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PROTEINS OF *Oryza latifolia* FROM BRAZILIAN PANTANAL: IMPACT ON THE USE OF AMINOACIDS AND WEIGHT GAIN OF ANIMALS

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ABSTRACT

The rice is one of the foods of larger worldwide consumption, and its relevance is perceived by diversity of species already identified, one of them, the specie *O. latifolia*, common of the Brazilian Pantanal, presents itself with one possibility to be introduced in the human feed, considering the cultivation and sustainable consumption. The rice, specially the specie *O. latifolia*, is considered one alternative viable, because it presents high nutritional potential and when combined with the legumes offer the ingestion of essential nutrients. The aim of the present study was available the protein quality and the digestibility of the specie *O. latifolia* in experimental model. The grains of *O. latifolia* were collected in the Serra do Amolar no Pantanal, Mato Grosso do Sul (MS), Brazil. Already the grains of *Phaseolus vulgaris* were purchased commercially in MS, Brazil. The grains of *O. latifolia* were dried, peeled, polished, crushed, sifted and boiled. For chemical composition was utilized methodology pre-established. In the protein analysis, *O. latifolia* was extract with sodium chloride, followed of the analysis of the aminoacids, after protein hydrolysis, in temperature controlled. Identification of the amino acids was given by High Performance Liquid Chromatography (HPLC). In biologic assay were used 32 rats (*Wistar*) distributed in 4 groups: Aproteic Group (1), Standard Group (casein) (2), Test Group (protein of the *O. latifolia*) (3), Test Group (protein of *O. latifolia* and of *P. vulgaris*) (4), after approve of the Ethics Committee on the Use of Animal, nº 167/2007. The food consumption and weight gain of animals were available, and collected the faeces and urine for the Nitrogenated Balance (BN), True Digestibility (TD), Biological Value (BV) and rates of protein utilization. The specie *O. latifolia* presented less quantity of the protein, ashes and fiber in comparison to *P. vulgaris*. On the other hand, the native rice demonstrated larger levels of the carbohydrates and lipids. The aminoacid composition reveled that *O. latifolia* and *P. vulgaris* had five limiting amino acids, being these isoleucine, lysine, threonine, leucine and the sulfur amino acids methionine + cystine and that felylalanine + tyrosine are the main amino acids present, followed by aminoacids lysine, leucine, valine, threonine among others to a lesser extent. Already the chemical composition of the diets revealed similar protein and caloric levels between *O. latifolia* and *O. latifolia* + *P.vulgaris*. The weight gain was more evident in the standard diet, followed by Test 1 and Test 2, likewise that the feed intake, ingested nitrogen and urinary. On the other hand, the Test 1 presented larger levels of fecal nitrogen, followed by Test 2 and standard diet. The BN and DV were more evident in the standard diet, followed by Test 1. The BV and protein use highlight for Test 2 and standard diet, followed by Test 1. The grains of *O. latifolia* presented revealed relevant nutritional content and aminoacid, which contributed significantly to the weight gain of the animals, possibly due to greater utilization of the liquid protein content, causing the species to be considered a viable alternative food for human consumption, as it provides high quality proteins, especially when combined with *P. vulgaris*.

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INTRODUCTION

Several world's population uses rice as its main food daily (IRRI 2016). Because of this, it is considered a key item in world food security. The use of wild plants is an important strategy for promoting food security and biodiversity conservation (Bortolotto *et al*. 2016). The use of biodiversity in a conscious and sustainable manner with respect to botanical species that are native foods is strongly encouraged by the Convention on Biological Diversity (CBD, 2016), specifically on actions related to food and nutrition (BRASIL, 2016; CBD, 2016). The name rice is attributed to about 24 species of the genus *Oryza* (*Poaceae*) which are distributed mainly in the tropical and subtropical regions of the world. The main species of rice consumed worldwide is *Oryza sativa* L. However, there are other species of the genus are also consumed by humans (Linares 2002; Bortolotto *et al*. 2016). Species such as *Oryza glumaepatula* Steud., *Oryza alta* Swallen, *Oryza grandiglumis* (Döll) Prod. and *Oryza latifolia* Desv. (Khush, 1997; IRRI 2016), (Khush, 1997; IRRI 2016) occur in Brazil, mainly in the Amazonian regions and Pantanal (Veasey *et al*. 2011). The Pantanal is a 140,000 km^2 sedimentary floodplain in Midlewest Region from Brazil and have approximately 2000 species of plants (Pott & Pott 1999) and some of them are relatives of cultivated plants such as *Oryza latifolia* and *Oryza glumaepatula* (Bortolotto *et al*. 2015). Species such as *O. latifolia* occur in the floodplain of the Paraguay river (BERTAZZONI; DAMASCENO-JUNIOR, 2011). Found on the islands and along the shores of Paraguay river, the Guatós Indians and the riverside population used some of these rice species in their diet. However, this use is in decline and is no longer part of the diet of these populations (MATTOS, 1875; BORTOLOTTO *et al*., 2015).

Oryza latifolia is a species that offers a good opportunity of studies due to its nutritional characteristics. It is important to stimulate the consumption of this type of rice in human food, because it is an ecologically sustainable and organic product and that has the capacity to gain more and more preference from consumers (BARATA, 2005). In fact, the scientific manuscript on this species of rice refers to the cultivation, genetic combinations and polymorphism (BERTAZZONI; DAMASCENO-JÚNIOR, 2011; LIU; LAFITTE; GUAN, 2004); there were no studies on its composition and / or nutritional quality. The study of the protein value of native foods, such as *O. latifolia* used by the communities of the Pantanal of the State of Mato Grosso do Sul, will help in understanding the relationship between biodiversity and nutrition. It is important to know the protein content of cereals and their combination with other foods such as legumes, since both contribute so much in the intake of essential nutrients (OGHBAEI e PRAKASH, 2015). According to FAO / WHO (1991), rice is the most important food for world food security, as it provides an excellent nutritional balance and high crop potential. The aim of the present work was to evaluate the protein quality and digestibility of the rice of the species *O. latifolia*, found on the river banks in the Brazilian Pantanal, using an experimental model of rats.

MATERIAL AND METHODS

Selection and Collection of Species

The rice grains of the species *O. latifolia* (Poaceae) were collected in the Serra do Amolar region of Pantanal, Mato Grosso do Sul, Brazil (18° 1'35.53 "S, 57° 28'53.34" W) and identified by the botanist Geraldo Alves Damasceno Junior of the Federal University of Mato Grosso do Sul (CGMS 53801). The species *Phaseolus vulgaris* L. (carioquinha beans) was purchased at a local supermarket in the city of Campo Grande, Mato Grosso do Sul, Brazil. Both compose the experimental diets used in the biological assay. *O. latifolia,* after drying in an oven with air circulation (40 ºC) until lower moisture of 10% were processed in a machine to remove the rice husk (200g of the material). Part of the peeled raw material was ground (Turratec® TE-102) and sieved (Tamis 60 mesh) constituting the whole base meal of the raw rice that was submitted to amino acid analysis and centesimal composition. A portion of processed O. *latifolia* and *P. vulgaris* were baked and then oven dried with air circulation (40 °C) until humidity less than 10%. Then both were crushed and sifted, obtaining the whole base flour of the cooked rice and whole-grain flour of the cooked beans. All the specimens were also submitted to the analysis of the centesimal composition for the ration of the test groups.

Chemical composition

The chemical composition of samples (raw brown rice, brown rice and cooked beans) were carried out by means of the determination of the humidity. For this, the samples were dried in an oven at 105 °C, according to the method described by the Instituto Adolfo Lutz (BRASIL, 2005). The protein quantification was determined by means of the total nitrogen content according to the method of micro Kjeldahl. The value found was multiplied by factor 5.95 and 6.25 for conversion of nitrogen into protein for rice and beans (Association of official analytical chemists (AOAC), 1995). Ethereal extract (lipids), ashes (fixed mineral residue) and carbohydrates (expressed as starch) were determined according to AOAC methodology (1995) and the crude fiber by the neutral detergent fiber method (Van de kamer; van ginkel, 1952).

Composition of Aminoacids

The protein content of samples of the species *O. latifolia* was extracted according to Macedo and Damico (2000), with 150 mL of sodium chloride (4% NaCl) for 1 hour. For the analysis of amino acids was used Pico-Tag amino acid analyzer (Waters System®). Protein hydrolysis was performed with hydrochloric acid (HCl 6M/1% phenol) at 106 ° C for 24 hours, and derivatization with 20 µL of methanol (CH_3OH), triethylamine, water and phenylisothiocyanate (7: 1: 1: 1, v/v) for 1 hour. The amino acids were identified by Reverse-phase High Performance Liquid Chromatography (RP-HPLC), comparing the amino acid retention times of the sample with the standards (Pierce) (HENRIKSON; MEREDITH, 1984).
For the determination of the amino acid values of the beans were used as reference parameters the values obtained by Mesquita *et al*. (2007).

Animals and Environmental Conditions

Thirty-two male Wistar rats (*Rattus norvegicus*), weaned at 21 days of age and 42.20 g of average weight, obtained at the animal house of the UFMS were randomly assigned to 4 groups: Group aproteic (1), Standard Group (2), Test Group *O. latifolia* (3), Test Group *O. latifolia* and *P. vulgaris* (4). Each animal received specific diet according to the group and water ad libitum. The animals were housed in individual metabolic cages and maintained in a clean and quiet environment with controlled light using a 12-hour light cycle (12 light hours and 12 dark hours) and maintained at 25 ± 2 ° C. The experiment was carried out in accordance with the International Declaration on the Rights of the Animals and after approval by the Ethics Committee on Animal Use of UFMS under registration number 167/2007.

Preparation of Experimental Diets and Centesimal Composition

Experimental diets were prepared according to parameters between the test and control groups. The environmental conditions, sex, age and weight of the animals were considered; as well as the diet used. We took into account the nutrient focus of study, as well as energetic ingredients that may influence weight gain, in order to obtain isocaloric and isoprotein diets (Carias; Ciocca; Havia, 1995). In the preparation of the diets, were considered the specifications of Vasconcelos *et al*. (2001) in order to provide a protein content of 10% for the standard groups (casein), test group 1 (protein contained in the flour prepared with *O. latifolia*), and test group 2 (protein contained in the mixture of *O. latifolia* and *P .vulgaris*).

Adjustments were made so that the experimental diets obtained content of 8% lipids, 9% sucrose, 5% mineral mixture, 2% vitamin mixture and the composition was supplemented with corn starch (62%) according to AIN-93 (REEVES; NIELSEN; FAHEY, 1993). The fiber content was also adjusted considering the chemical composition of the *O. latifolia* and *P. vulgaris* samples. In the aproteic diet (protein-free) corn starch was added in sufficient quantity to complete the composition (100%). To prepare the ration of test group 2, the ratio of 0.4: 1.6 was used for mixing the two ingredients based on the essential amino acid profile of each, compensating the limiting amino acids. Knowing the composition of native rice and beans, it is possible to highlight the proportional amount of proteins of each food in the rice and beans mixture. This diet described above was used to estimate the excretion of endogenous and metabolic nitrogen from the animals. After preparation of the rations, the centesimal composition was determined to confirm the concentration of the ingredients used and the formulations used.

the experiment were performed by weighing the animals and the feed offered, as well as leftovers on alternate days. The latter was also useful for determining the Protein Efficiency Ratio (PER) according to Pellet; Young (1980) and De Luca; Alexandre; Marques (1996). The data were recorded until the rats reached 51 days of age (De Luca; Alexandre; MARQUES, 1996). From the second week of experimentation, feces and urine were collected for the calculation of Nitrogen Balance (NB), True Digestibility (TD), Biological Value (BV) and biological utilization indexes of the protein (Pellet; Young, 1980; SGARBIERI, 1987; De Luca; Alexandre; Marques, 1996).

Statistical analysis

The data obtained were analyzed using Analysis of Variance (ANOVA), with significance level of 5%. Tukey's test ($p < 0.05$) was used to compare the averages, with a regression application according to Pimentel Gomes (2000).

RESULTS E DISCUSSION

Chemical Composition

The results of the analysis of the centesimal composition for the *O. latifolia* species in their raw and cooked whole form show lower values of proteins compared to cooked *P. vulgaris* (Table 1). Such low protein values may be related to the nutritional value of these foods, specifically *P. vulgaris*, so that among the macronutrients the protein content is the second that stands out in terms of quantity (EMBRAPA, 2015). The protein content obtained from the centesimal composition of the two modalities of *O. latifolia* evidenced that there was no statistical difference between nutrients, that is, the cooking process had little influence on the protein concentration. This condition may be strongly related to the presence of possible thermoset proteins. In fact, this information is of great relevance, since a great part of the antinutritional factors are thermosensitive proteins (SILVA; SILVA, 2000; GEMEDE; RATTA, 2014), which may suggest the absence of these factors in *O. latifolia*. The protein content stands out in relation to the other nutrients due to the combinatorial nutritional possibility.

Table 1. Chemical composition of the species *O. latifolia* (whole rice, raw and cooked) and *P. vulgaris* (carioquinha beans) both expressed in g / 100g

	O. latifolia				*P. vulgaris*	
	Raw Integral	Dry	Cooked Integral	Dry	Cooked Integral	Dry
Moisture	$9{,}62 \pm 0{,}09^a$	-	$8{,}35 \pm 0{,}08^a$	-	$4{,}02 \pm 0{,}09^b$	-
Ash	$1{,}30 \pm 0{,}05^a$	1,44	$1{,}37 \pm 0{,}10^a$	1,49	$3{,}74 \pm 0{,}06^b$	3,90
Lipids	$2{,}05 \pm 0{,}11^a$	2,27	$1{,}91 \pm 0{,}04^a$	2,08	$1{,}19 \pm 0{,}01^b$	1,24
Protein	$9{,}83 \pm 0{,}06^a$	10,88	$9{,}60 \pm 0{,}13^a$	10,47	$23{,}19 \pm 0{,}23^b$	24.16
Starch	$64{,}51 \pm 1{,}37^a$	71,38	$67{,}12 \pm 1{,}48^a$	73,24	$49{,}75 \pm 0{,}57^b$	51.83
Fiber	$13{,}15^a$	14,55	$11{,}76^b$	12,83	$18{,}12^c$	18.88
TCV (kcal)	$315{,}81^a$	349,47	$324{,}07^b$	353,56	$302{,}47^c$	315,12

TCV: Total Caloric Value. Values on the same line, followed by different letters, differ from each other ($p < 0.05$).

Biological Testing and Nutrition Assessment

The biological assay was performed according to a standard Pellet method; Young (1980), in this, was quantified the nitrogen excreted by the feces and urine of the animals by means of the method of Kjeldahl (AOAC, 1995). The control of the animals' body weight and feed consumption throughout

The association between *O. latifolia* and *P. vulgaris* provides a complement to the amino acid content found in these species. The presence of the amino acids methionine and cysteine present only in *O. latifolia*, while in *P. vulgaris* are found amino acids such as lysine. In this way there is a combination that leads to the formation of proteins of high biological value (PASSOS *et al*., 2014).

In terms of carbohydrate, the highest levels of starch were found in the samples of *O. latifolia* in comparison to *P. vulgaris*. Such results obtained are contrary to several studies that are dedicated to evaluate the centesimal composition of different species of the genus *Oryza*, as far as the content of starch (REDDY; BHOTMANGE, 2013; PASSOS *et al*., 2014). In this way, the obtained results reinforces that this macronutrient is predominant in this cereal (WANI *et al*. 2012). The species *O. latifolia* showed a superior lipid profile to *P. vulgaris*, a condition that reinforces the findings related to the nutritional composition of species of the same genus (WALTER; MARCHEZAN; AVILA, 2008; DEVI *et al*., 2015), In fact, part of the energy value of *O. latifolia* is attributed to the lipid content, a condition that differentiates it from the *P. vulgaris* species, which is nutritionally distinguished by its carbohydrate and protein profile (PHILIPPI, 2006; SEGURA-CAMPOS *et al*., 2014). The results obtained in our manuscript corroborating with studies that report the importance of the nutritional combination between species of rice and beans (PHILIPPI, 2006; PASSOS *et al*., 2014).

physiological conditions (WU, 2014; COUTINHO; MENDES; ROGERO and SILVA; MURA, 2014), with limiting amino acids being those with a chemical score lower than 1.0. Considering that the amount of amino acids was determined by calculating the chemical score for individuals between two and five years of age, it was possible to observe that the amino acids leucine and "methionine + cystine" had a lower chemical score in *O. latifolia*, in the same way as for the combination of *O. latifolia* and *P. vulgaris* (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 1991). On the other hand, the other classes of amino acids had a better chemical score (Table 2), a condition that must be taken into account for the adequate consumption of amino acids from cereals and grains and the maintenance of biological functions. The aminoacid profile of the species *O. latifolia* and the combination of "*O. latifolia* and *P. vulgaris*" shows that felylalanine + tyrosine are the main amino acids present, both with higher chemical scores. However, isoleucine, threonine, leucine, sulfur amino acids methionine + cystine and lysine were considered the limiting amino acids, except for the others, the presence of lysine as a limiting

Table 2. Amino acid composition of *O. latifolia, O. latifolia* and *P. vulgaris* (0.4: 1.6) and their chemical (EQ) scores

Aminoacids Essentials	*Oryza latifolia*		*Oryza latifolia* and *Phaseolus vulgaris*		FAO*
	Aminoacids (mg) / protein (g)	EQ	Aminoacids (mg) / protein (g)	EQ	
Phenylalanine + Tyrosine	189,70	3,01	128,40	2,04	63
Phenylalanine	163,00	nd		nd	-
Tyrosine	26,70	nd	nd	nd	-
Histidine	19,80	1,04	26,80	1,41	19
Isoleucine	18,60	0,66	28,83	1,03	28
Leucine	25,50	0,39	67,72	1,03	66
Lysine	40,50	0,70	83,59	1,44	58
Methionine + Cysteine	7,90	0,32	17,54	0,70	25
Methionine	1,90	nd	Nd	nd	-
Cysteine	6,00	nd	Nd	nd	-
Threonine	14,80	0,43	41,14	1,21	34
Valine	35,00	1,00	39,65	1,13	35

* FAO/WHO (1997) Theoretical standard, amino acid (mg) and protein (g) (essential amino acids for children 2 to 5 years of age); nd: not determined; values in bold represent the limiting amino acids.

The ash content showed that *P. vulgaris* species had a higher level of minerals when compared to *O. latifolia*. Both species possess rich mineral and vitamin composition, fact to which the nutritional properties of the two species are attributed (PHILIPPI, 2006; SEGURA-CAMPOS *et al*., 2014). Similar condition was observed with respect to the fibers, whose content was most evident in *P. vulgaris* in relation to *O. latifolia*, specifically of the whole raw species. The presence of the fibers highlights the nutritional relevance of these species, since the fibers are considered important components that act in the prevention and / or retardation of chronic noncommunicable diseases. In relation to the energetic value, there was no statistical difference between the different samples of *O. latifolia* and *P. vulgaris* ($p > 0.05$). The two species are characterized by the presence of different nutritional components such as carbohydrates and proteins that justify the energy content. These results were conclusive for the formulation of the rations used in the pre-clinical trial.

Aminoacid composition

When quantifying the amino acid content in *O. latifolia* as well as in the combination "*O. latifolia* and "*P. vulgaris*", it is possible to note that both had five limiting amino acids, being these isoleucine, lysine, threonine, leucine and the sulfur amino acids methionine + cystine (Table 2). These amino acids are considered fundamental for the maintenance of the

amino acid corroborates with studies by Sgarbieri (1996), Naves (2007) and Rohman *et al*. (2014) that demonstrated the presence of this amino acid class as a limiting factor in cereals, specifically in the *O. sativa* species in combination with *P. vulgaris* (Table 2). Sgarbieri (1996), Naves (2007) and Rohman *et al*. (2014) evidenced in their studies a high amount of sulfur amino acids, specifically methionine. The *O. latifolia* species were different in this regard, reinforcing the presence of the amino acids phenylalanine + tyrosine, as well as other more present classes such as phenylalanine, lysine, valine, tyrosine, leucine, histidine, threonine, methionine + cystine, cystine and methionine, with a chemical score ranging from 0.32 to 3.01. On the other hand, it was possible to note that the levels of isoleucine, leucine and threonine were below the standard values for essential amino acids, recommended by FAO/ WHO (1997) for children from 2 to 5 years of age. One of the most significant differences with respect to the amino acid concentration in *O. latifolia* was the phenylalanine value and its combination with tyrosine. On the other hand Naves (2007) in their study demonstrated inferior values of the combination in kind of the same genus. In this sense, it is important to observe that the phenylketonuric ones must be clarified as to the ingestion of this amino acid; however, according to the Brazilian *National Agency for Sanitary Surveillance* (ANVISA) labeling regulations (2005), the indication on the label is only necessary when it is a special food for diets with protein restriction. For the amino acid content of the combination of *O. latifolia* and *P. vulgaris*, the values were higher for lysine, leucine, threonine, valine, histidine and the combination of methionine + cystine. In these cases, chemical scores ranging from 0.70 to 2.04.These characteristics were conclusive for the preparation of the diets used in the preclinical test, especially with regard to the proportion to be used in the study of each component in order to supply the limiting amino acids (ratio of 0.4: 1.6) respectively to *O. latifolia* and *P. vulgaris*, in which methionine + cystine remained as limiting, that is, there was not sufficient complementarity to supply all the limiting amino acids of the species.

is directly related to the greater weight gain and, consequently, to the greater amount of feces observed in this group. Due to the differences in the composition of the diets in relation to the protein parameter (Table 4), the NI content of the animals whose diet contained as a protein source the *O. latifolia* was significantly higher in comparison to the *O. latifolia + P. vulgaris* mixture. When evaluating the amount of FN, it can be observed that the combination of *O. latifolia + P. vulgaris* proved to be nutritionally more adequate, since it presented FN values close to the value of the casein standard, which proves that this combination presented better digestibility compared to *O. latifolia*.

Table 3: Centesimal composition of the diet aproteic, standard, *O. latifolia* and *O. latifolia + P. vulgaris* used as diet in the protein biological assay

Components	Aproteic	Standard	*Oryza latifolia*	*Oryza latifólia* e *Phaseolus vulgaris*
Casein (%)	-	9,62±1,50^{a}	-	-
Rice flour (%)	-	-	9,23±0,67^{a}	1,86 ±0,26^{a}
Bean Flour (%)	-	-	-	7,43 ±0,26^{a}
Fibers (%)	8,65 ±2,76^{b}	7,32 ±1,11^{b}	9,51 ±0,11^{b}	5,82 ±1,87^{b}
Sucrose (%)	11,24 ±0,21^{b}	9,33 ±1,42^{a}	8,47 ±0,20^{a}	8,92 ±0,25^{a}
Saline Mixture (%)	3,81	3,42	3,49	3,47
Vitamin Blend (%)	0,95	0,85	0,87	0,87
Lipid (Soybean Oil) (%)	8,18 ±0,03^{b}	7,90 ±0,13^{b}	7,09 ±0,02^{a}	7,27 ±0,16^{a}
Starch (%)	69,83^{b}	63,00^{b}	66,29^{b}	64,86^{b}
Sodium benzoate (%)	0,10	0,09	0,09	0,09
Total Caloric Value (kcal)	397,90^{a}	398,90^{a}	399,77^{a}	397,71^{a}

Mean values ± standard deviation. Values on the same line, followed by different letters, differ from each other (p <0.05). Values in bold: consider the sum of the averages.

Table 4. Weight gain, consumption of ration, Stool quantity, Nitrogen intake (N) (g/ animal), Fecal nitrogen (FN) (g/animal) and Urinary nitrogen (UN) of rats submitted to the standard diet (casein), Test 1 (*O. latifolia*) and Test 2 (*O. latifolia + P. vulgaris*) for 29 days

Variables	Standard (Casein)	Test 1 (*O. latifolia*)	Test 2 (*O. latifolia + P. vulgaris*)
Weight gain (g)	72,39 ± 12,41	27,72 ± 8,55	13,74 ± 4,52
Consumption of diets (g)	262,85	185,43	135,85
Amount of feces (g)	26,74	25,14	17,17
N ingested (g)	3,75	2,63	1,91
N Fecal (g)	0,40	1,00	0,46
N urinary (g)	0,16	0,07	0,04

Mean values ± standard deviation

Chemical Composition of Diets

According to Table 3, the evaluation of the centesimal composition of the elaborated diets that were used in the preclinical test has the protein values of 9.62; 9.23 and 9.29 g / 100g and caloric values of 398.9; 399.7 and 397.7 kcal/100 g for the standard (casein), *O. latifolia* and *O. latifolia + P.vulgaris*. The centesimal composition of the rations demonstrates that despite the differences in the amounts of the components between the diets, the values did not show significant differences at the level of 5%, except for the moisture content. The indication of using isocaloric rations for the different groups and isoproteic was maintained, with the exception of the diet used for the aproteic group.

Biological testing and Nutrition Assessment

At the end of the 29 days of experiment, there was an average weight gain of 72.39g for the Standard Group, which served as a parameter for the evaluation of the weight gain for Test Groups 1 and Test 2. Regarding the consumption of ration, it was possible to notice a significant difference between the groups *O. latifolia* and *O. latifolia + P. vulgaris*, being the consumption more pronounced in the first one. Such condition

Similarly, for the UN, it was observed that the *O. latifolia* groups and the *O. latifolia + P. vulgaris* combination presented lower values when compared to the Standard diet, suggesting lower protein breakage and better organic utilization. Such results confirm the importance of this combination for the purpose of providing better nutritional quality. In addition, through this assay it was possible to validate that the amino acid proportionality standard and the digestibility are determinant of the protein quality (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 1991). Another important fact related to the quality of this nutrient is its association with the presence of one or more essential amino acids in adequate concentrations (GALLAND-IRMOULI *et al*., 1999). Nitrogen balance was similar in test systems 1 and 2, but was lower than in the standard (Table 5). The studies of the FAO (1991) are consistent with the process of identifying the factors that contribute to the lower digestibility of the proteins of vegetal foods in comparison to the proteins of animal origin, such as the presence of phenolic compounds, food fiber components, pigments, inhibitors of enzymes and others. In addition, it was possible to note that, according to FAO (1991), brown rice has in average 5.5% of protein of which 72.7% is of biological value; 96.5% digestibility and net utilization (NPU) of 70.2%.

Table 5. Nitrogen Balance (NB), Biological Value (BV), True Digestibility (TD), Protein Efficiency Ratio (PER), Net Protein Efficiency Ratio (NPR) and Net Protein Utilization (NPU) rice *O. latifolia* (Test 1) and of the mixture "*O. latifolia* + *P. vulgaris* (Test 2)" compared to standard casein

Grups	NB	BV (%)	TV (%)	PER	NPR	NPU (%)
Standard	3,30 ±0,01^{a}	95,47±0,16^{b}	92,18±0,31^{b}	3,09±0,53^{a}	3,68±0,53^{b}	58,60
Test 1	1,67±0,02^{b}	96,15±0,31^{b}	65,53±0,22^{b}	1,52±0,44^{b}	2,57±0,53^{a}	30,98
Test 2	1,52±0,01^{b}	97,65±0,36^{b}	81,33±0,31^{b}	1,15±0,38^{b}	2,29±0,38^{a}	29,57

Mean values ± standard deviation. Values in the same column followed by different letters, differ from each other ($p < 0.05$).

However, *O. latifolia* showed 83% of proteins, 96.15% of biological value, 65.53% of digestibility and 30.98% of NPU. These results demonstrate that for this rice species, the absorbed amino acids are possibly more retained by the organism compared to white rice (*O. sativa*), as well as for its combination with *P. vulgaris*. In this context, the fibers, especially the structural polysaccharides composed of cell walls and their protein interactions are able to reduce the accessibility of the protein to the proteolysis, reducing the digestibility (REEVES *et al*., 1993). Such influence is a condition of potential investigation so that the concentration of the dry sample showed 14.55%.

As for DV of *O. latifolia* and its combination with *P. vulgaris* in the studied proportion, it was verified that there was no significant difference between the diets. BN and VB are statistically the same, fact also evidenced when compared to the standard diet, with the exception of NB. This result suggests that the limiting amino acids of the proteins of the test groups did not interfere in the global use of the protein in relation to the proportion of nitrogen (protein) retained by the body of the total absorbed (SGARBIERI, 1987). The study of food digestibility is essential as it demonstrates the amount of nitrogen absorbed in relation to that offered in the diet. The evaluation of the digestibility of diets considering the combination of different genera of *O. sativa* and *P. vulgaris* was also studied by Cintra *et al*. (2007).

The results presented in our study reveal that the two diets were statistically different as compared to the standard diet with regard to PER and NPR, having lower values, a fact not evident when compared to the standard diet. This means that the species *O. latifolia* as well as its combination with the *P. vulgaris* species have lower quality than casein with respect to the promotion of growth and maintenance of the studied individuals, as evidenced by Monteiro *et al*. (2004) in their study. On the other hand, from the comparison between the test diets it was observed that *O. latifolia* has higher nutritional quality, a condition that allowed the development of the animals.

The use of *O. latifolia* native rice in formulations intended for the human diet as an alternative to obtaining nutritionally good quality protein requires the inclusion of other foods that are deficient in relation to essential amino acids, a recommendation given to many other food groups. It is also recommended to prepare them for consumption in the unnatural form, without polishing, to maintain their characteristics of brown rice of good classification, that is, to reduce the excessive breaking of the grains during the polishing process. In addition, it is an ecological product that can arouse interest for the local or national market, in the form of direct consumption or through the development of new products, especially in the area of gastronomy.

Conclusion

O. latifolia has considerable levels of proteins, carbohydrates, lipids and fibers, especially in its raw form. Its preparation may influence its nutritional content, although considered not significant. It is possible to deduce that Pantanal rice has nutritionally relevant amino acids, five of which have limiting characteristics. *O. latifolia* influenced significantly the weight gain of the studied animals, a result related to the increase of diet consumption and consequent of the eliminations. On the other hand, the species had lower digestibility in relation to its combination with *P. vulgaris* species. It was verified that there was greater use of the liquid protein of *O. latifolia*, a condition that allows a greater amount of amino acids to be retained by the organism, and consequent use. Pantanal rice should be encouraged for human consumption as a food option that provides good quality protein, whether or not it can be combined with *P. vulgaris*, a species that also offers significant levels of proteins and carbohydrates.

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