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Research Article

Does Genetic Background of Rainbow Trout Impact Growth and Feed Utilisation following Fishmeal Substitution by Partly Defatted Insect Meal (*Hermetia illucens*) or Microalgae Powder (*Arthrospira platensis*)?

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A growth trial was carried out to evaluate differences in feed utilisation and growth performance in response to substitution of fishmeal (FM) by either partly defatted insect meal or microalgae powder in different strains of rainbow trout. Three iso-nitrogenous and iso-energetic diets were fed to 495 juvenile rainbow trout with initial weight of 150 ± 37 g. Whereas, the control diet included 20% FM, the two experimental diets were free of FM. Instead of that, an equal amount of spray-dried Spirulina powder (SP) or partly defatted *Hermetia* meal (HM) was applied as alternative protein source. Feed utilisation and growth response were investigated in a commercial breed (TL) and three local strains (HK3, HK7, and HK8) over a period of 56 days using three replicates per diet. Diets were fed relative to fish body weight throughout at a constant rate of 1.0%. Although growth was comparable among diets, growth and feed utilisation differed between breeds. The strain TL tended to express the highest specific growth rate, however, associated with lower feed conversion. Protein utilisation was superior in local strains irrespective of diet, most pronounced between strains HK7 and TL. Due to large differences in initial body weights, compensatory growth might have affected the study's outcome. Both alternative protein sources showed to be adequate to fully replace FM in diets for rainbow trout. Improved adaption to diets including high levels of HM or SP might be achieved by selective breeding.

1. Introduction

Global aquaculture production is still expanding [1]. Among the major impediments to aquaculture production is the availability of sustainable sources of feed ingredients [2, 3]. Although declining, a significant share of world fisheries production is processed into fishmeal constituting a major protein source in a number of aquaculture diets, especially for piscivorous and carnivorous species. Aquaculture currently consumes around 70% of total global fishmeal production, and a predicted increasing demand will force further changes in feed formulations to maintain the sustainable aquaculture growth [1, 2, 4, 5]. Therefore, researchers have been intensively evaluating alternative protein sources [2, 6–8]. Nowadays, terrestrial plant-based products are the most common components in order to replace fishmeal due to their unrestrained market availability and low cost [8, 9]. Despite aquafeeds are formulated to meet fish nutrient requirements, various differences in nutrient composition between traditional marine-based resources

and plant-based diets may impact the two main determinants of growth-feed intake and feed efficiency-as well as other nutritional traits such as digestibility, nutrient retention, and fatty acid profile are observed [10]. Therefore, in carnivorous fish species, complete substitution of fishmeal often remains a challenge as it is frequently associated with reduced performance and fish health [11]. The rapid shift of formulated feeds towards increasing amounts of plant-based ingredients is also challenging future developments in fish breeding. Breeding programmes exist for most major farmed carnivorous fish species. Here, brood stock is selected and assessed mainly based on performance data as derived from marine resource-based feeding [12]. The adaption to the "novel" diets has not been fully realised in many carnivorous fish species under cultivation [13]. Salmonids like rainbow trout (*Oncorhynchus mykiss*) are carnivorous and evolutionarily and metabolically not very well adapted to plant-derived ingredients [11]. Although the potential of selective breeding to improve the utilisation of plant-based diets in rainbow trout has been shown in several studies [10, 14–16], aquafeed producers are looking for further protein alternatives. Additionally, some plant-derived aquafeed protein sources might rather be used for human consumption directly. Sourcing feedstuffs from agriculture for aquafeeds might, furthermore, contribute to the other ecological downsides such as global deforestation and nonsustainable resource consumption [17].

Being part of the natural diet of many fish species, insects, and microalgae (with inclusion of cyanobacteria like Spirulina) seems to be promising fishmeal substitutes receiving increasing attention in recent years [2, 18, 19].

With respect to insects, the black soldier fly (BSF; *Hermetia illucens*) exhibits the potential to act as major feed ingredient in aquaculture since it can be produced on a large-scale basis [20]. Furthermore, larval meals derived from BSF (especially defatted) show favourable nutritional compositions according to an essential amino acid profile, closely resembling the one observed in fishmeal, except for lysine and methionine [7, 21]. The European Union consequently approved the use of BSF-derived ingredients in aquafeeds in the year 2017 [22]. Several studies have shown that BSF is suitable to replace substantial amounts of fishmeal in diets for carnivorous fish without deteriorating growth or fish health (reviewed by [7, 23]). Regarding the use of microalgae in aquafeeds, many studies addressed Spirulina [24] as the most commonly cultured microalgae produced at commercial scale [25]. The nutritional profile is characterised by high levels of protein (40–70% per dry matter) containing all essential amino acids in a mostly well-balanced ratio as well as various vitamins, minerals, pigments, and polyunsaturated fatty acids [19, 24]. Previous studies showed various beneficial effects of Spirulina containing diets, especially on growth performance, meat quality, pigmentation, immunity, and disease resistance mainly in omnivorous fish species [24, 26]. Both ingredients, that is, BSF and Spirulina, have been already successfully investigated in rainbow trout. Some studies showed that up to 50% of dietary fishmeal could be replaced by defatted BSF meal without negative effects on growth [27, 28]. With regard to the application of Spirulina powder, substitution levels up to 75% have been found to be possible [29]. More commonly lower inclusion levels of up to 10% were studied [30–32].

Recently, the experimental diets originally formulated for the present study were used in another trial showing 100% fishmeal substitution by Spirulina which is well accepted by rainbow trout, however, accompanied with inferior growth and feed conversion ratios [33]. Except this and the aforementioned study, total fishmeal replacement of both novel ingredients has not been accomplished to our knowledge yet.

Rainbow trout is the most important carnivorous fish species in European aquaculture. A number of commercial selective breeding programmes continually aim at improving economically important performance traits [34]. According to our information, there is actually no breeding programme considering the adaption of cultivated fish (including rainbow trout) on diets totally substituting fishmeal by insect meal as well as microalgae to improve nutrient utilisation.

Therefore, the present study aimed to detect differences in adaption to a total fishmeal replacement by insect (BSF) or microalgae meals in local rainbow trout breeds that can be used as basis for further breeding programmes. In addition, a commercially improved strain was included as a reference.

2. Material and Methods

The experiment was conducted at the facilities of the Division of Animal Nutrition and Physiology at the University of Goettingen, Germany. The experimental protocol was in accordance with the guidelines on the care and use of experimental animals, i.e. [35], the German Animal Welfare Act [36], more specifically § 7a Section 2 Nr. 3 TierSchG.

2.1. Experimental Diets. Three iso-energetic and iso-nitrogenous diets were formulated to resemble the proximate composition of commercial trout feeds as well as to satisfy nutritional recommendations for rainbow trout (Table 1). Therefore, the control diet was designed to contain only feed ingredients typical for commercial trout feeds and to be in line with the essential amino acid (EAA) requirement recommendations for rainbow trout [37]. Fishmeal (FM) content was restricted to 200 g/kg. Wheat gluten and soy protein concentrate were used as main plant-derived protein sources, both to minimize antinutritional factors (ANF's) and to ensure optimal feed acceptance. Essential fatty acids were supplied by a mixture of fish oil and rapeseed oil. Titanium dioxide was used as inert marker in order to determine feed digestibility. Besides the control diet, two experimental diets (HM20, SP20) were formulated for substitution of fishmeal by either partially defatted *Hermetia* meal (HM), which was obtained from a commercial producer (Hermetia Baruth GmbH, Baruth/Mark, Germany) or commercial spray-dried Spirulina powder (SP; *Arthrospira platensis*). The diets HM20 and SP20 were fully devoid of fishmeal. For all diets, black iron oxide (Fe_3O_4) was used as

dye in order to compensate for the darkening effect of Spirulina powder and ensure equal pellet stain. This measure was necessary in order to avoid selective feeding behavior of the fish. All diets were produced as extruded pellets of either 3 or 4 mm in diameter by SPAROS Lda. (Olhão, Portugal). The final formulations of the diets were made using a database of feed compositions that included both the analyses of SPAROS Lda. as well as own analyses of the raw nutrients and the EAA of the *Hermetia* meal and Spirulina powder. The feed mixtures prepared using this calculation were analysed again before the start of the experiments in order to control the composition. The proximate composition of raw materials and experimental diets is presented in Table 1.

2.2. Experimental Design. The present study was part of a joint research project. All experimental fish used in the present study were derived from a previous on-farm feeding trial at the Division of Aquaculture and Aquatic Ecology at University of Goettingen, Germany (data unpublished) described as follows: Regionally adapted rainbow trout strains were kept at the experimental farm Relliehausen, University of Goettingen. Experimental fish were derived from autumn-spawning rainbow trout populations. In total, 28 full-sib families were produced by artificial reproduction from 8 populations. The offspring was grown to a body-weight (BW) of 31.2 ± 9.4 g. Subsequently, specimens were individually tagged by using passive integrated transponder tags (PIT-Tags) and equally distributed to 200 l tanks using a communal testing design. The fish were already fed the same experimental diets than in the present study (composition see 2.1.) with three replicate tanks per diet. Diet quantity was adjusted according to ambient water temperature (10–14°C) and fish biomass per tank following a comparable feeding table as applied by Stadtlander et al. [28]. Fish were hand-fed once a day with one third of the total daily feed ration. The remainder feed was supplied using an automatic band feeder (Fiap GmbH, Germany). After 90 days of feeding, the family-wise breeding values were estimated using the variable weight gain. Additionally, a commercially improved strain was included in the trial and subjected to the same experimental procedures. On the basis of the described performance testing, four different breeds of rainbow trout were identified to be used in the present study. One breed was a commercial strain (*TroutLodge* Inc., Bonney Lake, Washington, USA; TL) and three breeds were local strains reared at the university facilities (HK3, HK7, and HK8). Fish from TL and HK8 strain represented high performing breeds in the previous on-farm feeding trial, whereas HK3 and HK7 exhibited the low performance strains. Numbers as well as condition of experimental fish available for the present study were dependent on the previous section of the joint research project.

Fish were acclimatised to experimental conditions and feeding diets of the previous on-farm trial until visually assessed apparent satiation (hardly any individual could be attracted by the last pellets). The maximum realised feeding level of 1% BW for HK3, HK7, and HK8 (around 1.2% for TL) was used in the subsequent feeding experiment for all strains aiming to test whether differences in growth observed in the previous on-farm feeding trial could either be related to distinct nutrient utilisation rather than feed intake of strains when reared communally. Furthermore, the applied feeding level should also prevent the excess of unconsumed feed. At the onset of the experiment, all fish were individually weighed. To ensure comparable stocking densities and to account for differences regarding initial body weight (BW), numbers of fish had to be individually adapted resulting in less individuals per tank for the commercial strain (10 fish (TL) or 15 fish (HK3, HK7, and HK8) per tank. After a period of 28 days, fish were weighed group-wise to adjust feed supply. At the end of the experiment, fish were again individually weighed in order to calculate growth performance parameters. Prior to each weighing procedure, fish were fasted for 24 h. Feed utilisation and growth response were investigated over a period of 56 days making use of a closed in-door water recirculation system with 36 tanks (320 l/tank) and three replicate tanks per diet. Feed was supplied at a level of 1% BW based on feeding during the acclimatisation period. Meals were given by hand twice per day. Remaining feed was reweighed to account for accurate feed intake. The water temperature was kept constant at 15.5 ± 0.5°C. A photoperiod of 14 h light and 10 h dark was applied. Water quality parameters were monitored weekly to ensure compliance with reported reference data [38, 39].

2.3. Sampling and Chemical Analyses. At the beginning of the experiments, body composition of three fish per strain and diet (pooled sample; representing mean BW) was analysed serving a reference point for comparison with pooled samples taken at the end of the trial. The latter samples similarly comprised a random sample of three fish (resembling average BW) per replicate, strain, and diet. For sample collection, the fish were killed by an overdose of anaesthetic (Eugenol, 427 mg·L^{-1}, 5 min) and subsequently autoclaved (110°C, 160 min), homogenised (immersion blender), and stored at −20°C for further analyses.

To determine dietary digestibility, the remaining fish were subsequently fed for 14 consecutive days with their respective experimental diet according to the previous experimental protocol. Finally, all remaining fish were dissected in order to collect intestinal contents from the hindgut (rectum) (TL: n = 6-7/tank; HK8: n = 11-12/tank; HK3: n = 11-12/tank; HK7: n = 12/tank) proceeding similar to Percival et al. [40], except that only the last 2.5 cm proximal to the anus was used. Intestinal samples were freeze-dried for 70 hours and stored at room temperature using a vacuum desiccator until final analysis. Due to limited availability of intestinal material, replicate samples needed to be pooled, finally resulting in one pooled sample per strain and experimental diet.

For chemical analyses of dry matter (DM), crude ash (CA), crude protein (CP; $N \times 6.25$; Dumas-method), crude lipid (CL; Soxhlet-procedure, after HCl-hydrolysis), and phosphorus ingredients, diets, homogenised fish samples, and intestinal samples were analysed in duplicates according to the standards

Table 1: Formulation (% as feed basis), proximate composition (% in dry matter), and essential amino acid composition (g/16 gN or % in dry matter) of raw materials (FM = fishmeal, HM = *Hermetia* meal, SP = spirulina powder) and experimental diets.

	Raw materials			Diets		
	FM	HM	SP	Control	HM20	SP20
Ingredients [% as feed basis]						
Fish meal LT70 (NORVIK)[a]				20.00	—	—
Wheat meal[b]				14.00	12.20	12.50
Wheat gluten (VITAL)[c]				20.00	23.00	21.50
Hermetia meal[d]				—	20.00	—
Spirulina powder[e]				—	—	20.00
Soy protein concentrate[f]				20.00	20.00	20.00
Fish oil (sardine)[g]				10.70	10.55	10.70
Rapeseed oil[h]				10.70	9.35	10.70
Vit./min. premix[i]				1.00	1.00	1.00
$CaHPO_4$[j]				1.00	1.00	1.00
Carboxymethylcellulose				1.29	1.24	1.08
Titanium dioxide (marker)[k]				0.50	0.50	0.50
Fe_2O_3-black (dye)[k]				0.07	0.07	0.07
L-Lysine HCl (78% Lys)[l]				0.70	0.90	0.90
D,L-Methionine[m]				0.01	0.16	0.04
L-Tryptophan[l]				0.03	0.03	0.01
Proximate composition [% in DM]						
Dry matter (as feed basis)	93.30	94.47	92.00	94.60	93.70	94.00
Crude protein ($N \times 6.25$)[n]	75.46	60.84	68.91	47.99	47.71	48.62
Crude lipids	7.40	14.08	6.30	26.00	26.57	25.43
Crude ash	18.44	7.49	9.02	7.51	5.76	5.74
NfE[o]	—	17.59	15.76	18.50	19.96	20.21
Chitin	—	11.11	—	—	2.24	—
Gross energy [MJ/kg DM][p]	20.73	22.95	21.46	24.74	25.19	25.00
Essential amino acids		*[g/16gN]*			*[% in diet DM]*	
			NRC [37][q]			
Lysine	7.67	5.42	4.59	2.40	2.84	2.47	2.49
Methionine	3.69	1.24	2.05	0.70	1.14	0.93	1.00
(Cysteine)	0.85	0.80	0.94	0.40	0.74	0.75	0.74
Threonine	4.55	3.57	4.49	1.10	1.65	1.54	1.68
Valine	4.55	5.35	5.38	1.20	1.97	2.09	2.12
Leucine	7.10	6.24	7.99	1.50	3.38	3.21	3.53
Isoleucine	3.84	3.86	5.04	1.10	1.79	1.77	1.93
Phenylalanine	4.69	3.45	4.02	0.90	2.15	2.08	2.18
(Tyrosine)	3.69	7.04	3.94	0.90	1.53	1.77	1.60
Histidine	2.13	2.73	1.51	0.80	0.99	1.07	0.95
Tryptophan	1.14	1.31	1.41	0.30	0.34	0.33	0.33
Arginine	6.82	4.12	7.57	1.50	2.55	2.17	2.71

[a]Sopropêche, France. [b]Molisur, Spain. [c]Roquette, France. [d]Hermetia Baruth GmbH, Baruth/Mark, Germany. [e]*Arthrospira platensis* (feed grade); ERHARD ANDREAS GmbH–import & export, Bremen, Germany. [f]Soycomil P; ADM, The Netherlands. [g]SOPROPÊCHE, France. [h]JC Coimbra, Portugal. [i]Vitamin and mineral content in premix (g or IU/kg): vit. A, as retinyl acetate 948,37 IU; vit. D_3, 267000 IU; vit. E as α-tocopheryl acetate, 8.33; vit. K_3, 1.50; thiamine, 1.09; riboflavin, 1.39; pyridoxine, 0.96; pantothenic acid, 5.31; cyanocobalamin, 0.005; niacin, 7.61; biotin, 0.11; folic acid, 0.60; choline chloride, 113.40; inositol, 46.65; vit. C as L-Ascorbyl-2-polyphosphate, 356.80; betaine, 16.5; Ca, 86.80; P, 97.15; Mg, 30.82; Cl, 8.25; Fe, 5.02; Cu, 0.54; Zn, 1.45; Mn, 1.28; I, 0.10; Se, 0.02. [j]Phosphea, France. [k]Sigma Aldrich, USA. [l]Ajinomoto EUROLYSINE S.A.S, France. [m]ADISSEO, France. [n] protein content was not corrected for chitin. [o]NfE, nitrogen-free extract = 100 – (crude protein + crude lipid + crude ash). [p]calculated by energy conversion factors (MJ/kg): crude protein = 23.6; crude lipid = 39.5; NfE = 17.2. [q]NRC, National research council; recommended dietary levels of essential amino acids for rainbow trout.

defined by the Association of German Agricultural and Analytic Research Institutes [41]. Nitrogen-free extracts (NfE) were calculated by difference (NfE = 100 – (H_2O + CP + CL + CA)). Amino acid (AA) analyses were conducted using chromatographical methods [41]. Chitin content in HM was analysed by the Fraunhofer-IGB (Stuttgart, Germany) through the determination of *acetyl*-groups after total hydrolysis following the protocol of Hahn et al. [42].

2.4. Calculated Parameters. Growth and nutritional indices were calculated as follows:

$$\begin{aligned}
\text{Metabolic body weight}\left(\text{MBW}, \text{kg}^{0.67}\right) &= \left[\frac{\left(\text{BW}_{\text{final}}\,(\text{kg}) + \text{BW}_{\text{initial}}\,(\text{kg})\right)}{2}\right]^{0.67},\\
\text{Feed intake, metabolic}\left(\text{FI}_{\text{m}}, \frac{\text{g DM}}{\text{BW kg}^{0.67}}\right) &= \frac{\text{total feed intake (g)}}{\text{MBW}\left(\text{kg}^{0.67}\right)},\\
\text{Feed intake}\left(\text{FI}, \frac{\text{g DM/BW kg}^{0.67}}{\text{day}}\right) &= \frac{\text{FI}_{\text{m}}\left(\text{g DM/BW kg}^{0.67}\right)}{\text{days of experiment}},\\
\text{Specific growth rate (SGR, \%)} &= \frac{\left(\ln \text{BW}_{\text{final}}\,(\text{g}) - \ln\ \text{BW}_{\text{initial}}\,(\text{g})\right)}{\text{days of experiment}} * 100,\\
\text{Feed conversion ratio}\left(\text{FCR}, \frac{\text{g}}{\text{g}}\right) &= \frac{\text{total feed intake (g)}}{\text{total weight gain (g)}},\\
\text{Protein efficiency ratio}\left(\text{PER}, \frac{\text{g}}{\text{g}}\right) &= \frac{\text{total weight gain (g)}}{\text{total protein intake (g)}},\\
\text{Protein deposition (PD, \%)} &= \frac{\left[\left(\text{BW}_{\text{final}}\,(\text{g}) * \text{body protein}_{\text{final}}\,(\%)\right) - \left(\text{BW}_{\text{initial}}\,(\text{g}) * \text{body protein}_{\text{initial}}\ (\%)\right)\right]}{\text{total feed intake (g)} * \text{feed protein (\%)}} * 100,\\
\text{Phosphorus deposition (PhD, \%)} &= \frac{\left[\left(\text{BW}_{\text{final}}\,(\text{g}) * \text{body phosphorus}_{\text{final}}\,(\%)\right) - \left(\text{BW}_{\text{initial}}\,(\text{g}) * \text{body phosphorus}_{\text{initial}}\ (\%)\right)\right]}{\text{total feed intake (g)} * \text{feed phosphorus (\%)}} * 100.
\end{aligned} \tag{1}$$

2.5. Statistics. The statistical analysis of $\text{BW}_{\text{initial}}$, BW_{final}, SGR, FCR, PER, PD, and PhD was analysed using the mixed procedure of SAS version 9.3 (SAS Institute, Cary, NC, USA) using the following model:

$$y_{ijkl} = \mu + \alpha_i \times \beta_j + T_{ijk}\,(\alpha \times O) + b\left(W_j\right) + e_{ijkl}, \tag{2}$$

where y_{ijkl} is the respective dependent variable (e.g. SGR, FCR or PER), μ is the general mean, α_i is the fixed effect of feed group, $ß_j$ is the fixed effect of breed, b is the regression coefficient of the pre-experimental weight (W_j), T_{ijk} is the random effect of the tank, and e_{ijkl} is the random error term. Differences were tested applying the Tukey→Kramer test.

3. Results

3.1. Growth Performance and Protein and Phosphorus Utilisation. Survival of the fish during the experiment was 98.8%. All experimental diets were very well accepted as no quantity of feed was rejected.

Due to different growth response of strains and limited supply from fish from the previous on-farm feeding trial (see 2.2.), selection for similar initial BW was virtually impossible. Only similar initial BW for each strain within experimental diets could be nearly realised, except for the commercial breed (Table 2).

Dietary composition did not affect growth performance or protein utilisation whereas differences between breeds could be observed (Table 2). Fish from the commercial strain (TL) provided the highest SGR but without being significant. In contrast, FCR was, in general, inferior to TL in all diets compared to the local strains where HK7 was most efficient. The latter could also be observed regarding protein utilisation. HK3, HK8, and especially HK7 showed significant higher PER and PD, respectively. This was also reflected in bodyweight development with differences in final bodyweight between breeds which were less pronounced than in the initial weights. Regarding PhD, similar effects could be observed (HK7 significantly higher than TL). Additionally, PhD was also influenced by dietary composition. Fish fed the control diet showed significant lower PhD as compared to the fish fed diet HM20 or diet SP20 (Table 2).

3.2. Protein and Amino Acid Digestibility. As mentioned above (see 2.3.), due to the limited amount of gut material, samples from replicate tanks of each strain per diet had to be pooled. Therefore, statistical analysis of protein and AA digestibility was not feasible. Consequently, observations should be considered as preliminary indications rather than definitive results. While protein and AA digestibility seemed to be influenced more by diet and breed, DM digestibility was only influenced by breed (Table 3). The digestibility of CP, AA, and DM was generally higher in TL and HK3 than in HK7 and HK8. Nutritional effects could be observed in CP (Control, SP20 > HM20) as well as in the (semi-)essential AA

Table 2: Results* of feeding trial with one commercial strain (TroutLodge, TL) and three "local" strains (HK3, HK7, HK8) of rainbow trout fed either a fishmeal-based diet (control) or diets where fishmeal was fully substituted by *Hermetia*-meal (HM20) and spirulina-meal (SP20).

Diet	Breed	∅ $BW_{initial}$/fish (g)	∅ BW_{final}/fish (g)	SGR (%)	FCR (g/g)	PER (g/g)	PD (%)	PhD (%)
Control	TL	$223.80^{Ax} \pm 3.67$	299.92 ± 8.07	1.33 ± 0.06	1.19 ± 0.15	$1.69^{x} \pm 0.15$	$30.51^{x} \pm 2.57$	$21.39^{Ax} \pm 2.71$
	HK3	$133.51^{y} \pm 3.00$	275.17 ± 4.73	1.14 ± 0.03	0.93 ± 0.09	$2.62^{y} \pm 0.09$	$45.57^{yz} \pm 1.51$	$33.20^{Axy} \pm 1.59$
	HK7	$112.47^{z} \pm 3.00$	272.09 ± 5.22	1.13 ± 0.04	0.75 ± 0.10	$2.93^{y} \pm 0.10$	$48.48^{z} \pm 1.67$	$35.08^{Ay} \pm 1.75$
	HK8	$146.57^{y} \pm 3.67$	281.37 ± 5.71	1.16 ± 0.04	0.94 ± 0.11	$2.54^{y} \pm 0.11$	$37.76^{xy} \pm 1.82$	$25.44^{Ax} \pm 1.92$
HM20	TL	$199.96^{Bw} \pm 3.80$	$308.69^{x} \pm 7.12$	1.34 ± 0.05	1.02 ± 0.13	$2.18^{x} \pm 0.14$	$33.35^{x} \pm 2.27$	$37.07^{Bx} \pm 2.39$
	HK3	$133.11^{x} \pm 3.03$	$274.37^{y} \pm 4.79$	1.14 ± 0.03	1.05 ± 0.09	$2.67^{xy} \pm 0.09$	$42.84^{xy} \pm 1.53$	$50.60^{By} \pm 1.61$
	HK7	$106.33^{y} \pm 3.00$	$269.27^{y} \pm 5.44$	1.13 ± 0.04	0.65 ± 0.10	$3.02^{y} \pm 0.10$	$48.72^{y} \pm 1.73$	$52.68^{By} \pm 1.83$
	HK8	$154.90^{z} \pm 3.10$	$289.18^{xy} \pm 4.87$	1.23 ± 0.03	0.87 ± 0.09	$2.58^{xy} \pm 0.09$	$42.79^{xy} \pm 1.56$	$43.56^{Bxy} \pm 1.64$
SP20	TL	$195.86^{Bx} \pm 3.73$	293.62 ± 6.87	1.27 ± 0.05	1.06 ± 0.13	$2.04^{x} \pm 0.13$	$34.16^{x} \pm 2.19$	$42.07^{Bx} \pm 2.31$
	HK3	$127.98^{y} \pm 3.00$	281.95 ± 4.82	1.18 ± 0.03	0.92 ± 0.09	$2.71^{yz} \pm 0.09$	$41.60^{xy} \pm 1.54$	$47.63^{Bxy} \pm 1.62$
	HK7	$113.18^{y} \pm 3.00$	269.07 ± 5.20	1.11 ± 0.04	0.72 ± 0.10	$2.87^{z} \pm 0.10$	$46.06^{y} \pm 1.66$	$53.80^{By} \pm 1.75$
	HK8	$158.60^{z} \pm 3.00$	277.57 ± 4.76	1.15 ± 0.03	1.00 ± 0.09	$2.32^{xy} \pm 0.09$	$39.25^{xy} \pm 1.52$	$43.85^{Bx}v \pm 1.60$

*Least squares means ± SD (values corrected for differences in initial body weight), followed by different letters in the same column are significant different at a level of $p < 0.05$ (Tukey-Kramer test): capital letters (A, B, C) = diet effect within breeds, small letters (w, x, y, z) = breed effect within diets. $BW_{initial}$ = average bodyweight of fish at the beginning of feeding trial. BW_{final} = average bodyweight of fish at the end of feeding trial.

arginine (Control, HM20 > SP20), lysine, cysteine (both: Control, SP20 > HM20), valine, leucine, and isoleucine (all: Control > SP20).

4. Discussion

4.1. Growth Performance and Protein and Phosphorus Utilisation. All experimental diets were readily accepted (i.e., no feed refusal) by the fish even when FM was completely replaced by HM or SP. Similar observations were reported by Rosenau et al. [33] using the same diets (except HM20) and feeding level. The suitability of the diets was also confirmed as all experimental groups (except for the commercial strain TL) almost doubled their initial body weight. However, in earlier studies where partially defatted HM was fed to rainbow trout [23, 27, 28], higher feeding levels could be realised than in the present study ranging from 1.3 to 1.6% of BW. However, those authors reported lower levels of FM replacement (up to 50%). In this context, the outcome of the present study might be related to differences in initial BW or genetic background (which can affect feed intake as discussed below) of experimental fish when compared to the latter authors. As mentioned earlier, the FM content of experimental diets used in the present study was closer to formulations of commercial trout feeds than in the cited studies, where much higher levels were applied (20 vs. 60%). Consequently, the higher dietary FM content may have contributed to an increased feed attraction.

It has been reported that full substitution of FM with SP is only suitable in carp feed [26]. As yet, a maximum replacement in rainbow trout diets was shown to be possible only at 75% in combination with 25% soybean meal (corresponding to 45% SP in feed) and without negative effects on growth performance and feed intake [29].

A study by Rosenau et al. [33] using diets control and SP20 from the present study confirmed that FM can be totally replaced by SP causing no changes in feeding behaviour but in contrast was associated with reduced growth performance. Other investigations in rainbow trout applied only lower dietary SP concentrations, not exceeding 10% and FM replacement at similar levels [30–32] without any feed aversion. Therefore, observations on feed acceptance in the present study with an intermediate dietary SP of 20% are consistent with the aforementioned studies. Positive results in feed acceptance were also reflected in growth performance. Replacing fishmeal with the respective alternative protein source had no significant impact on growth parameters. For SGR and FCR, this was in line with previous studies in rainbow trout and partly defatted HM as well [23, 27, 28]. SGR in the aforementioned studies was somewhat higher than in the present one (1.4 vs. 1.1–1.3) but FCR was of similar range. In contrast, Dumas et al. [43] reported a significant inferior FCR when totally replacing FM by partly defatted HM. This may be attributed to different dietary composition as well as the quality of ingredients used (e.g., dietary lipid content, quality of HM, and replaced FM) that may influence final results [21]. In their review of a systematic meta-analysis on BSF in salmonid diets, Weththasinghe et al. [21] revealed a linear decrease in SGR as well as linear increase in FCR with the increasing level of FM replacement in rainbow trout, but it was not confirmed by the present study. The discrepancies with the present study are probably due to the fact that the authors also used studies in their data set in which both partially defatted HM and nondefatted HM were included in the meta-analysis. The absence of any effect on growth performance was confirmed by similar PER and PD when fed either the control diet or diet HM20 with total FM replacement. PER values were comparable to previous investigations [27, 28], except the commercial breed (TL) with inferior response. Renna et al. [27] could also not observe significant effects on PER when replacing FM by HM, whereas Stadtlander et al. [28] found a significant reduction in PER as well as PD (calculated as protein productive value PPV). This observation and the overall higher PD values in the latter study may be attributed to differences in quality of the applied HM and the replaced FM level as already discussed above for SGR and FCR.

Table 3: Apparent digestibility coefficients* of dry matter (DM), crude protein (CP) and (semi-) essential amino acids in different strains of rainbow trout fed either a fishmeal based (control) or fishmeal-free diets (HM20, SP20).

Diet	Control				HM20				SP20			
Breed	TL	HK3	HK7	HK8	TL	HK3	HK7	HK8	TL	HK3	HK7	HK8
DM	76.6	76.4	72.4	71.6	73.9	72.7	72.4	69.3	77.2	76.2	71.3	71.8
CP	89.9	89.5	84.8	82.5	86.1	84.9	84.4	79.5	88.1	87.5	81.7	81.2
Cys	87.1	86.9	78.6	77.5	85.8	85.0	81.0	77.3	88.6	88.6	81.6	80.6
Met	92.9	91.9	86.8	84.5	91.1	90.6	87.8	82.7	93.7	93.3	86.9	86.3
Thr	89.7	88.6	81.3	79.2	87.2	86.6	82.6	77.8	88.7	87.8	80.1	79.4
Val	89.8	89.5	82.8	80.4	88.1	87.0	84.2	80.4	87.5	86.2	79.0	77.9
Ileu	92.7	91.8	87.4	85.2	91.2	90.5	87.8	84.2	90.9	90.4	84.8	83.7
Leu	92.7	92.1	88.0	85.4	91.1	90.0	87.7	83.9	89.8	89.6	84.4	83.0
Tyr	92.8	92.8	87.4	84.5	92.2	91.6	89.3	86.0	91.4	91.7	85.1	85.0
Phe	94.2	93.7	90.0	87.9	93.9	93.1	91.3	88.1	94.1	94.1	89.4	88.8
Lys	92.2	91.3	86.0	83.0	89.4	88.3	85.4	79.7	92.1	91.9	84.3	83.3
His	91.8	91.1	85.1	83.1	89.7	88.5	86.0	83.0	91.8	92.2	85.5	85.1
Arg	94.7	94.5	90.5	88.2	93.7	93.2	91.1	87.5	85.9	85.9	80.6	80.1

*Values could not be tested for statistical differences due to limitation of replicates. HM20 = fishmeal was completely substituted by *Hermetia*-meal; SP20 = fishmeal was completely substituted by spirulina-meal.

Unfortunately, a limited number of studies are available regarding growth performance of rainbow trout feeding SP as comparable to the present study. Hernandez et al. [29] reported that substitution of 75% FM by SP caused no negative effects on SGR, FCR, and PER. However, SGR and PER in the reported study were superior to the present results, but FCR was of similar range. Differences likely existed due to smaller fish as well as higher feeding levels as applied by the latter authors. Another study by Teimouri et al. [30] also confirmed no negative effect on growth performance when replacing FM by SP, however, at a lower maximum level of inclusion (10%). In contrast, Rosenau et al. [33] observed inferior weight gain, SGR, and FCR when feeding similar diets (Control vs. SP20). The different results could be related to distinct genetic origin of the experimental fish affirmed by higher variances in final BW within the latter investigation, indicating deviant feed utilisation or feeding behaviour. The same author [44] also showed negative response in growth but not FCR in African catfish when using the same dietary composition. The differing life stage as well as fish species under investigation are expected to be responsible for these distinct results. Rosas et al. [26] considered the importance of fish species as the percentages of FM to be replaced which vary greatly according to species because the nutritional habits of the organisms studied strongly influence the digestibility, retention, and absorption of nutrients.

In general, the suitability of both alternative protein sources (HM, SP) and their respective diets (HM20, SP20) is highlighted by the fact that FCR values in all feeding groups of the current study ranged between 0.65–1.19, which is much lower than the estimated FCR average value of 1.3 for farmed trout [9, 45].

Differences in growth performance between the different strains due to the applied diet maybe attributed to some extent to varying genetic background as a result of selective breeding history.

Commonly, growth of fish from more selected strains is superior to less selected ones, especially towards their wild counterparts [34, 46]. For example, Janssen et al. [34] estimated a cumulative genetic gain in thermal growth coefficient of 200% and up to 900% cumulative genetic gain in harvest weight over the course of 8–20 selected generations. They also mentioned that up to 13% improvement in harvest weight per selected generation could be realized. Martens et al. [46] observed that the rapid growth of domestic fish was achieved through the combination of enhanced feed consumption as well as lower feed conversion ratio relative to wild fish. In this context, results of the present study are somewhat unexpected as the higher selected commercial breed TL showed on one hand higher SGR but on the other hand inferior FCR, PER, and PD. Higher SGR values could be slightly misleading and more attributed to higher initial bodyweights of TL fish. The latter is confirmed by the fact that fish from the less selected local strains (HK3, HK7, and HK8) sometimes gained more than double their initial bodyweight, whereas fish from TL only gained about 50%. Therefore, the authors of the present study also considered the influence of compensatory growth on the resulting performance. The current study was part of a joint project and fish were obtained from a previous on-farm feeding trial (see Section 2.2). In consequence, fish of the various breeds showed significant different initial bodyweights (especially between TL and HK7). All breeds were held together in the preceding on-farm feeding trial. Unfortunately, accurate feed intake of the respective strains could not be determined by the project partners. Martens et al. [46] mentioned that the main drivers of increased growth of domesticated strains include selection for greater appetite (i.e., motivation and feed consumption level) as well as feeding efficiency (i.e., mass gained per unit of feed) and could be responsible for the better condition of fish from TL when starting the recent study. Domestication of fish can also promote a more aggressive feeding and dominant social behaviour when selected for the improved growth [47]. Hyperphagia is, by far, the most common mechanism of growth compensation [48]. As all breeds in the present experiment were fed at the same feeding level, hyperphagia was mostly excluded. Nevertheless, hyperphagia in fish of the low performing strains (HK3 and HK7) might have occurred as a result of feeding level compensation from the previous on-

farm trial as well as due to the fact that fish were not reared communally, possibly alleviating strain-specific competitiveness. Won and Borski [49] found that hyper anabolism during refeeding is fuelled by an influx of metabolic substrates that are rapidly allocated to somatic growth through heightened mitogenic activity of the growth axis (e.g., increased growth hormone levels). However, hyperphagia alone may not account for the accelerated growth rate experienced during the compensatory growth. An increased growth hormone level can improve protein assimilation as well as FCR and the high substrate assimilation rates during the hyperphagia drive compensatory growth by partitioning resources specifically to the skeletal growth rather than to energy reserve deposition; the latter authors elucidated. The higher PER and PD values determined in the local breeds (HK3, HK7, and HK8) previously performing worse than the commercial strain TL indicated the presence of improved protein utilisation. This effect was more pronounced when the growth deficit between strains was higher at the beginning of the present feeding experiment (resulting from the previous on-farm trial).

Furthermore, results of Sanchez et al. [50] indicate that fish selected for enhanced growth rates, based on higher feed intake, might express their full growth potential only when fed ad libitum, and they might not be capable to compensate for equal or even lower feed or nutrient utilisation under restricted feed supply when compared to unselected fish. Feeding levels of 1% BW as applied in the present study have been somewhat stayed below apparent satiation of strain TL (see Section 2.2). Besides the impact of compensatory growth, the latter might additionally have contributed to the observed differences when compared to strains HK8, HK3, and HK7 which were fed close to apparent satiation. Furthermore, these observations might underpin the hypothesis that the strain TL had mainly been selected for high feed intake.

The observed differences between breeds regarding PhD are maybe related to similar context. Differences in PhD between diets could be due to distinct dietary phosphorus concentrations.

Phosphorus levels of diets HM20 (0.99% of DM) as well as SP20 (0.83% of DM) were next to the recommended dietary level for rainbow trout (0.7% of DM; [37]) whereas the control diet was of higher concentration (1.29% of DM). Riche and Brown [51] showed that an excessive dietary supply can reduce phosphorus utilisation.

4.2. Protein and Amino Acid Digestibility. In order to discriminate if the observed differences in growth performance were either caused by genetic background or improved feed utilisation related to compensatory growth, the digestibility of experimental diets was determined.

Obtaining digesta prior to its natural voidance as faeces directly from fish by stripping or dissection may result in the collection of an unknown amount of incompletely digested material probably causing underestimated ADC-values [37, 52]. However, none of the general applied procedures of fish faecal collection is without error [37, 53] and there is probably no perfect method. Bob→Manuel [53] noted that the most efficient method of faecal collection is yet to be established and standardisation of the techniques in faecal collection, pooling of faecal collection for chemical analysis, and time of collection of faeces is necessary. Thus, faecal collection always includes some degree of compromise, which affects the subsequent results and the comparability with other experiments. In this context, Hancz and Varga [54] argued that the most reliable way to obtain adequate and practically useful results is using the same system consequently while comparison of results obtained by different methodologies emerges lots of problems. The latter authors also mentioned that direct or active methods remained a viable procedure firstly for fish with a short and straight intestine like salmon and trout. Several studies suggested that faecal collection by dissection can provide reliable digestibility values if done correctly reducing drawbacks as much as possible [55–57]. In the present study, faecal samples were taken 12 hours after the last meal to ensure complete digestion of the food. The procedure was based on studies with salmonids [58]. Alternatively, the dissection method was used instead of stripping. As mentioned above, comparison of ADC values between studies applying different faeces collection methods is debatable. Nevertheless, ADC values of DM and CP are in the range of studies using similar HM [23, 27] but lower than that reported for SP [29]. Unfortunately, the latter study did not provide detailed information about the method of faecal collection that may explain the deviation.

Regarding the observed trend towards lower digestibility for DM and CP in diet HM20, previous studies suggested that the presence of chitin, a feature of the insect's exoskeleton, may be the reason for negative effects on ADC values, especially protein [27]. Furthermore, the relevance of amino acids bound by the chitin matrix in the insect's exoskeleton has to be considered, which could reduce the availability of protein in HM for protease enzymes or the activity of protease enzymes [21]. Even though chitin can compromise protein digestibility, no effect of the use of HM on ADC of DM and CP in rainbow trout was reported [23]. The maximum chitin level reached in the HM20 diet (2.24 g/100 g DM) was slightly above the 2% threshold. According to Renna et al. [27], a chitin level higher than 2% contributes to a decrease of CP digestibility. Nevertheless, the observed differences of ADC in the present study seem to be of marginal importance as no dietary effect on growth performance could be detected.

Considering digestibility related variations between breeds in the current study results in growth performance, especially in PD; it was expected that they were also caused by differences in the ADC of protein. But in contrast, the significant higher PD of HK7 compared to that of TL was not associated with a higher ADC of CP, and the opposite was observed (TL > HK7 for all diets). Therefore, it is unlikely that the higher PD of HK7 was due to better dietary adaption than an increased metabolic protein utilisation associated with compensatory growth (see Section 4.1.).

At this point, the authors would like to keep in mind again that due to the small amount of digesta samples in the present study, it was not possible to perform chemical analyses within each replicate. Therefore, samples had to be pooled, resulting in only one sample for each strain within each experimental

diet. Consequently, statistical analysis was not possible, thus only providing estimated ADC-values. Although no replicates were used, the ADC-values observed can be considered nearly representative as they were within the range reported in other studies (see above). In this context, further investigations are recommended to confirm the present results. To increase the amount of sampled faecal material and, hence, improve the determination of diet digestibility, alternative collecting methods may be considered. Using methods removing excretions from the fish tank soon after expulsion, the collection of passively excreted faeces by settling (Guelph system) or filtration (St-Pee system) can give good digestibility data [37, 53]. Both systems allow collecting faeces several times under the existing rearing conditions and, therefore, can increase the amount of sampling material compared to the dissection method (where fish can be sampled once only). Both methods rely on solid and nonfloating faeces particles to minimize either loss by nutrient leaching (due to break up of faecal material) or allow effective collection by settling as well as filtration. As characteristics of excretions depend on dietary formulation, faeces consistency should be tested before selecting the appropriate collecting method. Since neither of both aforementioned systems was available for the present study, the dissection method was selected to be an adequate option consequently as already discussed. Besides the sampling method, an increased feed ration might increase the amount of faeces excreted by the fish and therefore may offer the opportunity to obtain more sampling material when applying the dissection method. As the feeding level in the present study was observed to be close to apparent satiation, an increase of the feeding level would likely have led to an excess of uneaten feed rather than resulting in more sampling material. Different feeding levels in rainbow trout do not seem to influence digestibility [59] and should thus unlikely have affected results in the present study.

5. Conclusion

The present study has shown that FM can be completely substituted by either HM or SP in diets for rainbow trout without negative effects on growth performance. Both ingredients offer valuable alternative protein sources that can be used in more sustainable FM-free aquafeed formulations, even in carnivorous fish. Observed differences in protein utilisation between breeds appear to be related to compensatory growth rather than genetically fixed natural dietary accommodations. Hence, further improvement of feed acceptance as well as utilisation with high levels of HM or SP might be achieved by additional selective breeding.

Data Availability

The data supporting the results of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The study was carried out in accordance with the EU Directive 2010/63/EU for animals used for scientific purposes and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (ref. number: 33.9-42502-05-18A298).

Disclosure

Particular data were presented at the 74th conference of the Society of Nutrition Physiology [60].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S.W. and F.L. devised the study, the main conceptual ideas, and outline. C.D. and A.S. performed the suggested experiment and were responsible for numerical calculations and data analyses. R.S. conducted statistical analyses. Resources were provided by C.D., A.S., and J.G. C.D. wrote the draft for manuscript. A.S., S.W., and F.L. contributed to the final version of the manuscript. F.L. and A.S. were responsible for supervision and administration of the project. S.W. and F.L. realised the funding acquisition.

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