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NITROGEN BALANCE AND GROWTH IN YOUNG RATS GIVEN THE AMINES CADAVERINE, PUTRESCINE, HISTAMINE AND TYRAMINE IN FISH MEAL DIETS

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ABSTRACT

Young rats were given a combination of the amines cadaverine, putrescine, histamine and tyramine in fish meal diets. The amines were added at levels corresponding to 5% and 25% of the amounts of their origin amino acids in fish meal. No adverse effects on growth or nitrogen utilization were found.

INTRODUCTION

Amines may be formed by bacterial decarboxylation of free amino acids in stored fish and in fermented fish paste (Ritchie and Mackie, 1980; Fardiaz and Markakis, 1979). It has been suggested that histamine formed from free histidine in scombroid fish species is involved in the socalled scombroid fish poisoning in man (Strøm and Lindberg, 1945; Taylor, 1983). However, histamine alone seems to be less toxic than expected when given orally, and it is suggested that other amines, especially cadaverine derived from free lysine, may potentiate the histamine toxicity (Bjeldanes et al., 1978; Lyons et al., 1983).

The amines most commonly found in stored fish are, besides histamine and cadaverine, putrescine derived from arginine and tyramine from tyrosine.

Fish used for meal production may be stored for some time during transport and at the processing plant. It is of interest, therefore, to study whether a combination of amines in the diet causes any toxic or other untoward effects on experimental animals. The present study shows experimental data

on the effect of a combination of cadaverine, putrescine, histamine and tyramine on the nitrogen balance and the growth of young rats.

MATERIALS AND METHODS

Male Wistar rats, weighing about 60 g at the start of the experiments, were obtained from Møllegaard, Denmark. Three high quality fish meals were used as protein sources. They all contained about 700 g protein/kg (707, 706, 708 for fish meal 1, 2 and 3, respectively). The amino acid analyses are given in Table 1.

Table 1. Amino acid composition (g/kg protein)[1] of the fish meals used in Expt 1 (fish meal 1) and in Expt 2 (fish meal 2 and 3).

	fish meal 1	fish meal 2	fish meal 3
Aspartic acid	97	91	93
Threonine	48	44	44
Serine	46	42	42
Glutamic acid	142	134	131
Glycine	66	58	60
Alanine	66	61	60
Cystine	6	6	8
Valine	50	47	47
Methionine	27	27	28
Isoleucine	41	40	39
Leucin	81	75	76
Tyrosin	36	34	33
Phenylalanine	43	38	39
Lysine	95	82	82
Histidine	27	20	25
Arginine	62	57	55

[1] Tryptophan and proline were not determined.

The diets in Expt 1 contained about 150 g protein/kg and the diets in Expt 2 about 120 g. The other compounds of the diets were (g/kg): sucrose 200; soy bean oil 50; minerals 40; vitamins 10; cellulose powder 10; balance precooked dry potato starch (about 500). The amines mixed into the diets were the dihydrochlorides of cadaverine, putrescine and histamine, and the monohydrochloride of tyramine. Urea was used to balance the nitrogen content of the control diets, and sodium acetate to neutralize the added chlorides.

Protein (N · 6.25) was determined essentially as described by Crooke and Simpson (1971). Amino acids were determined in a Kontron Amino Acid

Analyzer Liquimat III after hydrolysis in 6M HCl for 20h, with norleucine as internal standard, using a Spectra-Physics integrator.

Experiment 1

Two groups of 10 rats were given fish meal 1 as the protein source. The added amounts of amines in the diet of the experimental group were 420 mg cadaverine, 220 mg putrescine, 125 mg histamine and 220 mg tyramine calculated as free bases/kg. These amounts are on a molar basis equivalent to about 5% of the amounts of lysine, arginine, histidine and tyramine normally found in high quality, non-scombroid fish meals. Further, 2.86 g NH_4Cl and 5.84 g sodium acetate/kg diet were added, the former on the assumption that about half the amides (from glutamine and asparagine) in fish protein might be hydrolysed to ammonia during fish storage. The control diets were without these additions but with 2.16 g urea and 4.13 g NaCl added per kg. The experiment lasted for 21 days. The rats were individually weighed at start and after 3, 7, 10, 14 and 21 days. The diets were given according to appetite.

Experiment 2

Groups of 7 rats were used, one experimental group and one control group for each of fish meals 2 and 3. The amine chlorides in the experimental diets corresponded to 1710 mg cadaverine, 910 mg putrescine, 540 mg histamine and 910 mg tyramine free bases/kg diet. The amounts were equivalent to about 25% of the corresponding amino acids normally found in fish meals. 5.75 g sodium acetate/kg diet were used. In the control groups 2.33 g urea and 4.06 g NaCl/kg diet were added.

The experiment fell in two parts. Part one was a nitrogen balance experiment lasting for nine days (four days preliminary period, five days collection period) (Njaa, 1963; Eggum, 1973). After completion of the balance experiment the rats were continued on the same diets for 23 days; during this time the diet allowance was gradually increased, but all rats were offered and did consume the same amounts of feed. Thus, including the balance experiment the growth part of the experiment lasted for 32 days.

RESULTS AND DISCUSSION

Table 2 shows weight gain data for both experiments, for Expt 2 also PER-values for the entire 32-day feeding period. Data from the nitrogen balance part of Expt 2 are presented in Table 3. There were no significant differ-

Table 2. Mean weight gain (W_n–W_0, g) for each group in Expt 1 (n = 10) and Expt 2 (n = 7) and PER (Protein Efficiency Ratio, weight gain/protein eaten) in the 32-days period in Expt 2.

Days	Expt 1		Expt 2			
	fish meal 1		fish meal 2		fish meal 3	
	control	+ amines[1]	control	+ amines[2]	control	+ amines[2]
3	13.5	13.1				
4			11.1	9.9	11.6	11.5
7	41.9	41.9				
9			23.7	24.0	25.2	25.7
10	62.6	63.0				
14	94.8	97.1				
16			55.8	57.5	59.3	59.8
17	115.9	115.6				
21	144.6	145.0				
23			90.0	89.1	90.9	94.1
30			122.8	122.9	122.8	125.4
32			133.6	134.0	132.5	136.6
PER			2.42	2.39	2.37	2.43
SD			0.13	0.12	0.29	0.09

[1] at a 5% level of corresponding amino acids.
[2] at a 25% level of corresponding amino acids.

ences observed between any of the parameters measured or calculated for the amine-containing diets and the controls. Also, the differences between the two fish meals compared in Expt 2 were insignificant. The slower weight gains observed in Expt 2 than in Expt 1 are probably explained by the difference in the protein contents of the diets.

There are few reported experiments with which the present may be compared. Experiments with fish meal diets with added cadaverine, putrescine, histamine and tyramine at levels similar to those used in Expt 2, except for a higher histamine level, showed retarded growth in mink pups in an eight week experiment (Norwegian Herring Oil and Meal Industry Research Institute, Bergen, personal communication).

Most of the reported experiments on the biological effects of amines are dealing with the alleged toxicity of histamine. A great volume of episodes have been reported where histamine found in fish and shellfish has been implicated (Taylor, 1983). There are, on the other hand, indications that histamine in itself has a low toxicity when given orally (for references see Bjeldanes et al., 1978 and Lyons et al. 1983). However, cadaverine and pro-

bably also other amines may potentiate histamine toxicity either by suppressing its metabolism in the intestine, or by increasing its rate of absorption. In the relevant studies histamine was administered by stomach tube, or its effect was studied with isolated gut segments.

The present experiments aimed at testing whether combinations of the four amines most frequently found in stored fish after bacterial spoilage gave toxic or other negative effects *per se* in growth and nitrogen balance studies in young rats. The results presented showed clearly that this is not so. Formation of amines causes losses in the corresponding amino acids and the reduced nutritive value in meals containing high levels of amines may be due to the resulting imbalance in the amino acid content. The amounts of amines used in Expt 2 were rather high, and stored spoiling fish having such concentrations of amines would surely be deemed putrid by organoleptic criteria.

Table 3. AD (Apparent Digestibility), TD (True Digestibility), NPU (Net Protein Utilization), Bal (Balance) and BV (Biological Value), %, from the balance experiment in Expt 2, mean value (n = 7), ±SD for AD and Bal.

	AD	TD	NPU	Bal	BV
fish meal 2					
control	82.0 (1.52)	92.5	78.7	59.2 (3.37)	85.1
+ amines	82.1 (1.77)	92.6	79.0	59.5 (2.47)	85.3
fish meal 3					
control	83.6 (2.73)	94.1	78.5	59.0 (4.32)	83.4
+ amines	83.4 (1.83)	93.9	80.5	61.4 (3.17)	85.7

$AD = 100 \cdot (1 - F/I)$
$TD = 100 \cdot (1 - (F - F'/I))$
$NPU = 100 \cdot ((I - (F - F') - (U - U')) / I)$
$Bal = 100 \cdot (1 - (F/I + U/I))$
$BV = 100 \cdot (NPU/TD)$

I = N intake in food, g
F/U = fæcal/urinary N output, g
F'/U'= fæcal/urinary N output on a protein-free diet, g

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