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Effects of 50 Percent Substitution of Soybean Meal by Alternative Proteins from *Hermetia illucens* or *Spirulina platensis* in Meat-Type Chicken Diets with Graded Amino Acid Supply

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Abstract

Alternative protein sources, such as insects or algae meals are in special focus of animal nutrition in order to replace soybean meal (SBM). As part of the multidisciplinary project "sustainability transitions" this study evaluated effects of replacing SBM by partly defatted larvae meal from the black soldier fly, *Hermetia illucens* or meal from the micro algae *Spirulina platensis* in broiler diets. The aim of the current study was to investigate the chickens' growth performance and the intestinal morphology as well as the health status. 288 one-day-old male growing chickens (Ross 308) from a commercial hatchery were randomly allotted to 48 pens (6 birds per pen) for the growth study with five diets and feed supply on free choice level. The control diet was based on wheat, corn and SBM. The experimental diets replaced 50% of SBM by the alternative proteins under study, both on a basic level of amino acid (AA) supplementation (Lys and Met added equal to the control diet) (diet HM and SM) and an extended level of AA fortification (Lys, Met, Thr, Arg, Val added) (diet HM+ and SM+). Response of chickens was evaluated by zoo-technical parameters, which were under weekly control (e.g. growth, feed intake, feed conversion ratio, mortality). After finishing the growth study birds from control and the experimental diets at the basic level of AA supplementation were slaughtered after 12 hour fastening and utilized for gut morphometric analysis and histological evaluation of the health status. Diets at a

basic level of AA fortification led to significant depression of growth, feed intake, feed conversion ratio, and protein conversion ratio, respectively. In addition, the acceptance of the *Spirulina* diet was lower ($p \leq 0.001$) as compared to the *Hermetia* based diet. However, the extended level of AA supplementation improved all parameters significantly. Diets without extended AA supply altered some morphological parameters of the intestinal wall, but the nutritional significance of this observation needs to be verified in AA balanced diets. The health state of chicken was not impaired by the diets under study.

Keywords

Growing Chickens, Feed, Growth Performance, Histology, Morphometry, *Spirulina platensis*, *Hermetia illucens*

1. Introduction

Soybean meal is the main feed protein source in diet formulation for pigs and poultry in EU. According to the projections of the Food and Agricultural Organization of the United Nations (FAO), the world's population will increase to nine billion people in 2050 [1] and it is expected that more food from animal origin will be needed [2]. According to [3], it is possible to feed the world`s population with a vegetarian diet; however such a change in consumer demands and preferences is not expected in the near future. In consequence, more protein feed for nutrient transformation by livestock is required while concurrently meeting the needs of human nutrition.

As alternatives to traditional feed proteins, insects have gained increased attention in Europe [4] because of their high protein content and adequate amino acid composition, which is widely comparable to conventional animal proteins, associated with various fatty acids, minerals and vitamins [5] [6] [7] [8]. Black soldier fly (*Hermetia illucens*) is one of the most promising species in terms of larval nutritional value [9]. [10] pointed out that *Hermetia* larvae meal is an excellent source of protein and energy in diets for growing chickens. They were able to show that the growth rate of chickens was improved when HM was applied as an ingredient of a complete broiler feed [11]. However, only limited information is available in order to optimize the use of *Hermetia* meal in broiler diets [10] [12]. Presently, in Europe, insect-based meals are not allowed to be included in livestock and poultry feeds [13]. Nonetheless, since 1 July 2017, processed insect protein has been authorized for the use in aquaculture by Regulation [14]. Therefore, the EU barriers are expected to be overcome in the future so that this promising protein source could be integrated into poultry diets.

An additional alternative protein source is blue-green algae meal from *Spirulina platensis*, which is widely considered as a potential livestock feed ingredient due to its high protein content and the abundance of vitamins, pigments and minerals [15] [16] [17]. *Spirulina* meal is suitable as a substitute for conventional

ingredients in poultry nutrition and has thus far been shown to yield no detrimental effects when up to 15% *Spirulina* meal was included in broiler feeds [18]. However, detailed investigations about the optimized application of Spirulina in chicken diets are scarce.

As part of the multidisciplinary project "Sustainability Transitions in food production: alternative protein sources from a socio-technical perspective", the present study aimed to evaluate the potential of *Hermetia* and *Spirulina* meal as alternative feed proteins for the substitution of soybean meal (SBM) in diets for meat type chickens. In a first step, 50% replacement of SBM was examined and the zoo-technical data are presented in the current study. According to earlier observations [19] [20], the intestinal morphology could also be affected at high inclusion rates of *Hermetia* or *Spirulina*. In consequence, histological analysis was an integrated part of the investigations.

2. Material and Methods

The experiments were conducted at the Division for Animal Nutrition Physiology at the Georg-August-University Goettingen and approved by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

2.1. Stock and Husbandry

A total of 288 day-old male growing chickens (Ross-308 line; WIMEX Agrarprodukte, Hatchery Rosefeld Germany) were randomly allotted to 48 floor pens (6 birds/pen) one day after hatching. Based on individual body weight (BW) of birds it was aimed to achieve a similar average BW at the experiment start for the five experimental treatments under study (Control diet, n = 12; 4 experimental diets, n = 9). In an environmentally controlled room with monochromatic (red) light for 23 hours, birds were bedded on wood shavings and had full access to feed and water. Climatic conditions were chosen according to the Ross management recommendations [21]. Experimental conditions were routinely checked twice daily with special attention to feeders, water supply, temperature and physiological state of chickens. Physiological state of chickens was measured by health and vitality of the birds. Growth data and feed consumption were recorded weekly.

2.2. Alternative Protein Sources

The investigated *Hermetia* meal was obtained from a commercial producer (Hermetia Futtermittel GbR, Baruth/Mark, Germany). Black soldier fly larvae were collected from a plant-based substrate (rye flour, wheat bran) after 20 days of fattening. Following 14 hours of drying at temperatures between 65˚C and 70˚C, the larvae were partly defatted with a screw press (Type AP08, Reinartz) and afterwards ground into a meal.

The *Spirulina platensis* microalga used in the study was a sun-dried commer-

cial *Spirulina* source powder obtained from Myanmar and declared to be free of GMO, irradiation, pesticides, colorants, preservatives and additives. As demonstrated by the nutrient composition of the protein sources (Table 1), the lipid fraction was not extracted from the algae meal. The microcystine content was analyzed by an external laboratory (TeLA GmbH, Geestland, Germany) and remained under the detection limit. The nutritional contents of the final alternative protein source products are shown in Table 1.

2.3. Diets and Feeding

Experimental diets were mixed and pelleted at the facilities of the Division for Animal Nutrition Physiology at the University of Goettingen. Pelleted diets were supplied *ad libitum* as starter (1 - 21 d) and grower diets (22 - 34 d). The starter/grower control diets were based on wheat (33/38%), corn (16/19%) and SBM (39/32%) as the main ingredients (Table 2). Experimental diets aimed to substitute 50% of the SBM in the control diet by the alternative proteins under study, both with a basic and an extended level of AA fortification. The basic level diets (HM and SM, respectively) were individually adjusted by Lys and Met supplementation so that they were equal to that of the control diet. The extended level of AA fortification (diet HM+ and diet SM+, respectively) aimed to yield an improved dietary AA balance according to an ideal AA ratio (IAAR) as currently recommended by [22]. Consequently, in these diets, beside Lys and Met, further

Table 1. Analyzed crude nutrient and AA composition of *Spirulina platensis* and *Hermetia illucens* meals as used for diet formulation.

Nutrient contents	Hermetia meal		Spirulina meal	
Moisture (%)	5.5		3.4	
Crude protein (% of DM)	60.8		58.8	
Crude ash (% of DM)	7.5		6.1	
Crude lipids (% of DM)	14.1		4.3	
Crude fibre (% of DM)	10.9		0.5*	
AA contents	**gAA/kgDM**	**gAA/16gN**	**gAA/kgDM**	**gAA/16gN**
Lys	32.97	5.42	22.97	3.91
Met	7.53	1.24	10.61	1.81
Cys	4.89	0.80	4.53	0.77
Thr	21.70	3.57	25.77	4.39
Arg	25.05	4.12	39.92	6.79
Val	32.58	5.36	34.50	5.87
Leu	37.95	6.24	47.23	8.04
Ile	23.47	3.86	29.81	5.07
His	16.58	2.73	7.51	1.28

*preliminary data due to difficulties in application of the standard procedure.

Table 2. Ingredient composition of experimental diets (g/kg as fed).

Ingredients/ Diets	Starter period (1 - 21 d)					Grower period (22 - 34 d)				
	Control	HM	SM	HM+	SM+	Control	HM	SM	HM+	SM+
Wheat	328.8	362.8	381.5	358.3	377.9	375.8	405.8	419.1	402.6	416.8
Corn	164.4	181.4	190.7	179.2	189.0	187.9	202.9	209.6	201.3	208.4
Soybean meal	390	195.0	195.0	195.0	195.0	320.0	160.0	160.0	160.0	160.0
Insect meal	-	145.4	-	145.4	-	-	119.0	-	119.0	-
Algae meal	-	-	118.2	-	118.2	-	-	97.0	-	97.0
Soybean oil	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5	78.5
Premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
DCP 40	11.0	12.0	12.0	12.0	12.0	10.0	8.0	10.0	8.0	10.0
$CaCO_3$	11.0	9.9	9.1	9.9	9.1	9.0	8.0	8.0	8.0	8.0
NaCl	3.0	1.7	1.7	1.7	1.7	3.0	2.0	2.0	2.0	2.0
Wheat starch	-	-	-	-	-	-	-	-	3.0	3.0
TiO_2	-	-	-	-	-	3.0	3.0	3.0	-	-
L-Lys·HCl	1.3	1.3	1.3	3.2	4.4	0.8	0.8	0.8	2.4	3.5
DL-Met	2.0	2.0	2.0	4.1	3.5	2.0	2.0	2.0	3.0	2.5
L-Thr	-	-	-	0.6	-	-	-	-	0.4	-
L-Arg	-	-	-	2.2	0.7	-	-	-	1.4	0.1
L-Val	-	-	-	-	-	-	-	-	0.5	0.2

HM = Hermetia meal diet with basic AA supply; SM = Spirulina meal diet with basic AA supply; HM+ = Hermetia meal diet with extended AA supply; SM+ = Spirulina meal diet with extended AA supply. [1]Added per kg of final diet: 2.1 g calcium, 0.8 g sodium, 5000 IU vitamin A, 1000 IU vitamin D3, 30 mg vitamin E, 2.6 mg vitamin B1, 4.8 mg vitamin B2, 3.2 mg vitamin B6, 20 µg vitamin B12, 3 mg vitamin K3, 50 mg nicotinic acid, 10 mg calcium pantothenate, 0.9 mg folic acid, 100 µg biotin, 1000 mg choline chloride, 50 mg Fe as iron-II-sulfate, monohydrate, 15 mg Cu as copper-II-sulfate, pentahydrate, 120 mg Mn as manganese-II-oxide, 70 mg Zn as zinc oxide, 1.4 mg I as calcium iodate, hexahydrate, 0.28 mg Se as sodium selenite, 0.55 mg Co as alkaline cobalt-II-carbonate, monohydrate and 100 mg butylhydroxytoluol.

AAs (Thr, Arg, Val) were supplemented. Analyzed nutrient composition of the diets is summarized in Table 3.

2.4. Recorded Parameters

2.4.1. Feed Analysis

All Analyses of ingredients and experimental diets were conducted according to the standards of German VDLUFA [24]. Nitrogen content of feed was measured using the DUMAS-method (TruMac®, Leco Instrument GmbH, Mönchengladbach). The calculation of fraction CP was based on factor 6.25 of the nitrogen content. The given AA contents of the final diets are based on the analyzed AA contents of the single protein sources. AA composition of the protein sources were analyzed through ion-exchange chromatography (Biochrom® 30, Biochrom Ltd. Cambridge, England) using acid hydrolysis with and without an oxidation step quantitative determination of sulphur-containing AAs. According to the

Table 3. Analyzed content of crude nutrients and selected AAs of experimental diets.

Diets	Starter period (1 - 21 d)					Grower period (22 - 34 d)				
	Control	HM	SM	HM+	SM+	Control	HM	SM	HM+	SM+
	Crude nutrients (g/kgDM)									
Crude protein	249.5	249.6	236.4	259.3	241.4	220.2	217.7	207.4	230.9	207.2
Ether extract	111.6	124.3	115.7	131.1	116.6	112.8	110.7	117.2	131.4	118.4
Crude fiber	45.2	49.4	33.4	47.1	31.1	40.4	41.1	28.5	41.7	30.4
Crude ash	65.6	60.1	58.1	60.4	59.2	61.6	55.6	55.2	56.5	53.5
N-free extract	528.1	516.6	556.4	502.1	551.7	565	574.9	591.7	539.5	590.5
AME_N (MJ/kgDM)[1]	14.4	15.2	15.4	15.3	15.4	14.8	15.5	15.6	15.6	15.6
	Amino acids (g/kg as-fed)[2]									
Lys	12.55	12.20	10.24	13.66	12.68	10.53	10.24	8.64	11.48	10.73
Met	4.99	4.96	5.13	7.01	6.60	4.63	4.68	4.82	5.6	5.31
Met+Cys	8.43	8.06	8.12	10.10	9.58	7.87	7.57	7.62	8.49	8.10
Thr	7.84	7.86	7.8	8.38	7.78	6.92	6.94	6.89	7.27	6.88
Arg	14.25	11.95	13.03	14.09	13.65	12.4	10.52	11.41	11.88	11.49
Val	9.34	10.43	9.85	10.40	9.83	8.31	9.21	8.73	9.68	8.92

HM = Hermetia meal diet with basic AA supply; SM = Spirulina meal diet with basic AA supply; HM+ = Hermetia meal diet with extended AA supply; SM+ = Spirulina meal diet with extended AA supply. [1]N corrected apparent metabolizable energy, calculated according to [23]; [2]Derived from analyzed AA content of the ingredients.

German standards, ether extracts were analyzed following HCl hydrolysis of the feed samples.

2.4.2. Performance Parameters

Feed intake (FI), BW and mortality were measured during the growth trial. Individual body weight and pen feed intake were recorded at weekly intervals. From these data, the feed conversion ratio (FCR) (see Equation (1)) and the protein conversion ratio (see Equation (2)) were calculated. Mortality was routinely checked twice daily.

$$FCR = \frac{DM_{intake}}{BW_{gain}} \quad (1)$$

$$PCR = \frac{CP_{intake}}{BW_{gain}} \quad (2)$$

whereby

FCR = feed conversion ratio (g/g)

PCR = protein conversion ratio (g/g)

DM_{intake} = dry matter intake (g)

BW_{gain} = body weight gain (g)

CP_{intake} = crude protein intake (g)

2.4.3. Histological Examinations

Verification of health status

For the histological evaluation of the health status, eight chickens per diet from selected treatments (control, HM and SM) were slaughtered and weighed after 12 hours fastening. Representative tissue specimen from liver, spleen, lung, heart, pancreas, kidney, glandular stomach, gizzard, caecum, colon and Bursa of Fabricius were fixed in formalin solution (4%), paraffin-embedded and cut into 4 µm tissue sections before being routinely stained with hematoxylin and eosin (HE) for microscopy. A certified specialist in veterinary pathology, comprehensively blind of different experimental treatments, examined histological specimens of all animals for pathological changes. Furthermore, the presence of inflammatory cell infiltrates within tissues/organs were semi-quantitatively evaluated by an ordinal scoring system (0 normal, (+) scarce, + mild, ++ moderate, +++ severe) according to [25].

Gut morphometric analysis

Twelve chickens per selected diet (control, HM and SM) were randomly quoted in order to conduct histological analyses of the intestine. Birds were killed by CO_2 inhalation and 10 to 15 min. later tissue samples (approximately 10 mm × 5 mm) were taken from three sections (the middle of the descending part of the duodenum, the middle of the jejunum behind Meckel's diverticulum and the middle of the left caecum). Tissue samples were flushed with saline and fixed in 10% neutral buffered formalin solution, dehydrated in ethanol (TP 1020, Leica, Germany) and embedded in paraffin (EG 1150, Leica, Germany). For microscopy, the 5-µm-thick sections were prepared by a microtome (HM 340E, Microm, Spain) and stained with hematoxylin and eosin making use of an automated multistainer ST 5020 (Leica, Germany). Morphometric analyses were performed in samples selected by systematic random sampling – every 30th section was chosen to obtain six sections of duodenal, jejunal or caecal tissue per bird. The slides were scanned in a MiraxDesk scanner (Carl Zeiss, Germany) and analyzed using the Pannoramic Viewer 1.15.4 software (3D-Histech, Hungary). The following measurements were performed: villi length, depth of the crypts of Lieberkühn, ratio of villus length to crypt depth and thickness of the tunica muscularis, respectively. The linear measurements were repeated 10 times per animal and the calculated mean value was applied for further assessments.

2.5. Statistical Analysis

Growth performance

Results are presented as means ± standard deviation (Mean ± SD). Statistical analysis was carried out using the software package SPSS (IBM SPSS Statistics, Version 24.0). One-way ANOVA were run using the Tukey-test and Games-Howell-test to identify significant differences between treatments ($p \leq 0.05$).

Gut morphometric analysis

For gut morphometric analysis, the data were analyzed using one-way ANOVA. Statistical analyses were performed using the Statistica 10.0 software

(StatSoft, USA). The significance of differences between means was evaluated by Duncan's test ($p \leq 0.05$).

3. Results

3.1. Growth Trial

Results of the growth trial are summarized in Table 4.

In the starter period, both the HM and SM diets led to a significant decline in dry matter intake, growth and feed efficiency as compared to the control diet and diets with extended AA supplementation (HM+ and SM+). The acceptance of the SM diet was lower ($p \leq 0.001$) as compared to the other diets (control, HM, HM+ and SM+). In contrast, diets HM+ and SM+ compensated for these observed depressions in all mentioned parameters. Diet HM+ did provide significantly superior results for BW, compared to the control group.

During the grower period, the HM and SM diets continued to yield significantly lower BW and feed intake rates. Feed- and protein conversion ratios were impaired as compared to the control, HM+ and SM+ diets. As was observed in

Table 4. Summarized performance results of the growth study in starter and grower period and over the whole 5 weeks trial of growing meat type chicken*.

Diet	Control	HM	SM	HM+	SM+	SEM	*p*
n	12	9	8**	9	9		
***Starter period* (*d*1-21)**							
Day old chicken (g)	47.5 ± 0.1	47.4 ± 0.1	47.3 ± 0.1	47.3 ± 0.2	47.3 ± 0.2	0.025	0.304
Final body weight (g)	960.9^{c} ± 50.5	693.2^{b} ± 30.1	504.6^{a} ± 31.0	1034.5^{d} ± 42.0	947.3^{c} ± 60.1	28.811	<0.001
Dry matter intake (g/d)	54.3^{cd} ± 3.8	47.1^{b} ± 3.7	37.7^{a} ± 3.4	58.0^{d} ± 4.1	52.6^{c} ± 4.6	1.164	<0.001
Feed conversion ratio (g/g)	1.25^{a} ± 0.11	1.53^{b} ± 0.13	1.70^{c} ± 0.1	1.23^{a} ± 0.07	1.23^{a} ± 0.04	0.030	<0.001
Protein conversion ratio (g/g)	0.31^{a} ± 0.03	0.38^{b} ± 0.03	0.40^{b} ± 0.02	0.32^{a} ± 0.02	0.29^{a} ± 0.01	0.007	<0.001
***Grower period* (*d*22-34)**							
Final body weight (g)	2173.7^{c} ± 112.2	1493.6^{b} ± 89.3	1062.5^{a} ± 64.8	2319.9^{d} ± 114.4	2121.8^{c} ± 121.6	69.568	<0.001
Dry matter intake (g/d)	145.0^{c} ± 8.0	124.8^{b} ± 11.1	96.0^{a} ± 4.5	138.0^{c} ± 9.9	144.4^{c} ± 9.0	2.928	<0.001
Feed conversion ratio (g/g)	1.44^{b} ± 0.07	1.88^{c} ± 0.21	2.05^{c} ± 0.18	1.29^{a} ± 0.07	1.48^{b} ± 0.04	0.045	<0.001
Protein conversion ratio (g/g)	0.32^{ab} ± 0.02	0.42^{c} ± 0.05	0.43^{c} ± 0.04	0.29^{a} ± 0.02	0.32^{b} ± 0.01	0.009	<0.001
***Whole trial* (*d*1-34)**							
Final body weight (g)	2173.7^{c} ± 112.2	1493.6^{b} ± 89.3	1062.5^{a} ± 64.8	2319.9^{d} ± 114.4	2121.8^{c} ± 121.6	69.568	<0.001
Average daily gain (g/d)	64.54^{c} ± 3.40	43.86^{b} ± 2.71	30.71^{a} ± 1.96	68.85^{d} ± 3.47	62.75^{c} ± 3.68	2.108	<0.001
Dry matter intake (g/d)	87.27^{c} ± 4.72	75.37^{b} ± 6.17	57.84^{a} ± 1.87	87.09^{c} ± 5.55	86.01^{c} ± 5.83	1.750	<0.001
Feed conversion ratio (g/g)	1.35^{b} ± 0.04	1.72^{c} ± 0.17	1.89^{c} ± 0.11	1.26^{a} ± 0.04	1.37^{b} ± 0.03	0.037	<0.001
Protein conversion ratio (g/g)	0.30^{a} ± 0.01	0.41^{b} ± 0.04	0.42^{b} ± 0.02	0.30^{a} ± 0.01	0.31^{a} ± 0.01	0.008	<0.001

HM = Hermetia meal diet with basic AA supply; SM = Spirulina meal diet with basic AA supply; HM+ = Hermetia meal diet with extended AA supply; SM+ = Spirulina meal diet with extended AA supply. *Mean ± standard derivation. **one box excluded, outlier in feed conversion ratio, detected with SPSS boxplot-test ($p \leq 0.05$). ^{abcd}Mean values with different superscript letters within lines are significantly different ($p \leq 0.05$).

the starter period, further AA fortification significantly improved all parameters also in the grower period. HM+ and SM+ results remained predominantly comparable to the control. Furthermore, HM+ yielded a significantly higher BW and a significantly improved FCR than the control group.

Considering the growth trial as a whole, the same tendencies as in the grower period are visible. SM and HM diets produce the significantly lowest BW and FI and correspondingly impaired feed- and protein conversion ratios as compared to the remaining diets. In addition, the same trend was observed for average daily gain (ADG). Here, HM+ surpassed the growth data and the ADG of the control diet and once again obtained a superior FCR. In all other parameters, HM+ and SM+ demonstrated similar results to the control diet. No significant diet effect was observed on mortality (2% - 4%) throughout the entire five-week trial.

3.2. Verification of the Health Status

Histological evaluation of all representative organ tissue specimens generally revealed none to mild unspecific findings which were similarly distributed over all dietary treatments involved. Those findings comprised predominantly scarce to mild multifocal lymphohistiocytic cell foci (see Table 5), mild fibrosis of the lamina propria (gizzard), and mild hyperemia in hepatic, pulmonary or colonic tissues. However, chickens fed with diet SM revealed a slightly pronounced proliferation of the bronchus-associated lymphoid tissue (BALT) within the lung, as compared to control diet and diet HM, respectively. On the other hand, scarce inflammatory cell infiltrates were only present in the pancreas of control animals.

Table 5. Average scores of inflammatory cell infiltrates within various meat type chicken organs after feeding of different protein diets.

Organ	Control (n = 8)	HM (n = 8)	SM (n = 8)
Liver	(+)	(+)	(+)
Spleen	0	0	0
Kidney	0	0	0
Pancreas	(+)	0	0
Heart	0	0	0
Lung (BALT)	0	0	(+)
Bursa of Fabricius	0	0	0
Proventriculus	+	+	(+)
Ventriculus (gizzard)	0	0	0
Caeca	(+)	(+)	(+)
Colon	++	+	+

Semiquantitative score (according to Gibson-Corley *et al.*, 2013): 0 none, (+) scarce, + mild, ++ moderate, +++ marked; HM = Hermetia meal diet with basic AA supply, SM = Spirulina meal diet with basic AA supply; BALT = bronchial associated lymphoid tissue.

3.3. Gut Morphometric Analysis

The tissue samples of duodenum, jejunum and caecum of all treatments under study of morphometric analysis exhibited a classical histological construction respective to the associated intestinal section. The general microscopic structure was typical for each section analyzed. A specific feature of the duodenum was the presence of leaf shaped villi and straight longitudinal in shape crypts. In the jejunum finger, shaped villi and predominantly lengthwise crypts were observed. The villi of the caecum were short and conical shaped, whereas the crypts became branched and more shallow compared to other fragments. The interior of the villi and spaces between crypts were filled with loose connective tissue containing numerous blood vessels and cells associated with the immune system – lymphocytes and plasma cells. There were no differences between the examined different sections of the same part of intestine across the entire analyzed treatments. The tunica submucosa and the tunica muscularis had a typical structure.

The summarized results of the gut morphometric analysis are shown in Table 6. The morphometric parameters characterizing the architecture of the mucosa varied slightly between the control diet and diets HM and SM. No significant differences were found in the length of the villi and the depth of the crypts in the duodenum and the caecum, whereas the differences were statistically significant between the experimental diets and control group in the jejunum. Figure 1

Table 6. Average morphometric parameters* of the intestinal sections of broiler chickens dependent on the diet under study.

Diets	Control (n = 12)	HM (n = 12)	SM (n = 12)	SEM	*p*
Duodenum					
Villus length (µm)	1570.6 ± 407.8	1684.0 ± 287.4	1419.6 ± 308.4	57.782	0.175
Crypt depth (µm)	208.8 ± 74.7	189.6 ± 54.4	161.1 ± 25.7	9.557	0.122
Villi:crypt ratio	8.24 ± 2.99	9.48 ± 2.68	9.03 ± 2.32	0.445	0.520
T. muscularis thickness (µm)	357.4^{b} ± 119.4	214.0^{a} ± 46.6	205.3^{a} ± 83.7	18.535	**0.001**
Jejunum					
Villus length (µm)	1234.8^{b} ± 185.6	1022.6^{a} ± 148.5	915.0^{a} ± 357.0	45.936	**0.011**
Crypt depth (µm)	180.1^{b} ± 60.5	153.2^{ab} ± 35.0	136.2^{a} ± 32.6	7.828	0.065 (tendency)
Villi:crypt ratio	7.49 ± 2.44	6.90 ± 1.31	6.84 ± 2.37	0.345	0.706
T. muscularis thickness (µm)	273.0 ± 114.7	255.5 ± 97.6	224.0 ± 59.0	15.499	0.438
Caecum					
Villus length (µm)	184.8 ± 29.2	182.3 ± 40.3	167.6 ± 18.2	5.115	0.341
Crypt depth (µm)	157.7 ± 21.8	157.1 ± 35.1	170.7 ± 63.9	7.188	0.693
Villi:crypt ratio	1.17 ± 0.14	1.20 ± 0.29	1.08 ± 0.33	0.044	0.536
T. muscularis thickness (µm)	518.7^{b} ± 163.8	360.1^{a} ± 137.8	336.5^{a} ± 95.7	25.830	**0.004**

HM = Hermetia meal diet with basic AA supply; SM = Spirulina meal diet with basic AA supply. *Mean ± standard derivation. [ab]Mean values with different superscript letters within lines are significantly different ($p \leq 0.05$).

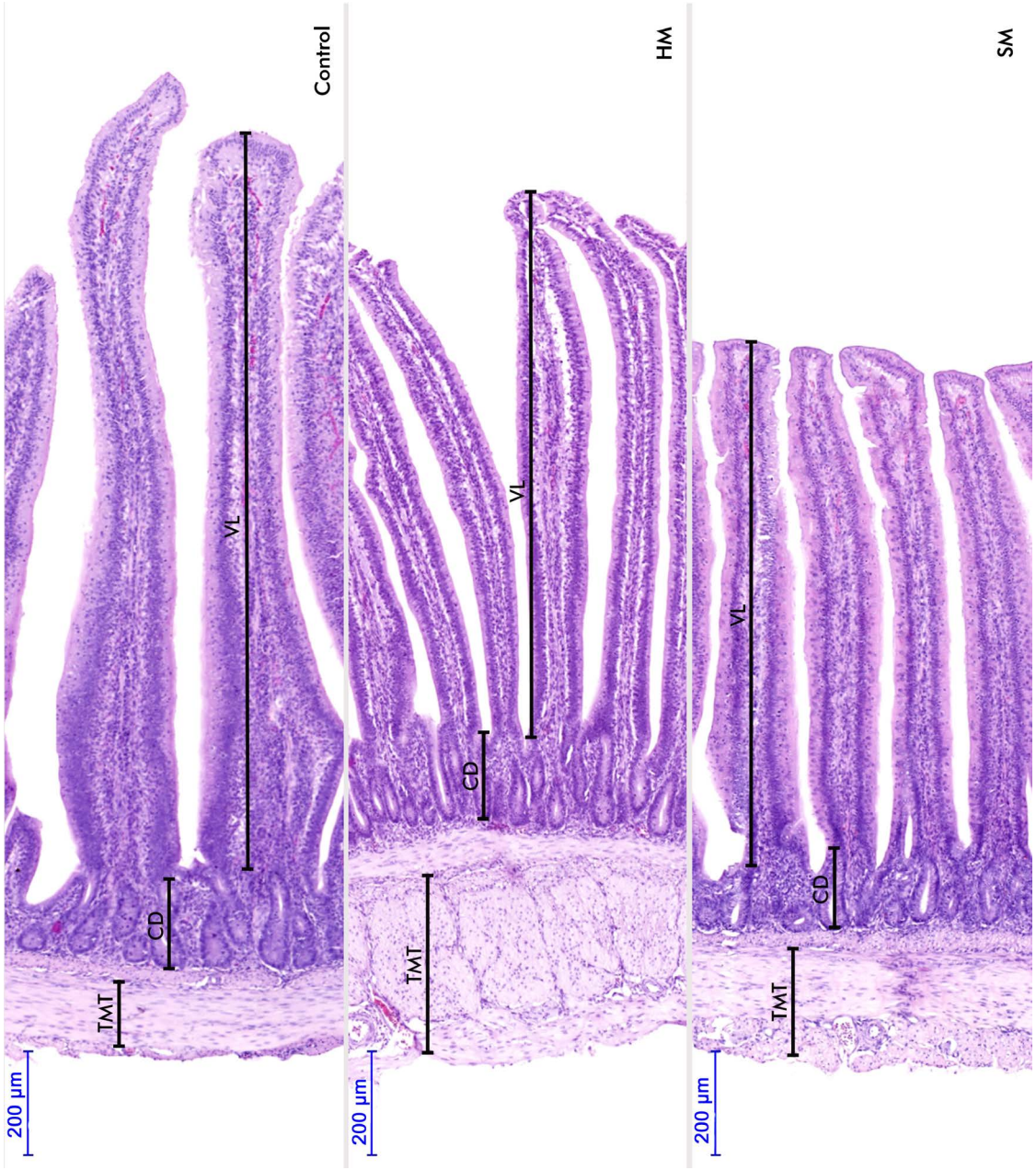

Figure 1. Histological section of the wall of jejunum and microstructure measures as applied. HE staining. Control = Control diet; HM = Diet with *Hermetia* meal at basic AA supply; SM = Diet with *Spirulina* meal at basic AA supply; VL = Villus length; CD = Crypt depth; TMT = Tunica muscularis thickness

shows the procedure of measuring microstructure parameters of the intestinal wall.

Dietary inclusion of *Hermetia illucens* and *Spirulina platensis* meals decreased the average length of villi ($p = 0.011$) in the jejunum. The HM and SM diets tended to reduce the depth of the crypts in the jejunum ($p = 0.065$). The ratio of the villus length to the depth of the crypt, referred to the intestinal integrity factor did not demonstrate any significant differences. Thickness of the tunica muscularis both of samples of the duodenum and of the caecum was significant-

ly higher in the control group as compared to diets HM and SM ($p = 0.001$ resp. $p = 0.004$). Few of enterocytes with symptoms of elevated apoptosis were observed in all dietary treatments under study.

4. Discussion

As demonstrated by parameters of growth response and feed efficiency, both partly defatted larvae meal from *Hermetia illucens* and meal of the microalgae *Spirulina platensis* are suitable alternative protein sources in diets for growing chickens at 50% substitution level of SBM. However, the basic level of AA supplementation as applied in the control diet was not sufficient both in diets HM and SM to create zoo-technical responses as comparable to the control diet. This observation indicated that an extended level of AA supplementation was required for an enhanced growth response when diets with 50% substitution of SBM are fed. Accordingly, a significant improvement of zoo-technical data was observed when AA supplementation was extended (diets HM+, SM+) and according to the currently assumed IAAR [22].

These observations for zoo-technical response were in general agreement with conclusions from actual N balance studies making use of diets with 100% substitution of SBM by *Hermetia* meal or *Spirulin*a meal [26]. In consequence, diets with extended AA supplementation yielded final BW similar (diet SM+) or superior ($p < 0.001$) to the control diet (diet HM+). Accordingly, [11] examined *Hermetia illucens* larvae as replacement for a full-fat SBM and yielded similar effects on growth response. In addition, [27] utilized 4.7% *Hermetia* meal in broiler diets with an additional supplementation of Lys and Met supplementation, but observed similar BW data during starter period as in the control. These results are quite similar to the observations in the present study, but extended AA supplementation (diet HM+) yielded even superior final BW as compared to the control. In case of *Spirulina platensis* as alternative protein source, [28] found no significant effect on BW when 14% and 17% *Spirulina platensis* were included in broiler feed. Accordingly, no significant effect on feed intake and BW was observed by [18], when 6% to 16% of *Spirulina* meal was included in chicken starter diets (1 - 21 d). As related to the current study, these observations are equivocal when considering the dry matter intake between control, SM+ and HM+ diet. [29] came to similar conclusions for the grower period (21 - 37 d) with 8% *Spirulina* meal in grower diets, but the tendency was obvious that BW decreased when the *Spirulina platensis* inclusion rate increased. A clear growth depression was observed with diet SM on a basic level of AA supplementation. [30] came to very similar conclusions making use of the microalgae *Staurosira* sp. from Lower growth rate (0 - 3 weeks of age) and impaired feed efficiency (0 - 6 weeks of age) were observed when SBM was partly (7.5%) substituted by the algae meal on a basic level of AA supplementation. However, if the same diets are supplemented with an extended level of indispensable AAs (Met, Lys, Arg, Ile, Thr, Trp, Val) as demonstrated in the current study, growing

chickens yielded zoo-technical parameters as the control diet or superior results with *Hermetia* meal. This observation is also supported by [11]. Additionally, FCR data over the whole period responded accordingly to the BW development in the present study. [28] found no effect on FCR when 14% and 17% *Spirulina platensis* were applied. In addition, the protein conversion ratio in the current study was very similar between control and diets with extended AA supplementation, but comparable results dealing with protein efficiency of chicken diets based on alternative proteins under study are scarce in the literature.

It is well-known that the diet might influence intestinal architecture [31] [32] [33]. Although feed proteins as provided from insects and algae are believed to be a good protein source and potential ingredient in poultry diets [9] [34] [35], there is a lack of knowledge corresponding to possible effects on gut health and intestinal morphology, respectively. However, information about this "black box" could be helpful to support current processes of authorization as feed for food producing animals. Morphometric measurements of villi length and the crypts depth of the intestine are widely used as one of the factors contributing to the maintenance of intestinal homeostasis [36]. The length of villi is an important factor which determines the surface area of an intestine and in consequence the efficiency of nutrient absorption [37]. In addition, the villi-crypt ratio corresponds to the exfoliation and regeneration cycle of the intestinal epithelium.

So far, the effect of micro algae on intestinal morphology has not been studied in poultry, but in pigs. However, experiments dealing with the influence of algae on the intestinal morphology did not yield equivocal results. [38] supplemented piglets' diet with *Laminaria digitata* and *Laminaria hyperborea* extract. The most favourable morphometrical results were observed in piglets fed with a mix of brown algae *Laminaria digitata* and *Laminaria hyperborean.* The piglets with *Laminaria digitata* in the diet created significantly shorter villi in the duodenum and jejunum as compared to the control treatment. This result is not in agreement with observations of [39], who found an increase of villi length and the decrease of crypts depth in jejunal and ileal tissues when *Spirulina platensis* and *Chlorella vulgaris* were applied in piglet diets.

In poultry, different effects on intestinal morphology have been obtained in studies with insect meals. In free-range growing chickens, graded portions of yellow mealworm larvae (*Tenebrio molitor*) did not influence the morphology of bird`s intestine [40]. However, [41] have demonstrated that a *Hermetia illucens* added diet caused a significant increase of villi length in the duodenum of laying hens.

In the present study, a substitution of 50% of SBM larvae meal of *Hermietia illucens* or microalgae *Spirulina platensis* did not yield a significant effect on villi length and crypts depth in the section of duodenum. However, in the section of jejunum both of the parameters declined significantly. It should be taken into account that digestion occurs in birds in the upper part of the small intestine including duodenum and the released nutrients are absorbed mainly in the lower

part of small intestine. In consequence, the majority of nutrient absorption occurs in the jejunum and the ileum [42] which could be impaired when both villi length and crypt depth are reduced. In addition, lower depth of the crypts is an indicator for the renewal rate of epithelial tissue. Both of these aspects give support for the assumption that an adverse effect on intestinal homeostasis could be created. However, this conclusion is hypothetical and cannot be supported by the observed growth response with diets HM and SM, which was much more related to the AA deficiency as created by the basic level of AA supplementation.

Moreover, a relationship between the type of diet and the thickness of intestinal tunica muscularis of the birds was observed. The basic level of AA supplementation in diets with *Hermetia illucens* and *Spirulina platensis* resulted in a significant decrease in thickness of the tunica muscularis in duodenum and jejunum. An atrophy of the small intestinal tunica muscularis layer may indicate a lower intestinal motility [43]. This observation was surprising, but needs further attention and validation in nutritional studies before such an effect of the protein source in chicken diets can be stated. Lower gut motility could also be discussed as a factor providing more time for processes of digestion and absorption. Results of birds with extended AA supplementation support the conclusion, that an alternative protein related adverse effect on digestive and absorptive potential of the small intestine in growing chicken is improbable.

Finally, the histological evaluation of all representative organ tissue specimens provided none or only scarce to mild unspecific changes. Therefore, it is important to point out that, notable differences in the general health status were not observed between the control and experimental groups. In consequence, alternative protein meals from *Hermetia illucens* and *Spirulina platensis* inclusion rate under study did not impair the healthiness of growing chickens.

5. Conclusions

50% replacement of SBM by partly defatted *Hermetia* or *Spirulina* meal in chicken diets depressed growth if AA were only supplemented at a basic level. However, with an extended level of AA supplementation growth parameters for both experimental diets were significantly improved. The morphological study of the intestinal wall yielded evidence for some detrimental effects on structure of mucosa and tunica muscularis in 50% SMB substituted diets at basic level of AA supplementation. A specific effect of the protein source could not be concluded because lower AA balance in the diets was an overlapping factor. In addition, no histological health implications could be found when including partly defatted *Hermetia illucens* or algae meal of *Spirulina platensis* in meat type chicken diets at 50% substitution of SBM. Comparing the two alternative protein sources under study it can be summarized, that diets with *Hermetia* meal achieved superior growth response and feed efficiency at both levels of AA supplementation. Nonetheless, both partly defatted *Hermetia illucens* and algae meal of *Spirulina platensis* are promising alternative protein sources in chicken

diets when the dietary AA balance is well adapted to the IAAR through an enlarged range of supplemented feed AAs.

However, further research in gut morphometric analysis is needed in order to verify the observed effects under conditions of an extended level of AA supplementation.

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