

Limits for single cell protein utilization in human feeding

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SUMMARY

Purines have been recognized in recent years as a limiting factor in Single Cell Protein (SCP) utilization by humans. Establishment of intake limits is prevented by differences between human purine catabolism and ordinary laboratory animals purine catabolism. Previously established limits have failed in long-term tests applied to a large number of subjects.

The determination of a natural safe limit of intake was presented based on concepts of "normal purine intake" and "maximum normal purine intake" in a homogenous population. Limits presented vary with each human group studied and are called "Natural Limits of Safety" (NLS).

SCP utilization above these limits, without new data, should be a subject of governmental decision involving weighing of possible health hazards against expected benefits.

INTRODUCTION

SCP utilization in human feeding in recent years has been frequently focused. The name SCP (Single-Cell Protein) was adopted in the United States and is intended as "a generic term for crude or refined sources of protein whose origin is unicellular or simple multicellular organism" (1).

It is believed that SCP utilization can contribute to fight protein-calorie malnutrition, a prevalent public health problem in developing countries (1, 2, 3, 4).

Recent papers on SCP have emphasized the high concentration of nucleic acid in these materials and the threats to the

health arising from it, however, a number of papers on this subject, published in Brazil (3, 9, 10, 11, 12, 13) and elsewhere (14, 16, 17, 18) failed to mention these questions.

Limits for human utilization have gained some attention in nutritional bibliography. Modern papers on SCP utilization have included common toxicity tests (14, 15, 18, 19, 20, 21, 22, 23, 24, 25, 42). Almost all experiments, however, have used laboratory animals which have a purine catabolism ending in allantoin. Among ureotelic animals, only a few produce uric acid as the end-product of purine degradation (26, 27, 28). Uricotelic animals would be controversial, because they have uric acid as end-product of protein catabolism too. The Dalmatian dog, an ureotelic animal, is an uric acid excretor only by tubular reabsorption failure, it does have uricase (29). Rarely, animals with purine catabolism similar to human catabolism were used in SCP evaluation. Pokrowsky (19), in a brief comment without further reference, said that Russian researchers used monkeys in toxicity tests on Petro-Yeasts. Calloway (22) mentioned toxicity tests in California, where monkeys and chimpanzees were used in nutritional and toxicity evaluation of SCP; these last experiments have not shown expected agreement between reactions in higher apes and in humans.

Studies using human subjects are increasing (4, 17, 22, 23, 24) and the more recent ones (22, 23, 24) have focused essentially the effects of overload with uric acid precursors.

The Protein Advisory Group (PAG, United Nations), since 1967, has been studying SCP toxicity, particularly, side-effects related to high nucleic acid content (5, 6). In 1968, PAG recommended that an ad hoc working group be established to prepare reports and policy guides for consideration at next PAG meetings (30). This ad hoc working group, prepared in June 1970, the publication of a PAG Statement N° 4 on SCP (31) where limits for human utilization are proposed. This statement was based, specifically on two documents (22, 23).

According to Tannembaum (1), "there appears to be a ceiling of safe intake of nucleic acids which will not lead to excessive concentration of blood uric acid or excretion of urinary uric acid". The ceiling of safe intake of nucleic acids appears to guide the previously mentioned documents that were used as basis for the PAG Statement on SCP.

MATERIAL AND METHODS

Gout and other diseases related to purine catabolism are believed to be "high-income group diseases"; this belief led us to examine values for purine intake in several income groups in the same community. If a significant difference were found, we could state a *natural limit of safety* (NLS), that is, consider the purine intake from SCP that increased the "low-income groups purine intake" to the level of "normal high-income groups purine intake" as not extremely hazardous, for many individuals over a large period.

For NLS formulation only bibliographic material was used:

- published nutritional surveys (38, 39);
- tables of purine contents in foods (35, 36);
- analytical data tabulated by Bressani (25) on purine content in common SCP sources.

The present approach, certainly, has some requirements that must be met:

- (i) it is necessary to quantify the different intake according to social and economical groups in many groups;
- (ii) it is necessary to quantify the different intake according to age groups within the social and economical groups, particularly those most frequently found in the programs of applied nutrition;
- (iii) it is necessary to know more about gout epidemiology within income groups;
- (iv) it is necessary to have an improved knowledge of food habits, and accurate data on purine content of food-stuffs consumed in regions where SCP utilization programs are being implanted;
- (v) it is necessary to admit —axiomatically— an identical purine tolerance for poor and rich people in the same area.

Discussion of the approach.

(i) It is necessary to quantify the different purine intake according to social and economical groups in many groups.

In spite of the traditional belief that gout and urate calculi are diseases of overnourished people, we have not found —in available bibliography— any data that showed purine intake levels for any region, separated or not by income group. Di-

rect data on purine levels of mixed diets were not found. Bayles (32) said that a purine intake of 100 mg per capita daily is obtained from a restricted diet. According to Wyngaarden (33), maximum dietary restriction reduces purine intake to 3 mg of purine-N, that is roughly 6-7 mg of purines per capita daily.

An approximate assessment of purine intake in two Brazilian rural areas was made (34). This was based on separate income groups according to published surveys (38, 39) and on data on purine values of several foodstuffs (35, 36). As pointed out in Table 1, not all food consumed was considered. Analytical data for some items showed absence of purines (between 1/3 and 1/2 of food consumed); for few foodstuffs no information was found regarding purine content and we have used purine contents for similar food (10-20% of food consumed); 30-40% of the items had their purine content listed in tables.

Nutritional surveys considered, prepared according to up to date recommendations, showed food intake per each income group and in grams per capita daily. For most food consumed, we have used a maximum global value and a minimum global value, due to discrepancies between the values in the literature.

As shown in Table 1 (note "f"), we have considered the maximum purine intake values. This allows maximum SCP supplementation through NLS.

Tables of special food substances have limited value because few common foodstuffs had their purine contents determined (35, 36).

(ii) It is necessary to quantify the different intake according to age groups within the social and economical groups, particularly those most frequently found in the programs of applied nutrition.

NLS determination for a total population can, under some conditions, be extrapolated to children. Except for a short period (breast feeding) children eat diets very similar to those of adults. NLS extrapolation from an all-aged population to children would be related to daily amounts of consumed food.

PAG limits derived from experiments with young adults, recommended doses for children lower correspondingly to weight (31).

Statements of a Natural Limit of Safety (NLS) for children would be more accurate if they were based on nutritional surveys that listed food intake by age and income groups.

(iii) It is necessary to know more about gout epidemiology within income groups.

Some data indicates that increasing family incomes —and correspondingly increased purine intakes— lead to larger probabilities of gout and related diseases (33, 37). Bonomo, in a Brazilian survey, states that “in spite of several gouty patients in low income groups, 80% of cases were found in medium and high income groups” (37). Other data on gout epidemiology are in agreement: European and North-American surveys put gout prevalence close to 0.3%; in New Zealand, where the greater protein consumer live, the prevalence in one pilot survey of adults was found to be 8.2% in men and 1.6% in women (33). Dietary influences can be foreshadowed from the fact that gout is rare in African populations but it is common in the American negro (33).

To increase purine intake of very low and low income groups to intake levels of high income groups, will probably increase gout prevalence; this fact must be taken as a minor problem when compared to hazards derived from protein malnutrition. We must consider also that in accordance to the scant data on gout epidemiology — diseases related to purine catabolism are not frequent diseases in the world.

The present approach supposes that prevalence of purine catabolism diseases in high income groups of the studied regions are not higher than prevalence of purine catabolism diseases in groups consuming similar protein diets.

(iv) It is necessary to have an improved knowledge of food habits and accurate data on purine content of foodstuffs consumed in region where SCP utilization programs are being implanted.

Discussion of items (i) and (ii) has probably lead to the understanding of requirement (iv). In simple words, it appears that few underdeveloped areas have adequate nutritional surveys. The common situation is to have little knowledge of regional food habits by age groups and of the purine content of its ordinary foodstuffs. Thus, in each area where

SCP supplementation is proposed, special nutritional surveys and a proper program of food analysis must be undertaken.

RESULTS

Calculations made (34), without consideration of ethary groups, put purine intake in Brazilian rural areas in the range of 144-490 mg per capita daily (Table 1). Large differences between income groups can be noted.

A finding is not restricted by poor analytical data; it refers to increases in purine intake as related to economical improvement. Our calculation (34) showed that purine intake in very low income groups is three to four times less than purine intake in high income groups and that purine intake in all groups is approximately 1.6 times greater than purine intake in very low income groups.

Data presented below, collected from an all-aged population can be improved. Assumed values for protein and purine content of food-yeast (SCP type considered) were calculated from Bressani's data (25). According to approximations previously mentioned, Table 2 shows NLS of two Brazilian rural regions.

Table 2, as seen, shows that improvement in protein intake varies with previous purine intake.

We must point out that, the Natural Limits of Safety are different if we design SCP supplementation exclusively for very low income groups or if we design the program to very low+low income groups. In general, programs of applied nutrition focus on low+very low income groups.

The present approach is represented in Figure 1 which shows its application to specific regions; the equation enclosed (*) is able to generalize concepts for every group for which proper data could be collected.

DISCUSSION

As seen (Introduction), statement of limits for SCP intake by humans can not be based on ordinary animal tests.

(*) $P = P_0 + 17 T$, where: P_0 is the previous purine intake (mg per capita per diem) of income group to which supplementation is designed; T is the amount of food-yeast (grams per capita daily) that puts purine level of supplemented diet to P level; 17 (mg of purines/grams of SCP) is the slope of the straight-line, fig. 1.

TABLE I
APPROXIMATE ASSESSMENT OF PURINE INTAKE IN TWO BRAZILIAN RURAL AREAS

Nutritional Survey	Income Groups (a) (*)	Persons in each Group (N ^o)	Items used by each Group (N ^o)	Food considered in the estimation			Sum (e)	Purine intake (mg per capita daily) (f)	Protein intake (g per capita daily)
				(b)	(c)	(d)			
Guaratiba GB 1966/67 (38)	v. l.	14	34	16	10	7	33	170	49.0
	l.	91	107	38	41	20	99	175	62.3
	m.	46	87	36	23	20	79	283	70.4
	h.	17	63	24	21	17	62	336	75.8
a. g.	168	130	51	38	29	118	262	64.7	
Piranema, Itaguaí, R.J. 1965 (39)	v. l.	36	42	13	18	11	42	144	39.9
	l.	58	65	23	22	19	64	183	50.4
	m.	25	62	23	22	16	61	222	70.5
	h.	10	46	22	14	9	45	490	108.7
a. g.	129	103	46	30	22	98	201	56.4	
Guaratiba + Itaguaí (g)	a. g.	297	—	—	—	—	—	235	61.2

- (*) v. l. = very low income group.
l. = low income group.
m. = medium income group.
h. = high income group.
a. g. = all groups.

(a) Expenses for food in a week per family member:

Guaratiba (38)	v. l.	NCr\$ 2.49,4 or US\$ 1.12
August, 1966	l.	NCr\$ 3.12,3 or US\$ 1.41
1 US\$ = NCr\$ 2.22	m.	NCr\$ 4.41,6 or US\$ 1.99
	h.	NCr\$ 7.01,3 or US\$ 3.16
	a. g.	NCr\$ 3.80,2 or US\$ 1.71

Itaguaí (39)
November, 1965
1 US\$ = NCr\$ 1.85

v. l.	NCr\$ 0.95	or US\$ 0.51
l.	NCr\$ 1.40	or US\$ 0.76
m.	NCr\$ 2.43	or US\$ 1.31
h.	NCr\$ 4.89	or US\$ 2.64
a. g.	NCr\$ 1.73	or US\$ 0.93

- (b) Considered "zero-in-purine" according to table values (35, 36).
(c) Values derived from tables (35, 36).
(d) Values not found; the used figures mean known values for similar food.
(e) Items not considered were consumed at negligible quantities.
(f) Sum of maximum values found or estimated; the methyl-purines were not considered.
(g) Average-weight based on the number of persons in each group from each area.

TABLE 2
NATURAL LIMIT OF SAFETY (NLS) - SCP SUPPLEMENTATION
EXCLUSIVELY FOR VERY LOW INCOME GROUPS

	Itaguaí	Guaratiba
Normal purine intake for very low income groups — —mg per capita daily— (A)	144	170
Normal purine intake for high income groups — mg per capita daily — (B)	490	336
Natural Limit of Safety (NLS): (B) — (A)	346	166
SCP mass (Food-Yeast) which contains this (B—A) purine quantity (*) — grams	20.3	9.8
Proteins contained in this Food-Yeast mass (**) — grams	8.0	3.7
Previous protein intake (see table 1), grams per capita daily	39.9	49
Per cent increase in protein intake	~20%	~7.5%

NATURAL LIMIT OF SAFETY (NLS) — SCP SUPPLEMENTATION
DESIGNED FOR "VERY LOW + LOW" INCOME GROUPS

	Itaguaí	Guaratiba
Normal purine intake for very low income groups — mg per capita daily	144	170
Normal purine intake for low income groups — mg per capita daily — (A)	183	175
Normal purine intake for high income groups — mg per capita daily — (B)	490	336
Natural Limit of Safety (NLS): (B) — (A)	307	161
SCP mass (Food-Yeast) which contains this (B — A) purine quantity (*) — grams	18.6	9.5
Proteins contained in this Food-Yeast mass (**) — grams	7.1	3.6
Previous protein intake (see table 1) { low class:	50.4	62.3
grams per capita daily { very low class:	39.9	49.0
Per cent increase in protein intake { low class:	14%	5.9%
{ very low class:	18%	7.3%

(*) SCP (Food-Yeast) with assumed 1.7% purine.

(**) SCP (Food-Yeast) with assumed 38% protein.

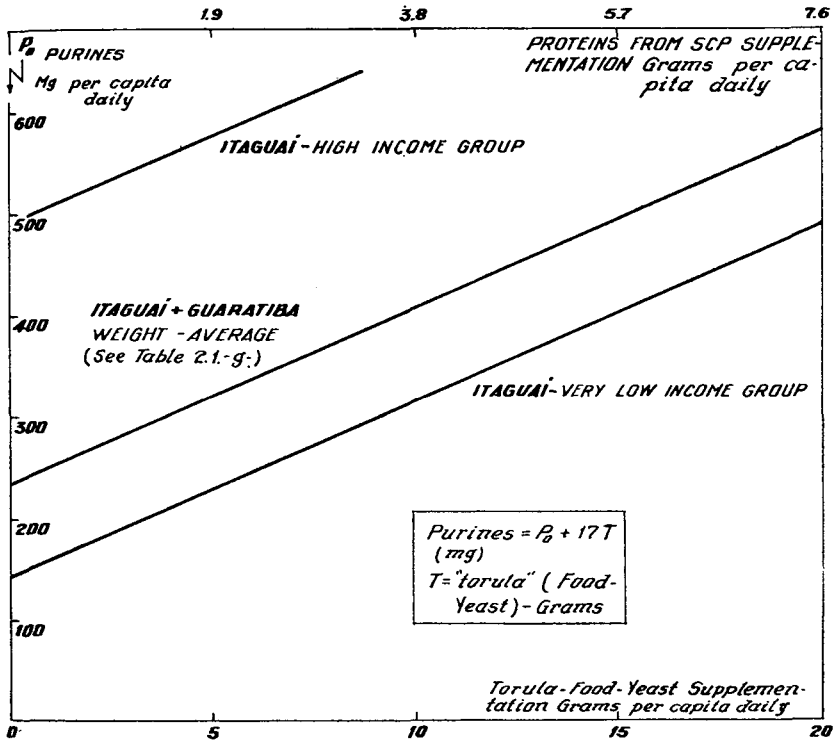


FIGURE 1

Experiments must use higher apes or humans. Except for a brief comment by Pokrowsky (19) and some experiments in California (22), it seems that exclusively human experiments were undertaken with the view of establishing limits to SCP human utilization.

A few conclusive references are made with respect to SCP limits in human nutrition. Bunker (40) found that postwar experiments in Germany indicate 10 per cent of the diet as the upper safe limit, but German law has fixed 5 per cent as the legal limit. The same Author (40), expressing his own opinion, states that "doses larger than 15 g or 1/2 ounce can be tolerated and that the ill-effects which appear are usually due to newly introduced material into the diet and a certain amount of gastric disturbances experienced by some people".

Fencel (41), based on data collected from a german ency-

clopedia, pointed out that clinical tests limited food-yeast intake to 20 g per capita daily; no further discussion is presented.

PAG limits are based on objective experiments: "The currently available information suggest that there should be a limit of two grams per day of the amount of nucleic acid introduced by SCP into the diet of an adult and correspondingly less by weight for children" (31).

Bibliographic referentes quoted in PAG Statement on SCP (31) refer to the establishment of intake limits and have used similar approaches. SCP was administered and uric acid blood levels were noted. Calloway's paper (22) states formally that "most authorities accept 6.0 mg/100 ml plasma as the lower limit for the high-risk population and most cases studied with reference to secondary gout had levels of 7 mg% or more".

Experiments of Calloway *et al.* (22) involved the feeding pure yeast ribonucleic acid with purine-free diets to healthy young men; data recorded were perfectly linear from 0 to 8 g/day. It appears that "50% of men can tolerate as much as 2 grams of nucleic acid in their daily diet without developing undesirably elevated plasma levels (Presumably women and young people could be given slightly larger amounts, but this has not been tested)". Calloway's limits assumed that the diet "did not contain other sources of nucleic acids". Curiously, this paper, in its last paragraph, points out: "The more prudent course would seem to be to develop methods for removing nucleic acids safely. The use of crude cells should be restricted to animal feed" (22).

Edozien *et al.* have also tested uric acid levels after SCP administration. In reviewing several studies of SCP intake by humans, Edozien concludes that "the cause of the wide diversity of views is not clear but variations in the nature of the yeast fed and the substrate on which they were grown are probably the principal factors" (23). These words could perhaps explain discrepancies in data presented in a excellent review prepared by Bressani (25). The final conclusion of Edozien's document states: "The acceptable maximum amount which could be safely tolerated is, therefore, less than 45 grams or the amount providing 3.0 g yeast RNA per day" (23).

As seen, our approach to indicating limits of SCP supplementation, does not use values for each community or for each age group. It will remain so until a large number of proper

surveys has been undertaken on actual purine intake by income and ethnic groups. From Calloway's (22) and Edozien's (23) documents we can note certain restrictions to the approach taken by the PAG Statement on SCP:

—To quantify the relationship between dietary purines and uric acid formation, only a few young adults were used and the longest test was a very short-term one;

—No evidence was presented to support the assumption that for children, a reduction related to body weight is correct;

—Calloway's and Edozien's approach leads to safe limits provided that the diet be an unrealistic one, that is, a purine-free diet.

Our approach does not suffer from the above mentioned restrictions:

—Until it becomes clear that generalization is possible, our approach requires that surveys for each region continue to gather tolerancy data for individuals living in different areas and with different food habits;

—Limits for children (not definitively calculated in our present example of NLS application) depend —for maximum accuracy— of children nutritional surveys; on these data we could apply the same concepts and the same calculations;

—Our approach, not based on short-term experiments, will indicate values derived from several years of life of people from high income groups.

It seems that PAG limits and other limits presented, in spite of their pioneer significance, do not assure an optimum of safety. Some undesirable side-effects have not their true frequency determined. Cumulative problems were not studied in humans.

It is evident that the statement of limits related to nucleic acid risks assumes that SCP intended for supplementation programs are safe relatively to other toxicological problems. The general evaluation of single-cell proteins has some well-defined quality criteria presented in the PAG Statement on SCP (31).

FINAL REMARKS

Our study presents an approach to the definition of natural limits of safety relative to SCP utilization in human nutri-

tion. Approximate values of SCP purine that can be included in diets of undernourished people living in two Brazilian rural areas are close to 160 mg per capita daily in one region and 350 mg per capita daily in the other, that is something as 10-20 g of food-yeast per capita daily.

The present approach can be applied to any community and to any group and is based on tolerated level for a length of time.

NLS will accomplish its finality as soon as more accurate data on purine content of food, on gout and related diseases prevalence and on food habits of the various regions be gathered.

The reduction of the nucleic acid in SCP and other means of obtaining SCP with a favorable ratio of protein to RNA are still at the stage of laboratory studies (7, 8), therefore, we think that the use of NLS in designing SCP supplemented programs is the safest manner to obtain good results.

RESUMO

Limites para utilização de proteínas microbianas na alimentação humana

As purinas têm sido assinaladas, nos últimos anos, como fatores limitantes da utilização de proteínas microbianas (SCP) por seres humanos. O cálculo dos limites de ingestão é dificultado pelas diferenças entre os catabolismos púricos de seres humanos e dos animais de uso comum nos laboratórios. Limites estabelecidos anteriormente não se originam de ensaios suficientemente prolongados e processados sobre um grupo adequadamente numeroso.

Apresenta-se uma determinação de um limite natural de ingestão com segurança baseada em conceitos de "ingestão púrica normal" e de "ingestão púrica normal máxima" em uma população homogênea. Os limites apresentados variam de acordo com o grupo populacional estudado e são chamados Limites Naturais de Segurança (LNS; "Natural Limits of Safety", NLS).

A utilização de proteínas microbianas (SCP) acima de tais limites, sem o surgimento de novos dados, deveria ser um assunto sujeito a decisões governamentais que julgassem os possíveis riscos para a saúde das populações envolvidas contra os benefícios esperados.

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