. Muscle fibers are large cells, with diameter up to 100 µm and length up to 12 cm (Feher, 2017). The cells are surrounded by an electrically inducible cell membrane, called sarcolemma, where the motor neurons provide an action potential to generate contraction. The cell itself consists of many myofibrils, which are arranged along the whole muscle fiber and bound to the sarcolemma at both ends to transfer a contraction to the entire cell. The muscle function requires ATP, which is provided by numerous mitochondria in the sarcoplasm (which is the cytoplasm of the muscle cell). Movement is controlled by the Ca^{2+} concentration in the sarcoplasm.

The skeletal muscle fibers can be classified in three categories according to their contraction speed and energy metabolism (Table 1). A skeletal muscle can contain all fiber types, and their proportion depends on the muscle function (Betts, 2017; Ono et al., 1993). All fibers have the ability to switch between the categories in response to internal and external environmental influences (van Wessel et al., 2010). It must be mentioned that the content of myoglobin determines the classification of meat; muscles with mainly type I and IIA fibers are described as red mead and muscles with mainly type IIB fibers as white meat. The *Pectoralis major* muscle of chicken is composed of type IIB fibers and therefore belongs to the white meat category (Ono et al., 1993).

Table 1: Properties of the different muscle fiber types.

Parameter	Fiber type		
	Type I	**Type IIA**	**Type IIB**
Movements	Persistent, with low tension	High tension and contraction speed	Very high tension and contraction speed
Contraction speed	Slow	Fast	Very fast
Energy metabolism	Aerobic	Primary aerobic, but also glycolytic	Primary glycolytic
Mitochondria content	High	High	Low
Myoglobin content	High	Medium	Low
Glycogen content	Low	Moderate	High
Creatine phosphate content	Low	High	High
Capillary density	High	Medium	Low
Fatigue	Slow	Medium	Fast
Color	Red	Red	White

The table is based on Berg (2013), Betts (2017), Gohil et al. (2013), and Mann et al. (2010)

Another important point, related to MQ and quality issues of broiler chicken breast meat, is the development of the skeletal muscle. It is well established that the total number of muscle cells is already defined in the embryo. In this state, the myoblasts (muscle cell precursors) generated by hyperplasia from mesodermal stem cells, are able to fuse to form the final number of myofibers (Fujimaki et al., 2013). Muscle growth after hatching is via hypertrophy, i.e., an increase in fiber diameter or an enlargement of the fiber (Halevy et al., 2006; Ono et al., 1993; Velleman, 2007). An increase in diameter is related to the growth of myofibrils or sarcoplasm, whereas enlargement means an increase in the number of sarcomeres (Ono et al., 1993). In this process, the myogenic stem cells, called satellite cells, are providing nuclei and therefore DNA and capacity for protein synthesis by fusion with an existing myofiber (Seale et al., 2001; Velleman, 2007). Satellite cells are arranged between the basal lamina and sarcolemma of the myofibers, where they stay quiescent until muscle regeneration is required (Fujimaki et al., 2013; Valentine, 2017). It must be noted that they can also act as precursors of other cell types, like osteocytes, adipocytes, or neurons, depending on the environmental signals and are able to show fibroblast properties (Fujimaki et al., 2013; Relaix et al., 2021; Seale et al., 2001). The growth of skeletal muscles after hatching through hypertrophy, the main affiliation of the fibers to fiber type IIB, and the heterogenic potential of satellite cells are critical points for the development of MQ abnormalities in breast muscles of fast-growing chicken breeds.

2.1. The transformation of muscle tissue into meat

In order to fully understand MQ, the development of muscle tissue into meat and the underlaying metabolic changes, which take place *post-mortem*, must be understood. In commercial slaughterhouses, broiler chickens are killed after electrical or gas stunning by neck cutting to bleed out which stops the supply of O_2 and the removal of metabolic waste of the muscle cells by an intact blood stream. The drop of O_2 leads to an anaerobic glycolysis in the muscle cell in order to produce ATP. The coenzyme NAD^+, needed for this process, is recovered by the formation of lactate out of the end product pyruvate, which is called the lactic acid fermentation. This process leads to an intramuscular accumulation of lactate and a drop in pH. The ATP obtained by this process is needed for normal muscle function: to maintain the polarization of the sarcoplasm by the sodium-potassium pump, to maintain a low Ca^{2+} level by a Ca^{2+} ATPase and to release myosin from the actin binding side (Toyoshima, 2009; Wise and Shadmehr, 2002). In muscle, glycogen is used as a source of glucose-1-phosphate, an intermediate of glycolysis, to produce ATP. The amount of glycogen stored in the muscle cell directly affects the drop of pH. Another source of ATP is creatine phosphate. Creatine is built in the liver, transported to muscle mitochondria, and phosphorylated. The phosphate of creatine phosphate can be transferred to ADP by a creatine kinase in order to restore ATP. The content of muscle glycogen and creatine phosphate differs between muscle fiber types and is higher in fast-switch fibers of type IIA and IIB (Table 1) (Gohil et al., 2013). Therefore , the depletion of ATP takes more time in these cells compared to type I cells and more lactate is produced due to a higher glycogen concentration (Choe et al., 2008). The lack of ATP finally leads to increased Ca^{2+} concentration and a permanent muscle contraction, called *rigor mortis*. The *post-mortem* proteolysis of myofibers and pH- and temperature-related protein denaturation finally cause a softening of the muscle afterwards (Koohmaraie, 1992; van Laack and Lane, 2000; Warner et al., 1997).

3. Meat quality

The term MQ is a wide-ranging term, which take into account different characteristics. The importance of these characteristics differs along the value chain. In general, MQ can be defined with all parameters describing individual meat properties, influences on processing parameters and the customer's acceptance of a product (Mir et al., 2017; Tondeur and Simons, 2019). Meat properties can be described by physical, biochemical, microbial, technical, hygiene, safety, sensorial and nutritional characteristics and even if the fundament of MQ is laid in the first parts of the value chain, like breeding and genetics, hatching, feeding, and farming, the quality becomes highly important from slaughter to consumption (Adzitey and Huda, 2011). Moreover, the importance of MQ characteristics differs between processors and consumers. For meat processing, functional properties, visual appearance of the carcass, bird's health and hygiene can be mentioned as the most important characteristics. Functional properties of meat are measurable and include pH, water-holding capacity (**WHC**) parameters (e.g., drip loss, cooking loss, freezing loss, marinade uptake), nutritional composition (e.g., content of fat, collagen), shelf-life, pathogen load, texture characteristics (e.g., share force, cohesiveness), protein solubility and fat-binding capacity. In contrast, for consumers aspects like nutritional composition, sensory perception (e.g., texture, taste, odor, visual appearance) and retail criteria (e.g., shelf-life, visual appearance, as well as costs and welfare labeling), can be seen as the most important ones, before and after purchase (Damez and Clerjon, 2012; Mir et al., 2017). In this thesis the main focus was on functional properties, visual appearance, nutritional characteristics as well as sensory and retail criteria.

The implementation of MQ is complex and depends on many interacting factors. For instance, genetic, hatching and farming conditions impact performance and related MQ issues. The composition of feed can affect pigmentation and nutritional value. The handling of the birds prior to slaughter can result in hematomas, which has an impact on the visual appearance. Pre-slaughter stress affects pH, water-binding capacity, color, and texture of the meat. The process of slaughter is also related to stress and electrical stunning can cause bleeding into the tissue (Mir et al., 2017; Tondeur and Simons, 2019). The handling of the carcass, like cooling time, affects the biochemical processes of a skeletal muscle to become meat, which is one major step influencing different MQ parameters like pH, WHC, shelf-life and sensorial aspects. The color of the meat depends on the protein denaturation and oxidation of myoglobin after slaughter. The final preparation of meat has a major impact on the sensorial properties. A main factor is the temperature of cooking and the nutritional content. It is described that a higher content of collagen leads to softer meat during cooking due to solubilization, whereas denaturation of

myofibril proteins leads to tougher meat. The denaturation of proteins during heating also affects WHC, which is increased with higher temperature (Hong and Lee, 2012; Murphy and Marks, 2000). Moreover, flavor is affected by different products of the Maillard reaction, as degradation of lipids and Maillard-lipid interactions depend on the cooking temperature and initial composition of nutrients (Jayasena et al., 2013).

Different quality issues, mainly impacting the breast meat as most valuable product for the industry, are described for modern broiler breeds in the literature and also by producers. The MQ abnormalities, which can be seen as a result of pre-slaughter stress and high growth rates, are presented in the next sections.

3.1. Meat quality abnormalities related to pre-slaughter stress

Some MQ abnormalities are related to pre-slaughter stress. These conditions are known as pale, soft, exudative (**PSE**) and dark, firm, dry (**DFD**) meat and can be observed in all species of livestock (Adzitey and Huda, 2011). The phenotype of these quality abnormalities shows important differences in meat pH, color, texture, WHC, and shelf-life compared to normal meat. The difference in color of PSE and DFD meat compared to normal meat can be seen in Figure 4.

These defects, finally seen in meat products, are largely depending on the time period during which livestock is exposed to stress conditions. PSE occurs after acute stress directly before slaughter (Petracci et al., 2004). The acute stress results in an increased metabolic activity, heat, and lactate production. After slaughter, lactate cannot be removed from the tissues due to the lack of blood circulation. The fast pH decline while the carcass temperature is still high results in a lower pH than normal and an increased protein denaturation. Ziober et al. (2010) reported an ultimate pH of broiler breast meat affected by PSE conditions of pH $\leq$ 5.7 and a pH between 5.8 and 6.1 for normal meat. The decrease in pH and increase in protein denaturation causes the typical characteristics of PSE meat. The majority of water in muscle is immobilized water (around 80%), due to steric effects and electronic attraction to proteins, mainly myofibrils (Warner, 2017). The denaturation of protein reduces the amount of immobilized water and increases free water in muscle cells. Moreover, the ability of muscle proteins to bind water is related to the net charge of the proteins. The lowest net charge is reached at the isoelectric point (**IP**) and in chicken breast, the IP is reached at around pH 5.5 (Cercel et al., 2015). Therefore, the water-binding capacity in PSE meat is lowered, resulting in an exudative condition. The increased loss of water also lowers the total weight of the meat. A high metabolic activity results in higher protease activity and protein denaturation which increases the softness of affected

fillets. The pale color is the result of a changed light scattering effect due to the protein denaturation and higher content of free intercellular water.

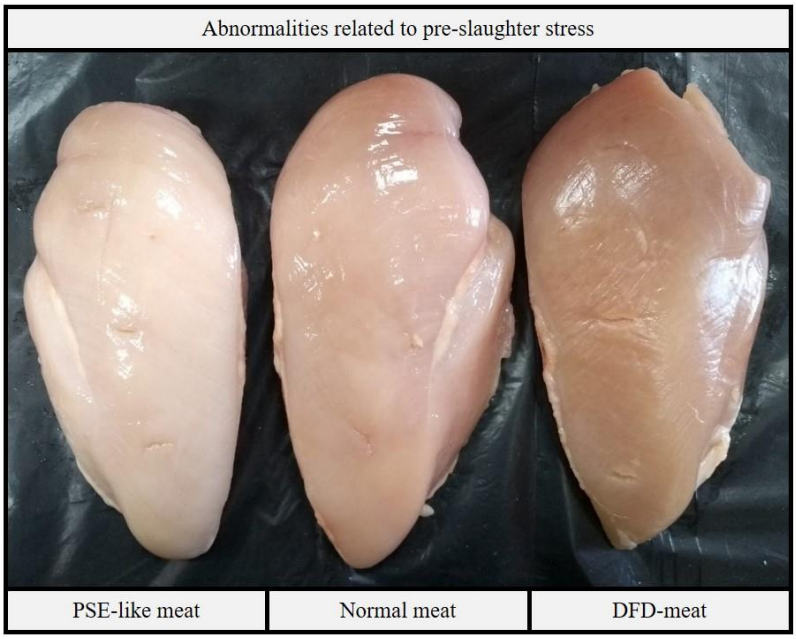

Figure 4: Meat abnormalities as effect of pre-slaughter stress in chicken breast meat. PSE: Pale, soft, exudative; DFD: Dark, firm, dry.

DFD meat is a result of chronic or long-term stress, like long transportation distances, long periods of feed deprivation, or high stocking density during transport (Adzitey and Huda, 2011). This type of stress condition leads to a depletion of stored glycogen in muscle and liver before slaughter. Therefore, less glycogen is available for the *post-mortem*, anaerobic lactic acid production, which results in higher pH values than normal (Adzitey and Huda, 2011). Ziober et al. (2010) reported an ultimate pH of ≥ 6.2 in DFD affected chicken breast meat. The ability of muscle proteins to hold water is increased in DFD meat, because of a higher net charge and decreased degradation of proteins. This results in dry and tough or firm meat. The dark color of the meat is caused by the higher ability of the muscle to absorb light and less light scattering. Therefore, DFD meat is often described as dark cutting for beef and sheep meat. Moreover, high pH lowers the shelf-life of the meat due to a lower inhibition of bacterial growth. In general, muscles consisting mainly of type I fibers are more sensitive to the manifestation of DFD condition and due to their low amount of glycogen. On the contrary, muscles of mainly type IIB fibers containing more glycogen are more sensitive to PSE condition (Adzitey and Huda, 2011).

Many factors are described as having an influence on stress condition which can result in these meat abnormalities. Genetics is one factor which can influence the stress sensitivity in livestock. In pigs, an underlying genetic point-mutation of the Ca^{2+} releasing ryanodine receptor in the membrane of the sarcoplasmic reticulum (**SR**) is known to negatively affect stress-susceptibility by an increased muscle contraction, which leads to a higher occurrence of PSE-meat. However, in poultry no such genetic modification is yet known (Bowker, 2017; Paião et al., 2013). An underlying genetic difference to affect the stress resistance and time for recovering after stress is also expected for the manifestation of DFD meat (Ponnampalam et al., 2017). For poultry, selection towards high growth-rates decreased the resistance towards stress conditions (Adzitey and Huda, 2011). For the manifestation of both defects, PSE and DFD, stressors during farming, transportation and slaughter are the main cause (Adzitey and Huda, 2011). Stressors can be fear, enhanced activity, weather extremes, feed restriction and stocking density. In order to solve these problems and decrease PSE and DFD conditions, breeding less stress-sensitive broiler chickens and an overall reduction of stress is recommended (Adzitey and Huda, 2011). In addition, slowing down biochemical processes by fast chilling of the carcass can prevent the manifestation of the PSE condition. Chilling of the carcass leads to a slower acidification by lactate production and therefore protein denaturation (Offer, 1991). Moreover, all other enzymatic activities, like proteolysis, are also inhibited. For DFD meat, an inverted effect is speculated by Ponnampalam et al. (2017) but not yet proven. For the industry, PSE and DFD conditions lead to high economic losses. Carvalho et al. (2014) calculated a loss of 5.1 million US $ for the Brazilian production in 2013 with a PSE incidence of 41%, and only by taking into account the poor WHC and the resulting weight loss. The overall occurrence of PSE in poultry in the different literature sources ranges between 5 to 70% (Dong et al., 2020; Karunanayaka et al., 2016). DFD meat in poultry is described to occur with 3 to 56% in some studies (Freitas et al., 2017; Teke et al., 2019). In the literature, the incidence of DFD and PSE condition is described to be season related. Indeed, a higher incidence of DFD meat was observed during winter and wet weather conditions, whereas the incidence of PSE meat increased during summer and dry weather conditions (Carvalho et al., 2014; Dong et al., 2020; Freitas et al., 2017; Petracci et al., 2004; Teke et al., 2019). However, in the reviews of Ponnampalam et al. (2017) and Gonzalez-Rivas et al. (2020) a higher incidence of DFD during heat stress and cold stress was described for cattle. Both phenomena are described to be related to excessive use of liver and muscle glycogen to adapt the energy needs during cold temperatures or self-restricted feed intake during heat stress. In general, the incidence of both

conditions differs between country, company, and farm as a result of different stressors and stress exposure prior to slaughter (Karunanayaka et al., 2016).

Recently a defect of the *Pectoralis minor* muscle with similarities to the PSE-like condition was described (Soglia et al., 2019b). Due the observed separation of the fiber bundles, it was named ‘gaping defect’. Albeit this phenotype shows similarities to spaghetti meat (cf. section 3.3.3), there are few common features. Similar to PSE-like fillets, muscles with the gapping defect showed lower pH, higher lightness and increased cooking loss and it is speculated to be related to the same *post-mortem* changes of the meat (Soglia et al., 2019b).

3.2. Growth-related meat quality abnormalities: Deep pectoral myopathy

In modern meat-type birds, the high growth rates and fast muscle development causes a high burden to the anatomy and metabolism of the birds. Birds become more sensitive to stress, and susceptible to the development of certain myopathies (Wilson et al., 1990). The term ‘myopathy’ describes a primary, degenerative disease of the skeletal muscle cells (Valentine, 2017). Deep pectoral myopathy (**DPM**) describes a degenerative muscle disease of the *Pectoralis minor* muscle caused by an ischemic necrosis. DPM was first described in the late 1960s in turkeys and then be observed in all meat-type birds being selected for high-growth rates and breast muscle yield (Bianchi et al., 2006; Dickinson et al., 1968; Siller, 1985). DPM is one result of the altered anatomy of the high yielded breast muscle in meat-type birds and is known to be triggered by wing-flapping, as it was experimentally shown for broiler chickens by Lien et al. (2012).

The normal function of the breast muscle in birds is to enable wing movement. The *Pectoralis minor* muscle lies between the sternum (bone) and the *Pectoralis major* muscle and is surrounded by inelastic fascia. During movement, blood flows towards the breast muscles and increases them in size. The proportional increase of the *Pectoralis minor* is up to 25% compared to a state of rest and results in high pressure in the tissue and a narrowing of blood flow since the muscle cannot expand in its surrounding area. As a consequence, the muscle becomes ischemic and hypoxic which can lead to necrosis (Bilgili and Hess, 2008; Siller, 1985). The phenotype of DPM is a result of its ischemic and necrotic condition and it can be classified in medium and severe cases. In medium cases, the affected muscles by ischemia show inflammation with edema, hemorrhages, and hyperemia, which results in a red color of the tissue (Figure 5). In later, severe stages, with chronic damage, the muscle becomes necrotic and green in color. The color is related to breakdown products of myoglobin and hemoglobin in the

tissue. Because of its green color during the necrotic stage, DPM is also called ‘green muscle disease’. Later on, the breakdown of the muscle tissue (atrophy) results in paleness and the tissue can be replaced by fibrotic or adipose tissue (Bilgili and Hess, 2008; Dinev and Kanakov, 2011; Harper et al., 1975; Siller, 1985).

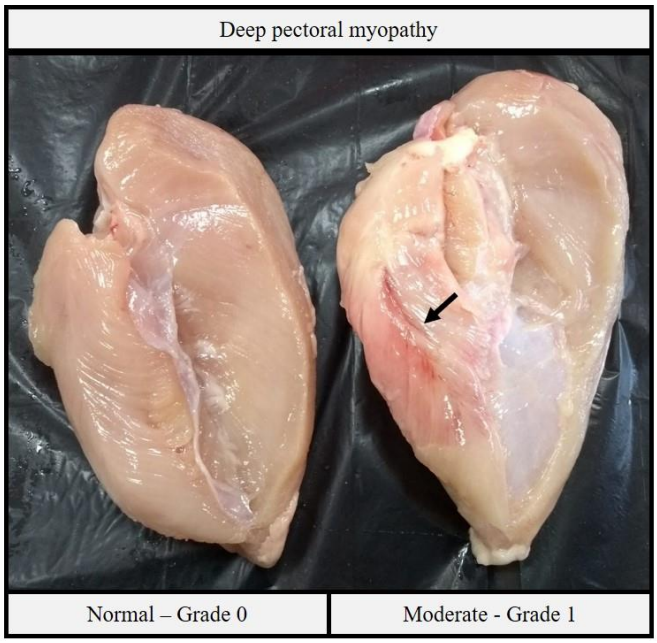

Figure 5: Appearance of the *Pectoralis minor* affected by moderate deep pectoral myopathy. A severe form was not detected in the trials described in the manuscripts.

DPM can occur in one or the two breast muscles, is mainly asymptomatic and does not apparently affect the health of the living bird. Therefore, affected birds cannot be sorted out during the growing period. Affected muscles are only observed at a later stage of the meat processing i.e., after deboning. The green color of the inner fillet generally leads to rejection by the consumer and is therefore removed during processing. This practice causes economic losses for the poultry industry. As reported by Lien et al. (2011), the loss for the US industry is about 16 million US $ per year and the author calculated a loss of 7,000 US $ per week for a processing plant with one million heavy broilers processed and a DPM incidence of 0.7%. The reported incidences for DPM in literature show a wide range. In a review of Siller (1985) incidences between 10 and 42% were described for broiler chickens, whereas Dinev and Kanakov (2011) described an incidence of 0.51%. Bianchi et al. (2006) gave incidences between 0 and 16.7% with an average of 0.84% in a commercial Italian processing plant, where 151 flocks of different breeds and slaughter ages were analyzed. Overall, the prevalence of

DPM increases with bird's weight and age. The sex of the birds also influences the occurrence of DPM, as more affected muscles could be found in male broilers until a weight threshold was reached for female ones (Bianchi et al., 2006; Bilgili and Hess, 2008; Harper et al., 1975; Siller, 1985).

In order to reduce the incidence of DPM in fast-growing meat-type birds, wing-flapping behavior induced by stress must be avoided. Wing-flapping is related to the farm management where excitement or stress of the birds, like abnormal sounds and lightings, human activity or feed and water restriction have to be control (Bilgili and Hess, 2008).

3.3. Novel breast muscle myopathies

During the last decade additional meat quality abnormalities were given high attention in the literature (Figure 6). These abnormalities, which mainly affect the *Pectoralis major* muscle of modern broiler chicken breeds, are described as breast muscle myopathies (**BMM**). Like DPM, these myopathies are related to high growth rates, breast weights and breast yields and show an increased incidence with bird's aging as a result of higher live weights. Three BMM were described until now: white striping (**WS**), woody breast (**WB**) and spaghetti meat (**SM**), which show similarities in underlying tissue changes, but with differences in the phenotype. All BMM are most commonly seen in broiler chicken, but WS and SM were also described in breast meat of turkeys (Zampiga et al., 2020). Moreover, several BMM can occur simultaneously in one fillet, but the most predominant co-occurrences are WS/WB and WS/SM (Bailey et al., 2020; Baldi et al., 2018; Petracci et al., 2019). In studies and under commercial conditions, BMM are mainly graded by their visual appearance or texture into different categories, but it must be kept in mind that all these gradings are highly dependent on the tester's personal experience and opinion.

Until now, no health or other concerns for humans regarding the consumption of affected meat were mentioned, but the appearance of BMM affects quality parameters, like visual appearance and texture. These quality parameters can be mentioned as the main reason for the unwillingness of the consumers to purchase affected meat. Affected fillets are not marketed as cut-ups, but downgraded and processed to other meat products or, in severe cases, are discarded. Therefore, the occurrence of BMM causes important financial losses and is still a great challenge for the poultry industry. The exact economic losses depend on the overall incidence, market prices, usage of affected fillets for further processing and, until now, mainly subjective out-sorting criteria of affected fillets. Zanetti et al. (2018) calculated the economic losses due

to the occurrence of WS and WB as 70,632.00 US $ per day (around 26 million US $ per year) in the Brazilian market with an underlaying condemnation rate of 0.8%. For the US market, Kuttappan et al. (2016) calculated a loss of 200 million US $ per year due to WS and WB myopathies. Due to the high economic relevance, a full understanding of the underlaying mechanisms, as well as strategies for prevention and handling solutions for affected meat is highly needed.

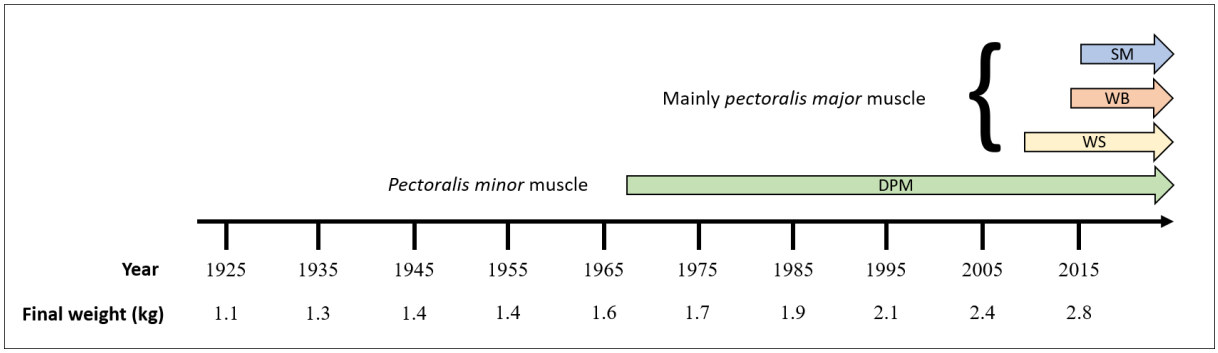

Figure 6: First recognition of breast muscle myopathies of fast-growing broiler chickens in the literature, including deep pectoral myopathy. Relationship between the occurrence of breast muscle myopathies and the growth-rate, breast yield, as well as age and slaughter weight are described in several literature sources. The final weights, given in the graphic, correspond to the average slaughter weights per year in the US. Weight data were published by the National Chicken Council (2019). The original data were provided by the United States Department of Agriculture (USDA). DPM: deep pectoral myopathy, WS: white striping, WB: woody breast, SM: spaghetti meat.

The metabolic underlaying mechanisms of BMM development in the breast muscles of fast-growing broilers, have been provided from histopathological and molecular findings as reviewed by Petracci et al. (2019). Excessive muscle growth causes hypertrophy of the muscle fibers, but without adequate growth of the supportive tissues, like blood vessels and satellite cells (Baldi et al., 2020). This leads to a hypoxic condition for the muscle cells, which could be worsen by the fact that the *Pectoralis major* muscle is containing mainly glycolytic type IIB fibers (Kuttappan et al., 2013b). During hypoxia reactive-oxygen species (**ROS**) are excessively generated in the cells, due to dysfunction of the respiratory chain in the mitochondria and other oxygen sensitive systems, like the activity of the NADPH oxidase (Ferreira and Laitano, 2016). Moreover, the lack of blood supply results in an accumulation of metabolic waste products, like lactate. Hypoxia, in turn, stimulates the formation of nitric oxide to increase the blood supply, but this could cause a worsening of the oxidative stress by the

formation of reactive nitrogen species. All this leads to cell damage, an altered cell metabolism, osmotic imbalance, inflammation and myodegeneration (Petracci et al., 2019). In a normal condition, a damaged muscle cell would be regenerated by removal of the damaged cell parts by leucocyte infiltration and merging of proliferating satellite cells directed by cytokines. Chronic hypoxic conditions interrupt this balance, which leads to fiber necrosis and replacement of the muscle fibers with fibrotic or adipose tissue. The formation of fibrotic and adipose tissue is described as being associated with the regulation of fibro/adipogenic progenitor cells during the reaction to different cell signals. For example, it is known that an increase of the cytokine TGFβ, which is released by macrophages during chronic inflammation, leads to muscle fibrosis (Jia and Sowers, 2019; Mahdy, 2019). However, the underlying mechanisms which lead to the expression of the various forms of BMM are still largely unknown and a recent genetic study suggests a complex multifactorial etiology (Petracci et al., 2019; Zambonelli et al., 2016).

3.3.1. White striping

The condition of WS was first described in 2009 and appeared as the first novel BMM in the literature (Kuttappan et al., 2012b). This myopathy is defined by the occurrence of stripes of adipose tissue in parallel with the muscle fibers, which is easily seen on the surface of the breast fillets (Bailey et al., 2015; Kuttappan et al., 2012b). Classically, this myopathy is visually graded by the thickness of the stripes as moderately or severely affected meat (Ferreira et al., 2014). Kuttappan et al. (2012b) classified the occurrence of stripes with a thickness of < 1 mm as moderately affected and with a thickness of > 1 mm as severely-affected, which represents the most common classification (Figure 7). It can be seen that the intensity of WS is often higher in the cranial parts of the fillets and close to the wing attachment point. Starting from there, the occurrence of the stripes spreads over the whole fillet with increased severity. In addition, the ventral side of the skin is usually more affected than the dorsal part (Ferreira et al., 2014; Kuttappan et al., 2013b). WS is also described to occur in several other parts of the carcass, including tenderloins (*Pectoralis minor* muscle), drumsticks and thighs (Baldi et al., 2020; Kuttappan et al., 2013b).

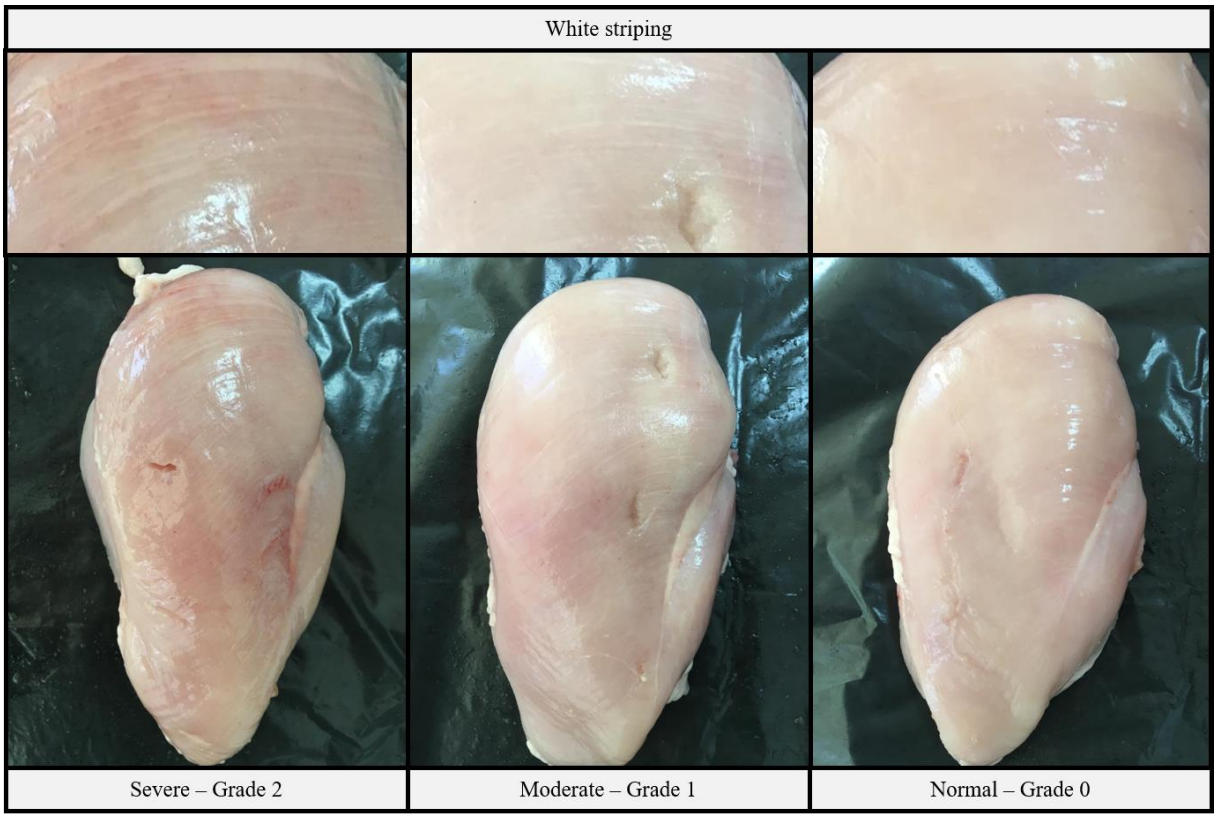

Figure 7: Appearance and visual, manual grading of white striping in breast fillets of broiler chickens. Normal – Grade 0: No or less thin stripes; Moderate – Grade 1: Occurrence of stripes with a size of mainly < 1 mm; Severe – Grade 2: Occurrence of stripes with a size of mainly > 1 mm. The zoomed pictures above the whole fillets show the cranial parts of the fillets.

The WS myopathy is more dominant in male broiler chickens (Kuttappan et al., 2013a; Lorenzi et al., 2014; Petracci et al., 2019) as a result of the connection between WS and growth performance. In some studies, the incidence and severity of WS increased with the slaughter age of the birds, as a possible consequence of higher weights. As a consequence, birds with higher slaughter weights showed more WS than those at lower weights but at comparable ages. On the other hand, WS is highly correlated with breast weights and breast yields as well as with greater thickness of the cranial part of the fillets (Bailey et al., 2015; Kuttappan et al., 2013a; Lorenzi et al., 2014). Moreover, high growth rates could promote the development of WS in broiler chickens, as it was pointed out that a broiler breeding line with slower growth rate during the starter period, but the same slaughter age developed less WS (Lorenzi et al., 2014; Zampiga et al., 2019a). Indeed, the influence of genetics on the occurrence of WS in fast-growing chicken breeds is controversially discussed. Some studies suggest a limited relationship between genetics and WS and indicate a high influence of environmental factors on the incidence of all BMM (Bailey et al., 2015, 2020), whereas other findings propose a strong influence of genetic

components on the occurrence of WS in fast-growing chickens and a correlation of WS with weight and breast yield (Alnahhas et al., 2016).

One of the main characteristics of WS is a change of fat and protein content in the muscle tissue. Affected muscles show higher contents of fat and collagen, whereas the overall protein concentration, especially of myofibrillar and sarcoplasmic proteins, is lowered (Kuttappan et al., 2013b; Mudalal et al., 2014; Petracci et al., 2014, 2019). In addition to the observed lipidosis and fibrosis, an increase in necrotic lesions of the muscle was observed and all these abnormalities increased with the severity of the condition (Kuttappan et al., 2013b). In connection with a lowered concentration of muscle protein in affected muscles, higher expression levels of the proteins MuRF1 and atrophin-1 were observed, which are related to muscle protein breakdown (Vignale et al., 2017). A lower concentration of the amino acids histidine (**His**), arginine and tryptophan in severely affected fillets were also reported, as well as an altered composition of fatty acids (Golzar Adabi and Demirok Soncu, 2019). Other changes observed in affected fillets include the loss of cross striations, variability in fiber size and lysis of fibers, infiltration of immune cells like macrophages, mineralization and vacuolation of the SR. Nevertheless, regeneration of the muscle fibers was also observed in affected muscles (Kuttappan et al., 2013b).

The reported impact of WS on MQ parameters were not uniform in the literature, but some main findings were seen in most studies. The pH of the fillets was higher in fillets affected by WS (Bowker and Zhuang, 2016; Petracci et al., 2013), which can be interpreted as a result of an increased lactate formation and glycogen breakdown during hypoxic conditions throughout muscle development. But contrary to the expectations which are resulting from the findings in DFD meat, the WHC is described to be lowered, leading to higher cooking loss and lowered marinade uptake (Mudalal et al., 2014; Petracci et al., 2013). The reason may be related to the lowered concentration of sarcoplasmic proteins which are the main drivers for WHC. The analysis of the color indicates a higher yellowness in severely affected fillets (Kuttappan et al., 2013a; Petracci et al., 2013). But interestingly, lightness and redness were not affected by WS in most of these studies.

The monetary value of WS on meat is mostly related to the visual appearance of the adipose stripes on the surface, which result in customer's unwillingness to buy fillets with any degree of WS. For the consumer, WS is connected with an increased content of fat and therefore a higher value of calories, as well as a bad appearance (Kuttappan et al., 2012b). Consequently, severely affected fillets are mainly used for further processing instead of direct marketing as

cut-ups. Because of the proven relationship between WS and growth rate, breast weight, breast yield and slaughter weight, the reported incidences in the literature show a high variability across the entire industry. Indeed, the incidence of WS is highly depending on region and growing conditions, slaughter age, analyzed broiler breed, and grading method (Baldi et al., 2020). Under commercial conditions, overall incidences between 12 and 72%, with 3 to 21% severe cases, have been reported (Baldi et al., 2020; Golzar Adabi and Demirok Soncu, 2019; Petracci et al., 2013, 2019). It was mentioned that nearly all broiler chickens in the US show signs of WS at nine weeks of age (Petracci et al., 2019). Under experimental conditions, the occurrence of WS can be even higher due to an optimized performance of the birds. Zampiga et al. (2019b) reported 82% fillets with WS (51% moderate and 31% severe affected) during a feeding trial.

3.3.2. Woody breast

The first report about a new myopathy, the WB, was in 2014 (Sihvo et al., 2014). This myopathy is characterized by a diffuse or focal hardness of the muscle. The hardness is often more pronounced in the cranial and ventral region of the fillets and a ridge-like bulge in the caudal area can be additionally seen in severely affected fillets. Furthermore, affected fillets show pale areas, as well as swollen and bulged parts. For some fillets, a viscous fluid on the surface, as well as petechia and hemorrhages can be detected (Cruz et al., 2017; Dalle Zotte et al., 2017; Hasegawa et al., 2021; Kuttappan et al., 2016; Sihvo et al., 2014).

In the literature, this myopathy is graded according to a variable number of categories, depending on the study. The classifications are mainly performed by a palpation of the fillets since this myopathy cannot be easily detected visually. The tactile grading can be based on the following parameters: flexibility and toughness. With a three-level grading scale, as shown in Figure 8, the fillets can be classified as normal, moderately and severely affected. Normal fillets show high flexibility and no detected hardness. Fillets with a moderate WB phenotype have hardened regions located mostly in the cranial part of the fillet and medium flexibility, whereas severe cases of WB show an overall hardness and limited flexibility.

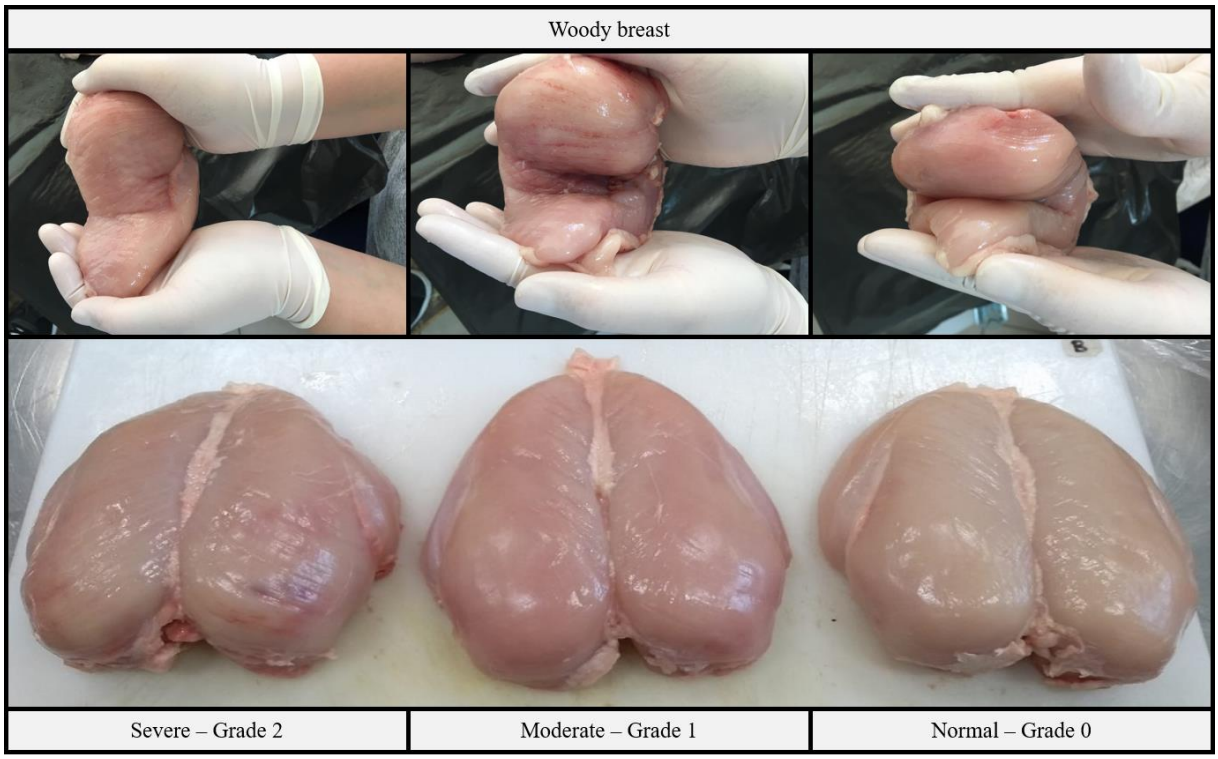

Figure 8: Appearance and manual grading of woody breast in breast fillets of broiler chickens by texture properties. Normal – Grade 0: Flexible fillet with no hard regions; Moderate – Grade 1: Medium flexibility with some hard regions located mostly in the cranial part; Severe – Grade 2: Limited flexibility, hardness can be detected over the whole fillet. Flexibility can be measured by folding the cranial towards the caudal part on the ventral side of the fillet.

Whereas lipidosis is the dominant characteristic in WS, fibrosis is the main pathological feature in WB-affected muscles, resulting in the observed hardened tissue (Kuttappan et al., 2016). Histopathologically, WB tissue shows necrosis, myodegeneration, fibrosis, lipidosis, edema and inflammation, but also regenerated sections. The muscle fibers are reduced in number and appear rounded, separated, in variable size, with a loss of striation. Giant and separated fibers were also described in breast muscle. The fillets show an increase in connective tissue, including granulation tissue and collagen-rich connective tissue, which causes the fibrotic phenotype. In addition, fibroblast infiltration was noticed. Furthermore, the tissue was infiltrated by lymphocytes, macrophages and heterophiles showing inflammation and phlebitis in affected muscle parts. The number of blood vessels was described to be generally reduced in affected muscles in early life of the birds. These changes indicate early chronic hypoxia. Moreover, an osmotic imbalance was described due to an increased SR and hyperplasia of mitochondria (Chen et al., 2019; Hasegawa et al., 2021; Kuttappan et al., 2016; Sihvo et al., 2014; Sihvo et al., 2018). An altered proteome was also seen in affected fillets. The proteins,

which were described to be differently expressed in WB fillets are mainly associated to the hypoxic adaption (e.g., increased Hypoxia-Inducible Factor-1, protein deglycase DJ-1, carbonic anhydrase III), fatty acid metabolism (e.g., increased lipoprotein lipase, serum albumin), energy metabolism (lowered creatine kinase M type and altered glycolytic enzymes) and muscle structure (e.g., myosin regulatory light chain 2) (Cai et al., 2018; Papah and Abasht, 2019). Moreover, an accumulation of lipofuscin was described, which also indicates hypoxic conditions and oxidative stress (Hasegawa et al., 2021).

The impact of WB on MQ is in many ways comparable to the WS condition, also the same variation is reported. The most effects were on pH, WHC, color, texture, and the composition of the meat. Affected fillets showed higher pH and lower WHC, and therefore higher drip loss and cooking loss, due to an altered muscle composition (Cai et al., 2018; Dalgaard et al., 2018; Dalle Zotte et al., 2017; Kuttappan et al., 2017; Soglia et al., 2016b; Trocino et al., 2015). Affected fillets had a lower content in protein, but higher values for moisture, fat, and collagen content (Soglia et al., 2016b; Wold et al., 2017). Similarly to WS, WB-affected fillets showed higher concentrations of Na^+ and Ca^{2+} (Soglia et al., 2016b). Moreover, a higher content of thiobarbituric acid reactive substances (TBARS) indicating increased lipid oxidation could be detected in affected fillets (Hasegawa et al., 2021; Soglia et al., 2016a). The color of WB-affected fillets was also analyzed, and they showed higher lightness, yellowness, and redness (Cai et al., 2018; Dalle Zotte et al., 2017; Wold et al., 2017). Interestingly, and contrary to the palpable hardness, the effects of WB on measurable hardness and especially share force are very controversially described in the literature (Petracci et al., 2019). This could be caused by the selection of different methods and regions for the measurement in diffusely or focally hardened fillets.

As with WS, limited genetic relationships between WB and the performance parameters weight and breast yield were found and the impact of environmental factors was determined as the most dominant one (Bailey et al., 2015, 2020). Because of the different findings when analyzing genetic aspects for WS, some controversial results can be also expected for WB. Independently of genetic analysis, studies investigating the relation between performance and incidence of WB showed clear correlation. The incidence of WB increased with age and weight of the birds and affected fillets were associated with higher breast weight and yield compared to normal fillets (Bowker and Zhuang, 2019; Chen et al., 2019; Dalgaard et al., 2018; Kuttappan et al., 2017). In this context, it should be noted that WB was observed in birds already at two weeks of age (Chen et al., 2019). Fast-growing chicken breeds developed more WB than old, slow-growing breeds, but the abnormality is also present in old breeding lines (Chen et al., 2019).

Moreover, the occurrence of WB is higher in male broilers and differences between broiler breeding strains were detected (Petracci et al., 2019; Trocino et al., 2015; Zhang et al., 2021).

Under commercial conditions, incidences of up to 20% were reported for the Brazilian market, whereas 60% were observed in Italy (Baldi et al., 2020; Petracci et al., 2019). Under experimental conditions, the WB occurrence shows a high variance. The lowest WB incidence of 5.6% was reported in a study of Gratta et al. (2019) in 48-d old birds, whereas the highest incidence of 96% was seen in 61-d old chickens in a study of Tijare et al. (2016). Depending on the degree of visual changes, like pale color, hemorrhages and bulging, fillets affected by WB are rejected by the consumer and cause economic losses. Indeed, they are sold to a lower price and are used for further processed and grounded products or, in the worst case, are discarded (Kuttappan et al., 2016). Moreover, fillets with WB condition have a lower nutritional value than normal fillets, because of the lower protein and higher collagen, fat, and moisture content (Petracci et al., 2019).

3.3.3. Spaghetti meat

In 2015, another BMM was reported for broiler chickens, first described as stringy and mushy meat (Bilgili, 2015). Later on, it was called SM due to the spaghetti-like appearance as a result of the poor muscle fibers cohesion and therefore the easy separation of muscle fibers in affected fillets (Baldi et al., 2018; Petracci et al., 2019; Tasoniero et al., 2020). The SM abnormality mostly occurs on the cranial, superficial part of the fillet with no affected deep layers.

In addition to the main histopathologic features which can be observed in all BMM, fillets with SM show a progressive loss of endo- and perimysial connective tissue and loose deposition of connective tissue (Baldi et al., 2018; Tasoniero et al., 2020), which could be a result of immature new collagen deposition in the muscle tissue (Bilgili, 2015). Moreover, reduced fiber number with rounded, sometimes split structure was observed and the tissue shows infiltration of adipose tissue and inflammatory cells (Baldi et al., 2018). Today it is not clearly known whether the condition only occurs after slaughter or in the living bird too. However, Baldi et al. (2018) reported that the loss of connective tissue could lead to an enlargement of intracellular spaces and higher loss of fluid *post-mortem*, which can result in the observed separation of the muscle structure. Moreover, it must be mentioned that a loose fiber structure was also described in 1990 in breast meat of high-weighted turkeys. Swatland (1990) speculated that the high-growth rates of turkeys result in a condition where the muscle fibers grow faster than their supportive tissue. Also, Baldi et al. (2018) reported a higher breast weight of affected fillets.

In broiler breast fillets, the myopathy can be graded as moderately and severely affected. Whereas small mushy parts, often with broken surface, can be seen on the cranial, superficial part in moderately affected fillets, severe SM show an easily detachable surface and free fiber bundles (Figure 9).

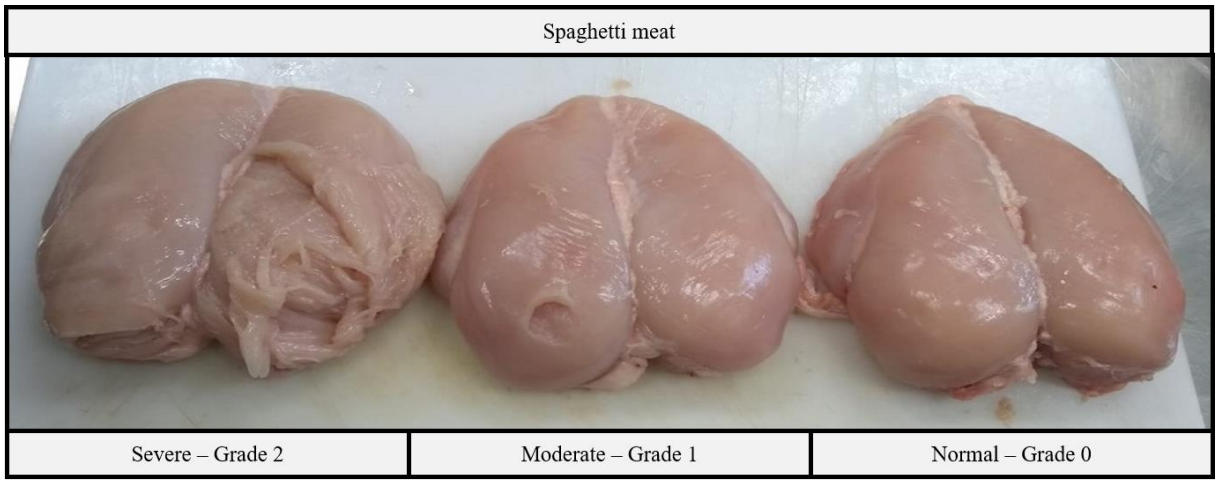

Figure 9: Appearance and grading of spaghetti meat in breast fillets of broiler chickens. Normal – Grade 0: No soft parts and intact surface on the cranial-superficial side of the filets; Moderate – Grade 1: Soft parts on the cranial-superficial side, often with small parts of broken surface (holes); Severe – Grade 2: Detachable surface and separated fiber bundles.

Like WS and WB, the occurrence of SM is a problem for the poultry industry, due to its unpleasant visual appearance when marketed as fresh meat. Therefore, affected fillets are often used in further processed products or discarded in severe cases, which results in economic losses (Baldi et al., 2018; Tasoniero et al., 2020). The soft texture and loose structure could also be a problem for the deboning machines used in an automated slaughterhouse. Moreover, altered meat quality parameters were reported by Tasoniero et al. (2020) and Soglia et al. (2019a) for fillets affected by SM. Affected fillets were heavier and showed higher pH, yellowness and drip loss compared to non-affected fillets. Most differences in the tissue composition were seen in the superficial part, with higher moisture and fat, but lower protein and ash content. Moreover, an increased content of Na^+ and Ca^{2+} was observed. NMR-results of the study of Soglia et al. (2019a) indicated less intermyofibrillary bound water and in the same study a higher content of protein carbonyls, i.e., a form of oxidative protein damage, was also reported in previously frozen muscles. Additionally, altered concentrations of amino acids (with higher concentrations of almost all canonical amino acids, i.e., proteinogenic amino acids), peptides (lower carnosine and anserine content) and metabolites of energy metabolism

(e.g., lower content of creatine, inosine monophosphate, lactate) and other molecules, like taurine, uracil, and β-alanine (**βA**) were observed in the SM abnormality.

Until now, the incidence of SM in fast-growing chicken breeds is not often reported in the literature. Nevertheless, Baldi et al. (2020) reported an incidence of 21% (4% moderate and 17% severe cases) for a commercial processing plant in Italy in a period between 2017 and 2018. In two feeding trials, incidences between 35% and 63% (moderate: 29% to 32%, severe: 6% to 31%) were measured after 35 and 43 d, respectively. The incidence was dependent on the broiler breed (Zampiga et al., 2019a,b). Moreover, it is reported that the SM condition is more prevalent in female birds, contrary to WS and WB which show a higher incidence in male broiler chickens (Petracci et al., 2019). A first analysis of the heritability of SM indicates limited genetic relationship between SM and weight and breast yield, but further studies are needed regarding this aspect (Bailey et al., 2020).

3.3.4. Current strategies to avoid novel breast muscle myopathies

So far, different methods were considered and tested in order to reduce the incidence and severity of BMM in broiler chickens. These methods include alternative feeding strategies and management. In summary, most of these methods aim at assisting the development of the supportive tissue, like blood vessel development, or improve the antioxidative status in the skeletal muscle tissue.

Feeding strategies with decreased or increased levels of amino acids are described for lysine, methionine, and arginine. An adapted feeding of these amino acids can help to improve the development of the supportive tissue. This can be done either by reducing the growth rate of the muscle fibers or by direct assistance of the surrounding tissue. It was believed that a supplementation of arginine above the usual values is able to increase the production of nitric oxide, which acts as a signaling molecules and promotes the relaxion of blood vessels to improve blood flow. In the literature, the effect of this feeding is not unanimous: in some sources, it led to a reduction of WB, WS and SM (Bodle et al., 2018; Zampiga et al., 2019b), but the opposite effect was also seen in other studies (Livingston et al., 2019a). Moreover, Livingston et al. (2019a) described an increased weight of broilers supplemented with additional arginine as well as glutamine. Therefore, a higher concentration of glutamine in the feed may also negatively affect the incidence of WB and WS. Furthermore, a reduced concentration of methionine and lysine in broiler feed compared to a usual diet can be used to reduce the high growth rates of broilers, to enable the adequate growth of the blood vessels and

other tissues. High growth rates together with the related factors final weight and age can be described as one of the most important factors for BMM development and related metabolic imbalance. Sachs et al. (2019) shows that the synthetic methionine to sulfur amino acids deficient diet can improve performance but also increase the occurrence of WS. After methionine, lysine is the second limiting essential amino acid for chicken. Therefore, lowering its concentration in feed can slow down the birds' growth rate. A decreased growth rate during the grower phase thanks to reduced lysine concentrations is reported to have a positive effect on the incidence of WS and WB with no effect on final weights, which was seen in the studies of Meloche et al. (2018) and Bodle et al. (2018). On the opposite, a higher concentration of lysine in feed leads to negative effects on the incidence of these BMM (Cruz et al., 2017).

A time-limited feeding can also lead to a reduction of the growth rate (Livingston et al., 2019b) and therefore BMM. In addition, anticoccidial treatments are mentioned to have a negative influence on the incidence of WB. The least WB was seen for vaccinated birds, but no negative control was given in the study of Aviagen (2019). It was seen that these effects are related to the growth curve, because chemical/inonophore anticoccidial treatment lowered bird weight compared to vaccination. Since BMM correlates with higher weights, the authors hypothesized that poor thermoregulation by using chemical/inonophores as anticoccidial treatment and the resulting heat stress could be a reason for higher WB incidences.

Alternative feeding strategies were focusing on the hypoxic status of the muscle fibers during BMM development. Dietary supplementation of antioxidants like vitamin C and E, a phytase-overdosing, and a supplementation with trace minerals and guanidine acetic acid (**GAA**), a precursor of creatine, have been tested. Increased dietary concentrations of vitamin E showed no effect on the occurrence of WS, whereas a supplementation of vitamin C alone or in combination with vitamin E was able to reduce WB (Aviagen, 2019; Bodle et al., 2018; Kuttappan et al., 2012a). Phytase-overdosing can be used to increase the concentration of inositol in the muscle which can act as antioxidant in the muscle cells after re-phosphorylation (Aviagen, 2019). In two studies, an over-dosing of phytase reduced WB in broiler chickens (Aviagen, 2019; Greene et al., 2019). A high concentration of trace minerals, i.e., copper, zinc, manganese, and selenium, in broiler feed may have a positive effect on the incidence of BMM. Indeed, they are used as cofactors of enzymes which act as defense systems against ROS, like the superoxide dismutase, glutathione peroxidase and metallothionine. But the effect of this feeding strategy on BMM is contradictory and not clearly described. Supplementation of the feeding with GAA improved performance as well as reduced the occurrence of WB and WS, by improving the energy metabolism of the cells (Aviagen, 2019; Córdova-Noboa et al., 2018).

Apart from feeding and unknown genetic effects, hatching and farming practice could have a big influence on the occurrence of BMM. One option to influence MQ could be the adaptation of hatchery and farm management methods. A sophisticated hatching system may improve the development of muscle tissue in the embryo, particularly when it provides an early access to feed and water (Aviagen, 2019). One the other hand, Livingston et al. (2019b) showed that a prolonged period of egg storage by 8 to 14 d prior to hatching increased the incidence of WS and reduced slaughter weight. On farm, practices like thinning (reduction of flock density by removing some broiler chickens) can improve performance prior to the final slaughter age, but they negatively affect the occurrence of BMM as they tend to cause higher growth rates and slaughter weights (Aviagen, 2019). High levels of CO_2 could increase the hypoxic condition of the birds and a hard litter could reduce blood supply to the breast muscle (Aviagen, 2019). Stress conditions, like disturbance by workers, heat and cold stress and other sudden changes, may lead to a higher metabolic activity of the birds, resulting in an increased hypoxic condition in the breast muscle (Boerboom et al., 2018). Otherwise, despite perfect growth conditions in the house and good performance parameters, the incidence of BMM increases with slaughter weight. Therefore, the influence of farming and hatching parameters on the occurrence of BMM needs to be further studied.

4. Histidine-related dipeptides in chicken skeletal muscle tissue

4.1. Properties of histidine

The amino acid His is a canonical amino acid in all species and an essential amino acid for most birds and mammals, including chicken (Konashi et al., 2000; McWilliams, 2002). This amino acid has a unique role in biological systems due to its imidazole side chain (Holeček, 2020). At physiological pH, His appears as a zwitterion with a deprotonated carboxylic group and protonated α-amino group (Holeček, 2020; Li and Hong, 2011). The imidazole side chain is an aromatic unit and exists in two tautomeric forms. The Nτ-tautomer is the dominating form, because of stronger intramolecular H-bonds compared to the Nπ-tautomer (Figure 10) (Li and Hong, 2011; Yannacone et al., 2020). Under physiological conditions, His is the only amino acid which can act both as proton donor and an acceptor and acts as an important pH buffer for biological systems, with a pKa of around 6.2 for free His and 6.5 for protein bound His (Holeček, 2020). Moreover, the imidazole side chain is able to bind cations of the first transition metal series and is able to scavenge free radicals (Holeček, 2020; Liao et al., 2013; Vistoli, 2015).

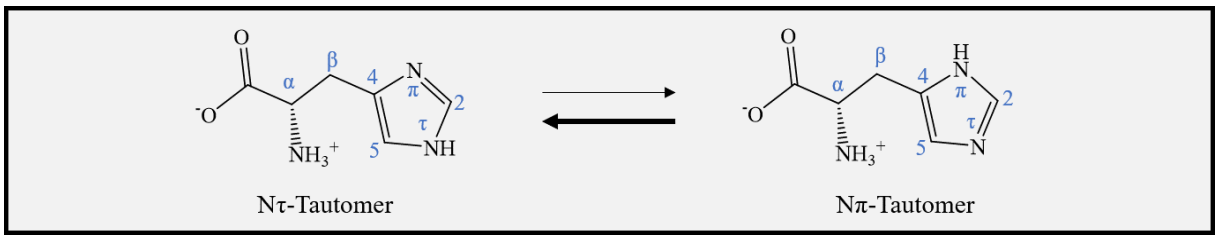

Figure 10: The tautomeric forms of histidine. The nomenclature is given according to the International Union of Pure and Applied Chemistry (IUPAC).

His plays a critical role for structure and catalytic activity in many proteins and it is present in the binding or active site of many enzymes (Brosnan and Brosnan, 2020; Hansen and Kay, 2014; Liao et al., 2013; Vistoli, 2015). Structure-wise, His promotes the formation of α-helices in the protein structure and the charge of its imidazole residue affects the stability of the helix (Armstrong and Baldwin, 1993; Banerjee et al., 2018). In active sites, it can act as a proton donor or acceptor during catalytic activity, like in the catalytic triad of proteases (Brosnan and Brosnan, 2020). In metallo-enzymes, His is able to coordinate metal cofactors, like in the carbonic anhydrase, or is a coordinating building block for the prosthetic heme group, which is used by catalase, hemoglobin, or myoglobin (Holeček, 2020).

In addition to its use as building block of proteins, the metabolism of His includes the catabolic pathway and the metabolism of histamine, the latter being an important signaling molecule. Moreover, His is a precursor of different peptides and derivates. The following paragraphs will give an overview of the different aspects of His metabolism.

4.2. The metabolism of histidine

Some derivates are described for His, including methylated and acetylated forms. Methylation of His occurs as a translational modification of proteins, mainly actin and myosin in muscle cells. In this case, the methylation is at the Nτ position to form 3-methylhistidine (**3MHis**). The His-derivate 3MHis can be used as marker for protein or muscle degradation, due to the fact that it is only excreted and not further metabolized (Brosnan and Brosnan, 2020; Holeček, 2020). Holeček (2020) describes that about 75% of 3MHis are released from skeletal muscle. Other sources of 3MHis include intestinal protein breakdown and dietary intake (Holeček, 2020). The methylation at the Nπ position, 1-methylhistidine (**1MHis**), can be catalyzed by using carnosine (**Car**) to form anserine (**Ans**), which will be further described in the next section. Both methylations of His are *S*-adenosylmethionine (**SAM**) dependent.

The catabolism of His includes two different pathways. The main degradation pathway, taking place mostly in the liver, is a non-oxidative catabolism towards the amino acid glutamate (Holeček, 2020; Poppe and Retey, 2003). The end-product of this pathway can be directly catabolized into intermediates of the tricarboxylic acid (**TCA**) cycle. This major catabolism of His involves four steps catalyzed by four different enzymes (Figure 11). In the first reaction, the α-amino-group of His is removed as ammonium to form *trans*-urocanate. This step is catalyzed by the enzyme histidine-ammonia lyase (**HAL**) which contains the uncommon electrophilic cofactor 4-methylideneimidazole-5-one (Cooke et al., 2009; Poppe and Retey, 2003). The free ammonium produced during the catabolism of amino acids is transferred to glutamate by the glutamine synthase to form glutamine, which can be further metabolized into uric acid and finally excreted to remove nitrogen from the organism (Berg, 2013; Salway, 2018). In the *stratum corneum* of the epidermis, *trans*-urocanate is the end-product of the His pathway, because of the absence of the further processing enzyme (Gibbs and Norval, 2011; Moro et al., 2020). It is assumed that in mammals *trans*-urocanate has a photoprotective function where it is reversibly converted into the *cis*-form of the molecule during the exposure to ultraviolet light (Barresi et al., 2011; Gibbs and Norval, 2011). *Cis*-urocanate can act as modulator of the immune system and has an immunosuppressive effect (Gibbs and Norval,

2011; Walterscheid et al., 2006). The enzyme urocanate hydratase (**UROC1**) catabolizes *trans*-urocanate to 4-imidazole-5-propionate by using NAD^+ as cofactor (Kessler et al., 2004; Keul et al., 1979; Rétey, 1994). During the reaction, a water molecule is added to the substrate to form the enol form of 4-imidazole-5-propionate as primary product. This enol form tautomerizes spontaneously to the more stable keto form (Kessler et al., 2004). In the third step of the pathway, the imidazole ring is broken by a hydrolytic cleavage of the Nτ-C_5 bond to form the intermediate formiminoglutamate (**Figlu**) (Su et al., 2016; Yu et al., 2006). The reaction is catalyzed by an imidazolonepropionase (**AMDHD1**), which is suggested to be Zn^{2+} dependent (Yu et al., 2006). The final step in the catabolism of His is realized by the enzyme formimidoyltransferase cycloamidase (**FTCD**). This enzyme is a bifunctional enzyme with two active sites, which catalyzes two consecutive but independent reactions (Kohls et al., 2000; Poppe and Retey, 2003). At one active site, the formimino-group of Figlu is transferred to tetrahydrofolate (**THF**,) the active form of folate, by a transferase reaction. During this reaction, glutamate is released. The second product N^5-formimino-THF is channeled between the active sites to form N^5, N^{10}- methenyl-THF and NH_3 as final products (Poppe and Retey, 2003). In the metabolism of folate, the N^5, N^{10}- methenyl-THF can be further metabolized into 10-formyl-THF and 5-methyl-THF, which are used as co-substrates in other important metabolic pathways like the synthesis of methionine out of homocysteine. In addition, the folate metabolism is coupled with the cobalamin metabolism via the reaction involving the enzyme methionine synthase (Shane, 2008).

The less frequent catabolism (Figure 11) occurs with high His concentrations (Brosnan and Brosnan, 2020). It starts with the activity of transaminases by using pyridoxal phosphate as cofactor. Two transaminase activities are described to use His as substrate. The prokaryotic histidine-pyruvate aminotransferase uses ketoglutarate as ammonium donor to form glutamate and imidazole pyruvate (Holeček, 2020; National Center for Biotechnology Information, 2021), whereas the second one, phenylalanine (His) aminotransferase, uses pyruvate as ammonium donor to form imidazole pyruvate and alanine (National Center for Biotechnology Information, 2021; Spolter and Baldridge, 1963). The imidazole pyruvate can be further metabolized to form either imidazole lactate by lactate dehydrogenase activity (National Center for Biotechnology Information, 2021) or imidazole acetate in an unclear step of decarboxylation to form imidazole acetaldehyde and subsequent aldehyde dehydrogenase activity (KEGG (gga00340)). Imidazole acetate can be used to form aspartate, which can be metabolized towards the TCA intermediate oxaloacetate by aspartate transaminase (Holeček, 2020).

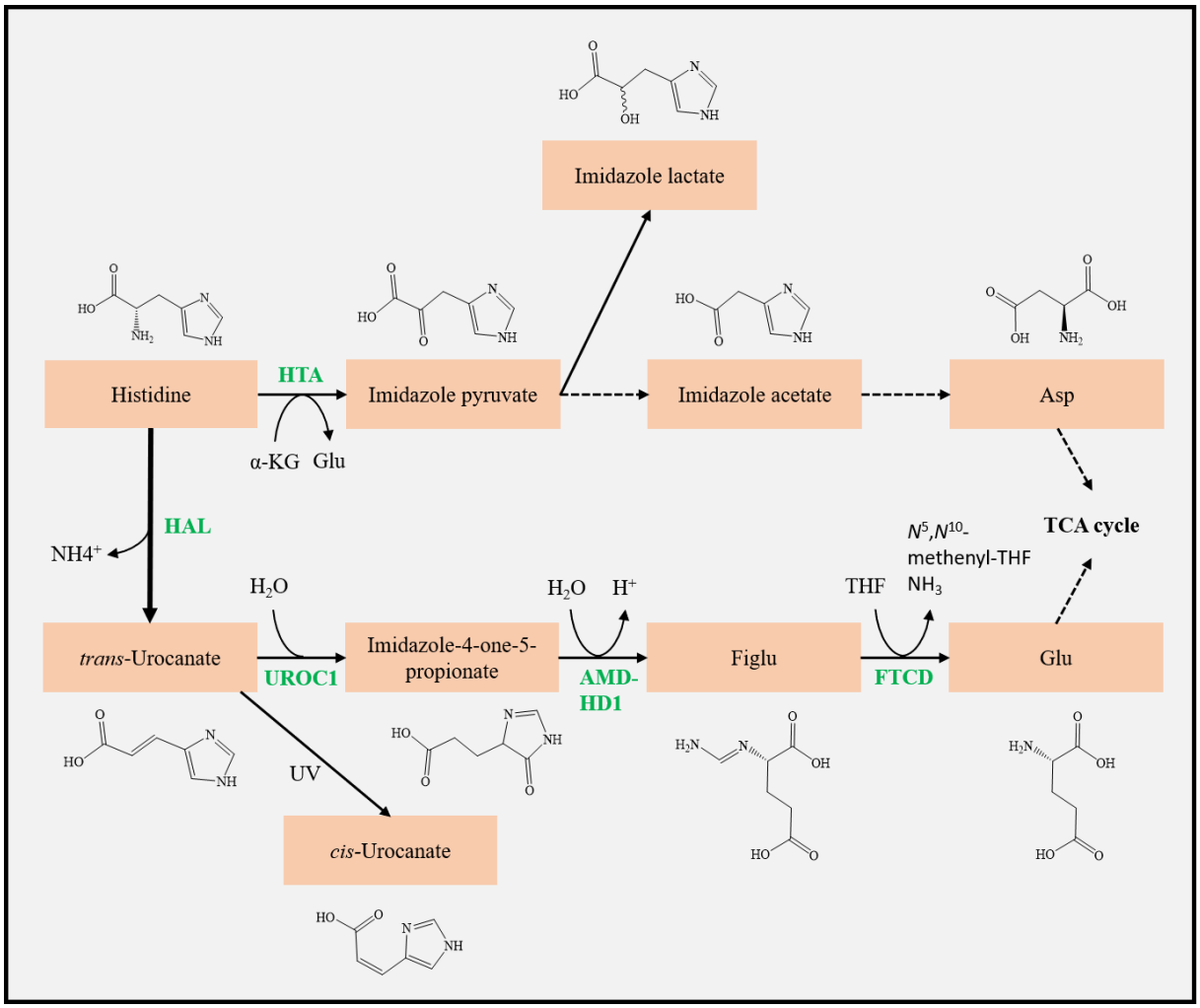

Figure 11: Histidine catabolism. The main catabolism is the one towards glutamate. The figure is based on KEGG (gga00340) and Holeček (2020). HAL: histidine-ammonia lyase, UROC1: urocanate hydratase, AMDHD1: imidazolonepropionase, FTCD: formimidoyltransferase cycloamidase, HTA: histidine transaminase, Figlu: formiminoglutamate, Glu: glutamate, Asp: aspartate, TCA cycle: tricarboxylic acid cycle, THF: tetrahydrofolate.

The signaling molecule histamine is formed by decarboxylation of His by the histidine decarboxylase (**HDC**). This reaction occurs mainly in histaminergic neurons of the brain, enterochromaffin-like cells of the stomach, as well as basophiles and mast cells of the immune system (Huang et al., 2018). Therefore, a wide range of physiological functions is regulated, including the secretion of hydrochloric acid, inflammatory response, sleep/wake rhythm, appetite, memory function and stress response, as well as blood circulation and vasodilation (Brosnan and Brosnan, 2020; Holeček, 2020). According to Maintz and Novak (2007), histamine can be degraded by two different pathways, by methylation or oxidation (Figure 12). The different enzymes used for the first step of histamine degradation differ in their expression site and excretion pattern. While the copper-containing diamine oxidase (**DAO**) is secreted, the

histamine-*N*-methyltransferase (**HNMT**), which uses SAM as methyl-donor, is a cytosolic enzyme. Both enzymes can be inhibited by their products. DAO catalyzes an oxidative deamination of histamine, which produces ammonia and hydrogen peroxide, which belongs to the group of ROS (Matlashov et al., 2014). The Nτ-methylation, which is catalyzed by HNMT, results in methylhistamine and can be further metabolized by DAO to form methyl-imidazole-4-acetaldehyde. Both aldehydes can be oxidized by aldehyde dehydrogenases using NAD^+ as a redox partner to form the carboxylic acid products (Maintz and Novak, 2007).

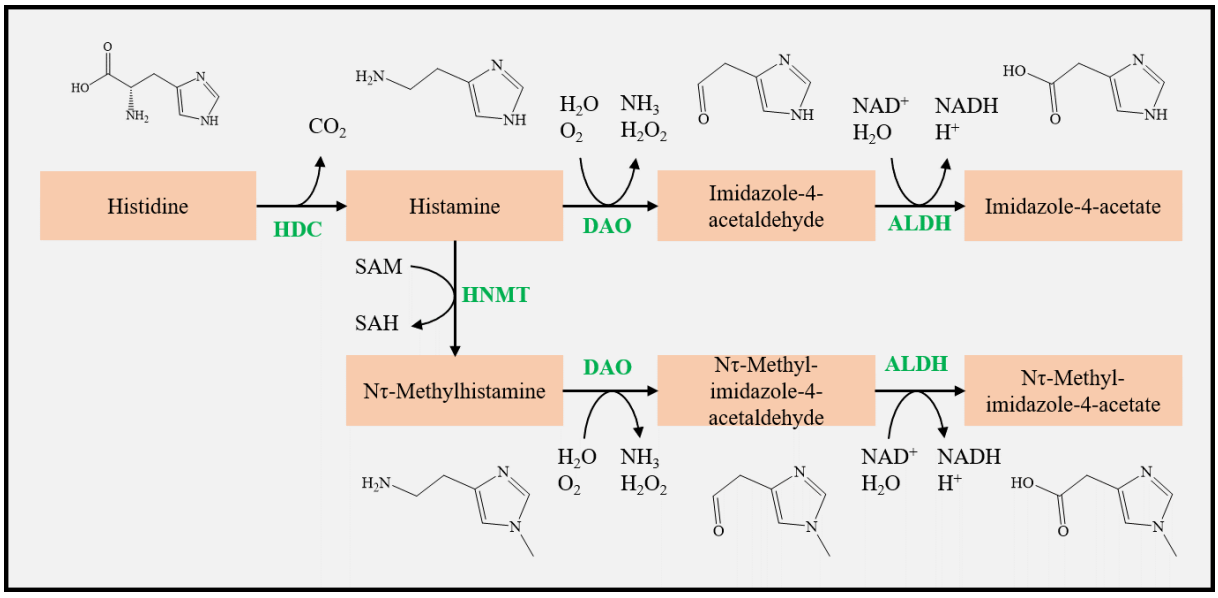

Figure 12: Histamine metabolism. The figure is based on KEGG (gga00340) and Holeček (2020). HDC: histidine decarboxylase, HNMT: histamine-*N*-methyltransferase, DAO: diamine oxidase; ALDH: aldehyde dehydrogenase, SAM: *S*-adenosyl methionine, SAH: *S*-adenosyl-homocysteine, NAD^+/NADH: nicotinamide adenine dinucleotide.

Knowledge of metabolic pathways is important, especially when it comes to supplementing His for specific goals. It should be mentioned that in general a supplementation of His is considered as safe for humans and, even though it is the precursor of histamine and can increase the histamine stores in specific cells, no allergic or toxic reactions are known. Nevertheless, it must be kept in mind that some harmful effect by feeding high concentrations of His are described for different animal species, including reduced growth rates and higher mortality (Harper et al., 1970; Ikezaki et al., 1996). As result of its catabolism, an increased supply of His changes the concentration of other amino acids in blood plasma of rats, with a decrease in branched-chain amino acids and an increase in glutamate, alanine, and glutamine, as well as an increase of total ammonia (Holeček and Vodeničarovová, 2019). Moreover, the requirement of folate or

cobalamin during catabolism can lead to a deficiency of these cofactors during His supplementation (Herbert and Zalusky, 1962; Holeček, 2020).

4.3. Carnosine and its derivates

His is a precursor of an important group of functional dipeptides, which can be naturally found in some parts of the brain (e.g., olfactory bulb), as well as in the skeletal and cardiac muscle tissue of vertebrates (Boldyrev et al., 2013; Drozak et al., 2010). One molecule of this group of nonproteinogenic histidine-containing dipeptides (**HCDs**) is Car, which was first described in meat extract and is build out of the amino acids His and βA (Boldyrev et al., 2013; Drozak et al., 2010; Vistoli, 2015). In addition, some methylated and acetylated forms exist, as well as dipeptides formed with histamine instead of His, and dipeptides with γ-aminobutyric acid as a second precursor instead of βA. In general, HCDs with γ-aminobutyric acid as a precursor are predominantly found in brain tissue, whereas HCDs with βA as a precursor are mainly found in muscle tissue (Drozak et al., 2010). The most important HCD, which can be found in high concentrations in skeletal muscle tissue of vertebrates, are Car and two methylated forms. The first methylated form is Ans, with a methyl-group at the Nπ position of the imidazole side chain, and the second derivate is ophidine (**Oph**), which contains the methyl-group at the Nτ position. According to Boldyrev et al. (2013), the average concentration and composition of HCD in the skeletal muscle of vertebrates varies between species and most vertebrate muscles contain two different HCDs. The HCD Oph is found mainly in marine mammals and snakes. Only few species such as humans form a single HCD, where only Car is present but in relatively low concentrations compared to many other species. On the other hand, in some species, e.g., in swine, all three HCDs are found. In some fish species with low or absent HCD concentrations, high amounts of free His were found (Boldyrev et al., 2013). This leads to the conclusion that the main functional unit is the imidazole side chain. In chicken, Car and Ans are both present in high amounts in skeletal muscle tissue, with higher concentrations of Ans as compared to Car (Boldyrev, 2007, 2013; Jung et al., 2013; Kai et al., 2015; Kojima et al., 2014; Yeum et al., 2010). It must be noted that the concentration of HCD is age and sex dependent. In humans, females show lower amounts of Car than males and after puberty, a drop in Car concentration is noticed (Boldyrev et al., 2013). Jung et al. (2013) also reported a gender effect for chicken, but female chicken had higher concentrations of Car in breast and thigh muscles and higher Ans concentrations in tights compared to male. According to some literature sources (Table 2), the concentration of Car and Ans in chicken muscle tissue ranges between 1220 to 5702 µg/g for Car and 2370 to 9830 µg/g for Ans, with a Ans:Car ratio between 1.6 and 5.3.

Table 2: Some reported carnosine and anserine concentrations in fresh chicken muscle tissue.

Source	Car (µg/g muscle tissue)	Ans (µg /g muscle tissue)	Ans:Car ratio	Breed	Age, d	Analyzed tissue
Boldyrev, 2007	2780	9830	3.5	not reported	not reported	Skeletal muscle
Yeum et al., 2010	2150	4440	2.1	not reported	not reported	Skeletal muscle
Jung et al., 2013	1614	8496	5.3	Korean native chicken[1]	140	Skeletal muscle
Kojima et al., 2014	4172	6479	1.6	Broiler (not specified)	56	Skeletal muscle
Kai et al., 2015	1434	5902	4.1	Chunky strain broiler	24	Skeletal muscle
Kopec et al., 2020	1220	2370	1.9	Hubbert Flex	28	Skeletal muscle
Lackner et al., 2021	2200 / 5702	2210 / 6773	2.6 / 3.1	Ross 308	35 / 54	Skeletal muscle

[1]Five chicken breeds. Abbreviations: Car, carnosine; Ans, anserine.

All HCDs show a wide range of biochemical properties which are mainly related to the imidazole side chain (Figure 13). One of the main properties is the ability to work as a buffer at physiological pH (Bellia et al., 2014; Boldyrev et al., 2013; Drozak et al., 2010). Chemically, the HCDs are polyprotic acids which show three pKa values. According to Jozanović et al. (2015) the first pKa for the carboxylic group was calculated as 2.57 for Car and 2.60 for Ans and the second one for the amino group of the βA side chain as 9.57 for Car and 9.51 for Ans. The pKa for the imidazole side chain is close to the physiological pH, at 6.71 for Car and 6.97 for Ans. Therefore, the two dipeptides are present as zwitterions under physiological conditions and the nitrogens of the imidazole side chain are responsible for the buffering activity (Boldyrev et al., 2013). Within the cells, HCDs are soluble buffering systems, which can reach most cellular compartments (Boldyrev et al., 2013). Under physiological conditions, this buffering system may play an important role during activity and anaerobic lactate formation, especially in type IIB muscle cells. A change in pH can be critical for many cell functions so it must be kept within an appropriate range around pH 7 and a pH buffering could result in a longer activity phase. The HCDs are also able to chelate metal ions of the first transition metal series, like copper and zinc, which are toxic for the cell in large amounts and can act as pro-oxidants (Boldyrev et al., 2013; Kawahara et al., 2020). Another important ability of HCDs is a broad

antioxidative activity (Bellia et al., 2014; Boldyrev, 2007, 2013). This is also mainly related to the imidazole side chain. The antioxidative ability is realized by different direct and indirect mechanisms. HCDs can directly scavenge free radicals, like ROS, and indirectly, can chelate pro-oxidative metal-ions like copper. Moreover, it was shown that they improve the enzymatic and non-enzymatic antioxidative activity by either an increasing or a restoring effect, or both (Boldyrev et al., 2013). The radical scavenging effect is expected to be realized by a delocalization, and therefore stabilization, of the radical and not by an oxidation of HCD, like it was seen for ascorbic acid (Boldyrev et al., 2013). Moreover, Car can be considered as a storage pool for His in the organism, which can be used for histamine formation during stress conditions (Boldyrev et al., 2013; Flancbaum et al., 1990). In addition, it was shown that Car acts as regulator for Ca^{2+} activity in muscle cells. Car is able to increase the contractility of cardiac muscle cells and interacts with the ryanodine receptor (i.e., a calcium channel in the membrane of the sarcoplasmic reticulum) which improves its open state (Roberts and Zaloga, 2000; Zaloga et al., 1997). HCDs can inhibit the formation of advanced lipoxidation and glycoxidation end-products by metal ion chelation, antioxidative activity, as well as quenching of reactive carbonyl species (Boldyrev et al., 2013; Hipkiss, 2000; Vistoli et al., 2017). The quenching mechanism is performed by different mechanisms on αβ-unsaturated carbonyls by a nucleophilic addition including the imidazole side chain and the primary amine group (Vistoli et al., 2017). Nevertheless, many other properties of HCDs are described in literature, including protein-interactions and modulation of nitric oxide metabolism (Boldyrev et al., 2013).

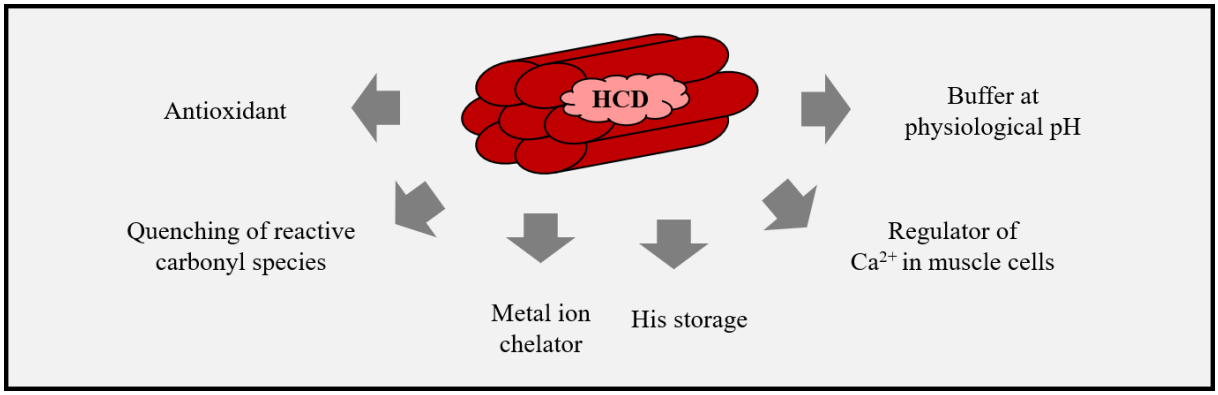

Figure 13: Main properties of histidine-containing dipeptides in the skeletal muscle tissue. HCD: Histidine-containing dipeptides (in this context: carnosine, anserine, and ophidine). The same properties are also seen for other related dipeptides, like acetylated and decarboxylated forms or His-dipeptides with γ-aminobutyric acid as second precursor.

The metabolism of HCSs is given in Figure 14 and contains two possible pathways. In HCD-containing tissues, the ligase carnosine synthase (**CARNS**) can be found, which belongs to the ATP-grasp family of enzymes. This enzyme, which uses Mg^{2+} as cofactor, can catalyze the formation of Car out of His and βA and hydrolyses ATP during this reaction. However, this enzyme shows a broad substrate acceptance, with less specificity than His and βA, and is able to catalyze the formation of other dipeptides like Ans and homocarnosine (Boldyrev et al., 2013; Drozak et al., 2010). Nevertheless, the regulation of generating the HCD Ans and Oph comes from the direct methylation of Car by methyltransferases (Bellia et al., 2014; Boldyrev et al., 2013). For Ans formation, the carnosine methyltransferase (**CARNMT**) was described in chicken as a histamine *N*-methyltransferase-like enzyme (Drozak et al., 2013). CARNMT is specific to Car as a substrate and uses SAM as a methyl donor. It must be mentioned that a methylation activity of Car is not found in the human body/cell, which only contains Car, and the methylation of Car to form Oph is expected to be performed by a different methyltransferase, which is currently unknown (Boldyrev et al., 2013; Kwiatkowski et al., 2018). While the formation of HCD is related to specific tissues, HCDs are degraded by a different enzyme in serum and tissues, which makes a direct therapeutic supplementation difficult: the carnosinases (**CN**) degrade Car in serum (CN1) and tissue (CN2). These enzymes belong to the family of M20/M28 metalloproteases but show some differences in their activity. The secreted CN1, which can be found in serum, brain, liver, and kidney, is more specific to Car than to other HCDs and homocarnosine, and it uses Zn^{2+} as cofactor. CN2 shows less specificity and is also known as a cytosolic non-specific dipeptidase. This enzyme uses Mn^{2+} as a cofactor and can be found in most tissues, but not in serum and cerebrospinal fluid (Bellia et al., 2014; Boldyrev et al., 2013). In addition to these two classes of HCD-degrading enzymes, which can be found in humans as well as in chicken, a third class is described for poikilothermic vertebrates, like salmon, and called anserinase (Yamada et al., 2005).

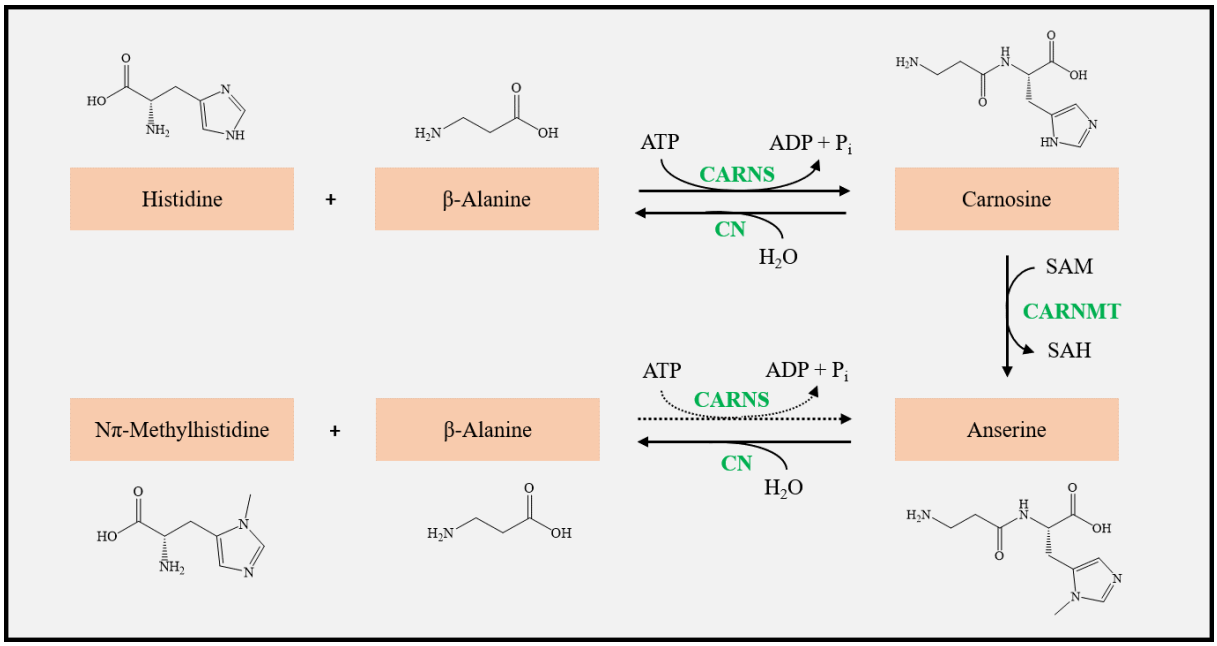

Figure 14: Metabolism of the histidine-containing dipeptides carnosine and anserine, which can be found in chicken. The figure is based on Boldyrev (2007, 2013). Both dipeptides are built in the cytoplasm of specified tissues (e.g., skeletal, and cardiac muscle, as well as brain tissue) and degraded in blood plasma and many other tissues. The main pathway to synthesize anserine is the methylation of carnosine, whereas the direct formation by using Nπ-Methylhistidine is less common. In the used nomenclature, Nπ-Methylhistidine corresponds to 1-methylhistidine. All metabolites are given in biological relevant L-conformation. CARNS: carnosine synthase, CN: carnosinase (β-alanine-histidine dipeptidase), CARNMT: carnosine methyltransferase, SAM: *S*-adenosyl methionine, SAH: *S*-adenosyl-homocysteine, ATP: adenosine triphosphate, ADP: adenosine diphosphate.

Car supplied via feed or food is actively absorbed in the intestine and can cross the blood-brain barrier (Bellia et al., 2014; Vistoli, 2015). Due to its broad functionality, Car supplementation has been tested for its therapeutic properties against some human diseases, like the Alzheimer disease, diabetes, or stroke (Boldyrev et al., 2013). Due to its metabolism and the degradation in blood and many tissues by carnosinase activity, the direct use as therapeutic agent represents a hurdle for the pharmacy (Bellia et al., 2014; Vistoli, 2015).

4.4. The metabolism of β-alanine

The second HCD precursor, the amino acid βA, is the naturally occurring, non-proteinogenic β-amino acid derivate of alanine, which makes it the simplest form of β-amino acids. This molecule is an intermediate of different metabolic pathways but βA is not used for protein synthesis. Besides its role as a metabolic intermediate, it is a precursor of metabolites like

HCDs. Moreover, βA is studied as a possible neuromodulator or neurotransmitter (Tiedje et al., 2010).

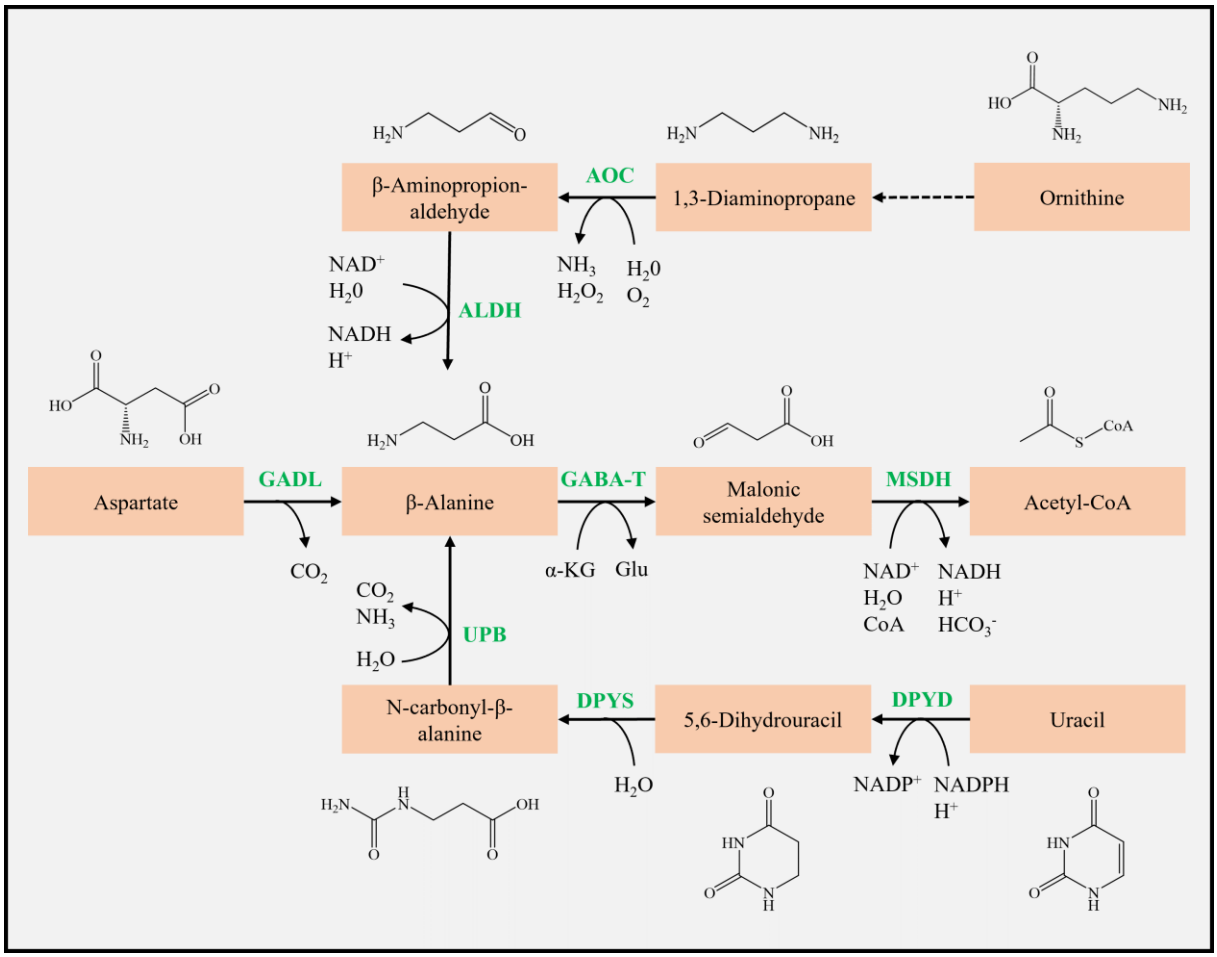

Figure 15: The metabolism of β-alanine. The Figure is based on KEGG (gga00410). AOC: primary amine oxidase, ALDH: aldehyde dehydrogenase, GADL: glutamate decarboxylase, GABA-T: methylpropionate transaminase, MSDH: malonate semialdehyde dehydrogenase, DPYD: dihydropyrimidine dehydrogenase, DPYS: dihydropyrimidinase, UPB: β-ureidopropionase, α-KG: α-ketoglutarate, Glu: glutamate.

Three anabolic pathways are mentioned in the literature for animals and are also present in chicken according to KEGG (gga00410, 19th May 2021, Kanehisa, 2019; Kanehisa et al., 2021; Kanehisa and Goto, 2000) and given in Figure 15. The first and main pathway for producing βA is the three-step synthesis out of the pyrimidine uracil (Mahootchi et al., 2020; Suidasari et al., 2015). This pathway includes a reduction of uracil by dihydropyrimidine dehydrogenase (**DPYD**), a hydrolytic cleavage of the ring system by dihydropyrimidinase (**DPYS**) and, as a final step, the hydrolytic reaction towards βA by β-ureidopropionase (**UPB**). All enzymes are

located in the cytosolic fraction of the cell (Ito et al., 2001). As second pathway, a decarboxylation of aspartate leads to the synthesis of βA. In birds, mammals and reptiles, this decarboxylation is catalyzed by the pyridoxal phosphate-dependent enzyme glutamate decarboxylase (**GADL**) (Mahootchi et al., 2020; Suidasari et al., 2015). The enzyme is unspecific and can also use cysteine sulfinic acid, cysteic acid and glutamate as substrates to produce hypotaurine, taurine and γ-aminobutyric acid, respectively (Mahootchi et al., 2020). Suidasari et al. (2015) also assumed the synthesis of βA out of ornithine as third pathway, because of the depletion of this metabolite and a correlated increase of βA by supplementation of pyridoxine in rats. The enzyme which catalyzes the synthesis of putrescine out of ornithine, ornithine decarboxylase, uses pyridoxal phosphate as a cofactor. In four additional steps, βA can be produced, including the synthesis of spermidine by spermidine synthase, a dehydrogenase reaction to produce 1,3-Diaminopropane, the catalytic activity of the primary amine oxidase (**AOC**) and the final step of an ALDH, respectively. Additionally, as mentioned in the previous section, the degradation of HCDs by CN also produces βA. The main catabolism of the β-amino acid is the transamination to malonic semialdehyde by methylpropionate transaminase (**GABA-T**), which uses α-ketoglutarate as a donor for the amino group (Ito et al., 2001). Malonic semialdehyde can be further metabolized into acetyl-CoA.

Research questions and objectives

Based on previous research, it has been established that the WB occurrence is correlated with a depletion of the antioxidants Car and Ans in breast muscle of affected broiler chickens (Abasht et al., 2016; Sundekilde et al., 2017). Later on, this observation was confirmed in the study of Soglia et al. (2019a), where depletion of Car and Ans were also reported in breast fillets of broilers affected by WS and SM conditions. These findings were interpreted as an increasing need for antioxidants during the hypoxic condition, which was described as an underlying condition of BMM. Moreover, it has been shown that the levels of the dipeptides Car and Ans in broiler breast meat depended on the supply of the precursor His in broiler feed (Kai et al., 2015). Therefore, the main objective of these studies was to evaluate the influence of dietary level of the essential amino acid His on the occurrence of BMM in broiler breast meat. Due to the fact that βA is the second precursor of the dipeptides Car and Ans, a combination of His with βA was also tested. In this thesis the following questions should be answered:

1. Is a supplementation of His above the commercially used His:Lys ratios able to increase the concentration of Car and Ans in the *Pectorals major* muscle?
2. Does an additional supplementation of βA further increase the concentration of the dipeptides or even have a stand-alone effect on these concentrations?
3. Is the performance of the broilers affected by any supplementation used in this study?
4. What impact do dietary His:Lys ratios alone or combined with βA have on the overall meat quality of broiler breast meat?
5. Does the addition of His with or without βA exert positive effects on BMM and other meat quality abnormalities?
6. Which impact has an increased His:Lys ratio in broiler feed on the overall metabolism of the chicken?

Two feeding trials were performed in order to address these questions. The first trial was conducted to test the influence of different His:Lys dietary ratios alone or in combination with βA on performance, dipeptide concentration in breast meat and blood plasma, as well as for generating samples for a metabolic profiling. These results are presented in Manuscript I and Manuscript III. The second trial highlighted the effect of a different His:Lys ration in feed on BMM and other MQ parameters. This trial is described in Manuscript II.

Manuscript I

Effect of feeding histidine and β-alanine on carnosine concentration, growth performance, and meat quality of broiler chickens

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ABSTRACT The high growth rates of modern broiler breeds increased the risk for novel breast muscle myopathies as serious quality issue, relevant for the industry. In affected muscles, a depletion of the dipeptides carnosine and anserine was reported. Therefore, this study was performed to test whether a supplementation of the precursors histidine and β-alanine, alone or in combination can increase the dipeptide content in the breast muscle and improve meat quality.

Ross 308 broiler chickens were supplemented with 3 different histidine:lysine ratios (0.44, 0.54, 0.64) of standardized ileal digestible amino acids (**SID**) combined with 0 or 0.5% β-alanine in total. The birds' performance was recorded at different ages: birds were slaughtered in 2 batches after 33 and 53 d of life. Meat quality was tested at different time points after slaughter on breast fillets stored aerobically. The concentration of the dipeptides and amino acids in blood plasma and muscle tissue was tested postmortem at 35 and 54 d. All performance and meat quality data, as well as peptide and amino acid concentrations, of the 2 × 2 × 3 randomized block design were analyzed separately for the influence of both supplements and for slaughter age. Moreover, the influence of storage time was analyzed separately for meat quality parameters. At both slaughter ages, lesser feed intake ($P \leq 0.005$) and breast yield ($P \leq 0.05$) were observed in the birds receiving β-alanine. A greater SID histidine:lysine ratio increased the carnosine concentrations in blood plasma ($P < 0.001$) and in skeletal muscle ($P < 0.001$), whereas β-alanine increased carnosine in plasma at 35 d only ($P = 0.004$). Anserine was increased in plasma and muscle of older birds ($P = 0.003$), whereas carnosine was reduced in muscle tissue ($P < 0.001$). The main impact on meat quality parameters was seen for the age of the birds and storage time of the fillets. In conclusion, the supplementation of histidine increased carnosine in breast muscle but both supplements showed only minor effects on meat quality.

Key words: broiler, carnosine, histidine, β-alanine, meat quality

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INTRODUCTION

Modern broiler breeds with a high-performance are fast-growing chickens with an optimized feed conversion ratio (**FCR**) to deliver white meat quickly to the market. The high growth rate and body weight are some of the most important triggers of meat quality issues like woody breast (**WB**) and white striping (**WS**) (Kuttappan et al., 2012; Petracci et al., 2013, 2019; Griffin et al., 2018; Chen et al., 2019). These problems lead to economic losses, especially at older slaughter ages, which are expected to amount from 26 million and 1 billion US$, depending on the incidence and country-specific market (Kuttappan et al., 2016; Zanetti et al., 2018). Therefore, an understanding of the underlying causes for such conditions is required for developing solutions.

The number of the muscle cells is determined during the development of the chicken embryo and later muscle growth corresponds to an increase in cell diameter (Smith, 1963). The increase in cell size during aging results in the displacement of the surrounding tissue by muscle fibers (Wilson et al., 1990) leaving less space for satellite cells and capillaries, which are also naturally less in Type IIB muscles, to which the *pectoralis major* belongs to (MacRae et al., 2006; Liu et al., 2012; Velleman, 2019). The reduced blood supply is associated with reduced nutrients and oxygen reaching the tissue cells as well as an accumulation of waste molecules

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(Petracci et al., 2019). The altered metabolic condition has adverse consequences such as oxidative stress and inflammatory processes and can result in myodegeneration with necrosis, lipidosis, and fibrosis which in turn may lead to the conditions of WB and WS in broiler chickens (Mutryn et al., 2015; Abasht et al., 2016; Zambonelli et al., 2016; Cai et al., 2018; Petracci et al., 2019; Velleman, 2019). In affected muscles, oxidative stress is considered as one of the major triggers during the development of breast myopathies (Petracci et al., 2019). To delay the outcome of breast myopathies, reducing oxidative stress can be helpful. Oxidative stress is defined as an imbalance of reactive-oxygen species (**ROS**) formation and antioxidative activity (Morry et al., 2017). The antioxidative system consists of enzymes as well as small antioxidative molecules (Chan and Decker, 1994). In muscle cells of most vertebrates, a pool of His-containing dipeptides (**HCD**) with antioxidant activity can be found in high concentration (Boldyrev et al., 2013). The most common and most described HCD is carnosine (**Car**), which occurs in high concentrations in skeletal muscles, in brain and some other organs of vertebrates (Bonfanti, 1999; Hipkiss, 2010; Boldyrev et al., 2013). The dipeptide Car is formed by the essential amino acid His and the β-amino acid β-Ala (**βA**). In chicken, Car as well as its methylated form anserine (**Ans**) can be found (Bonfanti, 1999; Boldyrev et al., 2013). The HCD have different biological functions besides antioxidative activities. These functions include pH buffering, metal-ion chelation, complexing of dangerous carbonyl compounds, antiglycating and anticross-linking activities on proteins and His storage (Bonfanti, 1999; Baran, 2000; Velez et al., 2008; Hipkiss, 2010; Boldyrev et al., 2013; Bellia et al., 2014). Therefore, HCD seem to have a protective function, especially during cell stress and ischemia (Hipkiss, 2010).

Depletion of His and related dipeptides was described in studies about the outcome of WB and WS (Abasht et al., 2016; Sundekilde et al., 2017; Golzar Adabi and Demirok Soncu, 2019; Soglia et al. 2019). We hypothesized, that supplementing His could increase the pool of HCD. The objectives were to test a standalone feeding of His and a combination of His with βA, to measure the HSD concentration in the *pectoralis major* muscle in context with performance and meat quality. In view of the high impact of age on the outcome of breast myopathies, all variables were assessed at a commercial slaughter age of 33 d and also in older birds with 53 d.

MATERIALS AND METHODS

Study Design

For the trial, a total of 2,208 one-day old male Ross 308 broiler chickens were used to test 6 dietary treatments in a randomized block design. The feeding trial was carried out by feedtest (Wettin-Löbejün, Germany). Study implementation and sampling followed animal welfare regulations for commercial fattening and the animal care legislation under the guidelines of the EU Directive 2010/63/EU. All chickens were obtained from Geflügelhof Möckern, Germany. In each of the 96 pens in the trial barn, 23 birds were placed randomly. The pens, with a size of 2 × 1.5 m (3 m^2), were arranged in 16 blocks inside the experimental house, placed along 4 lines. Each block contained one pen per feeding group with were arranged randomly to minimize the environmental impact. Each pen was equipped with a bell drinker and a round hanging feeder. Wood shavings were used as litter. Feed and water were given *ad libitum* during 4 feeding phases (Table 1). Lightning and temperature program were managed as given by the breeder's recommendation and welfare legislation. All birds were vaccinated against Newcastle Disease and Gumboro at d 15 of life. No further intervention by the veterinarian was needed.

Feed Analysis The composition of the basal diet is given in Table 1. The basal feed was calculated as commercial diet with a standardized ileal digestible (**SID**) His:Lys ratio of 0.44 (**CON**) and supplemented with His (L-Histidine Base, food grade ≥ 98.5%, Europepta, Hannover, Germany) and βA (3-aminopropanoic acid, ≥ 98.0%, Europepta, Hannover, Germany) to obtain ratios of 0.54 (**HIS1**) and 0.64 (**HIS2**) in the different His feeding groups. According to the studies of Hoehler et al. (2005) and personal information, supplemented AA, like His, are about 100% ileal digestible amino acids. Other groups with the same SID His:Lys ratios were additionally supplemented with 0.5% βA (**BA_CON, BA_HIS1** and **BA_HIS2**). The SID His:Lys ratios and βA concentrations per feeding group are detailed in Table 2. The main feed ingredients, as well as the final feed for each feeding group at each feeding phase, were analyzed for their content of amino acids by the AMINONIR service of Evonik Nutrition & Care GmbH (Hanau-Wolfgang, Germany) as described by Fontaine et al. (2001, 2002). The results from analyzing the main feed ingredients were used to formulate the final diets using the software Brill Formulation (version v2.08.002, Format Solutions Inc., Hopkins, MN). Moreover, the content of βA was analyzed by using the wet chemistry service AMINOLab of Evonik Nutrition & Care GmbH. The wet chemistry method is based on the official regulations of the European Union (European Commission, 2009).

Performance Records The birds were weighted penwise at the first day and before each phase-related change of feed. The feed was also weighted before and after each feeding phase and the performance variables such as bird weight, FCR, average daily gain (**ADG**), average daily feed intake (**ADFI**), and mortality were calculated as mean value per pen and treatment group. Weight, FCR, ADG, and ADFI were corrected for mortality by dividing the average body weight of each pen by the number of animals left after each feeding phase.

Carcass Analyses After 33 and 53 d of age, respectively, 4 birds per pen were randomly selected and slaughtered in a commercial slaughterhouse (Gönnataler

Table 1. Composition of the basal feed for each feeding phase.

Ingredient, %	Starter (1 to 10 d)	Grower (11 to 20 d)	Finisher 1 (21 to 33 d)	Finisher 2 (34 to 54 d)
Corn	49.9	57.9	60.7	64.5
Soybean meal 47% CP	37.2	32.6	29.2	25.7
Corn gluten meal 60% CP	4.00	-	-	-
Soybean oil	4.25	5.12	6.11	5.88
Monocalciumphosphate	1.75	1.52	1.32	1.24
Limestone ($CaCO_3$)	1.37	1.28	1.17	1.13
Premix blank poultry[1]	0.50	0.50	0.50	0.50
MetAMINO (DL-Met)	0.27	0.29	0.26	0.26
L-Lysine-HCl	0.21	0.17	0.16	0.18
Salt (NaCl)	0.24	0.26	0.26	0.25
Sodium bicarbonate	0.19	0.17	0.17	0.18
Choline Chloride 60%	0.01	0.01	0.03	0.02
ThreAMINO (L-Thr)	0.05	0.07	0.06	0.08
ValAMINO (L-Val)	0.01	0.04	0.04	0.05
L-Ile	-	0.0037	0.01	0.03
Nutrient composition as calculated (analyzed), %				
AMEn, kcal/kg	3000	3100	3200	3225
CP	24.3 (25.3)	20.3 (21.2)	18.8 (19.0)	17.5 (17.8)
Calcium	0.96	0.87	0.78	0.74
Phosphate	0.79	0.71	0.65	0.62
Composition of calculated SID[2] amino acids (total analyzed amino acids), %				
Lys	1.28 (1.47)	1.11 (1.21)	1.02 (1.11)	0.95 (1.04)
Met	0.61 (0.63)	0.55 (0.54)	0.52 (0.51)	0.50 (0.49)
Cys	0.32 (0.39)	0.27 (0.30)	0.25 (0.30)	0.24 (0.28)
Met + Cys	0.93 (1.02)	0.82 (0.83)	0.77 (0.81)	0.74 (0.76)
Thr	0.81 (0.98)	0.71 (0.81)	0.66 (0.75)	0.63 (0.71)
Trp	0.25 (0.29)[3]	0.22 (0.25)[3]	0.20 (0.23)[3]	0.18 (0.21)[3]
Arg	1.43 (1.63)	1.23 (1.34)	1.13 (1.24)	1.03 (1.13)
Ile	0.92 (1.07)	0.77 (0.85)	0.72 (0.80)	0.68 (0.75)
Leu	1.96 (2.25)	1.51 (1.66)	1.43 (1.58)	1.35 (1.47)
Val	1.01 (1.17)	0.88 (0.95)	0.82 (0.91)	0.77 (0.85)
His	0.56 (0.63)	0.48 (0.51)	0.45 (0.48)	0.42 (0.45)

[1]Composition of Premix Blank Poultry (per kg premix): Vitamin A (retinyl acetate) 2,000,000 IU; Vitamin D_3 (cholecalciferol) 500,000 IU; Vitamin E (dl-α-tocopherol) 10 g; Vitamin K_3 (menadione) 0.3 g; Vitamin B_1 (thiamin) 0.4 g; Vitamin B_2 (riboflavin) 1.5 g; Vitamin B_6 (pyridoxine-HCl) 0.7 g; Vitamin B_{12} (cyanocobalamin) 4 mg; Niacin 7 g; D-pantothenic acid 2.4 g; Choline chloride 92 g; Folic acid 0.2 g; Biotin 40 mg; Iron (as $FeSO_4*H_2O$) 16 g; Copper (as $CuSO_4*5\ H_2O$) 2.4 g; Manganese (as MnO) 17 g; Zinc (as $ZnSO_4*H_2O$) 12 g; Iodate (as KJ) 0.16 g; Selenium (as Na_2SeO_3) 30 mg.
[2]SID, standard ileal digestible.
[3]As calculated.

Table 2. Supplemented His and β-Ala concentrations in relation to the basal diet in the different dietary groups as analyzed.

	Feeding group					
Feeding phase	CON[1]	HIS1[2]	HIS2[3]	BA[4]	BA_HIS1[5]	BA_HIS2[6]
Supplemented His as analyzed, %						
Starter	nd[7]	0.12	0.25	nd	0.12	0.25
Grower	nd	0.11	0.21	nd	0.11	0.22
Finisher 1	nd	0.10	0.20	nd	0.10	0.19
Finisher 2	nd	0.10	0.18	nd	0.10	0.18
Reached SID[8] His:Lys ratio in the diet	0.44	0.54	0.64	0.44	0.54	0.64
Supplemented β-alanine as analyzed, %						
Starter	nd	nd	nd	0.47	0.49	0.50
Grower	nd	nd	nd	0.50	0.50	0.52
Finisher 1	nd	nd	nd	0.48	0.50	0.51
Finisher 2	nd	nd	nd	0.49	0.49	0.48
Reached β-alanine concentration in the diet, %	0	0	0	0.5	0.5	0.5

[1]His:Lys ratio 0.44 of standard ileal digestible amino acid.
[2]His:Lys ratio 0.54 of standard ileal digestible amino acid.
[3]His:Lys ratio 0.64 of standard ileal digestible amino acid.
[4]His:Lys ratio 0.44 of standard ileal digestible amino acid + 0.5% total β-alanine.
[5]His:Lys ratio 0.54 of standard ileal digestible amino acid + 0.5% total β-alanine.
[6]His:Lys ratio 0.64 of standard ileal digestible amino acid + 0.5% total β-alanine.
[7]nd, not detectable by analytical method (< 0.01%).
[8]SID, standard ileal digestible.

Putenspezialitäten GmbH, Altengönna, Germany). The carcasses were cooled to 2 to 4°C in the cooling facility of the slaughterhouse until processing. The carcasses were dissected manually after 12 h (33 d slaughter age) and 24 h (53 d slaughter age), respectively. The breasts, thighs, wings, and the rest of the carcass were weighted. The proportion of breast and thighs were calculated as percentage of the whole carcass.

Meat Quality Analyses

Sixty left fillets per treatment group were randomly selected for meat quality analyses and packed separately in plastic bags. They were shipped under temperature-controlled conditions (2−4°C) to the University of Bonn (Institute of Animal Science, Bonn, Germany). The fillets were aerobically packed individually in polypropylene trays with lids and stored at 4°C in low-temperature high-precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan), controlled by data loggers in intervals of 3 min (ES-CORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand). Meat quality data was collected 48, 96, 144, and 192 h after slaughter. At each of these investigation points, 15 randomly selected fillets per treatment group were analyzed. The analyses comprised a sensory assessment and physiochemical parameters. Water loss during cooking was measured at the beginning and end of storage (48 and 192 h, respectively). Every fillet was weighted after analysis, frozen at −20°C and used for measuring thiobarbituric acid reactive substances (**TBARS**).

Sensory Analysis of the Meat The sensory measurement was based on the method described by Albrecht et al. (2019a,b). In brief, a panel of 6 trained persons evaluated the meat quality and shelf life of the samples. A dichotomous purchase decision (1-yes / 0-no) was assessed for each fillet prior to the other analyses to avoid influences by smell. The percentage for a positive decision for each fillet was calculated. Afterward, the sensory evaluation of color, texture, and smell was conducted using a 3-point scale (3: good quality, 2: acceptable, and 1: unacceptable). Intermediate values (2.5 and 1.5) were allowed. These data were used to calculate the sensory index (**SI**) for every fillet according to Albrecht et al. (2019a). The acceptance level was set at SI ≤ 1.8 based on previous microbiological and sensory trials. The mean value for each group at each time point of storage was calculated. The mean values were plotted as a function of time and fitted to a linear model and the shelf life was also calculated according to Albrecht et al. (2019a). White striping was evaluated by a 3-grade scale, (0: no or little, thin white stripes, 1: moderate white stripes, and 2: numerous big white stripes). Unusual appearances of the fillets (e.g., hemorrhages, spaghetti meat, and spider veins) were noted.

Measurement of the pH The pH-value was measured on the surface of the fillets by using 2 calibrated portable pH-meters (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany; GPH114, GHM Messtechnik GmbH Standort Greisinger, Regenstauf, Germany). The value was measured in the caudal, middle, and cranial part of the fillets and the mean value was calculated for each fillet.

Water Loss During Cooking The parameter was measured by using the inner fillets of the breast samples. The inner fillets were carefully separated from the *pectoralis major* muscle and weighted, packed separately in plastic bags and cooked at 80°C in a water bath (Memmert, Schwabach, Germany) until the core temperature reached 72°C. The core temperature was tested with a food core thermometer (Testo, Lenzkirch, Germany). The inner fillets were weighted again after cooking. Cooking loss was calculated according to Albrecht et al. (2019a).

Freezing Loss The fillets were weighted before freezing and after thawing to calculate the freezing loss as difference of the weights divided by initial fillet weight given as percentage.

Thiobarbituric Acid Reactive Substance In the middle of the fillet, a part was cut out with a punch (8 × 4 cm, 32 cm^2). The surface was discarded and 7 g of each sample were minced (Moulinex XXL, DP800G, 1000 W, Moulinex Groupe SEB Deutschland GmbH, Frankfurt am Main, Germany) and finally homogenized on ice with 15 mL homogenizing buffer (7.5% TCA, 6.844 mL EDTA 0.5 M pH 8, 1 g propylgallat powder, Merck KGaA, Darmstadt, Germany) by using a dispersing device for 1 min (IKA Ultra-Turrax, TP 18/10, 220 V, 170 W, 20,000 1/min, with rod S 25 N -18 G, Janke & Kunkel GmbH & Co KG, Staufen im Breisgau, Germany). The 0.5 M EDTA solution for the homogenizing buffer was obtained by the dissolution of 46.53 g EDTA in 250 mL double distilled water. The pH was set by using NaOH. The homogenate was mixed with 10 mL homogenizing buffer. The samples were then centrifuged (15 min 2,000 rpm, 4°C; Thermo Scientific Heraeus Primo R, Thermo Fisher Scientific GmbH, Dreieich, Germany), filtered (What-man, grade 4, 125 mm, cellulose), and frozen at −80°C until TBARS measurement. A 2 μM malondialdehyde (**MDA**) solution (MDA ≥ 96%, 313.52 g/mol, Sigma-Aldrich, St. Luis, MO) was used to generate a standard range out of different, defined concentrations. One part of the samples was mixed with 2 parts TCA 10%. Eighty μL of this solution were mixed with 120 μL water and 200 μL 0.4% TBA solution. The samples were heated in a water bath for 60 min at 100.5°C, cooled in a centrifuge for 2 min (4,000 rpm, 4°C) and left on room temperature for 5 min. The samples were then directly pipetted in black bottom plate wells (FLUOTRAC 655076, 96 well-plate, Greiner Bio-One In-ternational GmbH, Kremsmünster, Austria) and fluorescence was measured at ex/em 515/553 (Synergy H1 Hybrid Multi-Mode Reader, BioTek Instruments, Inc., Winooski, VT).

Meat Color The color of the fillets was measured based on the CIE 1976 L*a*b scale with a large view spectrometer (MiniScan EZ 4500L, HunterLab, Marnau am Staffelsee, Germany). The measurement was done with a

wavelength between 400 and 700 nm, geometry of 45°/0° by using a D65 illuminant (6,500 K daylight). Each fillet was measured in the cranial, middle, and caudal region. The spectrometer was cleaned after each fillet. The mean value for the L*a*b values for each fillet was calculated.

Analysis of Amino Acids, Carnosine, and Anserine

The concentrations of the amino acids His and βA and the dipeptides Car and Ans were measured in blood plasma and the *pectoralis major* muscle. In addition, 1-methylhistidine (**1MHis**) and 3-methylhistidine (**3MHis**) were analyzed in the plasma samples. To obtain the samples, 10 randomly selected birds per treatment were slaughtered in the trial barn at 35 and 54 d of age, respectively. Blood samples were taken from all 10 birds. Per treatment, 5 birds were used to generate samples of the *pectoralis major* muscle.

Analysis of Blood Samples The samples were taken directly after decapitation from the neck vein. The preparation of the samples was carried out by feedtest (Wettin-Löbejün, Germany). The blood samples were transferred into Na heparin vacutainers for further processing. The blood was carefully mixed with the heparin by inverting the vacutainers and for 5 to 10 min on a roller mixer. The samples were chilled on dry ice for 2 min and centrifuged (1,500 *g*, 4°C, and 10 min). Afterward, 1.5 mL plasma were transferred to an empty vacutainer and freeze dried at −80°C. The vacutainer was weighted before and after freeze-drying. The samples were stored at −20°C until shipped on dry ice to Evonik Nutrition & Care GmbH. The amino acid profile of freeze-dried blood plasma was finally analyzed with an internal method of Evonik (AA 10-029 Version 4 *Laborvorschrift: Quantitative Bestimmung der freien Aminosäuren in physiologischen Flüssigkeiten*).

Analysis of Muscle Tissue Around 3 g tissue samples of the *pectoralis major* muscle were cut out of the middle of the cranial region directly after bleeding. The samples were directly frozen in liquid nitrogen, shortly stored at −20°C, shipped on dry ice to Metabolon Inc. (Morrisville, NC), and finally stored at −80°C. The analysis was performed by Metabolon Inc. About 200 mg of the tissue samples were used for the analysis and mixed with 200 μL of a standard solution (carnosine-d_4, anserine-d_4, histidine-$^{13}C_6$, β-alanine-$^{13}C_3$, ^{15}N) in 500 μL of acidified methanol containing 1% formic acid. The samples were homogenized by using glass beads and a tissue homogenizer (SPEX 2010 Geno/Grinder, SPEX SamplePrep). The samples were centrifuged, and the supernatant was used for liquid chromatography mass spectrometry/mass spectrometry (Agilent 1290/AB Sciex QTrap5500 LC MS/MS system, UHPLC BEH C18 column 2.1 × 100 mm 1.7 um). For separation, ion pair chromatography was used. The MS operated in positive mode by using electrospray ionization in MRM mode. The raw data were collected and processed by using the software Analyst 1.6.2 (SCIEX). Data were normalized to tissue weight. The used peak areas were measured against the areas of the used standards. A calibration curve, created by weighted least square regression, was used to determine the concentrations of the measured molecules.

Data Analyses

The data were analyzed by using Minitab18 (Minitab Inc., State Collage, PA). The significance for all tests was declared at $P \leq 0.05$. A tendency was declared at $P \leq 0.10$. All continuous data were tested for normal distribution with the *Anderson-Darling* method and the homogeneity of variances was tested by the *Levene's* test. All data of the 2 × 2 × 3 (Age x His:Lys ratio x βA supplementation) trial arrangement were analyzed for the factors His:Lys ratio and βA supplementation separately for both slaughter ages with the general linear model for ANOVA. With this test, the main effects of His and βA were tested as well as the interaction of both compounds, which is equivalent to a two-way ANOVA. The interaction term was removed when the *P*-value was not significant to adjust the *P*-values for the main effects. Additionally, the term for a main effect was also removed if not significant to improve the *P*-value for the other main effect. To find out which group differed, the one-way *Welch*-ANOVA protocol with the *Games-Howell* post-hoc test was applied for the His supplementations. Differences in βA supplementations were given by a *t*-test or Mann-Whitney-U test. Slaughter age was separately analyzed for all data as single factor by using a *t*-test or the nonparametric Mann-Whitney-U test. Moreover, the factor storage time was also separately analyzed for all meat quality parameters per feeding group. For this analysis the one-way *Welch*-ANOVA was used. To describe the statistical relevance of discrete data, measured during the sensorial evaluation of the fillets, the Chi-square test was performed. All data of the panel evaluation of white striping were pooled for this analysis. For the correlation of discrete data with other meat quality, the mean value of all testers was used to create continuous data. The correlation of different parameters was tested by using the *Spearman* method.

RESULTS

Performance

By comparing the His:Lys ratios and βA levels, as shown in Table 3, there were no interaction effects observed for all performance parameters; however, the main effects His and βA supplementation on mortality, ADFI and the proportion of breast and thigh per carcass were significant. The groups receiving 0.5% βA had lower ADFI than the groups without βA supplementation at both slaughter ages and tended to have lesser body weights and ADG. Breast yield was lower at both ages in broilers supplemented with βA, whereas thigh yield was

Table 3. Influence of the different supplements on performance and slaughter variables at different slaughter ages.

		SID[1] His:Lys ratio			β-Alanine			Probabilities		
Parameter	Age, d	0.44	0.54	0.64	0%	0.5%	SEM	His	β-Alanine	Int.[2]
Weight, g	1	42.8	42.8	42.8	42.8	42.8	0.04	0.927	0.822	0.928
Weight, g	33	2310	2319	2325	2336	2300	9.46	0.801	0.061	0.660
ADG, g/d		68.7	69.0	69.2	69.5	68.4	0.29	0.796	0.063	0.659
ADFI, g/d		98.1[b]	99.2[a]	99.9[a]	99.9[x]	98.3[y]	0.30	0.047 (0.046)[5]	0.005 (0.005)[5]	0.566
FCR[3], g/g		1.43	1.44	1.45	1.44	1.44	0.003	0.165	0.863	0.754
Mortality, %		1.7	2.5	2.6	2.2	2.4	0.33	0.284	0.570	0.164
Breast[4], %		27.8	27.6	27.9	28.1[x]	27.5[y]	0.08	0.393	<0.001 (<0.001)[6]	0.754
Thigh[4], %		30.8	30.9	31.0	30.8[y]	31.1[x]	0.06	0.528	0.004 (0.004)[6]	0.712
Weight, g	53	4402	4370	4380	4420	4348	19.20	0.785	0.064	0.467
ADG, g/d		82.2	81.6	81.8	82.6	81.2	0.36	0.783	0.063	0.478
ADFI, g/d		141.8	142.0	142.9	143.8[x]	140.7[y]	0.54	0.656	0.004 (0.004)[6]	0.223
FCR, g/g		1.72	1.74	1.75	1.74	1.73	0.005	0.172	0.419	0.959
Mortality, %		4.2[b]	8.1[a]	7.8[a]	6.7	6.8	0.68	0.044 (0.039)[6]	0.601	0.813
Breast[4], %		29.6	29.3	29.5	29.7[x]	29.3[y]	0.09	0.504	0.050 (0.048)[6]	0.544
Thigh[4], %		31.4	31.6	31.5	31.4	31.6	0.07	0.594	0.137	0.397

[a-c]Means within a row lacking a common superscript differ between used SID His:Lys ratios ($P < 0.05$).
[x-y]Means within a row lacking a common superscript differ between used β-alanine levels ($P < 0.05$).
[1]SID, standard ileal digestible.
[2]Int., interaction.
[3]FCR, feed conversion ratio.
[4]For breast and thigh evaluation the portion of breast and thigh per carcass weight was used.
[5]The interaction term was removed from the general linear model.
[6]The interaction term and one main term was removed from the general linear model.

higher only at 33 d of age. The influence of dietary SID His:Lys was limited to ADFI and mortality. The ADFI at d 33 was lower in treatments receiving 0.44 SID His:Lys compared to the higher levels of His. This was not seen in birds slaughtered at 53 d of age. The overall mortality after 53 d of age was lower when feeding a SID His:Lys ratio of 0.44 compared to higher ratios.

Amino Acid Profile

The age of the birds affected the concentrations of His, Car, and Ans in the breast muscle tissue. Broilers slaughtered at 54 d had greater His (35 d = 11.7 ± 1.4 μg/g, 54 d = 14.7 ± 1.3 μg/g, $P = 0.019$) and Ans (35 d = 6,071 ± 128 μg/g, 54 d = 6,585 ± 225 μg/g, $P =$

Table 4. Effect of the feeding groups on dipeptide and amino acid concentration in breast tissue and blood plasma.

		SID[1] His:Lys ratio			β-Alanine			Probabilities		
Parameter	Age, d	0.44	0.54	0.64	0%	0.5%	SEM	His	β-Alanine	Int.[2]
Concentration per total weight in breast tissue, μg/g										
His	35	3.0[c]	10.3[b]	21.8[a]	12.0	11.4	1.4	< 0.001 (< 0.001)[6]	0.720	0.533
β-Alanine		534[b]	104[a]	103[a]	172[y]	322[x]	32	< 0.001	< 0.001	0.048
Carnosine		2200[b]	4957[a]	5043[a]	4055	4078	222	< 0.001 (< 0.001)[6]	0.936	0.066
Anserine		5702	6337	6173	5910	6231	128	0.114	0.212	0.925
His	54	5.6[b]	18.8[a]	19.7[a]	14.1	15.3	1.3	< 0.001 (< 0.001)[6]	0.540	0.272
β-Alanine		288[a]	136[b]	137[b]	144[y]	230[x]	18	< 0.001 (< 0.001)[5]	0.004 (0.004)[5]	0.554
Carnosine		2210[b]	2226[b]	4184[a]	2514	3233	221	< 0.001(< 0.001)[6]	0.054	0.304
Anserine		6773	6045	6938	6315	6855	225	0.219	0.223	0.169
Concentration in blood plasma, μg/mL										
His	35	7.1[c]	14.5[b]	23.2[a]	15.4	14.4	0.11	< 0.001 (< 0.001)[6]	0.452	0.112
β-Alanine		20.5[a]	16.7[a]	14.5[b]	3.3[y]	31.2[x]	0.19	< 0.001	< 0.001	0.036
Carnosine		1.3[b]	4.0[a]	4.0[a]	2.5[y]	3.7[x]	0.03	< 0.001 (< 0.001)[5]	0.005 (0.004)[5]	0.563
Anserine		3.0[b]	4.5	4.4[a]	3.6	4.3	0.02	0.021 (0.020)[6]	0.163	0.687
1MHis[3]		2.8[c]	3.7[b]	5.1[a]	3.9	3.8	0.02	< 0.001 (< 0.001)[6]	0.737	0.425
3MHis[4]		0.7[c]	2.3[b]	3.2[a]	2.0	2.2	0.02	< 0.001 (< 0.001)[6]	0.457	0.420
His	54	8.7[b]	17.7[a]	21.0[a]	14.9	16.7	0.11	< 0.001 (< 0.001)[6]	0.302	0.531
β-Alanine		12.1	8.5	12.0	3.6[y]	18.1[x]	0.12	0.108	< 0.001 (< 0.001)[6]	0.496
Carnosine		1.1[b]	3.1[a]	3.5[a]	2.5	2.6	0.03	< 0.001 (< 0.001)[6]	0.784	0.425
Anserine		6.2	6.6	5.5	6.8	5.4	0.05	0.679	0.153	0.657
1MHis		3.0[b]	6.0[a]	5.7[a]	4.9	4.8	0.03	< 0.001 (< 0.001)[6]	0.776	0.614
3MHis		1.9[b]	4.8[a]	4.1[a]	3.3	3.9	0.03	< 0.001 (< 0.001)[6]	0.120	0.858

[a-c]Means within a row lacking a common superscript differ between used SID His:Lys ratios ($P < 0.05$).
[x-y]Means within a row lacking a common superscript differ between used β-alanine levels ($P < 0.05$).
[1]SID, standard ileal digestible.
[2]Int., interaction.
[3]1MHis, 1-methylhistidine.
[4]3MHis, 3-methylhistidine.
[5]The interaction term was removed from the general linear model.
[6]The interaction term and one main term was removed from the general linear mode.

0.003) concentrations, whereas Car (35 d = 4,067 ± 222 μg/g, 54 d = 2,873 ± 221 μg/g, $P < 0001$) was less concentrated than in birds slaughtered at 35 d. The content of βA was not affected by age (35 d = 247 ± 32 μg/g, 54 d = 187 ± 18 μg/g). While there were few interaction effects, there were significant main effects of dietary His and βA supplementation on the amino acid and dipeptide concentrations in breast tissue (Table 4). At both slaughter ages, the level of His in breast tissue was higher when higher SID His:Lys ratios were fed, whereas βA did not affect the His content. At 35 d of age, the His concentration in the breast tissue increased with each level of SID His:Lys ratio. At 54 d the dietary SID His:Lys level of 0.54 significantly increase breast tissue His, but this was not further improved in the 0.64 SID His:Lys treatment. The concentration of βA in breast tissue was greater when supplementing 0.5% βA compared to the control and lower when feeding higher SID His:Lys ratios of 0.54 and 0.64 compared to 0.44. Moreover, an interaction of His and ßA supplementation was observed for breast βA concentration in 34 d old birds. Car concentration in breast tissue was higher in the 0.54 and 0.64 SID His:Lys ratios than the control ratio of 0.44 at d 35, while only the 0.64 ratio increased Car compared to control at d 54. There was no effect of βA supplementation on detectable Car in 34 and 54 d old birds; however, at 54 d of age, there was a tendency for 0.5% βA supplementation to increase Car concentrations in breast tissue. The concentration of Ans in breast tissue was not affected by any treatment at any age.

Independently of age, the overall concentrations of all measured molecules in blood plasma were lower than in breast muscle, except the concentration of His (Table 4). Comparing the different slaughter ages, the content of Ans (35 d = 3.9 ± 0.2 μg/mL, 54 d = 6.2 ± 0.5 μg/mL, $P < 0.001$) and the methylated forms of His, 1MHis (35 d = 3.9 ± 0.2 μg/mL, 54 d = 4.9 ± 0.3 μg/mL, $P = 0.006$) and 3MHis (35 d = 2.1 ± 0.2 μg/mL, 54 d = 3.7 ± 0.3 μg/mL, $P < 0.001$), were higher in blood plasma of 54 d compared to 34 d old birds, whereas the βA concentration was significantly lower (35 d = 17.2 ± 1.9 μg/mL, 54 d = 10.4 ± 1.2 μg/mL, $P = 0.007$). The overall Car and His concentrations in blood plasma were not affected by age (Car 35 d = 3.1 ± 0.3 μg/mL, 54 d = 2.6 ± 0.3 μg/mL; His 35 d = 14.9 ± 1.1 μg/mL, 54 d = 15.7 ± 1.1 μg/mL). The effects of the different supplementations on the amino acid and dipeptide concentrations in blood plasma are also given in Table 4. The supplementation of βA showed no influence on the concentrations of His, as well as 1MHis and 3MHis in blood plasma at either age. At 35 d of age, the concentration of His and both methylated forms were increased with each higher SID His:Lys ratio. In 54 d old birds, the concentration was increased with supplementation of His, but without difference between the 0.54 and 0.64 of SID His:Lys ratios. Moreover, detectable βA in the blood plasma was higher with a supplementation of βA in the diet at both ages. In 35 d old birds, there was an interaction effect between dietary SID His:Lys ratios and βA supplementation on plasma βA level. At this age, the highest SID His:Lys increases βA in blood plasma. In 54 d old birds, there was no interaction effect or main effect of His supplementation on plasma βA concentration. The dipeptide Car was affected by the SID His:Lys ratio in feed at both slaughter ages. A higher concentration of plasma Car was observed in both treatments with 0.54 and 0.64 of SID His:Lys ratios compared to the control ratio of 0.44 at both slaughter ages, with no differences between the 2 higher ratio treatments. The amount of

Table 5. Coefficients of correlation (*Spearmann's rank correlation,* r_s) between amino acid and dipeptide concentrations in breast muscle and blood plasma.

		Parameter				
Parameter	Age, d	His	β-Alanine	Carnosine	Anserine	1MHis[1]
Concentration per breast tissue weight, μg/g						
β-Alanine	35	−0.668***				
Carnosine		0.468***	−0.630***			
Anserine		0.164	−0.116	0.139		
β-Alanine	54	−0.323*				
Carnosine		0.029	−0.358**			
Anserine		−0.412**	−0.214	0.494***		
Concentration in blood plasma, μg/mL						
β-Alanine	35	−0.193				
Carnosine		0.739***	0.044			
Anserine		0.372**	0.078	0.603***		
1MHis		0.776***	−0.125	0.623***	0.486***	
3MHis[2]		0.872***	−0.182	0.731***	0.453***	0.808***
β-Alanine	54	−0.044				
Carnosine		0.680***	−0.050			
Anserine		0.241	−0.028	0.455***		
1MHis		0.748***	−0.215	0.647***	0.537***	
3MHis		0.757***	−0.016	0.618***	0.386**	0.777***

The strength of correlation is given as low when $r_s \leq 0.1$, medium when $r_s \leq 0.3$ and high when $r_s \leq 0.5$.
P-values are ranked by using: *for $P \leq 0.5$, **for $P \leq 0.01$ and ***for $P \leq 0.001$.
[1]1-methylhistidine.
[2]3-methylhistidine.

βA in the feed showed an effect on detectable Car in blood plasma of 35 d old birds, as more βA resulted in higher amounts of this dipeptide. Finally, the highest SID His:Lys ratio treatment increased Ans in blood plasma of 35 d old birds, compared to the control ratio of 0.44, but no other effects of the supplementations were detected for this dipeptide.

The concentrations of all tested amino acids and dipeptides in the breast muscle and blood plasma were correlated. The *Spearmann's* rank factor r_s and the related significance are given in Table 5. A higher value of His in the breast tissue was related to a lower level of detectable βA at both slaughter ages. This correlation was stronger for 35 d old birds than for 54 d old ones. Moreover, Ans content in breast tissue was negatively correlated with the content of His in 54 d old birds. A positive correlation was found between His and Car at 35 d of age. The concentration of βA in breast tissue was correlated with Car at both ages, where more Car in breast tissues resulted in less βA. Car and Ans showed a moderate positive correlation relation in 54 d old birds. In blood plasma, the concentration of βA showed no correlation with any other analyzed molecule. Except βA, all molecules showed correlations between each other. The concentration of Car was strongly correlated with His, Ans, and both methylated forms of His at both ages. These correlations were all positive, indicating more of these molecules in blood plasma with more detectable Car. The same correlations were found for 1MHis and 3MHis. These molecules were related to all measured dipeptides and His. The positive correlation with His and Car and the methylated forms was strong. Ans was also positively related to His, Car, 1MHis, and 3MHis, except His measured in blood samples of birds with 54 d of age.

Meat Quality Analysis

The examination of the meat quality in breast fillets indicated few differences between the slaughter ages, different supplementations, or storage times. Most of the variation was noticed when comparing the slaughter ages. The meat quality data pH and TBARS for all fillets, analyzed after 48 and 192 h of storage, are shown in Figure 1, separated by feeding group.

When all storage times were pooled, the mean pH value of all analyzed fillets was higher for fillets of birds slaughtered at 53 d compared to 33 d, (pH 33 d = 6.16, pH 53 d = 6.32, $P < 0.001$), whereas groups-wise all groups, except of CON and HIS1 showed differences between the ages (pH HIS2: 33 d = 6.15, 53 d = 6.32, $P = 0.025$; pH BA_CON: 33 d = 6.13, 53 d = 6.34, $P = 0.002$; pH BA_HIS1: 33 d = 6.10, 53 d = 6.27, $P = 0.005$; pH BA_HIS2: 33 d = 6.15, 53 d = 6.41, $P = 0.001$). No impact of a βA or His supplementation on pH was seen after 48 h of storage at both slaughter ages. The fillets of birds slaughtered at 33 d of age and measured after 192 h of storage showed lower pH values ($P = 0.001$) when fed with 0.5% βA (pH = 6.51) than

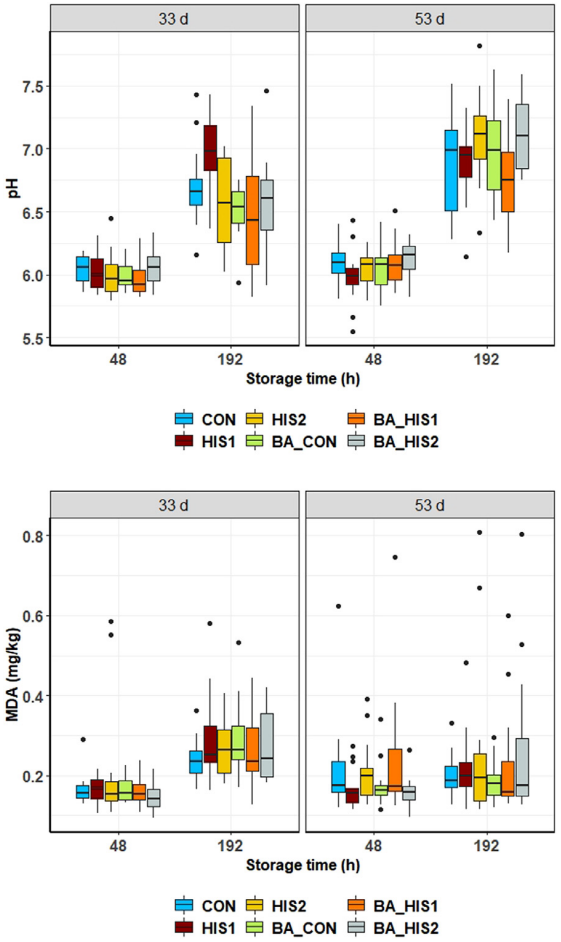

Figure 1. Measured pH and malondialdehyde equivalents in broiler breast fillets assessed at 33 and 53 d of age and at 48 and 192 h after slaughter. Abbreviations: BA_CON, His:Lys ratio 0.44 of standard ileal digestible amino acid + 0.5% total β-alanine; BA_HIS1, His:Lys ratio 0.54 of standard ileal digestible amino acid + 0.5% total β-alanine; BA_HIS2, His:Lys ratio 0.64 of standard ileal digestible amino acid + 0.5% total β-alanine; CON, His:Lys ratio 0.44 of standard ileal digestible amino acid; HIS1, His:Lys ratio 0.54 of standard ileal digestible amino acid; HIS2, His:Lys ratio 0.64 of standard ileal digestible amino acid; MDA, malondialdehyde equivalents.

fillets of birds without βA in the feed (pH = 6.75). Moreover, an interaction of SID His:Lys and βA was seen ($P = 0.008$). His supplementation showed an impact on fillets of 53 d old birds after 192 h of storage. These fillets had a lower pH ($P = 0.005$) when fed with a SID His:Lys ratio of 0.54 (pH = 6.83) compared to 0.64 (pH = 7.12). The supplementations at all other storage times did not affect the pH-value, but in all feeding groups the pH values increased at the end of storage (192 h) at both slaughter ages ($P \leq 0.007$) as shown in Figure 1.

The statistical analysis of TBARS in the breast tissue, as displayed in Figure 1, showed no influence of slaughter age. In all groups of birds slaughtered at 33 d of age, the concentration of MDA equivalents was higher after 192 h of storage compared to the fillets measured 48 h after slaughter ($P \leq 0.003$). No other differences in TBARS were noticed.

The analysis of the meat color by the L*a*b* scale showed no difference in lightness of the fillets between

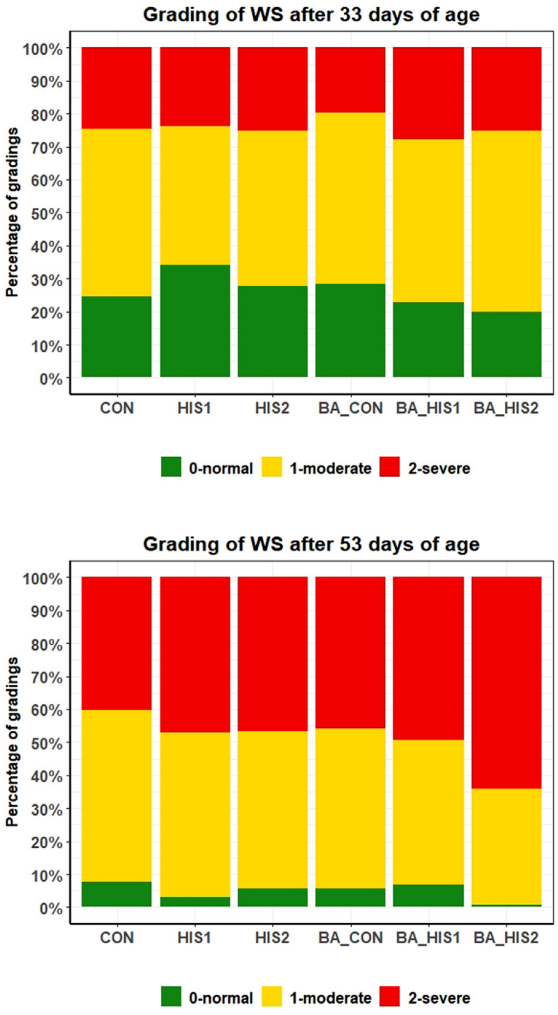

Figure 2. Percentages of fillets affected by the white striping condition. The fillets were graded as either 0-normal, 1-moderate affected, or 2-severely affected. Abbreviations: BA_CON, His:Lys ratio 0.44 of standard ileal digestible amino acid + 0.5% total β-alanine; BA_HIS1, His:Lys ratio 0.54 of standard ileal digestible amino acid + 0.5% total β-alanine; BA_HIS2, His:Lys ratio 0.64 of standard ileal digestible amino acid + 0.5% total β-alanine; CON, His:Lys ratio 0.44 of standard ileal digestible amino acid; HIS1, His:Lys ratio 0.54 of standard ileal digestible amino acid; HIS2, His:Lys ratio 0.64 of standard ileal digestible amino acid; WS, white striping.

the different His and βA levels in feed. Fillets showed higher redness and yellowness in older birds (a*: 33 d = 5.6, 53 d = 6.7, $P < 0.001$; b*: 33 d = 12.7, 53 d = 13.4, $P < 0.001$). This difference was seen in all groups for redness. Group CON and HIS1 were not different in yellowness, whereas all other groups showed higher values in older birds (data not shown). In fillets of 33 d old birds, the supplementation of βA reduced the redness of the fillets after 192 h (L*: βA 0% = 51.2, 0.5% = 52.0, $P < 0.001$). An interaction of βA and His supplementation was also detected ($P = 0.015$). The yellowness of the fillets was affected by His and βA supplementation, as well as interactions ($P = 0.035$) of these parameters for fillets of 33 d old birds and after 192 h of storage. The SID His:Lys ratios of 0.44 and 0.54 had a higher yellowness compared to 0.64 (b*: SID His:Lys 0.44 = 15.5, 0.54 = 15.7, 0.64 = 13.8, $P = 0.001$). In addition, a supplementation of βA also lowered the yellowness in these fillets (b*: βA 0% = 16.1, 0.5% = 13.9, $P < 0.001$). In fillets of 53 d old birds, the fillets showed higher values of yellowness with a ratio of 0,64 than with 0.44 (b*: SID His:Lys 0.44 = 14.9, 0.64 = 16.0, $P = 0.030$) after 192 h of storage. A change by comparing the storage times was only seen for yellowness, which was increased in all fillets stored 192 h compared to 48 h (33 d: b* 48 h = 11.2, b* 192 h = 15.0, $P < 0.001$; 53 d: b* 48 h = 13.0, b* 192 h = 15.3, $P < 0.001$) except of CON and BA_CON in fillets of 53 d old birds.

The overall water loss during cooking of the inner fillets was influenced by slaughter age (33 d = 16.0%, 53 d = 19.0%, $P < 0.001$). The fillets of 33 d old birds had greater cooking losses when SID His:Lys 0.64 was used as compared to 0.44 (SID His:Lys 0.44 = 16.5%, 0.64 = 18.3%, $P = 0.008$) and with βA supplementation (βA 0% = 16.9%, 0.5% = 18.3%, $P = 0.008$) with interaction of both factors ($P = 0.002$). The overall cooking loss was increased with storage time (48 h = 15.7%, 192 h = 19.2%, $P < 0.001$) and the same was seen by comparing the feeding groups (data not shown), with exception of HIS1 and BA_CON at 33 d of age. Thawing loss was only affected by slaughter age (33 d = 3.6%, 53 d = 2.8%, $P < 0.001$) and showed lower values in all feeding groups for fillets of 33 d old birds (data not shown).

The analysis of the WS condition showed a higher incidence of WS in the fillets of 53 d old birds. Indeed, 73.8% of the fillets of birds slaughtered at 33 d had moderate or severe cases of WS, whereas in the slaughter group after 53 d of age, 95.1% of the birds were affected by this condition ($P < 0.001$), as seen in Figure 2. Also, the proportion of severe cases was increased from 24.3% at 33 d of age to 48.9% in 53 d old birds ($P < 0.001$). Furthermore, the frequency of this condition was increased with storage time at both ages ($P \leq 0.019$). Indeed, 45.7% of the fillets of birds slaughtered at 33 d were graded as normal 48 h after slaughter (6.0% fillets of 53 d old birds) but after 192 h of storage, this percentage decreased to 13.7% (4.3% of 53 d old birds). Regarding the different SID His:Lys ratios and the supplementation of βA, an increased incidence of WS was noticed when 0.5% βA was fed, at both slaughter ages ($P \leq 0.013$). The various SID His:Lys ratios showed no effect in 33 d old birds by using the 2-way ANOVA. At 53 d of age, the number of fillets with WS condition increased with higher SID His:Lys ratios ($P < 0.001$). Indeed, with a ratio of SID His:Lys of 0.44, 6.8% of the fillets had no WS, whereas the percentage of normal fillets decreased to 4.9% with a ratio of 0.54 and reached only 3.1% with a 0.64 ratio. In addition, the analysis of the WS evaluation, as given in Figure 2, showed an effect of the feeding groups ($P < 0.001$) on the WS occurrence at both ages and the moderate feeding of His after 33 d of age had the maximal positive impact. Compared to CON, 9.4% more fillets of group HIS1 were graded as normal and severe cases were decreased by −0.9%.

Regarding the purchase decision of the sensory panel, it appears to be related to the storage time of the fillets at both ages ($P < 0.001$). The percentage of 'no'

increased up to 99% after 144 h of storage. Another influence on purchase decision was the age of the birds at slaughter. For the fillets stored 48 h and 96 h, a negative effect on the decision of birds slaughtered at 53 d of age was detected ($P < 0.001$). Indeed, 48 h after slaughter, 68% of the fillets of 33 d old birds and 91% of 53 d old birds were graded as 'no' and after 96 h, 89% of the fillets of 33 d old birds and nearly all fillets of 53 d old birds (99%) were graded in the same way. Fillets of 53 d old birds showed more hemorrhages and reddish or yellow parts at the surface than 33 d old birds. The thickness, the high incidence of white striping and the hardness of many fillets were also mentioned by the panel as reasons not to buy those fillets. When grading fillets of birds slaughtered at 33 d of age and 48 h after slaughter, the feeding group and the SID His:Lys ratio had an influence on the panel's decision ($P < 0.001$). The 0.44 ratio was related to 45% positively rated fillets, the 0.64 ratio to 30% and the birds supplemented with a 0.54 ratio showed the lowest approval of the panel with only 21%. Group-wise, fillets from CON and BA_HIS2 groups had a positive evaluation from nearly half of the panel, with 51 and 46% of positive grades respectively, followed by BA_CON with 39% and HIS1 with 28%. The lowest rating was given to fillets from HIS2 and BA_HIS1 (16 and 14%, respectively). The βA supplementation had no effect. Feeding groups, SID His:Lys ratios or βA supplementation had no influence on the decision concerning fillets of birds slaughtered after 53 d of age.

The SI values were lower with increased storage time and progressing spoilage at both ages ($P < 0.001$). No differences between the feeding groups were seen when analyzing the group-wise SI values at both ages. The calculated shelf life was higher in fillets of birds slaughtered after 33 d of age. The overall limit of shelf life was 131 h for fillets of 33 d old birds and 121 h for fillets of 53 d old ones ($P = 0.020$). When considering the feeding groups, the difference in shelf life of fillets at different ages was ranked as follows: BA_HIS2 (13 h) > CON (12 h) > BA_CON (11 h) > HIS2 (9 h) > BA_HIS1 (7 h) > HIS1 (5 h).

The correlation analysis of the meat quality parameters showed a strong connection between the measured values (Table 6). The pH increased with a higher fillet weight as well as a higher incidence of WS. The L* and b* values were increasing with the presence of WS. Fillet weight was positively correlated to WS as well as to yellowness. The SI value was positively correlated to the purchase decision. Moreover, purchase decision depended on redness and yellowness of the fillets, fillet weight, and WS. These parameters were negatively correlated with the purchase decision at both ages.

DISCUSSION

In this study, the overall performance of the birds was only slightly affected, and differences were limited to mortality, ADFI, as well as breast and thigh weight of the carcass. The increased mortality observed in the groups receiving His was limited to the 53 d old birds. This might be interpreted as a result of increased metabolic stress related to high His doses.

In former investigations, it was mentioned that a high supplementation of His could have harmful effects in terms of reduced growth rates and increased mortality in rats and other animal species (Harper et al., 1970; Ikezaki et al., 1996). However, it must be kept in mind that these studies used 1.6 to 15.9 times higher His concentrations than the maximum total His concentration used in the starter phase of group HIS2 and BA_HIS2 of our study. Therefore, the potential toxicity of His seems to be no reason for the increased mortality. The supplementation with His was also associated with increased feed intake until d 33, a finding that is contrasting to results obtained in humans, mice, and rats, indicating mostly a suppression in feed intake at higher His feeding levels (Moro et al., 2020). The greater ADFI in our study might be explained by a compensatory effect for other nutrients as the young chickens had to deal with the elevated amount of His in their metabolism. A compensation of a minor lack of nutrients by increased ADFI was already described by the National Research Council (1994). Feeding a high level of βA decreased ADFI at both ages. The increased supply of βA could stimulate the formation of Car and Ans from His, and thus reduce the need for downstream cofactors. The high depletion of βA by feeding high doses of His supports this notion. However, the amount of βA supplied was not related to the concentration of Car or Ans in muscle tissue, but to the Car concentration in the blood plasma of the young birds. Skeletal muscle is certainly not the only tissue in which high amounts of Car are needed (Manhiani et al., 2013), but other tissues such as liver, lung, and brain were not analyzed in this study. Jacob et al. (1991) also reported a suppression of ADFI by supplementing βA independent of supplementation with His after 4 wk of age. Another important finding in our study was the reduced breast yield at both ages when supplementing βA. In a study of Tomonaga et al. (2006), the addition of 1 and 2% βA, but not a supplementation of 0.5%, showed the same outcome, which was explained by reduced growth-performance and possible metabolic dysfunction. Opposite findings were reported by Qi et al. (2018), with 1.355 g/kg βA as optimal concentration for improving breast yield. Kralik et al. (2018) found no impact of His and βA supplementation, individually or combined, on breast yield of the carcass.

The main goal of this study was to assess the meat quality of chicken breast fillets containing a high concentration of Car. Therefore, the precursors of Car were fed to increase its concentration in the skeletal muscle. It was expected that the precursors cause separately a rise in the concentration of the dipeptides Car and Ans in the skeletal muscle and the combination of both increases it additionally. However, the results of this study showed that His, but not βA, was mainly affecting the Car concentrations, when a commercial diet was

Table 6. Coefficients of correlation (*Spearmann's rank correlation,* r_s) between various indicators of meat quality assessed 48 h after slaughter.

		Parameter									
Parameter	Age, d	pH	L*[1]	a*[2]	b*[3]	Fillet weigh, g	WLC[4], %	WLT[5], %	MDA[6], mg/kg	Sensory index	White striping
L*	33	−0.245*									
a*		−0.071	−0.217*								
b*		0.061	0.268*	0.328**							
Fillet weight, g		0.356**	0.256*	0.166	0.323**						
WLC, %		−0.001	0.156	0.155	0.081	0.096					
WLT, %		−0.157	0.406***	0.060	0.297**	0.124	0.295**				
MDA, mg/kg		−0.375***	0.209	0.066	−0.021	0.057	0.091	0.180			
Sensory index		0.301**	−0.308**	-0.115	−0.178	-0.018	0.015	−0.144	−0.232*		
White striping		0.302**	0.239*	0.209	0.308**	0.539**	-0.003	0.058	−0.196	−0.107	
Purchase decision		0.067	−0.198	−0.455***	−0.228*	−0.363***	-0.206	0.037	−0.298**	0.318**	−0.231*
L*	53	0.104									
a*		0.078	-0.365***								
b*		0.222*	0.433***	0.243*							
Fillet weight, g		0.273**	0.142	0.219*	0.251*						
WLC, %		−0.145	0.265*	0.037	0.164	0.044					
WLT, %		−0.087	0.188	0.118	0.034	−0.158	0.129				
MDA, mg/kg		−0.131	0.230*	−0.001	−0.046	0.001	0.189	0.271**			
Sensory index		−0.143	−0.415***	0.054	-0.308**	−0.176	−0.169	−0.276**	0.092		
White striping		0.217*	0.234*	0.272**	0.325**	0.352**	0.029	0.091	−0.037	−0.303**	
Purchase decision		−0.267*	−0.164	−0.367***	−0.210*	−0.321**	−0.047	−0.166	0.080	0.396***	−0.563***

The strength of correlation is given as low when $r_s \leq 0.1$, medium when $r_s \leq 0.3$ and high when $r_s \leq 0.5$. *P*-values are ranked by using: *for $P \leq 0.5$, **for $P \leq 0.01$ and ***for $P \leq 0.001$.
[1]L*, Lightness.
[2]a*, Redness.
[3]b*, Yellowness.
[4]WLC, water loss during cooking.
[5]WLT, water loss during thawing.
[6]MDA, malondialdehyde equivalents.

used as a basis. Also, no interactions of His and βA were detected.

In muscle tissue of the 33 d old birds, the 2 higher levels of His supplementation led to the same increase of Car. This indicates that the optimal supplementation level of His for synthetizing Car in the *pectoralis major* muscle was already reached at a SID His:Lys ratio of 0.54 in the diet. In 54 d old birds, Car increased in skeletal muscle only with the highest supplementation of His, indicating an increased His requirement with age. In blood plasma, a supplementation of His increased the concentration of Car at both ages without differences between the 2 supplemented levels. This may indicate an accumulation of this dipeptide in other tissues and an optimum supplementation of SID His:Lys 0.54. The correlation analysis also indicated a higher content of Car by increased His values in blood plasma, and for 35 d old birds, also in muscle tissue. Supplementation of His reportedly increases the Car concentrations in breast muscle tissue (Kai et al., 2015; Kralik et al., 2015). However, Kralik et al. (2018) described an increase in Car concentration in breast muscle tissue by feeding a combination of βA and His, but not with the stand-alone supplementations, thus deviating from the results of this study. According to other studies, βA could increase the concentration of HCD in muscle tissue (Tomonaga et al., 2012; Kralik et al., 2015; Qi et al., 2018). This was not observed in our study, as well as in the study of Kralik et al. (2018). Additionally, Łukasiewicz et al. (2015) indicated that supplementing 5 g/kg (= 0.5%) increased Ans but not Car in breast tissue. To our knowledge, this is the only study in which Ans was affected by βA. However, according to the results of these studies, the importance of βA for the synthesis of the dipeptides remains unclear. It may depend on the study design, the concentration of the supplement, and the age of the chicken at slaughter. We herein observed a distinct depletion of βA upon His supplementation indicating that βA is used to form dipeptides. But, as mentioned before, no impact of this amino acid on the concentration of Car and Ans was found in the analyzed breast tissue and blood plasma. It can be speculated that the natural supply of βA with the feedstuffs was already sufficient to increase Car in the skeletal muscle and additional ßA would not yield further increases in Car synthesis. This may indicate that His is the limiting factor for Car synthesis in chicken. A depletion of βA when supplementing His was also reported by Kai et al. (2015). For Ans, none of the supplementations tested herein affected its concentrations in blood plasma or breast tissue, but this dipeptide was more concentrated in 54 d old birds compared to 35 d old ones. Moreover, the Car concentration was lower in muscle tissue of the 54 d old birds. These findings might point to a slow transformation of Car into Ans. The positive correlation between Ans and Car, in particular in blood plasma, highlights the formation of Ans by the carnosine-methyltransferase.

The increased Car concentrations in muscle upon feeding higher doses of His were hardly affecting the meat quality of the chicken. More effects on meat quality were detected for age effect and storage time. Therefore, the changes observed for meat quality can be attributed to the changing composition of the muscle at different ages, and to storage and spoiling effects. In 53 d old birds, a higher incidence of hemorrhages, yellow and red parts, and hardness were reported by the sensory panel, thus explaining for being less inclined to buy such meat. The calculated correlations of purchase decision with redness, yellowness, WS, and fillet weight underpin these findings. Moreover, the panel described an increase in the hardness of the fillets from the 53 d old birds indicating a high portion of WB-affected fillets. During storage, the bacterial growth on the surface of the fillets leads to higher pH, and to changes in color (Gill, 1983; Faustman and Cassens, 1990; Albrecht et al., 2019b). Also, oxidative processes take place, leading to increased oxidative damage of cellular targets which is quantifiable by suitable assays, for example, the TBARS assay in case of lipids (Sujiwo et al., 2018).

Concerning the His supplementation, we observed decreased incidences of WS with a moderate supplementation of His (SID His:Lys of 0.54) in the group slaughtered at 33 d of age. This finding could be related to the increased concentration of Car and a moderate load of His in the muscle. In higher supplemented birds (SID His:Lys of 0.64), the greater supply of His, without changes in the concentrations of His-containing dipeptides, points to metabolic stress by the supplementation. In a report by Aviagen (2019) about all known breast muscle myopathies in chickens, a positive effect of increased His in feed was not proven when using a high ratio of His:Lys (0.70). The incidence of WS was correlated with most of the other quality parameters. A positive correlation was seen with the thickness of the fillets, which was higher in 53 d old birds. Taken together, the incidence of WS increased with fillet weight and age of the birds and thus confirms literature data as reviewed by Petracci et al. (2019). Lightness and yellowness were also increased with the incidence of WS. Baldi et al. (2018) reported an increase of yellowness and reduced redness in affected fillets, whereas Alnahhas et al. (2016) describes a higher lightness of affected fillets. Surprisingly, SI was lower with more WS only in 53 d old birds. In addition, redness was positively correlated in this slaughter group. The number of red parts of the fillets, what we attribute to handling and slaughter practice, was higher and more visible with more lightness. At both ages, the pH was positively correlated with WS in affected birds.

His is not well studied in context with the nutritional requirements of modern broilers. The recommended His:Lys ratio of Evonik (Evonik Nutrition & Care GmbH, 2016) is 0.33; this is well within the range proposed as optimum ratio, that is, between 0.31 and 0.41, in the literature for different growing periods (Han et al., 1991; Rostagno and Becker, 2005; Wecke and Liebert, 2013; Franco et al., 2017). This ratio can easily be reached by a standard diet. However, under certain metabolic circumstances such as oxidative stress, the requirements for His can be higher than those reported

for normal broiler performance. In this study, a high SID His:Lys ratio in a commercial broiler feed was related to greater Car concentrations in both skeletal muscle and blood plasma. Given that meat is the main source of Car in human nutrition, these results are of particular relevance. Taken together, the potential benefits of supplementing His for reducing the breast myopathy WS in young birds should be further studied together with other breast myopathies in broiler chickens.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PloS One 11:e0153750.

Albrecht, A., M. Hebel, C. Heinemann, U. Herbert, D. Miskel, B. Saremi, and J. Kreyenschmidt. 2019a. Assessment of meat quality and shelf life from broilers fed with different sources and concentrations of methionine. J. Food Qual. 2019:6182580.

Albrecht, A., M. Hebel, M. Mittler, C. Hurck, K. Kustwan, B. Heitkönig, D. Bitschinski, and J. Kreyenschmidt. 2019b. Influence of different production systems on the quality and shelf life of poultry meat: a case study in the German sector. J. Food Qual. 2019:3718057.

Alnahhas, N., C. Berri, M. Chabault, P. Chartrin, M. Boulay, M. C. Bourin, and E. Le Bihan-Duval. 2016. Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. BMC Genet 17:61.

Aviagen. 2019. Breast muscle myopathies (BMM). Accessed Apr. 2021. http://en.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/Breast-Muscle-Myopathies-2019-EN.pdf.

Baldi, G., F. Soglia, M. Mazzoni, F. Sirri, L. Canonico, E. Babini, L. Laghi, C. Cavani, and M. Petracci. 2018. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal 12:164–173.

Baran, E. J. 2000. Metal complexes of carnosine. Biochemistry (Mosc) 65:789–797.

Bellia, F., G. Vecchio, and E. Rizzarelli. 2014. Carnosinases, their substrates and diseases. Molecules 19:2299–2329.

Boldyrev, A. A., G. Aldini, and W. Derave. 2013. Physiology and pathophysiology of carnosine. Physiol. Rev. 93:1803–1845.

Bonfanti, L. 1999. Carnosine-related dipeptides in the mammalian brain. Prog. Neurobiol. 59:333–353.

Cai, K., W. Shao, X. Chen, Y. L. Campbell, M. N. Nair, S. P. Suman, C. M. Beach, M. C. Guyton, and M. W. Schilling. 2018. Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. Poult. Sci. 97:337–346.

Chan, K. M., and E. A. Decker. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. Nutr. 34:403–426.

Chen, L. R., M. M. Suyemoto, A. H. Sarsour, H. A. Cordova, E. O. Oviedo-Rondón, M. Wineland, H. J. Barnes, and L. B. Borst. 2019. Temporal characterization of wooden breast myopathy ("woody breast") severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. Avian Pathol 48:319–328.

Evonik Nutrition & Care GmbH. 2016. Recommendations for broilers. Accessed June 2021. https://animal-nutrition.evonik.com/product/feed-additives/downloads/recommendations_broiler_03_16.pdf

European Commission. 2009. Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L54/1 1–130.

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: a review. J. Muscle Foods 1:217–243.

Fontaine, J., J. Hörr, and B. Schirmer. 2001. Near-infrared reflectance spectroscopy enables the fast and accurate prediction of the essential amino acid contents in soy, rapeseed meal, sunflower meal, peas, fishmeal, meat meal products, and poultry meal. J. Agric. Food Chem. 49:57–66.

Fontaine, J., B. Schirmer, and J. Hörr. 2002. Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. J. Agric. Food Chem. 50:3902–3911.

Franco, S. M., F.d. C. Tavernari, R. C. Maia, V. R. S. M. Barros, L. F. T. Albino, H. S. Rostagno, G. R. Lelis, A. A. Calderano, and R. N. Dilger. 2017. Estimation of optimal ratios of digestible phenylalanine + tyrosine, histidine, and leucine to digestible lysine for performance and breast yield in broilers. Poult. Sci. 96:829–837.

Gill, C. O. 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. J. Food Prot. 46:444–452.

Golzar Adabi, S., and E. Demirok Soncu. 2019. White striping prevalence and its effect on meat quality of broiler breast fillets under commercial conditions. J. Anim. Physiol. Anim. Nutr. (Berl.) 103:1060–1069.

Griffin, J. R., L. Moraes, M. Wick, and M. S. Lilburn. 2018. Onset of white striping and progression into wooden breast as defined by myopathic changes underlying pectoralis major growth. Estimation of growth parameters as predictors for stage of myopathy progression. Avian Pathol 47:2–13.

Han, Y. M., H. Suzuki, and D. H. Baker. 1991. Histidine and tryptophan requirement of growing chicks. Poult. Sci. 70:2148–2153.

Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter. 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428–558.

Hipkiss, A. R. 2010. Aging, proteotoxicity, mitochondria, glycation, NAD and carnosine: possible inter-relationships and resolution of the oxygen paradox. Front. Aging Neurosci 2:10.

Hoehler, D., A. Lemme, C. Brito, and H. Rostagno. 2005. Standardized ileal amino acid digestibility of crystalline amino acids is close to 100% regardless of the standardization method. Poult. Sci. 84:39.

Ikezaki, S., A. Nishikawa, F. Furukawa, T. Enami, M. Mitsui, Z. Tanakamaru, H.-C. Kim, I.-S. Lee, T. Imazawa, and M. Takahashi. 1996. Long-term toxicity/carcinogenicity study of l-histidine monohydrochloride in F344 rats. Food Chem. Toxicol. 34:687–691.

Jacob, J. P., R. Blair, L. E. Hart, and E. E. Gardiner. 1991. The effect of taurine transport antagonists on cardiac taurine concentration and the incidence of sudden death syndrome in male broiler chickens. Poult. Sci. 70:561–567.

Kai, S., G. Watanabe, M. Kubota, M. Kadowaki, and S. Fujimura. 2015. Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. Anim. Sci. J. 86:541–546.

Kralik, G., M. Sak-Bosnar, M. Grčević, and Z. Kralik. 2018. Effect of amino acids on growth performance, carcass characteristics, meat quality, and carnosine concentration in broiler chickens. J. Poult. Sci. 55:239–248.

Kralik, G., M. Sak-Bosnar, Z. Kralik, O. Galovic, M. Grcevic, and I. Kralik. 2015. Effect of β-alanine and L-histidine on concentration of carnosine in muscle tissue and oxidative stability of chicken meat. Poljoprivreda 21:190–194.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677–2685.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.

Liu, G., F. Mac Gabhann, and A. S. Popel. 2012. Effects of fiber type and size on the heterogeneity of oxygen distribution in exercising skeletal muscle. PloS One 7:e44375.

Łukasiewicz, M., K. Puppel, B. Kuczyńska, M. Kamaszewski, and J. Niemiec. 2015. β-Alanine as a factor influencing the content of bioactive dipeptides in muscles of Hubbard Flex chickens. J. Sci. Food Agric. 95:2562–2565.

MacRae, V. E., M. Mahon, S. Gilpin, D. A. Sandercock, and M. A. Mitchell. 2006. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (gallus domesticus). Br. Poult. Sci. 47:264–272.

Manhiani, P. S., J. K. Northcutt, I. Han, W. C. Bridges, and P. L. Dawson. 2013. Antioxidant activity of carnosine extracted from various poultry tissues. Poult. Sci. 92:444–453.

Moro, J., D. Tomé, P. Schmidely, T.-C. Demersay, and D. Azzout-Marniche. 2020. Histidine: a systematic review on metabolism and physiological effects in human and different animal species. Nutrients 12:1414.

Morry, J., W. Ngamcherdtrakul, and W. Yantasee. 2017. Oxidative stress in cancer and fibrosis: opportunity for therapeutic intervention with antioxidant compounds, enzymes, and nanoparticles. Redox. Biol. 11:240–253.

Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genom 16:399.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Petracci, M., F. Sirri, M. Mazzoni, and A. Meluzzi. 2013. Comparison of breast muscle traits and meat quality characteristics in 2 commercial chicken hybrids. Poult. Sci. 92:2438–2447.

Petracci, M., F. Soglia, M. Madruga, L. Carvalho, E. Ida, and M. Estévez. 2019. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. Food Saf. 18:565–583.

Qi, B., J. Wang, Y.-B. Ma, S.-G. Wu, G.-H. Qi, and H.-J. Zhang. 2018. Effect of dietary β-alanine supplementation on growth performance, meat quality, carnosine content, and gene expression of carnosine-related enzymes in broilers. Poult. Sci. 97:1220–1228.

Rostagno, H. S., and B. G. Becker. 2005. Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements. 2nd ed. Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, Brazil.

Smith, J. H. 1963. Relation of body size to muscle cell size and number in the chicken. Poult. Sci. 42:283–290.

Soglia, F., A. K. Silva, L. M. Lião, L. Laghi, and M. Petracci. 2019. Effect of broiler breast abnormality and freezing on meat quality and metabolites assessed by 1H-NMR spectroscopy. Poult. Sci. 98:7139–7150.

Sujiwo, J., D. Kim, and A. Jang. 2018. Relation among quality traits of chicken breast meat during cold storage: correlations between freshness traits and torrymeter values. Poult. Sci. 97:2887–2894.

Sundekilde, U. K., M. K. Rasmussen, J. F. Young, and H. C. Bertram. 2017. High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine. Food Chem 217:151–154.

Tomonaga, S., K. Kaneko, Y. Kaji, Y. Kido, D. M. Denbow, and M. Furuse. 2006. Dietary beta-alanine enhances brain, but not muscle, carnosine and anserine concentrations in broilers. Anim. Sci. J. 77:79–86.

Tomonaga, S., M. Matsumoto, and M. Furuse. 2012. β-Alanine enhances brain and muscle carnosine levels in broiler chicks. J. Poult. Sci. 49:308–312.

Velez, S., N. G. Nair, and V. P. Reddy. 2008. Transition metal ion binding studies of carnosine and histidine: biologically relevant antioxidants. Colloids Surf. B Biointerfaces 66:291–294.

Velleman, S. G. 2019. Recent developments in breast muscle myopathies associated with growth in poultry. Annu. Rev. Anim. Biosci. 7:289–308.

Wecke, C., and F. Liebert. 2013. Improving the reliability of optimal in-feed amino acid ratios based on individual amino acid efficiency data from N balance studies in growing chicken. Animals (Basel) 3:558–573.

Wilson, B. W., P. S. Nieberg, R. J. Buhr, B. J. Kelly, and F. T. Shultz. 1990. Turkey muscle growth and focal myopathy. Poult. Sci. 69:1553–1562.

Zambonelli, P., M. Zappaterra, F. Soglia, M. Petracci, F. Sirri, C. Cavani, and R. Davoli. 2016. Detection of differentially expressed genes in broiler pectoralis major muscle affected by white striping - wooden breast myopathies. Poult. Sci. 95:2771–2785.

Zanetti, M., D. Tedesco, T. Schneider, S. Teixeira, L. Daroit, F. Pilotto, E. Dickel, S. Santos, and L. Santos. 2018. Economic losses associated with wooden breast and white striping in broilers. Semin. Cienc. Agrar. 39:887.

Manuscript II

Effects of feeding different histidine to lysine ratios on performance, meat quality, and the occurrence of breast myopathies in broiler chickens

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ABSTRACT In modern fast-growing broiler chickens, meat quality becomes increasingly important due to the occurrence of novel breast myopathies such as white striping (**WS**), woody breast (**WB**), and spaghetti meat (**SM**), compromising the sustainability of the poultry industry. Therefore, strategies for reducing the incidence of those myopathies are needed. This study focuses on the impact of different standard ileal digestible (**SID**) His:Lys ratios on growth performance, meat quality variables like pH, drip loss and pale-soft-exudative (**PSE**) meat as well as the incidence and severity of breast myopathies (WS, WB, SM), including deep pectoral myopathies (**DPM**). Thus, 440 male Ross 308 chickens were divided into 5 treatment groups with SID His:Lys ratios of 0.41, 0.45, 0.49, 0.53, and 0.57 in the feed, respectively. Performance was assessed on d 1, 10, 20, 33, and 38 of life. From each treatment group, 22 representative birds were slaughtered on d 38, 39, 40, and 41, respectively. All right fillets were examined 24 h after slaughter by 6 trained testers to assess the outcome of breast myopathies (3-point scale) and PSE-meat (presence and absence). Fillet weight, pH, and drip loss were recorded for selected fillets at different time points. The results of this trial showed no influence of the SID His:Lys ratios on growth performance or drip loss, whereas pH was slightly affected. The study showed a correlation between the occurrence of WB and WS ($P < 0.001$, normalized contingency coefficient = 0.576). A lower incidence of WB ($P = 0.008$) was observed in the group fed an SID His:Lys ratio of 0.45 compared with the group fed the lowest ratio of 0.41. For WS, a higher incidence was observed in broilers fed an SID His:Lys ratio of 0.49 ($P = 0.002$) and 0.53 ($P = 0.036$) when compared to 0.41. The occurrence of PSE was increased by feeding SID His:Lys at 0.51 ($P = 0.008$) compared to the lowest ratio. This study showed that the level of His in broiler feed had an impact on the occurrence of breast myopathies, but only WB could be decreased.

Key words: histidine, broiler, white striping, woody breast, PSE

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INTRODUCTION

Breast meat quality issues in chickens have been the focus of research for a long time. However, in the last decade the occurrence of novel breast myopathies of the *Pectoralis major* has increased in modern broiler chicken strains, which in turn has become an issue for the poultry industry. The novel myopathies include white striping (**WS**), woody breast (**WB**), and spaghetti meat (**SM**), all of which result in high economic losses (Kuttappan et al., 2016; Zanetti et al., 2018). The WS condition appears as white stripes parallel to the muscle fibers (Kuttappan et al., 2013), while WB is defined as fibrotic and diffusely hardened muscle (Sihvo et al., 2014). The condition of SM is described by poor muscle fiber cohesion and consequently the separation of muscle fiber buddles in affected fillets (Baldi et al., 2020). The incidence of these myopathies is related to fast growth rates and increases with slaughter age (Kuttappan et al., 2012; Petracci et al., 2013; Griffin et al., 2018; Chen et al., 2019; Petracci et al., 2019). Today it is known that the high growth-rate of modern broiler chickens may lead to excessive muscle hypertrophy. Without sufficient growth of the supportive tissues, like blood vessels, the rapidly growing muscles may become hypoxic, inflamed and eventually necrotic. These conditions are hypothesized to cause the aforementioned breast meat myopathies and both genetic as well as environmental factors during growth may contribute to their progression (Petracci et al., 2019; Bailey et al., 2020;

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Baldi et al., 2020). The most well studied meat quality issues in broilers are pale-soft-exudative meat (**PSE**) and the deep pectoral myopathy (**DPM**). A PSE condition is the result of a low *post-mortem* pH and consequently increased protein denaturation (Adzitey and Huda, 2011). This condition occurs when muscles undergo high metabolic activity directly before slaughter, which increases the formation of lactic acid that can no longer be removed by blood circulation (Bowker, 2017). Moreover, PSE can result from inadequate cooling of the carcass (Bowker, 2017). The DPM condition is a degeneration of the *Pectoralis minor* muscle (Bilgili, 2008), which has been correlated to a relatively high ratio of muscle growth to blood flow in the tissue. The condition can be exacerbated by wing flapping which puts pressure on the muscle, interrupting circulation and causing ischemic muscle necrosis. The overall result is a green appearance due to the breakdown of hemoglobin and myoglobin in the tissue (Bilgili, 2008). Another quality issue was recently termed as "gapping defect" in the literature, and described as a separation of muscle fiber bundles in the external part of the *Pectoralis minor* muscle (Soglia et al., 2019b).

Regarding the occurrence of hypoxia and oxidative stress in muscle tissue affected by breast myopathies, the antioxidant status of the muscle cells may be a predictor of these conditions. Some authors described a depletion of the dipeptides carnosine and anserine in fillets affected by WS, WB, or SM condition (Abasht et al., 2016; Sundekilde et al., 2017; Golzar Adabi and Demirok Soncu, 2019; Soglia et al., 2019a), which serve as natural antioxidants, pH-buffer and metal-ion chelator in skeletal muscle tissue (Boldyrev et al., 2013). In previous studies, dietary supplementation of the dipeptide precursor His significantly increased the concentration of carnosine in breast fillets of broilers (Kai et al., 2015; Kralik et al., 2015; Lackner et al., 2021). The recommended His:Lys ratio for broiler feed ranges between 0.31 and 0.41 in the literature for different growing periods (Han et al., 1991; Rostagno and Becker, 2005; Wecke and Liebert, 2013; Evonik Nutrition and Care, 2016; Franco et al., 2017), which is met by most commercial broiler diets. However, under special metabolic circumstances, like oxidative stress, the need for His could be higher. It is hypothesized that an increased supply of His in feed can improve the antioxidative status of the *Pectoralis major* and lower the incidence of breast muscle myopathies in modern, fast-growing broiler chicken strains. This study aimed at providing an insight into the effects of different standard ileal digestible (**SID**) His:Lys ratios in broiler feed on performance and meat quality, with special focus on breast myopathies.

MATERIALS AND METHODS

Trial Design

The feeding trial was conducted at the Banat University of Agricultural Science and Veterinary Medicine King Michel First from Timisoara, Romania. The trial was performed in accordance with legislation 43/2014 on the protection of animals used for scientific purposes (2014). The trial was approved by the National Sanitary Veterinary and Food Safety Agency (ANSVSA, 2015). A total of 440 one day-old male Ross 308 broiler chicks, were divided into 5 treatment groups to test different SID His:Lys ratios in the feed. One group of birds was used as control, with the commercially relevant basal SID His:Lys ratio of 0.41 (**HIS41**). The other groups were fed increasing SID His:Lys ratio of 0.45 (**HIS45**), 0.49 (**HIS49**), 0.53 (**HIS53**) and 0.57 (**HIS57**) by supplementing His to the basal diet. The feed was formulated in 4 phases: Starter, Grower I, Grower II, and Finisher as shown in Table 1. Feed and water were provided *ad libitum*. All birds were initially weighed and divided into 55 pens with eight birds in each pen. Eleven pen replicates were used for each of the 5 treatment groups, for a total of 88 birds per treatment group and pens were arranged in a randomized block design. Each pen was 0.8 m^2 with straw for litter. The pens were equipped with one feeder and water was supplied by an internal water system network. Room temperature was set as suggested by the breeder's recommendation (Aviagen, 2018). The trial site was equipped with a dynamic ventilation system and heaters (Aerotherm, Mauern, Germany). The positive pressure ventilation was achieved by single, variable-speed fans linked to temperature sensors which worked based on the measured temperature and age of the birds. The light regime was according to the breeders' recommendation (Aviagen, 2018). An examination by a veterinarian was carried out directly after arrival of the chicks. No further intervention by the veterinarian was necessary nor performed.

Experimental Diets The diets were formulated with Brill Formulation (version v2.08.002, Format Solutions Inc., Hopkins, MN). The basal diets were formulated to simulate a commercial broiler diet based on wheat, corn, and soybean meal (Table 1). All SID amino acids (**AA**) and nitrogen corrected apparent metabolizable energy were formulated according to the recommendation of Evonik Nutrition and Care, 2016. The lowest SID His:Lys ratio which could be achieved while meeting optimal levels of all other essential AA was 0.41 in Grower I, Grower II and Finisher phase. In order to provide a uniform His:Lys ratio throughout all feeding phases, His (L-Histidine Base, food grade ≥ 98.5%, Europepta, Hannover, Germany) had to be added in the Starter diet to obtain a SID His:Lys ratio of 0.41. The main feed ingredients, as well as the final feed for each feeding group at each feeding phase, were analyzed for AA content with the AMINO*NIR* service of Evonik Nutrition & Care GmbH as described by Fontaine et al. (2001, 2002). The analyzed results from the main feed ingredients were used to formulate the final diets. To generate higher SID His:Lys ratios for the treatment groups in the final diets, His was supplemented to this basal formulation (L-Histidine Base, food grade ≥98.5%, Europepta). According to the studies of Hoehler et al. (2005) and personal information, supplemented His can be used as 100% ileal digestible AA. The calculated and analyzed, supplemented His and SID His:Lys ratios of the different

Table 1. Composition of the basal diets fed during the different phases of the trial.

Ingredient, %	Starter (1 to 10 d)	Grower I (11 to 20 d)	Grower II (21 to 33 d)	Finisher (34 to 41 d)
Corn	37.5	33.5	23.4	16.3
Soybean meal	33.4	28.1	23.6	20.1
Wheat	20.0	30.0	44.5	55.0
Soybean oil	3.83	3.99	4.40	4.70
Limestone ($CaCO_3$)	1.75	1.74	1.56	1.54
Monocalciumphosphate	1.28	0.89	0.69	0.49
Premix Blank Poultry[1]	0.50	0.50	0.50	0.50
MetAMINO® (DL-Met)	0.37	0.29	0.26	0.25
L-Lys-HCl	0.33	0.27	0.28	0.29
Sodium bicarbonate	0.28	0.34	0.36	0.37
Salt (NaCl)	0.19	0.20	0.19	0.18
ThreAMINO (L-Thr)	0.14	0.10	0.11	0.12
ValAMINO (L-Val)	0.12	0.08	0.06	0.07
Gly	0.12	-	-	-
L-Arg	0.10	0.05	0.06	0.07
L-Ile	0.05	0.03	0.03	0.05
L-His	0.03	-	-	-
Choline Cloride 70%	0.01	0.01	0.01	0.01
Nutrient composition as calculated (analyzed), %				
AMEn, kcal/kg	3,035	3,083	3,131	3,167
Crude protein	22.5 (23.3)	20.3 (20.8)	19.0 (20.1)	18.0 (18.7)
Calcium	1.00	0.92	0.80	0.75
Phosphate	0.65	0.55	0.50	0.45
Composition of calculated SID[2] (total calculated / total analyzed) amino acids, %				
Lys	1.28 (1.41/1.44)	1.11 (1.22/1.26)	1.02 (1.12/1.17)	0.95 (1.05/1.05)
Met	0.64 (0.67/0.65)	0.54 (0.57/0.56)	0.50 (0.52/0.51)	0.48 (0.50/0.50)
Met + Cys	0.93 (1.02/1.01)	0.82 (0.90/0.90)	0.77 (0.84/0.85)	0.74 (0.81/0.82)
Thr	0.81 (0.94/0.91)	0.71 (0.82/0.82)	0.66 (0.76/0.78)	0.63 (0.72/0.71)
Arg	1.41 (1.54/1.56)	1.22 (1.34/1.37)	1.12 (1.23/1.29)	1.05 (1.16/1.15)
Ile	0.87 (0.97/0.99)	0.77 (0.86/0.88)	0.71 (0.79/0.84)	0.68 (0.75/0.77)
Leu	1.53 (1.72/1.73)	1.40 (1.56/1.58)	1.27 (1.42/1.48)	1.17 (1.31/1.30)
Val	1.01 (1.13/1.15)	0.89 (0.99/1.02)	0.81 (0.91/0.96)	0.77 (0.86/0.87)
His	0.53 (0.57/0.58)	0.46 (0.50/0.51)	0.42 (0.46/0.48)	0.39 (0.43/0.43)

[1]Composition of Premix Blank Poultry (per kg premix): Vitamin A (retinyl acetate) 2.000.000 IU; Vitamin D_3 (cholecalciferol) 500,000 IU; Vitamin E (dl-α-tocopherol) 10 g; Vitamin K_3 (menadione) 0.3 g; Vitamin B_1 (thiamin) 0.4 g; Vitamin B_2 (riboflavin) 1.5 g; Vitamin B_6 (pyridoxine-HCl) 0.7 g; Vitamin B_{12} (cyanocobalamin) 4 mg; Niacin 7 g; D-pantothenic acid 2.4 g; Choline chloride 92 g; Folic acid 0.2 g; Biotin 40 mg; Iron (as $FeSO_4*H_2O$) 16 g; Copper (as $CuSO_4*5\ H_2O$) 2.4 g; Manganese (as MnO) 17 g; Zinc (as $ZnSO_4*H_2O$) 12 g; Iodate (as KJ) 0.16 g; Selenium (as Na_2SeO_3) 30 mg.
[2]SID, standard ileal digestible.

treatment groups are given in Table 2. The pelleted feed was produced by Research Diet Service B.V. (Wijk bij Duurstede, Netherlands) and shipped to the trial barn site.

Growth Performance Analysis Individual bird weight was recorded at the beginning of the trial and at the end of each feeding phase. For weight estimation, each bird was weighed individually, and an average was calculated. Feed consumption for each pen was recorded

Table 2. Supplemented histidine concentrations in relation to the basal diet in the different dietary groups as calculated and analyzed by wet chemistry.

	Treatment group				
	Supplemented His as calculated (as analyzed), %				
Feeding phase	HIS41[1]	HIS45[2]	HIS49[3]	HIS53[4]	HIS57[5]
Starter	0.00 (0.03)	0.05 (0.08)	0.10 (0.12)	0.15 (0.16)	0.20 (0.22)
Grower I	0.00 (nd6)	0.04 (0.05)	0.09 (0.09)	0.13 (0.13)	0.18 (0.17)
Grower II	0.00 (nd)	0.04 (0.04)	0.08 (0.08)	0.12 (0.12)	0.16 (0.15)
Finisher	0.00 (nd)	0.04 (0.04)	0.08 (0.08)	0.11 (0.11)	0.15 (0.15)
SID[7] His:Lys ratio	0.41	0.45	0.49	0.53	0.57

[1]HIS41: standard ileal digestible amino acid His:Lys ratio of 0.41. The analyzed His concentration in the Starter phase of this group is the result of supplemented His to reach this ratio.
[2]HIS45: standard ileal digestible amino acid His:Lys ratio of 0.45.
[3]HIS49: standard ileal digestible amino acid His:Lys ratio of 0.49.
[4]HIS53: standard ileal digestible amino acid His:Lys ratio of 0.53.
[5]HIS57: standard ileal digestible amino acid His:Lys ratio of 0.57.
[6]nd, not detectable due to detection limit.
[7]SID, standard ileal digestible.

after each feeding phase. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were calculated from the average of these measurements. Mortalities were recorded daily including the weight and reason for each mortality. Growth performance variables like FCR were then corrected for mortalities.

Assessment of Meat Quality

Twenty-two birds per treatment were slaughtered on each of 4 consecutive days (d 38, d 39, d 40, and d 41). After electrical head-only stunning, birds were killed by exsanguination. Immediately after bleeding, the birds were individually scalded in a water-filled, electric scalding bath at 52 to 54°C for 2 min and afterward defeathered in a plucker machine. Head and claws, as well as the uropygial gland, sectioning of the skin at the neck and cloacal region, were removed manually. The carcass was cooled down afterwards to 2 to 4°C.

Drip Loss The assessment of Drip loss (**DL**) was performed on the left fillets of the22 birds per treatment group slaughtered at 38 d of age. Immediately after slaughter, the fillets were weighed and placed in a polyethylene bag under atmospheric pressure. The fillets were kept at 2°C for a total time of 192 h and all samples were weighed again at 24, 48, 96, 144, and 192 h after slaughter. DL was calculated at each time point as the difference in fillet weight as a percentage of the initial fillet weight after slaughter.

Measurement of pH The pH was directly measured in the first 2 h after slaughter and after 24 h of storage for all right fillets at all consecutive slaughter days. Additionally, the pH was measured after 48, 96, 144, and 192 h of storage in all fillets used for DL measurement. A calibrated pH-meter (Meat pH Meter portable waterproof HI99163, with electrode FC2323, Hanna Instruments Deutschland GmbH, Vöhringen, Germany) was used to measure pH in the caudal, middle, and cranial parts of the fillets and a mean value was calculated for each fillet.

Grading of Breast Myopathies The grading of breast myopathies was performed with all right fillets 24 h after slaughter of all birds slaughtered (88 fillets per treatment group). The occurrence of myopathies affecting the *Pectoralis major* muscle (WS, WB, and SM) was recorded with a visual grading system for WS and SM and a manual grading system for WB. Moreover, DPM was also visually recorded for the *Pectoralis minor* muscle. Each fillet was graded by 6 trained testers, evaluating independently from each other in a 3-score system (0-normal, 1-moderate and 2-severe). The WS was evaluated by the thickness of the occurring stripes as showed and described by Albrecht et al. (2019) and Petracci et al. (2019). To grade WB and SM, the grading method described by Petracci et al. (2019) was used. For DPM, the definition was 0-normal for an inner fillet color similar to the rest of the fillet. Grade 1-moderate was given to an inner fillet with a reddish color and 2-severe to a greenish color and necrotic appearance of the inner fillet.

Grading of PSE PSE condition was graded by the same trained panel of 6 testers independently from each other for the same fillets as used for the assessment of the other breast myopathies. PSE was graded by visually assessment of the meat color. Fillets with a normal color were graded as 0-normal. Fillets with a pale appearance were graded as 1-PSE. The soft texture was not used as criterium for analysis, because of the possible co-appearance of breast myopathies.

Data Analysis

All data were analyzed by using the Minitab 18 Statistical Software (Minitab Inc., State Collage, PA). The significance for all statistical tests was declared when $P \leq 0.05$. To determine the difference of continuous data (live weight, ADG, ADFI, fillet weight, DL, pH) per treatment group, the Minitab Statistical Software assistant One-way *Welch*-ANOVA protocol was used, with the *Games-Howell* post-hoc test. A two-way ANOVA was initially performed to test for potential time by treatment interactions for the parameters pH and DL, but this was not significant in all comparisons and therefore the one-way Welch-ANOA test was performed to analyze the influence of time and treatment group on these variables. For determining the incidence of the breast myopathies WS and WB, as well as the occurrence of PSE fillets, the data of all 6 testers were used as combined data set to reduce potential bias by individual testers and to characerize the underlying individual variation. The categorial data (WS, WB, PSE) per treatment group and among each grading category, were analyzed using the Chi^2-test. To determine the co-occurrence of WS, WB and PSE, normalized contingency coefficients ($\mathbf{C_{norm}}$) were calculated. The $\mathbf{C_{norm}}$ value was calculated by using the Chi^2 calculated by Minitab and the following equation (with m = minimum number of rows or columns, n = number of observations):

$$C_{norm} = \sqrt{\frac{m}{m-1} * \frac{Chi2}{Chi2+n}} \quad (1)$$

The gradings for WS, WB and PSE were analyzed by using the attribute agreement analysis protocol of the Minitab software. This analysis was used to describe the percentages of all testers matching in their ratings.

RESULTS

Performance

There were no significant treatment differences in live body weight, ADG, nor FCR of the birds observed at any age (Table 3). There was a significant treatment effect on ADFI in the Starter phase. At the end of this phase, feeding group HIS49 showed higher ADFI

Table 3. Growth performance variables of broiler chickens fed different His:Lys ratios.

Parameter	Age, d	Treatment group					SEM	Probability
		HIS41[1]	HIS45[2]	HIS49[3]	HIS53[4]	HIS57[5]		
Weight, g	1	43.0	42.9	43.0	43.1	42.9	0.055	0.828
Weight, g	10	336	331	337	330	331	1.44	0.495
ADG, g/d		29.3	28.8	29.4	28.7	28.8	0.143	0.487
ADFI, g/d		30.2	29.8	30.7[a]	29.3[b]	29.7	0.143	0.040
FCR[6], g/g		1.03	1.03	1.05	1.02	1.03	0.003	0.359
Weight, g	20	957	939	982	932	940	7.68	0.417
ADG, g/d		45.7	44.8	46.9	44.4	44.8	0.384	0.418
ADFI, g/d		57.2	56.6	57.5	55.9	56.6	0.360	0.764
FCR, g/g		1.25	1.26	1.23	1.26	1.26	0.006	0.548
Weight, g	33	1,939	1,909	1,931	1,924	1,917	14.1	0.887
ADG, g/d		57.5	56.5	57.2	57.0	56.8	0.428	0.888
ADFI, g/d		89.2	87.6	89.5	85.1	88.7	0.546	0.071
FCR, g/g		1.55	1.55	1.57	1.50	1.57	0.010	0.304
Weight, g	38	2,342	2,330	2,376	2,354	2,367	19.5	0.734
ADG, g/d		60.5	60.2	61.4	60.8	61.2	0.513	0.949
ADFI, g/d		98.6	96.9	99.5	94.9	98.6	0.618	0.166
FCR, g/g		1.63	1.61	1.62	1.57	1.62	0.010	0.426

[a-b]Means within a row with different superscripts differ between used standard ileal digestible His:Lys ratios ($P < 0.05$).
[1]HIS41: standard ileal digestible amino acid His:Lys ratio of 0.41.
[2]HIS45: standard ileal digestible amino acid His:Lys ratio of 0.45.
[3]HIS49: standard ileal digestible amino acid His:Lys ratio of 0.49.
[4]HIS53: standard ileal digestible amino acid His:Lys ratio of 0.53.
[5]HIS57: standard ileal digestible amino acid His:Lys ratio of 0.57.
[6]FCR: feed conversion ratio.

compared to HIS53. The overall mortality rate was 0% in the Starter and the Grower I phase. In Grower II, a mortality rate of 1.14% was recorded in groups HIS41, HIS53, and HIS57, corresponding to 1 bird per group, while the mortality rate was 2.27% in the HIS45 group. In the Finisher phase, the mortality rate was 0%, except the group HIS53 which had a mortality of 1.14%. It should be noted that the temperature in the barn during Grower II was sometimes above the guideline and comfort zone for the birds (max. 25°C), due to a heat wave in those weeks.

Meat Quality Analysis

No significant interactions between storage time and treatment group were observed for pH ($P = 0.205$) nor DL ($P = 0.264$). The pH of the fillets was significantly different between the treatment groups at 24 and 144 h after slaughter (Table 4). While group HIS45 showed a higher mean value than HIS53 after 24 h, group HIS45 showed higher values than HIS41 after 144 h of storage. The overall pH of the fillets was also influenced by storage time ($P < 0.001$): 0 h (6.21) and 192 h (6.24) > 144 h (6.10) > 96 h

Table 4. Meat quality variables of breast fillets from broiler chickens fed different His:Lys ratios.

Parameter	Storage time, h	Treatment group					SEM	Probability
		HIS41[1]	HIS45[2]	HIS49[3]	HIS53[4]	HIS57[5]		
Fillet weight, g	0	253	250	258	250	256	2.9	0.832
pH[6]	0	6.22	6.24	6.20	6.20	6.21	0.005	0.098
	24	5.96	5.97[a]	5.94	5.93[b]	5.93	0.004	0.005
	48	5.92	5.97	6.01	5.96	5.98	0.015	0.067
	96	6.06	6.03	6.01	6.02	6.02	0.014	0.883
	144	6.05[b]	6.13[a]	6.10	6.10	6.11	0.009	0.028
	192	6.20	6.26	6.24	6.25	6.22	0.011	0.384
DL[7], %	24	5.83	5.65	5.71	6.30	6.70	0.176	0.292
	48	7.30	7.13	7.30	7.68	7.98	0.177	0.551
	96	9.16	8.88	8.84	9.44	9.91	0.183	0.329
	144	10.61	10.20	10.17	11.00	11.21	0.190	0.300
	192	11.37	10.96	10.77	11.73	11.99	0.190	0.207

[a-b]Means within a row with different superscripts differ between used SID His:Lys ratios ($P < 0.05$).
[1]HIS41: standard ileal digestible amino acid His:Lys ratio of 0.41.
[2]HIS45: standard ileal digestible amino acid His:Lys ratio of 0.45.
[3]HIS49: standard ileal digestible amino acid His:Lys ratio of 0.49.
[4]HIS53: standard ileal digestible amino acid His:Lys ratio of 0.53.
[5]HIS57: standard ileal digestible amino acid His:Lys ratio of 0.57.
[6]pH was measured in the cranial, middle and caudal part of the fillets and an average was calculated for analysis. At 0 h and 24 h all right fillets (88 fillets per treatment group) were measured. The birds were slaughtered between 38 and 41 d of age with 22 birds per treatment group each day. For the measurement of pH after 48, 96, 144, and 192 h of storage the fillets used for drip loss measurement were analyzed.
[7]DL: Cumulative drip loss for each storage time. Drip loss was measured for 22 fillets per treatment group of birds slaughtered at 38 d of age.

(6.03) > 24 h (5.94) and 48 h (5.97). There were no effects of dietary treatment on the weight of the fillets immediately after slaughter nor at any measured time points of storage (Table 4). The parameter DL was however influenced by storage time ($P < 0.001$), independent of treatment: 0−24 h (6.0 %) > 48−96 h (1.8 %) > 24−48 h (1.4%) and 96−144 h (1.4%) > 144−192h (0.7%).

The visual analysis of the breast fillets yielded a less frequent occurrence of SM and DPM. SM was graded 23 times as 1-moderate and 5 times as 2-severe by individual testers. This complied to 0.74% of the 2439 total gradings (n (HIS41) = 2, n(HIS45) = 9 n(HIS49) = 6, n(HIS53) = 3, n (HIS57) = 3). DPM was graded as 1-moderate 30 times by individual testers, which corresponded to 1.23% of the total gradings (n(HIS41) = 6, n(HIS45) = 11, n(HIS49) = 3, n (HIS53) = 3, n(HIS57) = 7). However, these myopathies were not further analyzed due to their low occurrence. The fillets affected by WS and WB were not entirely consistent between all 6 testers: using the 3-score grading system (0-normal, 1-moderate, and 2-severe). The analysis of the agreement between testers showed that only 53% of WS gradings had a clear agreement between all testers, whereas WB grading had only 43% agreement within the same grading category. The evaluation of PSE was consistent for 77% of the fillets by using only 2 grading categories (0-normal and 1-PSE). The incidences of WS, WB and PSE are given in Figure 1. Compared to the control, the treatment groups HIS49 ($P = 0.002$) and HIS53 ($P = 0.036$) showed an increase in WS occurrence and fewer fillets graded as 0-normal. Indeed, group HIS49 had -8.94% fewer 0-normal fillets, while HIS53 had -3.48% fewer compared to the control. Group HIS49 also had 7.74% greater incidences of severe

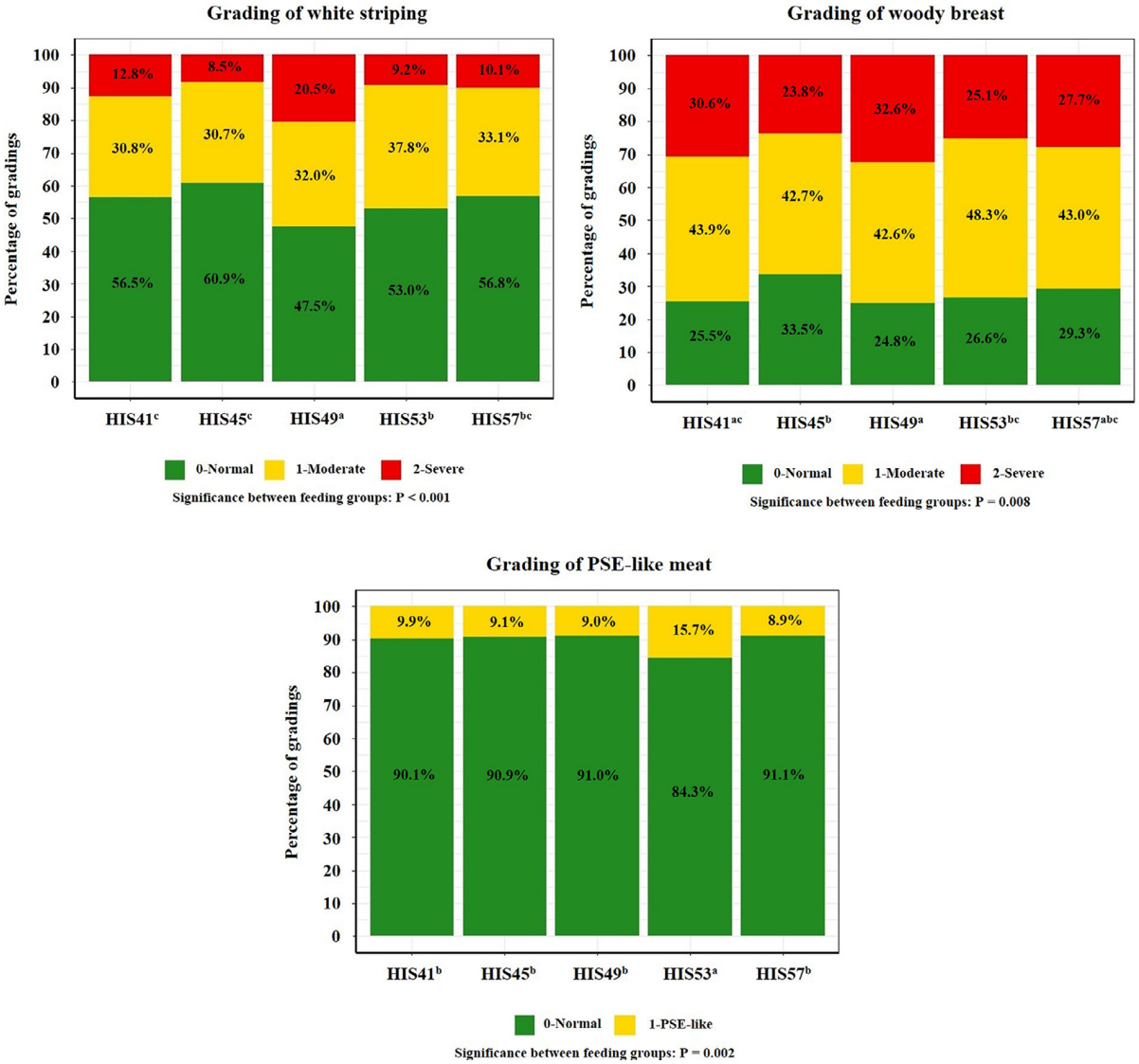

Figure 1. Percentages of fillets affected by the white striping, woody breast and of PSE conditions. All fillets were graded by 6 testers and the pooled data were used for analysis. All data were analyzed by using a Chi^2-test. Differences by groups, as given by the different superscripts, were analyzed by group-wise comparisons using the same test. Abbreviations: HIS41, standard ileal digestible amino acid His:Lys ratio of 0.41; HIS45, standard ileal digestible amino acid His:Lys ratio of 0.45; HIS49, standard ileal digestible amino acid His:Lys ratio of 0.49; HIS53, standard ileal digestible amino acid His:Lys ratio of 0.53; HIS57: standard ileal digestible amino acid His:Lys ratio of 0.57.

Table 5. Contingency table of white striping, woody breast, and PSE conditions of breast fillets from broilers fed different His:Lys ratios.

Separation criteria of the parameters	Occurrence of the quality issue per grading category, %				Probability	C_{norm}[2]
	WB[1] 0-normal	WB 1-moderate	WB 2-severe	Total		
WS[3] 0-normal	23.6	25.0	6.4	55.0	<0.001	0.576
WS 1-moderate	3.9	16.7	12.2	32.8		
WS 2-severe	0.5	2.4	9.3	12.2		
Total	28.0	44.1	28.0	100.0		
	WS 0-normal	WS 1-moderate	WS 2-severe	Total		
0-normal	49.2	29.5	10.8	89.5	0.826	0.018
1-PSE[4]	5.7	3.3	1.4	10.5		
Total	54.9	32.8	12.2	100.0		
	WB 0-normal	WB 1-moderate	WB 2-severe	Total		
0-normal	25.0	39.8	24.7	89.5	0.445	0.036
1-PSE	3.0	4.3	3.2	10.5		
Total	27.9	44.1	28.0	100.0		

The values are given in percentage of the overall gradings. The data of all testers were used for this analysis and a Chi^2-test was performed to determine the probability.
[1]WB, woody breast. The grading is given as 0-normal, 1-moderate, and 2-severe affected.
[2]C_{norm}: Normalized contingency coefficient. The contingency coefficient was calculated manually based on the Chi^2 calculated by Minitab®18 to evaluate the strength of the association between two gradings.
[3]WS, white striping. The grading is given as 0-normal, 1-moderate, and 2-severe affected.
[4]PSE, pale, soft, and exudative.

graded fillets, whereas in group HIS53, the main increase was seen in fillets which were graded as moderate with +7.00%. Moreover, group HIS53 showed a decrease in severe fillets by −3.52% compared to the control group. The occurrence of WS in group HIS49 was different compared to all other groups ($P \leq 0.002$), whereas HIS53 differed only from the control group and HIS45 ($P = 0.042$). In case of WB, HIS45 was different from the control group ($P = 0.008$), as more normal fillets (+7.96%) and less severe WB cases (−6.78%) were graded. No differences were noticed in moderate graded fillets. The treatment group HIS49 had more severe graded fillets (+8.8%) and less normal fillets (-8.7%) compared to the treatment group HIS45 (P = 0.001). Treatment group HIS53 had less severe cases (-7.5%) but more moderate graded fillets (+5.7%) than HIS49 (P = 0.037). The occurrence of PSE fillets was higher for group HIS53 compared to the other treatment groups (P ≤ 0.008). The correlations between the occurrence of WS, WB and PSE fillets were also studied by using gradings from all 6 testers (Table 5). No difference in the distribution of WS and WB gradings were seen by comparing them with the occurrence of PSE fillets. Indeed, when comparing the grading of WS and the gradings of WB, a moderate correlation was seen. The occurrence of WB was increased in fillets with higher occurrence of WS. However, it should be noted that both breast myopathies were also detected individually in some fillets.

DISCUSSION

In this study, increasing the dietary concentration of histidine did not significantly influence growth performance (ADG, ADFI, FCR) nor fillets' weight, with the exception of ADFI after the Starter phase. It is important to consider that the growth performance observed for birds in this study was below the expected target as suggested by Aviagen (2019b) for Ross 308 males for Grower II and Finisher phase, while FCR was higher than expected. This could have been related to the heat stress noted in the Grower II period, as the trial room was not sufficiently controlled during a summer heat wave and consequently, the room temperature was above the recommendation for a few days. It was reported that heat stress has negative impacts on bird's performance, health, and welfare (Lara and Rostagno, 2013; Rath et al., 2015; Nawab et al., 2018; Wasti et al., 2020). It can also be speculated that the heat stress during the trial influenced the response to the different dietary SID His:Lys inclusion levels because of the overall decrease in feed intake. Accordingly, the overall incidence of breast myopathies and the effect of the treatment groups may have been masked. In general, the results of the performance analysis were in accordance with previous studies. Kopeć et al. (2013) reported no differences in weight, FCR, fillet weight, nor mortality when comparing His:Lys ratios of 0.61 and 0.43 from 1 to 42 d. Moreover, Kai et al. (2015) reported no differences in weight, feed intake, and proportion of breast muscle for 14 d old female chicken fed a diet containing 0.70% His compared to a control diet with 0.35% His. Kralik et al. (2015) showed no difference in performance parameters of male broilers when supplementing 0.39, 0.49, 0.59, or 0.69% of His in the Finisher feed (22 −42 d), respectively. Likewise, the authors reported no influence on feed intake, breast weight and live body weight at 42 d of age for 2 different broiler strains. However, Edmonds and Baker (1987) showed a decrease in mean daily gain and feed intake in crossbred chickens when comparing a non-supplemented corn and soy-based basal diet to a basal diet supplemented with 4% additional His from 8 d of age until 16 d of age.

Conversely, a SID His:Lys ratio of 0.54 and 0.64 in the diet was shown to lead to an increase in the ADFI at 33 d of age compared to a control ratio of SID His:Lys 0.44 (Lackner et al., 2021). In the same study, this effect was no longer observed at 53 d of age. Mortality was also increased in broilers fed diets with a SID His:Lys ratio of 0.54 and 0.64 up to 53 d of age, whereas weight, FCR, ADG, and breast meat as percentage of carcass weight were not influenced by the SID His:Lys at both ages (Lackner et al., 2021). Kralik et al. (2018) reported the effect of a dietary supplementation of 0.25% His and 0.24% MgO. In their setup, MgO was used to activate ATP in a usable conformation as cofactor for the carnosine synthase. The supplementation was given between 22 and 42 d of age and resulted in higher weights. Indeed, FCR was also significantly lowered by His supplementation in that study, while breast meat yield was not influenced by His supplementation. To summarize, a high supplementation of His seems to have no or only limited impact on growth performance of broilers at a commercial slaughter age, which is comparable to the present study.

In previously published studies, it was shown that increased dietary His can increase the concentration of carnosine in skeletal muscle tissue of broilers, like the *Pectoralis major* muscle (Kai et al., 2015; Kralik et al., 2015; Lackner et al., 2021). This dipeptide is known as a pH buffer and therefore higher supplementation of the precursor His could have an impact on meat quality parameters. The water-holding capacity of meat is influenced by *post-mortem* pH changes, the sarcomere structure, as well as the proportion of fat, proteins, and other nutrients. After slaughter the muscle pH drops due to the formation of lactate in the muscle tissue. A low water-holding capacity of muscle proteins is reached at the isoelectric point of these proteins, which is given at a pH of 5.4 (Huff-Lonergan and Lonergan, 2005). The buffering effect of carnosine could be able to reduce the pH drop by lactate formation. Therefore, we speculated that higher dietary His could influences DL and pH of the breast meat. However, DL and pH were not affected by the different dietary SID His:Lys ratios in this study. The DL is influenced by many factors. Therefore, to evaluate the effect of a higher carnosine concentration, as result of a His supplementation in the diet, on DL a further dose-response study is needed. The same conclusion can be made for the influence of His and the resulting dipeptides on the pH of the fillets. The same results can be seen in the studies of Hu et al. (2009) by feeding 0.5% carnosine during the starter (1−21 d) or the Grower phase (22−42 d). Kralik et al. (2018) also reported no differences in pH, but a lower DL by feeding 0.24% His and 0.25% MgO. The only observed influence of the different dietary SID His:Lys ratios on meat quality parameters in this trial was on the incidence of breast myopathies and PSE. It was hypothesized that dietary supplementation of His and the resulting higher concentration of His-related dipeptides would improve the antioxidative status of the meat, therefore reducing muscle damage during hypoxia and decreasing the occurrence of breast muscle myopathies. In this trial, a lower incidence of WB was indeed observed by feeding a SID His:Lys ratio of 0.45, compared to the control at 0.41. In contrast, the incidence of WS was higher in treatment groups HIS49 and HIS53 compared to the control. In our previous study, the incidence of WS was reduced by feeding an SID His:Lys ratio of 0.54 compared to a ratio of SID His:Lys 0.44 and 0.64 at 33 d of age (Lackner et al., 2021). Aviagen (2019a) reported no reduction of breast myopathies by feeding an increased His:Lys ratio of 0.70 compared to a control diet with His:Lys 0.40. By comparing the results from the literature and the results of this study, no optimal ratio of His:Lys could be determined in regard to the incidence of breast myopathies. However, the sample size in the current study might have been suboptimal and limited the power of the trial. Therefore, additional studies are needed to analyze the impact of His supplementation on carnosine concentrations and its possible influence on breast myopathies. Another interesting finding was that regardless of the treatment groups, PSE was not related to the breast myopathies WB and WS, although both WB and WS myopathies were closely related (Table 5). Therefore, it can be speculated that WS and WB have a similar origin. It is known that both myopathies share some histopathological characteristics related to rapid muscle growth, such as hypertrophy, hypoxia, inflammation, and fiber necrosis (Petracci et al., 2019). Conversely, PSE may result from stress directly before slaughter or inadequate cooling of the carcass after slaughter (Bowker, 2017). This study also highlighted that the visual and manual grading systems for WS, WB and PSE are very subjective. The accepted number of grading categories and testers may need to be adjusted to improve this analysis. In order to make this analysis more objective, a valid automated assessment system is highly needed for the industry and future studies.

In conclusion, the dietary SID His:Lys ratios investigated in this trial had almost no influence on growth performance and DL and the pH differed only slightly between the feeding groups. In line with other studies, the results showed that a supplementation of His had ambiguous effects on the occurrence of breast myopathies, with few significant observable effects of the dietary treatments on the tested breast myopathies. An increased SID His:Lys ratio of 0.45 seems to decrease the incidence of WB, whereas the incidence of WS could not be reduced in this trial. Indeed, additional studies with a larger number of fillets could provide clearer information about the influence of His and the related dipeptides on the occurrence of breast myopathies.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PloS One 11:e0153750.

Adzitey, F., and N. Huda. 2011. Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences - a mini review. Int. Food Res. J. 18:11–20.

Albrecht, A., M. Hebel, C. Heinemann, U. Herbert, D. Miskel, B. Saremi, and J. Kreyenschmidt. 2019. Assessment of meat quality and shelf life from broilers fed with different sources and concentrations of methionine. J. Food Qual. 2019:6182580.

ANSVSA. 2015. Order no. 97/2015 of the national sanitary veterinary and food safety agency. Accessed July 2021. http://www.ansvsa.ro/wp-content/uploads/2020/08/Proiect_Ordin_modif_Ord_97_2015-11.08.2020.pdf?utm_source=rss&utm_medium=rss&utm_campaign=proiect-de-ordin-privind-modificarea-si-completarea-ordinului-ansvsa-nr-97-2015-pentru-aprobarea-normei-sanitare-veterinare-privind-procedura-de-autorizare-sanitara-veterinara-a-unitatilor-utilizatoa.

Aviagen. 2018. Broiler management handbook. Accessed July 2021. https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.

Aviagen. 2019a. Breast muscle myopathies. Accessed Apr. 2021. http://en.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/Breast-Muscle-Myopathies-2019-EN.pdf.

Aviagen. 2019b. Ross 308 and Ross 308 FF broiler performance objectives. Accessed May 2021. http://eu.aviagen.com/tech-center/download/1339/Ross308-308FF-BroilerPO2019-EN.pdf.

Bailey, R. A., E. Souza, and S. Avendano. 2020. Characterising the influence of genetics on breast muscle myopathies in broiler chickens. Front. Physiol. 11:1041.

Baldi, G., F. Soglia, and M. Petracci. 2020. Current status of poultry meat abnormalities. Meat Muscle Biol. 4:1–7.

Boldyrev, A. A., G. Aldini, and W. Derave. 2013. Physiology and pathophysiology of carnosine. Physiol. Rev. 93:1803–1845.

Bilgili, S. F.; and J. B. Hess. 2008. Arbor Acres update: green muscle disease. Reducing the incidence in broiler flocks. Accessed July 2021. http://ar.aviagen.com/assets/Tech_Center/AA_Technical_Articles/AAUpdateGreenMuscle.pdf.

Bowker, B. 2017. Developments in our understanding of water-holding capacity. Pages 77-113 in Poultry Quality Evaluation. M. Petracci and C. Berri, eds. Woodhead Publishing Series in Food Science, Technology and Nutrition, Sawston, UK.

Chen, L. R., M. M. Suyemoto, A. H. Sarsour, H. A. Cordova, E. O. Oviedo-Rondón, M. Wineland, H. J. Barnes, and L. B. Borst. 2019. Temporal characterization of wooden breast myopathy ("woody breast") severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. Avian Pathol 48:319–328.

Edmonds, M. S., and D. H. Baker. 1987. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: effects on growth and dietary choice in the chick. J. Anim. Sci. 65:699–705.

Evonik Nutrition & Care GmbH. 2016. Recommendations for broilers. Accessed June 2021. https://animal-nutrition.evonik.com/product/feed-additives/downloads/recommendations_broiler_03_16.pdf.

Fontaine, J., J. Hörr, and B. Schirmer. 2001. Near-infrared reflectance spectroscopy enables the fast and accurate prediction of the essential amino acid contents in soy, rapeseed meal, sunflower meal, peas, fishmeal, meat meal products, and poultry meal. J. Agric. Food Chem. 49:57–66.

Fontaine, J., B. Schirmer, and J. Hörr. 2002. Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. J. Agric. Food Chem. 50:3902–3911.

Franco, S. M., F.d. C. Tavernari, R. C. Maia, V. R. S. M. Barros, L. F. T. Albino, H. S. Rostagno, G. R. Lelis, A. A. Calderano, and R. N. Dilger. 2017. Estimation of optimal ratios of digestible phenylalanine + tyrosine, histidine, and leucine to digestible lysine for performance and breast yield in broilers. Poult. Sci. 96:829–837.

Golzar Adabi, S., and E. Demirok Soncu. 2019. White striping prevalence and its effect on meat quality of broiler breast fillets under commercial conditions. J. Anim. Physiol. Anim. Nutr. (Berl.) 103:1060–1069.

Griffin, J. R., L. Moraes, M. Wick, and M. S. Lilburn. 2018. Onset of white striping and progression into wooden breast as defined by myopathic changes underlying pectoralis major growth. Estimation of growth parameters as predictors for stage of myopathy progression. Avian Pathol 47:2–13.

Han, Y. M., H. Suzuki, and D. H. Baker. 1991. Histidine and tryptophan requirement of growing chicks. Poult. Sci. 70:2148–2153.

Hoehler, D., A. Lemme, C. Brito, and H. Rostagno. 2005. Standardized ileal amino acid digestibility of crystalline amino acids is close to 100% regardless of the standardization method. Poult. Sci. 84:39.

Hu, X., K. Hongtrakul, C. Ji, Q. Ma, S. Guan, C. Song, Y. Zhang, and L. Zhao. 2009. Effect of carnosine on growth performance, carcass characteristics, meat quality and oxidative stability in broiler chickens. J. Poult. Sci. 46:296–302.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci 71:194–204.

Kai, S., G. Watanabe, M. Kubota, M. Kadowaki, and S. Fujimura. 2015. Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. Anim. Sci. J. 86:541–546.

Kopeć, W., D. Jamroz, A. Wiliczkiewicz, E. Biazik, A. Pudlo, T. Hikawczuk, T. Skiba, and M. Korzeniowska. 2013. Influence of different histidine sources and zinc supplementation of broiler diets on dipeptide content and antioxidant status of blood and meat. Br. Poult. Sci. 54:454–465.

Kralik, G., Z. Kralik, I. D. Kušec, Z. Škrtić, and I. Kralik. 2015. Influence of dietary histidine, hybrid line and gender on chicken meat quality and carnosine concentration. J. Poult. Sci. 52:295–303.

Kralik, G., M. Sak-Bosnar, M. Grčević, and Z. Kralik. 2018. Effect of amino acids on growth performance, carcass characteristics, meat quality, and carnosine concentration in broiler chickens. J. Poult. Sci. 55:239–248.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677–2685.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.

Lackner, J., A. Albrecht, M. Mittler, A. Marx, J. Kreyenschmidt, V. Hess, and H. Sauerwein. 2021. Effect of feeding histidine and β-alanine on carnosine concentration, growth performance and meat quality of broiler chickens. Poult. Sci. 100:101393.

Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals (Basel) 3:356–369.

Nawab, A., F. Ibtisham, G. Li, B. Kieser, J. Wu, W. Liu, Y. Zhao, Y. Nawab, K. Li, M. Xiao, and L. An. 2018. Heat stress in poultry production: mitigation strategies to overcome the future challenges facing the global poultry industry. J. Therm. Biol. 78:131–139.

Petracci, M., F. Sirri, M. Mazzoni, and A. Meluzzi. 2013. Comparison of breast muscle traits and meat quality characteristics in 2 commercial chicken hybrids. Poult. Sci. 92:2438–2447.

Petracci, M., F. Soglia, M. Madruga, L. Carvalho, E. Ida, and M. Estévez. 2019. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. Food Saf. 18:565–583.

Rath, P., N. Behura, S. Sahoo, P. Panda, K. Mandal, and P. Panigrahi. 2015. Amelioration of heat stress for poultry welfare: a strategic approach. Int. J. Livest. Res. 5:1–9.

Romanian Parliament. 2014. Law no. 43/2014 on the protection of animals used for scientific purposes. Accessed July 2021. http://www.icbp.ro/resources/static_files/About/BUNASTARE/LEGE%20nr.%2043%20din%2011%20aprilie%202014.pdf.

Rostagno, H. S., and B. G. Becker. 2005. Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements. 2nd ed. Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, Brazil.

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Soglia, F., A. K. Silva, L. M. Lião, L. Laghi, and M. Petracci. 2019a. Effect of broiler breast abnormality and freezing on meat quality and metabolites assessed by 1H-NMR spectroscopy. Poult. Sci. 98:7139–7150.

Soglia, F., A. K. Silva, S. Tappi, L. M. Lião, P. Rocculi, L. Laghi, and M. Petracci. 2019b. Gaping of pectoralis minor muscles: magnitude and characterization of an emerging quality issue in broilers. Poult. Sci. 98:6194–6204.

Sundekilde, U. K., M. K. Rasmussen, J. F. Young, and H. C. Bertram. 2017. High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine. Food Chem 217:151–154.

Wasti, S., N. Sah, and B. Mishra. 2020. Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals (Basel) 10:1266.

Wecke, C., and F. Liebert. 2013. Improving the reliability of optimal in-feed amino acid ratios based on individual amino acid efficiency data from N balance studies in growing chicken. Animals (Basel) 3:558–573.

Zanetti, M., D. Tedesco, T. Schneider, S. Teixeira, L. Daroit, F. Pilotto, E. Dickel, S. Santos, and L. Santos. 2018. Economic losses associated with wooden breast and white striping in broilers. Semin. Cienc. Agrar. 39:887.

Manuscript III

PLOS ONE

RESEARCH ARTICLE

Effects of dietary supplementation with histidine and β-alanine on blood plasma metabolome of broiler chickens at different ages

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Abstract

Histidine is an essential amino acid for broiler chickens and a precursor for the dipeptides carnosine and anserine, but little information is available about its metabolism in modern, fast-growing broilers. We used untargeted metabolomics to investigate the metabolic changes caused by the use of different standardized ileal digestible His:Lys ratios in broiler diets with and without β-alanine supplementation. A total of 2204 broilers were randomly divided into 96 pens of 23 birds each. The pens were divided into 16 blocks, each containing one pen for all six feeding groups (total of 16 pens per group). These feeding groups were fed three different His:Lys ratios (0.44, 0.54, and 0.64, respectively) without and with a combination of 0.5% β-alanine supplementation. Five randomly selected chickens of one single randomly selected pen per feeding group were slaughtered on day 35 or 54, blood was collected from the neck vessel, and plasma was used for untargeted metabolomic analysis. Here we show that up to 56.0% of all metabolites analyzed were altered by age, whereas only 1.8% of metabolites were affected by the His:Lys ratio in the diet, and 1.5% by β-alanine supplementation. Two-factor analysis and metabolic pathway analysis showed no interaction between the His:Lys ratio and β-alanine supplementation. The effect of the His:Lys ratio in the diet was limited to histidine metabolism with a greater change in formiminoglutamate concentration. Supplementation of β-alanine showed changes in metabolites of several metabolic pathways; increased concentrations of 3-aminoisobutyrate showed the only direct relationship to β-alanine metabolism. The supplementation of β-alanine indicated few effects on histidine metabolism. These results suggest that the supplements used had limited effects or interactions on both His and β-alanine metabolism. In contrast, the birds' age has the strongest influence on the metabolome.

Introduction

Histidine (**His**) is an essential amino acid for many species, including poultry [1, 2]. With its unique aromatic imidazole side chain, His can function both as a proton donor and acceptor under physiological conditions and is important for the structure of proteins and for the catalytic activity of enzymes [3–7]. His and its related molecules can act as metal ion chelators, pH buffers, antioxidants, or extracellular messengers [3, 6, 8–11], which makes them important molecules under physiological conditions. The His metabolism consists of several known metabolic pathways: the main catabolism to glutamate, the catabolism through the intermediates imidazole pyruvate and imidazole lactate and the decarboxylation of His to form histamine [3, 6] which acts as a signaling molecule in many tissues and metabolic pathways [12]. Other metabolites include acetylated and methylated His forms. In vertebrates, His-related dipeptides can also be found in relevant concentrations in skeletal and cardiac muscle tissue and in some parts of the brain [9]. These dipeptides have important functions and can act as intracellular buffers, metal-ion chelators, and antioxidants [9]. In chickens, carnosine, synthesized from His and the non-essential amino acid β-alanine (**βA**), as well as its methylated form anserine, are the dominating His-related dipeptides [9, 13]. The second precursor amino acid βA is formed as an intermediate of the metabolic pathways of aspartate and uracil [14, 15].

Dietary recommendations for the ratio of His to lysine (**Lys**) vary from 0.31 to 0.41 in broiler chickens [16–20]. In commercial production, His is not considered a limiting factor, and thus receives little attention in feed formulation. Nevertheless, carnosine depletion has been described in birds suffering from breast muscle myopathies such as white striping, woody breast, and spaghetti meat [21, 22]. Therefore, higher feeding levels of His could help to increase the concentration of this important dipeptide and counteract the development of breast myopathies. However, less is known about the overall metabolism of broilers fed higher His concentrations and the effect of βA supplementation on metabolism. The metabolomic study described in this paper is based on a feeding trial in which the effects of three different standardized ileal digestible His:Lys ratios in the diets of fast-growing broiler chickens with or without βA supplementation were tested at different ages [23]. This previous study showed less influence of the His:Lys ratio or βA supplementation on meat quality parameters, but an increase in carnosine concentration with a higher His:Lys ratio in feed, but without βA supplementation having an effect on the concentration of this dipeptide. In addition, with generally little influence on the performance of the animals, it was shown that His feeding could lead to an increase in mortality in older animals. The current study aimed at complementing the previous study by identifying the main metabolites impacted by His or βA supplementation and the possible interaction of both. Additionally, the impact of birds' age on metabolism, especially His metabolism, should be further studied at different ages using an untargeted metabolomics approach. It was found that age had a major impact on broiler metabolism. The His:Lys ratio in feed mainly affected the targeted His metabolism, while a supplementation of βA had only marginal effects on a few different metabolites. A supplementation of βA, as opposed to His, had no effect on carnosine metabolism. In addition, it was found that with higher His:Lys rations in feed the concentrations of formiminoglutamate in blood plasma of broiler chicken was increased, which is a marker for folate or cobalamin deficiency [24, 25].

Materials and methods

Study design and sampling

The animals investigated in this study were kept and treated as for good farming practice but were not exposed to specific measures that could imply pain, suffering or damage. All

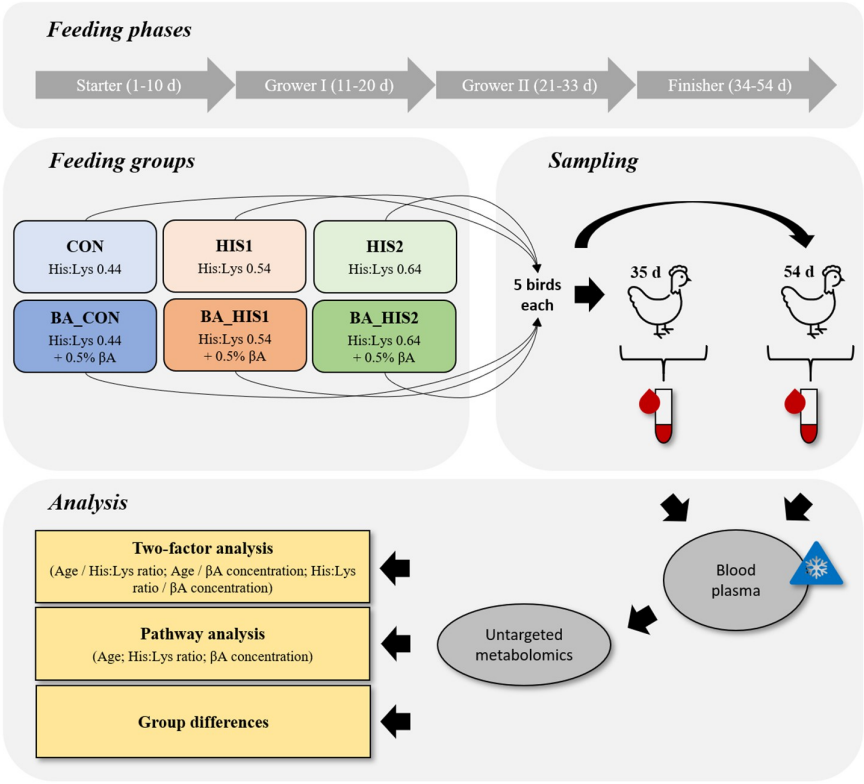

Fig 1. Study design. Abbreviations: His:Lys, standardized ileal digestible histidine:lysine ratio; βA, β-alanine; CON, group fed with a His:Lys ratio of 0.44; HIS1, group fed with a His:Lys ratio of 0.54; HIS2, group fed with a His:Lys ratio of 0.64; BA_CON, group fed with a His:Lys ratio of 0.44 + supplementation of 0.5% β-alanine; BA_HIS1, group fed with a His:Lys ratio of 0.54 + supplementation of 0.5% β-alanine; BA_HIS2, group fed with a His:Lys ratio of 0.64 + supplementation of 0.5% β-alanine.

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evaluations were performed on dead animals and the killing of animals for collecting samples is not defined as animal experiment according to the German animal welfare act (TierSchG). The study followed animal welfare regulations for commercial fattening and the animal care legislation of the German animal welfare act including the EU Directive 2010/63/EU. An overview of the study design is given in Fig 1. For the present metabolomic study, plasma samples collected in a feeding trial in which the effects of standardized ileal digestible amino acid His:Lys ratios of 0.44 (**CON**), 0.54 (**HIS1**), and 0.64 (**HIS2**) combined with or without a supplementation of 0.5% total βA (**BA_CON; BA_HIS1; BA_HIS2**) on performance, His-related dipeptide content, and meat quality were tested. This entire study, including the composition of the basal diet for each feeding phase and the supplemented His and βA concentrations relative to the basal diet, is described in detail in a previous publication [23]. Briefly, the study was conducted with 2,208 Ross 308 broiler chickens randomly allocated in 96 pens with 23 birds per pen. Each adapted feeding, of the six feeding groups, was administered to the birds in a randomized block design with 16 replicates (368 birds per group). The adapted feedings of different feeding groups were given over the whole fattening period in all feeding phases (Starter 1–10 d, Grower I 11–20 d, Grower II 21–33 d, Finisher 34–54 d). At 35 and 54 days of age, five different birds from a randomly selected pen per feeding group were randomly selected for

blood sampling. The same birds were also used for performance analysis and targeted analysis of molecules in the breast muscle, but not for further studies [23]. To take the samples, birds were stunned by hitting the head, which caused a lack of consciousness, and then killed in a slaughter funnel by severing the neck vessel. Approximately 7 mL of blood were immediately collected from the neck vessel during bleeding and placed in K_3 EDTA-containing vacutainers (polyethylene terephthalate, Greiner Bio-one, Frickenhausen, Germany) to prevent blood clotting. The blood was gently swirled by hand and briefly placed in a roller mixer. Samples were then centrifuged (1500 *g*, 4˚C, 10 min) and 200 μL of plasma were transferred to a pre-chilled 2 mL cryovial (polypropylene, Simport Scientific, Saint-Mathieu-de-Beloeil, QC, Canada). The tubes were immediately cooled in liquid nitrogen, stored at -80˚C, and further used for the metabolomics analysis.

Metabolomics analysis

For the metabolomics analyses, the samples were transferred on dry ice to Metabolon Inc. (Morrisville, NC, USA) where four methods were performed on their HD4 platform: Ultra-High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS), whereby two methods applied reverse-phase UPLC-MS/MS with electrospray ionization (ESI) in positive ion mode, one method used ESI in negative ion mode, and one method used hydrophobic interaction liquid chromatography UPLC-MS/MS with ESI in negative ion mode. Metabolites were identified using the Metabolon hardware and software. All metabolites were quantified as area-under-the-curve detector ion counts and normalized with respect to the raw area counts. For standardization of the measured values internal standards of Metabolon were used.

Data analysis

For the analysis, which takes more than one day, the original values were normalized in terms of raw area counts. For further statistical analysis all data were rescaled by the median of all 60 samples for a given molecule. Missing values were imputed with the observed minimum value for each detected metabolite after rescaling (4.8% of the overall values). A statistical heat map for age differences per feeding group (35 d and 54 d) and single group comparisons at each slaughter age was provided by Metabolon Inc. The heat map is based on the statistical differences of the respective group comparisons using pairwise comparison based on ANOVA contrasts on log-transformed data. The MetaboAnalyst 5.0 web tool (Wishart Research Group, University of Alberta, AB, Canada) was used for further analysis. The method used for two-factor analysis and pathway analysis was previously reported [26, 27]. Previously normalized and rescaled data were used for the analysis. Using MetaboAnalyst 5.0, metabolite data were transformed using the generalized log transformation. No additional filtering or normalization of the data was performed by the software. For the two-factor analysis, the study design of 'two-factor independent samples' was set, and two-way type I ANOVA analysis was performed to identify differences and interactions between the factors His:Lys ratio/ βA supplementation and His:Lys ratio/slaughter age in the diet and βA supplementation/slaughter age. False discovery rates (FDR) were set for the multiple test corrections with an adjusted p-value cutoff at 5%. For analyzing the metabolic pathways affected by His:Lys ratio in the diet, βA supplementation, or slaughter age as a factor, MetaboAnalyst 5.0 (Wishart Research Group, University of Alberta, Canada) was used based on the Kyoto Encyclopedia of Genes and Genomes database (KEGG, Kanehisa Laboratories, Institute for Chemical Research, Kyoto, Japan, https://www.genome.jp/kegg/). For metabolite identification, the ID numbers from the Human Metabolon Database (HMDB) were used because most metabolites were identified in this database. All

other analyzes were performed using the Minitab® 18 software (Minitab Inc., State College, PA, USA). For all analyses, significance was declared at $P \leq 0.05$.

Results

In total, 655 metabolites were identified. Metabolites were grouped into nine categories: Amino acids (n = 199), peptides (n = 27), carbohydrates (n = 29), energy metabolism (metabolites of the TCA cycle and oxidative phosphorylation, n = 12), lipids (n = 236), nucleotides (n = 46), cofactors and vitamins (n = 27), and xenobiotics (n = 79).

Influenced metabolites by treatments and age

To analyze possible effects of His:Lys ratio, βA supplementation, and slaughter age, as well as interactions of these factors a two-factor analysis was performed. In a two-factor analysis, no interactions were found between the His:Lys ratio and the βA-supplements in both slaughter ages. Therefore, both parameters were analyzed separately combined with slaughter age as factor. When the His:Lys ratio or the βA-supplements were analyzed together with the slaughter age, the most significantly different metabolites were detected for slaughter age (Figs 2A and 3A). The 12 metabolites that were significantly different for the His:Lys ratios are shown in Fig 2B. All these metabolites belong to His metabolism, including the dipeptides γ-glutamylhistidine and carnosine. The same metabolites differed significantly when the His:Lys ratio and βA additions at both slaughter ages were used as factors for two-factor analysis, except for *N*-acetyl-1methylhistidine, which was affected only by two-factor analysis of the His:Lys ratio and slaughter age. Four metabolites of His metabolism were influenced only by slaughter age: 1-methylhistamine (P = 0.023), *trans*-urocanate (P = 0.003), 1-methyl-5-imidazoleacetate (P = 0.022), and N-acetyl-3-methylhistidine (P <0.001). Two-factor analysis of βA supplementation and slaughter age identified 10 metabolites that were significantly affected by βA supplementation (Fig 3B). The metabolite βA showed differences for βA supplementation, slaughter age, and the interaction of both in blood plasma. The metabolites N-methyl-GABA, N-acetyltaurine, and creatine phosphate were decreased by 0.5% βA supplementation in broiler diets. The other affected metabolites found at higher concentrations by feeding 0.5% βA were 3-aminoisobutyrate, N2-acetyl-N6-methyllysine, leucylglycine, sphingomyelin d18:1/21:0, d17:1/22:0, d16:1/23:0, and the xenobiotics stachydrine and homostachydrine. The same metabolites differed significantly with βA-supplementation when using His:Lys ratio and βA supplementation as factors for two-factor analysis, except for sphingomyelin d18:1/21:0, d17:1/22:0, d16:1/23:0. Slaughter age had the greatest effect on metabolite concentration. Of all metabolites, 55.6% (n = 364) and 56.0% (n = 367) showed significant differences in the analysis of His:Lys and βA supplementation, respectively, in a two-factor analysis with age as the second factor.

Effected metabolic pathways by treatments and age

A metabolic pathway analysis was performed to analyze the effects of dietary His:Lys ratio, βA supplementation, and slaughter age on metabolic pathways. Analysis of the effects of His:Lys ratio in the diet and βA supplementation showed a small effect for these two factors. Variation of His in the diet mainly affected His metabolism. In addition, aminoacyl-tRNA biosynthesis was affected, but with less influence on the metabolic pathway (Fig 4A). Supplementation of βA affected βA-alanine metabolism, pyrimidine metabolism, pantothenate-CoA biosynthesis, arginine and proline metabolism, and glyoxylate and dicarboxylate metabolism. Propanoate metabolism was also affected, but with less impact on the metabolic pathway (Fig 4B). Unlike the His:Lys ratio in the diet and βA supplementation, where only a few metabolic pathways were affected, many metabolic pathways were affected by the age of the birds (Fig 4C). The

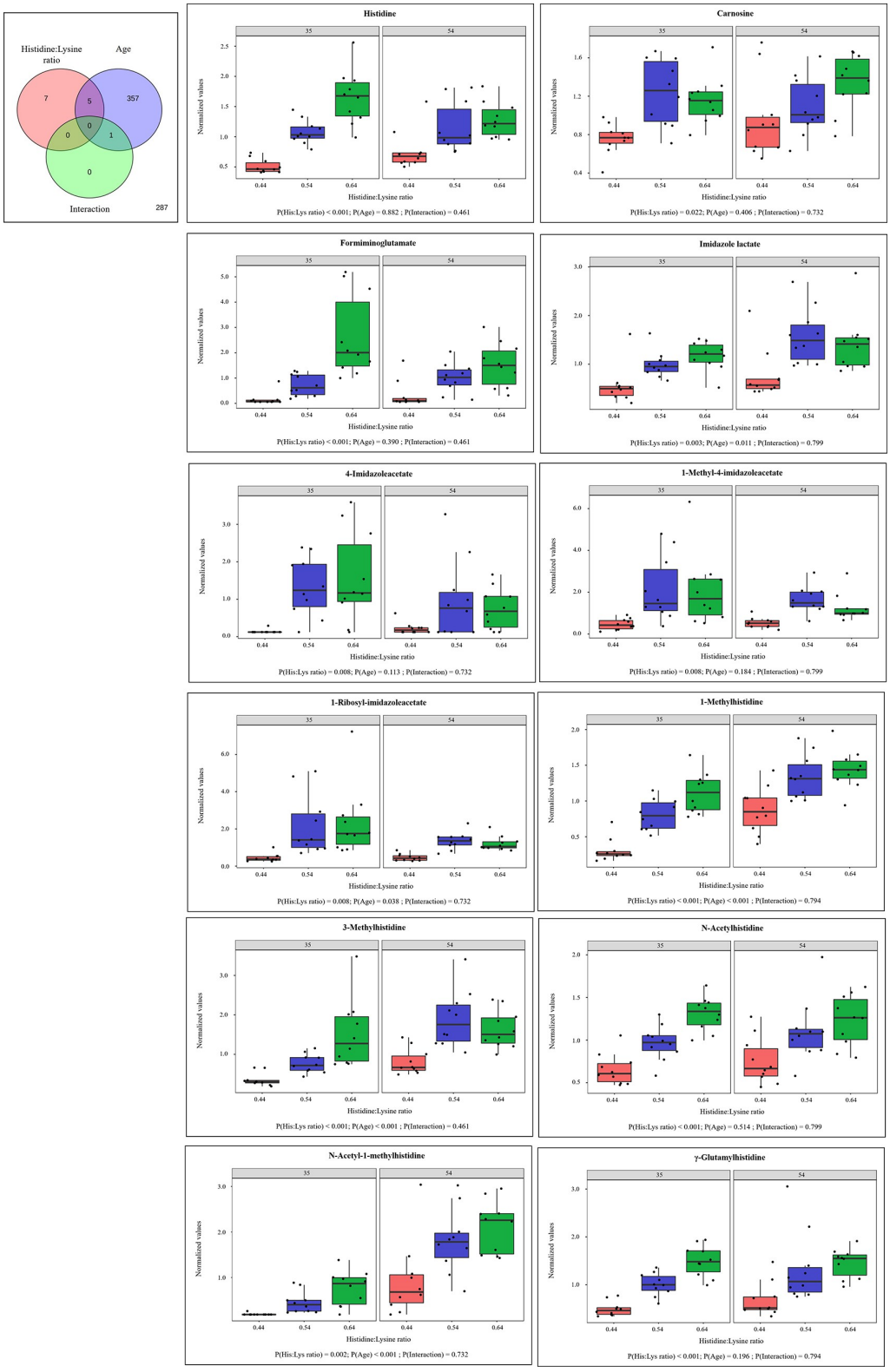
Histidine:Lysine ratio
Age
7
5
357
0
0
1
0
Interaction
287
Histidine
P(His:Lys ratio) < 0.001; P(Age) = 0.882 ; P(Interaction) = 0.461
Carnosine
P(His:Lys ratio) = 0.022; P(Age) = 0.406 ; P(Interaction) = 0.732
Formiminoglutamate
P(His:Lys ratio) < 0.001; P(Age) = 0.390 ; P(Interaction) = 0.461
Imidazole lactate
P(His:Lys ratio) = 0.003; P(Age) = 0.011 ; P(Interaction) = 0.799
4-Imidazoleacetate
P(His:Lys ratio) = 0.008; P(Age) = 0.113 ; P(Interaction) = 0.732
1-Methyl-4-imidazoleacetate
P(His:Lys ratio) = 0.008; P(Age) = 0.184 ; P(Interaction) = 0.799
1-Ribosyl-imidazoleacetate
P(His:Lys ratio) = 0.008; P(Age) = 0.038 ; P(Interaction) = 0.732
1-Methylhistidine
P(His:Lys ratio) < 0.001; P(Age) < 0.001 ; P(Interaction) = 0.794
3-Methylhistidine
P(His:Lys ratio) < 0.001; P(Age) < 0.001 ; P(Interaction) = 0.461
N-Acetylhistidine
P(His:Lys ratio) < 0.001; P(Age) = 0.514 ; P(Interaction) = 0.799
N-Acetyl-1-methylhistidine
P(His:Lys ratio) = 0.002; P(Age) < 0.001 ; P(Interaction) = 0.732
γ-Glutamylhistidine
P(His:Lys ratio) < 0.001; P(Age) = 0.196 ; P(Interaction) = 0.794
Normalized values
Histidine:Lysine ratio
35
54
0.44
0.54
0.64

Fig 2. Two-factor analysis by using standardized ileal digestible histidine:lysine ratio in feed and slaughter age as factor. (A) Venn diagram of dietary standardized ileal digestible histidine:lysine ratio and slaughter age as parameters. Differences in metabolite concentrations were analyzed using a 2-way ANOVA. Metabolites were separated according to the statistically significant effects of the parameter's standardized ileal digestible histidine:lysine ratio in the diet (0.44, 0.54, and 0.64, respectively), age at slaughter (35 d and 54 d, respectively), and the interaction of both. Significance was indicated at $P \leq 0.05$; (B) Boxplots of the significantly different metabolites by histidine:lysine ratio; One-way ANOVA results were generated using Minitab18®; Abbreviations: His:Lys, standardized ileal digestible histidine:lysine ratio.

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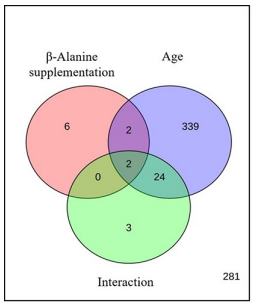
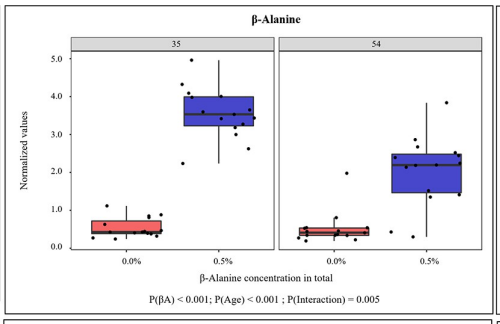
metabolic pathways affected included amino acid, nucleotide, lipid, cofactor, and carbohydrate metabolic pathways. In addition, His and βA metabolism were also strongly affected by the age of the birds. The metabolic pathways affected and the exact values of the effects, as well as the statistical relevance of each, are shown in Table 1.

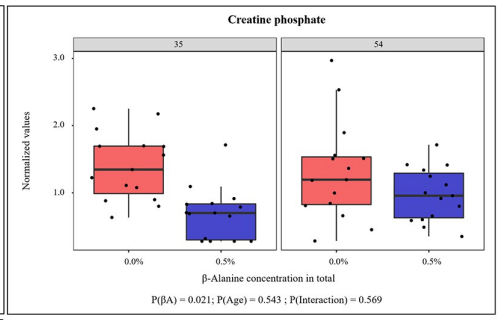
Effects of the different feeding groups on histidine metabolism

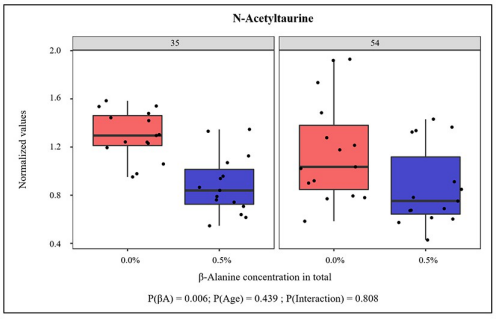
The group-wise analysis, as shown in the statistical heat map (Table 2), is intended to provide an overview of the differences in the His pathway due to the different feeding groups and slaughter ages for each group. Few metabolites of His metabolism had higher mean values in 54 d old birds than in 35 d old birds. The concentration of His in plasma was higher in the CON, HIS1, and BA groups at 54 d of age than in younger birds. Most changes between slaughter ages were observed in the methylated and acetylated forms of His. In addition, the metabolite N-acetyl-1-methylhistidine was affected by all age-related comparisons. The metabolite *trans*-urocante, which is related to the catabolism of His, was more concentrated in all groups supplemented with βA at 54 d of age. In contrast to the comparison of feeding groups, anserine was affected by slaughter age in HIS1, HIS2, BA_CON, and BA_HIS1. The concentration of βA was also age-dependent and decreased in the BA_CON and BA_HIS1 groups at 54 d of age. Many metabolites had higher concentrations when the experimental groups were compared with CON at both ages. As with the age comparison, many changes in methylated and acetylated forms of His were noted, but catabolism of His and histamine metabolism were also strongly affected in all groups that received a higher ratio of His:Lys compared with the control. The BA_CON group showed the least changes in the concentration of metabolites. After 35 d, only βA showed higher concentrations compared to CON. In addition, βA was less concentrated in HIS1 and HIS2 after 35 d, as well as in HIS1 after 54 d. The dipeptide carnosine showed no effect when comparing BA_CON and CON at both ages. The metabolites 4-Imidazole-5-propionate, glutamate, anserine, and histamine were not affected by the comparison of feeding groups at both ages. Relating the variation in the metabolome to variation in performance, as indicated by Lackner et al. 2021 [23], would have been interesting, but was not possible due to the small number of birds per group and the pen-wise recording of performance.

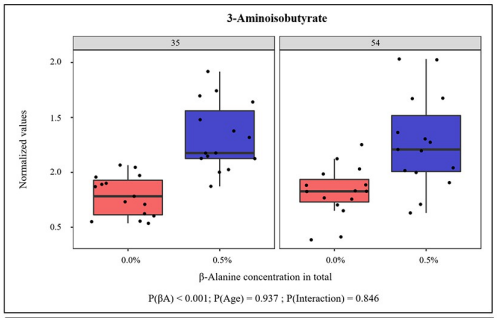
To analyze protein turnover and inflammatory conditions, some additional analyzes were performed. Protein turnover was analyzed by the 3-methylhistidine:creatinine ratio. The 3-methylhistidine:creatinine ratio increased with dietary His:Lys ratio ($P < 0.001$, His:Lys 0.44 = 0.63, His:Lys 0.54 = 1.07, His:Lys 0.64 = 1.51) and with βA supplementation ($P = 0.021$, 0% βA = 0.97, 0.5% βA = 1.17) using a linear model for ANOVA. Age had no significant effect on the ratio of 3-methylhistidine to creatinine. The ratio of kynurenine to tryptophan was used as a marker for inflammation. In a linear model for ANOVA, the ratio of kynurenine to tryptophan, as a marker of inflammation [28], was influenced only by age ($P < 0.001$, 35 d = 0.92, 54 d = 1.16), but not by experimental His or βA supplementation. In addition, interactions were found between age and βA supplementation ($P = 0.001$) and His:Lys ratio and βA

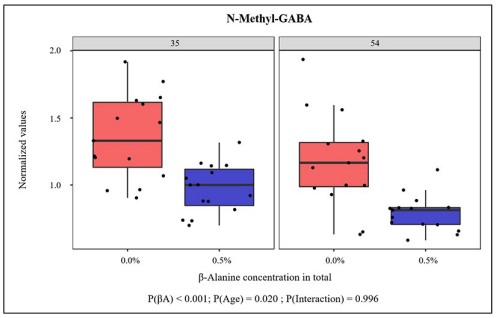

β-Alanine supplementation
Age
Interaction
6
2
339
0
2
24
3
281

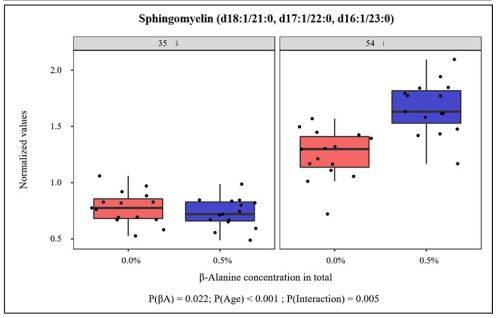

β-Alanine
35
54
Normalized values
β-Alanine concentration in total
P(βA) < 0.001; P(Age) < 0.001 ; P(Interaction) = 0.005

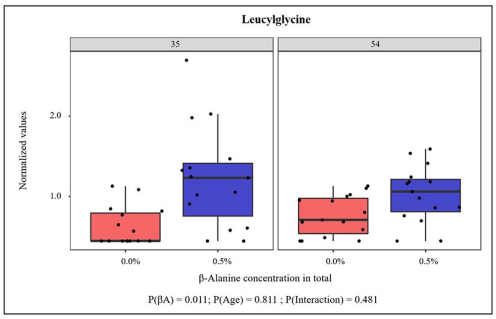

Creatine phosphate
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.021; P(Age) = 0.543 ; P(Interaction) = 0.569

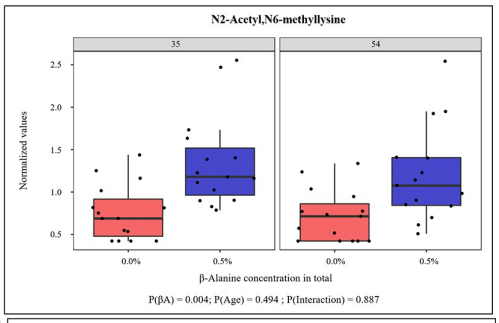

N-Acetyltaurine
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.006; P(Age) = 0.439 ; P(Interaction) = 0.808

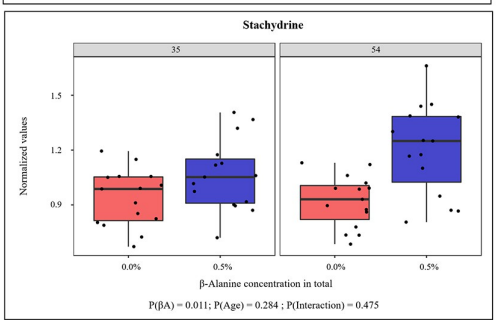

3-Aminoisobutyrate
35
54
Normalized values
β-Alanine concentration in total
P(βA) < 0.001; P(Age) = 0.937 ; P(Interaction) = 0.846


N-Methyl-GABA
35
54
Normalized values
β-Alanine concentration in total
P(βA) < 0.001; P(Age) = 0.020 ; P(Interaction) = 0.996


Sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.022; P(Age) < 0.001 ; P(Interaction) = 0.005


Leucylglycine
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.011; P(Age) = 0.811 ; P(Interaction) = 0.481


N2-Acetyl,N6-methyllysine
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.004; P(Age) = 0.494 ; P(Interaction) = 0.887


Stachydrine
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.011; P(Age) = 0.284 ; P(Interaction) = 0.475

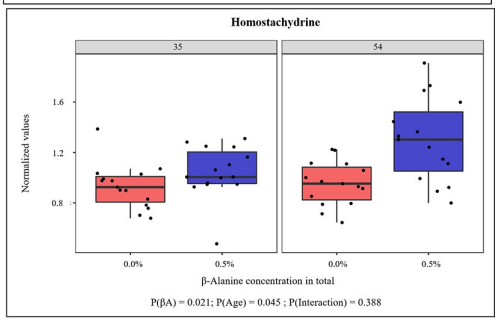

Homostachydrine
35
54
Normalized values
β-Alanine concentration in total
P(βA) = 0.021; P(Age) = 0.045 ; P(Interaction) = 0.388

Fig 3. Two-factor analysis by using β-alanine supplementation in feed and slaughter age as factor. (A) Venn diagram of dietary β-alanine supplementation and slaughter age as parameters. Differences in metabolite concentrations were analyzed using a 2-way ANOVA. Metabolites were separated according to the statistically significant effects of the parameters β-alanine supplementation in feed (0% and 0.5%), age at slaughter (35 d and 54 d of age), and the interaction of both. Significance was indicated at $P \leq 0.05$; (B) Boxplots of the significantly different metabolites by β-alanine supplementation. Abbreviations: βA, β-alanine.

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supplementation (P = 0.004). The results for the group-wise analysis of these metabolites were additionally reported in Table 2.

Discussion

Two-factor analysis of the His:Lys ratio and slaughter age, as well as metabolic pathway analysis for the His:Lys ratio, showed that the different ratios mainly affected His metabolism. Several metabolites of His metabolism were more abundant in the groups fed with higher His:Lys ratios, especially formiminoglutamate and imidazole lactate. The predominant metabolites of His catabolism are shown in the heat map (Table 2); formiminoglutamate which is the direct link between His and glutamate metabolism, was also found significant in the two-factor analysis (Fig 2B). It appears that oversupplied His is mainly catabolized towards glutamate and the citric acid cycle, as also stated for mammals [6]. Nevertheless, heat map analysis of glutamate and other metabolites did not reveal higher levels than those found at CON. Moreover, urocanate and 4-imidazole-5-propionate as precursors of formiminoglutamate were also not affected by the His:Lys ratios used, as analyzed by two-factor analysis (Fig 2B). This could indicate a fast-catalytic mechanism for formiminoglutamate and a slow turnover for glutamate. The greater concentration of formiminoglutamate could be the result of His loading by feeding His:Lys ratios of 0.54 and 0.64. A high concentration of formiminoglutamate in mammalian urine is also known to be a marker of folate or cobalamin deficiency [24, 25]. This is caused by the use of tetrahydrofolate (**THF**) by the enzyme formimidoyltransferase-cyclodeaminase. This enzyme transfers the formimine group to THF, resulting in glutamate as end product. It can be speculated that higher exposure to His, and thus higher turnover towards glutamate, results in lower THF levels and an increase in formiminoglutamate in the metabolism of broiler chickens. The cofactor cobalamin is also coupled to folate and His metabolism via the enzyme methionine synthase. This enzyme uses homocysteine and N5-methyl-THF to form

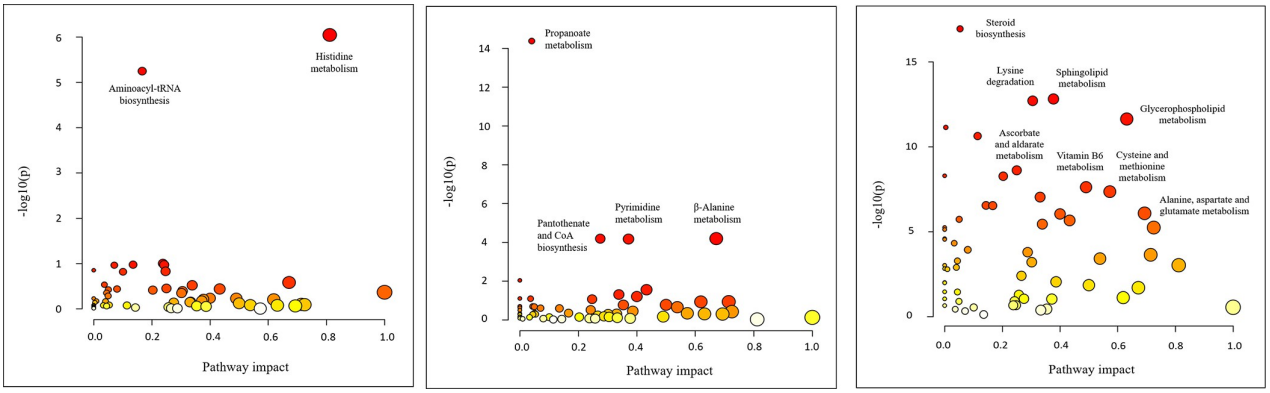

Fig 4. Summary of pathway analysis by using MetaboAnalyst 5.0. The color of the given dots are based on the P-value and the radius is based on the pathway impact value. (A) Pathway analysis by separating the data by standardized ileal digestible histidine:lysine ratios in the feed (0.44; 0.54 and 0.64, repectively); (B) Pathway analysis by separating the data by β-alanine supplementation in the feed (0% and 0.5%, repectively); (C) Pathway analysis by separating the data by slaughter ages (35 d and 54 d, respectively).

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Table 1. Impacted pathways by the different parameters as analyzed by pathway analysis.

Parameter	Metabolic pathway	Impact value	Probability
His:Lys ratio[a]	Histidine metabolism	0.81	<0.001
	Aminoacyl-tRNA biosynthesis	0.17	<0.001
βA supplementation[b]	β-Alanine metabolism	0.67	<0.001
	Arginine and proline metabolism	0.43	0.028
	Pyrimidine metabolism	0.37	<0.001
	Glyoxylate and dicarboxylate metabolism	0.34	0.049
	Pantothenate-CoA biosynthesis	0.28	<0.001
	Propanoate metabolism	0.04	<0.001
Slaughter age	Histidine metabolism	0.81	0.001
	Glycine/serine and threonine metabolism	0.72	<0.001
	Taurine and hypotaurine metabolism	0.71	<0.001
	Alanine/aspartate and glutamate metabolism	0.69	<0.001
	β-Alanine metabolism	0.67	0.020
	Glycerophospholipid metabolism	0.63	<0.001
	Cysteine and methionine metabolism	0.57	<0.001
	D-Glutamine and D-glutamate metabolism	0.50	0.014
	Vitamin B6 metabolism	0.49	<0.001
	Sphingolipid metabolism	0.38	<0.001
	Arachidonic acid metabolism	0.33	<0.001
	Lysine degradation	0.31	<0.001
	Ascorbate and aldarate metabolism	0.25	<0.001
	Pentose and glucuronate conversions	0.20	<0.001
	Terpenoid backbone biosynthesis	0.11	<0.001
	Steroid biosynthesis	0.05	<0.001

Slaughter age is repharing to the ages 35 d and 54 d, respectively

[a]His:Lys ratio: standardized ileal digestible histidine:lysine ratios in the feed (0.44; 0.54 and 0.64, repectively)

[b]βA supplementation: β-alanine supplementation in the feed (0% and 0.5%, repectively)

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methionine and THF. Therefore, a high concentration of formiminoglutamate could also be a consequence of cobalamin deficiency [29]. However, other THF-dependent metabolic pathways such as serine/glycine metabolism, methionine metabolism, or tryptophan metabolism showed no changes. The second intermediate of His catabolism detected when feeding a higher His:Lys ratio was imidazole lactate, which is formed from the previous metabolite imidazole acetate. It was pointed out that the minor catabolism of His to imidazole acetate occurs at high His concentrations and imidazole acetate can be further catabolized to imidazole lactate [3]. In addition, the intermediates imidazole-4-acetate and 1-methyl-4-imidazole acetate, the final metabolites of the two ways of histamine catabolism, were detected at higher concentrations when fed with higher ratios of His:Lys. The concentration of the His-related dipeptide carnosine was greater at the higher ratio of His:Lys, whereas no differences were detected for the methylated form anserine. However, these dipeptides are naturally degraded by carnosinase activity in blood plasma, whereas synthesis occurs in tissues such as skeletal muscle and brain [9, 30] where high concentrations of these two dipeptides have previously been reported following dietary supplementation with His [13, 23, 31]. In the study of Kai et al. 2015 [13] it was reported that no dipeptides were detectable in the blood serum of broiler chickens when fed a high-His diet, which is in line with our results. Indeed, in another analysis of the same blood samples, plus 5 additional samples per bird of the same study, carnosine was

Table 2. Statistical heat map of the histidine pathway and related metabolites, as well as inflammation marker and standards.

Metabolite	Slaughter age effects [a]						Feeding group effects at 35 d [b]					Feeding group effects at 54 d [c]				
	CON[d] 54 d/35d	HIS1[e] 54 d/35 d	HIS2[f] 54 d/35 d	BA_CON[g] 54 d/35 d	BA_HIS1[h] 54 d/35 d	BA_HIS2[i] 54 d/35 d	HIS1/ CON	HIS2/ CON	BA_CON/ CON	BA_HIS1/ CON	BA_HIS2/ CON	HIS1/ CON	HIS2/ CON	BA_CON/ CON	BA_HIS1/ CON	BA_HIS2/ CON
Histidine	1.30	0.98	0.78	1.70	1.15	0.76	2.16	3.37	1.10	2.28	3.42	1.63	2.04	1.44	2.02	2.01
1-Methylhistidine	1.94	1.58	1.17	3.94	1.73	1.40	2.22	3.20	0.79	2.44	3.27	1.80	1.93	1.60	2.18	2.36
3-Methylhistidine	1.70	2.26	1.21	2.93	2.68	0.92	2.00	4.28	0.86	1.99	3.61	2.66	3.05	1.48	3.14	1.96
N-Acetylhistidine	1.15	1.20	0.95	1.16	1.09	0.93	1.53	2.02	1.18	1.68	2.33	1.60	1.66	1.18	1.60	1.89
N-Acetyl-3-methylhistidine	1.00	2.97	2.41	1.36	3.15	2.24	1.00	1.59	1.00	1.20	1.00	2.97	3.83	1.36	3.79	2.24
N-Acetyl-1-methylhistidine	2.17	4.00	2.77	7.66	3.96	2.76	2.07	3.24	0.92	2.41	4.32	3.80	4.13	3.26	4.39	5.48
***trans*-Urocanate**	0.86	5.40	1.59	4.98	3.72	4.16	1.79	5.21	0.70	1.29	3.79	11.25	9.62	4.04	5.56	18.3
4-Imidazole-5-propionate	1.56	4.12	1.30	1.38	3.25	1.73	0.52	0.92	0.67	0.63	0.64	1.37	0.76	0.59	1.31	0.71
Formiminoglutamate	0.34	1.59	0.60	6.89	1.21	0.53	2.94	12.19	0.36	3.1	9.93	13.80	21.74	7.40	11.11	15.67
Glutamate	0.8	0.96	0.95	0.77	0.74	0.95	0.94	0.94	0.98	1.2	0.86	1.13	1.11	0.95	1.11	1.02
Imidazole lactate	0.88	1.75	0.89	2.16	1.38	1.55	1.65	1.78	0.65	1.28	1.69	3.30	1.81	1.61	2.03	2.99
Carnosine	1.09	0.91	1.31	1.43	0.86	1.03	1.50	1.37	1.09	1.92	1.83	1.25	1.65	1.43	1.52	1.75
Acetylcarnosine	1.10	0.79	1.11	1.80	0.98	1.16	1.93	1.74	1.04	1.66	2.04	1.38	1.76	1.71	1.48	2.16
Anserine	1.36	1.43	1.55	1.77	1.63	1.23	0.99	0.97	0.75	0.96	1.00	1.04	1.11	0.97	1.15	0.90
Histamine	1.00	0.45	0.61	0.62	0.87	0.55	2.11	1.39	1.24	2.2	1.39	0.95	0.85	0.77	1.90	0.77
1-Methylhistamine	0.97	0.25	0.64	0.82	0.34	0.84	12.86	4.63	1.72	7.23	4.95	3.35	3.04	1.45	2.53	4.26
1-Methyl-4-imidazoleacetate	1.24	0.58	0.74	1.08	1.05	0.51	5.66	3.80	1.12	3.96	5.72	2.66	2.26	0.97	3.36	2.38
1Mmethyl-5-imidazoleacetate	0.80	1.62	0.89	1.2	2.87	1.34	1.04	1.92	0.91	0.86	1.20	2.11	2.11	1.36	3.08	2.01
1-Ribosyl-imidazole-4-acetate	0.93	0.56	0.66	1.18	0.71	0.43	5.48	3.82	0.87	3.72	6.09	3.27	2.69	1.11	2.84	2.79
Imidazole-4-acetate	1.42	0.68	0.43	1.71	0.80	0.51	11.94	11.03	1.27	9.6	14.31	5.75	3.37	1.53	5.39	5.10
Acetylhistamine	1.66	0.80	0.55	1.34	1.34	0.55	5.20	4.32	1.44	2.63	4.22	2.52	1.44	1.17	2.12	1.41
β-Alanine	0.67	0.75	1.95	0.58	0.43	0.72	0.48	0.40	4.72	4.01	3.69	0.53	1.17	4.09	2.55	3.97
***gamma*-Glutamylhistidine**	1.06	1.14	0.95	1.66	1.48	0.99	2.00	3.36	1.16	2.4	3.17	2.14	3.01	1.81	3.35	2.96
Creatinine	1.14	0.98	1.07	1.24	0.99	1.03	1.15	0.96	0.85	0.99	0.94	0.99	0.9	0.92	0.86	0.85
Kynurenine	0.93	0.99	0.91	1.27	1.37	1.67	1.03	0.87	0.90	0.74	0.76	1.10	0.85	1.23	1.10	1.36
Tryptophan	0.97	0.89	0.87	0.89	0.97	0.96	0.97	1.01	0.99	0.99	0.95	0.88	0.91	0.91	0.99	0.94

For each metabolite, an average fold change (mean ratio difference between the two groups) was calculated based on age at slaughter and the effects of feeding group at both slaughter ages (at d 35 and 54, respectively). For a given comparison, the red and green colors indicate the relative significance increased and decreased concentrations, respectively, for each metabolite. Normalized and rescaled data were used for the analysis. Significance was declared by $P \leq 0.05$.

[a]The effects on the given metabolites by slaughter age (35d and 54 d) were analyzed for each feeding group

[b]The effects on the given metabolites at the slaughter age of 35 d were analyzed for each supplemented feeding group compared to the control group

[c]The effects on the given metabolites at the slaughter age of 54 d were analyzed for each supplemented feeding group compared to the control group

[d]CON: group fed with a standardized ileal digestible histidine:lysine ratio of 0.44

[e]HIS1: group fed with a standardized ileal digestible histidine:lysine ratio of 0.54

[f]HIS2: group fed with a standardized ileal digestible histidine:lysine ratio of 0.64

[g]BA_CON: group fed with a standardized ileal digestible histidine:lysine ratio of 0.44 + supplemented of 0.5% β-alanine

[h]BA_HIS1: group fed with a standardized ileal digestible histidine:lysine ratio of 0.54 + supplementation of 0.5% β-alanine

[i]BA_HIS2: group fed with a standardized ileal digestible histidine:lysine ratio of 0.64 + supplementation of 0.5% β-alanine

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significantly higher in plasma of birds fed a higher ratio of His:Lys at both ages [23]. In this analysis, anserine was also found in higher concentrations in plasma of birds fed a His:Lys ratio of 0.64 compared to 0.44 at 35 days of age [23]. The two-factor analysis shows that the metabolism of His depends not only on the His concentration in the diet but also on the age of the bird. The molecules involved were: *trans*-urocanate, 1-methyl-5-imidazoleacetate, 1-methylhistamine, and N-acetyl-3-methylhistidine. The fact that almost exclusively metabolites of His metabolism were affected by differences in the His:Lys ratio in the diet also suggests that such a metabolic study of the amino acid His is well suited to investigate the effects of His on the broiler chickens. In addition, it has to be mentioned that the metabolic pathway analysis revealed that His metabolism was affected by age.

Metabolomics analysis of βA supplementation revealed affected metabolites from several metabolic pathways, not all of which can be directly linked to βA metabolism. As expected, βA was also more concentrated in plasma samples from broilers fed 0.5% βA. Moreover, βA showed high impact on its associated metabolic pathways pyrimidine metabolism and pantothenate and CoA biosynthesis. The catabolism of βA generates malonate semialdehyde and acetyl-CoA. The first step is catalyzed by 4-aminobutyrate aminotransferase to form malonate semialdehyde. This enzyme is also known to catalyze the reaction of methylmalonate semialdehyde to 3-aminoisobutyrate [32]. It seems possible that the upregulation of this enzyme due to the high βA content in broiler feed could be the reason for the higher concentration of 3-aminoisobutyrate. All other metabolites involved, with the exception of the xenobiotics stachydrine and homostachydrine, could be generally related to βA metabolism but are not directly known to be part of this metabolism. Stachydrine and homostachydrine are mainly plant-derived compounds that are strongly associated with fruits [33]. Therefore, the higher concentrations of these metabolites could be a consequence of diet, but these metabolites were only detected significantly different in plasma samples from birds fed high levels of βA, which could indicate an interaction between βA supplementation and stachydrine metabolism in birds. The main reason for using βA supplementation in this study was to determine the effects of this metabolite on the metabolism of His, in which it is involved through the formation of the His-related dipeptides carnosine and anserine, which are found in high concentrations in chicken skeletal muscle [9]. The heat map analysis showed that βA-supplementation had no effect on the plasma concentrations of these dipeptides. This was also shown for both dipeptides in skeletal muscle tissue in the companion study [23]. Nevertheless, some metabolites of His metabolism after 54 d of age were affected by βA supplementation in the BA_CON group, but to a much lesser extent than in the other feeding groups. These results highlight previous findings that βA supplementation had no effect on carnosine and anserine concentrations in broiler chickens, even at higher His levels in the broiler diet, and that His therefore appears to be the limiting factor for dipeptide formation, which was also reported in a previous study [23]. It should be noted that both dipeptides are degraded in blood plasma by carnosinase activity in blood serum and their concentrations are low [30, 34]. A decrease of carnosine in blood plasma after βA supplementation was previously described [34]. The authors of this study also reported no changes of the anserine concentration in blood plasma when βA or His alone or in combination were added to the broiler feed.

Age showed the highest effect on the metabolism of birds; lipid, amino acid, vitamin, and carbohydrate metabolisms were mainly affected. The metabolism of His was also affected by the age of the birds, as well as βA metabolism, which was lowered in older birds. The metabolite βA can be formed by various metabolic pathways. Among them, decarboxylation of aspartate leads to direct formation of βA. This metabolic pathway was also influenced by age in the metabolic pathway analysis. When metabolites that affected this analysis were analyzed, aspartate was found to be lower in older birds. In addition, concentrations of spermidine and

spermine, which are also precursors of βA, were decreased at 54 d. Interestingly, age but not the His:Lys ratio in feed or a supplementation of βA was significant for the dipeptide anserine. In breast muscle tissue, anserine was also affected by age [23]. The age-related increase of anserine in blood plasma may indicate that methylation of carnosine in muscle is a slow process.

The two-factor analysis and heat map showed an effect on 3-methylhistidine by age and His:Lys ratio. In skeletal muscle, His is methylated and used as a building block for actin and myosin. When myofibrillar protein is degraded, 3-methylhistidine is released but cannot be further used for protein synthesis and is thus renally excreted. Approximately 84% of 3-methylhistidine is expected to be derived from broiler chicken muscle tissue, with actin being the major source [35]. Therefore, the metabolite in blood and urine can be used as a marker of muscle protein degradation and turnover [36–38]. In this study, the concentration of 3-methylhistidine increased with slaughter age in the CON, HIS1, BA_CON, and BA_HIS1 groups and all feeding groups with an increased His:Lys ratio of 0.54 and 0.64 (Table 2). By using creatinine as a standard that depends on muscle mass and age, the His:creatinine ratio can be used as a great indicator of muscle protein turnover by normalizing the effects of age and muscle growth. The results for the analyzed His:creatinine ratio possibly indicates a high turnover of muscle protein with high dietary His supply. Moreover, it could indicate that the birds used for sampling were affected of muscle defects such as white striping, woody breast, or spaghetti meat. Unfortunately, myopathies could not be assessed for technical reasons in our study, but a myodegeneration in affected muscles is described in previous literature [39]. Inflammation also occurs in muscle tissue affected by breast myopathies in chicken [39]. The ratio of kynurenine to tryptophan can be used as a marker of inflammation because of its dependence on the activity of indolamine-2,3-dioxygenase, an enzyme activated by cytokines [28]. The analyzed increased ratio for this inflammatory marker in older birds may indicate a higher incidence of breast myopathies [39]. However, it should be noted that the plasma concentration of these metabolites also depends on other metabolic factors and inflammatory processes, or the overall regulation of tryptophan metabolism [28].

In all interpretations of the results, it must be mentioned that this study had some limitations. For this metabolic study, only five samples were collected for each feeding group, and the analyzed values of these samples were pooled for statistical analysis according to the factors His:Lys ratio, βA intake, and slaughter age. It should also be noted that the missing values from the original analytical methods were annotated with the observed minimum value of the detected molecules after rescaling, which could affect some analyzes.

In conclusion, a higher supplementation of His or βA showed less effect on the metabolism of broiler chickens. The His:Lys ratio showed a direct influence on the targeted His metabolism, while the supplementation of βA affected a few metabolites of different metabolic pathways. A higher His:Lys ratio led to higher concentrations of formiminoglutamate in blood plasma, which could be an indicator for a folate deficiency. The dipeptide carnosine, which is built by His and βA, was only increased with a higher His:Lys ratio in broiler feed, whereas the methylated form anserine was increased by age. In general, age had the greatest influence on the metabolism of the birds.

Supporting information

S1 File. Rescaled and imputed raw data as used for analysis. The original values were normalized in terms of raw area counts. All data were rescaled by the median of all 60 samples for a given molecule. Missing values were imputed with the observed minimum value for each detected metabolite.
(XLSX)

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References

1. Munks B, Robinson A, Beach EF, Williams HH. Amino acids in the production of chicken egg and muscle. Poult. Sci. 1945; 24:459–464.
2. Wu G, Bazer FW, Dai Z, Li D, Wang J, Wu Z. Amino acid nutrition in animals: protein synthesis and beyond. Annu. Rev. Anim. Biosci. 2014; 2:387–417. https://doi.org/10.1146/annurev-animal-022513-114113 PMID: 25384149
3. Brosnan ME, Brosnan JT. Histidine metabolism and function. J. Nutr. 2020; 150:2570S–2575S. https://doi.org/10.1093/jn/nxaa079 PMID: 33000155
4. Liao S-M, Du Q-S, Meng J-Z, Pang Z-W, Huang R-B. The multiple roles of histidine in protein interactions. Chem. Cent. J. 2013; 7:44. https://doi.org/10.1186/1752-153X-7-44 PMID: 23452343
5. Vistoli G. Carnosine in the context of histidine-containing dipeptides. In: Preedy VR, editor. Imidazole dipeptides: chemistry, analysis, function and effects. Cambridge: Royal Society of Chemistry; 2015. p. 3–22.
6. Holeček M. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. Nutrients 2020; 12:848.
7. Armstrong KM, Baldwin RL. Charged histidine affects alpha-helix stability at all positions in the helix by interacting with the backbone charges. Proc. Natl. Acad. Sci. U S A. 1993; 90:11337–11340. https://doi.org/10.1073/pnas.90.23.11337 PMID: 8248249
8. Kessler AT, Raja A. Biochemistry, histidine. StatPearls, Treasure Island (FL); 2020.
9. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. Physiol. Rev. 2013; 93:1803–1845. https://doi.org/10.1152/physrev.00039.2012 PMID: 24137022
10. Wade A, Tucker HN. Antioxidant characteristics of L-histidine. J. Nutr. Biochem. 1998; 9:308–315.
11. Tabarean IV. Histamine receptor signaling in energy homeostasis. Neuropharmacology 2016; 106:13–19. https://doi.org/10.1016/j.neuropharm.2015.04.011 PMID: 26107117
12. Branco ACCC Yoshikawa FSY, Pietrobon AJ Sato MN. Role of histamine in modulating the immune response and inflammation. Mediators Inflamm. 2018; 2018:9524075. https://doi.org/10.1155/2018/9524075 PMID: 30224900
13. Kai S, Watanabe G, Kubota M, Kadowaki M, Fujimura S. Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. Anim. Sci. J. 2015; 86:541–546. https://doi.org/10.1111/asj.12322 PMID: 25521014
14. Suidasari S, Stautemas J, Uragami S, Yanaka N, Derave W, Kato N. Carnosine content in skeletal muscle is dependent on vitamin B6 status in rats. Front. Nutr. 2015; 2:39. https://doi.org/10.3389/fnut.2015.00039 PMID: 26835452
15. Mahootchi E, Homaei SC, Kleppe R, Winge I, Hegvik T-A, Megias-Perez R et al. GADL1 is a multifunctional decarboxylase with tissue-specific roles in β-alanine and carnosine production. Sci. Adv. 2020; 6: eabb3713. https://doi.org/10.1126/sciadv.abb3713 PMID: 32733999
16. Evonik Operations GmbH. AMINODat® 6.0; 2021.
17. Han YM, Suzuki H, Baker DH. Histidine and tryptophan requirement of growing chicks. Poult. Sci. 1991; 70:2148–2153. https://doi.org/10.3382/ps.0702148 PMID: 1956860
18. Rostagno HS, Becker BG, editors. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Universidade Federal de Viçosa, Departamento de Zootecnia; 2005.
19. Wecke C, Liebert F. Improving the reliability of optimal in-feed amino acid ratios based on individual amino acid efficiency data from N balance studies in growing chicken. Animals 2013; 3:558–573. https://doi.org/10.3390/ani3030558 PMID: 26479521
20. Franco SM, Tavernari FdC, Maia RC, Barros VRSM, Albino LFT, Rostagno HS, et al. Estimation of optimal ratios of digestible phenylalanine + tyrosine, histidine, and leucine to digestible lysine for performance and breast yield in broilers. Poult. Sci. 2017; 96:829–837. https://doi.org/10.3382/ps/pew305 PMID: 27647929

21. Abasht B, Mutryn MF, Michalek RD, Lee WR. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PLoS One 2016; 11:e0153750. https://doi.org/10.1371/journal.pone.0153750 PMID: 27097013

22. Soglia F, Silva AK, Lião LM, Laghi L, Petracci M. Effect of broiler breast abnormality and freezing on meat quality and metabolites assessed by 1H-NMR spectroscopy. Poult. Sci. 2019; 98:7139–7150. https://doi.org/10.3382/ps/pez514 PMID: 31529072

23. Lackner J, Albrecht A, Mittler M, Marx A, Kreyenschmidt J, Hess V, et al. Effect of feeding histidine and β-alanine on carnosine concentration, growth performance and meat quality of broiler chickens. Poult. Sci. 2021:101393. https://doi.org/10.1016/j.psj.2021.101393 PMID: 34530228

24. Brody T. Nutritional biochemistry: folate. San Diego, CA: Academic Press; 1994.

25. Yu S, Morris JG. Folate requirement of growing kittens to prevent elevated formiminoglutamic acid excretion following histidine loading. J. Nutr. 1998; 128:2606S–2608S. https://doi.org/10.1093/jn/128.12.2606S PMID: 9868217

26. Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res. 2009; 37:W652–60. https://doi.org/10.1093/nar/gkp356 PMID: 19429898

27. Xia J, Mandal R, Sinelnikov IV, Broadhurst D, Wishart DS. MetaboAnalyst 2.0-a comprehensive server for metabolomic data analysis. Nucleic Acids Res. 2012; 40:W127–33. https://doi.org/10.1093/nar/gks374 PMID: 22553367

28. Badawy AA-B, Guillemin G. The plasma [kynurenine]/[tryptophan] ratio and indoleamine 2,3-dioxygenase: time for appraisal. Int. J. Tryptophan Res. 2019; 12:1178646919868978–1178646919868978. https://doi.org/10.1177/1178646919868978 PMID: 31488951

29. Herbert V, Zalusky R. Interrelations of vitamin B12 and folic acid metabolism: folic acid clearance studies. J. Clin. Invest. 1962; 41:1263–1276. https://doi.org/10.1172/JCI104589 PMID: 13906634

30. Bellia F, Vecchio G, Rizzarelli E. Carnosinases, their substrates and diseases. Molecules 2014; 19:2299–2329. https://doi.org/10.3390/molecules19022299 PMID: 24566305

31. Kralik G, Sak-Bosnar M, Kralik Z, Galovic O, Grcevic M, Kralik I. Effect of β-alanine and L-histidine on concentration of carnosine in muscle tissue and oxidative stability of chicken meat. Poljoprivreda 2015; 21:190–194.

32. Tanianskii DA, Jarzebska N, Birkenfeld AL, O'Sullivan JF, Rodionov RN. Beta-aminoisobutyric acid as a novel regulator of carbohydrate and lipid metabolism. Nutrients 2019; 11:524. https://doi.org/10.3390/nu11030524 PMID: 30823446

33. Shibutami E, Ishii R, Harada S, Kurihara A, Kuwabara K, Kato S, et al. Correction: Charged metabolite biomarkers of food intake assessed via plasma metabolomics in a population-based observational study in Japan. PLoS One 2021; 16:e0250864. https://doi.org/10.1371/journal.pone.0250864 PMID: 33886678

34. Kopec W, Jamroz D, Wiliczkiewicz A, Biazik E, Pudlo A, Korzeniowska M, et al. Antioxidative characteristics of chicken breast meat and blood after diet supplementation with carnosine, L-histidine, and β-alanine. Antioxidants 2020; 9:1093.

35. Jones SJ, Aberle ED, Judge MD. Estimation of the fractional breakdown rates of myofibrillar proteins in chickens from quantitation of 3-methylhistidine excretion. Poult. Sci. 1986; 65:2142–2147. https://doi.org/10.3382/ps.0652142 PMID: 3822994

36. Williams AP. Amino acids determination. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition (Second Edition). Oxford: Academic Press; 2003. p. 192–197.

37. Young VR, Munro HN. Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Fed. Proc. 1978; 37:2291–2300. PMID: 350635

38. Berridge BR, van Vleet JF, Herman E. Cardiac, vascular, and skeletal muscle systems. In: Haschek WM, Rousseaux CG, Wallig MA, editors. Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition). Boston: Academic Press; 2013. p. 1567–1665.

39. Petracci M, Soglia F, Madruga M, Carvalho L, Ida E, Estévez M. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. Food Saf. 2019; 18:565–583. https://doi.org/10.1111/1541-4337.12431 PMID: 33336940

Overall conclusion and perspectives

Little is known about the nutritional requirements for His in broiler feed. The recommendations for this essential amino acid, given as His:Lys ratio, range from 0.31 to 0.41 in different literature sources (Evonik Nutrition & Care GmbH, 2016; Franco et al., 2017; Han et al., 1991; Rostagno and Becker, 2005; Wecke and Liebert, 2013). In a commercial diet, these recommended levels are easily reached. Recent studies pointed out that the occurrence of BMM reduces the concentration of the HCD's Car and Ans in the skeletal muscle tissue of broilers (Abasht et al., 2016; Soglia et al., 2019a). This could mean that these dipeptides play an important role in oxidative stress conditions, which were described as an underlaying process causing these muscular issues (Petracci et al., 2019). Therefore, modern, fast growing chicken breeds may have a higher requirement for His regarding MQ, to counteract the depletion during the condition of BMM.

The results of this thesis can be summarized in four main findings, regarding performance, the concentrations of Car and Ans, metabolomic status and MQ:

a) The performance analysis shows that a high SID His:Lys ratio in feed had only little significant influence on performance of fast-growing broilers. One critical change in performance was an increase in mortality after 53 d when using high SID His:Lys ratios during the first trial (Manuscript I). This effect was not seen at the youngest slaughter ages in both trials (Manuscript I and II). It must be noted that in some countries, like USA, heavier broilers are required by the industry, and as consequence birds are slaughtered at older ages (for US, see Introduction, Figure 2). Should this effect on mortality occur under commercial conditions, this would therefore be an exclusion criterion for increased His concentrations in broiler feed. Nevertheless, the death of only one animal had a high influence at the mortality rate in the trial condition and older birds have a greater mortality rate due to a higher weight and metabolic burden. So, the effect of a higher His concentration in broiler feed on mortality would need to be further studied. One other effect of a His supplementation in broiler feed was seen for ADFI at 33 d of age (Manuscript I). A SID His:Lys ratio of 0.54 and 0.64 increased ADFI. This could result in higher feed costs if using higher His concentrations in broiler feed under commercial conditions. An inconsistent effect on ADFI was also seen in Starter phase in trial 2 (Manuscript II). For this trial, the effect on performance at slaughter age may have been covered due to the specific heat stress conditions during the trial. A

supplementation of βA showed a contrary effect with lowered ADFI after 33 and 53 d of age (Manuscript I). This fact could be interesting regarding lowered feed cost. However, a supplementation of βA also reduced the breast yield of the carcasses at both slaughter ages, which is the most valuable part of the carcass (Manuscript I). When excluding mortality, a high SID His:Lys ratio as well as a supplementation of βA in feed seems to be safe to use in commercial broiler feed.

b) As an interim goal, in order to study the effect of high Car and Ans concentrations on BMM, it was shown that the concentration of Car increased when high SID His:Lys ratios were fed, which was also seen in previous studies (Kai et al., 2015; Kralik et al., 2015). Another finding was the absence of effect of the second Car and Ans precursor βA on the concentrations of HCDs in breast tissue of the broilers, neither alone nor in combination with His. This result differed from findings in other broiler feeding studies and in human-related studies, where βA has been reported to be the main factor for increasing Car (Artioli et al., 2010; Kralik et al., 2018; Perim et al., 2019; Qi et al., 2018). In conclusion, an additional supplementation of His was sufficient to increase Car in breast muscle tissue of fast-growing broiler chickens, whereas the concentration of Ans was only age dependent.

c) A supplementation of βA showed only marginal changes in broiler metabolism with effects on different metabolic pathways (Manuscript III). On the other hand, the SID His:Lys ratio in the feed showed an influence on the metabolic profile of the birds that was limited to His metabolism (Manuscript III). In particularly, the increased SID His:Lys ratios resulted in an accumulation of Figlu, which could be a hint to a folate or cobalamin deficiency during high supplementations. This assumption would however require further studies. In this study, age was the main factor in changes in broiler metabolism, including modifications in His metabolism (Manuscript III). Therefore, a deeper look at the age-related metabolic changes regarding nutritional needs is required.

d) The main goal of the performed studies was to assess the influence of His supplementation, alone or together with an additional βA supplementation, on MQ. The dietary supplementation of His showed only a very low impact on MQ parameters and the incidence of BMM was marginally influenced by the different SID His:Lys ratios in feed during both trials. Indeed, a small positive effect on the occurrence of WB and WS was observed by using a SID His: Lys ratio between 0.45 (Manuscript II) and 0.54 (Manuscript I) in broiler feed, while higher values showed no or even negative effects. However, it must be noted that the effects on BMM in the second trial (Manuscript II)

may have been masked by the heat stress as a result of the heat wave during the growing period. Moreover, a dose-response study with additional measurements of Car would be needed to clarify these observations.

The slight positive impact of high SID His:Lys ratios on the incidence of BMM should also be confirmed under commercial conditions to assess the commercially relevant effect. However, the higher concentration of Car in broiler breasts can be beneficial, because of the nutritional value for humans. In comparison to chicken and many other species, the skeletal muscle of humans contains only low amounts of HCD, but the concentration can be increased by nutrition (Boldyrev et al., 2013). Direct sources of Car for humans could be supplements and meat consumption. For meat consumption the concentration of biologically active Car depends on its bioavailability: e.g., concentration in meat source, absorption in gastrointestinal tract and target tissues (Jukić et al., 2021). Therefore, chicken meat with an increased content of Car could be a good source of HCD in human nutrition. Dipeptides like Car are studied to have different benefits for human health for instance by increasing exercise performance and muscle homeostasis, reduction of oxidative stress and inflammation in diverse tissues, preventing insulin resistance from worsening or by improving brain function (Jukić et al., 2021).

In general, the feeding of broilers (e.g., feed composition, feeding schedule, physical structure, quality, cost) is one of the most important parts of livestock production. An optimal diet formulation and a good quality of feed ingredients have a major impact on both performance and health of the birds. Thus, taking into account the quality of meat in the diet formulation can be a solution to reduce the incidence of novel breast myopathies and therefore improve the quality of chicken breast meat. In addition, it could be helpful to combine several feeding approaches to achieve the best tissue development and increase the antioxidative status of the muscle. Further studies are needed to identify the best and commercially practicable combination and concentrations of different feed ingredients. Such studies take time and the effects under commercial conditions may be too low or the improved feed may be too expensive for a profitable usage.

Indeed, the feeding scheme is accountable for a relatively small part of the possible impacts on MQ and most of the approaches are currently not efficient for a commercial application. This may lead to the observation that alternative management praxis may offer better perspective. General farm management and environmental influences during growth, as well as genetic and brooding condition, seem to have an influence on the occurrence of meat quality defects and a

deep dive analysis could be helpful to understand the development of the different MQ issues and find solutions to reduce them.

Until now, the only possible long-term solution of MQ problems is therefore a targeted genetic selection. This solution may take some time, due to the fact that new changes in pure-line elite birds take up to four years to be seen in the final broiler population (Tavárez and Solis de los Santos, 2016). Moreover, the origin of novel BMM is yet not fully understood and a multifactorial genetic impact is to be expected (Petracci et al., 2019; Zambonelli et al., 2016). To avoid BMM, until the fast-growing lines overcome these problems, the usage of slow-growing breeds can be a solution for the industry to reduce growth-related quality issues. But it must be kept in mind that those birds have a higher feeding demand per kg meat produced caused by a longer growing period on farm, including all negative side effects on sustainability and resource usage. Part of the industry where high animal welfare and high MQ are targeted to meet the customers' demand, slow-growing chicken can be a good alternative, but in order to fulfill the global increasing demand for poultry meat, fast-growing chicken breeds are still needed.

Nevertheless, prediction of combined effects is difficult knowing the multiple factors from breeding, hatching, farm and slaughter parameters affecting MQ. Hatching and farming factors could have a big impact on the appearance of BMM (Bailey et al., 2015, 2020). Stress-related quality issues are linked to farming, transport, and slaughter conditions. In fact, each parameter in the poultry value chain can have an impact on the final MQ. A solution to better understand the effects and the interaction of all different potentially impacting parameters would be a statistical big data approach may help to analyze cross-correlations of influencing factors. Therefore, to evaluate the impact of all possible parameters and find the most important ones, a wide-ranging approach is needed. So far, however, every part of the value chain is acting more or less independently, and very few data are collected, or collected but not used. The importance of data use and data exchange across the entire value chain is increasingly recognized by the broiler industry in order to improve economic performance and address new requirements such as animal welfare and sustainability. In order to break data silos and combine data along the whole production process, digital approaches and other precision livestock farming solutions are under development by different players of the value chain. In the future, the digitalization of poultry production will give us the opportunity to identify and analyze more and more relevant parameters for each farm individually. Based on real-time data and past experience, machine learning algorithms could be developed based on a large and comprehensive data set to improve and perhaps even predict the final MQ.

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References

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PloS One 11:e0153750.

Adzitey, F., and N. Huda. 2011. Pale soft exudative (PSE) and dark firm dry (DFD) meats : causes and measures to reduce these incidences-a mini review. Int. Food Res. J. 18:11–20.

Alnahhas, N., C. Berri, M. Chabault, P. Chartrin, M. Boulay, M. C. Bourin, and E. Le Bihan-Duval. 2016. Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. BMC Genet. 17:61.

Armstrong, K. M., and R. L. Baldwin. 1993. Charged histidine affects alpha-helix stability at all positions in the helix by interacting with the backbone charges. PNAS 90:11337–11340.

Artioli, G. G., B. Gualano, A. Smith, J. Stout, and A. H. Lancha, JR. 2010. Role of beta-alanine supplementation on muscle carnosine and exercise performance. Med. Sci. Sports Exerc. 42:1162–1173.

Aviagen. 2019. Breast muscle myopathies (BMM). Accessed Apr. 2021. http://en.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/Breast-Muscle-Myopathies-2019-EN.pdf.

Bailey, R. A., E. Souza, and S. Avendano. 2020. Characterising the influence of genetics on breast muscle myopathies in broiler chickens. Front. Physiol. 11:1041.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870–2879.

Baldi, G., F. Soglia, M. Mazzoni, F. Sirri, L. Canonico, E. Babini, L. Laghi, C. Cavani, and M. Petracci. 2018. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal 12:164–173.

Baldi, G., F. Soglia, and M. Petracci. 2020. Current status of poultry meat abnormalities. Meat and Muscle Biology. 4.

Banerjee, J., E. Radvar, and H. S. Azevedo. 2018. Self-assembling peptides and their application in tissue engineering and regenerative medicine. Pages 245–281 in Peptides and Proteins as Biomaterials for Tissue Regeneration and Repair. M. A. Barbosa and M. C. L. Martins, eds., Woodhead Publishing, UK.

Barresi, C., C. Stremnitzer, V. Mlitz, S. Kezic, A. Kammeyer, M. Ghannadan, K. Posa-Markaryan, C. Selden, E. Tschachler, and L. Eckhart. 2011. Increased sensitivity of histidinemic mice to UVB radiation suggests a crucial role of endogenous urocanic acid in photoprotection. J. Invest. Dermatol. 131:188–194.

Bellia, F., G. Vecchio, and E. Rizzarelli. 2014. Carnosinases, their substrates and diseases. Molecules (Basel). 19:2299–2329.

Berg J. M. , L. Stryer, J. L. Tymoczko, and A. Held. 2013. Stryer Biochemistry 7th edition. Springer Spectrum. Berlin Heidelberg, Germany.

Betts, J. G. 2017. Anatomy & physiology. Revision AP-2013-003(03/17)-LC. OpenStax College, Rice University, Houston, Texas.

Bianchi, M., M. Petracci, A. Franchini, and C. Cavani. 2006. The occurrence of deep pectoral myopathy in roaster chickens. Poult. Sci. 85:1843–1846.

Bilgili, S. F. 2015. Broiler Chicken Myopathies: IV Stringy/Mushy Breast. Accessed 2020. http://poul.auburn.edu/wpcontent/uploads/sites/13/2015/11/WOGS-FEB15.pdf.

Bilgili, S. F., and J. B. Hess. 2008. Green muscle disease: reducing the incidence in broiler flocks. Accessed Nov. 2021. https://eu.aviagen.com/assets/Tech_Center/Broiler_Breeder_Tech_Articles/English/AviagenBrief_Green_Muscle.pdf

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, and J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult. Sci. 97:3298–3310.

Boerboom, G., T. van Kempen, A. Navarro-Villa, and A. Pérez-Bonilla. 2018. Unraveling the cause of white striping in broilers using metabolomics. Poult. Sci. 97:3977–3986.

Boldyrev, A. A. 2007. Carnosine and oxidative stress in cells and tissues. Nova Science Publishers, New York.

Boldyrev, A. A., G. Aldini, and W. Derave. 2013. Physiology and pathophysiology of carnosine. Physiol. Rev. 93:1803–1845.

Bowker, B. 2017. Developments in Our Understanding of Water-Holding Capacity. Pages 77–113 in Poultry Quality Evaluation. M. Petracci and C. Berri, ed., Elsevier, Amsterdam, the Netherlands.

Bowker, B., and H. Zhuang. 2016. Impact of white striping on functionality attributes of broiler breast meat1, Poult. Sci. 95:1957–1965.

Bowker, B., and H. Zhuang. 2019. Detection of razor shear force differences in broiler breast meat due to the woody breast condition depends on measurement technique and meat state1. Poult. Sci. 98:6170–6176.

Brosnan, M. E., and J. T. Brosnan. 2020. Histidine metabolism and function. J. Nutr. 150:2570S-2575S.

Burt, D. W. 2005. Chicken genome: current status and future opportunities. Genome Res. 15:1692–1698.

Burton, P. M. 2008. Insights from diploblasts; the evolution of mesoderm and muscle. J. Exp. Zool. B Mol. Dev. Evol. 310:5–14.

Cai, K., W. Shao, X. Chen, Y. L. Campbell, M. N. Nair, S. P. Suman, C. M. Beach, M. C. Guyton, and M. W. Schilling. 2018. Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. Poult. Sci. 97:337–346.

Carvalho, R. H., A. L. Soares, D. C. Honorato, P. D. Guarnieri, M. R. Pedrão, F. G. Paião, A. Oba, E. I. Ida, and M. Shimokomaki. 2014. The incidence of pale, soft, and exudative (PSE) turkey meat at a Brazilian commercial plant and the functional properties in its meat product. LWT. 59:883–888.

Cercel, F., M. Stroiu, P. Alexe, and D. Ianiţchi. 2015. Characterization of myofibrillar chicken breast proteins for obtain protein films and biodegradable coatings generation. Agriculture and Agricultural Science Procedia. 6:197–205.

Chen, L. R., M. M. Suyemoto, A. H. Sarsour, H. A. Cordova, E. O. Oviedo-Rondón, M. Wineland, H. J. Barnes, and L. B. Borst. 2019. Temporal characterization of wooden breast myopathy ("woody breast") severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. Avian Pathol. 48:319–328.

Choe, J. H., Y. M. Choi, S. H. Lee, H. G. Shin, Y. C. Ryu, K. C. Hong, and B. C. Kim. 2008. The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Sci. 80:355–362.

Cooke, H. A., C. V. Christianson, and S. D. Bruner. 2009. Structure and chemistry of 4-methylideneimidazole-5-one containing enzymes. Curr. Opin. Chem. Biol. 13:460–468.

Córdova-Noboa, H. A., E. O. Oviedo-Rondón, A. H. Sarsour, J. Barnes, P. Ferzola, M. Rademacher-Heilshorn, and U. Braun. 2018. Performance, meat quality, and pectoral myopathies of broilers fed either corn or sorghum based diets supplemented with guanidinoacetic acid. Poult. Sci. 97:2479–2493.

Cruz, R. F. A., S. L. Vieira, L. Kindlein, M. Kipper, H. S. Cemin, and S. M. Rauber. 2017. Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels. Poult. Sci. 96:501–510.

Dalgaard, L. B., M. K. Rasmussen, H. C. Bertram, J. A. Jensen, H. S. Møller, M. D. Aaslyng, E. K. Hejbøl, J. R. Pedersen, D. Elsser-Gravesen, and J. F. Young. 2018. Classification of wooden breast myopathy in chicken pectoralis major by a standardised method and association with conventional quality assessments. Int. J. Food Sci. Technol. 53:1744–1752.

Dalle Zotte, A., G. Tasoniero, E. Puolanne, H. Remignon, M. Cecchinato, E. Catelli, and M. Cullere. 2017. Effect of "wooden breast" appearance on poultry meat quality, histological traits, and lesions characterization. Czech J. Anim. Sci. 62:51–57.

Damez J.-L., and S. Clerjon. 2012. Recent Advances in Meat Quality Assessment. Pages 161-175 in Handbook of Meat and Meat Processing 2nd edition. Y.H. Hui, ed., CRC Press, Boca Raton, FL, US.

Dickinson, E. M., J. O. Stevens, and D. Helfer, eds. 1968. Degenerative myopathy in Turkeys. Proceedings of 17th Western Poultry Disease Conference, Davis, CA, USA.

Dinev, I., and D. Kanakov. 2011. Deep pectoral myopathy: prevalence in 7 weeks old broiler chickens in Bulgaria. Rev. Med. Vet. (Toulouse) 162:279–283.

Dong, M., H. Chen, Y. Zhang, Y. Xu, M. Han, X. Xu, and G. Zhou. 2020. Processing properties and improvement of pale, soft, and exudative-like chicken meat: a review. Food Bioproc. Tech. 13:1280–1291.

Drozak, J., L. Chrobok, O. Poleszak, A. K. Jagielski, and R. Derlacz. 2013. Molecular identification of carnosine N-methyltransferase as chicken histamine N-methyltransferase-like protein (hnmt-like). PloS One. 8:e64805.

Drozak, J., M. Veiga-da-Cunha, D. Vertommen, V. Stroobant, and E. van Schaftingen. 2010. Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). J. Biol. Chem. 285:9346–9356.

Evonik Nutrition & Care GmbH. 2016. Recommendations for broilers. Accessed June 2021. https://animal-nutrition.evonik.com/product/feed-additives/downloads/recommendations_broiler_03_16.pdf

Farrell, D. 2012. The role of poultry in human nutrition. The nutritional benefits of chicken meat compared with other meats. Accessed Nov. 2021. https://www.fao.org/3/al714e/al714e.pdf

Feher, J. 2017. Contractile mechanisms in skeletal muscle. Pages 305–317 in Quantitative Human Physiology (Second Edition). J. Feher, ed., Academic Press, Boston.

Ferreira, L. F., and O. Laitano. 2016. Regulation of NADPH oxidases in skeletal muscle. Free Radic. Biol. Med. 98:18–28.

Ferreira, T. Z., R. A. Casagrande, S. L. Vieira, D. Driemeier, and L. Kindlein. 2014. An investigation of a reported case of white striping in broilers. J. Appl. Poult. Res. 23:748–753.

Flancbaum, L., J. C. Fitzpatrick, D. N. Brotman, A. M. Marcoux, E. Kasziba, and H. Fisher. 1990. The presence and significance of carnosine in histamine-containing tissues of several mammalian species. Agents and actions. 31:190–196.

Franco, S. M., F. d. C. Tavernari, R. C. Maia, Barros, Victor R S M, L. F. T. Albino, H. S. Rostagno, G. R. Lelis, A. A. Calderano, and R. N. Dilger. 2017. Estimation of optimal ratios of digestible phenylalanine + tyrosine, histidine, and leucine to digestible lysine for performance and breast yield in broilers. Poult. Sci. 96:829–837.

Freitas, A. S., L. M. Carvalho, A. L. Soares, M. E. S. Da Oliveira, M. S. Madruga, A. C. d. S. Neto, R. H. Carvalho, E. I. Ida, and M. Shimokomak. 2017. Pale, soft and exudative (PSE) and dark, firm and dry (DFD) meat determination in broiler chicken raised under tropical climate management conditions. Int. J. of Poult. Sci. 16:81–87.

Fujimaki, S., M. Machida, R. Hidaka, M. Asashima, T. Takemasa, and T. Kuwabara. 2013. Intrinsic ability of adult stem cell in skeletal muscle: an effective and replenishable resource to the establishment of pluripotent stem cells. Stem Cells Int. 2013:420164.

Gibbs, N. K., and M. Norval. 2011. Urocanic acid in the skin: a mixed blessing? J. Invest. Dermatol. 131:14–17.

Gohil, R., T. R. A. Lane, and P. Coughlin. 2013. Review of the adaptation of skeletal muscle in intermittent claudication. WJCD. 03:347–360.

Golzar Adabi, S., and E. Demirok Soncu. 2019. White striping prevalence and its effect on meat quality of broiler breast fillets under commercial conditions. J. Anim. Physiol. Anim. Nutr. (Berl.) 103:1060–1069.

Gonzalez-Rivas, P. A., S. S. Chauhan, M. Ha, N. Fegan, F. R. Dunshea, and R. D. Warner. 2020. Effects of heat stress on animal physiology, metabolism, and meat quality: a review, Meat Sci. 162:108025.

Gratta, F., M. Birolo, R. Sacchetto, G. Radaelli, G. Xiccato, C. Ballarin, D. Bertotto, A. Piccirillo, M. Petracci, L. Maertens, and A. Trocino. 2019. Effect of feed restriction timing on live performance, breast myopathy occurrence, and muscle fiber degeneration in 2 broiler chicken genetic lines. Poult. Sci. 98:5465–5476.

Greene, E., J. Flees, S. Dadgar, B. Mallmann, S. Orlowski, A. Dhamad, S. Rochell, M. Kidd, C. Laurendon, H. Whitfield, C. Brearley, N. Rajaram, C. Walk, and S. Dridi. 2019. Quantum blue reduces the severity of woody breast myopathy via modulation of oxygen homeostasis-related genes in broiler chickens. Front. Physiol. 10:1251.

Halevy, O., I. Rozneboim, S. Yahav, and Y. Piestun. 2006. Muscle development - could environmental manipulations during embryogenesis of broilers change it? 12th European Poultry Conference. (Abstr.)

Han, Y. M., H. Suzuki, and D. H. Baker. 1991. Histidine and tryptophan requirement of growing chicks. Poult. Sci. 70:2148–2153.

Hansen, A. L., and L. E. Kay. 2014. Measurement of histidine pKa values and tautomer populations in invisible protein states. PNAS. 111:E1705-12.

Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter. 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428–558.

Harper, J. A., P. E. Bernier, D. H. Helfer, and J. A. Schmitz. 1975. Degenerative myopathy of the deep pectoral muscle in the turkey. J. Hered. 66:362–366.

Hasegawa, Y., T. Kawasaki, N. Maeda, M. Yamada, N. Takahashi, T. Watanabe, and T. Iwasaki. 2021. Accumulation of lipofuscin in broiler chicken with wooden breast. Animal science journal = Nihon chikusan Gakkaiho 92:e13517.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003a. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509–1518.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003b. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Herbert, V., and R. Zalusky. 1962. Interrelations of vitamin B12 and folic acid metabolism: folic acid clearance studies. J. Clin. Investig. 41:1263–1276.

Hipkiss, A. R. 2000. Carnosine and protein carbonyl groups: a possible relationship. Biochemistry Biokhimiia. 65:771–778.

Holeček, M. 2020. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. Nutrients 12.

Holeček, M., and M. Vodeničarovová. 2019. Effects of histidine load on ammonia, amino acid, and adenine nucleotide concentrations in rats. Amino acids 51:1667–1680.

Hong, I.-K., and Y.-S. Lee. 2012. Textural properties and water-holding capacity of broiler breast meat cooked to various internal endpoint temperatures. Food Sci. Biotechnol. 21:1497–1499.

Huang, H., Y. Li, J. Liang, and F. D. Finkelman. 2018. Molecular regulation of histamine synthesis. Front. Immunol. 9:1392.

Damez J.-L., and S. Clerjon. 2012. Recent Advances in Meat Quality Assessment. Pages 161-175 in Handbook of Meat and Meat Processing 2nd edition. Y.H. Hui, ed., CRC Press, Boca Raton, FL, US.

Ikezaki, S., A. Nishikawa, F. Furukawa, T. Enami, M. Mitsui, Z. Tanakamaru, H.-C. Kim, I.-S. Lee, T. Imazawa, and M. Takahashi. 1996. Long-term toxicity/carcinogenicity study of l-histidine monohydrochloride in F344 rats. Food Chem. Toxicol. 34:687–691.

Ito, S., T. Ohyama, Y. Kontani, K. Matslida, S. F. Sakata, and N. Tamaki. 2001. Influence of dietary protein levels on beta-alanine aminotransferase expression and activity in rats. J. Nutr. Sci. Vitaminol. 47:275–282.

Jayasena, D. D., D. U. Ahn, K. C. Nam, and C. Jo. 2013. Flavour chemistry of chicken meat: a review. Asian Australas J. Anim. Sci. 26:732–742.

Jia, G., and J. R. Sowers. 2019. Increased fibro-adipogenic progenitors and intramyocellular lipid accumulation in obesity-related skeletal muscle dysfunction. Diabetes. 68:18–20.

Jozanović, M., N. Sakač, D. Jakobović, and M. Sak-Bosnar. 2015. Analytical characterization and quantification of histidine dipeptides, carnosine and anserine by modeling of potentiometric titration data. Int. J. Electrochem. Sci. 2015:5787–5799.

Jukić, I., N. Kolobarić, A. Stupin, A. Matić, N. Kozina, Z. Mihaljević, M. Mihalj, P. Šušnjara, M. Stupin, Ž. B. Ćurić, K. Selthofer-Relatić, A. Kibel, A. Lukinac, L. Kolar, G. Kralik, Z. Kralik, A. Széchenyi, M. Jozanović, O. Galović, M. Medvidović-Kosanović, and I. Drenjančević. 2021. Carnosine, small but mighty-prospect of use as functional ingredient for functional food formulation. Antioxidants (Basel) 10.

Jung, S., Y. S. Bae, H. J. Kim, D. D. Jayasena, J. H. Lee, H. B. Park, K. N. Heo, and C. Jo. 2013. Carnosine, anserine, creatine, and inosine 5'-monophosphate contents in breast and thigh meats from 5 lines of Korean native chicken. Poult. Sci. 92:3275–3282.

Kai, S., G. Watanabe, M. Kubota, M. Kadowaki, and S. Fujimura. 2015. Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. Animal science journal = Nihon chikusan Gakkaiho. 86:541–546.

Kanehisa, M. 2019. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 28:1947–1951.

Kanehisa, M., M. Furumichi, Y. Sato, M. Ishiguro-Watanabe, and M. Tanabe. 2021. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 49:D545-D551.

Kanehisa, M., and S. Goto. 2000. KEGG: kyoto encyclopedia of genes and genomes, Nucleic Acids Res. 28:27–30.

Karunanayaka, D. S., D. D. Jayasena, and C. Jo. 2016. Prevalence of pale, soft, and exudative (PSE) condition in chicken meat used for commercial meat processing and its effect on roasted chicken breast. J. Anim. Sci. Technol. 58:27.

Kawahara, M., Y. Sadakane, K. Mizuno, M. Kato-Negishi, and K.-I. Tanaka. 2020. Carnosine as a Possible Drug for Zinc-Induced Neurotoxicity and Vascular Dementia. Int. J. Mol. Sci. 21.

Kessler, D., J. Rétey, and G. E. Schulz. 2004. Structure and action of urocanase. J. Mol. Biol. 342:183–194.

Keul, V., F. Kaeppeli, C. Ghosh, T. Krebs, J. A. Robinson, and J. Rétey. 1979. Identification of the prosthetic group of urocanase. The mode of its reaction with sodium borohydride and of its photochemical reactivation. J. Biol. Chem. 254:843–851.

Kohls, D., T. Sulea, E. O. Purisima, R. E. MacKenzie, and A. Vrielink. 2000. The crystal structure of the formiminotransferase domain of formiminotransferase-cyclodeaminase: implications for substrate channeling in a bifunctional enzyme. Structure 8:35–46.

Kojima, S., H. Saegusa, and M. Sakata. 2014. Histidine-containing dipeptide concentration and antioxidant effects of meat extracts from silky fowl: comparison with meat-type chicken breast and thigh meats. FSTR. 20:621–628.

Konashi, S., K. Takahashi, and Y. Akiba. 2000. Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens. Br. J. Nutr. 83:449–456.

Koohmaraie, M. 1992. The role of Ca2+-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. Biochimie 74:239–245.

Kopec, W., D. Jamroz, A. Wiliczkiewicz, E. Biazik, A. Pudlo, M. Korzeniowska, T. Hikawczuk, and T. Skiba. 2020. Antioxidative characteristics of chicken breast meat and blood after diet supplementation with carnosine, L-histidine, and β-alanine. Antioxidants (Basel). 9.

Kralik, G., M. Sak-Bosnar, M. Grčević, and Z. Kralik. 2018. Effect of amino acids on growth performance, carcass characteristics, meat quality, and carnosine concentration in broiler chickens. J. Poult. Sci. 55:239–248.

Kralik, G., M. Sak-Bosnar, Z. Kralik, O. Galovic, M. Grcevic, and I. Kralik. 2015. Effect of β-alanine and L-histidine on concentration of carnosine in muscle tissue and oxidative stability of chicken meat, Poljoprivreda 21:190–194.

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2013a. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult. Sci. 92:811–819.

Kuttappan, V. A., S. D. Goodgame, C. D. Bradley, A. Mauromoustakos, B. M. Hargis, P. W. Waldroup, and C. M. Owens. 2012a. Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. Poult. Sci. 91:3230–3235.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J.-F. C. Meullenet, S. R. McKee, and C. M. Owens. 2012b. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240–1247.

Kuttappan, V. A., C. M. Owens, C. Coon, B. M. Hargis, and M. Vazquez-Añon. 2017. Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. Poult. Sci. 96:3005–3009.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013b. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.

Kwiatkowski, S., A. Kiersztan, and J. Drozak. 2018. Biosynthesis of carnosine and related dipeptides in vertebrates. Curr. Protein Pept. Sci. 19:771–789.

Lackner, J., A. Albrecht, M. Mittler, A. Marx, J. Kreyenschmidt, V. Hess, and H. Sauerwein. 2021. Effect of feeding histidine and β-alanine on carnosine concentration, growth performance, and meat quality of broiler chickens. Poult. Sci. 100:101393.

Li, S., and M. Hong. 2011. Protonation, tautomerization, and rotameric structure of histidine: a comprehensive study by magic-angle-spinning solid-state NMR. J. Am. Chem. Soc. 133:1534–1544.

Liao, S.-M., Q.-S. Du, J.-Z. Meng, Z.-W. Pang, and R.-B. Huang. 2013. The multiple roles of histidine in protein interactions. Chem. Cent. J. 7:44.

Lien, R. J., S. F. Bilgili, J. B. Hess, and K. S. Joiner. 2011. Finding answers to 'green muscle disease'. Deep pectoral myopathy, which is increasing in broilers reared for deboning, results from multiple genetic, physiological and management factors. Accessed Oct. 2021. https://www.wattagnet.com/articles/8761-finding-answers-to-green-muscle-disease

Lien, R. J., S. F. Bilgili, J. B. Hess, and K. S. Joiner. 2012. Induction of deep pectoral myopathy in broiler chickens via encouraged wing flapping. J. Appl. Poult. Res. 21:556–562.

Livingston, M. L., P. R. Ferket, J. Brake, and K. A. Livingston. 2019a. Dietary amino acids under hypoxic conditions exacerbates muscle myopathies including wooden breast and white stripping. Poult. Sci. 98:1517–1527.

Livingston, M. L., C. Landon, H. J. Barnes, and J. Brake. 2019b. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult. Sci. 98:217–226.

Lorenzi, M., S. Mudalal, C. Cavani, and M. Petracci. 2014. Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. J. Appl. Poult. Res. 23:754–758.

Mahdy, M. A. A. 2019. Skeletal muscle fibrosis: an overview. Cell Tissue Res. 375:575–588.

Mahootchi, E., S. C. Homaei, R. Kleppe, I. Winge, T.-A. Hegvik, R. Megias-Perez, C. Totland, F. Mogavero, A. Baumann, J. Glennon, H. Miletic, P. Kursula, and J. Haavik. 2020. GADL1 is a multifunctional decarboxylase with tissue-specific roles in β-alanine and carnosine production. Sci. Adv. 6:eabb3713

Maintz, L., and N. Novak. 2007. Histamine and histamine intolerance. Am. J. Clin. Nutr. 85:1185–1196.

Mann, C. J., E. Ayuso, X. M. Anguela, and F. Bosch. 2010. Skeletal muscle metabolism in the pathology and treatment of type 1 diabetes. Curr. Pharm. Des. 16:1002–1020.

Matlashov, M. E., V. V. Belousov, and G. Enikolopov. 2014. How much H(2)O(2) is produced by recombinant D-amino acid oxidase in mammalian cells? Antioxid. Redox Signal. 20:1039–1044.

McWilliams, S. R. 2002. Ecology of Vertebrate Nutrition. Accessed Nov. 2021. http://cels.uri.edu/docslink/McWilliams_pubs/ELS_Vert_Nutrition_2002.pdf

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili, and W. A. Dozier. 2018. Effects of reduced digestible lysine density on myopathies of the Pectoralis major muscles in broiler chickens at 48 and 62 days of age. Poult. Sci. 97:3311–3324.

Mir, N. A., A. Rafiq, F. Kumar, V. Singh, and V. Shukla. 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54:2997–3009.

Moro, J., D. Tomé, P. Schmidely, T.-C. Demersay, and D. Azzout-Marniche. 2020. Histidine: a systematic review on metabolism and physiological effects in human and different animal species. Nutrients. 12.

Mozdziak, P. 2019. Species of Meat Animals: Poultry. In Reference Module in Food Science, Elsevier, Amsterdam, the Neatherlands.

Mudalal, S., E. Babini, C. Cavani, and M. Petracci. 2014. Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. Poult. Sci. 93:2108–2116.

Murphy, R. Y., and B. P. Marks. 2000. Effect of meat temperature on proteins, texture, and cook loss for ground chicken breast patties. Poult. Sci. 79:99–104.

National Center for Biotechnology Information. 2021. PubChem Pathway Summary for Pathway PWY-5031, L-histidine degradation V, Source: BioCyc. Accessed Nov. 2021. https://pubchem.ncbi.nlm.nih.gov/pathway/BioCyc:META_PWY-5031.

National Chicken Council. 2019. U.S. Broiler Performance. Accessed 2021. https://www.nationalchickencouncil.org/statistic/us-broiler-performance/.

Offer, G. 1991. Modelling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. Meat Sci. 30:157–184.

Ono, Y., H. Iwamoto, and H. Takahara. 1993. The relationship between muscle growth and the growth of different fiber types in the chicken, Poult. Sci. 72:568–576.

Paião, F. G., L. M. Ferracin, M. Pedrão, T. Kato, and M. Shimokomaki. 2013. Skeletal muscle calcium channel ryanodine and the development of pale, soft, and exudative meat in poultry. Genet. Mol. Res. 12:3017–3027.

Papah, M. B., and B. Abasht. 2019. Dysregulation of lipid metabolism and appearance of slow myofiber-specific isoforms accompany the development of wooden breast myopathy in modern broiler chickens. Sci. Rep. 9:17170.

Perim, P., F. M. Marticorena, F. Ribeiro, G. Barreto, N. Gobbi, C. Kerksick, E. Dolan, and B. Saunders. 2019. Can the skeletal muscle carnosine response to beta-alanine supplementation be optimized? Front. Nutr. 6:135.

Petracci, M., M. Betti, M. Bianchi, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. Poult. Sci. 83:2086–2092.

Petracci, M., S. Mudalal, E. Babini, and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:3138.

Petracci, M., S. Mudalal, A. Bonfiglio, and C. Cavani. 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670–1675.

Petracci, M., F. Soglia, M. Madruga, L. Carvalho, E. Ida, and M. Estévez. 2019. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. Food Saf. 18:565–583.

Ponnampalam, E. N., D. L. Hopkins, H. Bruce, D. Li, G. Baldi, and A. E.-D. Bekhit. 2017. Causes and contributing factors to “dark cutting” meat: current trends and future directions: a review. Compr. Rev. Food Sci. Food Saf. 16:400–430.

Poppe, L., and J. Retey. 2003. Properties and synthetic applications of ammonia-lyases. Curr. Org. Chem. 7:1297–1315.

Qi, B., J. Wang, Y.-B. Ma, S.-G. Wu, G.-H. Qi, and H.-J. Zhang. 2018. Effect of dietary β-alanine supplementation on growth performance, meat quality, carnosine content, and gene expression of carnosine-related enzymes in broilers. Poult. Sci. 97:1220–1228.

Relaix, F., M. Bencze, M. J. Borok, A. Der Vartanian, F. Gattazzo, D. Mademtzoglou, S. Perez-Diaz, A. Prola, P. C. Reyes-Fernandez, A. Rotini, and Taglietti. 2021. Perspectives on skeletal muscle stem cells. Nat. Commun. 12:692.

Rétey, J. 1994. The urocanase story: a novel role of NAD+ as electrophile. Arch. Biochem. Biophys. 314:1–16.

Ritchie, H., and M. Roser. 2017. Meat and Dairy Production. Accessed 2021. https://ourworldindata.org/meat-production).

Roberts, P. R., and G. P. Zaloga. 2000. Cardiovascular effects of carnosine. Biochemistry Biokhimiia. 65:856–861.

Roser, M., H. Ritchie, and E. Ortiz-Ospina. 2013. World Population Growth. Accessed 2021. https://ourworldindata.org/world-population-growth.

Rostagno, H. S., and B. G. Becker. 2005. Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements. 2nd ed. Universidade Federal de Vi¸cosa, Departamento de Zootecnia, Vi¸cosa, Brazil.

Sachs, N. J., A. R. Hampton, K. K. Foster, M. Y. Pechanec, J. D. Henderson, A. J. King, and M. J. Mienaltowski. 2019. The effects of an alternative diet regimen with natural methionine ingredients on white striping breast myopathy in broiler chickens. Poult. Sci. 98:413–421.

Salway, J. G. 2018. The Krebs uric acid cycle: a forgotten Krebs cycle. Trends Biochem. Sci. 43:847–849.

Seale, P., A. Asakura, and M. A. Rudnicki. 2001. The potential of muscle stem cells. Dev. Cell 1:333–342.

Shane, B. 2008. Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food Nutr. Bull. 29:S5-16

Sihvo, H.-K., N. Airas, J. Lindén, and E. Puolanne. 2018. Pectoral vessel density and early ultrastructural changes in broiler chicken wooden breast myopathy. J. Comp. Pathol. 161:1–10.

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Siller, W. G. 1985. Deep pectoral myopathy: a penalty of successful selection for muscle growth. Poult. Sci. 64:1591–1595.

Soglia, F., L. Laghi, L. Canonico, C. Cavani, and M. Petracci. 2016a. Functional property issues in broiler breast meat related to emerging muscle abnormalities. Food Res. Int. 89:1071–1076.

Soglia, F., S. Mudalal, E. Babini, M. Di Nunzio, M. Mazzoni, F. Sirri, C. Cavani, and M. Petracci. 2016b. Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poult. Sci. 95:651–659.

Soglia, F., A. K. Silva, L. M. Lião, L. Laghi, and M. Petracci. 2019a. Effect of broiler breast abnormality and freezing on meat quality and metabolites assessed by 1H-NMR spectroscopy. Poult. Sci. 98:7139–7150.

Soglia, F., A. K. Silva, S. Tappi, L. M. Lião, P. Rocculi, L. Laghi, and M. Petracci. 2019b. Gaping of pectoralis minor muscles: magnitude and characterization of an emerging quality issue in broilers. Poult. Sci. 98:6194–6204.

Spolter, P. D., and R. C. Baldridge. 1963. The metabolism of Histidine. V. on the assay of enzymes in rat liver. J. Biol. Chem. 238:2071-2074.

Spomedial. 2003. Sportmedizin interaktiv lernen. Accessed 2021. http://vmrz0100.vm.ruhr-uni-bochum.de/spomedial/content/e866/e2442/e4687/index_ger.html.

Su, H., X. Sheng, and Y. Liu. 2016. Exploring the substrate specificity and catalytic mechanism of imidazolonepropionase (HutI) from Bacillus subtilis. Phys. Chem. Chem. Phys. 18:27928–27938.

Suidasari, S., J. Stautemas, S. Uragami, N. Yanaka, W. Derave, and N. Kato. 2015. Carnosine Content in Skeletal Muscle Is Dependent on Vitamin B6 Status in Rats. Front. Nutr. 2:39.

Sundekilde, U. K., M. K. Rasmussen, J. F. Young, and H. C. Bertram. 2017. High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine. Food Chem. 217:151–154.

Swatland, H. J. 1990. A note on the growth of connective tissues binding turkey muscle fibers together. Can. Inst. Food Sci. Technol. j. 23:239–241.

Tallentire, C. W., I. Leinonen, and I. Kyriazakis. 2016. Breeding for efficiency in the broiler chicken: a review. Agron. Sustain. Dev. 36.

Tasoniero, G., H. Zhuang, G. R. Gamble, and B. C. Bowker. 2020. Effect of spaghetti meat abnormality on broiler chicken breast meat composition and technological quality. Poult. Sci. 99:1724–1733.

Tavárez, M. A., and F. Solis de los Santos. 2016. Impact of genetics and breeding on broiler production performance: a look into the past, present, and future of the industry. Anim. Front. 6:37–41.

Teke, B., P. D. Akin, F. Akdag, M. Ugurlu, and B. Ekiz. 2019. The effect of season on dead on arrival rate and meat quality characteristics of broiler chicken transported in commercial slaughter conditions. Large Anim. Rev. 2019:147–151.

Tiedje, K. E., K. Stevens, S. Barnes, and D. F. Weaver. 2010. Beta-alanine as a small molecule neurotransmitter. Neurochem. Int. 57:177–188.

Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, and C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult. Sci. 95:2167–2173.

Tixier-Boichard, M., F. Leenstra, D. Flock, P. M. Hocking, and S. Weigend. 2012. A century of poultry genetics. Worlds Poult. Sci. J. 68:307–321.

Tondeur, W., and P. Simons. 2019. Broiler meat signals. A practical guide to improving poultry meat quality. Roodbont, Zutphen, the Netherlands.

Toyoshima, C. 2009. How Ca2+-ATPase pumps ions across the sarcoplasmic reticulum membrane. Biochim. Biophys. Acta Biomembr. 1793:941–946.

Trocino, A., A. Piccirillo, M. Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, and G. Xiccato. 2015. Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poult. Sci. 94:2996–3004.

Valentine, B. A. 2017. Skeletal Muscle. Pages 908-953.e1 in Pathologic Basis of Veterinary Disease. M. D. McGavin, and J. F. Zachary, Elsevier, Amsterdam, the Netherlands.

van Laack, R., and J. L. Lane. 2000. Denaturation of myofibrillar proteins from chickens as affected by pH, temperature, and adenosine triphosphate concentration. Poult. Sci. 79:105–109.

van Wessel, T., A. de Haan, W. J. van der Laarse, and R. T. Jaspers. 2010. The muscle fiber type-fiber size paradox: hypertrophy or oxidative metabolism? Eur. J. Appl. Physiol. 110:665–694.

Velleman, S. G. 2007. Muscle development in the embryo and hatchling. Poult. Sci. 86:1050–1054.

Vignale, K., J. V. Caldas, J. A. England, N. Boonsinchai, A. Magnuson, E. D. Pollock, S. Dridi, C. M. Owens, and C. N. Coon. 2017. Effect of white striping myopathy on breast muscle (pectoralis major) protein turnover and gene expression in broilers. Poult. Sci. 96:886–893.

Vistoli, G. 2015. CHAPTER 1. Carnosine in the Context of Histidine-Containing Dipeptides. Pages 3–22 in Imidazole Dipeptides. V. R. Preedy, ed., Royal Society of Chemistry, Cambridge, UK.

Vistoli, G., M. Colzani, A. Mazzolari, E. Gilardoni, C. Rivaletto, M. Carini, and G. Aldini. 2017. Quenching activity of carnosine derivatives towards reactive carbonyl species: focus on α-(methylglyoxal) and β-(malondialdehyde) dicarbonyls. Biochem. Biophys. Res. Commun. 492:487–492.

Walterscheid, J. P., D. X. Nghiem, N. Kazimi, L. K. Nutt, D. J. McConkey, M. Norval, and S. E. Ullrich. 2006. Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. PNAS. 103:17420–17425.

Warner, R. D. 2017. The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. Pages 419–459 in Lawrie´s Meat Science, F. Toldra, ed., Elsevier, Amsterdam, the Netherlands.

Warner, R. D., R. G. Kauffman, and M. L. Greaser. 1997. Muscle protein changes post mortem in relation to pork quality traits. Meat Sci. 45:339–352.

Wecke, C., and F. Liebert. 2013. Improving the reliability of optimal in-feed amino acid ratios based on individual amino acid efficiency data from N balance studies in growing chicken. Animals 3:558–573.

Wilson, B. W., P. S. Nieberg, R. J. Buhr, B. J. Kelly, and F. T. Shultz. 1990. Turkey muscle growth and focal myopathy. Poult. Sci. 69:1553–1562.

Wise, S. P., and R. Shadmehr. 2002. Motor Control. Pages 137–157 in Encyclopedia of the Human Brain. V. S. Ramachandran, ed., Academic Press, New York, US.

Wold, J. P., E. Veiseth-Kent, V. Høst, and A. Løvland. 2017. Rapid on-line detection and grading of wooden breast myopathy in chicken fillets by near-infrared spectroscopy. PloS One. 12:e0173384.

Yamada, S., Y. Tanaka, and S. Ando. 2005. Purification and sequence identification of anserinase. FEBS J. 272:6001–6013.

Yannacone, S. F., D. Sethio, and E. Kraka. 2020. Quantitative assessment of intramolecular hydrogen bonds in neutral histidine. Theor. Chem. Acc. 139.

Yeum, K.-J., M. Orioli, L. Regazzoni, M. Carini, H. Rasmussen, R. M. Russell, and G. Aldini. 2010. Profiling histidine dipeptides in plasma and urine after ingesting beef, chicken or chicken broth in humans. Amino acids. 38:847–858.

Yu, Y., Y.-H. Liang, E. Brostromer, J.-M. Quan, S. Panjikar, Y.-H. Dong, and X.-D. Su. 2006. A catalytic mechanism revealed by the crystal structures of the imidazolonepropionase from bacillus subtilis. J. Biol. Chem. 281:36929–36936.

Zaloga, G. P., P. R. Roberts, K. W. Black, M. Lin, G. Zapata-Sudo, R. T. Sudo, and T. E. Nelson. 1997. Carnosine is a novel peptide modulator of intracellular calcium and contractility in cardiac cells. Am. J. Physiol. 272:H462-8.

Zambonelli, P., M. Zappaterra, F. Soglia, M. Petracci, F. Sirri, C. Cavani, and R. Davoli. 2016. Detection of differentially expressed genes in broiler pectoralis major muscle affected by white striping - wooden breast myopathies. Poult. Sci. 95:2771–2785.

Zampiga, M., A. Meluzzi, S. Pignata, and F. Sirri. 2019a. Occurrence of breast meat abnormalities and foot pad dermatitis in light-size broiler chicken hybrids. Animals 9.

Zampiga, M., F. Soglia, G. Baldi, M. Petracci, G. M. Strasburg, and F. Sirri. 2020. Muscle abnormalities and meat quality consequences in modern turkey hybrids. Front. Physiol. 11:554.

Zampiga, M., F. Soglia, M. Petracci, A. Meluzzi, and F. Sirri. 2019b. Effect of different arginine-to-lysine ratios in broiler chicken diets on the occurrence of breast myopathies and meat quality attributes. Poult. Sci. 98:2691–2697.

Zanetti, M. A., D. C. Tedesco, T. Schneider, S. T. F. Teixeira, L. Daroit, F. Pilotto, E. L. Dickel, S. P. Santos, and L. R. d. Santos. 2018. Economic losses associated with wooden breast and white striping in broilers. SCA. 39:887.

Zhang, X., K. V. To, T. R. Jarvis, Y. L. Campbell, J. D. Hendrix, S. P. Suman, S. Li, D. S. Antonelo, W. Zhai, J. Chen, H. Zhu, and M. W. Schilling. 2021. Broiler genetics influences proteome profiles of normal and woody breast muscle. Poult. Sci. 100:100994.

Ziober, I. L., F. G. Paio, D. F. Marchi, L. L. Coutinho, E. Binneck, A. L. Nepomuceno, and M. Shimokomaki. 2010. Heat and chemical stress modulate the expression of the α-RYR gene in broiler chickens. Genet. Mol. Res. 9:1258–1266.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93:2970–2982.