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The Effects of Formaldehyde on Bacterial Growth in Mink Feed, Feed Consumption and Reproductive Performance of Adult Mink, and Growth of Mink Kits

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Chengfeng (Kathy) Li

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Major professor

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THE EFFECTS OF FORMALDEHYDE ON BACTERIAL GROWTH IN MINK FEED, FEED CONSUMPTION AND REPRODUCTIVE PERFORMANCE OF ADULT MINK, AND GROWTH OF MINK KITS

By

Chengfeng (Kathy) Li

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ABSTRACT

THE EFFECTS OF FORMALDEHYDE ON BACTERIAL GROWTH IN MINK FEED, FEED CONSUMPTION, AND REPRODUCTIVE PERFORMANCE OF ADULT MINK, AND GROWTH OF MINK KITS

By

Chengfeng (Kathy) Li

The objectives of this study were to determine the efficacy of formaldehyde (FA) in suppressing bacterial growth in mink feed and to determine the effects of FA incorporated into mink feed on feed consumption, reproductive performance and kit growth. Formaldehyde-treated diets (0, 550, and 1100 ppm FA) were kept refrigerated (4°C) for 7 days or incubated (30°C) for 24 hours to determine if FA would suppress bacterial growth. Results indicated that both concentrations (550 and 1100 ppm) of FA in the diets suppressed bacterial growth effectively. The feed consumption trial indicated that mink preferred untreated feed to FA-treated feed even though the presence of FA suppressed bacterial growth. In the reproductive trial, feed containing 1100 ppm FA decreased mink survival at birth, induced anemia and resulted in some kits displaying the undesirable "cotton fur".

To my husband, Cunqin,
my daughter, Sisi
and
my parents, Shishu Xiao and Keming Li

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INTRODUCTION

Formaldehyde (FA) has been commercially produced since the early 1900s and nearly seven billion pounds of FA are produced each year in the United States (1). Formaldehyde is one of industry's most important and widely used chemicals. It is used in the manufacturing of other industrial chemicals, agricultural products, and leather products. Formaldehyde is also used as a preservative in consumer products, such as cosmetics and household cleaning agents. In addition, it has medical applications as a disinfectant to kill viruses, bacteria, fungi, and parasites, and it may be also used as an antibacterial agent for proventing certain food products from spoilage by microbial contamination (2).

In 1996, the U.S. Food and Drug Administration (FDA) amended its food additive regulations to allow the use of FA as an antimicrobial agent in poultry feeds (3). FDA provided guidelines for the safe use of formaldehyde (37 percent aqueous solution) at the rate of 5.4 lbs per ton (2.5 kg per ton) as an antimicrobial feed additive for the purpose of keeping complete poultry feeds Salmonella spp. negative for up to 14 days, and at a level of 1.65 to 2.65 lbs per ton as an antimicrobial agent against bacteria, mold, and yeast in fishmeal and animal by-product meals.

Mink feed is often composed of poultry by-products which may be high in

bacteria upon arrival at the mink farm although part of the dietary ingredients may be frozen. During the summer season, this bacteria-contaminated feed is kept on the top of the cage for 24 hours (mink farmers usually feed mink once per day) which makes an ideal environment for bacterial growth. That is why mink farmers usually keep the diet refrigerated for no more than 3 days before discarding it. Many of the bacteria found in mink feed are potential disease producers. Young mink (kits) may be especially susceptible to bacterial infection during the "June Blues" period when kits make the transition from nursing to consuming adult feed placed in or on top of the cage. If FA can be used at levels approved by the FDA without adverse effects on mink and if it can reduce microbial activity in animal by-products and / or prepared feed, this may provide the mink farmer with alternative methods for preventing the spoilage of mink feed.

Under certain conditions, humans and animals may be exposed to FA by inhalation, ingestion, or skin contact which could result in adverse effects. Inhalation of high concentrations of FA has produced nasal tumors in rodents (4). Malignant and benign tumors of the stomach and intestines were observed in rats administered FA in a lifetime feeding study (5). Mink fed Pacific hake containing FA developed anemia and a condition called "cotton fur" because of reduced iron availability (6).

Thus, before FA is used as an antibacterial agent by mink ranchers, it is necessary to assess the toxicity of FA to mink. Although some data suggested adverse effects of FA on the respiratory and gastrointestinal tracts in animals as

mentioned above, the reproductive and teratogenic effects of FA have been poorly studied and there is little information on the use of FA-preserved poultry by-products in mink feed.

Therefore, we propose to test the hypotheses that:1) FA will not adversely affect mink reproductive performance, and 2) FA will suppress bacterial growth in mink feed. The objectives of this study are: (1) to determine the highest concentration of FA which mink will consume in feed; (2) to determine if mink will prefer FA-treated feed to untreated diet kept refrigerated for a period up to 7 days; (3) to determine the effects of FA on the breeding and reproductive performance of female mink as well as early growth and survival of the offspring; (4) to determine to what extent FA incorporated into mink feed will suppress bacterial growth in feed kept refrigerated for up to 7 days and in feed kept at 30°C for 24 hours.

LITERATURE REVIEW

PHYSICAL AND CHEMICAL PROPERTIES OF FORMALDEHYDE

Formaldehyde is a flammable, colorless, reactive, and readily polymerized gas at normal temperature and pressure. It has a molecular weight of 30.03, a boiling point of -19°C, and a melting point of -118°C. In the gaseous state, FA has a pungent odor. It is readily soluble in nonpolar solvents such as chloroform, ether, and toluene but undergoes solvation in polar solvents such as water or methanol (7). The most common commercial form of FA is a 30-50% (by weight) aqueous solution. Methanol or other substances are usually added to the solutions as stabilizers to reduce intrinsic polymerization (8).

Formaldehyde is a highly reactive chemical that undergoes many reactions at its polarized carbonyl group. The structure of FA is shown in Figure 1. Under atmospheric conditions, FA is readily photooxidized by sunlight to carbon dioxide as shown below:

$$HCHO \xrightarrow{O_2} HCOOH \xrightarrow{O_2} H_2O + CO_2$$

It reacts relatively quickly with trace substances and pollutants in the air, and its half-life in urban air, under the influence of sunlight, is short. In the absence of nitrogen dioxide, the half-life of FA is approximately 50 minutes during the day while in the presence of nitrogen dioxide, it is about 35 minutes (9). FA decomposes into methanol and carbon monoxide at

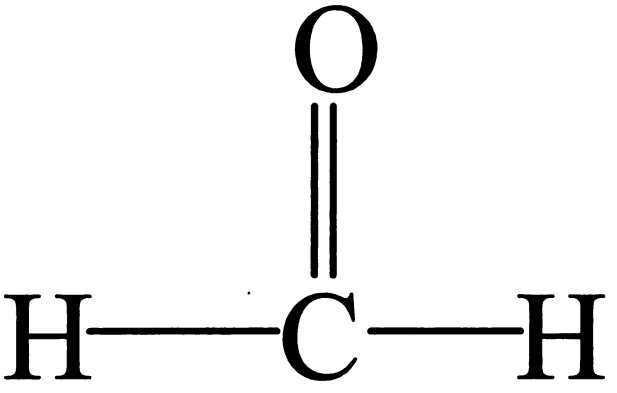

Figure 1. The Chemical Structure of Formaldehyde

temperatures above 150°C, although uncatalysed decomposition is slow at temperatures below 300°C. Synthesis of FA is accomplished typically by the oxidation of methanol in the presence of a copper or silver catalyst (9).

The formation of resinous products on reaction with other chemicals is one of the most useful characteristics of FA and is the reason for its immense importance in the synthetic resin industry. Under suitable conditions, the molecules of many compounds are linked together by methylene groups when subjected to the action of FA. Phenol- and urea-FA resins are polymethylene compounds of this type (10).

SOURCES OF FORMALDEHYDE

Formaldehyde is a normal metabolite in mammalian systems, and it is also present in food, either naturally or as a result of its use as a food additive. Formaldehyde is present in the environment as a result of natural processes and from man-made sources. It is formed in large quantities in the troposphere through the oxidation of hydrocarbons. Emissions of FA from industrial processes vary widely according to the type of industry. One example is automotive exhaust from engines without catalytic converters. Altschuller et al. (1961) reported that automotive exhausts contain FA at 29-43 ppm (11). Mobile sources (automobiles, diesel engines, and aircraft engines) emit approximately 666 million pounds of FA annually. Local concentrations may vary with traffic patterns and vehicular density. Minor natural sources include the decomposition of plant residues and the transformation of various chemicals emitted by foliage (10).

There are several indoor environmental sources that can result in human exposure

including cigarettes and other tobacco products, furniture containing FA-based resins, building materials containing urea-formaldehyde resins, paints, disinfectants, gas cookers and open fireplaces. Indoor areas of special importance are hospitals and scientific facilities where FA is used as a sterilizing and preserving agent (2).

ABSORPTION

The uptake of exogenous FA into the human body is accomplished by inhalation, ingestion, or dermal exposure. Formaldehyde is readily absorbed through the respiratory and gastrointestinal tracts while dermal absorption of FA appears to be very slight. Absorption of FA via the upper respiratory tract in dogs has been estimated to exceed 95% of the inhaled dose (12). Detailed studies on the distribution of ^{14}C-formaldehyde in the rat nasal cavity have confirmed that it is absorbed primarily in the upper respiratory system. Following a 6-hour exposure by inhalation, the amount of ^{14}C-formaldehyde absorbed appeared to be approximately proportional to the airborne concentration. The amount retained did not appear to vary following exposure (13). Following oral exposure of dogs to FA, formate levels in blood increased rapidly indicating rapid uptake and metabolism (14). Dermal absorption does not appear to be significant in comparison to inhalation or ingestion. For example, 24 hours after dermal application of 0.4-0.9 μg ^{14}C-formaldehyde / cm^2 on 5 male monkeys, most of the dose had been lost mainly by evaporation from the skin (52%) or it was bound to the surface layers of the skin at the application site (34%). Percutaneous penetration was very low, and was calculated to be, at most, 0.5% of the applied dose. The total body burden of a necropsied monkey, 24 hours after dermal dosing,

was 0.2% of the dose, confirming that aqueous FA does not penetrate the skin to any appreciable degree, even when applied directly to it (15).

DISTRIBUTION

Under normal conditions, the concentration of free FA is very low in animal and human tissues because of its rapid metabolism. In the blood of different species, the mean of endogenous FA concentrations were 2.24 ± 0.07 mg/kg (mean ± SE) in the rat (13), 2.42 ± 0.09 mg/kg in the monkey (16), and 2.61 ± 0.14 mg/kg in 6 human volunteers (4 males, 2 females) (17). Malorny et al. (14) intravenously infused 0.2 mol FA into dogs and cats and McMartin et al. (18) performed similar infusions in cynomolgus monkeys. In both studies, there was no accumulation of FA in the blood.

METABOLISM

Formaldehyde is a product of normal human metabolism, formed endogenously from serine, glycine, and other amino acids or substances containing methyl groups. It participates in one-carbon transfer in the intermediary metabolism of amino acids, purines, and pyrimidines. The biochemical transformations of endogenous and exogenous FA are similar and involve coenzymes and hydrogen transport systems that are normally present in all animals and bacteria (19-20).

The main reaction of FA appears to be an initial oxidation to formic acid in the liver and erythrocytes (20-23). Once formic acid (formate) is formed, it can undergo 3 reactions: oxidation to carbon dioxide and water, elimination in the urine as a sodium salt, or entrance

into the metabolic one-carbon pool. Formaldehyde may also enter the one-carbon pool directly.

In humans, the formation of formate from FA appears to involve an initial reaction with glutathione (GSH) to form a hemiacetal (24-25). The enzyme FA dehydrogenase, which is a cytosolic GSH-dependent enzyme, then oxidizes the hemiacetal to formic acid with nicotinamide adenine dinucleotide (NAD) as a hydrogen acceptor (25). This pathway may serve as an important defense mechanism in the detoxification of FA. Thus. depletion of GSH concentrations would inhibit FA metabolism and exacerbate FA effects on the tissues.

Instead of following the metabolic pathway described above, FA, being a highly reactive compound, can also interact with virtually every cellular constituent, including nucleic acids, histones, amino acids and proteins to form methylol adducts which can react further to form methylene linkages among these reactants (26-27). Reaction of FA is specific for single-stranded DNA because hydrogen bonding with the opposite strand hinders the reactivity of amino groups; thus, FA will not react with double-stranded DNA (28-30). Only FA cross-links of DNA and protein are stable (31). If permanent cross-links are formed between DNA reactive sites and FA, these links could interfere with replication of DNA and may result in a mutation.

Formaldehyde is rapidly metabolized to formic acid in humans, dogs, cats, rabbits, guinea pigs, rats, and monkeys (32-33). Heck and Casanova-Schmitz (17) showed that blood FA concentrations did not rise in human volunteers even immediately after inhalation exposure. In a study of formate-poisoned monkeys, there was no detectable increase in FA

concentration in samples of blood, urine, cerebrospinal fluid, freeze-clamped liver (at the temperature of liquid nitrogen), kidney, optic nerve, or brain (23, 32) at a time when formate concentrations were high. The time of semitransformation of FA into formic acid is only 1 minute in many animal species including man. Thus, free FA is not usually found in plasma or other body tissues in measurable quantities. Such endogenous FA as is produced may be reasonably presumed to be metabolized rapidly to formate or to enter the one-carbon pool. When exogenous exposure does occur, FA is likewise rapidly metabolized to formate and excreted, converted to CO_2, and / or incorporated into other molecules. The same pathways seem to occur in all mammalian species, but reaction rates differ among various species and tissues. For example, Den Engelse et al. (34) have shown that mouse (C3Hf/A) and hamster (Syrian Golden) lungs do not convert formate to CO_2 as efficiently as liver tissue does.

ELIMINATION AND EXCRETION

Elimination of formate is slower than its formation from absorbed FA and depends on the species. For example, elimination times of formic acid vary from 12 minutes in the rat and guinea pig to 67 and 77 minutes in the cat and dog, respectively (33). For man, the half-life of formate is 45 minutes (35). The 2 pathways of final elimination are exhalation or urinary elimination. Neely (36) administered FA intraperitoneally to rats and reported that 82% of the radiolabel was recovered as carbon dioxide and 13-14% as urinary methionine, serine and a cysteine adduct. Even after high FA uptake, the elimination of formate via the kidneys of rats is virtually negligible (37).

EFFECTS ON HUMAN BEINGS

Reproductive Effects

The teratogenic effects of FA and its reproductive toxicity have been poorly studied, according to contemporary standards. Only a few human studies have been conducted. Shumilina (38) studied the reproductive potential of women who were exposed to urea FA resins (1.25 to 3.75 ppm FA in air). The exposed women had a 3-fold increase in menstrual irregularities (47%) compared to non-exposed controls (18.6%), with dysmenorrhea being the most common effect. Pregnant exposed women tended to have higher rates of threatened abortion than pregnant non-exposed women, but full-term birth rates in both groups were comparable. Exposed mothers had babies of lower birth weight than non-exposed mothers. These data raise the possibility that FA may affect human reproductive processes. However, Hemminki et al. (39) examined the rate of spontaneous abortions in pregnant hospital workers engaged in sterilizing instruments with chemical agents. There was no increase in this rate associated with the use of FA.

Taskinen et al. (40) investigated the occurrence of spontaneous abortions among women working in laboratories in Finland and congenital malformations by a matched retrospective case-control study. The final population in the study of spontaneous abortions was 206 cases and 329 controls; that in the study of congenital malformations was 36 cases and 105 controls. The odds ratio for spontaneous abortion was increased among women who had been exposed to formalin for at least 3 days per week (odds ratio = 3.5; 95% confidence interval [CI] = 1.1-11). No association was observed between exposure to formalin and congenital malformations.

Genotoxic effects

The effects of FA on the frequencies of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes of people occupationally exposed to FA have been investigated in some studies. Both positive and negative results were obtained, but their interpretation was difficult because of the small number of subjects studied and inconsistencies in the findings (41).

Bauchinger and Schmid (42) reported an increased incidence of chromosomal aberrations in lymphocytes from 20 male paper makers exposed to FA for 2-30 years when compared with lymphocytes from 20 unexposed males in the same factory. In the study of Ballarin et al. (43), the frequency of micronuclei in respiratory nasal mucosa cells was investigated. At least 6000 cells from each individual were scored for micronuclei. A significant excess of micronucleated cells was seen in the exposed group (mean percentage of micronucleated cells, 0.90 ± 0.47; range 0.17-1.83 in exposed group; 0.25 ± 0.22; range, 0.0-0.66 in controls; $p < 0.01$). However, there were concurrent exposures to significant amounts of wood dust, and a dose-response with FA exposure levels was unable to be determined. These data suggest that FA can induce gene mutations in human cells *in vitro.* The mutations may be induced in part by a slipped mispairing mechanism occurring during replication, resulting from the formation of a DNA-protein cross-link (44). The FA-induced cross-links were repaired after 2 hours (45). However, in a study of workers exposed to FA in a factory manufacturing wood-splinter materials, short-term cultures of peripheral lymphocytes were examined from a group of 20 exposed workers (concentrations of 0.5-8.6 ppm FA for periods of 5-16 years) and a group of 19 control people. No significant

difference was observed between control and exposed groups with respect to any of the chromosomal anomalies scored in the study (46).

Carcinogenic effects

A large number of epidemiologic studies investigating potential associations of FA exposure with human cancer have been conducted over the past 20 years. Many critiques and reanalyses have also been published. Site-specific measurements of histopathology and DNA-protein cross-linking in rhesus monkeys (47-49) indicate the nasal cavity as the primary target, with the nasopharyngeal and tracheal regions also being somewhat susceptible. These observations also suggest that the nasal sinuses would not be a target since neither histopathologic effects nor DNA-binding were detectable there even at the highest FA exposure concentration in monkeys (6 ppm).

The study of British pathologists was extended and expanded by Harrington and Oakes (50), who added new entrants and traced new and previously studied subjects from 1974 through 1980. The population now included 2307 men and 413 women. Mortality from brain cancer was elevated among men (standardized mortality rate [SMR] = 3.3; 95% CI = 0.9-8.5; 4 deaths). No nasal cancer and no cancer of the nasal sinuses were seen.

This cohort was further evaluated by Hall et al. (51), who extended follow-up of mortality from 1980 through 1986 and added new members of the Pathological Society, resulting in 4512 individuals available for study. No significant increase was seen for cancer at any site.

Luce et al. (52) conducted a case-control study of cancer of the nose and paranasal sinuses in France to determine whether occupational exposure to FA was associated with an

increased risk of sinonasal cancer. Case (n = 207) and controls (n = 409) were interviewed to obtain detailed information on job history and other potential risk factors for sinonasal cancer. Occupational exposures to FA and 14 other substances or groups of substance were assessed by an industrial hygienist on the basis of information obtained during a personal interview at the hospital (for the cancer patients) or at home (for the healthy controls) on job histories. The results showed that no significant association was found between exposure to FA and squamous-cell carcinomas of the sinonasal cavities.

The cohort of Acheson et al. (53) was further followed-up from 1981 through 1989 by Gardner et al. (54). This follow-up included 7660 people employed before 1965 rather than the 7680 in the original study, because additional eligibility checks resulted in exclusion of 20 workers. The cohort also included 6357 workers who were first employed from 1965 onwards and who were thus excluded from the original report. The updated findings include 1 death from nasal cancer compared with 1.7 expected in this number of men during the follow-up period which gives no support to the original hypothesis based on animal experimental data that FA may be a nasal carcinogen in humans.

Andjelkovich et al. (55) reported on the mortality of a subset of a previously studied cohort of workers with potential exposure to FA in an automotive iron foundry in the United States. A cohort of 3929 men was followed from 1 January 1960 to 31 December 1989. For comparison, an unexposed group was also analyzed, which consisted of 2032 men who had worked during the same period but had not been exposed to FA. The results showed that there was no association between FA exposure and deaths from malignant or nonmalignant diseases of the respiratory system.

In summary, the evidence for increased risk of respiratory tract cancer in humans resulting from FA exposure is very weak. No studies have shown carcinogenic effects of FA in the liver of humans.

EFFECTS ON EXPERIMENTAL ANIMALS

Reproductive Effects

The teratogenic effects of FA and its reproductive toxicity for animals have also been poorly studied with only a few animal studies having been performed. These studies have indicated that whether administered to rodent species by the dermal, respiratory or oral route, FA did not exert adverse effects on reproductive parameters or fetal development (56-57).

Marks et al.(56) intubated pregnant CD-1 mice on days 6 to 15 of gestation with 1% aqueous FA at dose levels of 0, 74, 148, 185 mg/kg/day. On day 18, the mice were killed and the offspring examined. FA was lethal to 22 out of 34 pregnant mice at the highest dose (185 mg/kg/day), but no dose resulted in offspring malformations (fetus size, skeletal or visceral abnormalities). The number of resorption sites was increased and mean litter size was slightly decreased only in the highest dose group and these effects could be considered a consequence of maternal toxicity.

Four reports have been published from a study in which 3 groups of 12 female rats were exposed to levels of 0, 0.01, or 0.83 ppm FA in air for 24 hours/day, during a period extending from 10 to 15 days before mating and through gestation. Three males per dose level were also exposed prior to mating. Reproductive effects noted in this study did not

differ greatly between treated animals and controls and are poorly substantiated. There were no external malformations, macroscopic structural skeletal or visceral changes, and no developmental delays (58-61).

Sheveleva (62) exposed 3 groups of 26 pregnant albino rats to 0, 0.00004, or 0.004 ppm FA in the air for 4 hours/day, from days 1 to 19 of gestation. When 20 females from each group were sacrificed on day 20, the number of live fetuses was the same in each group and no external malformations were noted. All progeny from the remaining 6 rats per group appeared normal.

Pregnant hamsters were treated with dermal applications of 0.5 ml of 37% FA solution on day 8, 9, 10, or 11 of gestation (63). Fetuses were removed on day 15 and were weighed, measured, and examined for teratogenic effects. The resorption rate increased in the FA-treated groups, but treatment did not significantly affect body weight or length, and no malformations related to the treatment appeared. It was concluded that fetal risk due to topical exposure to FA was minimal in this model system. However, there is no information in this study on the amount of FA actually absorbed.

Groups of 25 mated female Sprague-Dawley rats were exposed to FA by inhalation at 2, 5 or 10 ppm (6 hours/day) on days 6-15 of gestation. At 10 ppm, there was a significant decrease in maternal food consumption and body weight gain. None of the parameters of pregnancy, including numbers of corpora lutea, implantation sites, live fetuses, dead fetuses and resorptions, or fetal weights were affected by treatment (64).

There are few data on the association between teratogenicity or adverse reproductive effects and formaldehyde exposure. The existing data do not suggest that formaldehyde, by

any route, produces significant teratogenic or reproductive effects. However, additional studies will be needed before any final assessment can be made. No mink reproductive studies with FA have been reported.

Mutagenic Effects

In mammalian cell mutation studies performed with Chinese hamster ovary tissue cultures, FA was not found to be mutagenic (65). FA (6.25 to 25 mg/kg bw) also did not significantly increase chromosomal aberrations in bone marrow and spleen cells, as shown from a micronucleus study in mice given 0.4% FA intraperitoneally (66). However, FA did produce DNA-protein cross-links in mouse L1210 cell cultures and in Chinese hamster V79 cells. The cross-links were repaired within 24 hours after removal of FA from the culture medium (67-68).

Dallas et al. (69) reported that male Sprague-Dawley rats were exposed to 0, 0.5, 3 or 15 ppm FA for 6 hours/day, 5 days/week, for 1 and 8 weeks. There was no significant increase in chromosomal abnormalities in bone marrow cells of FA-exposed rats relative to controls, but there was a significant increase in the frequency of chromosomal aberrations in pulmonary lavage cells (lung alveolar macrophages) from rats that inhaled 15 ppm FA. Aberrations, which were predominantly chromatid breaks, were seen in 7.6 and 9.2% of the scored pulmonary lavage cells from treated animals and in 3.5 and 4.8% of cells from controls, after 1and 8 weeks, respectively.

Carcinogenic Effects

Inhalation

After 18 months of a 2-year study, Swernberg et al. (70) demonstrated that 25% of rats exposed by inhalation to 15.1 ppm FA daily for 6 hours/day, 5 days/week for 18 months, had developed squamous carcinomas of the nasal turbinate. None of the rats exposed to 0, 2.1 and 5.6 ppm developed tumors, although 1 rat exposed to 5.6 ppm did develop squamous carcinoma of the facial skin overlying the nose. After the full 2-year period, 44.3% of the rats exposed to 14.1 ppm and 0.185% exposed to 5.6 ppm had developed squamous tumors, while rats exposed to 0 and 2.1 ppm did not develop any (71).

Tobe et al. (72) exposed male F-344 rats for 6 hours/day, 5 days/week, over 28 months, to 0.3, 2, or 14.2 ppm FA. Rhinitis accompanied by desquamation was found in all groups. In all FA-exposed groups, nasal epithelial hyperplasia and squamous metaplasia with hyperplasia were seen. In the 14.2 ppm FA group, squamous cell carcinoma was recognized in 14 rats and papilloma in 5 of 32 rats exposed.

Basal cell hyperplasia and/or squamous metaplasia were observed in the trachea-bronchial epithelium of C3H mice exposed to 41.7, 83.3, or 166.7 ppm FA, for 4 hours/day, 3 days/week, over 35 weeks, but not in untreated controls. Atrophic metaplasia was also observed in the highest dose group. There was no evidence of induction of pulmonary tumors at any dose (73).

A group of 88 male Syrian golden hamsters was exposed to 10 ppm FA for 5 hours/day, 5 days/week for life. There were 132 untreated controls available. No tumors of the nasal cavities or respiratory tract were found in either the controls or the animals

exposed to FA (74).

In addition, a few animal studies have shown an effect of FA on the liver. Gofmekler and Bonashevskaya (59) reported data related to histopathological changes in the liver of fetuses from rat dams exposed to FA (0.01 ppm) by inhalation. These hepatic changes included an increase in proliferation of epithelial cells in the bile duct and segmented forms in the hepatic sinusoids. Sanotskii et al. (75) exposed groups of pregnant and nonpregnant rats to 0, 0.3 or 5.0 ppm FA for 4 hours/day for 20 days. They reported that nonpregnant animals were more susceptible to the effects of FA than were pregnant animals. FA at 5.0 ppm resulted in altered hepatic function which was manifested by a decrease in urinary excretion of hippuric acid. However, no studies have shown carcinogenic effects of FA in the liver of animals.

Oral administration

A lifetime study was carried out by Soffritti et al. (76) in 1989. Formaldehyde was administered in drinking water to Sprague-Dawley rats beginning at various ages. Groups of rats received 10, 50, 100, 500, 1000, or 1500 ppm FA from 7 weeks of age for life; 2 control groups received 15 ppm methanol or nothing, respectively, in their drinking water. Two groups of breeder rats, 25 weeks old, were given FA at 0 or 2500 ppm for life. The offspring of these breeders were initially exposed to 0 or 2500 ppm FA via their mothers starting on day 13 of gestation and then received these levels in the drinking water for life. The survival rates in the treated groups were similar to those of controls. There was a dose-related incidence of leukaemia in the treated groups and a variety of malignant and benign tumors of the stomach and intestines in the treated animals. Although the incidence of

intestinal tract tumors was low, there were no comparable tumors in the control groups on this study, and some of these tumors were reported to be uncommon among historical controls.

In a 2-year drinking water study, FA was administered to Wistar rats at dose levels of 0, 1.2, 15, and 82 mg/kg/day for males and 0, 1.8, 21, and 109 mg/kg/day for females (77). There were no adverse effects on general health, survival, or hematological or clinical chemistry parameters. Body weight and food intake were decreased in the high-dose group. The administration of FA at doses of 82 and 109 mg/kg/day to males and females, respectively, caused severe damage to the gastric mucosa but did not result in gastric tumors or tumors of other sites.

Skin application

A study (78) was carried out on mice to test whether a FA solution applied to the skin induced malignant tumors as an initiator or promoter of cancer, or if it acted as a complete carcinogen. Two groups of 16 male and 16 female Oslo hairless mice received topical applications of 200 ul of 1 or 10% FA in water on the skin of the back twice a week for 60 weeks. All of the animals treated with 10% FA were necropsied and the brain, lungs, nasal cavities and all tumors of the skin and other organs were examined histologically. Virtually no changes were found in the mice treated with 1% FA. The higher dose induced slight epidermal hyperplasia and a few skin ulcers. There were no benign or malignant skin tumors or tumors in other organs in either group.

Hematopoeitic Effects

It was reported (6) that administration of Pacific hake containing FA led to decreased iron availability and the development of "cotton fur" in mink. The decrease in iron availability was confirmed by the low blood hemoglobin concentration (< 8g/100ml of blood). The deficiency of iron also caused a lack of pigmentation in the underfur of mink, which looked white and "cottony", and thus is called "cotton fur". Wehr et al. (79) postulated that FA in the Pacific hake was the cause of "cotton fur". When 600 ppm FA was added to a ration containing no fish, cotton fur was observed in 60% of the animals.

Helgebostad and Dishington (80) administered 175 female and 632 kit mink diets composed of 80% fish which contained from 50 ppm FA (provided only by the fish) up to 200 ppm (provided by fish plus additional FA) for the entire breeding period. They reported that diets containing 200 ppm FA had an appetite-decreasing and anemiogenic effect while the 50 ppm FA diets had no such effect on the females and kits. Thus, they concluded that the fish-induced anemia occurring in mink appeared to be unrelated to the quantities of FA found in fish comprising the diets of fur bearing animals. Trimethylamine oxide (triox), the precursor of FA which was widely distributed in marine organisms, was regarded as the dominant anemiogenic factor in raw fish diets by these authors.

EFFECTS ON MICROORGANISMS IN THE ENVIRONMENT

Mutagenic Effects

Formaldehyde has mutagenic activity in a variety of microorganisms. It was first demonstrated to be a bacterial mutagen over 40 years ago (81), inducing forward mutations

in *Pseudomonas fluorescens* and *Escherichia coli.* Crosby et al. (82) found that 4 mM FA induced 41% large insertions, 18% large deletions and 41% point mutations in the xanthine guanine phosphoribosyl transferase gene of *E. coli.* Treatment with 40 mM FA induced 92% point mutations consisting mainly of transitions at a single AT base pair. Although the extreme cellular toxicity of 40 mM FA brings the biological relevance of the results at that concentration into question, the data suggest that the types of mutations can differ at different concentrations. Crosby et al. (82) also treated plasmid DNA *in vitro,* amplified the plasmid in *E. coli*, and sequenced 11 of the resulting mutants. Five of these mutants involved single base pair substitutions, and 6 were frameshift mutations (addition or deletion of one base).

Formaldehyde was examined by O'Donovan and Mee (83) for bacterial mutagenicity using *Escherichia coli* WP2(pKM101) and WP2uvrA(pKM101), and *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and TA102 in the absence of any exogenous source of metabolic activation. They demonstrated that clear mutagenicity was seen for TA98, TA100 and TA102, and both *E. coli* strains when using pre-incubation exposure. Connor et al. (84) also showed a dose-related mutagenic response to formalin (40% FA with 15% methanol) in 4 *Salmonella typhimurium* strains. Control studies, using the same strains and 15% methanol alone, were negative.

Use as a Disinfectant

Formaldehyde is used as a disinfectant to kill viruses, bacteria, fungi, and parasites. Its virucidal property makes it indispensable for disinfection in the clinical field. It is an

important active substance in disinfectants that kill and inactivate microorganisms and is used in the prevention and control of communicable diseases and hospital infections.

The use of FA in consumer goods is intended to protect the products from spoilage by microbial contamination. At low concentrations, FA is present as a preservative to prevent the growth of gram-negative organisms in drugs and household products such as dishwashing liquids, shoe-care agents, waxes, and carpet cleaning agents. The amount of FA used is often based on the minimal inhibitory concentration (MIC) required to suppress particular microorganisms in a specific formulation. The FA MIC for gram-negative organisms such as *Pseudomonas aeruginosa* ranges from 20-550 ppm. The MIC for gram-positive organisms is about 250 ppm and it varies from 90-750 ppm for yeasts and molds (85). Under commercial conditions of poultry production, eggs, incubators, hatchery equipment, and poultry brooder houses are routinely disinfected with formalin to provide protection against bacteria such as *Salmonella* and *Pseudomonas* (86).

The Food and Drug Administration (FDA), in 1996, amended the food additive regulations to allow for the use of FA as an antimicrobial agent in poultry feeds (3). FDA provided for the safe use of formaldehyde (37 percent aqueous solution) at the rate of 5.4 lbs/ton (2.5 kg/ton) if used as an antimicrobial feed additive for keeping complete poultry feeds salmonella negative for up to 14 days, and at a rate of 1.65 to 2.65 lbs./ton if used as an antimicrobial agent against bacteria, mold, and yeast in feed composed of fishmeal and animal by-product meals.

BACTERIAL CONTAMINATION OF MINK FEED

Formaldehyde used as an antimicrobial agent may be of use to the mink farmer whose concerns are: 1) bacteria levels in raw animal by-products which are incorporated into the feed; 2) spoilage of feed after it has been placed on the feed board or grill on the top of the cage.

Many by-products of the food industry such as liver, fish, lung, tripe and poultry offal are used as components in the diets of mink. These by-products may be high in bacteria upon arrival at the mink farm although some feed ingredients may be stored frozen or in refrigerated or preserved chemically before they are incorporated into mink feed. This is especially true during the summer months since mink feed is typically placed on the wire top of the cage and it remains there until the following day when it's replaced with fresh feed. This practice could result in feed spoilage caused by microbial contamination. Thus, the types of major ingredients of mink feed, the process of mixing the raw materials into the feed, and the feeding technique employed make mink feed vulnerable to bacteriological spoilage. Even frozen feeds contain high numbers of microorganisms. Chou and Marth (87) demonstrated that frozen meat by-products and frozen liver contained an average of 5.5 million total bacteria, 175,000 enterococci, 10-30,000 coliforms and 400 yeasts and molds/gram feed. *Salmonellae* and coagulase-positive *Staphylococci* were recovered from 40% of the samples collected from the frozen meat by–products and frozen liver.

Although there is no satisfactory way to feed mink and not expose them to a wide variety of bacterial organisms, some methods in terms of decreasing bacterial growth have been developed for protecting animal feed from spoilage by microbial contamination. The

use of chemical preservatives is one of these methods. These chemicals should have antimicrobial properties and be relatively non-toxic and palatable, and have no adverse effects on the nutritional qualities of the feed and the growth of the animals.

Van Lunen et al. (88) conducted a study of the utilization of acid-preserved poultry offal by growing-finishing pigs. They prepared poultry offal hydrolysate using a mixture of sulfuric and formic acids for preserving these by-products. The results showed that acids remarkably suppressed aerobic bacterial growth and there were no *Salmonella spp* isolated from any sample. The authors concluded that acid-preserved poultry offal can be incorporated into the diet of growing-finishing pigs. However, negative linear trends were observed for growth rate and daily feed consumption with increasing levels of poultry offal hydrolysate. Poulsen and Jorgensen (89) also demonstrated that addition of large amounts of sulfuric acid-preserved fish in fur animal feed had an adverse effect on the metabolism of the animals.

There is no information on the use of FA-preserved poultry by-products for feeding mink. If FA can be used at levels approved by the FDA without effects on mink reproductive performance, body weight, feed consumption and early kit growth, while reducing microbial activity in animal by-products and / or prepared feed, this may provide the mink farmer with a viable method for preventing the spoilage of mink feed.

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CHAPTER 1

EFFECTS OF FEEDING FORMALDEHYDE-TREATED DIETS ON FEED CONSUMPTION AND REPRODUCTIVE PERFORMANCE OF FEMALE MINK

ABSTRACT

Two feed consumption trials were conducted to determine the highest concentration of formaldehyde (FA) in mink feed which would be tolerated by mink (*Mustela vision*) and to determine if mink would prefer FA-treated feed to non-treated feed kept refrigerated for a period up to 7 days which is 4 days longer than feed is normally kept. In addition, a long-term feeding trial was conducted to determine the effect of FA on mink reproduction and growth of offspring. In the first trial, diets containing 0, 1100, 1650, 2200, or 2750 ppm FA were fed to mink for 2 weeks. Food consumption was recorded daily and mink were weighed at the beginning of the trial and at the end of weeks 1 and 2. The results of this trial indicated that consumption of all the FA-treated diets was significantly lower than consumption of the control diet in a dose-related manner. Similarly, there was a dose-related decrease in body weights at the end of the 2-week period. In the second feed consumption trial, mink were fed diets containing 0, 550, or 1100 ppm FA. The results showed that consumption of the 1100 ppm FA diet was significantly lower than consumption of the control diet on days 1, 2, 4, 5, but body weight was not affected in this trial.

In the reproductive trial, mink were fed diets containing FA at concentrations of 0, 550, or 1100 ppm from 1 month prior to mating (January 27, 1997) until kits were weaned at 6 weeks of age (mid-June, 1997) for a period of approximately 135 days. In this trial, consumption of FA-treated diets had no effect on mating success, but kit survival at birth was adversely affected in the group consuming 1100 ppm FA. There were also significant decreases in hemoglobin concentration, hematocrit, mean

corpuscular volume, and mean corpuscular hemoglobin in the high-dose kits at 6 weeks of age. There were no significant differences in any of the parameters between the control animals and those consuming the 550 ppm FA diet.

INTRODUCTION

Raw animal by-products in mink feed are contaminated with large numbers of microorganisms. Even though it is impossible to eliminate all the risks associated with bacteria, there are ways to treat feed so that spoilage is reduced. One such way is the use of chemical preservatives, but it is imperative that these chemicals be relatively non-toxic, and palatable, and that they have no adverse effects on the growth and fur quality of the animals.

A preliminary study was conducted by Powell et al. (1) in which mink (*Mustela vision)* were fed diets containing copper-treated (0, 500, or 1000 ppm copper) eggs prior to and through breeding, gestation, lactation, and early kit growth. The results indicated that addition of copper-treated eggs to mink diets did not adversely affect any of the parameters examined and copper at a concentration of 1000 ppm was an effective agent to reduce bacterial and fungal growth in eggs.

Glowinska and Bieguszewaki (2) conducted an experiment with formic acid-preserved diet fed to ferrets. The results showed that feed containing formic acid at a concentration of 15,000 ppm had no effect on acid-base parameters and body weight of the animals, but it did decrease the digestibility of crude protein.

Formaldehyde has recently been approved for use as an antimicrobial agent in poultry feeds. The guidelines state that it can be used at the rate of 5.4 lbs per ton as an

antimicrobial feed additive for the purpose of keeping complete poultry feeds *Salmonella spp.* negative for up to 14 days, and at a level of 1.65 to 2.65 lbs per ton as an antimicrobial agent against bacteria, mold, and yeast in fishmeal and animal by-product meals (3). However, there is no information on the use of FA-preserved poultry by-products for feeding mink. Based on FA levels approved by the FDA for incorporation into feed (3), the effects of FA on mink feed consumption, reproductive performance, and offspring growth were examined.

The objectives of the present study were: 1) to determine the highest concentration of FA in mink feed which would be consumed by mink; 2) to determine if mink would prefer FA-treated feed to untreated feed kept refrigerated for up to 7 days because unpreserved food is typically kept refrigerated for no more than 3 days to prevent spoilage of food; 3) to determine the effects of FA on the breeding and reproductive performance of female mink and to study the early growth and survival of the offspring (kits) from the females treated with FA.

MATERIALS AND METHODS

Diet Preparation

For the initial feed consumption trial to determine what concentration of FA in the diet mink would consume, a control and 4 treatment diets were prepared using a basal mink diet (Table 1) to which was added quantities of a 37% aqueous FA solution (J. T. Baker Inc., Phillipsburg, NJ) to give concentrations of 0, 1100, 1650, 2200, or 2750 ppm. The highest dose level corresponds to the FDA-approved 2.65 lbs FA / ton. The feed and FA /

Table 1. Composition of Basal Mink Diets

Ingredients (%)	Trial		
	Initial Feed Consumption	Refrigerated Feed Consumption	Reproductive
Duck Offal[1]	22	26	26
Raw Eggs[2]	10	7	7
Beef Liver[3]	5	5	5
Fishmeal[4]	6	7	7
Cereal[5]	22	26	26
Water	26	29	29
Ground Whole Chicken[2]	9	–	–
Biotin[6] (mg/1000lb feed)	87	25	25

1 United Feeds Inc., Plymouth, WI.
2 MSU Poultry Science Research and Teaching Center, East Lansing, MI
3 ADA Beef Inc., Ada,MI.
4 Whole-herring meal, Sea Life Fisheries Inc., Product of Canada
5 XK-40 mink food, United Feeds, Inc., Plymouth, WI.
6 Biotin Premix 100, ADM Animal Health & Nutrition Division, Des Moines, IA

water combination were mixed by hand for 15 minutes to achieve homogeneity. For the feed consumption trial to determine if mink would consume feed refrigerated for 7 days and for the reproduction trial, 3 batches of feed were prepared by adding the appropriate amounts of FA to the water portion of the basal diet (Table 1) to give diets containing 0, 550, or 1100 ppm FA. These diets were mixed in a paddle mixer for 15 minutes to obtain a homogeneous mixture. Ten grab samples were taken from each treatment diet, placed in plastic bags, labeled with the diet code and date, and frozen in a freezer for subsequent FA analysis (National Environmental Testing Company, Chicago, IL). An additional sample was taken from the control diet for nutrient analysis (Litchfield Analytical Services, Litchfield, MI). The prepared mink diets were placed in plastic containers which were sealed with plastic lids. Each container contained a 3-day supply of feed for each treatment and was stored in a walk-in freezer until needed for feeding.

Feeding Trials and Animal Care

In the first feed consumption trial, which began on November 11, 1996 after a 1-week acclimation period, 45 6-month-old natural dark female mink were randomly assigned to 5 groups of 9 mink per group and placed on 1 of 5 dietary treatments (0, 1100, 1650, 2200, 2750 ppm FA) for 2 weeks. The mink were housed individually in an indoor animal room in wire mesh breeder cages measuring 76 cm L x 61 cm W x 46 cm H which were suspended above the floor. The room temperature was maintained above 40°F. The light was controlled by a timer set to simulate the photoperiod of that time of year. Mink were weighed at the beginning of the trial and at the end of weeks 1 and 2. Each day mink were

provided with a known quantity of thawed feed in excess of what they would normally eat. Feed remaining from the previous day was weighed to determine feed consumption and then was discarded.

In the second feed consumption trial, which began on April 28, 1997 following a 2-week acclimation period, 27 1-year-old female mink were randomly assigned to 3 dietary groups (0, 550, 1100 ppm FA). The mink were housed individually in an open-sided shed in mink grower cages (61 cm L x 30.5 cm W x 38 cm H) with attached wooden nestboxes (20 cm L x 16.5 cm W x 29 cm H). Mink were weighed at the beginning and end of the 7-day trial. Each mink was provided a known quantity of the refrigerated FA-treated feed on a daily basis for 7 days. Each day unconsumed feed was weighed to determine daily feed consumption.

In the reproductive trial, a total of 36 natural dark female mink (12 mink per treatment) were fed diets containing 0, 550, or 1100 ppm FA from January 27, 1997 (following a 1-week acclimation period) to weaning of the young at 6 weeks of age during mid-June, 1997. The mink were housed individually in an open-sided shed in mink breeder cages (76 cm L x 61 cm W x 46 cm H) with attached wooden nest boxes (38 cm L x 28 cm W x 27 cm H) bedded with wood shavings and excelsior. Mink were observed daily for signs of toxicity. Body weights were recorded at the start of the trial, at whelping, at 3 weeks post-partum, and at the termination of the trial.

Reproduction

Mating of females in the reproductive trial to untreated males began March 3, 1997 and ended March 28, 1997. Each female was introduced into the male's cage. After mating was completed, the females were immediately checked by taking vaginal aspirations. If motile spermatozoa were present in the aspiration, as observed under a microscope, the female was considered bred. The bred females were given an opportunity for a second and third mating the day following the initial mating and/or 8 days later. All the females were given the opportunity to mate every fourth day until a successful mating (presence of motile sperm in the vaginal aspiration) was obtained. Each female had at least 2 successful matings.

As the time of whelping approached, all nestboxes were cheched daily for newborn kits. The number of kits born alive or dead, their sex, and the presence of any external malformations were recorded. Body weights of the kits were recorded at birth, and at 3 and 6 weeks of age.

Blood Collection

Blood samples in the reproductive trial were collected from 6 adult female mink and 6 kits from each treatment before necropsy. The 18 females and 18 kits were anesthetized with 0.3 ml of ketamine hydrochloride (100 mg/ml; Fort Dodge Laboratory, Inc., Fort Dodge, IA) administed intramuscularly and weighed. Ten ml of blood from each animal were collected by cardiac puncture, 3 ml of which were placed in EDTA-containing tubes for various whole-blood measurements including differential leucocyte

counts, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total platelets (PLT), and mean platelet volume (MPV). The remaining blood was placed in heparin-coated tubes and centrifuged for 5 minutes. Approximately 2 ml of the plasma was removed from the centrifuged blood sample for determination of plasma chemistries including glucose, blood urea nitrogen, creatinine, calcium, chloride, potassium, sodium, albumin/globulin ratio, globulin, phosphorus, cholesterol, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine phosphokinase, alkaline phosphatase, and total bilirubin. All hematological and plasma chemistry analyses were performed by the Parke-Davis Pharmaceutical Research Clinical Pathology Laboratory, Ann Arbor, MI.

Necropsy

Necropsies of adult females and kits on the reproductive trial were performed on 2 different dates so that kits were between 6 and 7 weeks old. Six adult females and 6 kits were necropsied on June 5, 1997 and 12 adult females and 12 kits were necropsied on June 17, 1997.

After blood samples were taken from each animal, the females and kits were euthanized with carbon dioxide. The brain, lungs, heart, liver, kidneys, spleen, and adrenals were collected from each adult and weighed and the same organs plus thymus were collected from each kit and weighed. The tissues were fixed in a 10% formalin solution for histopathologic examination by Dr. James Render (Department of Pathology

and Animal Health Diagnostic Laboratory, Michigan State University). Fixed tissues were trimmed, embedded in paraffin, sectioned at 6 μm, adhered to glass slides, stained with hematoxylin and eosin, cover slipped and examined with a light microscope.

Statistics

Data were analyzed by using the SAS statistical package (SAS Institute Inc., 1994). Differences among treatments and days for the 7-day feed consumption trial were analyzed by factorial ANOVA. Multiple comparisons of treatment means and linear trends over time were based on Bonferronni's adjustment. Data from the initial feed consumption trial and the reproductive trial were analyzed by one-way ANOVA and differences between treatment means were based on Tukey's test. For the proportion data (kit sex ratio and kit survival), a normal approximation to a binomial distribution was used to derive a Z-test. Statements of significance are based on $p < 0.05$.

RESULTS

Concentration of Formaldehyde in Diets and Nutrient Analyses of Control Diets

The concentrations of FA in the 3 batches of mink diets are presented in Table 2. The results for the 3 batches were quite consistent in that actual FA concentrations were on average 62% of targeted concentrations. The nutrient analysis of the control diet is presented in Table 3.

Table 2. Concentrations of Formaldehyde in Mink Diets[1]

	Concentration of 37% FA Solution (ppm)					
Targeted dietary FA concentration[2]	0		1100	1165	2200	2750
Actual dietary FA concentration	14		615	960	1420	1910
Targeted dietary FA concentration[3]	0	550	1100	–	–	–
Actual dietary FA concentration						
1st Batch	24	300	679	–	–	–
2nd Batch	10	282	644	–	–	–

1 Actual concentrations of FA in mink diets were determined by National Environmental Testing Company, Chicago, IL.

2 Diets used for initial feed consumption trial.

3 Diets used for second feed consumption trial and reproductive trial.

Table 3. Nutrient Analysis of Control Diet[1]

Test	As Is Basis[2]	Dry Matter Basis
Fat (%)	8.19	19.15
Crude Protein (%)	17.64	41.25
Crude Fiber (%)	1.52	3.55
T.D.N.[3] (%)	39.6	92.6
Calcium (%)	0.82	1.91
Phosphorus (%)	0.59	1.38
Potassium (%)	0.38	0.89
Magnesium (%)	0.09	0.22
Sodium (%)	0.3	0.69
Ash (%)	3.69	8.64
Iron (ppm)	168	394
Manganese (ppm)	22	53
Copper (ppm)	4	10
Zinc (ppm)	44	103

[1] Analysis by Litchfield Analytical Services, Litchfield, Michigan
[2] Diet is 57.24 % moisture
[3] Total digestible nutrient

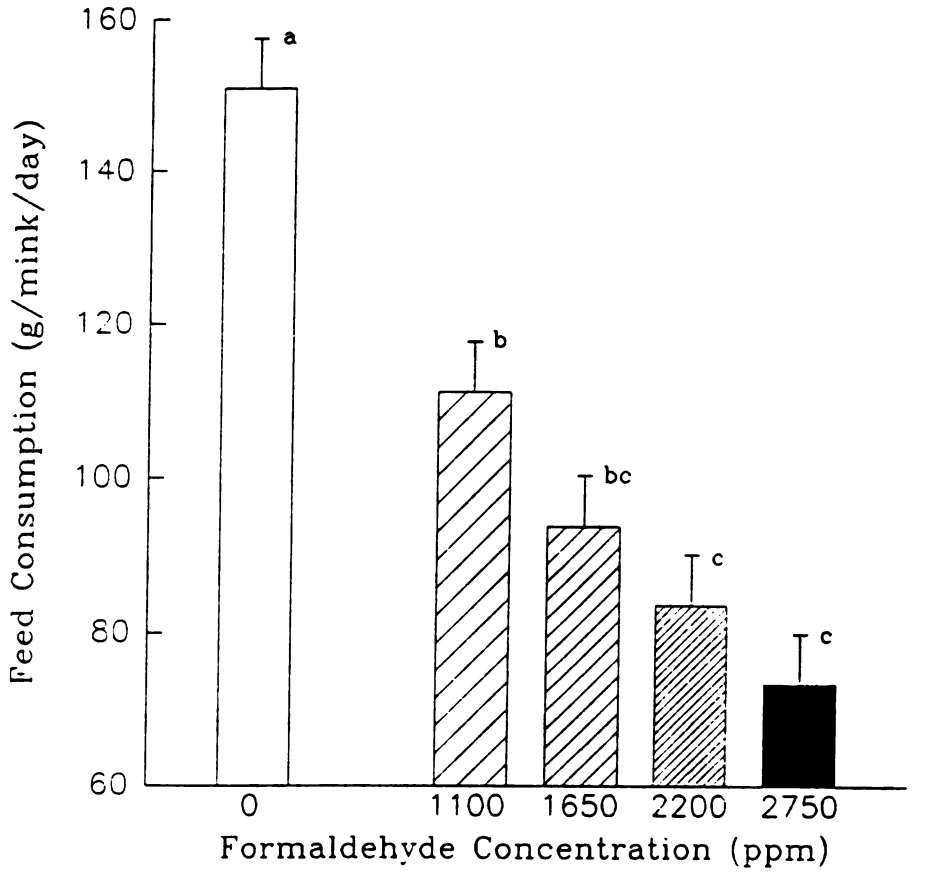

Figure 2. Average Daily Consumption of Formaldehyde-Treated Feed Over a 2-Week Period. Each bar represents the mean ± standard error of the mean of values for 9 animals per treatment. Means with different superscripts are significantly different at $p < 0.05$.

Feed Consumption and Body Weights

The mean feed consumption of each FA-treated diet in the initial trial is presented in Figure 2. In general, there was a decrease in the average amount of diet consumed per mink as the concentration of FA increased with the consumption of all FA-treated diets being significantly lower than consumption of the control diet. Consumption of the diet containing 1100 ppm FA was significantly higher than consumption of diets containing 2200 and 2750 ppm FA.

The percent body weight loss for adult female mink in this trial is shown in Figure 3. The percent body weight loss of mink consuming FA-treated diets significantly increased in a general dose-dependent manner as compared to the control animals. Furthermore, differences between animals in the 1100 ppm group and animals in the 2750 ppm group were significant.

In the second feed consumption trial, consumption of non-treated and FA-treated feed refrigerated for up to 7 days was analyzed on a daily basis (Figure 4). The mink on the 1100 ppm diet consumed significantly less feed than controls on days 1, 2, 4 and 5 while feed consumption of the 550 ppm diet was not significantly different from consumption of the control diet throughout the 7-day trial. When trend analysis was performed on the feed consumption data, the mink on the 550 ppm diet had decreasing feed consumption over the 7-day period while mink on the control and 1100 ppm diets had consistently high and low feed consumption, respectively. The percent body weight gain for adult female mink in this trial is presented in Figure 5. No significant differences were found in the mean of percent body weight gain among treatments.

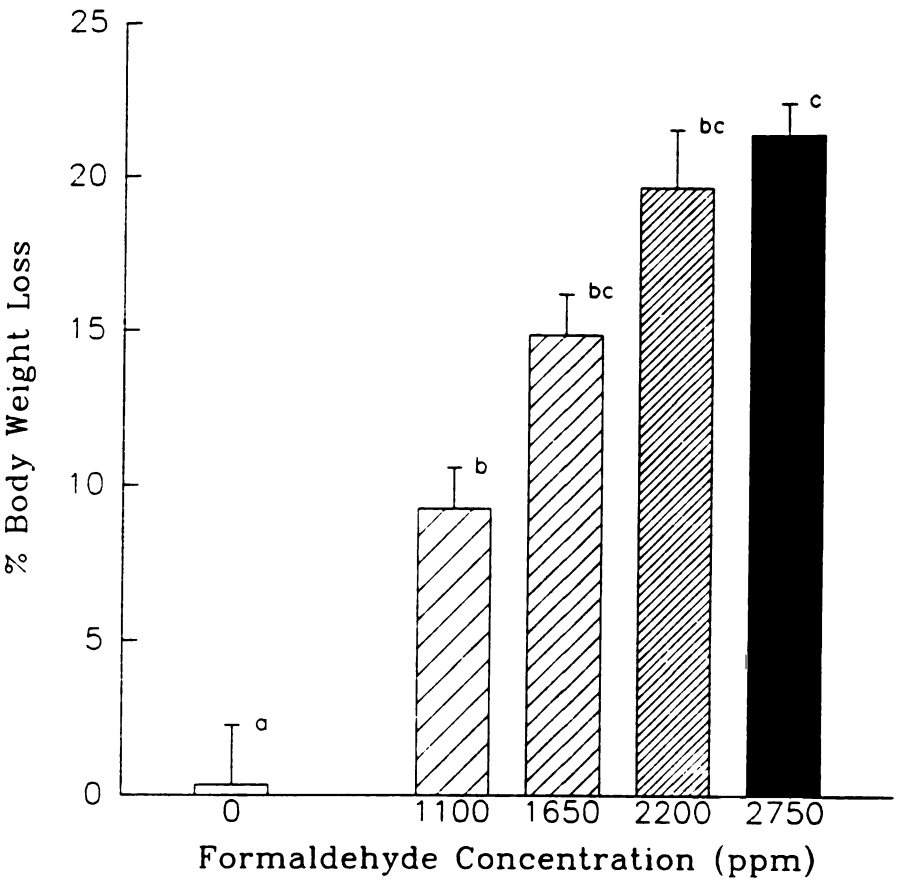

Figure 3. Percent Body Weight Loss for Female Mink Fed Various Concentrations of Formaldehyde-Treated Feed for 2 Weeks. Each bar represents the mean ± standard error of the mean of values for 9 mink per treatment. Means with different superscripts are significantly different at $p < 0.05$.

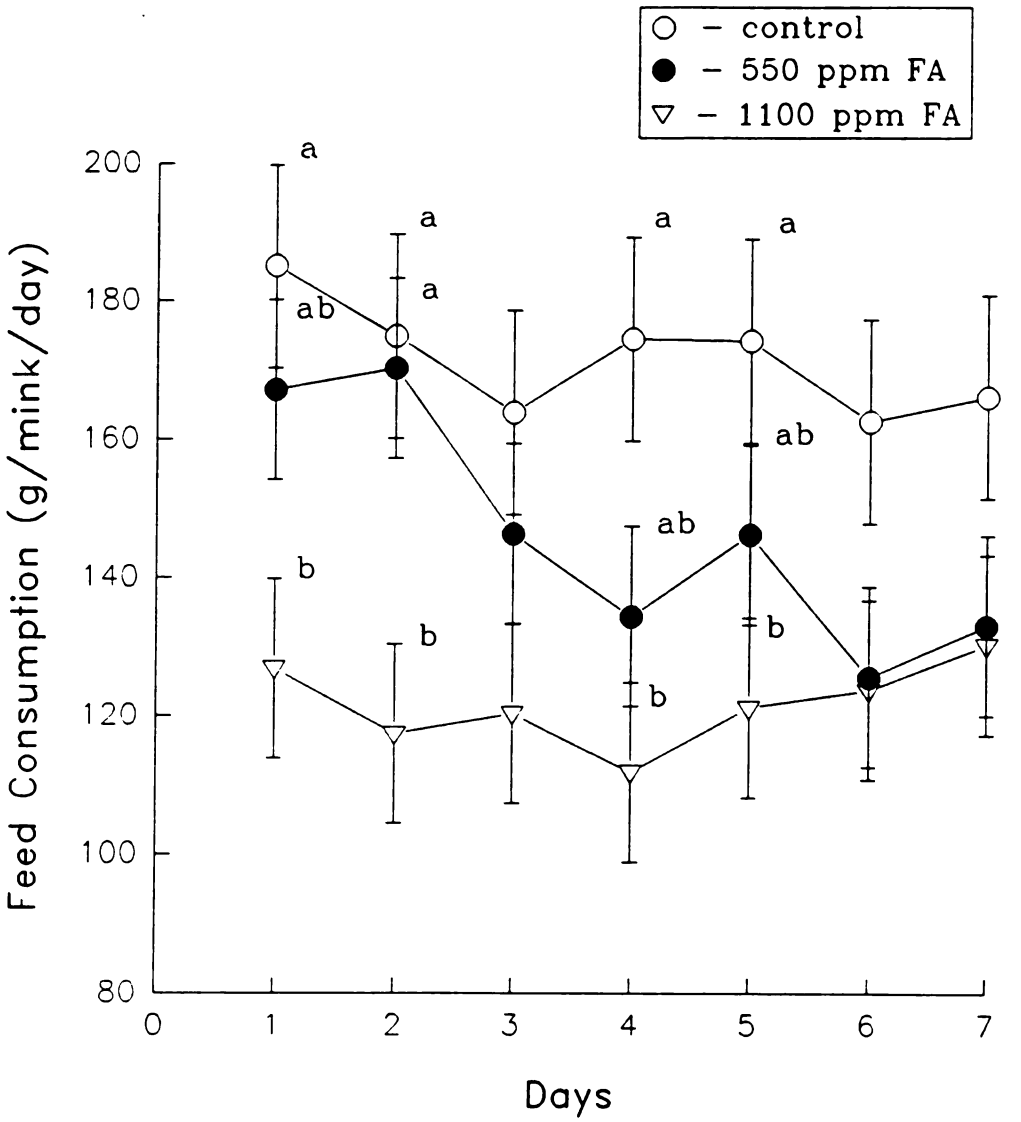

Figure 4. Daily Mean Feed Consumption of Formaldehyde (FA)-Treated Feed Refrigerated for Up to 7 Days. Each point represents the mean ± standard error of the mean of values for 9 animals per treatment. Means with different superscripts at each day are significantly different ($p < 0.05$).

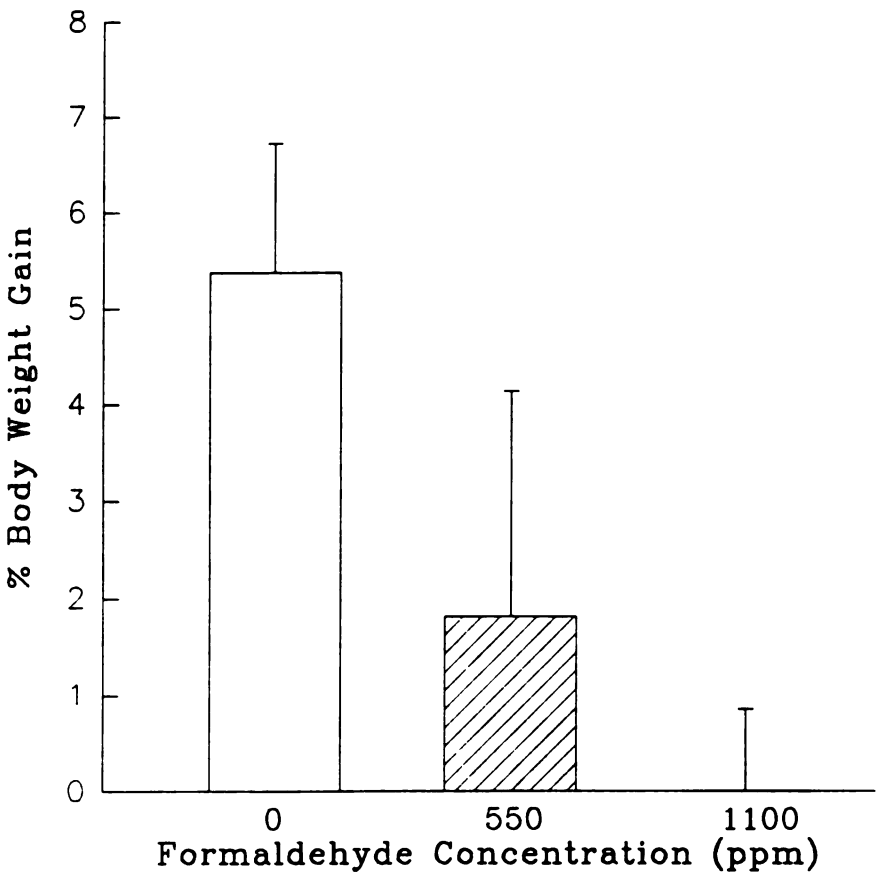

Figure 5. Percent Body Weight Gain for Female Mink Fed Various Concentrations of Formaldehyde-Treated Feed Refrigerated for Up to 7 Days. Each bar represents the mean ± standard error of the mean of values for 9 animals per treatment.

Reproductive Performance and Kit Survival

Data relating to the reproductive performance of the 36 female mink fed control and FA-containing diets prior to mating through weaning are presented in Table 4. Of the 36 females bred, 33 whelped. Of the 3 females that did not whelp, 2 were fed the 550 ppm diet and 1 was fed the 1100 ppm diet. The mean number of confirmed matings per female across the treatment groups ranged from 2.1 to 2.3. The average gestation lengths across the treatment groups ranged from 46.27 to 46.75 days. No significant differences were found in the means of kit sex ratio, number of kits born alive and dead, and litter size per dam. However, there was a significant difference in the percentage of kits alive at birth between the control and the 1100 ppm diet while there were no differences in kit survival between the dietary groups at 3 and 6 weeks of age.

During the trial, an adult female from the 550 ppm diet died from undetermined cause. Necropsy and histopathologic examination revealed multifocal splenic necrosis with associated splenomegaly, multifocal hepatic necrosis, and 3 markedly autolyzed fetuses in utero.

Nineteen out of 39 kits from the 1100 ppm diet had gray fur at the termination of the trial. Conversely, no control kits or 550 ppm kits had detectable gray fur (Figure 6).

Body Weights

The means of adult female body weights, body weight changes and percent body weight loss are presented in Table 5. There were no significant differences in these parameters although percent body weight loss in the 1100 ppm females was 21% compared

Table 4. Breeding and Reproductive Performance of Female Mink Fed Diets Containing Various Concentrations of Formaldehyde and Body Weights, Sex Ratios, and Survivability of Their Kits

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
	0 (Control)	550	1100
No. females	12	12	12
No. females whelped/no.mated	12/12	10/12	11/12
No. confirmed matings per female/ no. attempted matings	2.2/5	2.1/ 5.2	2.3/4.9
Gestation length (days)	46.75 ± 1.20[1]	46.60 ± 1.31	46.27 ± 1.25
Kit sex ratio (% males:females)	56:44	56:44	42:58
No. kits/dam			
Alive	5.67 ± 0.72	5.00 ± 0.83	5.33 ± 0.83
Dead	0.25 ± 0.18	0.33 ± 0.17	0.44 ± 0.34
Average litter size	5.92 ± 0.78	4.90 ± 0.86	5.00 ± 0.82
Kit survival (%)			
Birth[2]	96 [a]	92 [ab]	87 [b]
3 weeks	69(75)[3]	80	73(80)
6 weeks	66(75)	78	71(78)
Kit body weight (g)			
Birth (n=68,45,48)[4]	10.01 ± 0.30	10.01 ± 0.36	9.62 ± 0.35
3 weeks (n=47,39,40)	98.62 ± 5.81[a]	120.17 ± 6.91[b]	85.88 ± 6.83[a]
6 weeks (n=47,38,39)	245.23 ± 15.50[ab]	285.70 ± 18.43[b]	206.15 ± 18.21[a]

[1] Mean ± standard error of the mean

[2] Means in the same row with different superscript are significantly different ($p < 0.05$)

[3] Numbers in parentheses refer to percent survival if the one female in the control group which cannibalized all eight of her kits and the one female in the 1100 ppm FA group which cannibalized all five of her kits are removed

[4] Numbers in parentheses refer to sample size in the 0, 550, and 1100 ppm FA treatments, respectively

Figure 6. Effects of Formaldehyde on Kit Mink Fur. The kit on the left is a control animal and the kit on the right showing "cotton fur" is from the 1100 ppm FA group.

Table 5. Body Weights, Body Weight Changes, and Percent Body Weight Loss for Female Mink Fed Diets Containing Various Concentrations of Formaldehyde During the Reproductive Trial

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
	0 (Control)	550	1100
No. Females[1]	12	9	11
Body weight (g)			
Initial	1155.83 ± 52.33[2]	1117.89 ± 60.42	1149.00 ± 54.65
Terminal	993.50 ± 48.25	915.56 ± 55.72	884.00 ± 50.40
Change	– 162.33	– 202.33	– 265.00
% Body weight loss	12	14	21

[1] The sample size for 550 ppm group excludes 1 female which died and 2 which did not whelp and sample size for 1100 ppm group excludes 1 female which did not whelp.

[2] Mean ± standard error of the mean.

to an average loss of 13% for the controls and 550 ppm animals.

Body weights of kits at birth were not significantly different across the 3 dietary treatments. At 3 weeks of age, the mean kit body weight in the 550 ppm diet was significantly greater than mean body weights of kits in the control and the 1100 ppm diet. At 6 weeks of age, the mean body weight of kits in the 550 ppm group was significantly greater than mean body weight of the 1100 ppm kits (Table 4).

Organ Weights

The effects of FA on adult female organ weight are presented in Table 6. There were no significant differences in organ weights among the treatments.

Male and female kit organ weights are presented as actual and relative (% brain weight) weights in Tables 7 and 8, respectively. The mean actual spleen weight for males in the 550 ppm diet (Table 7) was significantly greater when compared to controls while no differences were noted between the 1100 ppm and the 550 ppm diets or the 1100 ppm and the control diets. Similarly, male relative spleen weight at 550 ppm as well as relative kidney weights were significantly greater than relative spleen and kidney weights of the control males. (Table 8).

Histopathology

There were no histologic lesions associated with consumption of FA-treated diet in any of the tissues examined microscopically from the adult female mink or their kits.

Table 6. Organ Weights (g) of Adult Female Mink Fed Diets Containing Various Concentrations of Formaldehyde

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Organ	0 (Control)	550	1100
Brain	7.70 ± 0.21[1]	8.04 ± 0.21	7.63 ± 0.21
Liver	34.85 ± 3.53	33.44 ± 3.53	38.38 ± 3.53
Spleen	2.43 ± 0.64	2.23 ± 0.64	3.21 ± 0.64
Kidneys	6.86 ± 0.38	6.71 ± 0.38	5.91 ± 0.38
Heart	5.79 ± 0.29	5.53 ± 0.29	5.73 ± 0.29
Adrenals	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01

[1] Mean ± standard error of the mean. Sample size is 6 for each treatment group.

Table 7. Actual Organ Weights (g) of 6-Week-Old Female and Male Kits Exposed to Various Concentrations of Formaldehyde During Gestation and Lactation and Through Consumption of the Diets Following Weaning

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Organ	0 (Control)	550	1100
Females			
Brain	8.95 ± 0.35[1]	8.45 ± 0.35	8.88 ± 0.35
Liver	15.07 ± 1.07	13.88 ± 1.07	16.13 ± 1.07
Spleen	1.73 ± 0.24	1.34 ± 0.24	2.18 ± 0.24
Kidneys	2.79 ± 0.19	2.74 ± 0.19	3.15 ± 0.19
Heart	2.22 ± 0.32	1.91 ± 0.32	2.69 ± 0.32
Adrenals	0.04 ± 0.003	0.04 ± 0.003	0.04 ± 0.003
Thymus	1.03 ± 0.20	0.85 ± 0.20	1.18 ± 0.20
Males			
Brain	10.15 ± 0.32	10.80 ± 0.32	10.31 ± 0.32
Liver	13.79 ± 2.13	22.24 ± 2.13	15.80 ± 2.13
Spleen	1.43 ± 0.20[a 2]	2.58 ± 0.20[b]	2.09 ± 0.20[ab]
Kidneys	2.88 ± 0.35	4.37 ± 0.35	3.31 ± 0.35
Heart	1.86 ± 0.34	2.87 ± 0.34	2.20 ± 0.34
Adrenals	0.04 ± 0.005	0.05 ± 0.005	0.03 ± 0.005
Thymus	0.78 ± 0.23	1.48 ± 0.23	0.90 ± 0.23

[1] Mean ± standard error of the mean. Sample size is 3.

[2] Means in the same row with different superscript are significantly different ($p < 0.05$)

Table 8. Relative Organ Weights (% brain weight) of 6-Week-Old Female and Male Kits Exposed to Various Concentrations of Formaldehyde During Gestation and Lactation and Through Consumption of the Diets Following Weaning

		Dietary Treatment		
		37% Formaldehyde Solution (ppm)		
		0 (Control)	550	1100
Female				
	Liver	167.92 ± 6.76[1]	164.21 ± 6.76	181.28 ± 6.76
	Spleen	19.17 ± 2.22	15.83 ± 2.22	24.33 ± 2.22
	Kidneys	31.13 ± 2.16	32.46 ± 2.16	35.57 ± 2.16
	Heart	24.64 ± 3.08	22.74 ± 3.08	30.11 ± 3.08
	Adrenals	0.41 ± 0.03	0.44 ± 0.03	0.43 ± 0.03
	Thymus	11.39 ± 2.20	10.22 ± 2.20	13.27 ± 2.20
Male				
	Liver	136.12 ± 16.78	205.11 ± 16.78	151.98 ± 16.78
	Spleen	14.13 ± 1.51[a 2]	23.88 ± 1.51[b]	20.10 ± 1.51[ab]
	Kidneys	28.35 ± 2.64[a]	40.31 ± 2.64[b]	31.96 ± 2.64[ab]
	Heart	18.37 ± 2.66	26.39 ± 2.66	21.17 ± 2.66
	Adrenals	0.36 ± 0.05	0.50 ± 0.05	0.33 ± 0.05
	Thymus	7.66 ± 2.01	13.58 ± 2.01	8.68 ± 2.01

[1] Mean ± standard error of the mean. Sample size is 3.

[2] Means in the same row with different superscript are significantly different ($p < 0.05$)

Hematologic Parameters

No statistically significant differences in hematologic parameters for adult female mink were found in any of the treatment groups (Table 9). In the kits, however, mean hemoglobin concentration, mean corpuscular volume and mean corpuscular hemoglobin were significantly lower in the 1100 ppm animals when compared to the other two groups and the hematocrit was significantly lower in the 1100 ppm animals when compared to the control group (Table 10).

Serum Chemistry Parameters

There were no statistical differences in the various plasma chemistry parameters for adult and kit mink in any of the treatment groups (Tables 11 and 12).

DISCUSSION

The purpose of the present study was to determine the effects of FA on feed consumption and reproductive performance of mink. If FA is to be used as an antimicrobial agent for preserving mink feed, then it must be palatable and have no adverse effects on the health of adult mink, reproductive performance, kit survivability and growth, and fur quality of kits.

Studies examining various chemical preservatives to control bacterial growth in animal products and by-products have been conducted. These have included a mink feeding study utilizing sulfuric acid-preserved fish silage during lactation and the growth period (4, 5), and the feeding of growing-finishing pigs acid-preserved poultry offal (6).

Table 9. Hematological Parameters for Adult Female Mink Fed Diets Containing Various Concentrations of Formaldehyde

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Parameters[1]	0 (Control)	550	1100
WBC ($\times 10^9$/L)	8.35 ± 2.18[2]	7.18 ± 2.18	7.43 ± 2.18
Neutrophils (%)	83.66 ± 2.75	84.24 ± 2.75	80.62 ± 2.75
Lymphocytes (%)	5.56 ± 2.59	6.09 ± 2.59	8.34 ± 2.59
Monocytes (%)	8.62 ± 1.36	7.43 ± 1.36	8.03 ± 1.36
Eosinophils (%)	0.18 ± 0.08	0.24 ± 0.08	0.37 ± 0.08
Basophils (%)	1.99 ± 0.42	1.99 ± 0.42	2.62 ± 0.42
RBC ($\times 10^{12}$/L)	8.28 ± 0.46	8.46 ± 0.46	7.30 ± 0.46
HGB (g/dl)	16.26 ± 1.17	16.94 ± 1.17	13.36 ± 1.17
HCT (%)	51.28 ± 3.90	53.72 ± 3.90	42.56 ± 3.90
MCV (fl)	62.14 ± 2.99	63.52 ± 2.99	57.32 ± 2.99
MCH (pg)	19.70 ± 0.90	20.06 ± 0.90	18.00 ± 0.90
MCHC (g/dl)	31.70 ± 0.27	31.58 ± 0.27	31.48 ± 0.27
PLT ($\times 10^9$/L)	884.80 ± 91.66	919.20 ± 91.66	1220.60 ± 91.66
MPV (fl)	12.87 ± 0.94	10.62 ± 0.94	11.74 ± 0.94

[1] WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = total platelets; MPV = mean platelet volume;

[2] Mean ± standard error of the mean. Sample size is 5 in each treatment. Clotted blood samples were excluded from analysis.

Table 10. Hematological Parameters for 6-Week-Old Kits Exposed to Various Concentrations of Formaldehyde During Gestation and Lactation and Through Consumption of the Diets Following Weaning

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Parameters[1]	0 (Control)	550	1100
WBC ($\times 10^9$/L)	4.83 ± 0.82[2]	4.74 ± 0.82	5.07 ± 0.82
Neutrophils (%)	50.96 ± 4.78	49.64 ± 4.78	54.04 ± 4.78
Lymphocytes (%)	40.22 ± 6.03	39.12 ± 6.03	32.02 ± 6.03
Monocytes (%)	5.98 ± 1.27	8.51 ± 1.27	7.19 ± 1.27
Eosinophils (%)	0.95 ± 0.31	1.43 ± 0.31	0.84 ± 0.31
Basophils (%)	1.87 ± 2.33	1.30 ± 2.33	5.90 ± 2.33
RBC ($\times 10^{12}$/L)	4.91 ± 0.25	4.71 ± 0.25	4.20 ± 0.25
HGB (g/dl)	10.58 ± 0.57[a 3]	10.37 ± 0.57[a]	8.15 ± 0.57[b]
HCT (%)	33.02 ± 1.81[a]	31.94 ± 1.81[ab]	25.36 ± 1.81[b]
MCV (fl)	67.54 ± 1.77[a]	67.72 ± 1.77[a]	60.14 ± 1.77[b]
MCH (pg)	21.64 ± 0.61[a]	22.02 ± 0.61[a]	19.30 ± 0.61[b]
MCHC (g/dl)	32.08 ± 0.42	32.52 ± 0.42	32.10 ± 0.42
PLT ($\times 10^9$/L)	969.80 ± 59.09	973.80 ± 59.09	1059.60 ± 59.09
MPV (fl)	7.54 ± 0.43	7.88 ± 0.43	8.34 ± 0.43

[1] WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = total platelets; MPV = mean platelet volume.

[2] Mean ± standard error of the mean. Sample size is 5 in each treatment. Clotted samples were excluded from analysis.

[3] Means in the same row with different superscript are significantly different ($p < 0.05$)

Table 11. Serum Parameters for Adult Female Mink Fed Diets Containing Various Concentrations of Formaldehyde

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Parameters[1]	0 (Control)	550	1100
GLU (mg/dl)	144.50 ± 22.80[2]	152.67 ± 22.80	191.33 ± 22.80
BUN (mg/dl)	15.67 ± 23.32	48.17 ± 23.32	48.67 ± 23.32
CREA (mg/dl)	0.48 ± 0.14	0.68 ± 0.14	0.78 ± 0.14
Na (mmol/L)	146.67 ± 2.66	147.50 ± 2.66	144.50 ± 2.66
K (mmol/L)	3.74 ± 0.58	4.10 ± 0.58	4.48 ± 0.58
Cl (mmol/L)	115.17 ± 3.37	115.50 ± 3.37	116.7 ± 3.37
A/G	0.82 ± 0.04	0.83 ± 0.04	0.72 ± 0.04
GLOB (g/dl)	3.85 ± 0.18	3.75 ± 0.18	4.05 ± 0.18
Ca (mg/dl)	9.32 ± 0.24	9.38 ± 0.24	9.05 ± 0.24
PHOS (mg/dl)	4.40 ± 1.69	6.83 ± 1.69	5.82 ± 1.69
CHOL (mg/dl)	321.50 ± 52.78	235.50 ± 52.78	327.50 ± 52.78
TP (g/dl)	7.02 ± 0.24	6.90 ± 0.24	7.00 ± 0.24
ALB (g/dl)	3.15 ± 0.10	3.15 ± 0.10	2.93 ± 0.10
ALT (u/l)	266.67 ± 71.77	323.00 ± 71.77	337.00 ± 71.77
ALKP (u/l)	149.83 ± 18.67	151.50 ± 18.67	161.50 ± 18.67
TBIL (mg/dl)	0.67 ± 0.11	0.70 ± 0.11	0.62 ± 0.11
AST (u/l)	466.60 ± 112.11	517.50 ± 102.34	464.17 ± 102.34
LDH (u/l)	3458.60 ± 700.14	2807.50 ± 639.14	1751.83 ± 639.14
CK (u/l)	4209.40 ± 1079.46	1587.50 ± 985.40	2159.33 ± 985.40

[1] GLU = glucose; BUN = blood urea nitrogen; CREA = creatinine; Na = sodium; K = potassium; Cl = chloride; Ca = calcium; A/G = albumin/globulin; GLOB = globulin; PHOS = phosphorus; CHOL = cholesterol; TP = total protein; ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; CK = creatine phosphokinase; ALKP = alkaline phosphatase; TBIL = total bilirubin;

[2] Mean ± standard error of the mean. Sample size is 6.

Table 12. Serum Parameters for 6-Week-Old Kits Exposed to Various Concentrations of Formaldehyde During Gestation and Lactation and Through Consumption of the Diets Following Weaning

	Dietary Treatment		
	37% Formaldehyde Solution (ppm)		
Parameters[1]	0 (Control)	550	1100
GLU (mg/dl)	116.33 ± 9.49[2]	139.50 ± 9.49	145.17 ± 9.49
BUN (mg/dl)	16.17 ± 2.86	14.00 ± 2.86	17.33 ± 2.86
CREA (mg/dl)	0.33 ± 0.05	0.33 ± 0.05	0.33 ± 0.05
Na (mmol/L)	145.00 ± 1.51	143.50 ± 1.51	145.17 ± 1.51
K (mmol/L)	4.02 ± 0.32	4.01 ± 0.32	4.36 ± 0.32
Cl (mmol/L)	113.50 ± 1.37	114.67 ± 1.12	114.67 ± 1.12
A/G	0.95 ± 0.02	0.90 ± 0.02	0.90 ± 0.02
GLOB (g/dl)	2.60 ± 0.10	2.57 ± 0.10	2.67 ± 0.10
Ca (mg/dl)	10.93 ± 0.47	11.08 ± 0.47	10.90 ± 0.47
PHOS (mg/dl)	8.65 ± 0.44	8.03 ± 0.44	8.45 ± 0.44
CHOL (mg/dl)	210.50 ± 24.08	239.33 ± 24.08	192.17 ± 24.08
TP (g/dl)	4.98 ± 0.15	4.85 ± 0.15	5.07 ± 0.15
ALB (g/dl)	2.40 ± 0.07	2.30 ± 0.07	2.42 ± 0.07
ALT (u/l)	47.00 ± 4.99	42.67 ± 4.99	57.33 ± 4.99
ALKP (u/l)	431.00 ± 45.57	433.33 ± 45.57	491.67 ± 45.57
TBIL (mg/dl)	0.30 ± 0.03	0.30 ± 0.03	0.23 ± 0.03
AST (u/l)	195.67 ± 19.72	169.33 ± 19.72	189.50 ± 19.72
LDH (u/l)	3539.00 ± 398.53	2601.50 ± 398.53	3169.00 ± 398.53
CK (u/l)	724.83 ± 152.87	699.67 ± 152.87	766.17 ± 152.87

[1] GLU = glucose; BUN = blood urea nitrogen; CREA = creatinine; Na = sodium; K = potassium; Cl = chloride; Ca = calcium; A/G = albumin/globulin; GLOB = globulin; PHOS = phosphorus; CHOL = cholesterol; TP = total protein; ALB = albumin; AST = aspartate aminotransferase; ALT = alanine ceminotransferase; LDH = lactate dehydragenase; CK = creatine phosphokinase; ALKP = alkaline phosphatase; TBIL = total bilirubin;

[2] Mean ± standard error of the mean. Sample size is 6.

The former study (4, 5) showed that incorporation of acid-preserved fish silage in mink diets had a serious effect on the metabolism of the animals and induced detrimental effects on early kit growth including increased mortality. The results of the latter study (6) demonstrated that growth of growing-finishing pigs was depressed when the animals were fed acid-preserved poultry offal. A preliminary study by Powell et al. (7) showed that FA incorporated into the diet of mink at 2750 ppm caused an unacceptable 47% reduction in feed consumption and 1375 ppm caused a 11% reduction in feed consumption compared to the controls.

In the present study, the results from the first feed consumption trial showed that incorporation of FA into the diet at concentrations over 1100 ppm had adverse effects on daily feed consumption and body weight (Figures 2 and 3). Based on these results, lower FA concentrations of 550 and 1100 ppm were chosen for subsequent mink studies.

The purpose of the second feed consumption trial was to examine if feed preserved with FA would be suitable (low bacterial count) for feeding mink if held beyond 3 days under refrigeration. Presently, we don't hold feed beyond 3 days because of increased bacteria count (spoilage). It was assumed that consumption of the control diet might decrease while consumption of FA-treated diets might remain stable when feed was kept refrigerator for longer than 3 days because mink farmers usually keep unpreserved mink feed in the refrigerator for no more than 3 days. However, the results (Figure 4) indicated that consumption of the control diet was consistent over the 7-day period and consumption of the 550 ppm FA diet decreased over the 7-day period, but was not statistically different, when compared to control consumption. Mink fed the 1100

ppm FA diet consumed significantly less feed compared to controls on days 1, 2, 4, and 5. Despite the decrease in feed intake by the mink on FA-treated diets, there were no significant changes in body weight in any of treatment groups (Figure 5). The results indicated that unpreserved mink feed can be kept in the refrigerator for up to 7 days without food consumption decreasing. The results also suggest that 1100 ppm FA has an immediate effect on feed consumption and might not be suitable in a commercial situation.

In the reproductive trial, feeding of 1100 ppm FA diet caused a decrease in kit survival at birth when compared to the control diets, but when mink were fed diets containing 550 ppm FA there were no adverse effects on any of the parameters examined which included the number of females whelped, gestation length, kit sex ratio, number of kits born alive, number of kits born dead, average litter size, kit survival, and kit body weight (Table 4). Similarly, pregnant dogs were fed diets containing FA (formalin in 40% solution) at concentration 125 or 375 ppm from day 4 through day 56 of gestation. None of the 212 pups examined showed anomalies. Some of these pups were returned to the breeding colony, and their offspring showed no abnormalities (8).

The concentrations of FA in the reproductive study did not adversely affect the body weights of the adult females (Table 5). This agrees with the results of a study by Powell et al. (7) in which mink body weights were not adversely affected by the use of FA-treated spent chickens in the diet which provided a concentration of 1100 ppm FA. In an another study by Bieguszewski and Lorek (9), foxes were fed diets in which 30 or 60% of the meat by-product portion of the diet was replaced with FA-treated fish (concentration not

specified). The results showed that there was no negative influence on body weight gains. They concluded that the feed preserved with FA did not affect the health of foxes.

There were no detrimental effects on the organ weights of adult females among treatment groups in the reproductive study (Table 6). However, the mean of actual spleen weights of male kits in the 550 ppm FA group was significantly higher compared to the control and the 1100 ppm FA groups (Table 7), and the means of relative spleen and kidney weights for male kits in the 550 ppm FA group were also significantly higher compared to the other 2 groups (Table 8). These increases may be a normal physiological response related to the "stress" of consuming the chemical (FA) in the diet or they could be due to the larger average body weights of the animals in the 550 ppm group. Although there were no statistically significant increase in the actual or relative weights of liver, heart, adrenals, and thymus for male kits in the 550 ppm FA group, these organ weights were numerically higher than those of the kits in the control diet.

Earlier investigations have demonstrated that mink fed large amounts of raw fish (Pacific hake and whiting) had a high incidence of anemia (10-14). The affected mink showed a "cotton fur" abnormality (characterized by a gray to white underfur), depressed body weight, light colored carcass, and anemia. Pelts from these mink are usually worthless to the fur industry. Havre et al. (15) concluded that one or more factors in the raw fish blocked the absorption of iron. Costley (16) indicated that FA which occurred naturally in raw-frozen, but not cooked-frozen or raw-unfrozen Pacific hake, significantly depressed absorption of iron. Wehr et al. (17) also concluded that FA rather than triox, the precursor of FA which was widely distributed in marine organisms, was the cotton fur causative

factor in Pacific hake. In the current study, mink feed did not contain Pacific hake, but different concentrations of FA were incorporated into mink diets to reduce bacteria growth. The results showed that 49% of the kits exposed to 1100 ppm of FA through gestation and lactation had developed cotton fur as compared to 0% of the controls (Figure 6). This agrees with the results of studies by Wehr et al. (17) in which FA was implicated as the cotton fur causative agent. However, in the present study, kits exposed to 550 ppm FA had normal fur color while in the Wehr et al. study (17), 600 ppm FA caused 60% cotton fur.

Stout et al. (13) reported that when cotton fur mink were transferred to the basal ration for a 6-month period, the new fur emerged normally pigmented following early summer shedding. The normal fur color was maintained in these adult animals even when they were replaced on the cotton-fur ration from July through the next winter's furring period. They concluded that only young mink develop the cotton fur condition and suggested that depigmentation of the fur occured only when the demands for fur growth coincide with the stress of body growth. These results agree with the results of the present study in that none of the adults females fed the FA-containing diets developed cotton fur.

Because FA depresses absorption of iron, the iron in the diet is unavailable to the mink resulting in anemia which is most frequently indicated by hematological parameters such as HGB, HCT, MCV, and MCH. A study by Skrede (18) showed that feeding mink raw blue whiting resulted in anemia in mink which was reflected by a decrease in HGB (9.1 g/100ml vs 12.6 for controls) and HCT (27.5% vs 37.0 for controls). In the current study, kits exposed to 1100 ppm FA in utero and during lactation had significantly lower HGB (8.15 g/dl) and HCT (25.36%) when compared to the control group (10.58g/dl and 33.02%;

Table 10), thus indicating exposure to FA resulted in anemia.

Duffy (19) demonstrated that a reduced MCV in the presence of modest anemia was suggestive of iron deficiency. A high positive correlation exists between the MCV and MCH (20). Either may be used as a parameter for initial evaluation while most patients with pronounced anemia due to iron deficiency will have low MCV and MCH (21). In the present study, there was significant decrease in MCV and MCH in the kits exposed to the 1100 ppm FA diet. These results suggested that there might be an iron deficiency in these kits due to the high FA concentration in their diets.

In summary, the results of the initial feed consumption trial indicated that dietary concentrations of FA higher than 1100 ppm would not be tolerated by mink. The second feed consumption trial was designed to test the hypothesis that untreated feed kept in the refrigerator for more than 3 days would not be readily consumed because of bacterial growth, but the addition of FA would keep the food from spoiling and thus it would be consumed. This trial indicated that mink actually preferred untreated feed through 7 days. This suggests that it might not be necessary to add FA as a chemical preservative to refrigerated feed and that mink farmers might be able to keep the diet refrigerated for a period longer than 3 days.

Formaldehyde, as an antimicobial agent incorporated into mink diets, was fed to mink 1 month prior to breeding through weaning of the young at 6 weeks of age. This treatment did not significantly impair mink reproduction and kit growth at 550 ppm, but it did affect mink kit survival at 1100 ppm. Furthermore, kits on the 1100 ppm FA diet had changes in hematological parameters suggestive of anemia and 49% of these kits developed

undesirable “cotton fur”.

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CHAPTER 2

SUPPRESSION OF BACTERIAL GROWTH IN FORMALDEHYDE-TREATED MINK FEED

ABSTRACT

Mink feed is an ideal environment for bacterial growth because of the raw animal by-products used in the typical mink diet. Many of these organisms are potential disease producers. FA may be used as an antimicrobial agent to kill bacteria, fungi, and parasites. Experiments were carried out to investigate the effect of incorporating different concentrations of FA into mink feed on growth of total and gram-negative bacteria. In the first experiment, feed containing 0, 550, or 1100 ppm of FA was refrigerated for up to 7 days. The number of colonies of total and gram-negative bacteria derived from the feed was examined each day utilizing enriched blood agar and MacConkey's agar, respectively. In the second experiment, diets containing the same concentrations of FA were incubated at 30°C for 24 hours and bacteria colonies cultured on enriched blood agar and MacConkey's agar were counted at 0, 12 or 24 hours of feed incubation. The results of the first experiment showed that counts of both total and gram-negative bacteria in the FA-treated (550 and 1100 ppm) feed were significantly lower than control counts on a daily basis. In the second experiment, FA at both concentrations was effective in significantly reducing bacterial counts in feed maintained at 30°C over the 24 hour period when compared to control counts.

INTRODUCTION

Varieties of raw animal by-products are used as components in the diet of mink. These by-products may be high in bacteria upon arrival at the mink farm due to distance of transportation or because of processing procedures. Chou and Marth (1) reported that

even frozen meat by-products contained high numbers of microorganisms. That is why mink farmers usually keep refrigerated feed for only 3 days. Kit mink may be especially susceptible to bacterial infection during the "June Blues" period when kits make the transition from nursing to consuming adult feed which is placed in or on top of the cage for a period of 24 hours (mink usually are fed once a day). The species of bacteria found in these various raw materials can vary considerably (1) and many of these organisms can cause serious illness. Bacterial enteritis is a common cause of death in mink and causative organisms include S*taphylococcus aureus* and *Escherichia coli* (2).

Mills and Radostits (3) reported that an epizootic of food poisoning occurred on a Saskatchewan mink ranch in June 1968. Of 5,200 mink, 3,150 were lost over a 10-day period because of bacterial enteritis and septicemia caused by hemolytic *E. coli* and hemolytic *Paracolon spp.* They suspected that high bacterial counts in mink feed might have been a factor in poor production and animal losses. An epizootic of mastitis in mink due to *S. aureus* and *E. coli* associated with food poisoning was reported by Trautwein et al (4). During the course of the epizootic, 2,000 kit mink and 480 adult mink of a total of 3,500 animals died within 10 days.

Numerous experiments have been conducted to determine if chemicals could be used to preserve mink food. These chemicals have included organic acids such as lactic, formic and propionic acid as well as inorganic acids such as sulfuric, hydrochloric and phosphoric acid. However, there is little information on the use of FA for preventing the spoilage of mink feed. It is known that FA, as an antimicrobial agent, has the ability to kill bacteria, fungi, and yeast (5). It has also been reported (see Chapter 1) that FA can be

incorporated into mink feed at a concentration low enough (550 ppm) so that feed consumption, body weight, mink reproductive performance and early kit growth is not deleteriously affected. If it can be demonstrated that concentrations of FA in this range are effective in decreasing bacterial growth, then the mink rancher has another option to protect mink feed from microbial contamination. Thus, the objective of the present study was to determine to what extent and for how long FA would suppress bacterial growth in feed kept refrigerated (4°C) for up to 7 days and in feed kept at 30°C for up to 24 hours.

MATERIALS AND METHODS

Experiment Design

Two experiments were conducted. In the first, diets containing 0, 550, or 1100 ppm of a 37% aqueous solution of FA were refrigerated (4°C) for up to 7 days. Three samples from each treatment diet were collected daily. A 1 gm portion of each sample was serially diluted and plated onto culture media which supported growth of either gram-negative (MacConkey's agar, MAC) or gram-negative plus gram-positive (enriched blood agar, EBA) organisms. Bacteria colony forming units (CFU) were counted in each dietary treatment at each sample time. In the second experiment, diets containing 0, 550, or 1100 ppm FA were incubated at 30°C for 24 hours. Three samples per treatment were collected immediately before incubation (0 hr), and after 12 and 24 hours of incubation at 30°C. Dilution of samples and counting of bacteria in the second experiment were the same as those in the first experiment.

Plating Samples

One gm portions of each sample were prepared for bacteriological analysis by homogenizing the sample in 9 ml sterilized 0.85% saline. The sample was then mixed for 2 minutes. The sample / saline mixtures were subsequently diluted by 1:10 serial dilutions ($10^{-1}, 10^{-2} \ldots\ldots 10^{-10}$) with 9 ml saline solution. A 10 µl and a 50 µl aliquot from the 10^{-10} dilution tube were removed and spread over half of the surface of an EBA and a MAC agar plate, respectively. The same procedure was repeated for the other half of the EBA and MAC agar plates. This procedure was repeated with the $10^{-9}, 10^{-8} \ldots\ldots 10^{-1}$ dilutions. All the plates were incubated at a temperature of 37°C for 20-24 hours after which the total bacterial and the gram-negative bacterial colony counts per gm product were assessed.

Statistics

A logarithmic transformation of the bacteriological data was made. Data were analyzed by using the SAS statistical package (SAS Institute Inc., 1994). Differences among treatments and days for each bacterial culture were analyzed by factorial ANOVA. Multiple comparisons of treatment means and linear trends over time were based on Bonferronni's adjustment. Statements of significance are based on $p < 0.05$.

RESULTS

The log values of gram-positive plus gram-negative bacterial CFU per gm of FA-treated feed refrigerated for up to 7 days are presented in Figure 7. The mean log values of bacterial counts in the various FA-treated diets (0 ppm, 550 ppm, 1100 ppm) were significantly different from each other on each of the 7 days. Linear trend analysis indicated

that the bacterial growth curves generated for the high-dose (1100 ppm FA) and the control sample were significantly different from the low-dose (550 ppm FA) sample. In the control sample, there was an increasing trend in the bacterial counts over time. The mean log values of bacterial counts in the low-dose sample remained quite low and stable over the 7-day period while bacterial growth in the high-dose sample decreased over the 7-day period.

The gram-negative bacteria CFU for the control and low FA diets on day 1 were significantly higher than CFU for the high FA diet, but CFU for the low FA diet were lower than control counts (Figure 8). On day 2, CFU for the control diet were significantly higher than the CFU for the low and high FA diets. On days 3 through 7, the mean log values of bacterial counts in the 3 treatment diets were significantly different from each other. Linear trend analysis indicated the growth curves for gram-negative bacteria over the 7-day period were not significantly different from one another.

The log values of CFU for gram-positive plus gram-negative bacteria in FA-treated feed incubated at 30°C for 24 hours are shown in Figure 9. The mean log values of bacterial counts in the various FA-treated diets were significantly different from each other at each sampling time (0 hour, 12 hours, 24 hours of incubation). The total bacterial counts in the control diet were the highest compared to the other two diets. Trend analysis indicated an increase in the bacterial counts of the control diet with a more rapid increase during the first 12 hours when compared to the last 12 hours. The bacterial counts in the high FA diet were the lowest. There were no linear trends in the low and high FA diets.

The patterns of gram-negative bacterial growth in FA-treated feed incubated at 30°C for 24 hours (Figure 10) were similar to the patterns for total bacterial growth. The control

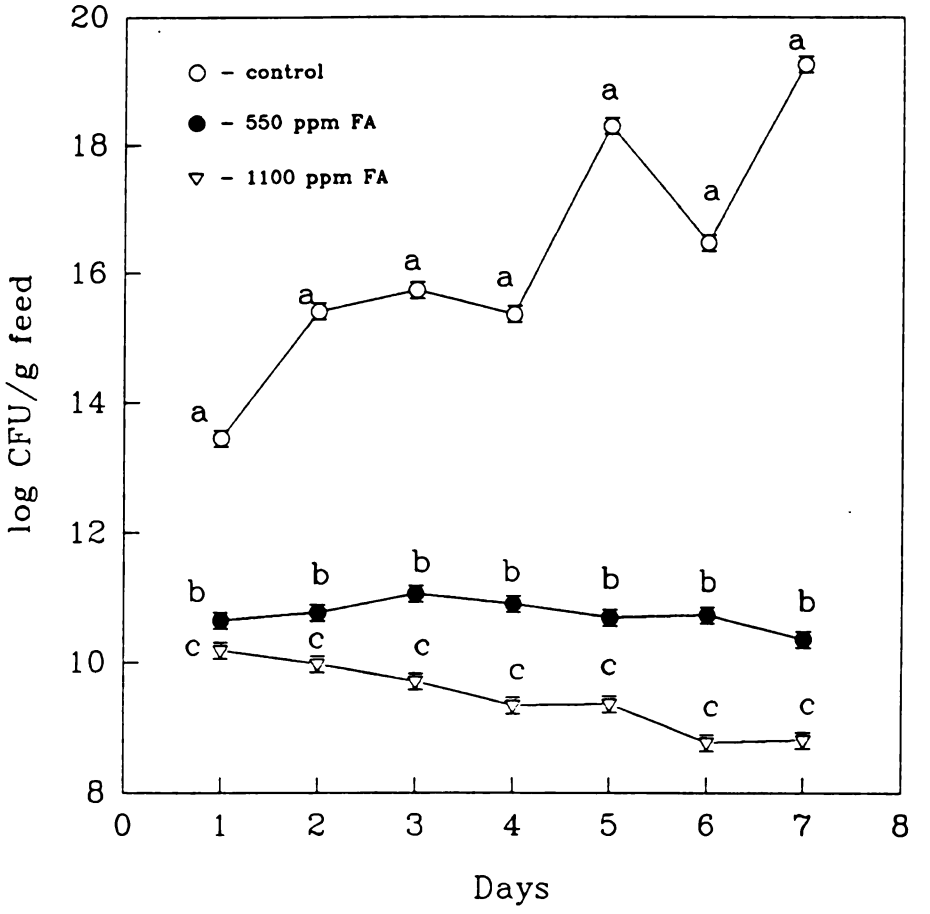

Figure 7. Bacterial Colony Forming Units of Gram-Positive Plus Gram-Negative Bacteria in Formaldehyde (FA)-Treated Feed Refrigerated for Up to 7 Days. Each point represents the mean ± S.E.M. of 3 samples. Means with different superscripts at each day are significantly different ($P < 0.05$).

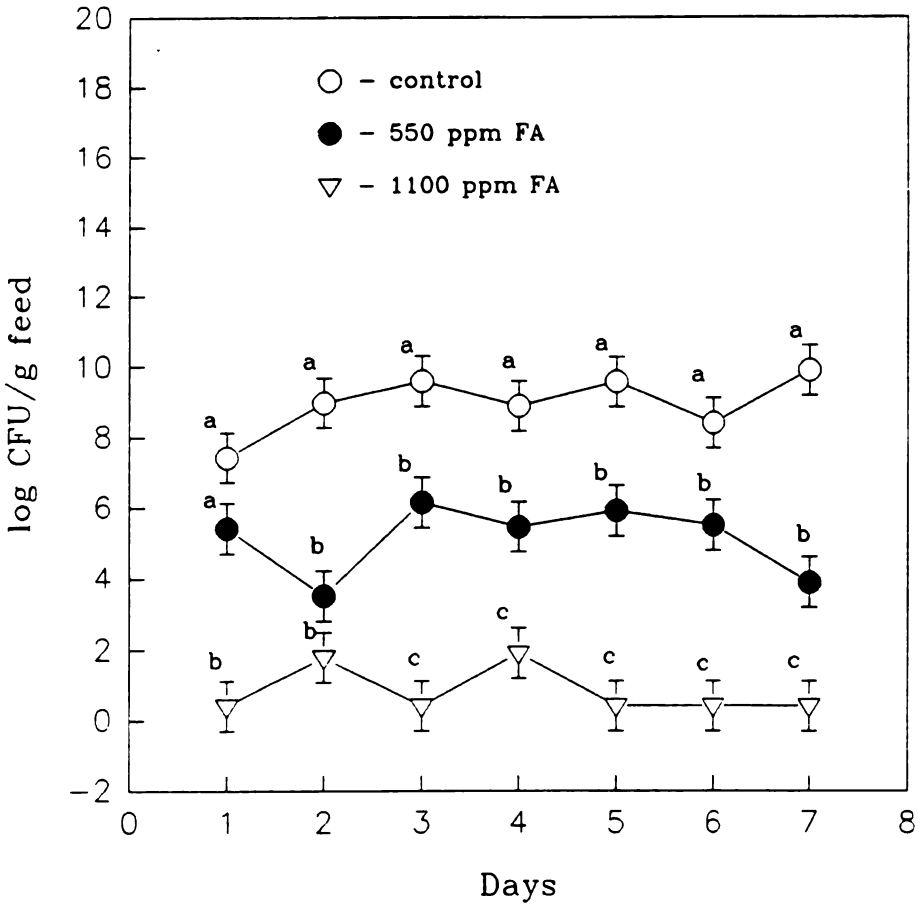

Figure 8. Bacterial Colony Forming Units of Gram-Negative Bacteria in Formaldehyde (FA)-Treated Feed Refrigerated for Up to 7 Days. Each point represents the mean ± S.E.M. of 3 samples. Means with different superscripts at each day are significantly different ($P < 0.05$).

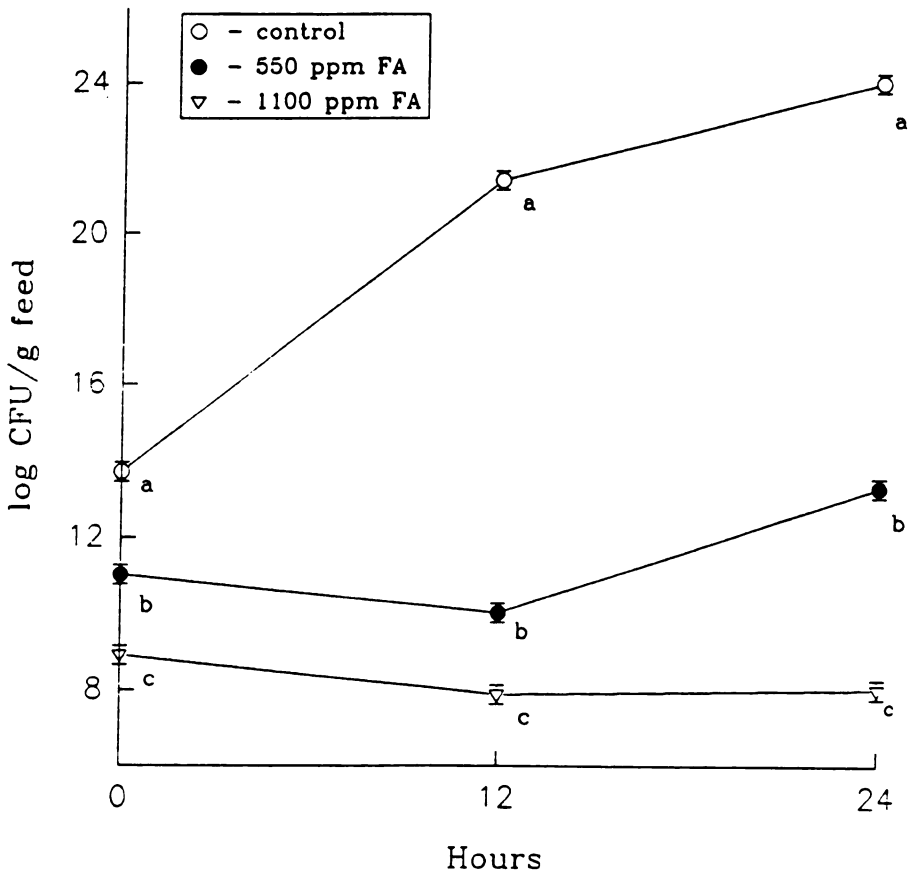

Figure 9. Bacterial Colony Forming Units of Gram-Positive Plus Gram-Negative Bacteria in Formaldehyde (FA)-Treated Feed Stored at 30°C for 24 Hours. Each point represents the mean ± S.E.M. of 3 samples. Means with different superscripts at each time are significantly different ($P < 0.05$).

diet had the highest counts at each sampling period followed by the 550 ppm diet with the 1100 ppm diet having the lowest counts. Counts for the control diet increased over the 24-hour incubation period while there was no increase in CFU in the 550 and 1100 ppm diets.

DISCUSSION

The results of the present study showed that both gram-negative plus gram-positive and gram-negative bacterial counts in the refrigerated control diet were always significantly higher than counts in the low and high FA diets indicating that FA was effective in depressing bacterial growth in mink feed (Figures 7 and 8). With increasing concentrations of FA, bacteria growth was suppressed to a greater degree. These results are similar to a preliminary study by Powell et al. (6) in which various concentrations (0 to 2750 ppm) of FA were incorporated into mink feed to examine whether FA would significantly suppress bacterial growth in the feed. The results of this preliminary trial showed that FA was effective in reducing bacterial numbers. Since FA is effective in keeping bacterial counts low in mink feed, it is possible that feed could be kept refrigerated for at least 7 days. The ability to keep food longer may decrease the cost of feeding and also reduce the incidence of food-borne disease in mink.

Feed in the second experiment was incubated at 30° C for 24 hours to mimic the situation which could be encountered when feeding mink during the summer. Bacteria in the unpreserved diet grew rapidly during the first 12-hour period at this temperature and then grew more slowly during the last 12-hour period (Figures 9 and 10). These results are similar to the finding reported by Chou and Marth (1). They examined microbiological

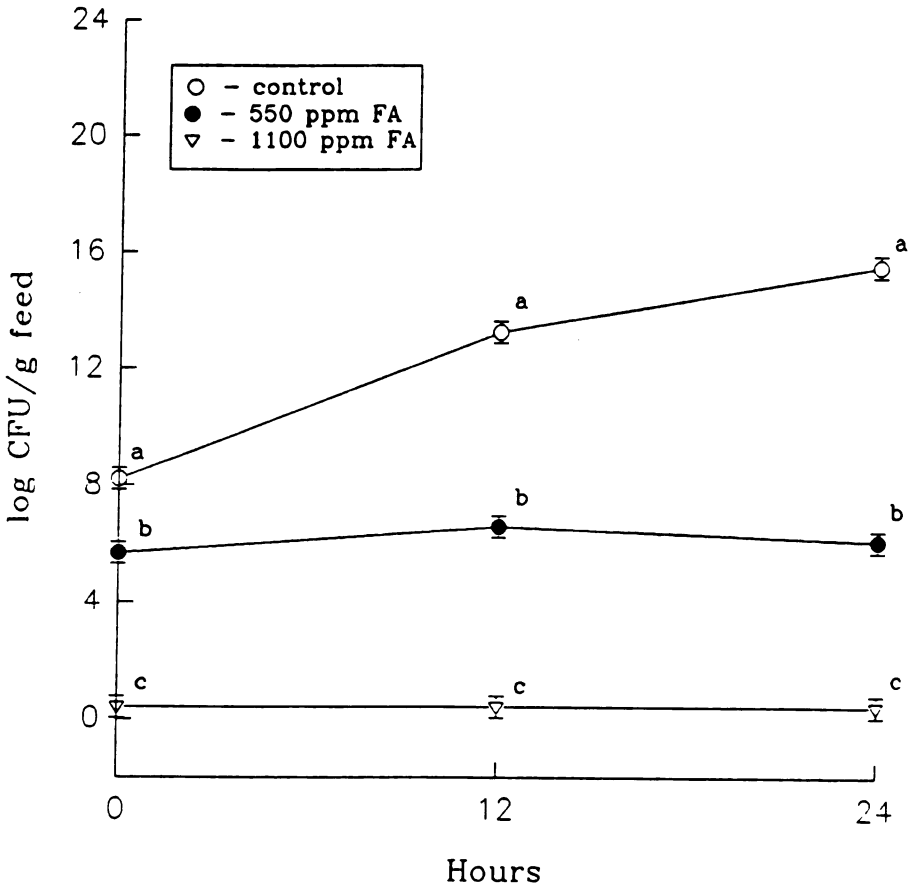

Figure 10. Bacterial Colony Forming Units of Gram-Negative Bacteria in Formaldehyde (FA)-Treated Feed Stored at 30°C for 24 Hours. Each point represents the mean ± S.E.M. of 3 samples. Means with different superscripts at each time are significantly different ($P < 0.05$).

characteristics of some frozen and dried feedstuffs and reported that after 12 and 24 hours of incubation at 30° C, all feedstuffs tested had rapid growth of bacteria during the first 12-hour period and a continued but slower growth during the second 12-hour period. In the present study, CFU in the low and high FA feed were considerably lower when compared to the control feed (Figures 9 and 10). This suggests that FA in the mink diet could delay the deterioration of mink feed placed on the animal's feed grill during warm environmental conditions. Urlings et al. (7) concluded that fur animal feed without any preservative agent would spoil within 20 hours when temperatures exceed 20°C. They suggested that an effective preservative against microbial spoilage was necessary for mink, or, alternatively, animals should be fed at least twice a day. Therefore, FA appears to be an effective preservative for preventing spoilage of mink feed.

Although bacterial counts in the high-dose diet were significantly lower than those in the low-dose diet during the 7-day and 24-hour trials, the concentration of FA in the low-dose diet still decreased bacteria activity efficiently when compared to the control diet. An additional consideration in choosing a dose of FA to preserve food is not only its effect on suppression of bacteria growth, but also its potential deleterious effects on the animal consuming the treated feed. Therefore, it could be concluded that the concentration of 1100-ppm FA in the mink feed is too high for the prevention of spoilage of mink feed because of its adverse effects on mink feed consumption and early kit growth, but the concentration of 550 ppm FA in the mink feed would be appropriate since it suppressed bacteria growth efficiently and did not have any detrimental effects on the adult and kit mink.

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SUMMARY AND CONCLUSIONS

The results of the present study suggest that both 550 and 1100 ppm FA in mink feed suppressed bacteria growth efficiently when feed was refrigerated for up to 7 days and when feed was kept at 30° C for 24 hours. However, since the 1100 ppm FA caused a decrease in feed consumption of adult mink and resulted in reduced survivability, anemia, and "cotton fur" in kits, it is unlikely that the concentration of 1100 ppm FA in the complete diet is a desirable concentration. Instead, the concentration of 550 ppm FA appears to be an appropriate concentration for preventing spoilage of mink feed. Therefore, it can be concluded that 1100 ppm of FA is too high to be incorporated into complete mink feed to suppress bacteria growth even though it is very efficient in reducing microbial activity in animal by-products and /or prepared feed and that the 550 ppm FA could be safely used for preventing the spoilage of mink feed.

A consideration for future studies would be to reduce the concentration of FA in complete mink feed from 550 ppm because it was noted that at this concentration, feed consumption of female mink was still lower when compared to control consumption, although not significantly. The ideal concentration of FA, in terms of mink well-being, would be the lowest concentration capable of significantly reducing bacterial growth in the mink diet. Another consideration would be to assess the effects of FA in mink exposed over their lifetime. It is possible that low concentrations of FA fed over a 2-year period

could cause deleterious effects in mink that would not be apparent in a 6-7 month study.