EFFECTS OF TEMPERATURE PELLETING AND SOME CHEMICALS
ON THE BIOLOGICAL CONTAMINATION OF FEEDS

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TABLE OF CONTENTS

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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INTRODUCTION

Food and feed for humans or animals should be nourishing, attractive and free of injurious biological contamination. Biological contamination in this report refers to _Salmonella_ and mold contamination in feeds.

Salmonellosis, a disease caused by _Salmonella_ organisms, is presently the most important disease transmitted from animal to man. Certain feed ingredients are contaminated with _Salmonella_ organisms and thus may result in contaminated mixed feeds. The latter acts as a vehicle in the transmission of _Salmonella_ to animals which then become healthy carriers and give rise to the production of contaminated foods of animal origin.

Mycotoxicosis is a condition caused by mycotoxins that are produced by certain molds which can grow on and in grain, forage or feedstuffs. Feed or food ingredients containing mycotoxins used in formulations can result in disease and serious economic losses due to reduced growth rate, impaired performance, disease and death.

The importance of controlling _Salmonellae_ and molds in feeds was emphasized by the studies of Erwin (1) for _Salmonella_ and the heavy death losses of turkey poults in England in 1960 due to turkey-x-disease. Later attempts have been made by other workers to control the two organisms by various means.

The purpose of this study was to investigate the effectiveness of pelleting of feed at different temperatures, and addition of chemicals to the feed, for _Salmonella_ or mold control in feeds.

REVIEW OF LITERATURE

Salmonella:

The Salmonella problem is believed by many to be one of the major food related health problems in the United States and other parts of the world. According to the reported cases, of paratyphoid and other salmonelloses compared with typhoid fever for the ten year period 1946 to 1955 in the United States (TABLE 1), it is apparent that, while typhoid fever gradually declined during this period, paratyphoid fever and other salmonelloses increased sevenfold (2). Greze (3) stated that, "Salmonella infections are

TABLE 1. TOTAL REPORTED CASES IN THE UNITED STATES, 1946 TO 1955[1]/

	Typhoid	Paratyphoid and other salmonelloses
1946	3,268	723
1947	3,075	951
1948	2,840	882
1949	2,795	1,243
1950	2,484	1,233
1951	2,128	1,733
1952	2,341	2,596
1953	2,252	3,946
1954	2,169	5,375
1955	1,704	5,447
Total	23,352	24,129

[1]/From Edward, P. R. (2).

of such proportions, it has been recommended the World Health Organization
establish an international reporting center to help combat the increasing
problem." It has been recognized as a common disease in human and animal
populations throughout the world. The increasing incidence of clinical
salmonellosis in human and nonhuman populations within the United States and
other parts of the world has been effectively documented (2, 4). It is
estimated that there are two million persons infected each year in the United
States. Reported Salmonella infections in the United States during the past
quarter century, other than typhoid fever, have increased from 504 in 1942
to 20,867 in 1965 (5).

Salmonella infections were long considered simply as zoonoses, i.e.,
animal diseases that can be transmitted to man. The present trend is to
consider them rather as anthropozoonoses, i.e. diseases transmitted directly
or indirectly by man or animals (6).

Numerous reports in the literature have clearly shown that feed ingredi-
ents which are used to formulate complete feeds for livestock and poultry are
frequently contaminated with Salmonella organisms. Edwards and Galton (7)
reviewed extensively the incidence of Salmonella organisms in man, animals of
all types, food for man and feed and/or feed ingredients for animals.
Salmonella organisms are ubiquitous in nature and have been isolated from every
variety of mammals, amphibians, birds and insects. The organism was named
after Dr. D. E. Salmon, who with Smith in 1885 isolated Salmonella cholerae-suis
from swine.

Salmonella In Animals:

In many parts of the world Salmonella dublin is endemic in cattle and
often has been transmitted to man through infected carcasses and milk. It is
known that many healthy cattle are either temporary or permanent carriers of

the organism, that large number of bacteria are excreted in the feces, and that mastitis occurs in infected cows, resulting in excretion of organisms in the milk (2). Rokey and Erling (8) reported a 33% mortality among the 85% young calves sick in an epidemic caused by Salmonella dublin. Granville (6) reviewed reported isolations of Salmonella in farm animals in Germany, Denmark and Belgium. Peterson and Coon (9) indicated that an explosive epizootic of Salmonella typhimurium infection in a 70-cow dairy was responsible for the death of nine cows and disease manifestations in 80% of the adult herd. These workers stated that total herd milk production dropped from 2,500 to 300 pounds per day. Recovery was generally quite rapid but was prolonged in those cows affected most severly.

When one considers the extremely large populations of domestic fowl and the frequency with which Salmonellae are isolated from them, birds probably constitute the largest single reservoir of these bacteria among animals (2, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15). Dogs and cats have been incriminated as carriers of Salmonellae (16, 17), and in some instances direct transmission of infection from dogs to man has been observed (2). Various surveys have indicated that 15 to 20% of normal household dogs may be infected with Salmonellae. Pets, domestic livestock, poultry, and laboratory animals have been involved in severe salmonellosis resulting from consumption of contaminated feeds (12, 18, 19). Salmonellae occur also in insects and other cold blooded animals. Cold blooded animals such as snakes (20), and tortoises (21, 22) have been found to carry Salmonellae, and Thomas (23) observed transmission of the bacteria from a tortoise to a child.

Salmonella In Animal Feeds:

Erwin (1) examined 206 bags of poultry feed consisting of products of nine commercial companies. He isolated S. oranienburg in three instances from

mash granules and concentrates. Boyer et al. (24) described an outbreak of
S. thomasville which was associated with the consumption of contaminated
feed. It was proven that S. thomasville, which caused 34% mortality in
poults, was from the feed eaten by the poults. Watkins et al. (25) reported
that out of 200 samples of animal byproducts cultured for Salmonella organisms,
37 were found to be contaminated. Shotts et al. (19) reported recovery of
Salmonella from 23% of animal feeds and from approximately 50% of animal feed
ingredients and rendered materials he studied. Burr and Helmboldt (26)
examined 436 animal byproducts and feed ingredient samples for the presence of
Salmonella. Fifty six (12.8%) samples were found to be contaminated with
Salmonellae. Pomeroy and Grady (27) while describing the isolation of Salmonella
organisms from feed ingredients stated that in the past 25 years a total of
over 100 serotypes have been isolated from chickens, turkeys and other avian
species in the United States. Many of the Salmonella serotypes isolated from
livestock and poultry have been isolated from feed ingredients. The bac-
teriological examination of 980 samples of animal byproducts from 22 states
and Canada indicated Salmonella contamination of 175 samples.

In an exhaustive investigation conducted by the Public Health Laboratory
Service of England (28) the frequency of Salmonella organisms, in compound
feedingstuffs, both meals and pelleted foods, and their incidence in the raw
ingredients incorporated in these foods were compared. In the first series
a total of 4,140 samples consisting of 1,284 raw ingredients, 1,742 meals or
mashes and 1,114 pelleted foods were examined. The highest incidence was
found in protein concentrate meals and meat and bone meal; milk products and
locust beans were free from Salmonella. Forty eight (2.8%) of the 1,742
samples of finished meals contained Salmonella as compared to 3 (0.27%) of
1,114 samples of pelleted foods. The process of pelleting, therefore,

apparently reduced Salmonella occurence when compared to meal or mash by 90%. In a second series of experiments in which 627 samples of protein concentrate meals were examined, 31 were found to be positive for Salmonella. The difference between the proportion of positive samples before and after the introduction of heat treatment was statistically significant. In a third series 130 samples of raw ingredients and 363 finished feed samples were collected from 12 manufacturers. Incidence of Salmonella was 13.1% of the 130 raw ingredient samples, 9% of 67 finished meal or mash samples and only 1.7% of the 296 pelleted foods. Salmonella senftenberg was the common serotype frequently isolated from samples analyzed in all the three series of experiments. Boyer et al. (11) showed a correlation between salmonellosis in turkey poults and chicks and contaminated feed. These workers isolated S. thomasville, S. newbrunswick, and S. kentucky from the livers and intestines of poults in one case and S. thomasville from an unopened bag of feed from the same lot that had been fed to those poults. In the second case, four serotypes were isolated from poults and two of the types were recovered from the feed.

According to the Salmonella Surveillance Summary 1964 (29), nonhuman isolations accounted for 5,164 recoveries of Salmonella which showed an increase of 1% over the previous year.

Pomeroy, according to Crane and Hansen (30), indicated 23.4% of 660 samples of feed ingredients were positive for Salmonellae. Most of the ingredients sampled were of animal origin. Pomeroy and Hansen (30) also reported a 2% incidence in 349 feed ingredient samples tested for Salmonellae at the Land-0-Lakes Laboratory. Out of 555 samples tested in 1961, 21 positives were recorded. The 1962 record showed an average 3.6% incidence of Salmonellae from a total of 757 tested samples. During 1963 and 1964, out of 269 carloads of meat meal sampled and tested, 74 were positive for Salmonellae. From

January 1965 through August 1965, 34 out of 132 carloads of meat meal samples were found to be positive (30).

During 1965, according to the Salmonella Surveillance Annual Summary (31), a total of 6,834 Salmonella isolations from nonhuman sources were reported representing an increase of 25% over the previous year. Of the 6,834 isolations, 56.2% were from poultry and fowl, 14.8% from domestic and wild animals, 12.2% from eggs and egg products, 5.4% from animal feed and 11.4% from other sources. Ellis (32) described the analysis of feed fed to seven-day old piglets. Two of 247 samples of feed taken at random for culturing yielded Salmonella. Steele (5) stated that during the first three years of National Salmonella Reporting to the Communicable Disease Center, 16,311 isolations were reported from nonhuman sources. Of these, 12,287 were from four domestic species; turkey 4,915 (40%), chickens 4,403 (36%), cattle 1,495 (12%), and swine 1,244 (10%).

A total of 7,709 recoveries of Salmonellae from nonhuman sources were reported during 1966, an increase of 12.8% over 1965. This probably, as stated in the report, reflected an increasing interest in surveillance of nonhuman reservoirs of Salmonellae (33). According to the report (33), there were 1,274 (16.5%) Salmonella isolations reported in 1966 from animal feed and feed ingredients as compared to 5.4% during 1965. Among the five most common serotypes isolated from animal feeds were S. senftenberg, 6.4%, S. anatum, 5.3%, and S. livingstone, 5%. Of 321 contaminated animal feeds that were identified in 1966, 314 (98%) were from animal or poultry byproducts. Only 2% were from vegetable protein supplement. In Canada, of the 1,048 non-human isolations reported in 1966, the most common sources were animal and poultry feeds, bone meal, and feed constituents.

Allred et al. (13) described a survey of feed mills in 26 states, made in

1966 to determine the <u>Salmonella</u> contamination rate in four categories of feed ingredients and three finished feed categories. A total of 12,770 samples was collected at 724 feed mills. Samples were taken from each category. The total samples, percentages of positives, and standard deviations reported were:

Grain   2,698 samples with 0.66 ± 0.19% +

Oil seed meal   2,629 samples with 2.28 ± 0.32% +

Fish meal   805 samples with 4.72 ± 2.18% +

Animal byproducts   869 samples with 31.07 ± 2.18% +

In the finished feed category:

Cattle feed   2,597 samples with 0.85 ± 0.22% +

Swine feed   1,567 samples with 3.13 ± 0.58% +

Poultry feed   1,605 samples with 5.23 ± 0.73% +

According to the 1967 <u>Salmonella</u> Surveillance Summary (34), a total of 8,794 recoveries of <u>Salmonellae</u> from nonhuman sources were reported, which represented an increase of 14.1% over 1966 and 28.7% over 1965. There were 1,541 (17.5%) isolations reported from animal feed and feed ingredients. Of 810 contaminated animal feeds identified in 1967, 807 (29.6%) were from feed containing animal or poultry byproducts and only 3 (0.4%) were vegetable protein supplements. The common serotypes isolated from feed included <u>S. eimsbuettel</u> (13.4%), <u>S. montevideo</u> (9.2%), <u>S. senftenberg</u> (9.0%), <u>S. anatum</u> (6.4%), and <u>S. oranienburg</u> (4.0%).

Tudor (35) stated that according to the U.S.D.A. releases of November 29, 1966 and March 10, 1967, intensive sampling of animal feed and feed ingredients in 26 states showed that feed ingredients of animal origin, including tankage, meat meal, feather meal and poultry byproducts and similar products were

frequent sources of Salmonella contamination in animal feed. Of 12,714 samples collected from over 700 basic feed mills, 5% of all the samples of ingredients and finished feeds and 42.1% of the plants showed detectable levels of Salmonella contamination. Animal proteins showed the highest incidence of Salmonella contamination (about 33%). Poultry feed had 6.3% whereas swine feed had 4.24% and cattle feed, which uses less animal proteins, had 1.32%. Tudor (35) also reported the results of a survey of New Jersey feed mills. Forty per cent of the feed mills and 11.88% of the samples showed contaminations. This was almost double the percentage of sample contamination for the entire country. Animal proteins had the highest incidences (52.3%), and poultry feed had 14.2% contamination.

Salmonella In Humans:

It is evident that numerous Salmonellae types are widely distributed throughout the animal population. It is not surprising, therefore, that the organisms are frequently present in food products of animal origin. The transmission of Salmonella infections from animals to man through contaminated animal food products has been well recognized. Literature regarding the transmission is voluminous. While Salmonellae obviously are indigenous to many animal food products, the role of the human carrier and the processing plant in the contamination of the final product must also be considered.

Felsenfeld and Young (36) reported that 26 of 56 outbreaks due to nonhost adapted Salmonellae were caused by human carriers. The frequent occurences of the normal human carriers have often been stressed (2, 4, 6, 7).

According to the 1964 Salmonella Surveillance Summary (29) a total of 21,113 isolations of Salmonellae from human sources was reported during 1964. This represented an increase of 13.7% over the previous year. There were 57 human deaths recorded as due to Salmonella infections. During 1965, 20,865

Salmonellae isolations from human sources indicated a decrease of 1.2% from 21,113 reported in 1964 (31). In 1966 a total of 20,040 isolations of Salmonella from humans were reported. Salmonella typhimurium and S. typhimurium var copenhagen were the most common serotypes, accounting together for almost one third of all isolations (33). According to the Salmonella Surveillance Annual Summary of 1967, (34) the reported human isolations of Salmonella were 19,723 during the year representing a 16% decrease from the previous year.

Control:

To study the efficacy of pasteurization before freezing, Woodburn et al. (37) inoculated precooked boned chicken packaged alone, with broth, or with white sauce with approximately one million cells per gram of S. senftenberg 775 W and Staphylococcus aureus. An electronic range or immersion in boiling water as the source of heat resulted in essentially sterile products. These workers stated that differences among the organisms in heat resistance were not pronounced under the conditions of the experiment. S. senftenberg appeared to survive in greater numbers than did S. typhimurium. The difference was highly significant for the electronic range but not for the boiling water treatment.

According to Granville (6) heating to a temperature over 60°C reduced the number of Salmonella by 80 to 90%. Preparation of feeds in pellets reduced the rate of infection from 9% to 1.7% in meals and mashes. Granville further stated that according to Zinn's recommendations for preventing recontamination of meals, treatment of bags with ethylene oxide gas was effective.

Various methods of control and prevention of Salmonella contamination in human food products, animal feed and ingredients, and salmonelosis in humans and nonhumans have been documented intensively by numerous workers (2, 4, 5, 7,

12, 38, 39, 40). Sanitation, pasteurization, heat treatment, chemical use, and radiation are among the methods recommended. Beta and gamma radiation are equally effective in killing Salmonella. Generally, Salmonella are more sensitive to radiation in the presence rather than in absence of oxygen (39).

Hobbs (41) while describing the manufacturing practices for meat and bone meals which are supposed to be the major source of Salmonella, stated that in the United States virtually all animal byproducts intended for feeds are treated by a dry rendering process. This entails the use of large steam jacketed vessels in which the ground material is placed; approximately 60 pounds steam pressure is utilized in the jacket and the material is agitated and vented as it is being heated. Hobbs further stated that according to the reports from the United Kingdom the heat treatment used for producing pellets kills most if not all Salmonellae in meals. Swahn and Rutquist (18) reported that heat sterilization was considered harmful for the product, but after warm pelleting with a steam pressure of 1.8 Kg and a temperature of 90°C (194°F) for six to eight minutes plus cooling time, only one of 37 samples from the preheater was positive for Salmonella and none of 229 samples of pellets was positive.

Ijichi et al. (42) found that a reduction in bacterial counts increased with increasing intensity of irradiation and decreased with increasing feed rate. At a feed rate of 100 ml. per minute and an incident ultra violet energy of 7.22×10^4 ergs per cm^2 per second, counts of S. typhimurium were reduced by a factor of 10^6 to 10^7. In a study with S. senftenberg in various dry meals, Mossel and Koopman (43) pointed out that chemical composition of the dry product seemed to play an important role in the degree of killing of the enteric bacteria used: fish meal and casein were the most and meat meal the least hostile to Salmonella at a given water activity.

Crane and Hansen (30) and Crane et al. (44) conducted studies to determine the effect of pelleting on Salmonella organisms. Meat meal samples prior to incorporation into the final feed were collected; four out of eight trials showed positive results at the time the feed was manufactured. Out of 25 feed samples, 14 were known to contain Salmonella, but after pelleting, all 25 were negative for Salmonella. According to Crane and Hansen, during 1965 pelleted and granulated formulas containing meat meal were routinely tested and while 28.8% of the incoming shipments of meat meal were found to be positive (for Salmonella organisms) none of the pelleted or granulated formulas were positive for Salmonella. While comparing expansion extrusion and pellet extrusion processes, Crane and Hansen pointed out the former process at 93^0 to 183^{0}C (200 to 350^{0}F) completely eliminated Salmonella organisms, whereas conventional pelleting at 70^0 to 83^{0}C (160 to 180^{0}F) did not. Further Crane et al. (44) described the results of Land-O-Lakes studies in which 102 separate tests were conducted on pelleted and granulated feed. No viable Salmonella organisms were recovered from feeds that were pelleted and granulated.

Mossel et al. (45), in a comparative study on decontamination of mixed feeds by radicidation and by pelletisation, reported that, pelletising was fortuitously accompanied by a most significant reduction in Enterobacteriaceae counts, under controlled conditions and could be relied on as a sole means of elimination of Salmonella. On the other hand, radicidation of mixed feed might require up to 1 M rad, for effective decontamination based on the criteria that 10 gram samples of treated material are consistently free from such bacteria.

Allred et al. (13) reported a pronounced effect of pelleting on the Salmonella content of poultry and swine feeds. The results of the experiments of these workers showed 6.29%, out of 1,813 meal samples analyzed, to be

positive for Salmonella; crumbles were 3.28% positive and pellets showed only 0.70% positive among 854 analyzed samples. Wilder (46) stated that many feed ingredients are heated during processing at temperatures high enough to kill the Salmonella. Sanitary handling practices in the processing plants will do much to keep recontamination to a minimum. Requirements for methods given for controlling or killing Salmonella organisms were: 1. must be effective in destroying Salmonellae, 2. must not cause a reduction in nutritive value of the feeds and 3. must be economical. Wilder (46) stated that: 1. irradiation, 2. chemical disinfection, and 3. heating are among the several different proposed methods for destroying Salmonellae in livestock feeds. He pointed out that irradiation has been shown to be effective but costs preclude its use for animal feeds at the present time. The present cost of 0.5¢ per pound, according to him, would be 12% or more of the cost of some feed ingredients. A gas sterilization method has also been developed in which propylene oxide was used. According to Wilder this treatment was effective but again costs preclude its use in animal feeds. He stated that the National Renderers Association and the Fats and Protein Research Foundation felt that the simplest and probably the least expensive method for killing Salmonella would be through the use of heat in a terminal heater. Wilder (46) gave the following conditions for destruction of Salmonella senftenberg:

1. Nine to ten per cent moisture uniformly distributed in feed.

2. A temperature of at least 210°F.

3. A residence time at that temperature of 2½ to 3 minutes.

Food and Drug Administration and the United States Department of Agriculture Programs for the Salmonella Problem:

Lennington, Food and Drug Administration Salmonella Project Officer (47), while giving the purpose of the Food and Drug Administration policy statement

on Salmonella in feed, said, "Veterinarians and microbiologists have for years insisted that as long as we have Salmonella contaminated feeds provided to food producing animals, we are maintaining a reservoir of infection back to man. We hope to clip the Salmonella chain by eliminating contamination in feed ingredients." McDanniel pointed out the Food and Drug Administration's goal in Salmonella control at that time was a significant reduction in contamination of animal byproducts. Contamination of these products was reported to be 33 to 50% compared with 0.5 to 3.5% in other feeds and feed ingredients (48).

While reporting the results of a recent survey conducted in five mid-western mills, at a meeting of the Salmonella committee of the United States Livestock Sanitary Association, Nape stated that feed mills can defend against Salmonella contamination of their feeds through purchasing programs, good house keeping, and good manufacturing sanitation practices. He stated, "The primary Salmonella cycle involves contaminated feed which infects animals and leads to Salmonella excretion. This may result in an increased problem when animals infect other animals directly or by contaminating the environment. The infected animal at slaughter contaminates the animal-origin foods through breaks in packing plant sanitation. These foods then serve as a source of infection for man and his domestic pets." He reported that 105 (23%) out of 448 samples from the feed processing lines in five feed mills yielded Salmonella organisms; 65% of the meat samples were positive. Three serotypes, S. montevideo, S. senftenberg, and S. eimsbuettel, were found most frequently in both feed mills and rendering plants (49).

Heat Resistance of Salmonella senftenberg:

Hobbs (41) described the thermal tolerance of Salmonella serotypes in dry-rendered animal byproducts with a view to post processing treatment. Dried meat and bone meal were inoculated with 10,000 organisms per gram of the heat

resistant strain of S. senftenberg 775 W, after heating to 68.3°C for 15 minutes the organisms could not be detected. Feather meal naturally contaminated with 0.5 per gram of S. montevideo required heating at 82.2°C for 15 minutes before the organisms could no longer be detected. It was concluded that decontamination by post processing heat treatment was economically impracticable in the United States, but the prevention of recontamination after processing was feasible and already being practiced successfully in some rendering plants.

Liu et al. (50) reported in a study of the thermal heat resistance of S. senftenberg 775 W, that significant differences in thermal heat resistances were found among five of the nine cultures tested. Chicks were infected by both smooth and intermediate derivatives of S. senftenberg 775 W. It has since been regarded as the standard Salmonella test organism for thermal resistance investigations.

Molds:

Heavy death losses of turkey poults in England in 1960 due to turkey-x-disease (51) focused attention of investigators on this problem. By 1962 the cause of turkey-x-disease had been traced to a lot of peanuts used in the feed. Some of these peanuts had been invaded by the fungus, Aspergillus flavus. Soon it was found that some strains of this fungus, when growing in certain materials including peanuts could produce a very potent toxin, aflatoxin (52).

Numerous reports in the literature have been cited regarding the occurence of toxicoses in livestock due to consumption of moldy grains and moldy feed. Albright et al. (53) found toxicoses in cattle fed moldy corn; Crump et al. (54) observed mycotoxicoses in dairy cattle when consuming legume hay found to be infected with Rhizoctonia leguminicola. Stevens et al.

(55) described 45 outbreaks of disease in turkeys with high mortality and constant post-mortem lesions. The birds died in good condition after a very short illness and mortality rates ranged from 10 to 70%.

Forgacs (56) stated, "Of the innumerable diseases that affect man and domestic animals, the mycotoxicoses are perhaps the most unfamiliar and least investigated, presumably due to a lack of proper techniques and interested scientists. This situation has prompted many to regard this elusive group of diseases as hypothetical, when in fact, there is abundant evidence to suspect mycotoxicoses as causal factors in disease of unknown etiology." Mycotoxicosis is poisoning of the host follows entrance into the body of one or more toxins of fungal origin. Mycotoxicoses affect animals and man. A careful review of literature describing mold intoxications in livestock in North America revealed sporadic outbreaks in animals that consumed fodder and other feedstuffs (57).

According to Sippel (58), the published findings of Carll et al. and of Forgacs and coworkers created an interest in mold intoxication in the United States. Forgacs and Carll (57) documented the work of various investigators which showed that mycotoxicoses could be the result of consumption of moldy grains and feedstuffs. Forgacs (51) stated that among the numerous fungi isolated from feed and litter where the poultry hemorrhagic syndrome was enzootic, the following fungi have been found toxic to chickens: Aspergillus clavatus, Aspergillus flavus, Aspergillus flavus oryzae, Aspergillus fumigatus, Paecilomyces varioti, Penicillium citricum, and several unidentified species of Penicillium.

Forgacs (59) collected a number of samples of feed, feed ingredients and litter from boiler houses from various parts of the United States where mycotoxicoses were enzootic. The samples were examined mycologically, and the fungal isolates were tested for toxicity in day-old chicks and older birds.

Forgacs (59) isolated <u>Aspergillus chevalieri</u>, <u>Aspergillus clavatus</u>,
<u>Aspergillus flavus</u>, <u>Aspergillus fumigatus</u>, <u>Aspergillus glaucus</u>, <u>Penicillium
citricum</u>, <u>Penicillium rubrum</u> and several others which were found to induce
mycotoxicosis in chickens.

Richardson et al. (60) and Richardson and Webb (61) fed chicks a
broiler mash that had been moistened and allowed to mold spontaneously for
two to ten weeks. These workers observed a growth depression in chicks which
were consuming diets that had molded from four to ten weeks. In another
study, Richardson et al. (60) found that soybean meal adjusted to 19% moisture
and allowed to mold for six weeks depressed growth rate in turkey poults, and
a significant decrease in the nutritive value of the meal was also observed.

The United States Department of Agriculture (62) reported that molds
can reduce production efficiency and cause deaths. The mold <u>Aspergillus
fumigatus</u> is said to cause several diseases in poultry and livestock. Certain
fungal infections from moldy feeds may cause abortion in farm animals by
shutting off the supply of nourishment to the fetus and thus destroying the
placenta. Hamilton (63) pointed out that animals which eat feeds contaminated
by mycotoxins suffer from a variety of debilitating and lethal conditions such
as hemorrhaging, anemia, cancer, photosensitivity, blindness, staggers,
hyperkeratosis, poor growth rate, and inefficient feed conversion. Hamilton
stated that in a survey of a feed mill and broiler operation 90% of the feed
samples were found to contain aflatoxins produced by <u>Aspergillus flavus</u>.

Control:

To control toxicoses caused by feeds and grains, various workers (56, 57,
63, 64) have recommended storing grains properly at lower moisture levels,
sanitation of bins, keeping feeds at lower moisture levels, and use of
chemicals in the field, in feeds and/or feed ingredients at the time of

processing.

Forgacs et al. (64) suggested that since the production of these mycotoxins is associated with fungal metabolism, a direct approach to the control of moldy feed toxicoses would be the inclusion of a suitable compound in the feed that would prevent fungal growth completely or at least modify the metabolism to prevent the production of mycotoxins. The same workers conducted studies to determine the efficacy of a number of compounds in inhibiting the growth of particular fungus isolates when inoculated into broiler mash. Feed was inoculated with toxin-producing fungi. Most of the lots contained initially various species of Aspergillus and Penicillium and to a smaller extent species of Fusarium and Mucorales. The inoculated feed was thoroughly mixed and brought to a moisture level which would accelerate fungal growth. The anti-fungal compound was added at a known level in an aliquot of the mash and finally mixed thoroughly into the feed. Samples were incubated at room temperature. The results of a series of experiments conducted by Forgacs et al. (64) with different chemicals indicated that at 21% moisture level, 8-hydroxyquinoline at a concentration of 300 ppm completely prevented fungal growth for 18 days and allowed only slight growth by 20 to 21 days. Para-chlorophenyl-(tricholo-methyl) thiosulfonate and ethyl-1, 3, 5-trimethyl-4-nitorso-2-pyrole-carboxylate were equally active. 2—bromo-5-nitrothiazole was as effective as 8-hydroxyquinoline at levels of 30 and 300 ppm. 2—chloro-5-nitropyridine at 30 and 300 ppm in chick feed was superior to 8-hydroxyquinoline. Forgacs et al. (64) reported that no significant toxicoses occurred among chicks fed on lots of the inoculated and incubated feed containing 8-hydroxyquinoline, para-chlorophenyl thiosulfonate, and ethyl-1, 3, 5-trimethyl-4-nitroso-2-pyrole carboxylate.

In an intensive study Forgacs et al. (65) mixed nine levels, 0 to 500 ppm,

of 8-hydroxyquinoline into nine lots of fungi-inoculated broiler mash, and incubated the lots at room temperature with regular observations for fungal growth. It was found that growth of a mixed inoculum of six fungi in moist broiler mash varied directly with concentration of 8-hydroxyquinoline up to 200 ppm. According to these observations 50 ppm appeared to be least active, and above 200 ppm fungus growth was entirely suppressed. Among chicks fed fungus-inoculated feeds, the symptoms of moldy feed toxicoses was reduced even when the ten ppm level of supplementary 8-hydroxyquinoline was used. Under simulated field conditions, it was observed that 500 ppm of 8-hydroxyquinoline added to fungus-inoculated dry broiler mash suppressed fungus proliferation and toxin formation.

In a study conducted to determine the effect of different levels of propylene glycol and pelleting on mold incidence in feed, Lange et al.** observed that the number of mold colonies was reduced by all levels of propylene glycol after the first week of feed storage. At the end of the fourth week of storage, only a 4% level of propylene glycol was effective. When compared to the control, a decrease of 99.7% and 93.0% at the end of the first and fourth week storage respectively was observed following the use of 4% propylene glycol. Pelleting and inclusion of propylene glycol resulted in a low incidence of mold.

**Unpublished data.

MATERIALS AND METHODS

Salmonella Studies:

In preliminary trials a chick grower formulation, (P-17) containing 5% meat scrap was used. Salmonella senftenberg 775 W, a thermal resistant strain as compared to other Salmonellae (50), was added at a level of approximately 3,000 organisms per gram of meat scrap. A batch of 200 pounds of feed was mixed well with the inoculated meat scrap in a horizontal twin ribbon mixer for five minutes. Samples of the inoculated meat scrap and of the mixed feed were collected. The mixed feed was subjected to steam conditioning and the mash was conditioned to two different temperatures. The first batch of feed was conditioned to a temperature of 50°C and pelleted. Samples of the conditioned mash and the hot pellets from the die were collected in sterilized pint jars. A second batch of the feed was processed in the same way, except the conditioning temperature was raised to 85°C.

In two other studies, four batches of feed were treated, processed, and sampled in the same manner as in the first study. A different feed formula containing 4% fat and 5% of the inoculated meat scrap was mixed for ten minutes and pelleted after conditioning to three temperatures, 52°, 80°, and 90°C.

Samples from all studies were analyzed for Salmonellae.

Preliminary trials were followed by a study on a larger scale. The pilot feed mill was first cleaned well and then contaminated by passing meat scrap inoculated with Salmonella organisms through the principal conveying systems.

The following operation procedure was followed for this Salmonella study:

I. Preparation of mill:

1. Cyclone outlets were covered with plastic and tape.

2. Mill was completely cleaned up.

3. One fan outlet was covered with three furnace filters.

4. Exit doors were wiped down, closed, and sealed with tape.

5. Warehouse entry was curtained off.

II. Contamination of mill:

1. Culture of Salmonella senftenberg 775 W was made.

2. Meat scrap to be used in feed was inoculated with approximately
   50,000 organisms per gram of the prepared Salmonella culture.

The collection of samples, mixing, and detailed procedure for the
Processing of the contaminated feed is discussed separately.

III. Mill clean up after study:

1. Ground grain containing Roccal® solution was passed through all
   conveyors and equipment connected in Part II.

2. Inside and outside surfaces of the mill equipment were sprayed with
   Roccal solution.

3. Complete area was sealed off for three days.

4. Swabs were collected to check for Salmonella presence.

5. All equipment was dismantled and cleaned.

6. Residual material was checked for presence of Salmonella; and
   residue not used in other work was destroyed.

IV. Personnel:

All personnel involved in this study were supplied with:

1. Face dust masks and

2. White coveralls or equivalent clothing.

3. Used filters from dust masks were destroyed.

®Trademark registered.

4. Clothing was washed with a sterilizing solution.

A chick starter formulation, (P-18) containing 10% meat scrap was used. Batches of this formulation were also treated with either 0, 3, or 6% added water. Prior to mixing into the final finished feed, the meat scrap was inoculated with approximately 50,000 organisms per gram of Salmonella senftenberg 775 W culture, and mixed thoroughly in the mixer, previously described, for five minutes. The level 50,000 organisms per gram of the Salmonella culture was selected so as to have a level of 5,000 organisms of the Salmonella per gram of the final finished feed. Three random samples of contaminated meat scrap were collected in sterile jars and plastic bags. The remainder of the material (approximately 454 Kg) was then circulated through the principal conveying units. The contaminated meat scrap was followed by 454 Kg of soybean meal. Three samples of soybean meal were collected at the point where material entered the batching bin. These represented the first, the middle, and the last materials passing through the conveying system. Then ground grain (approximately 454 Kg) was conveyed through the system, and samples were collected in the same manner as for soybean meal. The ingredients, including the contaminated meat scrap, soybean meal and ground grain were mixed in a horizontal twin ribbon mixer to produce the complete feed (P-18) for subsequent sampling. Samples of the mixed feed were collected at the mixer. The feed was then transfered to the pellet mill bin and subjected to pelleting at two conditioning temperatures, one near 50°C and the other near 80°C. Samples of the pellets after cooling for ten minutes in the cooler were collected separately. The other two batches of feed with 3 and 6% added moisture were processed in the same way as the first batch.

After all samples from the three batches of contaminated feed were collected, a batch of formula feed made with clean ingredients was transfered through the conveying system and subjected to pelleting. Samples of clean ingredients such as meat scrap, soybean meal and of mixed feed and the pellets

were collected in sterile jars.

In the second series (a duplication of the first) of the study, the procedures for treatment, processing, and sampling were repeated. All samples collected in both studies were analyzed qualitatively or quantitatively for Salmonella presence. The United States Department of Agriculture recommended procedure for the isolation of Salmonella organisms was employed (66). All analyses for Salmonella organisms were carried out under the direction of Prof. Foltz, Division of Biology, Kansas State University.

Mold Studies:

Samples analyzed for mold incidence in the feed were from three batches of a poultry layer formulation (P-40) processed at the Kansas State University pilot feed mill. Mold Curb®(MC), recommended for the control of mold growth, was used in this study. This material is a product of Kemin Industries, Inc., who identify propionic acid as the active ingredient, and sodium chloride, Propyl-p-hydroxybenzoate, Lactic acid, ethylmethyl phenylglycidate, calcium silicate, ethylorthoaminobenzoate, and ascorbyl palmitate to be inert ingredients.

The three batches of feed were treated with three levels of MC; zero, one, and two pounds per ton of feed. An aliquot of feed was mixed thoroughly with MC and then mixed in the complete feed. The first batch containing the zero level of MC was mixed at the mixer and transfered through the mill for further processing of the feed. Samples were collected before steam conditioning of the feed. The feed was first conditioned to a temperature near 50°C and was then pelleted. Pellets were cooled in vertical cooler for ten minutes and then discharged. The conditioning was then raised to approximately 70°C. Additional quantities of feed were pelleted, and pellets were cooled. For the third sample series, the feed was conditioned to approximately 90°C and pelleted. Samples of the conditioned mash, hot pellets,

and cooled pellets from all three temperature levels were collected in sterile plastic bags.

In a second study feed was mixed with one pound of MC added per ton of complete feed. The feed was conditioned to three different temperatures (50°, 70°, and 90^oC) in the manner previously described and pelleted. Samples of the mixed feed before conditioning, conditioned mash, hot pellets, and cooled pellets were collected as in the first study.

The processing and sampling procedures were repeated for a third study where the feed contained two pounds of MC per ton of complete feed.

In the second series of experiments, all three batches were treated, processed, and sampled in the same way as in the first series. All samples were stored in a cold room until they could be analyzed. In the analysis sub-samples of 11 grams each were taken aseptically and added to dilution bottles containing 99 milliliters distilled sterilized water. The mixture was then shaken vigorously. Standard plating procedures recommended by the American Public Health Association (67) were employed using potato dextrose agar adjusted to pH 3.5 with 10% tartaric acid (1 milliliter acid per 100 ml agar). A dilution series of 10^1 to 10^8 was followed. The poured plates were allowed to solidify and were incubated at 32° and 42^oC. Mold colonies were determined by visual counting using a colony counter after periods of three and five days. The average counts were calculated from data collected from all dilutions of each sample with its duplicate.

A separate study was conducted to determine the comparative effects of MC, sodium propionate and calcium propionate on mold incidence in feed. The feed formulation (P-40) was mixed thoroughly for ten minutes following addition of MC, sodium propionate or calcium propionate at a level of two pounds per ton. A control feed without chemicals was also prepared. Samples of the mixed

feed were collected in sterile plastic bags and stored in the cold room for a one week period. Samples were analyzed for mold colonies in the manner described above. Plates were incubated at 32° and 42^oC temperatures for three and five days.

In both the Salmonella and mold studies, the feeds were pelleted using a California Master Model Pellet Mill with a 3/16 inch pellet die. All samples in both the studies were collected in a sanitary manner to avoid cross contamination. The exact temperatures of the samples were recorded at the time of collection.

RESULTS AND DISCUSSION

Salmonella Studies:

Results of four series of experiments conducted in the preliminary study are summarized in TABLE 2. In the first series, samples collected before and after conditioning at 50°C (122°F) were found to contain Salmonella organisms. There were no Salmonella organisms in feed mash and hot pellets samples collected at pelleting temperatures of 83°, 65°, and 83°C respectively. Salmonella was isolated from all the samples collected in the second series. Positive tests for Salmonella might have been due to the high level of organisms (approximately 3,000 per g of feed) inoculated in the feed. As all the samples in the second study were positive for Salmonella, most probable numbers (MPN) were determined for the samples in a third study to determine if a reduction in Salmonella counts occurred due to pelleting. Decreases of 78.2 and 99.99% were observed in samples conditioned at 50° and 80°C respectively. The original unconditioned mash contained 1,100 organisms per gram. In the samples of hot pellets collected at 60°, 70°, and 80°C MPN indicated a decrease of 99.99% in the first two samples and a 100% destruction in the latter sample. MPN for the samples from the fourth series were also determined. The results were similar to those of the third series.

Quantitative and qualitative determinations for the presence of Salmonella in the samples collected in two subsequent studies are given in TABLES 3 to 7. MPN determined for the Salmonella organisms in the six random samples of inoculated meat scrap used to mix in the final feed, are given in TABLE 3. There were more than 1,100 organisms in five of six samples. One sample from the second study had only 240 organisms per gram. This may have been due to improper sampling or could be due to storage effect. All samples of meat scrap were collected when the material was prepared. Thus the meat scrap used

TABLE 2. PRELIMINARY TRIALS
SALMONELLA ISOLATIONS FROM CHICK GROWER FORMULATION (P. 17)

S. No.	Sample Description	I		II		III		IV	
		Temp.[a]	Qualitative	Temp.[a]	Qualitative	Temp.[a]	MPN	Temp.[a]	MPN
1	Feed mix before conditioning	---	(+)	---	(+)	---	1100/g	---	------
2	Feed mash after conditioning	50°	(+)	50°	(+)	50°	240/g	52°	46/g
3	Pellets (hot)	65°	(-)	65°	(+)	60°	0.15/g	---	------
4	Mash after conditioning	83°	(-)	82°	(+)	80°	0.023/g	---	------
5	Pellets (hot)	83°	(-)	82°	(+)	80°	00	80°	0.023/g
6	Pellets (hot)	---	---	---	---	70°	0.091/g	90°	00

[a]Steam conditioning temperature.

TABLE 3. SALMONELLA CONTENT IN SAMPLES OF CONTAMINATED
MEAT SCRAP BY MPN*TECHNIQUE

Meat scrap + cultured S.senftenberg 775 W	Study I	Study II
Sample No. 1	>1100/g	>1100/g
Sample No. 2	>1100/g	>1100/g
Sample No. 3	>1100/g	240/g

*MPN = Most Probable Number

in the second study had been prepared three days prior to the study. It has been reported by others that Salmonella levels decrease during storage under proper conditions.

As described in the materials and methods section, the contaminated meat scrap was followed by soybean meal and the soybean meal by ground grain to determine if contaminated meat scraps would also contaminate the soybean meal and grain. Results of Salmonella isolation from samples of these two ingredients from the two studies are reported in TABLE 4. Of the soybean meal samples tested, 83.3% were positive for Salmonella. All the ground grain samples analyzed were found to contain Salmonella.

The results summarized in TABLE 5 represent Salmonella isolations from samples of feed, collected at the mixer, that contained 10% contaminated meat scrap and three moisture addition levels. Feed samples with no added water showed a presence of more than 1,100 organisms per gram in the first study and only 2.0 organisms per gram in the second. It is suspected that such differences might be due in part to sampling problems and might also reflect loss of Salmonella viability. All samples of feed with 3% and 6% added water were positive for Salmonella.

The feeds described above were pelleted at two different temperatures. Results of Salmonella isolations for the pelleted feed (50^0 and 80^0C) from the two studies are summarized in TABLE 6. Viable Salmonella organisms were not recovered from any of the pelleted feed samples. This agrees with the results of studies reported by Public Health Laboratory Service England (28), Crane and Hansen (30) and Crane et al. (44). However Crane and Hansen (30), and Crane et al. (44) indicated that the expansion extrusion process completely eliminated Salmonella organisms whereas conventional pelleting did not. Thus better elimination of Salmonella organisms occured in these present studies.

TABLE 4. QUALITATIVE TESTS FOR PRESENCE OF SALMONELLA IN SOYBEAN MEAL AND GROUND GRAIN

SAMPLE DESCRIPTION	STUDY I	STUDY II
Soybean meal at batching bin - First material	(+)[1]	(+)
Soybean meal at batching bin - Middle material	(+)	(-)[2]
Soybean meal at batching bin - Last material	(+)	(+)
Ground grain at batching bin - First material	(+)	(+)
Ground grain at batching bin - Middle material	(+)	(+)
Ground grain at batching bin - Last material	(+)	(+)

[1] (+) indicates presence of Salmonella.

[2] (-) indicates absence of Salmonella.

TABLE 5. SALMONELLA IN MIXED FEED CONTAINING 10% CONTAMINATED MEAT SCRAP AND WITH THREE ADDED MOISTURE LEVELS

SAMPLE DESCRIPTION	STUDY I	STUDY II
Mixed ration with 0% added H_2O	>1100/g	2.0/g
Mixed ration with 3% added H_2O	(+)[a]	(+)
Mixed ration with 6% added H_2O	x[b]	(+)

[a](+) indicates presence of <u>Salmonella</u>.

[b]sample was not available.

TABLE 6. QUALITATIVE EXAMINATION FOR THE PRESENCE OF SALMONELLA IN PELLETS FROM MIXED FEED

SAMPLE DESCRIPTION	STUDY I	STUDY II
Pellets from ration containing 10% contaminated meat scrap and 0% added H_2O conditioned at low temperature (50°C)	(-)[a]	(-)
Pellets from ration containing 10% contaminated meat scrap and 0% added H_2O conditioned at high temperature (80°C)	(-)	(-)
Pellets from ration containing meat scrap and 3% added H_2O conditioned at low temperature (50°C)	(-)	(-)
Pellets from ration containing meat scrap and 3% added H_2O conditioned at high temperature (80°C)	(-)	(-)
Pellets from ration containing meat scrap and 6% added H_2O conditioned at low temperature (50°C)	(X)[b]	(-)
Pellets from ration containing meat scrap and 6% added H_2O conditioned at high temperature (80°C)	(X)	(-)

[a](-) indicates absence of Salmonella.

[b]samples were not available.

TABLE 7. QUALITATIVE TESTS FOR THE PRESENCE OF SALMONELLA IN CLEAN INGREDIENTS
AND A MIXED RATION PRODUCED FROM THEM

SAMPLE DESCRIPTION	STUDY I	STUDY II
Clean meat scrap	(-)[a]	(-)
Clean soybean meal	(-)	(-)
Mixed feed from clean ingredients	(+)[b]	(-)
Clean pelleted feed	(-)	(-)

[a] (-) indicates absence of _Salmonella_.

[b] (+) indicates presence of _Salmonella_.

Analyses for the presence of <u>Salmonella</u> in clean ingredients, mixed feed, and pelleted feed are reported in TABLE 7. Results indicate the recovery of <u>Salmonella</u> organisms from the mixed feed samples in only the first study. No <u>Salmonella</u> was isolated from the feed pelleted from these ingredients.

<u>Mold Studies</u>:

The average number of mold colonies found in samples from the three batches of feed treated with different levels of Mold Curb in two series of experiments are summarized in TABLES 8 and 9. Each treatment level resulted in a reduction in mold incidence. Reduction in the mold incidence in the pelleted feed samples from both experiments is graphically represented in Figures 1 through 8.

Discussion of the statistical analysis of the results will be in terms of logrithmically transformed data.

Analysis of variance on logrithmically transformed data indicated each level of MC had a highly significant (P<.01) effect on reducing the number of mold colonies in the sample plates incubated at 32^0 and 42^0C and examined after three days. The Least Significant Difference (LSD) was calculated and resulted in a value of 0.49975. The means for the three MC levels in ordered array were:

Feed with 0 level of MC	4.79615	a
Feed with MC @ 1 lb/ton	4.46031	a
Feed with MC @ 2 lb/ton	3.86264	b

Means of transformed data for mold incidence in samples conditioned at different temperatures are given in TABLE 10. A reduction in mold incidence occurred but statistically the effect of conditioning of feed at three

TABLE 8. FIRST SERIES OF EXPERIMENTS
MOLD COUNTS IN FEED, WITH DIFFERENT CONCENTRATIONS OF MOLD CURB, PELLETED AT DIFFERENT TEMPERATURES & PLATES INCUBATED AT TWO DIFFERENT TEMPERATURES FOR 3 & 5 DAYS

Sample	Sample Description	Feed Formula P-40	Temp. °C	Plates incubated at 32°C for		Plates incubated at 42°C for	
				3 days	5 days	3 days	5 days
1	Feed mix before condg.	W/O MC[a]		273,850	334,375	120,000	155,800
		W MC 1[b]		195,800	220,800	60,000	130,000
		W MC 2[c]		48,000	58,300	23,500	120,000
2	Feed mash after condg.	W/O MC	50	348,125	480,125	20,000	51,250
		W MC 1	50	111,875	161,875	10,000	38,750
		W MC 2	51	26,650	37,485	3,300	20,665
3	Feed Pellets (hot)	W/O MC	58	140,000	229,375	14,375	51,875
		W MC 1	63	7,500	102,500	6,250	45,625
		W MC 2	55	2,450	19,150	2,450	2,480

[a] Without Mold Curb.
[b] With Mold Curb @ 1 lb/ton.
[c] With Mold Curb @ 2 lb/ton.

Cont.

4	Feed Pellets (cooled)	W/O MC	32	64,375	93,125	6,875	27,500
		W MC 1	29	18,750	26,875	5,000	24,375
		W MC 2	35	11,650	25,000	3,300	14,950
5	Feed mash after condg.	W/O MC	69	56,875	176,250	61,250	317,500
		W MC 1	70	17,500	60,625	2,500	24,375
		W MC 2	71	4,150	5,000	000	2,480
6	Feed Pellets (hot)	W/O MC	72	69,375	170,625	9,375	206,250
		W MC 1	72	6,250	65,625	2,500	14,375
		W MC 2	74	3,300	40,830	000	1,660
7	Feed Pellets (cooled)	W/O MC	34	27,500	47,500	7,500	102,500
		W MC 1	31	26,650	37,500	4,375	11,250
		W MC 2	35	26,650	35,000	4,150	4,165

Cont.

8	Feed mash after condg.	W/O MC	90	20,625	50,625	51,650	101,660
		W MC 1	90	18,125	31,250	50,000	96,000
		W MC 2	90	3,300	30,000	47,500	90,000
9	Feed Pellets (hot)	W/O MC	88	99,375	275,000	30,800	60,000
		W MC 1	90	30,625	103,750	30,000	55,000
		W MC 2	92	10,000	20,000	26,650	50,000
10	Feed Pellets (cooled)	W/O MC	40	46,600	66,650	26,650	59,650
		W MC 1	32	33,300	50,000	12,500	26,875
		W MC 2	33	32,500	43,300	800	5,800

TABLE 9. SECOND SERIES OF EXPERIMENTS
MOLD COUNTS IN FEED, WITH DIFFERENT CONCENTRATIONS OF MOLD CURB, PELLETED
AT DIFFERENT TEMPERATURES & PLATES INCUBATED AT TWO DIFFERENT TEMPERATURES FOR 3 & 5 DAYS

Sample	Sample Description	Feed Formula P-40	Temp. °C	Plates incubated at 32°C for		Plates incubated at 42°C for	
				3 days	5 days	3 days	5 days
1	Feed mix before condg.	W/O MC[a]		75,000	95,000	45,000	137,500
		W MC 1[b]		73,000	87,500	29,000	82,500
		W MC 2[c]		55,000	70,000	26,000	72,500
2	Feed mash after condg.	W/O MC	50	129,165	197,500	143,300	145,000
		W MC 1	51	127,500	175,000	45,000	141,500
		W MC 2	50	83,300	135,000	37,500	107,500
3	Feed Pellets (hot)	W/O MC	58	82,500	85,000	148,300	175,000
		W MC 1	60	30,000	67,500	25,000	50,800
		W MC 2	56	14,165	61,650	5,800	49,950

[a] Without Mold Curb.
[b] With Mold Curb @ 1 lb/ton.
[c] With Mold Curb @ 2 lb/ton.

Cont.

4	Feed Pellets (cooled)	W/O MC	36	110,800	130,000	121,650	130,000
		W MC 1	34	50,800	75,000	45,000	112,500
		W MC 2	35	4,950	64,150	5,800	90,165
5	Feed mash after condg.	W/O MC	70	102,500	109,150	88,300	147,500
		W MC 1	70	70,800	85,000	87,500	137,500
		W MC 2	71	52,500	62,500	54,150	115,000
6	Feed Pellets (hot)	W/O MC	73	82,500	126,650	129,150	145,000
		W MC 1	72	52,500	57,500	42,500	110,000
		W MC 2	78	8,300	55,000	2,480	3,330
7	Feed Pellets (cooled)	W/O MC	38	77,500	79,100	102,500	174,150
		W MC 1	30	65,000	70,000	51,600	126,650
		W MC 2	35	8,300	40,000	6,050	80,000

Cont.

8	Feed mash after condg.	W/O MC	90	60,000	107,500	125,000	253,300
		W MC 1	90	50,000	92,500	99,150	162,500
		W MC 2	90	43,300	67,500	16,650	75,000
9	Feed Pellets (hot)	W/O MC	92	58,300	91,650	97,500	150,000
		W MC 1	90	52,500	82,500	59,150	80,000
		W MC 2	96	27,550	62,500	15,800	65,000
10	Feed Pellets (cooled)	W/O MC	37	80,000	110,000	67,500	130,000
		W MC 1	30	45,000	75,000	24,150	52,500
		W MC 2	37	5,800	39,150	15,000	36,650

TABLE 10. MEANS OF TRANSFORMED DATA FOR MOLD INCIDENCE IN SAMPLES
CONDITIONED AT DIFFERENT TEMPERATURES

Sample	Conditioned at 50°C	Conditioned at 70°C	Conditioned at 90°C
Feed mix before condg.	4.80403	4.80403	4.80403
Mash after conditioning	4.69643	4.16699	4.55070
Hot pellets	4.21925	4.85132	4.56337
Cooled pellets	4.26251	4.29597	4.31977

different temperatures was nonsignificant for plates incubated for three days.

There was a significant effect of incubation temperatures on mold growth as expected. The LSD calculated was 0.21236 and the means were 4.55082 at 32°C and 4.19524 at 42°C. This indicates profuse mold growth at 32°C incubation temperature; but since molds also developed at the higher temperature, a broad spectrum of molds was present.

Results of the colony counts on the plates incubated for five days showed a nonsignificant effect of different levels of MC on the mold growth. Since there was a significant effect following three days incubation, MC appears to be fungistatic and not fungicidal in its effect.

Conditioning of feed at different temperatures had a significant effect on mold growth in plates incubated for five days. Means for the number of mold colonies from the samples are summarized in TABLE 11.

Significant differences (P<.01) also occurred in plates incubated for five days. The effect of incubation temperature was significant (P<.01) with a LSD of 0.10291 at the 5% level. The means of transformed data for mold incidence in plates incubated at 32°C and 42°C were 4.86373 and 4.74503 respectively.

Results of mold incidence from a third study conducted to determine

TABLE 11. MEANS FOR THE NUMBER OF MOLD COLONIES FROM THE SAMPLES
OF THE FEED CONDITIONED AT THREE DIFFERENT TEMPERATURES

Sample	Conditioned at 50°C		Conditioned at 70°C		Conditioned at 90°C	
Feed mix before condg.	5.05830	a	5.05830	a	5.05830	a
Mash after conditioning	5.00751	a	4.76186	b	4.91137	a
Hot pellets	4.71820	b c	4.64004	b c	4.87837	a
Cooled pellets	4.72614	b d	4.67237	b d	4.66960	a b

LSD 0.05 = 0.26265

comparative effects of MC, sodium propionate and calcium propionate are

reported in TABLE 12. The grand means of the mold counts for each treatment

were as follows:

Control	4.906
Feed with Sodium propionate @ 2 lbs/ton	4.898
Feed with Calcium propionate @ 2 lbs/ton	4.870
Feed with Mold Curb @ 2 lbs/ton	4.826

Although there was a trend toward reduction in the mold counts of feed samples

treated with the different chemicals as compared to control feed, effects were

nonsignificant statistically at the 5% level.

TABLE 12. COMPARATIVE EFFECTS OF DIFFERENT CHEMICALS ON MOLD COUNTS

Sample Description	Feed Description	Plates incubated at 32°C for		Plates incubated at 42°C for	
		3 days	5 days	3 days	5 days
Feed Formula (P-40) mix before Conditioning	Control	65,000	90,000	65,000	83,300
		70,000	78,300	90,000	115,000
	Mold Curb* added @ 2 lbs/ton	48,300	63,300	45,000	63,300
		78,300	91,600	70,000	95,000
	Sodium Propionate @ 2 lbs/ton	80,000	93,300	85,000	100,000
		60,000	68,300	65,000	91,600
	Calcium Propionate @ 2 lbs/ton	76,600	98,300	63,300	80,000
		58,300	71,600	68,300	85,000

*Active ingredients: Propionic Acid

Inert ingredients: 80% - Sodium Chloride, Propyl p-Hydroxybenzoate, Lactic Acid, Ethyl Methylphenylglycidate, Calcium Silicate, Ethyl Orthoamino-benzoate, Ascorbyl Palmitate
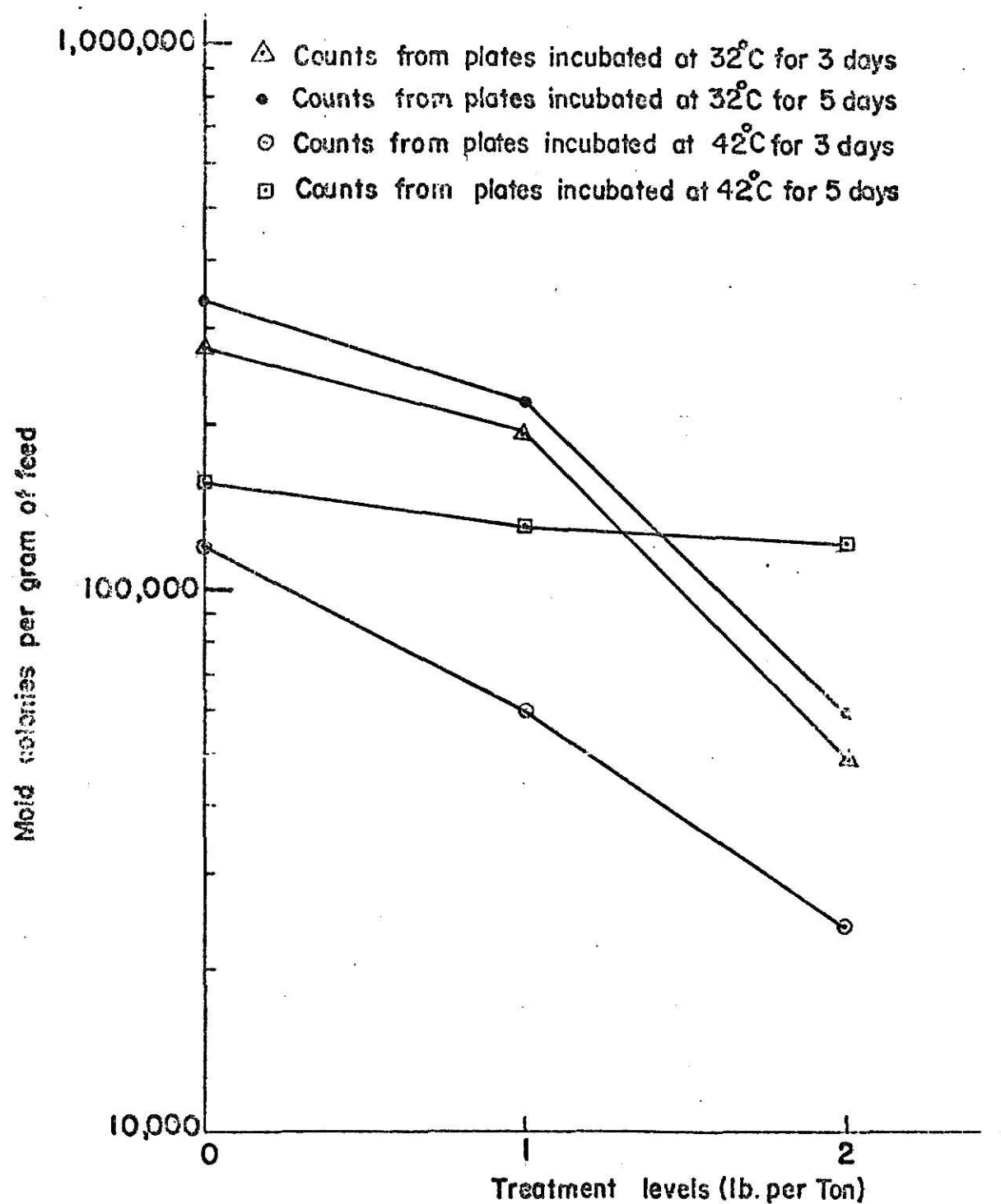
Fig.I. Effect of chemical (mold curb) treatment on mold colonies in mixed feed(first series of experiment).
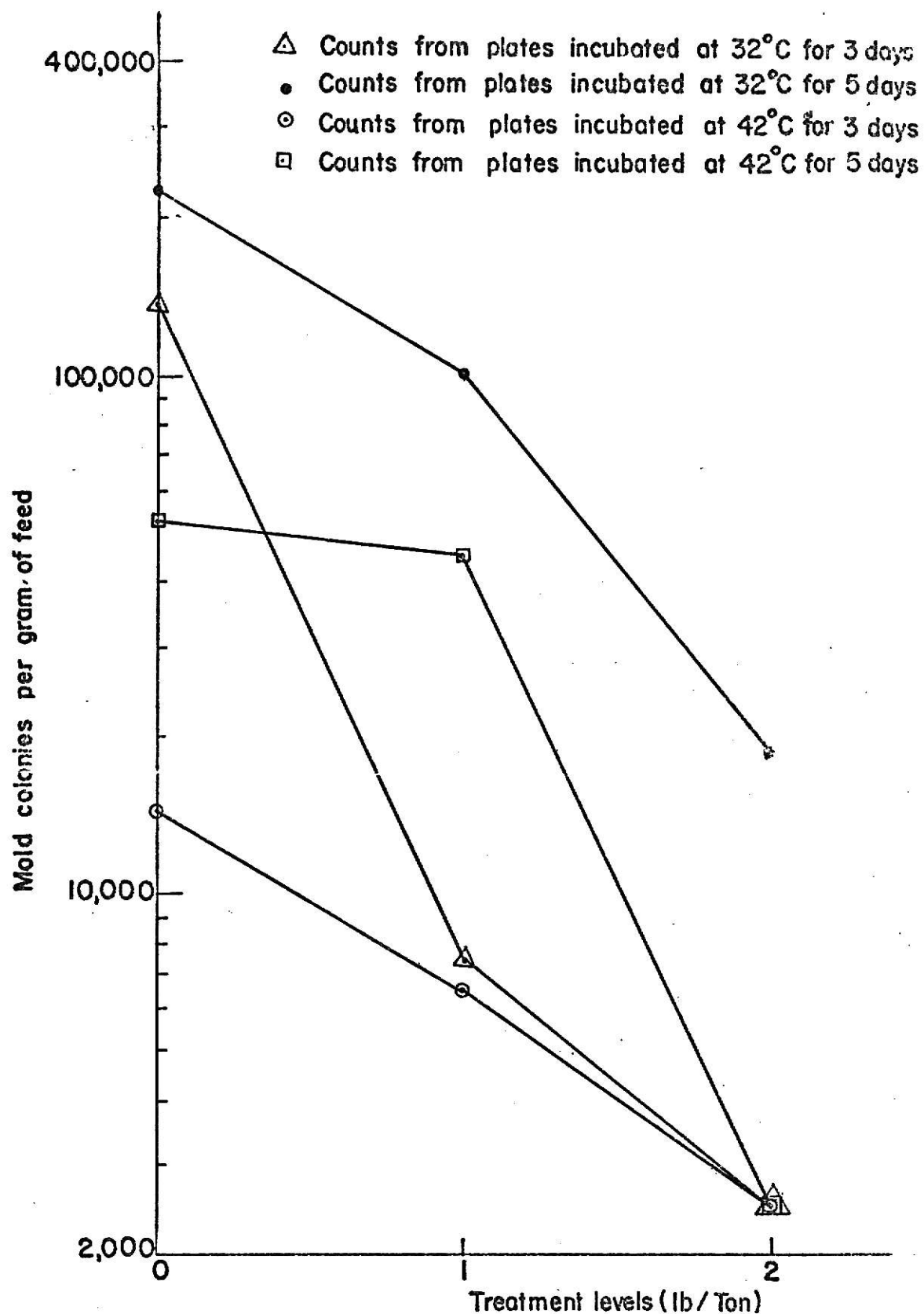
Fig.2. Effect of chemical (mold curb) treatment and pelleting (at a 50°C, conditioning temp.) on mold colonies in feed formula P-40 (first series of experiment).
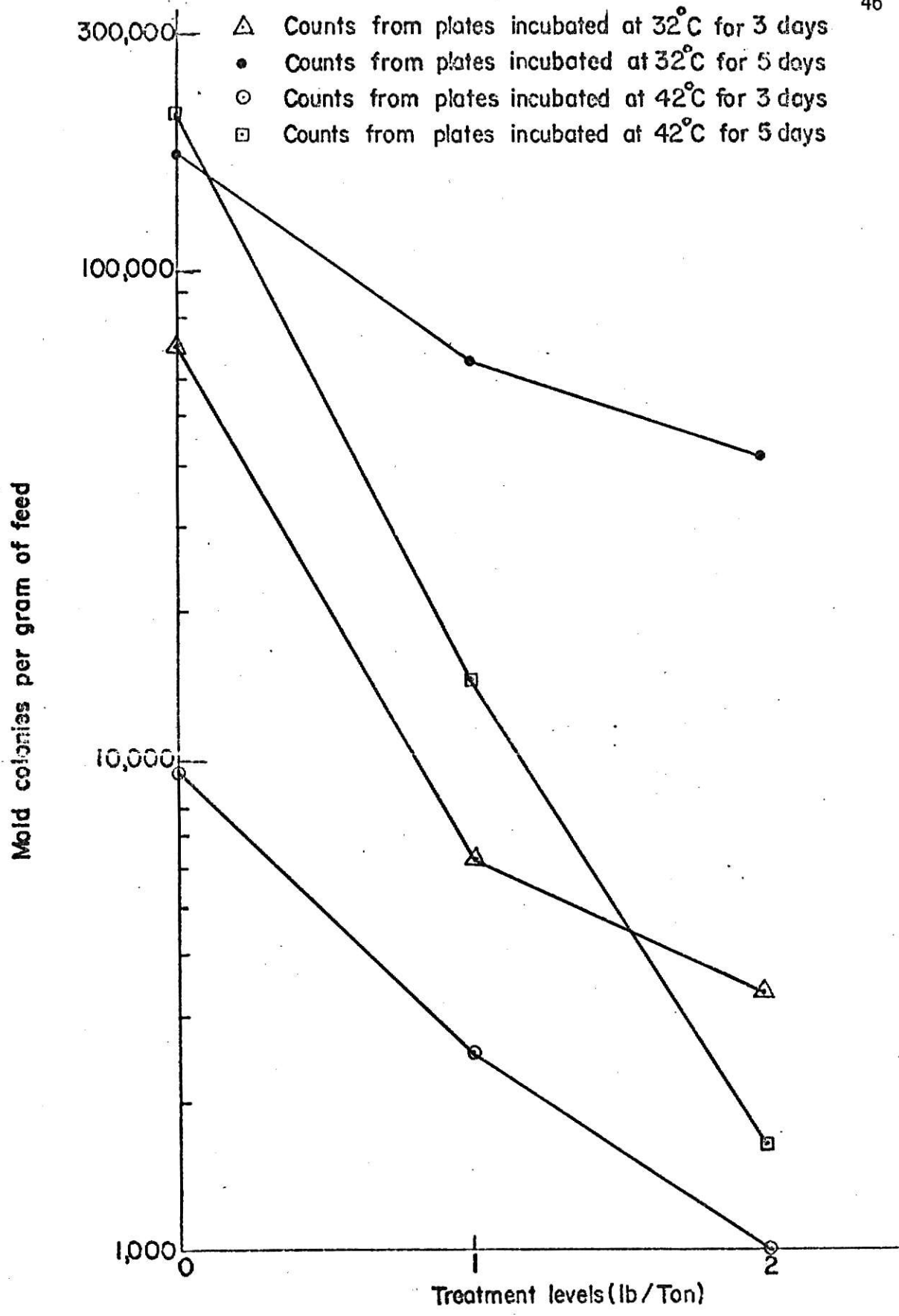
Fig. 3. Effect of chemical (mold curb) treatment and pelleting (conditioning temp. 70°C) on mold colonies in feed formula P-40 (first series of experiment).
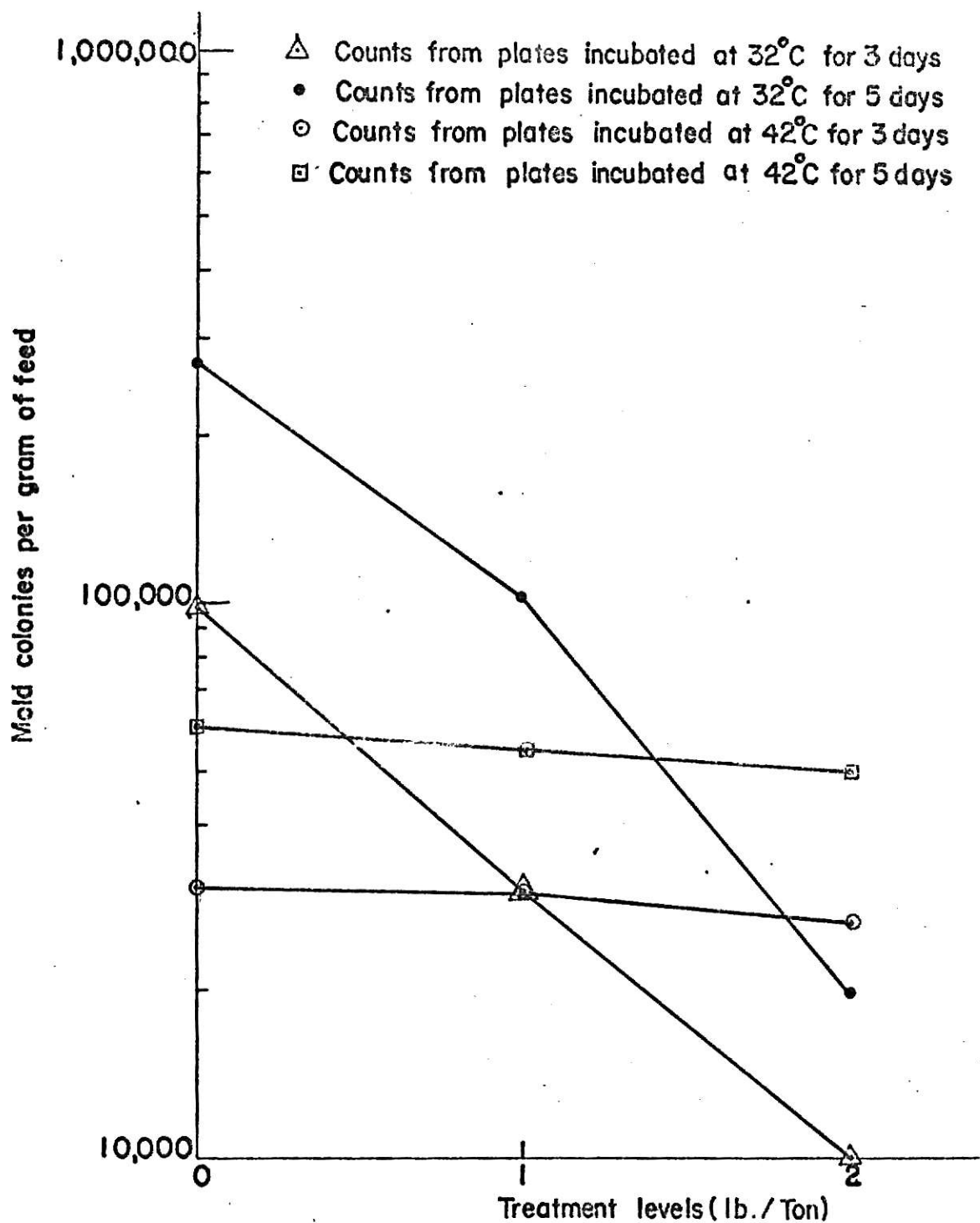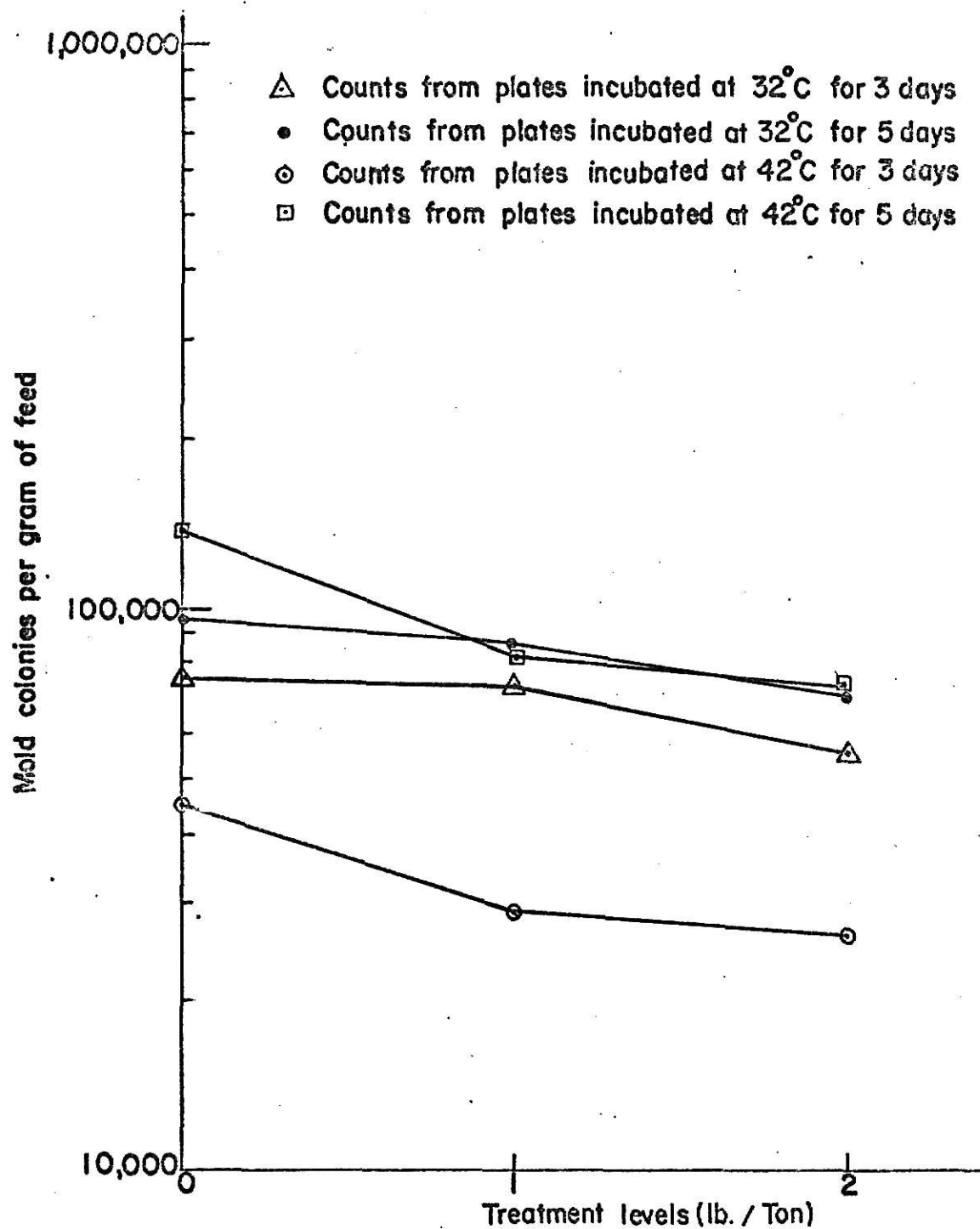
Fig.4. Effect of chemical (mold curb) treatment and pelleting (90°C conditioning temp.) on mold colonies in feed formula P-40°(first series of experiment).

Fig. 5. Effect of chemical (mold curb) treatment on mold colonies in mixed feed (second series of experiment).
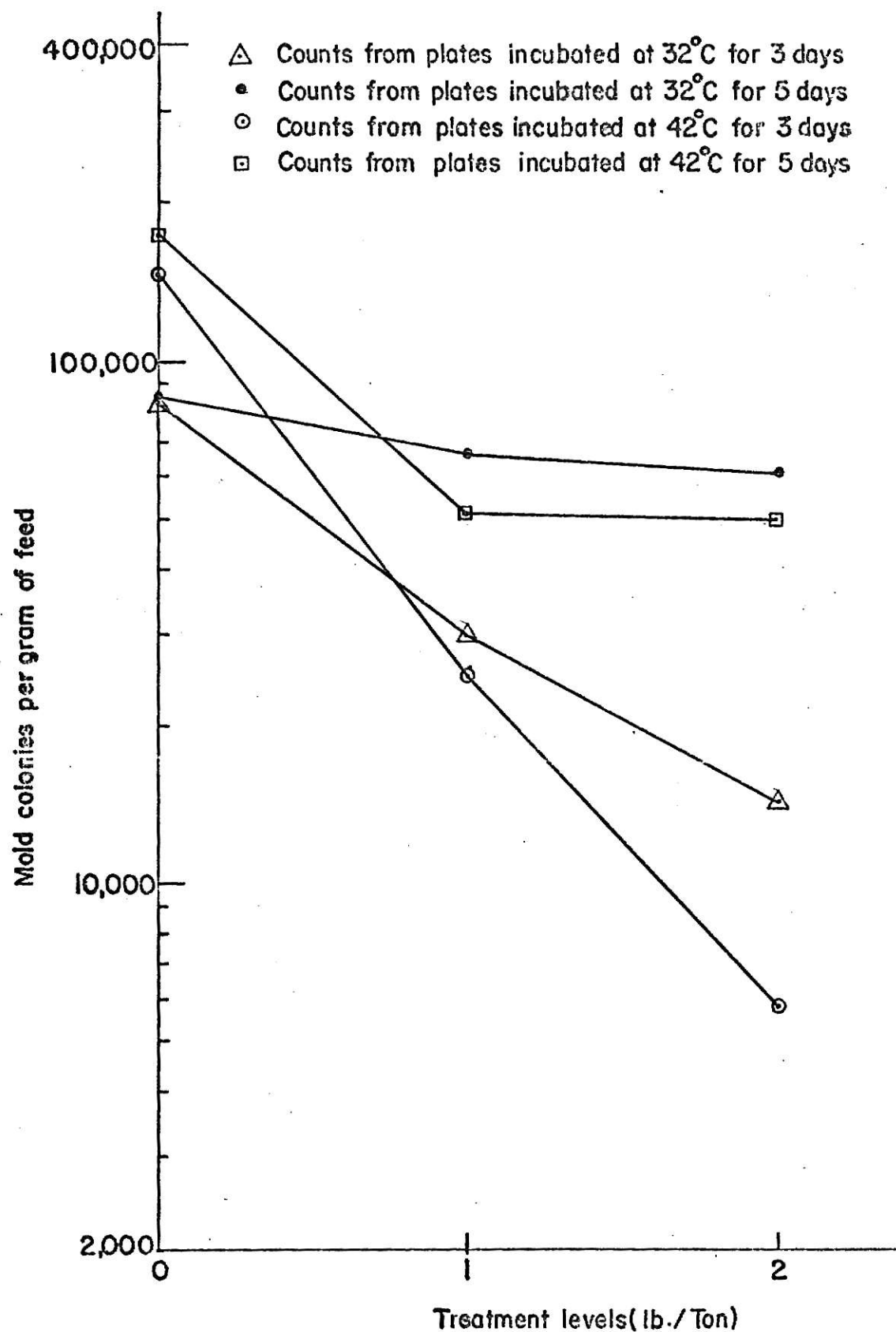
Fig.6. Effect of chemical (mold curb) treatment and pelleting (at a 50°C conditioning temp.) on mold colonies in feed P-40 (second series experiment).
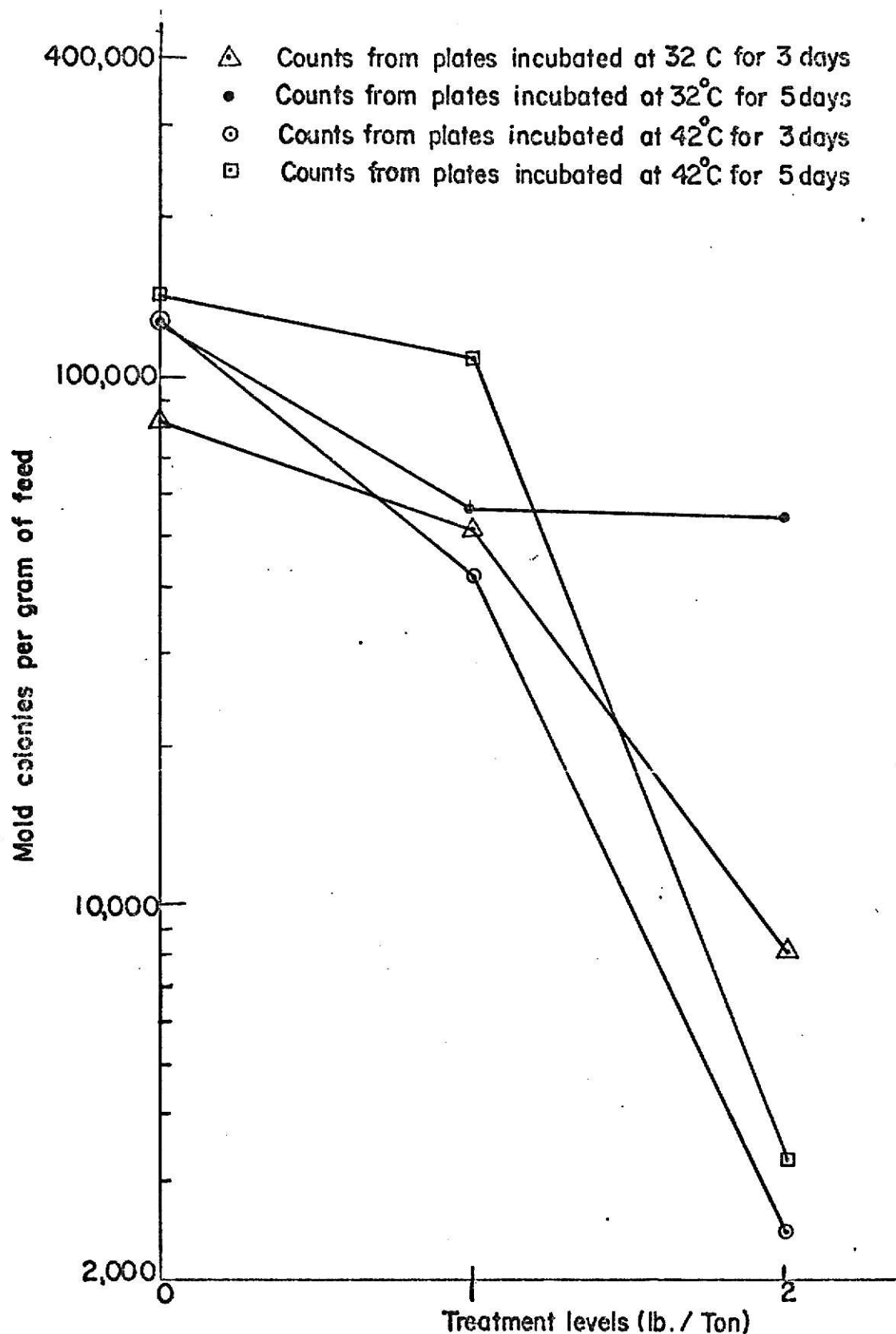
Fig. 7. Effect of chemical (mold curb) treatment and pelleting (conditioning temp. 70° C) on mold colonies in feed P-40 (second series of experiment).
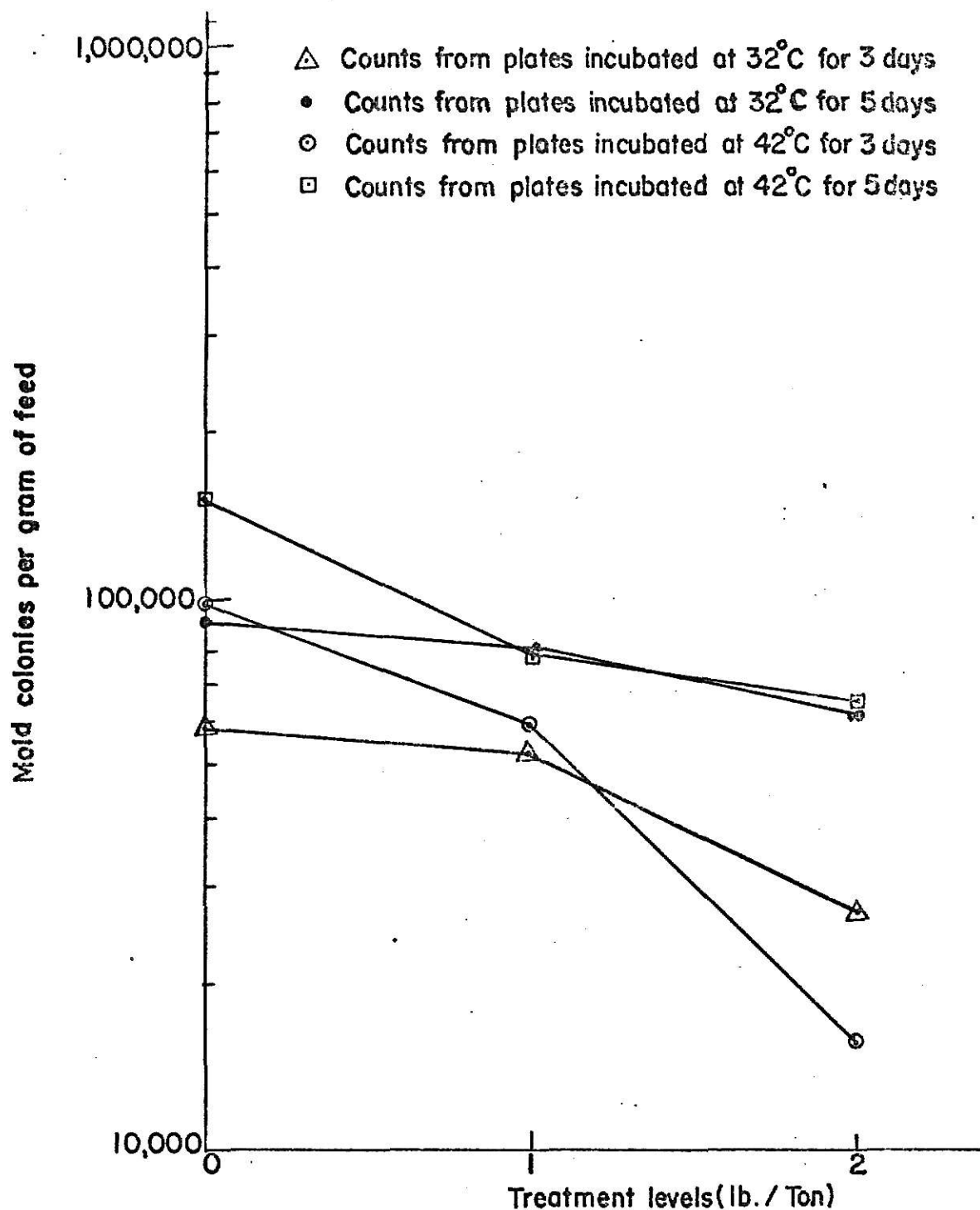
Fig.8. Effect of chemical (mold curb) treatment and pelleting (90°C conditioning temp.) on mold colonies in feed formula P-40 (second series of experiment).

SUMMARY

Salmonella organisms were isolated from all samples of the artificially contaminated meat scrap. Samples from soybean meal and ground grain which followed meat scrap through the feed mill conveying unit were also found to contain Salmonella organisms. Viable Salmonella organisms were found to be present in the unconditioned mixed feed made out of the above ingredients. Pelleting of the feed at 50 and 80°C completely eliminated Salmonella. Some samples of pelleted feed from preliminary studies showed the presence of Salmonella but as compared to the unconditioned feed samples there was a 99.99% reduction in the most possible number of the Salmonella organisms in samples pelleted at 70 to 80°C.

Data from three days incubation indicated a significantly lower number of mold colonies in samples treated with Mold Curb[®] at levels of one and two pounds per ton. Conditioning temperatures used in processing the feed resulted in significantly lower numbers of mold colonies in feed. This effect occurred in the samples incubated for five days. A profuse mold growth was found in the plates incubated at 32°C.

[®]Trade mark registered.

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EFFECTS OF TEMPERATURE PELLETING AND SOME CHEMICALS
ON THE BIOLOGICAL CONTAMINATION OF FEEDS

by

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MASTER OF SCIENCE

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The object of this study was to investigate the effects of different conditioning temperatures, pelleting, and some chemicals on Salmonella and mold contamination of feeds.

A chick starter formulation was artifically contaminated with a culture of Salmonella senftenberg 775 W organisms. The inoculated feed was conditioned at different temperatures and pelleted. Samples of different feed ingredients, of mixed feed before and after conditioning and pellets were analyzed for Salmonella. Procedure recommended by the United States Department of Agriculture for isolation of Salmonellae in animal products was followed. Salmonella organisms were isolated from meat scrap, other ingredients, and mixed feed. No viable Salmonellae were recovered from the samples which were pelleted at 50^0, 70^0, 80^0, and 90^0C temperatures.

Samples of the two series of experiments analyzed for mold content were from three batches of one ton each of a layer formulation processed at the pilot feed mill. Three batches of feed were treated with zero, one, and two pounds of Mold Curb,® a product containing propionic acid as an active ingredient and other inert ingredients, per ton of feed. All batches were individually subjected to pelleting at three different temperatures. Samples of the feeds were analyzed by standard plating procedures of the American Public Health Association.

Levels of Mold Curb® were found to have a significant effect on the mold growth. This effect occurred in sample plates incubated at 32^0 and 42^0C temperatures for three days, whereas their effects were nonsignificant for sample plates incubated for five days. This indicates that Mold Curb was fungistatic, not fungicidal in its effect. Conditioning of the feed at three different temperatures showed a significant effect on the mold growth. Effect of the incubation temperatures on the mold growth was significant.

An additional study was conducted in which one batch of feed was kept as

control and three batches were mixed individually with Mold Curb,® sodium propionate and calcium propionate at a level of two pounds per ton of feed. Samples of the four batches were analyzed for mold content. Effect of the four treatments on mold growth was found to be statistically nonsignificant although all treated samples had lower incidence of molds than the control.