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 (nd[6])	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.

		Treatment group						
Parameter	Age, d	HIS41[1]	HIS45[2]	HIS49[3]	HIS53[4]	HIS57[5]	SEM	Probability
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.

		Treatment group						
Parameter	Storage time, h	HIS41[1]	HIS45[2]	HIS49[3]	HIS53[4]	HIS57[5]	SEM	Probability
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

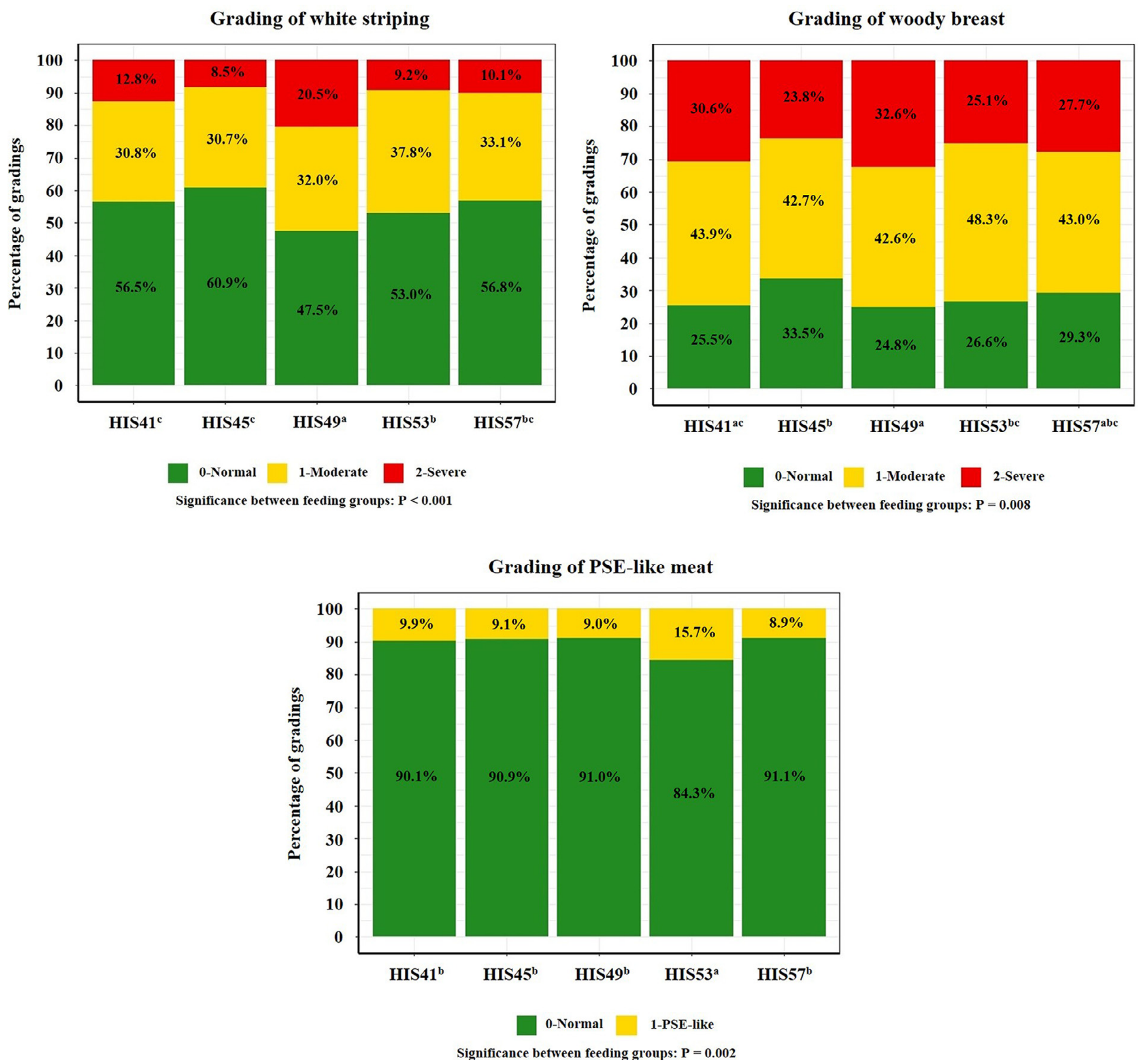

Figure 1. Percentages of fillets affected by the white striping, woody breast and of PSE conditions. All fillets were graded by 6 testers and the pooled data were used for analysis. All data were analyzed by using a Chi^2-test. Differences by groups, as given by the different superscripts, were analyzed 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.

	Occurrence of the quality issue per grading category, %					
Separation criteria of the parameters	WB[1] 0-normal	WB 1-moderate	WB 2-severe	Total	Probability	C_{norm}[2]
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 ($P \leq 0.008$). 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.

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