TRABAJOS DE INVESTIGACION

EFFECTS OF TEMPERATURE OF PROCESSING AND OF ISOLEUCINE FORTIFICATION ON THE NUTRITIVE VALUE OF BLOOD MEAL*

*Ana María L. Coser***, *L. Jokl and Enio C. Vieira*

Departamento de Bioquímica, Instituto de Ciencias Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG BRASIL

SUMMARY

A processing for cattle blood consisting of protein coagulation and drying at mild temperatures is described. The protein efficiency ratio and the food efficiency of this blood meal supplemented with isoleucine were similar to those obtained for casein. Fortification of commercial blood meal with isoleucine did not improve much its quality.

INTRODUCTION

Blood meal represents a protein source that has not been properly utilized in Brazil. In the slaughter houses, coagulation and drying of the blood proteins is performed at high temperatures for more than 9 h. The quality of the resulting blood meal, evidently, is lowered by this processing.

Experiments on feeding monogastric animals with blood meal showed that it is deficient in isoleucine (1-3). The deficiency symptoms did not appear when blood meal was supplemented with casein (4). The quality of blood meal was dependent on the temperature of processing (5,6).

Recibido el 18-6-76.

* This work was supported by grants from BNDE/FUNTEC-166 and from The From the Ford Foundation - DAP - 73 - 0820. This paper was extracted from the M.S. thesis of A.M.L. Coser.

** Present Address: Departamento de Morfología,, Instituto de Ciencias Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 2486 - 30000 Belo Horizonte, MG Brasil.

In this paper, a milder processing for blood is described. The nutritive value of the product and the effect of enrichment with isoleucine were evaluated.

MATERIAL AND METHODS

Commercial blood meal

Commercial blood meal was kindly supplied by Frigoríficos de Minas Geraia S. A., (FRIMISA). This is obtained by cooking the blood at over 100°C, 0.5 kg/cm^2 for about 9 h.

Experimental blood meal

Total bovine blood was collected at the time of bleeding and immediately fibrin was removed by vigorous shaking with a wooden stick. At the laboratory fibrin was mixed with the uncoagulated blood. Everything was cooked in an open pan until precipitation of the proteins occurred. This step usually took about 30 min. The liquid was removed and the proteins dried at 60°C for 5 h.

Chemical analysis

Moisture, total nitrogen, protein nitrogen, crude protein, real protein, ether extract, ash, and non-nitrogenous extract were determined according to methods described by AOAC (7). The methods os Spackman, Stein & Moore (8) and of Miller (9) were used for amino acid analyisis and tryptophan determination, respectively. Available lysine was determined by the method of Hall, Trinder & Givens (10).

Biological evaluation

Albino rats from the laboratory colony were used. The animals, 24-25 days old, were fed commercial ration for two days. They were then divided in five homogeneous groups of six rats (three males and three females) and maintained in individual cages. The initial average weight varied between 49.2 and 52.5 g. All animals received food and water ad

Table 1

Composition of diets based on commercial and experimental blood meals and on casein

Ingredients	Diets (g/kg)				
	A	B	C	D	E
Saline mixture*	50.00	50.00	50.00	50.00	50.00
Vitamin mixture*	10.00	10.00	10.00	10.00	10.00
Soybean oil	70.00	70.00	70.00	70.00	70.00
Cod liver oil	10.00	10.00	10.00	10.00	10.00
Cellulose	55.00	55.00	55.00	55.00	60.00
Starch**	671.70	671.70	656.70	656.70	670.20
Commercial blood meal	133.30	-	133.30	-	-
Experimental blood meal	-	133.30	-	133.30	-
L-Isoleucine	-	-	15.00	15.00	-
Casein***	-	-	-	-	119.10
Protein level (%)	10.62	10.69	10.76	10.77	10.75

* AOAC (7)
** Maizena, kindly supplied by Refinaçoes de Milho, Brasil S.A.
*** ANRC Reference protein (Sheffield Chemical, Norwich, N.Y.).

libitum for 28 days. Food consumption and weight gain were measured weekly.

Table 1 shows the composition of the diets which were isocaloric and isoproteic. The protein source for diets A and B were commercial and experimental blood meal, respectively. Diets C and D contained these meals supplemented with isoleucine. Casein was the protein source of diet E.

Protein efficiency ratio(PER) and food efficiency (FE) were calculated as follows:

$$PER = \frac{\text{weight gain (g)}}{\text{ingested protein (g)}}$$

$$FE = \frac{\text{weight gain (g)}}{\text{ingested food (g)}}$$

Apparent digestibility (D_{app}) was measured by adding ferric oxide (200 mg/100 g of diet), as a tracer, in the last week of the experiment. The feces were collcted daily, pooled for each animal at the end of the week, dried at 105°C for 24 h, and powdered. Total nitrogen was determined and the apparent digestibility was calculated with the equation:

$$D_{app} = \frac{\text{absorbed nitrogen (g)}}{\text{ingested nitrogen (g)}} \qquad (11)$$

Statistical analysis

Since the available rats showed variation in initial weight a covariance analysis for total food ingestion and weight gain was performed with the objetive of eliminating the effect of such difference on the results. The following mathematical model was used (12):

$$Y_{ij}{}_{adj} = Y_{ij} - b\ (X_{ij} - X)$$

and

$$Z_{ij}_{adj} = Z_{ij} - b\ (X_{ij} - X)$$

where

Y_{ij}_{adj} = adjusted weight gain for rat i on treatment j:
Y_{ij} = weekly weight gain for rat i on treatment j;
X_{ij} = initial weight for rat i on treatment j;
X = initial weight general mean;
b = positive effect of each gram of weight above the general mean;
Z_{ij}_{adj} = adjusted food ingestion for rat i on treatment j;
Z_{ij} = weekly ingestion for rat i on treatment j.

The analysis of variance was made using the data corrected by the covariance analysis. The comparison between the adjusted means was made through the minimal significant difference (m.s.d.) at 5% significance level.

$$m.s.d._{adj} = t_{5\%} \cdot \frac{QME_{adj}}{n}$$

where

QME = mean square of adjusted error;
n = number of observations

RESULTS

Production of blood meal

The final product of several batches was brick-red and tasteless and represented between 21.64 and 24.61% of the blood.

Chemical composition

Table 2 shows the chemical composition of commercial and experimental blood meal. Table 3 shows the amino acid composition of both products, including the level of available

Table 2

Chemical composition of commercial and experimental blood meal

Blood meal	Moisture (%)	% of dry matter					
		Total nitrogen	Protein nitrogen	Crude Protein	Real protein	Ether extract	Ash
Commercial	3.8	14.14	12.47	88.36	77.96	0.291	2.320
Experimental	5.7	14.20	12.72	88.80	79.53	0.477	3.107

Table 3

Amino acid composition of commercial and experimental blood meal
(g/100g)

Aminoacids	Commercial blood meal	Experimental blood meal
Lysine	7.32	7.29
Available lysine	3.46	5.42
Histidine	4.66	4.72
Arginine	3.63	3.42
Aspartic acid	7.74	7.43
Threonine	3.20	3.12
Serine	4.10	4.23
Glutamic acid	7.76	7.76
Proline	3.06	2.88
Glycine	2.54	2.88
Alanine	5.16	5.56
Valine	5.97	5.88
Methionine	0.78	0.82
Cystine	0.48	0.84
Isoleucine	0.47	0.44
Leucine	10.24	10.34
Tyrosine	1.76	2.32
Phenylalanine	6.12	5.58
Tryptophan	1.56	1.48

lysine. Attention is called to the difference in available lysine which corresponds to 47.2 and 74.3% of total lysine in commercial and experimental blood meals, respectively. Cisteine level was also higher in experimental blood meal.

Biological assay

Figures 1 and 2 show the average food consumption and

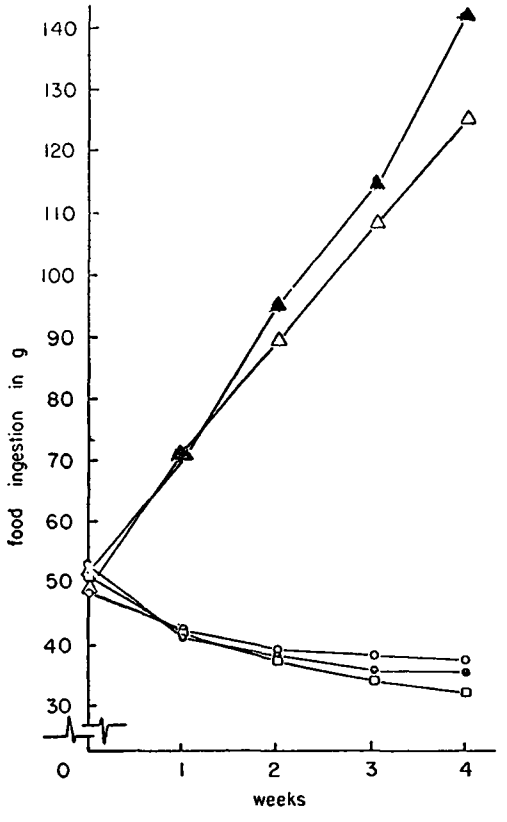

Fig. 1 - Average food ingestion of rats during four weeks on diets of different protein sources. Casein (▲—▲); experimental blood meal + isoleucine (△—△); commercial blood meal + isoleucine (○—○); experimental blood meal (●—●); commercial blood meal (□—□).

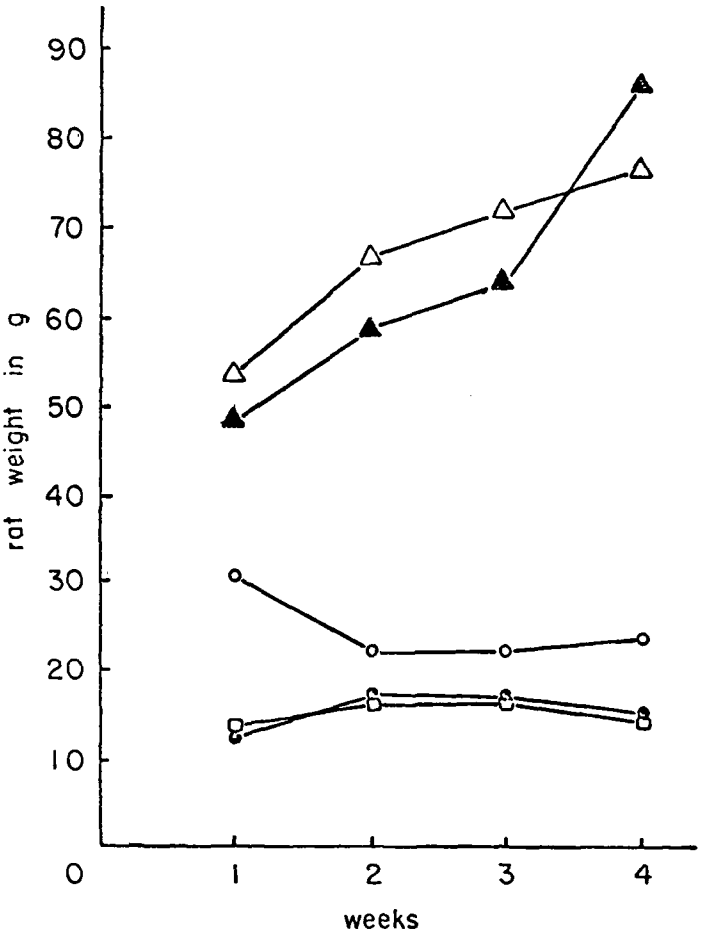

Fig. 2 - Average growth of rats during four weeks on diets of different protein sources. Casein (▲—▲); experimental blood meal + isoleucine (△—△); commercial blood meal + isoleucine (o—o); experimental blood meal (●—●); commercial blood meal (□—□).

weight gain, respectively, for the five groups of animals. The food consumption and weight gain were very poor in both diets based on commercial blood meal and on diet based on unsupplemented experimental blood meal. In the diet containing experimental blood meal fortified with isoleucine, however, the food consumption and weight gain were similar to the diet based on casein. In these two diets there was a correlation between food consumption and weight gain.

Table 4 shows weight gain, food consumption, food ef-

Table 4

Weight gain, food ingestion, food efficiency, protein efficiency ratio (PER) and apparent digestibility (Dapp) for diets whose protein source was commercial and experimental blood meal and casein. The figures represent the average of six rats (three males and three females).

Diets	Initial weight g±S.D.	Weight gain g±S.D.	Food Ingestion g±S.D.	Food Efficiency ±S.D.	PER ±S.D.	Dapp* ±S.D.
A	51.2 ± 4.54	-19.01 ± 4.34	60.31 ± 12.0	-0.308 ± 0.06	-3.012 ± 0.254	73.6 ± 0.36
B	52.5 ± 4.89	-18.76 ± 5.09	59.78 ± 10.1	-0.314 ± 0.082	-2.945 ± 0.581	91.2 ± 1.70
C	49.2 ± 4.02	-11.11 ± 5.90	100.01 ± 14.2	-0.113 ± 0.032	-1.092 ± 0.308	82.4 ± 3.66
D	49.6 ± 5.90	76.35 ± 22.68	268.99 ± 48.1	0.284 ± 0.036	2.639 ± 0.143	90.8 ± 1.89
E	51.5 ± 4.76	86.34 ± 29.07	255.16 ± 47.8	0.331 ± 0.056	3.080 ± 0.526	85.8 ± 4.09

Diet A - commercial blood meal as protein source
Diet B - experimental blood meal as protein source
Diet C - commercial blood meal + isoleucine as protein source
Diet D - experimental blood meal + isoleucine as protein source
Diet E - standard casein as protein source

* Non adjusted data

ficiency, protein efficiency ratio, and apparent digestibility for the five diets. There is no significant difference ($p>0.05$) between diets based on casein and isoleucine supplemented experimental blood meal for all variables measured. There is difference between these two and the other three diets. Nonsupplemented experimental and commercial blood meals showed no statistically significant difference. Concerning digestibility, there are signficant differences between diets based on supplemented and non-supplemented blood meals and on supplemented commercial and experimental blood meals.

DISCUSSION

The results show that processing blood under milder conditions improves its nutritive value when supplemented with isoleucine. Blood meal is deficient in isoleucine, since hemoglobin, its main protein, does not contain this amino acid.

Processing affects the protein quality of foods, specially when it is done by heat (13). There are evidences for methionine (14,15), lysine (16), and leucine and tryptophan (17) destruction or blocking in proteins, making them nutritionally unavailable.

A noticeable difference between commercial and experimental blood meal was the level of available lysine which represented 47.2 and 74.3%, respectively, of the total lysine content. Lysine, probably, is the most sensitive amino acid during processing. Dvorak et al. (18) showed that a three month storage of blood meal reduced the level of available lysine to 24% of the original lysine present.

The digestibility of blood meal depends on the temperature of processing (19). This would explain why the experimental blood meal had a better digestibility than the commercial one.

Experimental and commercial blood meal, without supplementation, showed negative values for protein efficiency ratio and food efficiency, with no significant difference between them. Isoleucine supplemententation improved the nutritive value of both products. However the results were more dramatic for the experimental blood meal. In the latter case, the nutritional value was nearly the same of casein.

Among the reasons for the better quality of fortified experimental blood meal when compared with fortified commercial blood meal, one should point out: (i) the low digestibility of commercial blood meal; (ii) the marked difference in available lysine.

The results showed that a milder processing in the preparation of blood meal improves, markedly its nutritive value when supplemented with isoleucine. The temperature of processing used does not exclude the possibility of contamination by microorganisms, since spores resist boiling for 30 min. This work shows, however, that a milder temperature may be found so that the resulting product will be more nutritious and at the same time bacteriologically safe.

Research on that line are under way in this laboratory.

RESUMO

Descreve-se um processamento para farinha de sangue bovina que consiste em coagulação e secagem das proteínas a temperaturas mais brandas. Os valores do coeficiente de utilização proteica e da eficiência alimentar da farinha experimental suplementada com isoleucina foram semelhantes aos obtidos com caseína. Fortificação da farinha de sangue comercial com isoleucina não melhorou muito sua qualidade.

BIOGRAPHY

1. Grau, C. R. & H. J. Almquist. Beef blood proteins in chick. **Poultry Sci., 23:** 486-490, 1944.
2. Poppe, S., H. Meier & H. Kristen. Uber den biologischen Wert einiger Futterproteine bei Küken. 3. Tierische. Futterstoffe. **Arch. Geflügelzucht. Kleintierk, 17:** 93-104, 1968.
3. Becker, D. F., I. D. Smith, S. W. Terrill, A. H. Jensen & H. W. Norton. Isoleucine need of swine at two stages of development. **J. Animal Sci., 22:** 1093-96, 1963.
4. Baars-Diehl, S. Fütterrungsversuche an wachsenden Ratten mit Tierkörper-und Blutmenhlen. **Deutsch. Tierärztl. Wochenschr., 75:** 279-281, 1968.
5. Miller, W. C. Low temperature blood meals as a source of essential amino acids for nutritive purposes. **Vet. Rev., 16:** 311-316, 1936 **(Nutr. Abstr. Rev., 6:** 1183, 1936-37).
6. Moegle, E. Herstellung von Blutmehl (in Württenberg). **Z. Fleisch. Milchhyg., 46:** 400-402, 1936.
7. Association of Official Agricultural Chemists. Offcial Methods of Analysis of the OAAC, 11th ed., Washington, D. C. The Association, 1970, 1015 pp.
8. Spackman, O. H., W. H. Stein & S. Moore. Automatic recording

apparatus for use in the chromatography of amino acids. **Anal. Chem., 30:** 1190-206, 1958.

9. Miller, E. L. Determination of the tryptophan content of feeding stuffs with particular reference to cereals. **J. Sci. Food Agric., 18:** 281-286, 1967.
10. Hall, R. J., N. Trinder & D. I. Givens. Observations on the use of 2, 4, 6 - trinitrobenzenesulphonic acid for the determination of available lysine in animal protein concentrate. **Analyst, 98:** 673-686, 1973.
11. National Academy of Sciences - National Research Council. Evaluation of Protein Quality, NAS-NRC, Publ. 1100, Washington, D. C., 1963, 74 pp.
12. Snedecor, G. W. & W. G. Cochran. **Statistical Methods.** Iowa State University Press, 6th ed., 593 pp.
13. Evans, R. & H. A. Butts. Studies on the heat inactivation of lysine in soybean oil meal. **J. Biol. Chem.; 175:** 15-20, 1948.
14. Horn, M. J., H. Lichtenstein & M. Womack. Availability of amino acids. A. Methionine-fructose compound and its availability to micoorganisms and rats. **J. Agric. Food Chem., 16:** 741-745, 1968.
15. Lindsay, R. C. & V. K. Lau. Reaction of glucose and methionine. **J. Food Sci., 37:** 787-788, 1972.
16. Carpenter, K. J. & B. E. March. The availability of lysine in groundnut biscuits used in the treatment of kwashiorkor. **Brit. J. Nutr., 15:** 403-409, 1961.
17. Sgarbieri, V. C., J. Amaya, M. Tanaka & C. O. Chichester. Nutritional consequences of the Maillard reaction. Amino acid availability from fructose-leucine and fructose-tryptophan in the rat. **J. Nutr., 103:** 657-663, 1973.
18. Dvorak, A., I. Vognarová, A. Havelková & Z. Hold. Availability of lysine from meat bone and meat meal. **Zivoc. Vyr., 10:** 341-346, 1965 **(Nutr. Abstr. Rev., 35:** 5454, 1965).
19. Honcamp, F., C. Eichler, M. Sachsse & H. Ch. Schulz. Untersuchungen über die Verdaulichkeit vom Blutmehl, Fleischmehl, und Fleischmehlabfälle, Tierkörpermehl sowie Walmehl und Waltierabfällen. **Z. Zücht. B. Tierzücht. Züchtungsbiol., 25:** 415-441, 1932.

ACKNOWLEDGEMENTS - The technical help of Maria E. Morais and the asistance in the statistics of Dr. Ivan B. Sampaio, are greatly acknowledged.