Utilization of Rye in Poultry Diets: Similarities to Malabsorption Observed in Steatorrhea

by

G. Leigh Campbell

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UTILIZATION OF RYE IN POULTRY DIETS: SIMILARITIES TO MALABSORPTION OBSERVED IN STEATORRHEA

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

Chicks fed rye were found to be comparable in many respects to other conditions where malabsorption occurs as a result of excessive microbial activity in the small intestine. Inclusion of rye in the diet reduced retention of amino aids and particularly fat. Chicks fed rye were also severely rachitic in some instances, indicating inadequate absorption of vitamin D_3. A microbial involvement was indicated by virtual elimination of the negative response when the chicks were raised in a germ-free environment, although the response was confounded by the irradiation treatment employed as a means of diet sterilization which alone exerted a positive effect. Streptococcus induced a similar effect to that observed in conventional chicks fed rye when administered to axenic chicks. Gamma irradiation of the rye most likely exerted a beneficial response by depolymerizing pentosan gums, which are apparently prone to irradiation-induced destruction. The results may be interpreted as positive evidence for the hypothesis that viscosity-increasing pentosan gums in rye predispose the young chick to excessive intestinal bacterial activity, which in turn mediates, in part at least, the poor performance of chicks fed rye. A possible mechanism by which this occurs is via excessive bile salt deconjugation in the small intestine which was indicated by a positive response to supplementary bile salts in the diet containing rye. A high level of calcium also tended to reduce fat loss in chicks fed rye diets, although this effect was not statistically significant. Further evidence indicating alteration of lipid metabolism in roosters fed diets containing rye was indicated by increased fecal fat loss, and a tendency of reduced plasma cholesterol levels and increased plasma cholesterol turnover rate. It was also observed that adult fowl exhibited excessive electrolyte loss when fed a diet containing rye, or alternately rye water extract. This appeared to be attributable to increased fecal mass and a limited capacity to extract electrolytes beyond a lower critical level. Increased excreta mass was due to increased dry matter loss, as well as a moderate increase in the water content of the wet excreta.

Practical studies, where the objective was to find a logical postion for rye in commercial diet formulation, indicated that rye may be fed to laying hens with no penalty in performance, provided the level in the diet is kept below approximately thirty per cent. The results of several chick trials indicated satisfactory performance with rye diets when supplemented with fish meal, additional salt, and soybean oil. However, the results were not sufficiently consistent to warrant recommendations regarding feeding rye to chicks.

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Thank you.

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1.0 INTRODUCTION

Rye has never been widely accepted as a useful feed ingredient for poultry as it has consistently been observed to reduce performance to a point where feeding rye is uneconomical regardless of price. The cause of the growth depression is not apparent from the usual indices of nutritional worth. Crude fibre, crude protein, ether extract and amino acid balance of rye do not differ markedly from wheat. Early workers in the area (Halpin et al., 1936) attributed the poor performance to the 'sticky' nature of rye, particularly when it was finely ground. This resulted in pasting of the vent, beak and toes, and the authors felt that the chicks responded by simply refusing rye on the basis of palatability.

Weiringa (1967) attributed the response (rats and swine) to the feeding of rye to a mixture of 5-n-alkyl resorcinols and 5-n-alkenyl resorcinols which could be removed by extracting with petroleum ether or acetone. Fernandez et al. (1973_c), however, were not able to duplicate these results using chicks; acetone extraction had no effect on body weight, feed efficiency or feces condition. On the other hand water extraction improved performance, and the water extract (lyophilized) added to a wheat diet was growth depressing. It also resulted in a sticky feces condition characteristic of feeding rye. From these results it was proposed that

water-extractable polysaccharide gums (presumably pentosans, although these were not measured specifically) were the causative agent causing growth depression. Addition of other water-soluble, viscosity-increasing gums such as pectin (Wagner and Thomas, 1977) to the diet resulted in a rye-type growth depression, which lends support to the argument that gums may cause the observed response.

Burnett (1966) implicated increased viscosity in the intestinal lumen when chicks were fed barley, particularly barleys grown in dry western regions (Willingham et al., 1960). It was proposed that B-glucans in the barley resulted in a highly viscous stable solution which interfered with digestion and absorption of nutrients, and that such a condition accounted for the poor nutritional value of some barley samples. Such an explanation can also be extended to the negative response of chicks fed rye, in this case the pentosans would be the viscosity-increasing agent. However this hypothesis cannot readily explain why chicks fed rye (or barley) tended to respond to a greater degree to a variety of antibiotics than did chicks fed other cereal grains (Fernandez et al., 1973 [a,b]; Misir and Marquardt, 1978 [a,b,d]; MacAuliffe et al., 1976 [a,b]; Patel et al., 1980). Furthermore, chicks fed pectin also responded to antibiotic treatment (Wagner and Thomas, 1977). Moran and McGinnis (1966) proposed that viscosity increasing agents, in this case the B-glucans in barley, supported an unfavorable

intestinal microflora that contributed to the negative response.

Although considerable progress has been made towards describing the nutritional effects rye has when fed to poultry, the basic question as to whether the gums themselves are growth depressing and the microbial effect is mainly coincidental, or whether the gums are directly responsible through changes in the nature of the digesta in the intestinal lumen has not been resolved. It is the intention of the author to attempt to explain the nutritional effects feeding rye to poultry has on the basis of the activity of the intestinal microflora. Physical effects pentosan gums may have will be dealt with only briefly. For this subject, the reader is referred to Lee (1981), Ward (1982), Antoniou (1980), and Misir (1978).

Practical feeding trials were also conducted to ascertain in what types of diets and under what conditions rye grain may be successfully incorporated into poultry diets.

2.0 REVIEW OF LITERATURE

2.1 Anti-nutritional effects attributed to substitution of rye grain for other cereal grains (wheat or corn) in poultry diets.

Early researchers (Halpin et al., 1936) attributed the anti-nutritional effects of rye to its sticky nature and concommittant reduction in palatability. More recently authors have indicated more specific ways by which rye mediates an anti-nutritional response. MacAuliffe and McGinnis (1971) reported results indicating that the metabolizable energy of rye declines curvilinearly as the level in the diet increases. They concluded that rye at 40% substitution either interferes with digestion of other dietary components or stimulates the growth of a flora that competes with the host for available nutrients. MacAuliffe et al. (1976_a) found that besides resulting in poorer growth, feeding diets containing rye caused severe rickets in turkey poults, despite seemingly adequate dietary levels of vitamin D, calcium and phosphorus. No such effect was observed in a corn diet containing the same level of vitamin-mineral fortification. This rachitogenic effect as measured by fat-free tibia ash in the poults fed rye could be alleviated by supplementation of the diet with vitamin D_3. While a dietary level of 1650 I.C.U. D_3/kg supported good bone

mineralization when corn provided the cereal base of the diet, it alone was insufficient for poults fed rye. Tibia ash of the turkeys fed rye was also improved by either addition of tallow to the diet (6.45%) or by the addition of penicillin (50 ppm). The authors suggested that since fat alleviates the severity of rickets it may be possible that rye interferes with absorption of vitamin D_3. In this regard fat most likely provides a transport vehicle for the D_3. They attributed the penicillin response to the suppression of an adverse microflora, which they felt somehow interfered with the absorption of D_3, perhaps by irritating the intestinal mucosa. MacAuliffe et al. (1976a) observed that rye diets gave a significant increase in growth in response to penicillin, with no further increase in bone mineralization. They speculated that the penicillin either exerted a dual effect, or had a sparing effect on other nutrients in addition to D_3.

Similar results to those noted with poults were obtained when rye diets were fed to broiler chicks (MacAuliffe et al., 1976_b). Diets containing 200 I.C.U. D_3/kg. were adequate when corn comprised the cereal component, however both tibia ash and growth were depressed when rye replaced corn. These effects could again be partially alleviated by supplementation with fat or procaine penicillin, and completely overcome with a high level of vitamin D_3 (2000 I.C.U./kg). Water extraction of the rye removed the factor

responsible for the rachitogenic effect, and an acid autoclave treatment partially relieved the condition. Growth rate but not bone ash, was significantly improved.

MacAuliffe and McGinnis (1976) fed chicks vitamin D_3-free diets containing either corn or rye to induce a rachitic response, then administered the chicks a single oral dose of vitamin D_3. The rachitic chicks fed the corn diet responded significantly better than the chicks fed rye, again indicating an anti-vitamin D factor in the rye. Exposing the chicks to ultraviolet light treatment for 10 minutes daily resulted in a significant improvement in growth rate, and a marked improvement in bone ash. MacAuliffe and McGinnis (1976) inferred that rye somehow interferes with absorption of vitamin D_3 from the gut, since it appeared to have no influence on the response to UV light, and hence no effect on endogenous biosynthesis of vitamin D_3 through any type of metabolic block. Zaviego et al. (1976) administered a single dose of ^3H vitamin D_3 to chicks fed either corn or rye diets, and were able to demonstrate reduced absorption where rye was fed.

Antoniou et al. (1980) could not demonstrate that the addition of vitamin D_3 improved chick performance when rye diets were fed. They felt the discrepancy between their results and previous work by other authors was due to their incorporation of fat into the diet (2.5 to 7.5%), and to the use of SCWL chicks as opposed to broiler stock. Antoniou et

al. (1980) did observe that at very high vitamin D_3 levels wheat-fed chicks, but not rye-fed chicks exhibited a toxic reaction. This was suggested as evidence for a factor in rye that complexes vitamin D_3.

Other nutrients, aside from D_3, have been reported to be adversely affected by the presence of rye in the diet. Tallow has been observed to be more poorly utilized by chicks than oil (Hakansson, 1974), however in the case of chicks fed rye based diets, this inability to use tallow as opposed to oil is exaggerated. The performance of chicks fed rye was improved to a greater degree as compared to chicks fed wheat when soybean oil replaced tallow in the diets (Antoniou et al., 1980). Divalent cations reduce fatty acid absorption by the formation of a nondigestible complex that is excreted (Hakansson, 1974). This effect was apparent in wheat-based diets (Antoniou et al., 1980) when dietary calcium was increased from 0.6 to 1.1% in diets containing either soybean oil or tallow as a fat source. The fat retention for both tallow and soybean oil declined at the higher calcium level - from 79.3 to 75.4% for the tallow supplemented diets, and from 84.4 to 81.1 for the soybean oil supplemented diets. Rye-based diets behaved differently. In this case fat retention for the soybean oil supplemented diets fell from 77.9 to 72.6%, which was similar to that observed for the wheat diet, however fat retention in the tallow supplemented diets improved from 58.4 to 67.4%. It was suggested from

these data that calcium has a positive effect in the case of tallow digestibility in rye diets. Marquardt and co-workers offer no specific explanation for this response, although they implicate pentosan gums which they suggest interfere with the absorption of all nutrients including fat, protein, calcium and possibly vitamin D_3 (Antoniou et al., 1980, Marquardt et al., 1979).

Marquardt et al. (1979) reported that the retention of most nutrients was depressed when chicks were fed rye as oppposed to wheat diets. Ether extract (27 vs 71%), total amino acids (65 vs 82%) as well as individual amino acid retention were depressed in rye as compared to wheat diets. Supplementation of the wheat or rye diets with a mixture of essential amino acids improved weight gain (372 vs 135%) and feed efficiency (71 vs 56%) to a greater extent in the case of wheat. Addition of penicillin to the diets resulted in improved retention of both total and individual amino acids, this effect again being greater for the rye as compared to the wheat diet. Neither penicillin nor supplementation with amino acids had an effect on ether extract retention. Marquardt et al. (1979) concluded that utilization of certain nutrients, particularly amino acids, was limited by factor(s) in the rye. They suggested that the penicillin depresses deleterious bacteria that compete with the host for the available nutrients, and poor utilization of amino acids is a major cause of the depressed performance in chicks fed rye.

Calcium, phosphorus and nitrogen retention were also depressed when rye was fed (Marquardt et al., 1979). It is difficult, however to attribute any performance depression to a lack of availability of these nutrients, since their retention will depend directly on growth rate. Absorbance in the case of calcium and phosphorus, and excretion (as uric acid) in the case of nitrogen, are controlled processes that reflect tissue needs. Thus any factor that interferes with growth, regardless of the mechanism, will increase excretion of calcium, phosphorus and nitrogen. This will be measured as reduced retention.

Chicks fed wheat or rye differ in their ability to use fish meal or meat meal as a protein source (Misir and Marquardt, 1978_a). Growth of chicks fed rye was improved to a greater extent than chicks fed wheat by providing fish meal as opposed to meat meal as the supplementary protein source. Rye diets were improved by additional protein (18 vs 26%) but only when the protein was furnished as fish meal. Penicillin supplementation also improved the rye diets, and its effect was greatest in rye diets containing a lower quality protein supplement (meat meal). Misir and Marquardt (1978) conclude that the chick's ability to digest and/or absorb nutrients was impaired in rye diets. They proposed that the high levels of unabsorbed nutrients subsequently provided a substrate source for the proliferation of a deleterious microflora, which then contribute to the deleterious response in an

unspecified manner. In retrospect it is difficult to conclude that these effects are specifically attributable to the protein source in question, fish meal versus meat meal. Fish meal is frequently higher in salt than meat meal, and salt has been shown to elicit a positive response at levels far beyond those recommended by N.R.C. when fed to chicks consuming rye-based diets (Lee, 1981). Fish meal has been reported to have an anti-microbial effect (Harrison and Coates 1964, 1972). Meat meal may contain substantial tallow which has been shown to be poorly utilized when diets also contain rye (Antoniou et al., 1980). The calcium-phosphorus differences between the two ingredients could also contribute to the response.

The mechanism by which gums cause such diverse effects as causing rickets, inducing poor retention of tallow and meat meal has not been established; most authors concede however, that it is likely the gum component that is the causative agent. Much of the evidence implicating gums is inferred from studies of the nutritional effects of feeding certain barley samples, in which B-glucans have led to an increase in intestinal viscosity (Burnett, 1966).

2.2. Evidence implicating the pentosan-gum component as responsible for the anti-nutritional effects of rye grain fed to chicks.

In many respects the adverse effects observed when rye is fed to chicks are similar to those that have been reported for barley diets, although the response is generally less severe. Like feeding rye to chicks, feeding barley may result in sticky droppings which contain more water than the excreta of chicks fed corn diets (Willingham et al., 1959). Nutrient retention in barley diets may be depressed in a similar manner to what has been reported for chicks fed rye diets. Potter et al. (1965) reported low digestibility for protein, fat, and nitrogen-free extract for chicks fed barley, which corresponds to the observations of Marquardt et al. (1979) regarding nutrient retention of chicks fed rye diets.

Although there is a tendency for poultry nutritionists to attribute the inferior performance of chicks fed barley to its high crude fibre content, a number of studies indicate that crude fibre per se may not be the major detrimental factor in barley. Removal of the hull, and the majority of the crude fibre component, usually results in only a marginal improvement in performance. Whereas whole barley had only 70% of the feeding value of corn, pearled barley (mechanically dehulled) achieved only 82 % of the corn value (Fry et al., 1958). Other studies with hulless barley have given similar results, in that hulless barley proved to be only 2% better than regular barley (Anderson et al.,1961). More recently researchers have attributed the poorer nutritional qualities of barley to soluble B-glucan that increases intestinal

viscosity, thereby causing problems for chicks fed barley diets (Burnett, 1966; White et al., 1981).

Several means of improving barley diets have been found to be successful, and the success of these treatments may be interpreted in terms of their effect on the B-glucan component of barley. In this regard the negative effects of barley may be at least partially alleviated by water treatment or enzyme supplementation of the diet.

Thomas et al. (1960) proposed that the mechanism by which a process of wetting and drying of grain improves its nutritional value may involve bacterial degradation of gums. They found that incubation of barley slurries with Bacillus subtilis improved the nutritional quality of barley. Bacillus subtilus was subsequently found to contain B-glucanases (Rickes et al., 1962). The observation that higher temperatures during soaking (Anderson et al., 1961) reduced the effectiveness of the water treatment supports the view that the improvement observed is the result of enzyme activity, however it does not rule out the possibility that the activity is the result of endogenous B-glucanase of the barley itself.

A variety of enzymes gave a beneficial effect when added to barley diets (Anderson et al., 1961, and Willingham et al., 1959). Enzymes that helped included crude bacterial, fungal, or malt enzyme preparations. Crystalline amylase was ineffective, which led Willingham et al., (1959) to propose

that impure enzyme sources are somehow protected, or that other enzymes besides amylase were present in the crude sources. The latter alternative would seem more likely. Surprisingly both groups found that crystalline bacterial protease had a beneficial effect, which is inconsistent with the basic premise that the gums are the causative agent, however, it is conceivable that plant proteins are involved in the formation of the viscosity-increasing gel structure. Of the enzyme sources studied Willingham et al. (1959) found fungal 'amylase' to be the most effective, however this high activity may have been due to contamination with other enzymes, since crude fungal sources are notoriously impure. Fungal enzymes sources are likely more effective than other sources for addition to feed due to their stability and activity at a fairly low pH. Many have a pH optimum around 4.5 and are stable at pH 2.5, enabling them to retain a substantial portion of their activity into the small intestine.

The improvement observed in growth rate due to enzymatic or simple water treatment of barley may be explained on the basis of improved nutrient retention. Leong et al. (1962) observed that a fungal enzyme supplement improved the metabolizable energy of western-grown pearled barley and regular barley 23.8 and 14.5%, respectively. Water treatment gave increases of 26.1 and 24.6% for the same samples. Similar results were reported by Potter et al. (1965) who

found that M.E. content of western-grown barley was increased 18% by the presence of fungal enzyme, and 22% by water treatment. The improvement in M.E. was attributed to an increased retention of protein and fat. The coefficients of digestibility of the protein, fat and nitrogen-free extract were respectively 45, 0, and 75% for untreated barley; 75, 76 and 81% for barley with enzyme and 84,84 and 78% for barley that had been water-treated.

Other observations would also suggest that the problems involved with feeding rye are in many respects similar to the situation for barley. Rye too has been reported to respond to water treatment and enzyme supplementation, however the improvement in the response has not been as consistent as for barley. Fry et al. (1958) observed that soaking ground rye in an equal weight of water overnight, followed by drying at 70 ^{o}C improved performance. Water soaking rye improved performance in the case of MacAuliffe and McGinnis (1971), but not in the case of Smith and McIntyre (1960). Fernendez et al. (1973_{c}) found that water extraction improved the performance of chicks fed rye. The water-extractable component was growth-depressing if lyophilized, however drying using heat (90 ^{o}C) appeared to reduce the response. Fernandez et al. (1973) concluded that the chick growth-depressing factor of the water extract was susceptible to heat.

The improvement of rye due to water extraction was

attributed to the pentosan gums (Fernandez et al., 1973_c) which would be partially removed by water treatment. The pentosan gum consists of a linear chain of B-D-xylopyranose molecules joined by 1-4 glycoside bonds, with L-arabinose units attached as single unit side-chains (Wolfrom and Tipson, 1959 quoted in Fernandez et al., 1973_c) The pentosan gums are sensitive to acid hydrolysis (Perlin, 1952), which possibly explains the positive response to acid-autoclaving reported by MacAuliffe et al. (1976_b). Mild acid hydrolysis preferentially removes the arabinose side units which permits association of the xylan and renders the complex insoluble. Autoclaving alone would have, predictably, no effect. In fact autoclaving alone has been observed to give a negative response in the case of barley since Willingham et al. (1960) observed that the nutritional value of 'eastern' barley was markedly lowered by autoclaving. The effect was attributed to the destruction of endogenous B-glucanase which would otherwise result in reduced viscosity in the gut.

Early attempts to improve rye by the use of enzyme supplementation failed (Smith and MacIntyre, 1960) or resulted in only a slight improvement (Moran et al., 1969). In the later study (Moran et al., 1969) little effect with either fungal or bacterial enzymes was evident. More recently, however, Patel et al. (1980) observed a substantial positive response to a 'pectic' enzyme (Irgazyme-100) in rye-containing diets indicating that rye, with appropriate

enzyme supplementation, will give a positive response similar to that noted with barley.

Barley has been observed to vary according to the region where it is grown (Willingham et al., 1960) with regard to the effectiveness of enzyme treatment. Barley grown in the midwest and east generally did not respond to enzyme supplementation to the same degree as did barley grown in the west. The authors, noting that the feeding value of eastern barley was markedly lowered by autoclaving, concluded that eastern barleys were higher in endogenous B-glucanase than western barleys. Given the hydroscopic nature of the B-glucan in barley it is tempting to speculate that the gums may play a role in water-retention in the seed; and that environmental conditions play a role in determining the level produced by the plant and the activities of the enzymes involved in its production and function.

In contrast to barley, few regional differences in the nutritional quality of rye have been detected. Moran et al. (1969) found very little difference between rye that was grown in the west or in the east, although there was a slight caloric advantage in favour of the eastern grown rye. Patel and McGinnis (1976) examined eight different samples of rye collected from widely varying locations including Washington, North Dakota, Minnesota and Michigan. They noted a consistent growth depression regardless of geographical area of production or protein content of the rye. It has been

suggested that soluble cell wall carbohydrates in rye may serve as freezing inhibitors for the seed, indicating that summer varieties of rye may have lower levels of pentosans than fall varieties. By lowering the temperature at which water in the seed will freeze, the gums are thought to aid in producing a high-intensity freeze that results in the controlled growth of ice crystals, less tissue damage and a higher capacity to withstand winterkill (Olien, 1965; Shearman et al., 1973).

The anti-nutritional effects of rye or barley may be explained on the basis of the gums and their interaction with the digestion and absorption process, although the mechanism by which the effects are manifested is not clear. It has been observed however that in many cases adding purified gums to diets (pectin, guar gum) causes a similar type of response as feeding rye or barley, which lends support to the contention that poor feeding quality observed for rye diets, and barley diets in some instances, is in fact due to the gum component.

2.3. The effects of gums when added to poultry diets and proposed mechanisms of activity based on their structure and viscosity-increasing characteristics.

Wagner and Thomas (1977) reported a rye type growth depression in chicks fed pectin. Incorporation of pectin into the diet resulted in growth depression and a sticky feces

condition characteristic of feeding rye. Like rye-fed birds, the chicks fed pectin responded to antibiotic supplementation of the diet. Vohra and Kratzer (1964) and Anderson and Warnick (1964) reported that several natural polysaccharides reduced growth of chicks, and that this growth depression could be alleviated with suitable enzyme supplementation.

In a later study, Kratzer et al. (1967) fed a number of polysaccharides to chicks at a level of 2% of the diet: cellulose, methylethyl cellulose, carboxymethyl cellulose, guar gum, carob gum, karaya gum and pectin. All the gums as well as carboxymethyl cellulose had a growth depressing effect. Guar gum reduced nitrogen retention, fat absorption and metabolizable energy. Guar gum, pectin, and carboxymethyl cellulose all reduced bone ash significantly when marginal levels of vitamin D_3 were fed. Carboxymethyl cellulose, and guar gum, which had the greatest effect on bone ash at the low level of vitamin D_3, also had the greatest effect on fat digestion. Kratzer et al. (1967) attributed the effects of the polysaccharides on bone calcification to interference of absorption of D_3 and they quoted Fisher et al. (1965) and Ricardi and Fahrenbach (1965) regarding the interference of pectin, carrageenan or guar gum with cholesterol utilization. Kratzer et al. (1967) indicated that absorption of vitamin D_3, also a steroid, may be similarly affected.

Attempts to explain the variety of effects incorporation of gums or 'fibre' in the diet may have can be divided into

two general categories - those explanations that implicate specific binding by the gum or fibre source in question and those explanations that rely on more general phenomenon to explain the effects, such as increases in viscosity or simply increases in fecal mass.

Nagyvary and Bradbury (1977) proposed a simple model in which an acidic polysaccharide such as pectin may bind a variety of anions including fatty acids or bile acids. The pectin may be converted to an anion exchanger by complexing with a cation, particularly a trivalent cation such as Al^{+3} or Fe^{+3}, which may then bind fatty acids or cholesterol. Furda (1979) demonstrated that pectinaceous fibres neutralized by trivalent cation (Fe^{3+}) were readily capable of binding fatty acid in vitro. Such a model does not directly account for the observation that vitamin D_3 absorption seems to be impaired in poultry fed rye in particular or gums in general. However, if the carboxyl groups involved in the binding protrude from a micelle, then it may be expected that the micelle would be bound to the fibre and hence excreted. Any fat-soluble vitamin such as vitamin D_3 (or cholesterol) carried in the micelle would also be excreted.

Other means of binding cholesterol have been proposed for intact fibre. Story et al. (1979) and McKay et al. (1979) indicated that bile acid binding is complex and likely depends on the source and composition of the fibre in

question. They concluded that binding is primarily hydrophobic, and implicate lignin as one binding component of the diet. Normand et al. (1979) indicated that hemicellulose may bind bile acids, although the mechanism was not specified. A novel theory proposed by Sandholm and Scott (1979) is that luminal digestive enzymes (the lipases, amylases and proteases) possess two binding sites. One is specific for the substrate, the other has affinity for sugar units protruding from the glycocalyx of the enterocyte which enables binding by the enzyme. This serves to reduce endogenous loss. Compounds such as phytohaemagglutins may bind to the same receptors, thereby causing loss of enzymes into the intestinal lumen. Excessive loss in this manner would reduce digestive capacity. Various sugars may have a similar effect - in this case the 'fibre' sugars compete with the enterocyte for the enzyme. Xylose and arabinose rank fairly high in their ability to cause this type of effect.

That such a broad variety of polysaccharides (pentosans, B-glucans or pectin gum) with different molecular structures cause similar effects, would suggest a general effect, such as increased viscosity, as opposed to specific molecular binding mechanisms. Reiser (1979) attributed the physiological effects of dietary fibre on the rate of nutrient absorption to the viscosity increasing capacity of dietary fibre. Jenkins et al. (1978) observed that the ability of guar gum, pectin, gum tragacanth, methylcellulose,

wheat bran and cholestyramine to lower blood glucose after a meal was significantly correlated with the viscosity of the fibre . Presumably absorption rate of other nutrients would be similarly affected.

Brown (1979) discussed transport in a gel network, and indicated that diffusion rate is not dramatically affected until the solute size approaches the average intersegmental distance of the polysaccharide chains. At this point there is a sharp drop in the diffusion rate. This implies the rate of association between enzyme and substrate (large molecules) will be affected prior to the absorption of the macromolecular component parts (smaller molecules), hence digestion may be affected more than absorption per se. Marquardt et al. (1979) observed that addition of purified amino acids to rye diets improved performance, however the retention of amino acids contributed by the rye portion of the diet was not enhanced. This suggests digestion, rather than absorption, may be the more limiting process. Diffusion of molecular conglomerates such as micelles may also be restricted moreso than other smaller components of the digesta. Burnett (1966) reported that feeding certain barleys resulted in the formation of a stable highly viscous condition in the intestine, which he hypothesized might interfere with digestion and absorption. Lee (1981) reported that a similar viscous condition developed in the intestine of rye-fed birds.

A final means by which fibre may reduce retention of nutrients is by simply increasing bulk. Lee (1981) indicated that salt retention was poor in birds fed rye. The low salt retention appeared to be simply related to fecal bulk - since chicks fed rye excreted a greater mass of excreta per unit feed and consequently their excretion of salt was correspondingly higher. The chick does not or cannot reduce the salt level in the feces below a certain critical level. Such a mechanism would not require specific binding effects of the fibre. It could also be applied to fat, or amino acids where only small reduction in digestibility coefficients and no increase in concentration of nutrients in the fecal matter are observed. In fact, it may be argued that demonstration of direct interference of digestion and absorption by a high fibre type, whether by binding or increased viscosity, would require increased levels of nutrients in the excreta.

In general, the incorporation of even low levels of gums in the diet bears close similarities to the condition observed when rye constitutes the cereal fraction of the diet. The similarities included reduced growth, the characteristic sticky droppings, reduced nutrient retention as well as the more specific rachitic effect.

2.4 Evidence suggesting that growth depression in chicks feed rye may be attributed to abnormal activity of the gut microflora.

While results of adding enzymes to rye diets have been variable, adding antibiotics has consistently been observed to alleviate the adverse effect, often to a considerable degree. Moran and McGinnis (1966), on observing that the growth depression in turkey poults fed barley could be partially alleviated by either an enzyme supplementation or the addition of oleandomycin, proposed that the barley gums adversely affected growth by supporting an 'unfavourable' microflora. Addition of oleandomycin or enzyme supplementation alone was not as effective as supplying both additives. Corn diets gave no significant response with either enzyme or oleandomycin supplementation. Moran and McGinnis (1966) conducted similar experiments feeding barley with or without oleandomycin and bacterial enzyme to turkeys 8 to 20 weeks of age. They found no difference in the growth or feed efficiencies of birds fed either corn or barley, and no effect of enzyme or antibiotic when added to the diet. They concluded that the intestinal microflora in older birds is not affected by the grain component of the diet, therefore the antibiotic had no effect.

Moran et al. (1969) fed an antibiotic mixture comprised of penicillin, chlortetracycline, tyrosine and oleandomycin to chicks in diets where the cereal base was rye or corn. The entire diet was either pelleted or fed as mash. There was a tendency for the chicks fed rye (pelleted diets) to give a slightly better response for gain, feed efficiency and

metabolizable energy than those fed corn. Overall performance on the rye diets, however, was good and roughly comparable to the corn diets. There was a marked response to pelleting the diet as opposed to feeding it in a mash form. This the authors attributed to adherence of feed on the chicks beaks, which could be prevented by pelleting the feed. Feed accumulation on the beak presumably reduced consumption.

MacAuliffe and McGinnis (1971) demonstrated that antibiotic supplementation to diets containing rye (penicillin and terramycin) produced a greater response for rye than for wheat diets. In one trial the growth depression was completely counteracted by adding 50 ppm penicillin; in a second trial the chicks fed rye did not achieve the performance of the chicks fed wheat. Similar results were obtained when beans, peas or lentils were fed at high levels (53.5%) (Goatcher and McGinnis, 1972). Growth of chicks fed autoclaved or raw dry beans was increased 50 and 200% respectively by dietary supplementation of antibiotics. The authors again implicated an unfavorable microflora in the intestinal tract as having an adverse effect that the antibiotics alleviate. The raw beans caused a type of diarrhea that the addition of the antibiotic did not alleviate. Kakade and Evans (1964, 1966) felt that the improvement in growth with rats fed dry beans with antibiotic could be attributed to increased digestibility or absorption of nutrients, or possibly inhibition of an enzyme that

liberates 'bound' toxic substances normally present in the bean.

Fernandez et al. (1973_b) fed chicks rye or corn diets supplemented with MD-bacitracin and 3-nitrophenylarsonic acid both singly and added in combination. Chicks fed rye diets exhibited greater growth response than chicks fed corn diets for any given drug combination. They also had a lighter shank colour, poorer feed efficiency and more sticky feces. The MD-bacitracin did not affect these latter traits. There appeared to be an interaction between MD-bacitracin and 3-nitrophenylarsonic acid, with a combination of these drugs giving a greater response than either alone.

Similar results were obtained in a second trial in which Fernandez et al. (1973_b) compared the growth response obtained when feeding rye, red Mexican beans or corn supplemented with procaine penicillin, MD-bacitracin and 3-nitro to chicks. Again the growth response to diets containing rye or beans was greater than for the corn based control diets. The supplements increased chick growth in the corn diets by 13%, in the rye diets 32% and in the bean diets 24%. Procaine penicillin appeared to be more effective as an additive than MD-bacitracin. As in the previously mentioned study there was evidence of a 3-nitro-bacitracin interaction, especially when bacitracin was used at a high level.

Patel and McGinnis (1976) supplemented diets containing varying levels of rye with procaine penicillin. The magnitude

of the response in growth was +34, +33, +31, and +24% respectively for diets containing 70, 55, 40 and 25% rye, indicating a lessening of respone as the level of rye was reduced. Diets containing wheat or corn and no rye did not give a significant response. In a second experiment, the response was +12 and +6% for wheat and corn, whereas the response on the rye diets ranged from +39 to +60%. Again feces condition was not affected by antibiotic supplementation.

Wagner and Thomas (1977) observed that pectin fed to chicks, like rye, caused a sticky fecal condition. Pectin was fed at 5 levels ranging from 0 to 6%, and the diets contained either 0 or 55 ppm penicillin. Penicillin supplementation of all diets containing added pectin gave a large positive response in feed efficiency and weight gain; it did not however, completely alleviate the negative response. The authors concluded that the negative effect of pectin was not completely due to the microflora, rather the microflora was only responsible for part of the depression, while the other mechanism remained unknown. Wagner and Thomas (1977) explained the poor response of chicks fed rye on the basis of its high "pectin" content (quoting McNab and Shannon, 1974). They proposed that, rye did not contain any unknown toxin, but rather enhanced the growth of an adverse microflora in the chick. They indicated that since pectin may also cause a rye-type response, the micro-organism(s) involved must be

either the microflora normally present in the intestine, or be derived from components in the diet besides the rye.

Wagner et al. (1978) determined weight gain and feed consumption for broiler chicks fed rye diets with or without antibiotic supplementation (procaine penicillin, chlortetracycline or bacitracin (55 ppm)). They determined weight gain and feed consumption at two day intervals for the 14 day experimental period in order to detect any changes in growth patterns during the first 2 weeks post-hatch. Chicks fed the antibiotic-supplemented rye diets exhibited a greater growth response, however the chicks fed the rye diet with no supplementation appeared to partially recover from the adverse affects of the rye after day eight, when the slope of their weight gain curve began to approach that of the supplemented diet. Wagner et al. (1978) did not analyze their data statistically for differences in the slope of the weight gain response, hence it is difficult to determine the significance of this observation. They interpreted their results to lend credence to the theory that chicks fed rye exhibit an adaptive response, and suggested that chicks fed rye develop an adverse microflora that may be transitory and diminish with time, possibly as a result of bacterial interactions or an immune response.

The evidence suggesting the microflora is responsible for the performance of birds fed rye is for the most part indirect, inferred from the ability of antibiotics to

alleviate the growth depression. Other studies have indicated more specific effects of the microflora in rye-containing diets. MacAuliffe et al. (1976) proposed that penicillin alleviates the rachitogenic effect in poults by depressing the growth of an adverse microflora that somehow interferes with the absorption of vitamin D_3. They also proposed that since penicillin gave a positive response beyond the growth improvement attainable with a ten-fold increase in the D_3 level, it either has a dual effect or it improves utilization of other nutrients that are normally depressed when rye is in the diet. MacAuliffe and McGinnis (1976) suggested that the intestinal microflora in the case of the rye diets may cause irritation of the mucosa and a reduction in the capacity to absorb some nutrients. Misir and Marquardt ($1978_{a,b,d}$) reaffirmed previous findings that penicillin supplementation of rye diets improve performance. Penicillin concentration greater than or equal to 160 mg/kg gave the maximal response. In a later study, Marquardt et al. (1979) found that penicillin enhanced retention of certain nutrients, particularly amino acids, and that this effect was more pronounced in rye than in wheat diets. The retention of fat was also depressed, however this was not alleviated by penicillin supplementation. Marquardt et al. (1979) and Misir and Marquardt ($1978_{a,b,d}$) proposed that rye contains factors which depress retention of certain nutrients, particularly protein and fat. The high levels of unabsorbed protein are

then available for the proliferation of the deleterious microflora. The bacteria in the case of rye diets compete with the host more successfully for available nutrients.

While numerous authors have implied that an adverse microflora is the direct causative agent reducing performance in rye diets, there is little hard evidence as to what these microfloral changes might be and what specific effects they might have on the physiology of the bird.

Wagner and Thomas (1978) enumerated the anaerobic microflora of 4 and 14 day old chicks fed diets containing rye, pectin (4.5%) or corn. Counts were determined for a 5 cm segment of the ileum distal to Meckel's diverticulum. The chicks fed diets containing rye or pectin had ileal counts that were 2 to 3 log cycles greater than those for chicks fed corn diets. The average counts per cm of ileum were 7.5×10^9, 1.1×10^{10}, 4.3×10^7, respectively for 4 day old chicks fed rye, pectin or corn; and 7.8×10^9, 8.4×10^9 and 4.1×10^7 respectively for chicks at 14 days of age fed the same diets. Wagner and Thomas (1978) found no consistent response to antibiotics in terms of microbial counts, in many cases total counts increased with antibiotic supplementation. The authors attributed this to the variable amount of digesta present in the ileum, for which they did not attempt to correct. Although the total bacterial counts were not affected by penicillin supplementation, there was a significant depression of the butyric acid-producing

component. When chicks were fed rye or pectin containing diets, the butyric acid and gas producing component was stimulated. The butyric acid-producing component varied inversely with a succinic acid-producing component, which was depressed significantly when pectin was fed. Penicillin supplementation to diets containing pectin or rye stimulated the succinic acid-producing component.

While total numbers of anaerobic bacteria were not affected by penicillin supplementation there was a marked depression in the count of spore forming bacteria, particularly when rye or pectin containing diets were compared with or without supplementation. Wagner and Thomas (1978) suggested that rye or pectin caused growth depression by stimulating the proliferation of a penicillin-sensitive, fermentative organism that produces as end-products substantial amounts of butyric acid and gas. They felt that the genus that best fits the observed characteristics was Clostridium, which possibly depressed the growth of chicks through production of a toxin. The "pectin" in rye, they proposed probably serves as a substrate for the bacterial proliferation. The evidence supporting this interpretation is not strong, since Wagner and Thomas (1978) presented only mean values for all microbial counts.

Untawale and McGinnis (1979) studied the effect of rye or beans on the adhesion of the microflora to the intestinal mucosa. Rye caused a significant increase in the counts of

lactobacilli and enterococci both in the intestinal lumen and on the epithelial wall of the intestine. The counts of viable enterococci were higher on the gut wall than in the lumen. Penicillin supplementation reduced the counts of lactobacilli and enterococci.

While there are several variations of the hypothesis that rye in particular or gums in general stimulate an adverse microflora that somehow mediates the anti-nutritional effects observed, there is really no direct evidence that conclusively demonstrates that this is the case. The mechanism by which rye, whether by way of the microflora or perhaps some other means, has such diverse effects as affecting vitamin D_3 metabolism, amino acid and fat retention is unresolved. This is not surprising considering that the problem of how antibiotics alone exert a growth-promoting effect when fed at low levels in animal feeds has not been answered conclusively. Nevertheless there appear to be many similarities between results of researchers working with rye and those dealing specifically with the microflora of birds and animals in general.

2.5 Antibiotics as growth-promotants in poultry diets

The feeding of low levels of antibiotics to poultry was observed to stimulate growth and feed efficiency in the late 1940's (Jukes, 1977). Although this improvement has been

consistently demonstrated over the years, the manner by which antibiotics have this effect has not been ascertained conclusively. Some authors (Luckey, $1978_{a,b}$) feel that antibiotics, at the low levels incorporated in animal feeds, have a direct effect on the animal rather than any suppressive effect on a particular component of the microflora. Luckey interprets the response to low levels of antibiotics as a result of hormesis - the stimulation by low doses of any potentially harmful agents. This argument is rejected by most authors, and there is general agreement that it is by suppression of a particular component(s) of the microflora that antibiotics exert their effect. Coates et al. (1963) found that germ-free chicks grew better than conventional chicks fed penicillin (45.5 mg/kg diet), whereas antibiotics did not elicit a stimulating effect in the germ-free birds. Such evidence directly implicates the microflora in the antibiotic response, and has been reviewed by Jukes (1977) and Visek (1978).

Eyssen et al. (1962) considered the properties of antibiotics used as growth promotants as the basis for a number of hypotheses concerning their beneficial effects. Bacitracin is effective, yet not absorbed, which they interpret as evidence indicating the gut is the site of action. Penicillin is also effective yet there is considerable doubt whether or not it reaches the distal segments of the G.I. tract since it is partially inactivated

by gastric juice and readily absorbed in the small intestine. High local concentrations are found only in the proximal parts of the gastrointestinal tract - the crop, the stomach and the small intestine. Eyssen et al. (1962) infer that the antibiotics must be having their effect in the anterior regions of the G.I. tract. Finally, most antibiotics commonly used as growth promotants in poultry diets share a common effectiveness against gram-positive organisms (Eyssen and DeSomer, $1963_{a,b}$). Several (eg. penicillin and virginiamycin) are specific for gram-positive organisms. Eyssen et al. (1962), noting that previous investigators had studied antibiotic-induced changes in the cecal and large intestinal flora, studied the effect of antibiotics on the crop microflora. They described the crop flora as purely lactobacillary which were highly sensitive to antibiotics that have a growth-promoting effect. Virginiamycin was particularly effective against lactobacilli both in vivo and in vitro. Eyssen et al. (1962) suggested that while lactobacilli are not normally pathogenic, they may be when present in sufficiently large numbers.

Stokstad et al. (1953) studied the magnitude of the growth response to aureomycin fed with various types of diets. The addition of either lactose or sucrose to the test diets gave a growth depression, with the depression being greater when lactose was fed. Chicks fed diets containing sucrose gave the greatest response to aureomycin. Eyssen and

De Somer (1963_b) determined growth rate at one day intervals for chicks fed sucrose containing diets (with gelatin and casein as protein sources). Growth was depressed at seven days. This depression was transitory, and was alleviated by inclusion of penicillin or virginiamycin, but not chloramphenicol or chlortetracycline. By 14 days of age, the rate of gain of both groups of chicks was similar, regardless of treatment. Eyssen and De Somer (1963_b) observed that the growth rate on diets without antibiotics was not improved when sucrose was replaced by a glucose-fructose mixture, but that glucose alone led to significantly better growth. They proposed that the fructose moiety is less well absorbed by the chick and consequently is available as a substrate for bacterial proliferation.

Eyssen and De Somer (1963_b) were able to demonstrate that the growth depression at one week of age coincided with a 3-fold increase in the level of fecal fat (dietary fat source was 3% corn oil), with 30 to 50% of the dietary fat being lost during the period of malabsorption. This transitory steatorrhea was most pronounced on diets containing sucrose or glucose plus fructose, less pronounced with glucose alone, and least pronounced in a starch diet. The fecal fat was reduced considerably in the affected birds when virginiamycin was fed. Eyssen and De Somer (1963_b) concluded that young chicks may be analogous to rats in which intestinal overgrowth is promoted by surgically creating a 'blind-loop'

in the small intestine that serves as a pouch to retain chyme and permit intestinal overgrowth. Rats with 'blind-loop' syndrome were observed (Eyssen and De Somer, 1963_b quoting Hoet et al., 1962) to excrete twice as much dietary fat as did the control rats, and this excessive fecal fat loss could be alleviated by administration of virginiamycin. They concluded that the chick suffers a transitory malabsorption syndrome that may include faulty absorption of other nutrients as is typical of steatorrhea. They envision gram-positive micro-organisms as provoking a polymalabsorptive state in which vitamins, minerals, protein, fat and carbohydrate are poorly absorbed.

Many studies report an improvement in nutrient retention when antibiotics are fed, or alternatively, when the germ-free condition is compared to a conventional environment. Nelson et al. (1963_b) found that the stimulation of growth by antibiotics was correlated with an increase in metabolizable energy, which they attributed to increased efficiency in the absorption of calorigenic nutrients. Supplee (1960) obtained markedly different results when poults were fed sucrose-based diets containing increasing levels of corn oil with or without antibiotic supplementation. The magnitude of the growth improvement depended on the presence of an antibiotic. Boyd and Edwards (1967) observed that germ-free chicks retained greater amounts of palmitic and stearic acids as compared to chicks

reared in a conventional environment. The environment in this instance did not appear to influence the chicks' absorption of oleic or linoleic acids however. Edwards and Boyd (1963) observed that when ^{47}Ca was fed considerably more appeared in the tibia of the germ-free than in the conventional bird wheras if the ^{47}Ca was given intraperitoneally there was no significant difference in its appearance in the tibia, suggesting interference of ^{47}Ca uptake. March and Biely (1967) investigated the hypothesis that antibiotics may favour a microflora that produce vitamins that are beneficial to the chick when a deficiency or borderline deficiency exists. They concluded that although the growth of chicks may be stimulated when an antibiotic is added to a diet deficient in B vitamin, the growth stimulation doesn't necessarily result from enhanced intestinal synthesis. Rather, they proposed that an increase in the absorptive capacity offered a more consistent explanation for the 'vitamin-sparing' effect of the antibiotic. March et al. (1972) observed that the metabolizable energy value of the diet declined after antibiotic was withdrawn. This coincided with a drop in growth rate and feed utilization.

Pensack (1963) verified the results of Eyssen and de Somer ($1963_{a,b}$) with regard to fat retention. Malabsorption was improved by the addition of either penicillin or chlortetracycline to the diet. In a subsequent study, Huhtanen and Pensack (1965_{b}) followed the development of the

intestinal flora of the young chick fed a sucrose-casein diet. The results from studies of the duodenal content at six days of age (when the chicks were undergoing the fat malabsorption) indicate that Streptococcus faecalis and a Lactobacillus sp were the predominating organisms at this time. As a function of time, the counts of Streptococcus faecalis increased to six days, then declined, thus implicating it as the causative organism. This was consistent with earlier work by Anderson et al. (1956) who observed a significant depression in growth and feed efficiency induced by feeding enterococci. This coincided with a reduction in coliforms and lactobacilli. Chlortetracycline in the diet reduced the number of enterococci and lactobacilli, and improved growth rate. The coliform count was not affected.

Addition of antibiotics to diets fed to chicks has a general beneficial effect in terms of growth rate, and this improvement may be attributed, in part at least, to superior nutrient retention in antibiotic-supplemented diets. In many respects chicks fed diets with no antibiotics resemble chicks fed rye diets, except that the malabsorption condition is worse in the latter instance. Streptococcus faecalis in particular seems capable of inducing a similar type of response, and has been studied as a growth-depressing agent in non-antibiotic supplemented diets using gnotobiotic chicks monoassociated with specific bacterial strains. Among the bacteria tested for their capacity to reduce growth when

monoassociated with gnotobiotic chicks, only Streptococcus faecalis has been consistently observed to cause the response.

2.6 Evidence implicating Streptococcus faecalis as responsible for the antibiotic growth effect in chickens.

Huhtanen and Pensack (1965_b) monoassociated germ-free chicks fed a casein-sucrose diet with Streptococcus faecalis. This resulted in a significant growth depression that could be alleviated by dietary penicillin. The growth depression was, however, considerably less than that exhibited by conventional chicks. The authors attributed this to other microorganisms and/or environmental factors that may have contributed to the response in the conventional environment. In particular they noticed a tendency for a synergistic effect between two bacterial strains. Huhtanen and Pensack (1965_b) also observed that penicillin (200 ppm) practically eliminated streptococci from the duodenum and small intestine of six day old conventional chicks.

Eyssen and de Somer (1965, 1967) studied the effects of pure and mixed cultures of Streptococcus faecalis, Lactobacillus as well as other components of the chick's intestinal microflora administered to germ-free birds fed a sucrose-containing diet. The response parameters were body

weight, small intestinal weight and fecal fat excretion. Of the organisms examined, only Streptococcus faecalis gave a consistent response, which was however less extensive than that observed in conventional birds. Streptococcus faecalis caused a limited growth depression, a moderate rise in fecal fat and an increase in small intestinal weight. The failure to obtain similar effects with other microorganisms was attributed to the inability to colonize the small intestine. With the exception of lactobacilli, other bacteria were largely confined to the lower ileum, the ceca and the colon. Streptococcus faecalis was found to be the predominant microorganism in the crop and small intestine during the first five to seven days (Ochi et al., 1964; Eyssen and de Somer, 1965; Huhtanen and Pensack, 1965_a), afterwhich lactobacilli gradually displaced Streptococcus faecalis. They noted, however, that even in gnotobiotic chicks monoassociated with Streptococcus faecalis, the moderate malabsorption tended to disappear during the second week even in the absence of lactobacilli, possibly as a result of an immune response.

An additional effect when a fecal filtrate from conventional birds was given to germ-free chicks or chicks monoinfected with Streptococcus faecalis was observed by Eyssen and de Somer (1965). The fecal filtrate alone caused an increase in fecal fat excretion, growth rate depression and increased intestinal weight. This effect was additive to

the effect obtained with Streptococcus faecalis. Antibiotics given to chicks exposed to both agents were not completely restored to the germ-free state, indicating that the filtrate material did not respond to antibiotics. Eyssen and de Somer (1965) could not identify the active principle in the fecal filtrate, however it was successfully propagated through a series of germ-free chicks, which supports their contention that it was most likely a virus. Cole and Boyd (1967) were also able to demonstrate that chicks (fed corn-soy diet) monocontaminated with Streptococcus faecalis exhibited reduced absorption of palmitic and stearic acids, as well as reduced retention of total fat. The absorption of linoleic and oleic acids was increased however. Gnotobiotic chicks monocontaminated with Escherichia coli, Staphylococcus or Lactobacillus showed no such effect.

Harrison and Coates (1972) noted that neither Streptococcus faecalis liquefaciens nor a bacteria-free fecal filtrate depressed growth when given alone; however growth was depressed when both were given. The diet in this case was a practical-type diet in which the major ingredients were maize, barley and defatted soybean meal. The bacteria-free filtrate increased gut weight when administered to germ-free chicks, whereas Streptococcus faecalis had an opposite effect. Given together, there was no difference from the germ-free chicks.

The ability of a collection of aerobic and anaerobic

bacteria isolated from the crop and cecum was reasessed by Fuller et al. (1979) for the capacity to depress growth in chicks. Aerobic bacteria always tended to depress growth, however only in the case of Streptococcus faecium was this significant. Fuller et al. (1979) suggest that previous authors (Huhtanen and Pensack, $1965_{a,b}$ and Eyssen and de Somer, 1967) were possibly mistaken in their identification of Streptococcus faecalis, which may have actually been Streptococcus faecium. While the most consistent growth depression has been attained when Streptococcus organisms and/or a fecal extract were administered in germ-free chicks, other studies have implicated different microorganisms, particularly Clostridium. Like Streptococcus, Clostridium are gram-positive, sensitive to many of the antibiotics that elicit a growth response, as well as 'normal' inhabitants of the intestines of the young chick. Timms (1968) determined microbial counts for the anterior and posterior small intestine of the chick. Lactobacillus, Streptococcus, Escherichia coli and Clostridium welchii were present in descending order of magnitude. Timms suggested that since Clostridium welchii are normally present at low concentrations, changes in population size may be particularly significant. Powell et al. (1974) observed that birds housed in a fumigated room could not be kept free from Clostridium perfringens, which indicates that chicks are universally exposed to these bacteria, which like streptococci constitute an element of the normal microflora.

2.7 Evidence implicating Clostridium perfringens as responsible for the antibiotic growth effect in chicks

Clostridium have been implicated by several authors as having the capacity to depress performance in young chicks. Like Streptococcus they are present in the young chick, and are also gram positive and susceptible to antibiotics used in feed formulations. Lev and Briggs (1956_a) observed that the predominant flora of newly hatched unfed chicks was Clostridium, with Escherichia coli sometimes present. They describe the Clostridium as non-hemolytic with physiological characteristics resembling Clostridium paraputrifican. In a second study, Lev and Briggs (1956_b) observed that the clostridium population was rapidly displaced on feeding, when the predominant bacteria became Escherichia coli and Aerobacter aerogenes. Lev et al. (1957) reported distinct differences in the presence and activity of Clostridium welchii in the first few days of a chicks life, and the presence or absence of Clostridium welchii correlated with the response to antibiotic. They also noted with penicillin supplement that Clostridium welchii was eliminated, whereas no differences were observed in the numbers of Lactobacillus, Steptococcus or coliforms.

In a later study Lev and Forbes (1959) monoassociated germ-free chicks with Escherichia coli, Lactobacillus, Streptococcus liquefaciens, and Clostridium welchii. An

intestinal microflora composed of Escherichia coli, Lactobacillus lactis or Streptococcus liquefaciens had no effect on growth rate, nor did penicillin supplementation of the diets improve growth rate. Clostridium welchii Type A as the sole intestinal flora did however depress growth, and this depression could be alleviated by procaine penicillin.

Eyssen and de Somer (1967) observed that Clostridium welchii developed profusely in the cecum and lower small intestine of the gnotobiotic chick. Unlike Streptococcus faecalis, Clostridium welchii did not inhabit the small intestine, nor did it cause any growth depression in this case. Similar results were obtained by Powell et al. (1974) who found no response of Clostridium perfringens administered to gnotobiotic chicks fed either commercial or purified starch-casein-gelatin diets. In addition, no correlation was observed between Clostridium perfringens and growth rate in conventional chicks. Similar results were obtained by Fuller et al. (1979) who found no growth depression when Clostridium perfringens was given to gnotobiotic chicks.

In contrast results supporting the contention of Lev and Forbes (1959) that Clostridium welchii may be responsible for the antibiotic growth effect were presented by Cole and Boyd (1967). They indicated that conventional chicks, as well as gnotobiotic chicks monocontaminated with Streptococcus faecalis or Clostridium welchii, or dicontaminated with both, exhibited the reduction in fat retention characteristic of

growth-depressed birds.

The evidence implicating Clostridium in the antibiotic growth response is variable. Differences among Clostridium welchii strains employed in the various experiments or differences due to environmental conditions may have contributed to the variation among studies. Environmental differences have been observed to affect the magnitude of the response to antibiotics. This difference in response is generally attributed to a lack of growth-depressing organisms in some environments and, to the evolution of growth-depressing organisms that are resistant to a particular antibiotic.

2.8 The effect of evironment on the magnitude of antibiotic response

The magnitude of a response to a particular antibiotic has been observed to depend upon the length of time it has been used in a particular environment, as well as the degree of contamination of the environment.

Waibel et al. (1954) observed a consistent response to antibiotic for a two year period, however during the third year the response became inconsistent and neglible. They suggested that the lessening of the response was due to a change in the microbial population caused by a decrease in

the moisture content of the room as a result of improved cleaning practices. Alternatively it was proposed that the harmful bacteria may have been eliminated by continued use of the antibiotic. Evidence supporting Waibel et al. (1954) with regard to a lessening of a growth response with time has been reported by Nelson et al. (1963_a) where it was observed that antibiotics used over an extended period gave only a small response, whereas antibiotics that had not been used previously gave a pronounced response. Penicillin, bacitracin, tylosine and erythromycin exhibited a lessening of response with extended feeding periods.

Lev et al. (1957) observed that improved growth attributable to antibiotics occurred in 'old' premises (ie. infected) whereas the response was neglible or non-existent in new premises. Similar results were reported by Edwards et al. (1960) who observed that chickens grown in a room immediately after it had been fumigated with formalin performed better than chicks grown in the presence of older birds, which served as a source of contamination. In a subsequent study, Edwards and Boyd (1963) demonstrated that the effect of contaminated quarters could be simulated by feeding diets containing one half or one per cent chicken feces. They report evidence suggesting that requirements for magnesium, zinc and methionine may be increased in the contaminated environment. The utilization of corn oil added at 10% of the diet may be likewise affected (Donaldson,

1962). While the addition of corn oil resulted in greater body weights in both 'new' and 'old' environments, the response was greater in the 'new' environment, suggesting that a factor, presumably bacterial in origin, was affecting fat utilization. Supportative evidence is provided by Young et al. (1963) who reported that the rearing of chicks in a fumigated lab significantly improved absorbability of lard fatty acids. This effect was accentuated if the fatty acids were fed in a high protein diet.

Such results demonstrate that environmental differences may contribute to the variability of response observed between experiments. Three factors would appear to be implicated: first, the environmental microbial population may vary in its ability to suppress growth; second, the environment may vary in its ability to support an 'adverse' microflora through differences in ventilation, moisture, etc; and third, the microflora may acquire resistance to the antibiotic employed. In addition to the effects imposed by the physical nature of the habitat, there is considerable evidence implicating the 'internal' environment; the gut content composition and its rate of passage, on the activity and composition of the microflora.

2.9 Dietary and internal factors affecting the gut microflora

Numerous authors have reported instances where inclusion of a particular dietary ingredient either modified the response to antibiotic or gave different results depending on whether the environment was conventional or germ-free. The original observation by Stokstad *et al.*, (1953) that diets incorporating sucrose gave a maximal response to aureomycin has since led to inclusion of sucrose in diets where maximization of the microbial response was desirable.

Other ingredients have been implicated in a growth effect through modification of the microflora. Harrison and Coates (1964) observed that fish solubles gave a similar pattern of response to penicillin. Like the antibiotic response, the growth response to fish solubles was variable and tended to diminish with time. The combined effect of fish solubles plus antibiotic was additive, from which Harrison and Coates (1964) inferred that the organisms affected by fish solubles and penicillin were different. Supportative evidence was obtained in a later study (Harrison and Coates, 1972) in which it was observed that fish solubles increased the growth of conventional but not germ-free chicks. The growth depression induced by administering either *Streptococcus faecalis* or a bacteria-free fecal filtrate could also be alleviated by introducing fish solubles into the diet.

Jayne-William and Hewitt (1972) noted a considerable difference in response depending on whether raw navy beans (*Phaseolus vulgaris*) were fed to conventional or germ-free

Japanese quail. Mortality of quail fed raw beans was high in the conventional environment. Hewitt et al. (1973) studied isolated fractions from navy beans in both the presence and absence of the gut microflora. A fraction containing trypsin inhibitor caused a 13.5% growth depression with chicks raised in a conventional environment, yet had no effect in the germ-free environment. A second fraction had only a slight effect in the germ-free environment, yet caused a 26.5% depression in the conventional environment. The nature of the effect of this second fraction was not determined. Raw meal caused only a 12% depression in the germ-free environment compared with a 48% depression in the conventional environment. Andrews and Jayne-Williams (1974) and Jayne-Williams and Burgess (1974) thought that trypsin inhibitors were not important in the negative response to navy beans. Rather, they proposed, phytohaemagglutinins (lectins) as the causative agent. They proposed that phytohaemagglutinins may induce toxicity by binding to the mucosa thereby impairing the body defense mechanisms resulting in normally innocuous bacteria (such as Escherichia coli) becoming invasive. Fuller (1975) observed that concanavalin A binds to crop epithelial cells, and in so doing, displaced lactobacilli.

Other studies have implicated more general mechanisms by which dietary factors may influence a microbial effect. Nairn and Bamford (1967), studying the susceptibility of birds

dosed with Clostridium perfringens in developing acute necrotic enteritis, noted that death incidence depended on diet to some extent. The suspect diet was identified as only "suspect commercial diet B". Bullen and Battery (1957) (cited in Nairn and Bamford (1967)) suggested that the proliferation of Clostridium perfringens, and the manifestation of the disease, may depend on the availability of carbohydrate as a substrate source.

Parish (1961) attempted to induce necrotic enteritis by administration of Clostridium welchii isolated from infected birds. Normally this was not successful, however when the birds were treated with mineral salts (chalk or sodium bicarbonate) and opium, their susceptibility to oral innoculation was substantially increased. Parish (1961) concluded that some dysfunction of the alimentary tract is a necessary predisposing cause of infection. Substantiating evidence was reported by Smith (1965_a) who observed that the addition of meat and bone meal to the diet increased Escherichia coli and Clostridium welchii at the expense of lactobacilli. Escherichia coli and Clostridium welchii suffered less destruction during passage through the gizzard when meat and bone meal were fed due to increased gizzard pH, which favoured their survival and ultimately their proliferation in the anterior regions of the small intestine.

Rate of passage also exerts an effect, as indicated by the response observed by Parish (1961) to opium. Wehman and

Lifshitz (1974) observed that injection of mecamylamine, which also retards passage rate, increased intestinal bacterial growth in rats. Furthermore Lifshitz et al. (1978) showed that mecamylamine treatment in rats caused a colonic flora to develop in the small intestine.

In conclusion it would appear that several factors may affect colonization of the intestinal tract by bacteria. Factors which predispose a chick to excessive colonization include: factor(s) which contribute to substrate availability for microbial proliferation (eg sucrose); factors which impair the body immune function and/or facilitate microbial invasion (eg lectins in navy beans); factors which slow rate of passage (as evidenced by opium or mecamylamine) enabling bacteria time to proliferate, and factors which increase the survival rate of bacteria passing through the acidic environment of the ventriculus and proventriculus (eg meat meal or carbonates) thereby increasing the bacterial 'load' reaching the small intestine. Other feed ingredients such as fish meal may also exert an anti-microbial effect, however the mechanism by which this occurs remains obscure.

The microbial population also undergoes changes with age of the host, particularly during the first few weeks of life, until the stable, adult flora is achieved. Changes in the flora may be correlated to some degree with the transitory malabsorption syndrome that occurs during early life of the chick.

2.10 The ecology of the microflora of the chick during the first two weeks after hatching

There is considerable confusion, particularly in the early literature, regarding both qualitative and quantitative features of the microflora of the chick. It should be recognized that many of the early studies were not complete in their assessment of the gut microflora, hence their interpretation of the population changes occurring during early life of the chick should be qualified.

The bacterial populations identified and studied by early workers were limited. Lev and Briggs (1956 $_{ab}$) observed that the gut flora in newly hatched chicks (unfed) was predominantly clostridia, with coliforms sometimes being present. The clostridia were described as non-hemolytic and physiologically similar to Clostridium paraputrificum. With feeding the flora quickly changed to what they described as an 'adult' flora consisting of predominantly Escherichia coli and Aerobacter aerogenes. In later studies lactobacilli and streptococci were added to the list of bacteria present in the intestine of the chick (Anderson et al., 1956, Lev et al., 1957). More detailed descriptions of the microflora of the chick were reported by Smith (1965$_{a,b}$), Timms (1968), Barnes and Impey (1970), Salanitro et al. (1978), and Barnes et al. (1980). Salanitro et al. (1978) was perhaps most complete in the identification of the microflora of chicks at

two weeks of age. Facultative anaerobes predominated in the duodenum and ileum (Streptococcus, Staphylococcus, Lactobacillus, Escherichia coli), however a variety of strict anaerobes was also present (Eubacterium, Clostridium, Gemmiger, Fusobacterium, Bacteroides). Strict anaerobes made up nearly the entire population of the ceca. While a simple description of the microflora is of academic interest, it is the changes in the population that occur with time that are of interest for the present discussion.

Smith ($1965_{a,b}$) described the flora of the alimentary tract in a variety of young animals. In most species the dynamics were as follows: initially there was extensive proliferation in the anterior stomach (or crop in the case of the chick), followed by partial destruction in the acidic posterior region. Multiplication recommenced in the small intestine such that progressively greater numbers of bacteria were found from the duodenum to the ileum. Smith (1965_{a}) observed that the early flora consisted of Escherichia coli and Streptococcus (12 - 48 hrs). Lactobacilli, although slower in colonizing, once established were the most common organism inhabiting the anterior region. A similar description is provided by Huhtanen and Pensack (1965_{a}). At one day of age large numbers of Escherichia coli were present in the cecum, the duodenum however was largely devoid of bacteria. Streptococcus faecalis became apparent in the duodenum at six days of age, then spontaneously disappeared

during the 14 - 28 day period.

A unique significance has been attached to both the lactobacilli and streptococci elements of the microflora. Eyssen et al. (1962) reported that Lactobacillus acidophilus was present in the crop 24 - 48 hours post-hatching, and establishment coincided with a drop in the pH of the crop to 3.8 - 4.2. Fuller and Brooker (1974) proposed that the low pH in the crop induced by the lactobacilli prevented the growth of potentially harmful bacteria, which otherwise proliferate in the crop and serve as a seed source for the rest of the gastrointestinal tract. In this synergistic relationship the lactobacilli benefit by receiving nutrients from the diet wheras the host benefits by a reduction in the numbers and activity of potential growth-depressing organisms. This function is facilitated by the capacity of the lactobacilli to bind to the crop epithelium, which enables a portion of the population to escape transit to the lower gastrointestinal tract, and serve as an innoculum source for incoming feed (Fuller, 1973). The nature of the adhesion by lactobacilli was studied by Fuller (1975), who obtained evidence indicating that lactobacilli adhered to the glycocalyx of the epithelial cells. Binding could be blocked by concanavalin A, from which Fuller inferred that the binding sites were glucose, mannose, fructose or arabinose.

A similar role to that of the lactobacilli has been ascribed to Streptococcus faecalis (Barnes et al., 1980). The

establishment of salmonella could be prevented when a mixture of facultative and strict anaerobes was administered to the chick, however lactobacilli alone failed to prevent establishment. The facultative anaerobes, particularly Streptococcus faecalis use the available oxygen, thereby lowering the redox potential and encouraging the growth of strict anaerobes in the lower tract. This is consistent with the observations of Ochi et al., (1964), who reported that the adult cecal flora normally required up to 30 days to become established.

Altering the sequence of bacterial colonization of the intestine has been exploited in an effort to depress potential growth-depressing organisms and induce an antibiotic type response without the antibiotic. Tortuero (1973) incorporated lactobacilli in the feed of chicks. An increase in the lactobacillus population was accompanied by an almost total disappearance of the enterococci. The chick responded with improved weight gain as well as a trend toward higher fat retention, although this later response was not statistically significant. Similar results were reported by Frances et al. (1978), who observed that a lactobacillus 'probiotic' product and zinc bacitracin were antagonistic with regard to their capacity to improve performance. This was attributed to lactobacilli being sensitive to zinc bacitracin. The lactobacilli reduced both coliform and total aerobic bacterial counts.

Several authors have attempted to attribute the depressed growth of conventional chicks to specific elements of the microflora. In many cases the bacteria implicated only reflect the particular fraction of the microflora that was studied in a particular experiment. Lev et al. (1957) could not find any differences in counts of lactobacilli, streptococci or coliforms with penicillin supplementation and concluded that clostridia may be responsible for growth depression in chicks, since the counts of this organism were lowered by penicillin. Eyssen et al. (1962) speculated in an early study, that while lactobacilli are not normally pathogenic, they may depress performance when present in sufficient numbers. Lactobacilli, which are generally considered a desirable bacteria, improved performance when fed to commercial layers at low levels, however at high levels (.0625 %) there was a consistent drop in performance (Miles, 1981).

Most results with gnotobiotic chicks indicate that Streptococcus faecalis or Streptococcus faecium are capable of reducing performance, although clostridia have also been implicated albeit less frequently. It should be noted that the microbial population of the young chick is most likely opportunistic to a large degree; that is the bacteria present are only transitory and will ultimately give way to specific bacteria geared to life in the intestine of the chick. In such a scenario it may not only be the presence of specific

bacteria that determines whether growth depression of the host occurs, but also its ability to colonize specific surfaces and achieve a sufficiently large population size.

While the capability of the bacterium to bind is important, Savage (1981) suggests that the overall ability of micro-organisms to colonize a particular surface may also be dictated by nutritional and environmental conditions. Salanitro et al. (1978) in citing unpublished data indicated that the diet had a profound effect, although the nature of the effect was not discussed.

Such findings suggest that a 'microbial balance' may affect the magnitude of the response of chicks to antibiotics. The microbial balance has been demonstrated on several occasions to affect performance, and there are reports of trends that indicate fat absorption may be affected. The 'deleterious microflora' proposed as the causative agent in the antinutritional effect in rye may not necessarily stem from a direct effect on a particular component of the microflora. It is also conceivable that normally innocuous bacteria are affected, which sways the microbial balance in favour of growth-depressing strains. Bacteria that have been implicated as causative of growth depression include Streptococcus, Lactobacillus and Clostridia all of which may be considered normal inhabitants of the chick gut. It seems possible that bacterial overgrowth, particularly in the small intestine results in

growth depression that may be induced by any of a number of 'normal' inhabitants of the chicks gut. In this sense it is the conditions favoring the establishment and proliferation of the microflora that must be regarded as the primary causative event in the observed growth depression in young chicks.

2.11 The effect of the microflora on the digestive physiology of the chick.

Several mechanisms have been proposed to explain the growth promotion response of antibiotics in terms of the effects the microflora has on intestinal morphology, and consequent digestion and absorption of nutrients.

Lev et al. (1957) suggested that antibiotics stimulated growth of the host by eliminating organisms that normally irritate and thicken the gut, thereby decreasing permeability and impairing absorption. They noted that Clostridium welchii produces a variety of enzymes including lecithinase, hyaluronidase and collagenase which may attach to and degrade mucosal cell surfaces. Palmer and Rolls (1978) also offered the explanation that uptake of calcium in germ-free birds proceeds more rapidly than in conventional birds due to a thinner gut wall. Larson and Hill, (1960) suggest that Streptococcus faecalis may produce tyramine by decarboxylation of tyrosine. Tyramine is a vasoconstrictor

that would conceivably reduce blood flow and absorption rate. Cole and Boyd (1967), attempting to explain the poorer absorption of palmitic and stearic acids by conventional birds, proposed that the microflora altered epithelial cells in such a manner as to affect their capacity for saturated fatty acid uptake. In general, however, the evidence supporting the hypothesis that mucosal cell absorptive capacity is affected is not strong.

Eyssen and De Somer (1963_b) compared the fat malabsorption syndrome of chicks to cases of steatorrhea in humans, and further speculated that the condition in chicks may resemble the malabsorption induced in rats by surgically creating a diverticulum (blind-loop) in the small intestine. Eyssen and De Somer argue that by analogy one may suppose that the malabsorption includes amino acids, vitamins and minerals. Boyd and Edwards (1967) offered the explanation that the microflora affects changes in the lumen contents, which result in poor micellar stability particularly when the micelles contain a high proportion of palmitic and stearic acids.

Combe et al. (1976) reviewed evidence supporting the contention that major differences occur between conventional and germ-free animals with regard to bile salt metabolism. In particular, deconjugated bile salts are reduced considerably in the germ-free as compared to the conventional state in the rat. They reported (citing Hoffman, 1961) that the

digestibility of fat depends to a great extent on the presence of sufficient quantities of conjugated bile salts. Free bile acids have a very limited capacity to form micelles, hence increasing their relative proportion reduces fat utilization. Combe et al. (1976) concluded that it is most probable that the microflora exerts its effect on fat digestion through the intermediary of bile salt metabolism. They further speculated that changes in bile salt metabolism affects transit time, histology of the small intestine and calcium absorption.

Visek (1978) reviewed evidence supporting the view that mucosal renewal in the intestine is related to microbial colonization, and the growth response to antibiotics may be at least partially due to a reduction in the requirement for mucosal cell synthesis. Feeding urea to chicks resulted in mucosal cell damage in localized area of the gastrointestinal tract, which Visek attributed to localized ammonia toxicity as a result of bacterial urease producing ammonia from urea breakdown. Visek also reported that feeding deconjugated bile acids produces similar, but more uniform changes in mucosal cell morphology. Differences in mucosal cell renewal rate between conventional and germ-free birds has been well established (Rolls et al., 1978; Cook and Bird, 1973).

In conclusion, the malabsorption syndrome exhibited by conventional chicks has been attributed to disruptions in mucosal cell morphology and a reduced capacity of the

epithelial cells to absorb digested nutrients, although evidence supporting this is lacking. A second hypothesis is that luminal digestion events are altered, particularly bile salt metabolism. This directly affects fat utilization, however it may also indirectly alter calcium absorption, transit time and mucosal cell morphology. Free bile acids may also increase mucosal turnover rate.

2.12 Fat digestion in the chick - Evidence implicating abnormal bile salt metabolism and/or insufficiency in the malabsorption of saturated fatty acids

There is considerable evidence indicating that young animals in general (Smallwood et al., 1972), and chicks in particular have problems coping with dietary fat under certain conditions. This has been related to bile acid metabolism in the chick. It is well established that chicks, under certain circumstances have problems coping with saturated fat. Renner and Hill (1960) reported that chicks have a depressed capacity to utilize tallow, which was overcome by 8 weeks of age. This effect was not apparent for corn oil or lard. In a subsequent study (Renner and Hill, 1961_a) it was observed that palmitic and stearic acids were virtually unutilized when fed singly. Absorption could be improved by feeding as triglyceride, particularly when the saturated fatty acid occupied the two position of the

glyceride molecule. The absorbability of the saturated fatty acids could also be improved by increasing the unsaturated fats in the diet. Substantiating evidence was reported by Young and Garrett (1963) who observed that increasing oleic acid in relation to palmitic acid resulted in a linear increase in palmitate absorption. Linoleic acid addition caused a similar response, however it was less pronounced. The absorption of saturated fatty acids also increased as the chain length decreased (Renner and Hill, 1961_b). Such evidence implies that the physical state of the fat in the gut may affect its utilization as there is improvement in fat digestion resulting from a variety of methods of liquefaction. Young and Garrett (1963) reported that the degree of utilization of a fat does not correlate well with its melting point, however Hakansson (1974) indicated that fats with melting points compatable with a liquid state at body temperature of the chick (42 C) were more readily digested than those present in a solid state.

Bile salt insufficiency on the part of the chick may be inferred from studies where the addition of bile salts or acids to the diet improved fat digestion. Fedde et al. (1960) noted poor utilization of beef tallow by young chicks, which could be improved with the addition of bile. Edwards (1962) observed a slight but nonsignificant improvement in fat digestion with cholic acid supplementation. Similar results were obtained by Gomez and Polin (1974) who observed that

cholic acid significantly improved the absorbability of all fats. In this case the improvement was small (2.1%) which the authors attributed to the high initial fat digestibility (92.7%). The largest net increase due to cholic acid was obtained with hydrogenated soya bean oil. The improvement in fat digestion coincided with increased M.E. of the diet. Gomez and Polin (1976) reported a greater response in fat digestion in a second study in which cholic acid, chenodesoxycholic acid and taurocholic acid increased apparent fat digestion by 8.4 percentage units in 4-7 day old chicks, and 10 percentage units in chicks 14-19 days of age.

An improvement due to supplementation with exogenous bile acids is not always apparent. Polin et al. (1980) fed chicks diets containing 4% tallow supplemented with a variety of bile acids. Cholic acid improved tallow absorption, but not significantly. Chenodeoxycholic acid improved tallow during days 0 to 7 only. In a second experiment cholic acid improved fat digestion by 8%, which is consistent with the results of other researchers.

The response of chicks to exogenous bile acids has been attributed to a bile salt insufficiency. Serafin and Nesheim (1967) indicated that very young chicks may be unable to replenish bile salts lost by excretion as readily as older birds, leading to bile salt insufficiency and a limited capacity to digest fat. There is some indication that components of the diet other than the fat source may affect

this response. Garlich and Neisheim (1964, 1965) observed that a soybean protein isolate caused severe fat malabsorption that could be rectified with either taurocholate or lecithin addition to the diet. Taurocholate at 0.3% or 0.6% improved fat digestion from 48 to 80 and 86 % respectively whereas lecithin (3%) improved fat digestion from 38 to 68 %. March et al. (1975) explained the variability in M.E. determinations for rapeseed meal as a consequence of an alteration in digestion for the entire diet. As a result, estimates in the M.E. value fluctuated widely. They also observed an increase in intestinal weight with rapeseed meal feeding, which could be reduced by feeding antibiotic. This the authors interpret as evidence for the involvement of the microflora in the response. It can be suggested from such results that chicks in general have difficulty coping with saturated fat sources, which in turn may be attributed to bile salt insufficiency. In support of this, it has been observed that chicks utilized unsaturated fats more effectively than saturated fats, and frequently respond to supplementation with exogenous bile acids. Certain ingredients, including rye, may affect fat retention only indirectly, with the microflora being the causative agent. Microbial alteration of bile salt metabolism may explain poor fat retention attributable to these ingredients.

2.13 Summary - Interpretation of the rye response in terms of alterations in the microflora and/or its activity.

Feeding rye to poultry produces growth depression which coincides with reduced apparent digestability for fat, amino acids, calcium and vitamin D_3. Feeding gums or gum-containing ingredients such as barley produce a response that is similar. Gums fed to chicks produce growth depression, and may also reduce fat retention, nitrogen retention and bone ash when vitamin D_3 is marginal. These responses may be corrected to some degree by incorporating appropriate enzymes in the diet, indicating intact gums are a prerequisite for the anti-nutritional effect.

Alternately, the negative response of the chicks fed rye may be alleviated by feeding antibiotics. Generally chicks fed rye give a greater response to antibiotics than chicks fed other cereal sources such as corn or wheat. Penicillin has been observed to reduce the rachitogenic effect of rye, as well as improve nutrient retention. Likewise, antibiotic supplementation improves performance of chicks fed diets containing purified gums beyond what would be anticipated with diets based on practical ingredients.

Such evidence supports the contention that the microflora directly mediates the anti-nutritional effects of gum-containing ingredients such as rye; with changes in the luminal contents induced by the gums being a necessary precondition. In many respects the differences observed when

chicks are fed rye diets parallel differences observed between conventional and germ-free chicks. Conventional chicks exhibit reduced growth, reduced fat retention (particularly for saturated fatty acids) and reduced calcium absorption. The bacteria implicated in this response are Streptococcus faecalis (or faecium) and Clostridium welchii, although supportative evidence for the latter is inconsistent.

The mode by which these bacteria depress growth and nutrient retention has not been resolved. One suggestion, consistent with most of the effects caused by the microflora, is that bacterial overgrowth in the upper regions of the small intestine alters bile salt metabolism. In particular, bile salts may be deconjugated, resulting in poor micelle formation and consequent fat malabsorption, poor calcium retention and alterations in small intestine morphology. Such processes may aggravate an already limited capacity of the chick to digest fat, particularly saturated fat sources, as evidenced by the frequently observed beneficial response to bile acids.

These effects may be exaggerated by locality, where environments harbouring deleterious bacteria may accentuate the effect. Various feed ingredients have also been observed to modify the response by affecting the ability of the bacteria to colonize various regions of the gastrointestinal tract. The presence of 'beneficial' bacteria such as

appropriate lactobacilli strains which antagonize growth depressing strains may also modify the response.

In conclusion, antibiotic-fed chicks tend to occupy an intermediate position between conventional and germ-free chicks. The conventional chick may be described as suffering a mild steatorrhea and malabsorption syndrome; the conventional chick fed rye may be one additional step in microbial activity and its consequences.

3.0 THE EFFECT OF MICROBIAL STATUS AND GAMMA IRRADIATION ON RETENTION OF NUTRIENTS IN CHICKS FED RYE DIETS

3.1 Introduction

The response of rye-fed chicks to antibiotics in excess of which is normally observed indicates that it is the microflora that directly mediate at least in part the antinutritional effects of rye. Two experiments were designed to test this hypothesis. In the first trial, rye or wheat diets were fed to chicks raised in a germ-free or a conventional environment. A second trial was conducted in which an attempt was made to induce the nutrient malabsorption observed in conventional rye-fed chicks by introducing specific bacteria into the germ-free environment. Additional treatments in the second trial were included to assess the effect of irradiating rye and wheat diets, since irradiation is normally employed as a means of diet sterilization for gnotobiotic work. The bacteria introduced to the germ-free chicks fed rye was a Streptococcus mixture isolated from 8 day old chicks. Streptococcus faecalis has been most frequently implicated in the antibiotic response, and in particular, in the increased fecal fat syndrome. While the demonstration of a rye-fed response with the addition of streptococci would indicate a bacterial involvement in the antinutritional effects of rye, it would not necessarily mean

that streptococci were the sole causative agent in the response.

3.2 Materials and methods

3.2.1 Trial management

3.2.1.1. Housing and care of gnotobiotic chicks

Gnotobiotic chicks were housed at the Veterinary Infectious Disease Organization,Saskatoon in presterilized rigid acrylic isolators (Canadian Cabinet, Nepean, Ont.) which employ high-efficiency particulate air (HEPA) filters on the intake and exhaust air. For Trial 3.1 the germ-free chicks (males) were housed individually in stainless steel cages. Each isolator housed six chicks per treatment, for a total of twelve chicks. Two isolators were employed, for a total of twelve birds per treatment. One isolator became contaminated and was not included in the experimental results. For Trial 3.2, no attempt was made to sex the chicks, since the probability of contamination increases with the number of eggs hatched in the isolator. The chicks were not confined within the isolator, but were placed in stainless steel cages for collection of excreta during the retention phase of the experiment.

3.2.1.2. Derivation of germ-free chicks

Naturally clean broiler strain eggs were incubated until the 19th day in a commercial incubator then sterilized using a peracetic acid treatment (N.A.S., 1970). This involved washing and brushing the eggs in warm water (35 - 37 ^{o}C) and detergent (sodium laurylsulphate), followed by washing and brushing the eggs in a 2% solution of peracetic acid (35 - 37 ^{o}C, with 0.1 per cent sodium laurylsulfate) adjusted to pH 6 with NaOH. The eggs were sprayed with peracetic acid again on placement into the isolator entry port, and allowed to sit in the entry port 25 min prior to introduction into the isolator. Two additional procedures were incorporated for Trial 3.2: the eggs were fumigated with formaldehyde gas in the incubator at one day, and prior to the peracetic acid treatment, they were cooled to room temperature for approximately an hour. It was observed that warm eggs had a poor survival rate during the sterilization treatment, which was thought to be due to cooling in the entry port and excessive uptake of the peracetic acid. The eggs were immediately dried after entry into the isolator and maintained at 37 ^{o}C in aluminum foil pans until hatching. The chicks were maintained at approximately 35 ^{o}C for the first week, after which the temperature was lowered over several days to normal room temperature.

3.2.1.3. Sterilization of isolators

Isolators were scrubbed with a germicidal soap (Hibitane), rinsed with water, swabbed with 95% ethanol, and thoroughly sprayed with a two per cent peracetic acid solution. During the spraying period the equipment necessary for the trial was also introduced (cages, heaters, paper towels, thermometers, water, etc.). The equipment was scrubbed, swabbed with ethanol, and where possible, autoclaved in autoclaveable plastic bags. Where the equipment would be damaged by the peracetic acid treatment, it was double bagged and the outer bag removed prior to spraying. For Trial 3.2 an additional sterilization treatment was employed in that the isolators were fumigated with formaldehyde gas prior to the peracetic acid treatment. In all cases the isolators were thoroughly evacuated and warmed prior to the introduction of the eggs.

3.2.1.4. Monitoring microbial contamination

Rectal swabs, as well as swabs of the litter, water, feed, and isolator walls were taken weekly during Trial 3.1. For Trial 3.2, swabs were taken routinely, whenever feed or water had to be introduced to the isolators. The swabs used were Culturette (McGaw Supply Ltd) sterile swabs containing Modified Stuart's Bacterial transport medium. The innoculum was transferred in a laminar flow hood to a variety of sterile media and incubated at 37 ^{o}C (24 - 72 hrs). The media employed were general purpose broth and agar, and included

trypticase soy, brain heart infusion, and thioglycollate broth to detect anaerobic growth. It was generally observed that where growth was apparent, the bacteria grew well in all media. For Trial 3.2 digesta samples were also collected at the termination of the trial and frozen in liquid nitrogen for ATP analysis, which may be considered indicative of bacterial activity (Lee et al.,1981).

3.2.1.5. Housing and care of conventional chicks

For Trial 3.1 conventional chicks (twelve cockerels per treatment) were housed individually in the University of Saskatchewan Poultry Unit. The cages had wire mesh floors which facilitated excreta collection during the digestibility study. For Trial 3.2 the chicks were housed in a Jamesway battery in which solid floors were installed to simulate the solid flooring of the isolator. The chicks were removed and placed in stainless steel cages for excreta collection for the digestibility study. For both trials the environmental temperature was regulated to approximate that of the gnotobiotic environment.

3.2.1.6. Isolation of Streptococcus and infection of the germ-free isolator

Chicks from which streptococci were isolated were

maintained on an antibiotic-free commercial chick starter until eight days of age, afterwhich they were sacrificed and gut contents collected for the isolation procedure. Mixed gut contents for groups of three chicks were diluted in the proportion of one g wet weight aliquot in nine ml reinforced Clostridium medium dilution fluid (Hirsch and Grinstead, 1954). This was repeated three times, for a total of nine chicks. Digesta was collected by milking jejunal and ileum lumen contents into a sterile Petri dish. Aliquots (0.1 ml) of diluted gut contents were plated on spread plates of modified thallous acetate tetrazolium glucose (TATG) agar (Barnes, 1956). This medium is specific for Streptococcus. The plates were incubated at 37 ^{o}C in a CO_2 atmosphere overnight. The following day 50 colonies were placed onto slants of the same medium. These were checked for purity microscopically, on the basis of streptococci being gram-positive cocci. Eight Streptococcus samples were checked for growth in TATG broth, then frozen in agar at -70 ^{o}C.

Prior to introduction to the isolator,innoculum from the frozen cultures was placed in 50 ml aliquots of TATG broth and incubated at 37 ^{o}C for 14 hrs in a carbon dioxide atmosphere. These were centrifuged at 10,000 g for 20 minutes and the supernatant decanted. The cells were resuspended in sterile saline (0.9 % NaCl) and cells from eight strains were combined in 30 ml saline. From this a 0.1 ml aliquot was taken and added to 4.5 ml sterile distilled water for

introduction into the isolator. The freshly prepared Streptococcus innoculum was administered to the chicks at seven days of age. The streptococci culture, contained in a plastic snap-top test tube, was swabbed with ethanol and sterilized with peracetic acid spray (2 %) in the previously described manner. Each chick was administered 0.2 ml of the mixture using a blunt-tip syringe.

Microscopic examination of cultures from the isolator revealed only gram-positive cocci, which was indicative of a pure culture being present.

3.2.2 The diets

The diets used in Trials 3.1 and 3.2 are listed in Tables 3.1 and 3.2. The diets were sterilized for Trial 3.1 by gamma irradiation (^{60}Co, 2.5 Mrads, Atomic Energy of Canada, Kanata, Ont.). For Trial 3.1 irradiated diets were fed to the germ-free chicks and non-irradiated diets were fed to the control chicks. For Trial 3.2 the gamma irradiation level was increased to 4 Mrad, and treatments were included to measure the response of conventional chicks to irradiated wheat and rye diets respectively. Chromic oxide (0.5%) was added to a portion of each diet for the digestibility phase of the trial. The diets for the gnotobiotic study were triple bagged in 200g lots using plastic bags. The outer bag was removed prior to introduction of the feed into the isolator entry

Table 3.1 Percent composition of experimental diets used in Trial 3.1

Ingredient	Diet 1	Diet 2
Rye (8.1% CP)	-	61.0
Wheat (14.3% CP)	61.0	-
Tallow	3.0	3.0
Corn Oil	1.5	1.5
SBM (48.0% CP)	30.0	30.0
Calcium carbonate	.5	.5
Calcium phosphate	2.5	2.5
Vitamin-mineral premix[1]	1.35	1.35
DL-methionine	.10	.10
L lysine	.05	.05
Calculated Analysis		
Crude protein (N x 6.25)	22.6	18.9
Calcium	.9	.9
Phosphorus	.8	.8
Methionine	.48	.48
Lysine	1.05	1.05

[1]Supplied the following per kilogram of diet: vitamin A, 16,500 I.U.; vitamin D_3, 1650 I.U.; vitamin E, 11I.U.; menadione, 3.3 mg; riboflavin, 4.4 mg; niacin, 55 mg; calcium pantothenate, 17.6 mg; vitamin B_{12}, 11.0 mg; choline chloride, 330 mg; ethoxyquin, 112.2 mg; zinc (as ZnO) 110.3 mg; manganese (as $MnSo_4$) 220.5 mg; iodized salt, 2500 mg

Table 3.2 Percent composition of experimental diets used in Trial 3.2

Ingredient	Diet 1	Diet 2
Rye (8.1% CP)	-	59.65
Wheat (14.3% CP)	59.65	-
Tallow	7.8	7.8
Soybean meal (48.0% CP)	29.3	29.3
Calcium carbonate	1.22	1.22
Calcium phosphate	1.46	1.46
Vitamin mineral premix[1]	.42	.42
DL methionine	.10	.10
L lysine	.05	.05
Calculated Analysis		
Crude protein (Nx6.25)	22.5	18.8
Calcium	.8	.8
Phosphorus	.7	.7
Methionine	.48	.48
Lysine	1.05	1.05

[1] Supplied the following per kilogram of diet: vitamin A, 13200 I.U.; vitamin D_3, 2250 I.U.; vitamin E, 16.5 I.U.; menadione, 2.25 mg; riboflavin, 10.5 mg; vitamin B_{12}, 16.5 µg; biotin, 82.5 µg; pantothenic acid, 16.5 mg; folic acid, .5 mg; niacin, 60 mg; iron, 60 mg; zinc, 90 mg; manganese, 90 mg; copper, 7.5 mg; iodine, .75 mg; cobalt, .15 mg; selenium, .15 mg; ethoxyquin, 188 mg; iodized salt, 2400 mg

port. It should be noted that the vitamin premix was increased beyond the normal recommendations (1.5 times recommendation by Scott et al. (1976) in Trial 3.1 and 1.5 times that used in commercial supplements in Trial 3.2). This was to allow for possible vitamin destruction by the irradiation treatment. Water for the gnotobiotic chicks was autoclaved in 1.0 l flasks (with screw top lids), again placed in autoclavable plastic bags. The bag was removed prior to the peracetic acid (2 %) spraying and introduction to the isolator. Water and feed were introduced into the isolator as needed. Both the conventional and germ-free chicks were fed and watered ad libitum.

3.2.3 Chemical analyses

Excreta samples were lyophilized and pooled to give the replications indicated for the corresponding analysis of variance. Both diet and excreta samples were finely ground using a Udy mill (1 mm screen) prior to analysis. Dry matter, ether extract, metatarsal bone ash and chromic oxide were determined according to procedures described by A.O.A.C. (1970). Samples were analyzed for total fat by the method of Marchello et al. (1971). This procedure incorporates an acid hydrolysis step which liberates any fatty acids present in the excreta as soaps. Gross energy values were determined using a Parr Adiabatic calorimeter. Samples were analyzed for

amino acids by procedures previously mentioned (Marquardt et al., 1979).

3.2.4 Calculation of nutrient retention and statistical analysis

Nutrient retention was calculated according to the formula:

$$((\text{level in feed} \times Cr_2O_3 \text{ feed}/Cr_2O_3 \text{ feces})/(\text{level in feed}))\times 100$$

All values were expressed on a dry matter basis prior to calculation.

Apparent metabolizable energy was calculated according to the formula:

$$((\text{gross energy(diet)}-(Cr_2O_3\text{(diet)}/Cr_2O_3\text{(excreta)} \times \text{gross energy(excreta)}))$$

All statistics were computed using SPSS (Nie et al., 1975) oneway analysis of variance, Tukey-HSD procedure, and t-test where appropriate. The level of probability accepted as being significant was $P<.05$ in all cases. All analyses were expressed on a dry matter basis unless otherwise noted in the results.

3.3 Results

3.3.1 Trial 3.1

Chicks fed non-irradiated rye diets and raised in a conventional environment exhibited a marked increase in level of excreta fat which caused a severe depression in both fat retention and metabolizable energy (Table 3.3). In comparison, the differences among the chicks fed rye in a germ-free environment, and chicks fed wheat in a conventional or germ-free environment were minimal. The chicks fed wheat in the germ-free environment did have significantly reduced levels of excreta fat compared to chicks fed wheat in a conventional environment, and tended to have reduced fat retention suggesting that chicks fed wheat respond similarly to environmental changes as chicks fed rye, albeit to a lesser degree. The rye fed chicks raised in a germ-free state also had significantly lower levels of excreta fat than chicks fed wheat in a conventional environment, and this reflected in a somewhat higher fat retention. This response, however, was not significant.

Substantial differences were also observed among treatments with regards to bone ash (Table 3.4). Conventional chicks fed non-irradiated rye exhibited severe rickets, which was reflected in the substantially reduced bone ash observed for these chicks. Significant differences were also observed in level of excreta soaps, however this appeared to be more closely related to environment than grain base of the diet. Germ-free chicks, whether fed wheat or rye, had less fatty

Table 3.3 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on excreta fat, fat retention and metabolizable energy for chicks fed wheat or rye diets (Trial 3.1), including summary of analysis of variance (mean squares)

Treatment	Excreta Fat[1] (%)	Fat Retention[1] (%)	ME[2] (kcal/kg)
Rye (CO, NI[2])	12.9^{a}	-13.9^{a}	2112^{a}
Rye (GF, I)	4.8^{b}	67.9^{b}	2915^{b}
Wheat (CO, NI)	6.8^{c}	61.5^{b}	3176^{b}
Wheat (GF, I)	3.0^{b}	75.9^{b}	3138^{b}
Summary of Analysis of Variance (Mean Squares)			
Treatment (3df)	133*	12930*	1893474*
Error (24df)	1.3	120	56446

a,b,c - different superscripts within a column denotes significant differences

1 - Excreta fat and fat retention are expressed on oven dry basis ME is expressed on air dry basis

2 - Abbreviations are as follows:conventional environment (CO), non-irradiated (NI), irradiated (I), germ-free (GF)

* denotes significant differences among treatments

acids present in the excreta as soaps than their conventional counterparts (Table 3.4).

Differences in amino acid retention among treatments (Tables 3.5 and 3.6) paralleled changes observed in fat retention, however the magnitude of the changes was less. Conventional chicks fed rye had a substantial reduction in retention for all amino acids. Again, in comparison, the differences among the other treatments were minor and not statistically significant. The germ-free birds fed rye tended to be somewhat inferior to the germ-free birds fed wheat, and superior to the conventional chicks fed wheat. The changes in amino acid retention among treatments were related to the level of the amino acids present in the excreta (Tables 3.7 and 3.8). Conventional chicks fed rye had substantially higher levels of excreta amino acids (total = 12.6%) than chicks in other treatments. In addition, conventional chicks fed wheat tended to have higher levels of excreta amino acids (total = 9.0%) than germ-free chicks fed wheat (total = 6.8%) or rye (total = 6.5%). There were however no differences in excreta amino acid levels for chicks fed wheat or rye in the germ-free state.

Performance data (Table 3.9) generally corroborates the results of the digestibility trials. Conventional chicks fed rye weighed significantly less than chicks in other treatments, and had significantly poorer feed conversion. Feed consumption was also depressed, however in this case the

Table 3.4 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on bone ash and excreta soaps for chicks fed wheat or rye diets (Trial 3.1), including summary of analysis of variance (mean squares)

Treatment	Bone Ash (%)	Excreta Soap(%)
Rye (CO, NI[1])	35.3^{a}	2.79^{a}
Rye (GF, I)	44.6^{b}	$.73^{b}$
Wheat (CO, NI)	48.0^{b}	2.71^{a}
Wheat (GF, I)	45.2^{b}	1.15^{b}
Summary of Analysis of Variance (Mean Square)		
Treatment (3df)	250.4^{*}	7.65^{*}
Error (24df)	12.1	.28

a,b means within a column bearing different superscripts are
* significantly different
1 denotes significant differences among treatments
for abbreviations see Table 3.3

Table 3.5 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on percent amino acid retention for chicks fed wheat or rye diets (Trial 3.1)

Amino Acid[1]	Wheat[1] (CO, NI)	Rye (CO, NI)	Wheat (GF, I)	Rye (GF,I)
Lysine	85.6^{a}	66.4^{b}	89.2^{a}	87.5^{a}
Methionine	90.9^{a}	79.0^{b}	96.2^{a}	92.1^{a}
Histidine	82.2^{a}	56.9^{b}	87.7^{a}	83.0^{a}
Arginine	87.1^{a}	69.5^{b}	90.9^{a}	84.1^{a}
Aspartate	82.7^{a}	60.8^{b}	85.0^{a}	83.6^{a}
Threonine	80.1^{a}	51.9^{b}	82.9^{a}	80.0^{a}
Serine	83.2^{a}	57.6^{b}	85.4^{a}	81.7^{a}
Glutamate	89.9^{a}	70.5^{b}	91.5^{a}	88.9^{b}
Proline	89.1^{a}	64.9^{b}	90.1^{a}	85.8^{b}
Alanine	81.5^{a}	56.9^{b}	86.8^{a}	84.9^{a}
Cystine	76.5^{ac}	61.5^{b}	86.0^{a}	68.5^{bc}
Valine	84.8^{a}	60.6^{b}	88.9^{a}	86.0^{a}
Isoleucine	86.6^{a}	62.8^{b}	90.1^{a}	87.6^{a}
Leucine	86.4^{a}	63.1^{b}	89.9^{a}	87.5^{a}
Tyrosine	86.3^{a}	55.7^{b}	90.2^{a}	86.6^{a}
Phenylalanine	87.6^{a}	64.2^{b}	90.9^{a}	88.0^{a}

^{a,b} - different superscript within a row denotes significant difference

[1] for abbreviations see Table 3.3

Table 3.6 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on amino acid retention for chicks fed wheat or rye diets (Trial 3.1) - summary of analysis of variance (mean squares)

Amino Acid	Treatment (3df)	Error(24df)
Lysine	842.7*	35.8
Methionine	411.2*	38.9
Histidine	831.7*	34.7
Arginine	732.4*	30.8
Aspartate	1003.5*	45.2
Threonine	1620.5*	50.9
Serine	1283.4*	48.2
Glutamate	732.6*	31.9
Proline	1071.1*	42.0
Alanine	1445.8*	30.5
Cystine	763.0*	86.5
Valine	1288.5*	38.7
Isoleucine	1217.0*	38.6
Leucine	1175.3*	34.4
Tyrosine	1956.2*	76.8
Phenylalanine	1155.5*	34.3

* denotes significant difference among treatments

Table 3.7 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on excreta amino acid levels for chicks fed wheat or rye diets (Trial 3.1)

Amino Acid[1]	Wheat[2] CO, NI	Rye CO, NI	Wheat GF, I	Rye GF, I
Lysine	$.54^{b}$	$.80^{a}$	$.39^{c}$	$.40^{c}$
Methionine	$.13^{ab}$	$.17^{a}$	$.05^{c}$	$.09^{bc}$
Histidine	$.33^{b}$	$.43^{a}$	$.22^{c}$	$.23^{c}$
Arginine	$.57^{b}$	$.76^{a}$	$.39^{c}$	$.37^{c}$
Apartate	1.09^{b}	1.56^{a}	$.91^{b}$	$.88^{b}$
Threonine	$.51^{a}$	$.71^{a}$	$.42^{b}$	$.40^{b}$
Serine	$.52^{b}$	$.69^{a}$	$.43^{bc}$	$.40^{c}$
Glutamate	1.43^{b}	1.96^{a}	1.15^{bc}	1.00^{c}
Proline	$.57^{b}$	$.79^{a}$	$.50^{bc}$	$.44^{c}$
Alanine	$.56^{b}$	$.77^{a}$	$.39^{c}$	$.36^{c}$
Cystine	$.27^{a}$	$.25^{ab}$	$.15^{b}$	$.26^{a}$
Valine	$.57^{b}$	$.80^{a}$	$.40^{c}$	$.38^{c}$
Isoleucine	$.46^{b}$	$.68^{b}$	$.33^{c}$	$.30^{c}$
Leucine	$.75^{b}$	1.10^{a}	$.54^{c}$	$.50^{c}$
Tyrosine	$.25^{b}$	$.43^{a}$	$.18^{a}$	$.18^{a}$
Phenylalanine	$.47^{b}$	$.69^{a}$	$.33^{c}$	$.31^{c}$

a,b,c - different superscripts within a row notes significant differences

1 - expressed on dry matter basis

2 - for abbreviations see Table 3.3

Table 3.8 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on amino acid retention for chicks fed wheat or rye diets (Trial 3.1) - summary of analysis of variance (mean squares)

Amino Acid	Treatment (3df)	Error (24df)
Lysine	.26*	.008
Methionine	.02*	.002
Histidine	.07*	.002
Arginine	.24*	.007
Aspartate	.72*	.024
Threonine	.15*	.003
Serine	.12*	.004
Glutamate	1.25*	.054
Proline	.17*	.007
Alanine	.25*	.004
Cystine	.02*	.004
Valine	.26*	.006
Isoleucine	.21*	.006
Leucine	.53*	.014
Tyrosine	.11*	.003
Phenylalanine	.22*	.005

* denotes significant difference among treatments

Table 3.9 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on body weight, feed consumption and feed conversion for chicks fed wheat or rye diets (Trial 3.1), including summary of analysis of variance (mean squares)

Treatment	Body Weight (g)	Consumption (g)	Feed/gain
Rye (CO, NI[1])	254.3[a]	628.8[a]	2.51[a]
Rye (GF, I)	424.5[b]	714.5[a]	1.68[b]
Wheat (CO, NI)	418.8[b]	703.9[a]	1.69[b]
Wheat (GF,I)	476.5[b]	697.5[a]	1.46[b]
Summary of Analysis of Variance (Mean Squares)			
Treatment (3df)	90753*	15546*	2.14*
Error (31df)	2070	5006	.06

[1] for abbreviations see Table 3.3

[a,b] - different superscripts within a column denote significant differences

* denotes significant differences among treatments

differences were not significantly different. The germ-free chicks fed irradiated rye tended to have greater feed consumption than either germ-free or conventional chicks fed wheat.

The effect of treatment on weight of gut segments was also determined (Table 3.10). Conventional chicks fed rye had substantially greater intestinal weight than chicks in other treatments. Conventional chicks fed wheat occupied a position midway between conventional chicks fed rye and germ-free chicks fed wheat or rye. There was no difference between germ-free chicks fed wheat or rye.

3.3.2 Trial 3.2

For Trial 3.2 the response of chicks fed irradiated rye was repeated at a higher irradiation dosage (4 Mrad). In addition germ-free chicks were infected with Streptococcus, and conventional chicks were fed irradiated rye or wheat in order to determine the response to irradiation treatment alone.

The results of Trial 3.2 generally corroborate the results of Trial 3.1. Chicks fed an irradiated rye diet in a germ-free environment gave a marked improvement in most parameters of performance relative to other treatments. Administration of streptococci to axenic chicks caused a significant increase in excreta fat (Table 3.11), which

Table 3.10 Effect of combined irradiation treatment (2.5 Mrad) and germ-free environment on gut weight for chicks fed wheat or rye diets (Trial 3.1), including summary of analysis of variance (mean squares)

Treatment	Jejunum	Ileum	Cecum	Colon
	(g/100 g body weight)			
Rye (CO, NI[1])	2.58[a]	2.03[a]	.78[a]	.47[a]
Rye (GF, I)	1.06[b]	.86[b]	.51[b]	.27[b]
Wheat (CO, NI)	1.56[b]	1.17[b]	.50[b]	.33[b]
Wheat (GF, I)	1.06[b]	.73[b]	.36[b]	.23[b]
Summary of Analysis of Variance (Mean Square)				
Treatment (3df)	4.82*	3.23*	.289*	.10*
Error (31df)	.14	.12	.03	.01

[1] for abbreviations see Table 3.3

[a,b] - different superscripts within a column denote significant differences

* differences among treatments are significant

Table 3.11 Effect of irradiation treatment (4 Mrad) and microbial status on excreta fat, fat retention and metabolizable energy for chicks fed wheat or rye diets (Trial 3.2), including summary of analysis of variance (mean squares)

Treatment	Excreta Fat[1] (%)	Fat Retention[1] (%)	ME[2] (kcal/kg)
Rye (GF,I)	5.64[a]	70.1[a]	3087[b]
Rye (Strep, I)	8.74[b]	53.5[b]	3027[ab]
Rye (CO, I)	9.71[b]	52.7[b]	3073[b]
Rye (CO, NI)	14.69[c]	23.3[c]	2817[a]
Wheat (CO, I)	7.18[a]	66.2[ab]	3219[bc]
Wheat (CO, NI)	9.56[b]	60.2[ab]	3358[c]
Summary of Analysis of Variance (Mean Square)			
Treatment (5df)	75.4*	2334.8*	311094*
Error (34df)	1.9	71.0	18857

1 oven dry basis
2 air dry basis
3 abbreviations are as follows: germ-free (GF), irradiated (I), Streptococcus infected (Strep), non-irradiated (NI), conventional (CO)
a,b,c,d - means within a column bearing different superscripts are significantly different
* denotes significant differences among treatments

resulted in significant depression in fat retention and an apparent depression in metabolizable energy. Feeding irradiated rye to conventional chicks gave a similar response to infection with streptococci. Excreta fat, fat retention and metabolizable energy were significantly improved over the non-irradiated diet fed to conventional chicks. Irradiation treatment of wheat diets resulted in a significant reduction in excreta fat also, which caused an apparent improvement in fat retention. The M.E. data did not support this trend however, and it may be that the range of differences was not sufficiently large to give an accurate estimation of digestibility differences. A second possibility is that the irradiation treatment may have adversely affected the digestion of other dietary components, which then confounded the apparent beneficial response in fat retention.

Metatarsal bone ash (Table 3.12) gave a similar pattern of response as the fat retention data. The highest value attained was for the irradiated rye diet fed to axenic chicks. Metatarsal bone ash was significantly depressed with the addition of streptococci (Table 3.12). Irradiated rye diets did not give a significantly different response between the conventional and germ-free environments, however chicks fed irradiated diets in a conventional environment did tend to have lower bone ash than chicks fed irradiated diets in a germ-free environment. As in Trial 3.1, the conventional chicks fed non-irradiated rye were severely rachitic. Both

Table 3.12 Effect of microbial status and diet gamma irradiation on metatarsal bone ash in rye and wheat fed chicks (Trial 3.2), including analysis of variance (mean squares)

Treatment	Excreta soaps (%)	Metatarsal Bone Ash (%)
Rye, CO, NI[1]	3.33^{a}	34.4
Rye, CO, I	1.92^{b}	44.3
Rye, Strep, I	2.48^{ab}	45.6
Rye, GF, I	2.04^{b}	48.1
Wheat, CO, NI	2.43^{b}	41.8
Wheat, CO, I	1.40^{b}	42.1

Summary of analysis of variance (Mean Squares)

Treatment	2.85^{*} (5df)	218.3^{*} (5df)
Error	.52 (34df)	19.4 (46df)

T-test[1] (Metatarsal bone ash)

			t
Wheat, CO, NI[1]	vs	Wheat, CO, I	.17
Rye, GF,I	vs	Rye, CO, NI	7.89^{*}
Rye, GF, I	vs	Rye, Strep, I	2.27^{*}
Rye, GF, I	vs	Rye, CO, I	1.47

[1] abbreviations are given in Table 3.11

[2] t-test was used to compare means of interest as there were significant differences in variation among treatment groups. The comparisons of interest had similar variation between treatments

^{a,b} denotes treatment means that were significantly different

groups of chicks fed rye in the conventional environment were more variable than the gnotobiotic chicks.

Excreta amino acid levels (Tables 3.13 and 3.14) corresponded to the results obtained for excreta fat; axenic chicks fed irradiated rye had consistently lower levels than chicks in the other treatments. Gnotobiotic chicks infected with streptococci, however, had higher levels of excreta amino acids, which were not significantly different from that of chicks fed irradiated rye in a conventional environment. Chicks fed non-irradiated rye had substantially higher levels of excreta amino acid than chicks in other treatments. Values for chicks fed irradiated rye, or streptococci infected chicks, were roughly comparable to those fed the wheat diets. For the most part, irradiation treatment had no significant effect on the excreta amino acid levels of chicks fed wheat; there was however a consistent trend towards reduced levels for the irradiated wheat diets, indicating that wheat too may respond to irradiation treatment, but to a lesser degree than rye. Changes in excreta amino acid levels lead to corresponding changes in overall amino acid retention (Tables 3.15 and 3.16). Irradiated rye fed to germ-free chicks resulted in superior amino acid retention compared to other rye treatments. Irradiated rye fed to conventional or streptococci-infected chicks tended to occupy an intermediate position, with amino acid retention being consistently lowest for non-irradiated rye fed to chicks in a conventional

Table 3.13 Effect of microbial status and diet gamma irradiation (4Mrad) on excreta amino acids in rye and wheat fed chicks (Trial 3.2)

Amino acid	Rye[1,2] CO,NI	Rye CO,I	Rye Strep,I	Rye GF,I	Wheat N,I	Wheat CO,I
Lysine	.76^{a}	.58^{b}	.47^{b}	.30^{c}	.55^{b}	.49^{b}
Methionine	.21^{a}	.16^{bc}	.17^{b}	.13^{c}	.18^{b}	.18^{ab}
Histidine	.31^{a}	.23^{b}	.18^{b}	.13^{c}	.23^{b}	.20^{b}
Arginine	.63^{a}	.40^{bc}	.38^{c}	.30^{c}	.50^{b}	.36^{c}
Aspartic acid	1.42^{a}	1.09^{b}	1.06^{b}	.79^{b}	1.08^{b}	.97^{b}
Threonine	.68^{a}	.55^{ab}	.50^{b}	.35^{c}	.53^{b}	.48^{bc}
Serine	.66^{a}	.48^{bc}	.49^{b}	.38^{c}	.52^{b}	.44^{bc}
Glutamic acid	1.55^{a}	1.14^{bc}	1.20^{b}	.80^{c}	1.18^{b}	1.01^{bc}
Proline	.57^{a}	.48^{a}	.54^{a}	.35^{a}	.44^{a}	.44^{a}
Alanine	.71^{a}	.57^{a}	.49^{b}	.24^{c}	.51^{ab}	.50^{ab}
Cystine	.33^{a}	.30^{a}	.31^{a}	.27^{a}	.35^{a}	.30^{a}
Valine	.82^{a}	.55^{c}	.50^{c}	.38^{d}	.67^{b}	.49^{c}
Isoleucine	.61^{a}	.39^{c}	.39^{c}	.27^{d}	.48^{b}	.43^{bc}
Leucine	1.17^{a}	.61^{b}	.59^{b}	.44^{b}	.97^{a}	.58^{b}
Tyrosine	.36^{a}	.27^{abc}	.21^{bc}	.15^{c}	.28^{ab}	.28^{abc}
Phenylalanine	.59^{a}	.41^{b}	.38^{b}	.25^{c}	.45^{b}	.36^{bc}

[1] for abbreviations see Table 3.11

[2] expressed on per cent dry matter basis

^{abcd} denotes means that significantly different within a row

Table 3.14 Effect of microbial status and diet gamma irradiation (4 Mrad) on excreta amino acids in rye and wheat fed chicks (Trial 3.2) - summary of analysis of variance (mean squares)

Amino Acid	Treatment (5df)	Error(34df)
Lysine	.175*	.006
Methionine	.004*	.0004
Histidine	.027*	.001
Arginine	.113*	.004
Asparatate	.345*	.025
Threonine	.088*	.007
Serine	.072*	.003
Glutamate	.474*	.046
Proline	.046*	.021
Alanine	.165*	.016
Cystine	.006*	.004
Valine	.193*	.004
Isoleucine	.102*	.002
Leucine	.600*	.034
Tyrosine	.037*	.004
Phenylalanine	.096*	.004

* denotes significant differences among treatments

Table 3.15 Effect of microbial status and diet gamma irradiation (4 Mrad) on amino acid retention in rye and wheat fed chicks (Trial 3.2) - summary of analysis of variance (mean squares)

Amino Acid	Treatment (5df)	Error (34df)
Lysine	194.9*	7.20
Methionine	71.0*	1.85
Histidine	174.5*	8.93
Arginine	99.9*	3.90
Aspartate	129.3*	10.94
Threonine	243.9*	20.28
Serine	210.0*	7.83
Glutamate	100.4*	6.49
Proline	98.3*	17.07
Alanine	325.6*	31.96
Cystine	171.8*	39.12
Valine	252.0*	5.56
Isoleucine	188.9*	3.10
Leucine	357.1*	11.79
Tyrosine	239.7*	27.11
Phenylalanine	182.0*	6.22

* denotes significant differences among treatments

Table 3.16 Effect of microbial status and diet gamma irradiation (4 Mrad) on amino acid retention in rye and wheat fed chicks (Trial 3.2)

Amino acid	Rye CO,NI[1]	Rye CO,I	Rye Strep,I	Rye GF,I	Wheat CO,NI	Wheat CO,I
Lysine	75.7[2]	82.9^{a}	84.6^{ab}	90.3^{c}	85.8^{b}	85.8^{ab}
Histidine	77.3	84.3^{a}	86.7^{ab}	90.0^{b}	87.5^{ab}	88.3^{ab}
Arginine	81.9	89.3^{a}	88.9^{a}	91.4^{b}	89.0^{ab}	91.1^{ab}
Aspartic acid	74.6	82.1^{ab}	80.8^{a}	85.8^{b}	83.6^{ab}	83.5^{ab}
Threonine	67.5	75.9^{a}	75.8^{a}	83.1^{b}	79.9^{ab}	79.6^{ab}
Serine	71.6	81.0^{ab}	78.6^{a}	83.6^{b}	84.0^{b}	84.8^{b}
Glutamic acid	83.0	88.5^{ab}	86.7^{a}	91.1^{bc}	91.6^{c}	92.0^{c}
Proline	84.5	88.0^{ab}	85.1^{a}	90.4^{bc}	92.8^{c}	92.0^{bc}
Alanine	70.3	78.0^{a}	79.4^{a}	89.6^{b}	83.2^{a}	82.0^{a}
Cystine	56.6	64.1^{a}	58.8^{a}	63.9^{a}	67.6^{a}	68.6^{a}
Valine	71.5	82.3^{a}	82.5^{a}	86.8^{b}	82.9^{a}	86.0^{b}
Methionine	79.4	85.1^{ab}	83.0^{a}	86.6^{b}	87.0^{b}	85.1^{ab}
Isoleucine	74.8	85.3^{a}	83.7^{a}	88.8^{b}	84.9^{a}	85.3^{a}
Leucine	70.8	85.7^{ab}	85.0^{ab}	88.8^{b}	82.1^{a}	88.1^{b}
Tyrosine	72.2	80.7^{a}	83.2^{ab}	88.3^{b}	83.7^{a}	82.3^{a}
Phenylalanine	77.6	85.7^{ab}	85.4^{a}	90.3^{c}	88.4^{bc}	89.6^{c}

[1] for abbreviations see Table 3.11

[2] the NI rye diet fed to CO chicks was not included in the analysis as the variation was substantially greater than for the other treatments. Since amino acid retention was obviously lower than the other treatments, it was felt it could be justifiably excluded

^{abcd} different superscripts within a row denote means that are significantly different

environment. Amino acid retention for chicks fed both wheat diet tended to be superior to that for either conventional or streptococci-infected chicks fed irradiated rye diets. Irradiation treatment did not improve retention in wheat diets, in fact there was a tendency for retention to be reduced for the irradiated wheat diets. This conflicts with the previously observed changes in excreta amino acids, however it should be noted that the differences for the most part were not significant.

The performance data (Table 3.17) generally agrees with the retention studies, with one exception. As expected, the conventional chicks fed the non-irradiated rye diet were substantially smaller than chicks fed all other diets. The chicks fed the irradiated rye diet in a conventional environment occupied a position midway between those fed the non-irradiated diet, and those fed the irradiated diet in the germfree environment. The discrepancy occurred in the case of the chicks infected with streptococci; they were significantly larger than the conventional chicks fed irradiated rye, and were in fact somewhat larger than the germ-free chicks. Based on the retention studies, it would be expected that these chicks would be comparable to chicks fed irradiated rye in a conventional environment. The numbers of birds studied, however were small, and while adequate for digestibility studies, were not necessarily large enough to give a good estimate of a growth response. The lack of a

Table 3.17 Effect of irradiation treatment (4 Mrad) and microbial status on body weight and gut size in rye and wheat fed chicks (Trial 3.2), including summary of analysis of variance (mean squares)

Treatment	Body Wt (g)	Duodenum	Jejunun	Ileum	Cecum	Colon
		(g/100 g body weight)				
Rye, CO, NI[1]	185.0[a]	2.64[a]	3.36[a]	2.79[a]	.89[a]	.47[a]
Rye, CO, I	315.9[b]	----------ND[2]----------				
Rye, Strep, I	368.1[c]	1.09[b]	1.43[b]	1.08[b]	.51[b]	.30[b]
Rye, GF, I	358.0[bc]	1.32[b]	1.44[b]	1.18[b]	.47[b]	.34[b]
Wheat, CO, NI	338.4[bc]	----------ND----------				
Wheat, CO, I	327.8[bc]	----------ND----------				
Summary of Analysis of Variance (Mean Squares)						
Treatment	104175 (5df)	4.89 (2df)	8.62 (2df)	6.41 (2df)	.37 (2df)	.05 (2df)
Error	1822 (105df)	.11 (18df)	.18 (18df)	.31 (18df)	.03 (18df)	.004 (18df)

[1] for abbreviations see Table 3.11
[2] ND - not determined

abcd denotes means within a column that are significantly different

growth depression response could conceivably be due to the fact that streptococci were not administered to the chicks until seven days of age and that a period of establishment was required before a full effect could be expressed. Irradiation of wheat diets resulted in an apparent improvement in body weight, but again this was not significant.

The weight of various segments of the gut (Table 3.17) indicated, as in Trial 3.1, that the germ-free environment resulted in a substantial reduction in gut weight as compared to the conventional environment. Addition of streptococci did not have any effect on gut weight as compared to the germ-free treatment, which is not consistent with previous reports of increased intestinal weights attributable to streptococci.

3.4 Discussion

The results indicate that a combination of irradiation treatment and germ-free environment renders performance of chicks fed rye or wheat diets similar. The germ-free chicks fed rye performed somewhat poorer than the germ-free chicks fed wheat, however this is probably due to the diets not being isonitrogenous or isocaloric. The levels of nutrients present in the excreta of germ-free chicks fed irradiated rye, including fat and amino acids, compared favourably to

wheat treatments and were in fact superior to chicks fed wheat in a conventional environment. There was a tendency for nutrient retention to be somewhat depressed for germfree chicks fed rye as compared to germ-free chicks fed wheat, however it appeared that this may be a simple bulking effect caused by a somewhat higher fibre level in the rye, rather than any residual anti-nutritional factor. The levels of nutrients present in the excreta were similar for the two treatments.

In terms of performance similar results were obtained in Trial 3.2 as for Trial 3.1. Again the germ-free chicks fed irradiated rye compared favourably with conventional chicks fed wheat (irradiated or not irradiated). The irradiation treatment alone elicited a pronounced improvement in the case of rye diets, which could be attributed to increased nutrient retention. Patel et al. (1981) proposed that gamma irradiation destroys unknown growth depressing factors in the rye. MacAuliffe et al. (1979) propose that gamma irradiation specifically destroys an unidentified vitamin D antagonist in rye. While results from the present study confirmed the effect of irradiation on bone ash values, it can be suggested that the irradiation effect is more general having a profound effect on retention of most nutrients.

The response of the chicks fed rye to infection with streptococci is consistent with the hypothesis that gums in the rye predispose chicks to excessive microbial growth,

which in turn mediates the depressed performance. Infection with streptococci resulted in a similar reduction in retention of amino acids and fat, a similar increase in excreta soaps and a similar depression in bone ash to that observed in chicks fed a rye diet in a conventional environment. Unfortunately, due to the effect of the irradiation treatment, it is not possible to ascertain from this data what fraction of the growth depression is attributable to the rye directly and what is attributable to the microflora. The magnitude of the irradiation response was not anticipated in these studies. It appears however that the combination of the irradiation and the elimination of the microflora effectively counteracts the depressed performance.

The effect of Streptococcus corresponds closely to the prediction of Eyssen et al. (1963_{ab}) that certain elements of the microflora provoke a polymalabsorptive state, in which retention of fat as well as other nutrients may be depressed. Later studies have indicated (Kim et al., 1966) that intestinal overgrowth induces steatorrhea by deconjugating bile salts. Recently Coates et al. (1981) have demonstrated that Streptococcus faecium SY1 deconjugated taurocholic acid and taurochenodeoxycholic acid. Such activity readily explains the depressed fat retention, and may also account for the depressed bone ash. Vitamin D, being fat soluble, requires adequate micelle formation to be effectively absorbed. In addition the undigested fatty acids complex

calcium (and other cations) rendering the complex insoluble and not available for absorption. Such a general explanation may account for the observed improvement in bone ash by antibiotic supplementation (MacAuliffe et al., $1976_{a,b}$; MacAuliffe and McGinnis, 1976) and seems to be more plausible than the explanation offered by McGinnis and co-workers in which it was argued that rye contains unidentified factors that directly and specifically affect vitamin D metabolism.

The reduction in retention of amino acids is not so readily explained on the basis of altered bile acid metabolism, since bile acids do not directly participate in protein digestion. It is conceivable that excessive quantities of fat in the gut lumen serve to "coat" undigested particles rendering digestion difficult (Mayes, 1979). Another possibility that cannot be discounted in the present study is that the increased nutrients in the excreta are simply due to the increased bacterial mass, rather than any effect on digestion in the upper G.I. tract. This appears to be more applicable in the case of protein than fat since the anaerobic flora will have limited capacity to synthesize fat de novo. Also roosters fed rye in conjunction with low fat diets have considerably lower levels of excreta fat than those seen in the present study.

Why rye should induce excessive bacterial growth is not clear. At least part of the effect may be due to a slowing of rate of passage by the viscosity-increasing gums present.

McKay (1980) has demonstrated that rye diets have a slower passage rate than similar wheat diets which would allow greater time for the microflora to proliferate. Gohl and Gohl (1977) have reported a similar response in barley-based diets, where B glucan was presumed responsible. A second possibility is that gums interact with polysaccharides protruding from the enterocyte, producing a region along the gut wall highly favourable to bacterial growth. This would explain the observation (Untawale and McGinnis, 1979) that bacteria adhering to the gut wall are increased when rye is fed. Streaming of gut contents is well established, and alterations in the physical nature of the digesta could promote excessive bacterial growth by permitting stagnation of the digesta in the area immediately adjacent to the gylcocalyx. Such an explanation may account for the effects of gamma irradiation, enzyme supplementation, and the ability to reduce the negative response of rye fed to chicks by antibiotics. Since irradiation disrupts the gel structure (Campbell et al., 1982), it would effectively destroy the conditions favourable to bacterial growth, thereby obliterating the potential for the excessive antibiotic response seen in chicks fed rye.

That streptococci appear to duplicate the rye effect indicates that bacteria of the chick's gut have a potential to reduce nutrient retention and performance. It does not necessarily mean that Streptococcus are the sole agent that

may induce this effect. Wagner and Thomas (1977) implicate clostridia in rye growth depression, and reported substantial quantities of gas in the chick intestine. In a later study Day and Thomas (1980) reported instances where 45.4% of the intestine of rye-fed chicks was inflated. We have observed in conventional chicks fed rye similar situations, where gas fills the entire cross-section of the lumen. It would seem possible, if not likely, that such gas formation would impair transport even further.

Overall, the results generally are consistent with the hypothesis that at least part of the depressed performance observed in rye fed chicks is attributable to the microflora. Unfortunately it is not possible to quantify what fraction of the depression is directly attributable to the rye itself,and what is attributable to the microflora, as the gamma irradiation procedure resulted in pronounced improvement in the feeding value of the rye diets. The magnitude of this response was not anticipated, and indicates that when working with rye diets in gnotobiotic studies, it is desirable to employ alternative sterilization procedures.

4.0 FACTORS AFFECTING FAT DIGESTIBILITY IN RYE DIETS : EFFECT OF GAMMA IRRADIATION, SODIUM TAUROCHOLATE SUPPLEMENTATION AND HIGH CALCIUM LEVELS

4.1 Introduction

The results of Trials 3.1 and 3.2 indicated that rye diets fed to chicks raised in a conventional environment resulted in severely depressed performance, including depressed nutrient retention. Fat retention was particularly low. Several experiments were undertaken to study means by which fat retention in chicks fed rye diets might be modified.

Gamma irradiation (6Mrad, ^{60}Co) has been reported previously to improve growth when chicks were fed rye, pectin, or guar containing diets (Patel et al., 1980). In pectin or guar containing diets performance was not further improved with pectic enzyme (Irgazyme-100) or penicillin additions. Since these treatments elicited a response when given alone, Patel and co-workers proposed that gamma irradiation or pectic enzyme produced structural changes in guar and pectin that somehow affected microbial activity. This effect, they proposed, was different from the rye growth depression. They noted an additive effect when penicillin was added to an irradiated rye diet, as well as failure of the gamma radiation to eliminate the sticky excreta in

rye-fed birds. From these data, they concluded that the factor responsible for sticky feces in rye-fed chicks was stable to gamma irradiation and/or pectin enzyme, and contend that it is therefore different from citrus pectin and the factor responsible for depressed growth in chicks fed rye.

Patel et al. (1981) studied the effects of gamma irradiation (6.2 Mrad) of laying hen diets, as well as supplementation with a commercial pectic enzyme and penicillin. While gamma irradiation successfully alleviated the depressed egg production observed when rye was fed, penicillin addition failed to improve egg production. Pectin enzyme had an effect during the first three week period, however this response was transitory and not evident in the subsequent five week period. Patel and co-workers concluded that the effect of rye on growth of chicks and on egg production in laying hens are somehow different, although they did not speculate on the mechanisms involved. The possible effect of gamma irradiation with regards to structural damage to the pentosan component of rye, or the effect on fat retention in the chicks was not examined.

The similarity between the fat malabsorption syndrome, chicks fed rye or gum and steatorrhea has been discussed previously. Kim et al. (1966) observed that addition of sodium taurocholate or tetracycline to the diets of dogs in which a blind-loop had been surgically created improved fat retention. In non-treated dogs steatorrhea of up to 56 per

cent of the dietary fat was noted after the creation of the blind-loop. This could be alleviated by the daily administration of 0.5g tetracycline, which caused fecal fat loss to drop to 7 per cent of the dietary fat. Alternatively, fat malabsorption could be alleviated by administration of the conjugated bile salt sodium taurocholate. It has been demonstrated that the fat malabsorption in rye fed chicks may be alleviated by antibiotics or a germ free environment, and induced in gnotobiotic chicks monocontaminated with streptococci. These observations indicate that chicks may have excessive bacterial growth with abnormal bile salt metabolism similar to the 'blind loop' syndrome. A differential response to added bile salts for tallow-containing diets with either wheat or rye as the cereal source would provide support for this contention.

Calcium and other divalent cations have also been implicated in increased fecal loss of fat through the formation of insoluble soaps that are excreted (Mattson et al., 1979). The results of Trials 3.1 and 3.2 indicated that excreta soap levels tended to be proportional to total excreta fat. It was not clear, however, as to the causative event, that is whether the soap formation was simply proportional to the excreta fat levels, or whether soap formation actually contributed to excreta fat loss. Comparison of fat digestion in a high tallow rye-containing diet both with and without supplemental calcium would clarify

the role of calcium when excreta fat levels are increased.

Three experiments were designed to study several means by which the poor fat retention observed in chicks fed rye might be modified. These included the effect of gamma irradiation of rye, the effect of added sodium taurocholate to rye versus wheat based diets, and the effect of high calcium levels in rye as compared to wheat diets. Gamma irradiated rye was also examined for evidence of possible structural damage to the pentosan component. To determine the effect of gamma irradiation, the response of chicks with regards to body weight, feed efficiency, fat retention and bone ash were determined at several irradiation levels (0 - 10 Mrad). The experiments studying the response of chicks to sodium taurocholate or calcium added to rye or wheat diets were retention studies conducted using chicks three weeks of age.

4.2 Materials and methods

4.2.1. Trial management

The chicks used in all trials were broiler type that were housed from one day of age in thermostatically controlled Jamesway battery brooders. For the gamma irradiation trial (Trial 4.1) six chicks (three males and three females) were randomly allotted per pen, and four pens allotted per dietary treatment. Both feed and water were supplied *ad lib.* for the

trial period, which commenced at one day of age and was terminated at 21 days of age. Excreta samples were collected twice daily during the 17 - 21 day period, and frozen in plastic bags for subsequent analysis.

To study the effect of supplementary sodium taurocholate in rye and wheat diets (Trial 4.2), male chicks only were maintained on an antibiotic-free chick starter until 21 days of age, afterwhich they were distributed to treatment groups. Four chicks were allotted per pen, and five pens allotted per treatment. During the trial period the chicks were pair fed by pen, which was accomplished as follows. The rye-fed chicks were starved overnight and offered feed the following morning. During the late afternoon the feed was taken from the rye-fed chicks and weighed back. The following day the chicks on the wheat treatment were paired by pen and offered feed equal to the consumption of the corresponding rye pen the previous day. The wheat-fed chicks normally consumed all the feed offered to them, hence consumption between the rye and wheat fed chicks was equal. There was however a one day delay between the rye and wheat treatments. The chicks were initially fed the unsupplemented wheat or rye diet for two days, following which there was a two day 'recovery' period when the chicks were given chick starter ad lib. The rest period was followed by the rye or wheat test diets supplemented with sodium taurocholate for two days. Excreta samples were collected daily during the test period and

frozen for later analysis.

The trial considering high calcium levels in rye and wheat diets (Trial 4.3) employed the same chicks and similar management as the sodium taurocholate trial. The chicks were rested several days on a commercial starter diet prior to the initiation of the test period.

4.2.2 The diets

'Puma' rye was fed in all three experiments, and the diets formulated are listed in Table 4.1. For the irradiation treatment rye was ground and irradiated (0, 2, 6, and 10 Mrad, Atomic Energy of Canada, Kanata, Ont.) prior to incorporation into the diet. An additional treatment was included in which the entire diet (6D) was irradiated at 6 Mrad dosage. Chromic oxide was added to the diets to facilitate retention determinations.

The experiments studying the response of chicks to sodium taurocholate or high calcium added to rye or wheat diets contained 80 per cent cereal grain and 20 per cent tallow. While this level of tallow far exceeds what is normally fed it was felt that effects on fat retention would be exemplified by challenging the chicks' capacity to utilize tallow.

4.2.3 Chemical Analyses

Table 4.1 Diets fed to chicks in Trial 4.1 - 4.3

Ingredient	Diet 1	Diet 2[2]	Diet 3[2]
Wheat	-	-	80.0
Puma rye	59.18	80.0	-
SBM (48% CP)	29.6	-	-
Tallow	7.9	20.0	20.0
Limestone	1.24	-	-
Dicalcium phosphate	1.48	-	-
Vitamin-mineral premix[3]	.45	-	-
DL-methionine	.10	-	-
L lysine	.05	-	-

1 Ground Puma rye was irradiated at 0, 2, 6 and 10 Mrad (^{60}Co) (Atomic energy of Canada) prior to incorporation into the diet. An additional treatment in which the whole diet (6 Mrad) was irradiated was also included

2 For supplemented diets in Trial 4.2, .5 per cent sodium taurocholate (United States Biochemical Corporation) was added to the diets. For supplemented diets in Trial 4.3, 10 per cent limestone was added to the diets. Approximate calcium level of these diets was calculated to be 3.5 per cent

3 The vitamin-mineral premix supplied the following per kilogram diet: vitamin A, 9000 I.U.; vitamin D_3, 1500 I.U.; vitamin E, 11 I.U.; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 11 mg; niacin, 24 mg; folic acid, .75 mg; biotin, 1 mg; choline, 500 mg; vitamin B_{12}, .01 mg; zinc, 60 mg; copper, 5 mg; manganese, 60 mg; selenium, .1 mg; iodized salt, 2500 mg

Excreta samples were lyophilized for Trial 4.1, and dried in a forced air oven (60 °C for 24 hours) for Trials 4.2 and 4.3. Both excreta and diet samples were ground in a Udy mill (1 mm screen) prior to analysis. Total fat for the diet and excreta were determined according to Van de Kamer et al. (1949). Chromic oxide was measured by the method of Fenton and Fenton (1979). For metatarsal bone ash determination, the bone was cleaned by boiling in water, extracted in ether for 24 hours, dried in a vacuum oven (105 °C for 16 hr) and ashed (600 °C for 24 hr). Bone ash was measured as the weight difference between the defatted and ashed sample. Excreta soaps were determined as the difference between total fat, as determined by the procedure of Van de Kamer et al. (1949), and ether extract (A.O.A.C., 1970).

The water extractable portion of the rye was examined for compositional changes that might be attributed to gamma irradiation. Fifty g ground rye (0 and 10 Mrad) were mixed with 100 ml distilled water (2:1) and permitted to soak (4 °C) for either one-half, 12 or 24 hours. The rye slurry was centrifuged (1800 g) and the supernatant hydrolysed (.57 N H_2SO_4 at 123 °C for 20 min) and analysed for component sugars by gas chromatography (Ward, 1982). Samples of the rye water extract were also analysed for sugars by descending paper chromatography, as verification for the gas chromatography. Paper chromatography was conducted on the hydrolysed water extract of rye (neutralized with barium carbonate) using

Whatman No.1 chromatography paper in a descending solvent system consisting of pyridine-ethylacetate-acetic acid in the proportions of 8:2:1. Exposure time was 48 hours, following which the resulting chromatograms were successively dried and dipped in silver nitrate-acetone solution (1 ml concentrated silver nitrate solution in 200 ml acetone), sodium hydroxide (0.5 N in ethanol) and sodium thiosulphate (5 %).

4.2.4 Calculation of nutrient retention and statistical analysis

For the gamma irradiation study fat retention was calculated using chromic oxide as an indicator as related in section 3.2.4. For the trials determining effects of sodium taurocholate and high calcium levels, quantification of excreta output relative to diet consumed was by total collection. All results were expressed on a dry matter basis prior to statistical analysis. All statistical analyses were computed using SPSS (Nie et al., 1975) oneway analysis of variance and Tukey-HSD procedure. For Trial 4.1, three week body weight was analysed as a 2 X 5 factorial design, with individual bird weight as the replication unit. Since sex had no significant effect, the weight data was re-analyzed as a one way analysis of variance. For Trial 4.2, excreta was collected from each of five pens daily for two days. Each day was treated as a replicate, thereby providing 10 replicates

per treatment. Analysis for Trial 4.3 was the same as Trial 4.2, except excreta was collected for only one day for each pen, hence there were five replicates per treatment. The limit of probability accepted as being significant was $P<.05$ in all cases.

4.3 Results

Gamma irradiation resulted in a significant improvement in body weight, feed consumption and feed conversion (Table 4.2). This was a curvilinear response that reached a plateau at 6 Mrad afterwhich additional irradiation had no beneficial effect. In fact there was a trend towards reduced performance at the 10 Mrad dosage, which possibly indicates nutrient destruction at high irradiation levels. Irradiation treatment of the entire diet (6D) resulted in no further improvement over treatment of the rye fraction alone. Again there was a trend towards reduced performance which may indicate a negative effect of irradiation on some nutrient components. At the 10 Mrad dosage a slight browning reaction was visually apparent, which may indicate a reduction in the nutritive value of dietary protein. The improvement in production parameters appeared to correspond to improvement in fat retention and bone ash, which also plateau at approximately 6 Mrad (Table 4.3).

The effect of irradiation treatment on the component

Table 4.2 Effect of irradiation treatment on consumption, three week body weight and feed conversion for chicks fed rye diets (Trial 4.1), including summary of analysis of variance (mean squares)

Radiation Level (Mrad)	Weight (g)	Consumption (g)	Feed/gain
0	237.5^{a}	3901^{a}	2.75^{a}
2	316.8^{b}	4556^{ab}	2.41^{ab}
6	416.0^{c}	5297^{c}	2.12^{b}
10	394.8^{c}	4804^{bc}	2.19^{b}
6D[1]	385.4^{c}	5056^{bc}	2.18b

Summary of Analysis of Variance (Mean Squares)

Treatment	127979* (4df)	1149600* (4df)	.266* (4df)
Error	3485 (11 df)	90490 (15 df)	.04 (15 df)

[1] For treatment 6D the entire diet was irradiated

a,b,c, - different superscripts within a column denotes significant differences

*significant differences among treatments

Table 4.3 Effect of irradiation treatment on excreta fat levels, per cent fat retention, and metatarsal bone ash for chicks fed rye diets (Trial 4.1); including summary of analysis of variance (mean squares)

Radiation level (Mrad)	Excreta fat[2] (%)	Fat retention[2] (%)	Bone ash (%)
0	13.35^{a}	53.0^{a}	48.9^{a}
2	12.67^{ab}	63.3^{b}	53.2^{b}
6	11.29^{b}	65.9^{b}	54.1^{b}
10	11.56^{ab}	59.4^{ab}	53.2^{b}
6D[1]	10.80^{b}	64.9^{b}	53.0^{b}

Summary of analysis of variance (mean squares)

Treatment	4.40^{*}(4df)	$.0111^{*}$(4df)	$.33^{*}$(4df)
Error	.85(15df)	.0012(15df)	.06(35df)

[1] for treatment 6D the entire diet was irradiated
[2] all values expressed on a dry matter basis
a,b different superscripts within a column denote significant difference
* significant differences among treatment means

sugars of the water extract indicate structural damage to the hemicellulose constituents of the rye (Table 4.4). The results indicate that levels of both arabinose and xylose increased with extraction time, irrespective of whether or not the diets had been irradiated. However irradiation also increased the amount of pentosans extracted, indicating that irradiation may solubilize otherwise insoluble hemicellulose components. The results of paper chromatography (Table 4.5) provide verification for the component sugars of the rye extract.

Addition of sodium taurocholate to a rye diet containing a high tallow level resulted in a marked improvement in fat digestion (Table 4.6). The wheat control diet was also improved but to a much lesser degree. The incorporation of high calcium into the diet also resulted in a different response between rye and wheat diets (Table 4.7). Fat retention for chicks fed the wheat diet was depressed with the high calcium level, however it had no significant effect in the case of the chicks fed rye. Furthermore, calcium in the rye diet tended to improve fat retention. The depression in fat retention in the wheat diet with a high calcium level correspond to a higher level of excreta soaps, however a similar increase in excreta soaps obviously did not have a negative effect when rye was fed.

4.4 Discussion

Table 4.4 Effect of irradiation treatment and extraction time on component sugars of rye water extract[1]

Treatment (Mrad)	Extraction Time (hrs)	Arabinose (%)	Xylose (%)	Mannose (%)	Galactose (%)
0	.5	5.27[2](.33)[3]	6.73(.42)	7.46(.46)	0.88(.06)
	12	5.80(.44)	8.36(.64)	6.38(.48)	1.21(.09)
	24	6.12(.53)	8.86(.76)	5.72(.49)	1.00(.09)
10	.5	6.18(.52)	9.08(.76)	5.71(.48)	1.01(.09)
	12	7.98(.78)	12.39(1.2)	6.48(.64)	1.17(.11)
	24	7.07(.80)	10.15(1.2)	5.12(.58)	.98(.11)

1 'rye water extract' refers to material extracted from ground whole rye when soaked in water for the specified time period

2 expressed as a per cent of water extract

3 expressed as a per cent of whole rye sample

Table 4.5 Component sugars of hydrolyzed rye water extract as indicated using paper chromatography

	C.P.[1](%)	Arabinose	Xylose	Galactose	Mannose
Rye	11.24	+++[2]	+++	+	+
Rye Extract[3]	21.41	+++	+++	++	+++
Extracted Rye[3]	9.45	+++	+++	+	tr.

1 crude protein (N X 6.25)
2 indicate approximate quantities as estimated visually
3 for explanation of rye water extract see Table 4.5. Extracted rye refers to whole rye with the water soluble component removed

Table 4.6 Effect on fat digestion of added sodium taurocholate in rye or wheat diets in chicks (Trial 4.2), including summary of analysis of variance (mean squares)

Treatment	Digestible fat (%)
Wheat	76.2^{b}
Rye	40.2^{a}
Wheat plus taurocholate	89.0^{c}
Rye plus taurocholate	77.1^{b}

Summary of Analysis of Variance (Mean Squares)

Treatment (3 df)	4338.2^{*}
Error (35df)	23.1

a,b,c, means within a column with different superscript are
* significantly different
significant differences among treatment means

Table 4.7 Effect of added calcium carbonate on fat digestion and excreta soaps in rye and wheat diets fed to chicks (Trial 4.3), including summmary of analysis of variance (mean squares)

Treatment	Digestible fat (%)	Excreta Soaps (%)
Rye	57.5^{a}	$-.8^{a}$
Wheat	88.1^{c}	$-.3^{a}$
Rye & $CaCO_3$	63.3^{a}	9.8^{b}
Wheat & $CaCO_3$	75.2^{b}	10.6^{b}
Summary of Analysis of Variance (Mean Squares)		
Treatment (3df)	909.8^{*}	193.3^{*}
Error (16df)	32.4	3.0

a,b,c, Values within a column bearing different
* superscripts are significantly different
significant differences among means

The results indicate that fat retention in tallow-containing rye diets may be modified by gamma irradiation, sodium taurocholate supplementation or the incorporation of high calcium levels.

Gamma irradiation caused a pronounced improvement in the nutritive quality of rye. The improved performance in terms of growth and feed efficiency coincided with improved fat retention as well as metatarsal bone ash. The response to irradiation in excess of 2 Mrad, where virtual sterility of the diet is achieved is indicative of structural changes in the rye that are beneficial to the chick. No additional benefit was achieved when the entire diet as opposed to just the rye portion was irradiated, indicating that in this particular diet it was the rye fraction alone that responded to treatment. MacAuliffe et al. (1979) attributed the positive response to gamma irradiation to inactivation of an unidentified vitamin D_3 antagonist, which exerted its effect by specifically binding D_3 thereby rendering it unavailable. Patel et al. (1980) reported that gamma irradiation effectively alleviated the sticky excreta condition induced when citrus pectin was incorporated into chick diets, whereas in rye diets the excreta condition was not altered. They proposed that the growth depressing factor in rye was therefore different from pectin. The results of the present study indicated that irradiation had a negative effect on excreta condition, however the composition of the water

extractable fraction of rye suggests an effect of gamma irradiation on the solubility of otherwise insoluble hemicellulose components. This would tend to support the contention that radiation treatment does affect the carbohydrate fraction; and may well cause its effect by rupturing intrachain bonds. It is likely that such damage need not be particularly severe to considerably reduce viscosity and thereby improve the overall performance of poultry fed rye.

The response to irradiation or alternatively, enzymatic treatment (Patel et al.,1980) supports the hypothesis that it is the viscosity characteristic of rye that is responsible for its anti-nutritional effects. In an earlier study (Wagner and Thomas, 1978), it was suggested that the 'pectin' in rye supported excessive bacterial growth by providing a substrate for proliferation. Since irradiation treatment would not effectively alter the suitability of the gums as a substrate, an alternate mode of action seems more plausible.

The pronounced response of the rye-containing diet to supplementary bile salts provides further support for the contention that chicks fed rye suffer a steatorrhea malabsorption syndrome similar to animals in which intestinal stasis has permitted bacterial overgrowth (Kim et al., 1966). Both respond to antibiotic supplementation, as well as to the feeding of conjugated bile salts. While such a hypothesis is appealing in that it provides a unifying concept by which the

various anti-nutritional effects of rye may be explained, it should be noted that it is not the only plausible explanation of the mechanism by which supplementary bile salts exert their effect. It is also conceivable that additional bile salts reduce micellar size, thereby permitting micellar diffusion to occur more readily. Diffusion of particles in a gel is thought not to be seriously impeded until the size of the particle approaches the 'mesh-size' of the gel, after which it declines rapidly (Brown, 1979). Thus if smaller micelles could be achieved by supplementary bile salts, it is possible that restraints on diffusion would be overcome.

An interesting relationship in the ratio of glycine conjugates to taurine conjugates has been reported with increased deconjugation in the small intestine in humans. (Sickinger, 1975). The ratio of glycine conjugates to taurine conjugates, which is normally 2-3 rises to 15-20. This is attributed to two factors; taurine is not absorbed as well as glycine and at least in humans, the taurine component is not synthesized, but rather derived from food. The taurine pool can be depleted rapidly and is functionally replaced by glycine. Tabaqchali et al. (1968) reported that taurine conjugates were selectively deconjugated, as compared to glycine conjugates. They interpret this finding to a greater capacity of the intestinal microorganisms to hydrolyze the taurine peptide bond. It is interesting to speculate that chickens, which rely on taurine conjugates

exclusively are perhaps quite sensitive to bacterial deconjugation of bile salts.

Addition of excessive calcium as limestone resulted in a considerable increase in the level of excreta soaps in both rye and wheat diets. This resulted in a reduction in fat retention for chicks fed the wheat diet however, it had no effect in the case of chicks fed rye. There was in fact an apparent improvement in fat retention, suggesting excess calcium may actually improve fat retention in the case of chicks fed rye. These results corroborate those of Antoniou et al. (1980), who observed that increasing calcium from 0.6 to 1.1 per cent of the diet improved tallow retention in rye diets, and depressed tallow retention in a similar wheat diet. Increasing the calcium level consistantly depressed retention of soybean oil, regardless of whether the cereal component was rye or wheat (Antoniou et al.,(1980)

The response of calcium added to tallow-containing rye diets is difficult to explain in terms of conventional thought on the influence of calcium on fat digestion. Mattson et al. (1979) studied the absorbability by rats of various triglycerides of stearic and oleic acids and the effect of the divalent cations calcium and magnesium. They observed that if stearic acid was esterified at the two-position, the resulting monosterin was well absorbed, whereas if it was esterified at the one or three positions it was released as free stearic acid, and formed poorly absorbed

soaps in the presence of calcium or magnesium. This explanation cannot readily account for the tendency for a slight positive response to high calcium in the case of the rye diets, which indicates that the calcium is probably exerting its effect at another level. Two possiblities are that the viscosity increasing characteristic of the rye gums are somehow altered in the presence of calcium (perhaps a salting out effect) or that the calcium is acting to stabilize the micelle perhaps through forming chelates between bile salt hydrophilic groups (carboxyl or sulphate) protruding from the surface of the micelle.

5.0 THE EFFECT OF RYE ON PLASMA CHOLESTEROL LEVELS, CHOLESTEROL TURNOVER AND FECAL FAT EXCRETION IN CHICKENS

5.1 Introduction

While induction of steatorrhea is a definite limitation in the possibility of rye becoming a useful feed ingredient for broilers, there are situations in the production of poultry where it is conceivable that fat malabsorption could be therapeutic and improve production, or at least be of minor consequence.

Patel et al. (1980) observed that diets containing rye, wheat or pectin caused a decrease in liver fat as compared to a corn diet. This was not simply due to reduced energy content of the diet, as substitution of 2% pectin for corn did not change the protein or energy value appreciably, yet liver fat was markedly reduced. These results were substantiated by chick experiments where it was observed that pectin and guar gum depressed liver fat and serum cholesterol, which Patel et al. (1980) suggested was due to pectin and pectin-like polysaccharides interfering with absorption of one or more nutrients from the gut. This explanation is consistent with the results of the current study. Patel et al. (1981) infer that hens fed rye may be less susceptible to fatty liver syndrome due to their reduced accumulation of liver fat. However, it seems doubtful that

inclusion of rye will prove to be a practical means of alleviating this condition since most of the evidence points to reduced nutrient retention that, while it may alleviate fatty liver, would certainly impinge on production capacity.

The results of Patel et al. (1981) may indicate that rye has some potential to affect fat metabolism, in particular to lower fat and cholesterol accumulation. A related area where rye has shown some promise is in broiler breeder pullet production, where it is essential to restrict body weight gain during the growing period. Inclusion of 40% Puma rye combined with a restricted feeding regime resulted in reduced abdominal fat and reduced body weight during the growing period, and a corresponding improved egg production during the subsequent laying period (McKay, 1980). The improvement in broiler breeder pullets fed rye observed by McKay (1980) would appear to be due to a reduction in fat accumulation and overall body weight in the rye fed birds.

Two experiments were designed to further study the effect of rye on fat and cholesterol metabolism. Plasma cholesterol levels were determined for chicks fed rye or wheat in a conventional and germ-free environment. Because of effect of rye on microbial activity, there is the possibility that its effect on cholesterol may be mediated at least partially by the microflora, particularly in view of the observed response with regard to fat digestibility. In addition, a trial was undertaken to study the effect of rye on cholesterol

turnover by following the rate of decline of plasma specific activity of injected ^{14}C - cholesterol. This was determined in adult roosters, since results with chicks would be confounded by differences in growth rate. For this trial no supplemental tallow was added to the diet, hence any changes in plasma cholesterol levels or turnover rate would reflect endogenous loss. Of particular interest was the possibility that roosters fed rye in a low cholesterol diet would exhibit an increased cholesterol turnover rate. Since a major means of cholesterol loss is via bile acids, an increase in turnover rate may imply increased bile acid loss, providing support for the postulation that bile salt insufficiency may be the primary cause of fat maldigestion in poultry fed rye.

5.2 Materials and methods

5.2.1 Trial management

Plasma cholesterol was determined for germfree and conventional chicks, from Trial 3.1, which were sampled at the termination of the trial. Blood samples were collected by heart puncture and plasma samples frozen for subsequent analysis. Details of trial management have been discussed previously (3.2).

For determination of plasma cholesterol levels turnover rate, and endogenous fat loss in adult roosters, ten SCWL

roosters of comparable body weight were housed individually in suspended wire cages. Five were assigned to wheat or rye treatments respectively. Each rooster was offered 100 g rye or wheat diet each morning, which was normally consumed entirely before the next feeding period. Water was supplied ad libitum. Blood samples were collected on a weekly basis via the wing vein prior to feeding. Plasma was collected and frozen for subsequent analysis. A four week period was allowed for equilibration of the ^{14}C - cholesterol and total body pool, afterwhich t1/2 was determined for the subsequent 13 week period. Excreta samples for fat analysis were collected weekly by suspending collection pans underneath the cages housing the roosters.

5.2.2 The diets

The diets fed to chicks from which plasma cholesterol levels were determined are given in Table 3.2. The diets fed to roosters are given in Table 6.3, and correspond to diets 1 and 3 without antibiotic supplementation.

5.2.3 Administration of ^{14}C - cholesterol and chemical analyses

Labelled cholesterol (50 uCi ^{14}C - cholesterol labelled in the four position, Amersham Corp.) was evaporated to

dryness under nitrogen, redissolved in absolute ethanol (1.0 ml) and diluted to 120 ml with sterile physiological saline (.75 % NaCl). Ten ml (4.2 uCi) were administered to each rooster via the wing vein. The lipid fraction of the thawed plasma samples was extracted (Folch et al., 1957), evaporated to dryness under nitrogen, and dissolved in the counting solution. The counting cocktail consisted of scintillation grade toluene and a commercial fluor mixture (PPO-dimethyl POPOP, Fisher Scientific Ltd.). Radioactivity was determined using a Beckman liquid scintillation counter (Beckmar Instruments, Inc.). Quench correction was by the channel-ratio procedure. Calculation of specific activity was based on total plasma cholesterol as determined by the procedure described by Mann (1961). Excreta samples were lyophilized, ground and analyzed for total fat according to the procedure of Marchello et al. (1971).

5.2.4 Calculation of cholesterol t1/2 and statistical analyses

Plasma cholesterol turnover rate (t1/2) was calculated for each rooster from a regression equation based on the logarithmic transformation of the weekly specific activity data, thus providing an unbiased estimate of the rate of decline of specific activity for individual birds. All statistical analyses were computed using SPSS regression and

oneway analysis of variance procedures (Nie et al., 1975)

5.3 Results

The results indicated (Table 5.1) no significant difference between plasma cholesterol levels of chicks fed rye or wheat in germ-free or conventional environments. The conventional chicks fed rye were significantly lower when one aberrant data point was excluded from the analysis. There were clearly no differences between rye and wheat in a germ-free environment, although there was an indication that the chicks fed wheat in a conventional environment had a somewhat lower level of plasma cholesterol than their counterparts in a germ-free environment.

Plasma cholesterol levels in roosters fed rye were lower ($P<.10$) than those of roosters fed wheat (Table 5.2). The half-life of plasma cholesterol was surprisingly long in both groups of roosters. There was a strong indication ($P<.10$) that cholesterol turnover was greater for the roosters fed rye. The levels of excreta fat, although considerably less than that observed for chicks fed rye or wheat, clearly indicate higher levels of excreta fat for the roosters fed the rye diet. Proportionally the increase in excreta fat was approximately the same as the increased plasma cholesterol turnover rate for roosters fed the rye as compared to the wheat diet. Feeding rye elicited

Table 5.1 Plasma cholesterol levels of chicks fed wheat or rye diets in conventional versus germ-free environments (Trial 5.1), including summary of analysis of variance (mean squares)

	Plasma Cholesterol (mg/dl)	
Conventional	$\bar{X}$	S.E.
wheat	195.8	8.7
rye	174.0[1]	11.3
Germ-free		
wheat	202.0	10.8
rye	205.0	6.2

Summary of analysis of variance (mean squares)	
Treatment (3df)	1264.4
Error (22df)	629.4

[1] The rye conventional birds had one aberrant value (223). When this value was eliminated the rye conventional chicks (X = 165.8) were significantly lower than the other treatments

Table 5.2 The effect of rye or wheat on plasma cholesterol, excreta fat and cholesterol turnover in adult roosters (Trial 5.2), including summmary of analysis of variance (mean squares)

	Plasma cholesterol (mg/dl)	excreta fat (%)	cholesterol t 1/2 (days)
Rye	99.5 ± 3.3	2.20 ± .25	23.3 ± 1.2
Wheat	106.4 ± 2.3	1.71 ± .04	29.0 ± 2.8
	Summary of analysis of variance (mean square)		
Treatment	2001.0 (1 df)[1]	4.0 (1 df)[2]	82.9 (1df)[1]
Error	678 (166 df)	.05 (65 df)	23.4 (8 df)

[1] significant at P<.10
[2] significant at P<.05

approximately a 30 per cent increase in excreta fat, and a corresponding 30 per cent increase in cholesterol turnover rate.

5.4 Discussion

Conventional chicks fed a rye diet had reduced cholesterol levels compared to germ-free chicks fed rye. Whether this was due to improved cholesterol uptake, or alternatively, to increased endogenous loss can not be ascertained directly from the chick data. Considering that fat retention is severely depressed in conventional chicks fed rye, however, it seems highly likely that absorption of cholesterol would be similarly affected. This contention is supported by a previous report (Zaviego *et al.*, 1976) where it was observed that absorption of vitamin D_3 was reduced by inclusion of rye in the diet. Cholesterol, which is also a steroid, would be similarly affected. The fact that plasma cholesterol levels are lowered also suggests, indirectly, that the factor responsible for the rachitic response of rye diets is not a specific anti-vitamin D_3 factor, but exerts its effect in a more general way, such as reduced fat retention.

Whether or not the lowered plasma cholesterol observed in chicks fed rye in the present study was specifically due to environment (germ-free or conventional) can not be determined

due to the confounding effect of the irradiation treatment (the chicks were fed non-irradiated diets in the conventional environment, and irradiated diets in the germ-free environment). Mokady (1973) demonstrated that in rats blood cholesterol was lowered by feeding pectin, and that the magnitude of the response was related to the molecular weight of the pectin. Similarly Patel et al. (1981) reported that rye reduced serum cholesterol in chicks, however 'pectinase' supplementation of the diet eliminated the cholesterol-lowering effect. Since gamma irradiation depolymerizes the pentosan complex (Campbell et al.,1982) it is likely that plasma cholesterol may be increased in irradiated versus non-irradiated rye diets.

Whether or not the microflora exerts an effect on plasma cholesterol is of interest in view of the proposed involvement of the microflora in the manifestation of the adverse effects of feeding rye to chicks. Patel et al. (1981) added penicillin to diets containing two per cent guar gum, and found no effect on serum cholesterol in chicks. There was however an indication of increased liver fat from which it may be inferred that fat retention was positively affected. The lack of response to penicillin in terms of serum cholesterol is surprising in view of the effects on fat retention, however Patel et al. (1981) only sampled three chicks per treatment which may be inadequate to demonstrate a response. There has been considerable variation in the levels

of serum cholesterol reported in the literature. Some examples of the ranges encountered are: 230 - 330 mg/dl (chick, Eyssen et al., 1969), 104 - 149 mg/dl (chicks, Patel et al., 1981) and 147 - 190 mg/dl (quail, Sutton et al., 1981). There appears to be some correlation between dietary cholesterol levels and serum cholesterol levels observed in the various trials.

Roosters fed either rye or wheat diets in the present study had substantially lower levels of plasma cholesterol than did the chicks. This may possibly be attributed to incorporation of tallow into the chick diets, but not in the rooster diets. Cholesterol turnover rate for the roosters thus represents endogenous loss as opposed to any differences that may occur with regard to cholesterol uptake from the diet. The trend toward increased plasma cholesterol turnover rate supports the contention that cholesterol loss may be increased for roosters fed rye as opposed to wheat diets. Whether the apparent increase in turnover rate was attributable to increased endogenous cholesterol loss or increased bile acid loss was not determined. Since the plasma cholesterol half-life was so long (20 - 30 days) excreta levels and hence activity, would be very low, and differences due to diet would be difficult to demonstrate.

Excreta fat was also increased for roosters fed rye as compared to wheat diets, however, overall the level of excreta fat was considerably less than that observed

for chicks (sections 3 and 4). Since roosters were fed a readily available fat source at a low level (2 per cent soybean oil), the excreta fat may be considered to represent endogenous fat loss, as opposed to unutilized dietary fat. The low excreta fat levels observed for both wheat and rye diets fed to roosters contrast sharply with the much higher levels (up to 15%) observed in chick diets, and indicate that fat digestion in tallow containing rye diets fed to chicks is severely impaired. The high fat content of excreta does not merely represent increased endogenous loss in the case of the chicks. A second point of interest is the close similarity between the change on excreta fat levels in the rye as compared to the wheat treatments, and the change occurring between cholesterol turnover rate of roosters fed rye or wheat diets. Both are approximately thirty per cent. If it is assumed that endogenous steroid loss constitutes a constant fraction of the excreta fat, then the increased level of excreta fat would more or less account for the observed change in turnover rate. This interpretation would have an important repercussion with regards to the poor fat utilization in chicks. If the poor fat utilization in chicks was initiated by bile salt deconjugation, increased excreta fat loss, and the concommittant loss of bile acids could conceivably aggravate the process by depleting the system of bile acids. Overall, the low levels of excreta fat, and implication of satisfactory utilization of dietary fat for

the roosters tends to indicate that in terms of practical poultry production the area where rye is most likely to find acceptance as a useful feed ingredient is in low energy, non-tallow containing diets. This would mean feeding rye to laying hens as opposed to broilers, where it is difficult to formulate a practical diet acceptable to commercial growers without comparatively high tallow levels. The report by Patel et al (1981) that inclusion of rye reduced liver fat, as well as the results of the present study indicating reduced cholesterol levels might indicate a response that may have some commercial value for raising heavy breeder hens, where obesity is problematic (McKay, 1980). Presently there is also considerable interest in the use of oat bran as a cholesterol lowering agent in humans, where soluble B glucans are believed to be responsible. The possibility that the effects rye has on fat metabolism may be utilized to some advantage certainly warrants further investigation, both in human and heavy breeder pullet nutrition.

6.0 THE EFFECT OF RYE OR RYE WATER EXTRACT ON EXCRETA MASS, WATER CONTENT AND ELECTROLYTE LOSS IN ADULT FOWL

6.1 Introduction

The most obvious physical change that occurs when rye is fed to poultry is the change in litter condition which tends to become wetter and stickier with inclusion of rye in the diet. While this would pose no particular management problem with caged laying hens, it was felt that the excessive water loss may be associated with ion loss, resulting in possible electrolyte shortages or acid base balance disturbances. Probably the phenomenon most closely resembling the condition observed in laying hens is the increased fecal 'bulk' observed when a variety of fibres are fed. The increase in fecal bile acids, nitrogen, fat, minerals, electrolytes and water which have been observed in humans are generally ascribed to physical effects of the fiber itself (Cummings et al., 1976, Southgate and Durnin, 1970; Kay and Truswell, 1977; Kritchevsky and Story 1974, Eastman et al., 1976). Thus the major effect of the viscosity increasing gum such as the pentosan complex in rye, or guar or pectin gums, would be to attract and 'hold' water and/or electrolytes thereby increasing the excretion of both (for review see Lee, 1981).

A second mechanism involving the gut microflora has also

been proposed. Stephan and Cummings (1980) present evidence suggesting an alternative means by which fibre may affect function in the human colon. They observed that some types of fibre were microbially degraded to a considerable extent, presumably in the colon, and that the changes in fecal composition reflected an increase in microbial mass. Addition of cabbage fibre, which is 92% degradable,resulted in an increase in stool weight (88.2 to 142.5 g/day), per cent moisture (69.4 to 74.6 %) and total nitrogen (1.5 to 2.1%). Stephan and Cummings believe that stimulation of microbial growth is a major means by which degradable fibre sources such as apple, carrot, guar gum or pectin cause observed change in fecal composition. They suggest wheat fibre and possibly cereals in general, may be an exception to this as their fibrous cell walls are resistant to microbial degradation. However a soluble pentosan complex,such as occurs in rye would be expected to behave as guar or pectin and provide a readily available microbial substrate. Evidence supporting this view (again in humans) is provided by Holloway et al. (1980), who observed that the majority of hemicelluloses, of which arabinose and xylose constituted the major component sugars, were degraded by the microflora. Again, the exception to this was the hemicellulose arabinoxylan of wheat bran that was relatively indigestable, presumably due to its lignification and resulting insolubility and resistance to microbial attack.

Stephen and Cummings (1980) attribute the increased water content of the stool of humans fed cabbage fibre to the increased bacterial mass which stimulated faster transit and led to less water being absorbed by the colonic mucosa. Such proposed mechanisms of bacteria-fibre interaction in changes in excreta characteristics may also be applicable to some degree to the changes observed in poultry excreta. The contribution of microbial cells to excreta composition has been determined to be 12% for excreta dry matter and 25% for amino acids (Parsons et al.,1981), which although less than that reported for humans still constitutes a significant fraction of the total excreta.

The normal microflora of the large intestine may also cause diarrhea when stimulated to a sufficient degree. This is evident in human studies in which patients suffer steatorrhea or 'malabsorption' syndrome, as digestibility studies with rye suggest occurs in chicks. In view of the similarity in excretory products, particularly fat, mechanisms involved in the manifestation of these diarrheas may be relevant to the conditions observed when rye is fed to poultry.

Malabsorption, if sufficiently severe, may have a profound effect on large intestinal water balance. Water moves passively in response to the net flux of electrolytes and dietary constituents. Thus an increase in semi-digested dietary components reaching the large intestine may, by

contributing to microbial substrate either indirectly reduce water absorption or, in extreme cases, cause secretion into the large intestine. Microbial degradation in the large gut results in the production of volatile fatty acids (VFA); primarily acetic, propionic and butyric acids, which contribute to increased osmotic pressure. The splitting of one molecule of glucose to two or three smaller molecules will favor the movement of water into the large gut, if the production of these acids excedes their capacity for absorption. The breakdown of larger molecules such as the pentosan complex would cause a substantial increase in particle numbers and digesta osmolality.

Volatile fatty acids are a major contributor to stool water osmolality in humans. Fordtran (1967) indicated that the bicarbonate concentration of stool water was only 30 meq/litre, and that there was an anion gap of 90 meq/litre which was attributed to VFA. Cummings et al. (1976) observed that total VFA excretion was increased on a high fibre diet; however VFA concentration was similar in both the control and high fibre treatments. Since VFA concentrations were unchanged, as one would predict if VFA were in part determining water content, the possibility that VFA production may be important in determining fecal bulk cannot be discounted. Volatile fatty acids have also been suggested to interact with fibre to produce a bulking effect.

Four experiments were conducted with laying hens and

adult roosters to determine whether feeding rye caused similar changes in excreta characteristics as have been reported for humans fed a variety of high fiber diets.In view of the possible contribution of VFA to osmotic pressure in the lower gut; acetic, propionic and butyric acids were determined in addition to excreta dry matter, total excreta mass relative to diet consummed, and excreta pH. Excreta loss of inorganic ions (sodium, potassium, calcium, and phosphorus) was assessed for roosters fed diets containing rye, rye water extract or water - extracted rye as compared to corn or wheat control diets. For these studies roosters were chosen as the experimental model as it was felt overall retention of electrolytes would not be confounded by level of productivity, as would be the case for laying hens or chicks. The effect of dietary antibiotic on VFA excretion for rye versus wheat diets fed to roosters was also determined.

6.2 Materials and methods

6.2.1 Trial management

For Trial 6.1 excreta samples were collected from laying hens fed diets containing 0.0, 19.5, 39.0, 58.5 or 78 per cent rye in order to determine the magnitude of the apparent laxative response induced by incorporation of rye in the diet. The hens were part of a production trial, and details

regarding trial management are given elsewhere (7.2.1). Excreta samples were collected from two cages per treatment, each of which contained eight hens.

Three trials were conducted using SCWL roosters as the experimental subject. In Trial 6.2 excreta composition was compared for a corn, a rye and a corn diet to which lyophilized water extract had been added. A second trial (6.3) was conducted in which wheat rather than corn substituted the reference diet, and a diet containing water - extracted rye was included. For Trial 6.4 VFA levels of excreta of roosters fed rye or wheat both with or without antibiotic supplementation were compared.

The roosters used in Trial 6.2 - 6.4 were housed in suspended wire mesh cages. In Trial 6.2 four cages each housing two roosters were assigned per dietary treatment. The roosters were offered 200 g test diet per cage daily, which was consumed entirely prior to the following day. The treatment period was four days, during which time excreta samples were taken. Trials 6.3 and 6.4 were conducted similarly to Trial 6.2, except that feed was supplied ad libitum. The roosters in Trial 6.4, however, were individually housed and five birds allotted per dietary treatment. Water was supplied free choice for all trials.

Metal trays were suspended beneath the cages to facilitate excreta collection during the test period. Excreta was collected each morning and temporarily stored in 125 ml

polyethylene bottles with snap - top lids. Droppings were taken on a continuous basis in order to avoid deterioration during the collection process. Excreta samples were stored on ice during the collection period (2-3 hrs), and several drops of concentrated mercuric chloride added as a precautionary measure against microbial degradation. The minimum amount of excreta required for subsequent analysis was 50 g.

The excreta samples were mixed thoroughly, and 25 g homogenized wet excreta taken for dry matter analysis. A second 25 g sample was diluted to 50 ml with distilled water, centrifuged and the supernatant kept frozen for future analysis.

Calculation of electrolyte loss in excreta relative to diet consumed necessitates the quantification of excreta mass relative to diet consumed. This was measured for Trial 6.1 by the addition of chromic oxide (0.4 %) to the test diets. The excretion coefficients for Trial 6.3 were determined by force - feeding one rooster from each cage 25 g test diet. Total excreta from that rooster was collected 24 hrs later, dried (24 hrs at 60 ^{o}C), and weighed.

6.2.2 The diets

The diets fed to laying hens in Trial 6.1 are those given in Table 7.1. The diets fed in Trials 6.2, 6.3 and 6.4 are given in Tables 6.1 to 6.3. In the preparation of rye water

Table 6.1 Per cent composition of experimental diets used in Trial 6.2

Ingredient	Diet 1	2	3
Corn	75.0	-	63.0
Rye	-	75.0	-
Rye extract	-	-	12.0
Soybean meal	15.0	15.0	15.0
Fish meal	3.0	3.0	3.0
Soybean oil	2.0	2.0	2.0
Alfalfa meal	1.75	1.75	1.75
Limestone	.75	.75	.75
Calcium phosphate	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0
Mineral premix[1]	.5	.5	.5

[1] rye extract refers to lyophilized water soluble fraction of whole rye grain

[2] supplied the following per kg diet: vitamin A, 8250 I.U.; vitamin D_3, 880I.U.; vitamin E, 5.5 I.U.; vitamin B_{12}, .01 mg; riboflavin, 2.2 mg; calcium pantothenate, 4.4 mg; niacin, 6.6 mg; choline chloride, 110 mg; DL methionine, .5 g; santoquin(50%), 250 mg; manganese oxide, 165 mg; zinc oxide, 55 mg, iodized salt, 4.8 g.

Table 6.2 Per cent composition of experimental diets used in Trial 6.3

Ingredient	Diet 1	2	3	4
Wheat	80.0	30.0	70.0	30.0
Rye	-	50.0	-	-
Rye water extract	-	-	10.0	-
Extracted rye	-	-	-	50.0
Soybean Meal	11.0	11.0	11.0	11.0
Fish	2.0	2.0	2.0	2.0
Soybean Oil	2.0	2.0	2.0	2.0
Alfalfa Meal	1.75	1.75	1.75	1.75
Limestone	.75	.75	.75	.75
Calcium phosphate	1.0	1.0	1.0	1.0
Vitamin premix[2]	1.0	1.0	1.0	1.0
Mineral premix[2]	.5	.5	.5	.5

[1] rye water extract refers to lyophilized water-soluble fraction of whole rye grain. Extracted rye refers to rye with water extractable fraction removed, again lyophilized

[2] see Table 6.1

Table 6.3 Per cent composition of experimental diets used in Trial 6.4

Ingredient	Diet 1[2]	2[2]	3[2]
Wheat	80.0	40.0	-
Rye	-	40.0	80.0
Soybean meal	11.0	11.0	11.0
Fish	2.0	2.0	2.0
Oil	2.0	2.0	2.0
Alfalfa meal	1.75	1.75	1.75
Limestone	.75	.75	.75
Calcium phosphate	1.0	1.0	1.0
Vitamin premix[2]	1.0	1.0	1.0
Mineral premix[2]	.5	.5	.5

[1] see Table 6.1
[2] Antibiotic supplemented diets contain 400g Pro-Strep '20' per 100 lb diet (procaine penicillin, streptomycin, Merck, Sharp and Dohme)

extract and extracted rye used in Trials 6.2 and 6.3 whole rye grain was finely ground and mixed in the proportion of 5 kg rye to 20 liters distilled water. This was mixed thoroughly and allowed to soak overnight (4 ^{o}C). The following morning the resulting slurry was centrifuged (250 ml tubes) at 8,000 rpm for 10 minutes, and the supernatant collected. For Trial 6.2 only, an attempt was made to partially purify the crude extract by precipitation of the protein component by heating the extract to 55 ^{o}C for 3 minutes. The heated extract was cooled on ice and recentrifuged at 8,000 rpm for 10 minutes. Since this procedure was found to remove only a fraction of the total protein it was not repeated for Trial 6.3. The water-extract, and the sediment for Trial 6.3, were lyophilized prior to incorporation into the test diets.

6.2.3 Chemical analyses

For dry matter analysis of fresh excreta samples, 25 g homogenized wet excreta (undiluted) was dried 24 hrs at 60 ^{o}C in a forced air oven,and per cent dry matter determined by weight difference. All electrolyte analyses were determined on the thawed supernatant of the previously extracted excreta samples. Volatile fatty acid analysis was determined by gas chromatography according to the procedure described by Erwin et al. (1961). Sodium, ammonia, phosphorus and calcium were

determined with the Technicon Autoanalyzer II (Model 7-70-140A). The chloride content of the excreta samples was measured with a chloridometer (Model IL 279, Instrumentation Laboratory, Inc.). Chromic oxide was analyzed according to AOAC (1970) by atomic absorption procedure (Perkin Elmer, model 303).

6.2.4 Calculations of electrolyte concentration, electrolyte loss relative to diet consumed, and statistical analyses

Since electrolyte concentrations were determined for the supernatant fraction, a dilution factor was necessary to express results in terms of electrolyte concentration originally present in the undiluted sample. This factor represents (water present in the diluted excreta / water present in fresh excreta). Multiplication of the electrolyte concentration of the diluted excreta supernatant by the dilution factor gives the concentration of the electrolyte present in the water fraction of the undiluted excreta sample. The dilution factor was calculated as follows:

$$\text{Dilution factor} = (50 - (25\ DM\ (.9))/(25\ WM)$$

where 50 = total volume of diluted sample (ml)

25 = weight of wet excreta in diluted sample (g)

DM = dry matter content expressed as a fraction of the undiluted excreta sample

0.9 = volume (ml) occupied by 1 g excreta dry matter. (This was determined for a typical excreta sample and assumed a constant. The correction is minor, and variation among samples would not introduce sgnificant error.)

WM = water content expressed as a fraction of the undiluted excreta sample

For Trials 6.1 and 6.3, the wet excreta weight per 100 g diet consumed was calculated as follows :

wet excreta weight per 100 g diet consumed = MDM/DM

where MDM = metabolizable dry matter, which equals (1 - excreta voided per 100 g diet consumed)

DM = dry matter expressed as fraction of the undiluted excreta sample

The excreta water/100g diet consumed equals

(wet excreta weight per 100 g diet consumed) (WM)

where WM = water content expressed as a fraction of the undiluted excreta sample

Knowing the volume of fluid excreted per 100 g diet consumed and the electrolyte concentrations permits electrolyte loss per 100g diet consumed to be calculated as follows :

electrolyte loss per 100 g diet consumed =
(volume of fluid loss per 100 g diet consumed) X
(electrolyte concentration)

Statistical analyses were computed using the pen as the experimental unit. For Trials 6.2 and 6.3 excreta was collected for four separate days. These samples were analysed separately and the mean for each pen taken for statistical analysis. All statistical analyses were computed using SPSS analysis of variance procedures (Nie et al., 1975). Tukey - HSD test and t-test were applied where appropriate. The limit of probability accepted as being significant was $P<.05$ in all cases.

6.3 Results

Substitution of rye for wheat in laying hen diets (Trial 6.1) resulted in increased excreta dry matter loss per 100 g diet consumed, increased excreta water content and increased total excreta mass (Table 6.4). The increase in the dry matter excreted represents a reduced digestibility of the rye diets that when combined with the increased water content contributes to the marked increase in total wet excreta. The increased total wet excreta expressed per 100g diet intake, indicates that hens fed rye may have some difficulty in maintaining electrolyte balance. Since water in the lower intestinal tract tends to follow electrolyte absorption, increased water loss may indicate a similar increase in electrolyte loss. The rise in water content of excreta in rye fed birds coincides with a rise in VFA levels and a corresponding reduction in excreta pH (Table 6.5).

Rye substituted for corn in diets fed to roosters (Trial 6.2) resulted in a significant increase in water content of the excreta (Tables 6.6), for which the birds appeared to compensate by increasing the relative absorption of ionic components (Table 6.7). This was apparent for Na^+, K^+ and Cl^- concentrations, all of which were significantly reduced for the rye treatments. Although not significant, a similar reduction was apparent for NH_4^+ and PO_4^{--} concentrations. The reduction in concentration of the major electrolytes would tend to suggest that the rye fed roosters are attempting to increase retention to overcome the consequence

Table 6.4 Excreta characteristics of laying hens fed rye (Trial 6.1), including summary of analysis of variance (mean squares)

Rye level (%)	DM excreted g/100 g diet consumed	Excreta Water (%)	Total Excreta g/100g diet consumed	Total Water Loss g/100g diet consumed
0	26.5[a]	80.7[a]	137.3[1]	110.8[1]
19.5	27.5[ab]	84.1[b]	173.0	145.5
39	29.3[abc]	85.4[b]	200.7	171.4
58.5	31.0[bc]	83.2[ab]	184.5	153.5
78	32.3[c]	84.0[b]	201.9	169.6

Summary of analysis of variance (mean squares)

Rye level (4 df)	20.8*	6.07*	--	--
Error	3.9 (14 df)	.60 (5 df)		

[1] calculated from excreta dry matter per 100 g diet consumed and excreta water content

a,b,c means within a column bearing same superscript are not significantly different

* denotes significant treatment effect

Table 6.5 Excreta volatile fatty acids and pH of laying hens fed rye (Trial 6.1), including summary of analysis of variance (mean squares)

Volatile Fatty Acid (meq/l excreta water)

Rye Level	Acetic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Total VFA	Excreta pH
0	12.3^{a}	4.2^{a}	1.3^{a}	1.4^{a}	19.2^{a}	6.70^{a}
19.5	17.8^{a}	5.7^{a}	2.0^{a}	1.3^{a}	26.7^{a}	6.78^{a}
39	43.8^{b}	17.8^{a}	$.6^{a}$	1.1^{a}	65.3^{b}	6.47^{ab}
58.5	40.9^{b}	21.1^{b}	$.7^{a}$	11.6^{b}	74.3^{b}	6.43^{ab}
78	47.6^{b}	18.5^{b}	6.1^{b}	14.2^{b}	86.4^{b}	6.14^{b}

Summary of analysis of variance (mean squares)

Rye level (4 df)	525.3^{*}	134.9^{*}	10.3^{*}	1770.4^{*}	1770.4^{*}	$.38^{*}$
Error	30.3 (5df)	4.3 (5df)	1.5 (5df)	88.3 (5df)	88.3 (5df)	.09

^{a,b} denotes significant differences among means within a column

* denotes significant treatment effect

Table 6.6 Excreta water content and pH of roosters fed corn, rye, or rye water extract (Trial 6.2), including summary of analysis of variance (mean squares)

Diet	Excreta Water Content (%)	Excreta pH
Corn	82.2[a]	6.46
Corn & rye extract	83.0[a]	6.40
Rye	85.0[b]	6.55
Summary of Analysis of Variance (Mean Squares)		
Treatment (3df)	8.36*	.0226
Error (12df)	.76	.0059

[a,b] different superscripts within column denotes significant differences

* denotes significant treatment effect

Table 6.7 Excretion of predominant ions by roosters fed corn, rye, or rye water extract (Trial 6.2), including summary of analysis of variance (mean squares)

Diet	Total VFA	Excreta Ions (meq/liter) K^+	Na^+	Ca^{++}	NH_4^+	Cl^-	HCO_3^-	PO_4^{--}
Corn	7.2	90.2^b	104.0^a	11.4^b	70.1	125.7^a	142.8	23.3^{ab}
Corn & rye extract	20.3	104.8^a	79.6^b	17.6^a	60.1	92.2^b	150.5	27.4^a
Rye	19.0	80.9^c	78.4^b	13.2^{ab}	63.4	65.1^c	151.9	18.9^b
Summary of Analysis of Variance (Mean Squares)								
Treatment (3df)	208.0*	580.1*	835.9*	40.2*	88.8	3693.4*	95.7	71.8*
Error (12df)	45.4	12.2	100.2	5.2	44.1	114.6	148.7	8.8

^{a,b} different superscripts within a column denote significant differences among means

* denotes significant treatment effect

of increased electrolyte loss on high rye diets. The question as to whether or not the attempt to compensate for increased fecal mass was sufficient is partially answered in Table 6.8. Although the coefficient relating excreta to consumption used for the calculation of excreta ions in terms of Meq/100g diet consumed are approximations (derived from Trial 6.1), the results tend to indicate the excreta loss of all ionic components is increased in the case of the rye-containing diet.

The rye extract added to the corn diet gave some indication of the rye type response, but this was not entirely consistent. Water content of the excreta was increased, but not significantly. Other ionic components, including Na^+, NH_4^+, Cl^-, Ca^{++} as well as 'unidentified' anions followed a similar pattern to that observed for the rye diet, however K^+ concentration was significantly higher in the corn plus rye extract diet than either the corn diet or the rye diet. This has been attributed to K^+ being concentrated in the rye extract (Lee, 1981).

A major difference between the laying hens (Trial 6.1) and roosters occurred with regards to excreta levels of VFA. Although, as in the laying hen trial, feeding rye (or rye water extract) resulted in a considerable increase in VFA concentration, overall contribution of VFA was much lower. There was also no reduction in excreta pH, as observed in laying hens fed rye, which corresponds to the lower level of

Table 6.8 The effect of rye or corn on the excretion of predominant ions by roosters (Trial 6.2), ncluding summary of analysis of variance (mean squares)

	Excreta ions (meq/100 g diet consumed)							
Diet	Total VFA	K^+	Na^+	Ca^{++}	NH_4^+	Cl^-	HCO_3^-	PO_4^{--}
Corn	.88[a]	11.0[a]	12.7	1.39[a]	8.54[a]	11.0[a]	17.4[a]	2.84
Rye	3.47[b]	14.8[b]	14.3	2.41[b]	11.53[b]	14.8[b]	27.7[b]	3.44
	+294[1]	+34.5	+12.5	+73.3	+35.0	+34.5	+59.2	+21.1
	Summary of analysis of variance (mean squares)							
Treatment (1df)	13.4*	28.2*	4.96	2.07*	17.79*	28.2*	211.1*	.72
Error (6df)	1.0	.39	1.16	.09	.72	.39	4.2	.14

[1] per cent increase of excreta ions induced by feeding rye

[a,b] different superscripts within a column denotes significant differences

* denotes significant differences among treatments

VFA. The poor correlation between level of VFA observed in the corn plus rye extract and the relatively low water content would also tend to suggest that VFA did not contribute significantly to osmotic pressure in this instance.

Roosters fed rye or rye extract in Trial 6.3 had a higher excreta water content and a greater excreta mass when compared to roosters fed a wheat or extracted rye diet (Table 6.9). This effect was only significant when treatments were pooled, nevertheless the overall trend is the same as Trial 6.2. Again, the roosters fed rye attempted to adjust for the increased excreta fluid loss by reducing ionic concentration through increased absorption (Table 6.10). Although not statistically significant this trend is apparent for K^+, Na^+, Ca^{++}, NH_4^+, Cl^- and PO_4^{--}. Addition of the water extract to the wheat diet gave a similar response to the rye diet, whereas extraction of the rye diet gave a similar response to the wheat diet. These results support the contention that the water extractable components of the rye may be responsible for the laxative effect of rye. As in Trial 6.2 addition of the water extractable fraction gave a somewhat different response than the addition of rye alone. In particular, both K^+ and Ca^{++} levels in the excreta were again increased over the other diets. The excreta water content of the wheat plus rye extract was in closer agreement with the rye diet than in Trial 6.2.

Table 6.9 Excreta characteristics of roosters fed wheat, rye, rye water extract or extracted rye diets (Trial 6.3), including summary of analysis of variance (mean squares)

Diet	WM excreted g/per 100g diet	Water Content (%)	PH
1. Wheat	139.8^{ab}	82.6	6.00^{ab}
2. Rye	175.5^{b}	84.8	6.10^{b}
3. Wheat & rye extract	167.6^{ab}	84.5	5.65^{a}
4. Extracted rye	125.5^{a}	81.7	5.95^{ab}
Summary of Analysis of Variance (mean squares)			
Treatment (3df)	2195.8^{*}	8.75	$.155^{*}$
Error (12df)	454.4	3.92	.036
'low gum' (1,4) versus 'high gum' (2,3) treatments			
t value (12df)[1]	3.65^{*}	2.50^{*}	1.09

^{a,b} different superscripts within a column denote means that
* are significantly different
[1] denotes different treatment effect
orthoganol contrasts (t-test) comparing pooled results of wheat and extracted rye versus rye and wheat plus rye extract

Table 6.10 The effect of rye, rye water extract and extracted rye on the excretion of predominant ions by roosters (Trial 6.3), including summary of analysis of variance (mean squares)

	Excreta Ions (meq/l excreta water)							
Diet	VFA	K^+	Na^+	Ca^{++}	NH_4^+	Cl^-	HCO_3^-	PO_4^{--}
1. Wheat	10.3	71.9	67.4	20.9	58.4	82.9	94.2	31.2
2. Rye	19.5	70.9	57.9	19.2	52.5	57.4	98.0	25.5
3. Wheat + rye extract	23.7	75.3	59.9	25.4	50.7	72.4	82.3	32.8
4.Extracted Rye	9.8	63.1	70.8	21.1	54.6	85.5	83.4	30.8
Summary of Analysis of Variance (mean squares)								
Treatment(3df)	189.2	105.5	150.3	27.2	43.8	651.6	245.5	40.6
Error(12df)	71.5	98.3	97.2	34.4	46.9	230.1	158.5	14.7
'low gum' (1,4) versus 'high gum' (2,3) treatment								
t value (12df)	2.73*	9.92*	1.13	.44	1.43	2.55*	.21	.95

[1] see Table 6.9
* denotes significant differences between treatments

The results of Trial 6.3 provide support for the hypothesis that feeding rye increases excreta fluid and excreta mass, and that the roosters attempt to compensate for this by increased electrolyte absorption. Expressed in terms of meq per 100 g diet consumed (Table 6.11), the results again indicate that the roosters fed rye or rye extract have some difficulty maintaining ionic equlibrium. When the pooled results of the wheat and extracted rye treatments are compared with the pooled results the rye and rye extract treatments, excretion of K^+, Ca^{++}, NH_4^+, HCO_3^- and PO_4^{--} are significantly increased. Sodium and Cl^- follow the same trend, however, the increase in this case was not significant. The per cent change in total excretion of electrolytes for the pooled treatments are presented in Table 6.12. These results are in close agreement with the values presented for Trial 6.2. Excreta VFA levels also concurred with those previously observed in Trial 6.2 - rye or rye extract increases VFA excreta levels. The VFA levels in Trial 6.3, as in Trial 6.2, were substantially lower than those observed in laying hens.

For Trial 6.4 only excreta VFA concentrations were considered. Roosters fed rye had significantly higher levels of excreta VFA's then roosters fed the same diet with wheat as the sole cereal source (Table 6.13), which is consistent with the increase observed in previous trials. Supplementation of the wheat or rye diets with a commercial

Table 6.11 Excretion of predominant ions by roosters fed wheat, rye, rye water extract, or extracted rye diets (Trial 6.3), including summary of analysis of variance (mean squares)

	Excreta Ions (meq/100g Diet Consumed)							
Total Diet	VFA	K^+	Na^+	Ca^{++}	$NH4^+$	Cl^-	HCO_3^-	$PO4^{--}$
1. Wheat	1.40	9.82^b	9.29	2.82	8.00	11.30^{ab}	12.96^b	4.24^{bc}
2. Rye	3.46	12.39^a	10.10	3.43	9.27	10.00^b	17.25^a	4.48^b
3. Wheat & rye extract	4.12	12.28^a	9.75	4.34	8.41	13.53^a	13.54^b	5.36^a
4. Extracted rye	1.26	7.82^c	8.85	2.64	6.73	10.69^{ab}	10.26^b	3.82^c
Summary of Analysis of Variance (mean squares)								
Treatment (3df)	8.38*	19.16*	1.19	2.36	4.47	9.36*	33.12*	1.68*
Error (12df)	2.24	.87	2.04	.92	1.45	2.43	2.97	.05
'low gum' (1,4) versus 'high gum' (2,3) treatments								
t value[1]	3.29*	7.54*	1.20	2.42*	2.45*	.99	4.39*	7.63*

a,b,c means within a column bearing same superscript are not significantly different

1 see Table 6.9

Table 6.12 Per cent increase in predominant excreta ions of roosters fed gum containing diets (Trial 6.3)

Excreta ions (meq/100g diet Consumed)	Wheat or [1] extracted rye	Rye or wheat + rye extract	Per cent change
Total VFA	1.33	3.79	+185.0
K^+	8.82	12.34	+ 39.8
Na+	9.07	9.93	+ 9.5
Ca^{++}	2.73	3.89	+ 42.3
NH_4^+	7.37	8.84	+ 19.9
Cl^-	11.00	11.77	+ 7.0
HCO_3^-	11.61	15.40	+ 32.6
PO_4^{--}	4.03	4.92	+ 22.0

[1] Excreta ions of roosters fed wheat or extracted rye were compared to those fed rye or wheat and rye extract

Table 6.13 Effect of rye or antibiotic on excreta volatile fatty acids in roosters (Trial 6.4), including analysis of variance (mean squares)

Rye Level	Volatile fatty acids (meq/liter) - Antibiotic	+ Antibiotic
0 Rye	11.5	6.9
40 Rye	28.2	10.6
80 Rye	22.0	14.8

Summary of Analysis of Variance (Mean Square)	
Treatment (5df)	317.7*
Error (24df)	50.1

Orthoganol Contrasts	
Effect of rye (24df) (0 rye versus 40 and 80% rye)	t= 3.53*
Effect of antibiotic (24df) (- versus + antibiotic)	t= 3.80*

* denotes significant treatment effect

antibiotic supplement ('Pro-strep) containing procaine penicillin and streptomycin resulted in significant reduction in the level of excreta VFA indicating that the higher VFA levels are a consequence of greater microbial activity in the case of roosters fed rye diets. Thus the greater increase for VFA relative to other ions observed in Trial 6.2 and 6.3 for rye as compared to wheat diets, would appear to be due to increased formation rate. The level of excreta VFA in Trial 6.4, as in Trial 6.2 and 6.3, was considerably lower than observed in laying hens (Trial 6.1).

6.4 Discussion

Across trials the results indicate that adult fowl may have some difficulty maintaining ionic equilibrium when rye is substituted for other cereal grains in the diet. This may be attributed in part to an increase in dry matter excreted when rye constituted the cereal base of the diet, that combined with the increased water content contributes to a marked increase in total excreta. The increase in excreta water, although moderate is sufficient to result in a change in excreta from solid to semi-solid. This is in agreement with Maxwell and Lyle (1957) who observed that only 5 per cent more water in the droppings was required to make them appear wet.

The excreta of birds is a variable mixture of fecal

material and urine, and changes in electrolyte composition of excreta of intact roosters will reflect the summation of intestinal events and any renal compensation that may occur. The increase in excretion of ionic components due to rye or rye water extract will be the result of changes in total body balance. The roosters demonstrated a limited capacity to cope with increases in excreta mass, caused by a reduced digestibility of dry matter and an increased water content. Although there was evidence of an attempt to reduce electrolyte concentration this was apparently insufficient to prevent increased total loss, at least over the short term. Increased ionic losses were demonstrated in rye or rye water extract containing diets when compared to wheat or corn diets.

Clear evidence of this phenomenon has been provided by Lee (1981) who demonstrated, using colostomized roosters, increased fecal ionic loss for which the roosters attempted to compensate by reducing urinary excretion. This was evident for sodium, potassium, and chloride. Lee demonstrated an increased requirement for salt when rye was the major cereal component. This effect was most evident for colostomized roosters, probably because birds with a colostomy or exteriorized ureters have a higher than normal salt requirement (Hart and Essex, 1942). However, chicks fed rye also had a high salt requirement, .60 per cent as opposed to .25-.35 per cent generally recommended (Lee,1981). The

present results showed indications of this effect also. Both sodium and chloride loss was increased in diets containing rye or rye water extract, although this response was not significant. It should be noted that the diets contained a comparatively high salt level, (.48%) which probably approached the requirement of intact roosters fed rye. Under such circumstances it may be difficult to demonstrate increased salt loss for the rye diets, as fecal loss would be compensated by urinary retention. However, the results are generally supportive of those obtained by Lee. Total ionic loss is consistantly increased in rye diets or diets containing rye extract, whereas the extracted rye diet elicited a similar response to the wheat diet. Bishawi et al. (1981) observed a similar phenomenon when guar gum (3 %) was added to chick diets. Analysis of the excreta revealed the same levels of nitrogen, calcium, potassium, sodium, and zinc as the control chicks. The level of lipids however increased from 16 to 24 %. Since guar gum would presumably reduce MDM, the overall ionic retention would be correspondingly affected. As excreta fat concentration was substantially increased, fat retention would be affected most seriously.

There is some doubt whether such effects are explicitly due to a 'toxic' effect of the rye, or are simply due to dietary dilution by a fiber source. (Lee, 1981) In this latter case the nature of 'fiber' occurring in the rye or rye water extract is such that water and electrolytes are

preferentially 'held' which contributes to excessive fecal loss. It is worthy of mention that early poultry rations contained much higher levels of salt than are currently recommended, and that the reduction in salt levels has coincided with the development of high density, low fiber diets. Halpin et al. (1936) included one per cent salt in rye-containing diets. More recently, experimental diets containing rye are often based on more modern nutrient recommendations and include only .25 per cent salt, which undoubtedly contributes to the negative effect observed when rye is fed to chicks (Lee 1981).

The results of the present study indicate that potassium was affected moreso than sodium or chloride. Whether this was due to increased urinary or fecal losses cannot be answered from these studies, however, Lee (1981) reported that colostomized roosters attempted to overcome excessive fecal sodium loss by increasing $Na^+ - K^+$ renal exchange. Ultimately this leads to increased urinary potassium loss.

The volatile fatty acid levels increase with inclusion of rye in the diet, however, at least in the case of roosters, are not sufficiently high to contribute to osmotic pressure. Laying hens fed rye, on the other hand, displayed quite high levels of excreta VFA. Although the total excreta VFA levels of laying hens were somewhat lower than those reported by Fordtran (1967) for human stool water, they were still sufficiently high for the VFA fraction to contribute

significantly to excreta osmolality. It seems unlikely that this difference between laying hens and roosters could be attributed to variations in microbial activity, however, it is conceivable that absorption procedes more efficiently in roosters than in laying hens. Argenzio et al., (1977), reported that the mechanics of VFA absorption involved bicarbonate exchange. Laying hens with a high demand for bicarbonate for egg shell formation may be forced to excrete more VFA as a bicarbonate conservation mechanism. In support of this argument it was noted that laying hens in Trial 7.2 had somewhat thinner shells when fed rye as opposed to wheat diets, although this was not significant. Reduced availability of bicarbonate for shell formation could have contributed to this effect, although alterations in calcium and vitamin D_3 utilization cannot be overlooked as possible contributing factors. Across trials excreta VFA levels do not correlate with excreta water, which combined with their low levels, indicates that reduced nutrient absorption does not induce an osmotic diarrhea in rye - fed birds.

The observation that feeding rye reduces the retention of most nutrients raises the question whether the response is in fact a specific effect of rye, or whether it is a general response arising from a somewhat higher fibre level for rye diets. It appears logical to suspect that the digestive system can extract nutrients from the digesta to a certain lower critical level. Thus inclusion of any non - digestible

diluent in the diet would induce similar changes to that observed for rye simply by contributing to digesta bulk, thereby adversely affecting retention. Such may be the case for electrolytes. In contrast to this, levels of excreta fat and amino acids tend to increase with incorporation of rye in the diet, which suggests that malabsorption of these components is primary.

A question that has not been answered is the possible relationship between dietary salt levels, and the fat malabsorption induced by feeding rye. Diarrhea observed in human patients suffering steatorrhea has been attributed to the formation of hydroxy fatty acids in the colon through microbial hydroxylation of undigested fatty acids (Soong et al., 1972). Such hydroxy fatty acids are structurally similar to ricinoleic acid, the active factor in castor oil (Levine, 1978). Free bile acids, too, have been implicated in certain diarrhea states (Levine, 1978). Although no experiments were conducted to study whether fat malabsorption may be involved in the wet litter caused by feeding rye, there were gross differences observed between trials that may suggest that fat level in rye diets may affect the wetness of the litter.

The reverse situation is also worthy of consideration. Increased levels of salt may affect fat digestion as the critical micellar concentration, the capacity of a given quantity of bile salts to solubilize fat, correlates with salt concentration (Hofmann and Borgstrom, 1962). Thus the

fat malabsorption observed in rye diets may be aggravated by a salt deficiency.

7.0 FEEDING RYE AS A REPLACEMENT FOR WHEAT IN LAYING HEN DIETS

7.1 Introduction

Most of the research pertaining to the use of rye in poultry diets has been designed to illustrate the nature of its growth-depressing properties rather than attempting to determine in what situations rye might be an acceptable cereal for poultry. From a practical standpoint the simplest means of introducing rye as a useful feed ingredient is to determine at what levels and in what types of poultry production the adverse effects of rye may be minimized.

A major problem with rye-based diets is the sticky, wet litter that would be unacceptable to a commercial producer. These litter problems can be avoided to a degree by feeding caged laying hens. Rye also makes more sense as a feed for laying hens rather than for growing birds from a nutritional standpoint as its protein and energy content are more compatable with laying hen requirements. Wagner and Thomas (1978) indicated that microbial hyperactivity in rye-fed chicks is transitory to a degree, and that birds may be able to suppress this toxic effect after an initial acclimatization period. Such results imply production depression in long term-feeding programs may not be as severe as noted in short-term broiler trials.

Two experiments were conducted with laying hens fed diets containing several levels of rye. The objective of Trial 7.1 was to determine whether the adverse effects of feeding rye could be controlled by limiting the level of inclusion of rye in the diet. A fall variety of rye (Puma) was substituted on a weight for weight basis for Glenlea wheat at several levels. No attempt was made to keep the diets isonitrogenous or isocaloric, however, an additional high rye diet was fortified with supplementary energy and protein to equal or exceed the all wheat diet to give an indication as to whether diet modification could alleviate any production depression. In Trial 7.2 the effect of feeding graded levels of a spring cultivar of rye (Gazelle), which again was substituted for Glenlea wheat was examined. In addition, protein-energy supplementation was extended over the range of rye levels to determine whether it could be effective in the lower regions of the scale. It was felt that supplementation at lower rye levels may provide one means of increasing the permissable level of rye in the diet as it would increase protein and energy intake without requiring an increase in feed consumption. The effect of replacing the tallow with a more readily digested fat source, soybean oil, was also examined.

7.2 Materials and methods

7.2.1 Trial management

For Trial 7.1, five month old commercial strain pullets (H&N) were individually weighed and randomly distributed to community cages, six pullets per cage and eight cages per dietary treatment. The cages were 80 cm by 40 cm colony cages, subdivided into two equal sections, sharing a common drinking cup and feeder. The lighting regime was a constant 16 hours of light per day. All pullets were initially fed the wheat control diet. Those on the rye treatments were gradually introduced to the rye diets to provide the pullets with an opportunity for acclimatization. Water was supplied ad libitum and weighed amounts of feed were added to the feeders as required to allow ad libitum consumption. Unconsumed feed was weighed back weekly. Feed conversion was calculated on a monthly basis. Egg production was recorded daily and egg weight data was collected for three consecutive days at three month intervals. Data collection was initiated when the pullets were in peak production and terminated after ten months, at which point the hens were again individually weighed.

For Trial 7.2, a five month laying hen trial was conducted using Shaver '288' laying hens that were in the latter phase of egg production (44 weeks old). Egg production was recorded for one month prior to initiation of the trial to permit allocation to treatment according to production level, thus eliminating any pre-trial differences between treatments. Housing of the hens was as for Trial 7.1 except

eight hens constituted a replicate, with five replicates being assigned per treatment. Care of the birds and collection of data for Trial 7.2 was similar to that described for Trial 7.1. Egg production, feed consumption, feed conversion were determined for each pen for the duration of the five month experimental period. Egg weight was determined for three consecutive days at the end of each 28 day period. Additional measurements for Trial 7.2 included shell strength, measured as shell elasticity and shell thickness, and tibia ash. Measurements concerning egg shell quality were made on three consecutive days at the termination of the experiment. Four eggs were randomly selected from each pen, and three measurements per egg taken. Tibia samples were also collected from five hens per treatment for tibia ash determinations at the end of the trial period.

7.2.2 The diets

The diets used in Trial 7.1 and 7.2, and their nutrient composition are listed in Tables 7.1 and 7.2. In Trial 7.1 Puma rye was substituted for Glenlea wheat at several levels, constituting 0.0, 19.5, 39.0, 58.5 and 78.0 per cent of the diet. Diet 6, containing 70 per cent rye, was fortified with additional energy and protein to marginally exceed all wheat diets. For Trial 7.2 Gazelle rye was substituted for Glenlea

Table 7.1 Per cent composition of experimental diets used in Trial 7.1

Ingredient	Diet 1	2	3	4	5	6
Rye (Puma)	-	19.5	39.0	58.5	78.0	70.0
Wheat (Glenlea)	78.0	58.5	39.0	19.5	-	-
Tallow	1.0	1.0	1.0	1.0	1.0	1.0
SBM	8.0	8.0	8.0	8.0	8.0	10.5
Meat meal	-	-	-	-	-	1.5
Fish meal	2.0	2.0	2.0	2.0	2.0	5.0
Limestone	3.9	3.9	3.9	3.9	3.9	3.8
Oyster shell	3.0	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5	0.75
Alfalfa meal	1.1	1.1	1.1	1.1	1.1	-
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0	1.0
Chemical analysis (calculated, air-dry basis)						
Crude protein (N X 6.25)	16.8	16.4	16.0	15.6	15.2	18.2
MEn (kcal/kg)	2746	2707	2668	2629	2590	2771
Calcium (%)	3.05	3.06	3.06	3.07	3.07	3.09
Phosphorus (%)	.64	.65	.67	.68	.69	.62
ME/CP	164	165	167	169	170	152

[1] Supplied the following per kilogram of diet: retinyl palmitate, 8260 IU; cholecalciferol, 880 IU; α-tocopherol, 2.5 IU; vitamin B_{12}, 0.011 mg; riboflavin, 8.9 mg; calcium pantothenate, 17.9 mg; niacin, 26.8 mg; choline chloride, 446.4 mg; santoquin, 250 mg; DL-methionine, 500 mg; manganese (as MnO), 330 mg; zinc (as ZnO), 110 mg; and iodized salt, 4650 mg

Table 7.2 Per cent composition of experimental diets used in Trial 7.2

Ingredient	Per cent composition							
	1	2	3	4	5	6	7	8
Rye (Gazelle)	-	23.3	46.7	70.0	-	23.3	46.7	70.0
Wheat(Glenlea)	77.5	54.2	30.8	7.5	70.0	46.7	23.3	-
Tallow	.5	.5	.5	.5	4.0	4.0	4.0	4.0
Soybean meal	10.5	10.5	10.5	10.5	14.5	14.5	14.5	14.5
Fish meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Oyster shell	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Dicalcum phosphate	.75	.75	.75	.75	.75	.75	.75	.75
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[2]	.5	.5	.5	.5	.5	.5	.5	.5
Chemical analyses (calculated, air-dry basis)								
Crude protein (N X 6.25)	15.9	15.4	15.0	14.5	16.9	16.4	16.0	15.5
ME_n (kcal/kg)	2752	2705	2659	2612	2836	2789	2742	2696
Calcium (%)	3.04	3.04	3.05	3.05	3.06	3.06	3.07	3.07
Phosporus (%)	.55	.55	.55	.55	.54	.54	.54	.54
ME/CP	174	176	177	180	168	170	171	174

[1] Treatment 9 and 10 correspond to treatments 5 and 8 with tallow replaced by soybean oil

[2] see Table 7.1

wheat at 4 levels, consisting of 0.0, 23.3, 46.7 and 70.0 per cent of the diet. Tallow was incorporated at 2 levels; 0.5 or 4.0 per cent, and two diets were included in which 4.0 per cent soybean oil replaced the tallow fraction. The diets containing 4 per cent dietary fat also contained additional protein furnished as soybean meal.

The diets were fed as mash for Trial 7.1, and pelleted in the case of Trial 7.2.

7.2.3 Chemical analysis

Shell thickness was measured using a micrometer. Shell elasticity was determined using a Marius deformation apparatus. In each case the mean of three measurements was taken for statistical analysis. For tibia ash determination, the tibia samples were cleaned, defatted and ashed for 4 hours at 550 C. The ashed samples were cooled, and per cent ash determined as weight difference between the ashed and defatted samples.

7.2.4 Statistical analysis

Statistical analysis was performed with SPSS (Nie et al., 1975) using analysis of variance procedure, and Tukey - HSD test when analysis of variance revealed significant differences among treatments. The limit of probability

accepted as being significant was $P<.05$.

7.3 Results

For Trial 7.1, both egg production and feed conversion showed a significant response that declined curvilinearly when the level of rye in the diet exceeded 39 per cent (Table 7.3). There were no significant differences among the diets containing 0.0, 19.5 or 39 per cent rye. Fortification of the diet containing 70 per cent rye gave only a marginal improvement over the 78.0 per cent rye diet, raising performance to approximately that of the 58.5 per cent rye diet. Rye had no effect on either consumption or egg size, although in both cases there was an apparent reduction as the levels of rye increased. The average hen weight gain (Table 7.3) during the experimental period corresponded to the pattern observed for egg production and feed efficiency. There were no differences from 0.0 to 39.5 per cent rye, afterwhich a sharp drop occurred as the level of rye increased to 58.5 and 78 per cent. The hens fed the rye diet supplemented with protein and energy responded similarly to diets containing the lower rye levels.

The productive performance of hens fed varying levels of Gazelle rye (Trial 7.2) and two levels of fat supplementation are shown in Table 7.4. Analysis of variance revealed no treatment effect for any of the parameters measured, with the

Table 7.3 Effect of graded levels of Puma rye fed to laying hens on egg production, feed conversion, consumption, egg size and body weight gain (Trial 7.1), including summary of analysis of variance (mean squares)

Rye Level	Egg Production (% hen-day)	Feed Conversion (kg/doz)	Consumption (g/day)	Egg Weight(g)	Weight Gain(g)
0.0	79.0^{a}	1.77^{a}	116.0^{a}	62.1^{a}	214^{a}
19.5	78.8^{a}	1.79^{a}	117.2^{a}	61.4^{a}	206^{a}
39.0	78.5^{a}	1.78^{a}	116.2^{a}	61.5^{a}	172^{ab}
58.5	72.9^{ab}	1.91^{ab}	115.8^{a}	61.7^{a}	94^{bc}
78.0	62.0^{c}	2.16^{c}	111.4^{a}	59.7^{a}	61^{c}
70 + S[1]	70.9^{b}	1.95^{b}	114.6^{a}	61.1^{a}	154^{ab}

Summary of Analysis of Variance (Mean Squares)

Treatment (5df)	365*	82163*	33.5	5.33	30022*
Error (42df)	24	11009	34.3	2.53	2989

a,b,c means within a column bearing the same superscript are not significantly different ($P<.05$)

1 refers to 70 per cent rye diet supplemented with additional protein and energy

* denotes significant differences among treatments

Table 7.4 Effect of supplementing diets containing graded levels of Gazelle rye fed to laying hens on egg production, egg weight, feed consumption and feed conversion (Trial 7.2), including summary of analysis of variance (mean squares)

Treatment	Egg Produduction (% hen-day)	Egg Weight (g)	Consumption (g/day)	Feed Conversion (kg/doz)
0 Rye, .5 Tallow	79.04	62.52	113.5	1.73
0 Rye, 4 Tallow	74.56	63.76	105.8	1.72
0 Rye, 4 Soybean oil	81.46	63.02	108.3	1.60
23.3 Rye, .5 Tallow	77.02	63.36	114.3	1.78
23.3 Rye, 4 Tallow	76.56	64.12	110.8	1.74
46.7 Rye, .5 Tallow	77.56	63.34	116.1	1.80
46.7 Rye, 4 Tallow	77.30	62.72	115.2	1.79
70 Rye, .5 Tallow	73.26	62.96	115.3	1.89
70 Rye, 4 Tallow	72.76	62.22	113.8	1.90
70 Rye, 4 Soybean oil	75.38	63.70	109.3	1.74
Summary of analysis of variance[1]				
Treatment (9df)	34.8	1.78	60.7	39.1
Error (40df)	38.6	3.40	26.5	19.7

[1] there were no significant treatment effects

exception of daily consumption. For the consumption data Tukey's test revealed no two means that were significantly different. Since neither rye level, fat level nor type (soybean oil vs tallow) had an effect on egg production, egg weight, consumption, feed conversion, shell thickness, elasticity or tibia ash, the data was pooled (Table 7.5) to distinguish trends attributed to rye level and tallow level. Diets supplemented with soybean oil were excluded from the analysis. The pooled data indicated similar trends in performance with high levels of rye to that of Trial 7.1, however the magnitude of the depression was much less severe. Egg production, feed conversion and egg weight showed the same trends of curvilinearity observed in Trial 7.1. The hens in Trial 7.2 gave some indication of an attempt to increase consumption as the level of rye increased, although this apparently was not sufficient to offset the minor depression in egg production, egg weight and feed conversion.

Increasing the tallow level from one half to four per cent did not improve performance in either the wheat or rye hens (Table 7.4). There is a tendency towards a reduction in egg production and feed conversion with a high tallow level in both rye and wheat diets. The supplementation of the zero rye and 70% rye diets with soybean oil gave egg production and feed conversion values that were numerically superior to diets containing either one half or four per cent tallow.

Egg shell thickness, shell elasticity and tibia ash

Table 7.5 Main effects of supplementing diets containing graded levels of rye fed to laying hens on egg production, egg weight, consumption and feed conversion (Trial 7.2)

	Egg production (% hen-day)	Egg weight (g)	Consumption (g/day)	Feed conversion (kg/doz)
Rye Level				
0 %	76.8	63.15	109.7	1.73
23.3 %	76.8	63.75	112.6	1.76
46.7 %	77.4	63.04	115.7	1.80
70.0 %	73.0	62.60	114.5	1.90
Fat Level[1]				
.5 %	76.7	63.05	114.8	1.80
4 %	75.3	63.21	111.4	1.79

[1] Diets supplemented with soybean oil excluded

followed a similar pattern to the egg production parameters (Table 7.6 - 7.7). Shell thickness declined with increasing rye level resulting in a corresponding increase in shell elasticity. Tibia ash also declined at the higher rye levels. There was no consistent effect of increasing the tallow level to four per cent, or substitution of the tallow for oil on shell thickness, shell elasticity or tibia ash.

7.4 Discussion

Substitution of Glenea wheat with Puma rye on a weight-for-weight basis resulted in a curvilinear response. Most production parameters, including egg production, feed efficiency and body weight, decline rapidly only if the level of rye is permitted to exceed 39 per cent. At levels below 39 per cent the differences are minimal or non-existent. The fact that this response is curvilinear indicates that rye is fully equivalent to wheat providing that the rye level is kept below the 'breaking point' of the response curve. In view of possible differences in content of the production-depressing agent, it may be desirable to allow a safety margin. Based on this data a reasonable maximum level may be considered to be approximately 30 per cent.

Fortification of diets containing high levels of Puma rye was not an effective means of alleviating the reduced performance, as it resulted in only a minimal improvement. In

Table 7.6 Effects of supplementing diets containing graded levels of rye fed to laying hens on egg shell thickness, shell elasticity, and tibia ash (Trial 7.2), including summary of analysis of variance (mean squares)

Treatment	Shell thickness (nm)	Shell elasticity (um)	Tibia ash (%)
0 Rye, .5 Tallow	14.10	27.92	61.31
0 Rye, 4 Tallow	13.72	30.00	63.85
0 Rye, 4 Soybean oil	13.80	30.90	58.03
23.3 Rye, .5 Tallow	14.12	29.26	62.67
23.3 Rye, 4 Tallow	13.78	29.44	61.48
46.7 Rye, .5 Tallow	13.94	30.02	60.75
46.7 Rye, 4 Tallow	13.80	29.78	60.73
70 Rye, .5 Tallow	14.36	27.50	58.20
70 Rye, 4 Tallow	13.24	32.66	61.66
70 Rye, 4 Soybean oil	14.36	28.92	59.59
	Summary of analysis of variance		
Treatment (9 df)	.56[1]	10.69[1]	16.78[1]
Error (40 df)	.33	9.04	8.85

[1] there were no significant differences among treatments

Table 7.7 Main effects of supplementing diets containing graded levels of rye fed to laying hens on shell thickness, shell elasticity, and tibia ash (Trial 7.2)

	Shell thickness (nm)	Shell elasticity (um)	Tibia ash (%)
Rye level			
0 %	13.91	28.96	62.58
23.3 %	13.95	29.35	62.08
46.7 %	13.87	29.90	60.74
70.0 %	13.80	30.08	59.93
Fat level[1]			
.5 %	14.13	28.67	60.73
4 %	13.63	30.47	61.93

[1] diets supplemented with soybean oil excluded

view that supplementation of the 70 per cent rye diet improved egg production only to equal the 58.5 per cent diet, yet improved body weight to equal the 19 and 39 per cent rye diets, it would appear that the additional energy and protein were directed towards increased body weight moreso than egg production. McNab and Shannon (1975) reported that nutrient retention, including ether extract, crude protein, organic matter, carbohydrate and amino acids, was depressed in colostomized hens fed rye as compared to wheat or triticale. This depression was only minor however and hens should overcome the nutrient deficiency by increasing consumption. Evidently this is not the case, since laying hens made no effort to increase consumption as the level of rye in the diet was increased. The poor performance of the hens fed high rye diets may be due to reduced digestibility and the failure, or inability, of the hens to increase consumption to compensate for the digestibility differences.

The results of Trial 7.2 where Gazelle rye was substituted for Glenea wheat are not in complete agreement with Trial 7.1 in that there is no distinct 'breaking point' beyond which performance rapidly declines with increasing rye level. However there is a consistent indication that this 'breaking point' occurs also in the second trial, yet it is not sufficiently large to be statistically significant.

There are several differences between the two trials that may have contributed to the different responses. First,

different strains of hens were used in the two trials; second, the hens were in a latter phase of production in the second trial; and third, the diets were pelleted for Trial 7.2. The hens in Trial 7.2 did increase consumption somewhat for the diets containing intermediate levels of rye, which may have partially offset any detrimental effect. This, combined with the lower requirement of hens during the second and third phases of production, probably minimized the adverse effect of feeding high levels of rye in Trial 7.2. Supplementation of the laying hen diets containing Gazelle rye, as occurred with diets containing Puma rye, was not effective in improving performance. However in the case of Trial 7.2 this may be attributed to the reasonable level of performance attained by the laying hens fed Gazelle rye, which left little room for improvement.

Considering that the proposed anti-nutritional component of rye are the pentosan gums, which have also been implicated in winterhardiness (Shearman et al., 1973; Olien, 1965), an attractive hypothesis concerning the differences between Trial 7.1 and 7.2 is that Puma rye is more detrimental to hens than Gazelle. However, more recent studies using broiler chicks have failed to reveal differences between Gazelle and Puma rye (Campbell and Classen, unpublished results).

Although not statistically significant there are numerous indications that the hens fed Gazelle rye were suffering similar effects to the hens fed Puma rye, or chicks fed Puma

rye. The effects were however much less severe. In particular, the shell thickness, shell elasticity, and tibia ash data suggest possible detrimental effects on calcium metabolism. Also soybean oil tended to give superior performance compared to tallow in the rye diets, which is possibly indicative of a similar effect on fat retention to that which occurs in chicks.

Patel and McGinnis (1980) reported that irradiation of rye prior to incorporating it in laying hen diets completely alleviated the negative response, whereas supplementation of the diets with penicillin or pectic enzyme had no effect. They concluded that the mechanism of growth depression induced by rye was somehow different in laying hens and chicks, since chicks respond readily to antibiotic or enzyme supplementation. Perhaps a more plausible explanation is that laying hens fail to respond to antibiotics because the adult microflora has stabilized and the antibiotic-sensitive strains responsible for growth depression no longer occur in significant numbers. The lack of response to pectic enzyme may be attributed to destruction of enzymatic activity in the more acid environment of the adult fowl as compared to the chick gut. The beneficial effect of gamma irradiation, which appears to reduce viscosity by depolymerizing polysaccharides, indicates that the viscous condition also has a detrimental effect on laying hens. The manifestation of this effect was probably somewhat different. Possibly laying

hens represent a condition where the gums alone depress performance, whereas for chicks it is a direct effect of the gums with a microflora effect superimposed on it. Although it is difficult to compare production performance of laying and growing birds, overall it seems that young birds are much more seriously affected by inclusion of rye in the diets.

The results of the present study suggest that the magnitude of the depression in performance of laying hens fed rye may be controlled by limiting the amount of rye in the diet. Both laying hen trials indicate that rye may be successfully fed to laying hens. The curvilinear nature of the response curve suggests that rye is fully equivalent to wheat provided that the level of rye is kept beneath the breaking point of the curve. The exact location of the breaking point is not clear. The results of Trial 7.1 indicates that it occurs in the region of 39 per cent, whereas the results of Trial 7.2 indicate that it occurred at 60 per cent, if it occurred at all. This interpretation regarding the curvilinear nature of the response of hens fed rye may also explain some discrepancies occurring in the results of earlier workers. Halpin et al. (1936) conducted two laying hen experiments in which they observed that inclusion of rye up to 45 per cent of the diet had no ill effect on laying rate. In contrast to this Fernandez, Kim, Buenrostro, and McGinnis (1973) reported that inclusion of 80 per cent rye caused a sharp drop in egg production and egg

weight, followed by a period of partial recovery. Such discrepancies may be explained on the basis that the adverse effects of feeding rye to laying hens are only apparent when the critical level is exceeded.

In addition to diets containing rye, the present study incorporated two diet modifications that have been demonstrated to partially offset the poor performance of chicks fed rye, and may induce a similar positive response in laying hens. These include the use of some fish meal in all diets (Antoniou, 1980) as well as the use of higher than usual salt (Lee, 1981). It may be prudent to incorporate such modifications if rye is to be used as a feedstuff on a commercial basis. A conservative estimate as to how much rye may be incorporated into a diet may be considered to be 30 per cent, although this may be higher under certain conditions. It is conceivable that the permissible level of rye in the diet may be increased by pelleting the diet and restricting its use to the latter stages of production. Increasing the energy and protein of the diet was clearly not an effective means of improving performance in diets containing a high level of rye.

8.0 FEEDING RYE AS A REPLACEMENT FOR WHEAT IN CHICK DIETS

8.1 Introduction

Several dietary modifications have been shown to be effective in reducing the adverse response to rye when included in diets fed to growing chicks. These modifications, that may not normally be used on a commercial basis but are feasible, consist of a higher than what normally would be considered optimal salt level, the inclusion of fish meal as a supplementary protein source, and the use of soybean oil as opposed to tallow to increase dietary energy. Lee (1981) has demonstrated the increased need for salt in chicks fed rye, which excedes NRC recommendations by a factor of two. Antoniou (1980) has demonstrated the effectiveness of fish meal as opposed to meat meal in rye containing diets. Soybean oil has been demonstrated to be a more effective source of fat than tallow (Antoniou, 1980; Misir, 1978) for chicks fed rye. A series of four short term experiments using Single Comb White Leghorn (SCWL) cockerels was undertaken to examine the effect of level of rye, dietary density, incorporation of antibiotic, and physical form of the diet in rye diets formulated to minimize the growth depression in chicks fed rye diets. For Trial 8.1 diets containing three levels of Puma rye both with and without penicillin supplementation were fed to growing chicks. Trial 8.2 was designed to determine whether adverse effects of feeding rye to chicks

could be overcome by increasing dietary density. In Trial 8.3 the possibility that the magnitude of the response of chicks fed rye to penicillin could be affected by dietary density was examined. One means of improving performance in low density diets is to pellet or crumble the diet. This was examined in Trial 8.4, where the sensitivity of chicks fed rye or wheat in response to size of grind and crumbling the diet was noted. In a fifth experiment broilers were finished to market age to assess the suitability of rye when fed to litter-raised birds, and the magnitude of the sticky litter problem under conditions similar to those employed in commercial practice. In addition the response of SCWL and broiler chicks to three weeks of age were compared for rye and wheat diets.

The parameters considered as indicative of performance for Trials 8.1 to 8.5 were body weight gain, feed consumption and feed conversion. Metabolizable dry matter was determined for Trial 8.1 as a general index of digestibility of the rye as compared to wheat diets.

8.2 Materials and Methods

8.2.1 Trial management

For Trials 8.1 to 8.4 SCWL cockerels were fed a commercial chick starter until one week of age. The chicks

were weighed prior to random distribution to pens to minimize pre-treatment weight differences. Six chicks constituted a treatment replicate, and six pens were allocated per treatment. The chicks were housed the duration of the trial period in thermostatically-controlled Petersime battery brooders. Feed and water were supplied ad libitum. Pen weights were taken at the start and finish of the experimental period. For MDM determination (Trial 8.1) excreta output was quantified using total collection for a three day period, afterwhich the excreta was dried in a force air oven (60 ^{o}C). Metabolizable dry matter was calculated (1 - excreta output/feed consumed). In all cases the trials were terminated when the chicks were three weeks old, for a trial duration of two weeks.

Trial 8.5 was divided into two periods. During the first period (0-17 days) both broilers and SCWL cockerels were housed in batteries as indicated for Trials 8.1 to 8.4. The chicks were fed a commercial starter diet for one week afterwhich growth, feed consumption, and feed conversion were determined for a 10 day period (7-17 days). For the second period of the experiment the broiler cockerels only were transferred to floor pens (4) and raised to market weight (7.5 wk). The broilers were individually weighed at the start and finish of the experiment. Feed consumption was recorded for each pen.

8.2.2 The diets

The diets fed in Trials 8.1 to 8.5 were chick diets in which Puma rye was substituted for Glenlea wheat. The rye and wheat samples (12.3 % crude protein versus 14.3 % crude protein) used compared favorably in composition, hence substitution on a weight-for-weight basis was possible with minimal changes in the composition of the final diet. For Trial 8.1 three levels of rye (Table 8.1) were substituted for wheat (0, 30 and 60 per cent of the diet). Procaine penicillin was used as an antibiotic source for the supplemented diets. The effect of substituting four levels of rye (0, 20, 40 and 60 per cent) at two densities was examined in Trial 8.2 (Tables 8.2 - 8.3). Density was manipulated by increasing fish meal and soybean oil at the expense of the soybean meal and wheat. In Trial 8.3 high and low density diets containing wheat or rye as the cereal base were compared both with and without penicillin (1.3 g procaine penicillin '10' per kg diet) supplementation. The diets fed were diets 1 and 4 (low density) and diets 5 and 8 (high density) used in Trial 8.2. In the case of Trial 8.4, where the effects of physical form of the diet were examined, the diets (Table 8.4) were similar to low density diets fed previously with two notable differences. The level of rye was increased to seventy per cent, and tallow replaced soybean oil. Grinding the rye or wheat component in all cases of the

Table 8.1 Per cent composition of experimental diets used in Trial 8.1

Ingredient	Diet 1[2]	Diet 2[2]	Diet 3[2]
Wheat (Glenlea)	60.0	30.0	-
Rye (Puma)	-	30.0	60.0
Soybean meal	20.0	20.0	20.0
Fish meal	9.0	9.0	9.0
Soybean oil	6	6	6
Dehydrated alfalfa	1.4	1.4	1.4
Calcium carbonate	1.1	1.1	1.1
Dicalcium phosphate	1.0	1.0	1.0
Vitamin premix [1]	1.0	1.0	1.0
Mineral premix [1]	.5	.5	.5
Chemical analysis (calculated)			
Protein (N X 6.25)	25.30	24.69	24.07
Ether extract	7.54	7.52	7.50
ADF	3.13	3.23	3.34
Ca	.98	.99	.99
P (total)	.69	.69	.69
Metabolizable energy (kcal/kg)	3101	3041	2981

[1] supplied the following per kg diet: vitamin A, 8250 I.U.; vitamin D_3, 880 I.U., vitamin E, 5.5 I.U., menadione, 1.1 mg; vitamin B_{12}, .01 mg; riboflavin, 2.2 mg; calcium pantothenate, 4.4 mg; niacin, 6.6 mg; choline chloride, 110 mg; DL methionine (98%), .5g; santoquin (50%), 250 mg; manganese oxide, 165 mg; zinc oxide, 14 mg; ferric sulphate ($7H_2O$), 31 mg; copper sulfate ($5H_2O$), 25.3 mg; iodized salt, 4.7

[2] diets 1,2,3 were supplemented with 'Pro - Pen' '10' (1.3 g/kg) for corresponding antibiotic supplemented diets (procaine penicillin, Merck, Sharp and Dohme)

Table 8.2 Per cent composition of experimental diets (low density) used in Trial 8.2

Ingredient	Diet 1	2	3	4
Wheat (Glenlea)	70.0	50.0	30.0	10.0
Rye (Puma)		20.0	40.0	60.0
Soybean meal	20.0	20.0	20.0	20.0
Fish meal	2.75	2.75	2.75	2.75
Soybean oil	2.0	2.0	2.0	2.0
Dehydrated alfalfa	1.0	1.0	1.0	1.0
Calcium carbonate	.75	.75	.75	.75
Dicalcium phosphate	2.0	2.0	2.0	2.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[1]	.5	.5	.5	.5
Chemical analyses (calculated)				
Protein (N X 6.25)	22.3	21.9	21.5	21.1
Ether extract	3.23	3.22	3.21	3.20
ADF	3.27	3.34	3.41	3.48
Ca	.88	.89	.89	.90
P (total)	.81	.81	.81	.81
Metabolizable energy (kcal/kg)	2902	2862	2822	2782

[1] see Table 8.1. Premix included 1 g methionine/kg , .5 g lysine/kg diet

Table 8.3 Per cent composition of experimental diets (high density) used in Trial 8.2

Ingredient	Diet 5	6	7	8
Wheat (Glenlea)	64.0	44.0	24.0	4.0
Rye (Puma)	-	20.0	40.0	60.0
Soybean meal	16.0	16.0	16.0	16.0
Fish meal	9.0	9.0	9.0	9.0
Soybean oil	6.0	6.0	6.0	6.0
Dehydrated alfalfa	1.4	1.4	1.4	1.4
Calcium carbonate	1.1	1.1	1.1	1.1
Dicalcium phosphate	1.0	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[1]	.5	.5	.5	.5
Chemical analyses (calculated)				
Protein (N X 6.25)	23.8	23.3	22.9	22.5
Ether extract	7.51	7.50	7.49	7.48
ADF	2.97	3.04	3.11	3.18
Ca	.97	.97	.98	.98
P (Total)	.84	.84	.84	.84
Metabolizable energy (kcal/kg)	3113	3073	3033	2993

[1] refer to Table 8.1. Premix included 1 g methionine/kg, .5 g lysine/kg

Table 8.4 Per cent composition of experimental diets used in Trial 8.4

Ingredient	Diet 1	Diet 2
Wheat (Glenlea)	70.0	-
Rye (Puma)	-	70.0
Soybean meal	20.0	20.0
Fish meal	2.4	2.4
Tallow	2.35	2.35
Dehydrated alfalfa	1.0	1.0
Calcium carbonate	.75	.75
Dicalcium phosphate	2.0	2.0
Vitamin premix[1]	1.0	1.0
Mineral premix[1]	.5	.5
Chemical analyses (calculated)		
Protein (N X 6.25)	22.10	20.63
Ether extract	3.56	3.52
ADF	3.27	3.51
Ca	.87	.89
P (total)	.81	.81
Metabolizable energy (kcal/kg)	2887	2746

[1] refer to Table 8.1. Premix included 1 g methionine/kg, .5 g lysine/kg diet

Table 8.5 Per cent composition of experimental diets used in Trial 8.5

Ingredient	Diet 1	2	3	4
Wheat (Glenlea)	70.0	10.0	64.0	4.0
Rye (Puma)[2]	-	60.0	-	60.0
Soybean meal[3]	20.0	20.0	16.0	16.0
Fish meal	2.75	2.75	9.0	9.0
Soybean oil	2.0	2.0	6.0	6.0
Dehydrated alfalfa	1.0	1.0	1.4	1.4
Calcium carbonate	.75	.75	1.1	1.1
Dicalcium phosphate	2.0	2.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[1]	.5	.5	.5	.5
Chemical analyses (determined)				
Protein (N X 6.25)	21.9	20.8	24.4	23.5
Ether extract	3.4	1.4	7.2	7.0
ADF	4.61	4.18	4.5	4.2
Ca	.95	.87	.98	1.11
P	.93	.81	.80	.73
Metabolizable energy (calculated) (kcal/kg)	2885	2765	3073	2953

[1] See Table 8.1, included 1g Pro-Strep '20'(procaine penicillin, streptomycin, Merck, Sharp and Dohme), 1g methionine, .5 g lysine per kg diet

[2] This was a different sample of Puma rye than used in preceding Trials 8.1 - 8.4 (11.2% CP vs 12.3% CP used previously)

[3] soybean meal in case of Trial 8.5 was 44% CP as opposed to 48% CP soybean meal used in Trials 8.1 - 8.4

diet was accomplished using a hammer mill. The diets fed in Trial 8.5 are depicted in Table 8.5. Rye was fed at 2 levels (0 and 60 per cent) at two densities. All diets in Trial 8.5 were supplemented with penicillin and streptomycin (1 g 'Pro-Strep' per kg diet).

The diets were fed as mash in Trials 8.1 to 8.4, with the exception of treatments indicated in Trial 8.4 where several treatments were pelleted and crumbled as indicated. All treatments in Trial 8.5 were pelleted and crumbled.

8.2.3 Statistical analysis

Statistical analyses were computed using SPSS analysis of variance procedure (Nie et al. (1975)). The design employed for Trial 8.1 was a 2 X 3 factorial, with three levels of rye both with and without penicillin supplementation. Trial 8.2 was analysed as a 2 X 4 factorial design, with four levels of rye at two dietary densities. For Trial 8.3 the design was a 2 X 2 X 2 factorial, with two grain types (rye vs wheat), two densities (high vs low) and two levels of antibiotic supplementation (- vs +). Trial 8.4 also constituted a 2 X 2 X 2 factorial, with grains (rye vs wheat), grind sizes (coarse vs fine) and physical form (crumbles vs mash) as the factors. The results of Trial 8.5 were analysed as two separate experiments. The first analysis compared the response of SCWL or broiler chicks fed rye or wheat in high

and low density diets (2 X 2 X 2 factorial design) and the second compared only broilers (7.5 wk) fed the rye or wheat diets (2 X 2 factorial design). The limit of probability accepted as being significant was $P<.05$.

8.3 Results

Substitution of rye for wheat in chick diets for Trial 8.1 resulted in a significant depression in both gain and feed conversion (Table 8.6). Procaine penicillin had no significant effect on gain or feed conversion, however in both instances there was evidence of a trend towards improvement with antibiotic supplementation. Metabolizable dry matter was also reduced as a function of the level of rye in the diet. Procaine penicillin however significantly improved MDM. The chicks did not attempt to overcome differences in MDM by increasing consumption, as there were no significant differences in consumption among the various treatments.

Reducing dietary density or increasing level of rye in chick diets in Trial 8.2 had a significant depressing effect on both body weight gain and feed conversion (Table 8.7). Increasing the level of rye in replacement for wheat gave a linear response in the high density diets, however in the low density diets there was evidence of curvilinearity. The chicks, as in Trial 8.1, failed to increase consumption to

Table 8.6 Main effects of supplementing high density wheat or rye diets with procaine penicillin on chick gain, consumption, feed conversion and MDM (Trial 8.1) including summary of analysis of variance (mean squares)

Rye level	Gain (g/pen)	Consumption (g/pen)	Feed Conversion (feed/gain)	MDM
0%	894.0	1489.7	1.67	.72
30%	855.4	1483.5	1.74	.69
60%	820.2	1495.3	1.83	.65
Procaine penicillin				
-	851.3	1489.7	1.75	.68
+	861.7	1489.3	1.73	.69
	Summary of analysis of variance (mean square)			
rye (2df)	16365.2*	420.3	.0755*	.0147*
antibiotic (1df)	971.5	1.8	.0042	.0009*
rye X antibiotic (1df)	103.3	1204.8	.0012	.0001
error (30df)	2237.0	2601.2	.0045	.0001

* denotes significant treatment effect

Table 8.7 Main effects of rye level and dietary density in diets fed to chicks (Trial 8.2), including analysis of variance (mean squares)

	Gain (g/pen)	Consumption (g/pen)	Feed conversion (feed/gain)
Rye Level			
0	969.3	1782.9	1.85
20	910.5	1740.2	1.92
40	890.3	1752.3	1.98
60	830.7	1746.2	2.12
Density			
Low	842.5	1774.0	2.12
High	957.9	1736.8	1.82

Summary of analysis of variance (mean squares)

Rye (3df)	39277.6*	4335.6*	.16*
Density (1df)	159967.4*	16688.6*	1.07*
Density X Rye (3df)	1217.4	3260.1	.01*
Error (40df)	1552.8	3058.6	.002

* denotes significant treatment effect

overcome density differences. The fact that the performance of chicks fed the high density rye diet (Diet 8) was similar to that of the low density wheat diet (Diet 1) indicates that supplementation of the rye diet was an effective means of increasing intake of protein and energy. The curvilinearity apparent in the response in the low density diets was not severe, and may be attributed to density alone, rather than a true density X rye interaction.

The results for Trial 8.3 are presented in Table 8.8. Both density and grain type had a significant effect on weight gain. Rye again depressed growth as did reducing dietary density. Antibiotic supplementation did not have a significant effect, although there was a trend towards improvement in gain, but not feed conversion. The improvement due to antibiotic was small and compared in magnitude with the results in Trial 8.1. The consumption data also follows the pattern previously observed in Trials 8.1 and 8.2. Chicks fed rye consumed less feed than chicks fed wheat in both high and low density diets. Density also had an effect on consumption, with chicks fed low density diets consuming more feed than those fed high density diets. The high density rye diets, which were similar in terms of protein and energy to the low density wheat diets, gave comparable performance to the low energy wheat diet, which is in agreement with the results of Trial 8.2.

The data indicate that relative overall performance of

Table 8.8 Main effects of dietary density and antibiotic supplementation on the performance of chicks fed rye diets (Trial 8.3), including summary of analysis of variance (mean squares)

	Gain (g/pen)	Consumption (g/pen)	Feed conversion (feed/gain)
Rye Level			
0	863.5	1498.6	1.73
60	779.2	1505.8	1.94
Density			
Low	792.5	1531.9	1.94
High	850.8	1473.5	1.73
Antibiotic			
-	817.6	1490.4	1.83
+	826.8	1513.3	1.84

Summary of analysis of variance (mean squares)

Rye (1df)	87420.0*	561.9	.50*
Density (1df)	43380.1*	38158.5*	.52*
Antibiotic (1df)	1891.8	5945.7	0
Rye X Density (1df)	2308.0	157.7	.02**
Rye X Antibiotic (1df)	2335.4	7530.2	0
DensityX Antibiotic(1df)	462.0	11094.6	.008*
Rye X Density X Antibiotic (1df)	3853.2	1756.8	.007*
Error (39df)	1243.0	3043.9	.0017

* denotes significant treatment effect

chicks in Trial 8.4 was poorer for chicks fed rye diets than occurred in previous trials in which low density, rye - containing diets (Table 8.9) were used. Substitution of rye for wheat resulted in depressed performance for gain and feed conversion, however it had no effect on consumption, which again indicates a failure on the part of the rye-fed birds to increase consumption in accordance with energy demands. Pelleting the diets significantly increased consumption for both the rye and wheat diets. The pelleting and grain interaction was also significant, indicating that pelleting resulted in a greater improvement for rye as compared to wheat diets. The improvement in consumption resulted in a trend towards a similar response in feed conversion and gain, although these were not significant in this case.

The results during the first period of Trial 8.5 comparing performance of SCWL and broiler chicks (Table 8.10) indicate a significant reduction in body weight and feed conversion attributable to incorporation of rye in the diet. The higher density diets resulted in a slightly higher weight gain, a significant reduction in consumption and a corresponding improvement in feed conversion. The strain differences of interest are the interactions apparent in Trial 8.5. The density X strain and strain X grain were highly significant for weight gain, but not significant for consumption or feed conversion. Both the laying strain and broiler strain chicks gave a similar pattern of response to

Table 8.9 Main effects of the physical form of the diet on the performance of chicks fed rye or wheat (Trial 8.4), including summary of analysis of variance (mean squares)

	Gain (g/pen)	Consumption (g/pen)	Feed Conversion (feed/gain)
Rye Level			
0	360.1	690.1	1.92
70	283.8	669.5	2.37
Pelleting			
-	308.8	664.5	2.19
+	335.2	695.1	2.10
Grind Size			
Coarse	323.9	683.2	2.14
Fine	320.0	676.4	2.15

Summary of analysis of variance (mean squares)

rye (1df)	69921.3*	5084.1	2.45*
pelleting (1df)	8374.1*	11224.1*	.10*
grind (1df)	184.1	546.7	0
rye X pelleting (1df)	1323.0	8694.1*	0
rye X grind (1df)	8.3	2552.1	.02
grind size X pelleting (1df)	290.1	114.1	.01
rye X grind X pelleting (1df)	161.3	444.1	0
error (40df)	676.3	2089.3	.01

* denotes significant treatment effect

Table 8.10 Effect of feeding rye or wheat in high and low density diets on layer and broiler strain chick weight gain, feed consumption and feed conversion (Trial 8.5), including summary of analysis of variance (mean squares)

Grain	Density	Strain	Weight gain (g)	Consumption (g)	Feed Conversion (feed/gain)
Wheat	Low	SCWL	94.9	173.7	1.83
Rye	Low	SCWL	91.7	184.2	2.01
Wheat	High	SCWL	101.4	167.9	1.65
Rye	High	SCWL	82.9	168.9	2.04
Wheat	Low	Broiler	265.6	404.4	1.52
Rye	Low	Broiler	228.9	390.7	1.72
Wheat	High	Broiler	281.0	386.5	1.38
Rye	High	Broiler	201.2	359.5	1.79

Summary of analysis of variance (mean squares)

density (1df)	41	3050*	.046*
grain (1df)	12610*	521	.964*
strain (1df)	257020*	494314*	.864*
density X strain (1df)	36	513	.004
density X grain (1df)	2202*	347	.120*
strain X grain (1df)	6289*	1855	.001
density X grain X strain (1df)	520	10	.000
error (36df)	198	487	.011

* denotes significant treatment effect

the various diets, however in the case of broilers this was more pronounced for weight gain, feed consumption and feed conversion (Table 8.11). The results obtained in Trial 8.5 differed from those previously observed in that the fortification of rye containing diets with additional protein and energy (high density) resulted in no significant improvement in performance. While the chicks fed wheat on high density diets tended to outperform those fed low density diets, the performance of rye - fed chicks given high density diets tended to be poorer than those fed low density diets. This is reflected in the highly significant density X grain interaction for feed conversion.

Body weight of broilers at 7.5 weeks (Table 8.12) indicated a similar response. Increasing density had little effect on the body weight of broilers fed wheat, however increasing density in the case of broilers fed rye resulted in poorer performance, as evident from the significant density X grain interaction. Both consumption and feed conversion also declined when rye was fed in a high density compared to a low density diet. Body weight gain actually decreased with respect to the high density rye diet during the latter production phase, as indicated by a weight gain plateau during the final week of the broilers fed this diet.

8.4 Discussion

Table 8.11 The effect of feeding rye in high or low density diets to layer or broiler strain cockerels expressed as a per cent of corresponding wheat diet (Trial 8.5)

Grain	Density	Strain	Weight gain[1]	Consumption[1]	Feed conversion[1]
rye	low	layer	96.7	106.1	109.8
rye	high	layer	81.7	100.6	123.0
rye	low	broiler	86.1	96.6	113.2
rye	high	broiler	71.6	93.0	129.7

[1] value presented in each case is the rye diet expressed as a per cent of the corresponding wheat diet

Table 8.12 Effect of wheat or rye in low and high density diets on body weight, feed consumption and feed conversion of broilers (Trial 8.5), including summary of analysis of variance (mean squares)

Grain	Density	Weight (kg)	Consumption[1] (kg)	Feed Conversion[1] (feed/gain)
Wheat	Low	2.23	4.43	1.99
Rye	Low	2.00	4.52	2.26
Wheat	High	2.18	4.28	1.96
Rye	High	1.58	3.88	2.46

Summary of analysis of variance (body weight)

	Mean square
Density (1df)	1.32*
Grain (1df)	5.15*
Density Grain (1df)	1.02*
Error (114df)	.14

[1] Consumption and feed conversion represent mean values since all birds for one treatment were housed in a single pen.

* denotes significant treatment effect

For the most part Trials 8.1 to 8.4 may be considered positive evidence that rye may be successfully fed to chicks with appropriate dietary modifications. Inclusion of rye in the diet lead to a linear depression for most parameters, however this response tended to be minor and readily responded to dietary supplementation with additional protein and energy furnished as fish meal and soy oil. Diets that were equivalent in terms of energy and protein gave similar performance regardless whether the cereal base was rye or wheat, indicating that substitution of rye for wheat simply increased the fibre level of the diet somewhat. Rye incorporated into low density diets gave some indication of a curvilinear response, however this could be attributed to low density alone rather than a specific effect of the rye component. Generally chicks fed rye made no attempt to increase consumption to overcome slight differences in dietary density, which necessitated the fortification of the diet to improve performance. Based on these results alone it would be concluded that rye could make up the entire cereal base of the diet. It would be necessary to increase diet density however, since apparently the chicks do not modify consumption to meet energy demands when fed rye diets.

The magnitude of the response of chicks fed rye to antibiotic supplementation was considerably less than previously reported. This may be attributed, in part at least, to the use of soy oil rather than tallow in most of

the diets, which reduces the response to antibiotics of chicks fed rye, as well as the use of fish meal in the diets (Antoniou, 1980). Inclusion of salt well beyond what is normally recommended may also have contributed to the lack of response, although the impact of this on the magnitude of the antibiotic response has not been ascertained. Sanitary quarters may also have reduced the potential for a larger response to antibiotic supplementation, since the chicks were housed in batteries that were dismantled, scrubbed and disinfected between trials. Also, there was no opportunity for contact and subsequent contamination from older birds. Overall, the results of Trials 8.1 and 8.3 indicate that in diets formulated to minimize the growth depression of rye diets, the potential for a response to antibiotics is also minimized.

The failure of chicks fed rye to increase consumption to overcome slight differences in nutrient density may be partially compensated by pelleting the diet. From the results of Trial 8.4 it may be suggested that chicks fed rye respond to a greater degree than chicks fed wheat to pelleting the diet. In Trials 8.1 to 8.4 there was no indication of interference of feed consumption by adherence of the rye to the beak that is sometimes apparent (Moran et al., 1969), nor was there a problem with pasted vents and excreta caking on the screens as is frequently encountered in experiments with rye diets. The litter for chicks fed rye was somewhat bulkier

than normal, however this effect in the case of these trials was of minor significance. The chicks in Trials 8.1 to 8.4 did not give the type of response that may be described as typical for rye based diets. Possibly this is attributable to the sample of Puma rye in these studies, which perhaps contained less of the pentosan component. This seems unlikely however, since other studies where a variety of rye samples were compared, little or no difference between rye samples (Patel and McGinnis, 1976) was observed. A more likely possibility is that other dietary components such as the fat and/or salt component, modify the response of chicks when a rye diet is fed.

The results of Trial 8.5 were clearly not in agreement with Trials 8.1 to 8.4. In this case increasing the density of the diet was not effective as a means of improving performance of either broiler or SCWL chicks fed a rye based diet, despite the inclusion of an antibiotic in the diet. One possible explanation for the difference in the response was the use of a different sample of Puma rye in Trial 8.5, as compared to Trials 8.1 to 8.4, although this seems unlikely. A second difference between the trials is that both the batteries and pens in Trial 8.5 were located in the poultry production unit at the University of Manitoba, whereas the chicks in Trials 8.1 to 8.4 used Animal Science Laboratory animal facilities. Previous authors have noted environmental differences on the magnitude of antibiotic response.

Considering the variation in response determined in Trials 8.1 to 8.4 and 8.5 it must be concluded that although feeding rye doesn't necessarily result in severe depression compared to wheat diets, it must be considered a high risk venture that is not suitable for commercial application. The discrepancies apparent in the present study point to a need for large scale all inclusive experimental work studying a variety of rye samples, locations, as well as various dietary manipulations on the magnitude of the depression of chicks fed rye. It is conceivable that under optimal conditions rye could be fed to growing birds.

9.0 GENERAL DISCUSSION

The effects observed when rye is fed to chicks parallel in many respects the condition of steatorrhea induced by excessive intestinal bacterial growth in other experimental animals, as well as humans. Normally bacterial proliferation is prevented at least in the anterior regions of the digestive tract by the acidity of the gastric juice, which reduces drastically the bacteria surviving passage. A second factor is the peristalsis that normally prevents bacteria from translocating from the lower bowel to the upper gastrointestinal tract (Tabaqchali and Booth, 1970). It would seem possible that the chick, with a relatively high gastric pH and frequent retrograde peristaltic action may be particularly susceptible to excessive bacterial activity. Bacterial growth in the crop may also subject the chick to a substantial bacterial 'load'in terms of bacteria with an opportunity to survive gastric passage.

Why rye should induce excessive bacterial growth (Wagner and Thomas, 1978, Untawale and McGinnis, 1979) is not clear. Gohl and Gohl (1977) observed that B-glucan (in barley) was capable of retarding the rate of passage, which they proposed would stimulate microbial hyperactivity. McKay (1980) was able to demonstrate a marked reduction in passage rate for rye-fed birds. Undoubtedly increased intestinal viscosity would reduce diffusion rate in the intestinal lumen which

would also confer a selective advantage on the microflora. Lifshitz et al. (1978) studied the effect of slowing rate of passage in low germ and conventional rats by injecting mecamylamine. Conventional rats given mecamylamine had increased intestinal bacterial growth, increased deconjugated bile salts and reduced absorption rates for most nutrients wheras the low-germ rats exhibited no such response.

The consequences of excessive intestinal bacterial growth, regardless of the causative event, are well defined. The concentration of conjugated bile salts decline, with the deconjugated forms predominant. Tabaqchali et al. (1968) observed in the presence of excessive microbial activity that the concentration of conjugate bile salts fell from 5-10 mM/1 to less than 5 mM/1, with the greatest reduction occurring in the case of taurine conjugates. The free bile acids do not participate optimally in micelle formation in a slightly acid environment (Hofman and Small, 1967). Moreover, they may be in a non-ionized form and passively absorbed along the entire small intestine (Dietschy et al., 1966; Hislop et al., 1967).

Bile salt deconjugation leads directly to fat malabsorption, with malabsorption of other nutrients being secondary. This includes fat soluble vitamins A, D, E and K, vitamin B_{12}, as well as electrolytes and possibly amino acids. Non-polar solutes, such as cholesterol and fat-soluble vitamins are absorbed neglibly, if at all, when micelles are not present (Hofman, 1961). Fat malabsorption may indirectly

affect water and electrolyte balance. Soong et al. (1972) indicated that undigested fatty acids resulted in the formation of hydroxy fatty acids that mediated the diarrheal condition in gluten-induced sprue. Both water and electrolyte metabolism was impaired, in particular the total potassium pool was reduced, as was calcium retention. Calcium combines with the unabsorbed fatty acids and is excreted in the feces as calcium soaps (Fink and Lazlo, 1957). Magnesium behaves similarly (Balint and Hirshowitz, 1961). Impaired fat digestion has been suggested to result in poor digestion of other feed components, which may be rendered indigestible by fat covering food particles (Mayes, 1979).

Similar changes to those occurring in humans suffering steatorrhea have been observed in nutrient retention in the case of chicks fed rye. They too suffer from poor retention of fats, amino acids and minerals. Fat in particular is severely affected and the rachitic state frequently observed tends to indicate that in some instances vitamin D absorption may be inadequate. The levels of excreta soap are also increased. Electrolyte imbalance has been implicated in the rye response, although most evidence indicates that this is most likely a direct effect of increased fecal mass. Under some instances, ie. when fat malabsorption is severe, undigested fatty acid or hydroxy fatty acid may contribute to this effect, although this has not been determined specifically for poultry.

Chicks fed rye also seem to respond to similar types of treatments as humans or experimental animals undergoing bacterially induced steatorrhea. Fat retention may be considerably improved by feeding appropriate antibiotics or alternatively,raising chicks in a germ - free environment. Feeding conjugated bile salts elicits a similar improvement. Antibiotics, or germ-free environment, also improve bone ash when rye - fed chicks are in a rachitic state. Moreover, addition of Streptococcus, which inhabit the small intestine, to germ-free chicks duplicates the response of chicks fed rye in a conventional environment. Ward (1981) also observed that fat digestion was improved by substitution of long-chain saturated fatty acids (as triglycerides) with unsaturated triglycerides or short-chain fatty acid triglycerides which resulted in improved fat retention. Replacing long chain saturated triglycerides with medium chain triglycerides is well known to be an effective remedy in steatorrhea, as their water solubility is 100 times greater than the long chain forms, and they are absorbed even in the absence of micellar solubilization (Zurier et al., 1965). The reported poor utilization of saturated fats compared to unsaturated fats by chicks may be explained on the basis of bile salt insufficiency. Generally unsaturated fats require a lower critical micellar concentration for effective solubilization by bile salts. In the case of rye, where bile salt metabolism may be disrupted, the effect would be more apparent for

saturated fat sources than for unsaturated fat sources, as has been demonstrated.

While the rye response is similar in many respects to steatorrhea induced by excessive bacterial proliferation, the evidence implicating the bacterial deconjugation of bile salts is mainly circumstantial, as the levels of conjugated versus deconjugated bile salts have not been determined specifically. The present study indicates that in the absence of bacteria, digestibility coefficients do not vary markedly between chicks fed rye or wheat, although this was in part confounded by the effect of irradiation treatment. The duplication of the rye response by addition of Streptococcus, which are gram-positive and hence susceptible to most, if not all, antibiotics effective as feed supplements provides further indication of the involvement of the microflora in the rye response. Recently, Coates et al. (1981) reported that Streptococcus faecium SY1 (isolated from chickens), with demonstratable growth depressing qualities, deconjugated bile acids both in vivo and in vitro.

Other explanations of the mechanics of the anti-nutritional effects of rye also lack definitive evidence as to what is the exact nature of rye induced growth depression. McGinnis and co-workers (MacAuliffe et al., 1976_{ab}; MacAuliffe et al., 1979; Patel et al., 1980, 1981) feel there are several factors affecting utilization in rye diets; one of which is an irradiation-sensitive Vitamin D

antagonist. This they propose as being separate from the factor which depresses nutrient utilization. Marquardt and co-workers (Ward, 1981; Antoniou, 1980; Misir, 1977) generally indicate a specific binding effect of the pentosan gum component; or alternatively, a reduction in absorption due to increased viscosity and consequent reduced diffusion rate. In a similar vein, the pentosan gums in the rye seed are suggested to manipulate water 'structure' (Shearman et al., 1973). Undoubtedly if this occurred in the gut it too would affect free diffusion and nutrient absorptive processes.

Such explanations alone cannot readily account for the variety of effects either supplementing diets with antibiotics or raising the chicks in a germ-free environment have. These effects include improved fat retention, amino acid retention, bone ash and reduced excreta soap levels. Based on these results it is the author's contention that, while the gum components likely exert a direct effect, this condition is severely aggravated in situations where bacterial overgrowth becomes apparent.

Based on SCWL chick growth trials conducted in this study, the bacterial effect is not always evident as indicated by the minimal response in two of the experiments to procaine penicillin supplementation. This may have been due to several factors including oil as opposed to tallow being used as a supplementary fat source, the comparatively

high level of salt or environment and the inclusion of fish meal in the diet. The performance of the chicks fed rye was relatively good, hence the magnitude of response in these particular studies left little room for improvement. Where there was a severe growth depression both the germ-free environment and irradiation improved performance.

Gamma irradiation of rye greatly reduces the capacity of carbohydrate components to increase viscosity (Campbell et al., 1982). The fact that it has a beneficial effect on pectin, guar, rye (Patel et al., 1980) as well as hulless barley (Classen et al., 1982), all of which are or contain viscosity-increasing components, provides additional evidence that irradiation destruction of viscosity increasing gums is responsible for the improvement. A more practical means of achieving the same effect may be to develop enzymes that will work efficiently in the micro-environment of the chick's gut. Of particular interest would be fungal enzymes which have optimal activity at the low pH predominant in the upper regions of the chick's digestive tract (Classen et al., 1982). Unfortunately, while there has been considerable effort directed towards describing the antinutritional effects of rye and how it is affected by other feed ingredients, thus far at least there has been no comprehensive study as to what combination of ingredients, including alternative fat sources, enzymes, irradiation and antibiotic treatments, that may minimize the rye response and

render rye a useful feed ingredient from a practical standpoint. The wide variation in response seen between trials observed both in the present studies, as well as those of Lee (1981), would indicate rye is, at present, unsuitable for widespread use in chick diets.

Rye would seem to hold more promise as a feed ingredient in laying hen diets. As opposed to chicks, which gave a linear response, results with laying hens indicate a curvilinear response with rye being fully equivalent to wheat providing the level is kept below the breaking point of the curve. The results of the laying hen trials gave conflicting results as to the location of the breaking point. A reasonable level for maximal inclusion of rye in laying hen diets may be considered to be 30 per cent, which should allow an adequate safety margin.

While laying hens may give a similar response to chicks in the sense that feed consumption and overall production is reduced, they do not necessarily respond to treatments shown to be effective in chick studies. Laying hens do not respond to antibiotic supplementation (Lee, unpublished data; Patel et al., 1981) or to enzyme supplementation, which Patel et al. (1981) interpret as evidence for an additional separate antinutritional factor in rye that is specific for laying hens. A more likely possibility would seem to be a lack of activity of the enzyme supplement in the more acid condition of the hens gut as compared to the chicks. Gamma irradiation

was an effective means of alleviating the depressed production in the case of laying hens (Patel et al., 1981), which would tend to indicate the same or similar functions are involved, although the manifestation of these effects may be somewhat different.

Gums in general have been shown in a variety of experimental animals to reduce rate of passage, reduce rate of nutrient absorption and alter fat metabolism. Such changes could be beneficial and used to some advantage in certain situations. In particular, slowing the rate of absorption of nutrients could possibly be used to advantage in broiler breeder diets, where an extension of the absorptive period could reduce the stress of the starvation in restricted feeding programs. The soluble pentosan complex may also provide a substrate source for microbial production of propionic acid, which being glucogenic would alleviate the ketotic condition associated with the starvation period. The effect rye appears to have on fat retention, endogenous fat loss, and plasma cholesterol levels may also be beneficial to obesity-prone broiler breeders. Of interest might also be whether similar effects occur when rye is fed to humans. The role of fibre in human diets as a means of lowering body fat as well as cholesterol levels is currently under intensive study. Rye, if studies with chickens are any indication, may prove a particularly effective fibre source for such purposes.

10.0 GENERAL SUMMARY AND CONCLUSIONS

Feeding rye to broiler chickens resulted in severely depressed growth which was attributed to malabsorption of most nutrients. Fat malabsorption was particularly severe, and chicks fed rye were often rachitic. The performance depresson could be alleviated by rearing the chicks in a germ-free environment and irradiating the diets. A similar condition to the conventional environment could be induced by associating axenic chicks with Streptococcus, although the response was much less severe. Overall the results were consistent with the hypothesis that chicks fed diets containing rye may suffer a bacterial-induced steatorrhea, possibly as a consequence of excessive bile salt deconjugation. Fat retention could be improved considerably by sodium taurocholate supplementation, and improved marginally by very high levels of calcium in the diet. Feeding rye also appeared to alter cholesterol metabolism as evidenced by reduced plasma cholesterol levels in chicks, and a tendency for reduced plasma cholesterol in roosters combined with an apparent trend towards increased cholesterol turnover rate.

Studies with adult fowl indicated that rye, and the water-extractable fraction in rye in particular, increased wet excreta mass as well as loss of electrolyte components. This appeared to be a general bulking effect caused by a

higher fibre level in the rye as compared to wheat or corn, rather than any type of toxicity per se. Roosters could not extract electrolytes from the excreta beyond a certain critical level which lead to increased losses when rye constituted the cereal base of the diet.

Studies intended to determine a position for rye in practical diet formulation indicated that rye may be incorporated in laying hens diets with no penalty in performance provided that the level of rye was not permitted to exceed about thirty per cent. Several trials with chicks fed diets containing soy oil, fish meal and high salt levels indicated only a slight depression for chicks fed rye diets, although the inconsistency observed between trials indicated the rye should not be fed to growing birds.

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