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# Intake of Nitrites and Nitrates from Processed Meats by Primary School Children

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Nitrites and nitrates are used as food additives in the processing of meat products. Nitrite in food is considered primarily to cause health problems because its presence both in food and in the body may lead to the formation of carcinogenic nitrosamines. The nitrates could be reduced into nitrites by the microflora in the oral cavity. To assess the risk of nitrites and nitrates intake from processed meat by primary school children, 378 primary school children, aged 8-10 years attending No. 4, Basic Education High School, Ahlone, were recruited in the study. Patterns of processed meat consumption were determined by 6-day food diary method and their body weights were measured. Nitrites and nitrates contents of ten items of processed meat which were commonly consumed were determined. The mean body weight was 27.2±7.3 kg. During the period of taking 6-day food diary, 164(43.4%) did not consume any processed meat products but 60(28%) consumed them more than 3 days. Nitrites and nitrates contents were 9.44 mg/kg and 67.15 mg/kg in three chicken meat products, 4.31 mg/kg and 23.06 mg/kg in four fish meat products, 5.97 mg/kg and 55.04 mg/kg in two pork meat products and 3.35 mg/kg and 43.5 mg/kg in one crab meat product, respectively. Mean exposure to the nitrite and nitrate (mg/kg body weight/day) from processed meat by children was 0.02±0.02 and 0.16±0.14, respectively. Intake of nitrites in 12(5.6%) children were more than Acceptable Daily Intake (ADI), i.e., 0.06 mg/kg body weight/day. Health education on low intake of processed meat should be encouraged.

## INTRODUCTION

Preservation of food products with additives is an ancient practice. In the late 19$^{th}$ century and early 20$^{th}$ century, food preservation was mainly confined to heat sterilization, in combination with the addition of salts and spices. As the society became more and more urbanized, the area of food technology including production, processing, and distribution of food products underwent a revolutionary change. The use of various additives to extend the shelf-life of food products has become widespread in food industry. In order to prevent indiscriminate use, regulations have been developed by many countries limiting the type, purity, uses and amounts of food additives permitted in food.

Nitrite and nitrate are widely used as food additives in the processing of meat products because of their antimicrobial action and their ability to give meat a characteristic pink color, texture and flavor. Cured meat products are the major source of nitrites in human dietary intake. Nitrate and nitrite can also be found in food as naturally occurring compounds, drinking water and vegetables being substantial source of nitrate intake. Interest in the dietary intakes of nitrate and nitrites has arisen from concerns about their possible adverse effect on health.[1]

The nitrate ion has a low level of acute toxicity, but if it is transformed into nitrite, it may constitute a health problem. Reduction to nitrite may take place in contact with metals in the presence of bacteria or enzyme nitrate reductase. It has

been estimated that 5-8% of the nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity.[2] The acceptable daily intake (ADI) for nitrate is 0-3.7 mg $kg^{-1}$ body weight (as nitrate ion).[3]

Nitrite has higher acute toxicity than nitrate and ADI is 0-0.06 mg $kg^{-1}$ body weight (as nitrite ion).[4] As an unstable ion, nitrite undergoes a series of reactions as soon as it is added to food. In the acidic environment, nitrite is converted into nitrous acid, which decomposes into nitric oxide. Nitric oxide, being an important product from the standpoint of color fixation in cured meat, reacts with myoglobin to produce a red pigment - nitrosomyoglobin.[4] The intake of nitrite is normally low compared with the dose that is acutely toxic but nitrite in food is considered primarily to cause health problems because its presence both in food and in the body may lead to the formation of carcinogenic nitrosamines[5] and clinical symptoms of metheamoglobinaemia.[6]

Although the food safety monitoring program has been carried out for a decade in Myanmar, there is the scarcity of studies concerning dietary risks due to consumption of nitrites and nitrates from processed meat by school children. Therefore, the aim of this study was to assess the dietary risk connected with the intake of nitrites and nitrates from processed meats by primary school children.

## MATERIALS AND METHODS

The study was descriptive and primary school children were chosen as the population of this study because they are assumed as the population group which is more likely to have above average consumption of processed meat products. Yangon was purposely selected because various kinds of cured meat products are available from markets and wholesale centers and No. 4, Basic Education High School, Ahlone was randomly selected. A total of 378 primary school children, 8-10 years of age attending the above-mentioned school were included in the study. Total study period was one year.

*Six-day food diary method*

Patterns of processed meat consumption were determined by 6-day food diary method. The children were explained how to fill the food diary. The food items with amount were noted starting from the first food or drink taken after waking up to the last food or drink taken before sleeping. To estimate the amount or portion of food taken, showing food photos with known weights or serving sizes was assisted. The food diaries were filled by the children themselves or with the assistance of their parents.

On next day, the validity of filling food diary was checked by the trained persons by interviewing. Portion sizes and amount of consumed meat products were confirmed by showing food photos. The pretest filling in food diary was carried out before the study and the explanations were made again. The participated students filled the food diaries for six days (including one holiday) and the invalid food dairies were deleted from the analysis. From the data of 6-day food diary, the patterns of processed meat products consumption were analyzed according to names of the food items and frequencies of them. Then, the intakes of processed meat products per day were estimated by showing the photos of them.

*Food sampling and sample preparation*

By the names of food items which were actually consumed by the participated students, altogether twenty processed meat products were identified as commonly consumed. Then, these foods were grouped on the base of meats which were made up of, e.g, chicken, fish, pork and crab meat products, etc. A total of ten items (four fish, three chicken, two pork and one crab) of processed meat products were purchased mostly from the shops of the school canteen and of the area where the students lived (if they were not available in the canteen). Generally, they were local made except fish-

tofu which is imported from Thailand. Food samples were prepared as consumption as soon as possible if they were raw. They were kept at the laboratory with a maximum storage period of 2 days. Prepared foods were frozen until analysis.

*Determination of nitrite and nitrate contents in the selected food samples*

The concentrations of nitrate and nitrite in foods were determined using standard, validated method (ISO/DIS 3091).[7] In summary, 5 grams of homogenized sample were kept in a hot borex solution containing activated charcoal, and proteins were precipitated by potassium ferrocyanide trihydrate and zinc acetate dihydrate. The deproteinated filtrate was required to determine both nitrate and nitrite contents in the sample. The extracted nitrates were reduced by metallic cadmium. A red color was developed by addition of sulphanilamide and *N*-1 naphthylethylene diamine dihydrochloride to the filtrate and photometric measurement at wavelength of 538 nm was done. Results were expressed as mg $kg^{-1}$ of sodium nitrate and sodium nitrite. The coefficients of variation (CV) for food matrices were 9.6% for nitrite, and 4.1% for nitrate, respectively. The mean recovery of known amounts of nitrite spiked into food samples was 97.1. Methods for determination of nitrite and nitrate concentrations were corrected for recovery. Nitrite and nitrate contents were determined in a total of 30 samples of ten food items (four fish, three chicken, two pork and one crab).

*Exposure estimate*

Body weights of the participated school children were measured to calculate the exposure of nitrite and nitrate from processed meat products. Weights were measured by means of bathroom scales placing on thin flat surface and zero adjustment was done before measuring.

An individual approach was applied: multiplying the amount (intake) of processed meat products per day and contents of nitrite and nitrate in the particular consumed food items, individual intake of nitrite and nitrate from those foods (expressed in mg $day^{-1}$) could be calculated. Then, it was divided by the individual body weight to give an exposure in mg $kg^{-1}$ body weight $day^{-1}$.

*Statistical analysis*

Data entry and analysis were done by using SPSS 11.5 for Windows. Consumption patterns of processed meat products and percent distributions of nitrite and nitrate intake from those products by primary school children were presented as frequency distributions. Nitrite and nitrate contents in studied samples of each meat were presented as mean±SD values. Acceptable Daily Intake (ADI) of 0.06 mg/kg body weight/ day and 3.7 mg/kg body weight/day were used as cut-off points for exposure estimation of nitrite and nitrate, respectively.

## RESULTS

Table 1 shows the consumption pattern of processed meat product by the studied students. During the period of taking food diary (6 days), among 378 students, 164 (43.4%) did not consume any processed meat products and 154(40.7%) consumed processed meat products less than three days but 60(28%) consumed them more than 3 days.

Table 1. Consumption patterns of processed meat products by primary school children

| | Percentage (No. of students) |
|---|---|
| *Days of consumption* | |
| <3 days | 84.1(318) |
| ≥3 days | 15.9(60) |
| *Means of consumption* | |
| As snacks | 64.0(137) |
| As dishes | 14.0(30) |
| Both | 22.0(47) |
| *Items of processed meat products* | |
| One meat product | 30.8(66) |
| Two meat products | 25.7(55) |
| Three meat products | 30.8(66) |
| More than 3 meat products | 12.7(27) |
| *Intake of processed meat products (gm/day)* | |
| <100 g | 82.7(177) |
| 101-200 g | 15.4(33) |
| 201-300 g | 1.4(3) |
| >300 g | 0.5(1) |

According to the 6-day food diary, only 214 students consumed processed meat products. Among 214 students, 137(64%), 30(14%) and 47(22%) had processed meat products as snacks, as dishes (with meals) and as both, respectively. Time of having these foods was mostly after the day's teaching. More than one tenth of the students (12.7%) were fond of having processed meat because they did not choose the items of them. The intake of processed meat products was <100 gm/day in the majority of the students (82.7%).

In Table 2, the mean values and standard deviation of nitrite and nitrate content in studied triplicate samples of meat products are shown. The mean nitrite and nitrate contents were 4.31±0.08 mg/kg and 23.06± 0.98 mg/kg in four fish meat products, 9.44±0.86 mg/kg and 67.15±3.87 mg/kg in three chicken meat products, 5.97±0.2 mg/kg and 55.04±1.25 mg/kg in two pork meat products, respectively.

Table 2. Mean±SD content of nitrite and nitrate in food samples (mg/kg of food)

| Items of food samples* | Nitrite | Nitrate |
|---|---|---|
| *Fish meat products* | | |
| Fish slice | 7.42±0.15 | 28.03±1.06 |
| Fish steak | 5.12±0.07 | 14.02±0.69 |
| Spicy fish slice | 2.89±0.05 | 17.05±0.98 |
| Fish-tofu | 1.82±0.05 | 32.87±1.20 |
| *Chicken meat products* | | |
| Minced slice | 21.72±2.02 | 136.94±7.5 |
| Chicken tendon ball | 1.33±0.08 | 39.76±3.9 |
| Sausage | 5.27±0.47 | 24.75±0.2 |
| *Pork meat products* | | |
| Minced slice | 1.82±0.09 | 49.2±0.09 |
| Sausage | 10.31±0.3 | 62.6±2.4 |
| *Crab meat product* | | |
| Crab steak | 3.35±0.3 | 43.5±1.3 |

*=Triplicate samples of each item

Among the studied four fish products, fish slice contained the highest amount of nitrite but the highest amount of nitrate was found in fish-tofu. Minced chicken slice contained higher amount of nitrite and nitrate than those of other two studied chicken products. The nitrite and nitrate contents in pork sausage were the highest among the studied processed meat products.

The grouped data of intake of nitrite and nitrate from processed meat products by 214 school children are shown in the Table 3. Intake of nitrite and nitrate from processed meat products was calculated by multiplying the intake amount of processed meat products (gm/day) and the concentration of nitrite and nitrates in the particular products (mg/kg of food). The mean±SEM of intake of nitrite and nitrate from processed meat products by 214 children were 0.57±0.04 (0.014-3.83) mg/day, and 4.15±0.26 (0.021-26.5) mg/day, respectively.

Table 3. Intake of nitrite and nitrate from processed meat products by primary school children

| | Percent (Number of students) |
|---|---|
| *Nitrite (mg/day)* | |
| <1 | 83.2(178) |
| 1-<2 | 15.4(33) |
| 2-<3 | 0.47(1) |
| ≥3 | 0.93(2) |
| *Nitrate (mg/day)* | |
| <1 | 17.8(38) |
| 1-<10 | 77.6(166) |
| 10-<20 | 3.7(8) |
| ≥20 | 0.93(2) |

Exposure of nitrite and nitrate from processed meat products of 214 school children are shown in Table 4. Exposure of nitrite and nitrate (mg/kg/day) was calculated by dividing the intake of nitrite and nitrate (mg/day) by body weight in kilogram of particular primary school children. Mean exposure to the nitrite and nitrate (mg/kg body weight/day) from processed meat by children was 0.02±0.02 and 0.16±0.14, respectively.

Table 4. Exposure of nitrite and nitrate from processed meat products of primary school children

| | Percent (Number of students) |
|---|---|
| Nitrite (mg/kg/day) | |
| <0.01 | 40.65(87) |
| 0.01-<0.06 | 53.7(115) |
| ≥0.06 | 5.6(12) |
| Nitrate (mg/kg/day) | |
| <0.1 | 48.6(104) |
| 0.1-<1.0 | 50.9(109) |
| ≥1.0 | 0.5(1) |

Based on current Acceptable Daily Intake (ADI) of 0-0.06 mg $kg^{-1}$ body weight $day^{-1}$ (as nitrite ion),[6] 12(5.6%) of children had higher exposure of nitrite than ADI. However, since the ADI for nitrate is 0-3.7 mg $kg^{-1}$ body weight $day^{-1}$ (expressed as nitrite ion),[6] all children of this study were less exposed to nitrate than ADI. Although mean intake of nitrite from meat products was not high, when the body weight was included in calculation of exposure, higher exposure to nitrite than ADI was found. It meant that the children with small body weight frequently consumed the processed meat products with high nitrite contents.

## DISCUSSION

Six-day food diary method followed by next day recall was used in this study to investigate the consumption patterns and intake of processed meat products. A trained interviewer checked the validity of food diaries by asking the individual students to recall all food and drinks for previous 24 hours. Parents were allowed to assist in writing food diaries and prompts for quantification of portion size by showing of food photos were used. This method could assess current or past diet and be repeated to gain measure of daily variation and improved precision, quick, and inexpensive. Bias caused by errors in memory, perception, conceptualization of food portion sizes was limited. Therefore, this method can be used in primary school children for dietary intake measurement.

Nitrate and nitrite are present in food naturally, or may be present as a result of the use of fertilizers on crops or from their uses as preservatives. In curing process, nitrate is partly reduced to nitrite by bacteria. Nitrites are essential for the pink color of cooked cured meat products and for the characteristics of cured meat flavor of some of these products. It is also essential for enhancing shelf life of meat products and in protecting against the bacterial spoilage and hazards caused by *Clostridium botulism*. Nitrites and nitrates are widely used in the production of cured meat products and in the preservation of fish in some countries. In most countries, the addition of nitrates and/or nitrites to meat and meat products are governed by legislation. The concentrations which are allowed in the food stuffs differ from country to country. In Canada, the content of nitrites in sausages is 26 mg $kg^{-1}$ and in ham 24 mg $kg^{-1}$.[8] In Estonia, the maximum permitted concentrations of residual nitrite (as $NaNO_2$) and nitrate (as $NaNo_3$) in commercial meat products are 100 and 250 mg $kg^{-1}$, respectively.[9]

The Estonia food safety monitoring program monitored and analyzed the cured meat products for nitrite and nitrate in 2004. The mean concentrations of sodium nitrite and sodium nitrate in cooked sausage were 30(<10-61) mg $kg^{-1}$ and 56(<5-160) mg $kg^{-1}$, respectively.[9] Therefore, the content of nitrate in pork sausage of this study (62.6±2.4 mg $kg^{-1}$) was slightly higher than that of Estonia's study. Moreover, in the Estonia's study, daily intake of nitrite and nitrate from meat products in 4 to 9 years old children were 0.83 (0.65-0.92) mg/day and 1.65 (1.3-1.8) mg/day, respectively.[9] Therefore, mean nitrate intake from meat products of 4.15±0.26 (0.021-26.5) mg/day in 8 to 10 years old school children of this study was slightly higher than that of Estonian children.

The European Directive 95/2/CE (1995) allows 150 ppm of nitrite (if alone) or 300 ppm when combined (nitrite plus nitrate), and the residual values should be less than 50 ppm (if alone) or 250 ppm (if combined). The Food and Drug Administration allows these compounds to be used as food additives as long as they are of food grade and are added only in the amount needed. The maximum amount of nitrite allowed in smoked and cured fish and meat is 200 ppm. There is no regulatory minimum in-going nitrite level for cured products that have been processed to ensure their shelf stability. However, 40-50 ppm nitrite are useful in that it has some preservative effect and color-fixing purpose.

By the time meats are consumed, they contain less than 50 ppm of nitrite. It is that commercially prepared meats in the USA contain about 10 ppm of nitrite when bought in a supermarket.[10]

In National Food Survey (United Kingdom, 1998), population average intakes (both adults and children) of nitrite and nitrate were 1.8 (1.6-11.9 mg/day) and 3.4 (1.8-3 mg/day, respectively.[11] In this study, nitrites intake from processed meat products was lower than that of United Kingdom National Food Survey (1998). However, their results were analyzed on Total Diet Study, i.e., all food groups (green and canned vegetables, fruits, dairy products, cereals, and beverages) were included. To estimate total intake, all sources of nitrate and nitrite in the diet should be taken into account. Since our data could only be analyzed on the processed meat products, if all food groups were included, the intake of nitrite could be more.

The Acceptable Daily Intake (ADI) is defined as the amount of a chemical, expressed on mg/kg body weight basis, which can be ingested daily over a lifetime without incurring any appreciable health risk and is based on an evaluation of available toxicological data. If the water concentration data could be included the individual exposure, it was valid to sum the contribution of nitrate water to that from food for each individual. Therefore, it can be said that higher exposure of nitrite from processed meat products than ADI is one of the public health concerns.

In conclusion, this study could not show the dietary risk of nitrite and nitrate at population level, but exposure of nitrite and nitrate from processed meat products in the selected primary school children could be found out. Among them, 5.6% children have exposed nitrites from processed meat products more than Acceptable Daily Intake. Since nitrates and nitrites could be exposed from other dietary sources, e.g, water and vegetables other than processed meats, health education on low intakes of processed meat should be encouraged.


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