# Protein Quality of Rice Drinks Fortified with Bovine and Porcine Blood Plasma

Calidad Proteica de Bebidas de Arroz Fortificadas con Plasma Sanguíneo de Bovino y Porcino

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Abstract. The future of nutrition in Colombia, and perhaps in other developing countries, will depend in large part on the ability of food technology to take full advantage of the food sources available in the country and to adapt and develop new products that will vary and complement the diets of the majority of the population at a low cost. The objective of this study was to evaluate the protein quality of rice-based drinks fortified with bovine and porcine blood plasma. Six treatments were prepared with different levels of fortification (14.5%, 18.5% and 29%). The effects of the plasma type and the addition levels on the protein content, the amino acid profile, and the in vitro digestibility of the drinks were observed. The AOAC method was employed for the determination of the protein content; the amino acid profile was created using HPLC. The protein digestibility was determined by subjecting a dispersion of the drink to the action of a multi-enzymatic solution. The protein content increased with the level of fortification. The drinks fortified with bovine plasma (104%) and porcine plasma (89%) presented a better protein quality index than the unfortified drink. The digestibility of the fortified drinks did not demonstrate significant improvements in comparison with the unfortified drink. The chemical score of the drinks fortified with porcine plasma (71.6) and bovine plasma (78.5) showed that the latter had the best nutritional quality.

**Key words**: Protein content, *in vitro* digestibility, amino acid profile, food design.

Resumen. El futuro de la alimentación en Colombia y quizás de otros países en desarrollo va a depender en gran parte de que la tecnología de alimentos sea capaz de aprovechar las fuentes disponibles de alimentos en el país y de adaptar y desarrollar nuevos productos que permitan variar y complementar la dieta de la mayoría de la población a bajo costo. El objetivo de este trabajo fue evaluar la calidad proteica de bebidas a base de arroz fortificadas con plasma sanguíneo de bovino y porcino. Se prepararon seis tratamientos con diferentes niveles de fortificación (14,5%; 18,5% y 29%). Se observó el efecto del tipo de plasma y los niveles de adición en el contenido proteico, perfil de aminoácidos y digestibilidad in vitro de las bebidas. Se empleó el método AOAC para la determinación del contenido proteico, el perfil de aminoácido se realizó por HPLC. La digestibilidad proteica se determinó al someter una dispersión de la bebida a la acción de una solución multienzimática. El contenido proteico aumentó al incrementar el nivel de fortificación. Las bebidas fortificadas con plasma de bovino (104%) y porcino (89%) presentaron mejor índice de calidad proteica que la bebida sin fortificar. La digestibilidad de las bebidas fortificadas no mostró una mejora significativa al compararla con la digestibilidad de la bebida sin fortificar. El computo químico para la bebida fortificada con plasma de porcino (71.6) y bovino (78,5) evidencia que esta última es de mejor calidad nutricional.

**Palabras clave**: Contenido proteico, digestibilidad *in vitro*, perfil de aminoácidos, diseño de alimentos.

The serious problems of malnutrition and animal protein deficiency that exist in various countries of the world have presented a challenge for national and international organizations (FAO, 2012). The heads of state and representatives of more than 185 countries have focused on the goal of reducing hunger by 50% by the year 2015 in light of the alarming evidence that 816 million people live in hunger and 170 million children under the age of 5 suffer from malnutrition worldwide (Lathan, 2002).

In Colombia, as in other countries, protein deficiency, hunger and malnutrition are not the result of low food availability but of the high levels of poverty, the inability to buy animal protein and poorly balanced diets that are largely dependent on cereals that, in many cases, form the nutrition base (Barboza *et al.*, 2005). An alternative for improving the nutritional state is the use of massconsumption cereals, such as rice, that form part of the daily diet of the population in the production of food fortified with protein. Fonseca and Villamarín (2004) reported that the fortification of new mass-consumption foods with an emphasis on children represents a line of action that is vital to the core of the national strategy for nutritional and food security in Colombia.

Although rice, both refined and un milled, represents an important energy source, it possesses an incomplete amino acid profile, with lysine being the limited amino acid, but also has significant quantities of methionine, isoleucine, and leucine (Table 1); and so, it has been

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recommended that rice be enriched with amino acids or animal protein sources in order to compensate for the deficient essential amino acids (González *et al.*, 2007).

Bovine and porcine blood contain close to 19% protein, easily digestible protein and quality amino acid composition, conferring a high biological value (Márquez *et al.*, 1995; Bracho *et al.*,2001; Montero *et al.*,2012); however, of all the blood components, plasma is the most used commercial product in food production due to the fact that its flavor and color are easily masked. Bovine plasma and porcine plasma contribute approximately 7.2 and 6.6 % of the protein,

respectively (Bracho *et al.*, 2001). All of the blood proteins are found in the plasma except hemoglobin, which for the most part is found in cellular fractions (Rodríguez *et al.*, 2011). Blood plasma contains all the essential amino acids for human nutrition as observed in Table 1, is rich in lysine but deficient in isoleucine and methionine, and so, should be administered in conjunction with other proteins that supplement this deficiency (Bracho *et al.*, 2001; Rodríguez *et al.*, 2011).

The recuperation and utilization of these proteins is very important if the high levels of global protein deficiency and the quantity of bovine and porcine that is butchered

 Table 1. Average values of the amino acids in rice and bovine and porcine blood plasma.

Amino acid	Rice grain	Bovine plasma	Porcine plasma
Isoleucine	4.1	2.56	2.25*
Leucine	8.2	5.96*	6.29
Lysine	3.8*	7.18	6.12
Methionine + cysteine	3.6	0.21**	0.53**
Phenylalanine +tyrosine	9.1	6.11	9.33
Threonine	4.1	5.34	3.95
Valine	5.8	3.85	4.12
Histidine	2.6	5.12	2.18
Chemical score	65.5	90.30	80.35

\* Amino acid is nutritionally limited when the considered food constitutes the only protein contribution to the diet = g of amino acids per 100 g of studied protein/ g of same amino acid per 100 g of reference protein x 100. \*\*Methionine value.

annually are taken into account. Similarly, blood protein with microbiological quality, obtained through the application of suitable procurement methods, thermal treatments, and posterior microbiological analysis of the plasma (Julio *et al.*, 2011; Benítez *et al.*, 2011), in addition to being an important source for nutrients, is important in terms of its recuperation because it would mean a reduction in the environmental contamination associated with the dumping of blood in the environment (Julio *et al.*, 2013) due to the high quantity of total solids (18%) and the elevated chemical oxygen demand (COD) of 500,000 mg/L (Rodríguez *et al.*, 2011).

Protein quality refers to the ability to provide the nitrogen and essential amino acid requirements of a body (Fennema, 1993). According to Suarez *et al.* (2006), the quality, or balance of a nutritional protein, depends on the content and type of the protein, on the nature and quantity of the essential amino acids it contains and on its digestibility; furthermore, it represents a measurement

of the efficacy with which it can be used by the body. An equalized protein, or one of high quality, contains essential amino acids in the approximate proportions established by the patterns of the FAO (Fennema, 1993).

Essential amino acids are those that cannot be synthesized by an organism, which implies that the only source for these amino acids is through their direct ingestion by diet. Those amino acids that are highly deficient with respect to the necessities are called "limiting" (Fennema, 1993). Cereal proteins are poor in lysine and occasionally lack tryptophan and threonine (Romo *et al.*, 2006), but contain significant quantities of methionine, leucine and isoleucine. On the other hand, blood plasma is generally deficient in methioline, leucine and isoleucine and possesses considerable contents of lysine and threonine (Bracho *et al.*, 2001).

Currently, for the evaluation of protein quality, it is more reliable to calculate the corrected chemical score using

multiplication with the digestibility of the protein, known as PDCAAS (Protein Digestibility Corrected Amino Acid Store). The World Health Organization and the United Sates FDA adopted PDCAAS in 1993 for the official analysis of protein quality values because it is based on the amino acid content of nutritional proteins, the digestibility, and the ability to supply indispensable amino acids at a quantity that sufficiently covers human requirements (González *et al.*, 2007).

As a result, there is a drive to take advantage of lowcost, protein-rich meat byproducts in the production of high-protein-content products using cereals, such as rice, that form part of the daily diet of many populations, especially in countries such as Colombia. This strategy has resulted in the alternative of fortifying rice based drinks with bovine or porcine plasma and new foods that contain significant, quality protein contents.

The future of food in Colombia, and perhaps in other developing countries, will largely depend on the ability of food technology to take advantage of the available foods and develop new products that diversify and compliment the diet of the majority of the population at low costs. Thus, the challenge is to direct techniques toward the improvement and increase of the nutritive value of foods destined for human consumption. For this reason, this study aimed to evaluate the protein quality of a rice drink fortified with bovine and porcine plasma.

### MATERIALS AND METHODS

**Obtaining samples**. The bovine and porcine blood were collected from the slaughterhouse in the municipality of Arjona- Bolívar and transported to the laboratory under refrigerated conditions (5 °C). The plasma was extracted following the methodology of Marquez *et al.* (2005) and preserved through freezing (Prandl *et al.*, 1994).

**Experimental design**. A 2x3 factorial design was developed with three replications in a random complete experimental design. Each sample was analyzed in duplicate. The response variables were: protein content, amino acid profile and protein digestibility of the product. The study factors corresponded to: type of added plasma (at two levels: bovine plasma and porcine plasma); and the levels of fortification (at three levels: 14.5%, 18.5% and 29% plasma); with the goal that a 250 mL serving of the drink supplied, at a minimum, 10%; 15%; and 20% of the daily requirement for protein, respectively, (RDA) as established by the FAO for preschool-age children (2-5 years). The refreshing

rice drink was produced according to the method of Montero and Muñiz (2001).

The treatments were:

- T1 = Rice drink with 14.5% bovine blood plasma.
- T2 = Rice drink with 18.5% bovine blood plasma.
- T3 = Rice drink with 29% bovine blood plasma.
- T4 = Rice drink with 14.5% porcine blood plasma.
- T5 = Rice drink with 18.5% porcine blood plasma.
- T6 = Rice drink with 29% porcine blood plasma.
- Control: Rice drink without blood plasma.

Analysis of variance (ANOVA) was applied to the data using SAS (2003) in order to detect differences between the means. The treatment means were compared using the LSD test. Differences at a probability level of 5% were accepted.

**Determination of essential amino acids**. High performance chromatography (HPLC) with a BAS<sup>®</sup> chromatographer (California, USA) and a Water 474 fluorescence detector was used. The fluorescence was detected with a wavelength of 340 nm and an emission length of 460 nm.

**Limiting amino acid** = g of amino acid per 100 g of studied protein/ g of the same amino acid per 100 g of reference protein. The FAO reference model, which is based on the needs for indispensable amino acids of preschoolers (2-5 years) is now considered the preferred reference protein, in place of the previously-used egg protein (Ayala *et al.*,2000).

**Chemical score**. mg of primary limiting amino acid per g of studied protein/mg of the same amino acid per g of reference protein x 100.

**Protein determination and quality index**. To obtain the protein content, the AOAC 976.05 method was employed (AOAC, 1997). The quality index was determined by the ratio between the age-determined protein requirement and the requirement of the most limiting amino acid of the studied protein (Ayala *et al.*, 2000).

*In vitro digestibility*. An *in vitro* method was applied in accordance with the protocol of Montero *et al.* (2012), subjecting a portion of the drink to the action of a multi-enzymaticsolution.

**Corrected chemical score (PDCAAS)**. Was determined by multiplying the ratio of the lowest amino acid by the digestibility of the protein (Suárez *et al.*, 2006).

**Sensory analysis**. To evaluate the acceptability, a panel of untrained tasters was used, composed of 50 preschoolers of both sexes, 2-5 years-old, who were selected at random at a kindergarten in Cartagena. A hedonic gratification scale of 5 points was used and the degree of satisfaction was measured, thereby determining the degree of acceptability of the drink fortified with 29% bovine plasma. The test was carried out in two sessions; each panelist was given samples (20 mL) of the rice-based drink without plasma (Control) and of the drink fortified with 29% bovine plasma (Julio *et al.*, 2011).

*Microbiological analysis*. The drink fortified with 29% bovine plasma was submitted to microbiological analysis according to the INVIMA standards (INVIMA, 1998). Total Mesophyll aerobic count (UFC/g): Plate count (INVIMA). NMP of fecal and total Coliforms: NMP-CBVB (INVIMA). Mold and yeast count (UFG/g): Plate count (BAM–FDA 8th ed).

### **RESULTS AND DISCUSSION**

The results indicate that the protein content varied according to the type of plasma and the level of fortification (Table 2). The protein content increased in proportion to the increase in the fortification level. However, an interaction was observed between the factors, indicating that the increase in the protein content of the drink with the increase in fortification level depended on the type of plasma.

The 29% bovine plasma treatment had a significantly higher (P<0.05) protein content (2.47%) than the porcine treatment (1.75%). The 18.5% bovine plasma treatment and the 29% porcine plasma treatment had equal protein contents. These results explain the observed interaction, indicating that not only the type of plasma but also the level of fortification were significant in explaining the protein content of the refreshing rice drink.

**Table 2.** Average values of the protein content (%) of the refreshing rice drink resulting from the different levels of fortification and plasma type.

	Plasma concentration			
Plasma type	Drink fortified with 14.5% plasma	Drink fortified with 18.5% plasma	Drink fortified with 29% plasma	Unfortified drink
Bovine plasma	1.26 cd ± 0.04	1.65 bc ± 0.09	2.47 a ± 0.31	0.3 ± 0.01
Porcine plasma	1.06 d ± 0.00	1.25 d ± 0.01	1.75 b ± 0.14	0.5 ± 0.01

Test performed in triplicate. Means with different letters differ significantly (P<0.05) according to Tuckey.

The increase in the protein content of the rice drink with plasma fortification coincides with the results reported by Barboza *et al.* (2005) who found that, when incorporating 40% plasmatic protein in a product formulated with soft corn, the protein content increased 3.54% in a product formulated without bovine plasma and 6.47% in a product that contained plasma.

Similar results were reported by Márquez *et al.* (1995) who found that, when adding bovine blood plasma to emulsified meat products, the protein content increased by up to 14.84%. Similarly, Benítez *et al.* (2008) observed that the protein content of a galantine formulated from flour and yuca increased considerably with the incorporation of bovine plasmatic proteins as a fortification ingredient.

Finally, there were no significant differences in the protein content of the porcine plasma treatments

at 14.5% and 18.5%, independent of the level of fortification, being the treatments with the lowest quantity of protein. The drink fortified with 29% bovine plasma presented the highest protein content (2.47%). According to the FAO, 1 g/kg for children aged 2 to 5 is considered an innocuous dose for reference proteins (FAO, 1991); considering a preschool-aged child (2-5 years) with a weight of 20 kilos, the daily protein requirement would be 20 g of protein; a serving of a drink fortified with 29% bovine plasma supplied 30% of the daily requirement for protein in a preschool-aged child (2-5 years), a higher contribution than a serving of milk (250 mL) with 18% of the daily requirements of the child. This shows that drinks fortified with 29% plasma are an alternative for helping to meet the protein requirements of infancy (Table 3).

Table 3 presents the protein contribution expressed in grams of a daily serving (250 mL) of the rice-based drink.

Plasma concentration				
Drink fortified with 14.5 % plasma	Drink fortified with 18.5% plasma	Drink fortified with 29% plasma	Unfortified drink	Cow milk <sup>1</sup>
3.12	4.12	6.17	0.75	2.5
2.65	3.12	4.37	0.75	3.5
	with 14.5 % plasma 3.12	Drink fortified with 14.5 % plasmaDrink fortified with 18.5% plasma3.124.12	Drink fortified with 14.5 % plasmaDrink fortified with 18.5% plasmaDrink fortified with 29% plasma3.124.126.17	Drink fortified with 14.5 % plasmaDrink fortified with 18.5% plasmaDrink fortified with 29% plasmaUnfortified 

Table 3. Protein contribution expressed in grams of a daily serving (250 mL) of the rice-based drink.

<sup>1</sup> Bracho *et al.,* 2001.

There were significant differences (P<0.05) in the contents of lysine, histidine, threonine, and aromatic and sulfated amino acids of the drinks fortified with bovine and porcine plasma (Table 4). These results coincide with those reported by Bracho *et al.* (2001) who also found significant differences in the contents of histidine, threonine and phenylalanine +tyrosine

when the content of essential amino acids in bovine and porcine plasma was evaluated. It was observed that the refreshing drinks fortified with bovine and porcine plasma fulfilled the sulfated amino acid and lysine requirements as suggested by the FAO (1991), although a small unbalance was seen in the isoleucine and leucine amino acids.

**Table 4.** Average essential amino acid values (g of amino acid/100 of protein) in the rice drinks fortified with bovine and porcine blood plasma.

Amino acid	Drink fortified with bovine plasma	Drink fortified with porcine plasma	FAO/WHO/ ONU* (1991)
Isoleucine	2.9 a	2.5 a	2.8
Leucine	6.2 a	6.5 a	6.6
Lysine	7.1 a	6.1 b	5.8
Methionine+cysteine	3.4 a	3.1 b	2.5
Phenylalanine+ tyrosine	6.7 b	9.3 a	6.3
Threonine	5.5 a	4.0 b	3.4
Valine	4.3 a	4.3 a	3.5
Histidine	5.2 a	2.2 b	1.9

\* FAO reference model, based on the needs for indispensable amino acids for preschoolers (2-5 years old).

Measurements with different letters in the same row differed significantly (P<0.05) according to Tukey.

Although no significant differences were found for the leucine and isoleucine contents of the drinks fortified with bovine and porcine plasma (Table 4), the chemical score of the amino acids had a value of 94 and 89 (Table 5) for the rice drink fortified with bovine plasma and porcine plasma, respectively.

The fortification of the refreshing rice drink with the plasma of different species complements the rice proteins and results in a product with a better balance of amino acids than the original premium materials. These results coincide with those reported by Barboza *et al.* (2005), who evaluated the effect of the incorporation of plasmatic protein on the protein quality of a product with corn, concluding that the combination of a cereal, such as corn, with plasma resulted in an increase of product quality and an appropriate equilibrium of amino acids, obtaining a food with a superior nutritive value, where the plasma contributed lysine to the corn and the corn contributed methionine to the plasma.

Two or more incomplete proteins, such as the proteins of rice and of the blood plasma of different animal

species, were combined in a manner that the deficiency of one or more amino acids of one was compensated for by the other. When complementary proteins are combined, they supply all the necessary essential amino acids for the human body, delivering a pattern of equilibrium of amino acids that is used efficiently (Williams, 1995).

**Table 5.** Limiting amino acids and chemical scores of the rice-based drink fortified with bovine or porcine plasma.

	Food		
Essential amino acids	Drink fortified with bovine plasma	Drink fortified with porcine plasma	
Isoleucine	1.03	0.89*	
Leucine	0.94*	0.98	
Lysine	1.22	1.05	
Methionine + cysteine	1.36	1.24	
Phenylalanine + tirosina	1.07	1.48	
Threonine	1.63	1.17	
Valine	1.22	1.22	
Histidine	2.73	1.15	

\*Nutritionally limited amino acid.

The nutritional value of a protein for a population of a determined age can be defined as the degree at which the ingestion is at a sufficient quantity to satisfy the nitrogen requirements of an individual and, at the same time, the requirements for each of the essential amino acids for the synthesis of tisular proteins, a concept represented by the protein quality index (Ayala *et al.*, 2000).

In this study, the protein quality index was evaluated taking into account the requirements for amino acids according to the FAO for preschool-aged children (2.5 years), with the result that the drink fortified with bovine plasma had a protein quality index value higher (104%) than the drink fortified with porcine plasma (98%) (Table 6).

**Table 6.** Ingestion of protein in accordance with the amino acid requirements of preschoolers and protein quality index.

	Drink fortified with bovine plasma	Drink fortified with porcine plasma		
Essential amino acids	(g kg <sup>-1</sup> d <sup>-1</sup> )			
Isoleucine Leucine	0.96 1.06	1.12 1.01		
Lysine	0.81	0.95		
Methionine+ cysteine	0.73	0.80		
Phenylalanine+tyrosine	0.94	0.67		
Threonine	0.61	0.85		
Valine	0.81	0.81		
Histidine	0.36	0.86		
Quality Index	104	98		

Index of protein quality = Requirement for protein of preschool-aged children\*/Requirements for aa more limited in subjects of the same age x 100.

This means that preschoolers must consume 1.06 g/kg/d of protein of the drink fortified with bovine plasma to satisfy the requirement for the most limiting amino acids, which in this case was leucine. This is correct if it is assumed that complete absorption exists in order to completely satisfy the requirements of each essential amino acid; but, if fecal losses are considered to be in the order 20%, the quantity of protein of the drink fortified with bovine plasma that a preschool-aged child must ingest is 1.27/g/kg/d. On the other hand, if it is assumed that the protein requirement for preschoolers is 1.10 g/kg/d, this quantity only provides 87% of any of the essential amino acids, limiting the synthesis of proteins in the body at these percentages.

On the other hand, in analyzing the drink fortified with porcine plasma, the values indicated that a preschooler must consume 1.12 g/kg/d of protein of the drink fortified with porcine plasma in order to satisfy the requirement for the most limiting amino acid, which in this case was isoleucine; if one considers fecal losses to be in the order of 20%, the quantity of protein of the drink fortified with porcine plasma that must be ingested by a preschool-aged child is 1.34 g/kg/d; that is to say, if one considers that the requirement of proteins for preschoolers to be 1.10 g/kg/d, this quantity only supplies 82% of any of the essential amino acids, limiting the synthesis of proteins in the body at this percentage.

The results obtained for the studied drinks showed that the highest digestibility value was seen in the

drink fortified with bovine plasma (83.56%) (Table 7), a value that was not significantly higher than that of the unfortified rice drink (82.02%); which demonstrated that, from the point of view of nitrogen absorption by the body, the addition of plasma did not make the drinks fortified with bovine or porcine plasma nutritionally superior. It is important to note that the plasma type influenced the protein digestibility of the product; the treatments fortified with bovine plasma presented significantly (P<0.05) higher percentages of digestibility when the fortification was carried out with porcine plasma; however, both results were below the digestibility values of chicken plasma (83-92) reported by Del Rio et al. (1980), this difference could be associated with the evaluation methodology employed and the pretreatments of the plasma samples.

In the calculation of the chemical score of the corrected amino acids for the digestibility (PDCAAS) of the protein of the fortified drinks, the lowest ratio corresponded to leucine (0.94) and isoleucine (0.89) for the drink fortified with bovine plasma and porcine plasma, respectively. The digestibility of the protein of the rice drink fortified with 29% bovine and porcine plasma was 83.56% and 80.48%, respectively; the corrected score for the protein digestibility of the drink fortified with bovine plasma was 78.5% and for the drink fortified with porcine plasma it was 71.6%; which means that the drink fortified with bovine plasma had a higher capacity for supplying indispensable amino acids in accordance with the ideal requirement for protein as proposed by the FAO.

**Table 7.** Average values of the *in vitro* digestibility (%) due to the different levels of fortification and plasmatic protein.

			Plasma con	centrations			
Plasma type	Drink fortified with 14.5% plasma	Drink fortified with 18.5% plasma	Drink fortified with 29% plasma	Unfortified drink	Bovine plasma	Porcine plasma	Casilan (protein pattern)
Bovine plasma	83.26 a ± 1.20	83.06 a ± 1.70	83.56 a ± 1.63	82 02 + 1.9	774+21	644+26	2 79 + 0 82
Porcine plasma	80.66 b ± 0.54	80.12 b ± 0.36	80.48 b ± 0.18	<u><u><u></u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	,,,, <u>,</u> ,,,,	0 I. I ± 2.0	2.75 ± 0.02

Tests carried out in triplicate. Means with different letters differ significantly (P< 0.05) according to Tuckey.

The results showed that, although the unfortified drink presented the highest mean, there was no significant difference in the acceptance of the unfortified drink and the drink fortified with 29% bovine plasma (Table 8).

The microbiological results showed that the microorganism counts for the fortified drinks were under the maximums permitted by the Codex Alimentarius (Table 9), guaranteeing the harmlessness of the product,

probably achieved with the adjustment of pH and thermal treatment. Furthermore, the results coincide with those

obtained by Benítez *et al.* (2008) with cookies improved with bovine blood plasma.

**Table 8.** Average values of the acceptability of the drink fortified with 29% bovine plasma and the unfortified drink.

Drink	Acceptability
Drink fortified with 29% bovine plasma	3.13 a ± 1.3
Unfortified drink	4.0 a ± 1.0

Means with different letters differed significantly (P<0.05). Nonparametric test. Multiple range test of Duncan.

Table 9. Microbiological	analysis of the fortified drink.
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Microorganism	Fortified drink	Codex alimentarius for the drinks
Aerobic count	< 3UFC mL <sup>-1</sup>	50 UFC mL <sup>-1</sup>
Mold count	< 1 UFC mL <sup>-1</sup>	8 UFC mL <sup>-1</sup>
Coliform count	< 1 UFC 100 mL <sup>-1</sup>	2 UFC 100 mL <sup>-1</sup>

# CONCLUSIONS

The type of added plasma and the levels of fortification significantly affected the protein contents of the refreshing rice drinks; however, the amino acid profile and the digestibility were only affected by the plasma type.

The rice drink fortified with bovine or porcine plasma did not significantly improve in digestibility, but a better amino acid balance was achieved when compared with the unfortified drink, as demonstrated in the protein quality index values, where the drink fortified with bovine plasma presented a value superior to that of the drink fortified with porcine plasma.

Likewise, the drink fortified with bovine plasma presented a superior value in regards to the capacity to supply indispensable amino acids at a quantity that is sufficient for covering the requirements of humans (PDCAAS); therefore, the drink fortified with bovine plasma had a better nutritional quality and can de recommended as a supplement for providing essential amino acids to preschool-aged children.

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