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Complete Replacement of Fish Meal with Potential Aquafeed Ingredients for Rainbow Trout in Iran

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Abstract

Iran, as one of the largest rainbow trout producers in the world, needs sustainable aquafeed resources to fulfill the requirements of this growing industry. Therefore, locally available canola meal, feather meal, blood meal and poultry by-product meal were evaluated on their suitability for feeding rainbow trout. Fish growth performance and apparent digestibility coefficients (ADCs) of nutrients were investigated for three casein-based fish meal-free diets, a practical feed including 10% spray-dried blood meal, 22% poultry by-product meal, 10% feather meal (GOLDMEHL®), 10% canola meal and 15.5% wheat flour in comparison to a commercial diet containing fish meal. The diets were allocated to aquaria in three replicates arranged in a random-block-design. Findings of the present study illustrated that growth performance remained unchanged ($p>0.05$) among fish fed fish meal-free diets and the commercial one. Formulating aquafeed using properly processed local feed ingredients such as poultry slaughterhouse by-products, canola meal, and crystalline amino acids can provide a sustainable solution to meet the feed requirements of the growing aquaculture industry on a regional scale. This research shows the potential for using locally available resources in aquafeed manufacturing.

Introduction

Excluding aquatic plants, about 66% of total aquaculture production is depending on feed, which is manufactured from a range of crops and plant co-products, caught wild fish, and fish trimming/terrestrial animal processing by-products (FAO, 2018). Globally, aquaculture production is continuously increasing and hence the need for aquafeed. The aquafeed sector experienced rapid growth in 2022, with a growth rate of 2.7%. This was notably higher than that observed in other farmed animal sectors, making it the fastest-growing segment within the feed industry. In 2022, approximately 53 million tons of aquafeed were produced globally and the share of Iran was 300 000 tons (Alltech, 2023). Fish meal and fish oil are the primary feed components in most aquafeeds, especially for cultured carnivorous finfish and marine shrimp species. In 2020, the aquaculture industry used about 86% and 73% of the total global produced fish meal and fish oil. However, global fish meal production from small pelagic species has a decreasing trend (FAO, 2022a). Due to the limited availability of fish meal and fish oil, as well as the rising demand for aquafeed, there is a growing need to explore alternative ingredients for future aquafeed formulations, especially from regionally abundant resources such as by-products from other industries. This approach is crucial to have a sustainable development of salmonid culture.

Among the most abundant resources in Iran that can be used as fish feed ingredients is canola meal. According to FAO (2022b), approximately 215 000 t canola seed was produced in Iran, equal to around 118 250 t canola meal in 2021. Besides canola meal, 1 994 000 tons of chicken meat was produced. When an average dressing percentage of 70% for chicken is supposed, as mentioned by Mountney and Parkhurst (1995), about 2 848 000 tons of live chicken was produced. This amount of live chicken can yield around 199 000 tons of raw feather (7%), 99 700 tons of fresh blood (3.5%), and 498 400 tons of other remains (17.5%). These available highly valuable protein sources in the country have a great potential to substitute fish meal. Currently, canola meal is not widely utilized as a feed resource for rainbow trout in Iran due to concerns about the presence of antinutrients in rapeseed cultivars (Burel *et al.*, 2000; Enami, 2011). Moreover, because of technical processing issues, rendering poultry by-products like feather and blood meals are not considered valuable resources for regional aquafeed manufacturers (Bahrevar & Faghani-Langroudi, 2015). In recent years, rainbow trout culture has been developed noticeably in Iran. As stated by FAO (2023), production of this species of salmonid reached approximately 194 000 tons in 2021 in that country, representing 20% of the total production of rainbow trout worldwide (around 952 700 t). Therefore, providing reliable protein feed components for this huge industry should be taken into consideration. Through a controlled feeding trial that assesses fish growth performance and digestibility, this study aims to evaluate the effectiveness of incorporating canola meal and rendered poultry by-products as terrestrial protein sources in experimental fish meal-free diets for rainbow trout. These diets were compared to a commercial rainbow trout feed, which contains fish meal. The results of this study have the potential to provide valuable options for addressing the challenges associated with sourcing sustainable and accessible terrestrial protein components as substitutes for fish meal in the growing rainbow trout industry in Iran..

Materials and Methods

Experimental Diets and Feed Preparation

This experiment was conducted with six diets (Table 1). Except for commercial (Com), the diets were formulated to meet the nutritional requirements of rainbow trout as recommended by NRC (2011) and match the lipid and protein content of the commercial diet. A casein-based semi-synthetic laboratory standard diet (Sem) was produced based on the Guelph Test Diet for trout with some modifications (Sugiura *et al*., 1998, Hardy & Barrows, 2002) as a highly digestible diet and also for testing the digestibility of test ingredients. FeM and CM diets were formulated to assess the impact of feather meal and canola meal on digestibility of the Sem diet as well as fish formance, in which 25% of casein in the standard Sem diet was substituted by either feather meal or canola meal on the basis of crude protein (CP), respectively. The plant-and-animal-based diet (PAD) was formulated with poultry slaughterhouse by-products and canola meal as a practical fish meal-free diet which can be compared to commercial diets for rainbow trout. The commercial diet (Com) was crumbled with a kitchen mixer and screened to 3-5 mm. To measure the digestibility of Com, this feed was ground, marked with titanium dioxide (TiO2) and then pressed again to have a commercial-repelleted (ComR) diet. It is notable to mention that Com and ComR were essentially identical in terms of their nutritional content. The commercial pellets before producing ComR diet as well as feed components were milled with a coffee grinder and passed through a 0.5-mm sieve. Before mixing, the rations containing canola meal (PAD and CM) were supplemented with exogenous microbial phytase at 4000 FYT per kg complete feed. All of the feed mixtures, except Com, were mixed precisely and structured with a dry pellet mill (Type 14-175, Amandus Kahl, Hamburg, Germany) to pass through a 4.0-mm die. The pellets were dried at room temperature for 24 hours and stored airtight at 4°C until use.

Fish Husbandry

Two-hundred-fifty-two juvenile rainbow trout, *Oncorhynchus mykiss*, averaging 30.3±3 g in initial weight and 14±0.76 cm in total length were bought from a local hatchery and allocated randomly to 18 experimental 57-l aquaria (14 fish per aquarium) connected to a semi-recirculating aquaculture system (RAS) at the indoor and windowless facilities with a 12 h light/12 h dark regime at Thünen Institute of Fisheries and Ecology in Bremerhaven, Germany. After 14 days of acclimatization and feeding with a commercial feed, the fish received one of the six experimental diets in three replicates in a random-block-design for 72 days. The fish were individually weighed after 24 hours of starvation at the beginning and end of the trial as well as every two weeks in order to adjust the feeding level to 1.5% biomass daily. The daily ration was offered regularly in two installments at 9:00 and 15:00. The fish were fed by hand and monitored to assure the meal was ingested completely. Otherwise, the feeding was stopped and the amount of remaining feed was recorded. Eventual cases of mortality were also recorded daily. Water inflow was adjusted at 3 l/min for each aquarium. Additionally, the aquaria were oxygenated with an air compressor via sand aerators. The system was equipped with biofilter, ultraviolet (UV) light and mechanical pad filtering.

Water temperature and dissolved oxygen content were measured by the probe weekly from each aquarium and daily from inflow and outflow basins two hours after feeding. The pH was measured also via probe and at the same time, NH_4^+, NO_2^-, and NO_3^- were controlled photometrically three times per week in the

Table 1. Composition of experimental diets (g.kg^{-1} dry matter)

	Diet[†]					
Ingredient	Sem	FeM	CM[‡]	PAD[‡]	Com[*]	ComR
Blood meal[1]				100.0		
Poultry by-product meal[2]				220.0		
Feather meal[3]		97.5		100.0		
Canola meal[4]			243.6	100.0		
Wheat flour				155.0		
Casein	400.0	300.0	300.0			
Gelatine	40.0	40.0	40.0			
Cellulose[5]	132.0	134.0	80.0	126.0		
Dextrin	90.0	90.0	30.0			
Pre-gelatinized corn starch[6]	107.4	110.2	109.7			
Fish oil	150.0	150.0	150.0	150.0		
Canola oil	49.0	45.0	18.0	1.0		
L-Lysine[7]				2.0		
DL-Methionine[8]		0.6	1.0	9.0		
L-Arginine[9]	1.9					
L-Threonine[10]				1.5		
L-Tryptophan[11]				1.0		
Carboxymethyl cellulose[5]				10.0		
Monocalcium phosphate	15.5	18.5	13.5	10.3		
Choline chloride 98%	1.0	1.0	1.0	1.0		
Vitamin C 35%	0.2	0.2	0.2	0.2		
Vitamin premix[12]	5.0	5.0	5.0	5.0		
Mineral premix[12]	3.0	3.0	3.0	3.0		
TiO2	5.0	5.0	5.0	5.0		5.0
Commercial diet[13]					1000.0	995.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[†]Sem: casein-based semi-synthetic laboratory standard diet; FeM and CM: diets where 25% of casein in Sem were substituted by feather meal or canola meal on the basis of crude protein, respectively; PAD = plant-and-animal-based diet; Com: commercial diet; ComR: commercial re-pelleted diet.

[‡]Supplemented with exogenous microbial phytase.

[*]According to the manufacturer the Com diet contained poultry by-product meal, soybean meal, feather meal, wheat, fish meal, canola oil, poultry fat, hemoglobin powder, fish oil.

[1]Spray-dried, provided by GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany.

[2]Poultry meal 64%, provided by GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany.

[3]GOLDMEHL®, provided by GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG, Diepholz, Germany.

[4]Provided by Teutoburger Ölmühle GmbH, Ibbenbüren, Germany.

[5]Provided by Mikro-Technik GmbH & Co. KG, Bürgstadt am Main, Germany.

[6]Provided by Kröner-Stärke GmbH, Ibbenbüren, Germany.

[7]Biolys®, provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany.

[8]MetAMINO®, provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany.

[9]Provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany.

[10]THREAMINO®, Provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany.

[11]TrypAMINO®, Provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany.

[12]Vitamin and mineral requirements of fish were met. Provided by Trouw Nutrition Deutschland GmbH, Burgheim, Germany.

[13]Skretting (Optiline F-3P, 6 mm)

inflow basin water. All experimental procedures were carried out in compliance with the European Directive 2010/63/EU, which governs the protection of animals used for scientific research purposes. An overview of the experimental conditions can be found in Table 2.

Sample Collection, Sample Preparation and Chemical Analysis

At the beginning of the trial, twenty fish were randomly selected from the fish stock as reference and anesthetized with an overdose of 2-Phenoxyethanol (Merck KGaA, Darmstadt, Germany), weighed and sacrificed by cutting the gill artery. Subsequently, they were stored at -21°C until further processing. This procedure was also used for the fish from each aquarium at the end of the experiment. The frozen fish bodies were defrosted in a fridge at 4°C overnight and autoclaved. The autoclaved fish samples were ground and frozen again at -21°C for at least 48 hours before freeze drying. To measure the digestibility of the diets, excreta was passively collected in the last six weeks by gently siphoning them from the bottom of the aquaria before feeding the fish in the morning and two hours after feeding. Collected feces were kept at -21°C until freeze-drying. A freeze drier was used to dry the autoclaved fish samples and the collected excreta. After drying, fish samples were ground with a laboratory grinder but the feces were with the coffee mill. The prepared samples were stored airtight at -21°C until further lab analyses.

Dry matter content was measured after drying in an electric oven at 103°C for four hours. For determination of ash content, samples were placed in a muffle furnace at 550°C for three hours. The gross energy content for the diets was determined by a bomb calorimeter. Crude lipid (CL) was analyzed by the Smedes extraction method with small modifications (Schlechtriem *et al*., 2003). The crude protein (CP = N% × 6.25), *in-vitro* digestible protein, crude fiber contents as well as amino acids of diets were measured by either SGS GmbH (Hamburg, Germany) and AGROLAB LUFA GmbH (Kiel, Germany), respectively, according to the European Union Regulations on methods of sampling and analysis for the official control of feed (EC/No 152/2009). The nitrogen-free extract (NFE) for the diets were determined by subtracting measured CP, CL, crude fiber and ash from 1000. The indigestible marker, TiO_2, was measured as described in detail by Zeller *et al*. (2015). The pH of diets was measured with the same probe for water pH measurements. The analytical data of experimental diets are presented in Table 3.

Calculations

Growth Performance

Weight gain (WG), feed conversion ratio (FCR), daily instantaneous growth rate (G_w) were calculated as follows:

WG [g] = (Final average body weight [g] + Mortality weight [g]) - Initial average body weight [g]

FCR = Dry feed fed [g] / WG [g]

G_w [$g.d^{-1}$] = [ln (Final average body weight in g + Mortality weight in g) – ln initial average body weight in g] / Number of trial days

Productivity and Digestibility

The comparative slaughter method (Jobling, 2001) was used to measure the nitrogen productive value (NPV) and lipid productive value (LPV) to evaluate the retention of those nutrients in fish body over the experimental period. Both NPV and LPV were calculated with the following formula for nutrient productive value (NutPV).

NutPV [%] = [(Final fish body nutrient in g – Initial fish body nutrient in g) / Total consumed nutrient in g] × 100

Where nutrient is either nitrogen (N) or crude lipid (CL).

The protein efficiency ratio (PER) was calculated with the formula by Hardy and Barrows (2002).

PER [g] = WG [g] / Dry crude protein fed [g]

The apparent digestibility coefficients (ADCs) for the nutrients, including crude protein (CP), crude lipid (CL), and organic matter (OM) were calculated as recommended by NRC (2011).

Table 2. Experimental conditions of the trial

Volume (*l*)	
whole system	1500
aquarium	57
Water inflow rate (*l*/min)	
Well water inflow into the system	8
Water flow to each aquarium	3
Water turnover rate (times/day)	
whole system	7.7
aquaria	75.8
Water source	Preprocessed well water
Number of aquaria per diet	3
Type of aquaria	rectangular glass, tapered bottom
Water temperature (°C)	
aquaria	13.30±0.20
system inflow	13.26±0.24
system outflow	13.27±0.23
Dissolved oxygen (mg/*l*)	
aquaria	9.30±0.40
system inflow	9.72±0.28
system outflow	9.56±0.28
pH	7.6±0.1
NH_4^+ mg/*l*	0.10±0.09
NO_2^- mg/*l*	0.23±0.21
NO_3^- mg/*l*	3.97±1.97
Photoperiod (light:dark)	12:12 with LED light

ADC = [1 – (TiO_2 concentration in feed) / (TiO_2 concentration in feces) × (Nutrient concentration in feces) / (Nutrient concentration in feed)] × 100

Statistical Analysis

The parameters were separately tested for normality and homogeneity of variances by Shapiro-Wilk and Levene tests, respectively. For each of the parameter it was also tested, whether the individual aquarium should be included as a random component. Since the effect of aquarium was not significant, we decided for linear models without random component (Zuur, 2011), but conducted one-way ANOVA. For the parameters where the normality assumption was violated, Kruskal-Wallis test was used. Tukey's HSD and Dunn tests were used to distinguish the statistical pairwise differences among means, respectively. Results were considered significant at $p<0.05$. All statistical analyses were conducted using R software version 3. 5. 1 (RCoreTeam, 2018).

Results

Growth and Feeding Efficiency Parameters

The growth performance and feeding efficiency criteria resulting from feeding the experimental diets are presented in Table 4. No significant differences ($p>0.05$) were observed for feed intake (FI), weight gain (WG), mortality weight (MW), feed conversion ratio (FCR) and daily instantaneous growth rate (G_w) among experimental diets. With the exception of PAD diet, all of the formulated diets resulted in a similar lipid productive value (LPV) to the commercial diet. Regarding the productivity of nitrogen, the fish consumed the commercial pellets showed significantly lower NPV than the casein-based semi-synthetic laboratory standard diets, but the plant-and-animal-based diet represented a same NPV to both commercial and casein-based semi-synthetic laboratory standard diets. Concerning the protein efficiency ratio (PER), commercial diet (Com) with the average 2.15±0.03 g

Table 3. Nutritional analysis of experimental diets (g.kg^{-1} dry matter

	Diet*					
	Sem	FeM	CM	PAD	Com	ComR
Dry matter	931	937	910	911	915	887
Gross energy (MJ/kg)	23.1	23.2	23.7	23.4	23.5	23.3
Crude protein	420	402	394	417	472	455
Digestible protein **	401	370	368	362	445	407
Crude lipid	176	180	190	193	193	198
Crude fiber	96	93	80	108	36	33
Crude ash	33	34	43	64	70	74
Nitrogen-free extract (NFE) †	275	291	293	218	229	240
Organic matter (OM) ‡	967	966	957	936	930	926
NFE:OM (%) ††	28.4	30.1	30.6	23.3	24.6	25.9
pH	4.20	4.47	5.14	5.83	5.93	5.96
Amino acids						
Alanine	16.3	19.3	16.5	25.1	28.1	24.5
Arginine	20.2	23.2	23.6	31.2	32.5	33.1
Aspartic acid/asparagine	31.7	32.4	28.1	32.8	44.6	38.9
Cysteine	1.7	6.5	3.6	9.3	8.1	7.1
Glutamic acid/glutamine	94.1	89.3	83.3	55.0	67.8	59.3
Glycine	18.6	27.5	21.1	30.8	32.6	28.9
Histidine	12.2	10.8	11.2	12.1	11.7	10.3
Isoleucine	21.8	22.8	18.8	18.4	20.4	17.6
Loucine	39.1	39.9	33.3	34.9	38.3	33.6
Lysine	34.2	29.8	29.0	24.9	26.8	23.4
Methionine	11.9	10.1	10.7	14.7	8.7	7.7
Phenylalanine	21.8	22.9	18.6	16.1	22.2	19.7
Proline	50.0	52.8	41.1	29.4	31.8	27.6
Serine	24.1	30.7	20.1	24.6	27.9	25.0
Threonine	17.4	18.1	15.6	19.4	18.4	16.2
Tryptophan	5.3	3.9	4.9	5.6	4.4	4.3
Tyrosine	18.9	17.3	15.1	10.1	12.9	10.4
Valine	27.5	30.4	23.5	25.1	28.6	25.0
TiO_2	5.9	5.7	5.5	5.6		5.6

*Sem: casein-based semi-synthetic laboratory standard diet; FeM and CM: diets where 25% of casein in Sem were substituted by feather meal or canola meal on the basis of crude protein, respectively; PAD = plant-and-animal-based diet; Com: commercial diet; ComR: commercial re-pelleted diet.

** *in-vitro* protein digestibility.

†Calculated by subtracting crude protein, crude lipid, crude fiber and ash from 1000.

‡Calculated by subtracting the ash content from 1000.

††Calculated by dividing NFE by OM and multiplying 100.

resulted in a less protein efficiency than the casein-based semi-synthetic laboratory standard diet with either feather meal (2.86±0.04 g) or canola meal (2.76±0.06 g). Moreover, the plant-and-animal-based diet and casein-based semi-synthetic laboratory standard diet represented no considerable differences in among themselves and with other experimental diets.

Digestibility

An overview of the apparent digestibility coefficients (ADCs) for the nutrients in the experimental diets is presented in Table 5. All of the formulated diets showed no significant differences with commercial for CP and OM digestibility. However, plant-and-animal-based diet obtained significantly different values with casein-based semi-synthetic laboratory standard diet (Sem) for CP ADC and with casein-based semi-synthetic laboratory standard diet with canola meal (CM) for organic matter ADC. The fish consumed casein-based semi-synthetic laboratory standard diet digested the CP to about 98% in contrast to plant-and-animal-based diet where 91% were digested. Considering the crude lipid ADCs, commercial and plant-and-animal-based diet did not differ significantly. The biggest and the smallest ADCs of crude lipid was observed for casein-based semi-synthetic laboratory standard diet with canola meal (94%) and casein-based semi-synthetic laboratory standard diet with feather meal (89%), respectively.

Discussion

Growth Parameters

The PAD diet formulated without any fish meal resulted in similar fish growth performance parameters to either commercial or casein-based semi-synthetic laboratory standard diets. Greiling *et al*. (2018) found that although untreated canola cake (mentioned as rapeseed by the author) involved some anti-nutritional factors, the moderate feeding level of 10.3% canola cake did not differ considerably WG, FI and FCR compared to the control group containing fish meal in rainbow trout. Furthermore, Lu *et al*. (2015) observed that 75% and 100% fish meal replacement with rendered poultry products such as poultry by-product meal, feather meal and blood meal did not negatively influence FI, WG and SGR in rainbow trout; however, those parameters deteriorated in the fish that consumed the diet with plant protein and rendered poultry by-product sources. This was not observed in the present study. Our findings PAD diet and casein-based semi-synthetic laboratory standard diet containing canola meal (CM) were in agreement with the work of Shafaeipour *et al*. (2008). They observed that the inclusion of 10% canola meal in the fish diet had no effect on FCR, WG, G_w and PER.

The fish that fed experimental diets did not show any significant differences in feed intake and this might have resulted in a similar WG and G_w. Morales *et al*.

Table 4. Growth parameters of rainbow trout achieved over the course of a 72-day feeding trial with six diets

Parameter	Diet*						Pooled P-value
	Sem	FeM	CM	PAD	Com	ComR	
Initial weight [g]	552.87±25.42^{a}	569.87±9.5^{a}	564.4±13.79^{a}	562.27±6.21^{a}	559.5±13.6^{a}	563.03±11.56^{a}	0.809
Final weight [g]	984.40±436.30^{a}	1299.07±91.62^{a}	952.43±228.98^{a}	1003.57±252.55^{a}	951.47±270.83^{a}	832.6±121.33^{a}	0.413
Mortality weight [g]	181.93±239.29^{a}	56.83±62.7^{a}	237.03±137.42^{a}	122.67±99.41^{a}	173.23±166.29^{a}	244.57±79.18^{a}	0.612
Average weight gain [g]	613.47±231.41^{a}	786.03±39.44 ^{a}	625.07±84.37^{a}	563.97±155.02^{a}	565.2±93.24^{a}	514.13±49.81^{a}	0.222
Feed intake [g]	550.91±138.65^{a}	684.06±31.35^{a}	573.97±65.4^{a}	560.72±89.37^{a}	557.42±87.18^{a}	506.3±49.18^{a}	0.262
FCR	0.93±0.11^{a}	0.87±0.01^{a}	0.92±0.02^{a}	1.02±0.14^{a}	0.99±0.01^{a}	0.99±0.05^{a}	0.251
G_w [g day^{-1}]	0.01±0.0^{a}	0.01±0.0^{a}	0.01±0.0^{a}	0.01±0.0^{a}	0.01±0.0^{a}	0.01±0.0^{a}	0.302
LPV [%]	65.86±3.76^{a}	69.55±3.27^{a}	61.96±5.78^{ab}	50.36±3.97^{b}	65.41±3.96^{a}	67.72±5.62^{a}	< 0.01
NPV [%]	43.50±5.10^{b}	44.51±0.79^{b}	43.14±3.43^{b}	37.20±3.72^{ab}	33.22±2.37^{a}	33.71±0.67^{a}	< 0.01
PER [g]	2.60±0.33^{ab}	2.86±0.04^{b}	2.76±0.06^{bc}	2.38±0.31^{ab}	2.15±0.03^{a}	2.23±0.11^{ac}	< 0.01

***** Sem: casein-based semi-synthetic laboratory standard diet; FeM and CM: diets where 25% of casein in Sem were substituted by feather meal or canola meal on the basis of crude protein, respectively; PAD = plant-and-animal-based diet; Com: commercial diet; ComR: commercial re-pelleted diet.
FCR, feed conversion ratio; G_w, daily instantaneous growth rate; LPV, lipid productive value; NPV, nitrogen productive value; PER, protein efficiency ratio.
The reported values are the mean of three replicates (*n* = 3) with their standard deviation (SD).
The means within one line not sharing a superscript letter are significantly different (p<0.05).

Table 5. Nutrient apparent digestibility coefficients for experimental diets determined at the end of a 72-day feeding trial

Parameter	Diet*****					Pooled P-value
	Sem	FeM	CM	PAD	ComR	
CL ADCs [%]	90.36±0.35^{b}	88.54±1.08^{c}	94.35±0.42^{d}	92.33±0.77^{a}	92.28±0.29^{a}	< 0.001
CP ADCs [%]	98.68±0.04^{b}	97.13±0.16^{ab}	97.36±0.15^{ab}	90.72±0.29^{a}	91.64±0.20^{ab}	< 0.05
OM ADCs [%]	79.84±0.22^{ab}	80.22±0.06^{ab}	82.08±0.30^{b}	73.31±0.74^{a}	80.72±0.70^{ab}	< 0.05

***** Sem: casein-based semi-synthetic laboratory standard diet; FeM and CM: diets where 25% of casein in Sem were substituted by feather meal or canola meal on the basis of crude protein, respectively; PAD = plant-and-animal-based diet; Com: commercial diet; ComR: commercial re-pelleted diet.
CP ADCs, crude protein apparent digestibility coefficients; CL ADCs, crude lipid apparent digestibility coefficients; OM ADCs, organic matter apparent digestibility coefficients.
The reported values are the mean of three replicates (*n* = 3) with their standard deviation (SD).
The means within one line not sharing a superscript letter are significantly different (p<0.05).

(1994) stated that feed intake can influence WG in fish. The statistically equivalent FCR in this experiment can be related to the non-significant differences in both FI and WG. The achieved results from feeding PAD diet, which 22% of its total digestible protein supplied from feather meal, agreed with previous works. Bureau *et al*. (2000) observed that the addition of 20% of the total digestible protein via feather meal did not deteriorate growth, feed efficiency, protein and energy gains in rainbow trout. Steffens (1994) recommended that a combination of poultry by-product meal and feather meal can be a good substitute for fish meal in rainbow trout feed if methionine and lysine were supplemented. In accordance with Kaushik and Seiliez (2010), it is essential to take into account the amino acid composition of aquafeed using fish meal alternatives. Therefore, in the current study, the PAD diet was carefully balanced by incorporating synthetic methionine, lysine, threonine, and tryptophan at an approximate rate of 1.4% DM of the complete feed. The addition of these small quantities of synthetic amino acids seems to have minimal impact on the overall cost of that diet, as the production of synthetic amino acids has become more widespread and cost-effective compared to before.

The apparent retention of nutrients in the fish body is a practical approach to assess the availability and balance of the respective nutrients (Hardy and Barrows, 2002). This can also be used to evaluate the eutrophication potential of aquafeeds. The casein-based diets were formulated from the point of their relative high digestibility compared to practical diets. Except PAD diet, casein-based semi-synthetic laboratory standard diets (Sem, FeM, CM) had a similar lipid retention when compared to the commercial diets although they had a relative lower crude lipid content. Moreover, the PAD diet showed notably a lower LPV than all of the experimental diets except the CM. This could be explained in part by the lower proportion of NFE to organic matter (OM) or NFE:OM in that ration rather than others and its lipid was mostly metabolized as an energy source. In proximate analysis, NFE is a prediction of easily available carbohydrates such as sugars, dextrins and starches as well as water-soluble vitamins (Hardy and Barrows, 2002; Kellems and Church, 2010). Even though carbohydrates are nonessential in fish feed, they comprise a cheap source of energy. With the shortage of carbohydrates in diets, proteins and lipids could be catabolized as an energy source for fish (Guillaume *et al*., 2001). Therefore, the absence of that energy source caused a low lipid accretion in the fish fed PAD.

The PAD diet resulted in a resembling NPV and PER with either commercial or casein-based semi-synthetic laboratory standard diets. The tested casein-based semi-synthetic laboratory standard diets (Sem, FeM, CM) had a significantly higher NPV than the commercial diets (Com, ComR). This is in accordance with Morales *et al*. (1994) who reported the NPV above 40% for the diets containing casein as a sole or part of the protein proportion. This can be explained by the higher protein contents in Com and ComR diets, which might increase nitrogen excretion more than retention and thereby lower NPV. Through increasing lipids or energetic compounds and decreasing the ratio of digestible protein to digestible energy, better FCRs and greater nitrogen utilization are expected in fish, particularly in salmonids (Guillaume *et al*., 2001). To explain how efficient a protein source supports the growth in fish, PER is applied (Jobling, 2001). The fish fed the Com diet had a lower final body weight per gram ingested protein compared to either FeM or CM. The gross energy contents in all the experimental diets were almost similar. It seems that the higher NFE fraction in the FeM and CM diets potentially led to “protein sparing”, meaning a decrease in catabolism of protein/amino acids for energy requirements (Bureau *et al*., 2002). The inclusion of 22% poultry by-product meal (PBM) in the current study was in line with Erturk and Sevgili (2003) who attained a nonsignificant difference for PER among the fish received up to 20% of that ingredient and the fish meal-based control diet.

Digestibility of Experimental Diets

Regarding crude lipid apparent digestibility coefficients (CL ADCs), both PAD and ComR diets had statistically similar coefficients but the CM had the maximum value. Generally, lipids have a good digestibility, particularly polyunsaturated fatty acids; however, low water temperature and high saturation level as well as the length of the carbon chain decrease digestibility (Guillaume *et al*., 2001). The CL ADC (94%) and CP ADC (97%) for the CM diet were higher compared to the findings of Dalsgaard *et al*. (2012) with the inclusion rate of 26% of the same oilseed meal in a fish meal-based diet which resulted in 80% and 88% for CL ADC and CP ADC, respectively. This is probably due to the synergetic effect of fatty acids from canola meal and casein on lipid digestibility. Morales *et al*. (1994) also obtained a higher lipid digestibility when they used both casein and fish meal as protein sources in the diet rather than only fish meal as a sole protein source. Although the fatty acid content in the experimental diets was not measured in our study, it seems likely that the fatty acid combination from casein and canola meal in the CM diet may have improved the lipid digestibility in that diet rather than the others. We observed the minimum CL ADC for the FeM (89%) when compared to the other diets. This can be explained from the contamination of feather meals with saturated fatty acids originating from poultry tissues. Avian lipids contain dominantly saturated and monounsaturated fatty acids (Guillaume *et al*., 2001). In spite of almost equal incorporation of feather meal in both PAD and FeM, the former had a higher lipid digestibility due in part to the inclusion of more diverse lipid sources and this may have a dilution effect on saturated fatty acids from contaminated feather meals. Austreng *et al*. (1979) found that the

unsaturated forms of fatty acids were utilized more efficiently than their saturated fatty acids. It should be also mentioned that feathers contain waxes. These lipids include a long-chain fatty acid associated with alcohol which are resistant to degradation and absorption in animals (McDonald *et al*., 2011). Even though wax esters include very hydrophobic fatty alcohol substrates, fish lipases hydrolyze them not as efficiently as triacylglycerols (Lie and Lambertsen, 1991).

The CP ADCs for crude protein were not significantly different among the experimental diets except for the PAD and Sem diets. The high digestibility of CP in the Sem diet was expected since the primary source of protein used was refined casein. The liberation of amino acids plays a crucial role in determining the digestibility of proteins. Consequently, the digestion of proteins is influenced by factors such as the type of protein, the bonds present within amino acids, and the interactions between these structural units of protein and other components (NRC, 2011). As a phosphoprotein, casein serves as the primary protein in milk and possesses excellent quality. However, it is limited in terms of methionine and cysteine content (McDonald *et al*., 2011; Hertrampf and Piedad-Pascual, 2000). The growth performance of the fish that consumed Sem, FeM, and CM diets remained undisturbed, and their liver appeared normal from the point of color and structure. The high values of CP ADC for these diets have already been noted in rainbow trout (Sugiura *et al*., 1998; Morales *et al*., 1994). The fish fed on PAD showed a lower CP ADCs (91%) than the Sem (98%). It is assumed that the higher fiber content in the PAD diet caused a reduced CP ADC. According to Jobling (1981), protein digestibility values are associated with the combination of feed formulas rather than individual feed compounds. He observed that an increase in indigestible carbohydrates in carnivorous fish species resulted in reduced protein digestibility. As a recommendation, he suggested that complex carbohydrates like starch and α-cellulose should be maintained at low levels to maximize weight gain in fish. Glencross (2009) reported that insoluble fibers have a negative impact on the digestibility of dry matter, energy, and protein in rainbow trout. Greiling *et al*. (2018), Saez *et al.* (2015), and Glencross *et al*. (2007) have all observed a positive effect of reducing fiber or dehulling on the apparent digestibility coefficient (ADC) of nutrients in rainbow trout. Since fibers cannot be fully degraded in rainbow trout, these materials facilitate the transition of digesta in digestive tract (Guillaume *et al*., 2001). This faster evacuation rate, however, leads to reduced contact between digestive enzymes and digesta. Additionally, Kozlowska *et al*. (2001) noted that dietary fiber dilutes nutrients in diets, which negatively affects their absorption. Despite the ComR diet having a higher protein content compared to other treatments, its CP ADC did not show a significant difference when compared to either PAD or Sem diets. This finding aligns with the observations made by Sugiura *et al.* (1998) and Shiau and Huang (1989), who also found no significant correlation between feed protein content and apparent protein digestibility.

The OM ADC did not differ notably between diets, except for the PAD and CM. These diets contained the minimum and maximum nitrogen-free extract (NFE), respectively. Moreover, the collected feces from the fish consumed CM diet were not so stable in water and this can lead to a higher OM ADC via leaching the soluble carbohydrates compared to other diets. Although trout has a very low amylase activity comparing to omnivores species (Hidalgo *et al*., 1999), it seems there is a direct relation between OM ADCs and NFE in diets meaning the quantity and composition of NFE portion as an energy-providing source affected the OM ADCs in experimental diets since the CM diet had both high NFE and OM ADC. Arnesen and Krogdahl (1995) found a direct association between the oat inclusion level and its starch digestibility as well as blood glucose level and liver glycogen in rainbow trout. Furthermore, by incorporating various sources of starch in the diet, that carbohydrate was utilized more efficiently in salmon and the glycogen content in the liver increased in line with the level of starch (Arnesen *et al*., 1995). It can be mentioned that higher NFE in the CM diet played a major role in OM ADC by providing more various carbohydrates from canola meal and the purified sources such as pregelatinized starch and dextrin in contrast to the PAD diet with the least NFE fraction. It can also be assumed that the higher lipid digestibility in the CM diet has improved its OM digestibility.

Conclusion

In this research, the PAD diet showed no significant difference with a commercial diet containing fish meal for all of the experimental parameters except for LPV; hence, it can be concluded that a sustainable fish performance can be achieved through formulating rainbow trout diets with terrestrial protein sources as well as synthetic amino acids. Since minor incorporation of synthetic amino acid was cost-effective, this has a potential to reduce the dependence of the aquafeed industry from marine fish stocks and foster the growing aquaculture industry locally, supply valuable food to the public and generate employment and income in rural areas.

Ethical Statement

All experimental procedures were conducted in accordance with European directive 2010/63/EU on the protection of animals used for scientific purposes.

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Author Contribution

Hamed Salehi planned the study, conducted experiments, organized lab work, analyzed data and wrote the manuscript. Stefan Reiser supervised experimental work and contributed to the manuscript. Mohammad Pourkazemi organized data collection in Iran. Ulfert Focken designed the study, supervised the experimental work, acquired funding and contributed to the manuscript.

Conflict of Interest

The authors confirm no conflicts of interest.

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