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Blood Catalase as an index of dietary protein quality

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Introduction

Biochemical methods based on blood analyses have been introduced to measure the protein quality in the recent years. Proteins as structural elements or as biocatalyst participate in every biological process and may predict its protein quality. Whitaker and Patrick (1971) found a correlation between plasma amino acid index and the biological value of the dietary protein. The urea content of the blood was inversely related to the protein quality (Münchow and Bergner 1968, Eggum 1971). A negative correlation between the biological value and the activities of serum arginase, ornithine carbamyl transferase and glutamic pyruvic transaminase was observed (Bergner 1977). Among the activity of metalloenzymes, the cytochrome oxidase

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was found to be positively correlated with the quantity of protein and the catalase also with the quality (EAA-index) however, the ceruloplasmin and the alkaline phosphatase showed no significant response (KIRCHGESSNER et al. 1977).

This paper deals with the influence of the dietary protein quality as measured in N-balance experiments with rats on the activity of blood catalase.

Materials and Methods

Protein sources

Twenty four commercial and laboratory prepared samples consisting of plant proteins, milk proteins, egg protein, amino acid mixture, yeast and various food products were selected for this investigation.

Pakistani national diet, four food dishes and wheat, maize, rice, barley, millet and sorghum breads were prepared according to Pakistani traditional cooking procedures and then freeze-dried and ground. All the samples were analysed for dry matter and N content and incorporated into N-free mixture (table 1).

Biological trials

The experimental procedure has been described by EGGUM (1973). Groups of five Wistar male rats weighing approximately 75 g were used. The preliminary period lasted for 4 days and the balance period for 5 days. The rats were weighed at the beginning of the experiments and divided into groups of five such that the average weights of the groups differed by no more than ± 0.5 g. Weighing was repeated at the end of preliminary and balance periods, access to feed and water was restricted 3 h before weighing. Each animal received 150 mg N and 10 g dry matter daily throughout the preliminary and balance periods. The N contents of the diet was adjusted by using a N-free mixture (Table 1) at the expense of autoclaved potato starch to be measured in N-balance experiment with rats.

Table 1

Composition (parts by weight) of the nitrogen free mixture

Potato starch (autoclaved)	767
Sucrose	90
Cellulose powder	52
Soybean oil	52
Mineral mixture [a]	40
Vitamin mixture [b] (mixed with autoclaved Potato starch)	20

a. To provide per kg diet: $CaCO_3$, 2.74 g; Calcium citrate, $Ca_3C_{12}H_{10}O_{14}$, 4 H_2O, 12.33 g; $CaHPO_4 2H_2O$, 4.51 g; K_2HPO_4, 8.75 g; KCl, 4.99 g; NaCl, 3.08 g; $MgSO_4$, 1.53 g; $MgCO_3$, 1.41 g; Ammonium ferric citrate (20.5 — 22.5 % Fe), 0.61 g; $MnSO_4H_2O$, 8.0 mg; $CuSO_4$, $5H_2O$, 3.1 mg; KJ, 1.6 mg; NaF, 20.3 mg; $AlNH_4(SO_4)_2$, $12H_2O$, 3.6 mg.

b. To provide per kg diet: Retinol equivalent, 1.2 mg; Cholecalciferol, 7.4 µg; Thiamin, 0.8 mg; Riboflavin, 2 mg; Nicotinamide, 8 mg; Pantothenic acid, 2 mg; α-tocopherol, 0,4 mg; Pyridoxine, 0.2 mg.

Catalase Assay

The catalase (EC 1.11.1.6.) activity was determined according to the method described by GRASSMANN and KIRCHGESSNER (1973). 20 µl of blood immediately taken from the cervical vein was mixed with 10 ml of ice-cold Phosphate buffer (1/150 M, PH 7.0). 0.5 ml of this solution was transferred to a cuvette containing 2.5 ml of substrate (buffer/H_2O_2 solution), mixed and time in seconds for a decrease in optical density from 0.450 to 0.400 at 240 nm was recorded. The activity was expressed as unit/µl of blood.

Results and Discussion

The result of the experiment of net protein utilisation (NPU) and blood catalase activity are shown in table 2.

Table 2

Blood catalase activity in relation to net protein utilisation of proteins for rats

Protein source	Net protein utilization		Blood catalase activity	
	(%)	(s)	(u/µl of blood)	(s)
Pakistani National diet	66	1.8	0.62	0.01
Wheat bread + meat and potato	65	0.8	0.61	0.03
Wheat bread + chick peas	60	1.4	0.60	0.04
Wheat bread + spinach and potato	55	1.8	0.59	0.03
Wheat bread + green peas and potato	61	1.5	0.58	0.03
Casein + methionine	87	1.6	0.84	0.05
Wheat flour	51	1.7	0.55	0.08
Wheat bread	52	0.3	0.54	0.03
Maize flour	58	1.7	0.56	0.05
Maize bread	53	0.5	0.55	0.03
Rice flour	71	1.2	0.69	0.04
Rice bread	72	2.4	0.71	0.03
Barley flour	62	1.5	0.59	0.04
Barley bread	58	1.7	0.58	0.05
Barley fraction	62	2.1	0.53	0.02
Millet flour	56	1.6	0.57	0.02
Millet bread	57	1.1	0.60	0.05
Sorghum bread	50	0.5	0.52	0.01
Egg protein	92	1.4	0.89	0.04
Amino acid mixture	82	1.9	0.81	0.06
Hydrolysed whey protein (conc)	76	1.5	0.75	0.04
Yeast	48	1.9	0.50	0.02
Cottonseed meal	51	1.1	0.54	0.02
Soybean meal	60	1.3	0.56	0.03

It is clear that there is a direct relation between blood catalase activity and the NPU of the protein of the diet. The relationship is given in the following regression equation $\text{NPU} = -4.31 + 108.1 \times$ blood catalase (Unit/µl of blood).

The following values were obtained: $r = 0.96$,

$$S = 3.12, Sb = 6.14$$

where S is the deviation from regression and Sb is the deviation of the regression

coefficient. The regression coefficient differs significantly from zero, as a t-test showed $P < 0.001$. The results are illustrated in figure 1.

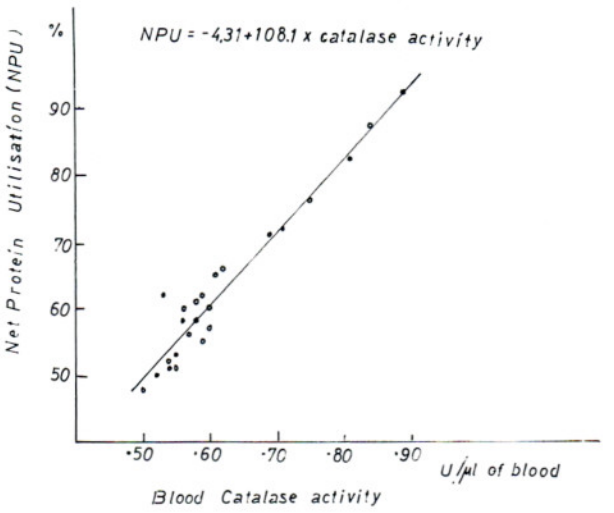

Fig. 1. Relationship of blood catalase activity and net Protein Utilisation

It is evident from the results that blood catalase activity may be regarded as a suitably rapid technique in the evaluation of dietary protein quality. The significant relationship between the NPU of the diet and the blood catalase activity thus confirms the findings of KIRCHGESSNER et al. (1977).

There is insufficient evidence so far to indicate the biological function of catalase, however, there is some indications that catalase, a haematin-protein complex is the principal enzyme destroying hydrogen peroxide in red blood cells and ist necessary for optimal health (METZLER 1977). Positive correlations exist between catalase activity and haemoglobin concentration of human blood (MILLER 1958) and between blood haemoglobin, erythrocyte values and quantity and quality of the dietary protein (KIRCHGESSNER et al. 1977). It seems possible that the blood catalase activity in the present experiment hat responded like haemoglobin with regard to the quality of dietary protein.

Summary

The influence of dietary protein quality on the activity of blood catalase was studied. Twenty four commercial and laboratory prepared samples consisting of animal protein, plant proteins and various food products were selected. Protein quality was measured in N-balance experiments with rats and compared with blood catalase activity. The results showed a highly significant positive correlation ($r = 0.96$) between net protein utilisation of the diet and the blood catalase activity. It is concluded that the estimation of blood catalase activity may be regarded as a rapid and efficient method for evaluating protein quality.

Zusammenfassung

Blutkatalase als ein Kriterium der Proteinqualität von Diäten

Es wurde der Einfluß der Proteinqualität von Diäten auf die Kataloseaktivität im Blut untersucht. Vierundzwanzig Handelspräparate und im Laboratorium hergestellte Proben aus animalischem Protein, pflanzlichem Protein und verschiedenen Nahrungs-

mitteln wurden dafür ausgewählt. Die Proteinqualität wurde in N-Bilanz-Versuchen mit Ratten ermittelt und mit der Katalaseaktivität des Blutes verglichen. Die Resultate waren hochsignifikant und ergaben eine positive Korrelation (r = 0,96) zwischen der Nettoproteinverwertung der Diäten und der Katalaseaktivität des Blutes. Die Bestimmung der Katalaseaktivität des Blutes wird als eine schnelle und zuverlässige Methode zur Bewertung der Proteinqualität betrachtet.

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