Scientific Research Publishing

Open Journal of Animal Sciences, 2018, 8, 12-26
http://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Improving the Dietary Protein Quality by Amino Acid Fortification with a High Inclusion Level of Micro Algae (*Spirulina platensis*) or Insect Meal (*Hermetia illucens*) in Meat Type Chicken Diets

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How to cite this paper: Neumann, C., Velten, S. and Liebert, F. (2018) Improving the Dietary Protein Quality by Amino Acid Fortification with a High Inclusion Level of Micro Algae (*Spirulina platensis*) or Insect Meal (*Hermetia illucens*) in Meat Type Chicken Diets. *Open Journal of Animal Sciences*, **8**, 12-26.
https://doi.org/10.4236/ojas.2018.81002

Received: October 1, 2017
Accepted: December 1, 2017
Published: December 4, 2017

Abstract

The objective of this study was to measure protein quality parameters (PPV, NPU and NPU_{std}) of chicken diets with complete substitution of soybean meal (SBM) by Spirulina meal (SM) or partly defatted Hermetia meal (HM). N balance experiments were based on the quantitative excreta collection method, divided into starter period (10 - 20 d) and grower period (25 - 35 d). The study utilized 70 all male meat type chicken (Ross 308). Data assessment applied the exponential N utilization model of the "Goettingen approach". The control diet was based on wheat, corn and SBM. In four experimental diets SBM was completely substituted by SM or HM, but fortified with feed amino acids (AA) both on a basic level of supplementation (Lys and Met added equal to the control diet) and on an extended level (Lys, Met, Thr, Arg, Val, Ile, His added). At a basic level of AA supplementation, complete replacement of SBM by SM or HM in chicken diets depressed dietary protein quality significantly ($p < 0.05$). However, the extended level of AA supplementation improved protein quality parameters of the diets with both of the alternative proteins significantly ($p < 0.05$), but still generally not on par with the control diet. The observed responses were accentuated when the well-known effect of N intake on protein utilization was eliminated through the standardization of N intake by application of the "Goettingen approach".

Keywords

N Balance, Growing Chickens, N Utilization Model, Amino Acids, Protein Quality, *Spirulina platensis*, *Hermetia illucens*

1. Introduction

Traditionally, soybean meal (SBM) is the major ingredient to meet protein and amino acid (AA) requirements in mixed diets for growing chickens. In addition, soybeans are also a basic component of human nutrition and therefore in competition with uses for raising livestock and poultry. To keep up with increasing food demand, derived from an increasing global population and consumption of meat [1], soybean production is expected to increase from today's 217 Mt to 390 Mt in 2050 [2]. However, increasing the amount of arable land used for cultivation is an unfeasible strategy due to the growing density of industrial and private building development as well as the associated negative consequences of accelerated soil erosion and desertification. Therefore, the search for alternative sources to close the protein gap in animal feeding is essential. The major challenge facing the poultry industry is the supply of feeds that will contain all the necessary dietary components for birds to grow efficiently.

Today, both the blue-green alga *Spirulina platensis* and the *Hermetia illucens* fly larvae are qualified as adequate sources of nutrients in poultry diets, due to their high protein contents as well as vitamin and mineral supplies [3]-[9]. Spirulina meal (SM) has been introduced as feed ingredient in poultry diets more than 20 years ago [10] [11]. It has already been demonstrated that *Spirulina platensis* can successfully be included in broiler diets [8] [9]. However, an inclusion rate higher than 20% of the overall diet can result in depressed growth rates, but up to 15% inclusion is well tolerated in chicken diets [11] [12]. Austic *et al.* [13] reported when SBM was replaced with the microalga *Staurosira* sp., 7.5% of overall diet, with a basic AA supplementation, decreased growth during the first three weeks of age and feed efficiency during 0 - 6 weeks of age could be observed. However, when diets were adequately supplemented with an extended level of indispensable AAs (Met, Lys, Arg, Ile, Thr, Trp, Val) the performance data were similar to those of the control group.

Insects have also been tested as a potential alternative to SBM and are becoming a very popular topic in poultry nutrition. Insects at different developmental stages are naturally ingested by wild birds and free-range poultry species [14]. Consequently, birds are evolutionary well adapted to insects as a part of their nutritional intake [15]. To date, the most widely studied insect meal, as an alternative protein source, is housefly larvae meal. Several studies have demonstrated that housefly larvae meal may be able to completely or partially replace groundnut meal [16], fishmeal [17] [18], or SBM [14] [19]. However, Hermetia meal (HM) qualifies as an interesting option because of its beneficial AA profile [20] [21]; the proportions of lysine and methionine are superior to SBM [21]. De Marco *et al.* [22] identified HM as an excellent source of digestible AAs for growing chickens, and Elwert *et al.* [23] concluded that full fat HM yields a protein quality similar to fishmeal. In addition, Maurer *et al.* [24] reported that HM may substitute up to 100% of soybean products in diets for layers. However, validated quantitative evidence regarding the application of *Hermetia illucens* in-

sect meal in broiler diets are scarce [22] [25]. Presently, in Europe insect-based meals are not allowed to be included in livestock and poultry feeds [26]; this in corporation with the scarce evidence on HM is currently hindering HM use in poultry diets. 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 similar to aquafeed [27].

To date, very high inclusion rates in chicken diets of both of the alternative protein sources in this study have rarely been studied. Specifically, it has to be pointed out that both feed protein quality and dietary amino acid efficiency have not yet been investigated. Consequently, as a part of the multidisciplinary project "Sustainability Transitions in the Food Chain" (Supported by the Lower Saxony Ministry of Science and Culture) the current study aims to quantify the dietary protein quality of meat type chicken diets where SBM is completely substituted with partly defatted Hermetia meal (HM) or Spirulina meal (SM). In addition, the potential to improve the feed protein quality through fortification of the dietary AA balance is investigated based on N balance studies.

2. Material and Methods

The N balance studies were conducted at facilities of the Division Animal Nutrition Physiology of Georg-August-University of Goettingen and permitted (03.2016/AZ15/2027) by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

2.1. Chemical Composition of Microalgae and Insect Larvae

The *Spirulina platensis* microalga powder used in the diets 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 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. HM was obtained from a commercial producer (Hermetia Futtermittel GbR, Baruth/Mark, Germany). The black soldier fly larvae were separated from the substrate (rye flour, wheat bran) after 20 days, dried for 14 hours at 65˚C to 70˚C, then partly defatted with a screw press, until finally the larvae were ground into a meal. The nutritional contents of the two final alternative protein source products can be found in **Table 1**.

2.2. Stock and Husbandry

All male one-day-old meat type chickens (Ross 308) were obtained from a commercial hatchery and housed using at climatic conditions according to the Ross management recommendations [28]. Monochromatic (red) light was delivered for 23 hours daily. Up until the start of the N balance procedure, the birds were housed with standard feeding and management practices on wood shaving litter

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

Nutrient contents	Spirulina meal		Hermetia meal	
Moisture (%)	3.4		5.5	
Crude protein (% of DM)	58.8		60.8	
Crude ash (% of DM)	6.1		7.5	
Crude lipids (% of DM)	4.3		14.1	
Crude fibre (% of DM)	0.49*		10.92	
AA contents	**mgAA/gDM**	**gAA/16gN**	**mgAA/gDM**	**gAA/16gN**
Lys	22.97	3.91	32.97	5.42
Met	10.61	1.81	7.53	1.24
Cys	4.53	0.77	4.89	0.80
Thr	25.77	4.39	21.70	3.57
Arg	39.92	6.79	25.05	4.12
Val	34.50	5.87	32.58	5.36
Leu	47.23	8.04	37.95	6.24
Ile	29.81	5.07	23.47	3.86
His	7.51	1.28	16.58	2.73

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

floor pens and only average weight birds were selected for the N balance trials. In total, the trials utilized 70 birds, divided into starter period (10 - 20 d; 35 birds) and grower period (25 - 35 d, 35 birds), respectively. At a trial start (starter period or grower period), the birds were first moved to metabolic cages adapted in size for the starter or grower period (25 × 30 cm; 80 × 80 cm), equipped with a wire floor, individual feeding troughs, and an automatic drinking system. Then the birds passed through an adaptation period of 5 days previous to the two consecutive collection periods (5 d each). For both of the age periods, separate birds were randomly allotted to the experimental diets.

2.3. Diets and Feeding

Experimental diets were mixed and pelleted at the facilities of the Division Animal Nutrition Physiology of the University of Goettingen. In both of the age periods, the 35 birds were randomly allotted into five pelleted diets resulting in 7 birds per diet. The starter/grower control diets were based on wheat (33/38%), corn (16/19%) and SBM (39/32%) as the main ingredients (Table 2). In the experimental diets, SBM was completely substituted by SM or HM starter/grower diets contained 21/17% SM or 26/22% HM in order to ensure recommended crude protein (CP) levels. The experimental diets were additionally fortified with supplemented feed AAs. Two of these diets were supplemented to the basic level (Lys, Met) equal to the control diet and two others were supplemented to an ex-

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

Ingredients/Diets	Starter period (10 - 20 d)					Grower period (25 - 35 d)				
	Control	HM (A)	SM (A)	HM (AA)	SM (AA)	Control	HM (A)	SM (A)	HM (AA)	SM (AA)
Wheat	328.8	416.9	449.6	408.9	442.6	375.8	455.5	493.5	449.7	488.6
Corn	164.4	208.5	224.8	204.5	221.3	187.9	227.7	246.7	224.8	244.3
Soybean meal	390	-	-	-	-	320	-	-	-	-
Spirulina meal	-	-	210	-	210	-	-	170	-	170
Hermetia meal	-	260	-	260	-	-	220	-	220	-
Soybean oil	78.5	78.5	78.5	78.5	78.5	78.5	65	55	65	55
Premix*	10	10	10	10	10	10	10	10	10	10
DCP 40	11	12	13	12	13	10	8	11	8	11
$CaCO_3$	11	9.1	9.1	9.1	9.1	9	7	7	7	7
NaCl	3	1.7	1.7	1.7	1.7	3	1	1	1	1
Wheat starch	-	-	-	-	-	3	3	3	3	3
L-Lysine·HCl	1.3	1.3	1.3	4.7	7.0	0.8	0.8	0.8	3.3	5.5
DL-Methionine	2.0	2.0	2.0	4.7	3.6	2.0	2.0	2.0	3.3	2.5
L-Threonine	-	-	-	1.2	-	-	-	-	0.7	-
L-Arginine	-	-	-	4.8	2.2	-	-	-	3.3	1.4
L-Valine	-	-	-	-	-	-	-	-	0.5	0.3
L-Isoleucine	-	-	-	-	-	-	-	-	0.5	-
L-Histidine	-	-	-	-	1.0	-	-	-	-	0.5

HM (A) = Hermetia meal with basic AA supply; SM (A) = Spirulina meal with basic AA supply; HM (AA) = Hermetia meal with extended AA supply; SM (AA) = Spirulina meal with extended AA supply; *Added per kg of final diet: 2.1 g calcium, 0.8 g sodium, 5,000 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.

tended level (e.g. Lys, Met, Arg, Thr, Val, Ile, His) according to current recommendations [29] to meet an ideal AA ratio (IAAR).

Feed was offered twice a day (08:00 and 20:00 h). At the beginning of the adaptation period, feed was supplied on free choice level to quantity the individual feed intake. During N balance studies, birds were slightly feed-restricted to minimize spillage. The individual feed supply was kept near to free choice level beginning at third day of adaptation period. Further adaptation of feed supply was necessary due to the fast growing birds. Further standardization of the protein quality parameters (see 2.6) ensured that the effect of varying feed intake did not impact on the derived protein quality data. Nutrient composition of the diets as analyzed is summarized in **Table 3**.

Table 3. Analyzed nutrient content of experimental diets.

Diets	Starter period (10 - 20 d)					Grower period (25 - 35 d)				
	Control	HM (A)	SM (A)	HM (AA)	SM (AA)	Control	HM (A)	SM (A)	HM (AA)	SM (AA)
	Crude nutrients (g/kgDM)									
Crude protein	249.5	244.4	212.9	248.9	225.5	222.9	219.9	203.3	241.4	202.3
Ether extract	111.6	146.9	117.7	142.6	119.6	118.3	118.0	93.0	120.2	87.8
Crude fibre	45.2	44.5	22.2	44.4	22.7	45.7	42.0	19.4	40.2	20.1
Crude ash	65.6	57.6	51.9	55.3	52.7	60.6	49.3	47.3	50.5	45.2
N-free extract	528.1	506.6	595.3	508.8	579.5	552.5	570.8	637.0	547.7	644.6
	Amino acids (g/kg as-fed)*									
AME_N (MJ/kgDM)**	14.4	16.1	16.3	16.1	16.3	14.8	16.0	15.9	16.0	15.9
Lys	12.6	11.0	7.4	13.6	11.8	10.5	9.5	6.4	11.4	10.0
Met	4.9	4.8	5.1	7.5	6.7	4.6	4.6	4.8	5.9	5.3
Met + Cys	8.4	7.4	7.5	10.1	9.1	7.9	7.2	7.2	8.4	7.7
Thr	7.8	7.3	7.2	8.5	7.2	6.9	6.7	6.4	7.3	6.4
Arg	14.3	9.0	10.9	13.7	13.1	12.4	8.3	9.7	11.6	11.1
Val	9.3	10.7	9.6	10.6	9.6	8.3	9.7	8.6	10.1	8.8
Leu	16.1	14.4	14.6	14.3	14.6	14.5	13.4	13.4	13.3	13.3
Ile	8.8	7.8	8.1	7.8	8.0	7.8	7.1	7.1	7.5	7.1
His	5.4	5.6	3.1	5.6	4.0	4.8	5.1	3.0	5.1	3.4

HM (A) = Hermetia meal with basic AA supply; SM (A) = Spirulina meal with basic AA supply; HM (AA) = Hermetia meal with extended AA supply; SM (AA) = Spirulina meal with extended AA supply; *Derived from analyzed AA content of the ingredients; **N corrected apparent metabolizable energy, calculated according to WPSA [30].

2.4. Collection and Sampling

Birds were individually weighed at the beginning of the adaption period, as well as at the beginning and the end of the corresponding collection periods. The feed intake was recorded daily. During the morning and afternoon feedings, excreta samples were collected, amounting to two samples per day in two consecutive collection periods of 5 days each (n = 14). Prior to excreta handling, feathers and spilled feed pellets were carefully removed from the samples. Spilled feed was quantified for correction of daily feed intake. Excreta were immediately frozen and stored at −20˚C until further analyses.

2.5. Laboratory Analyses

Dietary ingredients, experimental diets and excreta were analyzed according to the German VDLUFA standards [31]. In short, the feed ingredients and feed mixtures were ground to 1 mm. Excreta were carefully defrosted and thoroughly

homogenized for chemical analysis (DM, N). Nitrogen content of feed and excreta was measured by the Dumas method (TruMac®, Leco Instrument GmbH, Moenchengladbach, Germany) and fraction CP was calculated with a factor of 6.25. 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 was detected by ion-exchange chromatography (Biochrom® 30, Biochrom Ltd. Cambridge, England) using acid hydrolysis without and with an oxidation step for quantitative determination of sulphur-containing amino acids. According to the German standards [31], ether extracts were analyzed following HCl hydrolysis of the feed samples.

2.6. Nitrogen Balance Data

N balance data assessment was conducted according to current applications of the "Goettingen approach" [32]-[37] making use of the exponential N utilization model created by Gebhardt [38]. The basic function is an expression of body N retention dependent on N intake and feed protein quality, respectively:

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

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

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]

The genotype dependent model parameters for daily NMR (240 mg/$BW_{kg}^{0.67}$) and $NR_{max}T$ (4240 mg/$BW_{kg}^{0.67}$ and 3440 mg/$BW_{kg}^{0.67}$ for starter and grower period, respectively) were taken from earlier experiments where the same genotype was under study [39]. According to several recent reports [29] [32]-[37] [39] [40] [41] [42] [43], the dietary protein quality was evaluated by parameter (b) based on following equation:

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

Equation (3) is the result of logarithmization and transformation of Equation (1). Additionally, traditional parameters like the productive protein value (PPV) and net protein utilization (NPU) were applied to evaluate the complex dietary protein quality by taking into account the processes involved in digestion and post-absorptive utilization.

$$\mathrm{PPV}(\%) = \frac{\mathrm{ND}}{\mathrm{NI}} \tag{4}$$

$$\mathrm{NPU}(\%) = \frac{\mathrm{NR}}{\mathrm{NI}} \tag{5}$$

However, the traditional protein quality measures are not independent of the level of actual protein intake [44] [45] [46]. Consequently, a standardization of protein intake was conducted according to Thong and Liebert [45], providing NPU data which are independent of NI [42] [45] [46] [47] [48].

Accordingly, standardized net protein utilization ($\mathrm{NPU}_{\mathrm{std}}$) was calculated (Equation (6)) for equal daily nitrogen intake ($\mathrm{NI}_{\mathrm{std}}$: 3000 mg/$\mathrm{BW}_{\mathrm{kg}}^{0.67}$) to ensure the comparability of derived NPU data:

$$\mathrm{NPU}_{\mathrm{std}}(\%) = \frac{\mathrm{NR}_{\max} \mathrm{T}\left(1 - \mathrm{e}^{-b \cdot \mathrm{NI}_{\mathrm{std}}}\right)}{\mathrm{NI}_{\mathrm{std}}} \cdot 100 \tag{6}$$

Therefore, as according to Equation (6), protein utilization ($\mathrm{NPU}_{\mathrm{std}}$) is the parameter of importance in this study, where the original parameter "b" as derived from the N balance data according to Equation (3) is included in Equation (6) [45].

2.7. Statistical Analyses

Statistical analyses were conducted with SPSS software package (IBM SPSS Statistics, 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 ($p \leq 0.05$) the Games-Howell and Tuckey tests were applied.

3. Results

The results of the N balance trials are summarized in **Table 4**. In the starter period, both the HM (A) and SM (A) diets, with just the basic level of AA supplementation, yielded significantly lower body weights (BW) and feed intake rates (DM intake) as compared to the control diet. Accordingly, the lowest daily N balance was observed for the SM (A) diet and the HM (A) N balance was significantly higher than the SM (A) group; yet both groups remained significantly below the control diet. In contrast, the extended AA supplementation diets HM (AA) and SM (AA) appeared to be able to compensate for these observed depressions of body weight and feed intake, as the values did not differ from those of the control group. In addition, the N balance data showed similar conclusions. Generally, the SM (AA) and HM (AA) diets yielded results similar to the control diet, although the HM (AA) diet did provide superior, but insignificant, results compared to the control group in the starter period. As well, the HM (AA) diet led to a significantly ($p < 0.05$) higher daily N balance than the SM (AA) group.

During the grower period, HM (A) and SM (A) diets again yielded significantly lower body weights and feed intake rates as compared to the control diet. Accordingly, the lowest daily N balance was obtained feeding the SM (A) diet,

Table 4. Summarized results of the N balance experiments in starter and grower period of growing meat type chicken.

Diets	Control	HM (A)	SM (A)	HM (AA)	SM (AA)
Starter period (10 - 20 d)					
n	14	14	14	14	14
Mean BW (g)	$407^{c} \pm 124$	$201^{b} \pm 53$	$138^{a} \pm 44$	$417^{c} \pm 134$	$340^{c} \pm 105$
DM intake (g/d)	$49.1^{b} \pm 13.1$	$21.9^{a} \pm 8.8$	$14.3^{a} \pm 10.1$	$52.0^{b} \pm 13.6$	$45.2^{b} \pm 10.5$
N intake (mg/$BW_{kg}^{0.67}$/d)	$3572^{c} \pm 277$	$2461^{b} \pm 610$	$1740^{a} \pm 784$	$3884^{c} \pm 277$	$3406^{c} \pm 328$
N excretion (mg/$BW_{kg}^{0.67}$/d)	$1108^{ab} \pm 146$	$1099^{ab} \pm 350$	$844^{a} \pm 440$	$1333^{b} \pm 186$	$1203^{b} \pm 156$
N balance (mg/$BW_{kg}^{0.67}$/d)	$2463^{cd} \pm 163$	$1361^{b} \pm 308$	$896^{a} \pm 375$	$2550^{d} \pm 140$	$2203^{c} \pm 225$
Grower period (25 - 35 d)					
n	14	14	14	14	14
Mean BW (g)	$952^{b} \pm 228$	$762^{b} \pm 150$	$564^{a} \pm 83$	$895^{b} \pm 173$	$925^{b} \pm 202$
DM intake (g/d)	$93.7^{c} \pm 19.7$	$66.6^{b} \pm 16.3$	$41.8^{a} \pm 8.4$	$81.3^{bc} \pm 15.5$	$98.8^{c} \pm 18.5$
N intake (mg/$BW_{kg}^{0.67}$/d)	$3473^{c} \pm 245$	$2869^{b} \pm 366$	$1937^{a} \pm 240$	$3310^{c} \pm 273$	$3442^{c} \pm 288$
N excretion (mg/$BW_{kg}^{0.67}$/d)	$1220^{b} \pm 110$	$1299^{b} \pm 189$	$979^{a} \pm 175$	$1298^{b} \pm 120$	$1274^{b} \pm 142$
N balance (mg/$BW_{kg}^{0.67}$/d)	$2252^{d} \pm 166$	$1569^{b} \pm 212$	$957^{a} \pm 89$	$2012^{c} \pm 207$	$2168^{cd} \pm 187$

HM (A) = Hermetia meal with basic AA supply; SM (A) = Spirulina meal with basic AA supply; HM (AA) = Hermetia meal with extended AA supply; SM (AA) = Spirulina meal with extended AA supply; $^{a-d}$values within a row with different superscripts are significantly different ($p < 0.05$).

and similar to the starter period the HM (A) diet yielded a significantly higher daily N balance. As expected, the extended AA supplementation diets, HM (AA) and SM (AA) provided body weights and feed intake rates, similar to that of the control group. Daily N balances data responded accordingly; however, the control diet still achieved the highest daily N balance. This result is likely influenced by the level of N intake. Consequently, a final discussion of the results needs standardized data for N intake.

As demonstrated in Table 5, the N utilization parameters from both age periods deliver a similar trend to that observed in Table 4; that is the basic AA supplemented diets are inferior compared to the extended supplemented and control diets. As expected in the starter period, the HM (A) and SM (A) diets resulted in significantly lower PPV and NPU. However, both of these parameters are influenced by the level of N intake. Results for PPV and NPU data were significantly improved with the HM (AA) and SM (AA) diets. However, the control diet yielded higher PPV and NPU data, except the HM (AA) diet in the grower period. However, as already mentioned traditional parameters of protein utilization are also influenced by the level of N intake. When NPU is standardized (NPU_{std}), the results of comparison are modified. In this case, no significant difference was observed between the control and HM (AA) diet; though, the difference between the HM (AA) and control diets become significantly different from the SM (AA) diet.

Table 5. N utilization parameters of growing meat type chicken as derived from N balance data in starter and grower period.

Diets	Control	HM (A)	SM (A)	HM (AA)	SM (AA)
Starter period (10 - 20 d)					
n	14	14	14	14	14
PPV (%)	$69.1^{c} \pm 2.2$	$55.7^{a} \pm 5.7$	$51.9^{a} \pm 7.1$	$65.8^{b} \pm 2.9$	$64.7^{b} \pm 2.8$
NPU (%)	$75.8^{c} \pm 2.5$	$65.9^{a} \pm 6.4$	$67.6^{ab} \pm 8.5$	$72.0^{b} \pm 3.3$	$71.8^{b} \pm 2.9$
Model parameter b ($\times 10^{-6}$)*	$285^{c} \pm 14$	$196^{a} \pm 21$	$186^{a} \pm 21$	$278^{c} \pm 16$	$253^{b} \pm 19$
NPU_{std} (%)**	**$81.2^{c} \pm 2.5$**	**$62.6^{a} \pm 4.6$**	**$60.2^{a} \pm 5.1$**	**$79.8^{c} \pm 2.8$**	**$75.2^{b} \pm 3.7$**
Grower period (25 - 35 d)					
n	14	14	14	14	14
PPV (%)	$64.9^{d} \pm 1.8$	$54.7^{b} \pm 2.7$	$49.8^{a} \pm 4.2$	$60.7^{c} \pm 2.7$	$63.0^{cd} \pm 2.2$
NPU (%)	$71.8^{c} \pm 1.9$	$63.2^{a} \pm 2.9$	$62.4^{a} \pm 5.7$	$68.0^{b} \pm 2.6$	$70.0^{bc} \pm 2.4$
Model parameter b ($\times 10^{-6}$)*	$374^{d} \pm 28$	$262^{b} \pm 20$	$223^{a} \pm 19$	$324^{c} \pm 31$	$353^{d} \pm 30$
NPU_{std} (%)**	**$77.2^{d} \pm 3.1$**	**$62.3^{b} \pm 3.0$**	**$55.8^{a} \pm 3.3$**	**$71.1^{c} \pm 4.1$**	**$74.7^{cd} \pm 3.5$**

HM (A) = Hermetia meal with basic AA supply; SM (A) = Spirulina meal with basic AA supply; HM (AA) = Hermetia meal with extended AA supply; SM (AA) = Spirulina meal with extended AA supply; *Applied for NPU standardization based on: NMR = 240 mg/$BW_{kg}^{0.67}$/d; $NR_{max}T$ starter: = 4240 mg/$BW_{kg}^{0.67}$/d, $NR_{max}T$ grower: = 3440 mg/$BW_{kg}^{0.67}$/d; **Standardized daily N intake = 3000 mg/$BW_{kg}^{0.67}$; $^{a-d}$values within a row with different superscripts are significantly different ($p < 0.05$).

During the grower period, a similar ranking to that in the starter period was observed for diets HM (A) and SM (A). Accordingly, the extended AA supplementation in the HM (AA) and SM (AA) diets yielded superior N utilization as to the basic supplemented diets. The control and SM (AA) diets generated superior results for PPV and NPU and were not significantly different from one another. In addition, no significant difference was found between diets HM (AA) and SM (AA) in the grower period. Standardization of NPU data did not influence the comparison significantly; nonetheless, due to variation of N intake the difference between diets SM (A) and HM (A) became more pronounced (Table 4). Additionally, in contrast to the starter period diet HM (AA) yielded significantly lower NPU_{std} as compared to the control diet.

Generally, extended AA supplementation of both diets containing alternative protein sources improved dietary protein quality significantly. Misleading conclusions about dietary protein quality are prevented by the standardization of NPU data as described, and therefore, the discussion of dietary effects on achieved protein quality will only focus on NPU_{std} data.

4. Discussion

The general conclusion of this study is that partly defatted insect meal from *Hermetia illucens* (HM) larvae or the microalgae *Spirulina platensis* (SM) at inclusion rates of 26%/22% (starter/grower HM) or 21%/17% (starter/grower SM)

are acceptable in growing chicken diets when completely replacing SBM. The complete substitution of SBM was conducted to demonstrate the potential, but also the limitations, of the alternative proteins under study. In this context, we aimed to quantify the effects of extreme inclusion rates on the parameters of dietary protein quality in chicken diets by means of N balance studies.

Given these aims, we came to the conclusion that while feeding alternative protein sources with an AA supplementation level equal to that of the SBM control diet, a significant depression of feed protein quality was observed independent of the alternative protein source. These observations are in agreement with Austic *et al.* [13] who demonstrated that growing chickens grew slower (0-3 weeks of age) and had lower feed efficiency (0 - 6 weeks of age) when SBM was partly substituted by 7.5% *Staurosira* sp. at a basic level of AA supplementation. However, diets with 7.5% of the algae meal and supplemented with an extended level of essential AAs (Met, Lys, Arg, Ile, Thr, Trp, Val) yielded performance data equivalent to the control diet. Other studies [8] [49] have demonstrated that Spirulina meal fed birds reduced their feed intake in the starter period, but to a lower extent in the grower period [8]. This trend was supported by the current protein quality evaluation indicating that an age-related scenario is possible. Oluokun [20] also studied diets with HM as related to full-fat soybean meal diets and observed higher growth rates for HM meal diets, which match with our results in the starter period. Furthermore, Elwert *et al.* [23] reported similar results for starter chickens (1 - 10 d). In their study, the experimental diet, which included 4.7% HM with a fat content of 15% and the AA supplementation of Lys and Met, yielded no differences in body weight and feed intake compared to the soybean control treatment.

Looking more closely at the NPU_{std}, as protein quality parameter that is independent of variation in N intake, significantly lower protein quality during starter period was observed for the SM (AA) diet as compared to the control and HM (AA) diet. In contrast, during the grower period superior protein quality was observed for the SM (AA) diet and the results are not significantly different from the control diet. These conflicting results need further attention and are the focus of ongoing studies. However, the achieved dietary AA balance following extended AA supplementation is a factor of influence. Actual AA supplementation aimed to meet the current IAAR assumption according to Wecke and Liebert [29]. However, negative impacts of an individual AA excess on N utilization parameters cannot be excluded. Further investigation into the optimization of the dietary AA balance in chicken diets with an elevated inclusion level of alternative protein sources and feed AAs needs to remain a main priority.

Comparing the two alternative protein sources, it can be summarized that diets with HM tended to provide higher dietary protein quality as compared to the SM diets at both levels of AA supplementation. Nonetheless, both of the two alternative protein sources appear to be good candidates for replacing SBM, when the appropriate AA supplementation levels are considered.

5. Conclusions

Complete replacement of SBM by partly defatted Hermetia meal or Spirulina meal in chicken diets depressed dietary protein quality with only a basic level of AA supplementation. However, with an extended level of AA supplementation observed protein quality parameters of diets with both of the alternative proteins were significantly improved, but not generally on par with the control diet. This important response became more evident when the well-known effect of N intake on protein utilization was eliminated through the standardization of N intake by adequate model application. 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.

On this note, ongoing research to further optimize the dietary AA balance when 100% SBM is substituted by alternative proteins should remain a key priority.

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