The Absence of Antithiamin Factors in Radappertized Beef and Chicken

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Introduction

One of the auxiliary studies included in the U.S. Army Protocols for testing the wholesomeness of radappertized meats was that of determining if radappertized produced antithiamin factors in meats (1,2). The question arose from studies by Brin et al (3,4) in which rats were fed diets containing 35% (dry weight) radappertized pork. These diets, when not supplemented with thiamine, produced thiamine deficiency in rats. Repletion of the rats with levels of thiamin that were adequate for growth did not restore erythrocyte transketolase activity (ETK) to normal levels. Rats fed the same diets supplemented with thiamine grew normally and had normal ETK levels.

ETK has been demonstrated to be thiamine specific and a good measure of thiamine status in both rats and humans (e.g., Brin (%) and others (¢). In view of the lability of thiamine to heat and to irradiation as well as the relative absence of ETK repletion data with "low" levels of thiamine in rats, this study was considered important to the investigation of the wholesomeness of radappertized beef and chicken (7,8).

Methods

Study Outline. Charles River weaning rats, 156 per sex per diet group were housed individually in rooms with a light-dark cycle of 12 hours. All rats were fed a semipurified diet containing 20mg thiamin/kg for one week. After this acclimatization period, 24 rats were continued on the nondeficient control diet and the remaining 132 rats were fed the same diet not supplemented with thiamin. At deficiency, weight gain of less than 0.5g/day, 12 rats from each group, nondeficient control and thiamin deficient, were bled by cardiac puncture under penthram gas anesthesia for base line ETK and *in vitro* thiamin pyrophosphate ETK stimulation (TPP) and then sacrificed. The remaining 120 thiamin deficient rats were randomly divided into 10 groups of 12 rats which were fed five different diets each at two levels of thiamin, 3.75 and 20.0 mg/kg in the beef study and 3.0 and 20.0 mg/kg in the chicken study. The five diet groups, in addition to the remaining nondeficient semipurified diet group, were frozen, thermal, gamma and electron beef or chicken, and semipurified diet.

ETK and TPP. Blood samples, 1.5ml collected by cardiac puncture on day 0, 7, 14 and 28 of repletion, for ETK and TPP were drawn into EDTA-containing syringes. Hematocrit was determined in duplicate and the red blood cells were washed and stored frozen until assayed. The procedure followed was that reported by Smeets et al (9) as modified and adapted for the Autoanalyzer by Waring et al (10). Enzyme activity was expressed as:

$$\text{ETK} = \text{I.V./ml packed red cells}$$

$$\text{I.V.} = \text{u mole glyceraldehyde-3-phosphate produced/min at } 37^{\circ}\text{C}$$

Percent *in vitro* thiamin pyrophosphate stimulation as:

$$\text{TPP} = \frac{\text{ETK stimulated} - \text{ETK unstimulated}}{\text{ETK unstimulated}} \times 100$$

Thiamin Assay. Thiamin was assayed by themicrobiological method described by Pearson (11) using *Lactobacillus viridescens* for all meats, mixed and semipurified diets.

Beef. The beef was obtained from whole carcasses of fresh, chilled, US choice grade beef, deboned and defatted of surface and internal fat. A 100 kg portion (0.125-1.4 kg pieces) was mixed with 1 kg sodium chloride, 0.4 kg sodium tripolyphosphate and 3 kg chipped ice and then stuffed into casings. The diameter of the stuffed casings was between 9.45 and 10.1 cm. Stuffed casings were placed in a cookhouse and heated to an internal temperature of 68°-74°C for enzyme inactivation.

Chicken. Chicken was obtained from fresh broilers or fryers (1.1-1.6 kg each) and fresh hens (1.4-2.3 kg each), but the proportion of hens was not more than 15% of the total procurement. Breasts, thighs, and legs were skinned and deboned by hand. The flesh and skin were ground separately and blended in the approximate natural proportions of white meat, dark meat, and skin (85% minimum meat and 18% maximum skin). A 100 kg portion was mixed with 0.75 kg sodium chloride, 0.3 kg sodium tripolyphosphate and 3.0 kg chipped ice and then stuffed into casings for enzyme inactivation to an internal temperature of 73°-77°C.

Processing of Beef and Chicken. Three-fourths of the enzyme inactivated meats (beef or chicken) were vacuum packed in cans; the remainder was vacuum packed in flexible pouches. All meats were frozen immediately after packaging. Of the canned meats, one-third was retained as the frozen control, one-third was thermally processed (F_0=6, 115.6° for 160 min) and one-third was irradiated with Cobalt-60 gamma rays. The meat in the flexible pouches was irradiated with 10 MeV electrons. Temperature during irradiation was -40° to 5°C. Average irradiation dose was 59 kGy (5.9 Mrad) with a range of 47-71 kGy. Thermal and irradiation processed meats were stored at ambient temperature. The meats were procured and processed through commercial meat packing plants, but were irradiated in the US Army Natick Research and Development Command facilities. Further details on the procurements, packaging and processing were described in the Protocols (1,2).

Diets. The composition of the diets was as shown in Table 1. Proximate (12), calcium (13) and phosphorus (14) analyses were made. Fat and protein levels (dry weight) were adjusted in the semipurified diets to be similar to the meat diets. The calcium/phosphorus ratio in the beef diets was 1.32, in the chicken diets it was 1.29,

and in the semipurified diets it was 1.25. Diets were prepared no longer in advance than 48 hours prior to feeding and fresh diets in clear jars were fed at least every 48 hours. Chicken was ground through a 1/4-inch plate, but beef was heated to an internal temperature of 50°-60°C prior to grinding. Meats and their juices were than mixed with their respective dry, semipurified premixes in precalculated proportions to yield diets containing 35% dry weight meat. Average proximate and mineral analyses were as shown in Table 2.

Table 1: Diet Composition

	Semipurified	Beef or Chicken
Meat (dry weight)	-	35.0
Casein (vitamin free)	20.0 (21.8)[1]	-
Lard	10.0 (8.8)	-
Corn oil	5.0 (4.4)	-
Mineral mix[2]	4.0	4.0
Vitamin mix[3]	2.0	2.0
L-cystine	0.2	0.2
Choline chloride	0.2	0.2
Glucose	58.6	58.6
	100.0	100.0

1. ( ) For the chicken study.
2. The mineral mix contributed to the diet the following salts: in g/kg: $CaCO_3$, $CaHPO_4$, 22.21; $NaHCO_3$, 1.164; NaCl, 1.49; K_2SO_4, 6.728; $.H_2O$, 0.258; in mg/kg: $ZnCO_3$, 37.6; KI, 0.337; $FeSO_4 \cdot 7H_2O$,292; $CuSO_4 \cdot 5H_2O$,33.2; Na_2SeO_3, 0.33; Cr (Acetate)$_3 \cdot H_2O$,4.78; MoO_3,1.51; $CoSO_4 \cdot 7H_2O$,4.79.
3. The vitamin premix was made up in a cellulose carrier and contributed to the final diet the following vitamins in mg/kg: gelatin coated retinal (500 IU/mg) 26; Cholecalciferol (400 IU/mg), 5; DL-a-tocopheryl-acetate powder (250 IU/mg), 440; Menadione - sodium bisulfite trihydrate 1.0; Riboflavin 10; Pyridoxine .HCl 20; Niacin 60; Ca - D-Pantothenate 30; Folic Acid 2.0; Biotin 1.0; B_{12}, 0.1% triturate 30. Thiamin .HCl was incorporated into a second premix and added to the diets to achieve the specified levels.

Statistical Analyses. A packaged computer program, BMDP Biomedical Computer Program P2V (15) was used to perform a two-way analysis of variance (ANOVA) using food and vitamin levels as the grouping factors. The nondepleted semipurified diet control group was not included in ANOVA because it was, by inspection, obviously different. Comparisons between individual groups were by Dennett's method of multiple comparison (16) using appropriate mean square error values from the ANOVA. Analysis were made separately for each sex and for each of the collection periods, day 7, 14, and 28 of repletion.

Table 2: Average Proximate, Phosphorus and Calcium Analyses for Beef and Chicken

	Moisture %	Protein %	Fat %	Ash %	Phosphorus[1] %	Calcium %
Beef	59.2	23.4	14.1	2.04	0.229	0.005
Chicken	64.2	19.0	13.9	1.54	0.244	0.004

1. Includes added sodium tripolyphosphate.

Results and Discussion. In general, the data obtained with beef was similar to that obtained with chicken; therefore, separate discussions will not be presented. Growth and body weight data are shown in Table 3. Thiamine deficiency, as measured by growth cessation, occurred between the 14th and 16th day for both males and females on the chiamin deficient diet. Rats repleted on the meat diets regained their growth rate faster than those repleted on the semipurified diets. Within two weeks the weight of the meat-fed rats equalled or surpasses the weight of the nondeficient controls, particularly by the females.

Table 3: Initial and Final Body Weights During the 28 Day Repletion Period[1]

MALES

Diet	Beef Study 3.75[2]	Beef Study 20.0	Chicken Study 3.0	Chicken Study 20.0
Nondeficient	-	353±21[3] (0)[4]	-	378±38 (1)
Semipurified	335±25 (0)	327±34 (1)	350±35 (2)	347±33 (4)
Frozen	355±27 (1)	346±17 (1)	387±38 (1)	392±51 (3)
Thermal	351±24 (1)	341±17 (2)	383±32 (1)	399±26 (2)
Gamma	359±16 (1)	350±25 (1)	393±20 (0)	381±37 (2)
Electron	356±20 (2)	355±18 (1)	388±36 (2)	376±32 (2)
Av. Initial Wts: Nondeficient		185±12	190±13	
All Others		147±16	160±21	

FEMALES

Diet	Beef Study 3.75[2]	Beef Study 20.0	Chicken Study 3.0	Chicken Study 20.0
Nondeficient	-	196±12 (3)	-	240±19 (0)
Semipurified	188±20 (1)	196±27 (3)	232±28 (0)	235±31 (1)
Frozen	217±20 (1)	206±21 (1)	261±23 (0)	268±35 (1)
Thermal	210±24 (1)	217±21 (3)	258±31 (1)	262±25 (0)
Gamma	206±17 (1)	212±16 (2)	255±25 (2)	255±19 (1)
Electron	211±34 (1)	198±12 (4)	261±26 (3)	254±22 (3)
Av. Initial Wts: Nondeficient		133±9	152±10	
All Others		121±12	143±13	

1. There were 12 rats per diet group and all were bled by cardian puncture on day 7, 14, and 28.
2. mg thiamine/kg dry weight diet.
3. g ± SG.
4. ( ) number of rats that died as a result of cardiac puncture.

There was no consistent effect of vitamin level on repletion growth nor were there any differences among the differently processed beef or chicken items. Because of separations in time no attempt was made to compare beef to chicken data and, consequently, no significance should be placed on any differences between the two sets of data. When a semipurified diet containing 1.25mg thiamin/kg, the minimum level required for growth, was fed to thiamin deficient rats, growth was resumed at a nearly normal rate, but there was almost no recovery of ETK activity (7).

Erythrocyte transketolase activities are shown in Figure 1. ETK activity at thiamin deficiency was 20-25% of the nondeficient controls. After repletion ETK increased rapidly during the first seven days with smaller increases on day 14 and 18; however by day 28 the average ETK activity was still lower than the average of the nondeficient control ETK. The reason for this latter not uncommon observation may be related to red blood cell turnover rate (8). In general, there were significant differences ($P<0.05$) among the ETK activities due to vitamin level, but there were no significant differences among the differently processed beef or among the differently processed chicken items.

The average in vitro thiamin pyrophosphate ETK stimulation, which measures the relative unsaturation of the enzyme with cofactor, at deficiency was 23.1±13.5% for males and 30.0±22.0% for females in the beef study. In the chicken study, the TPP effect was 16.8±17.5% for males and 1.4±4.8% for females. At the same time the TPP effect for the nondeficient controls was 3.6±2.9% for males and 5.1±3.9% for females in the beef study, and 5.5±4.6% for males and 1.6±3.5% for females in the chicken study. After repletion all average values were less than 9% and were considered to be in the normal range, but standard deviations were high. TPP effects in excess of 15% are accepted as an indication of thiamin deficiency; however, this does not always obtain as has been reported by Brin and others (17, 18, 19). The magnitude of the TPP effect is influenced by sampling, handling, storage, and other factors, including the possibility of apotransketolase destruction during the hemolysis of the red blood cells (20). In this study, ETK was a better indicator of thiamin status than TPP effect.

Table 4: Thiamine Content of Beef and Chicken Meat[1]

	Frozen	Thermal	Gamma	Electron
Beef[2]	2.48±0.18[3]	0.52±0.04	0.59±0.10	1.15±0.11
Chicken	1.04±0.13	0.23±0.01	0.26±0.02	0.70±0.13

1. Meats were sampled three times and each sample was assayed at three different dilutions.
2. Cooked as described.
3. mg/kg dry weight ± SG.

Thiamin destruction by thermal and gamma processing in both beef and chicken was about 75%, but only about 40% in the electron processed meats (Table 4). Although it had been seen previously on occasion, the significance of less destruction by electron radappertization has not been explored and further discussion of this observation is not within the scope of this paper.

Conclusion. In these studies, ETK activity was a better indicator of thiamine status in rats than TPP effect and a thiamin level of greater than 1.25 mg/kg was required to restore ETK activity. Recovery of ETK activity in thiamin deficient rats was greater at 20.0 mg thiamin/kg than at 3.0 or 3.75 mg thiamin/kg diet. Among the thiamin deficient rats that were fed 35% enzyme inactivated beef or chicken, which was processed as frozen, thermal (F_0=6), gamma irradiated or electron irradiated (47-71 kGy), and supplemented with thiamin, there were no significant differences within each meat group in growth of ETK recovery during the 28 day repletion period. It was concluded that gamma and electron radappertization (or thermal processing) did not produce antithiamin factors in beef or chicken.

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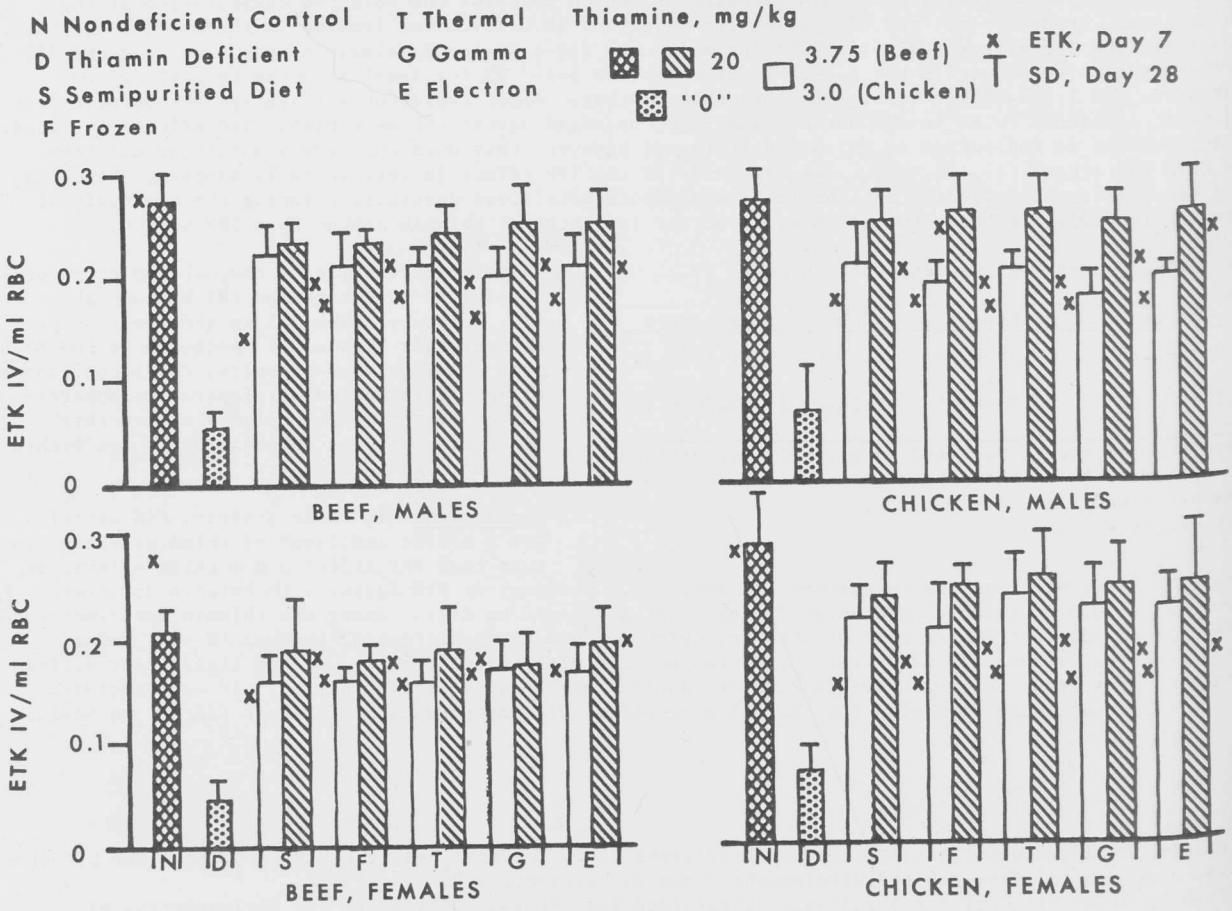

FIGURE 1. ERYTHROCYTE TRANSKETOLASE ACTIVITY FOR DAY 7 AND 28.