Received: 06 July 2024 | Revised: 18 July 2024 | Accepted: 19 July 2024

https://doi.org/10.56946/jzs.v2i2.425

Journal of Zoology and Systematics

Research Article

Coated Crystalline Amino Acid Supplementation May Assist Higher Soybean Meal Inclusion in Diets for Nile Tilapia (*Oreochromis Niloticus*) With Improved Protein Assimilation and Retention

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Abstract

The aquaculture industry has previously relied on high-quality fishmeal (FM) to fabricate diets of excellent standards. However, plant-based proteins such as soybean are more economic for low-value fish species, for example, tilapia and carp, and fishmeal use has been significantly lessened. Previous studies have addressed standard soybean meal (SBM) sources in aquafeeds seldom addressing essential amino acid correction. An eight-week feeding trial was conducted to evaluate the replacement of fish meal with soybean meal concentrate in Nile tilapia (*Oreochromis niloticus)* using a semi-purified diet against a Low Temperature (LT) fishmeal as a primary reference protein. Four diets with varying levels of soybean meal were evaluated and compared to a control diet of 100 % fish meal. Three diets containing 20, 40, and 80 % soybean protein concentrate (SBPC) were examined. The fourth diet consisted of 80 % SBPC and supplementation with two essential amino acids: lysine and methionine ($80SBPC^{AA}$) in a coated form. Daily growth values highlight similar rates when tilapia was fed 100FM and 40SBPC diets. No differences were observed in the final mean weights for all soybean-fed tilapia, but they were marginally lower than the 100FM control diet group. The 80SBPC diet showed the poorest feed conversion ratio (FCR) at 1.31 and protein efficiency ratio (PER) at 2.19. However, the $80SBPC^{AA}$ diet showed a significantly improved percent weight gain for tilapia compared to the 80SBPC-fed tilapia. PER was significantly higher at 2.47 and had a better Apparent Net Protein Utilization (ANPU) value of 28.51 % compared to un-supplemented 80SBPC-fed tilapia (25.48 %). These results confirmed that high-quality fishmeal can be substituted by up to 40 % SBPC alone and without any detrimental effects on growth or carcass composition in juvenile tilapia. Crystalline coated essential amino acid supplementation showed marginal improvement of performance for tilapia at high SBPC inclusion level.

Keywords: Tilapia, soybean protein concentrate, fishmeal, essential amino acids, growth and feed performance, protein retention

1. Introduction

Contemporary aquaculture encourages researchers to find sustainable alternatives to develop and improve the fish farming sector. The global demand for fishmeal (FM) is constantly rising and outweighs the industry's ability to produce it, being a finite resource. With global FM stocks under careful management and scrutiny, cost inflation can threaten the long-term sustainability and profitability of the aquaculture industry. Consequently, the prime focus is the strategic use of fishmeal in formulated diets for fish, with a focus on alternate sources [1]. A suitable substitute for FM is paramount, especially in diets for carnivorous fish feeds, but increasing interest also exists for warm water omnivore species that can still include appreciable fishmeal content. Replacement with suitable plant-based proteins is important, and the aquafeed sector has been using a myriad of such ingredients for many years, but most likely, these are sub-optimally included in many scenarios.

Development of feeds for commercially relevant fish species (inc. tilapia) has focussed on a protein economy and quality. Additionally, the quality of protein, which provides essential amino acids (EAAs), is crucial for fish growth and development [2]. It is important that a diet provides a balanced essential amino acids profile as well as individual levels. Collectively EAAs must meet the 'ideal protein' concept where all 10 essential amino acids are equally limiting. Fishmeal reflects such a balanced 'pattern 'and is ideal for matching most fish species' tissue requirements for maintenance and growth. For practical purposes, complementary proteins can be effectively blended to offer EAAs at the right level and proportion to each other in compound feeds for fish.

In this respect, soybean meal (SBM) has been widely applied as a significant substitute for fishmeal in aquafeeds. It has been shown that the use of SBM reduces the feed cost by 43 % in relation to a commercial FM dependent diet for Nile tilapia (*Oreochromis niloticus*) [3]. Other plant byproducts such as lupins, beans, peas, and pulses show much merit, but soybean continues to be popular on a global scale. The use of commercially processed SBM has proven successful in previous trials for tilapia. It has been stated that FM replacement by 22 % methylated soy protein isolates (MSPI) increases Nile tilapia fingerlings' final body weight [4]. The authors suggest a maximum replacement of 66 % MSPI without negative effects on tilapia health and performance. Similarly, it has been demonstrated that SBM, when replacing 75 % FM protein, increased Nile tilapia fry final body weight, attaining ~38.0 g, with a weight gain of over 3,000 %, and impressive Specific Growth Rate, (SGR) of 6.18 % day^{-1} [5]. Nevertheless, there is a debate among researchers regarding the benefit of SBM as a protein source for aquafeed for various reasons.

Poor feed efficiency, the presence of antinutritional factors, and negative impact on gut health have been described in some studies [6,7]. The authors also point out the different methods of processing SBM, diet formulation variation, and differences in fish species and culture systems [8]. A higher quality soybean meal arises from more advanced treatment to remove any Anti-Nutritional Factors (ANFs), resulting in a soybean meal concentrate with around 60 % protein content. Techniques such as heat treatment to denature thermally labile ANFs can be effective in reducing protein inhibitors (trypsin inhibitors), but too much heat application can cause bridging of protein, especially disulphide cysteine cross-linkages, and reduce protein digestibility. Other processing methods, such as the use of solvent extraction and dehulling, can reduce toxins and remove oil and fibre. Removing Non-Starch Polysaccharides (NSPs) like stachyose and raffinose can enhance protein levels to produce soyabean meal protein concentrates (SBPCs). These are deemed to be superior for monogastric animals and most fish reduce gut lesions like enteritis. Unfortunately, most types of standard soybean meal in many countries are inferior in value to modern aquafeeds and need to be further processed at cost.

A common association with SBM is the inadequacy of the essential amino acid profile (particularly lysine and methionine). Methionine and lysine are the first and second limiting essential amino acids, respectively, for most animal species. They are both vital for many important metabolic and physiological processes such as transport proteins in the blood and the immune system and muscle protein anabolic pathways for growth and development in fish. However, there is evidence the detrimental effects of soybean replacement are alleviated or perhaps reduced to insignificance with the supplementation of the two essential amino acids believed to be deficient in SBM. It was reported that SBM supplemented with methionine, lysine, and taurine can substitute up to 40 % FM in the diet for juvenile black sea bream (*Acanthopagrus schlegeli*) [9]. The replacement did not alter fish survival or SGR. Similarly, research has demonstrated a diet of 55 % SBM supplemented with 0.5 % L-lysine can realistically replace FM in a diet for Nile tilapia fingerlings with no harmful effects on animal performance [10]. These latter authors observed that tilapia fed with the supplemented SBM presented high values of final individual weight and length-specific growth rate (SGR, 1.15), feed conversion ratio (FCR, 1.61), protein efficiency ratio (PER, 0.62) and overall food intake.

More recently, investigations that compared standard-grade soybean meal and corn gluten meal, showed satisfactory results with juvenile tilapia assessed by the same metrics [11]. Others have assessed soybean meal inclusion with coated amino acids to allow synchronised absorption with intact protein from the intestinal tract to improve their assimilation and post-absorption protein biosynthesis. For example, workers have tested coated and uncoated crystalline amino acid fortification in formulating a low fishmeal diet for *Penaeus monodon* [12]. Their findings suggested that coated amino acids proved superior concerning growth, digestibility, body composition, haemolymph indices and nitrogen metabolism in shrimp.

The present study evaluates incremental inclusion levels of high-quality soybean protein concentrate meal (SBPC) at the expense of fishmeal with the addition of coated essential amino acids (EAAs) lysine and methionine at the highest inclusion level of 80 % fishmeal substitution. The aims were to assess the effects of increasing levels of SBPC on carcass composition and nutrient retention in the Nile tilapia (*Oreochromis niloticus*). Additionally, the research investigation assesses the effects of supplementing lysine and methionine-coated amino acids on fish performance. Coated amino acids are more slowly absorbed in fish's intestines than uncoated forms. This may offer better assimilation with intact dietary protein to synchronise amino acid absorption and their utilisation. Therefore, our study seeks to determine whether EAA-supplemented SBPC could be an economically viable FM alternative in practical feeds for tilapia towards achieving lower fishmeal-formulated diets.

2. Materials and methods

2.1 Diet design and formulation

A total of five test diets were produced for the feed trial (Table 1a). The basal control contained 100 % FM as the main protein source. The further four diets were formulated with levels of 20 % (20SBPC), 40 % (40SBPC), and 80 % (80SBPC) of FM being replaced with Soybean Meal Protein Concentrate (SBPC). The final diet consisted of 80 % FM replacement and an additional two essential amino acids: methionine and lysine ($80SBPC^{AA}$). This served to examine whether crystalline amino acid sources could mitigate the effects of increased SBPC inclusion. All diets were formulated to be isonitrogenous (37 % protein) and isolipidic (15 %). Test diets were produced by a single screw laboratory bench extruder using a 2 mm extrusion die and diet mixture preparation, pelleting and drying process as described by Bell and Davies, (2024) [11].

2.2 Experimental procedure

400 male tilapia (*O. niloticus*) fingerlings (first generation all XY from YY male, GMT and XX female tilapia) were supplied by FishGen Ltd (Camberley, England) and acclimated for two weeks in circular 40 L tanks that were part of a recirculating aquaculture system (RAS). Before the trial, fish were fed with a commercial diet (Skretting, Norway). The flow rate into each tank was ~4 L min^{-1}, with the overall RAS kept at pH 7-8 and 28 °C throughout the trial. The photoperiod was set at 14:10, light and darkness.

Within this trial, 335 male tilapias were used, with 20 fish randomly assigned to each triplicate tank per treatment. The feeding trial was carried out over 55 days, and tanks were fed 3 % of the total biomass per tank twice a day in two equal portions (1000 and 1700 h). Fish weights were measured every ten days after feed deprivation for 24 h to allow gut clearance, and the feed/body weight ratio was adjusted to reflect the weight gain increase every 10 days. For initial carcass analysis using terminal anaesthesia (MS222) followed by cranial percussion and destruction of the brain, 20 fish were culled in this manner. Also, at the conclusion of the trial, five randomly selected fish from each tank were again euthanised (MS222) and cranial percussion with destruction of the brain to confirm death for final carcass analysis.

2.3 Proximate analysis

Crude protein, lipid, moisture, and ash of diets and fish body samples were determined using standardised methods by the Official Methods of Analysis [13]. Samples were homogenised using a blender before analysis commenced. Moisture content was determined by heating samples at 105 °C for 48 h. Protein determination was carried out according to the Kjeldhal procedure by acid digestion and sequential distillation (Gerhardt Vapodest 3S, Königswinter, Germany). Crude protein content was calculated from the recovered Nitrogen using a 6.25 conversion factor (N*6.25). Crude lipid concentrations were determined using the Folch *et al.* (1957) method using chloroform and methanol extraction (2:1) [14]. For ash, the values were calculated by the percentage weight difference after samples being ignited at 550 °C for 16 h in a-

Table 1a. Test diet formulations for Nile tilapia (*Oreochromis niloticus*) showing level of fishmeal substitution and their corresponding proximate compositions (% as fed).

Ingredient (%)	**100FM**	**20SBPC**	**40SBPC**	**80SBPC**	**80SBPC^{AA}**
Fish meal[1]	45.00	36.00	27.00	9.00	9.00
Soyabean protein concentrate[2]	0.00	9.81	19.64	39.27	39.27
Wheat meal[3]	25.00	20.00	20.00	19.00	17.20
Blood meal[4]	2.00	2.00	2.00	2.00	2.00
Dextrin[5]	5.00	10.00	10.00	10.00	10.00
Potato starch	2.50	5.00	5.00	5.00	5.00
Oil (fish: corn oil, 3:1)[6]	10.05	10.94	11.83	13.33	13.33
Vitamin mix[7]	2.00	2.00	2.00	2.00	2.00
Mineral mix[7]	1.00	1.00	1.00	1.00	1.00
Carboxy-methyl cellulose[5]	2.00	2.00	2.00	2.00	2.00
α-cellulose[5]	6.35	4.35	2.33	0.00	0.00
Mono-calcium phosphate[5]	1.60	1.90	2.20	2.40	2.40
L-methionine[5]	-	-	-	-	0.90
L-lysine[5]			-	-	0.90
Proximate Composition (% as fed)					
Moisture	11.99	11.73	11.57	11.48	11.77
Protein	33.68	34.36	34.20	34.89	33.52
Lipid	15.65	15.69	15.40	15.53	14.96
Ash	7.36	6.88	6.66	5.50	4.94

Diet designation code: FM (Fish Meal), SBPC (Soybean Protein Concentrate meal), SBPC^{AA} (SBPC supplemented with methionine and lysine).

[1]Fishmeal: LT-94, Skretting, Norway.

[2]Central Soya Ltd, Michigan USA.

[3]Kalpro S™ Orans, Paris, France.

[4]American Protein Corporation, Des Moines, Iowa, USA.

[5]Sigma-Aldrich, St. Louis, Missouri, USA.

[6]Sevenseas, Hull, UK.

[7] PNP, UK.

muffle furnace until a constant weight is achieved as dried ash. A proximate analysis of diets is presented in Table 1a. Table 1 b shows the Essential Amino Acid (EAA) composition of the respective diets. EAA quantification was determined according to the protocol described by Davies et al. [15]. Amino acids were determined according to the method stated by Davies et al. (1997) [15]. *EAAs for tilapia are as reported by the NRC 2011 Nutrient Requirements for fish and shrimp and by Furuya et al. (2023) [16].

2.4. Growth performance and feed utilisation indices

The following indices and formulae are used as a measure of fish growth performance and feed utilisation during the feed trial:

1. Weight Gain = Final mean weight (g)– Initial mean weight (g)
2. ADG (Average Daily Growth) = Growth Gain (g) ÷ No of feeding days
3. SGR (Specific Growth Rate) = [(Ln Final Mean Weight (g) – Ln Initial Mean Weight) ÷ (Number of feeding days)] x100
4. FCR (Feed Conversion Ratio) = Feed Intake (g) ÷ Weight Gain (g)
5. ANPU – Apparent Net Protein Utilisation = [Final Retained Protein (g) – Initial Retained Protein (g) ÷ Protein Intake (g)] x100
6. PER (Protein Efficiency Ratio) = Weight Gain (g) ÷ Protein Intake (g)

2.5. Statistical analysis

Data values are expressed as mean values with their corresponding standard error. Datasets were analyzed using one-way analysis of variance and statistical differences were discerned using a post-hoc Tukey's test. Statistical significance was considered when P<0.05.

3. Results

3.1 Growth performance

Table 2 presents the growth performance of tilapia over the 55-

day feeding trial. The ADG values showed that 100FM-fed fish had the highest average daily growth rate (0.68 g fish-1 day-1) but the 40SBPC diet also performed equally well (0.62 g fish-1 day-1). The 80SBPC diet without EAAs supplementations yielded the lowest ADG value (0.56 g fish-1 day-1). The results indicated that the relationship between SGR and FCR is inversely proportional. Hence, the lowest value for the FCR was coupled with the highest rate of growth, indicating the most efficient use of food at this level. The 80SBPC-fed tilapia displayed numerically the highest FCR (1.31) for any treatment group. However, supplementation with essential amino acids methionine and lysine resulted in an approximate 8 % significant decrease or better FCR of the 80SBPCAA diet group 1.21). The tilapia receiving the 40SBPC diet has the highest APNU (Apparent Net Protein Utilisation) of 31.25 %. The 80SBPC diet recorded the lowest value for Apparent Net Protein Utilization (ANPU) at 25.48 %. Likewise, PER confirmed 40SBPC to have a higher value of 2.57, with the 80SBPC diet recording the lowest 2.19 and significant. The amino acid supplementation (80SBPCAA) gave a 12 % increase in PER in relation to the 80SBPC diet, which correlates to the same increase when measuring the ANPU performance metric.

Table 1b. Essential Amino Acid profile EAA (%) of experimental diets for tilapia.

Essential Amino Acids (EAAs)	100FM	20SBPC	40SBPC	80SBPC	80SBPC^{AA}	EAA tilapia*
Methionine	1.00	0.90	0.80	0.60	1.50	0.90
Lysine	2.82	2.70	2.62	2.44	3.33	1.43
Threonine	1.52	1.50	1.50	1.51	1.51	1.05
Phenyl alanine	1.50	1.56	1.66	1.84	1.84	1.55
Histidine	0.95	0.96	0.99	1.04	1.04	0.48
Arginine	2.35	2.38	2.46	2.61	2.61	1.18
Isoleucine	1.68	1.66	1.67	1.70	1.70	0.87
Leucine	2.82	2.81	2.85	2.93	2.93	0.95
Valine	2.27	2.18	2.12	2.01	2.01	0.78
Tryptophan	5.37	4.38	4.39	4.22	4.22	0.28

Table 2. Growth performance and feed utilisation of Nile tilapia (*Oreochromis niloticus*) after being fed increasing soybean protein concentrate meal and amino acid supplementation (Mean values +/- Pooled Standard Error of Mean, SEM).

	Diet					
	100FM	20SBPC	40SBPC	80SBPC	80SBPC^{AA}	SEM*
Initial mean weight (g^{-1} $fish^{-1}$)	17.99	17.77	17.21	17.76	16.70	0.37
Final mean weight (g^{-1} $fish^{-1}$)	49.26	46.34	48.09	43.55	44.36	1.71
Mean weight gain (%)	173.82	160.78	179.43	145.2	165.63	9.33
ADG (g $fish^{-1}$ day^{-1})	0.68^{a}	0.62^{b}	0.67^{a}	0.56^{c}	0.60^{b}	0.04
SGR (% day^{-1})	2.19^{a}	2.08^{ab}	2.23^{ab}	1.95^{c}	2.12^{b}	0.08
FCR	1.18^{c}	1.23^{b}	1.14^{c}	1.31^{a}	1.21^{b}	0.03
Total feed intake (g $fish^{-1}$)	36.9	35.14	35.20	33.78	33.47	0.96
Feed efficiency	0.85^{ab}	0.81^{b}	0.88^{a}	0.76^{c}	0.83^{b}	0.02
ANPU (%)	30.84^{a}	28.79^{b}	31.25^{a}	25.48^{c}	28.51^{b}	1.62
PER	2.52^{a}	2.37^{b}	2.57^{a}	2.19^{c}	2.47^{b}	0.11

*Different superscripts in each row indicate there is a significant difference ($P<0.05$). $\pm$ SEM. ADG – Average Daily Growth, SGR – Specific Growth Rate, FCR – Food Conversion Ratio, ANPU – Apparent Net Protein Utilisation, PER – Protein Efficiency Ratio. FM – Fish Meal, SBPC – Soybean Protein Concentrate meal, SBPC^{AA} – SBPC supplemented with methionine and lysine.

Table 3. Proximate composition of the pooled carcasses of test animals presented as a proportion of the whole fish (%).

	Initial	100FM		20SBPC		60SBPC		80 SBPC		$80SBPC^{AA}$		*SEM
Moisture	64.79	72.42	±0.22	72.07	± 0.19	72.01	± 0.06	72.65	± 0.08	72.35	± 0.10	0.19
Protein	19.68	15.71	± 0.13	15.63	± 0.08	15.51	±0.12	15.33	±0.14	15.38	±0.13	0.11
Lipid	10.53	8.35	± 0.16	8.45	± 0.18	8.78	± 0.08	7.99	± 0.29	8.77	± 0.55	0.23
Ash	5.77	4.23^{a}	± 0.1	4.16^{a}	± 0.04	4.12^{a}	± 0.06	3.88^{b}	± 0.11	3.84^{b}	± 0.05	0.12

*Different superscripts in each row indicate there is a significant difference ($P<0.05$). ± SEM. FM – Fish Meal, SBPC – Soybean Protein Concentrate meal, $SBPC^{AA}$ – SBPC supplemented with methionine and lysine.

3.2 Body Composition

The values obtained for moisture ranged from 72.0-72.65 % of the total biomass and were not deemed to be significantly different ($P>0.05$) (Table 3). The protein content had an inversely proportional relationship to the soybean content of the diet (15.71, 15.63, 15.51, 15.33, 15.38 %), but despite this obvious trend, it was not found to be significant (Table 3).

No significant differences in the carcass lipid concentrations were also noted ($P>0.05$). The ash content of tilapia progressively decreased (4.23, 4.16, 4.12 %) with an increasing soybean meal substitution (0-40 %) but was insignificant (Table 3). However, the 80SBPC and $80SBPC^{AA}$ diets caused a further significant decrease (3.88-3.84 %) in the whole-body carcass ash of tilapia compared to the other diet groups ($P<0.05$) (Table 3).

4. Discussion

Results indicate that substituting FM with up to 40 % SBPC in the Nile tilapia diet, replacing 80 % of fish protein did not show significant differences in the recorded parameters concerning growth or carcass composition when compared to the control diet. This study indicates that a 40 % fishmeal replacement could effectively support tilapia performance without compromising growth and feed utilisation efficiency. Elangovan and Shim, (2000) reported similar findings with tin foil barb (*Babodes altus)* [8]. Their results confirmed soybean meal could be included at up to 37 % of the diet, replacing approximately 33 % of the fish protein without significantly affecting the growth rate. However, it should be noted that tilapia is an omnivorous species and should be able to tolerate much higher inclusions of plant proteins as a main component of the diet, as explained by a comprehensive treatise on tilapia feeds and feeding [17].

For Anti-Nutritional Factors (ANFs) found within soybeans, it is required to expose soybeans to heat treatment processes before utilisation for monogastric animals. For example, this reduces the concentration of protease inhibitors such as trypsin inhibitors [18]. However, excessive heating may also reduce protein and amino acid digestibility and bioavailability, thus negatively impacting the fish's growth performance. The soybean meal concentrate used in the current trial had been effectively heat treated to denature specific ANFs such as trypsin inhibitor proteins. Another limiting factor in soy-based diets is the lower amounts of Essential Amino Acids (EAAs) compared to fishmeal, such as methionine and lysine, which can be the first and second limiting EAA. Methionine is considered the first limiting essential amino acid in some Nile tilapia-fed cereal-based diets, particularly soybean-meal-rich diets. Feed-grade lysine has also been supplemented in practical and experimental diets for Nile tilapia when fed corn meal. Lysine's primary metabolic role in protein synthesis is also the basis for it to be the primary reference Amino Acid (AA) in computing ideal AA ratios. Early studies have demonstrated that the effectiveness of using intact lysine from high-lysine corn protein concentrate was not significantly different from that of crystalline lysine in Nile tilapia [19]. The established methionine and lysine requirements for maintenance and utilisation efficiency for growth of two sizes of tilapia (*Oreochromis niloticus*) have been previously [20] and work on tilapia to examine the supplementation of key crystalline amino acids, namely methionine, lysine, tryptophan, and arginine [21]. These researchers used a high soybean meal base formulation (analogous to the current study) to assess the respective essential amino acid requirements by their individual deletion from the reference diet. Interestingly, tilapia did not require added tryptophan in their study. Compared to an un-supplemented group, these latter authors obtained maximum performance in balanced diets with essential amino acid supplementation (methionine, lysine, and arginine).

It should be noted by referral to Table 1b that in our current study, none of the diets except for the 80SBPC diet fell below the threshold requirements declared for tilapia [16,22] for all essential amino acids except for methionine (0.6%) compared to 1% for the FM control diet. Adding crystalline methionine to achieve 0.9% for the 80SBPC^{AA} diet restored some degree of performance. In the present investigation, the supplementation with methionine and lysine resulted in an improved growth rate for the higher soybean meal inclusion level tilapia. The 80SBPC diet, without additional amino acids and the highest soybean protein concentrate inclusion, caused the poorest FCR and SGR. On the other hand, the 80SBPC^{AA} diet, supplemented with the EEAs, showed better FCR and SGR compared to 80SBPC fed tilapia. Previously, it has been suggested that a diet that consisted purely of plant protein (e.g. soybean meal) could effectively replace a fishmeal diet without adverse effects, providing methionine was supplemented in the diet [23]. A similar similar study on Gibel carp *(Carassius gibelio)* which investigated diets with and without EAAs lysine and methionine supplementation give interesting findings [24]. These results illustrated the best SGR and final body weight was attained when fish were fed a diet containing lysine and methionine supplementation.

Crystalline Amino Acids (CAAs) can successfully be included at 60 g kg^{-1} in the Nile tilapia diet, as stated by Da Cruz et al. [25]. CAAs can stimulate protein synthesis coupled to lower fat deposition in Nile tilapia. The mRNA levels of mTOR and Raptor genes were found to be upregulated by CAA supplementation. This may explain the superior protein retention found in the present study in terms of both ANPU and carcass protein retention. Crystalline amino acids could, therefore, be used as a strategy to improve amino acid balance towards developing eco-friendly diets, avoiding fishmeal and providing a means for lower protein levels in feed formulations. Recently, a comprehensive review on the essential amino acid requirements for tilapia, focusing on precision protein nutrition and mentioning the role of supplementary EAAs in diets for optimum performance was undertaken [16]. Likewise, Xing *et al.* (2024) presented a comprehensive meta-analysis of literature data on essential amino acid (EAA) requirements of major farmed aquatic animal species (including tilapia) to re-define EAA requirements and provide ideal amino acid patterns for precision diet formulations [26].

In our study, the carcass ash content was found to be reduced progressively between diets with increased soybean supplementation and significant only at 80SBPC. This may be due to the declining mineral content associated with increasing soybean meal inclusion. Although we have adjusted for the phosphorous and calcium by adding supplementary inorganic salts as a mineral premix, the phytic acid within soybean chelates these minerals, especially phosphorous, within the diet. This leads to a lower availability of minerals to the fish and, consequently, may explain the reduced concentrations of ash noted in the carcass.

It should be noted that, in general, tilapia all responded well to each diet treatment, and growth rates were comparable to previous work on tilapia and with comparable feed utilisation parameter data [11]. We show that up to 40 % SBPC can replace FM without significantly detrimental effects on growth or carcass composition. Comparison between the data obtained for the 40SBPC diet and the 80SBPC diet treatments, respectively, indicates that there is a slight difference in carcass composition, notably protein and hence growth. It, therefore, indicates that the component nutrients, for example, crude protein and lipids, may not be fully bioavailable due to the incomplete action of digestive enzymes. Earlier findings of working with juvenile tilapia support such a view [27]. These authors stated that the higher the dietary inclusion of soybean meal, the lower the activity of proteases in the intestine and hepatopancreas, which constrains digestive efficiency.

In summary, our investigation demonstrates soybean protein concentrate can be used as a contributory protein source in diets for Nile tilapia up to a 40 % dietary inclusion threshold. Beyond this level, the soy diet appears deficient in available energy and amino acids, notably methionine. It would be pertinent to explore higher essential amino acids inclusion levels with fishmeal free diets for tilapia. Further research is warranted to determine the potential and optimum use of supplementary amino acids in alternative plant-based ingredients in diets for tilapia and other warmwater species like the African catfish, carp, pangasius and snakehead fish being farmed in many tropical and sub-tropical countries. Coated amino acids are more expensive but could mitigate dependency on fishmeal in aquafeed formulations to a greater extent. They can also prevent leaching losses of conventional supplementary amino acids from the diet and offer greater stability in the aqueous environment for feeds.

The optimal supplementation of crystalline amino acids in feeds for fish provides an opportunity to reduce formulation costs in a quite volatile commodity market for protein ingredients and the supply of fish meals. This should be undertaken for different-sized fish during the various production stages from juveniles (where protein levels are more stringent for essential amino acids) and towards harvest within finishing diets. This will further advance opportunities for a broader understanding of meeting sustainable aquaculture practices by extending plant-based proteins globally in fish diets. The full cost benefits must be considered concerning using commercially expensive crystalline amino acids in formulations where plant-protein ingredients are being further exploited.

5. Conclusion

The results confirmed that high-quality fishmeal can be substituted effectively to 40 % SBPC alone and without any detrimental effects on growth and feed performance or carcass composition for juvenile tilapia fed experimental diets. Crystalline coated essential amino acid supplementation showed marginal improvement of overall performance for tilapia at a high SBPC replacement of 80% fishmeal.

Acknowledgments

The authors are grateful to Tom Corry and Wayne Jackson for the practical aspects of this project and technical support assistance for the feeding trials.

Ethical approval

The animal husbandry techniques involved in the study were undertaken under the supervision of Professor Simon Davies in accordance with the Animal (Scientific Procedures) Act of 1986, License #Pil: 30/00080

Conflict of Interest

The authors declare that there is no conflict of interest relating to this research study.

Author contributions

Simon J Davies conducted the research, collected and analysed the data, wrote the manuscript, and edited it. Tom Corry and Wayne Jackson both collected the data. Matt E Bell analysed the data, wrote, edited, and reviewed the manuscript.

Data Availability statement

The data will be available upon justifiable request to the corresponding author.

Funding: Not Applicable

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How to cite this article: Davies SJ, Bell ME. (2024). Coated Crystalline Amino Acid Supplementation May Assist Higher Soybean Meal Inclusion in Diets for Nile Tilapia (Oreochromis Niloticus) With Improved Protein Assimilation and Retention. Journal of Zoology and Systematics, 2(2), 1-10.