Thermal Effects on Net Protein Ratio of Red Kidney Beans (*Phaseolus vulgaris* L)

Wu Wu,[a*] Woodie P Williams,[a] M Elizabeth Kunkel,[a] James C Acton,[a] Yun Huang,[b] Foster B Wardlaw[a] and Lawrence W Grimes[c]

[a] Department of Food Science, [b] Department of Poultry Science, [c] Department of Experimental Statistics, Clemson University, Clemson, SC 29634, USA

(Received 23 May 1995; accepted 4 March 1996)

Abstract: Net protein ratio (NPR), predicted-protein efficiency ratio (P-PER), relative NPR (RNPR), and corrected RNPR (CRNPR) of thermally processed red kidney beans were estimated in rats and compared to *in vitro* protein digestibility-corrected amino acid score (AAS_{IVDP}), and computed-protein efficiency ratio (C-PER). Thermal processing had a significant effect on protein intake, NPR, P-PER and CRNPR values of beans. Changes in protein intake suggest that heat processing had an effect on the palatability of the beans. Home-cooked beans and commercially canned beans had higher NPR values than beans autoclaved at 128°C for 20 min, while beans autoclaved at 121°C for 10–90 min had intermediate values. High correlation coefficients between P-PER and C-PER, CRNPR and C-PER, and CRNPR and AAS_{IVDP} ($r = 0{\cdot}990$, 0·992 and 0·960, respectively, $P < 0{\cdot}001$) were observed.

Key words: net protein ratio, corrective related net protein ratio, red kidney beans.

INTRODUCTION

The protein efficiency ratio (PER) assay is widely used for protein quality estimation and is the official assay in the USA and Canada (AOAC 1980). PER does not take into consideration protein maintenance requirements. It requires a minimum of 28 days to perform and as a consequence is not generally applicable for routine quality assurance use by the food industry (Bender and Doell 1957; Pellett 1978; Satterlee *et al* 1982). The net protein ratio (NPR) assay (Bender and Doell 1957) requires 10–14 days, includes a second group of animals which are fed a protein-free diet, and assumes that the weight loss of the protein-free group is a measure of the maintenance requirement of the rat. RNPR is relative NPR in which the NPR of the test diet is expressed relative to a value of 100 for the NPR of the reference diet. The adoption by AOAC of the RNPR method method was recommended by Happich *et al* (1984). (official first action) as an alternative to the PER Compared to the amino acid requirement for humans and rats, the RNPR values of proteins deficient in sulphur-containing amino acids for rat growth were multiplied by the factor of 1·5 to obtain corrected RNPR (CRNPR) values (Sarwar *et al* 1985). The CRNPR method does not discriminate against proteins with low sulphur-containing amino acid content, and may be the most suitable rat assay for routine use for evaluating protein quality (Sarwar 1987).

To evaluate *in vivo* protein quality of beans (*Phaseolus vulgaris*), PER, biological value (BV), true protein digestibility (TPD) and net protein utilisation (NPU) methods have been used frequently (Tobin and Carpenter 1978; Rockland and Radke 1981; Sgarbieri 1989; Marquez and Lajolo 1990; Marletta *et al* 1992).

The purposes of this study were to examine the effect of thermal processing of red kidney beans on protein intake, weight gain, net protein ratio (NPR), relative net protein ratio (RNPR), and corrected relative net protein ratio (CRNPR) and to compare CRNPR and *in vitro*

* To whom correspondence should be addressed. Current address: Department of Food Science, University of Arkansas, Fayetteville, AR 72704, USA.

J Sci Food Agric 0022-5142/96/$09.00

methodology (Wu *et al* 1994) for prediction of protein quality of thermally processed beans.

MATERIALS AND METHODS

Diets

Red kidney beans (*P vulgaris* L) were thermally processed as described previously (Wu *et al* 1994). Briefly, nine treatments were applied, viz raw bean (Raw), commercially canned beans (Can, Progresso, Kellogg's), common home-cooked beans (Home, boiling for 120 min), beans autoclaved at 121°C for 10, 20, 40, 60, 90 min (121–10, 121–20, 121–40, 121–60 and 121–90, respectively), and at 128°C for 20 min (128–20). The beans were forced air dried at 40°C overnight, ground to pass a 20 mesh screen, and stored in a freezer (−20°C) until used.

The ingredients of the diets were as reported by McDonough *et al* (1990). The composition of the mixed diets (g kg^{-1} dry matter basis) was about 100 protein (from beans), 100 fat, 20 vitamin mix, 35 mineral mix, 0·05 BHA, 50 cellulose and corn starch to equal 1000. A protein-free diet was used to estimate weight loss in animals and a diet with 100 g protein kg^{-1} from ANRC casein was the control (Table 1).

Animals

Forty-four male weanling Sprague-Dawley descended rats weighing 65 ± 5 g (Charles Rivers Laboratories, Research Triangle Park, NC, USA) were housed in individually suspended stainless-steel cages with filter paper suspended below the cages to minimise contamination of faeces with urine and to catch spilled food. The cages were placed in a care room at 22 ± 1°C and 65 ± 5% relative humidity, with a 12-h light, 12-h dark cycle. The rats were fed a standard pelleted diet for an acclimation period of 4 days, and then completely randomly distributed into 11 blocks each on the basis of equal mean body weights. The rats were provided distilled water *ad libitum* and fed the diets for 10 days with daily feed intake and weight gain recorded. Animal experimentation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1985).

Chemical analysis

Crude nitrogen, petroleum ether extractable material (total fat), ash, acid detergent fibre, and moisture for

TABLE 1
Composition of diets used in rat balance assay

Diet composition (g kg^{-1})[a]	*Diets*										
	Raw	*Home*	*Can*	*121–10*	*121–20*	*121–40*	*121–60*	*121–90*	*128–20*	*Casein*	*PF*
Raw	419·2	—	—	—	—	—	—	—	—	—	—
Home	—	423·7	—	—	—	—	—	—	—	—	—
Can	—	—	403·7	—	—	—	—	—	—	—	—
121–10	—	—	—	409·8	—	—	—	—	—	—	—
121–20	—	—	—	—	402·2	—	—	—	—	—	—
121–40	—	—	—	—	—	418·2	—	—	—	—	—
121–60	—	—	—	—	—	—	401·9	—	—	—	—
121–90	—	—	—	—	—	—	—	405·2	—	—	—
128–20	—	—	—	—	—	—	—	—	413·5	—	—
Casein	—	—	—	—	—	—	—	—	—	104·1	—
VitMix	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0
MinMix	35·0	35·0	35·0	35·0	35·0	35·0	35·0	35·0	35·0	35·0	35·0
Corn oil	91·4	90·2	90·1	90·2	89·6	90·1	89·3	90·0	89·6	100·0	100·0
Corn starch	457·9	430·6	450·7	444·5	452·7	452·7	453·3	449·3	441·4	740·4	844·5
Cellulose	—	—	—	—	—	—	—	—	—	50·0	50·0
BHA	0·05	0·05	0·05	0·05	0·05	0·05	0·05	0·05	0·05	0·05	0·05
Energy (kcal ME g^{-1})[b]	423·7	423·02	422·9	422·9	422·4	422·5	422·5	422·8	422·6	427·8	427·9

[a] Raw: raw bean diet; Home: home-made bean (boiled for 120 min) diet; Can: commercially canned bean (Progresso, Kellogg's) diet; 121(128)–(10–90): autoclaved bean (121(128)°C for 10–90 min, respectively) diets; MSE: mean square for error; casein: ANRC casein diet. Diet names used were same as their protein source names. VitMix: vitamin mixture (Cat No 904654, Nutr Bioch, Cleveland, OH, USA). MinMix: mineral mixture (AIN-76, Nutr Bioch, Cleveland, OH, USA). Corn oil: Mazola, adjusted to total 100. Cellulose: adjusted based on ADF of the feed. BHA: butylated hydroxyanisole (Cat No 1253, Sigma Chemical Co, St Louis, MO, USA).

[b] Calculated by Alwater factor of 4, 9 and 4 kcal g^{-1} for protein, fat and available carbohydrate, respectively.

diet and faeces were determined according to procedures described by the AOAC (1990). True nitrogen-to-protein conversion factors (T_t) for diet and faeces were obtained by using the amino acid composition and Kjeldahl nitrogen (Morr 1982; Wu *et al* 1995). The protein content was calculated by multiplying the Kjeldahl nitrogen content by F_t. The carbohydrate content was determined by difference.

Calculation and statistical analysis

Net protein ratio (NPR), predicted-protein efficiency ratio (P-PER), relative NPR (RNPR), and corrected RNPR (CRNPR) were calculated as follows:

$$\text{NPR} = (\text{WG} + \text{WL})/\text{P}_i \quad (1)$$

$$\text{P-PER} = 1{\cdot}17 \times \text{NPR} - 0{\cdot}71 \quad (2)$$

$$\text{RNPR} = (\text{NPR of test diet/NPR of casein diet})/120 \quad (3)$$

$$\text{CRNPR} = \text{RNPR} \times 1{\cdot}5 \quad (4)$$

P_i is the protein intake from diet, WG is the weight gain of animals fed test diet, WL is the weight loss of animals fed protein-free diet. Equations (1) and (2) followed Bender and Doell (1957). Equations (3) and (4) followed Sarwar *et al* (1985) and Sarwar (1987), in which a factor of 120 was used because non-methionine-supplemented casein was used as control.

The experiment was conducted in a completely randomised design. Data were analysed using analysis of variance and means were separated using the least significant difference (LSD) test procedures when significant ($P < 0{\cdot}05$) F values were obtained. Pearson correlation coefficients were used to compare the *in vitro* methodologies and *in vivo* corrected relative net protein ratio method for prediction of protein quality of thermal processed beans (Ott 1988).

RESULTS AND DISCUSSION

Protein intake and weight gain

In the NPR assay, rats favored the casein diet and consumed only small quantities of raw bean and protein-free diets. Rats fed the raw bean and protein-free diet initially exhibited an aggressive behaviour and progressively decreased their activity and body weight after a few days. Rats consuming thermally processed bean diets appeared healthy. Rats fed diets containing thermally processed beans had higher ($P < 0{\cdot}05$) protein intakes compared to rats fed diet containing raw beans. The change in protein intakes suggests that heat processing had an effect on palatability of the beans. Rats fed diets containing 121–40, 121–60, 121–90 and 128–20 beans had lower protein intakes than rats fed diets containing Home, Can, 121–10 and 121–20 beans (Table 2).

Rats fed the raw bean diet lost 55% more body weight than rats fed the nitrogen-free diet (22 g vs 14 g). These data were higher than the study reported by Almeida *et al* (1991), in which rats fed a raw bean diet lost 40% more body weight than rats fed a nitrogen-free diet (15 g vs 11 g). Rats fed the casein diet had a higher weight gain (44 g) than rats fed the processed bean diets ($\leq$6 g). Rats fed the mild heat-processed bean diets (Home, Can, 121–10 and 121–20) gained weight (6, 6, 5 and 3 g, respectively), whereas rats fed severely heat-

TABLE 2

Effects of thermal processing on protein intake (Pi), weight gain (WG), net protein ratio (NPR), predicted protein efficiency ratio (P-PER), relative NPR (RNPR) and Corrected RNPR (CRNPR) of red kidney beans

Diet[a]	*Pi (g)*[b]	*WG (g)*[b]	*NPR*[b]	*P-PER*[b]	*RNPR*[b]	*CRNPR*[b]
Raw	5·1c	−22c	−0·79d	−0·87d	−29·7d	−44·5d
Home	7·8a	6a	1·32a	1·31a	41·2a	66·3a
Can	7·4a	6a	1·34a	1·34a	45·1a	67·6a
121–10	7·8a	5a	1·22ab	1·22ab	41·1ab	61·7ab
121–20	7·6a	3ab	1·12abc	1·11abc	37·4abc	56·1abc
121–40	6·6b	1ab	1·17ab	1·16ab	39·2ab	58·8ab
121–60	6·6b	0b	1·03bc	1·03bc	34·7bc	52·0bc
121–90	6·6b	−1b	0·98bc	0·93bc	32·7bc	49·1bc
128–20	7·1ab	−1b	0·86c	0·86c	28·8c	43·2c
MSE	0·9	0·21	0·11	0·04	32·1	12·9
Casein	12·2	44	2·50	2·50	80·0	120·0

[a] Diets same as in Table 1.

[b] Means based on data from four rats for all diets. *t*-test was used for bean diets and not for casein diet. Means followed by different following letters within a column were significantly different ($P < 0{\cdot}05$).

processed bean diets (121-60, 121-90 and 128-20) neither gained nor lost (0, −1 and −1 g, respectively) weight during the experimental period.

Net protein ratio and predicted PER

While protein efficiency ratio (PER) reflects the ability of a protein to support weight gain, net protein ratio (NPR) reflects the ability of a protein to support weight gain and maintenance. Thermal processing significantly ($P < 0{\cdot}05$) affected NPR values of the beans (Table 2). The NPR for the raw diet was negative because rats fed the raw diet lost more body weight than rats fed the protein-free diet. In a previous *in vitro* study (Wu *et al* 1994) treatment Can gave an intermediate protein quality value as compared to other treatments. In this study, Can had the highest NPR value among the beans. This may have been due to the salt added in the canned beans which can increase palatability and thus protein intake. Rats fed diets 121-60, 121-90 and 128-20 had lower NPR values than rats fed the other processed bean diets (Table 2). Marquez and Lajolo (1990) found that whole cooked beans (*P vulgaris*) had poor digestibility (62·8%) and a low NPR of 2·27, which was similar to the NPR value for 121-10 in this report.

Predicted-PER (P-PER) values of beans (Table 2) were calculated from the NPR (Bender and Doell 1957). P-PER values of heated beans were 46-70% lower than C-PER values (Wu *et al* 1994). PER values were 0·97, 0·63, 0·55 and 0·82, respectively (corrected to casein PER = 2·5) for four varieties of beans (Sgarbieri *et al* 1979), and PER values were −0.87 for raw beans, 0·70 to 1·69 for cooked beans (Almeida *et al* 1991, corrected to casein PER = 2·5). These findings were similar to the P-PER values of this study (Table 2). The correlation coefficient of P-PER and C-PER was 0·99 ($P < 0{\cdot}001$), which suggests that both methods ranked the protein quality values in the same order, but the C-PER values may overestimate the protein quality of beans.

Corrected relative net protein ratio

As with PER, the NPR value is not based on a percentage or a unity scale. The relative NPR (RNPR) of a test protein is expressed as a fraction of the value obtained with a standard protein (casein) taken as unity or as a percentage as has been recommended by Happich *et al* (1984). The RNPR value of casein (low in methionine) is about 20% lower than that of methionine-supplemented casein (Sarwar *et al* 1985). The rat requirement for methionine + cystine is about 50% higher than that for humans. A corrected RNPR (CRNPR) for beans for rat growth can be computed by multiplying the RNPR values by a factor of 1·5 (Sarwar *et al* 1985). The RNPR values of heated beans were low and ranged from 27·6 to 43·3. After correcting the RNPR value by a factor of 1·5, the CRNPR values of the beans ranged from 43·2 to 66·2. Sarwar (1987) reported that the RNPR and CRNPR values of kidney beans were 49 and 73, respectively. This compared closely to the values of diets Home and Can (Table 2) in this study.

In vitro protein digestibility-corrected amino acid score (AAS_{IVDP}) and computed protein efficiency ratio (C-PER) of beans have been obtained (Wu *et al* 1994). A comparison of CRNPR value and the *in vitro* value was conducted. High correlation coefficients between P-PER and C-PER, CRNPR and C-PER and CRNPR and AAS_{IVDP} ($r = 0{\cdot}990$, 0·992 and 0·960, respectively, $P < 0{\cdot}001$) were observed. The CRNPR method considers the protein used for growth and maintenance, and does not discriminate against proteins with lower sulphur-containing amino acid content (Sarwar 1987). Therefore, CRNPR values of processed beans were considered acceptable parameters for evaluating protein quality of processed beans.

ACKNOWLEDGEMENT

This is technical contribution 4082 of the South Carolina Agricultural Experiment Station, Clemson University, SC, USA.

REFERENCES

Almeida N G, Barca A M C, Valencia M E 1991 Effect of different heat treatments on the antinutritional activity of *Phaseolus vulgaris* (Variety Ojo De Cabra) lectin. *J Agric Food Chem* **39** 1627-1630.

AOAC 1980 *Official Methods of Analysis of the Association of Official Analytical Chemists* (13th edn). Association of Official Analytical Chemists, Washington, DC, USA.

AOAC 1990 *Official Methods of Analysis of the Association of Official Analytical Chemists* (15th edn). Association of Official Analytical Chemists, Washington, DC, USA.

Bender A E, Doell B H 1957 Biological evaluation of protein: A new aspect. *Br J Nutr* **11** 139-148.

Happich M L, Bodwell L R, Hackler C E, Phillips G J, Derse P H, Elliott J G, Harpnagel R E Jr, Hopkins D T, Kapiszka E L, Mitchell G V, Parsons G F, Prescher E E, Robaidek E S, Womack M 1984 Net protein ratio data. AACC-ASTM collaborative study. *JAOAC* **67** 255-262.

Marletta L, Carbonaro M, Carnovale M C 1992 *In vitro* protein and sulphur amino acid availability as a measure of bean protein quality. *J Sci Food Agric* **59** 497-504.

Marquez U M L, Lajolo F M 1990 Nutritional value of cooked beans (*Phaseolus vulgaris*) and their isolated major protein fractions. *J Sci Food Agric* **53** 235-242.

McDonough F E, Steinke F H, Sarwar G, Eqqum B O, Bressani R, Huth P J, Barbeau W E, Mitchell G V, Phillips G J 1990 *In vivo* rat assay for true protein digestibility: Collaborative study. *JAOAC* **73** 801-805.

Morr C V 1982 A research note: Recalculated nitrogen conversion factors for several soybean protein products. *J Food Sci* **47** 1751-1752.

National Research Council 1985, *Guide for the Care and Use of Laboratory Animals.* NIH Publication, pp 85-23. National Institutes of Health, Bethesda, Maryland.

Ott L 1988 *An Introduction to Statistical Method and Data Analysis* (3rd edn). PWS-Kent Publ Comp, Boston, MA, USA.

Pellett P L 1978 Protein quality evaluation revisited. *Food Technol* **32** 60–79.

Rockland L B, Radke T M 1981 Legume protein quality. *Food Technol* **35** 79–82.

Sarwar G 1987 Digestibility of protein and bioavailability of amino acids in foods. Effects on protein quality assessment. *World Rev Nutr Diet* **54** 26–70.

Sarwar G, McDonough F E 1990 Evaluation of protein digestibility-corrected amino acid score method for assessing protein quality of foods. *JAOAC* **73** 347–356.

Sarwar G, Peace G W, Botting H G 1985 Corrected relative net protein ratio (CRNPR) method based on differences in rat and human requirements for sulfur amino acids. *JAOAC* **68** 689–693.

Satterlee L D, Kendrick, J D, Marshall H F, Jewell D K, Ali R A, Heckman M M, Steinke H F, Larson P, Phillips R D, Sarwar G, Slump P 1982 *In vitro* assay for prediction of protein efficiency ratio as measured by rat bioassay: Collaborative study. *JAOAC* **65** 798–809.

Sgarbieri V C 1989 Composition and nutritive value of beans (*Phaseolus vulgaris* L). *World Rev Nutr Diet* **60** 132–198.

Sgabrieri V C, Antumes P L, Almeida L D 1979 Nutritional evaluation of four varieties of dry beans (*Phaseolus vulgaris* L). *J Food Sci* **44** 1306–1308.

Tobin G, Carpenter K J 1978 The nutritional value of the dry beans (*Phaseolus vulgaris*): A literature review. *Nutr Abstr Rev* **48** 939.

Wu W, Williams W P, Kunkel M E, Acton J C, Wardlaw F B, Huang Y, Grimes L W 1994 Thermal effects on *in vitro* protein quality of red kidney bean (*Phaseolus vulgaris* L). *J Food Sci* **59** 1187–1191.

Wu W, Williams W P, Kunkel M E, Acton J C, Huang Y, Wardlaw F B, Grimes L 1995 True nitrogen conversion factor for diet and excreta in evaluating protein quality. *J Food Sci* **60** 854–857.