RICE AND BEANS – EFFECT OF MILK, MINERAL, AND VITAMIN A, ON WEIGHT GAIN, FOOD INTAKE, AND BODY PROTEIN RETENTION

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SUMMARY

Weanling rats were fed on diets of rice/beans (RB) with or without mineral (M) supplement; vitamin A (A); M + A (MA); or milk (L), containing from 3 to 14o/o protein. As determined by various measures, the best results were observed in the group fed the RB diet, supplemented with L. This group was followed by those receiving the MA and M diets. Protein utilization in the RB diet, as well as minimum protein requirement for maintaining body weight, depend on the supplementary nutrients. The mechanism of this effect may be related to the appetite of the animals and to the voluntary intake, which is influenced and regulated by the diet composition.

INTRODUCTION

Rice and beans (RB) are recognized as the most important basic foods consumed by the Brazilian population, who eat them sporadically, without any other adequate complementary food. Different proportions of rice/beans are habitually consumed in Brazil as documented by nutritional surveys; in the Northeast rural area (1) the total intake/individual/year was estimated to be 55.3 kg of cereals, and 46.1 kg of legumes and oleaginous. In earlier reports it was suggested that the RB mixture is limited in fat-soluble factors (2, 3). To design a food supplementation program it is relevant to observe the limitation of these nutrients. In a recent proposal in Brazil, for a model of basic minimum-cost diet for adults, the rice/beans daily intake was suggested to be: 100/200 g, or 140/120 g, respectively, for the southeastern and northeastern regions (4).

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Based on these data, the present investigation was designed to study the basic food system of rice/beans using experimental animal models, in order to determine the main limiting nutrients in rice/bean mixtures of 55/45o/o.

MATERIAL AND METHODS

Animals

A total of 250 Wistar weaning rats (21 days old) were housed individually, and given *ad libitum* different diets (shown in Table 1) for 25 days. The initial average body weight was 39 ± 1.8 g (mean ± SD).

TABLE 1

DIETS OF RICE/BEAN (RB) OR CASEIN (CAS), CONTAINING 3 TO 14o/o PROTEIN
(per 100 g diet)

Ingredients	Diet RB	RBM	RBA	RBMA	RBL	Cas	PF[5]
RB[1]	+[4]	+	+	+	+	+	+
Cas[1]	–	–	–	–	–	–	–
Milk (L)[2], g	–	–	–	–	–	–	–
Minerals (M)[3], g	–	4	–	4	–	4	4
Vitamin A (A), µg	–	–	6o/o	6o/o	–	–	–
Oil[3], g	–	–	–	–	–	2	2
Codfish oil, g	–	–	–	–	–	2	2
Vitamins[3], g	–	–	–	–	–	2	2
Starch[6], g	+	+	+	+	+	+	+

1 RB or Cas, amount to obtain diets containing 3 to 14o/o protein: RB – Rice, 55o/o, beans, 45o/o (protein of rice, 8o/o, beans, 24.8o/o) (Beans, cooked with water in autoclave, 115oC/40 min and lyophilized).
2 L – Milk (whole powdered, 25o/o protein), RB, 70o/o + L, 30o/o.
3 (Ref. 2).
4 (+) present, (–) absent.
5 PF– Protein-free diet.
6 Corn-starch to complete 100o/o of diet.

Ten animals/group were fed *ad libitum* each diet, containing from 3 to 14o/o protein.

Diets

Each individual diet was prepared so as to have a final protein content of 3, 7, 10 or 14o/o, which was estimated by calculating N x 6.25 (5).

Protein content of the diets is presented in per cents in Table 2, while their basic mineral composition can be appreciated in Table 3.

TABLE 2

PROTEIN CONTENT OF THE RICE AND BEANS (RB) DIETS SUPPLEMENTED WITH MILK (L): MINERALS (M) OR VITAMIN A (A), AND OF CASEIN (Cas)

Diet	Protein content (g/100 g)			
	3	7	10	14.5
RB[1]	2.5[2]	6.0	9.0	14.5
RBL	3.3	8.1	12.2	14.0
RBM	3.5	7.0	10.5	14.7
RBA	2.7	5.5	9.8	14.7
RBMA	3.9	8.7	10.5	14.4
Cas	2.8	7.0	10.5	13.1

1 As described in Table 1.
2 Measured as: Nitrogen x 6.25.
Protein content of PF diet was: 0.15 g/100 g.

TABLE 3

MINERAL COMPOSITION AND VITAMIN A OF THE RICE/BEAN (RB) DIETS SUPPLEMENTED WITH MILK (RBL) OR WITH MINERALS (RBM) COMPARED TO THE REQUIREMENTS FOR GROWING RATS (RGR)
(Expressed in mg/100 g)

	RB	RBL	RBM	RGR[3]
Calcium[1]	80	330	633	500
Iron[1]	4.71	3.39	15.7	3.5
Zinc[1]	3.12	3.23	3.62	1.2
Copper[1]	0.44	0.35	0.84	0.5
Magnsesium[1]	870	880	955	400
Manganese[1]	2.23	1.73	2.49	5
Phosphorus[1]	290	390	668	400
Vitamin A[2]	Trace	0.076	Trace	0.067

1 Determined in food.
2 Not analyzed (calculated from ICNND/INCAP Food Composition Table, 1961.
3 RGR — Requirements for growing rats (ref. 9).

Determinations

The following determinations were carried out, as stated:

1 – *Body weight and food intake* — The animals' weight and the food intake (FI) were recorded twice a week.

2 – *Protein efficiency* (6)

a) *NPR — Net protein ratio* — Calculated as body weight gain (BW) in the 10o/o protein group, plus the weight loss (WL) of the group, relative to protein intake (PI).

$$PER = \frac{BW + WL}{PI}$$

b) *PER — Protein efficiency ratio* — Calculated as body weight gain in the 10o/o protein group relative to protein intake.

$$PER = \frac{BW}{PI}$$

c) *RPV — Relative protein value* — From the regression line of the BW, using the 3 to 14o/o protein groups.

d) *MP — Minimun protein requirement* — This was determined from the regression line of the body weight slopes for each diet related to the protein content of the diet, at the point of x intercept.

e) *NPU — Net protein utilization* — Calculated from retained nitrogen (N) in the carcass, determined from the water content in the carcass before and after drying at 100oC, (H_2O). The calculation used was N = (3.09 + 0.032x) x (H_2O) (6), where x represents days of life. The protein was assumed to be: N x 6.25. The protein of the carcass of the test groups (P^d_{10}) of protein minus the carcass protein of the PF group (P^{PF}_0) was asumed to be the total protein retained (TPR). The NPU of each diet (NPUd) was calculated by $NPUd = \frac{TPR}{PI}$.

f) *TPR — Total protein retained* — The protein of the carcass of the test groups at 10o/o of protein (P^d_{10}) minus the carcass protein of the PF group (P^{PF}_0) was assumed to be the total protein retained (TPR):

$$TPR - \frac{P^d_{10} - P^{PF}_0}{}$$

3 – *Minerals* — Determined by spectrophotometry (Perkins Elmer 360) (7), and

4 – *Phosphorus* — Established following the method of the molybdenum blue complex at 700 nm (8).

RESULTS

The average body weight changes and P^d_{10} over 25 days, in rats fed the various 3 to 14o/o protein diets (RB, RBM, RBA, RBMA, RBL, CAS) are shown in Table 4.

TABLE 4

AVERAGE ENERGY INTAKE (EI/d) AND BODY WEIGHT VARIATION (BW) (g) IN RELATION TO PROTEIN OF DIET (Po/o), AND PROTEIN OF THE CARCASS AT 10o/o (P^d_{10}) OF 25 DAYS ON RATS FED DIFFERENT DIETS: RB, RBM, RBA, RBMA, RBL, CAS, COMPARED TO PF VALUES

Diet	EI/d Kcal	Regression of BW/Po/o	r[1]	P^d_{10}(g)[4]
RB[2]	25.4 ± 1.2(a)[3]	BW = 10.64 + 1.60 p(a)	0.96	9.13 ± 0.85(a)
RBM	35.1 ± 3.7(b)	BW = −12.29 + 2.44 p(b)	0.96	11.30 ± 0.95(b)
RBA	28.1 ± 1.8(a)	BW = − 7.62 + 1.51 p(a)	0.93	8.84 ± 0.81(a)
RBMA	33.8 ± 1.9(b)	BW = −23.30 + 4.40 p(c)	0.93	12.40 ± 1.08(b)
RBL	39.7 ± 4.2(c)	BW = −23.70 + 6.08 p(d)	0.98	16.80 ± 1.81(c)
CAS	40.1 ± 2.9(c)	BW = −10.57 + 7.64 p(e)	0.94	23.70 ± 1.81(d)
PF	23.1 ± 1.9(a)	BW = −10.7 g		7.2 ± 0.78(e)

(Mean ± SD).
n = 10.
1 Correlation.
2 Ref. Table 1.
3 Values followed by the same letter = NS; followed by a different letter = $p <$ 0.01 (in column) (t-Student).
4 Protein of the carcass of the test group at 10o/o of protein in the diet.

According to results, L was the best supplement followed by MA, and them by M. As the data reveal, vitamin A was not an effective supplement by itself, but together with M (AM) it was really effective compared to RB alone. Figure 1 shows the body weight variations of the animals fed on the different diets.

The minimums of protein capable of maintaining the body weight were 3.90, 5.38, 5.04, 4.95, 6.65 and 1.38o/o of protein for RBL, RBMA, RBM, RBA, RB and Cas, respectively (which correspond approximately to 0.39, 0.54, 0.50, 0.49, 0.66 and 0.14 g/day/rat). Figure 2 illustrates the regression lines of carcass protein for the different groups. By extrapolating the correspondent regression of Cas, we calculated the protein of the carcass of the animals fed on a protein-free diet (P^{Cas}_{O}). The carcass

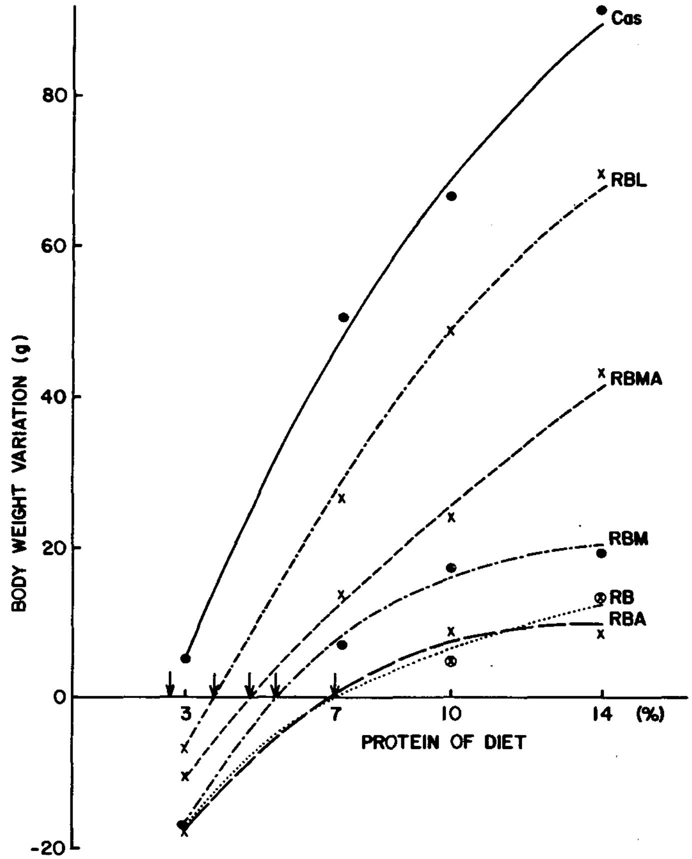

FIGURE 1

Body weight evolution (g) of rats fed diets RB, RBMA, RBM, RBL, RBA, CAS for 25 days and protein (o/o) (for details see Table 4)

protein of the animals fed on PF diet (P_0^{PF}) were compared to the calculated P_0^{Cas}.

P_0^{Cas} (8.72 $\pm$0.58) was significantly higher than P_0^{PF} (7.2 $\pm$ 0.78).

The values of RPV, NPU, NPR, PER, growth, FI and MP are depicted in Table 5 in relation to the casein-fed group. There was a significant difference among the groups: RB, RBA $<$ RBM, RBMA $<$ RBL ($p < 0.01$) (Student's "t" test). These data suggest that the best results were obtained with the RBL diet.

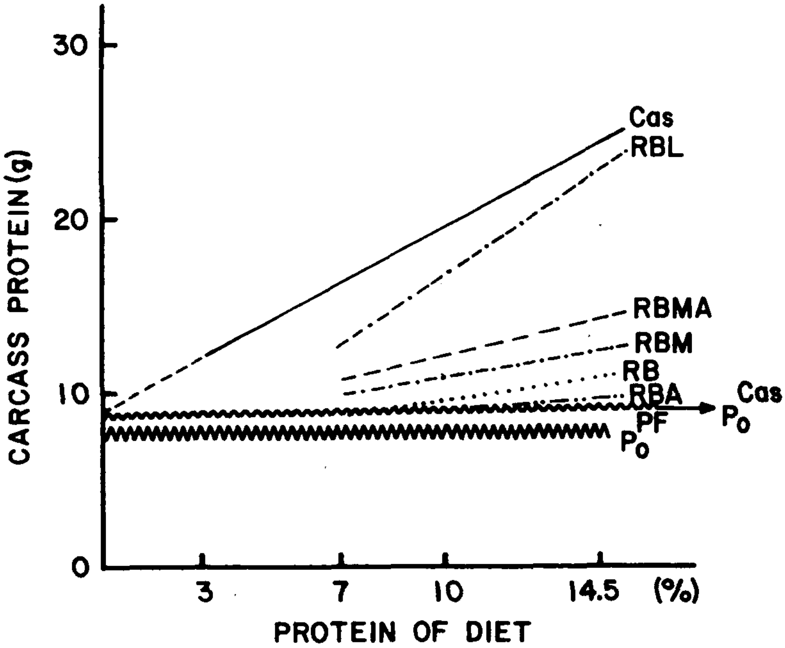

FIGURE 2

Regression lines of the carcass protein of the different group versus protein of diets. Rats fed diets: RB, RBMA, RBM, RBL, RBA, Cas, for 25 days. Values of P_0^{Cas} obtained by extrapolating the Cas regression line is compared to P_0^{PF} obtained with the experimental PF group. Data of P_0^{Cas}, 8.3 $\pm$ 0.58; P_0^{PF}, 7.2 $\pm$ 0.61 ($p < 0.01$).

DISCUSSION

Those animals fed on the rice and beans diet supplemented with milk, gained weight better than those without supplementation. Even other added nutrients such as M, A or MA had less effect than the diets assayed in this study. M and especially L supplements resulted in increased weight gain.

The regression lines of the weight changes versus protein level of the diet (Figure 1) were different for the RB diet itself, or when supplemented, suggesting that RB is effectively limited with respect to some of the minerals contained in the mineral mixture. Other important nutritional factors must be present in L. Vitamin A *per se* had no effect; however, AM had some supplemental effect compared to M alone. In the present study only vitamin A was used as supplement, since its deficiency constitutes a public health problem in Brazil, while vitamin D as food

TABLE 5

RELATIVE NUTRITIVE VALUE OF DIETS* RB, RBM, RBA, RBMA AND RBL DETERMINED FOR BODY WEIGHT (BW), FOOD INTAKE (FI), PER, NPR, RPB, TOTAL PROTEIN RETAINED (TPR), NPU, AND MINIMUM PROTEIN - RELATIVE TO CAS, 100°/o

Diet	Method[1]							
	BW	FI	PER	NPR	RPV	TPR	NPU	MP
CAS	100	100	100	100	100	100	100	100
RBL	73[b(2)]	80[b]	61[b]	72[b]	80[b]	72[b]	50[b]	36[b]
	± 6.8	± 5.1	± 5.8	± 5.8	± 4.2	± 8.2	± 6.8	± 2.1
RBMA	38[c]	56[c]	38[c]	72[b]	57[c]	54[c]	34[c]	26[c]
	± 4.1	± 2.9	± 2.9	± 1.2	± 5.2	± 1.9	± 3.4	± 1.8
RBM	26[d]	54[c]	30[c]	42[c]	32[d]	49[c]	23[c]	28[c]
	± 1.8	± 4.5	± 4.3	± 2.9	± 2.8	± 2.5	± 7.1	± 1.8
RBA	15[e]	40[d]	23[d]	72[b]	14[e]	38[d]	1[d]	26[c]
	± 2.1	± 2.2	± 2.8	± 6.1	± 6.2	± 5.5	± 8.2	± 2.1
RB	2[e]	37[d]	23[d]	40[c]	21[e]	39[d]	5[d]	20[d]
	± 7.4	± 5.4	± 7.1	± 5.6	± 7.2	± 5.9	± 7.2	± 2.9

n = 10.
* See description in Table 2.
1 Determination at 10°/o protein for BW, FI, PER, NPR, TPR, NPU.
2 Followed by different letters in the column - $p < 0.01$ (t-Student).

supplement is not relevant (probably because of our country's sunlight). Concerning vitamins E and K, there are no evidences of deficiencies in our country.

Actually, the increase in protein quantity for improved conditions (RBL, RBMA) enhanced the slopes of BW on P°/o in relation to the zero-point of P°/o, reaching to y-intercept with more negative values as shown in Table 4, because for very low protein levels the intersects of y-axis are different for high or low protein values. Therefore, determinations only at low levels of protein for RB could over-estimate the true quality, perhaps due to the conservation of certain amino acids, especially when in short supply (10). Then, the best measure of protein for growth is the slope between points of protein around the equilibrium.

The protein retained in the carcass is usually determined from the final body protein of each animal, corrected by the carcass nitrogen at the beginning of the experiment (11). It can also be estimated using the final body nitrogen of animals fed on a protein - free diet (12). Miller and Bender (13) introduced the body water determination as an easier method for the calculation of total nitrogen. Bressani *et al* (11) showed that there was a high correlation between the nitrogen content of the carcass and the nitrogen calculated through the water content of the carcass, as follows: $N = (3.09 + 0.032x) \times (H_2O)$, where x = days of life. In our previous studies (not published) the total nitrogen content of the carcass was determined by the Kjeldahl method and compared to the indirect method using the formula (11). The results obtained by both methods were not significantly different.

The fact that the obtained value of P_0^{Cas} was higher than the determined P_0^{PF}, might be explained by the enhancement of the basal metabolism of the animals fed on protein, as compared to those fed on PF. The difference $(P_0^{Cas} - P_0^{PF})$ might correspond to the greater loss of metabolic-endogeneous nitrogen in PF animals in relation to the extrapolated P_0^{Cas}

The MP required for maintenance was quite different among the diets, even considering that the protein quality for all the RB diets was fundamentally the same (obtained from rice and beans).

The differences among the MPs might be explained by the effect of additional factors required for protein utilization. The results suggest that minerals and other nutrients may be insufficient in RB. On the other hand, milk (L) was the best supplement for RB. Since in our previous investigation we observed that casein did not improve the results obtained with RB (3), the effect of milk supplementation may be related to other nutritional factors rather than protein quality.

We calculated the regression equation between TPR/day and PI/day, at 10°/o for all diets (Figure 3), obtaining the following equation: TPR/d = 0.22 + 0.70 x PI/d, with r = 0.82. For all diets with 7°/o and 10°/o protein (RB, RBM, RBA, RBMA, RBL, CAS) the analysis of TPR in relation to food intake (FI) and energy intake (EI) gave the relationships: TPR = 16.1 + 0.106 FI (the FI for maintenance would be 6.4 g/d); TPR = – 16.01 + 0.027 EI (r = 0.80) (The EI for maintenance would be 23.6 Kcal/d). Concerning only the casein groups, 3 to 14°/o of protein, the relation of TPR with FI presented a correlation of r = 0.999. These results suggest that *ad libitum* total food intake plays the primary role in protein retention. Therefore, L, as well as M or MA supplementation in the diet of RB are relevant factors in enhancing food intake by the animals, thus causing increase in the total energy intake.

In a previous paper (14) we suggested that increasing the relative proportion of Ca/Zn beyond 100, an adverse effect occurs, reducing the voluntary food intake and growth in rats.

Prasad (15) states that zinc is involved in many biochemical functions and there exists a competition with other metals as calcium for binding sites, while Yunice and Hsu (16) ovserved that zinc, as well as copper and manganese deficiency affect adversely the endocrine glands, either through decreasing dietary intake, or increasing excretion, and dietary intake can be influenced by several factors in the diet, such as the presence of phytic acid, fiber and high calcium intake.

Matuba *et al.* (17) showed that total zinc contents in the tibia of rats were decreased when calcium content in the diets was increased. On the other hand, Franz *et al.* (18) demonstrated that the addition of

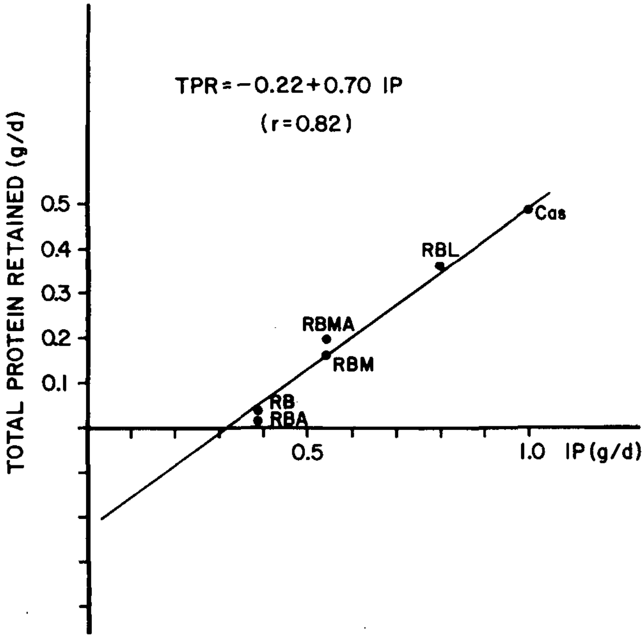

FIGURE 3

Regression equation between TPR/d and PI/d at 10°/o, for diets: RB, RBM, RBMA, RBA and CAS

zinc to a basal diet of rats (up to 14 ppm) enhanced the voluntary food intake.

In the diets used in this investigation, the Ca/Zn relationships for RB, RBL and RBM were 25, 102 and 175, respectively. It is possible that the better results obtained with RBL than with RBM are related to the proportion and quantity of Ca/Zn, around 100. Aside from this, the Ca/P relationship in RBL, RBM and RB were, respectively: 0.85, 0.94 and 0.27. Consequently, RB presented a very bad situation for calcium utilization (19).

The presence of vitamin A improved the effect of M supplementation, enhancing perhaps the body's mineral utilization, not by stimulating appetite.

Although the present results were determined in animals, the implication of these results is that the basic food system RB is deficient in nutrients. In extrapolating these conclusions to man, we suggest that populations consuming basically RB may require more attention in institutional nutrition supplementation programs.

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RESUMEN

ARROZ Y FRIJOLES – EFECTO DE LA LECHE, MINERALES Y VITAMINA A EN LA GANANCIA PONDERAL, INGESTA DE ALIMENTO Y RETENCION CORPORAL DE PROTEINA

Ratas recién destetadas fueron alimentadas con dietas de: arroz/frijoles (RB) suplementadas o no con minerales (M); vitamina A (A); M + A (MA), o leche (L), que contenían de 3 a 14º/o de proteína. Según se determinó a través de diversas mediciones, los mejores resultados se observaron en el grupo alimentado con la dieta RB suplementada con L, seguido por los grupos que recibieron las dietas MA y M. La utilización proteínica de la dieta de RB, así como el requerimiento mínimo de proteína para mantenimiento de peso corporal, depende de los nutrientes suplementarios. Puede que el mecanismo de este efecto esté relacionado con el apetito de los animales y con la ingesta energética voluntaria, la que es influenciada y regulada por la composición de la dieta.

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