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Apparent digestibility of dry matter, organic matter, protein and energy of native Peruvian feedstuffs in juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Trout production is a growing activity in recent years but requires new alternative sources of feed to be sustainable over time. The objective of this research was to determine the apparent digestibility coefficient (ADC) of dry matter (DM), organic matter (OM), crude protein (CP) and digestible energy (DE) of kañiwa (*Chenopodium pallidicaule* Aellen), kiwicha (*Amaranthus caudatus* L), quinoa (*Chenopodium quinoa* Willd), beans (*Phaseolus vulgaris* L.), sacha inchi, (*Plukenetia volubilis* L) and jumbo squid (*Dosidicus gigas*) meal in juvenile rainbow trout. The experimental diets were composed of a 70% basal diet and 30% of any raw materials. The ADC was determined by the indirect method using insoluble ash as a non-digestible marker. Jumbo squid, sacha inchi and quinoa showed the highest values of ADC (%) of DM (84.5, 73.5 and 69.7), OM (89.1, 78.4 and 72.9), CP (93.2, 98.0 and 90.3), and DE (4.57, 4.15 and 2.95 Mcal/kg DM), respectively. The ADC values for kañiwa, kiwicha and bean were significantly lower. In conclusion, quinoa meal and jumbo squid meal have an acceptable digestibility but sacha inchi meal is a potential alternative for rainbow trout feeding in the future.

Keywords: Digestibility, Jumbo squid meal, Quinoa, Rainbow trout feed, Sacha inchi

Background

Trout production in Peru has experienced considerable growth during the last 10 years (7.5 times), and Puno region produced 43,290 tons lately ([PRODUCE] Ministerio de la Producción 2017). Production of organic trout is also being considered due to the increase in demand for organic aquaculture.

Trout feeding requires feeds with high protein and energy content, which are mostly covered with fishmeal and fish oil (NRC (National Research Council) 2011). The availability of these raw materials is decreasing and therefore prices are increasing (FAO (Food and Agriculture Organization of the United Nations) 2016). The current trend of trout producers is to reduce the inclusion of fishmeal in the rations and replace it with vegetable raw materials but must supply essential nutrients for optimal fish performance (Glencross 2011). If an acceptable trout production with alternative raw materials is achieved, then it will increase profits and maintain its sustainability (Li et al. 2006).

The successful use of vegetable ingredients as a partial replacement for fish meal has been documented by several researchers (Gomes et al. 1995; Kaushik et al. 1995; Kumar et al. 2011; Lund et al. 2011; Lech and Reigh 2012). In fact, some diets that include proteins of vegetable origin are capable of producing acceptable growth comparable to traditional diets based on fishmeal (Gaylord et al. 2007; Davidson et al. 2013). However, it is necessary to identify and determine the nutritional value of newly available plant resources that can be used in the formulation of diets for fish.

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The Andean grains such as kañiwa (*Chenopodium pallidicaule* Aellen), kiwicha, (*Amaranthus caudatus* L) and quinoa (*Chenopodium quinoa* Willd) contain proteins of high biological value and are the main source of protein and energy in the diet of the Andean people (Repo-Carrasco et al. 2003; Repo-Carrasco et al. 2009; Molina-Poveda et al. 2015) since more than 80% of world production is carried out in Peru and Bolivia (Bazile et al. 2016). Likewise, beans (*Phaseolus vulgaris* L.) are an important source of nutrients in human nutrition (Barampama and Simard 1993; Prolla et al. 2010) and in aquafeeds (Rodríguez-Miranda et al. 2014).

Sacha inchi (*Plukenetia volubilis* L) produced in the Peruvian Amazonia has seeds rich in protein, oil and vitamins. The sacha inchi meal is an extruded by-product obtained after the extraction of its oil which is used for beneficial purposes in humans as improving the lipid profile of patients with dyslipidemia (Garmendia et al. 2011). Because of its high content of proteins of nutritional value (Ruiz et al. 2013), sacha inchi becomes an attractive alternative for animal feed.

The squid (*Dosidicus gigas*) is an abundant marine resource and a potential source of protein in Perú. The squid meal is produced based on the residues from the slaughtering process and has been used as an ingredient in shrimp diet (Córdova-Murueta and García-Carreño 2002).

Evaluating the digestibility of novel raw materials is important not only because it determines the proportion of nutrients that are available for trout but also because it also allows us to know the proportion of waste that is eliminated in the aquatic environment. In addition, it is necessary for identified sources of organic feed ingredients to develop organic feed formulations to produce organic aquacultured products for this burgeoning market.

The objective of the present study was to determine the apparent digestibility of dry matter, organic matter, crude protein and digestible energy of five organically certified plant protein sources (kañiwa, kiwicha, quinoa, beans, sacha inchi) and a marine protein source (jumbo squid) in juvenile rainbow trout.

Methods

Location, experimental design and analytical determination

All the experimental procedures were carried out in the Faculty of Veterinary of the National University of the Altiplano, Puno, Perú, at an altitude of 3828 m (15°49′29″S, 70°00′56″W). The digestibility test was performed in a water recirculation system (0.5 l/s) equipped with a closed water treatment system, gravel filter (STF Filtre System Leri Model 002737), activated carbon filter, biological filter (clays with Nitrifying Bacteria, Proline®) and UV filter (X-Ray UV Light Boyo®, China).

The system consisted of digestibility tanks (500 l capacity), each one provided with a sedimentation unit for fecal collection (Rodehutscord et al. 2000). The average water quality parameters were pH 8.6, temperature 12 °C (Peachimeter SI Analytics Lab 850®, Germany) and dissolved oxygen 6.2 mg/l (HI 9146 Dissolved Oxygen Meter HANNA®). A total of 198 juvenile rainbow trout from a commercial line (Troutlodge®, USA) of 130 days of age were used, with an initial weight of 92.6 ± 3.32 g and a total length (Ichthyometer, Aquatic Eco-Systems®) of 20.2 ± 0.42 cm (mean ± SD). The fish were randomly distributed in the digestibility tanks with an average load density of 4.4 kg/m^3. The small farmers used around stocking density of 10 kg/m^3. Before handling, the fish were placed in a smaller aerated tank containing tricaine methanesulfonate (50 mg/l) until they lost consciousness.

The chemical proximal composition of raw materials and experimental diets are shown in Tables 1 and 2, respectively. Seven diets were evaluated (basal diet and six experimental diets), and three tanks were considered per treatment (diet). The experimental diets were composed of a 70% basal diet and 30% of any of the five vegetable raw materials (kañiwa, kiwicha, quinoa, beans and sacha inchi) organically certified by Bio Latina, Perú and an animal raw material (jumbo squid), according to the methodology proposed by (Glencross et al. 2007). The basal diet was formulated considering the nutritional requirements for trout (NRC (National Research Council) 2011). The ingredients were mixed and then extruded (Khal® EE800, Germany). The apparent digestibility of the ingredients was determined by the indirect method using a non-digestible marker (Hyflo Super Cel®) according to (Rodehutscord et al. 2000). The test consisted of 10 days of habituation to the diet, to the environment and to the management and another 15 days of sample collection. The experimental diets were fed to the juveniles twice a day (10:00 and 16:00 h) until apparent satiety. After feeding, all waste of uneaten feed was collected and eliminated from the system and then excreta were collected directly from the sedimentation bottle. The experiment was carried out under a regime of natural light.

The novel ingredients, diets and feces, were analyzed according to the methodology of the (AOAC (Association of Official of Analytical Chemists) 2011). The dry matter was determined after drying for 4 h at 105 °C. In addition, gross energy (GE) was determined with a bomb calorimeter (Parr Instrument 6772® USA). The indigestible marker in the diets and feces were determined

Table 1 Chemical proximal composition of the novel raw materials

Parameters (%)	Kañiwa	Kiwicha	Quinoa	Bean	Sacha inchi	Jumbo squid
Dry matter	89.8 ± 0.26	91.0 ± 0.18	89.7 ± 0.27	90.2 ± 0.37	93.2 ± 0.20	90.5 ± 0.82
Organic matter	95.5 ± 0.01	96.1 ± 0.07	96.6 ± 0.02	96.0 ± 0.06	94.5 ± 0.02	87.9 ± 0.07
Crude protein	13.4 ± 1.03	15.4 ± 0.74	11.1 ± 0.76	21.9 ± 0.44	54.9 ± 1.82	72.0 ± 0.33
Crude lipid	4.5 ± 0.36	7.5 ± 1.14	6.7 ± 0.56	4.1 ± 0.19	9.9 ± 0.52	3.5 ± 0.97
Fibre	9.2 ± 0.25	7.5 ± 0.79	5.1 ± 0.25	4.8 ± 0.26	4.9 ± 0.87	2.2 ± 0.35
Carbohydrates[1]	58.2 ± 0.38	56.6 ± 0.90	63.4 ± 0.38	55.4 ± 0.47	18.0 ± 1.94	0.6 ± 0.51
Ash	4.5 ± 0.01	3.9 ± 0.07	3.4 ± 0.02	4.0 ± 0.06	5.5 ± 0.02	12.1 ± 0.07
Gross energy[2]	4.64 ± 0.04	4.61 ± 0.01	4.53 ± 0.01	4.44 ± 0.01	5.25 ± 0.02	5.08 ± 0.08

Values are means ± SD for three replicates
[1]CH = DM − (CP + CL + F + A)
[2]Analyzed by combustion (Mcal/kg DM)

Table 2 The ingredients and chemical composition of experimental diets

	Diets						
	Basal	Kañiwa	Kiwicha	Quinoa	Bean	Sacha inchi	Jumbo squid
Ingredients, %							
Fish meal	44.00						
Kañiwa		29.61					
Kiwicha			29.61				
Quinoa				29.61			
Bean					29.61		
Sacha inchi						29.61	
Jumbo squid							29.61
Soybean meal	24.40						
Corn meal	8.00						
Wheat middlings	14.00						
Fish oil	8.00						
Common salt	0.30						
Marker (Hyflo Super Cel®)[1]	1.00	0.30	0.30	0.30	0.30	0.30	0.30
Premix[2]	0.30	0.09	0.09	0.09	0.09	0.09	0.09
Basal diet		70.00	70.00	70.000	70.00	70.00	70.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition (%)							
Dry matter	93.3	93.4	92.1	93.3	93.1	91.9	90.4
Organic matter	87.4	89.4	88.8	89.4	89.2	88.6	86.8
Crude protein	40.0	32.4	34.1	32.8	35.4	44.1	48.9
Crude fat	28.9	25.1	25.1	27.2	21.9	26.3	26.3
Ash	12.6	10.6	11.2	10.6	10.8	11.4	13.2
Gross energy[3]	5.29	5.08	5.19	5.03	5.03	5.29	5.29

[1]Internal marker for determination of digestibility
[2]DSM Aquaculture Premix per kg of feed provided: Vitamins A = 14,000 UI, D3 = 2800 UI, E = 140 UI, K3 = 8 mg, B_1 (thiamine) = 18 mg, B_2 (riboflavin) 20 mg, Nicotinamide =150 mg, Pantothenic acid = 50 mg, B_6 (pyridoxine) = 15 mg, Biotin = 0.8 mg, Folic acid = 4 mg, C (ascorbic acid) = 600 mg, B_{12} (cyanocobalamin) = 0.03 mg, Choline = 600 mg; and Minerals: Manganese = 40 mg, Iron = 20 mg, Zinc = 20 mg, Copper 1.5 mg, Iodine = 1.5 mg, Selenium = 0.3 mg, Cobalt = 0.15 mg, BHT (butylated hydroxytoluene) = 120 mg
[3]Analyzed by combustion (Mcal/kg DM)

according to the methodology proposed by (Scott and Boldaji 1997).

Digestibility of dry matter, organic matter, protein and digestible energy

The apparent digestibility of DM, OM, CP and DE of the experimental diets were determined using the equation (I) proposed by (Forster 1999).

$$AD(\%) = 100\text{–}100 \times \left(\frac{MD}{MF}\right) \times \left(\frac{NF}{ND}\right) \quad (1)$$

where AD is the apparent digestibility (%), MD is the marker in the diet (%), MF is the marker in the feces (%), NF is the nutrient in the feces (%) and ND is the nutrient in the diet (%).

The digestibility of DM, OM, CP and DE of novel ingredients under study were estimated according to the equation (II) proposed by (Sugiura et al. 1998).

$$ADi\ (\%) = ADC_t + \left[\left(\frac{(1\text{-}s)Db}{s \times Dt}\right) \times (ADCt\text{–}ADCb)\right] \quad (2)$$

where AD_i is the apparent digestibility of the ingredient under study (%), ADC_t is the apparent digestibility coefficient of the evaluated diet, ADC_b is the apparent digestibility coefficient of the basal diet (%), D_b is the nutrients of the basal diet (%), D_t is the nutrients of the test diet (%), *s* is the proportion of the ingredient evaluated in the diet and 1-s is the proportion of the basal diet in the test diet.

Statistical analysis

The data analysis was performed using the analysis of variance procedure (ANOVA) in the SAS statistical program (SAS Institute Inc 2004). The digestibility means differences of the DM, OM, CP and ED among the kañiwa, kiwicha, quinoa, bean, sacha inchi and jumbo squid were compared by the Tukey test. The differences were considered significant at $P < 0.05$.

Results

The apparent digestibility of DM, OM, CP and DE among the kañiwa, kiwicha, quinoa, bean, sacha inchi and jumbo squid were significantly different among the experimental diets and among the new raw materials studied (Table 3, $P \leq 0.001$).

The experimental diets of jumbo squid, sacha inchi and quinoa had higher values of DM and OM digestibility than the diets containing kiwicha, kañiwa and beans. These results are consistent with the greater apparent digestibility of CP in diets containing sacha inchi meal (92.8%), jumbo squid meal (91.3%), quinoa meal (90.3%) and kiwicha meal (90.1%) and lower in the diets with kañiwa meal and bean meal. The DE value was also higher in the diets with jumbo squid meal (4.36 Mcal/kg DM) and sacha inchi meal (4.19 Mcal/kg DM) and lower in the diets with quinoa, kañiwa, kiwicha and bean meal.

Consequently, the apparent digestibility of DM and OM in novel raw materials was higher for jumbo squid and sacha inchi meals, followed by quinoa, kiwicha, kañiwa and bean meals. Consistent with the previous results, the apparent digestibility coefficient of CP was higher for sacha inchi (98.0%), jumbo squid (93.2%), quinoa (90.3%) and kiwicha (89.1%), followed by kañiwa meal (82.5%) and bean meal (71.6%). Consistently, jumbo squid (4.57 Mcal/kg DM) and sacha inchi (4.15 Mcal/kg DM) meals had also the highest values of DE, while quinoa (2.95 Mcal/kg DM), kiwicha (2.74 Mcal/

Table 3 Apparent digestibility of dry matter, organic matter, crude protein and digestible energy of diets and novel raw materials in juvenile rainbow trout (*Oncorhynchus mykiss*)

		Treatments						SEM[1]
	Basal	Kañiwa	Kiwicha	Quinoa	Bean	Sacha inchi	Jumbo squid	
Diets (%)								
Dry matter	72.9^{ab}	65.5^{d}	68.8^{cd}	72.1^{bc}	56.5^{e}	73.1^{ab}	76.3^{a}	1.11
Organic matter	76.6^{ab}	68.3^{d}	72.5^{c}	75.4^{bc}	60.4^{e}	77.2^{ab}	80.3^{a}	1.18
Crude protein	90.3^{b}	88.3^{c}	90.1^{b}	90.3^{b}	84.9^{d}	92.8^{a}	91.3^{b}	0.40
Digestible energy[2]	4.19^{b}	3.57^{c}	3.81^{d}	3.78^{c}	3.32^{e}	4.19^{b}	4.36^{a}	0.05
Novel raw materials (%)								
Dry matter		47.5^{d}	58.9^{c}	69.7^{b}	36.6^{e}	73.5^{b}	84.5^{a}	1.43
Organic matter		49.2^{e}	63.1^{d}	72.9^{c}	40.4^{f}	78.4^{b}	89.1^{a}	1.54
Crude protein		82.5^{d}	89.1^{c}	90.3^{bc}	71.6^{e}	98.0^{a}	93.2^{b}	1.14
Digestible energy[2]		2.25^{d}	2.74^{c}	2.95^{c}	1.75^{e}	4.15^{b}	4.57^{a}	0.09

Means with different letters in the same row differ significantly at $P < 0.05$

[1]Standard error mean ($n = 3$). The *P* value in all variables studied in diets and novel raw materials were statistically significant $P < 0.001$

[2]Expressed in Mcal/kg DM

kg DM), kañiwa (2.25 Mcal/kg DM) and bean (1.75 Mcal/kg DM) meals presented the lowest values.

Discussion

The apparent digestibility of DM, OM, CP and DE was significantly different between the raw materials of vegetable origin (sacha inchi, quinoa, kiwicha, kañiwa and bean) and that of animal origin (jumbo squid) used in this study. The lower digestibility of the DM and the OM of the vegetable ingredients can be explained by the higher content of fiber in its composition (4.8–9.2%) than in the jumbo squid meal (2.2%, Table 1). The levels of fiber in the vegetable ingredients are those that occur naturally, since these raw materials did not receive any previous treatment before the manufacture of the experimental feeds, except for sacha inchi meal that was extruded and defatted. The presence of antinutritional compounds such as saponins and tannins in plant feeds (Ruiz et al. 2013) also causes less nutrient digestibility (Gatlin et al. 2007). On the other hand, trout are carnivorous and therefore have a gut less specialized in the digestion of vegetable raw materials. However, since the extrusion was done to improve digestibility (Rodríguez-Miranda et al. 2014), the lower digestibility in the vegetable ingredients was attributed to higher fibre content. In fact, the digestibility of the protein depends on the methods of feed processing. The extrusion process improved the digestibility of the protein compared to pelleting in feed for trout (Fenerci and Sener 2005).

The digestibility value of the protein for squid meal in this study was similar to that reported for fishmeal (NRC (National Research Council) 2011; Gaylord et al. 2008). The higher protein digestibility of jumbo squid is attributed to the fact that feed from animal origin is with a high protein content (Córdova-Murueta and García-Carreño 2002) and also because trout are carnivorous fishes.

The sacha inchi meal had the highest values of digestibility among all the vegetable ingredients evaluated. This could be explained by the fact that it had been pre-processed (extrusion and extraction of oil). The integral seed of sacha inchi has around 24% CP and 42% fat (Gutiérrez et al. 2011), while the extruded and degreased meal used in this study had 54.9% CP, 9.9% fat and 5.25 Mcal/kg DM. In general, the CP and GE content of sacha inchi meal is similar to that of soybean meal solvent-extracted and cottonseed meal solvent-extracted. However, the results show that the digestibility of DM, OM, CP and DE for sacha inchi was higher than for soybean meal (NRC (National Research Council) 2011; Glencross 2011; Gaylord et al. 2008) and cottonseed meal (NRC (National Research Council) 2011; Gaylord et al. 2008).

The apparent digestibility of DM, OM, CP (%) and DE (Mcal/kg DM) of the 'Andean grains' were moderately high values, highlighting the apparent digestibility of DM and CP of quinoa meal (69.7 and 90.3, respectively). In a research carried out by (Muñoz et al. 2015) in juvenile fish *Oplegnathus insignis*, the apparent digestibility of DM and CP of quinoa was lower (64.1 and 80.2, respectively). In another study conducted by (Molina-Poveda et al. 2015) in shrimp *Litopenaeus vannamei*, they showed that fish meal can be replaced up to 45% with quinoa meal and only up to 15% with kiwicha meal without compromising the digestibility and performance. These results indicated to a greater digestibility of the CP in quinoa and lower in the kiwicha.

On the other hand, bean meal presented low digestibility values of DM, OM, CP and DE, and this can be attributed mainly to the presence of antinutritional factors in its composition (Lech and Reigh 2012) that reduce the nutritional quality. The antinutritional factors can, however, be eliminated with thermal treatment and soaking (Pfeffer et al. 1995). The digestibility of CP (71.6%) of the beans obtained in our study agrees with the studies carried out by (Tiril et al. 2009) for common bean (*Phaseolus vulgaris* L.) in juvenile rainbow trout (72.9%). In vegetable ingredients, it is difficult to decrease the content of indigestible carbohydrates, which reduces the nutritional value in fish (Krogdahl et al. 2010). The functional properties of aquaculture feeds containing 15% bean meal were improved with the extrusion process (120 °C and 18% humidity) by Rodríguez-Miranda et al. 2014.

In general, digestibility values of DM, OM, CP and DE in new raw materials showed significant differences. The results suggest that the juvenile rainbow trout was more efficient in the digestion of the protein and energy of the jumbo squid and sacha inchi meal and less efficient in 'Andean grains' and beans. Probably the digestibility energy of plant vegetable ingredients could have been affected by intrinsic factors or amylose/amylopectin ratio (Gomes et al. 1995; Gaylord et al. 2010). It is important to consider the value of the digestibility of raw materials in the preparation of diets for trout in order to reduce the release of fecal material and solids suspended in water (Davidson et al. 2013) as well as the feed wastage.

Finally, the most acceptable novel feeds for juvenile trout according to the digestibility values of the protein (%) and the digestible energy (Mcal/kg DM) are sacha inchi meal (98.0 and 4.15), jumbo squid meal (93.2 and 4.57) and quinoa meal (90.2 and 2.95), respectively. Also, kiwicha meal presents intermediate values, while kañiwa and bean meal presented digestibility values less recommendable to be included in the ration of juvenile trout.

Conclusion

This study represents the first nutritional assessment of apparent digestibility coefficient of kañiwa, kiwicha, quinoa, sacha inchi and jumbo squid. Our results suggest that quinoa meal and jumbo squid meal have an acceptable digestibility and can be used in feed formulation but sacha inchi meal is a potential ingredient for the feeding of juvenile rainbow trout. Further researches are needed to evaluate the influence of these ingredients on growth performance and feed utilization in rainbow trout.

Abbreviations

1-s: Proportion of the basal diet in the test diet; ADC: Apparent digestibility coefficient; ADCb: Apparent digestibility coefficient of the basal diet; ADCt: Apparent digestibility coefficient of the evaluated diet; CONCYTEC: Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica; CP: Crude protein; Db: Nutrients of the basal diet; DE: Digestible energy; DM: Dry matter; Dt: Nutrients of the test diet; GE: Gross energy; MD: Marker in the diet; MF: Marker in the feces; ND: Nutrient in diet; NF: Nutrient in feces; OM: Organic matter; *s*: Proportion of the ingredient evaluated in the diet; SD: Standard deviation

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Authors' contributions

AOC performed nutritional analysis discussion and wrote the initial version of the manuscript. RMPP performed the nutritional analysis. MECC contributed with study design. FHRH contributed in the data analysis, study design, discussion and writing. MJAA conceived and designed the study, supervised the work of AOC and contributed to the analysis, discussion and writing. All authors have reviewed and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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