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Effects of Cellulase from Trichoderma Viride on Wheat Bran Utilization and the Minerals Influenced by Cell Wall Components in Broiler Diets

Kee Thomas Nahm

EFFECTS OF CELLULASE FROM TRICHODERMA VIRIDE ON WHEAT BRAN UTILIZATION AND THE MINERALS INFLUENCED BY CELL WALL COMPONENTS IN BROILER DIETS

BY

KEE HONG NAHM

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

C. W. Carlson
Thesis Adviser Date

J. R. Romans
Head, Animal & Range Sciences Department Date

EFFECTS OF CELLULASE FROM TRICHODERMA VIRIDE ON WHEAT BRAN UTILIZATION AND THE MINERALS INFLUENCED BY CELL WALL COMPONENTS IN BROILER DIETS

Abstract

KEE HONG NAHM

Under the supervision of Professor C. W. Carlson

Two studies were conducted to evaluate the effect on the productive value of a diet for broilers containing high levels of wheat bran with a supplement of cellulase from Trichoderma viride. In both studies, broiler-type, mixed sex chicks were divided into replicate groups of 10 birds each and fed for 5 weeks. The wheat bran was defatted and added at 0, 10 and 20% levels. A fourth group received the 20% wheat bran plus a cellulase enzyme added at the level of 0.008%

The summarized data showed that the 20% wheat bran plus cellulase treatment had no significant effect on feed consumption and feed-to-gain ratio compared with the 10% wheat bran group.

Cellulase supplementation significantly increased dry matter content and ash content of the excreta and significantly improved the digestibilities of dry matter and all cell wall components of the excreta.

In the second part of the study, after the five week experimental period, the four diets with 1% chromic oxide were fed for five hours, after which the chicks were fasted for 14 hours. Feces were collected for 8 hours after withdrawal of the diet (13 hours after feed was offered). The excreta were collected in two parts, one voided in the

daytime (from 7:30 a.m. to 8:30 p.m.) and the other during the night (from 5:30 p.m. to 6:30 a.m. the next day) and analyzed separately.

Calcium, phosphorus, iron, zinc and copper associated with cell walls largely were solubilized by cellulase. Iron balance was negative in the 10% and 20% wheat bran groups. However, ferrous or ferric iron which is bound by bran was readily made available for absorption by cellulase.

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KHN

DEDICATION

This thesis is dedicated to the author's father and mother in KOREA. The homelife they provided the author during the early years of his life has resulted in a solid foundation that constantly increases in value with the passage of time.

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INTRODUCTION

As we proceed further into the 1980's, a review of the world food situation and global grain prospects is timely. World population growth was the single most significant factor providing impetus to rapid increases in demand for total grains over the past three decades. Although population growth was not evenly distributed, and many areas simply could not provide the grain to keep pace with population increases, it seems certain that the population factor accounted for about half to two-thirds of the increase in grain consumption over the past three decades (Barr, 1981).

The public tendency to view world food problems only in terms of crisis has done a profound disservice to the world's hungry. Food crises do occur but they often are only warning episodes of a critical underlying trend. We must understand and master how to save our current feeds and foods and to develop new feeds and foods. It has been recognized for a long time that biomass in the form of cellulose, hemicellulose and lignin provides a means of collecting and storing solar energy.

Before using this resource, it is necessary to convert it into a usable form. For this reason, a variety of strategies are being explored. One of the widely used approaches towards biomass utilization is the enzyme - catalyzed hydrolysis of cellulose and hemicellulose to low molecular weight compounds. Much work has been done on the use of an enzyme produced by the fungus Trichoderma viride. Fibrous material is generally not well assimilated by chicks.

Furthermore, the effect on the growth of chicks sometimes may be quite derogatory.

It has long been recognized that enzyme supplementation in barley diets can improve the nutritive value of barley based on the results of experiments, mainly with poultry. However, little is known as to how enzyme additions to high bran diets might affect the fiber digestibility and growth of chicks.

The objectives of the studies reported here were:

1. To compare the growth rate and feed utilization of chicks as influenced by cellulase added to a diet containing wheat bran.
2. To study of effect of cellulase on the digestibility of cell wall components and dry matter.
3. To examine how much digestion of each cell wall component and dry matter occurs in each segment of the GIT.
4. To investigate the extent to which minerals associated with cell walls are solubilized by cellulase in the GIT.
5. To determine whether cellulase has a role similar to that of phytate on fiber in the GIT.

Parameters measured were body weight change, feed intake and efficiency, gut dimensions, chromium turnover time, dry matter and ash content in each segment of the GIT and excreta and apparent digestibilities of dry matter and each cell wall component in the feed, feces and fiber fractions.

REVIEW OF LITERATURE

The structure and morphology of lignocellulosic residues.

The structure and morphology of cellulose was described as the cellulose chain conformation with its intrachain hydrogen bonds, the fibrillar morphology of native cellulose containing very thin elementary microfibrils, the acid hydrolysis of cellulose fibers to microcrystalline cellulose giving rodlike particle (micelles) of the same width as the fibrils, and the new but controversial helical model of the cellulose microfibrils.

Penetration measurements of native cellulose fiber wall using aqueous polymer solutions have given average pore dimensions of 5 A to 10 A. Accessibility measurements using deuterium and tritium oxide exchange have shown that native cellulose probably is deposited in a crystalline form (Ramby, 1969).

Bisaria and Ghose (1981) reported that cellulose, a linear homopolymer of anhydroglucose units linked by B(1→4) glucosidic bonds, does not occur in pure form in any natural resource. Even the seed hair of cotton, the most pure form of cellulose readily available in nature, contains about 10% by weight of non-cellulosic polysaccharides, proteins and mineral elements. In nature, cellulose is always associated with a variety of other polysaccharides, such as starch, pectin, lignin and a variety of hemicelluloses.

The hemicelluloses are polymers of galactose, mannose, xylose, arabinose, other sugars and their uronic acids. These are usually classified according to the sugar residue present, e.g. D-galactan,

D-mannan, D-xylan, L-arabinan, etc. However, they do not occur as homoglycans but rather as heteroglycans containing different types of sugar residues, often as short appendages linked to the main backbone chain (Atalla, 1979). The hemicelluloses rank next to cellulose as the most abundant natural organic chemical in the biosphere. The hemicelluloses are present in all layers of the plant cell wall, but are concentrated mainly in the primary and secondary layers, where they occur closely associated with cellulose and lignin (Bisaria and Ghose, 1981).

Adler (1977) reported that lignin, one of the major components of lignocellulosic residues, is a natural polymeric product arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: trans-p-coumaryl alcohol, trans-comiferyl alcohol and trans-sinapyl alcohol. Freudenberg and Neish (1968) said that a typical softwood (Spruce) lignin contains approximately 80% coniferyl alcohol, 14% p-coumaryl alcohol and 6% sinapyl alcohol. Hardwood lignin, on the other hand, contains similar amounts of coniferyl and sinapyl alcohol and a minor amount of p-coumaryl alcohol. According to Nimz (1974), there are three major intermonomer linkages in lignin: a) the arylglycerol-B-aryl ether type, b) the phenyl coumaryl structure and c) the biphenyl structures. B-aryl ether structures, pinoresinol structures and benzyl ether bonds are also present. This formula for lignin, like other formulas, shows only an arbitrary sequence of phenylpropane units from a very limited part of the lignin macromolecule.

Microorganisms involved in cellulose, hemicellulose and lignin-degrading enzymes.

The availability of high activity cellulose is the basis for a successful process for the enzymatic conversion of cellulose. Although many fungi and bacteria degrade cellulose, much of the products of growth are microbial cells and metabolic products such as carbon dioxide and methane, of limited use to animals. Bisaria and Ghose (1981) reported that fungi such as Trichoderma reesei (formerly called Trichoderma viride), Trichoderma Koningii, T. lignorum, Sporotrichum pulverulentum (formerly called Chrysosporum lignorum), Penicillium funiculosum, P. iriensis, Aspergillus wentii, Polyporus adustrus, Fusarium solani, F. lini, Sclerotium rolfsii, Eupenicillium javanicum, Schizophyllum commune and a few bacteria such as Clostridium thermocellum, Thermomono spora sp., Cellulomonas sp., and Streptomyces flavogriseus, are known for their ability to produce high activity cellulase capable of extensively degrading insoluble cellulose to soluble glucose in vitro.

In addition, Reese and Mandels (1980) reported that there are many organisms which produce cellulase which degrade only soluble cellulose derivatives such as carboxylmethyl cellulose (CMC). A few organisms produce very little residual cellulase of any type despite their active growth on insoluble cellulose. Cellulase is a complex of enzymes or enzyme-like factors and all components of the cellulase complex are not necessarily found in the culture fluid after growth of an organism.

Su and Paulavicius (1975) noted that some thermophilic organisms, such as an Ascomycete, Chaetomium thermophile var. dissitum, Humicola sp. and a Thermomonospora sp., also produce a cellulase enzyme system capable of degrading native cellulose.

Thermophilic organisms are being looked at as a source of thermostable cellulases; however, cellulases from thermophiles may not necessarily be more heat-stable than cellulase from mesophiles.

Most of the work on hemicellulases has been concerned with xylanases, since their substrate xylans constitute the largest proportion of hemicelluloses in pasture plants (Bisaria and Ghose, 1981). Xylanases have been detected in several rumen bacterial species, such as Bacillus firmus, B. pumilus, Bacteroides amylogens, Butyrivibrio fibrosolvens, Ruminococcus albus and Clostridium species (Nakanishi and Yasui, 1980).

Fungi such as Myrothecium verrucaria, Aspergillus oryzae, A. niger, A. Wentii, A. terreus, Coniphora cerebella, Trichoderma reesei, Chaetomium trilaterale and Penicillium janthinellum also produce xylanase enzymes (Nisizawa et al., 1971).

Crawford and Crawford (1980) reported that soft-rot fungi belonging to species of Paecilomyces, Allescheria, Preussia, Chaetomium and Stachybotrys, brown-rot fungi such as Poria monticola, P. cocos and Lanzites trabea, and white-rot fungi belonging to species of Coriolus versicolor, Polyporus anceps, Phanerochaete chrysosporium, Sporotricum pulverulentum, Aspergillus funigatus, Polyporus versicolor, P. hirsutus, Poria subacida, Polyporus abientinus, Gleoporus

dichrous and Lentinus edodes have been found to have the capacity to degrade lignin to varying degrees. Bacteria capable of degrading lignin or lignin related phenolic compounds include species of Nocardia, Streptomyces, Pseudomonas, Flavobacterium, Aeromonas, Bacillus, Agrobacterium and Xanthomonas.

Fractionations of enzymes and factors to influence the biological degradation of soluble cellulose.

Cellulase is a complex of enzymes containing mainly exo-glucanases and endo-glucanases, plus cellobiase which is B(1→4) glucosidase (Reese et al., 1950; Wood and McCare, 1972; Berghem and Pettersson, 1973; Eriksson, 1978; Ghose and Bisaria, 1979; Ryu and Mandels, 1980).

The most acceptable trend is to consider the cellulase system as a complex of several components as follows:

1. Endo-B(1→4)-glucan glucanases are present in several components varying in degree of randomness. One of these may be the enzyme that acts first on crystalline or highly ordered cellulose.
2. Exo-B(1→4)-glucanases are present in two major forms:
   a) B(1→4)-glucan cellobiohydrolase (CBH) removing cellobiose units from the non-reducing ends of the cellulose chain.
   b) B(1→4)-glucan glucohydrolase, which removes glucose units from the non-reducing end of the chain.
3. Cellobiase, B(1→4)-glucosidase, which converts cellobiose and other cellodextrins into glucose.

They reported that for the complete hydrolysis of insoluble cellulose, a synergistic action between these components is required. Since different cellulase preparations vary widely in the proportions of the different components, depending on source, growth conditions and harvesting and handling procedures, the rate and extent of their hydrolysis of cellulose substrates also vary widely.

The most intensive fractionation studies have been carried out on cellulases elaborated by Trichoderma reesei (Selby and Maitland, 1967; Nisizawa et al., 1971; Berghem and Pettersson, 1973; Reese and Mandels, 1980; Nevalainen et al., 1980), Trichoderma koningii (Wood and McCare, 1972; Halliwell and Griffin, 1973), and Sporotrichum pulverulentum (Eriksson, 1978) using standard protein separation procedures.

There are techniques such as solvent precipitation, ion-exchange chromatography, molecular sieve chromatography and isoelectric focusing. Several scientists demonstrated that the components are present in multiple forms, often as isoenzymes. Bisaria and Ghose (1981) reviewed the literature providing a comparison of cellulases from different microorganisms with respect to certain characteristic protein parameters such as molecular weight and isoelectric pH as shown in Table 1.

Ghose and Bisaria (1979) purified xylanases from Trichoderma reesei (Trichoderma viride) by DEAE - Sephadex Chromatography. Xylanases of the endo type [B(1→4)-D-xylan xylanohydrolase] are the only types that have been characterized. Although exo-xylanases

Table 1. Characteristic protein parameters of cellulase components isolated from different microorganisms

Organism	Cellulase components	Molecular weight	Isoelectric pH	References
T. reesei	Exo-glucanase	42000	3.79	Reese and Mandels, 1980
	Endo-glucanases			
	I	12500	4.60	
	II	50000	3.39	
	B-Glucosidase	47000	5.74	
T. koningii	Exo-glucanases			Wood and McCare, 1972
	I	62000	3.80	
	II	62000	3.95	
	Endo-glucanases			
	Cx_1	13000	4.73	
	Cx_2	N.A.	N.A.	
	Cx_{3a}	38000	4.32	
	Cx_{3b}	38000	4.32	
	Cx_4	31000	5.09	
	Cx_5	N.A.	6.28	
	B-Glucosidases			
	I	N.A.	5.53	
	II	N.A.	5.85	
S. pulverulentum	Exo-glucanase	48600	4.3	Eriksson, 1978
	Endo-glucanases			
	T_1	32300	5.32	
	T_{2a}	36700	4.72	
	T_{2b}	28300	4.40	
	T_{3a}	37500	4.65	
	T_{3b}	37000	4.20	
	B-glucosidase	N.A.	N.A.	

N.A. = not available

[B(1→4)-D-xylan xylohydrolase] have been found, purification was not accomplished. They showed that the molecular weights of the endoxylanases have been found to lie between 16,000 and 39,000, and the isoelectric pH of xylanases was on the acidic side, between 3.9 and 4.5.

Ghosh and Kundu (1980) reported that B-xylosidase, which has an action similar to the B-glucosidase component of the cellulase system, causes hydrolysis of B(1→4) bonds at the non-reducing ends of xylooligosaccharides and B(1→4)-aryl xylopyranosides.

Bisaria and Ghose (1981) reviewed the common range of cellulase components' molecular weights and isoelectric points. The most common range of molecular weights is probably 12,000 to 80,000. The smallest and largest molecular weights of endo-glucanases are 5,300 and 14,500, respectively.

B-glucosidases appear to have larger molecular weights than exo- and endo-glucanases; the largest molecular weight of B-glucanase found so far is 400,000. The isolectric points of all the components were on the acidic side, pH <6.3. The most common ranges of isoelectric pH seems to be as follows, exo-glucanases, 3.7 to 4.2; endo-glucanases, 3.3 to 6.2 and B-glucosidases, 5.5 to 5.9.

The Cellulase from Trichoderma reesei (Trichoderma viride)

Selby and Maitland (1967) fractionated culture filtrates from Trichoderma viride (T. viride) by gel filtration on Sephadex G-75 followed by ion-exchange chromatography on DEAE- and SE-Sephadex. The components essential for attack on cotton are carboxymethyl cellulase, a cellobiase and a third (C_1) component which has no action on

CM - cellulose, cellobiose or cotton. These components, which together can completely convert cotton into water-soluble products, lose their ability when separated and regain it quantitatively when recombined in their original proportions. Berghem and Pettersson (1973) isolated a cellulolytic enzyme ("C_1" enzyme which is the exo-glucanases) from a commercial cellulase preparation derived from culture filtrates of the fungus Trichoderma viride. The purification method is a four-step procedure including chromatography on Bio-Gel P-10, DEAE-Sephadex chromatography, isoelectric focusing and chromatography on Bio-Gel P-60. A yield of 144 mg enzyme was obtained per 100 g commercial cellulase. Crystalline cellulose (Avicel), phosphoric acid-swollen Avicel and cellotetraose were degraded by the enzyme and in each case the principal reaction product was cellobiose.

Nevalainen et al. (1980) reported that a mutant strain with increased production of cellulolytic enzymes was induced from the good cellulase producer, T. reesei QM 9414. Cellulase activities of the mutant in fermenter cultivations were increased two- to three-fold and B-glucosidase activity up to six-fold when compared to the corresponding activities produced by QM 9414.

Reese and Mandels (1980) indicated that the cellulases of Trichoderma were remarkably stable and resistant to inhibitors and other toxic components. Enzyme stability studies have been reinvestigated under the conditions used for cellulose hydrolysis (pH 4.8, 50°C, 24 hr). The cellobiohydrolase (CBH) component as measured on Avicel is less stable than other enzymes of the cellulase complex, and

is 60% inactivated by merthiolate (and other Hg compounds) under the above conditions. Endo- B(1→4)-glucanase is much more stable, and more resistant to merthiolate and other compounds. Under unshaken conditions the Avicelase of the Rutgers strain C30 shows greater stability to heat than that of other available strains. Biocides must be selected not only for their ability to prevent contamination, but also for their compatibility with cellulases.

Glutaraldehyde treatment greatly increased the enzyme size, lowered the pI values, and gave a slight shift in the pH activity curve.

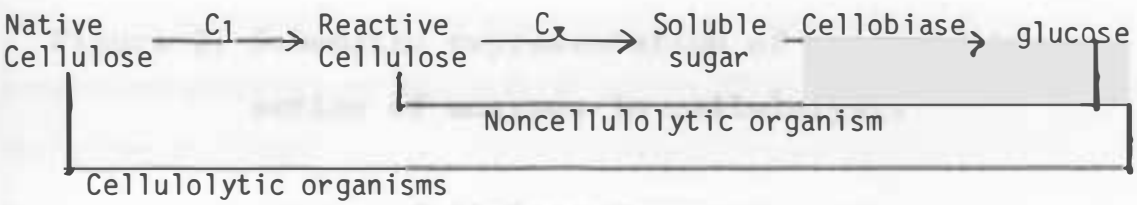
Ryu and Mandels (1980) said that strains of Trichoderma, particularly Trichoderma reesii and its mutants are good sources of extracellular cellulase suitable for practical saccharification. They secrete a complete cellulase complex containing endo- and exo-glucanases plus B-glucosidases (cellobiase) which act synergistically to degrade totally even highly resistant crystalline cellulose to soluble sugars.

Mode of action of cellulases and hemicellulases

The degradation of crystalline cellulose is a complex process requiring the participation of many enzymes.

Reese et al. (1950) suggested a route for the conversion of native cellulose to soluble sugar based on a two-step sequential (Figure 1).

Figure 1. Reese's concept.



They indicated that the ability to develop an enzyme capable of hydrolyzing the 1,4-B-glucosidic linkage found in cellulose and its derivatives is widespread among microorganisms. The classical enzyme "Cellulase", presumably converting native cellulose to sugars, consists of at least two systems. The first system designated as C_1, exists preliminary to hydrolysis of the straight chain by C_x. Throughout their work the assumption was made that C_x, the enzyme hydrolyzing the 1,4-B-glucosidic linkage in CMC (carboxymethyl cellulose), is also the enzyme attacking the same linkage in cellulose. This belief is based on the presence of C_x in the filtrates of cellulolytic organisms grown on cellulose.

Wood and McCare (1972) showed that the endo- and exo-glucanases have a strong synergistic action as indicated in Figure 2.

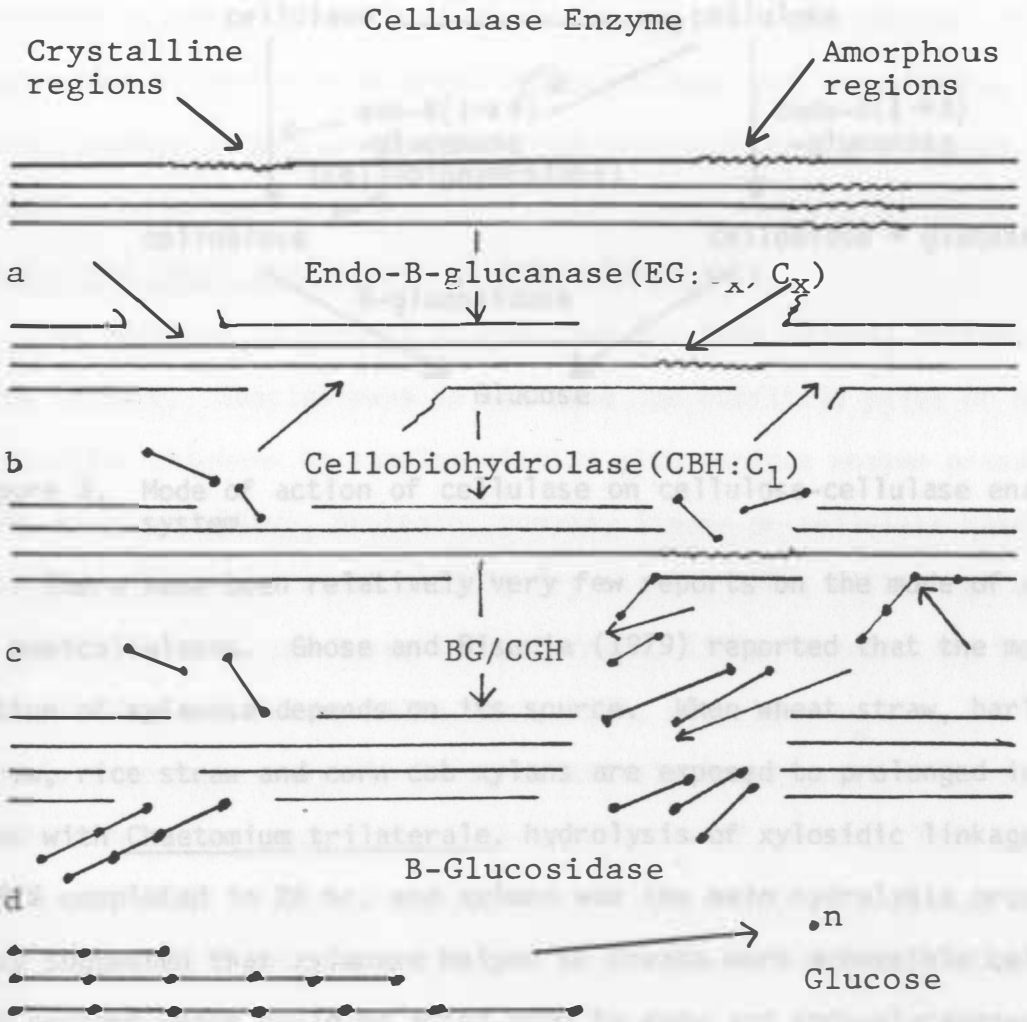
Eriksson (1978) suggested that:

a) Regions of low crystallinity in the cellulose fiber are attacked by endo-glucanases and free chain ends are created.

b) Exo-glucanases start the degradation from the chain ends by hydrolytically removing cellobiose.

c) Cellobiose is hydrolyzed to glucose through the action of B-glucosidase.

Figure 2. Schematic representation of synergistic action of enzymes in cellulolysis



The pattern is now understood (Ryu and Mandels, 1980) to be based on action of endo-B-glucanases, exo-B-glucanases and cellobiases. A simplified reaction and the mode of action of such cellulase complexes are shown in Figure 3.

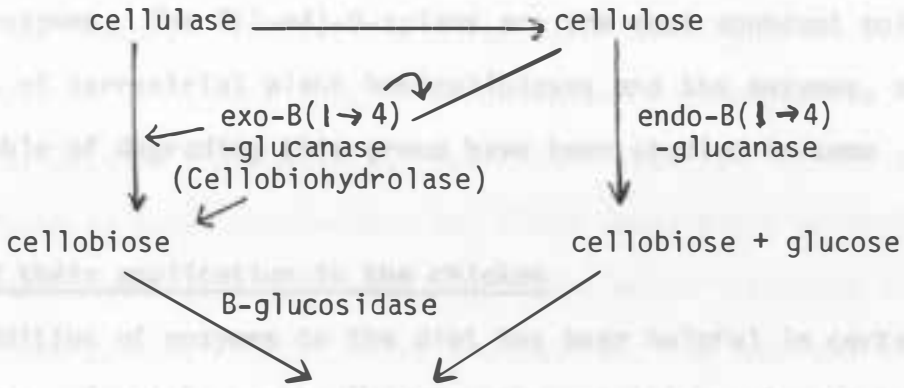


Figure 3. Mode of action of cellulase on cellulose-cellulase enzyme system.

There have been relatively very few reports on the mode of action of hemicellulases. Ghose and Bisaria (1979) reported that the mode of action of xylanase depends on its source. When wheat straw, barley straw, rice straw and corn cob xylans are exposed to prolonged incubation with Chaetomium trilaterale, hydrolysis of xylosidic linkage was 92% completed in 24 hr. and xylose was the main hydrolysis product. They suggested that xylanase helped to create more accessible cellulosic regions which could be acted upon by exo- and endo-glucanases by hydrolyzing the xylan component, thereby resulting in higher sugar production. Xylanase action was found to be most effective when acting synergistically with other hydrolytic components, viz. exo- and endo-glucanases.

Bisaria and Ghose (1981) noted that hemicellulases, like cellulase, also occur in two basic forms, i.e. exo-type and endo-type, the endo-type being more common. There are both types of L-arabinanases, D-glactanases, D-mannanases and D-xylanases among hemicellulose degrading enzymes. The B(1→4)-D-xylans are the most abundant polysaccharides of terrestrial plant hemicelluloses and the enzymes, xylanases, capable of degrading this group have been studied in some detail.

Enzymes and their application in the chicken

The addition of enzymes to the diet has been helpful in certain chick studies. Special ways to increase the nutritive value of barley for broiler chickens by supplementation with various enzyme preparations such as amylase, protease, gumase, lipase or cellulase have been demonstrated (Jensen et al., 1957; Fry et al., 1957; Willingham et al., 1959; Arscott and Rose, 1960; Burnett, 1965; Peterson and Sauter, 1968; Herstad and McNab, 1975; Gohl, 1977; Hesselman et al., 1982).

In experiments with poultry, relatively low digestibility coefficients have been reported for the nutrients of barley. This has been reported to be associated with the high levels of carbohydrates other than starch and disaccharides in the grain (Petersen, 1972) and with the presence, at least in certain barleys, of B-glucan in the hemicellulose fraction. The sticky droppings which occur when barley-containing diets are fed to chicks were also attributed to B-glucan (Burnett, 1965). The occurrence of sticky droppings which often is associated with high inclusions of barley in diets, has been decreased

by enzyme supplementation (Gohl, 1977, Qureshi et al., 1980).

Willingham et al. (1959) already demonstrated that a highly significant improvement in growth and feed utilization occurred when diets containing barley were supplemented (2g/lb feed) with enzymes from bacteria. However, crystalline α -amylase was completely inactive. Variety of barley had little influence on chick growth as influenced by enzymes from bacterial and fungal sources. No significant difference in chick performance was found among eight varieties. All eight were significantly improved by either water treatment or enzyme supplementation. Barley autoclaved wet was not improved by subsequent water treatment but was improved by dietary enzyme supplementation.

Herstad and McNab (1975) discovered that supplementing diets containing commercial barley with B-amylase produced slightly conflicting results in that there was an improved weight gain, feed conversion efficiency and digestibility value in two of three experiments. The digestibility and metabolizable energy values of American six row spring barley (Glacier) were significantly improved by enzyme supplementation. The effect of the enzyme on diets containing a high amylose barley (Glacier Pentlandfield) was positive but not significant. The digestibility of the high amylose barley is also low but the enzyme has less effect on the high amylose barley than on Glacier.

Quyeshi et al. (1980) reported on the effects of Trichoderma viride cellulase on cholesterol and lipid biosynthesis in chicks. When diets were supplemented with cellulase, low body weights were

corrected. Cholesterol synthesis was suppressed even more by inhibiting B-hydroxy-B-methylglutaryl-CoA (HMG-CoA) reductase, and the reduced conversion of acetate and mevalonic acid to cholesterol. Chicks fed barley and cellulase (0.008%) showed 90% lower cholesterol synthesis than controls. Plasma and liver cholesterol were decreased when the culture filtrate (cellulase) was added to the other cereal diets. Acetyl-CoA-carboxylase and fatty acid synthetase showed a two- to six-fold increase with cereals and a three- to eleven-fold increase with cereal plus cellulase supplemented diets. The drastic decreases of cholesterol biosynthesis observed indicate the presence of an inhibitor in cellulase, which may provide insight into the control mechanism for cholesterol production.

Hesselman et al. (1982) reported that B-glucanase supplementation (5g/kg feed) of chicken diets significantly improved feed consumption, weight gain and feed conversion ratio. Also B-glucanase increased dry matter content of the excreta and improved cleanliness of the cages. Enzyme supplementation of the diet as a dry preparation was slightly superior to adding B-glucanase through the drinking water.

Heger et al. (1983) compared the effectiveness of an enzymatic preparation (Mikrozyme) in diets for broiler chicks and growing rats. In the chick experiments, four levels of enzyme preparation (0, 0.1, 0.5 and 1.0% of diet) and two types of diets were used. Growth rate and feed efficiency of chicks were slightly improved by enzyme supplements. In comparison with the control group, the average daily weight gains and feed efficiency of chicks fed the diet supplemented with

0.05% enzyme increased by 7.5 and 3.7% respectively.

In balance experiments on growing rats, supplements of the 0.1 or 1.0% enzyme preparations to a cereal diet did not significantly affect true and apparent digestibility of nitrogen, biological value of protein or net protein utilization.

Parrish et al. (1960) demonstrated the presence of B-glucan composed of (1→3) and (1→4) linked glucose units in barley.

Aastrup (1979) noted that the viscosity of barley extracts is mainly caused by a soluble B-glucan.

Gohl (1978) reviewed an enzyme system in barley that involves endo-B-glucanase, causing a decrease in solution viscosity without any large development of reducing groups, and exo-B-glucanase, which liberates substantial amounts of glucose. The large increase in glucose parallels a decrease in viscosity. The vicosity of barley extracts is mainly caused by a soluble B-glucan. Also, other low-molecular carbohydrates, i.e., arabinose and xylose, were liberated during water-treatment, indicating that in addition to B-glucan other polysaccharides were also hydrolyzed.

In an earlier study, Gohl (1977) observed that the moisture content of feces increases with the levels of hydrocolloids in the feed, thus indicating that sticky droppings are caused by hydrocolloids present in barley. The moisture content of excreta increased with the viscosity produced by the barley. The hydrocolloid content of barley can be reduced through the action of endogenous enzymes during water-treatment or by addition of an enzyme capable of hydrolyzing B-glucan.

Addition of B-glucanase to barley caused a substantial increase in the dry matter content of excreta.

Willingham et al. (1959) also reported that chicks fed diets containing barley or wet-autoclaved barley had significantly more moisture and less ash in their feces than birds receiving diets containing corn, barley supplemented with enzymes, or water treated barley. Fecal material from birds fed diets containing regular or autoclaved wet barley adhered to the wire screen floors. This condition was less prominent when birds received enzyme supplemented diets, and did not occur when the diets contained corn or water treated barley. The increased ash content of feces is another measurement indicating improved feed utilization.

Tolerance of crude fiber in the chick gut

Fibrous material is generally not well assimilated by a growing chick; furthermore, the effect of fiber on the growth of a chick sometimes may be quite derogatory depending on the quantity of the fibrous material used in the diet. This effect is generally considered to be attributable to a reduction in the energy intake (Fraps, 1946; Peterson, 1950). Morris et al. (1932) reported that the amount of fiber in a chick ration could be increased to as much as 8 or 9% of the ration without showing harmful effects on mortality, rate of growth, feed consumption, age of mortality and egg production.

Halnan (1949) observed that it is not possible to set a precise limit for the level of fiber in poultry feed mixtures. It is apparent from the point of view of production that a level of about 7.5% would

be a desirable upper limit, allowing for a reasonable amount of nutrients for productive use after the maintenance requirements have been met.

Davis and Briggs (1947) discovered that from the addition of 5 to 15% of cellulose to a purified chick diet, a significant increase in the chick's growth resulted. In their later study (1948), they examined supplementary fiber levels in a purified chick diet and found that as high as 20% sawdust could be tolerated without ill-effect. Fisher and Weiss (1956) reported that the maximum possible intake of cellulose-containing diets was regulated by the extent to which the digestive tract could be distended. The addition of increments of cellulose to a basal diet up to a level of 24% elicited a linear increase in dry matter consumption after which the weight of feed consumed per chick was depressed.

Saito et al. (1959) studied the effect of cellulose in the diet on the growth of chicks. The addition of cellulose powder to the basal diet decreased the digestibility of the crude fat, nitrogen free extract and organic matter. Positive nitrogen balance in chicks was found to be the highest in the group fed at the level of 9.5% added cellulose. If the body weight of the chick given the basal diet alone was represented as 100, then the body weights of the chicks given the basal diet with added cellulose powder at levels of 3.5, 9.5, 16.5 and 26.5% were 120, 127, 134, and 130 respectively. Thus the addition of cellulose to a diet containing a low level of crude fiber indicates that there was a beneficial effect on chick growth.

Tasaki and Kibe (1958) said that alpha cellulose fed to birds was found to be degraded partially to beta cellulose in the digestive tract, but the beta cellulose thus produced was not absorbed but was excreted in the excreta. Gamma cellulose was excreted in the same amounts as given in the diet. The same amount of total cellulose ingested, including the different types of cellulose, was excreted in the feces, though a definite quantity of alpha cellulose was changed into beta cellulose in the digestive tract.

Kratzer et al. (1974) discovered that growth of chicks was depressed by approximately 30% when rice bran was used at 60% of the diet to replace corn as an energy source. There was no evidence that there was interference in trace mineral availability. The addition of casein or soybean oil to the diet containing 60% rice bran caused no improvement in growth. Autoclaving or steaming the rice bran caused a marked improvement in growth, indicating a destruction of the growth-depressing factor. The weight of the pancreas of chicks fed rice bran was significantly greater than that of control birds. The growth-depressing effect of the rice bran was noted both in diets containing fish meal or soybean meal as the source of protein.

Dvorak and Bray (1978) studied the effect of dietary cellulose level at various ambient temperatures on the voluntary feed intake of four-week-old chicks. Cellulose (as Solka Floc) was added to a basal diet at levels of 10, 20 and 30% in trial 1 and 15, 20 and 45% in trial 2. The addition of graded increments of cellulose elicited a linear increase ($P<0.05$) in feed intake and a linear depression

($P<0.05$) in growth rate. Cellulose tended to lower ($P<0.05$) gains per unit of basal consumed, indicating that the nondigestible material reduced the utilization of the basal portion of the diet. Differences in feed intakes at 15.6 and 23.9°C were not significant in trial 1, but chicks consumed more ($P<0.05$) feed at 7.2°C than at 23.9° in trial 2.

Halnan (1949) reported that some digestion of fiber occurs in the fowl. He found that, whereas the digestibility of the crude fiber in barley was nil in both a normal bird and one deprived of its ceca, with wheat and maize, the digestibility coefficients for fiber were 5.71 and 17.1%, respectively, for the normal bird, but 1.42% and nil for the bird deprived of ceca.

Halnan (1949) indicated in his review article that the digestibility coefficients for crude fiber were: pure cellulose, 0.0; whole barley, 0.0; ground barley, 0.24; whole wheat, 5.1; ground wheat, 6.78; whole maize, 19.72; ground maize, 19.76; raw peas, 7.03; oats, 9.25; raw white cabbage, 21.07; cooked cabbage, 15.68; and raw savoy cabbage, 14.7. The variation between different feeds, coupled with negative results obtained with filter-paper, shows that the digestibility of crude fiber is associated with its chemical composition and that cellulose per se is indigestible. He said that in the pigeon the digestion of crude fiber was not localized in a particular site but occurred throughout the gut, and that the digestive capabilities of the pigeon for crude fiber in maize, yellow peas, wheat and barley were greater than those of the hen. The duck and goose appear to

possess similar powers of digestion for crude fiber, but are less efficient than that of the hen.

Effect of dietary fiber on the gastrointestinal tract weight

Interspecific comparisons have indicated that amongst gallenaceous birds, the digestive system is larger in relation to body weight in species which eat more fibrous diets (Leopold, 1953; Moss, 1974).

Variations in gut size associated with variations in diet have also been found between different populations and in different seasons within species. The guts of California quail are longer in winter when the diet is more fibrous and less digestible than in the summer (Lewin, 1963) and the guts of wild red grouse, eating mainly heather, are longer than those of captive grouse fed on a concentrated pelleted diet (Moss, 1972).

Deaton et al. (1973) reported that the weight of the gizzard of broilers reared in cages is significantly less compared with that of broilers reared on litter floor pens despite the same nutritional regime being applied to both. Kubena et al.(1974) showed that the addition of 30 g pine shavings/kg in diets of broilers increased gizzard weight of broilers in cages.

Savory and Gentle (1976) observed Japanese quail fed on either a conventional diet (low fiber) or the same diet diluted with 200 g oak sawdust/kg (high fiber). The lengths of the colo-rectum, small intestine, two ceca and empty gizzard weight were measured at 10 and 20 weeks of age. When quail were fed on the low-fiber diet with 0, 200 or 400 g cellulose powder/kg, gut size at 20 weeks of age was

greatest when the most fibrous diet was fed and decreased concomitantly with dietary fiber concentration. They concluded that the main reason for the larger guts of birds fed high-fiber diets appears to be their greater food intake. With increasing dilution of the diet, greater weights and much greater volumes of food were necessary to meet requirements for energy and nutrients; so presumably the guts of these birds had to accommodate more material at a time and provide bigger areas for absorption than those receiving low fiber diets.

Deaton et al. (1977) also showed that gizzard weight could be influenced by dietary fiber. Caged layers were fed on diets containing 15, 30 or 60 g pine shavings/kg diet. As dietary fiber content increased, empty gizzard weight increased.

Cherry and Siegel (1978) found that the weights of the crop, gizzard, duodenum and the total gastrointestinal tract were, when expressed as a percentage of body weight, significantly heavier for male chicks from a low-weight than those from a high-weight line. Duodenal weights of female chicks from the low-weight line were also significantly larger than those from the high-weight line. Effects of the dwarfing gene on various components of the gastrointestinal tract, when expressed as percentage weights, were not significant with one exception. Dwarf females from the high-weight line had lower duodenum weights than the normals while the reverse was true in the low-weight line. In general, the proportionately larger intestinal weights appeared to correspond with a slower clearance of feed through the gastrointestinal tract and may be indicative of altered digestive or

absorptive functions resulting from selection for growth.

Kass et al. (1980) said that the empty weight of the stomach as a percentage of total GIT weight was decreased as dietary alfalfa meal increased ($P<0.01$). Organ weight, when expressed as a percentage of body weight, was increased for total GIT, small intestine, cecum and colon ($P<0.01$) as dietary alfalfa increased.

Effect of dietary fiber on the dry matter content of digesta in feces and in the GIT

Cooper and Tyler (1959a) investigated the physical properties of bran, and other feedstuffs, with regard to their ability to absorb and retain water. Two experiments have been described in which pigs were fed bran, fibrous cellulose, or different levels of powdered cellulose, added to normal pig rations. These experiments involved 24-hour fecal collections, and ultimate slaughter of the animals, together with analysis of fecal material and gut contents. The addition of bran or fibrous cellulose to a ration causes a reduction in the average percentage of dry matter in the feces, but tends to emphasize fluctuations in fecal dry-matter percentage, which are attributed to fluctuations in rate of passage through the large intestine. The behavior of the powdered cellulose was anomalous, giving rise to hard stools. It appeared to be almost inert in relation to water.

In another study (1959b) they showed the higher level (25%) also led to an increased laxative effect, effective as shown by more and larger stools. The availability of fibrous cellulose to absorb water is well known and this was clearly shown by a considerable lowering of

the percentage of fecal dry matter.

Another factor influencing the percentage of fecal dry matter is the residue of non-cellulosic dry matter. For example, two basal rations, one made up of ordinary feedstuffs and the other a synthetic diet, gave quite different percentages of fecal dry matter, the latter being much lower.

In their third report (1959c), they described an experiment in which pigs were fed rations containing four different levels of cellulose, each ration being fed successively at three different levels of water intake. The cellulose levels were superimposed on a highly digestible basal ration. It appeared that altering the level of water intake, while keeping the ration intake constant had only a very limited effect on the level of fecal dry matter percentage.

Savory and Gentle (1976) reported that the main reason for the larger guts of birds fed on the high-fiber diets appeared to be their greater food and water intake. Although the latter was not measured, it is likely that birds fed on high-fiber diets would have consumed more water than those fed on low-fiber diets.

Mongin and Sauveur (1974) observed that the water content varied along the digestive tract and was influenced by egg formation in all parts except the crop. The highest hydration rate always occurred 18 hours after oviposition (the last hour of night), while the lowest rate occurred at the beginning of egg formation. This hydration appears to be largely independent of water intake since the most water is imbibed just after oviposition and during albumin formation. They

suggested that the variations of water content result from a cycle of water secretion in the proventriculus, gizzard or both, and a cycle of absorption of water in the small intestine.

Mongin (1976) demonstrated that the dry matter present in the gizzard (5.30 $\pm$ 0.27 g) and in other segments of the small intestine (0.88 $\pm$ 0.062 g) was independent of the physiological stages of the egg formation and the day-night cycle. Only the dry matter in the crop follows a cyclic variation which is influenced by the photoperiod. He indicated that the crop was empty during the night while the gizzard contained a constant amount of dry matter. The water content of the crop did not change, but that of the gizzard was at a minimum just after the ovulation and at a maximum 18 hours later.

Van Soest et al. (1978) said that fine bran actually decreased fecal water and showed a barely significant effect upon passage. Salka Floc, also finely ground, is similar although it showed a larger fecal bulk due to its unfermentability. The different response between fine and course bran may account for some of the contradictory reports on the effects of fiber in studies where physical form may not have been adequately controlled.

Kass et al. (1980) found that percentage of dry matter in digesta of all sections of the gastrointestinal tract was negatively correlated with cell wall content of the diet.

Digesta rate of passage

Theoretical considerations

Uden (1978) suggested that rules of kinetics and exponential

functions for passage of digesta in the gastrointestinal tract (GIT) of most animals attempt to treat gastrointestine (GI) compartments, such as the forestomach and hindgut, as ideal pools. He indicated that the conditions necessary for compartmental analysis may not always be fulfilled. He developed his view on the limited conditions that the theory will only deal with kinetics of simple or multiple pools in an open system (with in- and outflow) and no reversible flow. Markers are given as a simple dose unless othewise stated.

Shipley and Clark (1972) noted that a pool has to be identified and the discharge from it must be possible to describe passage by exponential equations. A pool has a fixed volume and loses a simple tracer dose at a rate proportional to the remainder in the pool. The constant that can be derived from such a relationship is called the rate constant (K). Its dimension is a fraction or percent per unit time. In a simple pool model, the constant can be derived as the ratio of the input per unit time and pool size.

Eq. (1) $$K = \frac{\text{Input}}{\text{pool size}} = \frac{F}{W(\text{or } V)}$$

The input can be called flow (F) with the dimension of volume or weight per unit time and the pool size is expressed as volume (V) or weight (W).

1/K would give the mean marker retention time ($\bar{R}$) or the pool turnover time (T) (Eq. 2).

Eq. (2) $$\bar{R} = T = \frac{1}{K}$$

The halftime ($t_{\frac{1}{2}}$) is defined as the time when half of the marker

dose has passed out, i.e., the concentration of the marker is half that of time zero. It can be derived from Eq. 3 describing the kinetics of a simple pool.

Eq. (3) $$Y = Y_0 \cdot e^{-Kt}$$

where: Y = amount or concentration of marker in pool

Y_0 = amount or concentration at t = 0

when $Y/Y_0 = 0.5$, t = 1/2, this gives us;

$$\ln 0.5 = -K \cdot t_{\frac{1}{2}} \text{ and}$$

Eq. (4) $$t_{\frac{1}{2}} = \frac{0.693}{K}$$

In multiple pool systems where it may be difficult to sample and inject markers in each pool, it becomes more difficult to interpret marker excretion curves. The pools can be handled two at a time as it is possible to "look" into pool one when sampling pool two. The marker is introduced in pool one and the concentration in- or outflow from pool two is studied (Eq. 5).

Eq. (5) $$Y_2 = \frac{K}{K_1 - K_2} Y_{o_1} (e^{-K_1 t} - e^{-K_2 t})$$

The equation is derived as follows:

$$\frac{dY}{dt}_1 = -K_1 Y_1$$

which when integrated gives:

$$Y_1 = Y_{01} e^{-K_1 t}$$

$$\frac{dY_2}{dt} = -K_2 Y_2 + K_1 Y_1 = K_1 Y_{01} e^{-K_1 t} - K_2 Y_2$$

rearranging;

$$\frac{dY_2}{dt} + K_2Y_2 = K_1Y_{01}\, e^{-K_1t}$$

$Y_2 = Y_{02} = 0$; $t = 0$

$Y_1 = Y_{01}$; $t = 0$

K_1 = rate constant of pool 1.

K_2 = rate constant of pool 2.

Y_1 = concentration in pool 1.

Y_2 = concnetration in pool 2.

Interpreting left and right hand sides using Laplace transformations give equation (5) as above. The mean total retention time ($\bar{R}$) is derived by integration of the excretion curve over time divided by the total excretion.

Eq. (6) $$\bar{R} = \frac{\int tdY_2}{\int dY_2}$$

$$\int tdY_2 = A \int_0^{\infty} (te^{-K_1t} - te^{-Kt})dt$$

Integration by parts gives:

$$\int tdY_2 = A\,[1/K_1^2 - 1/K^2]$$

$$\int dY_2 = A \int_0^{\infty} (e^{-K_1t} - e^{-K_2t})dt = A[1/K_1 - 1/K_2]$$

Eq. (7) $$\bar{R} = \frac{1}{K_1} + \frac{1}{K_2}$$

Shipley and Clark (1972) proposed that the effect of number of pools and their relative size on the excretion marker is as shown Fig. 4(a-f).

As seen in Figure 4d with almost equal pool sizes, the asymptote of the curve will not become parallel with the slope of the line from

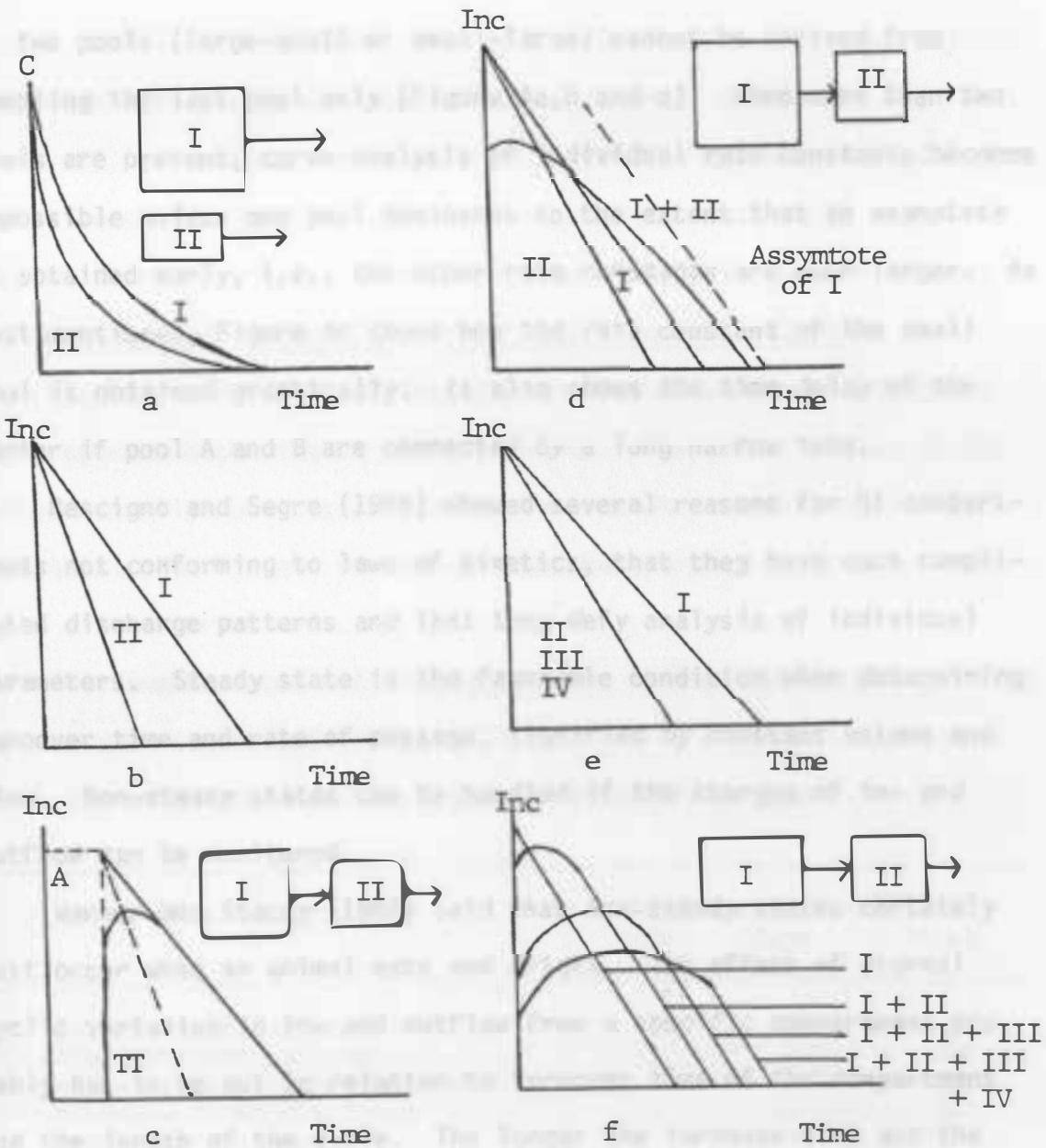


Figure 4 a-f. The effect of number of pools and their relative size on marker concentration in the effluent.

the largest pool until it approaches a very large value. The sequence of two pools (large-small or small-large) cannot be derived from sampling the last pool only (Figure 4a,b,and c). When more than two pools are present, curve analysis of individual rate constants becomes impossible unless one pool dominates to the extent that an asymptote is obtained early, i.e., the other rate constants are much larger. As just mentioned, Figure 4c shows how the rate constant of the small pool is obtained graphically. It also shows the time delay of the marker if pool A and B are connected by a long narrow tube.

Rescigno and Segre (1966) showed several reasons for GI compartments not conforming to laws of kinetics, that they have such complicated discharge patterns and that they defy analysis of individual parameters. Steady state is the favorable condition when determining turnover time and rate of passage, signified by constant volume and flow. Non-steady states can be handled if the charges of in- and outflow can be monitored.

Warner and Stacey (1968) said that non-steady states certainly must occur when an animal eats and drinks. The effect of diurnal cyclic variation in in- and outflow from a specific compartment probably has to be put in relation to turnover time of the compartment and the length of the cycle. The longer the turnover time and the shorter the cycle, the less effect it has on the accuracy of the obtained marker elimination curve.

In the animal, slowly or poorly mixed compartments probably exist (Sisson and Grossmann, 1953). Slow mixing of a pool would result in

an excretion curve looking like one from a two or multiple pool system. This can be demonstrated by putting a drop of dye in a glass container with a slow in- and outflow of water. Slowly the outflow water will get more and more colored as the dye spreads out and then gets diluted as more water runs through. This is probably a greater problem when using solid markers because of digesta stratification.

Chicken Gastrointestinal Tracts (GIT) and their length

The size and length of the digestive tract varies considerably among species depending on dietary habits. The midgut region of the chicken usually consists of one muscle stomach and the long small intestine. The hindgut of the chicken, however, is very short compared with other animals.

The length of the entire tract of the chicken may be 210 cm or more as seen in Table 2 (Duke, 1977).

Table 2. Length (cm) of the digestive tract of chickens.

	At 20 Days	At 1.5 Years
Entire digestive tract	85	210
Angle of beak to crop	7.5	20
Angle of beak to preventiculus	11.5	35
Duodenum (complete loop)	12	20
Ileum and jejunum	49	120
Cecum	5	18
Rectum and cloaca	4	11

Methods for calculating passage

Many scientists (Hoelzel, 1930, Hinton et al., 1969; Gohl, 1977)

used first appearance of a marker or time for excretion of a certain amount for studying passage rate. Grovum and Phillips (1973) showed intensive animal data as well as computer simulations. A two-compartment model for the sheep GIT describes nicely the biological significance of transit time (TT) (passages mainly through the small intestine) and the two rate constants (K_1 and K_2) for the rumen and the lower tract.

Blaxter et al. (1956) estimated that $\overline{R}$ or $\overline{t}$ in the foregut and hindgut is adequate. The method for calculating $\overline{R}$ was used to hand integrate the cumulative marker curve (including transit time) by adding the times for 5, 15, 25...95% excretion multiplied by 10, with the sum of the products to be divided by 100.

They derived their mean retention time ($\overline{t}$) in an analogous way by dividing the sum of the times of excretion of each individual particle of their particulate marker by the total number of particles recovered.

$$\overline{t} = \frac{\sum(n \cdot t)_i}{\sum n}$$

n = number of particles
t = time

Sibbald (1980) hypothesized that a procedure for measuring the rate of passage of feed through goats (Castle, 1956) could be used for making similar measurements with poultry. Six diets supplemented with stained particles of their major component (corn or wheat) were each fed for various periods of time to four adult Single Comb White Leghorn Cockerels. Cumulative particle excretion curves were prepared and used to estimate mean particle retention times.

There was a high level of variation in feed consumption both between birds and between the red, violet, and unmarked feeds. There were no apparent reasons for this, but undoubtedly the frequency of the excreta collections caused a higher than normal level of excitement in the flock. Variation in body weight was not associated with differences in feed intake. It seemed possible that some of the variation in the mean retention times might be a function of differences in feed intake, but the two variables were not significantly correlated. The results confirm that the methodology is applicable to poultry with certain limitations. Especially under conditions of continuous ad libitum feeding, the dilution of feed residues with urinary and metabolic excreta should be small and relatively constant. Thus it seems possible that the Castle (1956) technique would serve to measure the rate of passage of feed in full-fed birds.

Grovum and Phillips (1973) worked with flow through the small intestine which showed that continuous marker infusion is more convenient.

$$F = \frac{\text{Infusion Rate}}{\text{Marker concentration}} \text{ (Volume/Unit Time)}$$

If a gut segment is analyzed for the total amount of marker present, the flow can also be determined as distance traveled per unit time.

$$F_d = \frac{\text{Infusion rate(mg/h)}}{\frac{\text{Amount of marker}}{\text{Length of segment}}} \text{ (mg/cm)}$$

Another technique (Kass et al., 1980) of turnover time was

obtained simply by the values that were obtained by dividing the net chromium in the respective organ by the daily chromium intake and multiplying the result by 24.

$$\text{Chromium Turnover Time} = \frac{\text{Total chromium in segment}}{\text{Total chromium intake in 24 hours}} \times 24$$

Chromium as a marker in rate of passage studies

Chromic oxide is a popluar digestibility marker. Dansky and Hill (1952) reported that the index method gave more consistent and probably more accurate results of balance measurements in chickens under the conditions employed.

Chromic oxide has also been used for passage studies (Tuckey et al., 1958; McRae, 1974; Ghol, 1977; Cherry and Siegel, 1978; Kass et al, 1980; Mateos and Sell, 1981).

Jensen et al. (1957) followed the passage of meal supplemented with chromic oxide through chicks fed ad libitum and found that peak excretion of the marker occurred five hours after the meal with small quantities being excreted through ten hours after withdrawal of the marked feed.

Cherry and Siegel (1978) used ferric oxide as a marker and observed differences in the times of clearance from the alimentary canals of 7-week-old male and female chicks of two genotypes; the maximum time for clearance was about 22 hours. They concluded that some of the markers were difficult to identify in the excreta while others may have had rates of passage which differed from those of the feeds to which they were added.

McRae (1974) said that chromic oxide has a density of 5.25 and an extremely fine particle size and is probably not moving with the same speed as the solid digesta, especially not in herbivores.

Kass et al. (1980) found that the negative values obtained for apparent digestibility of all cell wall components in the stomach and small intestine show that chromic oxide is not a completely satisfactory marker for rate passage studies in swine.

In the experiment of Kaupp and Ivey (1923) in which lampblack was used as a marker, some of the excreta voided in the later stages was coated with lampblack while the inside of the excreta was not marked. They suggested that the marker adhered to the intestinal mucosa and was picked up by other feed as it passed through the alimentary canal.

Sibbald (1980) found that there was a relatively rapid excretion of red marker particles with 80% being voided within 8 hours. Further excretion was slower and was not completed until 44 hours after the feed change. The curve describing the excretion of violet particles tends to be sigmoid in shape. There were no violet particles excreted during the first 6 hours after introduction of the violet marked feed, but then excretion rate rose rapidly with 90% of the particles being excreted in the next 20 hours. Excretion of violet particles was complete 52 hours after feed introduction and 28 hours after removal.

Diet and the rate of food passage in chicks

Browne (1922) demonstrated that oats passed through the alimentary canal of fasted chicken hens in 5 to 6 hours. He found magenta stained bread dough, aluminum powder, gentian violet and

methylene blue to be unsuitable as dye indicators and suggested that the digestive fluids carried the stain forward faster than the solids.

Kaupp and Ivey (1923) used lampblack after observing that methylene blue and gentian violet caused some constipation and diarrhea. Using birds starved for 24 hours, they observed that food passed through the 71.5 inch alimentary canal of laying hens in 3 hours and 46 minutes; and through pullets in 3 hours and 52 minutes. Halnan (1949) reported that if a fasted bird was fed pills of oatmeal, barium sulphate and water, the first food arrived in the large intestine in 2 hours and the last food disappeared from the large intestine in 4 hours. In his experiments, the first appearance of food in different parts of the gut, timed from the commencement of the meal, was as follows: crop, within a few seconds; gizzard, 30 sec; duodenum, 15 min; cloaca, 2 hr.

He observed that food remained in the crop for 2 hours; Card et al. (1926) noted food to be retained for 3 to 11 hours; and Heuser (1944) reported 30 to 40 percent of the food still in the crop after 12 hours and entirely emptied after 24 hours.

Halnan (1949) suggested that it is unlikely that much digestion of crude fiber can occur owing to the rapidity of passage of food through the gut, coupled with the absence of cellulases in the fowl's digestive juices. There are only two possible sites in the gut, namely, the crop and the cecum, where food can remain sufficiently long to enable bacterial digestion of fiber to occur.

Fedorovski (1951) said that the first food entering the crop passes directly across the dorsal portion, through the proventriculus, and into the gizzard within 2 minutres. He added that using starved birds should result in the minimum food passage time and the 2 to 5 hours spread in food passage time observed on similar birds in these tests may be partially accounted for by the fact that the capsule could remain in the crop for some time.

Hillerman et al. (1953) said that age was a more important factor than production. Laying and non-laying hens were about the same; 3 hours and 42 minutes and 3 hours and 50 minutes, respectively. Penicillin in the feed slowed down the passage rate in turkeys and chickens slightly, since the antibiotic fed fowl averaged 3 hours and 15 minutes while the fowl on the normal mash averaged 2 hours and 57 minutes. Chicken hens and turkey hens were very similar in their feed passage rate. Environmental temperatures of 60^{o} and 90^{o} F caused very little difference in the time of food passage.

Monson et al. (1950) reported that the excretion time of chickens fed different carbohydrates varied in the following descreasing order: dextrin, sucrose, lactose.

Stokstad et al. (1953) found that sucrose diets pass more rapidly than glucose or starch diets through the gastro-intestinal tract of chicks. They also noted that chlorotetracycline retarded food passage in a sucrose diet but not in a starch or glucose diet.

Tuckey et al. (1958) observed that fat supplements at levels up

to 12 percent in the diet of growing chicks did not have a consistent effect on the rate of passage of feed through the digestive tract. A diet containing 12 percent lactose passed through the digestive tract faster than a similar diet containing either sucrose or cellulose. The fiber content of the diet did not affect passage time when the fiber was adjusted with ground oat hulls or ground cellulose. Passage time was similar in chicks fed diets containing ground wheat or ground corn as the cereal component. The inclusion of chlorotetracycline did not affect passage time with any of the diets tested. Rate of feed passage was retarded considerably if the birds were excited during the determination of passage time.

Cherry and Siegel (1978) found that the indigestible marker, ferric oxide, appeared earlier and disappeared later in the excreta of males from the low-weight line than in those from the high-weight line. Results with female chicks were similar, except that line differences in the time to the first appearance in the excreta were not significant. There were no significant differences in feed passage rates due to the dwarf gene.

For cumulative total and net particle excretions for wheat and wheat bran, independent of the levels of feed input, Sibbald (1979) discovered that the rates of excretion of feed residues were faster when measured by the net rather than the total values. For example, 10 hours after feeding, the percentage of wheat residues voided was 60% by the total data and 78% by the net data. (He calculated "net" values by subtracting from the weight of excreta dry matter the mean

weight of excreta voided by the negative control birds during the same time period. He said this correction is not entirely satisfactory because it fails to make allowance for the fact that the amount of metabolic plus endogenous waste voided by the negative controls was probably less than that voided by the fed birds.) The differences for the wheat bran were not as pronounced, but they followed the same trend. He noted that the bran passed through the alimentary canal more rapidly than the wheat but the times of final clearance were similar. He also demonstrated that the rates of passage of wheat and wheat bran are affected by the level of feed input but the completion of the excretion of feed residues is essentially complete within 24 hours after feeding.

An experiment was conducted by Mateos and Sell (1981) with White Leghorn hens to determine the influence of supplemental yellow grease and carbohydrate source on rate of food passge (ROP). The ROP was determined by utilizing either Cr_2O_3 or ^{144}Ce as nonabsorbable markers. First appearance of the markers in the excreta and percentages of the markers ingested that were recovered in the feces 9.5 to 11.5 hours after feeding were criteria used to determine ROP. The ROP varied with the composition of the diet. Diets containing starch had a slower ROP than diets containing sucrose (first appearance was 156 vs. 127 minutes, respectively). Also yellow grease supplementation decreased ROP from 150 to 133 minutes. The ROP of sucrose-containing diets was decreased more by fat supplementation than the ROP of starch-containing diets.

Similar trends were observed when ROP was measured as percentage of marker recovered in feces 9.5 to 11.5 hours after feeding. The results show that supplemental fat decreased ROP in chickens. This observation may help in understanding the nature of the extrametabolic or extracaloric effect of fat in poultry diets. With decreased ROP, the diet will be more thoroughly digested and absorbed and, thereby, more energy may be derived from a diet if fat is added than if not.

Correlation of GIT structure with function

According to Calhoun (1933), who has written an excellent monograph on the microscopic anatomy of the digestive tract of the fowl, the beak is the main prehensile structure. Food is retained in the mouth for only a short time. A complex system of salivary glands is present in the chicken, they are branched tubular glands. The amount of saliva secreted by the mature fasting hen in 24 hours varies from 7 to 25 cc., the average being 12 cc. The reaction is nearly always acid, the average pH being 6.75. Salivary amylase is always present. A small amount of lignin is also found.

The crop in the chicken is well developed (Warner et al., 1967). Mucus is secreted by the crop of the fowl, and amylase may be secreted as well. They said amylase found in the crop or on the crop mucosa may originate from the salivary glands, ingested food, bacteria in the crop, or from the crop mucosa itself.

Bolton (1965) believed that a significant amount of starch digestion occurred in the crop of chickens as a result of bacterial

action. Pritchard (1972) collected crop contents of chickens, killed the bacteria therein with chloroform and upon incubation of the contents found that sucrose was still digested; so nonbacterial digestion of carbohydrate apparently can occur in the crop. The reaction of the contents of the crop is always acid, approximately pH 5. Because of the small size of the cavity of the proventriculus, food cannot remain long in that location. Therefore the amount of gastric digestion taking place there must be slight.

Browne (1922) pointed out that the relatively dry nature of the gizzard contents makes it improbable that enzymic action goes on in that organ, all of which points to the duodenum as the probable seat of the main action of gastric juice. This view was strengthened by the fact that the reaction of the duodenum is nearly always acid and that the pancreatic juice and bile enter the duodenum not near its origin but at its posterior extremity.

In a bird deprived of food so as to clear both the crop and the gizzard (Halnan, 1949), the first two or three mouthfuls of food swallowed pass directly from the mouth to the gizzard, the time of passage varying from 15 to 30 seconds. In the act of swallowing, the head and neck are rapidly jerked backwards and forwards, and a negative pressure is set up in the esophagus. By-passing the crop entrance, it reaches the proventriculus, which it temporarily fills and distends. Here it remains for a short period; the length of stay, which is measured in seconds, apparently depending on the precise stage reached in the contraction cycle of the gizzard muscles. The

reaction of the gizzard is acidic, approximately pH 4.06.

After the gizzard has received its full complement of food, the character of the esophageal peristaltic wave changes, and the contraction wave stops at the entrance of the crop instead of passing on to the gizzard. During its passage through the gizzard, a certain amount of separation of the insoluble fibrous material from the starchy and more soluble material of the food takes place, and this partial retention of fibrous material may affect the digestibility coefficient of the crude fiber obtained in digestibility trials. On reaching the small intestine, the food mass undergoes digestion and absorption, and the residues are gradually propelled forward by the action of the muscles of the gut wall until they reach the rectum.

Browne (1922), by slaughter methods on hens given colored dough and dough mixed with sand and lead shot, showed that filling of the ceca occurs from the rectal contents, only the liquid portion of these taking part. He further noted that the nature of the cecal contents varied with the condition of the normal excreta of the hen. A gradual filling of the ceca by the semifluid rectal contents takes place, and the constricted entrances to the ceca prevent any material, other than liquid and finely comminuted solid particles, from entering the ceca. From time to time the ceca contract, expelling their contents.

Nakahiro et al. (1974) conducted experiments using 4-month-old Single Comb White Leghorn Cockerels, and the following results were obtained: 1) Most of cecal feces were excreted in the morning,

especially early in the morning. The amount of cecal content increased with time from evening till midnight. 2) The crude fiber content in the lower ileum and the rectum did not reveal any consistent tendency in relation to time, but the crude fiber content in the ceca showed a maximum value at 4:00-8:00 p.m., and thereafter decreased gradually. The ratio of chromic oxide to crude fiber showed almost the same tendency as to the crude fiber content, and both the crude fiber content and the rate were remarkably lower in the cecum than those in the lower ileum and the rectum. 3) This fact may indicate that a portion of intestinal ingesta, which is low in fiber content, is selectively introduced into the ceca. 4) The digestibilties of crude fiber, cellulose and pentosan were not changed by ligation of the ceca. These results indicate that the ceca of chickens may not play a significant role in the digestion of crude fiber.

In order to investigate the digestive and absorptive functions of ceca of chickens (Isshiki et al., 1974), 3 experiments were carried out using Simple Comb White Leghorn Cockerels, and the following results were obtained: 1) No significant difference was observed in the digestibility of nutrients between ceca of ligated and non-ligated chickens. 2) In 7-day- and 105-day-old chicks, cecal mucosa did not have both proteolytic and amyolytic activities, whereas cecal contents contained considerable contents of both activities.

Duke (1977) reported the pH of the intestine of the chicken to be as shown in Table 3.

Table 3. pH of the intestine of the chicken

Part of Intestine	pH		
	mean	low	high
Duodenum	6.31	5.64	7.10
Jejunum	7.04	6.12	8.01
Ileum	7.59	6.93	8.42
Right cecum	7.08	5.83	8.20
Left cecum	7.12	5.93	8.16
Rectum	7.38	6.29	8.18
Coprodeum	7.24	5.69	8.10

Mineral components in the plant cell wall

The mineral constituents of cotton and wood include all the inorganic elements needed by plants and fiber-degrading microorganisms for growth. The concentration of these elements varies greatly with the environment in which the fiber is grown (Cowling and Brown, 1969).

Jones et al. (1963) reported that the main functional groups in the organic components of plant cell walls that may be involved in binding or complexing metal cations are carboxyl in uronic acid (e.g., pectin) and carboxyl and hydroxyl in phenolic compounds (e.g., lignin). In a later study, Jones (1978) showed that some metal cations are associated with plant cell walls. Calcium is bound by pectin. Calcium pectate acts as a cementing agent in the cell wall. Binding of some metal cations by components of plant cell walls,

possibly by silica, is likely to reduce availability of the cations for intestinal absorption.

Studies of plants showing tolerance levels of zinc and copper that would be toxic to other plants provide a further example of the association of metal cations with cell walls. Zinc and copper were found to be concentrated in the cell wall fraction from the roots of the metal tolerant strains (Turner, 1970). Other studies involving chemical fractionation of the roots have revealed that the pectate extract from zinc-tolerant plants contained five to six times as much zinc as that from nontolerant plants. The mechanism of zinc tolerance is due to binding in the pectate fraction of the cell wall, thereby restricting the entry of zinc into the cytoplasm. The binding sites, or functional groups, are different for the two cations, zinc and copper (Peterson, 1969).

Studies of cell wall fractions from a grass, Holcus lanatus (Molley and Richards, 1971), have shown that pectin and lignin complex a large proportion of the calcium whereas magnesium is complexed only by lignin.

Ward and Harbers (1982) reported that distribution of calcium or phosphorus is relatively independent. The elemental maps for phosphorus and silicon are somewhat independent in their distribution. The structural source of the phosphorus that persists after harsh treatment is not understood.

A recent review article by Cunha (1983) stated that fiber and especially certain constituents in fiber have a weak to a strong

effect on the utilization of minerals such as calcium, phosphorus, zinc, iron, magnesium and possibly others. In a comparison of hemicellulose, pectin and cellulose, the cellulose was found to have the highest mineral binding capacity. He indicated that forage and many by-product feeds are high in fiber and differ in fiber constituents. The wide range of results on mineral requirement studies and recommendations being made may be partially due to the fiber level and its constituents in the diet used.

Kincaid and Cronrath (1983) demonstrated that a significant portion of calcium, phosphorus, iron, zinc and copper are associated with the fibrous portion of alfalfa and grass silage. Calcium, magnesium and copper in alfalfa were negatively correlated with percent neutral and acid detergent fiber and positively correlated with percent crude protein. For grass silage, phosphorus, potassium, magnesium and zinc were positively correlated with percent crude protein. Percentages of total minerals in alfalfa associated with neutral detergent fiber (NDF) were calcium 24%, phosphorus 31%, potassium 1%, magnesium 5%, copper 29%, zinc 31% and iron 77%. Percentages of total minerals located in the acid detergent fiber (ADF) were calcium 3%, phosphorus not detectable, potassium 3%, magnesium 2%, copper 43%, zinc 23%, and iron 19%. Additionally, copper and calcium in the NDF and calcium in the ADF decreased with higher NDF and ADF.

Effect of phytate on calcium, iron and zinc absorption

Much research has centered around the question of the availability of phosphorus present as phytin, or phytic acid. The latter compound

is an acid hexaphosphoric acid ester of inositol. It occurs as salts of calcium, iron, zinc and magnesium, the complex being referred to as phytin. Half or more of the phosphorus of most mature seeds and their products, notably wheat bran which is a rich source, is so combined. Thus the question of its availability is an important one in animal nutrition. There is no simple answer (Maynard et al., 1979).

Calcium and phytate

The various nutritional studies involving phytate have often produced conflicting results. For example, reduction in the absorption of calcium from the small intestine when phytate is present in the diet was attributed by McCance and Widdowson (1942a,b) to the formation of calcium phytate, a salt which they found was less soluble than calcium phosphate under the pH conditions likely to prevail in the small intestine (i.e. a change in pH from approximately 2 in the stomach to approximately 5-7 in the duodenum). Examination of the data of McCance and Widdowson, however, indicates that phytate can be hydrolyzed in the intestine, a phenomenon which appears to be inconsistent with their view that calcium may become unavailable for intestinal absorption because it is present as insoluble calcium phytate.

Because of the ability of phytate to bind multivalent cations, phytate has also been implicated as a dietary constituent which can prevent the intestinal absorption of a number of nutritionally essential elements such as calcium, magnesium, zinc and iron (McCance et al., 1943; Likuski and Forbes, 1965).

In 1939, Harrison and Mellamby isolated phytate from both oatmeal and commercial phytin (a Ca-Mg-K salt of phytic acid) and showed that feeding puppies either the acidic or the sodium form of this compound reduced the rachitogenic effect of the oatmeal. They concluded that phytate exerts its rachitogenic effect by binding the calcium of the diet, rendering it insoluble and thus reducing its availability in the intestine for absorption.

That this effect of phytate may be more complex is suggested by their finding that replacing phytic acid and sodium phytate with phytin or sodium phytate made alkaline by the addition of NaOH not only resulted in the elimination of the rachitogenic effect of phytate, but actually reduced the rickets over that in control animals.

Schwarzenbach (1952) reported that the binding of calcium by phytate and its inositol phosphate derivatives was determined by titrating the acidic forms of each compound with NaOH and $Ca(OH)_2$. From the differences in their titration curves, a measure of the differences in binding to calcium was obtained. At the same time, the pH at which precipitation of each calcium salt occurred was noted and the pH at which each of the salts become insoluble was thus established. Kaufman and Kleinberg (1971) also showed that a precipitate of calcium phytate was not observed until the pH was raised above approximately 5.4. This value is higher than the value of 3.0 reported by McCance and Widdowson (1942a), the value of approximately 4.0 to 5.0 observed by Harrison and Mellamby (1939).

No one has shown the true pH value at which calcium phytate is insoluble. Vohra and Kratzer (1966) suggested that phosphorus, whether as phytate, its decomposition products, or inorganic phosphate, readily forms a poorly soluble complex with zinc at the pH of the intestinal contents. Presumably this occurs with calcium and magnesium also.

Iron and phytate

Iron absorption is dependent on many complex and often interrelated factors, the important one being the body iron stores and the degree of erythropoises. Ascorbic acid may enhance absorption of dietary iron, especially in iron deficient subjects (Moore, 1955). In studies with human beings, it was reported (Apte, 1967) that the effects of phytic acid on iron absorption in cases of severe iron deficiency were variable. In some cases, the expected hemoglobin and red cell response occurred from a daily dose of 30 grams of ferrous sulfate, despite administration of 100 grams of phytic acid.

In others, no response occurred until use of the phytic acid was stopped. The results described (Apte, 1967) suggested that iron absorption is more dependent on calcium intake than on phytate intake. Calcium, iron, phosphate and phytates appear to be mutually interacting, changes in one affecting the absorption of the others. Studies by Cowan et al. (1956) on the effect of soluble phytate on iron absorption measuring total hemoglobin regeneration in nutritionally anemic rats, showed that sodium phytate and natural phytate had no effect on iron absorption. It was suggested (Apte, 1967) that

absorption rate of iron, calcium and phosphorus are dependent on the relative amounts of each of these substances in the diet. Iron absorption may fall below 3 percent in diets with a low calcium, high phosphorus and high phytic acid content. The limiting effect of phosphates and phytic acid on iron absorption may be reduced by excesses of iron and/or calcium. Ferric phosphate has a relatively poor biological availability for both humans (Cook et al., 1973) and rats (Amine et al., 1972). Ferric hydroxide is very insoluble at the pH encountered in the intestine and is low in biological availability to rats (McCance et al., 1943). Morris and Ellis (1976) showed that iron is in a soluble form and available for absorption when presented to the mucosal cells as monoferric phytate.

Much confusion and controversy regarding the effects of phytate on iron absorption exists.

Zinc and phytate

A number of investigators became interested in the relation between zinc and phytates.

Altsch (1957) showed that a zinc deficiency occurred not only among swine fed ordinary rations but also among poultry.

Some peculiarity of plant proteins was early recognized as a factor in the development of zinc deficiency. This was shown by the observation that purified rations containing casein produced good growth and normal skin in swine when the level of zinc was no greater than 10 ppm. However, whenever soybean rations were used, the level of zinc had to be increased to 80 ppm (Lease, 1961).

In one study (Oberleas et al., 1962) to evaluate the interrelationships between zinc, calcium and phytate, young pigs were fed a casein ration to which 1.4 percent phytic acid was added. The ration contained 14 ppm zinc and 1.5 percent calcium. The diet without phytic acid produced daily body weight gains of 1.68 pounds over a ten-week period.

An extension of the latter work by Oberleas et al. (1966) emphasized the inhibitory effect of calcium on zinc absorption in the presence of phytates. From the preceding work, it would appear that the deleterious influence of plant proteins on zinc absorption is related to their phytate content.

That the problem may not be that simple is suggested by a report by Dahmer et al. (1966) who found that the addition of histidine to a low zinc, soybean meal ration alleviated some of the zinc deficiency symptoms seen in chicks. Blood fibrin contains a large amount of histidine. For this reason, that protein was substituted for some of the corn in the ration. Even though the ration had a high level of calcium (1.3 percent) and only an average amount of zinc (30 ppm), none of the pigs fed the blood meal ration showed symptoms of parakeratosis. The swine fed the original ration, which did not contain the blood meal, developed parakeratosis.

Hydrolysis of phytate by enzymatic phytases

Phytates may be a calcium or magnesium salt of inositol hexaphosphoric acid, or phytic acid. They are characteristic and abundant constituents of whole cereals, grains and derived foodstuffs.

Potential hydrolysis by the enzyme, phytase, of phytic acid to inositol and six phosphoric acid molecules is an important factor in controlling the effect of phytic acid on iron, calcium and zinc metabolism. Phytase may be present in the diet and can destroy phytic acid even during the course of food preparation (Apte, 1967).

Intestinal bacteria are known to produce phytase in rats and phytase has been demonstrated in the gastrointestinal tract of albino rats, guinea pigs, chicks and human (Sullivan et al., 1966; Bitar and Reinhold, 1972). Its quantitative activity has not been thoroughly investigated, but the presence of phytase has been suggested as the reason why rats can utilize the iron, calcium and zinc of phytate rich cereals.

On how phytase activity might aid in iron absorption in either humans or rats, Morris and Ellis (1976) suggested that the pH optimum in both is near or slightly above 7.7. Thus, the pH environment for maximum hydrolysis of phytate would not be conducive to maximum availability of the resulting iron compounds, either ferric phosphate or ferric hydroxide.

Reinhold et al. (1974) showed that fermentation with yeast markedly increases the physiological availability of zinc in whole meal bread. The gains are attributed in part to the action of yeast in destroying phytate. However, the gain in solubility exceeded by several fold that to be expected if destruction of phytate was the only cause, so that other changes brought about by fermentation also must contribute to this observation.

Fiber, Independent phytate

While it is undoubtedly true that phytic acid impairs calcium absorption (McCance and Widdowson, 1942a,b), it now seems distinctly possible that fiber itself, independent of phytate, interferes with mineral absorption and metabolism (Cummings, 1978). Phytate is digestible (Sullivan et al., 1966) and like other digestible chelators, would ultimately liberate bound metal in digestion processes. On the other hand, metals bound by the indigestible residue, mainly fiber, remain unavailable for absorption. Although fiber may be attacked by the bacteria of the large gut with release of metals, absorption can no longer occur in this region and the metals will be lost in the feces. Consequently, the fiber content of feedstuffs has a great effect on availability of bivalent metals. Phytate would be important only to the extent that it escapes digestion (Reinhold et al., 1975).

Grasses are lower in lignin and cation exchange than alfalfa, but contain more digestible cellulosic carbohydrate (Van Soest, 1984). He said that cation exchange is associated positively with mineral absorption, hydration and rate of fermentation of the respective fiber sources. The exchange of high quality alfalfa cell wall is about 400-500 mg/kg of fiber which is equivalent to 50-75 g of calcium carbonate. He also noted that cabbage fiber is almost unlignified and has high exchange and the fastest rate of fermentation, and wood cellulose is at the other extreme in terms of low exchange and rate of fermentation.

Fiber and Calcium

Reinhold et al. (1975) said that the ability to bind calcium is a function of fiber concentration., They demonstrated that bran with a fiber concentration of 10.9% binds 5.4% of the calcium of a solution containing 10 mg/100 ml. Dephytinization brings about an increase in binding proportional to the increase in fiber concentration that accompanied removal of phytate. They showed that pure cellulose also binds calcium although with less activity than bran in terms of fiber content.

Ismail-Beigi et al. (1977) reported that addition of cellulose to a low fiber diet was accompanied by significantly increased losses of calcium in feces and the development of negative calcium balances in each of three participants. Plasma calcium concentration was also decreased by cellulose. They suggested that the high pectin intake resulting from the apple component of a fiber supplement did not make an important contribution to fecal losses of calcium.

When expressed as the fraction bound (Cummings, 1978), calcium binding by breadstuffs is less than that of zinc or iron, however, the quantity of calcium bound is far greater because of the much higher concentration of calcium present in digesta as compared with the trace metals.

Sandstead et al. (1979) showed that the apparent calcium requirements of volunteers was significantly increased by the addition of dietary fiber to a high protein diet. The 95% confidence limit of the regression line for calcium daily requirement ranged from 773 to 908

mg and was increased by the addition of dietary fiber to a range of 914 to 1057 mg. Calcium requirements were also higher in the high protein diet than in the low protein diet.

Fiber and Zinc

After it was observed that cellulose lowers absorption of zinc from the intestine (Becker and Hoekstra, 1971), hypogonadal dwarfism, which is a manifestation of zinc deficiency, was reported among adolescents in certain areas by Reinhold et al. (1975). The diet, which consisted mainly of whole meal wheat breads, supplied enough zinc to meet the daily requirements. It has been presumed that the availability of this zinc was low because of the high content of phytate (inositol hexaphosphate) in the diet. Recently this assumption has been questioned by Reinhold et al. (1976), and experiments in vitro in which phytate was removed from whole meal or bran by phytase showed that the binding of calcium and zinc was unaltered. The possibility that the fiber of whole meal bread plays a major role in binding metals in poorly available forms has been examined in two human subjects by Reinhold et al. (1976).

They showed that the amounts of calcium, zinc, iron and phosphorus were highly correlated with fecal fiber (acid detergent) and with fecal phosphorus.

Prior to the above research, Reinhold et al. (1975) demonstrated that crude fiber prepared from whole meal bread has a high affinity for zinc. Cellulose in the form of shredded filter paper also binds zinc, although less effectively than whole meal. They also showed

that removal of phytate from bran either by acid extraction or phytase action increased its affinity for zinc, a result of increasing fiber concentrations. Addition of phytic acid to the dephytinized preparations lowered the amount of zinc bound to roughly that in untreated bran. Phytic acid alone combined with only moderate amounts of zinc under the conditions of these experiments, but if both calcium and phytate were present, nearly all of the zinc was removed from the solution. However, calcium alone was nearly as effective in binding the zinc.

Ismail-Beigi et al. (1977) also indicated that fecal excretion of zinc increased during periods of high celluose consumption. They said concentrations of zinc in plasma declined in four of five instances following the consumption of the cellulose-apple supplements.

Branch et al. (1975) reported that zinc binds more strongly than calcium to dephytinized fiber in vitro.

Reinhold et al. (1975) also demonstrated that treatment of bran or wholemeals with acid modified their ability to bind zinc as it did for calcium. Under mild conditions at room temperature, binding increased. However, boiling with 1% HCl for four hours caused a decrease in binding to less than half of the original.

To study the effect of fiber in a diet which contains purified casein, diets containing wheat bran and derived fractions at a 10% equivalent level were fed to male, weanling rats (10/group). Harland et al. (1977) reported that kidneys from the wheat bran extract group exhibited significantly higher zinc levels than all other groups.

Feeding wheat bran and its fractions significantly alters body weight, and they said mineral metabolism may be altered by wheat bran as a result of a fiber-phytate-zinc interaction.

In contrast to the above reports, Sandstead et al. (1977) said that in their studies, no relationship between dietary fiber and fecal zinc or copper was found. Their research was done with four male volunteers fed mixed diets (basal) low in vegetable fiber but designed to meet individual needs. The diet contained an average of 3000 Kcal. of which 16% calories were from protein (70% from animal sources), 40% from fat and 44% from carbohydrate. After a 30 day increment, 30 g of soft white bran (WB), corn bran (CB) or soybean hulls (SH) were added to the diet for another 30 days. These materials had been screened. Zinc balance was positive in 5 of 6 periods on the basal diet, 3 of 4 periods with WB and 4 of 4 periods with CB. Copper balance was positive in 2 of 6 periods on the basal diet and 2 of 4 periods on the WB.

Fiber and Iron

There is a great deal of confusion and controversy regarding the effects of phytate on iron absorption. Results of studies on the effect of phytate on iron absorption in rats and chicks cannot be transposed to man, since the presence of phytase has not been confirmed in man.

Although many studies have been conducted on the effects of phytate on absorption of administered inorganic iron salts, few observations have been made of their effect on absorption of dietary iron (Apte, 1967).

The findings in rats that inorganic phosphates also inhibit iron absorption (Hegsted et al., 1949) make it difficult to state that the inhibitory effect of bran on iron absorption is due only to its high content of phytates.

The concept that iron absorption takes place from two independent pools, one heme iron pool and one pool of all non-heme iron compounds in the food (Hallberg and Bjorn-Rasmussen, 1972), implies that the inhibitory effect of bran on iron absorption from bread is not limited to an effect on iron in bread but will affect the whole non-heme iron pool in a meal. However, the inhibitory effect of bran on iron absorption has usually been ascribed to its high content of phytates. In a later study, Bjorn-Rasmussen (1974) found that fiber, not phytate, may have been responsible for the reduction in absorption of fortification iron when bran was introduced into white bread and consumed by humans.

Hallberg and Solvell (1967) reported that in humans, the majority of evidence indicates that phyates reduce the absorption of non-heme iron in the diet but have no effect on heme iron absorption.

In another trial of the same series, Bjorn-Rasmussen (1974) showed that with an increase in the bran content of white wheat flour from 0 to 10%, the content of inorganic phosphorus is about doubled from 22.7 to 43.2 mg/roll whereas the content of phytic phosphorus increases almost 20 times from 0.3 to 5.7 mg/roll. He confirmed that it may be more reasonable to ascribe the marked reduction in iron absorption when added 10% bran to the very marked increase in phytate

content than to the more moderate increase in inorganic phosphates.

Morris and Ellis (1976) found that the relative biological value of iron as the monoferric phytate, either isolated from wheat bran or as the synthetic product, was equal to the reference compound, ferrous ammonium sulfate. In contrast, the biological avilability of iron in the bran residue was significantly lower and the low biological availability of an insoluble form of ferric phytate was confirmed. They concluded that the major portion of the iron in wheat is monoferric phytate which has a high biological availability for the rat. They suggest that monoferric phytate in bran may be bound to cationic sites of proteins or other cellular components. Utilization of the iron may be through solubilization of the monoferric phytate by an ion-exchange type mechanism rather than by hydrolysis of the phytate as has been postulated.

Sandstead et al. (1979) again showed different results. They reported that the iron requirement apparently was not influenced by the addition of dietary fiber to the diet. Their lower requirement for iron with a low protein control diet might have been related to the timing of the observations. They studied the effects of a more modest increase in dietary fiber on mineral metabolism of volunteers who were fed diets similar to those eaten by many Americans.

Fiber and copper

The availability of copper in the plant cell wall has not been extensively investigated in the simple stomached animal.

Brenner (1970) said that the solubilities of three metals (zinc,

manganese, copper) in the rumen were found to be extremely low. Although over 50% of the zinc, manganese or copper in a dried grass ration was found to be water-soluble, over 90% of the zinc and managanese was liberated on cellulolytic digestion of ryegrass. However, in the rumen only 5-10% of the zinc and manganese and less than 20% of the copper were in a soluble form. They indicated that the strength of binding of the metals to the insoluble rumen residue appeared to be in the order Cu>Zn>Mn on the basis of the extent of dissociation of the metals on treatment with acid or with EDTA. Although large increases in the solubilities of zinc and manganese on passage of the digesta into the abomasum arise, the solubility of copper was reduced.

Perdomo et al. (1977) said that copper decreased in concentration in the NDF fraction with increasing maturity in all forages. In addition to this, apparent copper digestibility decreased in all grasses with increasing maturity.

Stoszek et al. (1979) indicated that copper metabolism in animals was influenced by the grass species grown as feed in muck soils. Consumption of tall fescue reduced liver copper stores and decreased plasma copper and ceruloplasmin oxidase activity to a deficiency status in less than 4 months. At the same time cattle fed quackgrass maintained normal blood copper and ceruloplasmin activity levels and increased liver copper stores. Quackgrass was lower than fescue in copper content.

Fiber and phosphorus

Vohra and Kratzer (1966) suggested that phosphorus, whether as

phytate, its decomposition products, or inorganic phosphate, readily forms a poorly soluble complex with bivalent metals at the pH of intestinal contents.

Reinhold et al. (1976) found that both fiber and phosphorus complex metals in the small intestine and that phosphorus which escapes absorption carries a quota of metals into the large gut and the feces. They reported that correlation between fiber and phosphorus in the feces is not as close or as consistent as that of either with the bivalent metals. Ismail-Beigi et al. (1977) also said that significantly increased excretion of phosphorus in feces occurred in one subject during the period of high fiber intake. On the other hand, two other subjects responded to the ingestion of fiber with a lowered output of phosphorus in feces.

They conducted two sets of experiments; in one set of experiments, fiber in the form of cellulose was added to a diet with a low fiber content and in the other set, cellulose was added to a diet containing larger amounts of fiber and phosphorus.

Their study indicated that a high intake of fiber and phosphorus (as phytate) produced disturbances of metabolism of calcium, magnesium and zinc similar to those resulting from consumption of Iranian breads. However, they found that changes in phosphorus excretion accompanying intakes of fiber were less consistent. They showed only slight binding of phosphate to cellulose under conditions that supported strong binding of divalent metals. They said that high intakes of fiber can explain to a considerable extent the impaired utilization

of zinc, calcium and magnesium, while phosphorus balance changed to negative in one subject among three subjects.

The previous studies on the effects of fiber on phosphorus absorption are somewhat confusing. Southgate et al. (1976) noted that the fecal excretion of sodium, phosphorus, potassium and iron were higher on a fiber supplemented diet, but the fecal excretion of zinc and calcium were not appreciably affected.

Cellulase and minerals associated with cell walls

Very little is known of the potential liberation of minerals associated with cell wall components by cellulase. Bremner (1970) found that enzymatic hydrolysis of the water-insoluble residue with a crude cellulase preparation (Trichoderma koningii) resulted in almost complete dissolution of the residual zinc. Only 2% of the zinc in the original ryegrass was associated with insoluble material after this hydrolysis. In contrast, digestion of the original water-soluble residue with a commercial hemicellulase preparation did not release zinc into solution. He used an in vitro system. A solution (5 ml) of a crude cellulase obtained from a culture filtrate of Trichoderma koningii was diluted with 0.2 M sodium acetate buffer (pH 4.8, 45 ml). It was then purified by extraction with 0.2% (w/v) dithizone in CCl_4. A sample (500 mg) of the water-insoluble residue was treated with this enzyme preparation (30 ml) at 45^{o} for 2 days, whereupon the mixture was filtered and suitable samples of residue and filtrate were taken for counting. He suggested it is probable that rumen fermentation could result in a similar liberation of the zinc from

the ryegrass as is obtained with cellulolytic enzymes in the rumen.

Kincaid and Cronrath (1983) also reported that minerals associated with cell walls largely are solubilized by cellulase or protease activity but not as readily as minerals in the soluble cell contents.

EXPERIMENTAL PROCEDURE

Three-week-old, broiler-type, mixed sex chicks were used in a study conducted for five weeks from January through March of 1981, and samples were analyzed until September, 1983. Chicks from three to six weeks of age were fed an 18% protein diet. At 7 weeks of age, diets were changed to 15% protein for a 2-week period.

The four experimental diets for the two studies were: 1) an 18% (15% from 7 weeks of age) protein corn-soy control diet, 2) control diet plus 10% wheat bran, 3) control diet plus 20% wheat bran, 4) control diet plus 20% wheat bran plus 0.008% cellulase (Table 4).

Each group of 10 birds was housed in a cage, 0.46 by 0.72 meter in size in a 24-pen battery which was electrically heated with raised wire floors. After six weeks of age, each group of birds were housed in a cage with wire floors, 0.92 by 1.52 meter in size. Feed and water were supplied ad libitum. The wheat bran was defatted and the enzyme was a culture filtrate from Trichoderma viride (Boerhinger, Mannheim, Gmn H, West Germany). The enzyme supplementation to the diet was as a dry preparation.

Weekly measurements of feed consumption were made and particular care was taken to account for all feed wastage by attaching an extra plate as a shield to catch all feed spilled by the chicks. This procedure also effectively minimized contamination of excrement with feed when collecting feces.

Part 1

After a five-week experimental period without a marker, birds in

Table 4. Composition of diets

Ingredients	Control	10% Wheat Bran	20% Wheat Bran	20% and Enzyme
	%	%	%	%
	Three to six weeks of age			
Corn	71.8	64	55.77	55.27
SBM	24.3	22.3	20.7	20.7
Wheat bran	-	10	20	20
Limestone	1	1.2	1.5	1.5
Dicalcium phosphate	2	1.5	1	1
Salt	0.5	0.5	0.5	0.5
Vitamin mix	0.5	0.5	0.5	0.5
D-methionine	-	-	0.03	0.03
Starch	-	-	-	0.492
Enzyme[a]	-	-	-	0.008
	Seven to eight weeks of age			
Corn	79.4	71.6	63.28	62.78
SBM	16.6	14.7	13	13
Wheat bran	-	10	20	20
Limestone	1	1.2	1.2	1.2
Dicalcium phosphate	2	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
Vitamin mix	0.5	0.5	0.5	0.5
D-methionine	-	-	0.02	0.02
Starch	-	-	-	0.492
Enzyme[a]	-	-	-	0.008
	Chemical analysis for seven to eight weeks of age			
Protein	15.38	15.01	15.88	15.07
Cell wall[b]	13.90	16.21	18.98	18.08
Hemicellulose	9.60	10.75	12.53	12.30
Cellulose	3.24	3.98	3.92	4.00
ADF[c]	4.30	5.46	6.45	6.50
Lignin	1.06	1.48	2.53	2.50

[a]Boerhinger, Mannheim, Gmn H, West Germany. [b]Cell wall (neutral detergent fiber fraction) represents ADF (acid detergent fiber fraction) plus hemicellulose fraction. [c]ADF (acid detergent fiber fraction) represents cellulose fraction plus lignin fraction.

a 24-pen battery on the four diets were supplemented with 1% chromic oxide and fed for 96 hours. The diets containing chromic oxide were fed for a 2-day preliminary period and for a 2-day collection period. Feces were collected three times daily from each diet group for two days at 2, 4 or 8 hours after feeding. At the end of 4 days, the feed was removed, and within each diet group, birds were randomly selected for slaughter at 2, 4 or 8 hours following removal of feed on the assigned day.

The entire gastrointestinal tract (GIT) was removed and ligated to form five compartments: the gizzard; the proximal small intestine, which is the duodenum and jejunum; the distal small intestine, which is the ileum; the cecum; and the rectum. The digesta from each compartment was removed for analysis and immediately weighed, placed in aluminum pans and freeze dried. The dried samples were ground using a 1 mm mesh Udy mill. Samples of dried feed, feces and gastrointestinal contents were stored in air-tight glass bottles until analyzed.

Part 2

After the five-week experimental period, chicks in 24 pens were fed the four diets with 1% chromic oxide for 5 hours after the chicks were fasted for 14 hours. Feces were collected for 8 hours after withdrawal of the diets (13 hours after feed was offered). The excreta were collected in two parts, that voided in the daytime (from 7:30 a.m. to 8:30 p.m.) and the other during the night (from 5:30 p.m. to 6:30 a.m. the next day) and analyzed separately. The relative amounts of elements excreted within 13 hours after the diet was

offered were determined.

The feces samples were immediately weighed, placed in aluminum pans and freeze dried. The dried samples were ground using a 1 mm mesh Udy mill. Samples of dried feed and feces were stored in air-tight glass bottles at 0°C until analyzed.

Samples were analyzed for calcium, phosphorus, iron, zinc and copper. After determination of NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) fractions, subsequent NDF and ADF fractions were analyzed for calcium, phosphorus, iron, zinc and copper.

Chemical Analysis

The estimation of chromium content

Samples were analyzed for chromic oxide content by the Bolin procedure (Bolin et al., 1952). Samples of 1 gram were weighed out; 10 ml of HNO_3 and 3 ml of $HClO_4$ were added to each. The mixture was heated for 20 to 30 minutes and then transferred to a 100 ml glass stoppered volumetric flask and made to volume with distilled water. The solution was mixed well and transferred to spectrophotometric tubes. Absorption was read against a water blank at 440 nm.

Determination of NDF, ADF, cellulose, hemicellulose and lignin

Samples were analyzed by the Van Soest procedure for neutral detergent fiber (NDF) content (Van Soest, 1967). Acid detergent fiber (ADF) content and lignin content were also analyzed by the Van Soest procedure (Van Soest, 1963). Hemicellulose content was calculated by subtracting the ADF content from the NDF content. Cellulose content was determined by subtracting the lignin content from the ADF content.

Samples were analyzed for NDF content by weighing out one gram of the sample and placing it in a beaker with 100 ml of neutral detergent solution. The beakers were placed on the digestion rack and brought to a rapid boil. A crucible was placed on a suction apparatus and the sample solution was added to the crucible. The beaker was washed out with boiling water and added to the crucible. The crucible was then placed in an oven at 100°C for 24 hours.

One gram samples were weighed out for ADF analysis and 100 ml of acid detergent fiber solution were added to them. The solution was heated to boiling and boiled for 60 minutes. The solution was filtered and the residue rinsed with water. The sample residue was dried for 3 hours in a 100°C oven.

Lignin content was determined by placing the acid detergent fiber portion of the samples in 25 ml of 72% sulfuric acid solution and extracted cold for 3 hours. As much of the acid was filtered off as possible. The samples were then dried overnight in the drying oven at 100° C.

Measurements of mineral contents in feed, feces and fiber fraction

Samples were analyzed for calcium (Ca), zinc (Zn), iron (Fe) and copper (Cu) content using atomic absorption spectrometry and phosphorus (P) content using spectrophotometry (A.O.A.C., 1965). Samples were placed in crucibles with 2 ml of concentrated HCl and warmed to dissolve the precipitate. The solution was transferred via a long-stem funnel into a 250 ml volumetric flask and made to volume with distilled water. The solution was filtered and the filtrate was used

for mineral analysis. Ca, Zn, Fe and Cu content were determined by atomic absorption spectrometry. P content was measured using spectrophotometry.

Statistical Methods

Part 1

Three-week-old, broiler-type chicks were assigned to 24 groups of 10 chicks for two replicates of 12 treatments. A completely randomized experiment with a 4 x 3 x 2 factorial arrangement (fed 0, 10, 20 or 20% wheat bran plus cellulase; slaughtered at 2, 4 or 8 hours after the last feeding; replication).

The data were analyzed statistically by analysis of variance by the following model:

$$Y_{ijk} = U + D_i + A_j + DA_{ij} + H_k + DH_{ik} + AH_{jk} + DAH_{ijk} + E_{ijk}$$

Where: U is overall mean.

D_i is effect of ith diet.

A_j is effect of jth replication.

DA_{ij} is interaction of ith diet and jth replication.

H_k is effect of kth hour.

DH_{ik} is interaction of ith diet and kth hour.

AH_{jk} is interaction of jth replication and kth hour.

DAH_{ijk} is interaction of ith diet and kth hour and jth replication.

E_{ijk} is random error associated with ith diet in jth replication measured in kth hour.

All effects in the model were assumed to be fixed except for

E_{ijk} which was considered normally distributed. A computer program (Barr et al., 1982) was used to compare treatment effects. Duncan's Multiple Range Test was used to determine treatment differences when the F-test was significant ($P<0.05$).

Part 2

Three-week-old, broiler-type chicks were assigned to 24 groups of 10 chicks for two replicates of 12 treatments. Four diets (0, 10, 20 or 20% wheat bran plus cellulase replicated) were tested in a complete 4 x 2 factorial arrangement.

The data were analyzed statistically by analysis of variance by the following model:

$$Y_{ij} = U + D_i + A_j + DA_{ij} + E_{ij}$$

Where: U is overall mean.

D_i is effect of ith diet.

A_j is effect of jth replication.

DA_{ij} is interaction of ith diet and jth replication.

E_{ij} is random error associated with ith diet in jth replication.

A computer program (Barr et al., 1982) was used to compare treatment effects. Duncan's Multiple Range Test was used to determine treatment differences when the F-test was significant ($P<0.05$).

RESULTS

Part I

Growth and feed utilization

The feed consumption data and calculated feed efficiencies are shown in Table 5. The 20% wheat bran plus enzyme treatment had no significant effect on feed consumption and feed-to-gain ratio compared with the 10% wheat bran group.

On the other hand, 20% wheat bran without the enzyme significantly increased feed consumption and depressed efficiency of feed utilization compared with the 0 and 10% groups ($P<0.01$). Average body weight at 8 weeks of age was significantly decreased with increasing fiber in the diet ($P<0.05$). Body weights measured at 8 weeks and body weight increases from 3 to 8 weeks did not show any difference between the groups fed 10% or 20% wheat bran or the 20% wheat bran with cellulase.

Gastrointestinal tract component dimensions

The effects of level of wheat bran on gut dimensions are shown in Table 6. Gizzard weight, as a percentage of body weight, was significantly influenced by diet. Adding wheat bran to the basal diet increased gizzard weight.

The group fed on the high-energy basal diet had the lowest gizzard weight ($P<0.05$). Cellulase supplementation showed no effect on gizzard weight.

There were no significant effects of diet on gut dimensions of the small intestine, cecum and rectum.

Table 5. Effect of wheat bran on growth and feed utilization

Diet	Body wt increase (3-8 weeks)	Body wt. at 8 weeks	Feed to gain ratio	Feed Consumption (3-8 weeks)
	g/wk	kg		g/day
0% Wheat bran	223.8	1.43[d]	2.47[c]	79.7[c]
10% Wheat bran	215.9	1.38[e]	2.57[bc]	80.7[bc]
20% Wheat bran	210.3	1.38[e]	2.83[a]	85.7[a]
20% Plus enzyme	212.3	1.36[e]	2.71[ab]	81.6[b]

[a-c]Mean values not having a common superscript are significantly different at the 1% level of probability.

[d-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 6. Effect of dietary wheat bran level on gastrointestinal tract measurements of chicks.

Diet[a]	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum
	% Body wt.	cm	cm	cm	cm
0% Wheat bran	1.28[b]	76.52	51.48	16.20	7.01
10% Wheat bran	1.50[c]	76.16	51.24	16.42	6.82
20% Wheat bran	1.53[c]	74.49	50.11	16.00	6.83
20% Plus enzyme	1.51[c]	75.27	50.63	16.01	7.20

[a]Each value is mean of 30 birds

[b-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

Dry matter and ash percentage of the digesta in the GIT

Dry matter percentage of the digesta in the GIT and excreta are summarized in Table 7. Supplementation with cellulase increased fecal dry matter content significantly ($P<0.05$). Dry matter percentage of the digesta in the GIT (Gastrointestinal tract) tended to be negatively correlated with cell wall content of diet and digesta in all segments. The percentage of dry matter in the digesta of the rectum was less than that of the gizzard and small intestine. The percentage of dry matter in the gizzard was greater than that of other portions of the GIT. The types of diet did not influence the percentage of dry matter in the cecum.

Chicks fed diets containing cellulase plus 20% wheat bran had significantly more ash in their feces than birds receiving the diet containing the 20% wheat bran (Table 8). Ash contents in feces were negatively correlated ($P<0.05$) with cell wall contents of the diet, but the ash contents of digesta in each segment except the rectum were non-significant among diets. Ash content tended to increase in the lower gut with cellulase supplementation.

Chromium Turnover Time

The rates of passage of digesta through the gizzard, prox. small intestine, distal small intestine, cecum and rectum and entire GIT of chicks fasted for 2, 4 or 8 hours as estimated by chromium turnover time (total chromium in segment/total intake in 24 hours) are summarized in Table 9.

Chromium turnover time (minutes) in the gizzard, prox. small

Table 7. Dry matter content (Percent dry matter by diet) in gizzard, prox. small intestine, dis. small intestine, cecum, rectum and feces of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

	Organ					
Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	40.03[a]	19.40	18.67	17.50	17.80	21.61[a]
10% Wheat bran	35.47[b]	19.23	18.30	17.00	16.50	19.18[ab]
20% Wheat bran	34.50[b]	18.47	16.73	17.13	14.40	17.27[b]
20% Plus enzyme	34.23[b]	19.57	17.53	17.60	16.61	21.59[a]

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 8. Ash content (% of dry matter) of digesta in gizzard, prox. small intestine, dis. small intestine, cecum, rectum and feces of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

	Organ					
Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	4.47	9.84	13.25	12.72	21.84[a]	21.88[a]
10% Wheat bran	4.40	8.95	12.03	12.79	19.41[b]	20.04[a]
20% Wheat bran	5.03	8.83	11.88	13.07	17.59[c]	18.59[b]
20% Plus enzyme	4.40	8.87	12.33	13.06	20.11[b]	20.74[a]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

intestine, distal small intestine, cecum, rectum and entire GIT of chicks tended to decrease as the level of wheat bran increased, but the differences were not significant. Addition of cellulase apparently showed longer retention time but there were no significant differences.

Table 9. Chromium turnover time [a](Turnover time by diet) in gizzard, prox. small intestine, dis. small intestine, cecum, rectum and entire gut of chicken fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Total
	min.	min.	min.	min.	min.	min.
0% Wheat bran	61.43	121.55	45.40	11.67	21.50	271.15
10% Wheat bran	50.77	104.40	53.35	8.83	17.65	234.30
20% Wheat bran	50.13	87.25	59.35	8.17	16.45	226.25
20% Plus enzyme	56.83	100.15	61.25	11.77	16.45	241.00

[a]Chromium turnover time = Total chromium in segment/total chromium intake in 24 hrs.

Apparent digestibility of dry matter and cell wall components

The digestibilities of dry matter, cell wall components and nitrogen are given in Table 10. The apparent digestibilities of dry matter, nitrogen and cell wall components were depressed with increased wheat bran level.

Cell wall digestibility in the 20% wheat bran plus cellulase diet was significantly more digestible than that in the 20% wheat bran

diet, however, a similar trend was noted for all other parameters except nitrogen. The hemicellulose in all four diets was more digestible than the cellulose in the respective diets.

Table 10. Effects of level of dietary wheat bran on apparent digestibilities of dry matter, cell wall, acid detergent fiber, hemicellulose, cellulose and nitrogen in chicks

	Percent digestibility by diet			
Diet component	0	10	20	20 + enzyme
Dry matter	81.67^{d}	79.30^{de}	73.21^{f}	75.33^{ef}
Cell wall	55.31^{a}	44.78^{b}	33.03^{c}	40.24^{b}
Acid detergent fiber	46.32^{d}	35.78^{de}	25.06^{e}	32.55^{de}
Hemicellulose	59.80^{a}	48.93^{b}	38.14^{c}	44.01^{bc}
Cellulose	42.44^{a}	31.02^{ab}	15.94^{c}	28.00^{bc}
Nitrogen	62.27	57.30	58.64	59.21

$^{a-c}$Means within the same row and within the same criterion without a common superscript are significantly different at the 5% level of probability.

$^{d-f}$Means within the same row and within the same criterion without a common superscript are significantly different at the 1% level of probability.

The contents of cell wall components (NDF, ADF, cellulose, hemicellulose) and nitrogen in the digesta and feces

The contents of cell wall components in each segment are given in Tables 11, 12, 13 and 15 and nitrogen contents are summarized in Table 14.

The proximal small portion, in all cases, had much lower con-

Table 11. Cell wall (CW) content in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces CW
	%	%	%	%	%	%
0% Wheat bran	35.23	10.00	20.52[a]	9.13[a]	36.40[a]	36.84[a]
10% Wheat bran	37.06	13.71	28.96[b]	11.54[ab]	41.70[b]	43.63[b]
20% Wheat bran	43.31	15.68	32.55[b]	14.96[b]	46.44[c]	47.51[b]
20% Plus enzyme	38.88	14.09	31.38[b]	14.95[b]	46.11[c]	45.53[b]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 12. Acid detergent fiber content (ADF) in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces ADF
	%	%	%	%	%	%
0% Wheat bran	13.16	4.01	7.21[a]	0.42[b]	12.50[a]	12.57[a]
10% Wheat bran	13.66	5.03	9.49[a]	0.22[a]	17.00[b]	16.93[b]
20% Wheat bran	16.87	6.12	10.83[b]	0.18[a]	18.64[c]	18.46[c]
20% Plus enzyme	15.50	5.33	10.05[ab]	0.20[a]	17.34[b]	17.62[bc]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

centrations of all cell wall components than other segments of the GIT. Contents from the rectum and feces showed similar cell wall composition. The percentage of all cell wall components from the gizzard was greater than that from other segments of the GIT.

Table 13. Hemicellulose (HC) content in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces HC
	%	%	%	%	%	%
0% Wheat bran	22.07	6.00[a]	13.12[a]	7.32	21.83[a]	21.28[a]
10% Wheat bran	23.40	8.68[b]	18.87[ab]	8.76	25.24[ab]	26.70[ab]
20% Wheat bran	26.44	9.48[b]	21.73[b]	9.31	29.33[b]	29.06[b]
20% Plus enzyme	23.29	9.17[b]	22.66[b]	9.32	27.01[ab]	27.91[ab]

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

The content of cell wall components of digesta reflected the content of all cell wall components in the feed.

The amount of NDF and hemicellulose in the cecum was greater than the ADF and celluose content in the cecum. ADF and cellulose were influenced in the cecum. Supplementation of the 20% wheat bran diet with cellulase gave highly significantly less NDF, ADF and hemicellulose in the feces.

The effect of cellulase added to the 20% wheat bran diet on the cellulose content of the feces was also pronounced as compared with

Table 14. Nitrogen content in each segment and apparent digestibilities (AD) of nitrogen (N) of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces N	ADN
	%	%	%	%	%	%	%
0% Wheat bran	1.63	-	-	8.85	4.08[a]	4.03[a]	62.27
10% Wheat bran	1.75	-	-	8.57	3.42[ab]	3.43[ab]	57.30
20% Wheat bran	1.66	-	-	8.21	3.25[b]	3.18[b]	58.64
20% Plus enzyme	1.71	-	-	8.33	3.13[b]	3.12[b]	59.21

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

non-cellulase supplementation, but the differences were not significant.

The apparent digestibility of nitrogen was decreased with increased wheat bran in the digesta in the rectum and feces, but there were no significant differences in the amounts of nitrogen excreted by the chicks fed diets containing 0, 10, 20 or 20% wheat bran plus cellulase. The percentage of nitrogen in ceca digesta was greater than that of other segment digesta and feces excreta, and the amount of nitrogen in the gizzard was less.

Table 15. Cellulose content in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces C
	%	%	%	%	%	%
0% Wheat bran	12.10	3.67	6.80[a]	0.36[b]	11.79[a]	11.24[a]
10% Wheat bran	12.85	4.48	8.15[a]	0.21[a]	16.45[b]	14.84[b]
20% Wheat bran	14.34	5.13	8.85[b]	0.17[a]	15.13[b]	15.36[b]
20% Plus enzyme	11.66	4.33	8.33[ab]	0.16[a]	14.00[b]	14.86[b]

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

Apparent digestibilities of dry matter in each segment

For the most part, the digestibilities of dry matter in the segments of chicks on the control diet were significantly greater than those of chicks on the diets containing wheat bran (Table 16). Except in the ceca, apparent digestibilities of the dry matter were signifi-

cantly reduced in chicks by the addition of wheat bran. The negative values obtained for apparent digestibilities of dry matter in the gizzard, proximal small intestine and ceca show that Cr_2O_3 is not a completely satisfactory marker for rate of passage studies in chicks.

For ceca digesta, there were no significant differences in dry matter digestibilties for chicks fed different levels of wheat bran. Cellulase supplementation increased apparent digestibilities significantly in each segment of the GIT and in the excreta.

Table 16. Apparent digestibilities[g] of dry matter in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	-70.85^{de}	24.70^{a}	62.93^{a}	-28.80	69.12^{ab}	81.67^{d}
10% Wheat bran	-78.26^{de}	0.56^{ab}	52.07^{ab}	-49.37	65.34^{bc}	79.30^{de}
20% Wheat bran	-87.21^{e}	-12.72^{b}	44.56^{b}	-73.41	60.23^{c}	73.21^{f}
20% Plus enzyme	-55.16^{d}	-9.92^{b}	54.28^{ab}	-62.47	65.01^{bc}	75.33^{ef}

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

[d-f]Mean values not having a common superscript are significantly different at the 1% level of probability.

[g]Apparent digestibility (%) =

$$100 - \left(100 \frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\ \text{Nut. in feces}}{\%\ \text{Nut. in feed}}\right)$$

Apparent digestibilities of NDF, ADF, cellulose and hemicellulose in each segment

Lower digestibilities of NDF, ADF, cellulose and hemicellulose in each segment and feces were obtained with increasing levels of wheat bran in the diet (Tables 17, 18, 19 and 20). The negative values obtained for apparent digestibilities of cell wall components in the gizzard and small intestine in this study indicate relatively greater retention of cell wall components than of the indicator, Cr_2O_3, in these portions of the GIT. Table 21 shows that interval from feeding to slaughter influenced the apparent digestibility of dry matter and cell wall components in all diets; digestibility decreased progressively as fasting interval increased.

NDF and hemicellulose digestibilities in ceca were very low or negative for the wheat bran diets including the cellulase supplementation diet. In contrast, ADF and cellulose digestions for the wheat bran diet were significantly higher than that of the control. ADF and cellulose were significantly more digestible than NDF and hemicellulose in the ceca.

The cellulase addition affected the digestibility of dietary fiber. Tables 17, 18, 19 and 20 show that the cellulase supplement resulted in significantly greater apparent digestibilities of NDF, ADF, cellulose and hemicellulose.

Part II

Calcium

Table 22 suggests that the percent of fecal excretion of calcium

Table 17. Apparent digestibilities of cell wall content in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	-279.33	44.94[a]	45.10[a]	27.87	21.42[a]	55.31[a]
10% Wheat bran	-306.67	14.68[b]	15.55[b]	16.69	15.25[ab]	44.78[b]
20% Wheat bran	-329.67	5.75[b]	5.90[c]	13.42	9.88[b]	33.03[c]
20% Plus enzyme	-221.00	9.82[b]	9.70[bc]	17.92	17.73[ab]	40.24[b]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 18. Apparent digestibilities of acid detergent fiber in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	-427.0	32.54[a]	35.85[a]	77.33[a]	23.25[a]	46.32[d]
10% Wheat bran	-345.0	8.23[b]	12.35[b]	92.39[b]	19.30[b]	35.78[de]
20% Wheat bran	-389.7	-7.96[c]	-3.85[c]	93.14[b]	13.45[b]	25.06[e]
20% Plus enzyme	-271.0	0.23[bc]	3.20[bc]	95.12[b]	19.43[b]	32.55[de]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

[d-e]Mean values not having a common superscript are significantly different at the 1% level of probability.

Table 19. Apparent digestibilities of cellulose in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	-478.3	24.30[a]	29.55[d]	85.99[a]	37.45[a]	42.44[a]
10% Wheat bran	-386.3	-0.99[b]	6.44[de]	91.07[ab]	17.27[b]	31.02[ab]
20% Wheat bran	-435.3	-13.92[b]	-5.56[e]	91.52[ab]	14.22[b]	15.94[c]
20% Plus enzyme	-296.3	-4.23[b]	0.43[de]	94.82[b]	28.33[ab]	28.00[bc]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

[d-e]Mean values not having a common superscript are significantly different at the 1% level of probability.

Table 20. Apparent digestibilities of hemicellulose in each segment of chicks fed 0, 10, 20 or 20% wheat bran plus enzyme

Diet	Gizzard	Prox. Small Intestine	Dist. Small Intestine	Cecum	Rectum	Feces
	%	%	%	%	%	%
0% Wheat bran	-294.3[b]	50.69[a]	49.28[a]	1.53[a]	43.25[a]	59.80[a]
10% Wheat bran	-288.3[b]	18.14[b]	20.60[b]	-22.82[b]	28.37[b]	48.93[b]
20% Wheat bran	-329.0[b]	13.01[b]	10.72[c]	-28.51[b]	15.45[c]	38.14[c]
20% Plus enzyme	-195.0[a]	15.51[b]	13.26[c]	-22.78[b]	27.38[b]	44.01[bc]

[a-c]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 21. Analyses of variance of apparent digestibilities of digesta components from gizzard, proximal small intestine, distal small intestine, cecum, rectum and feces of chicks fed 0, 10, 20 or 20% wheat bran plus cellulase

Sources of variation	df	Mean Square				
		DM[a]	CW[b]	ADF[c]	HC[d]	C[e]
		Gizzard				
Feed (F)	3	1107.987***	13179.758***	16872.910***	21668.410***	23951.707***
Hours after feeding (H)	2	125.863*	38904.688***	34372.438***	35833.563***	36783.469***
FH	6	183.267***	1961.114***	2101.473***	1905.288***	4297.723***
		Proximal Small Intestine				
F	3	1745.185***	1902.674***	2080.924**	1962.099***	1597.591***
H	2	1709.977***	162.916*	607.995	119.571	493.907***
FH	6	79.697**	2.204	67.098	22.685	45.177***
		Distal Small Intestine				
F	3	343.443**	9902.142***	1795.438***	1882.191***	1414.708***
H	2	153.459*	188.561**	465.354**	76.499*	810.643***
FH	6	53.209***	1.593	16.723*	11.435	23.174*

Table 21. Continued

Sources of variation	df	Mean Square				
		DM[a]	CW[b]	ADF[c]	HC[d]	C[e]
		Cecum				
Feed (F)	3	2211.017***	232.611***	402.735***	94.491***	85.406***
Hours after feeding (H)	2	2789.268***	1265.417***	91.105***	26.521*	15.362*
FH	6	845.188***	317.433***	92.393***	391.102***	46.085***
		Rectum				
F	3	723.064	175.561*	204.024*	578.513***	841.508**
H	2	12.478	45.163	40.205*	213.339***	48.953
FH	6	4.565	29.594	33.221**	9.810*	73.669***
		Feces				
F	3	81.048*	495.365***	467.953***	505.864***	610.582***
H	2	9.313	9.489	79.020*	162.313*	73.923*
FH	6	3.629	54.639	54.170***	6.182	80.142***

*P<0.05; **P<0.01; ***P<0.005;

[a]Dry matter; [b]Cell wall; [c]Acid detergent fiber; [d]Hemicellulose; [e]Cellulose

was increased by the addition of dietary fiber. However, a statistical analysis of the data showed none of the treatment variations to be significant ($P>0.05$).

Table 22. Effect of dietary wheat bran level upon calcium (Ca) content of fecal excreta

Dietary treatment	Ca consumption (analyzed)	Ca content in feces	% of fecal Ca
	g	g	%
0%	0.619	0.423	68.34
10%	0.620	0.474	76.45
20%	0.625	0.552	88.36
20% plus enzyme	0.622	0.482	77.41

Calcium distributions associated with fecal fiber fractions are shown in Table 23. Calcium in the different fiber fractions was very small compared to calcium contents of the original feed (Table 24). Calcium appeared to be solubilized readily from the plant cell wall. Wheat bran may not have had much effect of calcium absorption in this study.

Calcium associated with cell walls was solubilized by cellulase ($P<0.05$). Calcium in the NDF fraction was more solubilized ($P<0.01$) than that of the ADF fraction (Table 25). This table showed that NDF

Table 23. Measurements on distribution of fecal calcium (Ca)

Dietary treatment	Ca in feces or its fractions (g)			% of fecal Ca in different fiber fractions		Level[1] of significance
	Original feces (Analyzed)	NDF	ADF	NDF	ADF	
0%	0.423	0.00137	0.00021	0.33[ab]	0.05[a]	***
10%	0.474	0.00156	0.00040	0.43[ab]	0.09[ab]	***
20%	0.552	0.00172	0.00070	0.47[b]	0.11[b]	***
20% plus enzyme	0.482	0.00121	0.00038	0.25[a]	0.08[ab]	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[1]Significance of differences between the means of percent of Ca in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 24. Measurements on distribution of feed calcium (Ca)

Dietary treatment	Ca in feed or its fractions (g)			% of Ca in different fiber fractions of feed		Level[1] of significance
	Original feed (Analyzed)	NDF	ADF	NDF	ADF	
0%	0.619	0.03932	0.00088	6.35[c]	0.14[a]	***
10%	0.620	0.04050	0.00104	6.49[c]	0.16[b]	***
20%	0.625	0.04689	0.00102	7.51[d]	0.16[b]	***
20% plus enzyme	0.622	0.04650	0.00102	7.44[d]	0.16[b]	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[c-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Ca in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 25. Calcium (Ca) located in fiber fractions of feed and fecal excreta

Dietary treatment	Ca in fractions of feed (g)		Level[1] of significance	Ca in fractions of feces (g)		Level[1] of significance	% of fecal Ca in fiber fraction to Ca in feed fiber fraction		Level[1] of significance
	NDF	ADF		NDF	ADF		NDF	ADF	
0%	0.03932[c]	0.00088	***	0.00137	0.00021[c]	***	3.48[a]	23.69[c]	**
10%	0.04050[c]	0.00104	***	0.00156	0.00040[d]	***	4.98[ab]	38.34[c]	**
20%	0.04689[d]	0.00102	***	0.00172	0.00070[e]	***	5.45[b]	69.07[d]	**
20% plus enzyme	0.04650[d]	0.00102	***	0.00121	0.0038[d]	***	3.39[a]	36.93[c]	**

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[c-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Ca in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

fractions bind calcium with less affinity than the ADF fractions. Absolute amounts of calcium in the NDF fractions were much higher than that in the ADF fraction of feed or feces.

Phosphorus

Fecal excretions of phosphorus as affected by diet are summarized in Table 26.

Significantly increased excretion of phosphorus in feces occurred as a result of the 20% wheat bran diet treatment ($P<0.01$). The mean percent phosphorus availability from the diet containing 20% wheat bran appeared to be lower than the phosphorus availability from the diet without bran, but there were no significant differences.

Table 26. Effect of dietary wheat bran level upon phosphorus (P) content of fecal excreta

Dietary treatment	Phosphorus consumption (Analyzed)	P content in feces	% of fecal P
	g	g	%
0%	0.463	0.213[a]	46.00
10%	0.470	0.234[a]	53.40
20%	0.478	0.292[b]	60.88
20% plus enzyme	0.469	0.246[a]	52.45

[a-b]Mean values not having a common superscript are significantly different at the 1% level of probability.

The mean percent phosphorus absorptions from the diets containing 0 and 20% wheat bran were 54 and 39%, respectively. Table 27 demonstrated that phosphorus showed only a slight binding to the fiber

Table 27. Measurements on distribution of fecal phosphorus (P)

Dietary treatment	P in feces or its fractions (g)			% of fecal P in different fiber fractions		Level[1] of significance
	Original feces (Analyzed)	NDF	ADF	NDF	ADF	
0%	0.213	0.00136	0.00089	0.64[a]	0.42	***
10%	0.234	0.00191	0.00111	0.76[ab]	0.44	***
20%	0.292	0.00245	0.00133	0.84[b]	0.46	***
20% plus enzyme	0.246	0.00157	0.00104	0.64[a]	0.43	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[1]Significance of differences between the means of percent of P in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

portions.

Significant amounts of phosphorus were in the NDF fractions of feed ($P<0.005$) and the ADF fractions of feed had much smaller concentrations of phosphorus (Table 28).

Phosphorus in the NDF fractions of feed which were soluble in the chicks' GIT were significantly higher ($P<0.005$) than that in acid detergent fiber of feed (Tables 27, 28). Table 29 shows that an increase in solubility and availability of phosphorus in the diet was obtained by cellulase ($P<0.01$).

Iron

The effects of the different fiber levels on iron availability are summarized in Table 30. Iron availability from the high fiber diets was particularly poor. Iron balance was negative for the chicks fed 20% wheat bran. However, ferrous or ferric iron, which is bound by bran, was apparently made readily available for absorption by cellulase ($P<0.01$).

There was less iron in the ADF fractions than in the NDF fractions ($P<0.005$, Table 31). There was no effect of diet on the percent of fecal iron content.

Table 32 shows that significant amounts of iron bind directly to the NDF and ADF fractions in the feed. Adding over 10% wheat bran in the feed showed a very marked increase in iron content of the NDF fraction and a moderate increase in the iron content of the ADF fraction ($P<0.005$).

The percent of fecal iron content in the ADF fraction was in

Table 28. Measurements on distribution of feed phosophorus (P)

Dietary treatment	P in feed or its fractions (g)			% of P in different fiber fractions of feed		Level[1] of significance
	Original feed (Analyzed)	NDF	ADF	NDF	ADF	
0%	0.463	0.1256	0.0334	27.14[a]	7.22[a]	***
10%	0.470	0.1381	0.0417	29.40[bc]	8.88[b]	***
20%	0.478	0.1613	0.0445	33.76[c]	9.33[bc]	***
20% plus enzyme	0.469	0.1600	0.0452	34.12[c]	9.67[c]	***

[a-c]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of P in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 29. Phosphorus (P) located in fiber fractions of feed and fecal excreta

Dietary treatment	P in fractions of feed (g)		Level[1] of significance	P in fractions of feces (g)		Level[1] of significance	% of fecal P in fiber fraction to P in feed fiber fraction		Level[1] of significance
	NDF	ADF		NDF	ADF		NDF	ADF	
0%	0.1257[c]	0.0337[c]	***	0.00136[c]	0.00089[c]	***	1.08[a]	2.65[ab]	***
10%	0.1382[cd]	0.0417[cd]	***	0.00191[d]	0.00111[cd]	***	1.38[b]	2.66[ab]	***
20%	0.1614[d]	0.0446[d]	***	0.00245[e]	0.00133[d]	***	1.52[b]	2.97[b]	***
20% plus enzyme	0.1601[d]	0.0452[d]	***	0.00157[cd]	0.00104[cd]	***	0.98[a]	2.30[a]	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[c-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of P in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

negative iron balance except for the cellulase treatment (Table 33). While it is undoubtedly true that phytic acid impairs iron absorption, it now seems possible that fiber itself may also have a role.

Table 30. Effect of dietary wheat bran level upon iron (Fe) content of fecal excreta

Dietary treatment	Fe consumption (Analyzed)	Fe content in feces	% of fecal Fe
	μg	μg	%
0%	5490.12	4569.57[a]	83.19[ab]
10%	5345.24	5240.83[ab]	104.28[b]
20%	5386.03	5795.67[b]	107.60[b]
20% plus enzyme	5490.19	4273.43[a]	77.84[a]

[a-b]Mean values not having a common superscript are significantly different at the 1% level of probability.

Zinc

The results given in Table 34 show that the addition of wheat bran interfered in the availability of zinc. The concentrations of zinc in NDF and ADF fiber fractions of feces (Table 35) and of feed (Table 36) were very low, 1 to 2% and 4 to 5%, respectively. The availabilities of zinc in the fiber fraction are given in Table 37.

While over 60% of the zinc in the NDF fraction could be in the soluble form in the chicken GIT, the avilability of zinc in the ADF fiber fraction was variable, 20 to 60%. Enzymatic hydrolysis of the water-insoluble residue with cellulase resulted in significant dissolution of the residual zinc ($P<0.05$).

Table 31. Measurements on distribution of fecal iron (Fe)

Dietary treatment	Fe in feces or its fractions (μg)			% of fecal Fe in different fiber fractions		Level[1] of significance
	Original feces (Analyzed)	NDF	ADF	NDF	ADF	
0%	4567.57	2274.63	1358.05	49.49	29.53	***
10%	5240.83	2720.30	1764.38	48.71	31.59	***
20%	5795.67	3169.21	2099.12	54.34	35.98	***
20% plus enzyme	4273.43	2101.15	1399.30	48.99	32.63	***

[1]Significance of differences between the means of percent of Fe in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 32. Measurements on distribution of feed iron (Fe)

Dietary treatment	Fe in feed or its fractions (μg)			% of Fe in different fiber fractions of feed		Level[1] of significance
	Original feed (Analyzed)	NDF	ADF	NDF	ADF	
0%	5490.12	3486.87	1060.23	63.47[a]	19.31[a]	***
10%	5345.24	4154.30	1497.41	77.72[b]	28.01[b]	***
20%	5386.03	4792.18	1560.72	88.97[d]	28.92[c]	***
20% plus enzyme	5490.19	4775.99	1564.61	87.00[c]	28.50[bc]	***

[a-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Fe in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 33. Iron (Fe) located in fiber fractions of feed and fecal excreta

Dietary treatment	Fe in fractions of feed (μg) NDF	ADF	Level[1] of significance	Fe in fractions of feces (μg) NDF	ADF	Level[1] of significance	% of fecal Fe in fiber fraction to Fe in feed fiber fraction NDF	ADF	Level[1] of significance
0%	3484.86^{c}	1060.23^{c}	***	2274.63^{c}	1358.05^{c}	***	65.27^{b}	128.13^{cd}	***
10%	4154.30^{d}	1497.40^{d}	***	2720.30^{cd}	1764.28^{cd}	***	56.49^{ab}	117.84^{cd}	***
20%	4792.18^{e}	1557.68^{e}	***	3169.21^{d}	2099.12^{d}	***	66.14^{b}	134.71^{d}	***
20% plus enzyme	4775.98^{e}	1564.60^{e}	***	2101.15^{c}	1399.30^{c}	***	44.00^{a}	89.41^{c}	***

$^{a-b}$Mean values not having a common superscript are significantly different at the 5% level of probability.

$^{c-e}$Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Fe in NDF and in ADF estimated by the t-test. *$P<0.05$, **$P<0.01$, ***$P<0.005$

Zinc contents in the NDF fraction of the feed or feces were not significantly higher than that in ADF fractions. Table 37 may suggest that the binding strength of zinc to the NDF fraction might be weaker than that to the ADF fraction.

Zinc tolerance might be due to binding in the pectate fraction of the cell wall, thereby restricting the entry of zinc into the cytoplasm.

Table 34. Effect of dietary wheat bran level upon zinc (Zn) content of fecal excreta

Dietary treatment	Zinc consumption (Analyzed)	Zn content in feces	% of fecal Zn
	μg	μg	%
0%	2647.32	1722.31	65.06
10%	2730.17	2234.74	81.84
20%	2692.24	2629.33	97.66
20% plus enzyme	2659.10	1537.66	57.83

Copper

The results given in Table 38 show that fiber may not play a part in copper absorption. No significant relationship among percents of fecal copper was found although copper content in feces showed differences. Fecal copper in the different fiber fractions decreased in concentration compared with that of feed copper content in different fiber fractions, and there were significant differences ($P<0.005$) between copper contents of NDF and ADF fractions of feces and feed (Tables 39,40).

Table 35. Measurements on distribution of fecal zinc (Zn)

Dietary treatment	Zn in feces or its fractions (μg)			% of fecal Zn in different fiber fractions		Level[1] of significance
	Original feces (Analyzed)	NDF	ADF	NDF	ADF	
0%	1722.31	24.92	11.65	1.45	0.68[a]	N.S.
10%	2234.74	37.05	20.47	1.66	0.86[ab]	N.S.
20%	2629.53	45.17	30.43	1.72	1.16[b]	N.S.
20% plus enzyme	1537.66	22.65	14.87	1.47	0.91[ab]	N.S.

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[1]Significance of differences between the means of percent of Zn in NDF and in ADF estimated by the t-test. *$P<0.05$, **$P<0.01$, ***$P<0.005$

N.S., not significant ($P>0.05$).

Table 36. Measurements on distribution of feed zinc (Zn)

Dietary treatment	Zn in feed or its fractions (μg)			% of Zn in different fiber fractions of feed		Level[1] of significance
	Original feed (Analyzed)	NDF	ADF	NDF	ADF	
0%	2647.32	93.38	29.05	3.53[a]	1.10[a]	N.S
10%	2730.17	108.41	39.72	3.97[ab]	1.46[b]	N.S.
20%	2692.24	127.60	41.32	4.74[b]	1.53[b]	N.S.
20% plus enzyme	2659.10	126.55	41.67	4.76[b]	1.57[b]	N.S.

[a-b]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Zn in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

N.S., not significant (P>0.05).

Table 37. Zinc (Zn) located in fiber fractions of feed and fecal excreta

Dietary Treatment	Zn in fractions of feed (μg) NDF	ADF	Level[1] of significance	Zn in fractions of feces (μg) NDF	ADF	Level[1] of significance	% of fecal Zn in fiber fraction to Zn in feed fiber fraction NDF	ADF	Level[1] of significance
0%	93.37[a]	29.05[a]	***	24.92[a]	11.65[a]	***	26.68[ab]	40.04[a]	***
10%	108.41[ab]	39.72[b]	***	37.05[ab]	20.47[b]	***	34.20[b]	51.22[a]	***
20%	127.60[b]	41.31[b]	***	45.17[b]	30.43[c]	***	35.40[b]	73.58[b]	***
20% plus enzyme	126.54[b]	41.67[b]	***	22.65[a]	14.87[ab]	***	17.91[a]	33.66[a]	***

[a-b]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Zn in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

There were no significant differences in concentration of copper in different fecal fiber fractions, the range of percent copper content in the NDF fraction of feed was 45 to 49%, but 32 to 37% in the ADF fraction (Table 40).

The range in the solubility of copper of the NDF and ADF fractions was 55-45% and 73-55%, respectively (Table 41). Large increases in the solubility of copper were obtained by the cellulase treatment ($P>0.05$). That the percent of total feces copper content did not show any differences among dietary treatments is difficult to explain. The availability of metals along the alimentary tract may not necessarily be equated directly with their solubilities, but it is to be expected that some correlation would exist.

Table 38. Effect of dietary wheat bran level upon Copper (Cu) content of fecal excreta

Dietary treatment	Copper consumption (Analyzed)	Cu content in feces	% of fecal Cu
	μg	μg	%
0%	269.23	215.86[a]	80.18
10%	269.18	219.50[ab]	81.54
20%	270.14	235.40[b]	87.14
20% plus enzyme	269.32	205.27[a]	76.25

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

Table 39. Measurements on distribution of fecal copper (Cu)

Dietary treatment	Cu in feces or its fractions (μg)			% of fecal Cu in different fiber fractions		Level[1] of significance
	Original feces (Analyzed)	NDF	ADF	NDF	ADF	
0%	215.86	65.35	40.05	30.15	18.47	***
10%	219.50	72.72	45.97	34.37	21.51	***
20%	235.64	96.34	50.55	34.92	20.98	***
20% plus enzyme	205.27	59.35	39.38	28.63	13.37	***

[1]Significance of differences between the means of percent of Cu in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 40. Measurements on distribution of feed copper (Cu)

Dietary treatment	Cu in feed or its fractions (μg)			% of Cu in different fiber fractions of feed		Level[1] of significance
	Original feed (Analyzed)	NDF	ADF	NDF	ADF	
0%	269.23	119.43	85.60	44.35[c]	31.79[a]	***
10%	269.18	132.42	96.91	49.19[d]	36.06[ab]	***
20%	270.41	131.09	98.56	48.49[d]	36.45[b]	***
20% plus enzyme	269.32	130.84	99.48	48.58[d]	36.94[b]	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[c-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Cu in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

Table 41. Copper (Cu) located in fiber fractions of feed and fecal excreta

Dietary treatment	Cu in fractions of feed (µg)		Level[1] of significance	Cu in fractions of feces (µg)		Level[1] of significance	% of fecal Cu in fiber fraction to Cu in feed fiber fraction		Level[1] of significance
	NDF	ADF		NDF	ADF		NDF	ADF	
0%	119.42	85.60[a]	***	65.35[c]	40.05	***	54.75[a]	46.79[ab]	***
10%	132.42	96.91[b]	***	72.71[c]	45.97	***	54.93[a]	49.16[ab]	***
20%	131.09	98.56[b]	***	82.29[d]	49.44	***	62.77[b]	58.72[b]	***
20% plus enzyme	130.83	99.48[b]	***	59.35[c]	39.88	***	45.38[a]	27.86[a]	***

[a-b]Mean values not having a common superscript are significantly different at the 5% level of probability.

[c-d]Mean values not having a common superscript are significantly different at the 1% level of probability.

[1]Significance of differences between the means of percent of Cu in NDF and in ADF estimated by the t-test. *P<0.05, **P<0.01, ***P<0.005

DISCUSSION

Experiment

Part I

Supplementation of the 20% wheat bran diet with cellulase from Trichoderma viride resulted in a highly significant effect ($P<0.05$) upon feed consumption and feed conversion efficiency. The addition of cellulase did not give a significant growth response.

Perhaps the cellulase addition of 0.008% in this study was too low. The previous levels used were 2 g fungal amylase per lb feed (Willingham et al., 1959), 2 g α-amylase per kg feed (Hersted and McNab, 1975) and 5 g α-amylase per kg feed (Hesselman et al., 1982). However, Qureshi et al. (1980) showed that chicks fed barley with cellulase (0.008%) had a higher body weight and 90% lower cholesterol synthesis than those on the control diet.

Gizzard weight, when expressed as a percentage of body weight, and gut dimension did not show any difference when cellulase was added to the high fiber diet. The reason for the lack of changes in GIT dimensions by the cellulase has not been determined.

Presumably the gut of birds fed 20% wheat bran plus cellulase in this research did not have to accommodate more material at a time or provide greater areas for absorption than that of those receiving the control 20% wheat bran diets. However, Hesselman et al. (1982) reported that there were no significant differences in the weight of ceca expressed as a percentage of body weight between their enzyme treatment group and a non-enzyme treatment group.

Dry matter percentage of the digesta in the GIT was negatively correlated with cell wall contents of the diet in all segments except the ceca.

Cooper and Tyler (1959a,b,c) and Kass et al. (1980) showed that an increase in cellulose resulted in a decrease in percentage fecal dry matter and suggested that the time the feed remains in the GIT may influence total water excretion. As compared to chicks on the enzyme treated diet, the controls had significantly more moisture and less ash in their feces and digesta in all segments except the cecum. Hesselman et al. (1982) suggested that a dry matter increase in chick excreta produced by enzyme supplementation might be a result of enzymatic breakdown of B-glucan, cellulose and hemicellulose to glucose and of oligosaccharides to reducing sugars.

Ryu and Mandels (1980) said that cellulase acts to degrade totally even highly resistant crystalline cellulose to soluble sugar. The increase in ash content of feces produced by enzyme treatment is another measurement indicating improved feed utilization (Willingham et al., 1959).

Chromium turnover time (minutes) in the gizzard, small intestine and entire GIT of chicks fed the diet containing cellulase tended to increase compared with that of chicks fed the control 20% wheat bran diet, but the differences were not significant. Castle and Castle (1956) and Kass et al. (1980) suggested that the length of time digesta remain in GIT, where it is exposed to digestive enzymes and microfloral action, is greater when cell wall content in the diet is

reduced.

The addition of cellulase resulted in a higher percentage of fecal and digesta dry matter compared to that of the control plus 20% wheat bran diet. To my knowledge few animal experiments have been reported where dry matter content of feces have been related to the intake of hydrocolloids.

Gohl et al. (1978) reported that different types of diets which are rich in dietary fiber include plant hydrocolloids, and when consumed result in a lower percentage of fecal dry matter. Gohl (1977) said that most of the plant hydrocolloids accelerate digesta passage in various degrees, but some of the plant hydrocolloids have a retarding action. The reason for this different action seems obscure since there is no apparent chemical relationship between the hydrocolloids with each other. Two groups of hydrocolloids that are of a similar chemical structure are guar gum and carob gum. The principal component of both gums is a galactomannan with a linear chain of (1→4) linked B-D-mannopyranose units with α-D-galactopyranose units attached by (1→6) linkages. These are attached to every alternate mannose in the case of guar gum and to every fourth or fifth mannose in the case of carob bean gum. The two gums also act similarly in that they are the only hydrocolloids to accelerate digesta passage and at the same time increase the dry matter weight of stools.

Preece et al. (1954) described an enzyme system in barley that involves endo-B-glucanase, which causes a decrease in solution viscosity without any large development of reducing groups, and

exo-B-glucanase, which liberates substantial amounts of glucose. They suggested that the beneficial effects are due to changes in the composition of the B-glucan fraction.

The results of apparent digestibility of cell wall components in this study suggested that cellulase consists of a complex containing endo- and exo-glucanases plus B-glucosidases (cellobiase) which act synergistically to degrade highly resistant crystalline cellulose to soluble sugars in the chicken GIT, as proposed by Ryu and Mandels (1980).

Hemicellulose seemed to be more digestible than cellulose in all groups (Table 10). This difference is possibly due to the mild effect of acid in peptic digestion of hemicellulose (Uden, 1978).

Although cellulose is not hydrolyzed in the simple stomach, hemicellulose hydrolysis does occur. Hemicellulose digestion was 38.2% in the part of the GIT anterior to the cecum and colon of swine (Kass et al., 1980).

The apparent digestibility of nitrogen also increased with cellulase supplementation. The disappearance of nitrogen in the GIT is generally considered to be the result of deamination, whereas the appearance of nitrogen in the feces corresponds to bacterial synthesis of protein. There was no significant difference in the amount of nitrogen excreted by the chicks fed the various diets, indicating that microbial syntheses in the large intestines were similar. This is in contrast to the suggestion of Friend et al. (1963) that increased dietary fiber would affect increased nitrogen excretion.

The contents of cell wall components in the gizzards of chicks fed the cellulase supplemented diet in all cases were lower than that of chicks on the control diet. There are two major possible sites, the crop and gizzard, where feed is retained sufficiently long enough to enable cellulase digestion of cell wall components to occur and where the proper pH condition for cellulase activity exists.

The differences in cell wall components in the ceca in this study may indicate that a portion of intestinal ingesta is selectively introduced into the ceca, as reported by Nakahiro et al. (1974).

The negative values obtained for apparent digestibilities of dry matter and all cell wall components in the gizzard and proximal small intestine (Tables 16, 17, 18, 19, 20) show that Cr_2O_3 is not a completely satisfactory marker for rate of passage studies in chicks.

Kass et al. (1980) demonstrated that the negative values obtained for apparent digestibilties of all cell wall components in the stomach and small intestine of pigs indicate relatively greater retention of cell wall components than that of the indicator's, Cr_2O_3, in these portions of the GIT.

Our results (Table 21) demonstrated that the interval from feeding to slaughter influenced the apparent digestibilities of dry matter and cell wall components in all diets. Digestibilities decreased progessively as fasting intervals increased, probably due to the faster rate of passage of Cr_2O_3 than the digesta through the GIT. This agrees with the results reported by Sibbald (1979).

The results from this present study demonstrate positive effects

of cellulase treatment on the nutritive value of a high fiber diet for chicks. The positive response suggests that the beneficial effect was due to a change in the composition of the B-glucan fraction such as homoglycan and heteroglycan.

Part II

The apparent calcium excretion was increased by the addition of dietary fiber (Table 22), but there were no significant differences. Fiber had no apparent effect on calcium absorption in this study.

Calcium levels in the fiber fractions of feces and feed were very low. The available calcium content in the ADF fraction was significantly lower for each level of wheat bran supplementation than for that in the NDF fraction ($P<0.001$, Table 25). The amount of calcium excreted was highly correlated with the ADF fraction, and the binding of calcium to the ADF fraction could be expected to reduce availability of calcium for intestinal absorption.

Reinhold et al. (1975) reported that the ability to bind calcium is a function of fiber concentration. Bran with a fiber concentration of 10.9% bound 5.4% of calcium in a solution containing 10 mg/100 ml.

Sandstead et al. (1979) showed an apparent interference with the intestinal absorption of iron, zinc, calcium, magnesium and phosphorus in subjects who were fed diets rich in dietary fiber and phytate, high in vegetable protein, and relatively low in animal protein.

The percent of fecal excretion of phosphorus was not significantly affected by the addition of dietary fiber. Even the phosphorus

content in feces of chicks fed 20% wheat bran was significantly higher than that for the other dietary treatments (Table 26).

Changes in phosphorus excretion accompanying increased NDF or ADF were even less consistent (Table 27, 28).

Significantly different amounts of phosphorus were in the NDF fraction of feed ($P<0.005$) and were removed from the NDF fraction of feed in the chick's GIT (Table 27, 28, 29). A substantial increase in non-phytate phosphate could have reinforced the action of phytate in decreasing calcium, iron and zinc absorption from the gut (Reinhold et al., 1973).

Ismail-Beigi et al. (1977) demonstrated that only slight binding of phosphorus to cellulose supported strong binding of divalent metals, especially when the diet was rich in phosphorus. However, Ward and Harbers (1982) said that distributions of Ca and P are relatively independent of the cell wall. The element maps for phosphorus and silicon are somewhat independent in their distributions. Reinhold et al. (1976) noted that the correlation between fiber and phosphorus in the feces is not as close nor as consistent as that of calcium and magnesium.

When the chicks were fed the diets containing over 10% wheat bran, iron balance was negative (Table 30). Substantial amounts of ferrous or ferric iron are bound by bran (Tables 31, 32, 33) as reported by Reinhold et al. (1975). Bjorn-Rasmussen (1974) showed that there was a decrease in iron absorption from rolls baked with added bran in amounts equal to and greater than 3.3%. He found a

significant linear relationship between the ratio of absorption with/without added bran. He concluded that addition of approximately 7% of bran to wheat bread decreases iron absorption.

It is considered reasonable to ascribe the marked reduction in iron absorption to the content of phytate in bran. Bran also contains fair amounts of inorganic phosphates.

The findings in rats that inorganic phosphates also inhibit iron absorption (Hegsted et al., 1949) make it difficult to state that the inhibitory effect of bran on iron absorption is due only to its high content of phytates.

Morris and Ellis (1976) suggested that in the rat the monoferric phytate present in bran was readily available for absorption when isolated from the bran while other bran-iron-phytate complexes were not.

Although the present study was not designed to investigate the interactions among phytic acid, iron and fiber fractions, it is possible to conclude that iron binds directly to plant cell wall components, especially the ADF fraction, and that a bran-iron-phytate complex is formed, supporting the hypothesis proposed by Morris and Ellis (1976).

Excretion of zinc showed no significant changes as a result of any of the dietary treatments (Table 34).

Fiber fractions from wheat bran have an extremely low affinity for zinc (Tables 35, 36), and zinc bound to fiber fractions was less effectively solublized than other metals (Table 37). The lack of any relationship between dietary fiber and fecal zinc found in this study

agrees with the results reported by Sandstead et al. (1979).

There was no attempt made to illustrate the probable relationship between wheat bran content and copper absorption (Table 38), although significant amounts of copper were in the NDF and ADF fractions of feces and feed (Tables 39,40).

However, copper in fiber fractions may consist of two main fractions: a potentially available mineral fraction released for absorption as a result of fiber and protein digestion, and a complexed or bound fraction of low bioavailability (Table 41).

Bremner (1970) showed that the strength of binding of the metals appeared to be in the order of Cu>Zn>Mn on the basis of the extent of dissolution of the metals upon treatment with acid or with EDTA. The results of his study were strengthened by the finding that treatment with detergent had little effect on the solubilities of the copper in fiber fractions, and that the fiber fraction from wheat bran had no apparent effect on copper absorption in the chick's GIT.

Sandstead et al. (1978) reported that copper balance was improved when wheat bran was fed, possibly because the copper intake was simply increased.

The limited observations of the present studies neither support nor refute the reports of Reinhold et al. (1973, 1974, 1975, 1976) that bread prepared from high-extraction wheat flour can impair intestinal absorption of metals.

However, the present studies agree with the results reported by Sandstead et al. (1978) and Southgate et al. (1976).

The observations of the Reinhold group were made on subjects fed a middle Eastern diet, the composition of which was substantially different from the diets in the present experiments. The one exception where our findings appear to agree is on the effect of increase dietary fiber on iron extraction.

Our findings suggest that modest intakes of dietary fiber do not adversely affect chicks which consume a balanced ration that includes a proper intake of sources of organic and inorganic minerals.

An involvement of the residual calcium, phosphorus, iron, zinc and copper with complex carbohydrates in the cell wall of the plant is suggested by the action of cellulase. Significant amounts of metals associated with the cell wall were liberated by this treatment. As cellulolytic enzymes function in the rumen, it is probable that rumen fermentation could result in a similar liberation of the metals from the cell wall components (Bremner, 1970).

SUMMARY

Two studies were carried out to investigate the value of cellulase in a diet containing 20% wheat bran on solubilizing minerals associated with cell walls.

The following general conclusions were made based on the results obtained in terms of the parameters measured:

1. Supplementation with cellulase resulted in a highly significant effect on feed consumption and feed efficiency, but it did not show a growth response.
2. Gut dimension studies did not show any differences when cellulase was added to the higher fiber diet.
3. Chicks fed the control diet containing 20% wheat bran as compared to those fed the cellulase supplemented diet had significantly more moisture and less ash in their excreta and in the digesta of all intestinal segments except the ceca.
4. Chromium turnover time (minutes) in the gizzard, small intestine and entire GIT of chicks was not influenced by cellulase.
5. Apparent digestibilities of dry matter and all cell wall components were significantly higher from chicks fed cellulase than from chicks fed the control diets.
6. Much digestion of cell wall components by cellulase can occur in the crop and gizzard.
7. Significant amounts of calcium, phosphorus, iron, zinc and copper associated with cell wall components were solubilized by cellulase.

8. Iron balance was negative from the 10% and 20% wheat bran fed groups. Chicks fed the cellulase supplemented diets showed a positive balance. Ferrous or ferric iron which is bound by wheat bran was apparently made available for absorption by cellulase.

REFERENCES

Aastrup, S., 1979. The relationship between the viscosity of an acid flour extract of barley and it's B-glucan content. Carlsberg Res. Commun. 44:289-304.

Adler, E., 1977. Lignin chemistry - past, present and future. Wood Sci. Technol. 11:168-218.

Altsch, M. D., 1957. Zinc deficiency and dietary calcium in swine. Nutritional Reviews 15:334-337.

Amine, E. K., R. Neff and D. M. Hegested, 1972. Biological estimation of available iron using chicks or rats. J. Agr. Food Chem. 20:246-251.

Apte, S. U., 1967. Effect of phytate on iron absorption. Nutritional Reviews 25:218-222.

Arscott, G. H., and R. J. Rose, 1960. Use of barley in high-efficiency broiler rations. Poultry Sci. 39:93-95.

Association of Official Agricultural Chemists (A.O.A.C.), 1965. Official Methods of Analysis. 10th ed. Washington, D.C.

Atalla, R. H., 1979. Conformational effects in hydrolysis of cellulose. Advances in Chemistry Series 181. p. 55-69.

Barr, A.J., J. H. Goodnight, J. P. Sall, W. H. Blair and D. M. Chilko, 1982. A user's guide to SAS 1982. J. T. Helwig and K. A. Council, ed., SAS Institute Inc., P. O. Box 10066, Raleigh, North Carolina, 27605.

Barr, T. N., 1981. The world food situation and global grain prospects. Science 214:1087-1095.

Becker, W. M., and W. G. Hoekstra, 1971. The Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides. p. 209. Oxford.

Berghem, L. E. R., and L. G. Pettersson, 1973. The mechanism of enzymatic cellulose degradation. Eur. J. Biochem. 37:21-30.

Bisaria. V. S., and T. K. Ghose, 1981. Biodegradation of cellulosic materials (reveiws). Enzyme Microb. Technol. 3:90-104.

Bitar, K., and J. G. Reinhold, 1972. Phytase and alkaline phosphtase activities in intestinal mucosae of rat, chicken, calf and man. Biochem. Biophys. Acta 268:442-452.

Bjorn-Rasmussen, E., 1974. Iron absorption from wheat bread, influence of various amounts of bran. Nutr. Metabol. 16:101-110.

Blaxter, K. L., N. McC. Graham and F. W. Wainman, 1956. Some observations on the digestibility of food by sheep and on related problems. Br. J. Nutr. 10:69-73.

Bolin, D. W., R. P. King and E. W. Klosterman, 1952. A simplified method for determination of chromic oxide (Cr_2O_3) when used as an index substance. Science 116:634-637.

Bolton, W., 1965. Digestion in crop. Brit. Poult. Sci. 6:97-102.

Branch, W. J., D. A. T. Southgate and W. P. T. James, 1975. Binding of calcium by dietary fiber: its relationship to unsubstituted uronic acid. Proc. Nutr. Soc. 34:120A-127A.

Bremner, I., 1970. Zinc, copper and manganese in the alimentary tract of sheep. Br. J. Nutr. 24:769-782.

Browne, T. G., 1922. Some observations on the digestive system of the fowl. J. Comp. Path. Ther. 35:12-32.

Burnett, G. S., 1965. Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. Brit. Poult. Sci. 7:55-75.

Calhoun, M. L., 1933. The microscopic anatomy of the digestive tract of gallus domesticus. Ia. St. Col. Jour. Sci. 7:261.

Card, L.E., M. H. Keith and H. H. Mitchell, 1926. Facts obtained on rate of digestion on feed by hens. Illinois Station Report, 1926:80.

Castle, E. J., 1956. The rate of passage of food through the alimentary tract of the goat. 1. Studies on adult animals fed hay and concentrations. Br. J. Nutr. 10:15-23.

Cherry, J. A., and P. B. Siegel, 1978. Selection for body weight at eight weeks of age. 15. Feed passage and intestinal size of normal and dwarf chicks. Poultry Sci. 57:336-340.

Cook, J. D., V. Minnich, C. V. Moore, A. Rasmussen, W. B. Bradley and C. A. Finch, 1973. Absorption of fortification iron in bread. Am. J. Clin. Nutr. 26:861-872.

Cooper, P. H., and C. Tyler, 1959a. Some effects of bran and cellulose on the water relationship in the digesta and feces of pigs. Part I. The effects of including bran and two forms of cellulose

in otherwise normal rations. J. Agr. Sci. Camb. 52:332-339.

Cooper, P. H., and C. Tyler, 1959b. Some effects of bran and cellulose on the water relationship in the digesta and feces of pigs. Part II. The effects of adding different levels of fibrous cellulose to a highly purified ration. J. Agr. Sci. Camb. 52:340-347.

Cooper, P. H., and C. Tyler, 1959c. Some effects of bran and cellulose on the water relationship in the digesta and feces of pigs. Part III. The effect of level of water intake and level of cellulose in the ration on the dry matter content of the feces. J. Agr. Sci.Camb. 52:348-351.

Cowan, J. W., M. Esfahani, J. P. Salji and S. A. Azzam, 1956. Effect of phytate on iron absorption in the rat. J. Nutr. 90:423-427.

Cowling, E. B., and W. Brown, 1969. Structural features of cellulosic materials in relation to enzymatic hydrolysis. Cellulase and Their Application, p. 152. Advances in Chemistry Series.

Crawford, D. L., and R. L. Crawford, 1980. Microbial degradation of lignin. Enzyme Microb. Technol. 2:11-22.

Cummings, J. H., 1978. Nutritional implications of dietary fiber. Am. J. Clin. Nutr. 31:521-529.

Cunha, T. J., 1983. Proper mineral supplementation called complex matter. Feedstuffs, p. 39. Oct. 3, 1983.

Dahmer, E. J., R. H. Grummer and W.G. Hoekstra, 1966. Alleviation of zinc-deficiency in swine by feeding blood meal. J. Animal Sci. 25:890-898.

Dansky, L. M., and F. W. Hill, 1952. Application of the chromic oxide indicator method to balance studies with growing chickens. J. Nutr. 47:449-460.

Davis, F., and G. M. Briggs, 1947. The growth promoting action of cellulose in purified diets for chicks. J. Nutr. 34:295-300.

Davis, F., and G. M. Briggs, 1948. Sawdust in purified chick rations. Poultry Sci. 27:117-118.

Deaton, J. W., L. F. Kubena, J. D. May, F. N. Reece and B. D. Lott, 1973. A noted difference in gizzard weight between cage- and floor-reared broilers. Poultry Sci. 52:1217-1218.

Deaton, J. W., L. F. Kubena, F. N. Reece and B. D. Lott, 1977. Effect

of dietary fiber on the performance of laying hens. Brit. Poult. Sci. 18:711-714.

Duke, G. E., 1977. Duke's Physiology of Domestic Animals. Ninth Edition. p 313.

Dvorak, R. A., and D. J. Bray, 1978. Influence of cellulose and ambient temperature on feed intake and growth of chicks. Poultry Sci. 57:1351-1354.

Eriksson, K., 1978. Enzyme mechanisms involved in cellulose hydrolysis by the rot fungus Sporotrichum pulverulentum. Biotech. and Bioeng. 21:131-146.

Fedorovskii, N. P., 1951. Crop and stomach digestion of hen turkeys. Sovetskara Zootekhmia 1:50-58.

Fisher, H., and H. S. Weiss, 1956. Feed consumption in relation to dietary bulk and energy level. Poultry Sci. 35:418-423.

Fraps, G. S., 1946. Composition and productive energy of poultry feeds and ration. Texas Agri. Exp. Sta. Bull. 678.

Freudenberg, K., and A. C. Neish, 1968. Constitution and Biosynthesis of Lignin. Springer, Berlin. p. 196.

Friend, D. W., H. M. Cunningham and J. W. G. Nicholson, 1963. The production of organic acids in the pig. Can. J. Anim. Sci. 43:156-162.

Fry, R. E., J. B. Allred, L. S. Jensen and J. McGinnis, 1957. Improvement in the nutritional value of barley for chicks by enzyme supplementation. Poultry Sci. 36:919-921.

Ghose, T. K., and V. S. Bisaria, 1979. Studies on the mechanism of enzymatic hydrolysis of cellulosic substances. Biotech. and Bioeng. 21:131-146.

Ghosh, B. S., and A. B. Kundu, 1980. Induction of cellulases and hemicellulases by Tamarind (Tamarindus indica) kernal polysaccharide. J. Ferment. Technol. 58:135-141.

Gohl, B., 1977. Effects of hydrocolloids on productive value and feeding characteristics of barley. Dept. of Animal Husbandry. Agricultural College of Sweden, S-750 07 Uppsala 7, Sweden. pp. 2-23.

Gohl, B., 1978. Influence of water treatment of barley on the digestion process in rats. Dept. of Animal Husbandry.

Agricultural College of Sweden, S-750. 07 Uppsala 7, Sweden. pp. 1-17.

Gohl, B., S. Alden, K. Elwinger and S. Thomke, 1978. Influence of B-glucanase on feeding value of barley for poultry and moisture content of excreta. Brit. Poult. Sci. 19:41-47.

Grovum, W. L., and G. D. Phillips, 1973. Rate of passage of digesta in sheep. 5. Theoretical considerations based on a physical model and computer simulation. Br. J. Nutr. 30:377-390.

Hallberg, L., and E. Bjorn-Rasmussen, 1972. Determination of iron absorption from whole diet. Scand. J. Haemat. 9:93-111.

Hallberg, L., and L. Solvell, 1967. Absorption of hemoglobin iron in man. Acta. Med. Scand. 181:147-152.

Halliwell, G., and M. Griffin, 1973. The nature and mode of action of the cellulolytic component C_1 of Trichoderma koningii on native cellulose. Biochem. J. 125:587-594.

Halnan, E. T., 1949. The architecture of the avian gut and tolerance of crude fiber. Br. J. Nutr. 3:245-253.

Harland, B. F., R. G. O'Dell, C. L. Stone and L. Prosky, 1977. Metabolic aspects of altering fiber, phytate and zinc in wheat bran fractions fed to rats. Federation Proc. 36:1118.

Harrison, D. C., and E. Mellamby, 1939. Phytic acid and rickets producing actions of cereals. Biochem. J. 33:1660-1680.

Heger, J., Z. Frydrych and J. Varak, 1983. The effectiveness of an enzymatic preparation in chicks and rats. Biol. Chem. Vet. 25:35-42.

Hegsted. D. M., C. A. Finch and T. D. Kinney, 1949. The influence of diet on iron absorption. J. Exp. Med. 90:147-152.

Herstad, O., and J. M. McNab, 1975. The effect of heat treatment and enzyme supplementation on the nutritive value of barley for broiler chicks. Brit. Poult. Sci. 16:1-8.

Hesselman, K., K. Elwinger, M. Nilsson and S. Thomke, 1982. The effect of B-glucanase supplementation, stage of ripeness and storage treatment of barley in diets fed to broiler chickens. Personal communication, Swedish Univ. of Agric. Sci., S-750 07 Uppsala, Sweden. pp. 1-20.

Heuser, G. F., 1944. The rate of passage of feed from the crop of the hen. Poultry Sci. 24:20-24.

Hillerman, J. P., F. H. Kratzer and W. D. Wilson, 1953. Food passage through chickens and turkeys and some regulatory factors. Poultry Sci. 35:332-335.

Hinton, J. M., J. E. Leonard-Jones and A. C. Young, 1969. A new methods for studying gut transit times using radioisotope markers. Gut 10:842-848.

Hoelzel, F., 1930. The rate of passage of inert materials through the digestive tract. Amer. J. Physiol. 92:466-497.

Ismail-Beigi, F., J. G. Reinhold, B. Faraji, and P. Abadi, 1977. Effect of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. J. Nutr. 107:510-518.

Isshiki, Y., H. Yokota, Y. Nakahiro and I. Tasaki, 1974. Digestion and absorption in the cecum of chickens. Jap. J. Zootech. Sci. 45:488-493.

Jensen, L. S., R. E. Fry, J. B. Alred and J. McGinnis, 1957. Improvement in the nutritive value of barley for chicks by enzyme supplementation. Poultry Sci. 36:919-921.

Jones, J. H., 1978. Mineral components of plant cell walls. Am. J. Clin. Nutr. 31:594-598.

Jones. L. H. P., A. A. Milne and S. M. Wadham, 1963. Studies of silica in the oat plant. Plant Soil 18:350-361.

Kass, M. L., P. J. Van Soest, W. G. Pond, B. Lewis and R. E. McDowell, 1980. Utilization of dietary fiber from alfalfa by growing swine. J. Animal Sci. 50:175-191.

Kaufman, H. W., and I. Kleinberg, 1971. Effect of pH on calcium binding by phytic acid and its inositol phosphoric acid derivative and on the solubility of their calcium salts. Archs. Oral Biol. 16:445-460.

Kaupp, B. F., and J. E. Ivey, 1923. Time required for food to pass through the intestinal tract of fowls. J. Agr. Res. 23:721-725.

Kincaid, R. L., and J. D. Cronrath, 1983. Amounts and distribution of minerals in Washington forages. J. Dairy Sci. 66:821-824.

Kratzer, F. H., L. Earl and C. Chiaravanant, 1974. Factors influencing the feeding value of rice bran for chickens. Poultry Sci. 53:1795-1800.

Kubena, L. F., I. W. Deaton, J. D. May and F. N. Reece, 1974. A dietary method to correct a gizzard abnormality of broilers. Poultry Sci. 53:407-409.

Leopold, A. S., 1953. Intestinal morphology of gallinaceous birds in relation to food habits. J. Wildl. Mgmt. 17:197-203.

Lease, J. C., 1961. Zinc deficiency in chicks. Nutritional Reviews 19:111-113.

Lewin, V., 1963. Reproduction and development of young in a population of California quail, Condor 65:249-278.

Likuski, H. J. A., and R. M. Forbes, 1965. Mineral utilization in the rat. IV. Effects of calcium and phytic acid on the utilization of dietary zinc. J. Nutr. 85:230-234.

Mateos, G. G., and J. L. Sell, 1981. Influence of fat and carbohydrate source on the rate of food passage of semipurified diet for laying hens. Poultry Sci. 60:2114-2119.

Maynard, L. A., J. K. Loosli, H. F. Hintz and R. G. Warner, 1979. Animal Nutrition. 7th Edition. pp. 86,235.

McCance, R. A., C. N. Edgecombe and E.M. Widdowson, 1943. Phytic acid and iron absorption. Lancet 2:126-128.

McCance, R. A., and E. M. Widdowson, 1942a. Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol. 101:44-65.

McCance, R. A., and E. M. Widdowson, 1942b. Mineral metabolism on dephytinized bread. J. Physiol. 101:304-313.

McRae, J. C., 1974. The use of intestinal markers to measure digestive function in ruminants. Proc. Nutr. Soc. 33:141-150.

Molley, L. E., and E. L. Richards, 1971. Complexing of calcium and magnesium by the organic constituents of Yorkshire Fog (Thelcus lanatus). J. Sci. Food Agri. 22:397-400.

Mongin, P., 1976. Composition of crop and gizzard contents in the laying hen. Brit. Poult. Sci. 17:499-507.

Mongin, P., and B. Sauveur, 1974. Hourly water consumption and egg formation in domestic fowl. Brit. Poult. Sci. 15:361-368.

Monson, W. J., L. S. Dietrich and C. A. Elvehjem, 1950. Studies on the effect of different carbohydrates on chick growth. Proc. Soc. Exp. Biol. Med. 75:256-259.

Moore, C. V., 1955. The importance of nutritional factors in pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutr. 3:3-10.

Morris, E. R., and R. Ellis, 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753-760.

Morris, L., R. B. Thompson and V. G. Hiller, 1932. Crude Fiber in chicken rations. Poultry Sci. 11:219-225.

Moss, R., 1972. Effects of captivity on gut lengths in red grouse. J. Wildl. Mgmt. 36:251-263.

Moss, R., 1974. Winter diets, gut lengths and interspecific competition in Alaskan ptarmigan. Auk 91:737-746.

Nakahiro, Y., Y. Isshiki and I. Tasaki, 1974. Digestion of crude fiber in the cecum of the chicken. Jap. J. Zootech. Sci. 45:427-432.

Nakanishi, K., and T. Yasui, 1980. Uptake of B-xyloside by Streptomyces sp. during induction of xylanase. J. Ferment. Technol. 58:171-174.

Nevalainen, K. M. H., E. T. Palva and M. J. Bailey, 1980. A high cellulase-producing mutant strain of Trichoderma reesei. Enzyme Microb. Technol. 2:59-60.

Nimz, H., 1974. Beech lignin proposal of a constitutional scheme. Angew. Chem. Internat. Edit. 13:313-321.

Nisizawa, T., H. Suzuki and K. Nisizawa, 1971. Synthesis of cellulase induced by sophorose in Trichoderma viride cell. J. Biochem. 70:387-393.

Oberleas, D., M. E. Muhrer and B. L. O'Dell, 1962. Effect of phytic acid on zinc availability and parakeratosis in swine. J. Animal Sci. 21:57-61.

Oberleas, D., M. E. Muhrer and R. L. O'Dell, 1966. Dietary metal-complexing agents and zinc availability in the rat. J. Nutr. 90:56-62.

Parrish, F. W., A. S. Perlin and E. T. Reese, 1960. Selective enzymolysis of poly-B-D-glucans, and the structure of the polymers. Can. J. Chem. 38:2094.

Perdomo, J. T., R. L. Shirley and C. F. Chicco, 1977. Availability of nutrient minerals in four tropical forages fed freshly to sheep. J. Animal Sci. 45:1114-1119.

Petersen, V. E., 1972. The properties and value of various feed grains in poultry nutrition. J. Cereal Processing and Digestion. pp. 67-75.

Peterson, C. F., and E. A. Sauter, 1968. Enzyme sources and their value in barley rations for chick growth and egg production. Poultry Sci. 47:1219-1224.

Peterson, D. W., 1950. Some properties of a factor in alfalfa meal causing depression of growth in chicks. J. Biol. Chem. 183:647-653.

Peterson, P. J., 1969. The distribution of zinc-65 in Agrostis tenus sibth. and A. stolonifera L. tissues. J. Exptl. Botany 20:863-866.

Pomare, E. W., and K. W. Heaton, 1973. Alteration of bile salt metabolism by dietary fiber. Brit. Med. J. 4:262-269.

Preece, I. A., R. A. Aitken and J. A, Dick. 1954. Non-starch polysaccharides of cereal grain. VI. Preliminary study of the enzymolysis of barley B-glucosan. J. Inst. Brew. 16:497-507.

Pritchard, P. J., 1972. Digestion of sugars in the crop. Comp. Biochem. Physiol. 43A:195-205.

Qureshi, A. A., W. C. Burger, N. Prentice, H. R. Bird and M. L. Sunde, 1980. Suppression of cholesterol and stimulation of fatty acid biosynthesis in chicken livers by dietary cereals supplemented with culture filtrate of Trichoderma viride. J. Nutr. 110:1014-1022.

Ramby, B., 1969. Recent Progress on the Structure and Morphology of Cellulose. American Chemical Society, Washington, D.C. pp. 139-151.

Reese, E. T., and M. Mandels, 1980. Stability of the cellulase of Trichoderma reesei under use conditions. Biotech. and Bioeng. 21:131-146.

Reese, E. T., R. G. H. Sui and M. S. Levinson, 1950. The biological degradation of soluble cellulose derivatives and its relationship

to the mechanism of cellulose hydrolysis. J. Bacteriol. 59:485-497.

Reinhold, J. G., B. Faradji, P. Abadi and F. Ismail-Beigi, 1976. Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. J. Nutr. 106:493-503.

Reinhold, J. G., F. Ismail-Beigi and B. Faradji, 1975. Fibre vs. phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. Nutr. Rep. Int. 12:75-78.

Reinhold, J. G., K. Nasr, A. Lahimgarzadeh and H. Hedayati, 1973. Effect of purified phytate and phytate-rich bread upon metaboism of zinc, calcium, phosphorus and naitrogen in man. Lancet 1:283-288.

Reinhold, J. G., A. Parsa, N. Karimian, J. W. Hammick and F. Ismail-Beigi, 1974. Availability of zinc in leavened and unleavend whole meal wheaten breads as measured by solubility and uptake by rat intestine in vitro. J. Nutr. 104:976-952.

Rescigno, A., and G. Segre, 1966. Drug and Tracer Kinetics. Waltham, Mass.; Toronto; London: Blaisdell Publ. Company.

Ryu, D. D., and M. Mandels, 1980. Cellulases: biosynthesis and applications. Enzyme Microb. Technol. 2:91-102.

Saito, M., I. Tasaki, K. Kibe, H. Yamada and T. Igarash, 1959. Effect of various cellulose levels in the diet on the chick growth. Poultry Sci. 38:373-376.

Sandstead, H. H., L. M. Klevay, R. A. Jacobs, J. M. Munoz, G. M. Logan, S. J. Reck, F. R. Dintzis, G. E. Inglett and W. C. Shuey, 1979. Effect of dietary fiber and protein level on mineral element metabolism. Dietary Fibers Chemistry and Nutrition p. 147-156. Edited by G. E. Inglett and S. Z. Falkehag.

Sandstead, H. H., L. M. Klevay, J. M. Munoz, R. A. Jacobs, S. J. Reck, D. Tucker, G. M. Logan, L. Eelkema, G. E. Inglett, F. Dintzis and W. Shuey, 1977. Zinc and copper balance in humans fed fiber. Federation Proc. 36:1118.

Sandstead, H. H., J. M. Munoz, R. A. Jacobs, L. M. Klevay, S. J. Reck, G. M. Logan, F. R. Dintzis, G. E. Inglett and W. C. Shuey, 1978. Influence of dietary fiber on trace element balance. Am. J. Clin. Nutr. 31:3180-3184.

Savory, C. J., and M. J. Gentle, 1976. Effect of dietary dilution with fiber on the food intake and gut dimensions of Japanese quail. Brit. Poul. Sci. 17:561-570.

Schwarzenbach, G., 1952. Chelate complex formation as a basis for titration processes, Analyt. Chem. Acta. 7:141-155.

Selby, K., and C. C. Maitland, 1967. Separation of the components involved in the solubilization of cotton. Biochem. J. 104:716-724.

Shipley, A. R., and R. E. Clark, 1972. Tracer Methods for In Vivo Kinetics. New York and London: Academic Press.

Sibbald, I. R., 1979. Passage of feed through the adult rooster. Poultry Sci. 58:446-459.

Sibbald, I. R., 1980. The clearance time and rate of passage of feed residues. Poultry Sci. 59:374-377.

Sisson, S., and J. D. Grossman, 1953. The Anatomy of Domestic Animals. Philadelphia and London: W. B. Saunders Co.

Southgate, D. A. T., W. J. Branch, M. J. Hill, B. S. Drasar, R. L. Walters, P. S. Davies and I. M. Baird, 1976. Metabolic responses to dietary supplements of bran. Metabolism 25:1129-1135.

Stokstad, E. L. R., T.H. Jukes and W. L. Williams, 1953. Growth-promoting effect of aureomycin on various types of diet. Poultry Sci. 32:1054-1058.

Stoszek, M. J., J. E. Oldfield, G. E. Carter and P. H. Weswig, 1979. Effect of tall fescue and quackgrass on copper metabolism and weight gains of beef cattle. J. Animal Sci. 48:893-899.

Su, T. M., and I. Paulavicius, 1975. Enzymatic saccharification of cellulase by thermophilic actinomyces. Applied Polymer Symposium 28:221-236.

Sullivan, J. F., J. O'Grady and H. G. Langford, 1966. Composition of gastrointestinal secretions. Gastroenteral 48:438-446.

Tasaki, I., and K. Kibe, 1958. A study on the digestion of cellulose in poultry. Poultry Sci. 38:373-376.

Tuckey, R., B. E. March and J. Biely, 1958. Diet and the rate of food passage in the growing chick. Poultry Sci. 37:787-792.

Turner, R. G., 1970. The subcellular distribution of zinc and copper within the roots of metal-tolerant clones of Agrostic tenuis sibth. New Phytol. 69:725-730.

Uden, P., 1978. Comparative Studies on Rate of Passage, Particle Size and Rate of Digestion in Ruminants, Equines, Rabbits and Man. Cornell University. Ph.D. Thesis.

Van Soest, P. J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Ass. Official Agr. Chem. 46:829.

Van Soest, P. J., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constitutents. J. Ass. Official Agr. Chem 50:50.

Van Soest, P. J., 1984. Fiber Quality. Feed Management 35:36-38.

Van Soest, P. J., J. B. Robertson, D. A. Roe, J. Rivers, B. A. Lewis and L. R. Hackler, 1978. The role of dietary fiber in human nutrition. Cornell Nutrition Conference. pp. 5-12.

Vohra, P., and F. H. Kratzer, 1966. Influence of various phosphates and other complexing agents on the availability of zinc for turkey poults. J. Nutr. 89:106-112.

Ward, C., and L. H. Harbers, 1982. Effect of pH on extractability of calcium and oxalate from alfalfa leaflets. J. Dairy Sci. 65:154-160.

Warner, A. C. I., and B. D. Stacey, 1968. The fate of water in the rument. 2. Water balance throughout the feeding cycle in sheep. Br. J. Nutr. 22:389-410.

Warner, R. L., L. Z. McFarland and W. O. Wilson, 1967. Microanatomy of the upper digestive tract of the Japanese quail. Am. J. Vet. Res. 28:1537-1548.

Willingham, H. F., L. S. Jensen and J. McGinnis, 1959. Studies on the role of enzyme supplements and water treatment for improving the nutritional value of barley. Poultry Sci. 38:93-95.

Wood, T. M., and S. I. McCare, 1972. The purification and properties of C_1 component of Trichoderma koningii celluase. Biochem. J. 128:1183-1192.