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Research Article

Digestibility and Growth Response of Rainbow Trout (*Oncorhynchus mykiss*) Fed Coated and Non-Coated Crystalline Essential Amino Acids as Supplements in Plant-Based Feeds

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Abstract

The efficacy of using essential amino acids to support the inclusion of high plant based proteins in diets for rainbow trout was undertaken over a 56-day growth and feed utilisation study with juvenile (60g) rainbow trout (*Oncorhynchus mykiss*). Fish were fed diets where a predominantly white fishmeal based control diet was compared to diets where full fat soyabean meal (FFS) as well as maize gluten meal (MGM) were incorporated respectively, at significant levels (up to 75% of the fishmeal). Ten essential amino acids (EAAs) were supplemented in these diets in both standard and coated forms to evaluate their potential to rebalance the dietary EAA profiles more precisely. All diets were adjusted to be isonitrogenous and isolipidic. In addition to the growth trial, a low purified protein diet was fed to the fish separately (at a mean weight of 200g) to collect faeces for determining endogenous protein loss as well as the individual experimental diets described. As such, it was possible to calculate the true digestibility for essential amino acids. The results indicated that corrective amino acid fortification of high plant diets, especially MGM, could significantly improve trout performance and raise their nutritional value. This was confirmed by final body weight, specific growth rate, feed conversion ratio, and protein utilisation values. The control (animal protein blend/fishmeal protein) reference diet fed trout (Ref) gave superior and significant ($P<0.05$) growth performance with a 146% overall increase in body weight. Conversely, FFS (Full Fat Soyabean meal) fed trout was the most inferior performing group amongst the experimental diets, with only a 78% increase in weight gain over the same period. Supplementation of this basic formulation with a mixture of essential amino acids in crystalline form resulted in a moderate but significant ($P<0.05$) improvement in overall growth performance (86.80%) and, in particular for MGM+EAAS (Maize Gluten and Essential Amino Acid, uncoated) fed trout when in protected coated form, yielding an SGR of 125% ($P<0.05$). The true digestibility of the EAAs was similar to the values of each digestible EAA, but coated EAAs were slightly superior in their digestibility coefficients. Coated EAAs improved digestibility coefficients by 2–5% and FCR by 8% compared to crystalline forms and also improved their utilisation, leading to better trout performance with respect to growth, feed conversion efficiency, and nitrogen (crude protein) retention ($P<0.05$). The findings are discussed in the context of sustainable feeds and the circular bioeconomy for fish farming.

Keywords: Rainbow Trout; Plant-based Diets; Coated Amino Acids; Amino acid Digestibility; Feed efficiency

1. Introduction

The aquaculture industry is increasingly focusing on sustainable and cost-effective feed formulations, particularly for species like rainbow trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*). As such, the global aquaculture feed sector faces increasing pressure to reduce its reliance on fishmeal (FM) as a primary protein source in aquafeeds due to economic and environmental concerns [1]. Soyabean meal (SBM) has emerged as an established alternative due to its availability, cost-effectiveness, and favourable amino acid profile. However, SBM is deficient in certain essential amino acids (EAAs), such as methionine and lysine, and contains anti-nutritional factors (ANFs) that can impair growth performance and nutrient utilisation in carnivorous fish species like rainbow trout [2, 3]. Research indicates that up to 50% of fishmeal can be replaced with enzyme-treated soyabean meal without adverse effects on growth performance or feed conversion ratios in rainbow trout fry with considerations to anti-nutritional factors in diet ingredients [3]. Additionally, maize gluten meal and various other legume, pulse, and cereal derived protein concentrates have been utilised increasingly in compound aquaculture diets.

It has been stated previously that fish require a critical balance of essential amino acids (EAAs) rather than extrinsically dietary protein. The combined EAAs constitute the "ideal protein". This concept was first proposed by Wang and Fuller for pigs and is just as relevant for fish of all species [4, 5]. In fish, this can often be related to the composition profile of muscle or gonadal tissue and the relative essential amino acid index. To address this, supplementation of fish feeds with crystalline amino acids (CAAs) has been explored for many animal species, and also with fish [6]. Studies have demonstrated that incorporating CAAs, such as lysine, methionine, and threonine, into plant-based diets can effectively improve growth performance and protein retention in rainbow trout to achieve optimal performance and better feed efficiency in production [7].

Replacing fishmeal with plant-based ingredients like soyabean and maize gluten meals, supplemented with crystalline amino acids, offers a viable strategy for developing cost-effective and sustainable diets for many fish species such as salmon, rainbow trout and tilapia [7, 8, 9]. This approach not only maintains optimal growth performance and feed utilisation but also contributes to the sustainability of aquaculture practices. Supplementation strategies typically involve the use of either crystalline or coated amino acids (in a protected shell of protein). Crystalline amino acids (CAAs) are free-form compounds that are rapidly absorbed in the gastrointestinal tract, often leading to mismatches in amino acid availability relative to *in vivo* protein synthesis needs. This asynchronous availability can reduce the efficiency of nitrogen utilisation and potentially limit growth performance [9, 10]. Conversely, Coated (Co) Amino Acids (CoAAs) are encapsulated in protective layers of polymeric proteins designed to modulate their release and absorption rates, thereby aligning more closely with the temporal demands of protein synthesis [11]. These differences in absorption dynamics suggest that CoAAs may outperform standard CAAs in sustaining optimal growth and feed conversion rates in fish [12]. Furthermore, understanding the bioavailability and metabolic fate of supplemented EAAs is crucial to optimizing their use in aquaculture feeds. In fact, delaying EAA absorption can act to enhance their efficiency to better time their uptake along with the complete protein degradation during the main digestion phase in the midgut of fish [13]. Salmonid species like trout (*O. mykiss)* and Atlantic salmon (*S. salar)* have quite stringent demands for balanced essential dietary amino acids, and this becomes limiting when fishmeal is reduced at the expense of plant protein [14]. White fishmeal sources are often used in many organic and sustainable fish production systems, and currently there is an increasing use of lower specification fishmeal sources generated from trimmings and discards within the capture fisheries industry [15].

Consequently, the principal aims of this investigation were to evaluate the growth performance and feed utilisation, of rainbow trout fed a full fat soyabean meal (FFSBM) and maize gluten meal based diets supplemented with either crystalline or coated EAA mixtures. By comparing these supplementation strategies, we provide insights into optimizing plant-based diets for sustainable aquaculture practices in lower grade white fishmeal type diet formulations. Our experimental objectives to evaluate the optimal use of essential amino acids in diets for rainbow trout incorporated high plant protein concentrates in a classical growth trial. A consecutive digestibility evaluation for the essential amino acids was also performed, and an additional low protein diet was formulated in order to ascertain their 'true digestibility' by quantitating the endogenous losses to correct apparent digestibility coefficient values.

2. Materials and Methods

2.1. Diets

Six experimental diets were formulated for rainbow trout to evaluate the feasibility of replacing the fishmeal component with either full fat soyabean meal (FFS) or maize gluten meal (MGM) as major plant protein concentrates. The effect of using supplementary crystalline essential amino acids (CAAs and coated CoAAs) to restore dietary levels to those in the control white fishmeal and animal protein based diet was compared with EAA mixtures as well as coated (polymeric protein complex) versions for the plant substituted diets. All diets were formulated using FeedSoft^{TM} (Austin, Texas) feed formulation software with a designated rainbow trout database for nutrient requirements. These experimental diets are presented in Table 1, showing their respective ingredient composition profiles and nutrient analysis.

Diets were physically processed by blending the dry ingredients into a homogenous mixture with a Hobart A 120 industrial food extruder. The required supplementary oil for each diet was added gradually and after a few minutes of mixing, 350ml of water was added to prime the binder. Once a homogenous dough mixture was obtained, the diets were extruded through a mincer into 'spaghetti-like' strands and broken into smaller 3mm pellets. Pellets were dried by convection air in an oven at 70°C. After cooling, the diets were packed in sealed airtight containers and stored at 15°C until required. Prior to feeding the fish, the diets were further broken into smaller pellets (1-3mm diameter) to suit the gape size of juvenile and later production size rainbow trout for digestibility assessments.

2.2. Fish and feeding trial regime

720 all-female rainbow trout *(O. mykiss*) of mean weight 30g were randomly distributed (40 fish per tank) into each of 18 tanks (Six triplicate treatment groups) of 0.5m^3 capacity within a semi-closed recirculation system with incorporated mechanical and biological filter compartments. The water temperature was held at a constant 15°C throughout the growth trial period of 8weeks (56 days). A photoperiod of 12hrs light and 12hrs dark was maintained for the 8weeks period.

Table 1. Composition of experimental diets fed to juvenile rainbow trout (*Oncorhynchus mykiss*) (g/kg).

Ingredients	Ref diet	FFS	FFS+ EAAS	MGM	MGM+EAAS	MGM+EAAS coated
White fishmeal (66)	537.3	150	150	150	150	150
Full-fat soya bean (36)	-	566.3	555.5	-	-	-
Maize gluten meal (60)	-	-	-	283.3	235	235
Poultry byproduct meal	100	100	100	100	100	100
Blood meal	20	20	20	20	20	20
Fish oil (Marine)	81.8	15	15			
Wheat middling flour	205.9	103.4	88.5	311.8	308.2	308.2
Vitamin premix[1]	20	20	20	20	20	20
Mineral premix[2]	20	20	20	20	20	20
*Supplementary amino acids						
D.L. Methionine	-	-	5.4	-	4.8	4.8**
L Lysine	-	-	5.8	-	15.2	15.2*
L Threonine	-	-	3	-	5.5	5.5*
L Histidine	-	-	0.7	-	2.4	2.4
L Argine	-	-	3.9	-	13.1	13.1
L Isoleucine	-	-	1.3	-	4.2	4.2
L Leucine	-	-	3.1	-	-	-
L Valine	-	-	2.9	-	4.4	4.4
L Tryptophan	-	-	-	-	1.3	1.3
Dietary marker; chromic oxide (Cr_2O_3)	5	5	5	**5**	5	5

1, 2, Vitamin and mineral premixes by PNP Ltd (Premier Nutrition Products) and supplying the NRC 2011 requirements for rainbow trout. * Supplementary non-coated and coated amino acids were provided by Eurolysine, Avril Group, Amiens, France, AjiPro®-L; c-Lysine. **DL-Methionine 50%, coating materials 50% Shandong Fulikang Animal Nutrition Co., Ltd. Shandong, China.

Water quality parameters were kept constant within limits tolerable to rainbow trout for ammonia, nitrite, and nitrate with a 5% exchange per day with charcoal filtered mains water.

A feeding rate of 2% body weight per day was adopted and adjusted every two weeks on the basis of sample weight inventories. All rainbow trout were individually weighed at the start and end of the trial and bulk weighed at intermediate stages. On termination of the study, samples of fish (six per triplicate tanks) were taken for full proximate analysis to determine body composition. Rainbow trout were anaesthetised in buffered MS222 (200 mg/L), until loss of equilibria and response to human contact was observed. Subsequently, fish were subjected to lethal cranial percussion to ensure a humane endpoint.

2.3. Digestibility trial and faecal collection

In a separate trial for faecal collection, a selection of rainbow trout (450) originating from the primary growth trial were allowed to attain a mean weight of 200g on a standard commercial trout diet (Biomar Ltd). Subsequently, they were then returned to the original experimental diets for an acclimation period, fed to apparent satiation, reaching a mean weight of 250g. During a one-week faecal sampling period, rainbow trout were anaesthetised in buffered MS 222 (200 mg/L), until loss of equilibria and response to human contact was observed. Manual stripping of faeces was performed by hand, by lightly applying pressure to the hind portion of the abdomen. All twenty-five fish per triplicate tank (25 fish per treatment group) were sampled as described. After rainbow trout were sampled, they were reintroduced to their respective tanks for full recovery and again sampled after a period of three days and further feeding. Faecal material was collected in aluminum trays over ice and pooled by tank number. The faecal samples collected were freeze-dried and homogenised with a pestle and mortar to achieve a fine texture. It should be noted that rainbow trout were fed the separate low protein diet (Table 2) for endogenous protein loss determination in the same manner described above for faecal sampling. This diet was fed to the previous reference diet group only and fed for one week to obtain digesta representing low endogenous faecal protein losses.

2.4. Biometrics and calculations

From the mean initial and final weights and the values obtained for initial and final carcass protein content, specific growth rate (SGR) and apparent net protein utilisation (NPU) for each dietary treatment could be calculated. Specific growth rate (SGR), the rate of growth of an animal, is a sensitive index of protein quality under controlled conditions being proportional to the supply of essential amino acids. It is the main growth assessment indicator for fish performance used in the scientific literature. Note: Mean Growth Rate (MWG); Average Daily Growth (ADG); Specific Growth Rate (SGR); Food Conversion Ratio (FCR); Apparent Net Protein Utilisation (ANPU); Protein Efficiency Ratio (PER).

These were calculated according to the formulae presented below; Apparent Digestibility Coefficients (ADC) were determined using the equations listed below equation 1-7.

$$\text{MWG} = \text{final mean weight (g)} - \text{initial mean weight (g)} \quad \text{Equation (1)}$$

$$\text{ADG} = \frac{\text{Total growth (g)}}{\text{Number of feeding days}} \times 100 \quad \text{Equation (2)}$$

$$\text{SGR} = \frac{(\text{Ln final mean weight (g)} - \text{Ln initial mean weight (g)})}{\text{days fed}} \times 100 \quad \text{Equation (3)}$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \quad \text{Equation (4)}$$

$$\text{ANPU} = \frac{(\text{Final retained protein (g)} - \text{Initial retained protein (g)})}{\text{Protein intake (g)}} \times 100 \quad \text{Equation (5)}$$

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \quad \text{Equation (6)}$$

$$\text{ADC} = \left(1 - \frac{\text{\% indicator in feed}}{\text{\% indicator in faeces}}\right) \times \left(\frac{\text{\% nutrient EAAs in faeces}}{\text{nutrient EAAs in feed}}\right) \times 100 \quad \text{Equation (7)}$$

True protein and EAA digestibility were calculated using the formula above but based on corrections for the endogenous faecal N losses from feeding a low protein 2.00% diet as described in Table 2.

Table 2. Formulation of a low protein 2.00% diet for rainbow trout (*Oncorhynchus mykiss*) for endogenous faecal nitrogen correction and amino acid digestibility studies.

Ingredient	% Inclusion
Casein (85%CP)	2.22
Corn starch	25
Corn dextrin	25
Marine oil	12
Vitamins/minerals	3
Attractant*	2.78
α-cellulose	30

* The attractant used was a glycine betaine (betaine hydrochloride) Sigma–Aldrich, St Louis, MO, USA.

2.5. *Analytical methods and procedures*

Determination of chromic oxide

Chromic oxide is the substance most commonly considered in evaluating the digestibility of experimental fish diets. The following method is a modification of the one proposed by Furukawa and Tsukahara so as to handle micro samples in determining the chromic oxide content of feed and faeces [16].

Proximate composition

Proximate analyses of diets (Table 3) and trout carcasses were made following the usual procedures [17]. These describe the classical kjeldahl method for crude protein (N*6.25) and soxtherm extraction method for crude fat/oil extraction. Moisture and ash content of diets and fish were also determined by classical AOAC methods with slight modifications. These protocols were described in further detail for similar work conducted with rainbow trout [10].

Analysis of Amino acids in diets and faeces

Amino acids were analysed in feed ingredients and diets according to standard protocols for sample digestion and amino acid analyser protocols (Table 4). The AA analyses of feed (ISO 13903-2005) were performed with an HPLC (Alliance System, Waters, Guyancourt, France) after hydrolysis of 100mg dried samples with 6 N HCl at 110°C for 23 h under reflux in small, sealed ampoules. Derivatisation with ninhydrin was done by reaction with ninhydrin using spectrophotometric detection at 570 nm (440 nm for proline). This method has been the most widely performed method for several decades and is still commonly used. This method is officially approved by Commission Regulation (EU) No 152/2009. IEC requires derivatization of AAs, which can be performed by post-column derivatization using ninhydrin. Post column ninhydrin derivatization has been the preferred method for many years by a majority of laboratory protocols.

Table 3. Nutrient analysis of experimental diets (%).

Nutrient	Ref diet	FFS	FFS+ EAAS	MGM	MGM+EAAS	MGM+EAAS coated
Moisture	5.5	4.13	4.85	4.53	7.21	7.23
Protein (Nx6.25)	44.67	44.11	44.39	44.84	44.11	44.84
Lipid	12	13.73	13.5	12	12.5	11.92
Ash	16.67	12.45	12.67	11.06	10.56	9.95
N.F.E*	12.76	20.24	19.04	26.13	24.75	23.95
Fibre	2.11	3.83	3.67	2.68	2.62	2.58
Calcium	4.97	2.31	2.31	2.21	2.21	2.21
Phos.	2.63	1.48	1.46	1.4	1.39	1.39

*Nitrogen Free Extractives (NFE) 100- [moisture+ crude protein+ ash+ lipid]
Nutrient analysis following standard AOAC methods for moisture, protein (N x 6.25; Kjeldahl), lipid, fibre and ash on ingredients and diet/fish tissue samples.

Table 4. Essential Amino Acid EAA and (digestible EAA) profiles of experimental diets (percent of diet as fed basis).

Nutrient	Ref diet	FFS	FFS+ EAAS	MGM	MGM+EAAS	MGM+EAAS coated*
Methionine	1.24 (1.01)	0.71	1.24	0.83	1.24	1.24
		-0.52	-1.02	-0.74	-1.09	-1.04
Meth + Cyst	1.68	1.23	1.75	1.36	1.72	1.72
	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)	(N/A)
Lysine	2.98 (2.44)	2.43	2.98	1.51	2.98	2.98
		-1.64	-2.05	-1.15	-1.99	-2.05
Tryptophan	0.4	0.44	0.43	0.28	0.4	0.4
	-0.37	-0.31	-0.28	-0.23	-0.33	-0.41
Arginine	3.16	2.82	3.16	1.95	3.16	3.16
	-2.98	-2.14	-2.58	-2.12	-2.93	-3.36
Histidine	1.04	0.99	1.04	0.86	1.04	1.04
	-0.9	-0.7	-0.72	-0.74	-0.81	-0.95
Threonine	1.83	1.56	1.83	1.38	1.83	1.83
	-1.53	-1.03	-1.28	-1.15	-1.48	-1.53
Isoleucine	1.72	1.62	1.72	1.42	1.72	1.72
	-1.43	-1.11	-1.19	-1.14	-1.42	-1.48
Leucine	3.05	2.79	3.05	4.15	3.69	3.69
	-2.49	-1.34	-2.07	-3.53	-3.2	-3.19
Valine	2.1	1.84	2.1	1.79	2.1	2.1
	-1.66	-1.14	-1.41	-1.41	-1.64	-1.59
Phenylalanine	1.68	1.71	1.68	1.87	1.68	1.68
	-1.54	-1.61	-1.49	-1.82	-0.76	-1.64

2.6. Statistical analysis

The effect of diet was assessed using one way analysis of variance (ANOVA). The results for initial and final fish weights were subjected to statistical analysis by one-way analysis of variance. Where significant differences at the 5% probability level ($p \leq 0.05$) were found between dietary treatments, the means were compared using Duncan's Multiple Range Test. The data for carcass composition were based on pooled samples from each tank. Pooled Standard Errors of pooled means (± SEM) were calculated to identify the range of means as described by Standard Error = s/ √n (where: **s** = sample standard deviation n = sample size).

3. Results and Discussion

3.1. Growth performance

The results of this trial show the growth of rainbow trout was clearly affected by the different dietary treatments due to protein substitution and also the supplementation of essential amino acids (Table 5). The control (animal protein blend/fishmeal protein) reference diet fed trout (Ref) gave superior and significant ($P<0.05$) performance with a 146% overall increase in body weight in the experimental duration. Conversely, FFS (Full Fat Soyabean meal) fed trout was the most inferior performing group amongst the experimental diets, with only a 78% increase in weight gain over the same period. Supplementation of this basic formulation with a mixture of essential amino acids in crystalline form resulted in a moderate but significant ($P<0.05$) improvement in overall growth performance (86.80%) and, in particular for MGM+EAAS (Maize Gluten and Essential Amino Acid, uncoated) fed trout when in protected coated form, yielding an SGR of 125% ($P<0.05$). The other associated biometric values, such as daily live weight gain and protein intake, reflected these trends and significance.

3.2. Feed utilization

The feed utilisation parameters supported the growth performance metrics above are presented in Table 5. The feed conversion ratio FCR was better for the Ref diet fed trout (1.05) and poorer for FFS (1.74) ($P<0.05$). There was an improvement for the FFS+EAAS group. However, all diets based on maize gluten meal displayed very good FCRs with a trend towards improved feed conversion in diets supplemented with both standard and protected amino acid fortification (i.e. MGM+EAAS and MGM+EAAS coated. The net protein utilization (NPU) in trout receiving each diet gave a similar trend in terms of both growth and feed performance as with the Protein Efficiency Ratio (PER). However, it should be noted that NPU being a direct measurement of protein assimilation efficiency compared to PER which is based directly on protein to total biomass conversion and not direct protein gain alone. Ref diet fed trout attained 36% ANU and FFS and FFS+EAAS were 23.6% as compared with those fish fed diets MGM, MGM+EAAS and MGM+EAAS coated giving 26.46%, 29.21%, and 34.62% ANUs respectively ($P<0.05$). Plant dietary treatments (especially MGM) supplemented with standard and protected type of amino acids produced significantly ($P<0.05$) superior dietary protein utilisation for fish.

3.3. True digestibility coefficients for essential amino acids

The true digestibility coefficients are displayed in Table 6 for rainbow trout fed the experimental diets with corrections for endogenous protein loss from the low protein diet. It is obvious that there was a broad uniformity in the digestibility coefficients reported but a clear enhancement for diets where essential amino acids were added and in their coated forms. These true digestibility coefficients were used to present the digestible amino acid profile for the diets to complement the data for protein bioavailability in real terms compared to the apparent digestibility coefficient data more often presented in similar work with fish. It was observed that coated forms of tryptophan, arginine, and histidine gave superior and significant ($P<0.05$) improvements in coefficients in true digestibility over the standard EAA forms. All forms of supplemental essential amino acids in diets for rainbow trout yielded better digestibility values compared to the digestibility of comparable amino acids in the high plant diets and, in some cases, restored the coefficients to values found for trout fed the reference diet. In general, coefficients ranged from values of 82% to most at 88-90%. However, some very low values were recorded such as for leucine (52%) and tryptophan and threonine in the full fat soya treatment (70 and 71%) respectively indicating poor digestibility for this ingredient in un-supplemented EAAs. It should be noted that values are apparent true digestibility of individual amino acids +/- pooled standard error of mean. Values sharing the same superscripts are not deemed to be significantly different ($P<0.05$).

3.4. Carcass composition

On the basis of carcass composition (Table 7), the composition of the rainbow trout did not significantly differ ($P>0.05$) when assessed at the termination of the study after 8weeks on the experimental diets. However, it is interesting that the Ref diet control group and MGM+EAAS coated diets produced the highest protein carcass levels but was not deemed to be significant. This agreed with the better protein utilisation NPUs values observed for these fish and in alignment with performance. Generally, rainbow trout remained in a very similar weight class despite some differences in growth and feed performance over the experimental period. As such, this was not sufficient to drive any significant changes in the nutrient partitioning in the carcass as reflected by protein, lipid and ash (mineral retention) profiles. It should be noted that the diets were formulated to be iso-proteinic and isocaloric to maintain an optimal ratio of protein and energy intake during the 8-week trial.

Table 5. Growth performance and feed utilisation of rainbow trout (*Oncorhynchus mykiss*) over eight weeks.

Parameter	**Ref diet**	**FFS**	**FFS+ EAAS**	**MGM**	**MGM+EAAS**	**MGM+EAAS coated**	**SEM±**
Initial mean weight (g)	31.53	28.88	30.33	30.52	29.52	29.53	0.16
Final mean weight (g)	77.44^{a}	51.48^{e}	56.67^{d}	63.00^{c}	65.95^{bc}	66.48^{b}	1.49
% Increasing body weight	145.60^{a}	78.30^{e}	86.80^{d}	106.40^{c}	123.40^{b}	125.10^{b}	4.23
SGR %*	1.61^{a}	1.04^{d}	1.13^{d}	1.29^{c}	1.43^{b}	1.45^{b}	0.04
Feed intake (mg/fish/day)	861^{a}	680^{d}	740^{c}	770^{b}	780^{b}	760^{b}	9.82
Live wet gain (mg^{-1} day^{-1})	820^{a}	390^{e}	471^{d}	580^{c}	650^{b}	660^{b}	25.33
FCR^{a}	1.05^{e}	1.74^{a}	1.57^{b}	1.33^{c}	1.20^{d}	1.15^{d}	0.04
PER^{b}	2.05^{a}	1.30^{f}	1.47^{e}	1.68^{d}	1.89^{c}	1.94^{b}	0.05
Protein intake (mg^{-1} fish^{-1} day^{-1})	402^{a}	300^{b}	321^{b}	345^{b}	344^{b}	341^{b}	22.38
Protein retention (mg^{-1} fish^{-1} day^{-1})	140.05^{a}	70.90^{d}	76.00^{d}	91.30^{c}	100.5^{c}	118.10^{b}	5.98
Net protein utilisation (%)	35.95^{c}	23.64^{a}	23.66^{a}	26.46^{a}	29.21^{b}	34.62^{c}	0.89

*Specific growth rate, [a]Feed Conversion Ratio, [b]Protein Efficiency Ratio; Mean values in rows with different superscripts are significantly different (P< 0.05).

Table 6. The coefficient of digestibility for individual essential amino acids for respective diets.

Nutrient	Ref diet	FFS	FFS + ESSA	MGM	MGM + EAAS	MGM + EAAS (coated)	SEM±
Methionine	90.02^{a}	75.90^{c}	83.96^{b}	85.91^{b}	91.25^{a}	91.31^{a}	2.43
Lysine	89.14^{a}	75.17	75.47	77.61	85.83	87.13	2.58
Tryptophan	82.27^{b}	70.26^{c}	63.81^{d}	71.71^{c}	82.00^{b}	100^{a}	5.22
Arginine	88.80^{a}	79.97^{c}	81.13^{c}	85.25^{b}	89.61^{b}	92.85^{a}	2.07
Histidine	84.85^{a}	69.65^{d}	66.30^{d}	76.40^{c}	81.73^{b}	85.15^{a}	3.26
Threonine	88.46^{a}	71.35^{c}	72.43^{c}	82.36^{b}	86.65^{a}	88.56^{a}	3.22
Isoleucine	90.45^{a}	76.57^{c}	73.65^{c}	82.66^{b}	90.59^{a}	91.20^{a}	3.17
Leucine	88.55	52.05^{d}	70.55^{c}	87.24^{b}	90.43^{b}	90.65^{a}	6.37
Valine	87.87^{a}	69.83^{c}	70.83^{c}	82.12^{b}	86.98^{a}	81.58^{b}	3.19
Phenylalanine	100	100	100	100	100	100	0.00

Values are apparent true digestibility* (corrected for endogenous losses) of individual amino acids +/- pooled standard error of mean. Values sharing the same superscripts are not significantly different (*p<0.05*).

Table 7. Carcass composition (Proximate analysis % whole wet weight).

Nutrient	Initial+	Ref diet	FFS	FFS+ EAAS	MGM	MGM+EAAS	MGM+EAAS coated	SEM±*
Moisture	75.08	72.27	73.07	73.61	71.99	72.24	71.94	0.67
Protein	13.29	15.87	15.17	14.62	14.55	14.48	15.85	0.64
Lipid	10.1	8.6	8.99	8.98	10.59	10.16	9.08	0.78
Ash	2.18	2.71	2.23	2.44	2.35	2.57	2.75	0.17

+Composition of initial sample of fish removed at start of experiment. Mean values without superscripts were not significantly different (*p> 0.05*) *SEM pooled standard error of mean.

4. Discussion

This investigation on optimising diets for rainbow trout using higher plant based protein ingredients was justified to meet the global need for sustainability facing the aquaculture industry. This is now a priority research area to test various oilseed and pulses and cereal derived concentrates [18]. The reduction in fishmeal inclusion in contemporary diets places pressure to obtain sufficient balance to ensure the correct essential amino acid profile in commercial feed formulations. Our experiment demonstrated that coated crystalline amino acids can effectively compensate for amino acid deficiencies associated with higher maize gluten inclusion. For example, a marked improvement was observed for maize gluten meal because lysine is a well-known first limiting essential amino acid for this cereal derived protein concentrate. Its deficiency can compromise the ideal essential amino acid profile in livestock and is highly significant in swine nutrition when diets are high in this ingredient relative to soyabean meal [19]. This is similar for many fish and other animal studies where maize products have been utilised in formulated commercial diets and under experimental conditions [20].

Previous work has identified the influence of multiple amino acid supplementations on improving performance and feed utilisation in rainbow trout [2]. In that study, soyabean-containing diets replacing up to 66% of fishmeal were supplemented with crystalline amino acids. These consisted of methionine only; dual supplemented with two methionine and lysine levels; and finally, a group complex comprising methionine, lysine, tryptophan, threonine, arginine and, histidine. Results indicated that soyabean meal (SBM) was inferior to the reference fishmeal protein when SBM was used alone. No restoration in growth, feed efficiency and apparent net protein utilisation was obtained by either methionine only or with both methionine and lysine supplementation. However, multiple amino acid addition to the diet yielded an improved percentage weight gain, specific growth rate and a small improvement in apparent net protein utilisation for rainbow trout. Other work on rainbow trout was closer in concept to the current study [8] investigating the addition of crystalline lysine to correct its deficiency in wheat gluten meal protein. Wheat gluten meal has been used in salmonid diets but is constrained by lysine being a first limiting essential amino acid. Our present study here with maize gluten meal reinforces its use with protected essential amino acid fortification. Coating amino acids to determine the effects of supplementation of crystalline or coated lysine and/or methionine on growth performance and feed utilization has been tested on the Chinese sucker, *Myxocyprinus asiaticus*, [11] A fermented full fat soyabean meal (FSBM) was prepared by either not supplementing with lysine and methionine (NLM), or supplementing with both crystalline Lys and Met (LM), with coated Lys (CL), with coated Met (CM), or with both coated Lys and Met (CLM). Coating the two amino acids elevated growth performance and feed utilisation in this study.The supplementation of diets for pigs and poultry has long been deemed important in flexible diet formulations where corn or maize gluten meals have been the basis of diets with lower levels of soyabean and animal byproducts. Precision diets using more accurate nutrient data utilising digestibility coefficients of individual amino acids and protein form the basis of such feeds in younger animals (piglets and chicks) as well as later production stages [4]. In fast growing juvenile stages, protein accretion is much more critically dependent on essential amino acid supply and balance to meet the 'ideal protein' concept first proposed by Wang and Fuller [4, 21] in swine.

The most important single factor affecting the efficiency of protein utilisation is the profile of digestible essential amino acids entering the small intestine. Assuming a constant ideal amino acid profile in absorbed protein, the requirements for all amino acids can be estimated once the relative requirement for a single amino acid—typically lysine—has been established. The findings of the present study in rainbow trout support this principle. However, because a faecal stripping method was used to obtain faecal samples, the results are not directly comparable with studies employing total faecal collection protocols commonly used in other animal species. Previous researchers [19] have used the amino acid deletion method to arrive at an estimate of optimum dietary EAA pattern for rainbow trout. With 40% deletions of a single EAA from the control pattern. Based on N utilisation data, an estimate of optimum dietary EAA pattern for rainbow trout can be constructed. Using a linear formulation software, we compared the dietary EAA pattern for rainbow trout, based on amino acid composition of rainbow trout whole-body protein EAA requirements for rainbow trout [20].

Modern approaches to undertake precision diet formulations for fish must depend on accurate digestibility data being obtained, and this has been fraught with technical difficulties compared to terrestrial animal studies as is stated in previous work for rainbow trout to evaluate minimize nitrogen excretion and maximizing nitrogen retention, as in our current study [10]. However, the present investigation featured a separate trial to determine the intestinal endogenous amino acid excretion with the use of a low-protein diet. This diet was carefully formulated to contain 2% crude protein from casein as a purified diet. A low rather than a zero protein diet prevents over stimulation of endogenous protein due to gut enterocyte sloughing into the digesta resulting in over estimation of nitrogen losses. Assuming that residual amino acids supplied in a low-protein diet should be subtracted from the exogenous dietary derived faecal source, the true amino acid excretion was calculated. To our knowledge this current study to assess essential amino acid supplementation with fishmeal being replaced by soyabean meal and maize gluten is unusual in providing true amino acid digestibility data for trout in combination with supplementary essential amino acids.

A comprehensive review of fishmeal replacement by various alternative ingredients on growth performance of fish and adoptability has been recently published [12] and for aquaculture sustainability as reported for Nile tilapia to examine true and apparent digestibility of protein and amino acids in feed [23, 24]. As such, the practical applications of crystalline essential amino acids for both shrimp and fish diets are now gaining potential within the industry for maximum efficacy of plant ingredients and for enhanced performance, welfare and cost of production [25, 26]. With the emerging use of closed Recirculation Aquaculture Systems (RAS) for fish rearing there is an increased interest for low fishmeal formulated diets to meet the intensive production of salmonid species [27]. This will require the establishment of optimal use of protein and supplemental essential amino acids to improve nutrient digestibility and environmental impact of nitrogen excretion. The present study provides data for rainbow trout that can be useful to meet such concepts in practice.

Some investigations have been done to make economic savings by attempting to reduce the crude protein levels of diets by supplementation of the first, secondary and tertiary essential amino acids in advanced feeds for salmon, trout and for other fish species such as tilapia as proposed recently by with work of Bell and Davies [28]. Also, for channel catfish, *Ictalurus punctatus* [29]. In this way the 'ideal' protein ratio and quantitative levels of essential amino acids can be restored to some effect. The optimum protein to energy ratio of salmonid diets has been well established but there is more scope for refinement by lowering the protein levels in diets. This can lead to higher N and amino acid retention with less metabolic, physiological losses leading to adverse environmental impact on the aquatic medium [30] in different rearing systems.

5. Conclusion

It was demonstrated that partial replacement of fishmeal with soyabean meal and maize gluten meals could be achieved without serious impairment of growth performance and feed utilisation in trout, but higher levels of inclusion necessitated crystalline amino acid supplementation to mitigate nutritional constraints. Although the reference diet fed to trout produced the better overall growth performance and protein retention, plant ingredients like soyabean and maize could become more effective in linear least cost formulations for salmonid fish species like trout and salmon provided essential amino acids are provisioned. Meeting the circular economy with plant by products will necessitate more refined data on their digestibility. With the increasing exploitation of novel ingredients like SCPs and insect meals we need more precise digestibility data for different fish species in order to correct any dietary essential amino acid deficiencies. In this approach, using Linear Feed Formulation software can offer corrective amino acid supplementation strategies to optimise diet formulations. The cost benefits of using synthetic and crystalline essential amino acids must also be factorized in modern feed formulations. the comparative efficacy of coated versus non-coated crystalline amino acids on digestibility and nutrient utilization in modern aquafeeds.

Author contributions

Simon J Davies: Conceptualization & Leadership, Experimental Methodology, Formal Analysis, Writing – original draft. Matt E. Bell: Investigation, Data curation, Tables, Visualization, Writing – Review & Editing. Ayesha Tanveer: Resources, Supervision, Project Administration Formatting for Journal Submission. Sadia Nazir: Review & Editing, Validation of Data & Statistical Appraisal. All authors have read and approved the final manuscript for publication.

Ethical approval

The fish experimental procedures conformed to the European Community Directive (No. 2010/63/EU) (EC, 2010) and the UK Animal Scientific Procedures Act of 1986 (Home Office Licence PPL 30/2135). The investigations were approved by the local Institutional Animal Care Committee.

Conflicts of Interest

The authors report no conflicts of interest.

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Data availability statement

The data presented in this study are available on request from the corresponding author and lead author by access to original data obtained in the rainbow trout trial and project students theses.

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