IDENTIFICATION OF THE FIRST LIMITING AMINO ACID IN COOKED POLISHED WHITE RICE FED TO WEANLING HOLTZMAN RATS

Mellova Masrizal[1] and J. B. Lamb[2]

1. Pharmaceutical Science Department, Faculty of Mathematics and Natural Sciences, ISTN, Jakarta 12640, Indonesia
2. Animal Science Department, University of Nebraska - Lincoln, USA

E-mail: masrizaldt@yahoo.com

Abstract

Forty-eight male weanling rats (91 g) were utilized to study the nutritional adequacy of cooked polished white rice. Rats were individually housed, and allowed ad libitum access to one of six treatment diets. Treatment diets were 1) polished white rice plus 10% casein and 0.18% methionine, CAS, 2) polished white rice, WHR, 3) polished white rice plus 0.45% lysine, LYS, 4) polished white rice plus0.40% methionine, MET, 5) polished white rice plus 0.30% threonine, THR, 6) polished white rice plus 0.45% lysine, 0.40% methionine, and 0.40% threonine, COM. Rice was cooked prior diet formulation using a 3 to 1 ratio of water to rice. Vitamins (AIN-76) and AIN minerals were added to all diets to meet NRC (1978) requirements. Rats fed CAS diets were significantly heavier on d 21 (P<0.05) than rats on COM, LYS, MET, THR, or WHR diets, (219.9 vs. 171.6, 153.2, 153.2, 148.3, or 155.4 g respectively). Supplementation of the most deficient essential amino acids, lysine (LYS) or methionine (MET) did not improve (P>0.05) rat performance over WHR fed rats, Average daily gain (ADG) for CAS was 6.1 g/d and ADG for LYS and MET was 3.0 g/d. The addition of threonine (THR) significantly (P<0.05) reduced ADG when compared to WHR diets (2.7 vs. 3.0 g/d). When rats were fed to COM diet significant (P<0.05) improvement in ADG was observed compared to WHR fed rats (4.8 vs. 3.0 g/d). The increased gains achieved with COM diet and the poor gains observed with the single amino acid diets (LYS, MET, or THR) would suggest that polished white rice is limiting in more than one essential amino acid.

Keywords: amino acids, rice, supplementation, rat, colimiting

1. Introduction

Polished white rice was staple grain for a large portion of the world population. These populations depend on rice for the bulk of their energy and protein needs [1]. Processing or polishing rice removes approximately 25% of whole rice protein [2]. Cooking polished rice can change the viscoelastic properties, protein structure and protein digestibility. Ressureccion et al. [3] reported that uncooked rice protein had digestibility value of 100%, and cooking rice reduces protein digestibility values by 10%. Typical crude protein content of polished white rice is 7 to 8% and these levels do not fluctuate appreciably from variety to variety.

Rats fed polished white rice gained poorly when compared to rats fed a casein or albumin supplemented diet [4]. These finding would suggest that process rice diets are not nutritionally adequate to support maximal growth.

Given the worldwide importance of polished rice, further research is warranted to find ways to improve rice quality. A study was designed with the objectives of: 1) determine whether cooked polished rice is deficient in protein, and 2) if protein is limiting, identify which amino acid is first limiting to rat growth and development.

2. Methods

Forty-eight Holtzman (Sprague-Dawley, Madison, WT) male weanling rats (average BW =91g) were randomly assigned to individual metabolism cages and exposed to 12-h light-dark cycle and constant 25°C ambient temperature. Rats allowed ad libitum access to water and standard lab rat chow for 2 d environment acclimation period prior to going on a 21 d growth trial.

Following acclimation, rats were weight and randomly assigned to one of six dietary treatments (n = 8). Treatments were:

(1) Polished white rice plus 10% casein and 0.18% methionine, CAS
(2) Polished white rice, WHR
(3) Polished white rice plus0.45% lysine, LYS
(4) Polished white rice plus 0.45% methionine, MET
(5) Polished white rice plus 0.35% threonine, THR
(6) Polished white rice plus 0.45% lysine, 0.40% methionine, 0.40% threonine, COM

Table 1. Ingredient composition of experimental diets, DM basis

Ingredients (%)	Treatment					
	CAS	WHR	LYS	MET	THR	COM
White rice	84.82	95.00	94.55	94.60	94.70	93.85
Casein	10.00	0.00	0.00	0.00	0.00	0.00
Lysine	0.00	0.00	0.45	0.00	0.00	0.45
Methionin	0.18	0.00	0.00	0.40	0.00	0.40
Threonine	0.00	0.00	0.00	0.00	0.30	0.30
AIN vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00
AIN mineral mix	3.50	3.50	3.50	3.50	3.50	3.50
Limestone	0.30	0.30	0.30	0.30	0.30	0.30
Choline bitartrat	0.20	0.20	0.20	0.20	0.20	0.20

Rice was cooked prior to diet formulation using a 3 to 1 ratio of water to rice. Diets were formulated (Table 1), with the exception of CAS, to meet National Research Council amino acid requirements [5]. The CAS diet was formulated to be nutritionally adequate and supply 15% crude protein in the diet. Vitamins (AIN-76A) and AIN minerals were added to all diets to meet NRC requirements [5]. Throughout the study period rats were allowed ad libitum access to diets and water.

CAS = white rice plus 7% casein; WHR = white rice; LYS = white rice plus 0.45% lysine; MET = white rice plus 0.40% methionine; THR = white rice plus 0.30% threonine; COM = white rice plus 0.45% lysine, 0.40% methionine, and 0.30% threonine.

All rats were weighed at weekly intervals. Daily feed consumption was not recorded due to excessive wastage observed on under cage collection trays. All rats were feed approximately 18 g DM once daily at 08.00. This level appeared to allow rats 24 h ad libitum access to feed and minimized feed wastage. On day 21, all rats weighed, euthanized by carbon dioxide asphyxiation, and frozen.

Four rats from each treatment were freeze died for 10 d in a conventional virtis freeze mobile 25 freeze drier (Virtis Co., Inc., Garddiner, NY). Carcass dry weight and body water composition were calculated for each diet rat. An attempt was made to analyze rats for whole body nitrogen. Due to fat content of the rats, we were unable grind or chop rats fine enough to obtain a representative sample for kjeldahl analysis.

Data were analyzed using the GLM procedure of SAS [6]. The experimental design was a randomized complete block with or shelf level as the blocking term. Response criteria were weight gain, ADG, carcass dry weight, and body water content. Initial treatment BW bias. Orthogonal contrasts were used to separate treatment means. Contrast were 1) CAS vs. COM, 2) COM vs. WHR 3) WHR vs. LYS, 4) WHR vs. MET, and 5) WHR vs. THR.

3. Result and Discussion

Hegsted and Yuliano [7] analyze nine milled (polished) rice and suggested, based on chemical score, that the limiting essential amino acid of rice protein are lysine, threonine, methionine and tryptophan. In the present study, when single supplementation of these limiting amino acid (LYS, MET, and THR) did not increased rat gain (Figure 1) over those observed in unsuplemented polished white rice diets (WHR).

These finding were similar to those observed by Pecora and Hundley [8], for deficient essential amino acids (lysine, histidine, methionine, tryptophan, threonine, phenylalanine, valine, leucine, isoleucine, and arginine). They observed that when deficient amino acid was added individually to rice, rat growth responses were not improved over that obtained with unsupplemented rice died. The addition of THR significantly ($P<0.05$) reduces ADG compare to WHR (2.7 vs. 3.0 g/d). These authors offer no explanation why ADG was reduced with the THR diet.

When lysine, methionine and threonine (COM) were feed in combination, rats had significantly ($P<0.05$) higher gains than rats fed LYS, MET, THR, and WHR. Rats fed COM gained on the average 1.8 g/d more weight than did rats fed either LYS, MET, THR, and WHR diets (Figure 2). The increased gain is similar to that observed by Pecora and Hundley [8]. They observed that when rats fed combinations of the most limiting amino acids, only lysine in combination with threonine, significantly ($P < .5$) increased ADG. They observed a threefold increase in gains with lysine, threonine in combination. In the present study, combination of all three amino acid (COM) increased ADG by 160%. We can only speculate that the 160% increase with growth over LYS, MET, THR and WHR diets, was due to combination of lysine and threonine. The lower gains achieved with COM when compared to the work by Pecora and Hundley [8] was due to our diet being cooked. Resurreccion et al. [3] observed that cooking lowered the digestibility of polished rice protein by 10%.

All rats gain weight over this 21 d trial, which indicated that white rice protein is not totally nutritionally inadequate. It appears that polished white rice fed without supplement could provide ample protein and energy for maintenance and minimal growth (Figure 1).
Miller and Bender [9] observed that body nitrogen was highly correlated to percent body water, and dry body weight was highly correlated to protein accretion. Dried rat carcasses weights were not statistically different ($P>0.05$) between treatments, but visually, treatments appeared to follow the same trend observed BW gains and ADG (Figure 3). No differences ($P>0.5$) were observed between treatments when rat analyzed based on percent body water (Figure 4). However, treatments appeared to visually follow a similar pattern as that observed for BW gains, ADG, and dry carcass weight.

Figure 1. Growth performance of weanling rats fed cooked white rice supplemented with or without casein, lysine, methionine, threonine, or the combination of lysine, methionine, and threonine.

Figure 2. Average daily gains of weanling rats fed cooked white rice supplemented casein, lysine, methionine, threonine, or the combination of lysine, methionine, and threonine.

Figure 3. Carcass dry-weight of weanling rats fed cooked white rice supplemented with or without casein, lysine, methionine, and threonine[1].

Figure 4. Percent body water of weanling rats fed cooked white rice supplemented with or without casein, lysine, methionine, and threonine[1].

4. Conclusions

A large portion of the world population depends on rice for the bulk of their energy and protein needs. However, rice as a protein source may be limiting under certain conditions. Rats fed rice diet gained poorly when compared to a nutritionally adequate diet. Polished white rice is deficient of several amino acids and, no single amino acid is limiting to growth and development. The addition of lysine in combination with threonine to rice, can significantly improves growth and development. Cooked polished white rice diets appear to have a combination of amino acids that may be co-limiting growth and development.

Reference

[1] K. Murata, M. Nishikaze, M. Tanaka, J. Nutr. Sci. Vit. 23 (1977) 125.
[2] W.C. MacLean, G. L. Klein, F. L. Romana, E. Massa, G. G. Graham,. J. Nutr. 108 (1978) 1740.
[3] A.P. Resurreccion, X. Li., T. W. Okita, B. O. Juliano, Cereal Chem. 70 (1993) 101.
[4] M. Miyazaki, M. Horii, Proc. Int. Con. Nutr. 10 (1975) 354.
[5] NRC, Nutrient Requirements of Laboratory Animals. Third revised Ed. National Academy of Sciences – National Academy Press, Washington, DC. (1978)
[6] SAS, User's Guide: Statistic (version 6.03 Ed.). SAS Inst. Inc., Cary, NC. (1988)
[7] D.M. Hegsted, B. O. Juliano, J. Nutr. 104 (1974) 772.
[8] L. J. Pecora, J. M. Hundley, J. Nutr. 44 (1951) 101.
[9] D. S. Miller, A. E. Bender, Brit. J. Nutr. 9 (1955) 382.