Reprinted from JOURNAL OF FOOD SCIENCE, 1963, Vol. 28, No. 5, Pages 611–615

Nitrogen Balance of Dogs Fed Lime-Treated Corn Supplemented with Proteins and Amino Acids[a, b]

RICARDO BRESSANI AND EMELINA M. DE VILLARREAL[c]
Institute of Nutrition of Central America and Panama (INCAP) Guatemala, C. A.

(Manuscript received November 5, 1962)

SUMMARY

The effect on the nitrogen balance of young dogs of supplementing lime-treated corn with small amounts of black bean flour, skim milk, fish flour, and torula yeast was investigated. Although the diets were kept isonitrogenous, all supplements increased nitrogen retention significantly, as did supplementing with lysine and tryptophan. The gain was most marked for skim milk, fish flour, and torula yeast plus lysine, and of sufficient magnitude to be of practical significance for human feeding. The better the protein quality of the supplement, the greater the decrease in nitrogen retention after its removal.

It is well recognized that lime-treated corn protein is most deficient in the essential amino acids lysine and tryptophan (Bressani, 1960; Bressani *et al.*, 1958; Scrimshaw *et al.*, 1958). It is also well documented that corn is the most important staple of the rural Central American diet (Flores, 1961). Therefore, efforts should be made to find ways of improving the quality of corn protein. Synthetic amino acids are still too costly to be practical for the enrichment of cereal foods. However, the nutritive value of the proteins of lime-treated corn can be improved by supplementing them with other proteins that are rich in its limiting amino acids. This method has the further advantage that the enriched product will contain higher amounts of protein, as well as be improved in protein quality. The present work determines the effect on nitrogen balance of supplementing lime-treated corn with small amounts of lysine and tryptophan, or proteins rich in these two amino acids.

MATERIAL AND METHODS

The lime-treated maize flour was prepared by methods described previously (Bressani *et al.*, 1958). The supplements tested were: skim milk powder (supplied by UNICEF), torula yeast (Lake States Yeast Corporation, Rhinelander, Wisc.), deodorized fish flour (VioBin Corp.) and cooked black bean powder prepared by cooking beans in the autoclave for 10 minutes (Bressani *et al.*, 1962). The effect of adding these foods to lime-treated maize flour was studied by the nitrogen balance method in young mongrel dogs 4–5 months old. The basal diet was: 78% lime-treated corn, 7% corn gluten, 2% mineral mixture (Hegsted *et al.*, 1941), 10% hydrogenated vegetable fat, 1% cod liver oil, and 2% cornstarch. Three ml of a complete vitamin solution (Manna and Hauge, 1953) were added per 100 g of diet. The average nitrogen content of the basal diet was 1.77% with a calculated calorie content of 425 cal/100 g.

The nitrogen content of the corn gluten was 7.60%, of the skim milk 4.48%, of torula yeast 8.00% and of the fish flour 12.14%. The protein and amino acid supplements, added to the basal diet, replaced part or all of the nitrogen from corn gluten (supplied by Dr. E. L. Powell, American Maize Products Co., Roby, Ind.), and the composition of the diet was adjusted to 100% with cornstarch. Thus, all diets were approximately isonitrogenous and isocaloric.

The amounts added were 5% skim milk, 3% torula yeast and 4% fish flour. The amounts of lysine and tryptophan added to the diet were equal to the quantities found in 5% skim milk or in 3% torula yeast. The cooked dehydrated black beans contained an average of 3.44% nitrogen and were fed in two periods in an amount equivalent to about 32% and 22% of the daily nitrogen intake of the dog respectively. This quantity of beans represents the range in

[a] This investigation was supported by grant A-3811 from the National Institutes of Health.

[b] INCAP Publication I-235.

[c] Part of thesis by the junior author for a degree in Chemistry and Pharmacy from the Universidad Autónoma de El Salvador, El Salvador, C. A.

daily family consumption in Guatemala (Flores and García, 1960; Flores, 1961). The diets were fed for 10 days, allowing two days for adaptation and the remaining eight for two balance periods of 4 days each.

Four series of studies were carried out. The two dogs used in the first weighed an average of 4.71 kg at the start and 7.32 kg at the end of the series. The diets fed were: basal plus 5% skim milk, basal plus 0.17% L-lysine plus 0.025% DL-tryptophan, basal plus black bean flour, basal plus 5% skim milk, and basal plus 5% skim milk plus black bean flour, with basal diet feedings in between each supplement feeding. Three dogs were used in the second series of experiments in which the sequence of diets tested was: basal plus skim milk, basal plus 3% torula yeast, basal plus 3% torula yeast plus 0.14% L-lysine HCl, with basal diet feeding in between supplement feedings. The average weight of the animals was 7.00 kg at the start and 8.01 kg at the end of the series.

In the third series of tests 3 dogs were also used, weighing 5.06 kg at the start and 7.28 kg at the end of the series. The order of diets fed was: basal plus 5% skim milk, basal plus 3% torula yeast, and basal plus 4% fish flour, with basal diet feedings in between the other treatments. In the fourth series of experiments, 4 animals were fed the basal diet plus 5% skim milk, basal and basal plus 4% fish flour. The average weight at the start was 6.36 kg and 6.79 kg at the end of the experiment.

Protein and calorie intakes differed with each series of experiments but remained as constant as possible within each series. Feeding was carried out twice daily at 8:00 a.m. and 4:00 p.m., and water was available at all times. Feces and urine were collected twice a day and stored at 4°C until analysis was performed. Urine was collected with 1 cm^3 of concentrated acetic acid. The feces and urine were pooled every four days and after homogenizing they, as well as all diets fed, were analyzed for nitrogen by the Kjeldahl method.

RESULTS

Table 1 summarizes nitrogen-balance results of the first series of tests. The addition of 5% skim milk powder increased nitrogen retention significantly over the values obtained with the basal diet. The addition of 0.17% L-lysine plus 0.025% DL-tryptophan, the quantities of these two amino acids found in 5% skim milk, also improved nitrogen retention significantly; in fact, to the same level reached with skim milk. The addition of beans increased nitrogen retention, but supplementation with both skim milk and beans brought further improvement. The retentions of nitrogen decreased markedly and sometimes became negative with a return to the basal diet after feeding the basal plus any of the supplements.

Table 2 shows the results of individual periods as well as averages per treatment of the second series of experiments. The addition of skim milk again improved nitrogen retention significantly. The additions of torula yeast and torula yeast plus

Table 1. Nitrogen balance of dogs fed lime-treated corn supplemented with skim milk, lysine and tryptophan and black beans.[a]

Diet	Nitrogen						Average change in NR/NI [b] to basal diet	
	Intake	Fecal	Urine	Retained	Absorbed	Retained		
	mg/kg/day				% of intake		Before	After
Basal	578	165	398	15	71.4	2.6	....	....
B + 5% skim milk	643	179	327	137	72.2	21.3	.187	.283
Basal	560	194	405	— 39	65.3	— 7.0	....	....
B + L-lysine HCl + DL-tryptophan [c]	611	197	283	131	67.7	21.4	.284	.153
Basal	520	189	299	32	63.6	6.1	....	....
B + black beans [d]	635	254	250	131	60.0	20.6	.145	.302
Basal	394	143	289	— 38	63.7	— 9.6	..	....
B + 5% skim milk	457	133	215	109	70.9	23.8	.334	.189
Basal	370	124	228	18	66.5	4.9	....	....
B + 5% skim milk + black beans [e]	569	193	205	171	66.1	30.0	.251	....

[a] Average values from 2 dogs and 2 balance periods per dog per treatment.
[b] Nitrogen retention/nitrogen intake.
[c] Amino acid levels used: 0.17% L-lysine HCl and 0.025% DL-tryptophan.
[d] Amount of black bean flour fed equivalent to 32% of total nitrogen intake (weight/day 40–44 g).
[e] Amount of black bean flour fed equivalent to 22% of total nitrogen intake (weight/day 24–28 g).

Table 2. Nitrogen balance of dogs fed lime-treated corn supplemented with skim milk, torula yeast and torula yeast plus lysine.[a]

		Nitrogen						Average change in NR/NI[b] to basal diet	
		Intake	Fecal	Urine	Retained	Absorbed	Retained		
	Period	mg/kg/day				% of intake		Before	After
Basal	1	349	83	228	38	76.2	10.9	....	....
B + 5% skim milk	1	420	104	204	112	75.2	26.7	....	....
	2	416	103	172	141	75.2	33.9	....	....
	$\bar{x}$	418	103	188	127	75.3	30.4	0.195	0.284
Basal	1	425	135	323	— 33	68.2	— 7.8	....	....
	2	392	102	242	48	74.0	12.2	....	....
	$\bar{x}$	408	118	282	8	71.1	2.0	....	....
B + 3% torula yeast	1	447	131	219	97	70.7	21.7	....	....
	2	469	139	213	117	70.4	24.9	....	....
	$\bar{x}$	458	135	216	107	70.5	23.4	0.214	0.085
Basal	1	387	133	202	52	65.6	13.4	....	....
	2	309	100	157	52	67.6	16.8	....	....
	$\bar{x}$	348	117	179	52	66.7	14.9	....	....
B + 3% torula yeast + 0.14% L-lysine HCl	1	344	128	131	85	62.8	24.7	....	...
	2	341	117	134	90	65.7	26.4	....	...
	$\bar{x}$	343	122	132	89	64.4	25.9	0.110	0.175
Basal	1	307	112	186	9	63.5	2.9	....	....
	2	314	100	171	43	68.1	13.7	....	....
	$\bar{x}$	310	106	178	26	65.8	8.4	....	....

[a] Average per period of 3 dogs.
[b] Nitrogen retention/nitrogen intake.

0.14% L-lysine HCl as well as the amount of lysine found in 3% torula yeast, also increased nitrogen balance significantly, but the values were lower than that from skim milk addition. The torula-lysine addition was slightly more effective than torula alone in increasing nitrogen retention. Feeding of the basal diet after any supplemented period caused a decrease in nitrogen retention. The decrease was greatest after skim milk, followed by torula yeast plus lysine and torula yeast alone. This is indicated by the change in nitrogen retention/nitrogen intake upon adding and withdrawing the supplement.

Table 3 summarizes the average results obtained in the third series of experiments. The addition of skim milk and of torula yeast, increased nitrogen balance significantly, the increase being greater with skim milk, followed by the torula yeast. The fish flour supplement increased retention of nitrogen in the first period only, but the lower retention observed in the second was probably due to a decrease in nitrogen intake. Food intake with the basal diet was not maintained at a constant level, because the animals did not consume all that was offered, particularly at the end of the series. As before, nitrogen balance was decreased by feeding of the basal diet after any of the supplements and was lowest after skim milk.

Table 4 presents the results of the final series of studies. The addition of both skim milk and 4% fish flour significantly improved the nitrogen retention of dogs fed lime-treated maize flour. In this study, retention of nitrogen was superior for the fish flour supplement.

DISCUSSION

The results corroborate observations made with rats in which lime-treated corn flour was enriched with proteins of animal and vegetable origin (Bressani and Marenco, 1962; Bressani *et al.*, 1960). The improvement in nutritive value made by the protein added, as indicated by the increases in nitrogen retention, is probably due mainly to the contribution of lysine and tryptophan, the most limiting amino acids in lime-treated corn (Bressani, 1960; Bressani *et al.*, 1958; Scrimshaw *et al.*, 1958).

While it is true that nitrogen retention varies proportionally with nitrogen intake and that, in the experiments reported there was some variation in intake, this is not the primary cause of the effects noted. Nitrogen intake, although adjusted accord-

Table 3. Nitrogen balance of dogs fed lime-treated corn supplement with skim milk, torula yeast and fish flour.[a]

		Nitrogen						Average change in NR/NI [b] to basal diet	
		Intake	Fecal	Urine	Retained	Absorbed	Retained		
	Period	mg/kg/day				% of intake		Before	After
Basal	1	721	179	460	82	75.2	11.4	....	....
	2	738	188	437	113	74.5	15.3	....	....
	$\bar{x}$	729	183	448	98	74.9	13.4	....	....
B + 5% skim milk	1	813	179	350	284	78.0	34.9	....	....
	2	796	138	294	364	82.7	45.7	....	....
	$\bar{x}$	804	158	322	324	80.3	40.3	0.269	0.207
Basal	1	684	151	397	136	77.9	19.9	....	....
	2	665	159	378	128	76.1	19.2	....	....
	$\bar{x}$	674	155	387	132	77.0	19.6	....	....
B + 3% torula yeast	1	664	121	274	269	81.8	40.5	....	....
	2	684	159	307	218	76.7	31.9	....	....
	$\bar{x}$	674	140	290	244	79.2	36.2	0.166	0.117
Basal	1	552	135	308	109	75.5	19.7	....	....
	2	526	124	249	153	76.4	29.1	....	....
	$\bar{x}$	539	129	278	132	76.1	24.5	....	....
B + 4% fish flour	1	516	165	185	166	68.0	32.2	....	....
	2	393	142	180	71	63.9	18.1	....	....
	$\bar{x}$	454	153	183	118	66.3	26.0	0.015	....

[a] Average per period of 3 dogs.
[b] Nitrogen retention/nitrogen intake.

ing to the weight gain of the animals, could not be kept constant without force feeding, because the animals refused to consume all of the calculated unsupplemented diet. As suggested by Harper (1957–58), it may be harmful to the animal to consume large amounts of deficient proteins. Care should be taken in suggesting increased consumption of protein with amino acid deficiencies since the refusal of the unsupplemented diet is probably a defense mechanism.

Except for the bean supplement the decrease in nitrogen balance after removal of the supplement was greater the better the protein quality of the supplement. For example, the protein quality of skim milk is superior to that of torula yeast plus lysine and both are superior to torula yeast alone. Although torula yeast is a good source of lysine, only 80% is biologically available; this would account for the better quality when supplemented with lysine.

Table 4. Nitrogen balance of dogs fed lime-treated corn supplemented with skim milk and fish flour.[a]

		Nitrogen					
		Intake	Fecal	Urine	Retained	Absorbed	Retained
Diet	Period	mg/kg/day				% of intake	
Basal + 5% skim milk	1	472	103	275	94	78.2	19.9
	2	469	132	270	67	71.8	14.3
	3	497	132	269	96	73.4	19.3
	$\bar{x}$	479	122	271	86	74.5	17.9
Basal	1	491	125	323	43	74.5	8.7
	2	498	129	291	78	74.1	15.7
	$\bar{x}$	494	127	307	60	74.3	12.1
Basal + 4% fish flour	1	479	109	221	149	77.2	31.1
	2	481	110	216	155	77.1	32.2
	$\bar{x}$	480	109	218	153	77.3	31.9

[a] Each period represents the average of four dogs.

Although black beans are of a lower protein quality than skim milk or torula yeast, the findings with this supplement after its omission from the basal diet did not follow the pattern observed with skim milk, torula yeast with and without lysine. This was probably because of the larger increase in nitrogen intake when beans were fed and the significant decrease in intake when they were omitted. These results deserve further study because the rural populations in many areas in Latin America consume mainly corn and beans.

Nutritional surveys carried out in preschool children in Guatemala (Flores and García, 1960) have shown that some animal protein is consumed every two or three days, and in some cases every day. The effect of this sporadic supplement is not well known though it is assumed to be good. However, as indicated by the dog data, the change might be of little or no benefit to the extent that it reduces subsequent food consumption. The results are of practical importance for populations consuming diets which are poor on both quantity and quality of protein since lime-treated corn is the most important staple food in the rural diet of most Central American countries (Flores, 1961; Flores and García, 1960). Efforts should be made to find effective ways of improving its protein contribution.

REFERENCES

Bressani, R. 1960. Optimum levels of amino acid supplementation for lime-treated corn. *Federation Proc.* **19**, 320.

Bressani, R., and E. Marenco. 1962. The enrichment of lime-treated corn flour with proteins, lysine and tryptophan and vitamins. *J. Agr. Food Chem.* (submitted).

Bressani, R., and N. S. Scrimshaw. 1958. Lime-heat effects on corn nutrients. Effect of lime-treatment on *in vitro* availability of essential amino acids and solubility of protein fractions in corn. *J. Agr. Food Chem.* **6**, 774.

Bressani, R., N. S. Scrimshaw, M. Béhar, and F. Viteri. 1958. Supplementation of cereal proteins with amino acids. II. Effect of amino acid supplementation of corn-masa at intermediate levels of protein intake on the nitrogen retention of young children. *J. Nutrition* **66**, 501.

Bressani, R., E. Marenco, and A. T. Valiente. 1960. Enrichment of lime-treated corn flour with animal and vegetable proteins and with amino acids. Abstr. Fifth Intern. Congr. on Nutrition, Sept. 1–7, 1960. Washington, D. C. p. 59.

Bressani, R., L. G. Elías, and A. T. Valiente. 1962. The effect of cooking and of amino acid supplementation on the nutritive value of black beans (*Phaseolus vulgaris*, L.). *Brit. J. Nutrition* **17**, 69.

Flores, M. 1961. Food patterns in Central America and Panama. In: "Tradition Science and Practice in Dietetics." Proc. 3rd Intern. Congr. of Dietet., London, July 10–14, 1961, p. 23–27.

Flores, M., and B. García. 1960. The nutritional status of children of pre-school age in the Guatemalan community of Amatitlán. 1. Comparison of family and child diets. *Brit. J. Nutrition* **14**, 207.

Harper, A. E. 1957–58. Balance and imbalance of amino acids. *Ann. N. Y. Acad. Sci.* **69**, 1025.

Hegsted, D. M., R. C. Mills, C. A. Elvehjem, and E. B. Hart. 1941. Choline in the nutrition of chicks. *J. Biol. Chem.* **138**, 459.

Manna, L., and S. M. Hauge. 1953. A possible relationship of vitamin B_{12} to orotic acid. *J. Biol. Chem.* **202**, 91.

Scrimshaw, N. S., R. Bressani, M. Béhar, and F. Viteri. 1958. Supplementation of cereal proteins with amino acids. I. Effect of amino acid supplementation of corn-masa at high levels of protein intake on the nitrogen retention of young children. *J. Nutrition* **66**, 485.

Printed in the United States of America