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Protein quality of autoclaved cowpea varieties as influenced by anti-nutritional factors

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Abstract

One hundred and ten (110) weanling male winstar strain rats were used to determine the protein efficiency ration (PER) and net protein ration (NPR) of five cowpea samples. PER ranged from 1.06 in IT 81D-1137 to 1.91 in IT84E-1-108 while values in autoclaved samples ranged from 1.55 in IT81D-1137 to 2.78 in IT84E-1-108. for NPR, raw samples gave values that ranged between 2.44 and 3.57 in IT81D-1137 and IT84E-1-108 respectively. Autoclaved samples gave NPR values that ranged from 2.72 to 4.47 in IT 81D-1137 and IT84E-1-108 respectively. In the raw and autoclaved cowpea diets, trypsin inhibitor, lectin and tannic acid were negatively correlated with PER and NPR while HCN and phytic acid had positive relationship with these indicators of protein quality. In autoclaved samples cowpea variety IT84E-1-108 had the highest PER and NPR values showing that it would support growth to a reasonable extent.

Keywords: Protein efficiency ratio, net protein ratio, autoclaving, antinutritional factors, cowpea varieties.

Introduction

Dietary surveys in the developing countries have shown that maize (*Zea mays*) and cowpea (*Virgna unguiculata*) are the two most important sources of protein in rural diets (Ayleroyd and Doughty. 1982). From nutritional view point, cereal grains not only are low in total protein but are also deficient in some of the essential amino acids particularly lysine Leguminous seeds such as cowpea could supply the limiting amino acid in maize protein (Elias *et al.*, 1964).

In spite of their respectively high protein content it has been well established by several investigators (Bressani and Elias. 1974; Elias *et al.*, 1964) that the protein quality of legumes is low which has been attributed not only to their deficiency in sulphur – containing amino acids (Bressani *et al.*,1973) but also because they are poorly digested (Bressani and Elias. 1977). The problem of protein digestibility in legumes has been studied (Glick and Joslyn. 1970) and several theories proposed among which was the presence of antinutritional factors (Jaffe. 1968; Liener. 1973; Griffiths and Mosley. 1980).

Materials and Methods

Cowpea: Five cowpea varieties (IT82D-889, IT81D-113, IT84E-1-108, IT82E-16 and Ife Brown) supplied by the international Institute of Tropical Agriculture (IITA), Ibadan but multiplied with a view to obtaining fresh seeds were used for this study.

Protein determination: Protein contents of the test diets were assayed by adopting AOAC (1990) Kjeldahl technique. Antinutritional factors of the same cowpea varieties had been estimated (Oke, *et al.*, 1996).

Rats Feeding Trial: One protein-free diet (Table 1) and ten (10) test diets were fed to ten (10) weanling male Wistar strain rats per diet with average initial weights ranging from 39.25g to 43.87g. this was done in accordance with the National Council methods of 1963 and modifies by adopting the report of the international Union of Nutritional Sciences and United Nations University World Hunger Programme (Pellett and Young. 1980). Rats were housed individually in clean stainless steel metabolic cages. Cowpea samples (raw and autoclaved) replaced maized starch present in the protein free diet such that each diet supplied 10% crude protein on dry matter basis. Feed and water were given to the rats *ad libitum* during the 28-day experimental period. Feed intake and weight gain were measured.

Table 1: *Composition of Protein-Free Diet (%)*

Maize Starch	79.75
Maize Oil	10.00
Non-nutritive cellulose	5.00
Vitamin Premix	1.00
Mineral Premix	1.00
Oyster Shell	1.00
Bone Meal	2.00
Salt (NaCl)	0.25
Total	100.00

Every gram of vitamin premix contains: Vitamin A (1000 IU); Vit. D. (100 IU); Vit.E (10IU); Vit. K. (0.5MG); Thiamine (0.5mg); Riboflavin (1.0mg); Pyridoxine (0.4mg); Pantothenic acid (4.0mg); Niacin (4.0mg); Choline (200mg); Inositol (25mg); Para-amino benzoic acid (10mg); Vit. B12 (2micrograins); biotin (0.02mg) and Folic acid (0.2mg).

Statistical analysis: Randomized complete block two-treatment factor analysis of variance was employed to analyze the data. Duncan's Multiple Range Test was used to separate treatment means (Steel and Torrie. 1980). Relationship between the antinutritional factors and protein efficiency ratio together with Net Protein Ratio were established by carrying out regression analysis. Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) were computed as shown below.

$$PER = \frac{\text{Gain of Test Animal}}{\text{Protein Consumed}}$$

$$NPR = \frac{\text{Weight gain of Test animal + weight loss of Control animal}}{\text{Protein Consumed by Test Animal}}$$

Results and Discussion

Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) are indicators of protein quality based on weight gain and protein consumption. In the raw samples, PER ranged from 1.06 in IT81D-1137 to 1.91 in IT84E-1-108 with a mean of 1.32 while in the autoclaved samples. value ranged from 1.55 in IT81D-1137 to IT84E-1-108 with an average of 2.10. For NPR, raw samples gave values that ranged from 2.44 to 3.57 in IT81D-1137 and IT84E-1-108 HCN and phytic acid had positive relationship with these indicators of protein quality. All the correlation coefficient values were not significant except that relating trypsin inhibitor in raw samples to PER thus accounting for 38.99 variability among other antinutritional factors (Table 3, 4 & 5). The reverse relations between trypsin inhibitor on the one hands and PER cum NPR on the other hand (r=-0.62 and -0.50

respectively) were in conformity with the findings of numerous workers like Close *et al.*, (1946) and Kuppuswamy *et al.*, (1983). Although conflicting reasons for this relationship have been advanced, the most plausible could be the intestinal tract of animals as proposed by Liener (1980). This author opined that hepatic hypertrophy represents one of the primary physiological factors responsible for the poor growth response on a diet that contained raw legumes seeds. Since these indicators of protein quality depend on weight gain, poor growth would therefore undermine their values.

Table 2: *Composition of Protein-Free Diet (%)*

	Protein Intake (g)		Weight Gain (g)		PER		NPR	
Cowpea Variety	R	A	R	A	R	A	R	A
IT82D-889	6.00	7.37	7.36	15.39	1.23	2.08	2.64	3.21
IT81D-1137	6.15	7.21	6.53	11.14	1.06	1.55	2.44	2.72
IT84E-1-108	5.11	5.03	9.76	13.97	1.91	2.78	3.57	4.47
IT82E-16	5.91	6.79	6.7	12.87	1.13	1.90	2.57	3.24
Ife brown	7.05	7.80	8.87	17.35	1.26	2.22	2.46	3.31
Mean	6.05	6.84	7.84	14.14	1.32	2.11	2.74	3.40
Sex	±0.20	±0.30	±0.40	±0.67	±0.09	±0.13	±0.13	±0.18

Values are means of eight rats

R= Raw: A= Autoclaves

Table 3: *Prediction equations and correlation coefficients relating antinutritional factors to PER of cowpea protein*

Antinutritional Factor	Prediction equation			r-value
Trypsin Inhibitor	y=2.23-	0.06x+	0.001 x^2	-0.62
Lectin	y=-0.01+	0.04x-	0.0003 x^2	-0.34
Tannic acid	y=0.40+	6.58x-	10.41 x^2	-0.24
HCN	y=0.12+	0.86x-	0.14x^2	0.21
Phytic acid	Y=-42.00	+0.19x	-0.0002x^2	0.25
AUTOCLAVED				
Trypsin inhibitor				
Lectin				
Tannic acid	y=2.23	-0.06x+	0.001 x^2	-0.62
Lectin	y=-0.01	+0.04x-	0.0003 x^2	-0.34
Tannic acid	y=0.40	+6.58x-	-10.41 x^2	-0.24
HCN	y=0.12	+0. 86x-	-0.14x^2	0.21
Phytic acid	Y=-42.00	+0.19x	-0.0002x^2	0.25
AUTOCLAVED				
Trypsin inhibitor				
Lectin				
Tannic acid	y=0.77	-20.39x	-32.10 x^2	-0.03
HCN	y=2.30	-0. 37x	+0.19x^2	0.01
Phytic acid	Y=-25.6	+0.16x	-0.0002x^2	0.35

r values exceeding 0.051, 0.64 and 0.96 are significant at $P<0.05P<0.01$ and $P<0.001$ respectively, df=13

Table 4: *Prediction equations and correlation coefficients relating antinutritional factors to NPR of cowpea protein*

Antinutritional Factor	Prediction equation	r-value
Trypsin Inhibitor	$y=3.96 - 0.09x + 0.001 x^2$	-0.50
Lectin	$y=-0.66 + 0.11 x -0.001 x^2$	-0.40
Tannic acid	$y=1.54 + 8.15 x-12. 50 x^2$	-0.14
HCN	$y=0.10 + 1.90 x -0.32x^2$	0.27
Phytic acid	$Y=-57.20 + 0.26 x -0.0003x^2$	0.29
Phytic acid	$y = 28.20 + 0.18 - 0.0003x^2$	0.25

r values exceeding 0.051, 0.64 and 0.96 are significant at P<0.05P<0.01 and P<0.001 respectively, df=13

Table 5: *Stepwise Regression of antinutritional factors with PER and NPR values*

	PER		NPR	
coefficient of determination	R	A	R	A
due to trypsin inhibitor	38.99	-	26.33	-
due to lectin	13.20	-	23.83	-
due to tannic acid	15.03	64.79	7.75	64.51
due to HCN	11.01	0.06	21.75	4.17
due to phytic acid	21.77	35.15	21.08	31.32

The non-specific interference of lectin with absorption of nutrients across the intestinal wall would be reflected in the extent to which the protein digested. Hence, the inverse relation between samples (Jaffe, 1960, Jaffe and Camejo, 1961). Tannic acid similarly had inverse relations with PER and NPR in raw legumes as reported by Griffiths and Mosley 1980). This could be due to the inhibition of trypsin and alpha-amylase thus leading to increased pancreatic secretion of digestive enzymes and their exogenous loss (Griffiths and Mosley, 1980). The same sequence was recorded in the autoclaved samples which served as a reflection of reduced tannic acid level. The observed insignificant direct relationship of HCN and phytic acid to PER and NPR in the raw and autoclaved samples could be due to the detoxification of GCN when it entered the blood system (Lang. 1933; Wood and Cooley, 1956; Oke. 1973; Tewe. 1984) and the non-involvement of phytic acid in nitrogen digestibility (Savage *et al.*, 1964).

Conclusion

Autoclaving significantly improved the quality of cowpea protein with respect to PER and NPR. The influence of trypsin inhibitor in impairing protein quality in raw cowpea samples became glaring.

Complexing tannic acid with protein even in the autoclaved samples became obvious as a depressant of PER and NPR. In autoclaved samples, cowpea variety IT84E-1-108 had the highest PER and NPR values showing that it would support growth to a reasonable extent.

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