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PREDICTING DIETARY PROTEIN QUALITY BY AN *IN VITRO* METHOD

M. Akmal Khan*, Kausar Almas and H. M. Chaudhry

A rapid *in vitro* method using a multienzyme system, consisting of trypsin, chymotrypsin and peptidase for the estimation of protein digestibility was tested and the values were compared with *in vivo* true protein digestibility (TD) measured in N-balance experiments with rats. Twenty protein sources and food products were selected for the study. The results indicated that the pH at 10 minutes following the addition of proteolytic enzymes had a significant positive correlation ($r = 0.96$) with true protein digestibility in rats. The regression equation was $TD = 263.25 - 26.05 \times pH$ at 10 min. It is suggested that *in vitro* evaluation may be regarded as a good index for predicting the true digestibility of a variety of proteins and may prove a useful tool for those concerned with quality control.

INTRODUCTION

The nutritional quality of a dietary protein is determined by its amino acid composition, content and digestibility. Recently, the equations for predicting the protein quality of meat and meat products, wheat and legumes have been developed by using bioassay techniques (Almseyer *et al.*, 1974; Khan and Eggum, 1979; Khan *et al.*, 1979), which is an expensive and time consuming procedure. There is thus an urgent need for a faster *in vitro* method to generate protein quality data which has a high correlation with biological data.

Several workers have attempted to relate the quality of food protein with the pattern of amino acids released by *in vitro* enzyme digestion. The pepsin digest residue amino acid index (PDR) was reported to correlate with the net protein utilization (NPU) value of the variety of proteins (Sheffner, 1967). A pepsin pancreatin dialysis index (PPDD) has been reported to correlate with the biological value (BV) for a series of proteins (Mauron, 1970). More recently a multienzyme *in vitro* method has been developed to estimate apparent protein digestibility within 1 hour period (Hsu *et al.*, 1977).

*Department of Nutrition, University of Agriculture, Faisalabad, Pakistan.

The purpose of this research was to find the relationship between true protein digestibility measured in N-balance experiments with rats and *in vitro* values obtained by using a multienzyme system.

MATERIALS AND METHODS

Protein sources and foods tested: Twenty commercial and laboratory prepared samples consisting of milk proteins, meat proteins, plant proteins and various food products (Farex and wheat porridge) were selected for this study. Five food dishes and wheat and barley breads were prepared according to traditional cooking methods and then freeze-dried and ground. All the samples were analysed for dry matter and nitrogen content and incorporated into N-free mixture (Table 1).

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 gm; calcium citrate, $Ca_3C_{12}H_{10}O_{14}$, $4H_2O$, 12.33 gm; $CaHPO_42H_2O$, 4.51 gm; K_2HPO_4, 8.75 gm; KCl, 4.99 gm; NaCl, 3.08 gm; $MgSO_4$, 1.53 gm; $MgCO_3$, 1.41 gm; ammonium ferric citrate (20.5–22.5% Fe), 0.61 gm; $MnSO_4$ H_2O, 8.0 mg; $CuSO_4$, $5H_2O$, 3.1 mg; KI, 1.6mg; 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, 8.8 mg; riboflavin, 2 mg; nicotinamide, 8 mg; pantothenic acid, 2 mg; tocopherol, 0.4 mg; pyridoxine, 0.2 mg.

In vivo true digestibility determination : Rat feeding trials were conducted to determine the *in vivo* protein digestibilities of various food proteins. The experimental procedure has been described by Khan and Eggum (1978).

Groups of five Wistar male rats weighing approximately 75 gm 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 each so that the average weights of the groups differed by no more than ± 0.5 gm. Weighing was repeated at the end of preliminary and balance periods; access to feed and water was restricted 3 hours before weighing. Each animal received 150 mg N and 10 gm dry matter daily throughout the preliminary and balance periods. The N content 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. True protein digestibility was determined as follows :

$$\text{True digestibility (TD)} = \frac{\text{dietary N–(faecal N–metabolic N)}}{\text{dietary N}} \times 100$$

In vitro protein digestibility method : The *in vitro* digestibility was determined by a multienzyme system according to the method of Hsu *et al.* (1977). The enzymes used for *in vitro* digestion were porcine pancreatic trypsin (Type IX); bovine pancreatic chymotrypsin (Type II) and porcine intestinal peptidase (Grade III). All samples were ground to a fine powder. Glass distilled water was used in preparing solutions and for washing all apparatus.

Fifty milliliters of aqueous protein suspensions (6.25mg/ml) in distilled water were adjusted to pH 8.0 with 0.1N HCl and/or NaOH, while stirring in a 37°C water bath. The multienzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase/ml) was maintained in an ice bath and adjusted to pH 8.0 with 0.1N HCl and/or NaOH. Five milliliters of the multienzyme solution were then added to the protein suspension which was being stirred at 37°C. A rapid decline in pH was observed. This was caused by the freeing of carboxyl groups from the protein chain of amino acids by the proteolytic enzymes. The pH drop was recorded over a 10 minutes period using an automatic recording pH meter.

RESULTS AND DISCUSSION

Table 2 illustrates the pH values of various food samples after 10 minutes incubation with proteolytic enzymes and *in vivo* true digestibilities in rats.

Table 2. pH after 10 min. incubation and *in vivo* true digestibility of some common foods

Protein source	pH after 10 min. incubation	True digestibility (%)
Casein + methionine	6.30	98
Casein + barley	6.75	93
Casein + sorghum	7.15	77
Wheat flour	6.40	94
Rice + lentils	6.50	91
Rice + sorghum	7.20	78
Wheat bread + chick peas	6.60	92
Soybean meal	6.60	91
Barley flour	6.70	88
Barley bread	6.80	85
Potato (boiled)	7.00	80
Cauliflower	7.15	78
Rapeseed meal	7.00	85
Meat and bone meal	7.10	75
Rye flour	7.10	77
Green beans	7.35	68
Black gram (cooked)	6.70	90
Farex (baby food)	6.60	94
Rice pudding	6.40	96
Wheat porridge	6.40	97

The relationship is given in the following regression equation :

$$TD = 263.25 - 26.05 \times \text{pH at 10 min.}$$

The following values were obtained : $r = 0.96$, $s = 2.47$ and $sb = 1.78$; where s is the deviation from regression and sb is the deviation of the regression coefficient. The regression coefficient differed significantly from zero, shown by t-test ($P < 0.01$). The linear regression curve describing the equation using the pH values at 10 minutes is shown in Fig. I.

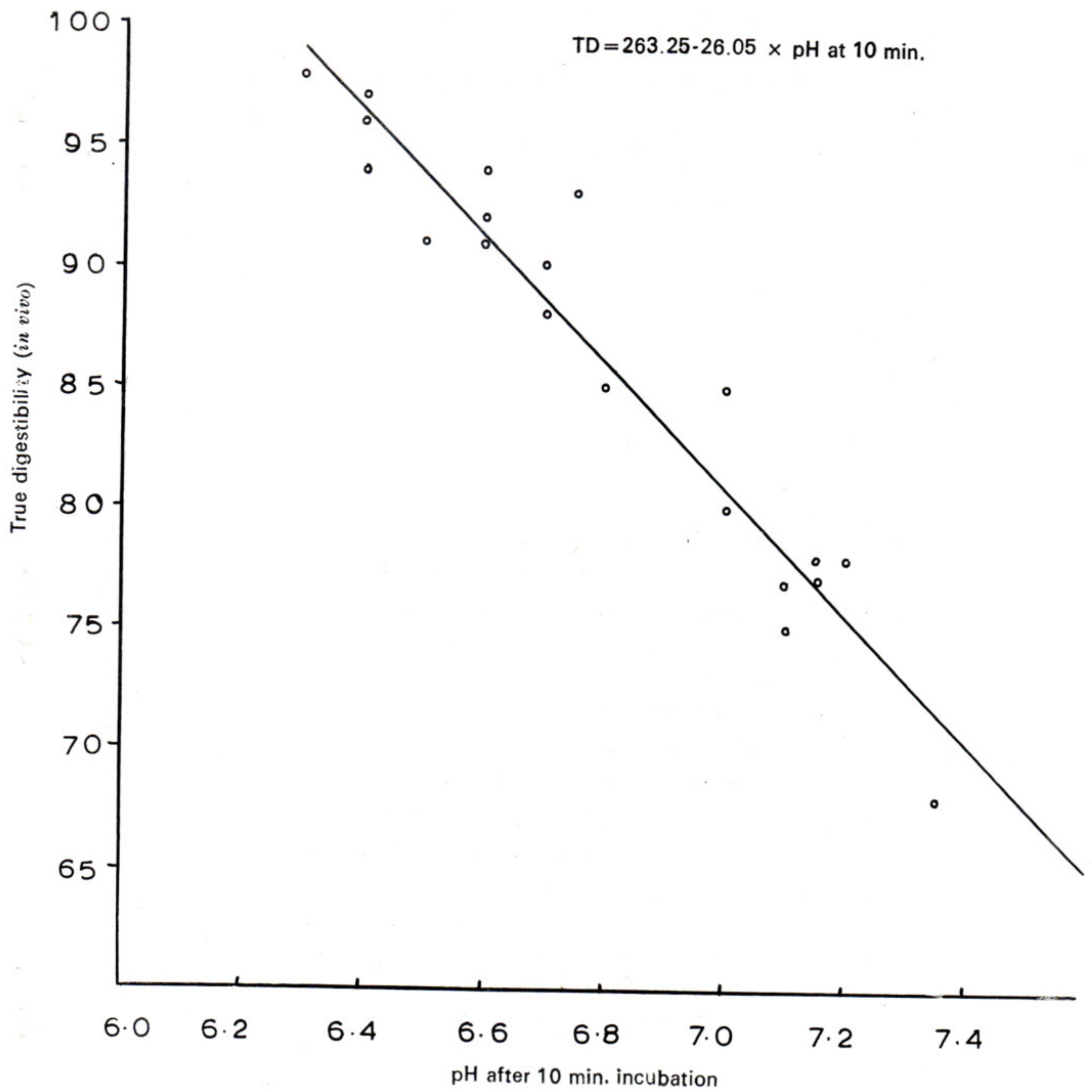

Fig. I. Relationship of pH at 10 min. and true rat digestibility.

It is evident from the results that the pH at 10 minutes following the addition of proteolytic enzymes may be regarded as an index for predicting the TD of proteins. The significant relationship between the pH values after 10 minutes and the TD of proteins thus confirms the findings of Hsu *et al.* (1977). They also demonstrated that the multienzyme method was not affected by food lipids and buffering salts commonly found in foods and was sensitive enough to be able to detect the presence of trypsin inhibitor in soy flour, chlorogenic acid in leaf protein concentrate and the changes in protein digestibility that occurred during the processing of specific foods.

The *in vitro* multienzymatic digestion for the estimation of true protein digestibility is a rapid method with a high degree of sensitivity and can be applied in routine food quality control.

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