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Effect of Ammoniated Casein in the Diet on the Growth of Weanling Rats

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Effect of ammoniated casein in the diet on the growth of weanling rats was studied using feeds containing 11.4, 20, 30, and 40% casein which had been subjected to treatment in the dry state with ammonia gas. No significant differences in growth were found at any protein level between controls fed untreated casein and those fed ammoniated casein containing 66.4 mmol of ammonia/100 g of casein. Of a total of 44, one rat fed ammoniated casein (30%) died after 10 days, presumably of unrelated causes. No other adverse nutritional effects were observed over the time period studied. The kidney weight/body weight ratios after 35 days on the diets were correlated to the protein level ($r = +0.701$) but were not affected by the ammonia. There was no significant difference between the observed protein efficiency ratio of the ammoniated casein and that of the control; values were 2.88 ± 0.14 and 2.80 ± 0.02, respectively.

The absorption of ammonia gas by dry isoelectric casein has been shown to be an effective method for converting this protein into a water-soluble form similar to ammonium caseinate (Girdhar and Hansen, 1974). Such treatment markedly improves the physical and chemical properties of the casein but results in the retention of 1.0–1.8% nitrogen as ammonia in the product. Although treatment with ammonia gas has previously been proposed for fumigating citrus fruit and corn (Bothast et al., 1973) and for decontaminating aflatoxins from peanut and cottonseed meal (Gardner et al., 1971), little is known regarding the mechanism of action of ammonia on the protein or the nutritive value and possible toxic effects of the treated material.

According to a review of the status of the safety of ammonium ions (National Technical Information Service, 1973), ammonia salts are generally recognized as safe (GRAS) and should not be expected to produce adverse effects if ingested in moderate amounts. However, large amounts of ammonia salts are toxic and may cause acidosis and liver and kidney damage.

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The purpose of this study was to gain information about the nutritive value of ammoniated casein and to determine if the feeding of diets containing elevated levels of this product as the sole source of protein had any detrimental effects on the growth of weanling rats.

MATERIALS AND METHODS

Ammoniated casein was prepared by placing 3000-g batches of vitamin-free casein under vacuum in a chamber (31.25 cm × 27.5 cm) connected to a water aspirator. After 10–15 min, the pump was closed off and dry ammonia gas was admitted such that a slightly reduced pressure was maintained over a period of approximately 50 min. During the process, the temperature of the casein increased from 25 to 50 °C. The treated casein was then heated to 60 °C and degassed under vacuum for 60 min in order to remove excess ammonia from the casein particles. After degassing, the powder contained 0.93% additional nitrogen due to residual adsorbed ammonia. A 5% aqueous solution had a pH of 7.4.

Nitrogen was determined on the original and ammoniated caseins and on all the mixed diets by using the macroKjeldahl procedure (AOAC, 1970). Total nitrogen in the control samples was converted to protein by use of the factor 6.38. Nitrogen due to residual ammonia was determined by difference and was not included in the calculation of the protein content.

Male, 35 to 46 g, weanling albino rats of the Sprague-Dawley strain were obtained commercially. The rats were

Table I. Composition of Basal Diets Used in Feeding Studies (%)

	Diet[a]			
	1	2	3	PER[e]
Vitamin-free casein[b]	20	30	40	11.44
Dextrose	59	49	39	67.56
α-Cellulose	13	13	13	13
Corn oil	2	2	2	2
Vitamin mix[c]	2	2	2	2
Mineral mix[d]	4	4	4	4

[a] All dietary components were purchased from ICN Pharmaceuticals, Cleveland, Ohio, except for the Mazola brand corn oil which was purchased at a local supermarket. [b] With or without NH_3 treatment. [c] Composition of the vitamin mix in g/100 g of diet: vitamin A concentrate (200 000 IU/g), 0.009; vitamin D concentrate (400 000 IU/g), 5×10^{-4}; α-tocopherol, 0.01; ascorbic acid, 0.09; inositol, 0.01; choline chloride, 0.15; menadione, 0.0045; *p*-aminobenzoic acid, 0.01; niacin, 0.009; riboflavin, 0.002; thiamin hydrochloride, 0.002; calcium pantothenate, 0.006; biotin, 4.0×10^{-5}; folic acid, 1.8×10^{-4}; vitamin B_{12}, 2.7×10^{-6}. [d] Composition of the mineral mix in g/100 g of diet: cupric sulfate, 3.11×10^{-5}; ferric ammonium citrate, 0.061; manganese sulfate, 8.04×10^{-4}; ammonium alum, 3.69×10^{-4}; potassium iodide, 1.62×10^{-4}; sodium fluoride, 0.002; calcium carbonate, 0.274; calcium citrate, 1.23; calcium biphosphate, 0.451; magnesium carbonate, 0.141; magnesium sulfate, 0.153; potassium chloride, 0.499; dibasic potassium phosphate, 0.875; sodium chloride, 0.308. [e] Diet used to determine the protein efficiency ratio.

Table II. Protein Efficiency Ratio of Ammoniated Casein

	Observed	Corrected[a]
Control	2.80 ± 0.02[2]	2.50 ± 0.02
treated	2.88 ± 0.14	2.57 ± 0.12

[a] Correction to a standard casein value of 2.50. [b] Mean ± standard error of the mean (SEM).

divided into six treatment groups of four. A second lot of rats, 33 to 39 g in weight, was divided into two treatment groups of ten for determination of the protein efficiency ratio (PER) in accordance with the official method (AOAC, 1975). The animals were caged individually in raised stainless steel wire screen cages and allowed food and tap water ad libitum. The animal room was light controlled with 12-h periods of light and darkness.

The animals were fed the basal diet shown in Table I. The ammoniated casein was examined for nutritional quality by feeding at the 10% protein level for 4 weeks. Diets containing higher levels of ammoniated casein were fed for 5 weeks. Body weight and food intake were recorded daily and the rats were observed for any signs of aberrant behavior. Control diets at all protein levels contained untreated vitamin-free casein from the same lot used to prepare the ammoniated casein.

At the end of the 5-week period, all animals were sacrificed, and, after dissection, the kidney and liver weights were determined. Statistical analyses were carried out as described by Ostle (1956).

RESULTS

The PER value of protein as measured with rats is accepted by the U.S. Food and Drug Administration as a standard for protein quality (AOAC, 1975). In the present study, the PER determination for ammoniated casein was made using a diet containing casein with 8.4 mmol of ammonia/100 g of feed. The observed and corrected PERs of the control and ammoniated caseins are shown in Table II; there were no significant differences ($P > 0.05$) between these values.

Since higher levels of residual ammonia in the diet might affect growth at elevated protein levels, diets containing 20, 30, and 40% ammoniated casein were fed for 5 weeks. The results are summarized in Table III.

No abnormalities in condition or behavior of the rats occurred during the experimental period but there was one death after 10 days in the group fed the diet containing 30% ammoniated casein. It was concluded that death was due to circumstances other than the diet because there were no deaths or abnormal behavior among the rats fed a higher level of ammoniated casein.

Although the weights of the livers showed no statistically significant differences, the ratios of kidney weights to total body weights could be correlated with the protein level ($r = +0.701$) and were significantly larger in both groups receiving the diets containing 34.5% protein.

Body weight gains were statistically evaluated after all groups had been on the diets for 5 weeks. By analysis of covariance, it was first shown that the weight gain of each rat over the time period studied was not dependent on the initial weight of the rat, so an analysis of variance was suitable for data analysis. The results are shown in Table IV. The data show that there are no significant differences in growth rate at any protein level due to residual ammonia in the diet. In addition, there were no significant effects on growth brought about by the variable protein levels in this 5-week study.

DISCUSSION

Ammonia is a normal metabolite of various nitrogenous compounds and is constantly present in all organisms. Ammonia can produce deleterious effects when normal detoxification processes are impaired by disease or protein deprivation or when ammonia is introduced too rapidly in excessive amounts (Visek, 1968). Stevens et al. (1975)

Table III. Effects of Ammoniated Casein in Diets Fed to Four Male Rats/Group for 5 Weeks

Diet	Total protein in feed, %	Ammonia/ 100 g of feed, mmol	Body weight gain, g/rat	Feed intake, g/rat	Feed efficiency, g wt gain/g feed	Liver weight, % of body weight[a]	Kidney weight, % of body weight[a]
20% casein							
Control	16.9	0	182 ± 6	485	0.38	3.74 ± 0.08	0.87 ± 0.02
Ammoniated		15	177 ± 8	457	0.39	3.98 ± 0.09	0.90 ± 0.03
30% casein							
Control	25.9	0	169 ± 6	440	0.38	3.95 ± 0.32	0.95 ± 0.03
Ammoniated		23	182 ± 11[b]	547[b]	0.33[b]	4.16 ± 0.23[b]	0.90 ± 0.05[b]
40% casein							
Control	34.5	0	176 ± 16	498	0.35	4.02 ± 0.27	0.99 ± 0.02[c]
Ammoniated		28	163 ± 13	454	0.36	4.00 ± 0.14	1.03 ± 0.02[d]

[a] Mean ± SEM. [b] Average of three rats. [c] Significantly different ($P < 0.05$) from the 20% casein group according to Student's *t* test. [d] Significantly different ($P < 0.01$) from the 20% casein group according to Student's *t* test.

Table IV. Analysis of Variance Effect of Ammoniated Casein in the Diet on Growth after 35 Days

Source of variation	Degree of freedom	Mean squares	Significance
Between treatments	(5)	(224.534)	
NH_3 effect at 16.9% protein	1	60.500	ns
NH_3 effect at 25.9% protein	1	1.9405	ns
NH_3 effect at 34.5% protein	1	325.125	ns
Effects due to variable protein levels	2	735.104	ns
Error	17	438.356	
Total	22		

showed that ammonia intoxication was a very real hazard in the rehabilitation of severely protein-deprived rats, whereas an ammonia load was well tolerated in rats receiving an adequate protein level containing the proper balance of essential amino acids. Kulasek et al. (1975) have shown that tolerance of rats to ammonia injected intraperitoneally as ammonium chloride increased proportionally to the nitrogen content of the diet. The LD_{50} was 0.565 mmol of ammonia/100-g body weight for animals receiving 0.8% nitrogen whereas for those receiving diets containing 11.1% nitrogen, the LD_{50} rose to 0.792 mmol of ammonia/100-g body weight. These authors showed that the faster rate of ammonia detoxication was due to increased urea synthesis resulting from increased amounts of substrate and available energy and changes in the activity of the ornithine cycle enzymes.

Our investigation has shown that over the time period studied, no deleterious effects were produced in rapidly growing rats by ingestion of moderate amounts of ammonia from diets containing ammoniated casein as the sole source of protein, even though animals on the diet containing 10% protein (1.55% total nitrogen) were ingesting as much as 2.1 mmol of ammonia (100-g body weight)$^{-1}$ day^{-1} in the initial stages of the experiment. The ability of our animals to withstand an ammonia load 3.8 times greater than that reported by Kulasek et al. (1975) is explained by the fact that our animals ingested the daily ammonia dose over a 24-h time period rather than being challenged by a single injected dose.

Our findings are in contrast to those of Reid (1972) who reported that the feeding of diets containing 20% ammoniated cottonseed meal, equivalent to 0.17% ammonia nitrogen, produced a significant decrease in the growth rate of broiler chicks. However, the metabolic pathway for the excretion of nitrogen in avian species is completely different from that of mammals (Visek, 1968, 1974).

The significantly larger kidney weights we found in both treatment groups receiving 34.5% protein, although indicative of a slight acidosis (National Technical Information Service, 1973), are to be expected since high-protein diets have been observed to increase kidney size (Halliburton and Thomson, 1965) and addition of ammonium chloride to the diet has been shown to increase kidney weight (Thomson and Halliburton, 1966). Since there was no significant difference between the average kidney weights of the control group and the group receiving ammoniated casein in the diet at each of the three higher protein levels, residual ammonia in the diet at the levels fed in our studies apparently was not sufficient to cause kidney enlargement.

The ammoniation of casein and other dry isoelectric proteins is an energy conserving process for producing soluble proteins for industrial food applications (Girdhar and Hansen, 1974). The findings that the ammoniation process does not alter the PER of the casein and that ammoniated casein can be fed at high protein levels over a 5-week time period without adversely affecting growth warrant the more detailed studies required to establish this product as a safe food ingredient (Food Protection Committee, Food and Nutrition Board, 1959, 1970).

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