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Does graded substitution of soy protein concentrate by an insect meal respond on growth and N-utilization in Nile tilapia (*Oreochromis niloticus*)?

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ABSTRACT

Besides fishmeal, soybean protein is the most common protein source in aquafeed. However, the sustainability of soybean production is criticized. Due to the rapid development of aquaculture, the increasing demand for high value feed proteins has initiated research into alternative and more sustainable proteins for aquafeeds. In order to evaluate one promising alternative protein source, a growth study (56 days) was conducted with juvenile Nile tilapia (*Oreochromis niloticus*, 35 g on average) with a partial substitution of soybean protein by a partly defatted insect meal from black soldier fly larvae (*Hermetia illucens*). Growth performance, feed utilization and dietary protein quality parameters were evaluated based on analysed body protein deposition.

A control feed (8% fish meal, 37% soy protein concentrate (SPC)) and three iso-nitrogenous and iso-energetic experimental feeds with 25, 50 or 100% replacement of SPC by partly defatted *Hermetia* meal (HM) were formulated. All feeds supplied essential amino acids as recommended for Nile tilapia. Growth response and protein utilization were examined in a semi-closed in-door water recirculation system. A comparative slaughter technique was applied to generate N deposition data for protein quality evaluation based on the standardized net protein utilization (NPU_{std}) according to the "Goettingen approach". All feeds were very well accepted. Replacement of SPC by HM up to 50% improved feed protein quality and result in similar specific growth rate and feed conversion ratio, respectively. However, a higher inclusion rate of HM tended to impair growth, but not the observed protein quality. In conclusion, the replacement of SPC by partly defatted HM up to a level of 50% had no negative effect on growth performance and improved the dietary protein quality of tilapia feeds under study. Insect protein from *Hermetia illucens* could be a promising option to make aquafeed formulation more flexible and sustainable.

1. Introduction

During the last decade world fish aquaculture production has still consistently grown up to now 80 million tons (FAO, 2018). According to FAO, the aquaculture market is expected to grow by 50% between 2010 and 2030. The most pronounced increases are expected for tilapia, carp, and Pangasius/catfish, respectively. Global tilapia production is predicted to nearly double between 2010 and 2030. Therefore, the sustainability of production will be a great challenge (World Bank, 2013).

With a current production of 4.2 million tons in 2016, Nile tilapia is in fourth position of the most farmed fish in the world (FAO, 2018). More than 80% of global tilapia production is based on commercial aquafeed, which is expected to increase up to 95% by 2020 (FAO, 2011). The predominant protein source in commercial tilapia feed (20–60%) is soybean meal (FAO, 2011; Ng and Romano, 2013). But the sustainability of soy cultivation is criticized with regard to forest clearance and excessive use of pesticides (Rumpold and Schlüter, 2014; Sánchez-Muros et al., 2016a). Therefore, there is actually an increasing interest in insect meals as an alternative protein source (Sánchez-Muros et al., 2016a). Nutrient composition studies have indicated that insect meals are rich in high value protein (Finke, 2002, 2013; Rumpold and Schlüter, 2014). The European Commission permits proteins from insects (insect PAPs) in feed for aquaculture (Commission regulation (EU), 2017/893). However, before these feeds can be utilized commercially information on safety issues, ecological, economical and nutritional benefits needs to be addressed.

In Europe, one of the most promising insect species for commercial exploitation is the black soldier fly (BSF), *Hermetia illucens* (EFSA, 2015; Lock et al., 2016). Although several studies have already investigated the potential of BSF as a fish meal substitute in fish feed (Belghit et al., 2018; Devic et al., 2017; Kroeckel et al., 2012; Li et al., 2017; Lock

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Table 1
Formulation (% as feed basis), proximate composition (% in dry matter, DM), and essential amino acid composition (g/16 g N or % DM) of the raw materials (HM = *Hermetia* meal, SPC = soy protein concentrate) and experimental feeds used in the feeding trial.

	Raw materials			Feeds[k]			
	SPC	HM		Control	HD25	HD50	HD100
Ingredients [% as feed basis]							
Fishmeal[a]				8.00	8.00	8.00	8.00
Wheat meal				37.60	37.80	37.80	37.90
Wheat gluten[b]				6.00	7.10	8.30	10.70
Hermetia meal[c]				–	9.25	18.50	37.00
Soy protein concentrate[d]				37.00	27.75	18.50	–
Fish oil[a]				4.00	3.35	2.75	1.50
Soybean oil				4.00	3.35	2.75	1.50
Vit./Min. Premix[e]				1.00	1.00	1.00	1.00
$CaHPO_4$				0.70	0.70	0.70	0.70
$CaCO_3$				0.70	0.70	0.70	0.70
Carboxymethylcellulose[f]				1.00	1.00	1.00	1.00
Proximate composition [% DM]							
Dry matter (as feed basis)	91.56	94.47		90.75	90.92	91.11	91.47
Crude protein (N × 6.25)[g]	74.09	60.84		42.96	42.82	42.74	42.61
Crude lipid	0.36	14.08		10.97	10.91	10.96	10.94
Crude ash	6.38	7.49		7.49	7.62	7.74	7.98
NFE[h]	19.17	17.59		38.58	38.65	38.56	38.47
Chitin	–	11.11		–	1.07	2.13	4.24
Gross energy [MJ/kg DM][i]	20.92	22.95		21.11	21.06	21.05	20.99
Essential amino acids	*[g/16 g N]*		*[% DM]* NRC (2011)[j]				
Lysine	6.14	5.42	1.56	2.23	2.14	2.05	1.86
Methionine	1.24	1.24	0.48	0.60	0.60	0.60	0.60
(Cysteine)	1.24	0.80	0.37	0.58	0.56	0.54	0.50
Threonine	3.98	3.57	1.05	1.56	1.51	1.47	1.39
Valine	4.33	5.35	1.50	1.78	1.82	1.87	1.97
Leucine	7.59	6.24	1.90	3.05	2.95	2.86	2.67
Isoleucine	4.39	3.86	0.88	1.71	1.66	1.62	1.53
Phenylalanine	4.90	3.45	1.05	1.99	1.90	1.81	1.64
(Tyrosine)	3.64	7.04	0.50	1.30	1.50	1.69	2.07
Histidine	2.54	2.73	0.48	1.00	1.01	1.01	1.02
Tryptophan	1.00	1.31	0.28	0.44	0.46	0.48	0.53
Arginine	7.60	4.12	1.18	2.79	2.53	2.28	1.78

[a] Herring; BIOCEVAL GmbH & Co. KG, Cuxhaven, Germany.
[b] Gluvital 21,000; Cargill GmbH, Hamburg, Germany.
[c] Hermetia Baruth GmbH, Baruth/Mark, Germany.
[d] Soycomil R; Denkavit Futtermittel GmbH, Warendorf, Germany.
[e] Deutsche Vilomix Tierernährung GmbH, Neuenkirchen-Vörden, Germany; Vitamin and mineral content in premix (g or IU/kg): vit. A, as retinyl acetate 750,000 IU; vit. D3, 45,000 IU; vit. E as α-tocopheryl acetate, 9.0; menadione, 0.6; thiamin mononitrate, 0.5; riboflavin, 0.72; pyridoxine as pyridoxine HCl, 1.8; pantothenic acid as Ca-pantothenate, 1.37; cyanocobalamin, 0.01; niacin, 9.19; biotin, 0.007; folic acid, 0.12; choline chloride, 120.05; inositol, 0.05; vit. C as L-Ascorbyl-2-polyphosphate, 10.15; $CaCO_3$, 650.63; KCl, 1.00; NaCl, 1.00; $MgSO_4$, 1.00; $FeSO_4*H_2O$, 32.00; CaI_2O_6, 0.21; ZnO, 6.40; MnO, 2.30; $CuSO_4*5H_2O$, 2.40; Na_2SeO_3, 0.11.
[f] Carl Roth GmbH + Co. KG, Karlsruhe, Germany.
[g] protein content was not corrected for chitin.
[h] NFE, nitrogen-free extract = 100 - (crude protein + crude lipid + crude ash).
[i] calculated by energy conversion factors (MJ/kg): crude protein = 23.6; crude lipid = 39.5; NFE = 17.2.
[j] NRC, National Research Council; recommended dietary levels of essential amino acids for tilapia.
[k] HD = Hermetia feeds: SPC was substituted by either 25, 50 or 100% (HD25, HD50, HD100, respectively).

et al., 2016; Magalhães et al., 2017; Muin et al., 2017; Sealey et al., 2011; Stadtlander et al., 2017; St-Hilaire et al., 2007), to the best of our knowledge no comparable studies are available that consider the substitution of soybean protein solely by BSF-meal in aquafeed. Taking into account this novel aspect, the current study aimed to evaluate the efficiency of partially defatted insect meal from BSF larvae as a substitute for soybean protein concentrate in tilapia feeds.

2. Materials and methods

The experiment was conducted at the facilities of the Division Animal Nutrition Physiology at Georg-August-University of Goettingen, Germany. The experimental protocol was designed according to the guidelines of the current European law on the care and use of experimental animals (European Directive, 2010/63/EU, came into effect in Germany with § 7a Section 2 Nr. 3 TierSchG).

2.1. Experimental feeds

Four iso-energetic and protein equivalent feeds were formulated to resemble the proximate composition of commercial tilapia feeds (Table 1). The control feed was designed to contain only feed ingredients that are typical for commercial tilapia feeds. The formulation was in line with the essential amino acid (EAA) requirement recommendations for Nile tilapia (Furuya et al., 2006, 2012; NRC, 2011) as well. Fishmeal (FM) content was held constant at 80 g/kg. Differing from commercial practice, soy protein concentrate (SPC) was used as main plant-derived protein source. The latter aimed for minimization of anti-nutritional factors (ANF´s) and to ensure optimal feed acceptance. Essential fatty acids were supplied by a mixture of fish oil and soybean

oil (ratio 1:1). Feed ingredients were analysed for crude nutrients and EAA to assist in final feed formulation.

The three experimental feeds were formulated for graduated substitution of SPC by partially defatted *Hermetia* meal (HM), obtained from a commercial producer (Hermetia Baruth GmbH, Baruth/Mark, Germany). Substitution took place at 25% (HD25), 50% (HD50), and 100% (HD100) of SPC by HM. *Hermetia* meal was produced as follows: *Hermetia* larvae were collected from the substrate (rye, wheat bran) by sieving, killed at 80 °C and subsequently dried for 14 h at a low temperature (66–70 °C). Dried larvae were then partially defatted by a screw press (Type AP08, Reinartz GmbH & Co. KG, Neuss, Germany) and finally milled to yield a fine meal.

To prepare the experimental feeds the milled dry ingredients and oils were thoroughly mixed with a precision laboratory mixing system (Type M20MK, Loedige Ltd., Paderborn, Germany). Tap water was added to the mixtures to attain an appropriate consistency for pelleting (temperature < 45 °C, 4 mm diameter) while using a professional pellet-press (Lister Petter Ltd., Gloucestershire, UK). After pelleting, the feeds were dried at 40 °C for 24 h in a ventilated oven (Model 800, Memmert GmbH & Co. KG, Schwabach, Germany) and finally stored in a cool, dry and dark room at 2 °C. Before starting the experiments, the proximate nutrient composition in the final feeds was analysed to inspect whether the nutrient composition was as expected based on calculations. The proximate composition of raw materials and experimental feeds is presented in Table 1.

2.2. Nile tilapia feeding trial & experimental conditions

The trial was conducted in a semi-closed in-door water re-circulating system with 16 circular plastic tanks (320 L per tank). Each tank was continuously supplied with a mixture of

fresh water (approximately 10%) and biologically filtered water. Water temperature (25.0 ± 0.2 °C) and photoperiod (14h light : 10h dark) were regulated. Water quality parameters were monitored weekly in the effluent tank water to ensure compliance with reported reference data (DeLong et al., 2009).

All-male juvenile *O. niloticus*, originating from the Lake Manzala (Egypt) population, were obtained by mating yy-males with normal females (Kronert et al., 1989; Müller-Belecke and Hörstgen-Schwark, 2000) at the Division of Aquaculture and Aquatic Ecology at Georg-August-University of Goettingen, Germany. Fish were acclimatized to experimental conditions for several weeks, using a homemade standard feed. At the start of the experiment the fish were individually weighed and selected according to similar body weight (BW). Four replicate groups per feed (20 fish per tank) were included in the 56-day growth experiment. Fish were hand fed twice daily. To attain optimal feed conversion, feed supply was set at approximately 2.2% of the total tank BW per feeding. After a period of 28 days and at the end of the experiment, fish were individually weighed again to calculate growth performance parameters. The fish were fasted 24h prior to each weighing.

2.3. Sampling and chemical analyses

At the trial start, the body composition of 10 fish (representing the mean BW) was analysed for reference which was compared to pooled samples at the end of the trial. The final pooled whole body composition samples consisted of three average BW fish per replicate per feed. For both start and final samples, the fish were killed by an anesthetic overdose (Eugenol, 427 mg L^{-1}, 5 min), autoclaved (110 °C, 160 min), homogenized (immersion blender) and stored at −20 °C for subsequent analyses. Chemical analyses of dry matter (DM), crude ash (CA), crude protein (CP; N × 6.25; Dumas-method), Crude lipid (CL; Soxhlet-procedure, after HCl-hydrolysis) for ingredients, feeds and homogenized fish samples were conducted in duplicates according to standards defined by the Association of German Agricultural and Analytic Research Institutes (VDLUFA, 2017). N-free extracts (NFE) were calculated by difference (NFE = 100 − (H_2O + CP + CL + CA)). Amino acid (AA) analyses were conducted using chromatographical methods according to EU standard methods (Commission regulation (EC) No 152/, 2009). Chitin in HM was analysed by the Fraunhofer-IGB (Stuttgart, Germany) through the determination of *acetyl*-groups after total hydrolysis as described by Hahn et al. (2018).

2.4. Calculated parameters

Growth and nutritional indices were calculated as followed:

Metabolic body weight (MBW, $kg^{0.67}$) = [(final body weight (kg) + initial body weight (kg)) ÷ 2]$^{0.67}$

Feed intake (FI, g DM/BW$kg^{0.67}$/day) = total feed intake (g) ÷ MBW ÷ days of experiment

Specific growth rate (SGR, %) = 100 × [(ln BW_{final} (g) – ln $BW_{initial}$ (g)) ÷ days of experiment]

Feed conversion ratio (FCR, g/g) = total feed intake (g) ÷ total weight gain (g)

Protein efficiency ratio (PER, g/g) = total weight gain (g) ÷ total protein intake (g)

Protein deposition (PD, %) = [(BW_{final} (g) × final body protein) − ($BW_{initial}$ (g) × initial body protein)] ÷ (total feed intake (g) × feed protein) × 100

The applied model of protein utilization initially established by Gebhardt (1966, 1980) has been further developed and adapted for several growing species (Dorigam et al., 2017; Liebert and Benkendorff, 2007a,b; Thong and Liebert, 2004; Wecke et al., 2016) using the basic functions as given in Eqs. (1)–(3):

$$NR = NR_{max}T \times (1 - e^{-b \times NI}), \quad (1)$$

$$ND = NR_{max}T \times (1 - e^{-b \times NI}) - NMR, \quad (2)$$

$$b = [\ln NR_{max}T - \ln(NR_{max}T - NR)] \div NI, \quad (3)$$

where NR is daily N-retention [mg/$BW_{kg}^{0.67}$]; ND the sum of daily N-deposition; NMR the daily N-maintenance requirement and $NR_{max}T$ the theoretical maximum for daily N-retention [mg/$BW_{kg}^{0.67}$]. The model parameter b represents the slope of the exponential function and depends on dietary protein quality, but is independent from daily N-intake (NI, [mg/$BW_{kg}^{0.67}$]). Finally, parameter e is the basic number of natural logarithm (ln).

Previous investigations with juvenile tilapia (Liebert et al., 2006) derived the model parameters NMR (70 mg/$BW_{kg}^{0.67}$/day) and $NR_{max}T$ (388 mg/$BW_{kg}^{0.67}$/day) for the genotype reared in this study. The power of 0.67, used to assess the metabolic body weight corresponding to protein metabolism in growing warm-blooded animals, was also utilized in the present study because of the inconclusive database for fish (Jobling, 1981, 1994). To evaluate the nutritional value of dietary protein, the parameter net protein utilization, NPU (Bender and Miller, 1953; Miller and Bender, 1955) was applied. NPU is defined as the proportion of dietary N that is retained in the body. The retained N is expressed as the sum of N-deposition plus the N-maintenance requirement. NPU in the recent study was applied as standardized NPU making use of Eq. (1) and resulting in Eq. (4):

$$NPU\ (\%) = [NR_{max}T \times (1 - e^{-b \times NI})] \div NI \times 100 \quad (4)$$

Making use of the model parameter b according to Eq. (3), NPU data are derived for a given N-supply according to Eq. (4). In general, NPU is dependent both on protein quality and N-intake, which may result in a misleading evaluation of the dietary protein quality at varying level of protein supply and consequently requires a standardization of the

intake level. In the current study, NPU data were standardized (NPU_{std}) by applying a fixed N-intake (referred to as an average daily N-intake and observed = 540 mg/$BW_{kg}^{0.67}$). Further details about the "Goettingen approach" have been reviewed by Liebert (2013, 2017).

2.5. Statistics

Statistical analyses were performed using the R statistical software package (version 3.0.2; The R foundation, available from www.r-project.org).

Data were analysed by one-way ANOVA using the lm procedure, according to the model:

$$Y_{ij} = m + P_i + e_{ij}$$

where Y is the single observation, m the general mean, P the effect of the diet (i = HD25 or HD50 or HD100 feeds) and e the error.

Significant differences among means ($p < 0.05$) were determined by the Tukey multiple range test. Homocedasticity and Normality of data were verified by Levene´s Test and Kolmogorov-Smirnov-Test, respectively.

3. Results

3.1. Proximate composition of ingredients and feeds

Differences in proximate composition were analysed for both of the investigated feed ingredients – SPC and HM (Table 1). SPC was higher in CP and NFE (N-free extracts), but lower in CL and CA as compared to HM. EAA concentrations in CP were lower in HM, with the exceptions of methionine, valine, histidine and tryptophan.

The proximate nutrient composition of final feeds was very similar. The substitution of SPC by HM did not provide an obvious limitation in EAA supply in the final feeds. In consequence, crystalline amino acids were not supplemented. The chitin content increased according to the inclusion rate of HM.

3.2. Growth performance

Survival of the fish during the experiment was 99.5%. Mortalities only occurred due to aggressive behavior and were not related to diet.

All experimental feeds were well accepted as supported by the observed feed intake (Table 3). Higherfeed intake was observed for feed HD100 ($p < 0.001$). At SPC replacement levels of 25 or 50%, BW_{final}, SGR, FCR as well as PER responded similar to the control (Table 2). Feed HD100 did not differ significantly from the control as well, but FCR and PER were lower ($p < 0.05$) as compared to feeds with abated substitution rates for SPC.

Table 2
Growth performance of juvenile Nile tilapia fed the control and experimental feeds over 56 days (mean ± sd, n = 4).

	Control	HD25[6]	HD50[6]	HD100[6]
BW_{start}[1]	35.0 ± 0.3	34.8 ± 0.3	35.1 ± 0.2	34.9 ± 0.2
BW_{final}[2]	71.1 ± 2.0	72.9 ± 1.7	72.6 ± 1.4	68.9 ± 3.2
SGR[3]	1.27 ± 0.04^{ab}	1.32 ± 0.04^{a}	1.30 ± 0.04^{ab}	1.21 ± 0.08^{b}
FCR[4]	1.86 ± 0.07^{ab}	1.79 ± 0.05^{a}	1.80 ± 0.05^{a}	2.02 ± 0.15^{b}
PER[5]	1.38 ± 0.05^{ab}	1.43 ± 0.04^{a}	1.43 ± 0.04^{a}	1.28 ± 0.09^{b}

Values with different superscripts are significantly different ($p < 0.05$).
[1] BW_{start} = initial body weight (g).
[2] BW_{final} = final body weight (g).
[3] SGR, specific growth rate (% per day) = [ln (BW_{final}) - ln (BW_{start})] ÷ experimental days × 100.
[4] FCR, feed conversion ratio = feed intake (g) ÷ weight gain (g).
[5] PER, protein efficiency ratio = weight gain (g) ÷ protein intake (g).
[6] HD = Hermetia feeds: SPC was substituted by either 25, 50 or 100% (HD25, HD50, HD100, respectively).

3.3. Nitrogen utilization and protein quality

N-deposition data were significantly improved by substituted diets irrespective of the inclusion level (Table 3), but can only be explained by elevated N-intake in feed HD100. The derived dietary protein quality (NPU_{std}) was higher when feeds HD25 and HD50 were fed ($p < 0.05$). At a complete substitution of SPC (HD100) the observed NPU_{std} was not significantly impaired.

4. Discussion

4.1. Proximate composition of ingredients and feeds

The proximate composition of this study´s partially defatted HM was in the range of economically produced HM utilized in previous studies (Kroeckel et al., 2012; Lock et al., 2016; Renna et al., 2017). The chitin content (10.5% as feed basis) was similar to pre-pupae data (9.2% as feed basis) reported by Kroeckel et al. (2012). The chitin content of HM as obtained by Renna et al. (2017) was somewhat lower (4.7% as feed basis) and differences may be due to the absence of a validated analytical procedure. A validated procedure would be required to gain more insight into the nutritional properties of chitin in insects (Veldkamp and Bosch, 2015) including the importance of non-protein nitrogen (NPN) in the chitin fraction.

Due to the NPN content in chitin a modified N-conversion factor (< 6.25) for calculating the crude protein content in whole larvae or processed larvae meals is recommended (Janssen et al., 2017). As a comprehensive database is currently lacking the common N-conversion factor (6.25) was applied in the present study. As reported elsewhere (Finke, 2007) no correction of dietary CP from chitin was conducted. In contrast, several reports (Kroeckel et al., 2012; Sánchez-Muros et al., 2016b; Stadtlander et al., 2017) recommend corrections. Considering the actual inclusion rates of HM (9.3, 18.5 and 37.0%) only 0.5, 0.9 and 1.8% CP in DM could possibly be attributed to the chitin fraction.

4.2. Growth performance

Insect meal derived from *Hermetia illucens* is one of the most studied alternative proteins in aquafeed. Due to the substrate variation in insect rearing, differing processing technologies, fish species and developing stage, the published results are remain mostly conflicting (Henry et al., 2015; Makkar et al., 2014). In Nile tilapia, first observations revealed promising effects(Devic et al., 2017; Muin et al.,2017).

Insects need to be further processed in order to ensure an optimal handling for feed manufacturing (Veldkamp et al., 2012) and a continuous control of quality (Sánchez-Muros et al., 2014). Several current studies examined commercially produced HM in aquafeeds (Belghit et al., 2018; Kroeckel et al., 2012; Lock et al., 2016; Magalhães et al., 2017; Renna et al., 2017). Depending on fish species levels ranging from 19.5 to 100% of dietary FM could be replaced by HM without significant adverse effects on growth performance.

The impact of chitin is a major concern and results are often inconsistent. In turbot levels above 33% dietary HM reduced feed acceptance, possibly attributed to the presence of chitin (Kroeckel et al., 2012). In Atlantic salmon (Lock et al.,2016) and European seabass (Magalhães et al.,2017) no negative effect both on digestibility of CP or AA and lipid fraction were observed. No effect on lipid digestibility was found in rainbow trout as well, but apparent CP digestibility responded inconclusively (Renna et al., 2017). An inferior apparent nutrient digestibility (CP, CL, CA, AA) was reported elsewhere (Belghit et al., 2018) when 85% of dietary protein originated from HM. The same study once showed no effect on protease (trypsin) activity but a markedly lower activity of leucine aminopeptidase. This brush border enzyme breaking down peptides into AA in the proximal and mid

Table 3
Dietary protein quality and N-utilization of tilapia fed the control and experimental feeds (mean ± sd, n = 4) over 56 days.

	Control	HD25[6]	HD50[6]	HD100[6]
FI[1]	7.79 ± 0.05[a]	7.82 ± 0.04[a]	7.78 ± 0.03[a]	8.11 ± 0.03[b]
N-intake[2]	535.75 ± 3.15[a]	535.91 ± 2.80[a]	532.36 ± 1.77[a]	552.75 ± 1.75[b]
N-deposition[2]	109.36 ± 3.60[a]	121.45 ± 3.19[b]	118.72 ± 3.05[b]	121.71 ± 7.19[b]
N-retention[2,3]	179.36 ± 3.60[a]	191.45 ± 3.19[b]	188.72 ± 3.05[b]	191.71 ± 7.19[b]
PD[4]	20.41 ± 0.74[a]	22.66 ± 0.61[b]	22.30 ± 0.57[b]	22.02 ± 1.33[ab]
NPU_{std}[5]	33.40 ± 0.75[a]	35.64 ± 0.61[b]	35.30 ± 0.56[b]	34.93 ± 1.36[ab]

Values with different superscripts are significantly different ($p < 0.05$).
[1] FI = Feed intake ($gDM/BW_{kg}^{0.67}/d$).
[2] ($mg/BW_{kg}^{0.67}/d$).
[3] Sum of daily N-deposition and daily N-maintenance requirement ($70\,mg/BW_{kg}^{0.67}$).
[4] PD = protein deposition (%).
[5] NPU_{std} = standardized net protein utilization (%), based on the average daily N-intake as observed = $540\,mg/BW_{kg}^{0.67}$.
[6] HD = Hermetia feeds: SPC was substituted by either 25, 50 or 100% (HD25, HD50, HD100, respectively).

intestine, where the majority of proteins are digested and absorbed. The authors argued that the reduced activity of this enzyme might be due to the content of chitin in HM-based diets. Investigations in tilapia (Shiau and Yu,1999) observed impaired lipid digestibility and reduced growth performance when feeds were supplemented with 2% chitin. Considering the dietary chitin level of up to 3.9% in the current study, impaired growth performance parameters with feed HD100 could be related to this dietary factor.

The highest tolerable inclusion level of HM causing no reduction of growth performance throughout this study was 18.5%. This inclusion level is slightly lower as compared to previous studies (Kroeckel et al., 2012; Lock et al., 2016; Magalhães et al., 2017; Renna et al., 2017). Several factors like fish species and varying palatability of the substituted ingredient (FM versus SPC) have to be considered in this context. It is also suggested that processing procedure of the insect meal could be an important factor impacting on growth response (Lock et al.,2016). In addition, when replacing SPC by HM the plant based content of phytate in the final diet declines. In consequence, beneficial effects on growth can be expected as was demonstrated in Nile tilapia by (Liebert and Portz, 2005).

4.3. Nitrogen utilization and protein quality

Protein quality evaluation by NPU_{std} based on the application of the "Goettingen approach" is a complex measure. The procedure considers both digestibility and post-absorptive utilization of the ingested nitrogen compounds including the N from the chitin fraction. An important advantage of using NPU_{std} for protein quality evaluation is the independency from N-intake.

The number of studies on N-utilization and protein quality of feeds containing commercially produced defatted HM is very limited. Most studies only include parameters, like productive protein value (PPV), which are not independent of protein intake. In Atlantic salmon, the observed PPV (40–44%) was not impaired by the inclusion of HM (Lock et al., 2016). But PPV significantly decreased in juvenile turbot when the HM inclusion exceeded 33% of the feed (Kroeckel et al., 2012). In juvenile European seabass, increasing the incorporation level of HM led to decreased PER of the diets (Magalhães et al., 2017). However, the apparent digestibility of protein was not affected, suggesting that only the efficiency of post-absorptive utilization was impaired. Results of the present study were in line with Lock et al. (2016), who observed no negative effect of HM inclusion in salmon feed on PD and protein quality.

The EAA content of the current experimental feeds covered the recommendations for Nile tilapia. The observed superior N-utilization of SPC substituted feeds indicates that protein/AA availability from HM surpassed the plant protein source SPC. Whether this observation was more related to protein digestibility or post-absorptive utilization remains speculative. The significance of the achieved dietary AA balance in HM containing feeds needs to be verified in future investigations.

Phytate is not eliminated during SPC production (Peisker, 2001).The role of a lowered phytate concentration in SPC substituted feeds remains unclear and is worth to be investigated in detail as well.

5. Conclusion

Results of the present study show that the replacement of SPC by partly defatted HM up to a level of 50% (18.5% in total diet) improve the dietary protein quality of the tilapia feeds. Growth performance of Nile tilapia remains similar as well. Nonetheless, FCR and PER are negatively affected at a 100% replacement level of SPC. This observation may be related also to chitin content. However, the effect is less pronounced on protein utilization. This aspect should be examined in further detail. Overall, the present study suggests that insect protein derived from *Hermetia illucens* could be an option to make aquafeed formulation more flexible and sustainable.

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