Bovine Blood Components: Fractionation, Composition, and Nutritive Value

Renata T. Duarte, Miriam C. Carvalho Simões, and Valdemiro Carlos Sgarbieri*

Food Chemistry and Applied Nutrition Center, Institute of Food Technology (ITAL), Av. Brasil 2880, CEP 13073-001, Campinas, São Paulo, Brazil

The objective of this research work was to fractionate bovine blood, collected hygienically in a slaughterhouse, into blood plasma protein concentrate, red blood cell concentrate, globin isolate, and a carboxymethylcellulose−heme iron (CMC−heme) complex. All four fractions were studied for proximate composition and amino acid and mineral contents. The nutritive value of plasma protein concentrate and globin isolate was comparatively studied using rat bioassays. The amino acid content in plasma protein concentrate is well balanced and produced net protein utilization and net protein ratio equivalent to 95% those of casein. Globin isolate (∼91% protein) is deficient in isoleucine and S-containing amino acids and was unable to support rat growth at 10% concentration in the diet. Red blood cell concentrate and the isolated CMC−heme complex were good sources of bioavailable iron. Iron availabilities for CMC−heme and whole blood cell concentrate, related to ferrous sulfate as 100%, were 64 and 70%, respectively.

Keywords: *Bovine blood; protein nutritive value; iron bioavailability*

INTRODUCTION

Bovine blood is made up of 80.9% water, 17.3% protein, 0.23% fat, 0.07% carbohydrate, and 0.62% minerals (Alencar, 1983). Blood composition is similar to meat composition except for the iron (36.3 mg/100 g of blood), which is ∼10 times the concentration in meat (Wismer-Pedersen, 1979; Alencar, 1983).

Blood can be fractionated into plasma (65−70%) and cellular mass (35−40%) on a volume basis (Halliday, 1973). For this fractionation, one normally uses anticoagulant to complex calcium and avoid coagulation (Gunstone, 1980).

The plasma contains 7.9% protein, and the main plasma proteins are the albumins (3.3%), immunoglobulins, α- and β-globulins (4.2%), and fibrinogen (0.4%) (Halliday, 1975; Howell and Lawrie, 1983). Dehydrated plasma contains, on average, 7% moisture, 80% protein, 7.9% minerals, and ∼1% fat (Tybor et al., 1975; Piske, 1982).

The cellular fraction contains all of the hemoglobin and most of the iron in the form of heme-Fe.

One of the drawbacks of using the blood cellular fraction as food ingredient for humans is the red color of hemoglobin and the metallic flavor attributed to this protein. Therefore, some investigators (Tybor et al., 1975; Autio et al., 1984; Lee et al., 1991) have proposed methods for breaking down hemoglobin into globin (its protein component) and the heme, which is a rich source of iron.

Blood protein has been used as food ingredient both for its functional properties (Caldironi and Ockerman, 1982a,b; Maddipati and Marnett, 1987; Faraji et al., 1991; Lee et al. 1993) and as a nutritive complement, mainly for cereal proteins (Delaney, 1975; Del Rio de Reyes et al., 1980; Wismer-Pedersen, 1979).

A number of studies have indicated whole blood or red blood cells as supplemental sources of iron, mainly because of its high bioavailability (Walter et al., 1993; Viteri et al., 1995; Hurrell et al., 1991).

The purpose of the present study was to establish a methodology to fractionate bovine blood into a plasma protein concentrate, a globin isolate, a cellular fraction, and a complexed carboxymethylcellulose−heme (CMC−heme) fraction. These fractions were studied with regard to their composition and nutritive value both as a source of protein and as a hematinic iron for the rat.

MATERIALS AND METHODS

Blood Collection. Bovine blood was collected in a slaughter house near Campinas under hygienic condition. Blood was collected by the use of a hollow knife (Iraí, Rio Grande do Sul, Brazil) by direct puncture into the artery (arcus aortae) immediatly after stunning in a receptacle containing, as anticoagulant, a concentrated sodium citrate solution to make the final concentration 0.33% (w/v) (Tybor et al., 1973; Hayashi et al., 1991).

Fractionation Procedures. The general procedures for fractionation of blood into its main components are shown in Figure 1.

Noncoagulated blood was centrifuged (5000*g*, 10 °C, 15 min) to obtain the plasma and the cellular fraction. The plasma was concentrated by ultrafiltration (cellulose membrane, MW cutoff 10 000 Da, Millipore, Bedford, MA) and dehydrated by spray-drying. The cellular fraction was processed in different ways: (a) resuspension in distilled water and dehydration in a spray-dryer (Niro atomizer, type F11 D-A-P, Copenhagen, Denmark), inlet temperature = 160 °C and outlet temperature = 80 °C; (b) resuspension in distilled water (1:3 cell mass to water) following agitation for 1 h for hemolysis. Acidification to pH 2.5 was accomplished with 1 N HCl solution, followed by addition of 0.8% CMC (Hercules 12M31 PD, Wilmington, DE) solution (1:7) of hemolyzed cell suspension to CMC solution. The mixture was centrifuged (Beckman, model J-21B, Fullerton, CA) at 5000*g* and 10 °C for 20 min, and the precipitate was collected and washed with distilled water to

* Author to whom correspondence should be addressed (fax 55 19 242 4585; e-mail sgarb@fea.unicamp.br).

10.1021/jf9806255 CCC: $18.00
Published on Web 12/16/1998

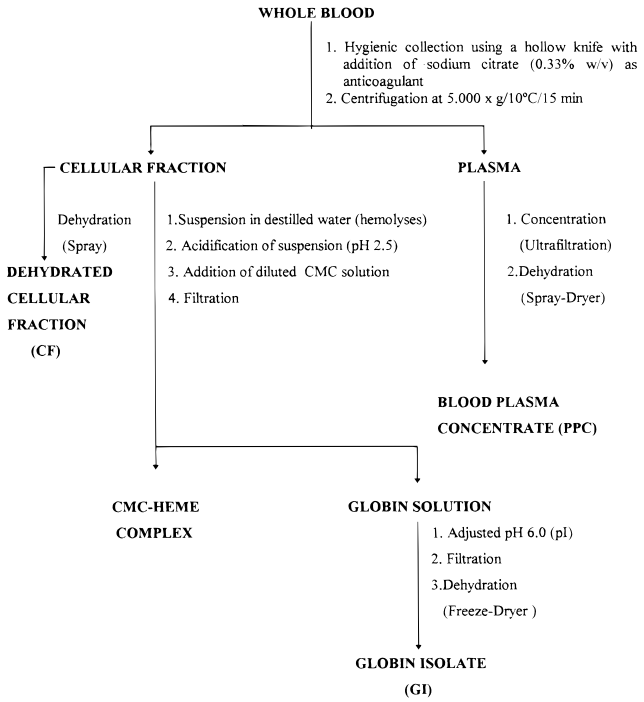

Figure 1. Flow diagram of blood component fractionation.

obtain a CMC−heme complex. The supernatant containing the discolored globin was adjusted to pH 6.0 (p*I*), and the precipitate was isolated by centrifugation and lyophilized (Dura-Top, Dura-Dry, FTS Systems, New York).

Chemical Determinations. Water, total nitrogen, and ash contents of the various blood fractions were determined according to AOAC procedures (AOAC, 1990). The conversion factor of 6.25 was used to convert total nitrogen to protein. Total lipids was determined according to the method of Bligh and Dyer (1959). Carbohydrate was calculated by difference, subtracting from 100 the sum of all other components, and also according to the method of Dubois et al. (1958). Calcium, phosphorus, potassium, sodium, magnesium, zinc, and copper were determined after the samples had been ashed at 450 °C in an oven and then dissolved in 5% nitric acid solution, according to the method of Angelucci and Mantovani (1986). For iron determination, organic matter was treated with nitric acid and hydrogen peroxide until complete digestion and then dissolved in 5% HCl solution (Slavin et al., 1975). Quantification of the mineral elements was done in a plasma emission spectrometer (ICP-2000 Baird, MA) simultaneous version at a frequency of 40 MHz and 1000 W with concentric pneumatic nebulator and a low flux flame. Sample entrance flow was 3 mL/min and argon flow 5 mL/min (IMO Industries Inc., ICP-2000). Chloride ion was determined according to the method of Angelucci and Mantovani (1986) by precipitation of chloride with silver nitrate in the presence of nitric acid. Residual silver was titrated with standard solution of ammonium thiocyanate using ammoniacal ferric sulfate as an indicator.

Amino acid composition was determined in acidic hydrolysates (6 N HCl, 110 °C, 22 h) in an automatic amino acid analyzer (Dionex DX 300, Sunnyvale, CA) equipped with a cation exchange column following derivatization with ninhydrin. Tryptophan was determined according to the calorimetric procedure of Spies (1967).

Total dietary fiber was determined only in the CMC−heme complex according to the method of Prosky et al. (1984).

Hemoglobin concentration was determined in 20 μL of blood collected from each rat's tail, which was immediately transferred to a test tube containing 5 mL of Drabkin solution (AOAC, 1984). After agitation, absorbance was measured at 540 nm in a Hitachi spectrophotometer (Hitachi U-2000, Tokyo, Japan). Absorbance reading was converted to grams per deciliter of hemoglobin, multiplying by a factor obtained by comparison with the absorption of a standard solution (10 g of Hb/dL).

Statistical Analysis. Analysis of variance (ANOVA) and *F* test for comparison of treatments were applied in all rat assays. The Tukey test was also applied for comparisons of means at 5% probability level ($p \leq 0.05$). Software utilized for data analysis included the Statistical Analysis System (SAS) and the Minitab.

Protein Nutritive Value. Protein nutritive value was determined by using the techniques of nitrogen balance and net protein ratio (NPR).

Forty-two rats of the Wistar strain were randomly distributed in groups of seven, average weight per group = 438.2 g. Composition of the diets was according to recommendations of AIN-93 (Reeves et al., 1993) except for protein concentration, which was fixed at 10% w/w of the diet. The lower protein concentration in the experimental diets was compensated by increased proportion of starch. The following sources of protein were compared: plasma protein concentrate, globin isolate, 1:1 mixture of plasma protein concentrate and globin isolate, 2:1 mixture of plasma protein concentrate and globin isolate, and casein. A protein-free diet was also prepared in which the dietary protein was replaced by starch. Data obtained from the protein-free diet group were used for correction of endogenous nitrogen excretion (nitrogen balance) and body weight change (NPR). The assay had a duration of 14 days. The first 5 days was considered an adaptation period for the rats to the diet and to the environment of the experimental room.

Nitrogen intake and fecal and urinary nitrogen excretion were determined from day 6 to day 12, for the nitrogen balance. The whole period of 14 days was considered for calculation of protein consumption, body weight change, and NPR.

Temperature in the experimental room was maintained at 21 ± 2 °C, with alternated periods of light−dark of 12 h.

Bioavailability of Iron. Bioavailability of iron in the whole blood cell fraction and in the CMC−heme complex was evaluated according to the hemoglobin depletion−repletion method utilized by Cabral and Sgarbieri (1992). Fifty weanling male rats, specific pathogen-free (SPF) of the Wistar strain, from the Experimental Animal Center of University of Campinas, were used in the experiment.

For the depletion phase, the 50 rats were maintained for 45 days in a basal diet with the composition of AIN-93 (Reeves et al., 1993) except for the absence of iron in the mineral mixture until a drop of ∼50% of their hemoglobin was measured. Twenty-eight of the 50 initial rats with hemoglobin ≤7 g/dL were used in the repletion phase. Laboratory temperature was maintained at 21 ± 2 °C with alternated periods of light−dark of 12 h.

For preparation of diets and during the experiments, all utensils were of glass or stainless steel previously decontaminated by washings with 25% (v/v) nitric acid solution by immersion for 2 h and then 1% NaEDTA solution following washings with deionized water to eliminate unaccounted sources of iron.

The following experimental diets were used in the repletion phase: (1) basal diet with 36 mg of Fe/kg of diet added in the form of ferrous sulfate ($FeSO_4 \cdot 7H_2O$, analytical grade, Merck, Darmstadt, Germany), which was the control diet; (2) diet containing cellular fraction to furnish 36 mg of elemental iron/kg of diet; (3) diet prepared with the complex CMC−heme to furnish the same 36 mg of Fe/kg of diet; (4) the basal diet was also used as a negative control.

The animals were distributed in groups by randomized blocks, according to the guidelines of Montgomery (1991). All treatments (seven rats per group) received during 14 days diet and deionized water ad libitum.

Biological utilization of iron was determined according to the hemoglobin regeneration efficiency method (HRE%) of Mahoney et al. (1974), using the expression

$$\text{HRE\%} = [[\text{mg of HbFe (final)} - \text{mg of HbFe (initial)}] \times 100]/\text{mg of Fe (ingested)}$$

Hemoglobin iron (HbFe) was calculated by considering blood as 6.7% of rat weight and 1 g of hemoglobin containing 3.35 mg of Fe (Thannoun and Mahoney, 1987); therefore

$$\text{mg of HbFe} = \text{rat wt} \times 6.7/100 \times \text{g of Hb/100 mL} \times 3.35/\text{g of Hb}$$

RESULTS AND DISCUSSION

The flow diagram for the fractionation of blood components is shown in Figure 1. For human consumption it is important that blood collection be made with maximum care to keep microorganism counts as low as possible. According to Gordon (1971) and Graham (1978) microorganism counts should be in the range $10-1 \times 10^2$/mL of blood.

Another important consideration is to avoid coagulation and hemolysis, if one wants to use the plasma or plasma protein concentrate as a food ingredient. Among the various anticoagulants, sodium citrate at 0.3−0.5% seems to be the most adequate (Halliday, 1973, 1975). Phosphoric acid salts, although very efficient, have their use restricted by legislation of many countries. On the other hand, sodium chloride tends to cause too much hemolysis (Piske, 1982).

For deconjugation of hemoglobin and decoloration of globin, the method of Autio et al. (1984) with slight modification was used as shown in Figure 1. This method was chosen because it is practical, offers a good recovery of the globin, and permits the utilization of both the globin and the complexed CMC−heme as food ingredientes.

According to Halliday (1973), the plasma fraction represents, by volume, 65−70% and the cellular mass, 30−35% of blood. On the other hand, plasma protein represents only one-third of total blood protein (Rusig, 1979).

In this investigation it was found that 71.44% of total blood protein was in the cellular fraction.

Yields of the various fractions, in grams per liter of blood, were 47.5 for plasma, 52.9 for globin 52.9, and 33.5 for the CMC−heme complex.

Sato et al. (1981), using a CMC ion exchange column to decolorize hemoglobin, obtained 70 mg of globin/g of CMC used. In this investigation 860 mg of globin was obtained per gram of CMC used in the process.

Table 1 presents the proximate composition of the four bovine blood fractions. Moisture content ranged from 2.24 to 7.24%, and it was higher in the CMC−heme complex and in plasma protein concentrate (PPC) and lower in globin isolate (GI) and cellular fraction (CF). Protein concentration ranged from 47.5 (CMC−heme fraction) to 91.4% in the GI; PPC and the CF were intermediate in protein concentration. Ash was highest in PPC (7.26%) and lowest (0.5%) in the CMC−heme complex. Intermediate values were found for GI and CF fractions. Total lipid was fairly low, ranging from 0.04 to 1.45%, the lowest value for GI and the highest for PPC. Carbohydrate was highest (43.97%) in the CMC−heme complex, followed by the cellular fraction (17.27%) and lowest (2.36%) in GI.

The high carbohydrate content of CMC−heme and CF must be represented mainly by added CMC, free sugars, and glycoprotein components of biomembranes. The protein contents of GI, PPC, and CF fractions were similar to reported values (Tybor et al., 1975; Delaney, 1975; Del Rio de Reyes, 1980).

Table 1. Proximate Composition of Bovine Blood Fractions

component[a] (%)	fraction			
	PPC	GI	CF	CMC−heme
water	6.67	2.24	2.29	7.24
protein (N × 6.25)	79.54	91.40	78.16	47.48
ash	7.26	3.96	2.05	0.50
total lipid	1.45	0.04	0.23	0.81
carbohydrate[b]	5.08	2.36	17.27	43.97

[a] Results represent mean values of three determinations. [b] Carbohydrate was determined by difference from 100% after the other components were summed.

Table 2. Mineral Composition of Bovine Blood Fractions

element[a] (mg/100 g)	fraction			
	PPC	GI	CF	CMC−heme
calcium	60.60	9.50	8.27	1.00
magnesium	10.20	0.31	4.02	0.33
phosphorus	101.40	2.42	63.80	7.48
sodium	2496.30	152.50	505.00	101.10
potassium	330.60	3.40	94.50	2.73
chloride	3668.40	1454.20	245.13	126.29
iron	ND[b]	14.20	253.00	115.00
zinc	1.50	3.56	3.80	0.56
copper	1.50	0.85	0.09	2.89

[a] Results are mean values of three determinations. [b] ND, not determined.

Regarding mineral content, the distribution of various elements in the blood fractions is shown in Table 2. Comparatively, sodium and chloride were high in PPC, GI, and CF. Iron was not detected in PPC, was in relatively low concentration (14.2 mg/100 g) in GI, and was very high (253 and 115 mg/100 g fraction) in CF and the CMC−heme complex, respectively. Copper is fairly high in PPC and GI but much higher (2.89 mg/100 g) in the CMC−heme complex. Probably the much higher copper content of the CMC−heme fraction is due to binding of copper ion to CMC.

From a nutritional point of view, iron is by far the most important mineral element in blood. According to Piske (1982), blood contains 3 times the iron concentration of bovine meat.

The amino acid composition of the blood fractions, the object of this study, appears in Table 3. The essential amino acid profile of the four fractions can be compared with the FAO/WHO/UNU (1985) reference profile. The most complete protein fraction is PPC, with no limiting essential amino acid in comparison with the reference standard. GI is low in Ile and sulfur-containing amino acids, whereas CF and the CMC−heme complex are low in Ile, S-containing amino acids, and also in tryptophan. If one calculates the amino acid chemical score, it will be >1.0 for PPC and essentially 0 for GI, CF, and CMC−heme protein, suggesting that these three fractions could not support growth if used in the diet as the only source of protein. Similar results were reported for PPC and GI by Tybor et al. (1975) and Sarwar (1987).

The results of a nitrogen balance (NB) assay, expressed by various calculated indexes of protein quality, are shown in Table 4.

NB, the expression of ingested nitrogen that was retained in the rat body, was significantly higher ($p \leq 0.05$) for casein than for PPC. GI presented the lowest nitrogen retention of all protein sources tested. The 1:1

Table 3. Amino Acid Composition of Bovine Blood Fractions

amino acid[a] (mg/100 g of protein)	fraction				
	PPC	GI	CF	CMC−heme	ref[b]
Val	6.73	8.44	8.5	10.67	3.5
Ile	3.35	0.14	UN[c]	UN	2.8
Leu	9.34	14.80	13.92	12.98	6.6
Thr	6.60	5.50	5.11	4.56	3.4
half-Cys	1.68	UN	UN	UN	
Met	0.86	0.14	0.36	2.15	
total S-AA	2.54	0.14	0.36	2.15	2.5
Tyr	4.78	2.39	2.39	2.18	
Phe	5.16	7.62	8.19	9.29	
total Ar-AA	9.94	10.16	10.58	11.47	6.3
His	4.18	7.64	6.38	3.80	1.9
Lys	7.47	10.04	10.37	10.46	5.8
Try	1.18	1.03	UN	UN	1.1
Asp	9.80	9.77	11.03	12.18	
Ser	6.67	7.00	5.47	3.26	
Glu	14.08	6.10	8.09	9.68	
Pro	4.74	3.77	3.24	2.58	
Gly	3.39	4.09	4.51	4.67	
Ala	5.00	10.16	9.47	8.35	
Arg	3.30	1.75	2.07	2.24	

[a] Results are means of three determinations. [b] FAO/WHO/UNU (1985). [c] UN, undetected.

Table 4. Nitrogen Balance Data of Bovine Blood Protein Fractions

protein source[b]	determined indices[a]			
	NR (g)	TD (%)	TBV (%)	TNPU (%)
CAS	1.47 ± 0.15[a]	94.77 ± 0.77[a]	90.37 ± 1.69[a]	85.65 ± 1.78[a]
PPC	1.13 ± 0.10[b]	94.85 ± 1.35[a]	85.46 ± 1.25[b]	81.05 ± 1.21[b]
GI	0.20 ± 0.04[e]	91.83 ± 0.80[c]	65.99 ± 4.60[d]	60.60 ± 4.17[e]
PPC/GI (1:1)	0.42 ± 0.06[d]	92.82 ± 1.95[b]	77.37 ± 3.69[c]	71.81 ± 3.53[d]
PPC/GI (2:1)	0.62 ± 0.09[c]	95.03 ± 2.11[a]	79.54 ± 5.02[c]	75.55 ± 4.46[c]

[a] Results are mean ± SDM of seven animals per treatment. Different superscript letters (columns) indicate statistically different results ($p \leq 0.05$). [b] CAS, casein; PPC, plasma protein concentrate; GI, globulin isolate; NB, nitrogen balance; TD, true protein digestibility; TBV, true protein biological value; TNPU, true net protein utilization.

and 2:1 mixtures of PPC/GI exhibited significantly lower nitrogen retention than PPC and casein but substantially higher nitrogen retention than GI. As expected, the retention improved as the proportion of PPC increased in the mixture.

Digestibility was statistically identical for casein (CAS), PPC, and the 2:1 mixture of PPC/GI but inferior for GI and for the 1:1 mixture of PPC/GI. True biological value (TBV), the expression of absorbed nitrogen that was retained in rat organism, was highest for casein and lowest for GI. Biological value for PPC was statistically inferior to that for casein but was superior to the values found for the mixtures (1:1 and 2:1) of PPC/GI ($p \leq 0.05$).

The true net protein utilization (TNPU), for all dietary protein sources tested, showed statistical differences among themselves. Casein was the best, followed by PPC, then 2:1 PPC/GI, 1:1 PPC/GI, and finally the GI fraction.

Considering that protein digestibility was high in all fractions, the differences in NPU were mainly due to high differences in nitrogen retention (NR).

According to Peng (1979) and Tackman et al. (1990), lack of balance in the amino acid profile results in amino acid imbalance in blood plasma and the central nervous system, causing repression in the ingestion of diet and growth retardation of the rats. According to this study, the following decreasing order of importance was established for amino acid imbalance: isoleucine, tryptophan, leucine and valine, histidine, phenylalanine, threonine, lysine, arginine.

Table 5. Protein Consumption, Body Weight Gain, and Net Protein Ratio of Rats Fed Different Protein Diets[a]

protein source	protein consumption (g)	body wt gain (g)	NPR
CAS	15.55 ± 1.55[a]	53.86 ± 5.75[a]	4.24 ± 0.13[a]
PPC	12.55 ± 1.16[b]	38.23 ± 4.21[b]	4.01 ± 0.14[b]
GI	4.23 ± 0.23[e]	0.19 ± 0.11[e]	2.88 ± 0.27[d]
PPC/GI (1:1)	6.27 ± 0.48[d]	11.20 ± 2.04[d]	3.71 ± 0.32[c]
PPC/GI (2:1)	7.99 ± 1.01[c]	20.98 ± 4.35[c]	4.12 ± 0.20[ab]

[a] Different superscript letters (columns) indicate statistically different results ($p \leq 0.05$). CAS, casein; PPC, plasma protein concentrate; GI, globulin isolate.

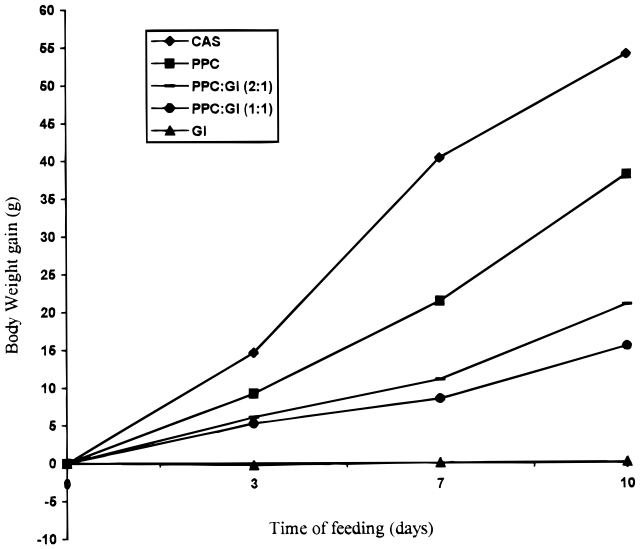

Figure 2. Growth curves for rats fed diets containing different sources of protein.

Protein consumption, body weight gain, and NPR for rats fed casein and blood protein fraction as the only source of dietary protein are presented in Table 5.

Figure 2 illustrates the growth curves for the rats fed ad libitum the different sources of protein.

By comparing the data of Table 5 and Figure 2, it becomes apparent that both essential amino acid balance and ingestion of diet are important for protein nutritive value. As it happened for NB, NPR was highest for casein and lowest for the GI diet. Comparson of the blood protein diets PPC and 2:1 PPC/GI, they were identical and superior to 1:1 PPC/GI. Comparison of Table 5 with Figure 1 reveals that growth was directly proportional to protein intake, being highest in casein followed by PPC, 2:1 PPC/GI, 1:1 PPC/GI diets. GI diet (smallest intake) did not promote growth but did maintain the initial body weight of the rats. These findings agree with reported values on blood protein nutritive value by Penteado et al. (1979) and by Tackman et al. (1990) that limitation of essential amino acids in the protein severely limits diet intake and growth.

Results of the iron bioavailability assay are reported in Tables 6 and 7.

Table 6 shows body weight gain and hemoglobin recovery for rats on the control (basal diet supplemented with ferrous sulfate) and on diets supplemented with the CF or the CMC−heme complex. A group of rats was maintained on the low-iron (basal) diet, and the results are also shown in Table 6, for comparison.

Table 6. Body Weight and Blood Hemoglobin Changes[a] of Anemic Rats during Repletion Phase (14 Days)

	body wt		hemoglobin (g/dL)	
diet	initial	final	initial	final
basal[b]	203.8 ± 26.9	241.1 ± 25.2	7.24 ± 0.63	6.62 ± 0.62[c]
control[c]	202.6 ± 16.0	253.0 ± 20.7	6.84 ± 0.64	13.33 ±0.99[a]
basal + CF	202.5 ± 25.3	236.4 ± 31.8	6.77 ± 0.50	11.08 ± 1.16[b]
basal + CMC−heme	201.0 ± 8.3	241.4 ± 12.0	6.53 ± 1.01	10.17 ± 1.19[b]

[a] Values are mean ± SDM for groups of seven rats. Different superscript letters (columns) indicate statistically different results ($p \leq 0.05$). [b] Basal diet = AIN-93 diet, withdrawing the iron from the mineral mixture. [c] Control diet = basal diet with 36 mg of Fe from $FeSO_4 \cdot 7H_2O$/kg of diet added.

Table 7. Ingested Iron, Hemoglobin Iron, and Hemoglobin Regeneration Efficiency (HRE%) of Anemic Rats Fed Three Different Diets

	diet[a]		
determination	control	basal + CF	basal + CMC−heme
ingested Fe (mg)	9.73 ± 0.51	8.60 ± 1.24	8.82 ± 0.84
initial HbFe (mg)	3.23 ± 0.26	3.23 ± 0.52	3.10 ± 0.58
final HbFe (mg)	7.93 ± 0.92[a]	6.19 ± 1.12[b]	5.78 ± 0.72[b]
HRE (%)	47.8 ± 7.0[a]	33.7 ± 8.0[b]	30.8 ± 6.4[b]
HRE (% control)[b]	100.0[a]	70.5[b]	64.3[b]

[a] Different superscript letters (rows) indicate statistically different results ($p \leq 0.05$). [b] Hemoglobin regeneration efficiency relative to ferrous sulfate (= 100%).

There were no statistical differences in the growth of rats fed the various diets for 2 weeks. Nevertheless, rats fed the control diet gained relatively more hemoglobin ($p \leq 0.05$) than the rats receiving diets supplemented with CF or CMC−heme as the source of iron. There were no statistical differences between the two experimental sources of iron.

Ingestion and retention of iron and hemoglobin regeneration efficiency (HRE%) for all three sources of dietary iron are shown in Table 7. Initial HbFe and ingested iron did not differ statistically ($p \leq 0.05$) for the various diets; however, final HbFe and HRE% were statistically higher ($p \leq 0.05$) for ferrous sulfate-containing diet (control) than for the CF- and CMC−heme-containing diets. HRE% values for the two blood iron sources, although slightly higher for CF than for the CMC−heme complex, were not statistically different and represented 65−70% of the iron sulfate bioavailability.

According to Fritz et al. (1970), a normal level of hemoglobin for rats is in the range 12−17.5 g/dL; therefore, in our experiment, the ferrous sulfate diet was the only treatment that permitted full hemoglobin regeneration in 14 days. Ferrous sulfate is considered the best source of iron for the rat and is normally used as reference in iron bioavailability studies (Fritz et al., 1970; Ranhotra et al., 1981).

The HRE of 48% encountered in this work for ferrous sulfate was similar to 50.6% reported by Cabral et al. (1992), and the heme-Fe bioavailability of 65−70% relative to ferrous sulfate was also similar to 60% reported by Zhang et al. (1989), for bovine meat iron. Jansuittivechakul et al. (1985) reported HRE values of 60 and 37%, relative to ferrous sulfate, for bovine meat and hemoglobin, respectively, in a repletion phase of 10 days. Walter et al. (1993) found that fortification of biscuits with 6% bovine CF improved the iron status of school children.

The results reported in this paper lead to the following conclusions. It is possible to obtain food grade blood fractions to be used as ingredients in human foods, mainly as sources of iron and protein. Blood PPCs are of good nutritional quality and can be obtained by ultrafiltration and dehydration by spray-drying. GI can be obtained in ∼60% yield from hemoglobin after erythrocyte hemolysis, acidic deconjugation, and precipitation of heme-Fe complexed to CMC with an iron recovery >80%. Globin can be recovered with a purity >91% after isoelectric precipitation at pH 6.0 (p*I*). Both whole CF and the CMC−heme complex showed high iron bioavailability and can be used for iron supplementation in foods when color is not a limiting factor. Globin alone does not promote rat growth, but by virtue of its high lysine and adequate tryptophan contents could be used as complement with cereal protein sources.

LITERATURE CITED

Alencar, F. A. Estudos da recuperação das proteínas do plasma bovino por complexação com fosfatos e a sua utilização em produtos cárneos. Campinas, SP, Brasil. Thesis dissertation, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, 1983.

Angelucci, E.; Mantovani, D. M. B. *Minerais em Alimentos: Manual Técnico*; ITAL/SBCTA: Campinas, 1986; 131 pp.

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Agricultural Chemists: Gaithersburg, MD, 1984.

AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Agricultural Chemists: Gaithersburg, MD, 1990.

Autio, K.; Kiesvaara, M.; Mälkki, Y.; Kanko, S. Chemical and functional properties of blood globin prepared by a new method. *J. Food Sci.* **1984**, *49*, 859−862.

Bligh, E. C.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can J. Biochem. Physiol.* **1959**, *37*, 911−917.

Cabral, M. M. V. N.; Sgarbieri, V. C. Hemoglobin repletion in anemic rats fed a new bean cultivar (*Phaseolus vulgaris* L.) as source of iron. *J. Nutr. Sci. Vitaminol.* **1992**, *38*, 481−491.

Caldironi, H. A.; Ockerman, H. W. Incorporation of blood plasma proteins into sausage. *J. Food Sci.* **1982a**, *47*, 405−408.

Caldironi, H. A.; Ockerman, H. W. Bone and plasma protein extracts in sausages. *J. Food Sci.* **1982b**, *47*, 1622−1665.

Delaney, R. A. M. The nutritive value of porcine blood plasma concentrates prepared by ultrafiltration and spray drying. *J. Sci. Food Agric.* **1975**, *26*, 303−310.

Del Rio de Reyes, M. T. E.; Constantinides, S. M.; Sgarbieri, V. C.; El Dash, A. A. Chicken blood plasma proteins: physicochemical, nutritional and functional properties. *J. Food Sci.* **1980**, *46*, 1782−1784.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebbers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1958**, *28*, 350−356.

FAO/WHO/UNU. *Energy and Protein Requirements*; Report of the Joint FAO/WHO/UNU Expert Consultation Technical Report Series 724; Geneva, 1985.

Faraji, H.; Decker, E. A.; Aaron, D. K. Supression of lipid oxidation in phosphatidylcholine lipossomes and ground pork by spray-dried porcine plasma. *J. Agric. Food Chem.* **1991**, *39*, 1288−1290.

Fritz, J. C.; Pla, C. W.; Roberts, T. Biological availability in animals of iron from common dietary sources. *J. Agric. Food Chem.* **1970**, *18*, 647−651.

Gordon, A. Animal blood as a source of proteins in food products. *Food Trade Rev.* **1971**, *41*, 29−30.

Graham, A. The collection and processing of edible blood. *CSIRO Food Res. Q.* **1978**, *38*, 16−22.

Gunstone, J. W. Using blood plasma. *Nat. Provisioner* **1980**, *23*, 20−28.

Halliday, D. A. Blood−A source of proteins. *Process Biochem.* **1973**, *8*, 15−17.

Halliday, D. A. Blood—A source of proteins. *Process Biochem*. **1975**, *10*, 11−12.

Hayashi, T.; Biagio, R.; Saito, M.; Todoriki, S.; Tajima, M. Effect of ionizing radiation on sterility and functional qualities of dehydrated blood plasma. *J. Food Sci.* **1991**, *56*, 168−171.

Howell, N. K.; Lawrie, R. A. Functional aspects of blood plasma proteins. I. Separation and characterization. *J. Food Technol.* **1983**, *18*, 747−762.

Hurrell, R.; Reddy, M. B.; Dassenko, A.; Cook, J. D.; Shepherd, D. Ferrous fumarate fortification of chocolate drink powder. *Br. J. Nutr.* **1991**, *65*, 271−283.

IMO Industries Inc. Analytical Instruments Division. *Spectrometer User's Guide*; ICP 2000 Baird, 1990.

Jansuittivechakul, O.; Mahoney, A. W.; Cornfoth, D. P.; Hendricks, D. G.; Kangsadalampai, K. Effect of rat treatment on bioavailability of meat and hemoglobin iron fed to anemic rats. *J. Food Sci.* **1985**, *50*, 407−409.

Lee, C. C.; Love, J. A.; Johnson, L. A. Sensory and physical properties of cakes with bovine plasma products substituted for egg. *Cereal Chem*. **1993**, *70*, 18−21.

Lee, Y. Z.; Johnson, L. A.; Love, J. A.; Johnson, S. Effects of processing and usage level on performance of bovine plasma as an egg white substitute in cakes. *Cereal Chem*. **1991**, *68*, 100−104.

Maddipati, K. R.; Marnett, L. J. Characterization of the major hydroperoxidase-reducing activity of human plasma. *J. Biol. Chem*. **1987**, *262*, 17398−17403.

Mahoney, A.; Van Orden, C. C.; Hendricks, D. G. Efficiency of converting food iron into hemoglobin by anemic rat. *Nutr. Metabol*. **1974**, *17*, 223−230.

Montgomery, D. C. *Design and Analysis of Experiments*, 3rd ed.; 1991; p 134.

Peng, Y. S. Studies on the severity of various amino acid imbalances in the young male rat. *J. Nutr*. **1979**, *109*, 1916−1924.

Penteado, M. V. C.; Lajolo, F. M.; Santos, N. P. Functional properties of isolated bovine blood proteins. *J. Sci. Food Agric.* **1979**, *30*, 809−815.

Piske, D. Aproveitamento de sangue de abate para alimentação humana—Uma Revisão. *Bol. Inst. Tecnol. Aliment*. **1982**, *19*, 253−308.

Prosky, L.; Asp, N. G.; Furda, I.; Devries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of total dietary fibers in foods products and total diets: interlaboratory study. *J. Assoc. Off. Anal. Chem*. **1984**, *67*, 1044−1052.

Ranhotra, G. S.; Gebroth, J. A.; Torrence, F. A.; Rock, M. A.; Winterringer, G. L. Bioavailability of iron in iron-fortified fluid milk. *J. Food Sci.* **1981**, *46*, 1342−1344.

Reeves, P. G. P. G.; Nielsen, F. H.; Fahey, G. C. *Purified Diets for Laboratory Rodents*; Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76. A rodent diet. *J. Nutr*. **1993**, *123*, 1939−1951.

Rusig, O. Studies on comminuted meat products. Philosophy Doctor Thesis, Easter School in Agricultural Science, University of Notthingham, Nottingham, 1979; 271 pp.

Sarwar, G. Digestibility of protein and bioavailability of amino acids in foods. *World Rev. Nutr. Diet.* **1987**, *54*, 26−70.

Sato, Y.; Hayakawa, S.; Hayakawa, M. Preparation of blood globin through carboxymethyl cellulose chromatography. *J. Food Technol.* **1981**, *16*, 81−91.

Slavin, S.; Peterson, G. E.; Lindahl, P. C. Determination of heavy metals in meats by absorption spectroscopy. *At. Absorpt. Newsl.* **1975**, *14*, 57−59.

Spies, J. R. Determination of tryotophan in proteins. *Anal. Chem*. **1967**, *39*, 1412−1415.

Tackman, J. M.; Tews, J. K.; Harper, A. E. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. *J. Nutr*. **1990**, *120*, 521−533.

Thannoun, A. M.; Mahoney, A. W. Effect of meat-bread mixtures on bioavailability of total dietary iron for anemic rats. *Cereal Chem*. **1987**, *64*, 399−403.

Tybor, P. T.; Dill, C. W.; Landman, W. A. Effect of decolorization and lactose incorporation on the emulsification capacity of spray-dried blood protein concentrates. *J. Food Sci.* **1973**, *38*, 4−6.

Tybor, P. T.; Dill, C. W.; Landman, W. A. Functional properties of protein isolate from bovine blood by a continuous pilot process. *J. Food Sci.* **1975**, *40*, 155−159.

Viteri, E.; Alvarez, E.; Batres, R.; Torun, B.; Pineda, O.; Mejia, L. A.; Sylvi, J. Fortification of sugar with iron sodium ethylenediaminotetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations. *Am. J. Clin. Nutr.* **1995**, *61*, 1152−1163.

Walter, T.; Hertrampf, E.; Pizarro, F.; Olivares, M.; Llaguno, S.; Letelier, A.; Vega, V.; Stekel, A. Effect of bovine-hemoglobin fortified cookies on iron status of schoolchildren: a nationwide program in Chile. *Am. J. Clin. Nutr.* **1993**, *57*, 190−194.

Wismer-Pedersen, J. Utilization of animal blood meat products. *Food Techol.* **1979**, *33*, 76−80.

Zhang, D.; Hendricks, D. G.; Mahoney, A. W. Bioavailability of total iron from meat, spinach (*Spinacea oleracea* L.) and meat-spinach mixtures by anaemic and non-anaemic rats. *Br. J. Nutr.* **1989**, *61*, 331−343.

Received for review June 9, 1998. Revised manuscript received September 18, 1998. Accepted September 22, 1998. We acknowledge FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for financial support for this research.

JF9806255