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The Graded Inclusion of Algae (*Spirulina platensis*) or Insect (*Hermetia illucens*) Meal as a Soybean Meal Substitute in Meat Type Chicken Diets Impacts on Growth, Nutrient Deposition and Dietary Protein Quality Depending on the Extent of Amino Acid Supplementation

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Abstract

Three consecutive growth experiments were conducted to evaluate growth performance, whole body analyses and protein quality parameters from chicken diets with 50%, 75% and 100% substitution of soybean meal (SBM) by Spirulina meal (SM) or partly defatted Hermetia meal (HM). Each of the experiments was divided into a starter period (1 - 21 d) and a grower period (22 - 34 d). One-day-old male growing chickens (Ross 308) were randomly allotted to 48 floor pens making use of 6 birds/pen (Exp.1) or 7 birds/pen (Exp. 2, 3), and the experiments included a control diet (n = 12) and four experimental diets (n = 9). Experiment 1 examined a 50 % replacement of SBM by the alternative proteins under study, both on a basic and an advanced level of amino acid (AA) fortification to meet the recommended ideal amino acid ratio (IAAR). In experiment 2, 75% (starter diet) and 50% (grower diet) replacement of SBM was investigated. Experiment 3 investigated the effects of complete SBM substitution by SM or HM in starter and grower diets. In the second and third experiment diets with both of the alternative proteins and the control diet were AA supplemented to meet the current IAAR. In a further step, the calculated first limiting AA (LAA) was reduced to 80% of its requirement recommendation to allow for further evaluation of the individual AA efficiency according to the "Goettingen approach". Different levels (50%,

75%, or 100%) of replacing SBM by HM or SM in chicken diets depressed dietary protein quality ($p < 0.001$) and zoo-technical parameters ($p < 0.001$) with only a basic level of AA supplementation. This effect was much more pronounced in diets with SM. However, with an extended level of dietary AA supplementation zoo-technical parameters ($p < 0.001$), crude protein deposition ($p < 0.001$) and protein quality parameters ($p < 0.001$) were significantly improved. HM diets with an advanced level of AA tended to provide higher dietary protein quality and growth performance as compared to all SBM replacement levels through SM with different levels of AA supplementation and control diets.

Keywords

Growing Chickens, Amino Acids, N Utilization Model, Growth Performance, Body Analyses, Alternative Proteins, Feed Protein Quality

1. Introduction

Soybean meal (SBM) is currently the main protein and amino acid (AA) source to meet requirements in mixed diets for growing chickens. Yet, alternative proteins like insect or algae meals have moved into the focus of animal nutritionists, due to the limited acceptance of SBM imports from overseas in several European countries. Today, the blue-green alga *Spirulina platensis* and *Hermetia illucens* larvae meals are seen as adequate ingredients in poultry diets because of their high protein contents and additional supply of vitamins and minerals [1]-[7]. *Spirulina platensis* is a prokaryotic multicellular cyanobacterium. Spirulina algae belong to the photosynthetic organisms and grow only in warm climate and high light intensity. Natural environmental conditions are alkaline salt lakes as well as basic fresh waters. *Hermetia illucens* (black soldier fly) is a widespread fly and belongs to the family of *Stratiomyidae*, which is a member of the order *Diptera*. The larvae of Hermetia are able to utilize a wide range of nutrient sources and develop rapidly between 20˚C - 30˚C. Generally, both of the alternative proteins have the potential for SBM substitution. However, there are some notable limitations in the incorporation of Spirulina meal (SM). It has been demonstrated that elevated inclusion rates (20+%) of SM in chicken diets may yield decreased feed acceptance and growth depression, while 15% SM inclusion is well tolerated [8] [9]. The observed high variability in crude nutrients and AA composition of SM is one explanation for varying results [10]. On the other hand, numerous studies have reported the successful incorporation of insect meal in chicken diets. Oluokun [11] completely substituted full-fat soybeans by larvae meal from *Hermetia illucens* in broiler diets without negatively effecting growth performance. The feed conversion ratio (FCR) has even been significantly improved in 30-day-old male broilers through the complete replacement of SBM by non-defatted larvae of *Tenebrio molitor* [12]. Additionally, Hwangbo *et al.* [13]

reported higher growth performance and slaughter yield when SBM was replaced by 10% or 15% dried house fly larvae meal. At present, insect-based meals are not authorized for livestock and poultry feeds in Europe [14]. Nonetheless, the EU legislative barriers are expected to be overcome in the near future so that this promising protein source could be integrated into poultry diets, as was the case with aquafeed in 2017 [15]. As a part of the multidisciplinary project "Sustainability Transitions in the food chain" (Supported by the Lower Saxony Ministry of Science and Culture), the objective of this research focuses on replacing soybean meal (SBM) by partly defatted larvae meal from *Hermetia illucens* (HM) or blue-green micro algae *Spirulina platensis* (SM) in broiler diets. Both of the alternative protein sources have high protein contents and a balanced amino acid (AA) composition. The current experiments evaluate the potential of substituting 50%, 75% and 100% of SBM by either HM or SM in diets for meat type chickens during the entire fattening period (starter and grower periods). The effect of diet on zoo-technical parameters, whole body analyses and protein quality parameters were evaluated.

2. Materials and Methods

Three consecutive growth studies were conducted at the Division Animal Nutrition Physiology of Goettingen University and approved by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

2.1. Alternative Protein Sources

The alternative protein sources under study were derived from two different batches of SM (SM1, SM2) and one batch of HM. SM1 was a sun dried commercial Spirulina source obtained from Myanmar and declared to be free of GMO, irradiation, pesticides, colorants, preservatives and additives. As demonstrated by the nutrient composition (**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. SM2 as applied in experiment 3 was a spray dried product (dried for 4 - 6 seconds at an inlet temperature of 180˚C and outlet temperature of 75˚C) obtained from China and also GMO free, non-irradiated and free of pesticides. SM2 was cultivated in plastic-lined ponds filled with a water culture. After harvesting, the Spirulina was rinsed, filtered and spray-dried. The batch of HM was provided 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 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. Nutrient contents of the protein sources are summarized in **Table 1**.

Table 1. Analyzed nutrient composition of alternative protein sources under study.

Nutrient content	Spirulina meal (SM) (Exp. 1 + 2)		Spirulina meal (SM) (Exp. 3)		Hermetia meal (HM)	
Moisture (%)	3.4		8.0		5.5	
Crude protein (% of DM)	58.8		68.9		60.8	
Crude ash (% of DM)	6.1		9.1		7.5	
Crude lipids (% of DM)	4.3		6.3		14.1	
Crude fiber (% of DM)	0.49*		0.49*		10.92	
Amino acid (AA) content	**mgAA/ gDM**	**gAA/ 16gN**	**mgAA/ gDM**	**gAA/ 16gN**	**mgAA/ gDM**	**gAA/ 16gN**
Lys	22.97	3.91	31.64	4.59	32.97	5.42
Met	10.61	1.81	14.09	2.05	7.53	1.24
Cys	4.53	0.77	6.49	0.94	4.89	0.80
Thr	25.77	4.39	30.93	4.49	21.70	3.57
Arg	39.92	6.79	52.14	7.57	25.05	4.12
Val	34.50	5.87	37.09	5.39	32.58	5.36
Leu	47.23	8.04	55.05	7.99	37.95	6.24
Ile	29.81	5.07	34.70	5.04	23.47	3.86
His	7.51	1.28	10.39	1.51	16.58	2.73

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

2.2. Stock and Husbandry

Each of the experiments was divided into a starter period (1 - 21 d) and a grower period (22 - 34 d). One-day-old male growing chickens (Ross 308) were randomly allotted to 48 floor pens making use of 6 birds per pen (Exp.1) or 7 birds per pen (Exp. 2, 3). Average body weights (BW) per pen were similar at the start of each experiment. Under environmentally controlled conditions (temperature, monochromatic red light for 23 hours) birds were bedded on wood shavings and had unlimited access to feed and water. Experimental conditions were checked routinely, twice daily, paying special attention to feed and water supply, temperature and the state of birds' health. Growth data and feed consumption were recorded weekly.

2.3. Diets and Feeding

Each experiment included a control diet (n = 12) and four experimental diets (n = 9). Pelleted diets were manufactured at the facilities of the Division Animal Nutrition Physiology, Goettingen University, and fed at a free choice level. The control diets were based on wheat, corn and SBM as the main ingredients. Experiment 1 examined the 50 % replacement of SBM by the alternative proteins under study, both at a basic and an advanced level of AA fortification (Table 2). For the basic level (diets HM+ and SM+), Lys and Met supplementation was equal to the control diet. The advanced level of AA fortification (diets HM+AA

Table 2. Ingredient composition and analyzed nutrient content of starter and grower diets in experiment 1.

Diets	Starter diets 50% SBM replacement					Grower diets 50% SBM replacement				
	C	HM+	SM+	HM+AA	SM+AA	C	HM+	SM+	HM+AA	SM+AA
					Ingredients (g/kg as—fed)					
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	390.0	195.0	195.0	195.0	195.0	320.0	160.0	160.0	160.0	160.0
Hermetia meal	-	145.4	-	145.4	-	-	119.0	-	119.0	-
Spirulina 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*	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	3.0	3.0	3.0
L-Lysine·HCl	1.3	1.3	1.3	3.2	4.4	0.8	0.8	0.8	2.4	3.5
DL-Methionine	2.0	2.0	2.0	4.1	3.5	0.2	0.2	0.2	3.0	2.5
L-Threonine	-	-	-	0.6	-	-	-	-	0.4	-
L-Arginine	-	-	-	2.2	0.7	-	-	-	1.4	0.1
L-Valine	-	-	-	-	-	-	-	-	0.5	0.2
					Analyzed 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	117.4	131.4	118.4
AME_N (MJ/kgDM)**	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)****					
Lys	12.6	12.2	10.2	13.7	12.7	10.5	10.2	8.6	11.5	10.7
Met	4.9	5.0	5.1	7.0	6.6	4.6	4.7	4.8	5.6	5.3
Met+Cys	8.4	8.1	8.1	10.1	9.6	7.9	7.6	7.6	8.5	8.1
Thr	7.8	7.9	7.8	8.4	7.8	6.9	6.9	6.9	7.3	6.9
Arg	14.3	12.0	13.0	14.1	13.7	12.4	10.5	11.4	11.9	11.5
Val	9.3	10.4	9.9	10.4	9.8	8.3	9.2	8.7	9.7	8.9
Leu	16.1	15.7	15.9	15.6	15.8	14.5	14.1	14.3	14.1	14.2
Ile	8.8	8.6	8.8	8.6	8.8	7.8	7.6	7.7	7.6	7.7
His	5.4	5.7	4.3	5.7	4.3	4.8	5.1	3.9	5.1	3.9

C = control; HM+ = HM with basic AA supply; SM+ = SM with basic AA supply; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; *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; **N corrected apparent metabolizable energy, calculated according to WPSA [18]; ***derived from analyzed AA content of the ingredients.

and SM+AA) aimed to yield an improved dietary AA balance according to the currently assumed ideal AA ratio (IAAR) [16]; therefore, in addition to Lys and Met, further AAs (Thr, Arg, Val) were supplemented. In experiment 2, 75% (starter diet) and 50% (grower diet) replacement of SBM was investigated. Taking into account results from experiment 1, diets with either of the alternative proteins and the control diet were AA supplemented as summarized in **Table 3**. The control starter diet was completed with Lys, Met and Thr. The control grower diet also contained Val. The AA supplementation of HM and SM diets as well as the control diet (**Table 3**) aimed to achieve the IAAR [16]. In a second step, the calculated first limiting AA (LAA) was reduced to 80% of its recommended requirement to allow for further evaluations of the individual AA efficiency according to the "Goettingen approach" [17] and in order to verify its limiting position. In consequence, Met was limited for both the starter and grower HM diets. In SM diets, Lys was reduced as the calculated first LAA in the starter diet and Met was reduced in the grower diet. Experiment 3 investigated the effects of complete SBM substitution by SM or HM in starter and grower diets. Individual diet supplementation with AAs is summarized in **Table 4**. As compared with experiments 1 and 2, all diets were AA supplemented at the extended level to meet the IAAR [16]. According to the procedure in experiment 2, the concentration of the calculated first LAA of the experimental diets was reduced to 80% of its recommendation (HM: Met; SM: Lys) in order to verify the limiting position and to allow further evaluations of individual AA efficiency.

The analyzed nutrient compositions in **Table 2** demonstrate that in experiment 1 crude protein (CP) contents in dry matter (DM) tended to be lower in SM diets. Crude fiber contents ranged between 31.1 and 49.4 g/kg DM (starter diets) or 28.5 and 41.7 g/kg DM (grower diets). Crude ash (CA) contents were very similar in starter diets (58.1 to 65.6 g/kg DM) and grower diets (53.5 to 61.6 g/kg DM). Calculated AME_N data show that diets with alternative protein achieved a higher energy density, which is not related to the analyzed crude fat content. According to the elevated CP content in the alternative proteins, the larger amounts of wheat and corn in the diet composition increased the energy content of the final diets. This effect was not compensated by the reduction of soybean oil. At the basic level of AA supplementation, HM+ and SM+ diets contained lower levels of Lys, sulphur containing amino acid (SAA) and Arg as compared to the control diet. In addition, SM+ and SM+AA diets were lower in His content indicating that His supply could be a limiting factor for these diet compositions.

The analyzed nutrient compositions in **Table 3** demonstrate that in experiment 2 CP contents tended to be similar in the starter as well as in the grower diets. Crude fiber contents ranged between 31.4 g/kg DM to 49.6 g/kg DM (starter diets) or 38.1 g/kg and 57.7 g/kg DM (grower diets). CA contents were very similar in starter diets (55.1 g/kg DM to 63.0 g/kg DM) and in grower diets (54.1 g/kg DM to 61.1 g/kg DM). According to experiment 1, the calculated AME_N data demonstrates that diets with HM or SM have a higher energy density

Table 3. Ingredient composition and analyzed nutrient content of starter and grower diets in experiment 2.

Diets	Starter diets 75% SBM replacement					Grower diets 50% SBM replacement				
	C	HM+AA	SM+AA	HM-LAA	SM-LAA	C	HM+AA	SM+AA	HM-LAA	SM-LAA
					Ingredients (g/kg as—fed)					
Wheat	326.7	390.3	392.5	391.6	394.8	360.2	396.5	398.8	397.7	399.9
Corn	163.4	195.1	196.2	195.8	197.4	180.1	198.3	199.4	198.9	200.0
Soybean meal	390	97.5	97.5	97.5	97.5	330.0	165.0	165.0	165.0	165.0
Hermetia meal	-	217.1	-	217.1	-	-	122.5	-	122.5	-
Spirulina meal	-	-	221.0	-	221.0	-	-	124.7	-	124.7
Soybean oil	78.5	58	52	58	52	91.0	80.0	76.0	80.0	76.0
Premix*	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
DCP 40	11.0	8.0	11.0	8.0	11.0	10.0	8.0	9.0	8.0	9.0
$CaCO_3$	11.0	11.0	9.0	11.0	9.0	8.0	8.0	7.0	8.0	7.0
NaCl	3.0	1.0	0.8	1.0	0.8	2.5	1.5	1.0	1.5	1.0
Wheat starch	-	-	-	-	-	3.0	3.0	3.0	3.0	3.0
L-Lysine·HCl	2.5	4.2	5.8	4.2	2.3	1.8	2.8	3.6	2.8	3.6
DL-Methionine	3.6	4.2	3.5	2.1	3.5	2.6	2.9	2.5	1.2	0.8
L-Threonine	0.3	0.1	-	0.1	-	0.1	0.03	-	0.03	-
L-Arginine	-	3.5	0.2	3.5	0.2	-	1.5	-	1.5	-
L-Histidine	-	-	0.6	-	0.6	-	-	-	-	-
L-Valine	-	-	-	-	-	0.7	-	-	-	-
					Analyzed crude nutrients (g/kgDM)					
Crude protein	247.8	268.6	262.2	255.4	251.1	236.9	224.4	254.9	237.6	237.1
Ether extract	102.2	111.0	85.2	107.1	83.9	117.1	120.6	114.5	117.6	110.3
AME_N (MJ/kgDM)**	14.4	15.3	15.3	15.3	15.3	15.0	15.5	15.5	15.5	15.5
					*Amino acids (g/kg as—fed)****					
Lys	13.5	14.3	13.5	14.3	10.8	11.5	12.0	11.5	12.0	11.5
Met	6.5	7.1	7.1	5.1	7.1	5.3	5.6	5.6	3.9	3.9
Met+Cys	10.0	10.1	10.0	8.1	10.0	8.5	8.6	8.5	6.8	6.8
Thr	8.1	8.0	8.8	8.0	8.8	7.1	7.1	7.6	7.1	7.6
Arg	14.2	14.3	14.2	14.3	14.2	12.6	12.2	12.5	12.2	12.5
Val	9.3	11.0	11.4	11.0	11.5	9.1	9.4	9.6	9.4	9.6
Leu	16.1	15.6	17.5	15.6	17.6	14.6	14.3	15.4	14.3	15.4
Ile	8.8	8.6	9.9	8.6	9.9	7.9	7.7	8.5	7.7	8.5
His	5.4	5.9	4.6	5.9	4.6	4.9	5.2	4.1	5.2	4.1

C = control; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; HM-LAA = HM with 80% of limiting AA; SM-LAA = SM with 80% of limiting AA; *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; **N corrected apparent metabolizable energy, calculated according to WPSA [18]; ***derived from analyzed AA content of the ingredients.

due to the increased wheat and corn contributions to the ingredient composition, which increased the energy content of the final diets.

In Table 4 the analyzed nutrient compositions show that in experiment 3 CP contents tended to be very similar in the starter as well as in the grower diets. Crude fiber contents were between 18.2 g/kg DM to 52.3 g/kg DM (starter diets) and 15.4 g/kg DM to 39.1 g/kg DM (grower diets). The lowest crude fiber contents in starter and grower diets were found in the Spirulina diets. CA contents ranged between 53.6 g/kg to 63.4 g/kg DM (starter diets) and 50.9 to 61.9 g/kg DM (grower diets). Calculated AME_N data show that diets with HM or SM had a higher energy density because more wheat and corn contributed to the ingredient composition and therefore increased the energy content of the final diets. In the case of SM diets, the analyzed crude fat content was lowest when substituting out 100% of SBM; also the starch and carbohydrates in Spirulina lead to increased energy contents of the final diets.

2.4. Recorded Parameters

2.4.1. Feed Analysis

Analyses of feed ingredients and mixed diets were conducted according to the standards of VDLUFA [19]. Feed nitrogen analyses were carried out using the DUMAS-method (TruMac®, Leco Instrument GmbH, Moenchengladbach) and CP was calculated using the factor 6.25 on nitrogen content. Amino acids were analyzed by ion-exchange chromatography (Biochrom® 30, Biochrom Ltd. Cambridge, England) using acid hydrolysis with and without an oxidation step for the quantification of sulphur containing amino acids. Crude fat was analyzed following HCl-hydrolysis.

2.4.2. Performance Parameters

Feed intake (FI), BW and mortality were measured during the growth trial. Individual BW and pen feed intake were recorded at weekly intervals and feed conversion ratio (FCR; g dry matter intake/g gain of BW) was calculated from these data. Mortality was routinely checked twice daily.

2.4.3. Whole Body Analysis and Nutrient Utilization Parameters

For individual body composition analysis, 4 birds per treatment with a representative average BW were selected at the end of the trial, killed by CO_2-inhalation after 24 h feed deprivation, packed in air-tight plastic bags and frozen at −20˚C until further analysis. The carcasses were autoclaved (4 hours at 110˚C, pressure about 1 bar) and homogenized. A sample of approximately 500 g/bird was utilized for body nutrient analyses (DM, CA, and CP) according to the standards of VDLUFA [19]. The difference between the nutrient content at the end of the trial and of analyzed birds at the start (data from Pastor [20]) was applied to quantify nutrient deposition data. Energy deposition was calculated based on 23.7 kJ/g body protein and 39.8 kJ/g body fat [21].

Table 4. Ingredient composition and analyzed nutrient content of starter and grower diets in experiment 3.

Diets	Starter diets 100% SBM replacement					Grower diets 100% SBM replacement				
	C	HM+AA	SM+AA	HM-LAA	SM-LAA	C	HM+AA	SM+AA	HM-LAA	SM-LAA
					Ingredients (g/kg as—fed)					
Wheat	326.3	439.5	478.2	441.2	480.5	359.9	456.5	485.3	457.9	487.2
Corn	163.2	219.8	220.1	220.6	240.3	180.0	228.2	242.6	229.0	243.6
Soybean meal	390	-	-	-	-	330.0	-	-	-	-
Hermetia meal	-	250.0	-	250.0	-	-	210.0	-	210.0	-
Spirulina meal	-	-	230.0	-	230.0	-	-	200.0	-	200.0
Soybean oil	78.5	42.0	10.0	42.0	10.0	91.0	62.0	34.0	62.0	34.0
Premix*	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
DCP 40	11.0	8.0	12.0	8.0	12.0	10.0	7.0	11.0	7.0	11.0
$CaCO_3$	11.0	11.0	9.0	11.0	9.0	8.0	8.0	6.0	8.0	6.0
NaCl	3.0	1.0	1.0	1.0	1.0	2.5	0.5	0.5	0.5	0.5
Wheat starch	-	-	-	-	-	3.0	3.0	3.0	3.0	3.0
L-Lysine·HCl	2.5	6.1	6.2	6.0	2.7	1.8	4.8	4.7	4.8	1.8
DL-Methionine	2.2	2.3	1.0	1.2	1.0	1.7	1.8	0.6	0.8	0.6
L-Threonine	0.3	0.8	-	0.8	-	0.1	0.6	-	0.6	-
L-Arginine	-	5.5	-	5.4	-	-	4.2	-	4.2	-
L-Histidine	-	-	0.7	-	0.7	-	-	0.3	-	0.3
L-Leucine	-	0.8	-	0.7	-	-	-	-	-	-
L-Isoleucine	-	-	-	-	-	-	0.8	-	0.8	-
L-Valine	-	-	-	-	-	0.7	0.1	-	0.1	-
L-Cystein·HCl × H_2O	2.0	3.4	2.8	2.1	2.8	1.3	2.6	2.0	1.4	2.0
					Analyzed crude nutrients (g/kgDM)					
Crude protein	246.4	259.9	240.3	253.8	261.2	218.6	230.8	222.8	218.9	229.3
Ether extract	104.2	103.0	46.8	105.8	47.5	116.9	104.3	66.3	114.0	65.1
AME_N (MJ/kgDM)**	14.4	15.4	14.9	15.4	14.9	15.0	15.9	15.4	15.9	15.4
					*Amino acids (g/kg as—fed)****					
Lys	13.5	14.4	13.5	14.4	10.8	11.5	12.3	11.5	12.3	9.2
Met	5.1	5.1	5.1	4.0	5.1	4.4	4.3	4.4	3.4	4.4
Met+Cys	10.0	10.1	10.0	8.1	10.0	8.5	8.6	8.5	6.9	8.5
Thr	8.1	8.0	8.7	8.0	8.8	7.1	7.1	7.9	7.1	7.9
Arg	14.2	14.3	14.2	14.3	14.2	12.6	12.2	12.8	12.2	12.9
Val	9.3	10.5	10.8	10.5	10.8	9.1	9.4	9.8	9.4	9.8
Leu	16.1	15.0	17.2	15.0	17.3	14.6	13.0	15.8	13.0	15.8
Ile	8.8	7.7	9.6	7.7	9.6	7.9	7.7	8.7	7.7	8.7
His	5.4	5.5	4.6	5.5	4.6	4.9	5.0	3.9	5.0	3.9

C = control; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; HM-LAA = HM with 80% of limiting AA; SM-LAA = SM with 80% of limiting AA; *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; **N corrected apparent metabolizable energy, calculated according to WPSA [18]; ***derived from analyzed AA content of the ingredients.

2.4.4. Protein Evaluation Using the "Goettingen Approach"

Protein deposition data were utilized for the evaluation of dietary protein quality parameters based on an exponential N-utilization model [17] [22] [23] [24] [25] [26]. Equations ((1) and (2)) summarize the essential aspects of the procedure:

$$NR = NR_{\max}T\left(1-\mathrm{e}^{-b*NI}\right) \tag{1}$$

$$ND = NR_{\max}T\left(1-\mathrm{e}^{-b*NI}\right)-NMR \tag{2}$$

whereby

NR = daily N retention (ND + NMR) [mg/$BW_{kg}^{0.67}$]

ND = daily N deposition or N balance [mg/$BW_{kg}^{0.67}$]

NMR = daily N maintenance requirement [mg/$BW_{kg}^{0.67}$]

$NR_{max}T$ = theoretical maximum for daily N retention [mg/$BW_{kg}^{0.67}$]

b = model parameter for the slope of the function between NI and NR, depending on the dietary protein quality

NI = daily N intake [mg/$BW_{kg}^{0.67}$]

e = basic number of natural logarithm [ln]

Both the model parameter NMR (240 mg/$BW_{kg}^{0.67}$) and $NR_{max}T$ (3840 mg/$BW_{kg}^{0.67}$) are derived from experiments with fast growing chickens of equal genotype [27]. For evaluation of the whole growth period, an averaged $NR_{max}T$ (3840 mg/$BW_{kg}^{0.67}$) was applied. Following the logarithmic transformation of Equation (1), model parameter "b" provides a NI independent measure of dietary protein quality according to Equation (3):

$$b = \frac{\left[\ln NR_{\max}T - \ln\left(NR_{\max}T - NR\right)\right]}{NI} \tag{3}$$

Additionally, the net protein utilization (NPU) was calculated, for a complex dietary protein quality evaluation, by taking into account both digestion and post-absorptive utilization:

$$NPU\left(\%\right) = \frac{NR}{NI}*100 \tag{4}$$

However, traditional protein quality measures are not independent of the level of realized protein intake [28] [29] [30]. Consequently, a standardization of protein intake was conducted by the "Goettingen approach", as according to earlier studies [17] [29] [30] [31] [32], providing NPU data which are independent of NI. According to Equation (5), standardized net protein utilization data (NPU_{std}) were calculated from equal daily nitrogen intake NI_{std} (3000 mg/$BW_{kg}^{0.67}$):

$$NPU_{std}\left(\%\right) = NR_{\max}T\frac{\left(1-\mathrm{e}^{-b*3000}\right)}{3000}*100 \tag{5}$$

The parameter "b" as derived from the exponential model (Equation (3)) is a prerequisite for this standardization procedure.

2.5. Statistical Analysis

Statistical analyses were conducted with SPSS software package (IBM SPSS Sta-

tistics, Version 24.0) and results are presented as means ± standard deviation. One-way analysis of variance (ANOVA) tests were performed to compare means of the primary N balance data. To verify the variance homogeneity and identification of significant differences ($p < 0.05$) the Games-Howell and Tuckey tests were applied.

3. Results

According to the main components of the investigations, the results are summarized in three sections.

3.1. Body Nutrient Composition

Body nutrient compositions of birds at the end of the growth trial are shown for each experiment in Table 5.

In experiment 1, a significant effect on CP content in DM was only observed between the control and SM+AA diet. Birds fed with the control diet yielded the highest CP content, but not significantly different to the SM+, HM+ and HM+AA diets. The lowest CP content was observed with SM+AA diet. The same ranking was found in reverse order for the ether extract (EE) contents in DM, indicating the highest EE content in birds from the SM+AA diet group significantly differing from control diet birds. CA content in DM was lowest for the SM+AA diet birds, but only significantly different from the HM+ and SM+ diets birds.

In experiment 2, the highest CP content and the corresponding lowest EE content in body DM was also found in control diet birds, but the values did not significantly differ from birds fed the SM+AA diet. HM-LAA diet yielded the lowest CP content in the whole body analysis, but did not differ significantly in body CP contents of birds fed SM-LAA and HM+AA diets. Numerical differences were found between the SM+AA, HM+AA and SM-LAA diets, respectively. Both of the HM diets and the SM-LAA diet achieved superior EE contents in body DM. The CA body content was not significantly different between diets.

In experiment 3, corresponding with observations in experiment 1 and 2, the highest CP content in body DM was found for control diet birds, differs significantly from all birds except those fed the SM+AA diet. Generally, the CP content of body DM did not significantly differ between the HM and SM diets. The body EE content was lowest in birds fed the control diet, as was also observed in experiments 1 and 2. Birds fed the HM and SM diets did not differ significantly in body EE contents of DM. The SM-LAA diet birds yielded the highest CA content and only differed significantly from HM+AA birds; while all the other groups did not differ significantly from one another nor from SM-LAA and HM+AA fed birds.

3.2. Zoo-Technical Data and Nutrient Deposition

Results of the growth study are summarized in Table 6. Within each of the ex-

periments, the initial BW was very similar between diets. Enabling the conclusion to be drawn that the diets had an effect on the final BW as these data significantly differed.

In experiment 1, the AA balanced diet HM+AA yielded a superior final BW that was significantly higher than birds fed the control diet; however SM+AA birds were similar to that of the control group. The basic level of AA supplementation (SM+ and HM+ diets) depressed growth significantly. The SM diet

Table 5. Average body nutrition composition of birds at the end of the 5 weeks growth trials dependent on the diet under study.

			Nutrient content		
		(%)	(g/kgDM)		
		DM	**CP**	**EE**	**CA**
Day old chicken		22.54 ± 0.18	687.9 ± 12.1	226.9 ± 11.3	85.2 ± 1.1
Experiment	**Diets**	n = 4	n = 4	n = 4	n = 4
1	Control	29.91^{a} ± 1.71	570.2^{b} ± 21.0	354.0^{a} ± 24.2	75.8^{ab}± 4.4
1	HM+	31.14^{ab} ± 0.76	522.1^{ab} ± 24.1	395.1^{ab} ± 26.3	82.8^{b}± 2.7
1	SM+	29.90^{a} ± 0.73	536.3^{ab} ± 20.2	379.1^{ab} ± 22.2	84.5^{b}± 2.4
1	HM+AA	31.69^{ab} ± 1.36	531.4^{ab} ± 27.7	394.8^{ab} ± 34.8	73.8^{ab}± 7.4
1	SM+AA	32.92^{b} ± 1.13	493.7^{a} ± 19.5	433.1^{b} ± 20.9	73.2^{a}± 2.8
SEM		0.354	7.229	7.866	1.386
p		**0.013**	**0.005**	**0.010**	**0.005**
2	Control	29.66^{a} ± 0.55	592.3^{c} ± 26.9	329.6^{a} ± 29.8	78.1± 3.0
2	HM+AA	31.99^{ab} ± 0.69	535.0^{ab} ± 20.4	392.7^{bc} ± 19.9	72.4± 3.3
2	SM+AA	30.54^{ab} ± 1.53	557.5^{bc} ± 14.6	365.3^{ab} ± 17.8	77.2± 6.4
2	HM-LAA	32.99^{b} ± 0.70	511.1^{a} ± 16.7	417.1^{c} ± 21.1	71.8± 4.9
2	SM-LAA	31.48^{ab} ± 1.69	532.7^{ab} ± 18.0	398.2^{b} ± 20.4	69.1± 3.1
SEM		0.348	7.438	8.251	1.166
p		**0.008**	**<0.001**	**0.001**	**0.050**
3	Control	30.47^{a} ± 0.28	603.6^{b} ± 19.1	321.0^{a} ± 22.5	75.5^{ab}± 4.6
3	HM+AA	33.01^{ab} ± 1.07	535.2^{a} ± 13.4	396.7^{b} ± 16.7	68.2^{a}± 3.3
3	SM+AA	31.54^{ab} ± 1.91	563.3^{ab} ± 48.7	365.8^{ab} ± 53.7	70.9^{ab}± 6.1
3	HM-LAA	33.33^{b} ± 0.61	507.8^{a} ± 9.8	423.0^{b} ± 11.7	69.2^{ab}± 2.2
3	SM-LAA	31.45^{ab} ± 1.54	543.4^{a} ± 22.7	378.8^{ab} ± 26.2	77.8^{b}± 3.6
SEM		0.345	9.020	9.805	1.189
p		**0.028**	**0.002**	**0.003**	**0.022**

DM = dry matter; CP = crude protein, EE = ether extract; CA = crude ash; C = control; HM+ = HM with basic AA supply; SM+ = SM with basic AA supply; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; HM-LAA = HM with 80% of limiting AA; SM-LAA = SM with 80% of limiting AA; SEM = standard error of the mean; p = p-value; $^{a\text{-}c}$ means with different superscript letters within columns of individual experiments are significantly different ($p < 0.05$).

Table 6. Zoo-technical and corresponding nutrient deposition data dependent on the diet under study.

	Experiment 1: (1 - 34 d) 50% SBM replacement						Experiment 2: (1 - 35 d) 75%/50% SBM replacement						Experiment 3: (1 - 34 d) 100% SBM replacement					
Diets	**C**	**HM+**	**SM+**	**HM +AA**	**SM +AA**	SEM *p*	**C**	**HM +AA**	**SM +AA**	**HM -LAA**	**SM -LAA**	SEM *p*	**C**	**HM +AA**	**SM +AA**	**HM -LAA**	**SM -LAA**	SEM *p*
n	**12**	**9**	**8***	**9**	**9**		**12**	**9**	**9**	**9**	**9**		**12**	**9**	**9**	**9**	**9**	
Growth performance data																		
$BW_{Initial}$ (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**	51.6 ± 0.3	51.9 ± 0.2	51.7 ± 0.4	51.7 ± 0.1	51.8 ± 0.1	0.035 **0.171**	45.5 ± 0.1	45.5 ± 0.1	45.5 ± 0.1	45.5 ± 0.1	45.5 ± 0.1	0.015 **0.745**
BW_{Final} (g)	2174^{c} ± 112	1494^{b} ± 89	1063^{a} ± 65	2320^{d} ± 114	2122^{c} ± 122	69.568 **<0.001**	2368^{bc} ± 153	2530^{c} ± 100	2327^{b} ± 75	2317^{b} ± 93	1780^{a} ± 165	39.975 **<0.001**	2177^{b} ± 104	2397^{c} ± 103	2053^{b} ± 102	2320^{c} ± 72	1548^{a} ± 128	44.785 **<0.001**
DM intake (g/d)	87.3^{c} ± 4.7	75.4^{b} ± 6.2	57.8^{a} ± 1.9	87.1^{c} ± 5.6	86.0^{c} ± 5.8	1.750 **<0.001**	93.1^{b} ± 5.9	93.7^{b} ± 3.4	87.3^{b} ± 3.9	89.0^{b} ± 4.9	75.6^{a} ± 6.3	1.174 **<0.001**	93.8^{c} ± 3.7	88.7^{b} ± 3.6	86.2^{b} ± 4.0	86.8^{b} ± 2.3	69.9^{a} ± 6.3	1.308 **<0.001**
FCR (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**	1.37^{b} ± 0.06	1.29^{a} ± 0.03	1.30^{ab} ± 0.05	1.33^{b} ± 0.02	1.49^{c} ± 0.05	0.013 **<0.001**	1.45^{b} ± 0.06	1.25^{a} ± 0.03	1.42^{b} ± 0.03	1.26^{a} ± 0.02	1.54^{c} ± 0.05	0.017 **<0.001**
Nutrient deposition																		
CP deposition (g/d)	11.05^{d} ± 0.58	7.16^{b} ± 0.44	4.95^{a} ± 0.31	11.64^{d} ± 0.58	10.23^{c} ± 0.60	0.371 **<0.001**	12.02^{bc} ± 0.79	12.52^{c} ± 0.50	11.44^{b} ± 0.38	11.28^{b} ± 0.46	8.56^{a} ± 0.81	0.215 **<0.001**	11.94^{cd} ± 0.58	12.64^{d} ± 0.55	10.86^{b} ± 0.55	11.70^{c} ± 0.37	7.82^{a} ± 0.66	0.252 **<0.001**
EE deposition (g/d)	6.92^{c} ± 0.36	5.51^{b} ± 0.33	3.58^{a} ± 0.22	8.73^{d} ± 0.43	9.08^{d} ± 0.53	0.293 **<0.001**	6.74^{a} ± 0.44	9.28^{c} ± 0.37	7.57^{b} ± 0.25	9.31^{c} ± 0.38	6.49^{a} ± 0.61	0.185 **<0.001**	6.39^{b} ± 0.31	9.45^{d} ± 0.41	7.11^{c} ± 0.36	9.85^{d} ± 0.31	5.52^{a} ± 0.46	0.249 **<0.001**

C = control; HM+ = HM with basic AA supply; SM+ = SM with basic AA supply; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; HM-LAA = HM with 80% of limiting AA; SM-LAA = SM with 80% of limiting AA; BW = body weight; DM = dry matter; FCR = feed conversion ratio; CP = crude protein; EE = ether extract; *one box excluded, outlier in feed conversion ratio, detected with SPSS boxplot-test ($p \leq 0.05$); SEM = standard error of the mean; p = p-value; [a-d] means with different superscript letters within the same row for each experiment separately are significantly different ($p < 0.05$).

supported the lowest growth rate, corresponding with a low DM intake (DMI) and an impaired FCR. The extended AA supplementation in the HM+AA and SM+AA diets appeared to compensate for AA imbalances and supporting FCR data are similar or superior (HM+AA) to the control. Accordingly, the observed crude protein deposition (CPD) was highest with the HM+AA diet, but not significantly different from the control. However, fat deposition (EED) was significantly elevated in birds fed the AA fortified diets.

In experiment 2, the diets with extended AA supplementation (HM+AA and SM+AA) led to final BW of birds that were not significantly different from the control group, but did differ from each other, with the insect meal diet showing superior results. DMI and FCR were not significantly different between the alternative protein source groups when supplemented at an extended level. The most efficient FCR was observed with the HM+AA diet and was significantly improved to that of the control. When the expected LAA was reduced, the SM-LAA diet resulted in the highest FCR and was significantly different from all other diets; whereas the HM-LAA diet resulted in a significant difference from the HM+AA diet, in addition to differing from the SM-LAA, but did not differ from the control. The reaction of CPD data and final BW was very similar across diets. HM diets produced the highest EED, which was significantly higher than

the control and SM diets.

In experiment 3, zoo-technical data responded very similarly to experiment 2. However, DMI of birds with a 100% substitution rate of SBM were significantly lower, even when the extended AA supplementation was applied. FCR data between +AA diets with either of the protein sources were significantly different, where the 100% substitution of SBM by SM yielded lower feed efficiency compared to the substitution by HM. Nonetheless, FCR for the SM+AA diet was similar to that of the control group. Compared to the HM+AA diet, the HM-LAA diet yielded no significant response for zoo-technical data, but CPD significantly declined ($p < 0.001$) while EED remained unaffected at the highest observed level. Fat deposition was significantly enhanced with the utilization of HM in the diets, compared to the other diets. However, reducing the supply of LAA in the SM-LAA diet impaired zoo-technical data significantly compared to all other diets. Accordingly, CPD and EED were also significantly lowest across the treatments.

3.3. Protein Quality Parameter (NPU_{std})

Assessment of dietary protein quality results in Table 7 are focused on the model parameter "b" and the derived NPU_{std}, yielding the most complex measure of the achieved effect on feed protein quality

Throughout all three experiments, the protein quality of the control diets remained very similar and no significant effect between experiments 1 - 3 was observed.

In experiment 1, the basic level of AA supplementation, which was equal to the control diet, significantly declined the feed protein quality with either of the alternative protein sources. In contrast, the extended level of AA supplementation in diet HM+AA yielded superior NPU_{std}; it was not significantly higher than the control diet results. However, with the SM+AA diet protein quality was significantly lowered.

In experiment 2, no significant difference was found amongst the control diet, HM+AA diet and SM+AA diet. However, diets with a lower supply of the expected LAA yielded significantly lower protein quality, confirming that the expected LAA was indeed the actual LAA. This information is of special importance for further calculations with the "Goettingen approach".

In experiment 3, the superior protein quality ($p < 0.001$) was found with the HM+AA diet and the SM+AA diet yielded lower protein quality ($p < 0.001$), even compared to the control group, despite the extended AA supplementation. Both diets with a reduced supply of the expected LAA resulted in lower protein quality ($p < 0.001$), indicating the limiting position as expected.

4. Discussion

Based on zoo-technical data collected in these experiments, we could demonstrate that an extended AA supplementation according to the IAAR [16], both

Table 7. Protein quality parameter.

Experiment	Diets	n	Protein quality parameter	
			Model parameter b ($\times 10^6$)**	NPU_{std} (%)***
Exp. 1 50% SBM replacement	C	12	$225^{c} \pm 7$	$62.9^{c} \pm 1.3$
	HM+	9	$162^{a} \pm 15$	$49.1^{a} \pm 3.7$
	SM+	8*	$151^{a} \pm 9$	$46.7^{a} \pm 2.1$
	HM+AA	9	$228^{c} \pm 7$	$63.4^{c} \pm 1.4$
	SM+AA	9	$215^{b} \pm 5$	$60.9^{b} \pm 0.9$
SEM			4.930	1.078
p			**<0.001**	**<0.001**
Exp. 2 75% / 50% SBM replacement	C	12	$230^{c} \pm 11$	$63.7^{c} \pm 2.0$
	HM+AA	9	$225^{c} \pm 5$	$62.9^{c} \pm 1.0$
	SM+AA	9	$223^{c} \pm 8$	$62.3^{c} \pm 1.6$
	HM-LAA	9	$213^{b} \pm 3$	$60.4^{b} \pm 0.7$
	SM-LAA	9	$191^{a} \pm 14$	$55.7^{a} \pm 3.0$
SEM			2.374	0.488
p			**<0.001**	**<0.001**
Exp. 3 100% SBM replacement	C	12	$230^{c} \pm 10$	$63.7^{c} \pm 1.9$
	HM+AA	9	$249^{d} \pm 8$	$67.3^{d} \pm 1.4$
	SM+AA	9	$215^{b} \pm 5$	$60.9^{b} \pm 1.0$
	HM-LAA	9	$233^{c} \pm 4$	$64.3^{c} \pm 0.8$
	SM-LAA	9	$189^{a} \pm 7$	$55.3^{a} \pm 1.4$
SEM			3.023	0.604
p			**<0.001**	**<0.001**

C = control; HM+ = HM with basic AA supply; SM+ = SM with basic AA supply; HM+AA = HM with extended AA supply, SM+AA = SM with extended AA supply; HM-LAA = HM with 80% of limiting AA; SM-LAA = SM with 80% of limiting AA; *one box excluded, outlier in feed conversion ratio, detected with SPSS boxplot-test ($p \leq 0.05$); **applied for NPU standardization based on: NMR = 240 mg/$BW_{kg}^{0.67}$/d and $NR_{max}T$ = 3840 mg/$BW_{kg}^{0.67}$/d; ***standardized N intake = 3000 mg/$BW_{kg}^{0.67}$/d; SEM = standard error of the mean; p = p-value; $^{a-d}$ means with different superscript letters within columns of individual experiments are significantly different ($p < 0.05$).

for the HM and SM diets, led to superior growth responses and feed conversion. In the case of HM based diets, the response also exceeded the control level ($p < 0.001$ in experiment 1 and 3). Accordingly, Oluokun [11] examined diets with HM as related to full-fat soybean meal diets and observed higher growth rates for HM meal diets. Although the diets were not exact to the diets used in this study, Elwert *et al.* [33] also reported similar growth responses in chickens (1 - 10 d) for their SBM control group and their experimental diet, which included a lower inclusion of HM (4.7%) and only the supplementation of Lys and Met. Although these superior responses were monitored at the extended AA supplementation level, the basic level of AA supplementation in experiment 1, which

was equal to the control diet, proved insufficient to produce acceptable growth performance and feed efficiency responses in meat-type growing chicken. This effect was obvious with either of the alternative proteins, but was much more pronounced in diets with SM, generally indicating that a higher level of AA supplementation is required. Nevertheless, Spirulina diets with extended AA supplementation yielded growth data similar to that of the control diet. Evans *et al.* [8] also observed no significant effects on BW (21 d) with microalgae meal integrated at a rate between 6% and 16% in chicken diets. Venkataraman *et al.* [34] yielded similar conclusions with a 14% and 17% *Spirulina platensis* inclusion rate in their diets, but they found that BW was significantly depressed with 21% Spirulina in the diet. We did not observe such a dose-effect with higher inclusion rates. In experiment 3, 23.0% (starter) and 20.0% (grower) Spirulina powder in the diets yielded no depression of final BW when AA supplementation balanced the AA supply. In direct correspondence to growth response the feed acceptance could be a factor of importance [8], but only in experiment 3 with 23.0% (starter) and 20.0% (grower) Spirulina powder in the diets did feed intake decline ($p < 0.001$) as compared to the control diet. This effect was very weak and only numerical in experiments 1 and 2. Regarding the feed conversion ratio no significant effect between the control and SM+AA diets was found in experiments 1-3. Accordingly, Venkataraman *et al.* [34] observed no decline of feed efficiency with SM at 14% and 17% inclusion rates. The application of HM in diets with an extended AA supplementation improved ($p < 0.001$) FCR in each of the experiments reported. However, it should be noted that an extended AA supplementation to balance the observed AA deficiencies according to the current IAAR [16] is a significant precondition. Feeding alternative protein sources without an adequate AA supplementation is also the main premise found in the N balance studies conducted by Neumann *et al.* [32] with broiler chickens and complete substitution of SBM by HM or SM as well as the study by Austic *et al.* [10].

Regarding the final body composition, in each of the experiments the highest CP content (57 to 60 percent of DM) was observed in birds fed the control diet. Accordingly, the body fat content was lowest in control birds and ranged between 32 and 35 percent of DM. This is likely because the control diets in both of the age periods (starter and grower) were lowest in AME_N content. Experimental diets with either of the alternative protein sources yielded a shift in dietary crude fat content. This effect was most pronounced in diets with HM due to the high fat content of the partly defatted insect meal. However, it was not the aim of the experimental design to compensate for this obvious effect on energy concentration in the diets by adapting plant oil content in the final mixtures. Consequently, the observed significant responses on body composition data were not surprising. Therefore it is more interesting to focus further discussion on the nutrient deposition data, which take into account that varying body composition is an important factor influencing zoo-technical data. From this point of view, the superior CPD in birds fed HM+AA diets needs to be highlighted. This effect was

demonstrated in each of the experiments, but as compared to the control diet the observed advantage was only numerical. Except in experiment 2, birds fed the SM+AA diets produced lower CPD ($p < 0.001$) than birds of the control group. Diets with HM and SM with a basic level of AA supplementation in experiment 1 achieved only very low daily CPD, more than 50 percent below the control, indicating that the basic supply of feed AAs was insufficient as already demonstrated by the zoo-technical results. In addition, as expected according to the final feed mixtures, fat deposition was highest in birds with HM+AA diets according to the increased energy content in these diets. However, the diet construction cannot be identified as an influencing factor for the level of daily CPD. In consequence, the slight energetic oversupply in HM and SM diets ensured that no energetic limitation occurred for dietary protein utilization.

Looking more closely at the observed dietary protein quality, the standardized net protein utilization (NPU_{std}) indicates that the control diet yielded very similar results ($p > 0.05$) between the three experiments and in consequence the control diet is a reference diet across experiments. As reported earlier, the protein quality parameter NPU_{std} is a measure of protein quality independent of individual variation of N intake. It is clearly demonstrated in our study that diets with an alternative protein and extended AA supplementation level yield superior protein quality. This observation is also supported by a current report by Neumann *et al.* [32], who assessed the dietary protein quality based on N balance studies in growing chickens. Generally, an extended AA supplementation is required in chicken diets containing either of the alternative protein sources under study at a high level of SBM substitution. It is also obvious from the NPU_{std} data that improvements in balancing of the AA supply in SM diets needs further attention, given that the observed protein quality was generally below that with the HM diets. This observation could also be attributed to a lower protein digestibility of algae meal. However, microalgae do not contain cellulose in the cell wall, but a thin, unstable shell of murein (peptidoglycans) which does not act as a barrier for proteolytic enzymes during digestion [35]. The observed *in vitro* protein digestibility ranged between 70% and 85% [36] when pepsin and subsequently pancreatin incubation was applied. However, fresh Spirulina has been shown to be more digestible as compared to sun-dried or freeze-dried meal [36]. To date there is no relevant study currently available dealing with the digestibility parameters of algal biomass in chicken diets. Carbohydrates and fibers in the Spirulina biomass could affect the digestibility and also create gastro-intestinal disturbances, flatulence or fluid retention [37]. It would be important to know whether the cell walls are digestible themselves or only fragile enough to make the cell content readily accessible to digestive enzymes. Schiavone *et al.* [38] measured the apparent total tract digestibility both of partly defatted HM and highly defatted HM. The later achieved significantly lower digestibility coefficients of ether extract (0.98 vs. 0.93); however no significant effect was observed for the digestibility coefficient of crude protein (0.62 vs. 0.62) and

organic matter (0.69 vs. 0.64) of partly defatted and highly defatted HM, respectively. These reported low protein digestibility values suggest conflicting evidence of the actual protein digestibility of HM when taking into account our observations of complex protein quality with a high inclusion rate of insect meal and extended AA supplementation. We conclude that digestibility parameters as derived by differences in technique and chemical separation of fecal N should not be over-interpreted. In consequence, more research is needed to overcome the inconsistencies in observations.

Experiments 2 and 3 were also designed, to provide preliminary information about individual AA efficiency in diets with a high substitution rate of SBM by either of the alternative proteins under study. Therefore, the supply of the potential LAA was reduced to measure protein deposition under circumstances of its validated limiting position. Following reduced LAA supply, zoo-technical parameters responded significantly (**Table 6**) and dietary protein quality (**Table 7**) as well. Reducing Lys supply in Spirulina diets to 80% of its recommendation yielded clear responses, also indicating the importance of balancing this AA in Spirulina based diets. Effects were less pronounced following the reduction of Met supply in Hermetia diets, but were still significant as compared to the +AA diet. In consequence, the dietary AA efficiency of the individual LAA can be directly derived and utilized for further applications of the "Goettingen approach" as discussed elsewhere [17] [22] [23] [24] [25] [26]. Further investigations are needed to exploit the complete potential of AA supplementations in order to achieve an optimal AA balance in diets with a high substitution level of SBM by either of the protein sources under study. In addition, based on observed AA efficiency data more focus should be on improving the AA efficiency in such alternative diets. In this context we also have to point out, that the influence of different batches of the alternative proteins under study is an important additional factor which needs to be investigated in more detail before final conclusions about optimal AA supplementation in chicken diets with high inclusion rate of these proteins are validated.

5. Conclusion

At only a basic level of AA supplementation, graded substitution (50%, 75% or 100%) of SBM by partly defatted larvae meal of *Hermetia illucens* or algae meal of *Spirulina platensis* depressed the protein quality of diets for growing chickens and zoo-technical parameters as well. This effect was much more pronounced in diets with algae meal. However, an extended level of AA supplementation according to the IAAR yielded significantly improved growth responses, protein deposition and dietary protein quality, but still generally not on par with the control diet. Comparing the two alternative protein sources, diets with the insect meal provided superior results. Insect meal based diets with an advanced level of AA supplementation yielded superior responses on growth, feed conversion ratio, protein deposition and protein quality, even significantly at a 100% substitu-

tion level of SBM. The quality of the achieved dietary AA balance was a more important factor for the observed protein quality than the substitution level. In consequence, both partly defatted larvae meal of *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 recommendations through an enlarged range of supplemented feed AAs.

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