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Assessment of protein quality, organ weight and tibia length of experimental animals fed with quinoa (*Chenopodium quinoa*)

Avaliação da qualidade proteica, peso de órgãos e comprimento da tíbia e animais experimentais alimentados com quinoa (Chenopodium, quinoa)

Resumo

Objective: The aim of this work was to evaluate the protein quality and the effects of quinoa (*Chenopodium quinoa*) ingestion on liver, spleen and tibial length in experimental animals. ***Method:*** For analysis of protein quality and feed efficacy, a 28-day biological assay and 18 Wistar rats were used, calculating the protein efficiency coefficient (PER), net protein ratio (NPR), chemical score corrected for protein digestibility (PDCAAS), utilization of liquid protein (NPU), in vivo digestibility and food efficiency coefficient (CEA) (AOAC). ***Results:*** Significant differences were found between PER, NPR, NPU and CEA values for quinoa (0.54; 2.58; 26.22 and 0.05, respectively) and casein (2.04; 3.84; 59 .9; 0.2, respectively). As for the proximate composition, values of 11.31% of protein, 11.28% of moisture, 2.04% of ash, 7.9% of lipids and 67.47% of carbohydrates were found. No significant differences were found between digestibility values of casein (96.93%) and quinoa (92.2%). The PDCAAS value found for quinoa was 0.97. There were significant differences in relation to organ weights, except for the weight of the tibia, with casein having greater weight. ***Conclusion:*** The lowest PER, NPR, NPU and CEA values demonstrated that quinoa protein had a lower quality than milk, but that the digestibility and PDCAAS values prove that it has good protein quality and an interesting amino acid profile, requiring further studies to assess its nutritional value.

Keywords: *Chenopodium quinoa*. Protein quality. Digestibility.

Resumo

Objetivo: O objetivo deste trabalho foi avaliar a qualidade proteica e os efeitos da ingestão da quinoa (*Chenopodium quinoa)* no peso do fígado, baço e comprimento da tíbia de animais experimentais. ***Método***: Para análise da qualidade proteica e eficácia alimentar, utilizou-se ensaio biológico de 28 dias e 18 ratos linhagem *Wistar*, calculando-se coeficiente de eficácia proteica (PER), razão proteica líquida (NPR), escore químico corrigido pela digestibilidade proteica (PDCAAS), utilização da proteína líquida (NPU), digestibilidade *in vivo e* coeficiente de eficácia alimentar (CEA) (AOAC). ***Resultados***: Foram encontradas diferenças significativas entre valores de PER, NPR, NPU e CEA da quinoa (0,54; 2,58; 26,22 e 0,05, respectivamente) e da caseína (2,04; 3,84; 59,9; 0,2, respectivamente). Quanto à composição centesimal, foram encontrados valores de 11,31% de proteína, 11,28% de umidade, 2,04% de cinzas, 7,9% de lipídios e 67,47% de carboidratos. Não foram encontradas diferenças significativas entre valores de digestibilidade da caseína (96,93%) e quinoa (92,2%). O valor de PDCAAS encontrado para quinoa foi 0,97. Houve diferenças significativas em relação aos pesos dos órgãos, exceto o peso da tíbia, sendo que a caseína obteve maior peso. ***Conclusão***: Os menores valores de PER, NPR, NPU e CEA demonstraram que a proteína da quinoa teve qualidade inferior à do leite, mas que os valores de digestibilidade e PDCAAS provam que ela apresenta boa qualidade proteica e perfil aminoacídico interessante, sendo necessários mais estudos para avaliar seu valor nutritivo.

Palavras-chave: *Chenopodium quinoa*. Qualidade proteica. Digestibilidade.

INTRODUCTION

The real quinoa (*Chenopodium quinoa*), a plant from the Andes Mountains, has a promising economic value and is considered a potential component in animal feed and human food. Due to its high nutritional quality, it has attracted the attention of researchers in various parts of the world.[1,2] Scientists are showing interest in nutritional quality, due to its high protein content (≈15%), with a right balance of essential amino acids, high content of vitamins and also nutraceutical compounds, such as flavonoids.[2,3] Its seeds contain high levels of photochemicals, such as phenolics, peptides or oligosaccharides. Proteins containing large amounts of lysine and methionine represent four main classes.[2,4] The amounts of fiber in quinoa seeds are greater than those previously determined for wheat or rice and are comparable to those determined for pulses.[2,4] This seed is an excellent source of vitamins B, E and C as well as minerals such as Ca, Fe, Mg, Mn, P, K and Zn.[2,3]

It is known that *Chenopodium quinoa* has been cultivated in the Andean region for thousands of years. Over the past 30 years, it has been introduced in many countries in Europe, North America, Asia and Africa.[4] It has been proven that, due to its wide genetic variability, quinoa can be adapted effectively and easily to a wide range of environmental conditions. [2,4]

Quinoa is known as pseudocereal because, although it does not belong to the Gramineae family, it produces seeds that can be ground into flour.[2,5-8] However, the amino acid profile of quinoa is much superior to other cereals.[9] It has the amino acid lysine in its composition, in considerable quantity,[8] being considered a pseudocereal whose protein has high quality and is comparable to the casein in milk.[5-8] And yet, it has other advantages over other cereals, as it has high amounts of vitamins such as riboflavin, niacin, thiamine, B6, and minerals such as magnesium, zinc, copper, iron, manganese and potassium.[10,11]

Another interesting feature of quinoa is the absence of gluten in its composition, an important feature for the treatment of celiac disease.[2,12] The celiac disease (CD) is an immune-mediated enteropathy that occurs through the ingestion of gluten in genetically susceptible individuals. The antigenic portion of gluten protein for celiac patients is predominantly wheat gliadin, which has a high content of the amino acids glutamine and proline, as well as barley hordein and rye secalin. There is also an antigenic potential of oat avenin, which has an intermediate content of the amino acids glutamine and proline, and which can determine CD.[13] Celiac patients, when consuming gluten, have intense diarrhea and foul odor, considerable weight loss and nutritional deficiencies, since have decreased nutrient absorption.

The treatment of celiac disease consists of permanently introducing a gluten-free diet, and therefore the following cereals and their derivatives should be excluded from the diet: wheat, rye, barley, malt, oats. Thus, quinoa is an excellent option for people with this disease.[12]

Another advantage of quinoa protein is that it has some desirable characteristics from a food processing point of view, as it has adequate gelatinization property, water absorption and emulsification capacity, in addition to stability (resistance to retrogradation).[1-3,5-7] These are very interesting aspects for the development of new products, including the development of products for individuals with celiac disease, since the biggest problem is the replacement of cereals that contain gluten by other raw materials that do not. is the absence of these characteristics.[11]

Given the above, the purpose of this work was to evaluate the protein quality of real quinoa (*Chenopodium quinoa*) and the effects of its ingestion on liver, spleen weight and tibia length in rats.

METHOD

Animal experiments were conducted following the Ethical Principles in Animal Experimentation, recommended by the Brazilian College of Animal Experimentation (COBEA). The research was approved by the Animal Experimentation Ethics Committee – CETEA of the Federal University of Minas Gerais, under registration number 149/2008.

The work was carried out in Experimental Nutrition and Bromatology laboratories at the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM) – Diamantina, Minas Gerais.

Determination of quinoa proximate composition

To determine the moisture content in quinoa samples, the methodology described by Instituto Adolfo Lutz[5] and AOAC[14] (Association Of Official Analytical Chemists) was used.[14] Protein, lipid and fixed mineral residue contents were determined by the methods recommended by the AOAC;[14] the digestible carbohydrate and fiber content was calculated by the percentage difference.

Determination of amino acid profile

Initially, milling (1 millimeter) was carried out, followed by degreasing (1 milliliter of ethyl ether, repeated 3 times), to then undergo hydrolysis – except for the determination of tryptophan, when the SPIES method was used.[15]

For analysis of the amino acid profile, the methods according to Spackman et al.[16] were used.

Biological test

Eighteen Wistar rats (AOAC) were used.[17] According to the AOAC methodology, mentioned above, six experimental animals must be used per treatment, which must remain in individual wired cages, receiving water and food *ad libitum*. Thus, the animals were divided into three groups: standard (DP), which received a diet with casein; non-protein (LN), which received a nitrogen-free diet (this group was used to calculate NPR - net protein ratio and true digestibility - D); and the quinoa experimental group (DQ), which received a diet with quinoa flour as a protein source. With this type of management, it is possible to obtain the animals' weight, feed and amount of ingested protein with six repetitions for each treatment, for 28 days, which is conclusive for this study.

For protein quality assessment, data were collected up to 14 days; and to assess the development of organs and tibia length, the experiment lasted up to 28 days, according to AOAC.[17] The animals were from the vivarium of the Federal University of Viçosa-MG, with an initial average weight of 98.44 grams and were heavy on the seventh, fourteenth, twenty-first, and twenty-eighth days of life. The bowls were filled whenever they were empty. The amount of ingested protein was calculated, considering the protein content of the diet. To calculate the consumption of the animals, the weight of the full feeder was used, discounting the weight of the empty feeder and leftover paper.

Diet preparation

The protein sources used to prepare the diets were real quinoa and casein. Commercial casein was obtained from Rhoster® Indústria e Comércio Ltda., as well as fiber (cellulose), mineral mixture, vitamin mixture, L-cystine, choline bitartrate, dextrinized starch (Table 1). Real quinoa was obtained from the local trade in Diamantina and Belo Horizonte – Minas Gerais, in the form of flour. Diet composition was based on AIN-93G[18] with protein content changed to 9-10%. The diets were homogenized in an industrial mixer. After preparation, the protein content of each diet was determined by the semi-micro Kjeldahl method, using the 6.25 factor to obtain the protein content. The diets were placed in polyethylene bags, properly labeled and stored in a refrigerator.

Table 1. Composition of diets (gram/100grams), offered to rats for 28 days. Diamantina - Minas Gerais, 2008.

	Casein (grams)	LN* (grams)	Quinoa (grams)
Protein source	10,58	0	79,56
Sucrose	9	9	9
Soy oil	7	7	1,4
Cellulose (Fiber)	5	5	5
Mineral mixture	3,5	3,5	3,5
Vitamin mix	1	1	1
L-cystine	0,3	0,3	0,3
Choline bitartrate	0,25	0,25	0,25
Dextrinized	13,2	13,2	0
Maize starch	50,17	60,75	0

* Free of nitrogen

The diets used for the biological assay were isoprotein (9.0 to 10.0 %) and isocaloric (356 to 360 Kcal), so that there was no interference in the diet consumption and in the ingested protein content on the results of the experiment. The carbohydrate contents were obtained by percentage difference after determining moisture, protein, lipids and minerals.

Determination of proximate composition of diets

The determination of the proximate composition of the diet was carried out according to item 2.1.

To measure the amount of quinoa to be purchased for the experimental trial, nutritional information from real quinoa® flour labels and food composition tables were used.[19,20]

Protein Efficacy Coefficient (PER)

The calculation of PER (*protein efficiency ratio*) was performed in accordance with AOAC.[17] This method relates the weight gain of animals with protein intake.

PER was calculated by the following equation:

$$PER = \frac{\text{test group weight gain (g)}}{\text{Protein consumed by the test group (g)}}$$

Net Protein Ratio (NPR)

The calculation of NPR (*net protein ratio*) was performed according to the AOAC.[17] This method relates the weight gain of the test group with the weight loss of the aprotein group and the protein consumed by the test group.[21]

The NPR was calculated with the following equation:

NPR = test group weight gain (g) + aprotein group weight loss (g)

protein consumed by the test group (g)

Determination of *in vivo* digestibility

True digestibility was calculated by evaluating the amount of nitrogen ingested in the diet, through the consumption of diets on the days when feces were collected; nitrogen excreted in feces and metabolic nitrogen loss in feces, which corresponds to fecal nitrogen in the non-protein diet group, which quantifies nitrogen from endogenous, non-food sources.

To calculate the digestibility, the diets were marked with indigo carmine, in the proportion of 200 milligrams of indigo carmine/100 grams to mark the beginning and end of feces collection. Feces were collected from the 8th to the 13th day, placed in individual containers and stored in a refrigerator. They were weighed wet and then placed to dry in an oven at 105°C overnight; after, they were weighed dry and the values recorded. Subsequently, they were ground in a mini-processor and nitrogen was determined using the semi-micro Kjeldahl method, with samples in triplicate, according to AOAC.[14]

The calculation of true digestibility was performed according to the following equation:

(%) Digestibility = NI – (NF – NFK) x 100

NI

NI = Nitrogen ingested by the test group.

NF = Test group fecal nitrogen.

NFK = Fecal nitrogen from the non-protein diet group.

Determination of the chemical score corrected by protein digestibility (PDCAAS)

The essential amino acid score (EAE) was estimated, which corresponds to the proportion of the most limiting (first limiting) amino acid of the test food in relation to the essential amino acid requirements of two- to five-year-old children (preschoolers), used as a reference standard according to FAO.[22]

EQ = mg amino acid/gram protein test

mg of amino acid/gram of standard protein

From the EQ values, the protein value of quinoa was calculated using the PDCAAS - Protein Digestibility-Corrected Amino Acid Score,23 a method recommended by the FAO22 and which corresponds to the EQ product by the true digestibility of the protein.

PDCAAS (%) = (EQ* x Dv**) /100

*EQ= Chemical Score (CS)

**Dv= True Digestibility (TD)

Food Efficacy Coefficient (CEA)

To calculate CEA, the following formula was used: CEA= animal weight gain (g)/total intake (g). This formula assesses the efficiency of the diet in promoting body weight gain, that is, the food as a whole, and not just the efficiency and quality of proteins.[24]

Determination of NPU

From the NPR value, the NPU index (net protein utilization) was estimated, in order to evaluate the protein retention by the organism, having been obtained by the following equation:[21]

Tibia, liver, spleen and tibial length weight

On the 28th day of the experiment, the animals were anesthetized and sacrificed. Afterwards, the liver, spleen and tibia were removed. All organs were weighed and the length of the tibia was also determined.

Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) and Tukey's test at 5% probability, using The SAS System version 9.00 (2002).[24]

RESULTS AND DISCUSSION

Evaluation of the proximate composition of quinoa flour and experimental diets

The mean values of moisture, protein, lipids, ash and digestible carbohydrates plus fiber from the quinoa flour were 11.28%, 11.31%, 7.9%, 2.04% and 67.47%, respectively. According to Ordinance 354/96 DETEN/MS,[25] the moisture content found for quinoa is adequate, as this value cannot exceed 15%.

It is observed that quinoa has a higher protein content than other cereals and legumes. The results of the protein content of quinoa showed that it has a higher protein content than other cereals and pulses such as rice (2.5%), corn (6.6%), beans (4.8%) and lentils (6.3 %).[20]

The values found in this study corroborate those found by Vilche et al.,[26] who reported that quinoa protein values ranged from 10 to 18% and 4.5 to 8.75% in fat. These values are similar to those obtained by Speahr[27] and Tavano & Amistá,[28] who found, respectively, 11.72% moisture, 5.50% fat, 14.81% protein, 3.38% ash and 60.95 % carbohydrate and 12.9% protein, 8.83% lipid and 1.63% ash, both for quinoa flour.

As for the composition of the experimental diets, it was observed that they were isoprotein (9 and 10%), isocaloric (356 to 360 Kcal), isolipid (7 to 7.9%) and isoglycidic (77.35 to 79.5%) .

Diet, protein and weight gain

There were no statistical differences between dietary and protein intake between the test group and the standard group (Table 2).

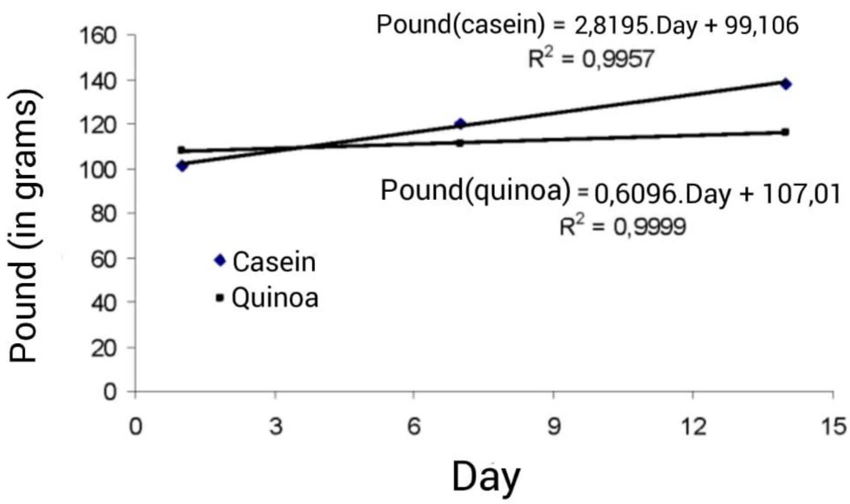
Table 2. Average diet and protein consumption of rats for 14 days long. Diamantina – Minas Gerais, 2008.

Groups	Diet consumption (grams)	Protein consumption (grams)
Casein	175,27ª	17,52ª
Quinoa	151,24ª	14,36ª

a, b - Means followed by the same letter do not differ from each other, by Tukey's test at 5% probability.

Analyzing the weight gain of the experimental animals, there were significant differences, in which the standard group had the greatest weight gain, as shown in Figure 1.

Figure 1. Average weekly weight of rats during 14 days of treatment, submitted to standard diets (diet with casein) and diet with quinoa.



Diamantina - Minas Gerais, 2008.

It is observed that the average weight of rats submitted to the standard diet (casein) presented an average daily gain rate of 2.8195g, while the average weight of rats fed a diet with quinoa presented an average daily gain of 0.6096 grams.

Silva et al.,[29] in a research with soy protein, found similar results, in which soy protein promoted less weight gain than casein, even though the consumption of feed and protein did not differ between the groups. Naves et al.,[30] in a study with corn protein, found a lower consumption of diet and protein from conventional corn compared to the standard group; but with a consequent lower weight gain in the test group, as in this study.

Food Efficacy Coefficient (CEA)

As for CEA, there were differences between animals fed a diet containing quinoa (0.05) and those fed a casein diet (0.20), with the latter having better efficacy.

In a study carried out with mesquite, a legume, authors found a CEA 0.12,[31] below that of casein, but higher than that of quinoa. Given the above, it is observed that the diet with quinoa is less efficient, that is, it does not promote efficient weight gain when compared to casein.

NPR (net protein ratio), NPU (net protein utilization), PER (protein efficiency ratio) and in vivo digestibility values

An average NPR value of 2.55 (69.86% in relation to casein) was found for the quinoa diet. Mendes et al.[32] found higher values: NPR of 3.98 (79.48% in relation to casein).

Studies with cooked beans and a fermented soybean product found NPR values of 2.9 and 3.13 respectively, both with higher values than quinoa.[15,30] These values can be explained by the fact that beans are cooked and soy is fermented, which facilitates absorption. Mendes et al.[32] found relative NPR values for different soybean varieties ranging from 51.18 to 68.63%, close to that found for quinoa.

The NPU found for quinoa was 26.22 (43.72% in relation to casein) and for casein, 50.96. The NPU (net protein utilization) is calculated from the NPR, in order to assess the protein retention by the body.[21]

In studies with other proteins of vegetable origin, it was observed that corn had an NPU of 43.2%, black beans 34.74% and soybeans 53.9%.[31,33,34]

The present study found a PER value for quinoa of 0.54 (26.47% in relation to casein), low when compared to the value obtained by Alves et al.,[35] who found a PER value for quinoa of 2.87.

When compared with other proteins of vegetable origin, studies found PER values for maize of 0.68 and a relative PER of 15.59%[36] and for beans 1.12 and a relative PER of 28.98%.[37] Vale to emphasize that it is described in the literature that the NPR is better to assess protein quality than the PER, as it takes into account the endogenous nitrogen, and the PER only takes into account the weight of the animal and the protein ingested.

There was a significant difference between the NPR, PER and NPU of quinoa and casein, with the latter group showing higher values (Table 3).

Digestibility is a measure of the percentage of proteins that are hydrolyzed by digestive enzymes and absorbed by the body in the form of amino acids or any other nitrogenous compound. It is a determinant of the protein quality of the diet. When certain peptide bonds are not hydrolyzed in the digestive process, part of the protein is excreted in feces or transformed into metabolism products by microorganisms in the large intestine.[33] Quinoa flour digestibility was 92.20%, and relative digestibility was 95, 12%, not statistically different from casein digestibility (Table 5), thus demonstrating that this protein is easily broken down and, consequently, highly absorbed. Alves et al.[35] found a value for quinoa digestibility of 98%; Mendes et al.[32] found a value of 85.95%. It is noteworthy that quinoa has greater digestibility than other cereals and legumes that do not exceed 90% in its entirety,[35] such as oats, which has

76%, rice 75%, whole wheat 79%, the germ of wheat 65%, whole corn 76%, corn germ 60% and some legumes and tubers such as soybeans, which have 78% digestibility, beans 60% and potatoes 74%.[34]

Table 3. Mean "in vivo" digestibility, NPR, PER, NPU of the 14-day experiment. Diamantina - Minas Gerais, 2008.

Groups	True Digestibility	Relative Digestibility	NPR	NPR relative (%)	NPU	NPU relative (%)	PER	PER relative (%)
Casein	96,93^a	100	3,65^a	100	59,96^a	100	2,04^a	100
Quinoa	92,20^a	95,12	2,55^b	69,86	26,22^b	43,72	0,54^b	26,47

a, b - Means followed by the same letter do not differ from each other, by Tukey's test at 5% probability.

Comparing with the digestibility of other foods of vegetable origin, Luján et al.[38] found a digestibility value for beans of 84.88; Mendes et al.[32] reported for other cereals, such as oats and rice, values of 87.84 and 92.12, respectively.

Tibia, liver, spleen and tibial length weight

As for the weight of the tibia of the rats, there was no significant difference between the two evaluated groups. Regarding liver weight, spleen weight and tibia length, there were differences, and the values of the control group were higher than those of the test group, in the experiment with 28 days (Table 4).

Table 4. Mean Organ Weight (grams) and Tibia Length (centimeters) of the experimental animals. Diamantina - Minas Gerais, 2008

Groups	PB*	PF**	PT***	CT****
Casein	0,52^a	8,3^a	0,46^a	3,4^a
Quinoa	0,38^b	6,6^b	0,39^a	3,1^b

* - Peso do baço (spleen weight)
** - Peso do fígado (liver weight)
*** - Peso da tíbia (tibia weight)
**** - Comprimento da tíbia (tibia length)
a,b - Data followed by the same letter in the same column do not differ from the pattern by Tukey's test.

In a biological trial testing the effect of a probiotic on the modulation of serum cholesterol levels and liver weight in rats fed a diet rich in cholesterol and folic acid, carried out over 28 days, authors found an average weight of livers in fed rats with a standard casein diet (AIN-93G) of 14.7g.[38] Guzmán-Silva et al.,[39] testing the effect of rations with or without food supplement and vitamins and minerals during the growth period, report that in 28 days after receiving a diet containing casein, vitamins and minerals (AIN-93G), the animals had an average liver weight of 12.08g. Both studies above found values higher than those found for the same group in the present work, which was 8.3 grams for rats fed a standard casein diet (AIN-93G).

As for the weight of the spleen, Guzmán-Silva et al.,[39] in their study, reported an average weight of 0.87grams in rats receiving a diet containing casein, vitamins and minerals (AIN-93G) for 28 days, a value greater than the found in the present study, which was 0.52 grams.

The mean length of the tibia found was 3.4 centimeters and 3.1 centimeters for casein and quinoa, respectively.

Rocha et al.[40] found tibial length values of 3.55 cm for rats in the control group (fed with a normoprotein diet) and 3.06 cm for rats in the test group. Prazeres et al.[41] found a tibia length value of 3.59cm for rats, both experiments lasted 30 days and presented values greater than those of the present study.

As for the weight of the tibia, Rocha et al.[40] reported a weight of 4.023g for rats in the control group in which the protein source was casein (80%), fed for 30 days. This value, when compared to the control group (casein) found in the present work, which is 8.3 grams, is lower.

PDCAAS

After calculating the chemical score, the amino acid that obtained the lowest value was methionine + cysteine, as quinoa did not present any limiting amino acid. All values found were greater than 1 (Table 5); PDCAAS was then calculated, and the value found for quinoa was 0.99. Alves et al.[35] found a quinoa PDCAAS value of 1.07, similar to that found in this study.

Table 5. FAO/OMS (FAO / WHO) standard, amino acid profile and real quinoa chemical score. Diamantina – Minas Gerais, 2008.

Essential Amino acids	FAO / WHO Standard (milligrams/ grams protein)	Amino acid real quinoa (milligrams/grams protein)	Chemical score real quinoa
Histidine	19	32,71	1,72
Isoleucine	28	45,09	1,61
Leucine	66	71,61	1,08
Lysine	56	61,89	1,10
Methionine + Cysteine	25	26,52	1,06
Phenylalanine+Tyrosine	63	77,8	1,23
Threonine	34	45,09	1,32
Tryptophan	11	21,22	1,92
Valine	35	65,42	1,86

Monteiro et al.[6] found the following PDCAAS values for different soybean varieties, ranging from 0.75 to 0.8; while Silva et al.[29] found the PDCAAS value for soybeans of 0.88. Naves et al.[30-33] found a value of 0.82 for corn. All studies cited here present values lower than those found for quinoa. This demonstrates that quinoa, when evaluated for digestibility and PDCAAS, can be considered a good quality protein.

CONCLUSION

It is concluded that quinoa is a good alternative for food, as it has adequate protein content, digestibility and PDCAAS, as it did not present any limiting amino acid. However, further studies should be carried out, since this protein presented values of PER, NPR, NPU and CEA lower than casein, as well as the weight of organs. It is noteworthy that quinoa has some advantages over cereals and legumes, such as higher protein content, better digestibility and amino acid profile.

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