

# TRABAJOS DE INVESTIGACION

# **Nutritional factors affecting the consumption of leguminous seeds**

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## **SUMMARY**

For the purpose of stimulating consumption of leguminous seeds, several nutritional factors that affect their intake were studied. Biological trials were carried out in rats, offering them the opportunity to choose between corn and beans. Consumption of both corn and beans was recorded separately, in order to obtain the corn to beans ratio. Results indicate that without the addition of nutrients, the corn to bean ratio was 3.6:1; this proportion is significantly better than that normally consumed by people in the rural areas of Guatemala, which is 7.3:1. The optimum ratio for the higher protein complementation found previously by the "mixed feeding" technique was 2.6. The addition of other nutrients enhanced the relative consumption of beans, improving the ratio and significantly increasing the utilizable protein. Consumption of beans per se is stimulated by the addition of vitamins, minerals and calories; response to the addition of methionine needs the presence of the other nutrients. The dietary treatment applied to corn also effects favorably the consumption of beans.

The use of cowpea instead of black beans followed the same tendency, except that the protein quality of the diets was higher. It was also found that protein digestibility or lysine availability could not account for all of the improvements observed. It is therefore concluded that availability of leguminous seeds is an important factor that possibly impairs a higher consumption of this food commodity.

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## INTRODUCTION

From the nutritional point of view, leguminous seeds are a very important staple food in the diet of many people of various areas of the world (1). In Latin America beans hold an outstanding position because this food forms part of the cultural pattern of its people, and also because of its value as a protein source. Recently, great interest has arisen in regard to the more efficient utilization of this food item, since it could be of significant help in meeting protein needs. This is true provided their consumption could be increased, their protein quality improved, and if they would not cause harmful or adverse physiological effects (2, 3).

The absolute amounts of corn and beans consumed in some Central American countries indicate that bean intake is relatively low as compared to corn. This fact explains the low nutritive value of this habitual diet in terms of protein quality and quantity (4). Although the ratio of corn to beans varies according to the dietary habits of each country, it can be concluded that it is high; in Guatemala, for example, the usual diet contains 7.3 parts of corn to one of beans (4). The best ratio found from experimental data is about 2.6 to 1 (5, 6); this corresponds to a mixture where 50% of the protein is contributed by corn, and 50% by beans. In terms of weight, this represents 72 g of corn and 28 g of beans.

These results have been explained on the basis that in this combination, beans partially provide the amounts of lysine and tryptophan that limit corn protein quality, while corn contributes part of the methionine in which beans are deficient.

The above-mentioned data indicate that the actual human population bean intake is below that which could result in a better protein-quality diet.

It has been suggested that the low digestibility of beans, and the flatulence caused by their ingestion, are the responsible factors for the relatively low amounts of this food consumed by people subsisting on cereal-legume diets (1-3).

The question we attempted to answer, assuming that availability was not a limiting factor, was to determine the existence of other factors responsible for the relatively low intake of beans by humans. Tests were thus carried out in ex-

perimental animals, bearing in mind the probability that information derived from these studies, would be applicable to humans.

## MATERIALS AND METHODS

The leguminous seeds used in all experiments were a variety of black beans (*Phaseolus vulgaris*, L.), and a variety of cowpea (*Vigna sinensis*); both cultivars were grown at INCAP's Experimental Farm in Guatemala. The raw material was stored at 4°C until used.

A cooked bean flour from each legume grain studied was prepared according to the technique previously described (7) and stored under refrigeration until needed. Lime-treated corn flour was then prepared from corn, following procedures already described (8).

Young rats, of the Wistar strain from the INCAP colony, 21 days of age, were used as the experimental animals. Several groups were then formed, of 8 rats from both sexes, each one. The mean initial weight of the animals ranged from 40 to 50 g, and the mean weight among the various groups differed by not more than 1 g. They were placed in individual all-wire screen cages with raised screen bottoms. A "separate feeding" technique was used in all the growth experiments, as follows. The animals were allowed to eat as they wished from two feeder cups placed in the cage, one containing corn, and the other cooked beans or cowpea. As shown under "Results", the corn and legume grain diets were modified by adding vitamins (9), minerals (10), additional calories as 5% refined vegetable oil, and their respective limiting amino acids. In the case of corn, 0.30 g% of L-lysine HCl and 0.10% of L-tryptophan were added; bean protein was supplemented with 0.30 g % of DL-methionine. This was done so that the rat could choose the food prepared to make it more suitable from the nutritional standpoint. Intake from each cup, as well as weight gain were recorded weekly for a period of 28 days. Animals had free access to water at all times.

The protein efficiency ratio and the percentage of utilizable protein were calculated from the protein content in each diet and their individual intakes. Thirty-two-day-old rats were used for the protein digestibility studies. Nitrogen deter-

minations in the diets and feces were done by the Kjeldhal method (11). Available lysine was determined according to the procedure described by Conkerton and Frampton (12), and *in vitro* digestibility was performed through digestion with pepsin in an acid solution (11).

## RESULTS

Table 1 summarizes the results obtained in the first experiment; as the data reveal, when the animals were fed corn and beans without any dietary treatment (Group No. 1) the ratio of corn to beans was 3.58, with a utilizable protein of 3.11. PER values are not shown because they were calculated from diets with different protein content, parameter which would affect this method of protein evaluation. Instead, results are expressed as utilizable protein, a measurement for both quality and quantity of this nutrient.

When both foods were individually supplemented with a mixture of vitamins, minerals and additional calories (5% refined soybean oil), the ratio was similar to that of the control group (N<sup>o</sup> 1), but the utilizable protein was significantly higher. In this case, intake of both foods increased, with corn consumption augmenting proportionally more than that of beans with respect to the control diet.

The addition of the three groups of nutrients to beans only, stimulated significantly both bean and corn intakes. In this case, the ratio obtained was 2.08, with a utilizable protein of 6.24. Although to a lesser degree, the same effect was observed when only corn was supplemented. Here, the results obtained were due more to an increased bean intake than to that of corn, when compared with the control group and the other groups included in the study.

The results obtained by improving the protein quality of corn and beans are shown in Table 2. It is interesting to note that the individual addition of lysine and tryptophan to corn, and of methionine to beans, did not change the consumption of these two foods as compared with the animal groups fed the corn and bean diets free of amino acid supplementation. As a matter of fact, a slight decrease in individual food intakes was observed.

TABLE 1  
EFFECT OF VARIOUS DIETARY TREATMENTS ON THE FREE INTAKE OF MAIZE AND BEANS  
(PHASEOLUS VULGARIS L.) FED TO YOUNG RATS

| Group<br>N <sup>o</sup> | Food  | Dietary treatment    | Intake        |       | Average<br>weight gain<br>g/28 days | Utilizable<br>protein***<br>% |
|-------------------------|-------|----------------------|---------------|-------|-------------------------------------|-------------------------------|
|                         |       |                      | g/rat/28 days | Ratio |                                     |                               |
| 1                       | Maize | —                    | 188 ± 12.9*   | 3.58  | 27 ± 1.8*                           | 3.11                          |
|                         | Beans | —                    | 53 ± 10.7     |       |                                     |                               |
| 2                       | Maize | Vit. + Min. + Cal.** | 238 ± 13.6    | 3.87  | 58 ± 5.2                            | 5.15                          |
|                         | Beans | Vit. + Min. + Cal.   | 61 ± 6.1      |       |                                     |                               |
| 3                       | Maize | —                    | 257 ± 14.6    | 2.08  | 89 ± 7.6                            | 6.24                          |
|                         | Beans | Vit. + Min. + Cal.   | 124 ± 12.5    |       |                                     |                               |
| 4                       | Maize | Vit. + Min. + Cal.   | 250 ± 16.5    | 2.65  | 80 ± 5.9                            | 6.19                          |
|                         | Beans | —                    | 94 ± 8.0      |       |                                     |                               |

\* Standard error.

\*\* Vitamins + Minerals + Calories.

\*\*\* Protein content in the diet x relative nutritive value to casein, as measured by the PER method.

**TABLE 2**  
**EFFECT OF VARIOUS DIETARY TREATMENTS ON THE FREE INTAKE OF MAIZE AND BEANS**  
**(PHASEOLUS VULGARIS L.) FED TO YOUNG GROWING RATS**

| Group<br>N <sup>o</sup> | Food                       | Dietary treatment                   | Intake        |       | Average<br>weight gain<br>g/28 days | Utilizable<br>protein<br>% |
|-------------------------|----------------------------|-------------------------------------|---------------|-------|-------------------------------------|----------------------------|
|                         |                            |                                     | g/rat/28 days | Ratio |                                     |                            |
| 1                       | Maize                      | —                                   | 188 ± 12.9*   | 3.58  | 27 ± 1.8*                           | 3.11                       |
|                         | Beans                      | —                                   | 53 ± 10.7     |       |                                     |                            |
| 2                       | Maize                      | Lys. + Try.                         | 184 ± 17.7    | 3.86  | 32 ± 1.8                            | 3.81                       |
|                         | Beans                      | Met.                                | 47 ± 12.0     |       |                                     |                            |
| 6                       | Maize                      | Lys. + Try. + Vit.<br>+ Min. + Cal. | 272 ± 10.7    | 2.26  | 98 ± 7.0                            | 6.68                       |
|                         | Beans                      | Met. + Vit. + Min.<br>+ Cal.        | 120 ± 13.6    |       |                                     |                            |
| 7                       | Maize                      | Lys. + Try. + Vit.<br>+ Min. + Cal. | 291 ± 11.3    | 2.94  | 80 ± 6.1                            | 6.10                       |
|                         | Beans                      | Vit. + Min. + Cal.                  | 59 ± 3.6      |       |                                     |                            |
| 8                       | Maize                      | Vit. + Min. + Cal.                  | 256 ± 13.9    | 2.28  | 90 ± 6.7                            | 6.55                       |
|                         | Beans                      | Met. + Vit. + Min.<br>+ Cal.        | 112 ± 9.5     |       |                                     |                            |
| 9                       | Maize                      | —                                   | 199 ± 20.5    | 1.03  | 134 ± 9.7                           | 9.12                       |
|                         | Casain diet<br>(22% prot.) | Vit. + Min. + Cal.                  | 194 ± 20.7    |       |                                     |                            |

\* Standard error.

**TABLE 3**  
**EFFECT OF VARIOUS DIETARY TREATMENTS ON THE FREE INTAKE OF MAIZE AND COWPEA**  
**(VIGNA SINENSIS) FED TO YOUNG GROWING RATS**

| Group<br>N <sup>o</sup> | Food    | Dietary treatment                   | Intake        |       | Average<br>weight gain<br>g/28 days | Utilizable<br>protein<br>% |
|-------------------------|---------|-------------------------------------|---------------|-------|-------------------------------------|----------------------------|
|                         |         |                                     | g/rat/28 days | Ratio |                                     |                            |
| 1                       | Maize   | —                                   | 207 ± 14.2*   | 4.00  | 32 ± 3.4*                           | 3.29                       |
|                         | Cowpea  | —                                   | 52 ± 7.4      |       |                                     |                            |
| 2                       | Maize   | Vit. + Min. + Cal.                  | 292 ± 12.3    | 3.28  | 104 ± 5.9                           | 7.29                       |
|                         | Cowpea  | Vit. + Min. + Cal.                  | 89 ± 7.2      |       |                                     |                            |
| 5                       | Maize   | Lys. + Try.                         | 220 ± 18.9    | 5.50  | 42 ± 2.9                            | 4.34                       |
|                         | Cowpea  | Met.                                | 40 ± 9.2      |       |                                     |                            |
| 6                       | Maize   | Lys. + Try. + Vit.<br>+ Min. + Cal. | 340 ± 24.4    | 3.50  | 120 ± 6.7                           | 7.36                       |
|                         | Cowpea  | Met. + Vit. + Min.<br>+ Cal.        | 98 ± 16.7     |       |                                     |                            |
| 9                       | Maize   | —                                   | 253 ± 22.3    | 1.70  | 116 ± 10.3                          | 7.64                       |
|                         | Soybean | Vit. + Min. + Cal.                  | 153 ± 17.8    |       |                                     |                            |

\* Standard error.

**TABLE 4**  
**PROTEIN QUALITY OF DIETS BASED ON MAIZE-BLACK BEANS AND MAIZE-COWPEA**  
**AS SELECTED BY YOUNG GROWING RATS**

| Food   | Dietary treatment        | Intake        |       | Average weight gain, g/28 days | Utilizable protein % | Protein digestibility |               | Available lysine g % | Digestible lysine*** % |
|--------|--------------------------|---------------|-------|--------------------------------|----------------------|-----------------------|---------------|----------------------|------------------------|
|        |                          | g/28 days/rat | Ratio |                                |                      | In vitro*             | In vivo       |                      |                        |
| Maize  | Vit. +<br>Min. +<br>Cal. | 238 ± 13.6**  | 3.87  | 58 ± 5.2**                     | 5.15                 | 74.0                  | 71.5 ± 0.69** | 0.454                | 78.8                   |
| Beans  | —                        | 61 ± 6.1      |       |                                |                      |                       |               |                      |                        |
| Maize  | Vit. +<br>Min. +<br>Cal. | 292 ± 12.3    | 3.28  | 104 ± 5.9                      | 7.29                 | 78.1                  | 74.5 ± 0.57   | 0.454                | 82.6                   |
| Cowpea | —                        | 89 ± 7.2      |       |                                |                      |                       |               |                      |                        |

\* Digestion with an acid pepsin solution.

\*\* Standard error.

\*\*\*  $\frac{\text{Lysine intake} - \text{Lysine excreted in feces}}{\text{Lysine intake}} \times 100$ .

Data in Table 2 reveal that when both amino acids and other nutrients were added to the two foods, a higher intake of both occurred, resulting in a 2.26:1 ratio and a utilizable protein of 6.68. Stimulation of corn or beans intake can be achieved through individual improvement of the protein quality of each food in the presence of other nutrients; this can be appreciated in Groups N<sup>o</sup> 7 and N<sup>o</sup> 8. It is of interest to observe, however, that addition of the limiting amino acids stimulates bean intake, as when beans are supplemented, a measure that induces an increase in both bean and corn intakes.

Based on these results, it seems that when allowed to choose its own food, the rat does so by balancing the quality of the ingested protein.

The last group was allowed to eat corn and a casein diet prepared to simulate beans in regard to its protein content. As compared with the other groups, this group gave the best performance in every measure.

Table 3 summarizes the results obtained with cowpea (*Vigna sinensis*), species that is known to have a higher protein quality than black beans (13). As the data reveal, the ratio of corn to cowpea follows the same trend observed in the previous experiments (see Tables 2 and 3). However, in those cases where consumption of cowpea was stimulated, higher weight gains and utilizable protein were obtained when comparing them with those induced by black beans. When soybean flour was utilized instead of cowpea, the results showed still higher values for utilizable protein, a finding that was to be expected due to its better protein quality. An important observation in this experiment was that cowpea intake did not reach higher levels than those obtained with black beans. However, in the cowpea experiment, weight gains surpassed those of the bean study. This finding probably indicates that other factors are also responsible for limiting the consumption of *Phaseolus vulgaris*. In order to determine if protein digestibility was the factor that induced the highest response obtained with cowpea, another experiment was carried out. Results of this study are shown in Table 4, and again indicate that the ratio of corn to beans is similar in both cases. Despite this finding, the differences in weight gain and utilizable protein obtained were significant.

tly higher with the cowpea diet, although protein digestibility and lysine availability were not significantly greater for the cowpea diets.

## DISCUSSION

The results obtained in the first experiment, related to the animals fed corn and beans without any dietary treatment, are of interest. The animals, in this case, tried to balance the protein quality of the diet ingested, since the ratio obtained was close to 2.6:1, a figure that corresponds to the optimum ratio found when corn and beans were offered in mixed diets (5, 6). Calculation of the ingested limiting amino acids of those two protein foods corroborate this aspect, since the amount of lysine, tryptophan and total sulfur amino acids was found to be close to the requirements of the rat, expressed as mg of amino acid per gram of nitrogen, or as a percentage of the diet. The higher protein utilization observed when both foods were supplemented with vitamins, minerals and calories was expected, considering that the presence of these nutrients stimulates a higher intake of corn and beans and are also needed for better utilization of the protein absorbed.

It is probable that the increased consumption determined when only beans were supplemented, was due to the fact that the animal tried to fulfill its requirements for the other nutrients, particularly calories. On the other hand, the stimulus observed in the corn intake when the bean intake was increased, may have been caused by the need of the animal to maintain the best ratio of corn to beans, as far as the protein quality is concerned. This effect was also found in the fourth treatment where consumption of corn was similar to that of the previous group, while the intake of beans was lower. The data on the effect of amino acid supplementation deserve important considerations. First of all, it is clear that if an increased intake of beans is to be achieved by improving its protein quality, the addition of the other nutrients is compulsory; this aspect is important not only from the viewpoint of consumption, but also as far as protein utilization of the diets is concerned. However, what is more significant from the practical standpoint, is that corn and bean intake is sti-

mulated by nutrient supplementation, but not by amino acids alone. This is a very important finding, since calculation on the ingestion of the limiting amino acids of this diet, clearly showed that although the amino acid requirements were covered, the protein utilization was of the same order as that observed in the diet without nutrient supplementation. We also deem it of interest to point out that the addition of the deficient amino acids to the two foods, that is, lysine and tryptophan to corn, and methionine to beans, did not produce better effects than those previously observed when lysine and tryptophan were added to a mixed corn-bean diet (14).

The response of the animals fed the casein diets was probably due not only to its highest digestibility as compared with beans, but also to the different amino acid patterns of those two proteins. The better results obtained with cowpea in comparison with beans are difficult to explain. Nevertheless, it is possible that they may be the consequence of the lower concentration of antiphysiological factors in cowpea, in comparison to that of *Phaseolus*. It has recently been shown that even after cooking *Phaseolus* may contain hemagglutinating activity (15).

Since the main purpose of this work was to assess the effect of nutritional factors that influence the consumption of leguminous seeds, several statements can be inferred from the results. First, it was clear that the rat chose its food by balancing the quality of the protein ingested; furthermore, the stimulus in the bean consumption may be different when this food is offered as the only nutrient source. It is also evident that this "stimulus" is not related to the dietary treatment applied both to beans and corn. An additional increment in the bean intake beyond that obtained by means of the dietary treatment, can only be achieved through the identification of other adverse physiological factors present in the leguminous seeds.

These results also show that, when available, rats tend to consume more beans, since in this series of studies, availability was not a factor that reduced intake. Furthermore, in all cases the ratio which could be modified tended towards the best ratio for maize and beans, which is 2.6:1.

Finally, it is also important to note that no adverse effects were observed when the animals ingested more beans.

In the introductory section of this paper, it was stated that one of the objectives of the experimental work described was to determine the presence, in beans, of factors that could limit the intake of this food in humans, provided that beans were available and at low cost. The argument can be advanced that it is difficult to extrapolate data obtained in experimental animals to humans. However, there is evidence (16) which shows that the mixtures of corn and beans that prove to be of optimum quality for humans, are also optimum for the rat when applying techniques similar to those described in the present paper.

#### RESUMEN

##### Factores nutricionales que afectan el consumo de leguminosas

El propósito del presente estudio, efectuado en ratas, fue determinar la posibilidad de inducir en estos animales un mayor consumo libre de frijol y caupí cocidos, a través de estímulos nutricionales agregados a las semillas leguminosas, o al maíz.

Las pruebas biológicas realizadas consistieron en brindar a las ratas la oportunidad de escoger libremente entre el consumo de maíz y frijol, ofrecidos en comederos individuales. El consumo de los dos alimentos se midió individualmente para poder calcular la relación de consumo de cereal a leguminosa, y establecer la forma en que esta relación era influenciada por la adición de otros nutrientes.

Los resultados indicaron que sin el agregado de nutrientes, la relación de consumo de maíz a frijol era de 3.6. Esta proporción es nutricionalmente mejor de la que normalmente ingieren los pobladores del medio rural de Guatemala, en cuyo caso la relación es de 7.3. Usando otros métodos experimentales, se logró establecer que, en ratas, la relación de mayor valor nutritivo era de 2.6.

La adición de otros nutrientes incrementa el consumo de leguminosa, mejorando la relación y aumentando significativamente la cantidad de proteína utilizable. El consumo de leguminosas per se es estimulado por la adición de vitaminas, minerales y calorías; la obtención de una respuesta favorable al agregado de metionina, requiere la presencia de otros nutrientes. Varios tratamientos nutricionales aplicados al maíz indujeron también un incremento en el consumo de frijol.

El uso de caupí en vez de frijol negro, aplicando los mismos tratamientos usados con el *Phaseolus*, siguió las mismas tendencias, salvo que la calidad proteínica de la dieta fue superior a la preparada con frijol. Se

encontró, asimismo, que en este caso, la digestibilidad de la proteína o la disponibilidad de la lisina no podían explicar la superioridad nutricional del caupí. Con base en los hallazgos, se llegó a la conclusión de que la disponibilidad de leguminosas comestibles es un factor importante que posiblemente limita el mayor consumo de este alimento.

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