THE EFFECTS OF SODIUM HYDROXIDE TREATMENT AND OTHER FACTORS ON THE UTILIZATION OF DPW BY THE CHICKS

by

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ABSTRACT

Broiler performance was studied with diets containing 10, 15 and 20% dried poultry waste (DPW) or sodium hydroxide treated DPW. Feeding trials in evaluating the utilization of non-protein nitrogen in the DPW, the supplemental energy requirement, the effects of amino acid supplementation, and antibiotic supplementation on the utilization of nutrients in the diets containing DPW were carried out. Metabolism studies were conducted to determine the availability of protein and minerals in DPW.

Studies showed that DPW when added to the broiler diet at levels between 10-20% would support growth related to the dietary energy. Inclusion of DPW in the diet did not affect growth but lowered the feed efficiency when compared to the control diets containing similar energy and protein contents. Alkali treatment at various concentrations markedly improved the growth and feed efficiency of diets containing various levels of DPW.

The metabolizable energy values of untreated DPW or DPW treated with 2, 3 or 5% sodium hydroxide were determined to be 827, 1155, 1245 and 1205 kcal/kg dry matter respectively.

Supplementing the basal diets which were suboptimal in protein level with uric acid, urea, diammonium citrate or from the NPN in the DPW did not improve the growth of chicks. Nitrogen retention was reduced by the supplementation and uric acid excretion was increased indicating that these nitrogen sources would not be utilized for growth by chicks.

The availability of total nitrogen and true protein of untreated DPW, DPW treated with 2, 3 or 5% sodium hydroxide were 63.6 and 50.5%; 83.2 and 66.1%; 83.3 and 70.2%; and 90.4 and 72.2% respectively. The availability of calcium and phosphorus in untreated DPW was 53.8 and 19.8% respectively.
The calcium and phosphorus availabilities were not significantly affected by the alkali treatments. The sodium availability of untreated DPW was 84.4%, which was decreased to 41.8%-48.3% by the alkali treatments.

Supplementing the basal diets with 0.2% methionine significantly improved the growth of chicks fed the control diet but did not affect those fed the DPW diets (untreated or alkali treated). Growth of chicks fed the DPW diets were better than that of the control (without supplementation). These indicated that the amino acids in DPW could be utilized for growth.

Increasing the energy level of the diets by adding 2% fat improved the growth and nitrogen utilization of chicks indicating the poorer performance of chicks fed DPW diets was related to its energy dilution effect.

In the absence of antibiotics, the dietary fat (tallow) utilization was impaired with the inclusion of DPW in the diet. The metabolizable energy of the diets was directly related to fat utilization. Antibiotics addition completely ameliorated the adverse effect of DPW on fat utilization and markedly increased the M.E. of the diets and improved the growth and feed efficiency. Antibiotics supplementation had no effect on the nitrogen and minerals retention of the diets.
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It is the author's wish to dedicate this thesis to the honor of his mother, Mrs. C.C. Kwok, and his wife, Katie Kwok.
INTRODUCTION

In the past two decades, the trend toward confinement production has been well-established in the swine, dairy and particularly in the poultry industries. The concentration of animals in fewer and larger operation permits greater efficiency in the animal production as well as economy of operation. The accumulation of droppings creates a problem not only for the poultrymen—but also to the quality of the environment in which the poultry industry is concentrated. Disposal of animal manure becomes an increasing cost to the operation due to concentration of animals and poultry units near metropolitan areas and the need to transport waste over greater distance for land use. Also the animal wastes create water, air and soil pollution. Recently, a machine was developed which may be practical for the dehydration of poultry and livestock waste. This provides a means for easy handling of the manure as well as preventing contamination of the environment. The dried product can be sold as fertilizer or can be recycled by incorporating into the feed. However, the high cost of handling manure has reduced its competitive position as fertilizer. Conversely, the rising cost of feed ingredients increases the possibility of including animal waste in the feed. Dried poultry waste (DPW) has been shown to contain considerable amount of nutrients. Such nutrients in DPW have definite nutritive value for animals. It has been demonstrated that poultry litter and DPW can be utilized by the ruminants. The inclusion of DPW in the poultry rations has also been studied by several investigators.

Results indicate that DPW is low in metabolizable energy due to the high fibre content. It has been shown that alkali treatment can effectively degrade the fibre and cell wall constituents thus increase the digestibility of the fibre for ruminants. Smith et al. (1969) also showed that treating cow waste with alkali increased the digestibility of dry matter of the manure for
cattle. Therefore, DPW was treated with various levels of sodium hydroxide solution in an attempt to reduce the fibre content. The nutritive values of DPW and alkali-treated DPW were analysed in the following study.

Feeding trials including a determination of the dietary level of waste that chicks can utilize, evaluation of utilization of non-protein nitrogen and amino acids in the DPW and of supplemental energy requirement were performed. Metabolism studies were conducted to determine the availability of protein and mineral in the DPW with chicks. Finally, the effect of antibiotic supplementation on the utilization of nutrients in DPW was studied.
A. Factors Affecting the Composition of Poultry Wastes

The two broad types of wastes produced by poultry enterprises are:
a) caged poultry manure, the waste collected from the birds (such as layers) which are confined to wire cages or batteries, and b), poultry houses litter which is the solid waste composed of base bedding material and excreta. Dried poultry waste (DPW) refers to the manure collected from the cages or batteries and thermally dehydrated to a moisture content of not more than 15%. The average composition and range of variation of caged poultry manure and poultry house litter are shown in Table 1 (Blair & Knight, 1973a; El-Sabban et al., 1969).

Generally the moisture content of the floor litter is significantly lower as compared to fresh manure. The crude protein content of the litters, on dry matter basis, is higher than that of the manure, presumably due mainly to the contribution of wasted feed particles, bacterial synthesis, and to a minor extent the direct contribution of the base material. An important factor is that litter base tends to lower the moisture content, thus reducing the nitrogen loss. The base material is a major contributing factor for the higher crude fibre content of the litters. However, the total ash content of the manure is higher than any of the litters.

Poultry manure and litter vary widely in both physical and chemical composition due to the effect of various factors. These factors include: kind of feed, type of birds, age of the birds, number of birds per unit area, amount of litter, climatic condition during litter or manure production, methods of handling and storage of the waste.

The true protein content of the poultry waste is fairly constant while
Table 1. Chemical composition of poultry manure and poultry litter.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Poultry Manure</th>
<th>Poultry Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Dry matter (before drying)</td>
<td>35.1</td>
<td>——</td>
</tr>
<tr>
<td>(after drying)</td>
<td>89.0</td>
<td>82.7-95.0</td>
</tr>
</tbody>
</table>

Composition of Dry Matter

<table>
<thead>
<tr>
<th></th>
<th>Poultry Manure</th>
<th>Poultry Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Crude protein (N x 6.25)</td>
<td>25.3</td>
<td>15.2-38.8</td>
</tr>
<tr>
<td>True protein</td>
<td>10.5</td>
<td>8.1-14.9</td>
</tr>
<tr>
<td>Uric acid</td>
<td>6.3</td>
<td>2.8-11.4</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.8</td>
<td>0.9-3.0</td>
</tr>
<tr>
<td>N.F.E.</td>
<td>35.6</td>
<td>26.4-45.1</td>
</tr>
<tr>
<td>Available carbohydrate</td>
<td>6.7</td>
<td>2.2-13.9</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>13.8</td>
<td>10.7-19.3</td>
</tr>
<tr>
<td>Ash</td>
<td>26.5</td>
<td>18.8-40.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>7.8</td>
<td>4.9-12.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.2</td>
<td>1.7-2.8</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.42</td>
<td>0.1-0.96</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.37</td>
<td>0.04-2.09</td>
</tr>
<tr>
<td>Iron</td>
<td>0.2</td>
<td>0.1-0.42</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.63</td>
<td>0.4-1.03</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.93</td>
<td>0.56-1.39</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>61</td>
<td>47-94</td>
</tr>
<tr>
<td>Bromide (ppm)</td>
<td>16</td>
<td>7-22</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>291</td>
<td>190-405</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>325</td>
<td>210-448</td>
</tr>
</tbody>
</table>

1. Data taken from Blair and Knight, 1973; and El-Sabban et al., 1969.

2. The values, otherwise stated, are expressed in percentage.
the non-protein nitrogen content (NPN), uric acid in particularly, varies rather widely. Uric acid is the factor that largely accounts for the variability in total N or crude protein of the waste. The true proteins in the wastes represent the undigested protein residues in the feed, endogenous protein from the birds, dead microorganisms and feather debris and the non-protein nitrogenous compounds are mainly from metabolic wastes excreted in urine. The amount of nitrogen in waste is influenced by the type of feed given the birds. Creek and Vasaitis (1961) showed that the amount and percentage of dietary nitrogen excreted as uric acid increased with protein level in the diet. Kubena et al. (1973) also observed that total nitrogen and total amino acids in the excreta increased with increasing dietary amino acids from 80% to 120% of the requirement. Type of protein used in the feed also affects the nitrogen excretion. Neshein and Carpenter (1967) observed that the proportion of the total urinary N contributed by uric acid varied with the type of protein, and was greater with heat-damaged proteins. Thomas et al. (1969) also showed that the proportion of total dietary nitrogen excreted as urate was higher with feeding groundnut meal (generally considered as poor quality protein) than with good-quality fishmeal.

The manure and litter collected from broilers appear to contain more crude protein than those collected from layers. The two major fractions of crude proteins, true protein and non-protein nitrogen, were also significantly higher in broiler waste (El-Sabban et al., 1969). This can be explained on the basis that broiler rations contain a higher protein content than layer rations. Broilers are grown in larger numbers per unit area when compared to layers, thus producing more manure. Also, the higher moisture content of the wastes produced from layers and longer periods of accumulation of the hen excreta permit greater loss of nitrogen in the form of ammonia. Age is also
a factor since Kubena et al. (1973) demonstrated that total nitrogen in the excreta increased with the age of the birds, from 5 to 8 weeks of age. The total ash content is markedly higher in the layer excreta, especially the calcium and phosphorus content, due to the higher levels of these minerals used in the laying ration (El-Sabban et al., 1969).

Various factors such as bird density, litter depth, and poultry house conditions (ventilation, insulation and house temperature) affect the dry matter and the nitrogen contents of the waste material. El-Sabban et al. (1969) showed that the crude protein content is positively correlated with the dry matter content of the waste. It is probably under moist, cool conditions which favor anaerobic break-down and release of nitrogen as ammonia. Burnett and Dondero (1969) showed that extended storage of a small batch of manure containing 75% moisture resulted in a rapid decrease in the uric acid followed by the formation of the odorous substances, ammonia and aliphatic amines. However, Kubena et al. (1973) showed that when excreta was maintained under aerobic conditions there was no appreciable degradation of amino acids or loss of nitrogen. Perkins and Parker (1971), and Biely (1972) reported that the length of time that manure was kept in the house before collection had little effect on the composition of the manure if the house was well-ventilated and air-conditioned.

Various treatments and processing of the waste had a significant effect on the chemical composition. Dry heating significantly reduced the gross energy and nitrogen content (Manoukas et al., 1964; Shannon and Brown, 1969; Caswell et al., 1975). The amount of loss in nitrogen was found to be correlated with the drying temperature (Shannon and Brown, 1969) and the length of drying period (Kubena et al., 1972). The greatest loss of energy and nitrogen occurred when the sample was dried at 40° C in a vacuum oven.
(Shannon and Brown, 1969). Fontenot et al. (1971) showed that the loss of crude protein was reflected in losses of a similar magnitude for protein and non-protein nitrogen and a large loss in ammonia. Acidifying the litter to pH 6.0 prior to dry heat processing decreased the nitrogen loss substantially. Other treatments resulting in loss of nitrogen include dry-heating followed by addition of paraformaldehyde and fumigation with ethylene oxide (Caswell et al., 1975). Freeze drying, autoclaving and steam cooking, treatment with B-propiolacetone are reported to have little effect on the total and uric acid nitrogen or other nutrients of the waste (Shannon and Brown, 1969; Fontenot et al., 1971; Biely, 1972; and Caswell et al., 1975).

B. Poultry wastes as feeds for ruminants

1. **House litter**: Bhattacharya and Fontenot (1965) and Parigi-Bini (1969) reported that when poultry litter was used to replace isolated soy protein or soybean meal, partially or completely as the sole source of supplemental protein in the ration, digestibility of dry matter and crude protein of the rations by sheep decreased significantly with each increase in litter level. This indicates that the poultry litter is less digestible than soybean meal or isolated soy protein. On the other hand, Bhattacharya and Fontenot (1966) showed that substituting the basal ration of alfalfa hay and shelled corn with 25% and 50% of peanut hull or woodshaving broiler litters, there was little effect on the crude protein digestibility of the rations. Ammerman et al. (1966) reported that the apparent digestion coefficients of dry matter, nitrogen and crude fibre for lambs fed rations containing 65% citrus pulp base poultry litter were in general higher than those fed the basal mixture of Bermuda grass hay and corn meal.

When poultry litter supplied 25 and 50% of the total nitrogen in the
ration, digestibility of crude protein in the litter, calculated by difference, was 67 and 65% respectively as compared to 71% for isolated soy protein. The digestibility of crude protein in the litter was 58% with 100% of nitrogen supplied from the litter (Bhattacharya and Fontenot, 1965). Other reports showed that the apparent digestibility of crude protein from poultry litter with sheep varied from 65 to 82% (Ammerman et al., 1966; Bhattacharya and Fontenot, 1966; McInnes et al., 1968; Jeroch et al., 1970).

Positive nitrogen retention was obtained in sheep fed diets containing various levels of poultry litters. When 25 or 50% of the dietary nitrogen was supplied by the litter, nitrogen retention was not significantly lower than when soy protein supplied all of the dietary nitrogen (Bhattacharya and Fontenot, 1965). Furthermore, Ammerman et al. (1966) reported that animals receiving the poultry litter diet retained higher proportion of dietary nitrogen than those fed the basal hay and corn meal mixture. Parigi-Bini (1969) showed that although excretion of nitrogen in feces was greater on the poultry litter diet, urine was not affected. These data indicated that poultry litter nitrogen can be utilized efficiently by ruminants, especially when the level of litter nitrogen does not exceed 50% of the total nitrogen intake.

Poultry litter can be a source of energy for ruminants also. Studies in which woodshaving and peanut hull litters were substituting for 25 and 50% of the hay-corn grain ration Bhattacharya and Fontenot (1966) reported that the average digestibility of energy of the litters was 64% (calculated by difference). Average broiler litter contains 2440 kcal. of digestible energy per kg, 2200 kcal M.E. per kg and 59.8% TDN (dry basis). Parigi-Bini (1969) also reported similar M.E. value for poultry litter (2217 kcal/kg, D.M.). Brugman et al. (1968) showed that the apparent digestibility of
energy in laying house litter by cattle was 59.2%.

Litter has been estimated by different investigators to contain 16.2 to 24% digestible crude protein, (Muftic et al., 1968; Jeroch et al., 1970), starch equivalent of 35.8, and digestible energy of 2440 kcal/kg. These values compared favorably with those of good quality hay such as alfalfa. In addition, poultry litters are a good source of calcium and phosphorus (McInnes et al., 1968; Muftic et al., 1968).

Fontenot et al. (1971) noted that there were no significant differences in nitrogen utilization among animals fed broiler litter processed by the different methods including autoclaving, dry heating at 150°C for four hours, and dry heating after acidification. Caswell et al. (1975) also reported that dry heating, dry heating after the addition of paraformaldehyde and fumigation with ethylene oxide did not significantly affect the apparent digestibilities of the ration containing the treated litter with respect to dry matter, crude protein, crude fibre, nitrogen free extract and ether extract.

Broiler litter was successfully ensiled with corn forage which resulted in a sharp increase in crude protein content (Fontenot and Webb, 1974). The use of 15 or 30% litter (dry basis) in silage improved dry matter intake and nitrogen retention and had no marked effect on apparent digestibility of dry matter.

Palatability of the rations containing poultry litter can be a problem during the initial period of feeding. Southwell et al. (1958) observed that within the first few days of the test, palatability of the grain-litter mixture decreased as the amount of poultry litter was increased from 15 to 30%. Steers became accustomed to poultry litter within 3-4 weeks and thereafter acceptability was satisfactory. When cattle had access to rations containing
different proportions of broiler litter (cafeteria style) the acceptability of rations decreased as the level of litter in the ration increased (Fontenot et al., 1971). Fontenot et al. (1966) reported that during the early period of feeding a mixture containing shelled corn and 25% litter with no hay considerable digestive disturbances including marked diarrhea was encountered, hence a limited amount of long hay was necessary.

Noland et al. (1955) were among the first to report that with equal energy intake, rate of gain of fattening steers fed poultry litter was similar to cattle fed cottonseed meal. Southwell et al. (1958) showed that rate of gain of steers fed a fattening ration containing 30% corn cob broiler litter supplying all the supplemental nitrogen was similar to that of control steers. Fontenot et al. (1966) showed that rate of gain of steers fed a fattening mixture containing 25% peanut hull or woodshaving broiler litter was similar when compared to the control. The relative value of feeding different base litters in fattening mixtures at 25 and 40% levels was studied by Drake et al. (1965). Feeding litter with the four base materials, peanut hull, corn cob, grass hay and soy-bean hull resulted in similar performance.

Ray and Child (1964) reported similar performance for fattening steers fed rations containing 25% rice hull or rice hull-broiler litter which supplied the roughage portion; whereas beef calves and yearlings fed cottonseed hull and rice hull poultry litter as roughage in the finishing ration gained more rapidly and efficiently than steers fed prairie hay (Ray and Cate, 1966). Ray and Child (1965) reported that beef cows and calves were successfully wintered on tall fescue pastures supplemented with a mixture of 20% corn and 80% oat straw broiler litter.

Fontenot et al. (1966) reported that the carcass grade and dressing percent tended to be lower for the steers fed the poultry litter. However,
there were no significant difference in the carcass characteristics. Feeding broiler litter did not adversely affect the taste of meat (Fontenot et al., 1966; Fontenot et al., 1971).

Performance of gestating-lactating ewes fed a ration containing ground chicken litter was reported to be similar to that of ewes fed soybean meal ration (Noland et al., 1955). Merino wethers were given a mixture of wood-shaving broiler litter and wheat during periods of drought by McInnes et al. (1968). The mean body weight of the group was not significantly different from the group fed only wheat.

2. **Dried poultry waste (DPW) or caged manure:** The partial or complete replacement of barley or groundnut cake in the concentrate with DPW resulted in decreased digestibilities of dry matter and crude protein of ruminant rations (Lowman and Knight, 1970; Jayal and Misba, 1971). El-Sabban et al. (1970) and Bucholtz et al. (1971) also reported that the crude protein digestibility of rations containing autoclaved caged manure or DPW were significantly lower compared to the ration containing soybean meal as the sole source of protein. However, Rodriguez-Guedas (1966) showed that replacing 60% of corn meal in the concentrate with DPW increased the digestibilities of dry matter and crude protein of the diets with sheep. Bull and Reid (1971) indicated that adding graded levels of air-dried manure to a nitrogen deficient diet resulted in increases in digestibilities of dry matter, total dietary protein and total carbohydrate with steers.

Lowman and Knight (1970) determined the digestibilities of nutrients in DPW either directly by feeding 100% DPW or calculated from the regression equations on digestibilities of the proportion of DPW in the diet. The mean of the direct and extrapolated coefficients for energy was 60% and for total nitrogen was 77.2%. Thus, DPW was calculated to contain a digestible energy
value of 2170 kcal/kg D.M. and 20% apparent digestible crude protein. Bull and Reid (1971) showed that the digestibility of crude protein in DPW, calculated by difference, to be between 73.3 - 82.3% while Jayal and Misba (1971) reported 68.8%.

Even though the crude protein in the manure appeared to be less digestible than that of soybean meal the nitrogen from DPW is reported to be utilized as efficiently as the nitrogen from soybean meal by ruminants. El-Sabban et al. (1970) showed that the nitrogen retention in sheep fed rations containing autoclaved or cooked caged manure as the sole source of protein were similar to those fed soybean meal. Bucholtz et al. (1971) reported similar nitrogen retention by sheep fed DPW or soybean meal as supplemental nitrogen source.

Studies have shown that the main nitrogenous compound (uric acid) contained in the litter and manure can be utilized by rumen microorganisms. Rodriguez-Guedas (1966) calculated that 55% of the uric acid was broken down in the rumen. Oltjen et al. (1968) observed that uric acid was broken down in the rumen at a slower rate than urea. In their studies there was a trend towards more efficient non-protein nitrogen utilization when uric acid was used, compared to urea. This is further supported by Fontenot and Webb (1974) who observed that retention of nitrogen was 23% of intake from steers fed uric acid compared with 18% for those fed urea.

Bull and Reid (1971) showed that the calcium and phosphorus in manure are readily available to the ruminant and are well-utilized. In their study, over 90% of absorbed calcium and 70% absorbed phosphorus from manure were being retained by the steers.

Bull and Reid (1971) concluded that palatability of caged manure is not a serious diet problem as long as the dried manure contained less than 20%
moisture. Bucholtz et al. (1971) observed that cows fed the concentrate containing DPW refused part of the feed during the first few days of the experiment. When a more gradual change-over was made, there were no acceptability problems. Bull and Reid (1971) suggested that an adoption period of 7-21 days is needed before achieving maximum consumption of diets containing DPW. Moreover, it was shown that the use of the ensiled high moisture corn as the basal grain with DPW could shorten the adoption time and resulted in a more consistent intake. The high-moisture corn completely controlled dust and particle separation in the mix; in addition, the acidity and aroma of the ensiled corn masked any odor apparent in DPW.

Bucholtz et al. (1971) reported that average daily gain of yearling steers fed the soybean meal supplemented ration was significantly greater than the groups supplemented with DPW (32% in the ration), half DPW + half soybean meal, or half DPW + half urea; but economic value based on feed cost per unit weight gain was best for the \( \frac{1}{2} \) DPW - \( \frac{1}{2} \) urea supplemented group. Meregalli et al. (1971a) indicated that steers fed a concentrate containing 25% DPW in place of soybean meal and bran in the basal ration, with equivalent protein and energy content, grew as fast as those on the basal ration although the feed efficiency was slightly poorer. El-Sabban et al. (1970) reported that the performance of steers fed autoclaved manure ration were not significantly different from those fed soybean meal. Bulls fed concentrate with 21% DPW grew slightly faster than those fed concentrate with 25% sunflower oil meal even though both were equal in energy and crude protein (Meregalli et al., 1971b). Growth and feed intake of bullocks on the diet with all the soy and fishmeal replaced by DPW were the same as the control (Oliphant, 1974). It was shown in a number of reports that feeding DPW or caged manure did not adversely affect the carcass characteristic and meat acceptability (El-Sabban et al., 1970; Bucholtz et al., 1971; Oliphant, 1974).
Bucholtz et al. (1971) fed lactating dairy cows with concentrate mixtures containing up to 30% DPW. Milk production and production persistency from animals fed waste containing diets were normal and satisfactory compared with animals fed nitrogen from soybean meal or NPN from silage. Studies on feeding DPW to dairy cattle by the Agricultural Development and Advisory Service of the U.K. Ministry of Agriculture (Blair and Knight, 1973b) showed that a ration including 20% DPW and fed at the same rate as the control ration gave a production equivalent to that of the control. When the DPW ration was fed a rate of 20% above the control, it resulted in an increase of five percent more milk. Bull and Reid (1971) observed that cows would consume enough manure to meet their protein needs when it was the sole source of supplemental nitrogen in an otherwise low-nitrogen diet. These workers suggested that DPW could serve as the sole source of supplemental nitrogen for cows producing up to 28 kg milk per day. Furthermore, feeding DPW did not affect the milk quality and flavor or animal health.

Lowman and Knight (1970) fed a range of diets in which DPW replaced barley at levels of 0-100%. They demonstrated that an equal combination of barley and DPW can support medium to high rate of growth with sheep. If the cost of DPW was half of that of barley, 100% DPW was the cheapest feed for maintenance, and for liveweight gain 50% and 50% barley was the most economical combination. Rodriquez-Guedas (1966) reported that growth of lambs fed pea straw and concentrate containing 35% carob bean meal or DPW were similar.

Zorita et al. (1966) fed ewes during the second half of gestation and 40 days of lactation with pelleted concentrate containing 60% DPW. Birth weights and daily weight gain of the lambs and weight pattern of ewes were considered normal. In a latter study Zorita et al. (1967) showed that milk
yield and weight change of ewes fed concentrate with 50% DPW were not different from the group fed standard concentrate.

Hence, it can be seen that the nutritive values of litter and caged manure for the ruminants are similar. These excretion products can be an economic sources of energy as well as nitrogen and minerals for ruminants.

C. Poultry wastes as feed for swine

Geri (1968) fed young pigs with diets containing 7-10% poultry manure to replace some bran in a balanced feed for 4 weeks. Those fed manure containing diets had lower daily weight gain and higher feed intake per kg gained. The younger pigs (17 kg) given manure were not as healthy as the control (many developed diarrhea). When antibiotics and vitamin B_{12} were supplemented and the manure diet was given to older pigs (32 kg) daily gain and feed efficiency were slightly better than the control. Results obtained at the Harper Adams Agricultural College (Blair and Knight, 1973b) indicated that five percent DPW could be included in swine rations without influencing growth rate and feed efficiency. There was a depression in live weight gain and feed efficiency with 10% DPW in the feed. Perez-Aleman et al. (1971) and Denisov et al. (1975) calculated a significant linear relationship between the amounts of manure added to the conventional diet on growth and feed efficiency. Growth was reduced by 0.02 kg/day, feed efficiency by 0.25 unit and killing out percentage by 0.96% for every 10% addition of manure. However, these workers concluded that it might be economical to include DPW at a level of 10% in the diet, which would save 3% of the conventional diet.

In spite of its adverse effect on growth, feeding manure decreased the backfat thickness and increased the meat:fat ratio which might improve the overall grading of the carcasses (Perez-Aleman et al., 1971; Osterc, 1972;
Denisov et al., 1975). Since the manure contains large amounts of fibre and ash and therefore relatively low digestible energy, Osterc (1972) concluded that the use of DPW for finishing swine was justified but with an adjustment of the ration to balance its low energy value.

D. Poultry wastes as feeds for poultry

Calvert et al. (1971) compared the nutritive value of DPW, or biodegraded hen manure with soybean meal. Chick weight was substantially less when soybean meal in the basal diet was replaced by 22% biodegraded manure or DPW but growth was slightly better with DPW than cellulose. This suggests that DPW does not seem to be of any value when substituted for soybean meal in the chick diet. Hodgetts (1971) also concluded from the results of the experiment with laying hens that DPW seems to have little or no nutritional significance and merely acts as a filler or diluent in the ration.

When levels of DPW up to 20% were fed to broiler chicks from 7 to 28 days Rinehart et al. (1973) reported that there was a linear increase in feed consumption and a depression in feed conversion with limited effect on weight gain. Broiler fecal volume increased in a direct relationship with consumption of DPW, which suggested a low nutrient utilization of the material. Furthermore, it was shown that the substitution of 5 and 10% DPW in a low protein, low lysine control diet presented no amino acid type response. Also the metabolizable energy content of DPW was shown to be zero with chicks. These results therefore indicate that DPW has no value for the young chick.

Sloan and Harms (1973) showed that body weight and feed conversion were depressed progressively as the level of DPW was increased from 5-20% in a basal containing 24% protein. They compared the substitution of sand at levels of 5 and 10% to the same levels of substitution with DPW. Improved results
were obtained using sand over DPW. The authors suggested that some factor is presented in the DPW (perhaps uric acid) that masks the bird's ability to eat to meet its energy requirement, thus having a depressing effect upon growth and feed utilization.

In spite of these findings, favorable results have been obtained by other workers. Wehunt et al. (1960) added broiler manure, hen manure and soybean meal to provide additional 1.5 or 3% crude protein (N% x 6.25) to the basal containing 15% protein, with dextrose and cellulose in the diets being adjusted to equalize energy. Growth was improved by the supplementations. However, the chicks receiving supplemental nitrogen from manure required more crude protein per unit gain in body weight than those received from soybean meal suggesting the crude protein (or nitrogen) in the manure was less efficiently utilized than soybean meal. Nevertheless, all lots required about the same amount of true protein per gm. gain, indicating the true proteins in the manure were about equally efficient as compared with soybean meal.

Similar growth responses from adding DPW to diets which were sub-optimal in protein or non-essential amino acids were obtained by McNab et al. (1972), Lee and Blair (1972) and Lee and Blair (1973). McNab et al. (1972) added 5-20% DPW to a basal diet, which contained suboptimal level of non-essential amino acids but sufficient essential amino acids to meet the minimum requirement, and the diets were isocaloric. Growth of the chicks fed the DPW diets were equal to chicks fed a standard broiler ration and consistently better than those fed the low-protein basal diet. Similarly, adding 20% DPW to a semi-purified diet containing only 10% of the essential amino acids resulted in an improvement in growth, which was similar to the effect of adding an isonitrogenous amount of glutamic acid (Lee and Blair, 1972; 1973).
However, the feed conversion efficiency of the DPW diets was lower than that of diets supplemented with glutamic acid, showing that the amino acids of the protein in the DPW were not utilized as efficiently as glutamic acid.

In another study, Lee and Blair (1973) showed that the overall feed conversion efficiency of a broiler starter diet containing five percent DPW was better than that of an isonitrogenous-isocaloric control. This suggested that the true protein present in DPW can be utilized by the chick, and also some of the energy of DPW may have been utilized since the M.E. of DPW was assumed to be nil when formulating the diets.

Using an artificial anus Yoshida and Hoshii (1968) showed that the digestibility of N in the broiler excreta by hen was 52.8%. However, McNab et al. (1974) using similar technique and fed with 99.5% DPW showed that true digestibility of the total N in the DPW was 70.5%. The true protein in the DPW was 64.2% digestible while the digestibility of uric acid was very high, up to 91%. The digestibilities of amino acids were the lowest with valine (24.7%) and highest with cystine and serine and an average value of 54.4%. Polin et al. (1971) observed that only 34% of the total nitrogen in DPW was used as a protein source by laying hens.

The primary deficiency in DPW is the low metabolizable energy content. Pryer and Connor (1964) showed that about 1/3 of the gross energy contained in feces could be utilized by the chicken (M.E. value of 1.09 - 1.11 kcal/gm D.M.). Polin et al. (1971) using white Leghorn chicks showed that the M.E. of DPW was 1.29 or 1.40 kcal/gm; depending on the mathematical approach used to evaluate the data. Shannon et al. (1972) reported that the M.E. values of several samples of DPW varied from 0.64-1.27 kcal/gm D.M., with a mean of 0.97 kcal/gm. From these M.E. values, it is noted that the DPW nutrient profile was quite similar to other fibrous feedstuffs, including bran and alfalfa meal.
Part of the variability in M.E. value can be attributed to the different formulation of the original diets fed to the birds and variations in the quantity of feed spillage in the manure. Presser and Ousterhout (1972) demonstrated that excreta produced from birds fed low fibre-high calorie diets were somewhat better utilized when recycled. Young and Nesheim (1972) showed that DPW from hens fed 19.8% wheat bran had a M.E. value of 0.18 kcal/gm as compared to 0.66 kcal/gm for DPW from hens fed a standard laying ration, which supports findings of Presser and Ousterhout (1972).

Ousterhout and Presser (1971) showed that recycling manure resulted in a 25% utilization of the total dry matter nutrients in the manure. Young and Nesheim (1972) found that 30% of the dry matter was digested. Shannon et al. (1972) demonstrated the digestibilities of organic matter and dry matter of DPW to be about 18.6 and 24.4%. These figures are close to the figure suggested by the M.E. value. The total carbohydrate content of DPW is about 50% or less but only 1/7 is shown to be available to the bird (Blair, 1974).

McNab et al. (1974) reported that the apparent digestibilities of calcium and phosphorus varied widely with 1.2 - 45.3% for calcium and 7.5 - 46.2% for phosphorus. Parker et al. (1959) reported that the available phosphorus, as determined by the method of the A.O.A.C. (1945), in broiler manure was 94% and in hen manure was 88%.

The low digestibility of the nutrients and available energy in the manure can be explained by the fact that in modern high-energy poultry rations, 70%80% of the energy yielding components and other nutrients of the diet are digestible and may be metabolized by poultry. The remainder of the gross energy, indigestible components of the diet, or dietary components not retained by the animal are found in the excreta. The high fibre and ash, and low fat and digestible carbohydrates contents in the excreta account for the
low M.E. value. Nevertheless, the protein contained in the DPW can be utilized by the chicken.

Non-protein nitrogen (NPN) in poultry excreta is 47 to 64% of the total nitrogen, of which 30 to 60% is uric acid. Davidson and Thomas (1969) partitioned the total nitrogen into 19% as true protein, 5% as free and bound amino acids, 60% as uric acid, 9% as ammonia, 1.7% as urea and 1.2% as creatine and creatinine.

Wiseman et al. (1956) noted that when chickens were fed folic acid-deficient diets containing antibiotics, there was an increase in numbers of folic acid synthesizing coliform bacteria primarily *Aerobacter* species. Since the *Aerobacter* species found in the intestinal contents were able to utilize uric acid, Wiseman proposed that antibiotic supplement would result in better growth of chicks through encouraging the increase in numbers and activity of bacteria so removing a potentially toxic substance, uric acid, from the intestine. Results presented by Bare et al. (1964) gives support to the above hypothesis. Adding two percent uric acid to the basal diet significantly depressed the growth of the chicks and this growth depression was alleviated by supplementing with antibiotics. Chemical analysis of the intestinal content revealed an increased degradation of uric acid in the tract of the "uric acid-antibiotic" fed chicks. They proposed that uric acid depresses growth by acting as an irritant, since it is an insoluble waste product that occurs in high level in the intestinal tract when ingested by the chick and thus, interferes with the absorption of nutrients from the intestine. Concurrently, Lau and Wiseman (1964) showed that the addition of uric acid to a synthetic medium reduced the in vitro production of riboflavin by cultures of bacteria isolated from the intestinal contents of rats and from human feces. Therefore, the growth depressing effect of uric acid
could result from the inhibition of the synthesis of vitamins or other unidentified nutritional factor for the chick by the intestinal microflora without affecting the bacterial growth.

Lee and Blair (1972) supplemented the basal diet which contained crystalline essential amino acids only with uric acid and obtained no growth response; in fact a slight depression in growth was observed. Baker and Molitoris (1974) showed that uric acid supplementation to a basal crystalline amino acid diet deficient in non-specific nitrogen did not elicit a growth response. On the other hand, Stapleton and Biely (1975) did not obtain a growth depression as Bare et al. (1964) did when two percent of uric acid was added to a 20% protein chick diet. They concluded that dietary uric acid was not, in itself, a factor in depressing weight gain of chicks; yet it is not utilized by the chick to any extent.

Sullivan and Bird (1957) first demonstrated that the chick can use diammonium citrate and urea when fed low protein diets, provided adequate essential amino acids are present. Using purified diets designed to supply a balanced sufficiency but not an excess of essential amino acids the supplementation with diammonium citrate, triammonium citrate or urea resulted in a growth response (Featherstone et al., 1962; Blair et al., 1972; Lee and Blair, 1972). Plasma amino acids levels were increased by the supplementations, and carcass composition studies showed that the additional weight gains by the birds receiving these nitrogen sources were accompanied by deposition of protein indicating the chick is able to convert the nitrogen from these compounds to amino acids and proteins. It is therefore concluded that nitrogen from ammonium compounds or urea is likely to be useful in partially satisfying the non-essential amino acid requirement of the birds only when the diets contained a balanced sufficiency but not excess of essential amino
acid together with a deficiency of non-essential amino acids or non-specific nitrogen. However, Allen and Baker (1974) showed that the organic ammonium compounds and urea were less efficacious than L-glutamic acid or a mixture of dispensable amino acids.

The use of non-protein nitrogen sources for growth by chicks on free amino acid diets is well established; but, the responses in a practical diet remain questionable. Only Miller (1973) showed that supplementing the 12% protein basal diet which contained fishmeal as the sole source of protein, with ammonium compounds or glutamic acid at level equivalent to 3% protein in most cases improved the growth of chicks. According to Blair and Waring (1969) chicks responded to diammonium phosphate at the 1.5% dietary level supplemented to a practical diet which supplied all the essential amino acids at minimum required level and 19.5% protein, but not at the 3% level (equivalent to 2.3% protein). Other workers (Moran et al., 1967; McNab et al., 1972; Balloun and Kazemi, 1975; Trakulchang and Balloun, 1975a) showed that at low levels of inclusion (less than 3%) diammonium citrate might not have any effect on growth. At higher levels (5% or above) it depressed growth, reduced feed consumption and nitrogen retention in most instances. March and Biely (1971) in adding one or two percent urea to diets containing 20, 22 or 24% protein obtained depression in growth of chicks from day old to 8 weeks of age. Kazemi and Balloun (1972) showed that nitrogen retention was markedly reduced when urea or diammonium citrate was included in the diet.

Most of the published data have shown that the nitrogen from non-specific sources can be effectively utilized by the laying hens for egg production. Young et al. (1965) showed that the addition of diammonium citrate equivalent to three percent protein to a practical 13% protein diet improved egg production. This was equal to that obtained with a 16% intact protein diet.
Reid et al. (1972) indicated that supplementation of the low-protein basal diets which contained the essential amino acids to meet the requirement with non-specific nitrogen such as ammonium sulfate, diammonium phosphate or citrate improved egg production and the efficiency of conversion of essential amino nitrogen into egg protein.

Moran et al. (1967) did not obtain an improvement in egg production with diammonium citrate supplementation to the diet containing sufficient essential amino acids but deficient in nitrogen. In another trial, the addition of diammonium citrate equivalent to 5% protein in a 10% protein practical laying diet depressed egg production significantly. Fernandez et al. (1973) also obtained a depression in egg production when diammonium citrate or phosphate was added to a 11.5% protein practical diet or a semi-purified diet that contained all essential amino acids to meet the N.R.C. requirements but inadequate in non-essential amino acids or non-specific nitrogen.

The effects of urea supplementation on egg production are inconclusive. Chavez et al. (1966) and Moran et al. (1967) reported that urea supplementation had no effect on egg production while Fernandez et al. (1973) showed a graded response in egg production to urea supplementation at 0.65 and 1.25% levels.

Early studies with poultry manure indicated that poultry feces contained an unidentified growth factor for chicks. Rubin et al. (1946) reported that broiler chicks from day old to 6 weeks of age fed diets containing 5% urine-free hen feces in place of equal amount of corn in the basal showed improved growth over those fed the basal. Elam et al. (1954) showed that the addition of fish solubles, antibiotics or autoclaved poultry litter suspension (after filtered through cheese-cloth) to the diet improved the chick's growth. Additional response was obtained when the litter suspension was fed in
combination with the antibiotics. Hydrolysed poultry manure was demonstrated to be as effective as or even superior to fish soluble or distiller's solubles in supplementing the chick diet with unidentified growth factors required by chicks for optimum growth (Fuller, 1956; Wehunt et al., 1960). Rubin et al. (1946) believed that the factor was probably synthesized in the lower portion of the gut where absorption is not very good or perhaps in the voided feces.

Flegal and Zindel (1971) observed that the body weight gain of White Leghorn chicks was not influenced when up to 20% of DPW was included in the diet. The diets tested were similar in true protein content but no iso-caloric. Feed efficiency was inversely related to the level of DPW in the diet indicating the energy dilution effect of DPW. However, broiler chicks could tolerate only 5% DPW in the diet without adverse effect on growth and feed efficiency. Growth and feed efficiency were improved by adding 4.5% to a diet containing 20% of DPW.

Biely et al. (1972) indicated that DPW has a definite value as a broiler feed ingredient. Inclusion of DPW at 5-20% levels could lower the cost per pound of gain when compared to the control ration even though growth and feed efficiency of chicks on the DPW diets were slightly depressed.

Bhargava and O'Neil (1975) demonstrated that by adjusting for the energy and protein content in diets DPW up to the 20% level had no adverse effect on growth to 8 weeks of age, edible meat ratio or carcass grade of broilers. The addition of lysine or methionine singly or in combination did not affect growth response. In another study, when balanced for the amino acid content of the diets by supplementing with lysine, methionine and arginine, Trakul-chang and Balloun (1975b) showed that the additional protein from 10 to 20% to the diets effectively increased growth. Cunningham and Lillich (1975) reported that only the group receiving 38.2% DPW had growth depression in a study
with various DPW levels in isocaloric and iso-proteinous diets. The dressing percentage, carcass quality and meat flavor were not affected by feeding high levels of DPW. Trakulchang and Balloun (1975b), however, showed that DPW at 10% of the diet did not affect the weight gain or feed efficiency but at 20% performance was significantly reduced even though the diets were isocaloric and iso-proteinous.

Trakulchang and Balloun (1975c) continued recycling the excreta from the chicks fed the DPW diets. Diets containing 0, 10 and 20% were originally formulated to be equivalent in energy, protein, calcium and phosphorus content. DPW recycling, at both 10 and 20% dietary levels significantly depressed weight gain of birds 4 to 8 weeks of age. As the number of recyclings increased calcium content in the excreta decreased linearly so that the calcium : phosphorus ratio of the diet was decreased from a ratio of 1:1 during the first cycle to 0.34:1 after the 4th recycle. This may be a factor in the growth depression previously discussed.

Quisenberry and Bradley (1969) were among the first to report that including 10 and 20% DPW in the diets had no adverse effect on the egg production, egg weight, feed efficiency and mortality of the laying hens. Flegal and Zindel (1971) showed no significant effect on egg production from the inclusion of up to 30% DPW in the diets without adjusting for the energy content; but feed efficiency was inversely related to the level of DPW in the ration. Including 40% DPW in the diet significantly depressed the egg production, which was not alleviated by the addition of fat, although it improved the feed efficiency of the diet. Birds receiving rations containing more than 10% DPW did not have body weight increases comparable to the control during the laying period. Hodgetts (1971) added 10% DPW in the basal and observed no reduction in egg production and feed efficiency; however, the feed cost
was reduced by the DPW inclusion.

Flegal and Dorn (1971) reported that egg production of groups fed diets with 12.5 and 25% DPW as a replacement for corn in the control was not different from the control although feed intake was increased. Young and Nesheim (1972) also observed that egg production or egg weight was not affected by the inclusion of 22.5% DPW in the diet but feed conversion and body weight gain were adversely affected. It was noted that the laying hens increased food intake to achieve a constant daily metabolizable energy intake. Fecal volume increased directly in proportion to the level of DPW and energy content of the diets. These authors concluded that DPW is a low energy, low protein material with an apparent utilization of not more than 30%. At dietary levels up to 25% it does not affect egg production. Conversely, Trakulchang and Balloun (1975b) observed that DPW at these levels depressed egg production and feed efficiency and increased mortality even though diets were isocaloric and iso-proteinous.

Blair and Lee (1973) supplemented the basal laying diet containing 11.5% protein with 9.7% autoclaved DPW. Feed intake, egg production, total egg mass, mean egg weight and Haugh score were increased by such supplementation. Rinehart et al. (1973) showed that egg production was increased by 6.1% when DPW was added to the diet containing 80% of the amino acid requirement of the laying hen. These reports illustrate that laying hens can utilize the amino acids and protein contained in the DPW. Rinehart et al. (1973) concluded that DPW had 30-35% of the value of corn for laying hens.

In general, egg size tended to decrease as the level of DPW increased in layer diets. However, the shell thickness and Haugh units were not affected by feeding DPW (Flegal and Zindel, 1971; York et al., 1970). The storage quality, color, odor, taste and/or microbial content of eggs produced from
hens fed up to 30% DPW were not significantly different from the control (Flegal et al., 1970; York et al., 1970).

Ousterhout and Presser (1971) and Young and Nesheim (1972) calculated that the recycling of DPW in laying ration could reduce the disposing problem by no more than 25%. This would still leave 75% of the manure to be disposed off by other means.

E. Effect of feeding poultry wastes on animal health

There are risks due to residues in the poultry excreta in causing health hazards in animal receiving rations containing the waste material. The first is the disease risk. Freshly voided excreta, particularly from caged stock, can contain viable organisms such as clostridia, salmonella, corynebacteria and mycobacteria (Alexander et al., 1968; Kraft et al., 1969). Many of these bacteria are normal inhabitants of the intestine of poultry and while they are not pathogenic to poultry they are known to cause blackleg, gas gangrene and enterotoxemia in cattle. However, the Salmonella and Arizona species are not highly resistant to heat in litter of normal moisture content and are unable to survive in built-up litter or in silage (Tucker, 1967; Alexander et al., 1968; Messer et al., 1971). Fontenot et al. (1971) and Caswell et al. (1975) observed that autoclaving, dry heating at 100° C for up to 48 hours, or treatment with B-propiolactone or ethylene oxide (though not able to completely sterilizing the litter) was effective in pasteurizing the waste. Shannon et al. (1972) examined a range of poultry manure dried by a variety of commercial procedures and concluded that the number of organisms were so small that the risk of a disease is low. Furthermore, no disease problems have been reported from the inclusion of poultry wastes in rations for cattle, sheep and poultry.
The main risk from residues in wastes is from chemicals and drugs. Griel et al. (1969) reported abortion in cows fed dried litter from a roaster operation. The roaster feed had 0.15–0.23 kg of 14% dienestrol diacetate premix per ton and bioassay of the litter showed it had extremely high estrogenic activity.

A musty taint in eggs and broiler meat due to the presence of anisoles in litter has been reported by Curtis et al. (1972). The trouble was attributed to the phenolic compounds added to timber as antifungal agents, which were subsequently converted microbiologically to anisoles in the litter. Therefore poultry litter which is known to have originated from treated timber is probably not suitable for feeding to animal stock.

Copper toxicity was observed in ewes fed poultry litter containing high levels of copper (Taylor, 1971; Webb et al., 1973). However, in a study by Lowman and Knight (1970), DPW with a copper content almost double that of barley (73 vs 48 ppm) showed a low digestibility of copper with sheep (24.2%). Hence, the available copper content of DPW could be equal to or lower than other feedstuffs. Moreover, the copper problem will not likely be as severe in cattle since sensitivity to copper is less than that in sheep.

No marked levels of DDT, DDE or other pesticides were detected in poultry litter sampled from various locations (Fontenot et al., 1966; Messer et al., 1971; Fontenot et al., 1971). Feeding DPW or broiler litter did not result in pesticide residues or chlorinated hydrocarbon compounds accumulation in liver and fat of fattening cattle (Fontenot et al., 1966; El-Sabban et al., 1970; Fontenot et al., 1971).

No residues were detected in the various organs of lambs fed litter which was shown to contain amproleum and arsenic residues from the feed (Brugman et al., 1969). Webb and Fontenot (1972) found residues of penicillin, Chlortetra-
cycline, nicarbazin and amproleum in broiler litter. Steers fed as high as 50% of such litter for 121 or 198 days did not show an increase in amprolium, nicarbazin and chlortetracycline level in tissues.

Little work has been reported for the effect of waste recycling on health problems in poultry. Varghese and Flegal (1972) demonstrated that continuous recycling DPW to pullets did not alter the levels of arsenic, mercury, copper and zinc in the tissues, eggs and feces.

These data indicate that the medicinal and pesticide residues in poultry excreta do not appear to cause a serious problem. Besides, drug residues in caged manure would probably be of little consequence since laying hen rations contain little, if any drug additives.

F. Effect of intestinal micro-organisms on nutrients utilization

When young chicks were given fresh chicken feces or suspension of fresh dropping, the growth rate may be lowered and mortality may increase. This growth depression can be alleviated by the addition of antibiotics to the feed. Heating at 100° C or autoclaving will also eliminate the growth depressing effect of fresh feces (Kratzer et al., 1951; Warden and Schaible, 1961; Yates and Schaible, 1961). This indicates that the microorganisms present in the feces may be responsible for the growth depression from feeding the feces. Furthermore, the chick's requirement for phosphorus and magnesium were higher when 0.5 or 1.0% fresh droppings was included in the feed or by administering orally a water suspension of feces. Feeding sterilized chicken feces did not affect the nutrient requirements (Edwards and Boyd 1963). On the other hand, many studies showed that feeding antibiotics might lower the requirement for several nutrients. These studies suggest that the micro-
organisms in the intestine interfere with the nutrient utilization and subsequently affect the growth. The influence of intestinal micro-organisms on host nutrition has been discussed quite thoroughly by Jayne-William and Fuller (1971).

Comparison of the activities of intestinal proteases in germfree and conventional chickens shows that gut microflora exerts little or no effect on the enzyme activities (Lepkovsky et al., 1964). However, many reports have shown that dietary antibiotics can improve protein utilization in chickens (Anderson et al., 1952, Machlin et al., 1952; Slinger et al., 1952; West and Hill, 1955). When the protein content of the diet is below that required for maximum growth, growth of chickens is improved by feeding antibiotics, which indicates that the intestinal micro-organisms compete with the host as the amount of protein available becomes limiting. On the other hand, when fed a diet containing ample readily digestible protein (26%) there is no difference between germfree and conventional birds in their efficiency of protein utilization (Miller, 1967).

Young et al. (1963) observed that fatty acids were absorbed better by chicks when reared in a fumigated laboratory or fed antibiotic than in a contaminated environment. Furthermore, germfree environment appears to increase the retention of total fat by the chick (Boyd and Edwards 1967).

The influence of the microflora on the absorption of fat and fatty acids depends on the type of fatty acids. Absorption of the saturated fatty acids, palmitic and stearic acids, are lower in the conventional chicks than in germfree chick, but the absorption of the unsaturated fatty acids, oleic and linoleic acids, are not affected by the presence of microflora (Boyd and Edwards 1967, and Cole and Boyd 1967). Hence fats containing high proportions of saturated fatty acids, such as tallow, will be affected to a greater extent
than those containing high proportion of unsaturated fatty acids, such as corn oil, by the presence of the microflora.

Intestinal micro-organisms do not seem to affect fat absorption by reducing the lipase activity since the lipase activity in the intestine was found to be similar between germfree and conventional chicks (Lepkovsky et al. 1964). Studies have shown that intestinal bacteria such as *Clostridium welchii*, *Streptococcus faecalis* and certain other bacteria, mostly strict anaerobes are able to split conjugated bile acids in taurine or choline and the constituent acid can be further degraded (Rosenberg 1969 and Flock et al. 1972). This would severely affect the function of bile salt in fat digestion and absorption.

The hydrolyzed products of lipolysis at the duodenum, i.e. monoglycerides and free fatty acids, are incorporated into micelles and then uptake and esterification of fats by the intestinal mucosal cell occur. Bile salts are obligatory for micelle formation. The function of bile salts in the duodenum and jejunum depends not only on concentration but also on chemical form. Free bile acids are incapable of incorporating products of lipolysis into micelles. In vitro studies suggest that free bile acids may be inhibitory to several mucosal cell functions including active transport of sugars and amino acids and reesterification of fat in the mucosa (Rosenberg 1969 and Holt 1972). Therefore bacteria proliferate in the proximal intestine and free bile acids will be formed. Since free bile acids do not function as micelle forming agents and the effective concentration of bile salts will fall below those required for micelle formation, fat absorption will be impaired.

Lindblad et al. (1954) and Simco and Stephenson (1961) showed that the addition of antibiotics to diets would elicit greater growth response with diets containing 0.4 - 0.6% calcium and 0.2% phosphorus than with higher
levels of the minerals. Body weight of chicks fed these low levels of calcium and phosphorus with antibiotics often were similar to those fed 1% calcium and 0.2% phosphorus but without antibiotics. The percent bone ash of chicks fed the suboptimal levels of calcium was also increased by the antibiotic feeding. Kirchgessner and Friesecke (1965) showed that the retention of calcium and phosphorus was increased by feeding with aureomycin. However, the effect of antibiotics on calcium and phosphorus balance and retention was not apparent when the diets were adequate in these minerals (Ahuja et al. 1971). Hence, it appears that the influence of antibiotics on calcium and phosphorus utilization in the chicks is marked only when the diets contain suboptimal or inadequate amount of these nutrients.
METHODS AND MATERIALS

Experiment 1. Effect of sodium hydroxide treatment on the chemical composition of DPW.

A batch of DPW obtained from Fraser Valley Organic Ltd. was used to study the effect of sodium hydroxide treatment on the nutrient value of poultry waste. The waste was collected from commercial broilers raised on heated concrete floor with no bedding materials and dried in a gas heated drum drying system. Equal weights of water or 2, 3, 4, 5 and 7% sodium hydroxide solution was added to the DPW, thoroughly mixed and dried under a heater (about 40°C) for 2 days. The dried samples were ground and analysed for: acid-detergent fibre content by a rapid micro-digestion method described by Waldern (1971); total nitrogen (using the macro Kjeldahl method - AOAC 1965); and uric acid content by a modified method described by Stapleton & Biely (1975). The true protein and non-protein nitrogen content of the DPW samples were determined by the procedure described by Hawk et al. (1954) who adopted it from Folin & Wu for the determination of non-protein nitrogen in the serum. The method used was as followed: 1 gm of the DPW samples was weighed into a 50 ml Erlenmyer flask and 25 ml of 0.2 N sulfuric acid was added. The mixture was boiled gently on a water bath with occasional stirring for 30 minutes. The flask was removed from the water bath, 10 ml of 10% sodium tungstate solution was added and placed at room temperature for another 30 minutes with occasional stirring. The solution was then filtered, the filtrate and the residue were analysed for nitrogen by the macro-Kjeldahl method. The amount of nitrogen in the residue was multiplied by a factor of 6.25. This is the true protein in the sample while the amount of nitrogen in the filtrate is the non-protein nitrogen content of the sample.
One sample of untreated DPW and the sample treated with 2% NaOH were hydrolyzed with 3N hydrochloric acid for 16 hours at 121°C. The hydrolysate was analysed for amino acid contents by an automatic amino acid analyser.

The pH of the DPW samples and percentage of sodium hydroxide residual in the DPW were determined by stirring 1 gm of the sample in 20 ml of distilled water and the pH of the solution was measured. It was then filtered and the filtrate was titrated with standard hydrochloric acid using phenol red as the indicator. The sodium hydroxide equivalent present in the DPW sample was calculated and expressed as the percentage of the original sodium hydroxide added to the DPW.

**Experiment 2.** Performance of broiler chicks fed of untreated DPW or DPW treated with various concentrations of sodium hydroxide and the M.E. values.

Untreated DPW or DPW treated with 2, 3 and 5% sodium hydroxide solution were included in diets at levels of 10, 15 and 20%. All diets were formulated for equivalent true protein content. The composition of the diets are shown in Table 2. The DPW sample contained approximately 13% true protein and the M.E. was estimated to be 1000 kcal/kg. The control 1 diet contained 23% protein and a calculated M.E. value of 2959 kcal/kg, which was comparable to the diet containing 10% DPW; The control 2 diet was formulated to provide a protein content and M.E. value similar to those diets containing 15 and 20% DPW.

Broilers chicks (five days of age) were used with each experimental diet fed to three replicates of eight birds each. Birds were randomly assigned to electrically heated battery brooders and feed and water were supplied ad libitum. Chicks were weighed at the beginning of the test period and weekly thereafter during the three week study. Body weight gain and feed conversion
Table 2. Composition of the diets in Experiment 2.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Control 1</th>
<th>Control 2</th>
<th>10% DPW</th>
<th>15% DPW</th>
<th>20% DPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground wheat</td>
<td>61.5</td>
<td>30.0</td>
<td>46.5</td>
<td>41.5</td>
<td>36.5</td>
</tr>
<tr>
<td>Ground barley</td>
<td>--</td>
<td>33.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DPW^2</td>
<td>--</td>
<td>--</td>
<td>10.0</td>
<td>15.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Soybean meal</td>
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<td>27.5</td>
<td>28.0</td>
<td>28.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Meat meal</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
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<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
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<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
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<tr>
<td>Ground limestone</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Micronutrients^3</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Calculated M.E. kcal/kg</td>
<td>2959</td>
<td>2754</td>
<td>2919</td>
<td>2805</td>
<td>2697</td>
</tr>
<tr>
<td>% True protein</td>
<td>23.01</td>
<td>22.96</td>
<td>22.85</td>
<td>22.83</td>
<td>22.83</td>
</tr>
</tbody>
</table>

1. The same formulation was used in Experiment 5, 6 and 7 for preparing the basal diets.
2. The DPW used was untreated or treated with 2, 3 or 5% sodium hydroxide.
3. The micronutrients supply per kg. of feed;
   riboflavin, 6.6 ug.; calcium pantothenate, 8.8 mg.; niacin, 22 mg.;
   choline chloride, 220 mg.; amprolium, 500 mg.; ethoxyquin, 500 mg.;
   manganese sulfate, 308 mg., zinc oxide, 60.5 mg.; copper sulfate,
   31 mg.; iodized salt, 0.25%.

Unless otherwise stated, the premix also contained 11 mg. of zinc bactracin per kg. of feed.
ratios were calculated at weekly intervals.

Broiler chicks (three weeks of age) were used to determine the M.E. of untreated DPW, and DPW treated with 2, 3 and 5% sodium hydroxide solution. DPW samples were substituted in the basal ration at 20% or 30% with 0.3% chromic oxide marker added to each diet. Each treatment consisted of three replicates with 8 birds each. The birds were fed the test diets for three days. A sample of feces was collected on the 4th, 5th and 6th days from each group, pooled together and freeze-dried. The feed and feces were analysed for gross energy by oxygen bomb calorimetry, chromic oxide content by the method of Czarnocki et al. (1961); and nitrogen content by the macro-Kjaldehl method. The metabolizable energy values for the basal and DPW diets were calculated according to the method of Hill and Anderson (1958).

\[
E \text{ diet} = \text{combustible energy per gm of diet dry matter}
\]

\[
E \text{ excreta} = \text{combustible energy in excreta per gm of diet dry matter}
= \text{combustible energy/gm excreta} \times \frac{\text{Cr}_2\text{O}_3 \text{ per gm diet}}{\text{Cr}_2\text{O}_3 \text{ per gm excreta}}
\]

\[
N = \text{Nitrogen retention per gm of diet dry matter}
= \frac{N}{\text{gm diet}} - \frac{N}{\text{gm excreta}} \times \frac{\text{Cr}_2\text{O}_3 \text{ per gm diet}}{\text{Cr}_2\text{O}_3 \text{ per gm excreta}}
\]

\[
\text{M.E. kcal/gm diet} = E \text{ diet} - E \text{ excreta} - 8.22 \text{ N}
\]

\[
\text{M.E. of DPW (kcal/gm)}
= \frac{\text{M.E./gm test diet} - (\text{M.E./gm basal diet} \times \% \text{basal in test diet})}{\% \text{DPW in test diet}}
\]

Experiment 3. Utilization of the non-protein nitrogen from the DPW or from the various nitrogenous compounds by broiler chicks.

A basal diet (20% protein) was formulated to supply suboptimal level of protein for maximum growth of broiler chicks. Two DPW diets were prepared,
each containing 20% of untreated DPW or DPW treated with 2% NaOH solution. Non-protein nitrogen supplemented diets were prepared by substituting alphacel in the basal with uric acid, urea or diammonium citrate which was equivalent in nitrogen content to NPN in the DPW diets. The composition of the diets are shown in Table 3. All diets were equivalent with respect to their true protein and calculated M.E. contents. A high protein control diet containing the same amount of metabolizable energy as the experimental diets but contained 23% protein was also used.

Day old broiler chicks were used in this experiment with each treatment consisting of five replicates with 5 birds each. Chicks were randomly assigned to in electrically heated raised floor battery brooder. Experimental diets were fed to chicks (4 weeks), with the body weight gain and feed consumption recorded weekly.

When the chicks were 3 weeks old they were used for nitrogen retention determination. Chicks of each treatment were starved for 16 hours, then fed the experimental diet for 12 hours and starved again for 12 hours; afterwards they were returned to the ad-libitum feeding. Feces were collected during this 24 hours feed-and-fast period, freeze-dried and weighed. The feed consumption was also recorded. The nitrogen content of the feed and feces was determined according to macro-Kjeldahl method (A.O.A.C.); uric acid content of the DPW diets and the feces collected from each treatment were determined according to modified method described by Stapleton and Biely (1975).

**Experiment 4. Availability of total nitrogen, true protein and minerals in DPW and sodium hydroxide treated DPW.**

The availability (or true digestibility) of total nitrogen, protein, calcium, phosphorus and sodium in the untreated DPW or DPW treated with 2, 3
Table 3. Composition of diets used in Experiment 3

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diets</th>
<th>High Protein</th>
<th>Basal Control</th>
<th>Uric</th>
<th>Urea</th>
<th>DAC&lt;sup&gt;1&lt;/sup&gt;</th>
<th>DPW&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>30.0</td>
<td>48.0</td>
<td>48.0</td>
<td>48.0</td>
<td>48.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Barley</td>
<td></td>
<td>33.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>--</td>
</tr>
<tr>
<td>DPW&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>20.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td></td>
<td>27.5</td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>Meat meal</td>
<td></td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
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<td>1.0</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tallow</td>
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<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td></td>
<td>--</td>
<td>2.0</td>
<td>1.0</td>
<td>1.22</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>NPN sources&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td>--</td>
<td>--</td>
<td>1.0</td>
<td>0.78</td>
<td>2.0</td>
<td>--</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ground limestone</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Micronutrients&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Calculated

- M.E. (kcal/kg) 2754, 2825, 2825, 2825, 2825, 2765
- True protein (%) 22.96, 20.08, 20.08, 20.08, 20.08, 20.0
- Total N (%) 3.67, 3.21, 3.57, 3.57, 3.57, 3.57

1. Diammonium citrate.
2. DPW used was untreated or treated with 2% NaOH.
3. Non-protein nitrogen was supplied by uric acid, urea or diammonium citrate.
4. The micronutrient premix used was same as in Experiment 2.
or 5% NaOH solution was determined according to the method of Bragg et al. (1969) with slight modification.

According to the method of Bragg et al. (1969) the birds were fed the marker diet immediately after feeding the purified diet or the test diet. However, it was found in a preliminary study, that some of the undigested material from the previous diet tended to mix up (in the intestine) with the ingesta from the marker diet as it was fed immediately following the test diet. Therefore, the procedure was modified to allow sufficient time for evacuation of the excreta of the previous diet from the intestine before feeding the marker diet. The marker diet was used to indicate the complete excretion of the feces from the previous diet.

The composition of the purified diet (nitrogen free-mineral free) used consisted of glucose 81.0%, alphacel 8.8%, corn oil 10.0% and choline chloride 0.2%. Each of the DPW samples was mixed with the purified diet at 1 : 1 ratio in the test diets. The marker diet used was a practical starter diet containing 0.3% ferric oxide.

Sixty three week-old broiler chicks were used in the digestibility determinations. The test birds were fed a commercial starter diet from one day to 21 days of age. They were then randomly assigned to 12 groups with 5 birds per group and three groups per dietary treatment. The total weight of birds in each group was similar. Chicks were housed in electrically heater batteries with stainless steel feeders and waterers. The birds were fed the marker diet on the first day and the feed was withdrawn in the afternoon (6 p.m.) of the second day. These birds were starved for 14 hours, followed by feeding the purified diet for four hours. Birds were fasted again for four hours and fed the marker diet. Excreta from the purified diet (containing no ferric oxide) were collected. The same procedure was repeated on the following day.
with the test diets (50% DPW + 50% purified diet) replacing the purified diet. Excreta from the test diets were again collected. Weight of the test diets consumed were measured. Water was limited during the starvation period to avoid excessive consumption of water and consequently production of extremely wet feces.

The feces collected from each group were freeze-dried and the weight was individually measured. The test diets, and the feces from the purified diet and test diets were analysed for total nitrogen by the A.O.A.C. method; true protein as described before; and calcium and sodium contents by atomic absorption spectrometry following wet digestion with perchloric and nitric acid by the method of Johnson and Ulrich (1959). The phosphorus content of all samples was determined with a spectrophotometer following development of color with ammonium molybdate.

The availability of each individual nutrient was calculated according to the following formula:

\[
\% \text{ nutrient availability} = \frac{\text{Total consumed} - (\text{total in feces from test diet} - \text{total in feces fed})}{\text{Total consumed}} \times 100
\]

The four samples of untreated DPW or DPW treated with 2, 3 and 5% sodium hydroxide were also analysed for phytate phosphorus and phytic acid according to the method of Wheeler and Ferrel (1971).

Experiment 5. Effect of amino acid supplementation on the performance of chicks fed diets containing untreated DPW or alkali-treated DPW.

The amount of available lysine, arginine, methionine + cystine and threonine in the control and DPW diets used in Experiment 2 were calculated
and are shown in Table 4. The amino acids in the untreated DPW were assumed to be 50% available while that of the alkali-treated DPW were about 70% available, and the amino acid compositions of the two samples of DPW determined in Experiment 1 were used to calculate the amount of available amino acids contributed by the 20% DPW in the diets. It was noted that both of the control diets (control 1 and 2) were suboptimal in the sulfur-containing amino acids while the DPW diets contained higher levels of the sulfur-containing amino acids than the controls. All diets were suboptimal in available lysine as compared to the level recommended by NRC (1971) nutrient requirements. In this experiment, three basal diets were prepared with the composition of Control diet 2 and 20% DPW diets as in Experiment 2 (see Table 2). One of the DPW diets contained untreated DPW while the other contained 3% sodium hydroxide treated DPW. The basal diets were without or with 0.2% D-L methionine, or with 0.2% D-L methionine + 0.1% L-lysine or with 0.2% D-L methionine + 0.2% lysine supplement. The high-energy control (Control)1)s as in Experiment 2 but supplemented with 0.2% D-L methionine and 0.1% lysine was also used for comparing the performance of chicks fed the medium-high energy and low energy diets. There were a total of 13 dietary treatments included in this study.

Three hundred and ninety day-old broiler chicks were randomly assigned to electrically-heated battery brooders with 10 birds per replicate. Three replicates were used per dietary treatment. The birds were given the test diets and water ad libitum for four weeks. Body weight and feed consumption were measured weekly.

**Experiment 6.** Effect of increasing the energy content of the diets with supplementary tallow on performance of chicks fed the diets containing untreated DPW or alkali-treated DPW.
Table 4. Available amino acid content of the diets (calculated$^1$)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>N.R.C. Requirements</th>
<th>Levels in Diets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control 1</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.40</td>
<td>1.42</td>
</tr>
<tr>
<td>Lysine</td>
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<td>1.16</td>
</tr>
<tr>
<td>Methionine + cystine</td>
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<td>0.69</td>
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<tr>
<td>Threonine</td>
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<td>0.82</td>
</tr>
<tr>
<td>Protein (true)</td>
<td>23.0</td>
<td>23.01</td>
</tr>
</tbody>
</table>

1. The available amino acid content of the ingredients was based on the figures provided by the Agricultural Extension Service, University of Arkansas, 1966.

2. The available amino acid content of the untreated DPW was estimated by multiplying the determined amino acid content of a sample of DPW by a factor of 50%.

3. The available amino acid content of the NaOH treated DPW was estimated by multiplying the determined amino acid content of the 2% NaOH treated DPW by a factor of 70%.
Three basal diets as in the previous experiment containing a metabolizable energy value of approximately 2750 kcal/kg were prepared. One of the diets contained no DPW, while the other two contained either 20% untreated DPW or 20% alkali-treated (3% NaOH) DPW. The energy content of these diets were increased by approximate 100 kcal/kg by adding 2% animal fat to the diets with the soybean meal contents being adjusted so that all diets were equivalent in the true protein contents. No antibiotics were added to the diets. All diets were supplemented with 0.2% D-L methionine and 0.1% lysine. The composition of the diets are shown in Table 5.

Day old broiler chicks were randomly distributed to each of the battery brooders. Three replicates of 10 chicks each were used per dietary treatment. The test diets and water were supplied ad libitum for four weeks. Body weight and feed consumption of each group were measured at weekly interval.

Sample of feces was collected from each replicate and freeze-dried when the chicks were seventeen days old. Crude fat content by extracting the sample with anhydrous diethyl ether for sixteen hours using the Soxphlet apparatus was determined for feed and feces. The gross energy content of the feed and feces were determined by bomb calorimetry using the Parr plain jacket oxygen bomb and the nitrogen content was determined using the Kjeldahl method. The acid insoluble ash content of the feed and feces was determined according to the method described by Vogtmann et al. (1975).

The apparent digestibility of fat, metabolizable energy value and nitrogen retention of the diets were calculated using the following formulas:

\[
\text{App. digestibility of fat} = 1 - \frac{\% \text{Fat in excreta}}{\% \text{Fat in feed}} \times \frac{\% \text{ash in feed}}{\% \text{ash in excreta}}
\]

\[
\text{M.E.} = \text{GE}_F - \frac{\% \text{ash in feed}}{\% \text{ash in excreta}} \times \frac{\% \text{ash in feed}}{\% \text{ash in excreta}} - 8.22 (N_F - \frac{\% \text{ash in feed}}{\% \text{ash in excreta}})
\]
Table 5. Composition of diets used in Experiment 6.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Low-fat Basal Diets</th>
<th>Fat-supplemented Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DPW&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ground wheat</td>
<td>30.0</td>
<td>36.5</td>
</tr>
<tr>
<td>Ground barley</td>
<td>33.0</td>
<td>--</td>
</tr>
<tr>
<td>DPW</td>
<td>--</td>
<td>20.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.5</td>
<td>28.0</td>
</tr>
<tr>
<td>Meat meal</td>
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<td>3.0</td>
</tr>
<tr>
<td>Cereal grass</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tallow</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>1.5</td>
</tr>
<tr>
<td>Ground limestone</td>
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<td>1.0</td>
</tr>
<tr>
<td>Micronutrients&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td><strong>100</strong></td>
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</table>

Calculated chemical composition

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<th>Control</th>
<th>DPW&lt;sup&gt;1&lt;/sup&gt;</th>
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<tr>
<td>M.E. (kcal/kg)</td>
<td>2755</td>
<td>2720</td>
<td>2844</td>
<td>2810</td>
</tr>
<tr>
<td>Protein (true) %</td>
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<td>23.14</td>
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<td>23.06</td>
</tr>
<tr>
<td>Crude fibre %</td>
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<td>7.17</td>
<td>3.90</td>
<td>7.10</td>
</tr>
<tr>
<td>Calcium %</td>
<td>1.26</td>
<td>2.0</td>
<td>1.27</td>
<td>2.01</td>
</tr>
</tbody>
</table>

1. The DPW used was untreated or treated with 3% NaOH.

2. The micronutrient premix used was same as in Experiment 2 except without zinc bacitracin and supplemented with 2 g. D-L methionine and 1 g. L-lysine monohydrochloride per kg. of feed.
where \( GE_F \) = gross energy of the feed kcal/gm D.M.

\( GE_E \) = gross energy of the excreta kcal/gm D.M.

\( N_F \) = Amount of nitrogen per gm D.M. of feed

\( N_E \) = Amount of nitrogen per gm D.M. of excreta

\[
\% \text{ N retention} = 1 - \frac{\% \text{ N in excreta}}{\% \text{ N in feed}} \times \frac{\% \text{ ash in feed}}{\% \text{ ash in excreta}}
\]

**Experiment 7.** Effect of antibiotics supplementation on the performance of chicks fed with the DPW diets.

The three basal diets containing no antibiotics from Experiment 6 were again used in this experiment. In addition, each of the three basal diets was supplemented with zinc bacitracin at 44 mg/kg and procaine pencillin at 11 mg/kg to give the "antibiotic-fed" diets.

Day old broiler chicks were allotted to groups of 10 chicks each in electrically-heated brooder batteries. Each experimental diet was fed to triplicate lots of chicks from day old to 4 week of age. The groups fed the antibiotic supplemented diets were kept separated from the control groups so as to avoid possible contamination between the dietary treatments. Body weight and feed consumption were measured weekly.

Samples of feces from each lot were collected and freeze-dried on the 7th, 15th and 27th day. The feed and feces were analysed for crude fat content by extracting with anhydrous diethyl ether for four hours with the Goldfisch apparatus. The gross energy content of the samples was determined by bomb calorimetry and nitrogen by Kjeldahl method. The calcium content of the feed and feces were determined by atomic absorption spectrometry following wet digestion with perchloric and nitric acid. The phosphorus content was
determined by the spectrophotometry method as described before.

Acid insoluble ash was used as the index substance for calculating the digestibility coefficients and metabolizable energy values. The apparent fat digestibility, M.E. and percent nitrogen retention of the diets fed to the chicks at 1, 2 and 4 week of age were calculated according to the formulas in the previous experiment. In addition, calcium and phosphorus retention of the chicks at 2 week of age with the different dietary treatments were also determined.
RESULTS AND DISCUSSION

Experiment 1. Effect of sodium hydroxide treatment on the chemical composition of DPW.

The chemical composition of the samples of DPW untreated or treated with various levels of sodium hydroxide solution are shown in Table 6. Sodium hydroxide treatment slightly reduced the acid-detergent fibre (cellulose and lignin) content of DPW; however, a significant decrease was observed only at the 7% level of NaOH treatment. The alkali treatment significantly reduced the true protein content of the DPW at levels above 2%, and at 7% the effect was most pronounced showing a severe destruction of protein. The non-protein nitrogen and uric acid contents of the DPW were also reduced in relation to the level of sodium hydroxide used. The destruction of true protein, non-protein nitrogen and uric acid are apparent following treatment due to the strong odor of ammonia observed.

The amino acid composition of a sample of untreated DPW and the sample treated with 2% sodium hydroxide solution is shown in Table 7. Most of the amino acids were not affected by the treatment. Lysine, arginine, serine, proline, leucine and cystine showed slight reduction while alanine showed an increase with the treatment.

When 2% sodium hydroxide was added to the DPW all of the sodium hydroxide had reacted with the material with only a slight increase in pH of the sample. Treatments with 3 to 5% NaOH significantly increased the NaOH residue in the DPW sample. Therefore, levels above 5% showed an undesirable effect.

DeGroot and Slump (1969) observed that treatment of food proteins with alkali might induce chemical changes which were attended with the occurrence
Table 6. Effect of sodium hydroxide treatment on chemical composition of DPW

<table>
<thead>
<tr>
<th>Chemical composition (%)</th>
<th>Level of (%) Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Acid-detergent fibre</td>
<td>24.3</td>
</tr>
<tr>
<td>True protein</td>
<td>14.6</td>
</tr>
<tr>
<td>Non-protein-nitrogen (% N x 6.25)</td>
<td>11.7</td>
</tr>
<tr>
<td>Uric acid</td>
<td>3.5</td>
</tr>
<tr>
<td>Percent residual of sodium hydroxide</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Table 7. Amino acid composition of a sample of untreated DPW and the sample treated with 2% NaOH.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>% in sample</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated DPW</td>
<td>Alkali-treated DPW</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>0.48</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.29</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1.11</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.0</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>0.49</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>0.64</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.59</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>0.88</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0.93</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.58</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.25</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td>1.49</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.33</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.54</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.84</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.33</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.51</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>
of a new peptide, lysinoalanine, and with decreased content of cystine and to a lesser extent, lysine and serine. Cystine was decreased by 50% or more in samples of casein and isolated soy protein treated at pH 12.2 (0.2 M NaOH) and further decreased by raising the temperature above 40° C during drying. At lower NaOH concentrations (pH below 10) the effect on the amino acid content was not great. In this study, the effect of NaOH treatment on the amino acid content of DPW is in agreement with results of DeGroot and Slump (1969). However, the degree of reduction is less than that observed by the above authors even though the concentration of NaOH used in this study is higher (2% vs 0.8% or 0.2 M). There is an increase in alanine content in the NaOH treated DPW. This may be due to the formation of the lysinoalanine during the treatment followed by acid hydrolysis or other chemical changes. A primary change in proteins by alkali treatment, as proposed by DeGroot and Slump (1969), is the formation of dehydroalanine residues from cystine and serine residues. This compound may react with the E-amino group of lysine to give lysinoalanine.

Experiment 2. Performance of broiler chicks fed various levels of untreated DPW or DPW treated with various concentrations of sodium hydroxide and the M.E. values.

The effects of inclusion of untreated or sodium hydroxide treated DPW at levels of 10, 15 and 20% in the diets on the growth, feed consumption and feed conversion ratios are shown in Table 8.

The growth and feed efficiency of chicks fed the control 2 diet, (low energy control) were significantly lower than those on control 1 (high energy control). Including 10% DPW in the diet did not affect the performance of chicks, although the feed conversion ratio was slightly higher than the high energy control. As the untreated DPW in the diet increased from 10% to 20%,
Table 8. Effect of including untreated and sodium hydroxide treated DPW in diets on 3 weeks body weight gain, feed consumption and feed conversion ratio.

<table>
<thead>
<tr>
<th>Level of NaOH Treatment (%)</th>
<th>Body Weight Gain (g.) (^1)</th>
<th>Total Feed Consumption per bird (g.)</th>
<th>Feed Conversion Ratio (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Control 2</td>
<td>10% DPW</td>
</tr>
<tr>
<td>0%</td>
<td>426.2 (^{abcd})</td>
<td>375.6 (^{a})</td>
<td>432.9 (^{cd})</td>
</tr>
<tr>
<td>2%</td>
<td>--</td>
<td>--</td>
<td>470.4 (^{de})</td>
</tr>
<tr>
<td>3%</td>
<td>--</td>
<td>--</td>
<td>434.8 (^{cd})</td>
</tr>
<tr>
<td>5%</td>
<td>--</td>
<td>--</td>
<td>457.6 (^{de})</td>
</tr>
</tbody>
</table>

1. Body weight gain = final body weight -- initial body weight
2. Feed conversion ratio = g. of feed/g. of gain.
3. Different subscripts indicate significant difference (P < 0.05).
growth of the chicks was progressively decreased. Body weight gain of the chicks fed the 15% DPW diet (402 g) was between those of the high-energy control (426.2 g) and low-energy control (375.6 g). This is directly related to the calculated M.E. content of the diet, which is below that of high-energy control but above that of low-energy control. Body weight of chicks fed 20% DPW (388.1 g) was much below that of the high-energy control but similar to those of low-energy control. Therefore, the poor growth of chicks fed the 20% untreated DPW diet, as compared with the high-energy control further supports the effect of dietary energy on growth. When compared to a similar energy control diet, addition of untreated DPW as high as 20% did not adversely affect growth. The feed conversion ratio progressively increased as the level of untreated DPW in the diet increased from 10-20% and was significantly greater at 20% DPW than that of the low-energy control, indicating that the diets containing high levels of untreated DPW were utilized less efficiently when compared to the control. However, higher feed consumption at the 15 and 20% DPW supported growth equal to or better than that of the low-energy control.

All levels of sodium hydroxide treatment significantly improved the growth and feed conversion ratios of the chicks fed the diets containing high levels of DPW (above 10%). Chicks fed the sodium hydroxide treated DPW grew as well as those fed the high-energy control and in some cases had better growth. All NaOH treated DPW diets supported growth better than the low-energy control. This is due to the increase in feed consumption with equal or better utilization of feed by the chicks fed NaOH treated diets compared to the low energy control. These results suggest that the NaOH treatment significantly improved the M.E. content of the DPW. The improved energy level is further confirmed by the comparison of M.E. values for the untreated and sodium hydroxide treated DPW as shown in Table 9.
Table 9. Effect of sodium hydroxide treatment on metabolizable energy value of DPW

<table>
<thead>
<tr>
<th>Level of NaOH treatment (%)</th>
<th>Metabolizable Energy Value (kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20% inclusion in the basal</td>
</tr>
<tr>
<td></td>
<td>30% inclusion in the basal</td>
</tr>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>0%</td>
<td>725</td>
</tr>
<tr>
<td></td>
<td>930</td>
</tr>
<tr>
<td></td>
<td>827</td>
</tr>
<tr>
<td>2%</td>
<td>1165</td>
</tr>
<tr>
<td></td>
<td>1145</td>
</tr>
<tr>
<td></td>
<td>1155</td>
</tr>
<tr>
<td>3%</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>1291</td>
</tr>
<tr>
<td></td>
<td>1245</td>
</tr>
<tr>
<td>5%</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>1110</td>
</tr>
<tr>
<td></td>
<td>1205</td>
</tr>
</tbody>
</table>
The M.E. values of 0, 2, 3 and 5% sodium hydroxide treated DPW were 827, 1155, 1245, and 1205 kcal/kg respectively. Addition of 2% sodium hydroxide significantly improved the M.E. of the DPW, but no further increase with higher NaOH dosage was observed. The value of 1155 kcal/kg for the 2% NaOH treated group is relatively low when compared to grains such as barley and oats even though these grains have a similar protein content compared to DPW. The high fibre content of the DPW reduces the available carbohydrate in the DPW. It is apparent that the sodium hydroxide treatment may degrade the plant cell walls and increase the enzymatic digestion which resulted in an improvement in the utilization of nutrients contained in the DPW and in the M.E. content.

Results of this study indicate that DPW added to the broiler diet at levels between 5 and 20% will support broiler performance related to the dietary composition, especially energy. Chemical treatment (NaOH) improved the nutritive value of DPW in which the major improvement is credited to increased available energy. The treatment does not appear to have any adverse effects on the bird with NaOH concentration up to five percent.

Experiment 3. Utilization of the non-protein nitrogen from the DPW or from the various nitrogenous compounds by broiler chicks.

The body weight gains, feed consumption and feed conversion efficiencies of the chicks fed the experimental diets for four weeks are shown in Table 10. The body weight gains of chick fed the basal diet were not different from those fed the 23% protein diet. Apparently, the chicks tended to consume more feed so as to satisfy their protein requirement, as their food consumption was higher than those fed the high protein diet. The growth rates of chicks fed the diets containing 20% untreated DPW, alkali treated DPW or 1% uric
Table 10. Body weight gains, feed conversion ratio and feed consumption of chicks fed the different diets for 4 weeks in Experiment 3.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Body weight gains (g.)</th>
<th>Feed consumption per bird (g.)</th>
<th>Feed Conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-protein</td>
<td>530&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1012&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal control</td>
<td>531&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1123&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uric acid</td>
<td>530&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1136&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea</td>
<td>497&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1066&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diammonium citrate</td>
<td>394&lt;sup&gt;a&lt;/sup&gt;</td>
<td>893&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td>521&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1199&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaOH treated DPW</td>
<td>534&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1221&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. Different subscripts denote significant difference (P < 0.05).
acid were not significantly different from the control, while those fed the 
urea supplemented diet were significantly lowered. Growth of chicks fed the 
diet supplemented with 2% diammonium citrate were greatly depressed. Feed 
consumption was increased by the inclusion of 20% untreated or alkali treated 
DPW in the diets. Addition of uric acid to the basal diet did not affect the 
feed consumption of chicks, but addition of urea and diammonium citrate signi-
ficantly depressed the feed consumption, with diammonium citrate producing the 
greatest effect. Addition of the various NPN sources did not affect the feed 
efficiency (g. feed/g. gain). The DPW diets had poorer feed efficiencies when 
compared with the control. Chicks fed the diet containing 20% NaOH treated 
DPW tended to have better growth and feed efficiency, though not statistically 
significant than those fed the diet containing untreated DPW.

The percent nitrogen retention, g. N retained/100 g. of feed, and g. of 
uric acid excreted/100 g. of feed consumed of each treatment are shown in 
Table 11. The percent nitrogen retention of the DPW diets or the NPN 
supplemented diets was lower than the control, but the amount N retained per 
100 gm. of feed consumed by chicks fed these diets was not different from 
the control. Inclusion of DPW in the diet or addition of the non-protein 
nitrogen to the basal diet increased the uric acid excretion.

The control diet was suboptimal in protein content for maximum growth 
of broiler chicks. If the chick can utilize the non-protein nitrogen for 
synthesis of amino acids and protein, supplementation of such sources of 
nitrogen to the control should have stimulated a growth response. However, 
no favorable responses were obtained with the additional amount of nitrogen 
from the non-protein nitrogen fraction of the DPW or from the various 
nitrogenous compounds added to the diets. The lack of response may be due 
to two factors: firstly, the diets were low in metabolizable energy, so
Table 11. Effect of feeding DPW and non-protein nitrogen supplemented diets on nitrogen metabolism of the chicks.

<table>
<thead>
<tr>
<th>Diets</th>
<th>N Retention %</th>
<th>N (g.) retained per 100 g. feed</th>
<th>% uric acid in feed</th>
<th>Uric acid (g.) excreted per 100 g. feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-protein</td>
<td>40.5</td>
<td>1.42</td>
<td>--</td>
<td>4.35</td>
</tr>
<tr>
<td>Basal control</td>
<td>39.0</td>
<td>1.20</td>
<td>--</td>
<td>3.38</td>
</tr>
<tr>
<td>Uric acid</td>
<td>35.7</td>
<td>1.28</td>
<td>1.02</td>
<td>4.33</td>
</tr>
<tr>
<td>Urea</td>
<td>36.3</td>
<td>1.28</td>
<td>--</td>
<td>4.15</td>
</tr>
<tr>
<td>Diammonium citrate</td>
<td>37.0</td>
<td>1.22</td>
<td>--</td>
<td>4.36</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td>36.6</td>
<td>1.30</td>
<td>0.98</td>
<td>4.30</td>
</tr>
<tr>
<td>NaOH treated DPW</td>
<td>36.1</td>
<td>1.27</td>
<td>0.87</td>
<td>4.11</td>
</tr>
</tbody>
</table>
that the chicks may have increased feed consumption to satisfy their energy needs, thereby consuming enough protein; secondly, the non-protein nitrogen may not be utilized by the chick.

Shannon and Blair (1969) and McNab et al. (1974) using colostomised hens, showed that the percentage of absorption of diammonium citrate and uric acid were 99 and 91.2% respectively. Martindale (1975) also observed little urate excretion in feces of colostomised hens fed the 20% DPW diets. These studies show that NPN are completely available to the birds; yet the amount of nitrogen retained per 100 gm of feed consumed by chicks fed the non-protein nitrogen supplemented or DPW diets were not increased. This indicates that additional amount of nitrogen from NPN was not retained by the chick but simply excreted in the urine. The increase in uric acid excretion by approximately the same amount of uric acid or equivalent consumed when fed NPN diets supports this suggestion. Martindale (1975) also showed that urate excretion was greater during DPW feeding by approximately the amount consumed. When a dose of $^{14}$C-urate was introduced into the crop of the bird 98.8% of the radioactivity was recovered from the urine in the first 24 hours with the remaining activity found in the feces. Hence, results show that none of the urate present in DPW can be utilized by the growing chicks or by the laying hens.

In vitro studies with chick liver homogenate, Lee and Blair (1972) showed that NH$_4^+$ from the diammonium citrate can be incorporated into α-oxoglutaric acid to form glutamic acid by means of glutamic dehydrogenase. However, in vivo studies by Lee et al. (1972) in which they supplementing 11.07% diammonium citrate to the basal diet containing essential amino acids as the sole source of nitrogen did not cause induction of glutamate dehydrogenase. Compared with the control, no increase in transaminases such as aspartate transaminase,
and alanine transaminase was found in the livers of birds given the semi-synthetic diets, suggesting that supplying non-essential nitrogen does not result in further induction of these enzymes. Literature results to date have shown that non-protein nitrogen sources were shown to be utilized successfully only if the diets were deficient or lack non-specific nitrogen. A dietary inadequacy of non-essential nitrogen is improbable when ordinary feed-stuffs are employed. Therefore, though it has the biochemical potential of converting the nitrogen from non-specific nitrogen sources into amino acids, the chick does not seem to gain any benefit in vivo from the inclusion of non-protein nitrogen in protein-based diets.

Feeding as low as 0.78% urea to the chick in this experiment was shown to have deleterious effect on chick's growth. The toxic effects of urea ingestion probably result from ammonia produced by hydrolysis of the urea by bacterial urease activity in the intestine. Using different agents which were able to suppress gastrointestinal urease activity in rats, chick and other simple stomach species, Visek (1962) and Visek (1968) showed that growth and feed utilization of the animals were significantly improved. They suggested that ammonia produced from urea hydrolysis affects cell regeneration as well as leads to profound histological changes on the intestine of some species.

Antibiotics have been shown to be effective in suppressing the ureolytic activities in the intestine. Dintzis and Hastings (1953) observed that high dietary levels of penicillin, oxytetracycline and sulfaguanidine greatly reduced fecal bacterial count and at the same time completely suppressed urea hydrolysis. Visek et al. (1959) showed that 100 ppm of penicillin or chlortetracycline in the diet could significantly reduce the ureolytic activities in the gastrointestinal tract of the rat. Harbers et al. (1963) also showed that the addition of 100 ppm of chlortetracycline enhanced the
four week gains of chicks and lowered the urease activity and ammonia concentration in the intestine. In these experiments, diets contained 11 ppm of zinc bacitracin, however, no improvement in growth was obtained with this level of antibiotics in the urea supplemented diet.

Olsen et al. (1963) showed that the inclusion of up to 12% diammonium citrate in a semi-purified basal diet with isolated soy-protein or casein as the sole protein source resulted in progressive increases in the concentration of glutamine and a significant decrease in glutamic acid in the plasma. The observed decrease of plasma glutamic acid is probably a reflection of the use of glutamic acid for the formation of glutamine with the dietary ammonia. Hence, the chick, like other species, utilized glutamine as a carrier of ammonia in the blood. The level of glutamine in the blood depends on the balance between amount of ammonia ingested, the rate of formation of glutamine, the rate at which glutamine is utilized for uric acid or other synthesis, and the rate of uric acid excretion. When the amount of nitrogen ingested, in the form of protein, diammonium citrate, urea or glutamic acid is increased, uric acid excretion is increased correspondently. (Olsen et al. 1963, Kazemi and Balloun 1972, Creek and Vasaitis 1961). However, when the intake of nitrogen is high enough that excretion rates approach or exceed the mean tubular secretory capacity of the kidneys, it results in a significant increase in plasma glutamine concentration and consequently accumulation of ammonia in the blood. This, in turn, adversely affects the feed intake. Indeed, chicks fed the diets supplemented with urea or diammonium citrate consumed less feed than those fed the basal diet which may explain the depression in growth obtained with these diets.

Olsen et al. (1963) observed that the depression in weight gain by diets containing high level of diammonium citrate were not improved by the addition
of glutamic acid to these diets. These workers suggested that the decreased weight gain could not be attributed to a diversion of glutamic acid needed for protein synthesis that was diverted to glutamine formation. On the other hand, Bloomfield et al. (1969) observed a significant protective effect of glycine and glycine + glucose against ammonia intoxication in chicks. These protective agents exert their effect by enhancing uric acid synthesis. They suggested that the third step in uric acid synthesis, that is, formation of glycaminamide ribotide from glycine and phosphoribosylamine may be the limiting step in chicks under stress from ammonia load. The Agricultural Research Council (1963) suggested that 1% glycine is adequate in practical-type diets. With higher dietary nitrogen levels the glycine requirement may be greater than 1%, which may be related to the involvement of glycine in the detoxification of excess dietary nitrogen as uric acid. In fact, Snetsinger and Scott (1961) found that over 2% glycine was required to overcome the growth depression effect resulting from excesses of single amino acids. Blair et al. (1972) found that increasing the glycine level in the basal crystalline amino acids diet containing 10% diammonium citrate from 1% to 1.6% slightly improved the growth of the chicks though not reaching statistical significance. The amount of glycine contained in the basal and the DPW diets was 1.17% and 1.25% respectively, although adequate according to NRC recommendation, may become limiting under ammonia load from the non specific nitrogen supplementation and consequently causing depression in growth.

Experiment 4. Availability of total nitrogen, true protein and minerals in DPW and sodium hydroxide treated DPW.

The availabilities of total nitrogen, true protein, calcium, phosphorus and sodium in the untreated DPW and in the DPW samples treated with 2, 3 and
The figures on the digestibilities of total nitrogen and true protein of the untreated DPW obtained with chicks in this study are in close agreement with those obtained by Yoshida and Hoshii (1968) and McNab et al. (1974) although they determined availability with colostomized hens. The total nitrogen in the DPW showed higher digestibility than that of true protein, indicating the non-protein fraction of the total nitrogen is more digestible than the true protein.

The very low digestibility of the true protein is expected. The true protein in the DPW consist mainly of undigested protein residues, dead microorganisms, endogenous proteins (enzymes and dead cells) from the bird and to a less extent of spilled feed and feather debris. The undigested protein residues can be partitioned into those that have escaped enzymatic attack during digestion and those that are truly indigestible with the enzymes. When these proteins are refed to the chicks most of them can be easily digested but the truly indigestible ones remain undigested. This accounts for the low digestibility of the true protein in DPW. Davidson and Thomas (1969) observed that about 55% of the true protein in the excreta from layers was soluble in phenol-acetate-water solution. They suggested that this portion may be the endogenous protein from sources such as the intestinal flora, tissue, secretion, etc. while the remaining portion which was contained in the residue represents the truly indigestible protein from the food. This offers an explanation for the 50% digestibility of protein in the DPW obtained in this study.

As early as the beginning of this century workers have found that the digestibility of food proteins, especially those from plant origins, in the alimentary tract of monogastirc animals is largely independent of the proteins
Table 12. Availability of total nitrogen, true protein, calcium, phosphorus and sodium in untreated and NaOH treated DPW.

<table>
<thead>
<tr>
<th>Level of NaOH</th>
<th>Availability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Total N</td>
</tr>
<tr>
<td>0</td>
<td>63.6*</td>
</tr>
<tr>
<td>2</td>
<td>83.2</td>
</tr>
<tr>
<td>3</td>
<td>83.3</td>
</tr>
<tr>
<td>5</td>
<td>90.4</td>
</tr>
</tbody>
</table>

* Significant different (P < 0.05).
themselves but may be determined by the constituents of foods other than protein, the cell wall components in particular. It was postulated that since the plant cell walls are not always easily permeable to the digestive juices, it renders the proteins comparatively inaccessible to the digestive juices, thus in part explaining the possibility of poor utilization (Mendel and Fine 1911). In fact, Mendel and Fine showed that isolated proteins from wheat, corn and barley were utilized much better than the same proteins in the intact plant materials and were as thoroughly utilized as the nitrogen components of fresh meat. Seidler et al. (1964) observed that the digestibility of protein in oats with Leghorn cockerels was decreased with increasing fibre content from 0.14% (dehusked) to 16.8% in the grains. The sunflowers meals containing increasing amount of cellulose (from 10 to 26%) were also shown to have a decreasing percentage of digestible protein (Sirbu et al. 1972). These observations confirmed the above theory. Furthermore, in this study, the DPW treated with various levels of sodium hydroxide showed a marked improvement in the protein digestibility (50% for untreated vs 70% for alkali-treated), which gives further support to the above theory. The alkali-treatment is known to breakdown the cell wall components and structure and increase the digestibility of dry matter and nutrients in the roughage by ruminants (Chandra and Jackson, 1971; Singh and Jackson, 1971; and Saxena, 1971). The alkali-treatment, therefore, by acting on the cell wall components increases the access of the substrates to the enzymatic or microbial attack and hence increases the digestibility of the nutrients.

The cell wall components (cellulose and lignin) per se may also exert an unfavorable influence on the nutrient digestibility, that is when it cannot be accused of rendering the nutrients inaccessible to the digestive agents. Piekarska (1964) and Rao and Sunderavalli (1970) observed that rats fed
semi-purified casein diets containing 10-20% fibre or crystalline cellulose showed increased fecal nitrogen excretion and hence the apparent protein digestibility of the casein in the diet was reduced. With chicken a similar adverse effect on the protein digestibility of the diets was observed when the diets contained high levels of fibre, either from powdered crude lignin, from straws or from orchardgrass (Kibe et al., 1964; Keys et al., 1970; and Vlcek and Pazourek, 1970). This can be related to the laxative effect of fibre in the diet. Since the fibre is generally indigestible by the monogastric animal, the increased bulk of non-assimilable material in the large intestine and possibly in the small intestine has an effect on transit time so that there is less time for digestion and absorption. Mechanical factors, such as particle size of the fibres may also influence the rate of passage through the intestine (Mendel and Fine, 1911 and Morgan, 1934).

A large proportion of the nitrogenous material of normal feces could be bacterial since Bell et al. (1959) found that as much as one third of the total solids of human feces is bacteria. The bacterial proteins are known to have low digestibility. In vitro studies, Baker (1943) showed that the iodophile bacteria were resistant to digestion by pepsin and varied in degree to trypsin digestion depending on the strains of bacteria. Reed et al. (1949), and McNaught et al. (1954) showed that the true digestibility of proteins of the bacterial preparation from the rumen of sheep was 63-74% with growing rats. Kaufman et al. (1957) demonstrated that the average digestibilities of dried E. coli and Lactobacillus arabinosis cells were higher, 83.3 and 89% respectively, but still below that of caesin. Therefore, the relatively low digestibility of bacterial cells can also explain part of the low protein digestibility of DPW. The figure of protein digestibility of DPW treated with alkali is comparable to that of the bacterial protein.
This may indicate that the alkali treatment did not effectively affect the bacterial cell wall. Zalabak et al. (1972) demonstrated that when a protein preparation containing *Bacillus megaterium* was treated with small amounts of lysozyme or egg white, solubility of the protein and its digestion with trypsin was increased. Therefore, apparently the bacterial cell wall has to be lysed, either by mechanical means or by enzymes (lysozyme), before the proteins are readily available to the animal.

The availability of calcium and phosphorus in the DPW samples determined in this study is much lower than the values reported by Nwokolo and Bragg (1976) with soybean meal (SBM), rapeseed meal (RSM), cottonseed meal (CSM), and palm kernel meal (PKM), as shown in Table 13. This can be explained by the high levels of crude fibre and phytic acid in the DPW as compared with the other feedstuffs. These two factors are known to affect the mineral availability dramatically.

Moran (1934) found that ingestion of cellulose by test subjects caused an increase in the excretion of calcium and phosphorus in the feces. Begin et al. (1960) and Griffith (1961) showed that the apparent calcium digestibility was decreased with increasing dietary cellulose levels from 3-12%. Nwokolo and Bragg (1976) in correlating the digestibility of calcium and phosphorus with the crude fibre content in the feedstuffs, demonstrated a significant inverse relationship between crude fibre and calcium digestibility ($r = -0.73$) or phosphorus digestibility ($r = -0.91$). Therefore, the influence of fibre on calcium and phosphorus digestibility was well-illustrated. It is believed that the fibre affects the availability of minerals by similar mechanisms as discussed above.

Phosphorus of plant origin has been generally considered poorly available. A large proportion of the phosphorus in the plant materials, particularly cereals and cereal-by-products exists as phytin, the calcium-magnesium
Table 13. Effect of crude fibre and phytic acid content on calcium and phosphorus availability of various feedstuffs.\(^1\)

<table>
<thead>
<tr>
<th>Content</th>
<th>Feedstuffs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.5</td>
</tr>
<tr>
<td>Phytic acid(^3)</td>
<td>0.85</td>
</tr>
<tr>
<td>Phytate-P</td>
<td>0.24</td>
</tr>
<tr>
<td>Total P</td>
<td>0.87</td>
</tr>
<tr>
<td>Non-phytate P in total P (%)</td>
<td>72.4</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.39</td>
</tr>
<tr>
<td>Ca:P ratio</td>
<td>0.45:1</td>
</tr>
<tr>
<td>Availability (%)</td>
<td>89.3</td>
</tr>
<tr>
<td>phosphorus</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>85.6</td>
</tr>
</tbody>
</table>

1. Data taken from Nwokolo and Bragg (1976).
2. The values, otherwise stated, are expressed in percent in the feedstuffs.
3. Phytic acid (%) = Phytate P (%) x 3.55.
4. Non-phytate P in total P (%) = \(1 - \frac{\text{phytate P (\% in feedstuff)}}{\text{total P (\% in feedstuff)}}\) x 100
salt of inositol hexaphosphoric acid. Nwokolo and Bragg (1976) found highly significant inverse relationship between the phytate content and the calcium availability \( (r = -0.93) \) or the phosphorus availability \( (r = -0.93) \) of the feedstuffs.

The extent of the phytin phosphorus that is available for utilization by different species of animals at various ages still remains debatable. Certain authors have reported it is utilized to a limited extent. Others have considered it is highly available to animals. Ashton et al. (1960) fed \( ^{32}P \) labeled calcium phytate and observed that approximately 20% of the phytate phosphorus was retained by four-weeks old chicks. Hence these authors concluded that the chicks can utilize one-fifth of the phytate phosphate. Temperton and Cassidy (1964) reported chicks retained approximately 60% of the phytate phosphate and only 50% of the non-phytate phosphorus. The wide disagreement that existed between investigators on the ability of the chick to utilize phytate phosphate, was explained by Nelson (1967) to be due to the variations in the source of phytate phosphorus used, criteria of response, age of the test animals, and calcium and vitamin \( D_3 \) levels in the experimental diets.

Nelson (1967) in a review showed that there is no evidence showing that phytate is absorbed and utilized intact by any animal species. In order to be utilized, phytin phosphorus must be hydrolyzed to yield inorganic phosphate. However, this phosphorus compound cannot be hydrolysed by the normal enzymes of the digestive juices of animals. The enzyme phytase, which is present in certain feed ingredients and possibly secreted by the intestine, specifically hydrolyses it to phosphate and inositol. Phytases from all sources act only on soluble phytate (Taylor 1965). Hence, availability of phosphorus from free phytic acid and sodium phytate, both of which are highly soluble,
was shown to be far greater than that of calcium phytate which is insoluble (Maddaiah et al., 1963; Waldroup et al., 1964). Therefore, the extent to which phytates are hydrolyzed depends largely on their solubility, which in turn depends on the ions with which they are associated (mainly Ca$^{++}$, Mg$^{++}$, Mn$^{++}$, K$^{+}$ and H$^{+}$ in natural phytates) and on the level of calcium or the Ca : P ratio in the diet.

Vandepopuliere et al. (1961) reported that plant source phosphorus was readily available for growth in chicks when fed at an optimum Ca : P ratio of 1:1. Waldroup et al. (1964b) demonstrated that the combination of low dietary calcium or low Ca : P ratio (0.8:1) significantly improved the utilization of calcium phytate by growing chicks. However, wider Ca : P ratios, apart from 1:1 would depress the utilization of phytic acid phosphorus or the calcium-phytate phosphorus (Harms et al., 1962; and Waldroup et al. 1964b). Nott et al. (1967) also reported that phytin phosphorus availability for laying hens was reduced by high levels of calcium in the diet. Conversely, diets high in phosphate (inorganic) promote the breakdown of phytate. This may be brought about by increasing the solubility of the phytate by removing, as insoluble phosphate, calcium which would otherwise combine with and precipitate phytate.

Assuming the phytate-phosphorus is completely unavailable and the non-phytate phosphorus is completely utilisable, then the percent availability of phosphorus in the feedstuff will be equal to the percent of non-phytate phosphorus in total phosphorus. However, data presented by Nwokolo and Bragg show that availability of phosphorus from feedstuffs were always higher than the percent of non-phytate phosphorus in total phosphorus of the feedstuff by about 20% (see Table 13). This indicates that at least 20% of the available phosphorus was from the phytate source. In this study, the DPW
contained 30% of total phosphorus as non-phytate phosphorus but has phosphorus availability of 20%, indicating the phytate-phosphorus in the DPW was completely unavailable. This discrepancy can be explained by the difference in the calcium content of the materials. DPW contains considerably high level of calcium (2.39%) and high Ca : P ratio (1.76:1) as compared with the other feedstuffs although their phosphorus contents are similar. The high level of calcium in the DPW may render the phytate phosphorus unavailable by the mechanism mentioned above. Furthermore, the high fibre content of DPW may depress the availability of phosphorus.

Calcium has been shown to have an adverse effect on the availability of phytate phosphorus. Conversely, the phytate molecule has been observed to reduce the availability of calcium. Hoff-Jorgensen (1946) observed that addition of phytic acid to the diets of dogs depressed both the absorption as well as retention of calcium. Maddaiah et al. (1963) also showed that sodium phytate in the diet reduced calcium retention in chicks and rats.

Maddaiah et al. (1964) in vitro study, showed that at physiological pH range the phytate anion (H₄phy⁻⁸) would be formed. It appears this anion is involved in the complex formation with the cations such as Ca⁺⁺, Zn⁺⁺, Mn⁺⁺ and Mg⁺⁺ in the intestine and rendered them unavailable. On this basis, one mole of phytic acid will chelate 4 moles of calcium or 1% dietary phytate will chelate 0.24% dietary calcium in the intestine.

Assuming the calcium not chelated to phytate is completely available while the phytate calcium is indigestible, and the phytic acid in the feedstuffs is fully chelated with calcium, then the availability of calcium in soybean meal, rapeseed meal, cottonseed meal, palm kernal meal are calculated to be 49, 46, -77 and 6% respectively. All of these are lower than the actual determined values while that of DPW is 66%, similar to the determined value.
This means that the phytic acid in the former four feedstuffs are only partially chelated with calcium, while the phytic acid in DPW is completely chelated with calcium. Therefore an attempt was made to calculate the degree of saturation of the phytic acid with calcium in the feedstuffs. As 14.4% of calcium in soybean meal is not available and soybean meal contains 0.39% calcium, the amount of phytate calcium in soybean meal is 0.056% (14.4% x 0.39%). Therefore 0.85% phytic acid in the soybean meal chelated 0.056% calcium or 1% to 0.066% calcium. If the phytic acid is saturated with calcium, 1% dietary phytic acid will chelate 0.24% calcium. Therefore the phytic acid in the soybean meal would be 36% (0.24% ÷ 0.066%) saturated.

Similarly, using the same procedure, phytic acid in rapeseed meal, cottonseed meal, palm-kernel meal and DPW are calculated to be 52, 20, 33 and 138% saturated.

The degree of saturation of the phytic acid with calcium can be related to the relative abundance of calcium and other cations, Mg$^{2+}$ in particular. The calcium and magnesium contents in the feedstuffs and their ratios are shown in Table 14. It appears that the degree of saturation of phytic acid correlates very well with the ratio of calcium to magnesium in the feedstuff. Cotton-seed meal has the lowest Ca : Mg ratio; that is, containing relatively more magnesium with respect to calcium shows the lowest degree of saturation of the phytic acid and vice versa. Maddaiah et al. (1964) found that the stability of the complex formed between phytic acid and calcium was lower than that formed with magnesium. Therefore, in the presence of a comparatively excess amount of Mg$^{2+}$, it will successfully displace the Ca$^{2+}$ from chelation with phytic acid, hence the degree of saturation of phytic acid with calcium is lower and more calcium is available. Conversely, when calcium is abundant it will successfully compete with Mg for chelation, as in DPW.
Table 14. Calcium and magnesium contents of the feedstuffs in relation to the degree of chelation of phytic acid with calcium.

<table>
<thead>
<tr>
<th>Feedstuffs</th>
<th>Calcium %</th>
<th>Magnesium %</th>
<th>Ca:Mg ratio</th>
<th>% saturation of phytic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBM</td>
<td>0.39</td>
<td>0.41</td>
<td>0.95:1</td>
<td>36</td>
</tr>
<tr>
<td>RSM</td>
<td>0.85</td>
<td>0.47</td>
<td>1.80:1</td>
<td>52</td>
</tr>
<tr>
<td>CSM</td>
<td>0.26</td>
<td>0.56</td>
<td>0.46:1</td>
<td>20</td>
</tr>
<tr>
<td>PKM</td>
<td>0.36</td>
<td>0.44</td>
<td>0.81:1</td>
<td>33</td>
</tr>
<tr>
<td>DPW</td>
<td>2.39</td>
<td>0.42</td>
<td>5.69:1</td>
<td>138</td>
</tr>
</tbody>
</table>

1. Data taken from Nwokolo and Bragg (1976).
It is interesting to note that both rapeseed meal and cotton-seed meal contain the same amount of phytic acid yet the latter shows lower calcium availability than the former. Suppose that a similar amount of calcium is chelated to the phytic acid in both feedstuffs and since cotton-seed meal contains a lower amount of calcium, then the proportion of total calcium that is chelated to phytic acid will be higher in cotton-seed meal, which explains the lower calcium availability.

In summary, the availability of calcium in the feedstuffs is affected by the three factors; viz., the phytic acid content, degree of chelation of phytic acid with calcium and the calcium content. With the same calcium content, increasing the phytic acid content, and/or increasing the degree of chelation will decrease the availability, while with the same phytic acid content, higher calcium content will increase the availability.

The calcium, phosphorus, phytate phosphorus and phytic acid content of the DPW and alkali-treated DPW samples are shown in Table 15. The calcium and phosphorus availabilities of the alkali-treated DPW were not significantly different from that of the untreated DPW, due to the high variability among the replicates. Since the alkali treatment did not affect the phytic acid content of the material, it is obvious it could exert no effect on the calcium and phosphorus availability.

The sodium in the DPW is considered to be readily available. However, the percent availability of sodium in the alkali treated DPW were much lower. This is likely because most of the sodium hydroxide added to the DPW had reacted with the material to form complex compounds which were not available, so lowering the proportion of available sodium in these samples.

In the previous experiment, when chicks were fed the 20% alkali-treated DPW diet, especially that treated with 5% sodium hydroxide, a considerably
Table 15. Effect of sodium hydroxide treatment on the calcium, phosphorus and phytic acid contents of DPW.

<table>
<thead>
<tr>
<th>Content (%)</th>
<th>Level of NaOH (%) Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.39</td>
</tr>
<tr>
<td>Total P</td>
<td>1.36</td>
</tr>
<tr>
<td>Phytic acid</td>
<td>3.34</td>
</tr>
<tr>
<td>Phytate P</td>
<td>0.94</td>
</tr>
<tr>
<td>Phytate P in total P (%)</td>
<td>69.2</td>
</tr>
<tr>
<td>P availability (%)</td>
<td>19.8</td>
</tr>
<tr>
<td>Ca availability (%)</td>
<td>53.8</td>
</tr>
</tbody>
</table>
higher quantity of sodium was consumed compared to the control. Normally with this quantity of sodium consumed it may create stress on the chicks. However, no adverse effect on growth and health of feeding the alkali-treated DPW diet was observed. This is due to the low availability of the sodium in the alkali-treated DPW, hence, even though the chick has consumed the excessive amount of sodium from these materials not all of them were available to the chicks sufficiently high enough to create the sodium stress.

Experiment 5. Effect of amino acid supplementation on the performance of chicks fed diets containing untreated DPW or alkali-treated DPW.

Body weight gain, total feed consumption and feed conversion ratio of chicks fed the control and the DPW diets with or without supplementation with methionine alone or with methionine and lysine for four weeks are shown in Table 16.

When the basal control 2 diet was supplemented with 0.2% D-L methionine or 0.2% methionine + 0.1% lysine, growth of the chicks were improved (507 g, 582 g and 576 g, respectively). The improvement in growth was attained when 0.2% methionine was added to the diet. Additional supplementation with 0.1% lysine did not result in additional improvement. Therefore, it is suggested that methionine is the critical essential amino acid in the control diet.

Addition of 0.2% methionine to the control diet significantly increased the feed intake. This is in accordance with the results obtained by various investigators showing that deficiencies of single amino acids caused marked reductions in voluntary food intake (Baker 1974, review). Furthermore, the growth of chicks fed the supplemented control 2 diet was similar to that of the control 1 which contained a higher energy level. This indicates that
Table 16. Effect of amino acid supplementation on 4 week body weight gains, total feed consumption and feed conversion ratio of chicks fed the control and the DPW diets.

<table>
<thead>
<tr>
<th>Level of Supplementation</th>
<th>Average 4 week Body Weight Gains$^1$ (g.)</th>
<th>Total Feed Consumption per bird$^1$ (g.)</th>
<th>Feed Conversion Ratio$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Control 2</td>
<td>Untreated</td>
</tr>
<tr>
<td>0</td>
<td>--</td>
<td>506.6$^a$</td>
<td>544.4$^{abc}$</td>
</tr>
<tr>
<td>0.2% met</td>
<td>--</td>
<td>581.8$^{de}$</td>
<td>523.7$^{ab}$</td>
</tr>
<tr>
<td>0.2% met + 0.1% lys</td>
<td>576.3$^{cde}$</td>
<td>575.7$^{cde}$</td>
<td>541.8$^{abc}$</td>
</tr>
<tr>
<td>0.2% met + 0.2% lys</td>
<td>--</td>
<td>552.9$^{bcd}$</td>
<td>533.1$^{abc}$</td>
</tr>
</tbody>
</table>

1. Different subscripts indicate significant difference (P < 0.05).
without amino acid supplementation, the methionine deficiency in the low-energy control diet (control 2) limited the chick's ability to consume enough feed to meet its energy requirement. When the amino acids were balanced, the chick was able to consume higher amounts of the lower energy diet (1055 g vs 1006 g in control 1) and hence resulted in corresponding growth.

Feed efficiency of the non-supplemented control 2 diet was poorer than that of the balanced control 1 diet. This is because of the methionine deficiency which lowered the protein or other amino acid utilization. Supplementing this basal diet with methionine significantly improved the feed efficiency which was comparable to the high-energy control 1 diet.

The control 2 diet was calculated to be suboptimal in lysine with respect to the chick's requirement. However, growth was improved by supplementing with 0.2% methionine only; adding 0.1% lysine to the methionine supplemented control diet did not resulted in further improvement in growth. This is because of the lower energy content of the diet, which stimulated the feed consumption so that the chick had consumed enough amount of lysine to satisfy its need. These results are in agreement with Harper and Rogers (1965) finding that if feed intake could be maintained in rats fed an imbalanced diet by adjusting the protein: calorie ratio of the diet growth would be improved and equivalent to that of control.

Increasing the level of supplementation of lysine from 0.1 to 0.2% tended to reduce the body weight gain (575 g vs 553 g) and feed intake (1057 g vs 997 g) although differences were not statistically significant. This shows the signs of amino acid imbalance. The diet containing 0.2% lysine would be slightly in excess of lysine with respect to other amino acids, particularly arginine. Excess of dietary lysine is shown to cause a reduction in voluntary food intake, which explains the depression in growth, as reported

Growth of chicks fed the basal DPW (untreated or alkali-treated) diets were higher than those fed the control 2 diet. In addition, growth of the chicks fed these diets were comparable to those fed the balanced high energy control diet. This was expected because the DPW diets contained higher levels of the sulfur-containing amino acids than the control diet, hence, no amino acid deficiency effect was demonstrated. Furthermore, the lower energy content of these diets facilitated higher feed intake and consequently the chicks could consume enough of the amino acids to meet the requirements. Therefore, the addition of methionine and lysine to the diets containing untreated or alkali treated DPW, did not result in additional growth. Moreover, these supplementations did not increase the feed intake, further confirming that methionine was not deficient in the DPW diets.

The chicks fed the untreated DPW diet, in general, grew slower than those fed the control diets or the alkali-treated diet. The feed efficiency of this diet was the lowest even though all diets were formulated to be similar in energy and true protein content. This is due to the low digestibility of true protein (50%) in the untreated DPW as determined from the previous study. The DPW was calculated to furnish 2.6% true protein in the diet, but actually it contributed only 1.3% available protein. Therefore the untreated DPW diet contained less than 21.7% available protein as compared to the 23% in the control. This explains the poorer growth and lower feed efficiency with the untreated DPW diet. The alkali-treatment markedly improved the protein availability of DPW (from 50 to 70%), which brought about the improvement in growth and feed efficiency. Nevertheless, the protein digestibility of alkali-treated DPW was still below that of wheat and soybean meal, which generally are over 90% availability. Thus the alkali-treated DPW diet was
slightly suboptimal in protein content. Although it did not affect the growth, the feed efficiency was reduced as compared with the balanced control.

The feed consumption of chicks fed the DPW diets were higher than those fed the supplemented control 2 diets. Since the diets are calculated to be equivalent in energy content, it is probably because of the lower protein content in the DPW diets which stimulated greater feed consumption. Therefore, the feed consumption of chicks is influenced by dietary balance.

In general, the amino acid profile of DPW is similar to that of the cereal grains such as wheat or barley. When high levels of DPW are used to replace wheat or corn in the diets they are likely to be deficient in lysine and methionine as in the case of wheat or corn based diets. In fact, in studies by Stapleton and Biely (1975), the supplementation of both the wheat and corn based control diet and the diet containing 20% DPW with lysine and methionine resulted in improvements in growth of similar magnitude. However, the sample of DPW used in this study contained much higher level of methionine and cystine, about three times that of the DPW sample used by Stapleton and Biely or in the wheat. Thus the DPW diets used in this study contained relatively adequate level of the sulfur-containing amino acids as compared to the wheat-based control. Supplementation of the DPW diets with methionine did not result in growth response, indicating the methionine in the DPW was utilized by the chick for growth so that no deficiency was to be demonstrated. This is in accordance with the findings of Bhargava and O'Neil in which addition of methionine or lysine either singly or in combination to the DPW diets, which calculated to contain sufficient methionine and lysine to meet the requirements, did not affect growth response. Therefore, when balanced for amino acid content inclusion of high level of DPW in the diet does not affect the growth.
DeGroot and Slump (1969) showed that severe treatment of isolated soy-protein with sodium hydroxide (pH 12.2) caused destruction of cystine and lysine which resulted in lowering the nutritive value of the protein. Alkali treatment also reduced the true digestibility of the proteins with rats. However, in this study, the results from treating DPW with alkali were in opposite to that of DeGroot and Slump. The alkali treatment increased the digestibility of protein in the DPW. It also improved the nutritive value of DPW even though it had slight adverse effect on the cystine content of the DPW. The differences in the response to alkali treatment can be attributed to the difference in nature of the materials treated. When high protein materials are treated with the alkali, it will react directly with the proteins causing chemical and structural changes which may be undesirable. On the other hand, when materials containing high levels of fibres are treated with the alkali, it reacts with the structural substances, bringing about changes which result in increased utilization of the materials.

Experiment 6. Effect of increasing the energy content of the diets with supplementary 'fat' (tallow) on performance of chicks fed the diets containing untreated DPW or alkali-treated DPW.

The 2nd, 3rd and 4th week body weight gains of chicks fed the experimental diets are shown in Table 17. The body weight gains of chicks fed the different diets were similar during the first two weeks of age. At the end of the 3rd week improvement in weight gain was observed with chicks fed the higher energy diets with 2% additional fat. The growth response was more pronounced at the end of the 4th week. Addition of 2% fat to the three basal diets improved weight gains by similar magnitude among the diets. There was no difference in growth among the diets with the same energy level.
Table 17. Effect of fat supplementation on body weight gains, feed consumption and feed conversion ratio of chicks fed the control and DPW diets.

<table>
<thead>
<tr>
<th>Age (week)</th>
<th>Low Fat Basal Diets</th>
<th>Fat Supplemented Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Untreated DPW</td>
</tr>
<tr>
<td>2</td>
<td>195&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>180&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>360&lt;sup&gt;a&lt;/sup&gt;</td>
<td>373&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>588&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>574&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Total Feed Consumption per bird (g.)

<table>
<thead>
<tr>
<th>Low Fat Basal Diets</th>
<th>Fat Supplemented Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1095&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1169&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Overall Feed Conversion Ratio

<table>
<thead>
<tr>
<th>Low Fat Basal Diets</th>
<th>Fat Supplemented Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1. Body weight gain = Body weight at the end of the week — day-old body weight

2. Different subscripts on the same line indicate significant difference (P<:0.05).
Feed consumption and feed conversion ratios of chicks fed the different diets are also shown in Table 17. With the exception of the untreated DPW diet, addition of 2% fat to the control or the alkali-treated DPW diet did not affect the feed consumption but improved the feed efficiency. The feed efficiency of the alkali-treated DPW diets was improved to a greater extent (differed by 0.11 unit) than that of the control (differed by 0.06 unit). Conversely, addition of 2% fat to the untreated DPW diet increased the feed consumption but did not affect the feed efficiency.

The results obtained in this study are in agreement with the findings of many investigators in that addition of fat, both animal or vegetable, improved body weight gain and feed efficiency (Potter et al., 1960; Vermeersch and Vanschoubroek 1968). However, Richardson et al. (1958) showed that the addition of fat to the diets mainly improved the feed efficiency with little effect on the growth. In fact, the growth responses to supplemental fat were related to the M.E. content of the diet. Begin (1961) showed that with 22% protein in the diet, a level of 2,970 kcal/kg. was the minimum energy level required for maximum growth. With the M.E. above this level (2970-3200 kcal/kg) increasing the energy content by adding fat did not result in an increase in growth; whereas, with the dietary energy below this level, increasing the energy by adding fat would improve the growth. Feed efficiency was directly related with the energy content of the diets. Therefore, the three basal diets used in this study were suboptimal in energy content, and increasing the energy by adding 2% fat increased the caloric intake, which resulted in higher growth.

Although the dietary treatments were formulated to contain similar energy content at the two energy levels, the diets containing untreated DPW or alkali-treated DPW always showed poorer feed efficiency than the control diet.
at the two energy levels. This indicates that the nutrients in the diet, especially the energy were less efficiently utilized by the chicks when fed with DPW and may be attributed to the higher level of crude fibre contained in the DPW diets compared to the control (7% vs 3.9%). However, many authors have shown that the only deleterious effect of including high level of fibre in the diets was due to its energy dilution properties. When the energy content of the diets containing high levels of fibre were compensated for by adding fat, growth and feed efficiency were not impaired (Richardson et al. 1958; Potter et al., 1960; Begin, 1961). Therefore some other factor besides fibre may be responsible for the lowering in the feed efficiency when fed with DPW in the diets.

Since fat contributed a significant portion of the energy in the DPW diets, any factors that affect the fat utilization will significantly affect the M.E. content of the diets as well as the feed efficiency. Therefore the apparent fat digestibility and M.E. values of the diets were determined and shown in Table 18. The apparent digestibility of crude fat (mainly from tallow) in the control diets were between 75-78.8%. This is close to the figures of 70-73% for tallow obtained by Young (1961), March and Biely (1957), and Vermeersch and Vanschoubroek (1968). However, the apparent digestibilities of crude fat in the DPW diets were markedly lower than that of the control diets, with the lowest in the untreated DPW diets. This shows that DPW impaired the digestion of fat in the diet.

Saito et al. (1959) showed that the digestibility of crude fat tended to decrease from 87.2% with no added cellulose in the diet (containing 2.7% crude fibre) to 84% with 3.5% and 83% with 9.5% cellulose added. Begin (1961) and Polin et al. (1971) found that fat utilization was not affected by including various levels of cellulose in the diet. Therefore the higher level of
Table 18. Apparent fat digestibility, metabolizable energy values and nitrogen retention of the different diets in Experiment 6.

<table>
<thead>
<tr>
<th>Low Fat Basal Diets</th>
<th>Fat Supplemented Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Apparent Fat Digestibility (%)</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Metabolizable Energy Kcal/kg (as fed basis)

<table>
<thead>
<tr>
<th></th>
<th>Formulated 1</th>
<th>Corrected 2</th>
<th>Determined</th>
<th>Difference 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2755</td>
<td>2720</td>
<td>2720</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2755</td>
<td>2561</td>
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<td></td>
<td>2710</td>
<td>2561</td>
<td>2590</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>2844</td>
<td>2810</td>
<td>2810</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>2844</td>
<td>2599</td>
<td>2738</td>
<td>246</td>
</tr>
<tr>
<td></td>
<td>2892</td>
<td>2564</td>
<td>2744</td>
<td>66</td>
</tr>
</tbody>
</table>

Nitrogen Retention (%)

|                     | 45.7 | 38.7 | 38.0 | 54.5 | 40.7 | 42.1 |

1. The M.E. values of the diets were calculated using the figure of 7700 kcal/kg for the M.E. of tallow.
2. The M.E. values of the diets were calculated after correcting for the variation in the fat digestibility of the diets.
3. The difference between the formulated value and the determined value.
fibre in the DPW diets cannot totally explain the marked reduction in the fat digestibility, though it may have some effect.

Werner and Lutwak (1963) reported that fat absorption from intestine of rat diminished in a linear fashion with increasing dietary calcium levels. With chicks Fedde et al. (1959) observed that lowering the dietary calcium level to 0.33% increased the digestibility of tallow from 77% to 91%. On the other hand, when the dietary calcium level was increased to 3%, digestibility of tallow decreased to 71%. The effect of dietary calcium on fat utilization appears to be in relation to the kind of fat in the diet. Calcium in the diet was found to have no influence on the digestibility of low-melting fats while it markedly decreased the digestibility of high-melting fats and hydrogenated fats (Edwards et al., 1960). However, Hakansson (1974) showed that with laying hens, the digestibility of all the fats was lower with the higher calcium (4.34%) than with the lower calcium (2.87%). This decrease affected all the fatty acids but especially stearic and palmitic acids. Since tallow contains higher proportion of these saturated fatty acids in the fat as compared with other vegetable oils (Renner and Hill, 1961), the digestion and absorption of tallow is likely to be affected to a greater extent by the dietary calcium level.

Marchand-Bédard (1957) observed that when chicks were fed tallow, only a small percentage of the fecal fat was in the form of neutral fat, indicating the tallow was readily hydrolysed by the intestinal enzymes. However, the greater part of fat excreted was in the form of insoluble soap and free fatty acids. This suggests that soaps formed from saturated fatty acids were not absorbed. Calcium probably interacts with the free saturated fatty acids after hydrolysis in the gut. Dissociation of the calcium-tallow complex, in vitro, was shown to occur between pH 4.8 and 6.4 (Hunt et al., 1961).
The pH of the duodenum and jejunum, where most of the calcium is absorbed, is between 5.6 and 6.3, and increases thereafter to about 7.0 in the cecum. Therefore the calcium could have been absorbed in the duodenum without forming insoluble soap. However, with higher dietary calcium intake, the proportion of dietary calcium absorbed is reduced (Morrissey and Wasserman, 1971) so that more of the calcium will be free to form insoluble soap in the lower portions of the gut. Hence, as the dietary calcium level is increased, more of the saturated fatty acids from the tallow will be converted into soaps and excreted, consequently the digestibility of these fats is decreased.

The DPW diets were calculated to contain higher level of calcium (2.0%) compared to the control (1.26%) due to the high amount of calcium contributed by the DPW. It is reasonable to believe that the lower utilization of fat (tallow) in the DPW diets was caused by the higher dietary calcium level. On the contrary, Polin et al. (1971) indicated that DPW at 8 or 16% in the diet did not affect the fat utilization of the diets. In their study, corn oil was used. Since corn oil contains a very low level of the saturated fatty acids the utilization of corn oil would not be affected nearly as much by the calcium level. Therefore, no significant effect on fat utilization would be anticipated from feeding DPW together with vegetable oils.

Fedde et al. (1959) and Sibbald and Slinger (1963) found that the level of dietary inclusion of fat did not appear to affect the utilization of fats. Williams et al. (1959) demonstrated that the digestibility coefficient of fat from the basal feed ingredients were lower than for added fat. Hence, the increase in apparent digestibility coefficient of fat in the diet with increases in dietary fat levels as observed by Whitson et al. (1943) and Williams et al. (1959) is due to the higher utilization of the added fat. When corrected for the low digestibility of fat in the basal ration the added
fat was shown to be equally utilized at the various levels studied (Williams et al. 1959). In this study, the apparent digestibility of the fat tended to increase with the fat levels in the diet, but the increase was not large enough to have any significant effect.

The M.E. values of the diets determined with chicks between 2 and 3 week of age are shown in Table 18. The determined values for the control diets were close to the calculated values. This indicates that the M.E. value of tallow with 75% digestibility would be close to the value of 7700 kcal/kg used in the formulation. With the higher fat control diet, the determined value was slightly higher than the calculated value. This is probably due to the higher fat digestibility (78.8) as compared to that of the lower fat control (75%). The M.E. values of the DPW diets at both fat levels were much lower than the calculated values. The low fat digestibility appears to be the major factor related to these low values. Assuming the M.E. value of the tallow was directly in proportion to its digestibility, then with 55.6% digestibility the M.E. value would be 5708 kcal/kg. Using this M.E. value, the M.E. of the untreated DPW diet containing 8% tallow would be 2561 kcal/kg. Similarly, the corrected calculated values for the untreated DPW diet with 10% fat, alkali-treated DPW diets with 8% fat or with 10% fat would be 2599, 2590 and 2738 kcal/kg respectively. These values resemble the determined value. Therefore the M.E. value of the diets were in direct relationship with the fat digestibility of the diets.

Addition of 2% more fat to the diets increased the M.E. contents of the diets except with the untreated DPW diet. With the untreated DPW diet, addition of 2% more fat to the diet resulted in only a very small increase in the M.E. content, which was due to the slight decrease in fat digestibility with the increased fat level.
The feed efficiencies of the diets, therefore, were directly related to the fat digestibility and the actual M.E. content of the diets. Due to the lower fat digestibility and hence lowered M.E. content, the feed efficiency of the diets containing DPW would be reduced. Increasing the fat digestibility by specific factors or raising the M.E. content by adding more fat would improve the feed efficiency.

Feed consumption of chicks fed the DPW diets at the two energy levels were higher than the control diets. This is probably due to the lower M.E. contents of these diets. In general, addition of 2% fat to the diets caused a significant increase in the M.E. content of the diets, but appeared to have no effect on the feed consumption. This is similar to the finding by Begin (1961) that the energy level of the diets had little effect on the feed consumption. As the cellulose level of the diet increased with a corresponding decrease in energy level, the chicks did not appear to increase their feed intake to compensate for their energy needs. The bulk alone was shown not to be the limiting factor in feed intake since the diet containing 21% cellulose promoted a volume intake that was significantly greater than any of the other cellulose diets (containing 3-18% cellulose). This indicates that it would have been physically possible for these intermediate cellulose groups to have consumed more feed, yet they failed to do so. Therefore factors other than the energy level influence the feed intake. Since the M.E. content per unit of volume was progressively reduced as cellulose was added to the diet, the decrease in caloric density could become a factor. In this study, the DPW diets had lower caloric density (due to higher bulk of the DPW) compared to the control diets which might have stimulated the greater feed consumption. The increase in energy content by adding 2% fat might not be high enough to overcome this effect.
The increase in caloric intake may affect the protein utilization. The nitrogen retention of diets determined between the 2nd and 3rd week of age for the chicks are shown in Table 18. In general, addition of 2% fat to the diets improved the N retention which was correlated with the metabolizable energy content of the diets. Various studies have shown that as the energy level of the diet increased the N retention also increased up to the optimal level of energy. Additional increases in energy beyond this level would result in no further increase in N retention and may decrease it when the energy level was too high (Sanslone and Squibb, 1963; Farrell, 1974; and Velu, 1974). Summer et al. (1964) demonstrated that energy concentration of 3050 kcal/kg was the optimal level for maximum N utilization. Therefore the diets used in this study were suboptimal in energy. It appears that about 3000 kcal/kg is the optimal level of the energy in the diet for maximum growth and nitrogen utilization.

The chicks fed the DPW diets showed much lower N retention than those fed the control diets. This can be attributed to the lower energy content of the diets which impaired the N utilization, the low digestibility of protein in DPW and probably zero utilization of the non-protein nitrogen in the diet. Assuming the protein in the DPW was 50% digestible and the digestible protein contained in the DPW diet was retained by the chick to the same extent as with the control diet (45.7% retention) while the non-protein nitrogen from the DPW was not utilized at all, then the N retention of the DPW diet was calculated to be 38%, which is exactly the same as the determined value. Therefore, the proteins of the DPW diets were utilized as efficiently as that of the control diets provided the energy levels were similar. This also indicates that the protein in the DPW, aside from its low digestibility, the digestible portion is well utilized by the chicks.
Because of the high calcium content in the DPW diets, utilization of the tallow in the diets was impaired and hence the M.E. contents of the diets were markedly lowered. As a result, the nitrogen utilization and feed efficiency were reduced. If the utilization of the fat in the DPW diets was improved then the performance of chicks fed such diets would be comparable to the control. This leads to the objective of the next experiment.

Experiment 7. Effect of antibiotics supplementation on the performance of chicks fed with the DPW diets.

The body weight gains of chicks fed the basal diets supplemented with antibiotics were always higher than those fed the basal diets at the end of 1, 2, 3 and 4 week of age (Table 19). The antibiotic supplementation increased the weight gains of chicks at the end of the 4th week by 47, 54 and 57 g with the control, the untreated DPW diet and the alkali-treated DPW diet, respectively, or by an average of about 8.5%. Apparently, the antibiotic supplementation elicited a slightly greater growth response when fed with the DPW diets. With or without the supplementation, weight gains of chicks fed the DPW diets were higher than those fed the corresponding control diets at the end of the 4th week. The antibiotic supplemented alkali-treated DPW diet group showed the highest weight gain. This is probably due to the higher feed consumption by these groups as seen in Table 20. The total feed consumption of the antibiotic-fed groups was not different from that of the controls. The overall feed efficiency of the chicks on these diets was also improved by the antibiotic supplementation. The improvement in feed efficiency was greater with the groups fed the DPW diet than with the control.

The digestibilities of fat in the diets with chicks at 1, 2 and 4 week of age are shown in Table 21. Irrespective of the antibiotic supplementation,
Table 19. Effect of antibiotics supplementation on body weight gains of chicks at 1, 2, 3 and 4 week of age.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Cumulative Body Weight Gain (^1) (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>70.7(^a)</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>80.6(^{bc})</td>
</tr>
<tr>
<td>Difference</td>
<td>10.0</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
</tr>
<tr>
<td>DPW Basal</td>
<td>70.8(^a)</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>83.0(^c)</td>
</tr>
<tr>
<td>Difference</td>
<td>12.2</td>
</tr>
<tr>
<td>NaOH treated DPW Basal</td>
<td>74.2(^{ab})</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>82.9(^c)</td>
</tr>
<tr>
<td>Difference</td>
<td>8.7</td>
</tr>
</tbody>
</table>

1. Body weight gain = Body weight at the end of the week –day-old body weight
2. The difference in body weight gain between the antibiotic fed groups and the groups fed the basals.
3. Different subscripts within the same column indicate significant difference at the 0.05 probability level.
Table 20. Total feed consumption and feed conversion ratio of chicks fed diets for 4 weeks in experiment 7.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Feed Consumption (g.)</th>
<th>Feed Conversion Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1059&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1221&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1267&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>NaOH Treated DPW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1235&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1250&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.12</td>
</tr>
</tbody>
</table>

1. Overall feed conversion ratio = \( \frac{\text{Total feed consumption for 4 weeks}}{\text{4 week body weight gain}} \)

2. Difference subscripts in the same column indicate significant difference (\( P < 0.05 \)).

3. The difference in feed conversion ratio between the antibiotic fed groups and the groups fed the basals.
Table 21. Effects of age and antibiotics feeding on fat digestibility of the diets in Experiment 7.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Apparent Fat Digestibility (%)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Basal</td>
<td>42.5</td>
<td>64.2</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td>Antibiotic</td>
<td>60.3</td>
<td>74.4</td>
<td>84.3</td>
</tr>
<tr>
<td></td>
<td>Difference</td>
<td>17.8</td>
<td>10.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td>Basal</td>
<td>47.1</td>
<td>53.0</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>Antibiotic</td>
<td>62.7</td>
<td>76.9</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>Difference</td>
<td>15.6</td>
<td>23.9</td>
<td>7.8</td>
</tr>
<tr>
<td>NaOH treated DPW</td>
<td>Basal</td>
<td>43.1</td>
<td>57.8</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>Antibiotic</td>
<td>61.8</td>
<td>75.2</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>Difference</td>
<td>18.7</td>
<td>17.4</td>
<td>9.0</td>
</tr>
</tbody>
</table>

1. The difference in fat digestibility between the antibiotic fed groups and the groups fed the basals.
the digestibility of fat (mainly from tallow) increased with age of the chicks. A marked increase in fat digestibility was observed between the 1st and 2nd week. Between the 2nd and 4th week there was still a gradual increase in fat digestibility. This is generally in agreement with the findings of Fedde et al. (1959), Renner and Hill (1960), Carew et al. (1972) and Hakansson (1974). In reviewing the results obtained by these workers, it was shown that the digestibility of tallow at one week of age was between 40-53% and increased to 70-79% at the 2nd week; at the 8th week the tallow digestibility was 76-82%. The results from this study indicate that maximum digestibility of tallow may be attained at the 4th week of age and beyond this age there would be little increase in the digestibility.

The effect of age on fat digestibility varies with the kind of fat fed to the chicks. With corn oil or lard, the digestibility of fat only increased slightly or showed no change from the 1st to 2nd week or with older age (Carew et al., 1972; Fedde et al., 1959; and Renner and Hill 1960). Hakansson (1974) demonstrated that palmitic and stearic acids showed the largest increases in digestibility with age whereas the unsaturated fatty acids increased only slightly with age. This may explain the differences in the effect of age on the various kind of fats. The digestibility of vegetable oils, which contain lower proportion of the saturated fatty acids is less affected by the age factor, and vice versa.

During a period early in the life of the chicks many workers (Pensack and Huhtanen, 1963; Eyssen and DeSommer, 1963 and Carew et al. 1972) observed that the chicks consumed feed normally, but apparently developed a transitory syndrome of malabsorption of feed nutrients as expressed by reduction in growth rate, impairment in feed utilization and increased output of fecal material. Fat excretion, in terms of percent fat in the excreta, was especially in-
creased during this period, and reached the peak at 5–9 days of age. Spontaneous improvement in growth rate and intestinal absorption subsequently occurred after this initial period. These observations show that the newly-hatched chick has not developed fully the physiological ability to absorb fat. However, this appears to develop rapidly after the first ten days of life. The development of the ability for fat absorption seems to relate to the type of fat ingested. Carrew et al. (1972) showed that with corn oil, the quantity of fat excreted approached its minimal value almost immediately after the first week so that no difference was observed between 9–15 days of age. With tallow, the fat excretion tended to decline progressively until the end of the 2nd week or further.

When antibiotics were added to the diets, the fat digestibilities were increased at all ages. However, a marked increase in fat digestibility occurred at the 1st week, and thereafter the increase was more gradual and tended to diminish with age. This is in accordance with Pensack and Huhtanen (1963) and Eyssen and DeSomer (1963) showing that antibiotic feeding reduced fecal fat excretion and resulted in increased weight gain and improved feed efficiency during the critical period of malabsorption.

Huhtanen and Pensack (1965a) found that enterococci, mainly \textit{Streptococcus faecalis} predominated in the duodenum of the chicks at 3 days of age and were maximum at 6 days, then gradually decreased to 28 days of age, being largely replaced by anaerobic types. Hence, the period during which the chick was undergoing unutrient malabsorption appeared to coincide with the period of increase in numbers of \textit{Str. faecalis} organisms in the duodenum, thus suggesting that this organism was involved in the malabsorption phenomenon. In fact, inoculation of germ-free chicks with \textit{Str. Faecalis} was shown to cause a depression in growth and rise in fecal fat excretion but no such
effects were observed with other organisms such as lactobacilli and enterobacteriaceae. Administration of antibiotics was found to eliminate the organism and ameliorate the growth depression (Huhtanen and Pensack, 1965b; Eyssen and DeSomer, 1967). Tortuero (1973) also observed that suppressing the \textit{Str. faecalis} population during the early period of the chick's life by the implantation of \textit{Lactobacillus acidophilus} or by antibiotic feeding increased fat digestibility, and resulted in increased weight gain and better feed efficiency. Therefore all these findings indicate that antibiotics stimulate chick growth by their action against micro-organisms which interfere with the absorption of feed nutrients, especially during the early part of the chick's life.

Eyssen and DeSomer (1963) indicated that the growth-promoting effect of antibiotics was restricted to the critical period of a few days early in the chick's life. However, in this study, the growth promoting effect of antibiotics and their effect on fat digestibility were still encountered up to 4 weeks of age. Therefore, besides inhibiting the undesirable organisms during this critical transitional period the antibiotics also acted in the later stage to promote higher nutrients absorption by the mechanisms as discussed in the literature review.

Although the fat digestibility was improved by the antibiotic feeding, it was further increased by increasing age. Thus the digestibility of fat (tallow) was affected by the age as well as by the microbial action. The effects of age and the antibiotic feeding appeared to be additive.

There was no difference in the fat digestibility between the basal diets (without antibiotics) at the 1st week of age. However, at the 2nd week and onward, the digestibility of fat in the DPW diets were again found to be lower than that of the control diet. This difference tended to decrease.
and became insignificant at the end of the 4th week. The effect of high calcium level on fat digestibility was not apparent at the first week probably because the chick's ability to absorb fat was limited. At the older age (4th week) the chick's ability to absorb fat was much improved and probably was able to overcome the insoluble-soap formation and absorb a larger amount of free fatty acids. Hence, it is only between these two periods that the effect of high calcium level on fat digestibility was demonstrated. All groups fed with antibiotics showed similar fat digestibility at the various ages. This indicates that antibiotic feeding overcame the effect of high calcium level on fat digestibility, probably by increasing the calcium absorption.

The M.E. values of the diets determined at the various age (shown in Table 22) were correlated with the fat digestibility. Hence at each age, feeding antibiotics would increase the M.E. values of the diets and M.E. also increased with the age of the chicks. Nelson et al. (1963) observed that when growth was stimulated by antibiotics an increase in M.E. of the diet was also obtained. The authors attributed this effect to antibiotics facilitating the absorption of calorigenic nutrients, which in this study proved to be the fat.

At four week of age the M.E. values of the alkali-treated DPW diets (without or with antibiotics) were equivalent to the corresponding control diets. However, the M.E. values of the untreated DPW diets were always lower than the other diets, especially in comparison to the alkali-treated DPW diets even though treated DPW exhibited the same degree of fat digestibility. It is probably because of the low digestibility of nutrients in the untreated DPW and also the lower M.E. value of the untreated DPW (lower than the value of 1000 kcal/kg used in the formulation). This indirectly confirmed that the
### Table 22. Effect of age and antibiotics feeding on the metabolizable energy values of the diets in Experiment 7.

<table>
<thead>
<tr>
<th>Diets</th>
<th>M.E. kcal/kg (as fed basis)</th>
<th>Formulated</th>
<th>Determined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2755</td>
<td>2510</td>
<td>2680</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>2755</td>
<td>2653</td>
<td>2786</td>
</tr>
<tr>
<td>Difference</td>
<td>--</td>
<td>143</td>
<td>106</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2720</td>
<td>2008</td>
<td>2347</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>2720</td>
<td>2259</td>
<td>2520</td>
</tr>
<tr>
<td>Difference</td>
<td>--</td>
<td>251</td>
<td>173</td>
</tr>
<tr>
<td>NaOH treated DPW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2720</td>
<td>2242</td>
<td>2574</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>2720</td>
<td>2480</td>
<td>2726</td>
</tr>
<tr>
<td>Difference</td>
<td>--</td>
<td>238</td>
<td>152</td>
</tr>
</tbody>
</table>
alkali-treatment improved the M.E. of the DPW.

The nitrogen, calcium and phosphorus retention of the diets are shown in Table 23. It was evident that antibiotic supplementation did not significantly affect the utilization of these nutrients in the diets. This is presumably because the diets contained adequate levels of these nutrients so that no beneficial effects would be exercised by the antibiotics as discussed in the literature review.

The N retention of the DPW diets were lower than that of the control diets, reflecting the lower utilization of nitrogen in the DPW diets. However, the alkali-treated DPW showed better nitrogen utilization than the untreated DPW.

The percent of calcium and phosphorus retained by the chicks fed the DPW diets was lower than that of the control. This is because of the low digestibility of these minerals in the DPW and also due to the high levels of these minerals contained in the diets which reduced the mineral absorption (Morrissey and Wasserman 1971). The untreated DPW diets contained the higher level of calcium and phosphorus (shown in Table 23) and showed lower retention of these minerals as compared with the alkali-treated DPW diets.

The feed efficiency of the diets (represented by the feed conversion rations) at weekly intervals was studied and shown in Table 24. The feed efficiency of the control diet was improved similarly throughout the entire experimental period. However, the feed efficiency of the DPW diets (untreated or alkali treated) were markedly improved after the second week, as shown by an increase in difference in the feed conversion ratios between the antibiotic fed groups and the groups fed the basals. This is due to the marked improvement in fat digestibility and M.E. contents after this age. Although the antibiotic feeding significantly improved the fat digestibility and M.E.
Table 23. Effects of antibiotics feeding on nitrogen, calcium and phosphorus retention of chicks fed the experimental diets at 2 week of age.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Nitrogen Retention %</th>
<th>Calcium</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% in diet</td>
<td>Retention %</td>
<td>% in diet</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>58.2</td>
<td>1.29</td>
<td>44.5</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>55.7</td>
<td>1.29</td>
<td>43.1</td>
</tr>
<tr>
<td>Mean</td>
<td>57.0</td>
<td>--</td>
<td>43.8</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPW Basal</td>
<td>49.2</td>
<td>2.49</td>
<td>16.9</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>48.6</td>
<td>2.49</td>
<td>20.9</td>
</tr>
<tr>
<td>Mean</td>
<td>48.9</td>
<td>2--?</td>
<td>18.9</td>
</tr>
<tr>
<td>NaOH treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPW Basal</td>
<td>54.0</td>
<td>1.96</td>
<td>39.6</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>49.5</td>
<td>1.96</td>
<td>43.6</td>
</tr>
<tr>
<td>Mean</td>
<td>51.8</td>
<td>--</td>
<td>41.6</td>
</tr>
</tbody>
</table>
Table 24. Feed conversion ratio of chicks fed the experimental diets at weekly intervals in Experiment 7.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Feed Conversion Ratio (Weekly)</th>
<th></th>
<th></th>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 1</td>
<td>1- 2</td>
<td>2- 3</td>
<td>3- 4</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.26</td>
<td>1.71</td>
<td>1.81</td>
<td>2.08</td>
<td>1.80</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1.19</td>
<td>1.63</td>
<td>1.71</td>
<td>1.99</td>
<td>1.74</td>
</tr>
<tr>
<td>Difference</td>
<td>0.07</td>
<td>0.08</td>
<td>0.10</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Untreated DPW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.31</td>
<td>1.82</td>
<td>2.02</td>
<td>2.37</td>
<td>2.01</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1.28</td>
<td>1.79</td>
<td>1.92</td>
<td>2.19</td>
<td>1.91</td>
</tr>
<tr>
<td>Difference</td>
<td>0.03</td>
<td>0.03</td>
<td>0.10</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>NaOH treated DPW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.28</td>
<td>1.87</td>
<td>2.02</td>
<td>2.25</td>
<td>1.99</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>1.23</td>
<td>1.82</td>
<td>1.87</td>
<td>2.09</td>
<td>1.87</td>
</tr>
<tr>
<td>Difference</td>
<td>0.05</td>
<td>0.05</td>
<td>0.15</td>
<td>0.16</td>
<td>0.12</td>
</tr>
</tbody>
</table>

1. Overall feed conversion ratio = \( \frac{\text{Total feed consumption for 4 weeks}}{\text{4 week body weight gain}} \)

2. Different subscripts in the same column indicate significant difference (P < 0.05).

3. The difference in feed conversion ratio between the antibiotic fed groups and the groups fed the basal.
content of the DPW diets to the level comparable to the control, the feed efficiency of these diets was still inferior to the control. It was probably the low digestibility of protein in the DPW that was responsible for the lower feed efficiency of diets containing it. Therefore, the antibiotics markedly increased the fat digestibility, however there was no increase in the digestibility of protein in DPW.
SUMMARY AND CONCLUSION

Samples of DPW were treated with 2, 3, 4, 5 and 7% sodium hydroxide solution and their chemical compositions were determined. Untreated DPW and alkali-treated DPW were included at 10, 15 and 20% levels in the chick diets and the performance of broiler chicks fed such diets were studied. Feeding trials in evaluating the utilization of non-protein nitrogen in DPW by chicks and metabolic trials to determine the availability of protein and minerals in DPW were performed. Furthermore, the effects of supplementations of lysine and methionine, dietary fat, and antibiotics on the performance of chicks fed the DPW diets and on the utilization of nutrients in the diets were studied. The following results were observed.

1. The alkali treatments had little effect on the acid-detergent fibre content of DPW except at the highest level (7%). The true protein, non-protein nitrogen and uric acid contents of DPW were reduced in relation to the level of sodium hydroxide used. Most of the amino acids in DPW were not affected by the 2% NaOH treatment except lysine, arginine, serine, proline and cystine which showed slight reduction while alanine showed an increase.

2. DPW when added to the broiler diet at levels between 10-20% would support growth related to the dietary energy. Inclusion of DPW in the diet did not affect growth but lowered the feed efficiency compared to the controls containing similar energy and protein contents.

3. Alkali treatment at various concentrations markedly improved the growth and feed efficiency of diets containing various levels of DPW.

4. The M.E. values of untreated DPW and DPW treated with 2, 3 and 5%
the growth, feed efficiency and nitrogen utilization indicating the poorer performance of chicks fed DPW diets was related to its energy dilution effect.

10. In the absence of antibiotics, the dietary fat (tallow) utilization was impaired with the inclusion of DPW in the diet. The metabolizable energy of the diets were directly related to the fat utilization. Feeding of antibiotics completely ameliorated the adverse effect of DPW on fat utilization and markedly increased the M.E. of the diets, hence improved the growth and feed efficiency.

11. Antibiotic supplementation had no effect on the nitrogen and minerals retention of the diets.

These studies showed that when balanced for amino acids and energy, inclusion of high levels of DPW in the diets did not affect growth. However, feed efficiency of the diets containing DPW was impaired due to the low digestibility of protein in the DPW and reduced utilization of fat (tallow) in the diet in the presence of DPW. Nevertheless, the impaired fat utilization can be ameliorated by the antibiotic feeding and the low digestibility of protein can be increased by sodium hydroxide treatment.

The low M.E. value of DPW, low nutrient availabilities and variability in composition of the product may mean that it has limited value as a source of nutrients. However, a low level of inclusion (about 10%) is desirable since this may lower the cost of feed per unit gain and also increase the growth of chicks through stimulating feed intake due to its energy dilution effect. Treatment with sodium hydroxide significantly improved the nutritive values of DPW as a feed ingredient for chicks.
NaOH were determined to be 827, 1155, 1245 and 1205 kcal/kg D.M. respectively.

5. Supplementing the basal diet which was suboptimal in protein level (20%) with 2.25% protein equivalent from uric acid or from NPN in the DPW did not improve the growth of chicks. Growth was depressed by urea or diammonium citrate supplementation. Nitrogen retention was reduced and uric acid excretion was increased by the supplementation indicating that these nitrogen sources would not be utilized by the chicks.

6. The availability of true protein of untreated DPW was found to be 50.5%. Therefore, the low digestibility of protein in DPW is responsible, in part, for the poorer feed efficiency of diets containing DPW as compared to the controls. The alkali treatments markedly improved the protein digestibility (about 70%). This explains the improvement in performance of chicks obtained with the treatments.

7. The availability of calcium and phosphorus in untreated DPW was 53.8 and 19.8% respectively. The alkali treatments did not affect calcium and phosphorus availabilities significantly. The availability of sodium in untreated DPW was 84.4%, which was decreased to 41.8-48.3% by the alkali treatment.

8. Growth of chicks fed the 20% DPW basal diets (untreated or alkali-treated) were higher than those fed the basal control diet. However, no growth response was obtained from supplementing the DPW basal diets with methionine and lysine. These results indicate that DPW diets were not deficient in amino acids and the amino acids in the DPW could be utilized by the chicks for growth.

9. Increasing the energy level of the diets by adding 2% tallow improved
LITERATURE CITED


Nwokolo, E.N. and D.B. Bragg, 1976. The influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. (in press)


