THE INFLUENCE OF CONTINUOUS RECYCLING DEHYDRATED POULTRY ANAPHAGE TO LAYING HENS ON VARIOUS HEAVY METALS IN TISSUES, EGGS AND EXCRETA

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ABSTRACT

THE INFLUENCE OF CONTINUOUS RECYCLING DEHYDRATED POULTRY ANAPHAGE TO LAYING HENS ON VARIOUS HEAVY METALS IN TISSUES, EGGS AND EXCRETA

By

Karingattil Sam Varghese

Several research reports have shown that animal excreta has nutritional value. Therefore, utilizing animal excreta as a feedstuff could provide an additional feed ingredient plus a reduction of livestock pollution potential.

Dehydrated poultry waste (DPW or poultry anaphage) has been successfully fed to ruminants and poultry. However, a few research reports have indicated that possible health hazards, such as diseases transfer, toxic metals and accumulation of drug residues, may result from feeding animal wastes. The purpose of this experiment was to determine the effect of continuous recycling DPW in laying hen rations on heavy metals in tissues, eggs and excreta.

Five hundred eighty-eight (588) pullets (20 weeks old) were divided into three groups and were fed rations which contained no DPW (Control), 12.5 percent DPW or 25 percent DPW (replacing an equal amount of corn). All other ingredients were the same in all the rations fed. The birds were fed

these rations for 33 cycles, each cycle comprised a period of approximately 12 days. At the end of the 12 day period, the fecal samples were collected, dehydrated and used in preparation of the ration for the next cycle and fed to the same birds at the levels mentioned above.

Samples of tissues (muscle, liver and kidney), eggs or excreta were collected from birds fed the respective rations from the 25th cycle and thereafter at alternative cycles until termination at the 33rd cycle. These samples were then analysed for the heavy metals, mercury, copper and zinc by standard techniques and statistically analysed.

The results indicated that there was no significant difference found in the concentration of mercury in muscle, liver or eggs due to the level of DPW fed in the ration (0%, 12.5% or 25%). However, mercury concentration in the excreta of birds fed the 3 rations were significantly different ($P < .0005$). Statistical analysis of the data for the various tissues, eggs and the excreta showed that the concentrations of mercury were significantly different due to the recycling effect of DPW.

The results of the copper analysis in the various tissues, eggs and excreta indicated that there was no significant difference in the concentration of copper in any of the samples analysed either due to the level of DPW fed or due to the recycling of DPW.

The results of zinc analysis for muscle indicated that there was a significant reduction ($P < .05$) in the concentration of zinc when compared to the concentration of zinc in muscles of birds fed the control ration. No significant differences were observed in any tissues, eggs or excreta due to the effect of continuous recycling of dehydrated poultry waste for zinc concentration.

In this experiment it was observed that in general continuous recycling of dehydrated poultry waste in a laying hen ration did not tend to cause an accumulation of mercury, copper or zinc in muscle, liver, kidney, eggs or excreta.

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DEDICATION

This Thesis is hereby dedicated to my beloved parents, Mr. K. J. Varghese and the late Mrs. Kunjamma Varghese for their wonderful encouragement and for their many sacrifices which led me to achieve this honour of having a Doctoral degree.

Sam K. Varghese

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INTRODUCTION

Historically, livestock and poultry farmers have disposed of animal wastes by spreading it on agricultural land. They thus accomplished two objectives--comparatively sanitary disposal, plus, deriving the benefits of organic fertilizer. Today, due to the "population explosion", the movement of people to suburban areas which were formerly agricultural land has thus, limited available land for spreading animal wastes. Therefore the disposal of animal waste has become a severe problem for livestock and poultry producers. In addition, the livestock and poultry industries have expanded all over the world and have become highly characterized with large numbers of animals confined to small acreages.

Several studies have been conducted to find an efficient, acceptable and economical system of manure disposal and/or utilization. It has been shown that animal wastes have nutrient value. Nutritionists have, therefore, introduced the concept of recycling animal waste via animal rations in an effort to decrease pollution and further utilize it as a feed ingredient.

If dehydrated poultry waste (DPW), also called anaphage, can be used successfully and safely as a feed ingredient, it

may well become a partial substitute for protein in poultry and animal rations. Thus, dehydrated poultry waste could possibly be used extensively in animal rations in countries where grains and protein sources are inadequate or unavailable.

The inclusion of excreta in animal rations may cause certain apprehensions in the minds of both producers and consumers of livestock and poultry products. Firstly, there may be injurious effects to the well being of animals fed such rations due to various pathogens and disease carrying organisms which may be present in the excreta. This risk may be reduced, however, by proper handling and sterilization. Currently, there are many feed additives used in most commercial feeds and some of them may not be totally utilized by the animals to which they are fed and may be found in the excrement. Another concern is that in a system utilizing continuous recycling of animal excreta, the substance goes through the digestive tract of animals many times. Hence, toxicological problems may be suspected. Lastly, meat, milk, eggs and their resulting products for human consumption may contain harmful residues due to refeeding excreta.

Dehydrated poultry waste has been sold under the trade name Toplan in England and used as a feed ingredient in animal rations. However, in the United States, its use is not yet permitted by the Food and Drug Administration.

The purpose of this study was to evaluate the status of the heavy metals, mercury, copper and zinc, in an experiment where dehydrated poultry waste (DPW) was recycled continuously in laying hen rations. The tissues studied were muscle, liver and kidney, plus eggs and excreta.

REVIEW OF LITERATURE

Food ingested by an animal passes through the digestive tract and undergoes many chemical and physical changes. The digestive portion of food is absorbed and utilized. That which escapes digestion passes out as excreta. The digestibility and utilization of any food depends upon several factors such as the type of food ingested, the size of particles, the total intake, the rate of passage, the digestion coefficient, the retention time, etc. Research work in the past has shown that these factors are interrelated. In ruminants, Shalk and Amaden (1928) studied the effect of size and specific gravity of individual particles in the reticulo-rumen. They concluded that low specific gravity of hay and concentrates in the reticulo-rumen resulted in the accumulation of the majority of the fibrous digesta become saturated and partly decomposed, the specific gravity of the particles increased and they tended to sink in the ventral regions of the reticulo-rumen and this increased their chances of passage to the omasum and abomasum. King and Moore (1957) also reported such a relationship of specific gravity of particles to rate of passage.

The relationship between voluntary intake and rate of passage was studied by many workers in ruminants (Balch,

1950; Blaxter, 1955; Castle, 1956, etc.).

A relationship between the type of ration and rate of passage has also been demonstrated. Balch (1950) indicated that rate of passage of roughage and the concentrate fraction of the ration was not the same. Rodrigue et al. (1960) demonstrated that digestibility of forage affects rate of passage; while Campling et al. (1961) pointed out that straw has a slower rate of passage than does hay. Eng et al. (1964) reported that as the amount of hay in the ration increased, the rate of passage increased. They also reported that the movement of corn was hastened by an increase in rate of the hay portion of the ration.

The digestion coefficient of various rations has also been reported. Philipson (1942) studied the digestion pattern of different types of carbohydrates in the rumen of sheep. He observed that glucose, fructose, and cane sugar underwent rapid fermentation while maltose, lactose and galactose were fermented less rapidly. Bondi and Meyer (1943) studied the chemical nature and digestibility of roughages and reported that the digestibility for soluble pentosans was from 64.0 to 66.2 percent while the insoluble hexosans digestibility ranged from 74.0 to 76.5 percent.

Elam et al. (1962), in an investigation with sheep, reported that the total digestibility of ground hay was 60 percent and that of barley was only 40 percent. They observed a highly significant decrease in dry matter

digestibility with an increased plane of nutrition.

Cellulose digestion throughout the gastrointestinal tract was studied by Philipson et al. (1942), Gray et al. (1947), Hale et al. (1947), Gray et al. (1958), etc. in ruminants. They have reported that 40 to 45 percent of the cellulose present in fodder was digested in the rumen and that some digestion also occurred in other sections of the G. I. tract. Hale et al. (1947) reported that soluble nutrients disappeared from the rumen predominantly during the first 6 hours of a 12 hour period. Only a small amount of cellulose was digested during this first phase of digestion. A larger and more rapid digestion of cellulose occurred during the second 6 hour period.

Scott, Nesheim and Young (1969) reported that the digestion of protein in laying hens is only 55 percent efficient, while broilers are only 65 percent efficient in protein utilization. They also have reported a sex difference in the efficiency of utilizing various nutrients.

Absorption of nutrients, and their utilization in the body, generally take place once food is digested. However, certain nutrients are also excreted into the digestive tract as food passes down the tract. This includes nutrients from sources such as saliva, degeneration of epithelial cells, microbial products, etc. and enhances level of nutrients in the excreta (Yang, 1964).

Feeding Ruminal Excreta in Ruminant Rations

Total digestion coefficient studies in various species have demonstrated clearly that a portion of the nutrients present in the feed escapes utilization by the animals. These may appear in the excreta and it may be possible that the nutrients could be utilized by animals if fed a second time. McAnally (1942) suspended oat straw and oat straw fecal residues in silk bags in the rumen of sheep. He observed 52 percent digestion of the original straw and an additional 12 percent digestion of the fecal residues. In this study, it was shown that apparently 6 percent of the original straw fiber escaped digestion upon the initial pass through sheep.

McAnally (1942) and Dehority and Johnson (1961) have shown that the cellulose portion of a diet can be divided into two categories; a potentially digestible portion and an indigestible portion. The fecal cellulose contains both of these portions and potentially digestible cellulose can be well-utilized with ruminant by refeeding.

Anthony and Nix (1962) fed cattle manure back to cattle (steers) in a trial (to minimize the wet fecal residues) at about 40 percent of the weight of the concentrate mixture. They reported excellent rate of gain (3 pounds per day) for the yearling steers and indicated that cattle manure was economically promising as a feed ingredient.

In 1967, Anthony tried a new venture by mixing cattle manure with Coastal Bermuda grass hay to make a high dry matter silage. He studied the nutritive value of this product and compared it against the manure concentrate portion. In feeding this silage to steers, he concluded that dry matter, crude protein and cellulose digestion coefficients were essentially the same for the two rations that were fed.

Anthony (1967, 1968) developed the "wastelag" concept for using cattle manure with the potential of a feed. He showed that wastelag represents a flexible system of removing manure daily, blending it with hay and storing it as silage. Singh and Anthony (1968) suggested that yeast fermentation of wastelag increases the nutritive value of this product and claimed about 68 percent of manure dry matter seems recoverable from the final product. Further, they reported that while feeding this product they succeeded in obtaining as good daily gain similar to those of the control group. In addition, they reported in this study that the incidence of parakeratosis was reduced when manure was incorporated in the ration.

Smith et al. (1969) studied the influence of chemical treatments on digestibility of ruminant feces. In this study, sheep consumed corn silage rations containing 25 percent of the total dry matter as either untreated or

3 percent sodium peroxide treated orchard grass cattle feces. The addition of 3 percent sodium hydrogen peroxide to feces increased the average digestibility of the dry matter 29 percent, nitrogen 25 percent, cell wall 55 percent, cellulose 41 percent and hemi-cellulose 90 percent when compared to that of the untreated feces.

Anthony (1970) studied the effect of cooking on utilization of manure in rations and concluded that there were very little differences in the feeding value between the cooked and the uncooked feces.

Anthony (1971) concluded that the advantage of using cattle manure is twofold. First of all, he indicated that sanitation for confined cattle can be enhanced to the benefit of man's environment and secondly he pointed out that efficiency of producing beef for food can be improved by utilizing cattle manure in their rations.

Tinnimit et al. (1972) studied the palatability and nutritive value of different types of animal waste in four different trials with sheep. They reported acceptability of cattle manure for sheep was satisfactory. They used a range of 20-80 percent cattle manure mixture in a mixed ration for growing sheep and this furnished 40-90 percent of the nitrogen requirement. Cattle manure, they indicated, was satisfactory in sheep trials and animals fed these rations were all in positive nitrogen balance. They reported good utilization of the manure in these sheep rations.

Cattle Manure Fed In Rations Of Non-ruminants

Hammond (1942) conducted a series of trials using cow manure in the rations of growing chickens. He reported that cattle manure has a marked beneficial effect on growth in chicks if it is added to diets deficient in riboflavin. He further observed a factor in cow manure which promoted comb growth both in female and male chickens.

Bohstedt, Grummer and Ross (1943) fed cattle manure in practical swine rations in which a vegetable protein source was used. They reported that the results of this study shed a new light showing cattle manure was a good supplement in such a ration. Rhode Island Red chickens were fed cow manure and rumen dried contents as a partial substitute for alfalfa meal in poultry ration by Hammond (1944). He reported that rumen contents and cow manure dried at 21-160°C and supplemented with vitamin A and riboflavin were highly satisfactory. Palafox and Rosenberg (1951) fed cow manure in laying rations and reported egg production was satisfactory in birds that received manure in their rations.

Durham et al. (1966) used an air dried, all concentrate, cattle feedlot manure in pullet and laying hen rations at 10, 25, and 40 percent replacement levels for milo in a basal ration where milo comprised 70 percent of the ration. In this study, they noticed that an increased level of manure caused increased feed consumption. However, no significant

differences were observed in mortality or egg production.

Cattle manure has been successfully used in feed for catfish (Durham et al., 1966). High levels of feedlot manure (50 percent) added to a basal diet were used in this study and a good rate of gain of the catfish was reported. Care was taken not to deplete the oxygen supply in the pond during the study.

Chemical Composition Of Poultry Litter

Noland, Ford and Ray (1955) analyzed poultry litter which was not heat treated and showed that of the total nitrogen present, 19.2 percent was from uric acid.

Eno (1962) reported poultry droppings contained 63 to 87 percent uric acid nitrogen. He indicated that as the moisture increases in manure, decomposition takes place faster and a portion of the ammonia will be lost in this way.

In 1965, Chance analyzed poultry litter from broiler and laying houses. The estimated crude protein nitrogen was 4.84 percent for the broiler litter and 1.51 percent for the hen litter. The range of crude protein values was 24.44 to 32.66 percent for the broiler litter and 9.38 to 14.98 percent for the hen-house litter.

Bhattacharya and Fontenot (1966) analyzed peanut hull and wood shaving broiler litter. The crude protein value for the peanut hull litter was 32 percent and for the wood

shaving litter was 30.2 percent. Calcium and phosphorous levels were 2.77 percent and 2.86 percent, respectively, for the peanut hull litter while the level in wood shaving litter for these two minerals was 2.48 and 2.26 percent, respectively. Analysis of these two litters for amino acid composition demonstrated that of the amino acids, the methionine level was lowest in both type of litters.

Brugman et al. (1968) reported sterilization of poultry litter reduced its nutritive value. El-Sabban et al. (1969) reported the chemical composition of poultry litter. They indicated factors, such as type of birds, bird density, kind of litter base material, litter depth, ventilation, insulation, storing time, temperature at which litter was dried, can influence the chemical composition of poultry litter.

Michigan investigators have done extensive studies with dehydrated poultry manure and wet chicken manure. Benne (1970) showed the crude protein value of poultry manure (dried vs wet) ranged from 3 to 40 percent in analyses conducted from 1966 to 1970. Robertson and Wolford (1970) analyzed samples of chicken manure from Huron County, Michigan, and reported that the values were in the range of 1.00 to 1.50 percent for nitrogen, 0.68 to 0.71 percent of phosphorous, 0.70 to 0.74 percent for potassium, 2.79 to 3.01 percent calcium, 0.00009 to 0.00011 percent for copper

and 0.13 to 0.16 percent for zinc on an as received basis.

Flegal and Zindel (1970a) reported that the analysis of DPW (dehydrated poultry waste) showed an average of 1.73 percent protein nitrogen and 2.14 percent non-protein nitrogen. Average calcium and phosphorous levels were reported as 7.78 percent and 2.56 percent, respectively. The copper and zinc levels reported were 0.0051 percent and 0.0423 percent, respectively. The amino acid composition of the DPW indicated that glutamic acid was highest and the methionine was the lowest. The values were 14.09 percent and 0.82 percent, respectively, on the basis of true protein. Fontenot, Webb, Tucker, Harmon, and Moore (1971) used several treatments to sterilize poultry litter and concluded that a high heating temperature reduced the crude protein value of the litter.

Sheppard, Flegal, Dorn, and Dale (1971) used five different temperatures ranging from 300 to 700°F and reported that an inverse relationship existed between the heat and the resulting total protein. However, the difference was not statistically significant. Flegal, Sheppard, and Dorn (1972) studied the storage effect of caged layer manure on crude protein content and concluded that crude protein was considerably reduced by long term storing. In a period of 0-98 days, they observed that the crude protein value decreased from 30.3 percent to 18.3 percent.

Flegal et al. (1972) have also reported the effect of continuous recycling dried poultry waste on its chemical composition. When continuously recycling DPW in laying rations, it was reported that the range of crude protein varied from 18.3 percent to 39.5 percent.

Nesheim (1972) studied the amino acid composition of the dried poultry manure. Methionine and lysine level in the manure were the lowest, while glycine and glutamic acid were found highest. The total amino acids present in the poultry waste (commercially dried sample) was 10.9 percent. In this study he also reported that when dehydrated poultry manure was included in a laying ration, the excreta obtained contained more dry matter than birds fed the control ration.

Biely (1972) reported that crude protein value of DPW obtained from caged layers was 31.08 percent, while the DPW obtained from the growing birds showed the crude protein value to be 20.11 percent. The zinc concentration of DPW averaged 0.005 percent for the above two sources. Copper level of DPW averaged 0.0026 percent.

Feeding Poultry Excreta In Ruminant Rations

Sheep

Noland et al. (1955) used ground chicken litter as a source of nitrogen in rations for lactating ewes and lambs. The two other sources of nitrogen used were soybean and

ammoniated molasses. These investigators obserwed that litter-fed ewes maintained similar weights to those of sheep fed soybean meal nitrogen. However, they reported that sheep which were fed the ammoniated molasses did not gain as rapidly as those fed the soybean ration. In this study, the chicken litter used was not sterilized. Nevertheless, no digestive or nervous disorders were noticed in ewes or lambs fed rations that contained poultry litter.

Bhattacharya and Fontenot (1965) determined the efficiency of utilization of broiler litter nitrogen by sheep. Four different levels of nitrogen were supplied by litter to sheep in this study, and the autoclaved litter contained a crude protein value of 32.6 percent. In three metabolism studies with yearling wethers, poultry litter nitrogen replaced 0, 25, 50, and 100 percent of the nitrogen of a purified ration that contained isolated soybean protein as the nitrogen source. The results indicated that the apparent digestibility of crude protein in the rations decreased significantly with each increase in litter nitrogen level above the 25 percent level.

Ammerman et al. (1966) reported the feasibility of using poultry litter in ruminant rations. In a digestibility study, of various nutrients in poultry litter (dried citrus pulp was used as an absorbant in the poultry litter) and citrus pulp fed to lambs, they reported that the results

showed poultry litter had a much higher digestion coefficient than that of the citrus pulp and the difference was highly significant ($P < 0.01$).

Long, Bratzer, and Frear (1969) used lambs in an investigation of the nutritive value of hydrolysed and dried poultry waste. They studied the effect of source of nitrogen upon energy and nitrogen utilization in this trial. The experimental animals were fed semipurified diets which were isocaloric and isonitrogenous. The sources of nitrogen were from soybean oil meal, hydrolized poultry waste, and cooked poultry waste. They reported that the apparent digestion coefficient for protein from the hydrolized poultry waste was the only significant difference noted for the different treatments.

El-Sabban et al. (1970) fed autoclaved and cooked poultry litter to wethers as a nitrogen source. The control animals were fed a ration containing soybean meal as the source of nitrogen. They reported a highly significant difference in the apparent digestibility of nitrogen from soybean meal as compared to that from the litter.

Thomas (1970) replaced 32 percent of a soy-corn basal diet with dried poultry manure and these two rations were fed to sheep. The dry matter digestibility of the manure substituted ration was reported to be 58 percent and that of the soy-corn basal diet to be 62 percent.

Lowman and Knight (1970) fed dried poultry manure to sheep. They substituted dried poultry manure at the expense of barley, as 25 percent poultry manure plus 75 percent barley, 50 percent poultry manure plus 50 percent barley, 75 percent poultry manure plus 25 percent barley, and 100 percent poultry manure in different treatments. The dry matter digestibility of the diets gradually fell from that of barley to that of the dried poultry manure in a highly significant straight line ($P < 0.01$). The group of sheep fed 100 percent dried poultry manure, however, did dispose of 57 percent of the dry matter. This study, thus, indicated the possibility of disposing of poultry manure by feeding it to ruminants.

Fontenot *et al.* (1971) studied the effect of heat treatment for sterilization of poultry litter and concluded that heat treatment reduced the crude protein value of poultry litter (chemical analysis). However, their work with lambs showed that acidifying the litter before heating decreased nitrogen loss considerably and did not alter nitrogen utilization.

Bucholtz *et al.* (1971) fed sheep rations that contained 20 to 44 percent dehydrated poultry excreta to study the acceptability, digestibility, and utilization of nitrogen of the rations. They reported that the acceptability of the ration that contained the dehydrated poultry waste was good.

When comparing rations for dry matter digestibility, the soybean ration showed 65 to 70 percent dry matter digestibility and dry matter digestibility of ration containing DPW was 58 to 71 percent. Nitrogen digestibility of soybean meal rations was greater than that of DPW diets while nitrogen digestibility of diets containing DPW was greater than that of the diets that contained dairy feces. All sheep fed the rations were in positive nitrogen balance.

Tinnimit et al. (1972) used dehydrated caged layer feces in growing sheep as 20 to 80 percent of a mixed ration. They indicated that DPW was well accepted by sheep. They also reported that regression analysis gave dry matter and organic matter digestibility of 53 and 64 percent, respectively, for dehydrated caged layer feces.

Beef Cattle

Noland et al. (1955) reported air dried chicken manure can be incorporated successfully in steer rations. Southwell et al. (1958) demonstrated that 20 to 30 percent chicken litter fed in rations for growing steers was quite satisfactory.

Fontenot et al. (1963) fed broiler litter that contained peanut hull and wood shavings in fattening steer rations at the level of 25 percent each. There was little difference in rate of gain between steers fed the ration that contained peanut hull litter and the control ration. The feed

efficiency was highest for steers fed the peanut hull broiler litter. Further, they mentioned that inclusion of broiler litter did not result in any adverse effect on the flavor of the meat of animals that were fed rations that contained litter. In another trial, these workers reported that weanling steer calves fed poultry litter (which had wood shavings as the base material) required an adjustmental period of 5 to 7 days for good feed consumption. They observed no difference thereafter in feed intake or average daily gain between animals fed the rations that contained litter and a control fed group. Brugman et al. (1964) fed poultry litter to beef cattle and reported a protein digestibility of 77 percent for the litter used.

Drake, McClure, and Fontenot (1965) fed poultry litter that contained peanut hull and wood shavings as the base material to heavy steers. In the first trial, steers were fed rations that contained 25 percent peanut hull litter (lot 1), 25 percent wood shaving litter (lot 2), and a control protein supplement (lot 3). Feed efficiency was highest for the peanut hull litter fed steers and lowest for the control fed group. They also reported no change in the taste of meat obtained from steers which had been fed rations that contained litter.

Bucholtz et al. (1971) fed dehydrated poultry waste (DPW) as the only source of protein, 1/2 DPW plus 1/2

soybean meal, 1/2 DPW plus 1/2 urea, and compared them to diets that contained either urea or soybean meal as the source of protein to yearling steers. Average daily gain for the soy supplemented group was significantly greater than for the groups supplemented with DPW (3.35 lb. vs. 2.75 lb.), 1/2 DPW plus 1/2 urea (3.03 lb.), 1/2 DPW plus 1/2 soy (2.88 lb.), but was not significantly greater than for the urea supplemented group (3.10 lb.).

Harmon, Fontenot, and Webb (1972) demonstrated with steers that 10 to 15 percent inclusion of molasses to high levels of poultry litter (25 to 50 percent) considerably increased the food intake.

Dairy Cows

Bucholtz et al. (1971) fed dehydrated poultry waste to lactating dairy cattle in a comparison to other sources of nitrogen. They reported in a preliminary trial that dairy cows did not consume a grain mixture containing 50 percent DPW and, hence, they used 30 percent DPW in grain mixture in this study. They reported total feed consumption varied from 15 to 20 pounds per cow per day. Cows, however, utilized the nitrogen from DPW well.

Thomas and Zindel (1971) conducted a trial with lactating dairy cows to compare utilization of different nitrogen sources. In this study, they reported that cows only consumed diets that contained 15 percent DPW during the

first week of the trial. However, the cows consumed 30 percent DPW in a ration thereafter.

In 1972, Thomas et al. used DPW to provide 23, 61, and 90 percent of total dietary protein as well as 11, 25, and 50 percent total dry matter intake, respectively, when fed to lactating dairy cows. They reported that the cows fed DPW produced more milk than those fed an inadequate protein level and amounts equal to those produced by cows fed usual protein supplements.

Bull and Reid (1971) reported that three non-lactating Holstein cows, 6 to 7 months gestation, were offered a ration that contained air dried chicken manure (ADM) to study the acceptability, intake and use of ADM by cows. The studies with dairy cows showed that ADM can be used as a source of supplemental nitrogen for cows fed low-protein basal diets. They reported further that this study revealed nitrogen, calcium and phosphorous in ADM were rapidly available and well-utilized by these animals.

Feeding Poultry Excreta In Poultry Rations

Rubin, Bird, and Rotchild (1946) reported a growth promoting factor in laying hen feces fed to chicks and indicated that it was similar to the factor found in cow manure. Chicken feces collected void of urine showed a higher rate of this growth factor for growing chickens than did feces

containing urine.

Elam et al. (1954) fed chicks an autoclaved water suspension of poultry litter in a corn-soybean meal basal diet. They reported that growth was increased by the addition of the litter preparation. Jacobs et al. (1954) also confirmed a growth promoting factor in poultry litter fed in chicken rations.

Fuller (1956) fed hydrolyzed poultry litter while Wehunt et al. (1960) included hydrolyzed poultry litter and poultry manure in chick rations. These workers further confirmed an unidentified growth factor in poultry litter. Wehunt et al. (1960) concluded that feed efficiency was poor when poultry litter was used at high levels in chicken rations.

Warden and Schaible (1961) and Yates and Schaible (1961) reported inclusion of fresh, dried autoclaved caged layer manure in chick rations. They demonstrated growth depression of chicks when fresh manure was incorporated in the basal diet. However, dried and autoclaved manure in chick and turkey poult rations improved growth rates.

Durham et al. (1966) fed poultry manure in laying rations at 10, 25, and 40 percent levels. The feed efficiency for the laying ration was reduced as the level of manure increased in the diet. The pullets that received 10 percent manure in the diet produced significantly more eggs than all

other groups; but, feed consumption was increased in this group when compared to the feed consumption of the control fed group.

Howes (1968) and Quisenberry and Bradly (1968 and 1969) reported the possibility of recycling poultry manure in poultry rations. Quisenberry and Bradly (1969) fed poultry litter and caged layer manure at 10 and 20 percent levels in layer rations. The results indicated that with the exception of one of these diets, the nutrient recycled diets were equal to or superior to the control ration for maintaining body weight, hen day egg production, feed efficiency, and egg size.

Flegal and Zindel (1969) reported satisfactory usage of dehydrated poultry waste in laying hen rations. Flegal and Zindel (1970a) fed diets which contained 5, 10, and 20 percent of DPW to broiler chicks. The results indicated a reduction in the body weight at four weeks of age for those chicks that consumed the diets that contained 10 and 20 percent DPW. In Leghorn-type chicks, no influence of DPW was seen on four week body weights when up to 20 percent DPW was included in the diet. Feed efficiency was inversely related to the level of DPW in the diet. However, these investigators observed that four week weight gains in broiler chicks that were fed additional fat in the ration which contained 20 percent DPW were equal to those of chicks fed a control diet.

Flegal and Zindel (1970b, 1970c, and 1971) fed DPW at different levels in laying hen rations. In general, the birds receiving diets containing 10 and 20 percent DPW showed a better feed efficiency than hens that were fed a control ration that contained no DPW. Flegal and Zindel (1970c and 1971) studied the effect of feeding high levels of DPW (10, 20, and 30 percent) with respect to feed consumption, egg production and egg quality. They reported that hens fed diets that contained DPW at the 10 percent level had the highest egg production. Nevertheless, they reported no significant differences ($P > .05$) were observed in egg production between hens fed any of the diets that contained DPW and those receiving a control ration. York et al. (1970) studied the effect of DPW on quality changes in shell eggs during storage. They concluded that including 10, 20, or 30 percent DPW in ration of hens had no significant deleterious effect on the quality of shell eggs as measured by Haugh units, storage weight loss, color, odor and/or microbial content.

Calvert (1970, 1971) used poultry manure in an indirect way as a feed for chicks. He used the housefly for biodegradation of the chicken manure, harvested the pupae (which were later dried and ground) and incorporated that product in chicken rations. He indicated good weight gain of chicks fed this protein source.

Hodgetts (1971) determined the metabolizable energy value of dried chicken manure in laying hen rations by two different methods; namely, Bolton's available carbohydrate method, and the "classical determination". The metabolizable energy value obtained for the DPW by the former method was 1955 Kcal/Kg, while for the later method, it was 893 Kcal/Kg. Polin et al. (1971) reported the metabolizable energy value for the dried poultry waste used in a laying hen ration as 1392 Kcal/Kg. Nesheim (1972), however, showed a much lower value for the metabolizable energy value of DPW. In one trial, this value was 675 Kcal per Kg of air dry sample (study with cockerels), while in the second trial, the metabolizable energy value was 205 Kcal and 490 Kcal/Kg. He pointed out that the metabolizable values in the laying hen rations were quite variable and suggested that this could be attributed to several factors such as the feed used, feed spillage, and bacterial action occurring in the excreta after it was avoided. Shannon, Blair, and Lee (1972) reported that the metabolizable energy value for poultry manure in the laying hen ration was about 970 Kcal/Kg.

Bergdoll (1972) conducted an evaluation of DPW in rations for starting, growing, and laying birds. He reported that the birds readily ate rations that contained DPW and further pointed out that the average livability was better

for birds that were fed the rations that contained DPW than for birds that were fed the control rations which contained no DPW. He observed that growing birds needed a preliminary period to get used to the ration that contained DPW and, hence, recommended starting with rations that had low levels of DPW and gradually increasing the level up to 30 percent in the ration. In this study the optimum level of DPW in the laying hen ration was reported to be 10 to 15 percent. When the level of DPW exceeded 20 to 25 percent of the laying ration, feed conversion was adversely affected.

Flegal et al. (1972) reported on the effect of continuous recycling dried poultry waste in the diet of 20 week old Leghorn chickens for 31 cycles (recycled approximately every 12 days). At the end of this period, hen-housed egg production of the birds (fed the diet) that contained 12.5 percent DPW was slightly higher (62.4 percent) than that of birds fed the control diet (59.6%). Egg production of the birds that were fed the diet that contained 25 percent DPW was 59.2 percent. The birds fed the ration that contained 25 percent DPW consumed 12.6 percent more feed than did the birds that were fed the rations that contained either 0 or 12.5 percent DPW.

Biely (1972) included DPW at levels of 5, 10, 15, and 20 percent in broilers rations to evaluate the nutritive value of DPW. He reported that as the level of DPW

increased, the feed efficiency decreased. He also reported that when the ration fed contained 20 percent DPW growth of broilers was depressed by 6.7 percent when compared to the growth rate of the control fed group.

McNab et al. (1972) and Lee and Blair (1972) reported that dried autoclaved poultry manure was added to a purified ration and this response was equal to that of chicks which received the purified ration that contained L-glutamic acid. They suggested this effect of manure was not of the uric acid nitrogen but, rather, a portion of the nitrogen (1/3) which had the property of L-glutamic acid. Lee and Blair (1973) further reported that on the basis of their results 5 percent poultry manure could be added to a broiler starter diet without affecting growth rate. However, Rhineheart et al. (1973) recently reported that DPW has no value for young broilers.

Dried poultry waste has also been included in turkey rations. Fadika, Wolford, and Flegal (1973) reported that levels up to 30 percent of DPW fed to growing turkeys did not significantly affect body weight gains. All rations used were made isocaloric. They reported feed efficiency was inversely related to the amount of anaphage incorporated in the diet.

Feeding Dried Animal Waste In Swine Rations

Bohstedt, Grummer, and Ross (1943) reported that for swine, cattle manure may have a helpful supplementary effect on practical rations in which only vegetable protein concentrates were used.

Digs and Baker (1965) fed dried pig feces to growing hogs at levels of 15 and 30 percent of a growing ration. They reported that pigs fed diets containing 0, 15, and 30 percent feces required 3.63, 3.62 and 4.65 pounds of feed per pound of gain, respectively. The daily rate of gain was reported to be 1.71 pounds for the animals fed the control ration, 1.56 pounds and 1.53 pounds for the rations that contained feces. They also indicated that meat from these animals did not show any unfavorable taste in a test conducted by a test panel.

Geri (1968) fed dried poultry manure in growing pig rations at 7 and 10 percent levels. He demonstrated that growth rates of animals fed the rations containing feces were, in general, equal to that of the control group.

Phelps (1969) conducted studies with poultry litter in rations of sows, growing and finishing pigs. In growing animals, a high level of poultry litter (45 percent) was used. He concluded that pigs up to 34 Kgs. live weight should not be fed more than 20 percent litter in the ration but that after this stage a 45 percent inclusion of litter

was possible and could be economical.

Perez-Aleman et al. (1971) investigated the utilization of poultry litter in rations. They fed poultry litter up to 30 percent in various rations. For every 10 percent inclusion of litter, they observed a reduction in growth rate of 0.02 Kg. per day and a reduction of feed conversion efficiency by 0.25 units. They concluded that if poultry litter were cheap it might be economical to include it in swine rations at the 10 percent level.

Orr et al. (1971) reported studies using dried swine feces and dried poultry waste in swine finisher rations. In the first trial, they observed inclusion of 22 percent dried swine feces resulted in poor growth of pigs. However, they reported feed consumption of the ration that contained feces was satisfactory. Supplementing the above ration with 0.1 percent lysine and 0.1 percent methionine resulted in improved performance (growth) but, over the entire trial, feed consumption was reduced 7 percent and daily gain was reduced 55 percent compared to the animals fed the control ration.

In a second trial, Orr et al. (1971) reported that incorporation of 20 percent DPW in a basal diet fed to growing swine caused a 15 percent decrease in feed consumption. They concluded from these experiments that dehydrated poultry waste was low in the critical amino acids and, hence, was of little value in swine rations.

Drugs, Pesticide, and Metal Residues In Poultry Excreta and Their Influence On Animals Fed Rations Containing Excreta

Animal feeds (commercial), in general, may contain many feed additives. Scott, Nesheim, and Young (1969) have listed the general categories of feed additives utilized. They include:

A. Antibiotics, arsenicals, nitrofurans

B. Coccidiostats

C. Broad spectrum, absorbable antibiotics

D. Worming drugs

E. Chemicals used to potentiate curative properties of antibiotics

F. Miscellaneous drugs such as reserpine, asperine and other tranquilizers

G. Antifungals

H. Flavoring agents

I. Pellet binders

J. Enzymes

K. Carotenoid sources, etc.

Calvert (1973) has reported that 57 feed additives or their combinations have been approved for use in poultry rations; 12 for nutritional purposes and 31 for their medicinal properties.

Analyses of poultry litter by various workers (Eno, 1962; El-Sabban et al., 1969; Flegal and Zindel, 1969; Benne, 1970; Robertson and Wolford, 1970; Fontenot et al.

1971; Nesheim, 1972; Biely, 1972; Flegal and Dorn, 1972) indicate high levels of ash are present in poultry litter. Brugman et al. (1964) reported that poultry litter was assayed for various drug residues such as arsanalic acid, zoaline, unistat, nicarbasin, furans, and sulfaquinoxaline. Arsanalic acid level in poultry litter was 0.0048 percent, while all other residues analysed in the litter showed negligible values.

Chance (1965) incorporated poultry litter in beef cattle rations. The animals fed the litter rations showed some digestive disturbance such as diarrhea. This disorder, they suspected, was due to the inclusion of poultry litter in the ration.

Brugman et al. (1968) reported that amprolium and arsenic were found in poultry litter. Nevertheless, when the same poultry litter was fed in a basal ration to sheep, chemical analysis of heart, spleen, twelfth rib, kidney, kidney fat, liver and brain revealed no residues of amprolium or arsenic.

Griel et al. (1969) reported that feeding grain, supplemented with dried, but unprocessed poultry litter obtained from a roaster operation, resulted in abortion in a beef breeding herd. The roasted feed was commercially mixed and had 0.33 to 0.51 pounds of 14 percent dienosterol diacetate premix per ton. Chemical assay of feed and the

litter did not reveal any drug residues but bioassay of the litter indicated esterogenic activity which was greater than 10 g. DES equivalents per 100 g. of litter.

Morrison and Peterson (1969) reported that analysis of poultry litter from a broiler house indicated the presence of 15 to 30 ppm of arsenic. Long et al. (1969) also reported approximately 17 mg. arsenic per Kg. of poultry litter.

El-Sabban et al. (1969) analyzed 32 samples of broiler litter and 22 samples of litter from laying hens for arsenic. They reported broiler litter averaged 11 mcg. of arsenic per gm. while the laying hen litter averaged 29 mcg. per gm.

El-Sabban et al. (1970) fed autoclaved, cooked and dried poultry waste from caged layers to steers. Although levels of chlorinated hydrocarbons and arsenic in the rations or the waste products of the layers were not known, feeding the waste product did not seem to increase the level of chlorinated hydrocarbons in the fat of the steers. They mentioned that no detectable residues of lindane, aldrin, dieldrin or heptachlor were found in the fat from the steers. However, they indicated that steers fed the ration that contained dried poultry waste had higher ($P < 0.05$) arsenic levels in their livers (0.38 ppm) than those fed autoclaved poultry waste, soybean oil meal or a urea control ration.

Messer et al. (1971) analyzed poultry litter and feed obtained from different commercial poultry farms in Ohio for various drug residues and pesticides. They reported that the arsenic content was in the range of 0.3 to 16.6 mg./Kg. with a mean value of 14.6 mg./Kg in the litter. They concluded that the arsenic content of the litter was dependent to a large extent upon the use of the arsenicals in the feed. For pesticides and other drugs (Furazolidone, Zoaline, Diethyl, Stilbestrol, DDT, and DDE), they reported values were negligible.

Fontenot et al. (1972) assayed livers and omental fat tissues of steers which were fed rations that contained 25 percent and 50 percent broiler litter for lindane, heptachlor, aldrin, heptachlor epoxide and DDE, Dieldrin, Endrin, ortho-para DDT, para para DDD and para para DDT. There was a slight increase in the tissue levels of lindane from the animals that were fed the rations that contained litter when compared to the tissue levels of lindane of the control fed animals. For most of the other pesticides, the liver values obtained were negligible. They concluded that there was no serious pesticide residue problem from feeding broiler litter as a feed for ruminants.

Webb and Fontenot (1972) fed rations that contained 25 percent and 50 percent poultry litter to beef cattle for a continuous period of 121 days. The amprolium level of the

litter was reported to be 27.3 ppm. Beef loin muscles were obtained from the animals fed the control ration and the two levels of litter and assayed for amprolium. The amprolium level of the loin muscle of animals fed the control ration was 0.014 ppm while the amprolium in the same tissue from the animals fed diets that contained 25 percent litter was 0.012 ppm. The concentration of amprolium in the loin muscle of the animal fed diets that contained 50 percent litter was 0.009 ppm. Liver amprolium levels were 0.0015, 0.0038, and 0.0008 ppm, respectively, for the control, 25 percent litter fed and 50 percent litter fed animals. In a second trial with steers, similar results were obtained and, hence, they concluded that amprolium did not accumulate in tissues of steers as a result of feeding poultry litter at high levels.

Webb and Fontenot (1972) also studied the effect of feeding poultry litter which contained known amounts of chlortetracycline residues (12.5 ppm) to steers at 25 and 50 percent of a steer ration. Kidney fat from steers from the two litter rations showed low levels of chlortetracycline activity (0.034 ppm and 0.041 ppm, respectively). However, chlortetracycline activity of the liver was negligible for all livers tested, regardless of dietary treatment.

Fontenot et al. (1972) fed different levels of broiler litter to ewes for extended periods of time. Ewes fed a

control ration (no poultry waste), 25 percent litter and 50 percent litter in the basal ration, were compared. The lambing performance was similar for ewes fed the different rations. On day 137 of the experiment, one ewe, which received the ration that contained litter, died and upon necropsy the cause was diagnosed as copper toxicity. The average copper content of the litter analyzed 17.8, 57.1, and 109.1 ppm copper, respectively. At the end of 254 days, death from copper toxicity was reported to have occurred in 64 percent of the ewes fed 50 percent litter and 55 percent of the ewes fed 25 percent litter. The liver copper levels at death or slaughter were significantly higher for the ewes fed diets that contained 25 or 50 percent poultry litter than for those fed the control ration. However, there was no significant difference reported for animals fed rations that contained 25 percent or 50 percent broiler litter in their liver copper status. In a second trial, Fontenot et al. (1972) fed the same levels of litter (25 and 50 percent) in ewe rations for a period of 15 months. Up to 14 months, no ewes were reported to have symptoms of copper toxicity. During the 15 month, however, symptoms of copper toxicity appeared in both groups fed the litter rations. It was reported that in the group which received the ration that contained 25 percent litter, two animals died and the group which received the ration that contained 50 percent liter, four ewes died.

Michigan investigators (Bucholtz et al., 1971; Tinnimit et al., 1972; Thomas et al., 1972) have fed different levels of dehydrated poultry manure in rations of steers, sheep and dairy cows. They observed no toxic effects in any of the animals studied. Thomas et al. (1972) indicated that there were no abnormal levels of calcium, phosphorous, sodium, potassium, zinc, iron, copper, or manganese found in the kidneys or livers of sheep fed rations that contained 25 or 50 percent dried caged layer feces for a period of 88 days. They concluded that fattening sheep could be fed rations that contained considerable amounts of dehydrated caged layer feces.

Calvert (1973) studied the excretion pattern of arsenic in sheep by feeding dried broiler manure containing 42 mg./Kg. arsenic. The levels of broiler manure fed were 0, 7, and 14 percent in a basal ration. The total intake of arsenic accounted was less than 0.01, 14.3 and 25.1 mg., respectively, for the 0, 7, and 14 percent dietary level of broiler litter consumed. The total excretion of arsenic was 83.2 and 90.8 percent, respectively, for sheep that were fed the diets that contained 7 and 14 percent broiler manure. The feces excretion as a whole was 76 percent while the urinary excretion was 23.9 percent. It was indicated that only 2.4 mg. arsenic was retained by the sheep, or 0.08 mg./Kg. if the arsenic was evenly distributed in the body of a 30 Kg. sheep.

Mercury In Feeds and Tissues Of Animals

Underwood (1971) indicated mercury occurs widely in low concentrations in the biosphere and has long been known as a toxic element. At room temperature, mercury has a significant vapor pressure (0.001 mm at 18°C), which rises markedly at higher temperatures (0.27 mm at 100°C).

Stock et al. (1933 and 1939) reported that fruits, vegetables, cereal grains, and animal tissues contain mercury in the range of .005 to .035 ppm. In animal fat, the level of mercury was reported in the range 0.07-0.280 ppm. Fish were reported to have contained 0.020-0.180 ppm mercury.

Pappas and Rosenberg (1966) analysed wheat samples from various parts of the United States and reported values ranging from 0.013-0.127 ppm. In Japan, where organomercurials have been extensively used in rice cultivation, Tomizawa (1966) reported comparatively high levels of mercury in rice from unsprayed fields (0.227-0.238 ppm).

Howie and Smith (1967) studied the mercury content in different human tissues. They obtained dead bodies of normal individuals who were not exposed to mercury contaminations. They reported that the mean concentration fell between 0.500 and 2.500 ppm Hg (dry basis). The concentration of mercury in liver, kidney, and lungs was reported to be higher than in other tissues.

Lunde (1968) reported a mean mercury concentration of 0.180 ppm (range 0.030 to 0.400 ppm) for 12 commercial fish meals from different sources.

Gomez (1972) reported the concentration of mercury in chicken muscle was .025 ppm. The mercury level of liver and egg was reported as 0.030 and 0.035 ppm, respectively.

Sell et al. (1973) analysed eggs from five different farms situated at different geographical areas of North Dakota for mercury concentration. They reported highly significant ($P < 0.01$) variations in the concentration of mercury in eggs received from a specific farm at a specific time. The ranges of mercury found in eggs from different farms were 10 to 420 ppb; 11 to 124 ppb, 22 to 93 ppb, and 2 to 6 ppb. The average concentration of the liquid portion was reported to be 14 ppb.

Gilch (1932) fed seeds, which were treated according to directions with different commercial dressings containing mercury, to hens. When given 150 g of seed per day the hens suffered no adverse effects.

Loliger (1955) fed hens with seed which was treated with a commercial dressing, probably a phenyl mercury compound. He found that in this way the hens could be fed 5 mg of the organic compound per day without sustaining injury. An oral, single dose of 30 mg. of the compound produced serious symptoms of poisoning, which, however, the animals survived.

Heuser (1956) reported that hens which were fed a mixture of sweet corn (1/3 treated and 2/3 untreated with hydroxymercury Ceresol) for 4 months had no signs of poisoning.

Carnaghan and Blaxland (1957) fed Cerasan-M treated wheat and barley grains to laying hens. Each hen received 150 g seeds for a period of 6 weeks. After 6 weeks the average mercury content in the liver of hens that were fed the mercury treated seeds was 1 ppm which the authors regarded as an insignificant figure from a toxicological point of view.

Miller et al. (1959a) reported that they observed a strain difference in the retention of mercury in the liver and kidney of chickens when they were injected with phenyl mercury acetate (PMA) or mercury chloride (MC). Mercury retention was reported greater in a strain selected for resistance to lymphamatosis than in two susceptible strains.

Miller et al. (1959b) injected phenyl mercury acetate at 3.0 mg Hg/Kg of body weight in strains of chickens selected for leukosis resistance and leukosis susceptibility and their reciprocal crosses. In this study, the results also indicated that the resistant strains retained more (18 percent) mercury in the kidneys after a 96 hour period than the susceptible strain (kidney retained 6.6 percent). They further reported a dose of 15 mg. mercury (as PMA) per

Kg of body weight injected in chickens caused only an occasional death within 96 hours. However, an injected dose of 18 mg. mercury (as PMA) per Kg of body weight increased mortality.

Miller et al. (1960) stated that for a single oral dose of phenyl mercury acetate, 60 mg/Kg body weight was lethal for hens. Miller et al. (1961) reported that for a single oral dose of ethyl mercury chloride, LD50 for hens was 20 mg Hg/Kg of body weight.

Swensson et al. (1959) studied the excretion of mercury compounds (mercury nitrate, phenyl mercury acetate, and mercury chloride) after a single injection in rats. They reported that immediately after the injections, the mercury concentration of the blood was very high. Nevertheless, they observed a drop in blood mercury after a period of 5 to 10 minutes and after that the compounds were excreted very slowly. The two organic compounds were, to a large extent, bound to the erythrocytes, whereas the inorganic compound was transported in plasma. The presence of mercury was demonstrated in urine immediately after injection of all the three compounds. Mercury nitrate and phenyl mercury acetate were deposited chiefly in the kidneys, whereas, the methyl mercury hydroxide appeared to be distributed more uniformly throughout the body.

Smart and Lloyd (1963) studied the effect of feeding mercury dressed seeds (mercury-6ppm) to chickens. Methyl mercury dicyandiamide dressed wheat was fed to laying hens for a period of 8 weeks and the mercury content of eggs, liver, muscle, and kidney were studied. The average concentration of mercury reported for the various tissues of birds which were fed non-treated grain was: muscle 0.005 ppm, liver 0.050 ppm, and kidney 0.050 ppm. Birds which were fed treated seeds had mean mercury concentrations as follows: eggs 10.9 ppm, muscle 3.4 ppm, and kidney 7.5 ppm.

Tejning and Vesterberg (1964) reported that one hen was fed exclusively with seed dressed with a methyl mercury compound at 14 µg Hg/g for 4½ months. They reported the hen "appeared healthy" when it was killed for mercury analysis. The mercury content of the brain was 14 ppm, kidney 43 ppm, and liver 42 ppm.

Miller et al. (1967) reported a single dose of mercury [phenyl mercury acetate (PMA) or mercury chloride (MC)] administered by various routes indicated that the accumulation of mercury in the liver and kidney of chickens increased as the amount given increased. Further, they reported that at the highest dose of phenyl mercury acetate or mercury chloride used (20 mg and 30 mg/Kg body weight, respectively) mortality was 25 percent. They concluded that the amount of mercury retained from oral doses was essentially

directly proportional to the dietary dose. Twice the amount of mercury accumulated in livers and kidney from chickens given PMA orally as in those from chicken given MC orally.

Swensson and Ulfvarson (1969) indicated that cocks were fed wheat dressed with different mercury compounds with a concentration of up to 80 mg/Kg of wheat. Cocks fed the diet that contained methyl mercury developed paralysis disturbances and incordination within 2 weeks. They suggested that the mercury methyl compound was excreted much more slowly than other compounds and gives a much higher concentration in the body on continuous administration. However, they indicated that when administration was discontinued, mercury concentration in the body decreased rapidly.

Jensen and Jernelov (1969) studied the mercury concentration in fish. They observed that the mercury present in fish was in the form of methyl mercury. These workers suggested that living organisms have the capacity to methylate mercury compounds present in pollution. It was reported that mono and dimethyl mercury (CH_3 Hg plus and CH_3 Hg CH_3) can be produced. Jensen and Jernelov (1969) studied the conversion of methyl mercury to dimethyl mercury. They kept known quantities of mercury (125 µg) in an open flask which contained two dead fish for a period of 7 weeks

and observed the conversion of methyl mercury to dimethyl mercury. They suggested the formation of volitile dimethyl mercury (b.p. 94°C) may be a factor in the redistribution of mercury from the disposal of industrial wastes that contain mercury into lakes or streams.

Kiwimae et al. (1969) fed White Leghorn hens for 140 days with wheat treated with methyl mercury hydroxide and mercury (II) nitrate. The daily administration was 400 or 1600 μg of mercury. The results indicated that the concentration level of mercury in eggs depended on the level of mercury in the food. The total mercury found in hens fed the mercury (II) nitrate was for muscle 0.16, liver 2.65, and kidney 3.16 mg/Kg. For the phenyl mercury hydroxide these values were, respectively, for muscle, liver, and kidney 0.23, 4.80, and 10.0 mg/Kg.

Curley et al. (1971) reported that mercury treated grain obtained by a farmer from a local granary in New Mexico resulted in mercury poisoning in hogs. One of the hogs that received the treated grain in the ration was slaughtered after a period of 3.5 months and the farmer's family ate the meat. The family members who consumed the pork showed symptoms of mercury poisoning within 3-4 months time. On chemical analysis it was confirmed that the human poisoning was due to organic mercury. Mercury was found in tissues of hogs which were fed the contaminated seed.

It was also reported that mercury was found in the urine, seman, and cerebro-spinal fluid of humans who ate the contaminated pork.

Copper In Feeds and Tissues Of Animals

The presence of copper in plants and animal tissue was recognized more than a century ago. Scott et al. (1969) reported the copper content in whole egg was 2.5 ppm, liver and glandular meal 90 ppm, meat and bone meal 12 ppm, fish (halibut) 2.5 ppm, yellow corn 4.5 ppm, soybean meal 20 ppm, wheat grain 7.8 ppm, and dried whey 50 ppm.

Robertson and Wolford (1970) reported that chicken manure contained a copper level of 0.00009 percent to 0.00011 percent on an as received basis. Flegal et al. (1972) reported the copper concentration found in dehydrated poultry waste, obtained from laying hens, was 0.0051 percent. Biely (1972) reported that dehydrated poultry waste obtained from pullets and laying hens in British Columbia had an average copper concentration of 0.0025 percent and 0.0021 percent, respectively.

Cunningham (1937) reported for a range of species studied, a higher proportion of body copper existed in the liver than in other tissues. Beck (1956) reported that the liver copper concentration of the domestic fowl (mature, normal diet) was 14.8 ppm (range 10-31 ppm).

Goldberg et al. (1956), in a trial with chicks, administered 50 mg of copper daily for one week, 75 mg copper daily during the second week, and then 100 mg copper daily until signs of anemia, toxicity, or death occurred. They reported the symptoms in these birds were weight loss, weakness, anorexia, lethargia, hunched up posture, and anemia. Eight of the 23 chickens tested hematologically developed anemia (combs became very pale). There was no elevation of icterus index in any of these birds.

Barber et al. (1957) reported that copper concentration in tissue from swine fed a control ration with no supplemental copper was: liver 13.5 ppm, kidney 6.36 ppm, spleen 1.35 ppm, muscle 0.88 ppm, and fat 0.66 ppm. However, it was indicated that a great variation in these values was observed for individuals.

Hall and Mackay (1931) reported that feeding rabbits 2 mg of normal copper acetate in each gram of diet for 105 days caused a pigmentation in the liver of most animals. Some animals showed cirrhosis of the liver.

Ferguson (1943) reported that dairy cows that were daily fed 2 grams of copper sulphate orally for a period of 18 weeks remained in excellent condition and in good health throughout the experimental period. There were no toxic symptoms or loss of appetite.

Kidder (1949) reported that when an overdose of copper sulphate was fed to a five hundred pound steer, the steer

developed a chronic copper poisoning and died after 122 days on a daily drench containing 5 grams of copper sulphate. Postmortum examination indicated general icterus, dirty, yellow-colored fat, hemolysis, hemoglobinuria, enlarged kidneys and spleen and yellowish liver.

Scott and Jenson (1952) showed that the growth of young poults that were fed practical starting diets was increased as much as 55 percent by the addition of the antibiotic chlortetracycline hydrochloride (11 mg/Kg). In 1965, Scott and Peter reported that antibiotics which contain copper compounds (chlortetracycline, copper sulphate, etc.) improved growth rate in poults in the magnitude of 8 to 13 percent.

Bull et al. (1955) reported copper toxicity in sheep which were grazed on natural pastures that contained Heliotropium Europaeum. Animals (1 to 7%) died with liver damage. A continuous loss (up to 55%) was reported during range grazing (second year). Haematogenous Jaundice was present in almost half the animals which died. Clinical pathological examination indicated that the sheep commonly exhibited a fall in hemoglobin and a rise in bilirubin in the blood. Chemical analysis for copper in the liver showed that the level of copper increased about 80 percent above the normal level. The animals that died from copper toxicity had liver copper levels that averaged 1000 ppm.

Wallace et al. (1960) added high levels of copper to corn-soybean type of rations fed to swine to determine the efficacy of feeding high copper levels and the possible interrelationship of copper, zinc and high levels of protein. After six experiments were conducted they concluded that copper levels of 250 ppm and above caused copper toxicity in swine. Two hundred ppm of copper did not affect growth but hemoglobin level was significantly reduced. One hundred - 150 ppm copper was generally non toxic. One pig that was fed 250 ppm copper died as a result of copper toxicity and postmortum examination revealed internal hemorrhages and pronounced icterus throughout the body fluids and tissues, particularly in the liver. High levels of zinc (500-1000 ppm) fed alone or with copper did not influence pig performance. As the protein level of a diet that contained 750 ppm of copper was increased (15-20 and 25%) copper toxicity as measured by weight gain, feed conversion and hemoglobin levels was reduced.

Cox and Harris (1960) and Magee and Matron (1960) indicated that zinc interferes with copper metabolism by decreasing the utilization and increasing the excretion of copper when fed to rats. However, they pointed out that zinc has little effect on copper absorption.

Hill and Matron (1961) reported that chicks which were fed copper and iron supplements in the ration had increased

hemoglobin as intake of these two elements was increased. Further, when iron was added to the same diet, less copper was needed for a given concentration of hemoglobin or vice versa.

Kowalezyk, Pope and Sorensen (1962), in an evaluation of chronic copper poisoning in sheep, allowed free choice-trace mineral salt, indicated that during the course of chronic copper intoxication, ingested copper was parially absorbed into the body and stored in the liver and kidneys. A portion of copper was eliminated in bile and urine, and the unabsorbed part was eliminated in the feces. As the intake of copper salt continued, the tissue storage of copper increased with a concomitant slow increase in blood copper concentration. Further supplementation of copper resulted in a sudden breakdown of the elimination mechanism with subsequent damage to the liver and kidneys, a significant rise in blood copper concentration, icterus, hematuria, hemoglobinuria and finally in death of the sheep.

Bunch et al. (1963) using pigs, compared the effect of feeding different compounds of copper (copper oxide, copper sulphate and chlortetracycline) on growth performance. All these compounds increased body weight compared to a basal diet which contained no supplemented zinc. Significantly higher levels of copper were found in the liver of pigs that received added cooper than in the liver of those which did

not. Also, the concentration of copper increased significantly in the loin muscle. There was significantly less iron in the liver of pigs that were fed the copper supplemented diet. Addition of zinc and iron to the copper supplemented diet promoted additional growth. However, when there was no zinc added, no effect was found due to the copper supplementation. Hemoglobin levels was lowered in the presence of added copper in the absence of supplemented iron.

Taylor and Thomke (1964) reported increased liver copper in pigs fed rations that were supplemented with copper (250 ppm). They reported that the level of liver copper was definitely influenced by dietary copper. They observed a wide variation in liver copper of pigs fed all experimental diets. In the control fed group, the liver copper ranged from 5.1-21.0 ppm while the liver copper of the treatment fed group ranged from 24-400 ppm. They indicated possibly this phenomenon was due to dietary fat absorption and the liver function with respect to fat metabolism.

Cartwright and Wintrobe (1964) indicated that copper entering the body from the intestine was bound to albumin and was distributed to liver, bone marrow, kidney, and other tissues. Owen (1965) suggested that ceruloplasmin delivered copper to the body tissues.

Hill et al. (1964) reported that mercury interaction with copper was different from that of zinc. In the copper deficient chick, when mercury was added, growth was improved; but when copper was added, poor growth due to mercury was noticed. They indicated an interrelationship of copper and mercury and suggested that it was not an antagonism, but rather could be due to a "total heavy metal ion effect".

Hill and Starcher (1965) indicated that reducing agents such as ascorbic acid, DPPD (2-3 diphenyl-p phenylnediamine) and BAL (dimercapto propanol) could aleviate copper deficiency in chicks. This they suggested, could be due to an internal metabolism of the element, rather than to an inhibitory effect on absorption.

Suttle and Mills (1966a) reported that the addition of 750 ppm copper to a diet of fattening pigs caused toxicity in 9 out of 12 animals. They reported jaundice, increased serum copper (five fold) and high aspartate transaminase levels in these animals at 4 weeks. At 6 weeks they noticed that jaundice and aspartate transaminase levels returned to normal, suggesting adaptation to high copper intake. However, growth depression and microcitic-hypochromic anemia persisted. Addition of 500 ppm zinc or 750 ppm iron in the presence of 750 ppm copper eliminated jaundice and produced serum copper and aspartate transaminase concentration similar

to those of the control fed group. Only supplemented iron protected against anemia.

Suttle and Mills (1966b) reported that, in the absence of zinc and iron supplements, 425 ppm copper in the growing pig ration caused severe toxicosis. They indicated simultaneous addition of zinc and iron eliminated all signs of toxicosis. Further, they mentioned that, in one of the experiment, high calcium levels (1.7%) in a basal diet that contained 30 ppm zinc probably induced zinc deficiency with favored copper toxicity.

Evans and Wiederanders (1967) suggested that blood copper can be divided into four fractions such as erythrocyte components, erythrocuprin and non-erythrocuprin, and plasma components, albumin and ceruloplasmin. They analysed total plasma copper, indirect copper, and ceruloplasmin oxidase activity for human, pig, rat, sheep, cattle, dog, peacock, and turkey plasma. Total plasma copper was found to be lowest in the turkey and peacock. The turkey had the lowest erythrocyte copper. No ceruloplasmin activity was detected in either turkey or the peacock.

Dowdy and Matrone (1968a) conducted 3 experiments with sheep, chicks, and rats to study the biological interaction between copper and molybdenum. They reported that copper and molybdenum can form a complex with a molar ratio of 4:3.

Dowdy and Matrone (1968b) studied the biological availability of the copper-molybdenum complex with lambs and baby

pigs. It was found that serum copper rose in response to dietary copper supplements provided as copper sulphate, copper citrate, sodium molybdate or as the copper:molybdenum complex. The intestinal absorption of copper was not affected by molybdenum even when fed as a complex. However, the level of ceruloplasmin activity showed that the copper:molybdenum complex was inhibited and the utilization of this complex was reduced when the ceruloplasmin level obtained was compared to other sources of copper alone, or copper citrate plus sodium molybdate. They concluded that copper:molybdenum complex was biologically unavailable to the animals.

Milne and Weswig (1968) used rats to study the effect of supplementary copper on blood and liver copper containing fractions. The levels of copper fed were 1, 10, 50, 100, and 200 ppm in the form of copper sulphate. Copper in the blood was determined as erythrocyte copper and plasma copper. Most of the plasma copper was in the protein ceruloplasmin, and the remainder in a loosely bound form. The ceruloplasmin values were depressed in the low copper rations and remained constant with copper intakes greater than 10 ppm. In the liver of normal rats (fed 18 ppm copper), the distribution of copper was reported as follows: debris 12.8 percent, mitochondria 13.5 percent, microsomes 17.9 percent, and soluble fraction 54.8 percent.

Marcilese et al. (1969) studied the effect of dietary molybdenum and sulphate upon copper metabolism in sheep. In diets that contained 0.4 percent inorganic sulphate or a combination of 50 ppm molybdate plus 0.4 percent sulphate, plasma clearance of intravenously injected Cu^{64} and its uptake by the liver and incorporation into ceruloplasmin were studied. When both sulphate and molybdate were present in the diet, a reduced uptake of radio copper by the liver and an impairment in copper utilization for ceruloplasmin synthesis was observed. Stable copper in the liver and in the ceruloplasmin fraction of plasma was significantly reduced in sheep that received both sulphate and molybdenum. They observed a metabolic interference with copper by sulphate and molybdenum in the liver. This, they suggested, was due to an impairment of copper uptake by liver cells or to a primary intracellular metabolic disturbance in the synthesis of copper-protein compounds including ceruloplasmin, or to both. Dietary inorganic sulphate, in the absence of supplemental molybdenum, had no effect upon copper metabolism.

Starcher (1969) reported that studies with chicks showed that more copper was absorbed in the duodenum than in the proventriculus. This he indicated, was due to the fact that a copper binding protein which has a molecular weight of 10,000 is present at the duodenal site and is needed for the absorption. He also indicated that the antagonism of

other metals such as zinc, cadmium, and silver have been shown to have a competition in binding with the copper binding protein and thus compete with copper for absorption.

Thomas et al. (1972) fed dehydrated poultry waste at 25 and 50 percent of the ration of growing sheep. They reported the average copper level of liver tissues of the animals that were fed rations that contained DPW was 55 µg/g on a wet basis. The kidney copper level of animals fed the control ration with no DPW was 3.28 µg/g compared to the kidney copper levels of 4.31 and 3.29 µg/g from sheep that were fed rations that contained 25 and 50 percent DPW.

Zinc in Feeds and Tissues of Animals

Zinc is present in almost every tissues of animals and plants. Scott et al. (1969) indicated that the zinc concentration in the chicken is about 35 mg on a fat free basis. Scott et al. (1969) also have reported that when chickens are on a 60 ppm dietary intake of zinc then tissues (on fresh basis) have a concentration of zinc as follows: muscle 7.6 µg/g, liver 26.0 µg/g, kidney 35.8 µg/g. Underwood (1971) indicated that the zinc concentration in liver and kidney is more or less the same in man, monkey, rats and pigs. He also indicated that the concentration of zinc in different

muscle varies with their color and their functional activity. In a normal hen's egg, he suggested the zinc content varies from 0.7 to 1.0 mg, most of which is present in the yolk.

Sadasivan (1951 and 1952) reported that when rats were fed zinc supplements at 0.5 percent in the ration food, intake was not reduced but growth rate was appreciably reduced. However, when the ration feed contained 1 percent zinc oxide, a lowered food intake resulted.

VanReen and Pearson (1953a) fed high levels of zinc (0.3 to 0.7 percent zinc) in purified rat diets. Diets that were supplemented with zinc in the form of zinc oxide resulted in a depression of growth which was linear with dose rate over a range from 0.3 to 0.7 percent zinc. Further, supplementation of the toxic diet with crude liver extract overcame the effect of zinc. Enzyme changes found in liver homogenates indicated decreased catalase and cytochrome oxidase activity in the toxic state as compared to controls fed the unsupplemented, purified diet. VanReen (1953b) indicated that a dietary level of 500-700 mg percent zinc in rat diets caused marked reduction in liver catalase and cytochrome oxidase activity. He indicated that copper feeding along with toxic zinc levels resulted in an increase of liver catalase and cytochrome oxidase activities when compared to normal values. The addition of copper to the

diet did not, however, influence the growth inhibition produced by diets that contained high zinc levels.

Feaster et al. (1954) fed steers zinc in the form of zinc carbonate at 1000 ppm. A control group was fed basal ration which contained 50 ppm zinc. Both the groups were fed these levels for more than a year. In both the cases, most of the zinc was excreted in the feces while a small portion was excreted in the urine. They also reported that further studies indicated that the accumulation of retained zinc in general, was higher in pancreas, liver, pitutary, kidney, and adrenal than other tissues.

Stevenson and Earle (1956) studied the relationship of zinc and calcium in the pig diet with parakeratosis and rate of growth. The results indicated that in diets for growing pigs that contained up to 1 percent calcium, the minimum zinc content needed to prevent parakeratosis was between 44 and 80 ppm.

Pensack et al. (1956) studied the tolerance of broilers and layers to high levels of zinc in their rations and concluded that growth and appetite depression occurred only when the level of zinc exceeded 3000 ppm.

Luecke et al. (1957) reported that pigs that were fed rations that contained 0.5 to 1.90 percent calcium and 0.61 percent phosphorous had depressed growth rate and 40 to 100 percent parakeratosis. However, the addition of 50 ppm

zinc to this diet resulted in an increased growth rate, improved feed efficiency and complete prevention of symptoms of parakeratosis.

Lewis, Hoekstra, and Grummer (1957) reported that when the zinc level was restricted in the diet of pigs (28 ppm) and calcium levels were decreased from 1.2 percent to 0.5 percent, increased weight gains and the incidence of parakeratosis was reduced. Mortality was also prevented. High intake of zinc (1028 ppm) caused a significantly higher concentration of zinc in the pancreas, liver, and hair of pigs when compared to those animals that received low level of zinc (100 ppm).

Brink et al. (1959) studied the tolerance and the characteristics of zinc toxicosis in weanling pigs. Levels of zinc supplementation (as reagent grade zinc carbonate) up to 0.8 percent of the diet were fed for 42 days in a corn-soy meal ration. They reported that the addition of 0.1 percent zinc to the diet was the maximum level tolerated. Higher levels produced symptoms of zinc toxicosis. Toxic symptoms included depressed rate of gain, feed intake and efficiency of gain. Other toxic symptoms reported were arthritis, extensive hemorrhage in the axillary space, gastritis, catarrhal enteritis, congestion of the mesentry and hemorrhages in the ventricles of the brain, lymph nodes, and spleen. Mortality occurred within 21 days after the

start of the treatment. Hemoglobin values were not affected. Further, they reported that calcium added at 1 percent of the diets as dicalcium phosphate had no effect upon the level of zinc tolerance or upon toxicity symptoms.

Hoeffer et al. (1960) studied the effect of added zinc, iron, and copper (50 or 75 ppm, 100 ppm, and 125 ppm, respectively) to pig rations that contained calcium levels of 0.55, 1.05, and 1.30 percent. Parakeratosis occurred at all calcium levels that were fed but the addition of zinc prevented parakeratosis.

Cox and Harris (1960) and Magee and Matrone (1960) have studied the effect of high levels of zinc on copper and iron metabolism. Supplements of iron had no apparent effect on the cytochrome oxidase activity of rats fed a high zinc diet which indicates that copper was the important factor connected with the regression of the enzyme activity.

Roberson and Schaible (1960a) fed different zinc compounds (zinc sulphate, zinc oxide and zinc carbpnate) in semipurified diets to chicks at levels of 10 and 20 ppm to study the availability of these compounds. They reported the availability of these compounds was approximately the same at the levels (10 and 20 ppm) studied.

Roberson and Schaible (1960b) studied the effect of calcium and phosphorous on the zinc requirements of chicks. They reported that addition of 0.5 to 1.0 percent calcium

to a slightly zinc deficient ration containing a normal level (1.23%) of calcium depressed growth, feed efficiency and made zinc deficiency symptoms more severe. It was also reported that an additional 20 ppm zinc could not overcome the effect of 1.0 percent additional calcium. When the diet contained 80 percent zinc and 2.23 percent calcium, the growth of chicks was found to be normal. The addition of 0.5 percent phosphorous to the diet containing 0.6 percent phosphorous and a total of 36 ppm of zinc, exerted no adverse effect.

Ritche et al. (1963) fed zinc supplement (100 ppm) in a basal ration alone or plus supplemental copper (250 ppm), to pigs. The pigs had a significant growth increase when zinc was added to the copper supplemented ration, as compared to when zinc was fed alone. O'Dell et al. (1964) reported that phytic acid markedly decreased the biological availability of zinc. Preformed calcium phytate has little effect on zinc availability. They reported that there was a clear interaction between calcium-zinc-phytate which decreased biological availability.

Heth and Hoekstra (1965) studied the antagonistic effect of calcium on the absorption of zinc from a practical diet fed to rats. They reported that increased dietary calcium significantly increased ($P < 0.01$) the initial rate of zinc-65 absorption following oral administration. A rapid fecal excretion of zinc occurred following

administration (via feed). Calcium in the diet significantly increased ($P < 0.01$) the percentage of zinc absorption.

Heth et al. (1966a) reported that zinc absorption was significantly reduced in rats when phosphorous was supplied at 1 percent as dietary inorganic phosphorous but not when the level was 0.3 to 0.5 percent.

Heth et al. (1966b) studied the possibility of a calcium-zinc interaction existing at sites other than the intestine. Zinc-65 was injected into laying hens receiving an isolated soy protein diet containing 65 ppm zinc and 2.25 percent calcium. During the first four weeks post-injection, about one-half of the administered zinc-65 was lost from the hens. Four weeks post-injection the birds were subdivided into four groups. Dietary calcium and zinc varied between groups. Lowered zinc (10 ppm compared to 65 ppm) significantly decreased excretory zinc but not egg zinc-65 output.

Ott et al. (1966a) conducted four experiments with lambs to determine the levels of dietary zinc which could be tolerated without affecting performance. They reported that when the zinc level of the diet was above 1.5 g/kg of the diet, depressed feed consumption resulted; while 1.0 gm of zinc per kilogram of diet caused reduced gains and decreased efficiency. When the lambs were force-fed 4 to 6

gm of zinc daily, water consumption and palatability were reduced. Prolonged consumption of 4 to 6 gm of zinc daily caused death but no other symptoms were observed.

Ott et al. (1966b) fed 0.9 gm/kg zinc in the diet (provided as zinc oxide) and caused reduced gains and low efficiency in steers and calves (male and female). However, zinc levels of 0.5 gm/kg of the diet and lower had no detrimental effect. Zinc levels of 1.7 g/kg of the diet and higher caused reduced feed consumption and depraved appetite, characterized by excessive salt and other mineral consumption and wood chewing.

Ott et al. (1966c) indicated that zinc added to the diet of sheep at levels of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 g/kg resulted in the following respective zinc values for liver: 621, 614, 512, 553, 488, 530, and 350 mcg/g ($P < 0.01$), a linear effect. Respective values for kidney were 59, 68, 56, 84, 70, 67, and 66 mcg/gm. A reducing effect in the liver was noticed in the sheep for the concentration of zinc. The respective zinc, copper and iron concentration in liver from animals fed zinc at different levels were as follows: 0 gm zinc/kg--35, 48, and 32 mcg/g; 0.5 gm zinc/kg--38, 32, and 29 mcg/gm; 2 gm zinc/kg--427, 18, and 49 mcg/gm; 4 gm zinc/kg--398, 19, and 276 mcg/gm. Further, they reported that high levels of zinc altered rumen metabolism, probably through its toxic effect on rumen microorganisms.

Ott et al. (1966d) indicated that high levels of dietary zinc resulted in accumulation of high levels of zinc in blood, liver, pancreas, kidney and bone, and lesser accumulation in the hair, spleen, lung, and heart of the beef cattle. They also reported that a high level of zinc in the liver was accompanied by increased liver iron and decreased liver copper levels. When high levels of zinc were removed from the diet, the serum zinc decreased to almost normal levels within six weeks.

Thomas et al. (1972) fed growing sheep diets that contained 25 and 50 percent dehydrated poultry waste. They reported the zinc level in the tissues, such as, liver and kidneys, did not increase considerably. The sheep fed a control ration (no DPW) had zinc concentration in the kidney of 20.1 μg/g, while for the two levels of DPW rations fed to sheep, zinc levels were 17.9 and 17.0 μg/g, respectively.

EXPERIMENTAL PROCEDURE

Five hundred and eighty-eight (588), 20 week old White Leghorn pullets were randomly assigned to three different treatments (rations) starting November, 1970.

The rations fed were:

1. Basal,
2. Basal plus 12.5 percent dehydrated poultry waste (DPW) in the place of corn, and
3. Basal plus 25 percent DPW in the place of corn.

The composition of the rations can be seen in Table 1.

For the initial experimental feed, droppings were collected from hens fed a standard cage laying ration and dried according to the method described by Surbrook et al. (1970). Thereafter, at intervals that averaged 12 days each, droppings were collected from the hens fed each of the rations that contained DPW, dehydrated (as above) and incorporated into their respective diets. Thus, feed that contained DPW was fed to the birds and the droppings were collected, dehydrated and fed back to the same birds at the levels shown. Therefore each 12 day period was considered as a cycle.

From the onset of the 25th cycle, three birds from each treatment were kept separate, and eggs and excreta (feces)

Table 1. Composition of Diets

Ingredient	Percent of Diet 1	2	3
Corn	68.05	55.55	43.05
Soybean meal, 49%	16.20	16.20	16.20
Alfalfa meal, 17%	2.50	2.50	2.50
Meat & bone meal, 50%	3.50	3.50	3.50
Fish meal, 60%	1.50	1.50	1.50
Limestone, ground	6.00	6.00	6.00
Dicalcium phosphate	1.00	1.00	1.00
Salt	0.25	0.25	0.25
Fat, stabilized	0.50	0.50	0.50
Dried poultry waste	0.00	12.50	25.00
Vitamin-trace mineral premix*	0.50	0.50	0.50
Total	100.00%	100.00%	100.00%
Calculated protein, %	17.00	17.46	17.92
Fat, %	3.68	3.46	3.24
Fiber, %	3.07	4.25	5.36
Calcium, %	3.00	4.15	5.30
Phosphorus			
Total, %	.73		
Available, %	.51	.89	1.25
Metabolizable energy, cal/lb	1320	1065	702.4

*The vitamin-trace-mineral premix contained the following per lb. of premix: Vitamin A, 800,000 U.S.P. Units; Vitamin D_3, 250,000 I.C. Units; Riboflavin, 700 mg; Pantothenic acid, 1,200 mg; Niacin, 2,500 mg; Choline chloride, 39,000 mg; Folic acid, 100 mg; Vitamin B_{12}, 1.20 mg; Vitamin E, 500 I.U.; Menadione sodium bisulfite, 150 mg; Manganese, 1.287%; Iodine, .0201%; Copper .081%; Cobalt, .0051%; Zinc, 1.00%; Iron, .5025%.

were collected from these birds the last four days of each cycle (three eggs from each bird were collected in each cycle). Therefore, excreta and eggs were taken from the same birds throughout the experimental period. Plastic (whirl-pak) bags were used under each cage for the collection of the fresh excreta. Excreta thus collected from individual birds was homogenized using a spatula and set on a tray in the poultry house for drying. All excreta samples collected were air dried 3 to 6 days. After drying, the excreta samples were broken by hand into smaller pieces and then ground in a Wiley-mill using a 20-mesh screen. Care was taken always to properly clean utensils before grinding another sample.

The ground excreta samples were collected in properly identified Whirl-pak bags and stored at -20°C until further analysis.

Eggs gathered from individual birds were brought to the laboratory, separately broken open, and the contents homogenized using a blender. After homogenizing, the egg samples for individual birds were collected in properly marked Whirl-pak bags and stored at -20°C until further analysis.

On the last day of the 25th cycle, three birds from each treatment (0 percent DPW, 12.5 percent DPW and 25 percent DPW) were removed from their respective cages at

random, marked for identification, and sacrificed by cervical dislocation. Birds were posted and observed in general for any abnormalities. Then the liver, kidney, and breast muscle were removed. These organs were stored at -20°C until further analysis.

Birds were continuously observed for gross abnormalities or toxic symptoms during the period of this study. Mortality records were maintained.

At the end of alternate cycles, eggs, excreta, and tissues from birds of each treatment were obtained as described earlier. At the end of the last cycle (33rd) birds, from which eggs and excreta were collected for the experimental period, were sacrificed (by cervical dislocation) and tissues removed for analysis as outlined above.

At the termination of the experiment, all the birds were sacrificed and examined by three veterinarians from the Diagnostic Laboratory at Michigan State University.

The kidney samples from the three birds from each treatment were not adequate for the various analysis on an individual basis and, hence, the kidney samples were pooled. For all other tissues, eggs, and feces, an adequate sample was obtained for individual analysis.

The determination of mercury was carried out by the method of Hatch and Ott (1968) using a Coleman 50 Mercury Analyser. The following procedures were used for the

digestion of the various samples. (The average recovery of known quantities of mercury in this study was 80 percent).

Digestion Procedure

1. Blend (1 g.) tissue with 4.5 ml trichloroacetic acid for 10 minutes.
2. Centrifuge at 2,000 G for 15 minutes.
3. Separate layers, pour off supernatent into a beaker and save.
4. Add ppt. from centrifuge to 5 ml con. H_2SO_4 (cooled to 5°C in an ice bath).
5. Add 0.5 ml 30% hydrogen peroxide to sulfuric acid, digest and let stand overnight (15 hrs); should give a light yellow solution.
6. Add supernatent from centrifuge step to the acid digest.
7. Read on Mercury Analyzer.

Copper and zinc determinations were done for the tissues, eggs, and excreta using the technique of Atomic Absorption Spectroscopy on a Jarrel Ash atomic absorption spectrophotometer (Model 82-500, Jarrel-Ash Co., Waltham, Mass.). The results, thus obtained, were analysed statistically by Analysis of Variance (Michigan State University Computer Center (1969), by Dunnett Multiple Comparison Procedure (1955), and the standard error for means (Steel and Torrie, 1960) were tested.

RESULTS AND DISCUSSION

Mercury

The level of mercury found in the muscles of chickens fed the control ration with no dehydrated poultry waste (DPW) and the rations that contained DPW are given in Table 2. The average mercury value for the muscle from chickens fed the control ration was 0.075 ppm. The range of values obtained for mercury in the muscles from chickens fed the rations that contained 12.5 percent DPW was 0.069-0.098 ppm and the average value of mercury found in the muscles of this group of birds for the different cycles was 0.075 ppm. The range of mercury found in muscles of chickens fed the ration that contained 25 percent DPW was 0.049-0.101 ppm while the average value of mercury in muscles of those birds for the various cycles was 0.069 ppm.

Statistical analysis of these data indicated that there was no significant difference in the values of mercury levels for muscles due to the different levels of DPW used in the ration. Also, there was no significant difference observed for the muscle mercury of birds fed the various dietary treatments with respect to the different cycles.

Table 2. Effect of Continuous Recycling DPW in Laying Hen Rations on Muscle Mercury (Wet Basis)

	Muscle Mercury PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	0.069±.008[1]	0.049±.005	0.059
27	----	0.070±.001	0.057±.008	0.064
29	----	0.067±.010	0.101±.008	0.084
31	----	0.098±.002	0.075±.007	0.087
33	0.075±.006	0.069±.008	0.063±.008	0.069
Avg. level	0.075	0.075	0.069	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.00039	2	0.00019	0.4024	0.673
Cycle	0.00368	4	0.00092	1.9200	0.137
Error	0.01246	26	0.00048		

[1] ± Standard error of mean.

The mercury status of liver for the birds fed the various dietary levels of DPW and different cycles is presented in Table 3. Mercury level for the liver of birds fed the control ration was 0.142 ppm. The liver mercury of birds that were fed diets that contained the 12.5 percent DPW averaged (for the five different cycles) 0.108 ppm (range 0.062 to 0.194 ppm). The mercury level for the livers of birds fed the diet that contained 25 percent DPW ranged from 0.054 to 0.117 ppm while the average mercury level for liver from birds in the various cycles averaged 0.085 ppm. The cycle averages of liver mercury (irrespective of DPW level in the ration) for the 25th-33rd cycle, respectively, were 0.138 ppm, 0.072 ppm, 0.066 ppm, 0.089 ppm, and 0.129 ppm. Statistical analysis of this data by Analysis of Variance indicated that the levels of mercury in liver from the different cycles were significantly different ($P < 0.05$). Statistical analysis of this data by the Dunnett multiple comparison procedure showed that values for 27, 29, and 31st cycles are significantly different ($P < 0.05$) than the values for the 25th cycle.

Table 4 shows the concentration of mercury in the kidneys of the birds that received the three different rations and the kidney mercury level of those birds sacrificed at each cycle. Kidney mercury level averaged 0.125 ppm for the birds which received no dehydrated poultry waste

Table 3. Effect of Continuous Recycling DPW in Laying Hen Rations on Liver Mercury (Wet Basis)

	Liver Mercury PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	0.194±.017[1]	0.082±.024	0.138a[2]
27	----	0.080±.026	0.063±.023	0.072b
29	----	0.069±.010	0.054±.004	0.066b
31	----	0.062±.009	0.117±.022	0.089b
33	0.142±.026	0.133±.004	0.108±.008	0.129a
Avg. level	0.142	0.108	0.085	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.00572	2	0.00286	1.5532	0.231
Cycle	0.02207	4	0.00552	2.9964	0.037[3]
Error	0.04788	26	0.00184		

[1] ± Standard error of mean.

[2] Significant at $P < 0.05$ by Dunnett's Multiple Comparison Procedure.

[3] Significant ANOVA.

Table 4. Effect of Continuous Recycling DPW in Laying Hen Rations on Kidney Mercury (Wet Basis)

	Kidney Mercury PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	0.200	0.200	0.200
27	----	0.225	0.150	0.188
29	----	0.115	0.150	0.133
31	----	0.100	0.150	0.125
33	0.125	0.125	0.165	0.138
Avg. level	0.125	0.153	0.163	

in the ration. For the birds that received 25 percent dehydrated poultry waste in their rations, the kidney concentration of mercury for the different cycles ranged from 0.150-0.200 ppm with an average of 0.163 ppm for the five different cycles. The mercury in the kidneys of the birds which received the 12.5 percent DPW in their ration ranged from 0.100 to 0.225 ppm in the different cycles with an average value of 0.153 ppm for the entire period of the experiment.

Mercury levels of the eggs produced by the birds fed the three different rations and the five different cycles are given in Table 5. The mercury content of the eggs produced by the birds which were not fed DPW averaged 0.073 ppm. The concentration of mercury in the eggs produced by the birds that received the ration that contained 12.5 percent DPW ranged from 0.062 to 0.129 ppm and averaged 0.089 ppm for the five different cycles. The mercury in eggs from the birds which received 25 percent DPW in their ration ranged from 0.049 to 0.117 ppm and the average mercury value for the different cycles was 0.072 ppm. However, these differences were not statistically significant.

The average concentrations of mercury in the eggs for cycles 25-33 was 0.123 ppm, 0.089 ppm, 0.065 ppm, 0.065 ppm and 0.063 ppm, respectively. Statistical analysis

Table 5. Effect of Continuous Recycling DPW in Laying Hen Rations on Egg Mercury (Wet Basis)

	Egg Mercury PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	0.129±.018	0.117±.005	0.123a[2]
27	----	0.105±.037	0.074±.002	0.089b
29	----	0.081±.013	0.049±.005	0.065b
31	----	0.066±.002	0.064±.004	0.065b
33	0.073±.015	0.062±.012	0.054±.004	0.063b
Avg. level	0.073	0.089	0.072	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.00289	2	0.00144	2.5215	0.100
Cycle	0.1742	4	0.00436	7.6135	<0.0005[3]
Error	0.01487	26	0.00057		

[1] ± Standard error of mean.

[2] Significant at P < 0.01 by Dunnett Multiple Comparison Procedure.

[3] Significant by ANOVA.

of the data by Analysis of Variance indicated that there was no significant difference due to dietary treatment in the concentration of egg mercury. However, a highly significant difference in the mercury content of eggs for the different cycles was obtained ($P < 0.0005$). Statistical analysis by the Dunnett (1955) procedure showed that the concentration of mercury in eggs of birds fed the test diets in cycles 27, 29, 31, and 33 was significantly different ($P < 0.01$) than the concentration of mercury for eggs of birds which received the test diets in the 25th cycle.

Mercury content of the excreta from the birds fed the control ration and the two levels of DPW are presented in Table 6. The excreta from birds fed the control ration had an average mercury content of 0.075 ppm. The excreta mercury from birds that were fed the rations that contained 12.5 percent DPW averaged 0.046 ppm while that from the birds fed 25 percent DPW averaged 0.051 ppm for the different cycles. The ranges of mercury found in the extreta of birds fed 12.5 percent and 25 percent DPW were 0.017-0.068 ppm and 0.020 to 0.072, respectively. The combined average mercury concentration in the excreta from birds fed the ration that contained DPW for each cycle was 0.055, 0.044, 0.065, 0.059, and 0.037 ppm for the 25th, 27th, 29th, 31st, and 33rd cycles, respectively.

Table 6. Effect of Continuous Rec ycling of DPW in Laying Hen Rations on Excreta--Mercury (Air-Dry-Basis)

Excreta Mercury PPM

Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	0.068±.010[1]	0.042±.002	0.055a[2]
27	----	0.033±.008	0.054±.010	0.044b
29	----	0.059±.017	0.072±.009	0.065a
31	----	0.052±.005	0.065±.008	0.059a
33	0.075±.010	0.017±.006	0.020±.006	0.037b
Avg. level	0.075a[3]	0.046b	0.051b	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.00661	2	0.00330	11.6539	<0.0005[4]
Cycle	0.01742	4	0.00436	7.6135	<0.0005[4]
Error	0.00738	26	0.00028		

[1] ± Standard Error of Mean.

[2] Significant at P < 0.01 by Dunnett's Multiple Comparison Procedure.

[3] Significant at P < 0.01 by Dunnett's Multiple Comparison Procedure.

[4] Significant by ANOVA.

Statistical analysis of these data indicated that mercury levels of excreta obtained from the birds fed the different dietary treatments were significantly different ($P < 0.0005$). Analysis of data by the Dunnett (1955) procedure showed that the average concentrations of mercury in excreta of birds fed the test diets were both significantly lower ($P < 0.01$) than that found in the excreta of birds fed the control ration.

Further, testing by Analysis of Variance also indicated that there existed a highly significant difference for the mercury concentration of excreta produced during the different cycles ($P < 0.0005$). Dunnett's test indicated that the average concentrations of mercury found in the excreta from birds in the 27th and 33rd cycles were significantly lower ($P < 0.01$) than that from excreta of birds in the 25th cycle.

The results suggested that the concentration of mercury in various tissues, eggs, and excreta was different for the different treatments. Some of the differences were found to be highly significant.

Dehydrated poultry waste was the only ingredient present in the test diets which was different from the control feed. A portion of the corn was replaced in the test diets with DPW. Since DPW was the only different ingredient in the test diets, the only possible source of

variation in the concentration of mercury in tissues from the ration should have been due to this ingredient. In the same manner, the difference in the concentration of mercury in tissues of the birds that received the two test diets should have been due to the level of DPW (12.5 vs 25%) used in the diets.

The results indicated that in general there was no accumulation of mercury due to the different dietary treatments in the tissues, eggs, or in the excreta. However, statistical analysis of the data showed highly significant differences in the concentration of mercury in the above tissues and eggs from the birds that received the various rations. The concentration of mercury in general decreased in tissues and eggs of birds fed the test diets in different cycles studied. Hence, the significant difference suggested by statistical analysis was not an accumulation but rather a reduction in the level of mercury in the tissues and eggs from the birds that were fed the test diets.

A major portion of mercury present in the control ration could be assigned to the grain portion since about 70 percent grain was used in the control ration. The level of mercury in grain depends upon various factors such as different disease and pest controls used on the crop and storage of grain. The concentration of mercury in natural feed is very low (Underwood, 1971) and hence the control

poultry ration should have had a low level. In the test rations the DPW used originally (in the first cycle) was obtained by feeding the same control ration to a group of caged layers. The following changes may have taken place in the mercury in the control ration when it was converted to DPW. A portion of the mercury present in the control ration might have disappeared due to its absorption (Hill et al., 1964; Starcher, 1969) and utilization or accumulation in the liver, kidney, feathers, brain, central nervous system, and cerebro-spinal fluid, etc. (Miller et al., 1959a; Swensson, 1959; Smart and Lloyd, 1963; and Kimiawe, 1969) or may have been excreted in eggs (Smart and Lloyd, 1963; Kimiawe, 1969) or in urine (Curley, 1971). The excreted mercury in the feces may then undergo a second phase of losses due to more exposure and also fermentation (Jenson and Jernelov, 1969). Excreta was left for a period of 12 days in the poultry house before further processing.

A third type of loss of mercury (a major loss) could have occurred during the conversion of the excreta into a dried product. During this process a considerable loss of mercury was possible since the excreta was dried by a very high temperature which has been shown to volitilize mercury (Jenson and Jernelov, 1969; Underwood, 1971). Thus, DPW obtained in this manner may have a lower percentage of

mercury than that found in the control feed. Therefore, when an equal quantity of corn was removed from the test diets and replaced by DPW, lower mercury concentration in the test diets could have resulted. Hence, of those birds which were fed the three different rations, it could have been expected that the maximum ingested mercury came from the control diet. The results (Table 6) indicate that the excreta mercury from the birds fed the test diets was lower than the feces mercury from birds fed the control ration which was consistent with the hypothesis mentioned. Thus, the concentration of excreta mercury for the birds fed the control ration and the test rations was 0.075 ppm (33rd cycle), 0.068 ppm and 0.042 ppm, respectively (25-cycle). For muscle mercury a similar trend can be seen for the different cycles studied. The egg and liver mercury concentration showed similar trends but not in every cycle.

Copper

Table 7 indicates the level of copper found in the muscle of birds fed the control ration and the rations that contained 12.5 and 25 percent DPW. The average concentration of copper found in the muscle of birds fed the control ration with no dehydrated poultry waste was 0.82 ppm. The average concentration of copper in muscle of birds that

Table 7. Effect of Continuous Recycling DPW in Laying Hen Rations on Muscle Copper (Wet Basis)

Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	Cycle avg.
		Muscle Copper PPM		
Cycle				
25	----	0.95±.19[1]	0.83±.26	0.89
27	----	0.68±.16	0.75±.07	0.72
29	----	0.68±.04	0.84±.07	0.76
31	----	0.66±.01	0.79±.08	0.73
33	0.82±.10	0.87±.18	0.72±.02	0.79
Avg. level	0.82	0.77	0.79	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.0032	2	0.0016	0.0570	0.945
Cycle	0.1191	4	0.0298	1.0655	0.393
Error	0.7265	26	0.0279		

[1] ± Standard error of mean.

received the 12.5 percent DPW in various cycles was 0.77 ppm; and the average muscle concentration of copper found in birds fed 25 percent DPW was 0.79 ppm. Statistical analysis of the data indicated that there was no significant difference in muscle copper concentration due to the different rations. The concentration of muscle copper changed from cycle to cycle during the entire period for the muscles from the birds fed the ration that contained dehydrated poultry waste. These differences were not found to be significant. The range of copper concentration for the different cycles of muscles which were obtained from group 2 (12.5 percent DPW) was from 0.66 to 0.95 ppm. For the 3rd group (25 percent DPW) the range in muscle concentration of copper for the different cycles was from 0.72 ppm to 0.84 ppm.

The concentration of copper in the liver tissue of birds which received the control ration and the two levels of dehydrated poultry waste can be seen in Table 8. Average liver concentration of copper for group 1 (control ration) was 3.88 ppm, group 2 (12.5 percent DPW) 3.76 ppm, and group 3 (25 percent DPW) 4.07 ppm for the five cycles. The range of copper found in the livers of birds fed the ration that contained 12.5 percent DPW varied from 3.07 ppm to 4.21 ppm. The range of copper found in the livers of birds fed the ration that contained 25 percent DPW was

Table 8. Effect of Continuous Recycling DPW in Laying Hen Rations on Liver Copper (Wet Basis)

	Liver Copper PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle avg.
25	----	3.07±.81[1]	4.44±.17	3.75
27	----	4.10±.36	4.44±.27	4.27
29	----	3.71±.09	4.19±.05	3.95
31	----	4.21±.13	3.72±.31	3.96
33	3.88±.34	3.73±.02	3.58±.11	3.65
Avg. level	3.88	3.76	4.07	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	0.8375	2	0.4187	1.1015	0.347
Cycle	1.3376	4	0.3344	0.8796	0.490
Error	9.8840	26	0.3802		

[1] ± Standard error of mean.

from 3.58 ppm to 4.44 ppm. The combined average liver copper level of the birds fed the two levels of dehydrated poultry waste for each cycle, in the order from 25th through the 33rd cycle, was 3.75, 4.27, 3.95, 3.96, and 3.65 ppm, respectively. There was no significant difference in liver copper concentration due to dietary treatment or the various cycles.

Table 9 shows the level of copper in the kidneys of birds fed the three dietary treatments during the 25th cycle through the 33rd cycle. The average concentration of copper in the kidney of the birds that received no dehydrated poultry waste was 3.16 ppm. The kidney copper level of birds which were fed the 12.5 percent DPW in the ration averaged 3.20 ppm. The average value of copper found in the kidney of birds which received 25 percent DPW in their ration was 3.20 ppm. The kidney copper level ranged from 2.46 to 3.64 ppm for the birds which were fed 12.5 percent DPW. The range in copper level of the kidney for the birds that received the ration that contained 25 percent DPW was from 2.97 to 3.40 ppm. The combined average values of copper concentration in the kidney in each cycle from the birds fed the two levels of DPW for cycles 25 to 33 were 3.52, 3.32, 3.30, 3.09, and 2.89 ppm, respectively.

The level of copper in egg contents from the bires fed the different rations is given in Table 10. The average

Table 9. Effect of Continuous Recycling DPW in Laying Hen Rations on Kidney Copper (Wet Basis)

	Kidney Copper PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	3.64	3.40	3.52
27	----	3.37	3.28	3.32
29	----	3.29	3.31	3.30
31	----	3.22	2.97	3.09
33	3.16	2.46	3.04	2.89
Avg. level	3.16	3.20	3.20	

Table 10. Effect of Continuous Recycling DPW in Laying Hen Rations on Egg Copper (Wet Basis)

	Egg Copper PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	1.10±.06[1]	0.89±.12	0.99
27	----	0.79±.13	0.78±.06	0.79
29	----	0.52±.07	1.08±.12	0.80
31	----	1.19±.08	0.93±.02	1.06
33	0.76±.04	-.75±.09	1.13±.14	0.94
Avg. level	0.76	0.87	0.96	

Statistical Analysis

Source	Sum of Squares	D.P.	Mean Square	F-Value	Significant Level
Level	0.1282	2	0.0641	1.2995	0.290
Cycle	0.3488	4	0.0872	1.7665	0.166
Error	1.2833	26	0.0494		

[1] ± Standard error of mean.

copper concentration in egg was 0.76 ppm for the birds fed the control ration while the average levels of copper in eggs of birds fed the two levels of dehydrated poultry waste were 0.87 ppm and 0.96 ppm, respectively. The range of copper in eggs from birds which received 12.5 percent DPW in the ration was 0.52 to 1.19 ppm. The egg concentration of copper for birds that received 25 percent DPW in the ration ranged from 0.78 ppm to 1.13 ppm. The combined average copper concentration in eggs of birds for cycles 25-33 was 0.99 ppm, 0.79 ppm, 0.80 ppm, 1.06 ppm, and 0.94 ppm, respectively.

The levels of copper found in the excreta from chickens that received the various rations throughout this experiment can be seen in Table 11.

The average concentration of copper in the excreta of the birds that were fed the control ration was 66.9 ppm. The average level of copper found in the excreta of birds that were fed the ration that contained 12.5 percent DPW was 74.8 ppm. The birds which were fed the ration that contained 25 percent DPW had an average copper level in the excreta of 75.27 ppm. There was no statistical difference in the excreta copper concentration due to dietary treatment or cycle. The ranges of copper found in the excreta of the birds that received the two rations that contained DPW were 64.60-82.13 ppm (12.5 percent DPW) and 69.87-78.20

Table 11. Effects of Continuous Recycling DPW in Laying Hen Rations on Excreta Copper (Air Dry Basis)

	Excreta Copper PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	82.13±3.08[1]	78.20±.13	80.15
27	----	81.90±0.68	69.87±.48	75.88
29	----	78.80±4.00	75.70±.56	77.25
31	----	66.30±2.62	77.73±2.44	72.01
33	66.90±1.60	64.60±6.14	74.87±3.68	68.79
Avg. level	66.90	74.75	75.27	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	14.0759	2	7.0379	0.1724	0.843
Cycle	434.4767	4	108.6192	2.6610	0.055
Error	1061.2780	26	40.8184		

[1] ± Standard error of mean.

ppm (25 percent DPW), respectively. The average concentration of copper in the excreta of birds that received the two rations that contained dehydrated poultry waste was (25th to 33rd cycle) 80.15, 78.88, 77.25, 72.01, and 68.79 ppm.

The results suggested that the highest concentration of copper in tissues of birds fed the three different rations was in the liver. The results of this trial also indicated that the level of copper in kidney was close to that of the liver, while muscle copper was found to be the lowest. This trend is in good agreement with what has been reported in the literature. The liver copper and the kidney copper levels of birds that received the control ration were in good agreement with what has been reported by Beck (1956) and Scott et al. (1969).

The results of this trial did not indicate any significant accumulation of copper in any tissues, eggs, or in the excreta. There was, however, an overall slight increase in kidney, eggs, and in excreta copper. Dehydrated poultry waste was considerably higher in copper concentration than the corn it replaced in the ration, and hence, inclusion of this in the test diets should have increased the copper intake by the birds in each cycle. An approximate copper intake by the birds fed the test diets can be calculated from the 27th through the 33rd cycles since the excreta

concentration of the copper was known for these cycles. This can be calculated using the following formula.

$$(A + B) - C = X$$

where A = concentration of copper from the vitamin mineral premix,

B = concentration of copper in the excreta,

C = concentration of copper in corn, and

X = concentration of copper in the test diet.

Thus, for the birds fed the ration that contained 12.5 percent DPW, copper in the ration in the 27th cycle was calculated to be 14.38 ppm. The calculations are as follows.

A = 4.67 mg/kg copper

B = 82.13 mg/kg copper in the excreta (12.5 kgs DPW/ 100 kgs feed)

C = 4.5 mg/kg copper in corn (12.5 kgs/100 kgs feed)

$(A + B) - C = X$ (467 mg + 1027 mg) - 56 mg =

1438 mg/100 kg or 14.38 ppm.

In the same manner, copper in rations was calculated during the 29th, 31st, and 33rd cycles for the 12.5 percent DPW ration and these values amounted to 14.34, 13.96, and 12.39 ppm. The calculated copper in the 25 percent DPW rations during the 27th - 33rd cycles amounted to 23.09, 21.02, 22.37, and 22.98 ppm, respectively. The birds which received the control ration had a supplemented copper of 4.67 ppm. It was thus indicated that generally there was

about three times more copper in the test diet (12.5 percent) of birds which received the group 2 ration and a five-fold increase was seen with rations which contained 25 percent DPW. Nevertheless, feeding of these two rations to birds did not cause a corresponding increase of copper in tissues, eggs, or in the excreta. Since the copper did not accumulate in tissues or in other organs studied, it could be suspected that the additional copper ingested by birds was not utilized. Since copper was absorbed once or more from the DPW ration it was not any more available to the birds and hence was excreted. Nevertheless, the concentration of the copper in the excreta did not show any accumulation from cycle to cycle.

In every cycle there is a possibility that copper from the DPW origin may arise from a just previous cycle or also from an earlier cycle. Thus, there is a possibility that copper from the DPW portion might have undergone the digestion process and other biodegradation and physical process a number of times and this may lead towards a better availability of at least a portion of copper present in the DPW. If this occurred during this trial, the utilization of that portion of copper was not by the tissues studied. Perhaps this might have enhanced the level of copper in other tissues such as skeleton, feathers, etc. Liver copper increases concomitantly with higher intake of copper in

certain species, especially in bovine (Dick, 1954). Milne (1968) also reported that in rat's liver copper does not increase as the intake increases, unless a very high level of copper is ingested. In this trial, the copper intake was not of that high magnitude. Evans (1967) has shown that the avian species has low plasma copper and ceruloplasmin activity. Wideranders (1968) has suggested that the avian species may have an altogether different copper metabolism. Hence, the results shown in this study (no accumulation of copper) may be due to these factors. Thomas et al. (1972) reported that by feeding DPW to sheep even up to 50 percent of the ration had not elevated the copper concentration significantly in liver or in kidney of sheep.

Zinc

The concentration of zinc in muscle of birds that were fed the control ration (group 1), the ration that contained 12.5 percent DPW (group 2), and the ration that contained 25 percent DPW (group 3) is shown in Table 12. The average levels of muscle zinc for groups 1 to 3 were 6.69 ppm, 6.20 ppm, and 5.29 ppm, respectively. Statistical analysis by Analysis of Variance indicated that these values were significantly different ($P < 0.05$). Analysis of the same data by Dunnett's procedure (1955) indicated that the level of

Table 12. Effect of Continuous Recycling DPW in Laying Hen Rations on Muscle Zinc (Wet Basis)

Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	Cycle Avg.
		Muscle Zinc PPM		
Cycle				
25	----	7.25±.30[1]	6.81±1.39	7.03
27	----	6.09±1.02	4.86±0.61	5.47
29	----	6.07±0.66	5.25±0.96	5.66
31	----	6.35±0.81	4.78±0.69	5.56
33	6.69±.51	5.23±0.33	4.77±0.15	5.56
Avg. level	6.69a[2]	6.20a	5.29b	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	11.5049	2	5.7704	3.8068	0.035[3]
Cycle	14.1847	4	3.5462	2.3394	0.982
Error	39.4113	26	1.5158		

[1] ± Standard error of mean.

[2] Significant at $P < 0.05$ by Dunnett's Multiple Comparison Procedure.

[3] Significant by ANOVA.

zinc in muscle of birds fed the group 2 ration was not significantly different than the control, while that in group 3 was found to be significantly lower ($P < 0.05$) than that of the birds which received the control ration. However, there was no significant difference in muscle zinc due to the continuous recycling effect of the poultry waste.

The concentration of zinc in the liver from the birds that were fed the three rations can be seen in Table 13. The average concentration of zinc in the livers from the birds that were fed diets 1 to 3, respectively, was 21.77 ppm, 23.17 ppm, and 20.94 ppm. The average concentration of zinc (cycles 25-33) in livers of birds for each cycle which were fed the rations that contained DPW was 21.64 ppm, 23.49 ppm, 24.41 ppm, 22.26 ppm, and 19.57 ppm. There was no significant difference in liver zinc level due to ration or cycle.

The average kidney level of zinc from the birds that were fed the different rations is presented in Table 14. The birds that were fed the control ration had an average concentration of 21.6 ppm zinc in the kidney. The birds which were fed the diet that contained 12.5 percent DPW had an average concentration of zinc in the kidneys of 22.0 ppm. The average level of zinc found in kidney of birds that received the ration which contained 25 percent DPW was 19.9 ppm.

Table 13. Effect of Continuous Recycling DPW in Laying Hen Rations on Liver Zinc (Wet Basis)

	Liver Zinc PPM		
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW
Cycle			Cycle Avg.
25	----	20.55± .651[1]	22.72± .931	21.64
27	----	27.32±3.521	19.66±1.245	23.49
29	----	21.70± .483	27.12±2.331	24.41
31	----	26.67±2.380	17.85±1.821	22.26
33	21.77±.460	19.62± .719	17.33±1.095	19.57
Avg. level	21.77	23.17	20.94	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	59.4120	2	29.7060	1.9293	0.165
Cycle	123.5820	4	30.8955	2.0066	0.123
Error	400.3247	26	15.3971		

[1] ± Standard error of means.

Table 14. Effect of Continuous Recycling DPW in Laying Hen Rations on Kidney Zinc (Wet Basis)

	Kidney Zinc PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	22.0	22.0	22.00
27	----	23.5	22.0	22.8
29	----	25.3	19.9	22.6
31	----	17.8	21.6	19.7
33	21.6	21.6	13.8	19.0
Avg. level	21.6	22.0	19.9	

Zinc concentration in the eggs from the groups of birds that were fed the various rations is given in Table 15. Average concentration of zinc in eggs of birds that were fed the control diet was 13.0 ppm. Birds that were fed the diet that contained 12.5 percent DPW had an average zinc concentration of 12.5 ppm in the eggs. The average concentration of zinc in the eggs from the birds that were fed the diet that contained 25 percent DPW was 13.2 ppm. The cycle average concentration of zinc in the eggs was 16.3 ppm (25th cycle), 12.9 ppm (27th cycle), 11.4 ppm (29th cycle), 11.9 ppm (31st cycle), and 12.2 ppm (33rd cycle).

Statistical analysis of the data showed that there was no significant difference in egg zinc due to ration or cycle.

Table 16 shows the average level of zinc in the excreta of birds that were fed the three different rations. The birds fed the control ration had an average concentration of 383.0 ppm of zinc in the excreta. The birds that were fed 12.5 percent DPW in their rations had an average level of 412.2 ppm zinc in the excreta for the different cycles, while those birds that were fed 25 percent DPW in their rations had an average excreta zinc concentration of 399.1 ppm for the different cycles. Cycle averages of the excreta zinc level of birds fed the two rations that contained DPW (from 25th to 33rd cycle) were 426.1, 414.9, 420.4, 377.6, and 387.2 ppm, respectively.

Table 15. Effect of Continuous Recycling DPW in Laying Hen Rations on Egg Zinc (Wet Basis)

	Egg Zinc PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	15.6± .75[1]	17.0± .12	16.3
27	----	12.6±1.26	13.1± .29	12.9
29	----	10.5±1.07	12.2± .08	11.4
31	----	13.3±1.47	10.5±1.10	11.9
33	13.0±.79	10.3± .16	13.3±1.71	12.2
Avg. level	13.0	12.5	13.2	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	1.9252	2	0.9626	0.2458	0.784
Cycle	29.3780	4	7.3445	1.8750	0.145
Error	101.8420	26	3.9170		

[1] ± Standard error of mean.

Table 16. Effect of Continuous Recycling DPW in Laying Hen Rations on Excreta Zinc (Air-Dry Basis)

	Excreta Zinc PPM			
Level of DPW fed	0 Percent DPW (control)	12.5 Percent DPW	25 Percent DPW	
Cycle				Cycle Avg.
25	----	411.9±38.36	440.2±23.12	426.1
27	----	483.3±76.21	346.6±19.95	414.9
29	----	430.0± 0.816	410.7±17.673	420.4
31	----	363.8±36.09	391.4±28.28	377.6
33	383.0±26.50	372.1±44.92	406.6± 5.566	387.2
Avg. level	383.0	412.2	399.1	

Statistical Analysis

Source	Sum of Squares	D.F.	Mean Square	F-Value	Significant Level
Level	2506.6236	2	1253.3118	0.2145	0.808
Cycle	9119.1947	4	2279.7987	0.3902	0.814
Error	151895.9987	26	5842.1538		

Analysis of variance of the data indicated that there was no significant difference in the level of zinc in the excreta of birds due to ration fed or cycle.

The zinc concentration in the tissue of birds fed the control ration indicated that the highest tissue concentration of zinc was in the liver. Kidney concentration of zinc was close to that of the liver, while muscle zinc was the lowest of the three tissues studied. The values obtained for the liver concentration and the muscle concentration of zinc were similar to the value reported by Scott et al. (1969). The zinc values obtained in this study for the eggs of birds fed the control ration were in agreement with that reported by Underwood (1971).

The muscle zinc concentration of birds fed the three rations indicated a significant difference due to the level of DPW fed. The average value for the cycles studied showed that the zinc concentration was lower in muscle of birds fed the ration that contained DPW than in that of the birds fed the control ration. Further, the level of zinc was lower in muscles of birds fed the 25 percent DPW ration than in muscle of birds fed the 12.5 percent DPW ration in all cycles studied. Thus, the significant difference indicated by the Dunnett's procedure was not an accumulation, but rather a decrease in zinc level in the muscle due to the addition of DPW in the rations. The test diets should have

a higher calculated concentration of zinc due to the inclusion of DPW in those rations. The control ration contained added zinc of 50 ppm. The calculated values of added zinc in the test diets were as follows (vitamin premix plus the zinc from the DPW): for the 12.5 percent DPW rations the calculated added zinc was 101.5 ppm, 110.4 ppm, 103.8 ppm, and 95.5 ppm, respectively, for cycles from 27 to 33; for the 25 percent DPW ration, the calculated added zinc for different cycles (27 to 33) were, respectively, 160.0 ppm, 136.7 ppm, 152.7 ppm, and 147.9 ppm. These calculations were similar to those for copper, except that in this case, only the zinc in the vitamin premix and in the DPW were considered. Even though the concentration of zinc in the test diets were considerably higher than that of the control ration, no significant accumulation of zinc was found in eggs, tissues, or in the excreta. Pensack et al. (1956) pointed out that broilers and layers have a high tolerance for zinc. They reported that growth and appetite depression occurred only when the level exceeds 3000 ppm. High levels of zinc have also been used in swine rations without any adverse effects by many workers (Brink et al., 1959; Ritche et al., 1963, etc.). Calcium and phosphorous, at high levels, have decreased availability of dietary zinc (Luecke et al., 1957; Lewis et al., 1957; Roberson and Schaible, 1960b, etc.). O'Dell et al. (1964) has reported

that phytic acid markedly decreased the biological availability of zinc. A clear interaction between calcium-zinc phytate has been shown by these workers. The higher zinc concentration of the diets was not of a high magnitude and hence did not show any accumulation in the tissues studied. If this level was absorbed and utilized by the animals, then the other possible accumulation of that portion of zinc could be in skeleton, feather, or such tissues.

In continuous recycling of the dehydrated poultry waste, the concentration of zinc in the excreta from the birds fed the test rations was higher than from the control. Further, it was quite evident that the birds which received the 25 percent DPW in rations excreted more feces than the birds which received the other two rations. Nesheim (1972) reported a similar trend when birds were fed rations containing DPW. No accumulation of mercury, copper, or zinc was found in tissues, eggs, or in excreta of birds which were fed the test diets. Nevertheless, if one considers the volume of excreta from these birds, possibly this would explain the reason for any accumulation of these metals in excreta. Even though the quantity of excreta increased in those birds receiving the high level of DPW, a constant quantity (12.5 and 25 percent) of DPW was used in every cycle. Thus, there is a possibility of diluting the concentration of these metals in the excreta of those birds

which produced more volume of the excreta. Thus, in succeeding cycles, the concentration of these metals might continually be reduced. This may be the reason that on a unit basis there was no accumulation of these metals in the excreta of the cycles studied for the birds which received the test diets.

SUMMARY

An experiment was conducted to study the effect of recycling dehydrated poultry waste (DPW) in laying hen rations on the concentration of metals mercury, copper, and zinc in three rations. Five hundred and eighty-eight (588) pullets (20 weeks old) were randomly assigned to three rations. One group of birds was fed a commercial type layer ration; the other two groups of birds were fed rations that contained DPW at 12.5 or 25% (replacing equal amounts of corn). All other ingredients were the same in all the three rations used.

The experiment lasted for approximately 400 days or 33 cycles. Each cycle period was approximately 12 days. At the end of the 12 day period, the fecal samples were collected, dehydrated and used in preparation of the ration for the next cycle to the same birds at the levels mentioned above. At the onset of the 25th cycle, three birds were assigned from each group for collection of excreta and eggs from every other cycle (up to 33rd cycle) and then sacrificed for tissue samples. In addition, three birds from the groups fed the rations that contained the DPW were sacrificed at the end of the 25th cycle and thereafter at

the end of alternative cycles. Samples of muscle, liver, and kidney from these birds were removed and stored until further analysis.

The results were statistically analysed by Analysis of Variance, Dunnett's Multiple comparison procedure, and by Standard Error.

The results indicated that there was no significant difference found in the concentration of mercury in the muscle, liver, or egg due to the level of DPW used in the ration (0, 12.5 or 25%); however, mercury concentrations in the excreta of birds fed the 3 rations were significantly different. Statistical analysis of the data for the various tissues, eggs, and the excreta showed that the concentrations of mercury in these organs were significantly different due to the recycling effect of DPW.

The results of the copper analysis in the various tissues, eggs, and excreta indicated that there was no significant difference in the concentration of copper in any of these either due to level of DPW used or due to the recycling of DPW in various rations.

The results of zinc analysis in different tissues, eggs, and excreta, indicated that there was no significant difference in the concentration of zinc in any of these due to the recycling of DPW. However, there was a significant difference in the zinc level due to the level of DPW

(0, 12.5, and 25%) used in these rations. Statistical analysis of the data indicated that the zinc concentration in the muscle of birds fed the 25 percent DPW were significantly lower than that of the control group.

In general, continuous recycling of dehydrated poultry waste in a laying hen ration in this experiment did not tend to cause an accumulation of mercury, copper or zinc in muscle, liver, kidney, egg or excreta.

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