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THE EFFECTS OF FEEDING MOLDED GRAIN AND VITAMINS TO *COTURNIX* QUAIL

by

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INTRODUCTION

The presence of mold growth on grain and the production of toxins by these molds has been suggested by Frazier (1) as the major problem confronting grain handlers. Due to the susceptibility of farm animals to these toxins, this problem of the grain handlers becomes a direct area of concern for the person feeding livestock. It was the purpose of this study to determine the severity of feeding molded grain, and to see if the addition of vitamins could have a compensating effect on livestock receiving such grain.

The evaluations made were accomplished by feeding Coturnix quail from hatching to maturity. The diets used contained corn purchased from a commercial source that was sterilized and then innoculated with a strain of Aspergillus flavus or Penicillium sp. In each of the diet classifications, vitamins were added at three levels. The effects of the molds, of the vitamins, and interactions of the effects of molds and vitamins were measured in terms of weekly weight gain, total weight gain, feed conversion, death losses, liver and bile duct tissue changes and egg production.

LITERATURE REVIEW

Mold growth on grain has always been a problem of the farmer and grain handler. The predominant storage molds are strains of Aspergilli and Penicilli (2). Since the identification of aflatoxin, much work has been completed with A. flavus. Not as well publicized, but also toxic, are the penicillium toxins. P. rubrum has been linked to "hepatitis X" in the dog and moldy corn toxins in swine and cattle (3).

The possibility of aflatoxin being produced by the growth of A. flavus mold is always present, and the possibility of A. flavus growth is quite probable. A. flavus is a constituent of the microflora of living or dead plants and animals as well as air and soil throughout the world. These molds grow when the substrate is suitable and the temperature is between 6° and 46°C with the optimum being 36° to 38°C (4). A temperature of 30° to 35°C was optimum for toxin production when the moisture was above 18% (5). Even if toxins are not produced the growth of mold reduces the value of grain. Molds use the most available nutrients and may reduce the feed value by 10% or more (2).

Commercially, aflatoxin is usually not present in large quantities in grain. Aflatoxin seldom causes acute toxic symptoms, but is in all probability, present at the chronic level. Since aflatoxin damage is permanent a short period of aflatoxin consumption will produce effects which will be present for the rest of the animal's life (2). First symptoms exhibited by all species suffering from aflatoxicosis were reported to be loss of appetite and reduced growth rate. These were the only physical signs until 1 to 4 days prior to death (6). Ducklings receiving aflatoxin gained significantly less than control birds (7). Chicks fed aflatoxin gained 30% less during an

8-week feeding trial than control birds (8). Feeding 10 ppm of aflatoxin to broilers reduced weight gain by more than 50% (9). In laying hens a significant decrease in egg production and loss in weight occurred due to aflatoxin (10). Pigs fed aflatoxin at the level of 465 ppb or greater had significantly lower weight gain and feed efficiency than the control group. Similarly, steers fed greater than 700 ppb of aflatoxin had lower weight gain and poorer feed conversion than the control groups (11). Other mycotoxins may produce outward characteristics other than those just described. A commercially processed feed contained sufficient amounts of an estrogenic toxin to produce greatly enlarged uteri in rats, and it also produced the "estrogenic syndrome" when fed to an experimental herd of swine (12).

Once aflatoxin is consumed much of the toxin passes out of the body. Seventy to 80% of a labeled aflatoxin when fed to rats was excreted through urine, feces, and CO_2 within the first 24 hours after feeding (13). When aflatoxin is consumed it affects the internal organs in a definite manner. The organ most consistently affected in all species is the liver. Other organs can show changes such as the kidneys and the adrenals (14). When ducklings were fed aflatoxin, the liver became a pale yellow color with subcutaneous hemorrhages and some bile duct proliferation (14) (8) (15). Rats are also quite susceptible to aflatoxin. Several workers have found that administration of 0.015 ppm in the diet or 0.4 mg or more total dose of aflatoxin produced liver degeneration with eventual liver cancer (16) (17) (18) (19) (20).

When aflatoxin G_1 was used rather than aflatoxin B_1, as in previous reports, a normal liver pattern was found except for an occasional small hyperplastic nodule (21). Two reports disagreed on the effects of aflatoxin on the weight of internal organs. Armbrecht and co-workers (22) found the liver-to-body ratio was significantly greater for birds receiving aflatoxin than for the controls. Carnaghan and co-workers (8), however, found no differences in organ-to-body weight ratios for heart, liver, spleen, adrenals, kidneys and gonads in chickens.

The discussion thus far has shown the effects of toxin upon laboratory and farm animals, but has not discussed the possibility of aflatoxicosis in man. The effect of a single dose of aflatoxin has yet to be proven, but it was found that monkeys, another primate, suffered from aflatoxicosis with liver and kidney degeneration (23). The actual mode of action of aflatoxin appears to be that aflatoxin interacts with DNA and thus inhibits messenger RNA with an overall change in the DNA-to-RNA ratio (24) (25) (26) (8).

Attempts to counteract the effects of aflatoxin have not been successful. Aflatoxicosis was more severe when a low casien diet was fed rather than one containing a high level of protein (27). Genetics appear to be a method of partially alleviating the problem of aflatoxicosis. When a susceptible chicken and a resistant chicken were crossed, the progeny produced were as resistant as the most resistant parent (11). This points to the possibility that susceptibility to aflatoxin poisoning may be a recessive trait. It also indicates physiological differences in ability to detoxify or eliminate the aflatoxin. Sheep are naturally resistant to aflatoxin, being the only animal thus studied with resistance (14).

The problem of aflatoxin poisoning will be present for a long time to come. As long as warm and moist weather conditions, faulty or inadequate storage facilities, and human error or ignorance combine to produce favorable circumstances for fungal growth, aflatoxin will continue to be found in food and feedstuffs (4).

Vitamins

The possibility of vitamins having a compensating effect for animals receiving aflatoxin was studied using inositol, Vitamin E, choline and Vitamin B_{12} supplementation to 30 week old laying hens. These additions had no effect upon fatty livers (28). This area was investigated since the deficiency symptoms reported for some vitamins are similar to the symptoms of aflatoxin poisoning. Effects of vitamin deficiencies on fowl are described in many publications. The deficiency symptoms of each vitamin are described briefly in "Nutrient Requirements of Poultry" (29).

Generally, a deficiency of a vitamin causes a decrease in weight gain and a decrease in feed consumption. A vitamin A deficiency produces an increase in mortality and a change in the kidney such that it becomes creamy white. Another vitamin deficiency symptom similar to that of aflatoxicosis is that seen in a pantothenic acid deficiency. Liver damage occurs when this vitamin is lacking in the diet. When vitamin B_{12} is lacking, a fatty liver and fatty kidneys will be produced (29).

Similarities between other vitamin deficiencies and aflatoxicosis exist and suggest the need to investigate the possibility of reducing the severity of aflatoxins by maintaining higher physiological levels of vitamins.

MATERIALS AND METHODS

Trial I

Corn to be used in this trial was autoclaved at 20 pounds per square inch gauge (1.5 Kg/cm^2) for a period of 20 minutes to destroy all fungi. During this process water was added giving a final moisture content of about 20%. The grain was taken from the autoclave and immediately placed in a plastic bag at a depth of 4 inches and allowed to cool. After cooling, one-half of the grain was innoculated with a strain of *Aspergillus flavus* while the other half was treated with a strain of *Penicillium sp*. Both molds were isolated from grain samples.

Beakers of water were placed at various locations in the bags of grain to keep a relative humidity of near 100%. The bags were then sealed and placed in a room with a temperature of 74 to 80°F. The bags were opened at intervals to allow oxygen to enter and enhance mold growth. After two weeks the grain was ground and dried prior to blending into the diets.

Coturnix quail were chosen as the laboratory animal to be used in the feeding trial due to their availability, economy and early maturity. The duration of the trial was from hatching to 4 weeks.

A diet was formulated (Tables 1 and 2) to supply growing quail with a level of nutrients equal to or greater than that reported in the literature to be required. In many cases requirements have not yet been established for the *Coturnix* quail. In these cases estimates were made drawing from information already determined for other types of fowl.

The diets to be tested consisted of a control, a diet containing *Aspergillus flavus* and a diet containing *Penicillium sp*. The control contained 10% autoclaved corn while the mold groups contained 10% of the respective

TABLE I. DIET CONSTITUENTS

Ingredients	% of Diet
Soybean meal	39.85
Corn	38.73
Wheat midds	6.37
Dehydrated alfalfa meal	2.45
Fish meal	1.96
Meat scraps	4.90
Distillers solubles	1.96
Dried whey	1.96
Dicalcium phosphate	1.23
Salt	0.49
Trace mineral premix[a]	0.05
Vitamin premix[b]	0.05
Total	100.00

[a]Manganese 10%
Iron 10%
Calcium 14% maximum
Calcium 12% minimum
Copper 1%
Zinc 5%
Iodine .3%
Cobalt .1%

[b]Vitamin D 480 ICU/kg
Niacin 30 mg/kg

TABLE II. CALCULATED NUTRIENT CONTENT OF THE DIET

Protein	28.68	%
Lysine	1.63	%
Cystine and methionine	0.769	%
Fiber	4.2	%
Metabolizable energy	2684.0	kcal/kg
Calcium	1.12	%
Phosphorus	0.986	%
Carotene	5.5	mg/kg
Vitamin D	480.0	ICU/kg
Vitamin E	16.8	IU/kg
Vitamin K	0.2	mg/kg
Biotin	0.23	mg/kg
Choline	1710.0	mg/kg
Folacin	0.52	mg/kg
Niacin	60.12	mg/kg
Pantothenic acid	11.05	mg/kg
Pyridoxine	7.21	mg/kg
Riboflavin	3.22	mg/kg
Thiamine	5.65	mg/kg
Vitamin B_{12}	3.55	mg/kg

molded corn. The A. flavus group contained 100 ppb of aflatoxin. Within each group three levels of vitamins were fed. These levels (Table 3) consisted of the vitamins as they occurred in the formulated diet, level 1; the addition of the calculated requirement of vitamins, level 2; and twice the calculated level of vitamins, level 3. Using C, A, and P to represent control, Aspergillus flavus and Penicillium sp. respectively and the vitamin levels previously described, the following diets were formulated: C-1, C-2, C-3, A-1, A-2, A-3, P-1, P-2, and P-3. These diets were fed to triplicate groups with 14 birds per group. The quail were weighed weekly as was the unconsumed feed. All birds dying during this study were submitted to the veterinary necropsy laboratory for examination. Upon completion of the study three live birds were removed from each pen and submitted to veterinary necropsy for observation of liver and bile duct tissue changes. The hearts and livers from six additional birds per pen were removed and weighed.

Trial II

Quail, from the control pens of Trial I, were divided into two groups and fed an all grain diet using the molded grain from Trial I. One group received the Aspergillus flavus molded grain while the other group received grain molded with Penicillium sp. The quail were sustained on their respective diets for ten days. On the tenth day six birds from each diet group were taken for examination.

Trial III

The grain for this trial was prepared in a manner similar to Trial I. The grain was autoclaved and treated with an isolated strain of Aspergillus flavus or Penicillium sp. after which it was placed in a plastic bag and

TABLE III. VITAMIN LEVELS
PER KILOGRAM FOR TRIAL I

Vitamin	Unit	Level 2 Additions	Level 3 Additions
A	IU	1600.0	3200.0
D	ICU	480.0	960.0
E	IU	10.0	20.0
K	mg	0.7	1.4
B_{12}	mg	0.003	0.006
Niacin	mg	60.0	120.0
Pantothenic acid	mg	30.0	60.0
Riboflavin	mg	3.6	7.2
Choline	mg	1900.0	3800.0
Thiamine	mg	2.0	4.0
Pyridoxine	mg	4.0	8.0
Folacin	mg	0.9	1.8
Biotin	mg	0.3	0.6

sealed. During the incubation period the bag containing the *Penicillium sp.* was accidentally opened allowing other types of mold spores to enter the bag. By the end of the incubation period a number of mold strains were visually present. The grain was similar to grain molded in a farm or commercial structure. Due to the presence of the various molds, the treatment was, thereafter, referred to as a mixed mold diet. Both bags of grain were ground after three weeks of incubation and dried to be incorporated into diets.

The basal diet was identical to that of Trial I (Tables 1 and 2). The vitamins present in the basal diet were again considered as level 1; the addition of the calculated vitamin requirements as level 2; and three times the addition of calculated requirements of vitamins as level 3 (Table 4). The autoclaved grain for the control, or molded grain for the remaining diets, was added as 20% of the total diet. The *A. flavus* group received 200 ppb of aflatoxin. The control, *Aspergillus flavus* and the mixed mold represented by C, A, and M, respectively plus the three vitamin levels previously discussed produced the following diets: C-1, C-2, C-3, A-1, A-2, A-3, M-1, M-2, and M-3.

Coturnix quail were again used in this trial with the duration from hatching through the thirteenth day of egg production, being approximately seven weeks. All diets were fed to triplicate groups of twelve birds per group. Birds were weighed weekly for the first four weeks of the trial. From the second through the fourth week feed was weighed at each feeding with unconsumed feed being weighed back weekly. All deaths were recorded but the dead birds were not taken for examination due to their high degree of deterioration. At the end of the fourth week three birds per pen were submitted for internal analysis to the veterinary necropsy laboratory. The remainder

TABLE IV. VITAMIN LEVELS PER
KILOGRAM FOR TRIAL III

Vitamin	Unit	Level 2 Additions	Level 3 Additions
A	IU	1600.0	4800.0
D	ICU	480.0	1440.0
E	IU	10.0	30.0
K	mg	0.7	2.1
B_{12}	mg	0.003	0.009
Niacin	mg	60.0	180.0
Pantothenic acid	mg	30.0	90.0
Riboflavin	mg	3.6	10.8
Choline	mg	1900.0	5700.0
Thiamine	mg	2.0	6.0
Pyridoxine	mg	4.0	12.0
Folacin	mg	0.9	2.7
Biotin	mg	0.3	0.9

of the birds were continued on their respective diets. The production of eggs was determined by sexing the birds and reporting the eggs produced as eggs per hen. These were recorded both as daily and as total egg production.

RESULTS AND DISCUSSION

Trial I

The death losses showed only slight differences. The control groups were similar with the major difference being the week during which the deaths occurred. All control groups had an average death loss of 0.33 birds per pen the first week, while the C-1 pens had an additional 0.33 deaths per pen during week 2 and the C-2 fed birds had an average death loss of 0.33 bird during week 3.

The groups receiving A. flavus molded grain had more total deaths than either of the other groups. As can be seen from Figure 1 these deaths occurred only in diets A-1 and A-3 with the largest death loss being in the A-3 group.

In the groups fed grain molded with Penicillium sp. the death loss was highest in the P-1 diet and decreased as vitamin levels increased. The P-1 diet had deaths in weeks 1, 2, and 4 while birds in the P-2 and P-3 groups died only in weeks 1 and 2.

Feed consumption, measured as feed per chick day, varied greatly from week to week with the greatest consumption being during week 3. As can be seen from Table 5 and Figure 2 little variation in feed consumption occurred in any groups other than those containing the penicillium mold. The birds receiving the P-1 diet consumed less feed each week than either the P-2 or P-3 diets. During the 4 weeks the P-1 groups consumed from 4 to 11% less feed per chick day than groups P-2 or P-3. The table shows a cumulative average feed per chick day that was 7% less than that of P-2 or P-3.

Feed conversion for this trial shows little consistent variation between vitamin levels or mold groupings. The conversion for groups A-1 and P-1 was

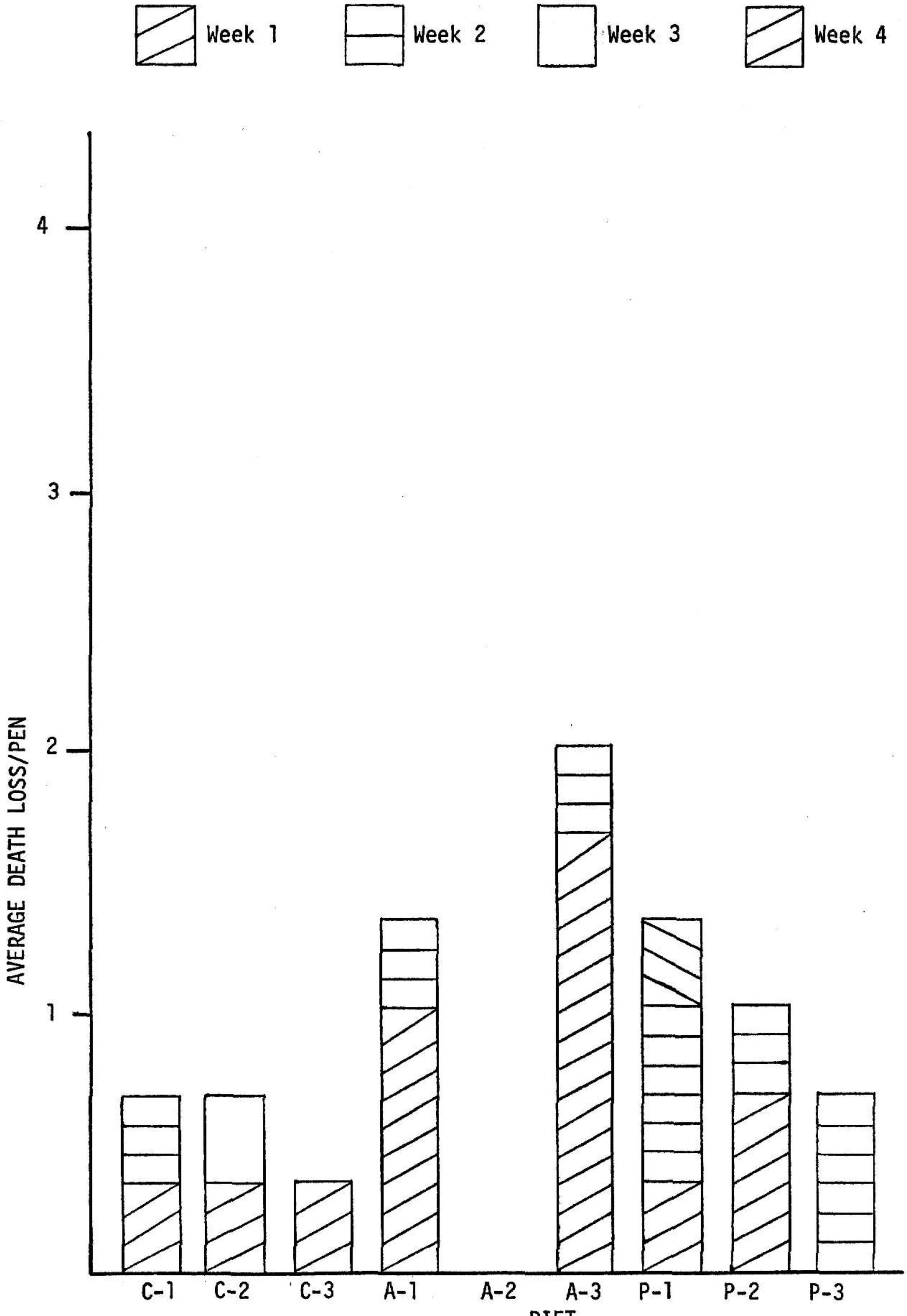

Fig. 1 TRIAL I AVERAGE DEATH LOSS/PEN

TABLE V. AVERAGE FEED PER CHICK DAY
IN GRAMS DURING TRIAL I

Treatment	Week 1	Week 2	Week 3	Week 4	Cumulative Average
C-1[a]	5.36	9.09	11.73	10.31	9.12
C-2[b]	5.63	9.09	11.30	10.36	9.10
C-3[c]	5.53	8.48	11.18	10.01	8.80
A-1[d]	5.76	9.32	11.69	10.25	9.26
A-2[e]	5.62	9.08	11.86	10.57	9.28
A-3[f]	6.06	9.37	11.52	10.40	9.34
P-1[g]	5.75	8.37	11.23	10.01	8.84
P-2[h]	5.86	9.02	12.38	10.66	9.48
P-3[i]	5.98	9.41	11.84	10.55	9.45

[a]Control-Vitamins present in diet

[b]Control-Vitamins present in diet plus addition of approximate vitamins required

[c]Control-Vitamins present in diet plus addition of twice the vitamins required

[d]Aspergillus molded grain-Vitamins present in diet

[e]Aspergillus molded grain-Vitamins present in diet plus addition of approximate vitamins required

[f]Aspergillus molded grain-Vitamins present in diet plus addition of twice the vitamins required

[g]Penicillium molded grain-Vitamins present in diet

[h]Penicillium molded grain-Vitamins present in diet plus addition of approximate vitamins required

[i]Penicillium molded grain-Vitamins present in diet plus addition of twice the vitamins required

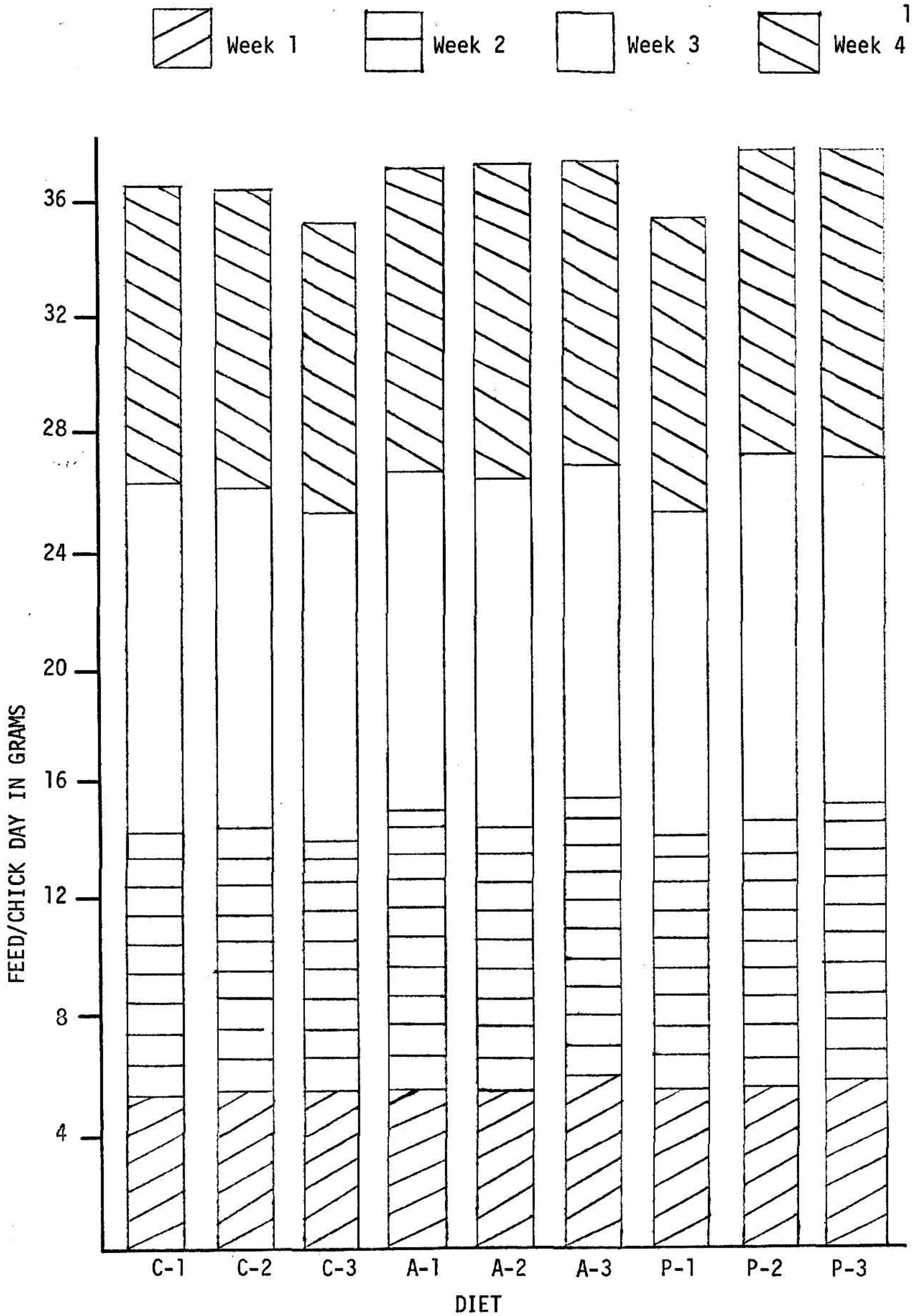

Fig. 2 TRIAL I AVERAGE FEED PER CHICK DAY

poorer than the higher vitamin level groups during weeks 1 and 3, but was essentially the same during weeks 2 and 4. The differences for week 2 were significant ($P < .05$) for mold-vitamin interaction and significant ($P < .10$) for the effects of mold. As can be seen from Table 6 feed conversion of the P-1 group for the 4 week cumulative average was poorer than for the P-2 or P-3 groups.

The variable showing the largest and most consistent difference was weight gain. Measured as both weekly weight gain and total weight gain, it is apparent (Tables 7 and 8) (Figure 3) that birds fed grain molded with *Penicillium sp.* responded to the addition of vitamins to the diet.

During the first week of the trial the birds fed the control diets gained approximately the same with the C-3 group doing somewhat poorer. Groups receiving grain innoculated with *A. flavus* seemed to be responding to the addition of vitamins with the A-3 birds gaining 16% more during the first week than the A-1 group. Birds receiving penicillium molded grain responded to the doubling of the vitamins but were only slightly affected by the addition of higher levels of vitamins. The P-2 group gained 15% more than the P-1 group while the P-3 treatment was 16% better than P-1.

Week 2 produced significant differences ($P < .10$) for weekly weight gain and cumulative weight gain. The control groups showed only small differences with the C-3 group gaining most. The cumulative weight gains for the control diets were nearly equal at this time. The apparent trend started during week 1 for the group receiving aflatoxins did not continue. Only a slight difference existed in average cumulative weight gain with A-2 and A-3 having 5 and 2% advantages respectively over the A-1 group. Groups receiving *Penicillium sp.* molded grain showed the greatest variation during the second

TABLE VI. AVERAGE FEED CONVERSION (GRAMS OF FEED PER GRAM OF GAIN) DURING TRIAL I

Treatment[a]	Week 1	Week 2	Week 3	Week 4	4-Week Cumulative Average
C-1	2.21	2.74	3.92	3.49	3.09
C-2	2.24	2.83	3.97	3.54	3.15
C-3	2.41	2.41	3.43	3.42	2.92
A-1	2.65	2.75	4.12	3.45	3.24
A-2	2.32	2.71	3.60	3.49	3.03
A-3	2.34	3.00	3.77	3.52	3.18
P-1	2.68	2.59	4.09	3.52	3.22
P-2	2.33	2.69	3.79	3.60	3.10
P-3	2.34	2.74	3.87	3.48	3.11

[a]See footnotes table V

TABLE VII. AVERAGE WEEKLY WEIGHT GAINS IN GRAMS DURING TRIAL I

Treatment[a]	Week 1	Week 2	Week 3	Week 4
C-1	16.97	23.23	21.01	21.82
C-2	17.56	22.45	20.15	22.14
C-3	16.18	24.70	22.80	18.33
A-1	15.36	23.84	20.04	23.96
A-2	17.19	23.94	23.10	20.69
A-3	18.21	21.92	21.41	21.27
P-1	15.07	22.77	19.66	22.17
P-2	17.66	23.47	22.80	19.02
P-3	17.95	24.07	21.48	21.57

[a]See footnotes table V

TABLE VIII. AVERAGE CUMULATIVE WEIGHT GAINS IN GRAMS DURING TRIAL I

Treatment[a]	Week 1	Week 2	Week 3	Week 4
C-1	16.97	39.87	60.88	82.69
C-2	17.56	40.02	60.17	82.31
C-3	16.18	40.87	63.68	82.00
A-1	15.36	39.20	59.24	83.19
A-2	17.19	41.15	64.25	84.93
A-3	18.21	40.15	61.55	82.92
P-1	15.07	37.85	57.50	79.51
P-2	17.66	41.13	63.93	82.94
P-3	17.95	42.02	63.50	85.07

[a]See footnotes table V

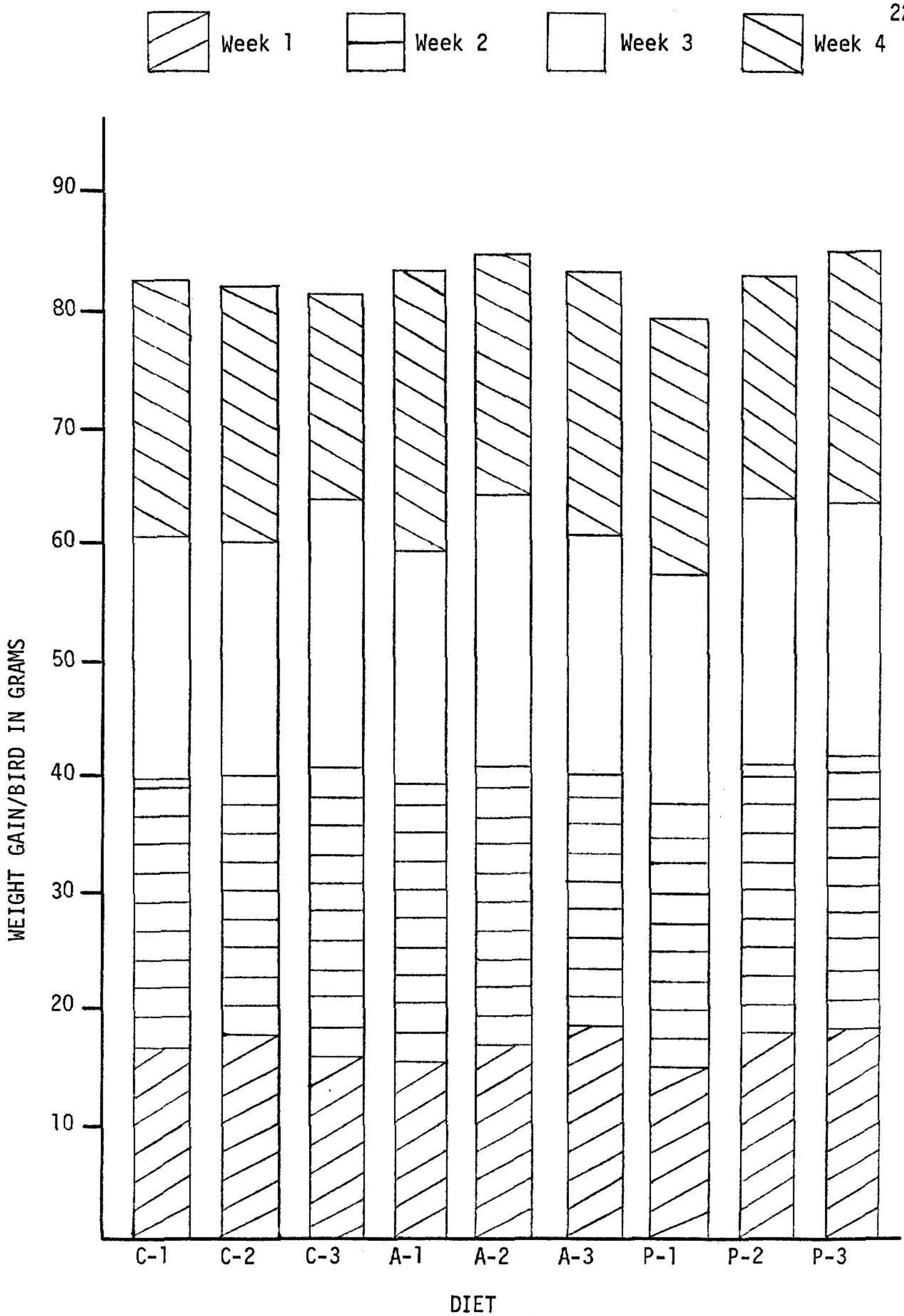

Fig. 3 TRIAL I WEEKLY AND CUMULATIVE WEIGHT GAIN/BIRD

week with a continuation of the trend started in week 1. This response to vitamin supplementation can be seen in Figure 3. The percentage increases for this week were lower than in week 1 with P-1 gaining 3 and 5% less than P-2 and P-3 respectively. The cumulative average weight gain represented an 8 and 10% growth increase by P-2 and P-3 respectively when compared to the P-1 groups. Growth due to the highest level of vitamins continued to be only slightly greater than the medium vitamin level.

Gains during the third week were more inconsistant in all treatments when compared to the previous 2 weeks. The lower weight gains for the lowest vitamin level groups continued and were significantly different ($P < .05$) for total weight gain. The control diets continued to be approximately equal with the C-3 group gaining the greatest amount. The aflatoxin fed birds varied in gains from the previous week with a large increase in weekly weight gain by the A-2 group. This trend, however, did not continue at the second addition level of vitamins. Lower weight gains were recorded for the A-3 group than the A-2 group. Continued response to vitamins was found for the penicillium fed groups with a 14% higher weight gain for the P-2 than P-1. The P-3 group did not have additional weight responses during this period and were only 8% better than the P-1 group for weekly weight gain. P-2 and P-3 were approximately equal in total weight gain with a 10 and 9% increase respectively over the P-1 group.

The fourth week resulted in weight gains from 18.33 to 23.96 grams with the lower value corresponding to groups that in the previous 3 weeks were gaining the most and highest gains were by groups which had previously gained a lesser amount. In the control groups, the C-3 fed birds gained much less during week 4 than either of the other control diets thus producing a 4 week cumulative weight gain with a variation of only 0.69 grams for the 3 control diets. The A-1 birds gained more weight than A-2 or A-3. A-2 had lower gains when compared to the average of the previous weeks. From the average cumulative weight gain table, it is apparent the A-2 group gained more than A-1 or A-3 while the A-1 and A-3 groups were essentially equal with only 0.27 grams difference. Fourth week gains for P-1, P-2 and P-3 groups indicated slower gains for the previous fastest growing group. P-1 had the greatest gain during week 4. From Figure 3 and the cumulative weight gain table, it is apparent that vitamin additions in the penicillium groups produced a response with a large increase due to the initial addition of vitamins and a smaller additional increase from the highest vitamin level.

Results of weekly weight gain for the four week period seemed to contradict results established during the first 3 weeks. This, however, should not be surprising. The strain of Coturnix quail used in this trial had a mature weight of 95 to 115 grams which they normally achieve in 4 to 5 weeks with egg production in 5-1/2 to 6 weeks. With the early maturity and low mature weight the rapid growing birds were, at the end of the third week, at a point such that rate of gain was decreasing. The slower growing birds were at this time still lower on the growth curve and thus continued growing at a faster rate than the previously faster growing birds.

Tissue changes due to the mold toxins were measured as to degree of change found on both gross and microscopic examination (Tables 9 and 10). The groups receiving the A. flavus molded grain were the only birds showing a response to the vitamin additions. The gross liver, microscopic liver and microscopic bile duct examinations, indicated changes were lessened as vitamin levels increased. No differences were found in organ-to-body weight ratios for the heart and liver (data not shown).

TABLE IX. GROSS LIVER ANALYSIS FOR TRIAL I

Treatment[a]	N[b]	C[c]	1[d]	2[e]	3[f]
C-1	1	5	2	1	0
C-2	3	3	2	1	0
C-3	3	6	0	0	0
A-1	0	6	2	2	2
A-2	1	6	2	0	0
A-3	1	3	3	2	0
P-1	4	3	1	1	0
P-2	7	1	1	0	0
P-3	4	3	1	1	0

[a]See footnotes table V

[b]Normal

[c]Congested

[d]Slightly yellow

[e]Yellow

[f]Very yellow

TABLE X. MICROSCOPIC ANALYSIS FOR TRIAL I

Treatment[a]	Liver			Bile Duct		
	N[b]	F-1[c]	F-2[d]	B-1[e]	B-2[f]	B-3[g]
C-1	4	3	0	2	0	0
C-2	2	6	0	1	0	0
C-3	5	3	0	1	0	0
A-1	0	4	5	1	4	1
A-2	1	4	3	3	1	1
A-3	0	6	3	2	2	0
P-1	2	6	1	3	0	0
P-2	6	1	2	2	0	0
P-3	1	3	3	6	0	0

[a]See footnotes table V

[b]Normal

[c]Fat globules scattered throughout parenchyma

[d]Most cells contain fat globules

[e]Slight increase in number of ducts in periportal areas

[f]Hyperplastic cells seen away from periportal areas

[g]Advanced hyperplasia

Trial II

Liver and bile duct changes, the only variable studied in Trial II, were greatly affected by molded grain (Table 11). Gross examination indicated some liver changes for the P-4 diet and no apparent liver changes for the A-4 diet. The P-4 fed birds contained microscopic liver changes in all instances with half of the birds examined showing a high number of liver cells containing fat. These birds had no apparent bile duct deterioration. All A-4 birds had microscopic liver changes and bile duct changes with the degree of change being from a slight increase of bile ducts in the periportal areas to advanced hyperplaia. From this study it appeared that each of these toxins were specific for the internal changes they produced.

Trial III

Death loss during experiment three was an important indicator of the effects of adding vitamins to diets containing mold toxins (Table 12) (Figure 4). Losses during the first week were not large in any group. The highest average loss per pen was 0.66 bird. The second week resulted in high death losses in many groups with the highest loss being in the A-1 group. Birds from all diets except C-3 had at least 0.33 deaths per pen with the A-1 group having 1.67 deaths per pen. Week 3 produced significant differences ($P < .005$) in death losses. Only A-1 and A-2 had death losses during this period. The diets containing A. flavus had significantly more ($P < .005$) death losses than control or mixed mold groups. The lowest level (A-1) of vitamins had significantly greater ($P < .005$) losses than vitamin levels 2 or 3. Week 4 also produced significant differences ($P < .005$) with the only deaths being in the A-1 group. The A-1 group had an average death loss of 4.33 birds per pen with continued deaths into weeks 3 and 4. During

TABLE XI. LIVER AND BILE DUCT
ANALYSIS FOR TRIAL II

Treat-ment[a]	N[b]	1[c]	2[d]	3[e]	F-1[f]	F-2[g]	F-3[h]	B-1[i]	B-2[j]	B-3[k]
P-4	3	0	1	2	2	1	3	0	0	0
A-4	6	0	0	0	4	1	0	3	2	1

[a]P-4--Total diet of grain molded with Penicillium sp.
A-4--Total diet of grain molded with Aspergillus flavus

[b]Normal

[c]Slightly yellow

[d]Yellow

[e]Very yellow

[f]Fat globules scattered throughout parenchyma

[g]Most cells contain fat globules

[h]Very high concentration of fat globules

[i]Slight increase in number of ducts in periportal areas

[j]Hyperplastic cells seen away from periportal areas

[k]Advanced hyperplasia

TABLE XII. *DEATH LOSS DURING TRIAL III*

Treatment	Total Deaths
C-1[a]	5
C-2[b]	3
C-3[c]	0
A-1[d]	13
A-2[e]	5
A-3[f]	6
M-1[g]	6
M-2[h]	1
M-3[i]	1

[a]Control-Vitamins present in diet

[b]Control-Vitamins present in diet plus addition of approximate vitamins required

[c]Control-Vitamins present in diet plus addition of three times the vitamins required

[d]Aspergillus molded grain-Vitamins present in diet

[e]Aspergillus molded grain-Vitamins present in diet plus addition of approximate vitamins required

[f]Aspergillus molded grain-Vitamins present in diet plus addition of three times the vitamins required

[g]Mixed mold-Grain molded with various molds with vitamins present in diet

[h]Mixed mold-Grain molded with various molds with vitamins present in diet plus addition of approximate vitamins required

[i]Mixed mold-Grain molded with various molds with vitamins present in diet plus addition of three times the vitamins required

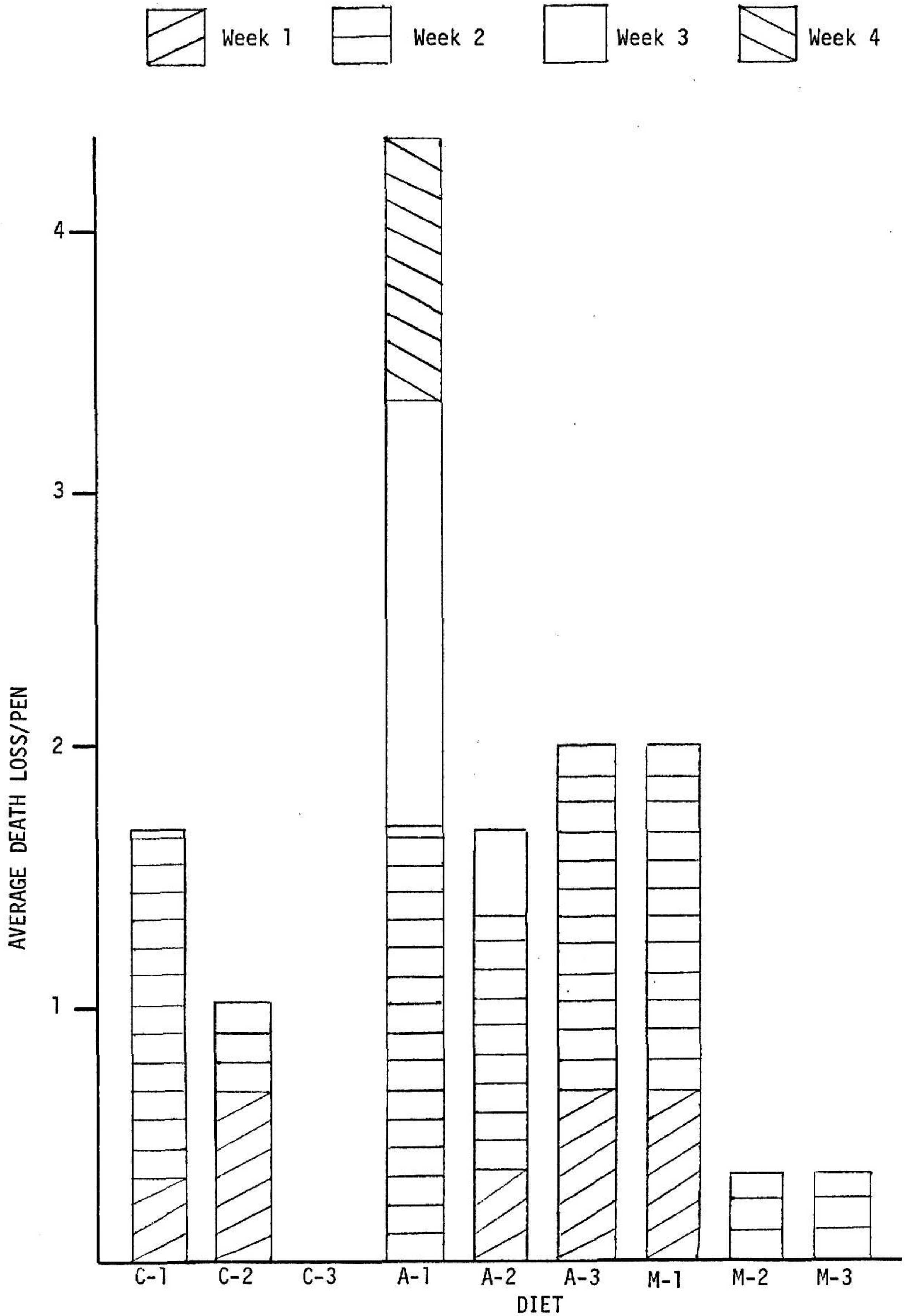

Fig. 4 TRIAL III AVERAGE DEATH LOSS/PEN

this study, the A-2 and A-3 groups had only 1.67 and 2.0 average death losses per pen respectively with the deaths occurring early in the study. The addition of vitamins helped to decrease death losses for quail receiving aflatoxin contaminated diets.

Feed consumption was not determined for the first week of the trial due to the inaccuracy which would have been associated with such a measure. Since the feed was placed on egg flats the first week to encourage eating, much of the feed was lost on the floor of the pen and the feed remaining at the end of the first week contained a large portion of fecal material.

During the second week groups on the lowest vitamin level consumed less feed than groups fed higher vitamin levels, except the M-1 group (Table 13) (Figure 5). No definite trends were formed.

Week 3 resulted in smaller differences in consumption between control and mixed mold groups receiving different vitamin levels. Treatments of vitamin levels at twice and 4 times the required had essentially the same feed consumption in the groups receiving the *A. flavus* molded grain while the A-1 group consumed 8% less.

Consumption during the final week of the study was inconsistent in all groups. The *A. flavus* treatments responded to vitamins with the A-2 and A-3 groups consuming 4 and 10% more respectively than A-1 fed birds.

Since feed consumption was not measured during week 1, feed conversion was not calculated. The remaining 3 weeks (Table 14) produced no significant differences in feed conversion and no meaningful trends. Values during the fourth week indicated a drastic decrease in feed efficiency. The M-2 group had a feed conversion of 11.36 grams of feed per gram of gain.

TABLE XIII. AVERAGE FEED PER CHICK DAY IN GRAMS DURING TRIAL III

Treatment[a]	Week 2	Week 3	Week 4
C-1	7.83	10.80	11.78
C-2	8.36	11.10	12.80
C-3	8.19	11.42	11.75
A-1	7.25	9.93	11.32
A-2	8.16	10.82	11.80
A-3	6.85	10.87	12.83
M-1	7.88	11.02	12.96
M-2	7.83	10.72	11.31
M-3	8.73	10.92	12.01

[a]See footnotes table XII

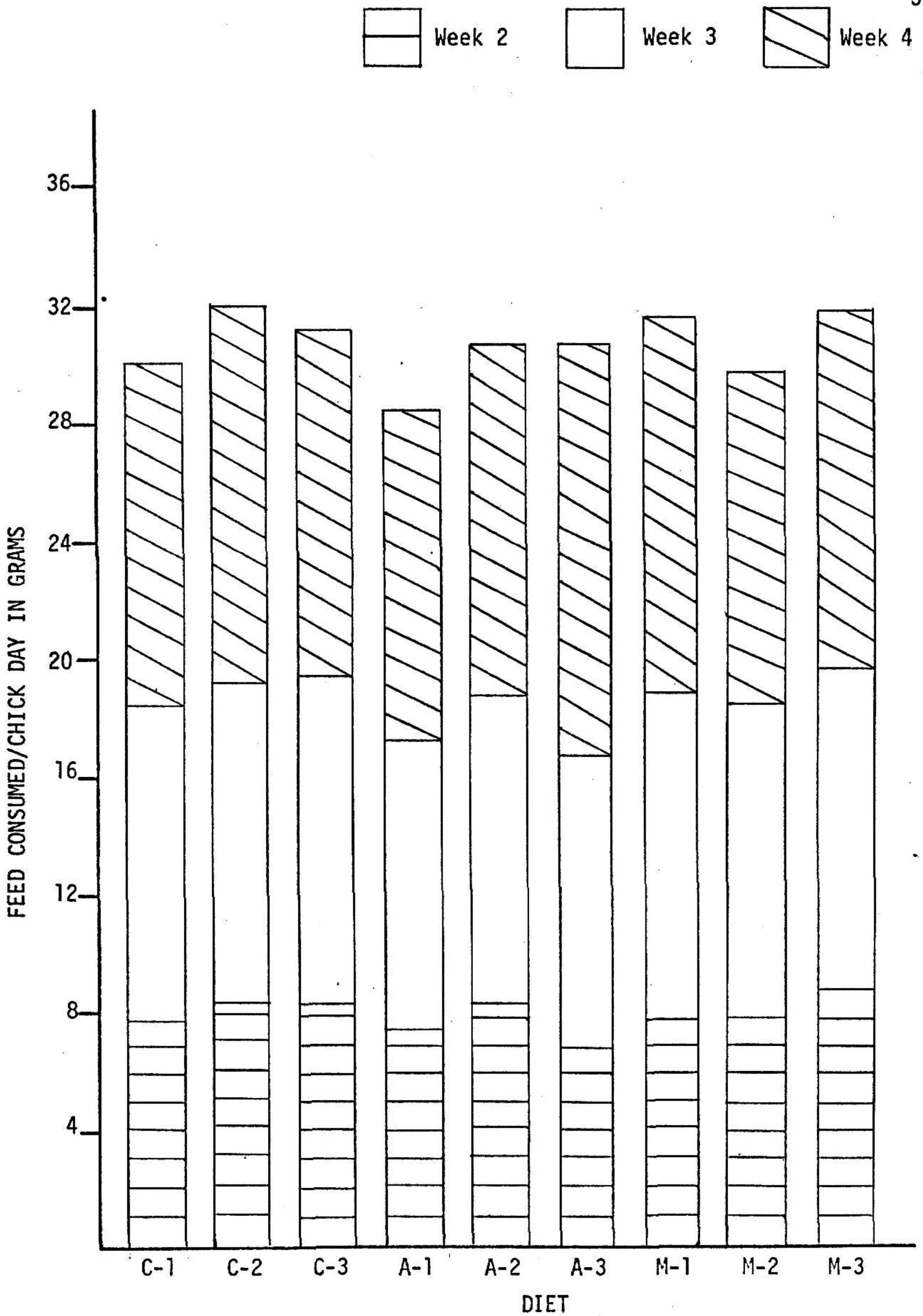

Fig. 5 TRIAL III AVERAGE FEED/CHICK DAY

TABLE XIV. AVERAGE WEEKLY FEED CONVERSION IN GRAMS DURING TRIAL III

Treatment[a]	Week 2	Week 3	Week 4
C-1	2.97	3.44	4.96
C-2	2.88	3.56	6.91
C-3	2.84	3.45	5.83
A-1	3.01	4.04	4.94
A-2	3.08	3.60	4.79
A-3	2.47	3.55	6.15
M-1	2.84	3.47	5.10
M-2	2.83	3.86	11.36
M-3	2.98	3.26	5.88

[a]See footnotes table XII

During the final week of the trial the M-2 group gained little and had poor feed conversion. This group, however, did not decrease in feed consumption. During the 5th week of this study a heavy death loss occurred in the pens of the M-2 birds. On examination of the dead birds, a yellow-orange gel was found in the body cavity similar to that reported in aflatoxicosis of swine (30). The birds that survived in these pens continued to have an unhealthy appearance with little egg production.

Weight gains, both weekly and total (Tables 15 and 16) (Figure 6) were significantly different during certain periods of this study. Week 1 resulted in significantly less ($P < .05$) weight gain by birds receiving the _A. flavus_ molded grain than the control birds and significantly less ($P < .05$) weight gain by birds receiving the lowest level of vitamins. A response to vitamins occurred when comparing the first to the second level of vitamins but little difference was found due to the third vitamin level.

Weight gains were approximately equal among all vitamin levels for the control and mixed mold groups during the second week. Birds receiving the aflatoxin responded to vitamin additions with increased weight gains of 9 and 14% for the A-2 and A-3 groups respectively when compared to the A-1 birds. For the total average weight gain the _A. flavus_ fed birds gained significantly less ($P < .05$) than other groups, and the lowest vitamin level gained significantly less ($P < .05$) than higher vitamin levels.

During the third week weight gains were similar to those of week 2. The control and mixed mold groups had little variation in weight gain. The birds receiving aflatoxin responded to vitamin additions with greatest increases in gains from the initial addition of vitamins. Slight additional response occurred from the highest addition of vitamins. Birds receiving the A-2 diet

TABLE XV. AVERAGE WEIGHT GAIN IN GRAMS DURING TRIAL III

Treatment[a]	Week 1	Week 2	Week 3	Week 4
C-1	12.39	18.63	21.99	16.71
C-2	15.06	20.28	21.77	14.08
C-3	15.14	20.17	23.25	14.17
A-1	11.34	16.92	17.37	16.52
A-2	14.11	18.58	21.08	17.70
A-3	12.47	19.64	21.57	15.86
M-1	12.74	19.92	22.20	17.71
M-2	15.08	19.46	20.02	10.91
M-3	14.56	20.53	23.84	14.84

[a]See footnotes table XII

TABLE XVI. AVERAGE CUMULATIVE WEIGHT GAIN IN GRAMS DURING TRIAL III

Treatment[a]	Week 1	Week 2	Week 3	Week 4
C-1	12.39	31.02	53.01	69.71
C-2	15.06	35.33	57.11	71.19
C-3	15.14	35.31	58.56	72.72
A-1	11.34	28.26	45.64	62.16
A-2	14.11	32.69	53.77	71.47
A-3	12.47	32.10	53.67	69.54
M-1	12.74	32.66	54.86	72.63
M-2	15.08	34.54	54.57	65.48
M-3	14.56	35.09	58.93	73.76

[a]See footnotes table XII

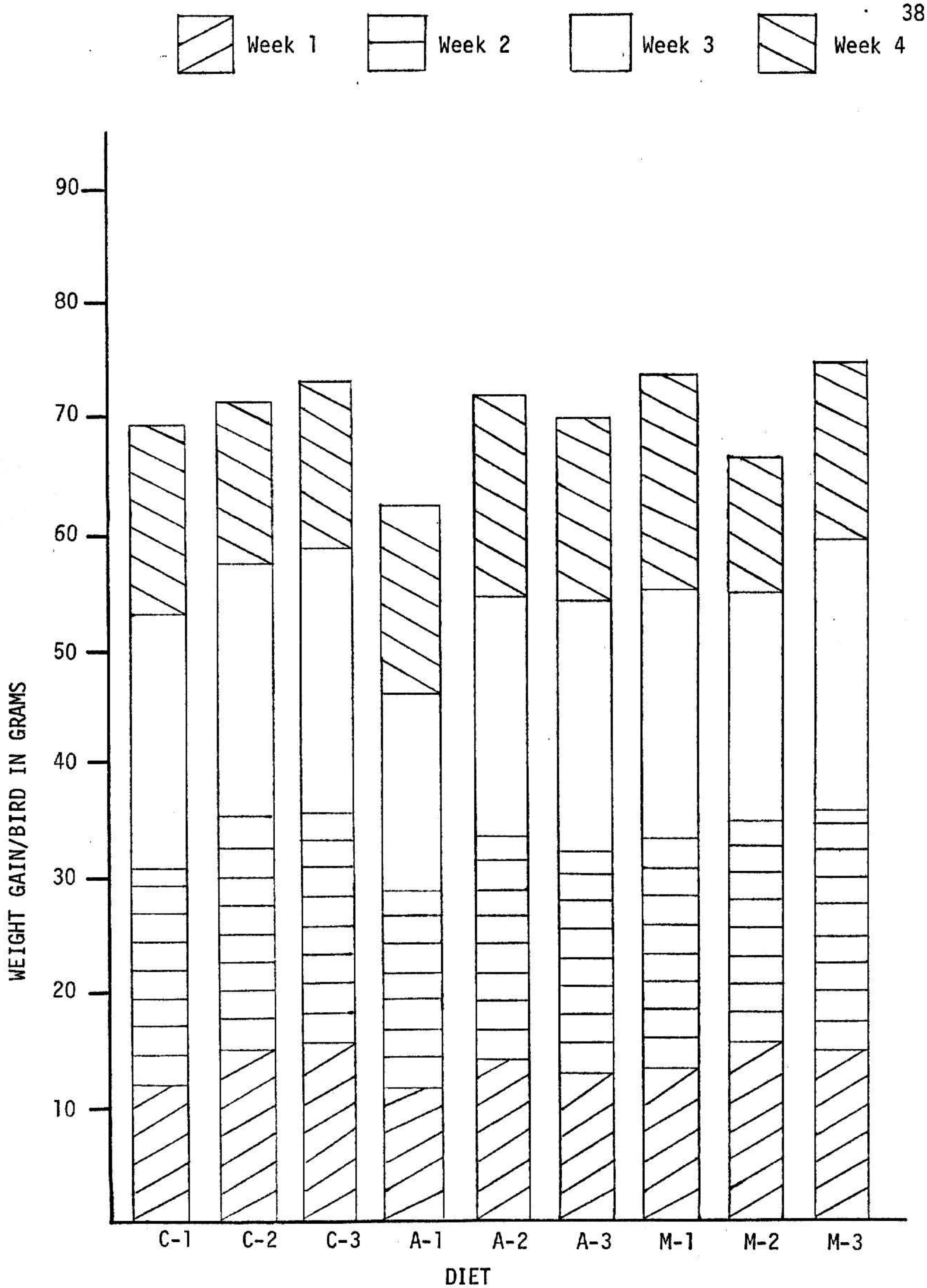

Fig. 6 TRIAL III WEEKLY WEIGHT GAIN AND TOTAL WEIGHT GAIN PER BIRD

gained 18% more than the A-1 while those receiving the A-3 diet gained 19% more than the A-1 fed birds. Total weight gain for the first three weeks indicated a response to vitamins by both the control and the A. flavus fed birds. Vitamin additions produced a 9% increase in weight gain by control groups and a 15% increase in weight gain by the A. flavus groups. The mixed mold treatment, M-3, gained 7% more than M-1 but the M-2 fed birds gained less than the M-1 birds.

Inconsistent weight gains occurred in all groups during the fourth week. Average weekly weight gains decreased in all groups. The birds gaining the most rapidly in the previous 3 weeks gained the least during this week. Total weight gains, accordingly, were similar and did not show effects of previous weeks. The M-2 group showed the greatest decrease in gain with a final average cumulative weight gain below that of the M-1 or M-3 groups.

The effects during the fourth week of this trial were similar to those of trial I and were apparently due to the factors discussed for the previous trial. The fastest growing birds were apparently closer to maturity and thus gained at a slower rate.

Birds examined for internal tissue changes (Table 17) had an increase in internal disorders when molded grain was fed. Bile duct deterioration in both numbers and severity decreased as vitamins increased in the mixed mold treatment. Internal changes were not observed in the other treatments in this trial.

Egg production data was measured to determine variation in sexual maturity due to mold toxins and vitamin additions. Methods of measurement were age at 25% production and total eggs per hen for the 13-day collection period. From Table 18 it can be seen that feeding aflatoxin at the lowest

TABLE XVII. LIVER AND BILE DUCT ANALYSIS FOR TRIAL III

Treatment[a]	F-1[b]	F-2[c]	F-3[d]	F-4[e]	B-1[f]	B-2[g]	B-3[h]
C-1	5	0	0	0	4	0	0
C-2	6	1	0	1	2	1	0
C-3	4	1	1	0	2	2	0
A-1	3	3	1	0	3	3	0
A-2	3	1	1	0	5	1	0
A-3	2	3	1	0	3	2	0
M-1	2	2	3	0	4	3	1
M-2	1	4	1	1	6	0	0
M-3	5	1	2	0	2	1	0

[a] See footnotes table XII

[b] Fat globules scattered throughout parenchyma

[c] Globules in most cells, some vacuoles seen

[d] Fat vacuoles quite prominent throughout

[e] Very high concentration of fat vacuoles

[f] Slight increase in number of ducts in periportal areas

[g] Hyperplastic cells seen away from periportal areas

[h] Advanced hyperplasia

TABLE XVIII. EGG PRODUCTION FOR TRIAL III

Treatment[a]	Number of Hens	Age at 25% Production In Days	Total Eggs/Hen
C-1	9	46	1.66
C-2	11	39	4.36
C-3	11	41	6.55
A-1	5[b]	--[c]	0.20
A-2	8	38	7.88
A-3	14	42	5.43
M-1	10	38	6.30
M-2	13	44	3.31
M-3	14	40	8.48

[a]See footnotes table XII

[b]Low number of hens due to high death loss

[c]Did not reach 25% production

TABLE XIX. CUMULATIVE EGGS PER HEN DURING TRIAL III

Treatment[a]	Oct.11 Per. 1	Oct.12 Per. 2	Oct.13 Per. 3	Oct.14 Per. 4	Oct.15-16 Per. 5	Oct.17-18 Per. 6	Oct.19 Per. 7	Oct.20 Per. 8	Oct.21-22-23 Per. 9
C-1	.11	.22	.22	.44	.88	1.22	1.55	1.55	1.66
C-2	.09	.36	.54	.72	1.18	1.90	2.36	2.72	4.36
C-3	0	.18	.27	.63	1.55	2.73	3.19	4.11	6.55
A-1	0	0	0	0	0	.2	.2	.2	.2
A-2	.25	.38	.63	1.13	2.26	3.89	4.64	5.27	7.88
A-3	0	.07	.14	.35	.92	1.85	2.21	3.07	5.43
M-1	.6	.8	1.0	1.4	2.0	3.1	3.7	4.4	6.3
M-2	.08	.16	.24	.32	.40	1.25	1.63	2.07	3.31
M-3	.21	.35	.63	1.47	2.68	4.32	4.89	5.83	8.48

[a]See footnotes table XII

vitamin level greatly decreased the age of sexual maturity. The A-1 group did not achieve 25% production and produced only 0.2 eggs per hen during the entire collection period.

The A-2 and M-1 birds were the first to reach 25% production with all diets other than A-1 reaching this degree of maturity within the following 8 days. Total egg production per hen was highest for the A-2 and M-3 groups. In most cases age at 25% production and total eggs per hen were closely related with the earliest maturing birds producing the most eggs.

SUMMARY

Three feeding trials were conducted with Coturnix quail to examine the effects of mycotoxins when fed as the total diet or in a complete mixed feed with different levels of supplemental vitamins. Control diets were fed at each vitamin level. Results showed increases in death losses when mycotoxins were fed. Death losses were significantly ($P < .005$) higher for the lowest vitamin levels.

Feed consumption was less for the lowest vitamin levels and the feed conversion was correspondingly poorer.

Weight gain data showed differences with the Penicillium sp. fed birds showing a response to vitamin additions during trial I while aflatoxin fed birds showed greatest responses in trial III. Birds receiving moldy grain plus additional vitamin supplementation were similar to control birds.

When high toxin levels were fed, internal changes appeared directly correlated to toxin source. The penicillium toxin produced fatty livers with no apparent bile duct damage while the birds receiving aflatoxin had little liver fat but had highly degenerated bile ducts.

Egg production from the group receiving aflatoxin and the lowest level of vitamins was the lowest of any of the groups. When vitamins were added to the diet, egg production was equal to or greater than the highest production by a control group.

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THE EFFECTS OF FEEDING MOLDED GRAIN AND VITAMINS TO COTURNIX QUAIL

by

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Mold growth on grain and toxin production by these molds has long been a problem of grain handlers and farmers. This work was accomplished to see if the severity of the toxin on livestock could be lessened by the addition of supplemental vitamins to the diet.

In the first trial the penicillium toxin produced more severe results than the aflatoxin. Birds receiving the penicillium toxin and what was considered the approximate vitamin level required, consumed less feed, had a poorer feed conversion and gained less weight. When vitamins were added at 2 times and 3 times the required level, the birds receiving the penicillium toxin gained as much weight as the control birds and were essentially the same in feed consumption and feed efficiency.

In trial II it was established that high levels of aflatoxin fed for a short period of time cause a high degree of bile duct degeneration with little liver fat build-up. Whereas, the feeding of a penicillium toxin produced fatty livers with no bile duct changes.

Trial III indicated that 200 ppb of aflatoxin in the diet produced higher death losses, lower feed consumption, poorer feed efficiency, lower weight gain and much lowered egg production for the birds receiving a diet with approximately the required level of vitamins. When vitamins were added as 2 times or 4 times the required, the aflatoxin fed birds were equal to or superior to the birds receiving the control diet in all of the above mentioned variables.