

THE EFFECT OF ENZYME SUPPLEMENTATION
ON THE BIOAVAILABLE ENERGY AND PROTEIN AND LIPID
DIGESTIBILITIES OF WHEAT, BARLEY, OATS, AND RYE GRAIN FOR
THE YOUNG BROILER CHICK.

A Thesis
Submitted to the Faculty
of
Graduate Studies
The University of Manitoba
by
Orville David Friesen

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science
Department of Animal Science
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THE EFFECT OF ENZYME SUPPLEMENTATION ON THE
BIOAVAILABLE ENERGY AND PROTEIN AND LIPID
DIGESTIBILITIES OF WHEAT, BARLEY, OATS, AND RYE GRAIN
FOR THE YOUNG BROILER CHICK

BY

ORVILLE DAVID FRIESEN

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

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ABSTRACT

A series of studies were designed to examine the effect of enzyme supplementation on the apparent metabolizable energy (AME_n) and apparent digestibilities of protein (APD) and lipid (ALD) of wheat-, barley- (hulled and hulless), naked oat- and rye-based diets fed to young broiler chickens.

Two chick bioassays were compared to determine the effect of enzyme supplementation on the AME_n of hulless barley and APD of hulless barley-based diets. In the first method, where barley was substituted for sucrose, enzyme supplementation increased ($P < .05$) the AME_n and APD of barley diets containing either corn oil or tallow as a dietary fat source. In the second method, hulless barley was substituted for an entire semi-practical diet and the energy balance of the diets was compared. Enzyme supplementation increased the AME_n of hulless barley ($P < .05$) with small improvements ($P > .05$) in APD. Increasing dietary levels of test grain substituted for wheat resulted in depressed growth performance compared to the control wheat diet. The antinutritive β -glucans (barley, oats) and pentosans (rye) had the most pronounced effect on ALD ($P < .01$). Enzyme supplementation increased ($P < .01$) AME_n , APD, and ALD for all test cereals. Enzyme supplementation resulted in 4, 7, 42, 33, and 14% improvements in the 70% wheat-, barley-, hulless barley-, oats- and rye-based diets, respectively, compared to their nonsupplemented counterparts.

Two enzyme dose-response studies using a 60% rye diet observed maximum response at 3.2 g enzyme/kg diet which yielded 71, 193 and 43% improvements in feed consumption, weight gain and feed to gain ratio, respectively, compared to the nonsupplemented rye control diet. In 60% rye diets, enzyme addition (3.2 g/kg diet) increased the dietary AME_n and

APD by 23 and 12%, respectively, compared to the nonsupplemented diet. The AME_n and APD of the 60% wheat control diet were increased 9.7 and 4.6%, respectively, by enzyme addition. The present studies suggest that the hydrolysis of β -glucans and pentosans by enzyme supplementation increases the AME_n, APD, and ALD of hulless barley, naked oats and rye for young broiler chicks.

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My sincere appreciation and thanks to my supervisor Dr. W. Guenter for his patience, guidance and advice throughout my M.Sc. program. I would also like to extend thanks to the members of my advisory committee, Dr. R. R. Marquardt and Dr. M. Ballance for their guidance and advice. Special thanks to Dr. B. A. Rotter for her valuable advice and help during the early experimental phase of my program. I would also like to express appreciation to Dr. E. H. Stobbe for reading this thesis and making helpful comments.

Special thanks to the Natural Sciences and Engineering Research Council for in part funding this research.

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My most special thanks and appreciation to my wife, April for her unfailing support and sacrifice throughout my studies. This thesis is dedicated to her and our two beautiful daughters, Kari and Allyson.

FOREWARD

The preparation of this thesis followed a manuscript format. Manuscript I (Experiment 1) was 1 of 4 studies published in "Poultry Science" in a paper having Dr. B. A. Rotter as the senior author and O. D. Friesen as the second author (see below). O. D. Friesen's contribution to this publication was carrying out and analyzing the data for this experiment and assisting in the writing and editing of this paper. O. D. Friesen's advisory committee felt that the data from this experiment was well suited to publish along with Dr. B. A. Rotter's data from 3 other experiments. Manuscript I in this thesis is not taken from this publication and was solely written by O. D. Friesen. Manuscripts II and III have been published in "Poultry Science". Experiment 2 (Manuscript 1) will not be published.

MANUSCRIPT I (Experiment 1):

ROTTER, B.A., FRIESEN, O.D., GUENTER, W. and MARQUARDT, R.R. 1990.
Influence of enzyme supplementation on the bioavailable energy of
barley. Poultry Sci. 69:1174-1181.

MANUSCRIPT II:

FRIESEN, O.D., GUENTER, W., MARQUARDT, R.R. and ROTTER, B.A. 1992.
The effect of enzyme supplementation on the apparent metabolizable
energy and nutrient digestibilities of wheat, barley, oats and rye
for the young broiler chick. Poultry Sci. 71:1710-1721.

MANUSCRIPT III:

FRIESEN, O.D., GUENTER, W., MARQUARDT, R.R. and ROTTER, B.A. 1991.
The effects of enzyme supplementation on the nutritive value of rye
grain (*Secale cereale*) for the young broiler chick. Poultry Sci.
70:2501-2508.

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ABBREVIATIONS

AME _n	- Nitrogen-corrected apparent metabolizable energy
AME _n (H)	- Nitrogen-corrected apparent metabolizable energy (Scott et al. 1982)
AME _n (S)	- Nitrogen-corrected apparent metabolizable energy (Sibbald and Slinger, 1962)
APD	- Apparent protein digestibility
ALD	- Apparent lipid digestibility
ME	- Metabolizable energy
DM	- Dry matter
MJ	- Mega-joule
kg	- kilogram
CP	- Crude protein
CF	- Crude fat
EE	- Ether extract
BW	- Body weight
FC	- Feed consumption
F/G	- Feed to gain ratio
Fat Ret.	- Fat retention
G/F	- Gain to feed ratio
N	- Nitrogen
h	- Hours
SEM	- Standard error of means
PE	- Protein/energy
CO	- Corn oil
TA	- Tallow
Ca	- Calcium
P	- Phosphorus
Lys.	- Lysine
Met.	- Methionine

INTRODUCTION

High energy and protein density diets have been utilized to compensate for the young broiler chick's limited feed intake capacity and to maximize growth during the starter phase. Consequently, the primary dietary energy sources used in commercial broiler diets have been corn (maize) and wheat, the traditional "high energy" cereals. Barley, rye, and oats containing lower levels of bioavailable energy (NRC, 1984) have received very limited use in broiler chicken diets. High dietary concentrations of these grains have consistently resulted in poor bird growth performance and unmanageable litter conditions due to sticky droppings compared with corn- or wheat-based rations.

Conventional (hulled) barley and oat cultivars contain high fibre and accompanying low level of metabolizable energy (ME) compared with corn or wheat (Aman and Hesselman, 1984). Hulless barley varieties containing lower fibre levels have been developed in an attempt to increase the barley ME (Rossnagel et al., 1981). While the hull does contribute to a lower ME content, the primary growth depressing factors in barley are the highly viscous, water soluble β -glucans (Burnett, 1966; White et al., 1981; Hesselman and Aman, 1986). Henry (1985) found the mean water-soluble β -glucan content of barley, oats and wheat varieties to be 2.89, 2.13, and .67%, respectively. Barley β -glucans increase digesta viscosity and consequently decrease the absorption of nitrogen and carbohydrate (Burnett, 1966; Hesselman and Aman, 1986). The relative levels of β -glucans in barley are influenced by genetic factors and environmental growing conditions (Willingham et al., 1960; Aastrup, 1979b; Bourne and Wheeler, 1984). Although containing low β -glucan concentrations, rye grain does not support acceptable chick growth performance which is

attributed to the presence of high levels of water soluble pentosans (Fengler and Marquardt, 1988b). Fengler and Marquardt (1988b) showed that the addition of *Trichoderma viride* cellulase to either a pentosan solution or a water extract of rye decreased the viscosity of the solution and resulted in increased fat and dry matter retention and decreased excreta viscosity. More recently, Bedford et al. (1991) observed that the intestinal viscosity of chicks fed rye-based diets was highly correlated with the concentration of high molecular weight carbohydrates in gut contents. The addition of a pentosanase reduced the concentration of these complexes resulting in reduced viscosity.

Growth performance of chicks fed barley, oats, or rye grain has been improved through the use of dietary enzyme supplements. Partial hydrolysis of the β -glucans in barley and oats and pentosans in rye results in a substantial decrease in extract viscosity and improvement in chick performance (Gohl et al., 1978; Patel et al., 1980; Broz and Frigg, 1986a,b; Hesselman and Aman, 1986; Broz, 1987a,b; Elwinger and Saterby, 1987; Classen et al., 1988a; Fengler et al., 1988; Edney et al., 1989; GrootWassink et al., 1989; Rotter et al., 1989a,c). There are, however, very few reports on the ME of enzyme supplemented cereals.

Leong et al. (1962) reported 23.7 and 14.5% increases in the ME of pearled and regular (hulled) barley, respectively, when supplemented with a crude fungal amylase suggesting that the primary site of enzyme action is in the inner portions of the kernel (endosperm) where the majority of β -glucans are located (Preece and Mackenzie, 1952b). Potter et al. (1965) observed similar responses to an uncharacterized fungal enzyme supplement and attributed the increase in the ME of the barley to increased digestibilities of protein, fat and nitrogen-free extract. Increases in the ME of barley-based diets have also been attained with using α -amylase preparations (bacterial and fungal) (Herstad and McNab, 1975; Mannion, 1981). Moran et al. (1969) showed 6.4 and 2.9% increases in the ME of Tetrapetkus and Western rye, respectively, when supplemented with a crude fermentation product (*Aspergillus*). Broz (1987b) found the ME of a 60%

rye diet increased 6.0 and 7.4% when supplemented with pectinase (*Aspergillus niger*) and cellulase complex (*Trichoderma viride*), respectively. Other studies have also documented improvements in bioavailable energy when rye-based diets are supplemented with cellulolytic (Scholtyssek and Knorr, 1987) or pentosanase enzyme preparations (Pettersson and Aman, 1989).

The ME of a feedstuff may be determined by a variety of methods including prediction equations and balance trials using both young chicks (Hill and Anderson, 1958; Scott et al., 1982) and mature birds (Sibbald and Slinger, 1963; Farrell, 1978; Campbell et al., 1986b; Sibbald, 1986). Two types of chick bioassays have been used to determine the ME of feedstuffs. Hill and Anderson (1958) developed a bioassay in which the test feedstuff is substituted for glucose and the energy balance of the two diets are compared. In the second method, originally developed by Potter and Matterson (1960), the energy balance of a semi-practical diet supplemented with the test feedstuff is compared to the energy balance of the diet by itself. Several studies have shown the ME of cereal grains to be somewhat dependent on the age of bird used in the bioassay with higher ME values observed with mature birds (Coates et al., 1977; Farrell et al., 1983). The methodology used in the ME determination of a feedstuff may also influence the ME value obtained (Miller, 1974; Sibbald et al., 1962; Pesti, 1984; Pesti et al., 1986).

Previously, the literature has shown that the nutritional value of barley, oats and rye may be improved substantially through the use of dietary enzyme supplements, however there is little data indicating the specific changes seen in the bioavailability of individual nutrients such as apparent metabolizable energy (AME_n), lipid and protein. The objective of the present research was to determine the effect of dietary enzyme supplementation on the bioavailable energy (AME_n) and the apparent digestibilities of protein (APD) and lipid (ALD) of diets containing various cereal grains (wheat, barley (hulled and hullless), naked oats, rye) and fat sources (corn oil, tallow) when fed to the young broiler

chicken. Due to a lack of information on rye, greater emphasis in this research will be placed on this cereal. Enhanced nutrient utilization should translate into improved chick growth performance with respect to body weight and feed conversion efficiency. Barley, oats and rye grain are well suited for grain production in the western Canadian prairie environment. Consequently, if enzyme supplementation of diets based on these grains results in nutrient utilization and accompanying bird growth performance up to the current industry standard of wheat based diets, the Canadian broiler producer may be able to enhance the economic position of his/her operation by incorporating these lower priced cereal grains into a commercial broiler feeding program.

LITERATURE REVIEW

Comparison of the Major Cereal Grains

Nutrient Composition

The major cereals, corn, wheat, barley, oats and rye, vary widely in terms of their nutrient content and bioavailable energy level (Table 1). Corn and wheat, traditionally termed high energy cereals, contain AME_a levels of 13.10-15.75 MJ/kg DM and 12.16-14.98 MJ/kg on dry matter basis (DM), respectively. These bioavailable energy levels are considerably higher than those seen for barley, oats and rye. Barley and oats contain crude fibre levels 2-4 fold higher than wheat or corn which typically contain 2-3.2% crude fibre. The high fibre content is due to the presence of the fibrous husk, which decreases the overall bioavailable energy content of the grain. However, even naked oats (Ref. 11, Table 1) are found to have a relatively high crude fibre content. Rye grain has a low crude fibre content and yet has low levels of bioavailable energy. This depression in energy is due to the presence of highly viscous pentosans which impede nutrient digestion and absorption (Fengler and Marquardt, 1988a). Barley and oats have also been shown to contain relatively high levels of β -glucans, a viscous polysaccharide having similar effects to the pentosans in rye (Burnett, 1966). Corn and wheat, on the other hand, contain negligible levels of these polysaccharides. As seen in Table 1, the average crude protein (CP) content of corn was the lowest (9.3% CP), with rye having an intermediate value of 11.4% CP. The remaining cereal grains contain similar levels of crude protein (Wheat, 12.9%; Barley,

Table 1. Comparison of the crude protein, bioavailable energy and composition of various cereal grains.

Reference ¹	Cereal Grain	CP ²	AME _{N3}	CF ⁴	EE ⁵	Ash
		(%)	(MJ/kg DM)	(%)	(%)	(%)
1	Corn	9.9	15.75	2.5	4.3	-
	Wheat	11.5	13.05	2.7	2.0	-
	Barley	13.0	11.05	5.7	2.0	-
	Oats	12.8	10.67	12.1	4.7	-
	Rye	13.8	10.99	2.5	1.7	-
2	Corn	-	14.08	-	-	-
	Barley	-	10.41	-	-	-
	Oats	-	10.51	-	-	-
	Wheat	-	12.16	-	-	-
3	Wheat	17.4	13.71	3.5	2.6	1.7
	Barley	13.9	10.59	6.1	2.5	2.4
	Oats	12.5	12.89	11.7	6.0	3.2
	Rye	11.8	10.68	2.9	2.0	1.6
4	Barley	11.3	10.89 ⁶	-	1.5	2.7
	Maize	8.2	13.20	-	3.2	1.2
	Oats	10.0	10.94	-	4.9	2.7
	Wheat	10.4	12.08	-	1.4	1.8
5	Barley	-	13.68 ⁷	-	-	-
	Wheat	-	14.98	-	-	-
6	Corn	-	13.10	-	-	-
	Wheat	-	12.89	-	-	-
	Barley	-	9.96	-	-	-
7	Wheat	14.3	14.52	3.2	2.1	2.0
	Barley	12.2	12.76	5.4	2.4	2.8
8	Oats	10.5	11.90	-	3.4	-
	Barley	12.6	12.60	-	1.0	-
	Wheat	12.5	13.60	-	2.0	-
9	Barley	12.9	15.04 ⁸	4.2	1.6	2.5
	Wheat	13.6	15.45	2.0	1.5	1.4
	Maize	9.7	15.88	2.3	3.6	1.9
	Oats	13.3	12.18	9.4	2.1	3.3
	Rye	11.9	14.78	2.0	1.2	1.8
10	Corn	9.3	13.18	-	-	-
	Wheat		12.59	-	-	-
	Barley		11.20	-	-	-
11	Oats (naked)	17.24	13.31	10.50	6.9	2.1
12	Corn	-	14.02 ⁹	-	-	-
	Wheat	-	12.59	-	-	-
	Corn	-	14.52	-	-	-
	Barley	-	11.21	-	-	-
13	Oats	11.1	-	-	6.6	2.9
	Oats (dehulled)	13.5	-	-	8.6	2.3

Table 1. Continued.

Reference ¹	Cereal Grain	CP ²	AME _m ³	CF ⁴	EE ⁵	Ash
		(%)	(MJ/kg DM)	(%)	(%)	(%)
14	Wheat	10.5	-	-	2.6	1.5
	Rye	7.9	-	-	2.1	1.8

¹ References: 1) NRC, 1984; 2) Potter and Matterson, 1960; 3) Sibbald and Slinger, 1963; 4) McNab and Shannon, 1974; 5) Sibbald, 1975; 6) Petersen *et al.* 1976; 7) Coates *et al.* 1977; 8) Davidson *et al.* 1978; 9) Wiseman *et al.* 1982; 10) Boldaji *et al.* 1985; 11) Maurice *et al.* 1985; 12) Boldaji *et al.* 1986; 13) Pettersson *et al.* 1987a; 14) Pettersson and Aman, 1988.

² Crude protein.

³ Apparent metabolizable energy (nitrogen corrected).

⁴ Crude fibre.

⁵ Ether extract.

⁶ AME_m values obtained using one year old laying hens.

⁷ AME_m values obtained using mature roosters.

⁸ AME_m values obtained using young pigs.

⁹ AME values are not nitrogen corrected.

12.7%; Oats, 12.6%). Of these five cereals, corn and oats have the highest ether extract levels with oats being somewhat higher than corn. Ash content values reflect those trends seen for crude fibre with barley and oats having the highest levels.

Broiler Chicken Growth Performance

Growth performance of chicks fed corn, wheat, barley, oats or rye follows the same trend as seen for bioavailable energy levels (Table 2). Wheat and corn consistently support superior growth and feed conversion efficiency compared to the other cereals. Corn, having a higher metabolizable energy level than wheat also yields a somewhat higher growth rate and improved feed conversion efficiency. Broz and Frigg (1986b) found that a corn-based diet supported increases of 8.8 and 5.4%, respectively, for body weight and feed conversion efficiency compared to a similar wheat-based diet. Feeding barley and oats resulted in similar growth rates, which are considerably lower than that seen for wheat. For example, Sibbald and Slinger (1963) reported that replacing wheat with barley or oats resulted in decreases of 9.8 and 24.9% for body weight and 12.2% for feed conversion efficiency, respectively, compared to the wheat-based diet. Other studies have demonstrated that these high fibre grains yield similar growth performance levels for broiler chicks (Broz & Frigg, 1986a,b). Pettersson et al. (1987a) demonstrated suggest that dehulled oats are of a higher nutritive value compared to whole oats, suggesting that the fibre does play a role in decreasing the potential nutritive value of a cereal grain. Although rye grain is a low crude fibre grain, it has a poor nutritive value and presently receives little or no use in commercial broiler diets. Broz and Frigg (1986b) reported rye-based diets resulted in depressions in body weight and feed conversion efficiency of 42.2 and 26.9%, respectively, compared to a wheat-based diet. This level of performance was considerably lower than that seen for barley and oats

Table 2. Comparison of growth performance of chicks fed various cereal grains.

Reference ¹	Cereal Grain	BW ²	FC ³	F/G ⁴
		(g)	(g)	(g/g)
1	Wheat	193	-	2.23
	Barley	174	-	2.54
	Oats	145	-	2.54
	Rye	166	-	2.60
2	Oats (naked)	493	672	1.51
	Corn	545	735	1.45
3	Maize	541	-	1.68
	Barley	338	-	2.35
	Oats	350	-	2.25
4	Maize	483	800	1.66
	Wheat	444	778	1.75
	Barley	401	737	1.84
	Oats	404	744	1.84
	Rye	279	629	2.27
5	Oats	612	983	1.61
	Oats (dehulled)	713	1034	1.45
6	Wheat	582	900	1.55
	Rye	364	723	1.99

¹ References: 1) Sibbald and Slinger, 1963; 2) Maurice et al. 1985; 3) Broz and Frigg, 1986a; 4) Broz and Frigg, 1986b; 5) Pettersson et al. 1987a; 6) Pettersson and Aman, 1988.

² Body weight.

³ Feed consumption.

⁴ Feed to gain ratio.

in the same study (Table 2, Ref.4). Sibbald and Slinger (1963) to the contrary reported that rye supported growth rates similar to barley and superior to oats. Their study, however, employed 2 week old birds whereas Broz and Frigg (1986b) initiated their experiment with 8 day old birds. The viscous polysaccharides in barley, oats and rye are most detrimental to young birds and thus, the age difference in part may have contributed to the different results.

Physical Characteristics of Beta-glucans and Pentosans

The low metabolizable energy of barley, oats and rye relative to other cereal grains such as corn and wheat for poultry diets has been attributed to the inhibition of nutrient digestion and absorption by the highly viscous compounds present in these cereals. Several authors have suggested that the viscous compounds in barley are the β -glucans (Rickes et al. 1962; Potter et al. 1965; White et al. 1983). The studies of Preece and MacKenzie (1952b) showed that water extracts of oat flour contained primarily glucan with 13% contaminating pentosan (xylose and arabinose). This data was substantiated by Wood et al. (1978) who observed that gum extracted by alkali from oats (*Avena sativa*) contained 70-87% β -glucan. Table 3 gives a summary of the β -glucan contents of various cereal grains. The range in total β -glucan content is 3.0-7.2% for barley, 2.5-5.7% for oats and .34-1.4% for wheat. Rye and triticale also contain significantly lower levels of β -glucans (1.93-2.4% and .34-1.2%, respectively) than barley or oats.

Mixed link β -glucans (β 1-3,1-4 linkages) form part of the endosperm and in barley account for 70-75% of the endosperm cell walls (Fincher, 1975; Forrest and Wainwright, 1977), the remainder being mainly arabinoxylans (about 25%), mannose-containing polymers (glucomannans), cellulose, protein and phenolic compounds (Salomonsson et al, 1984). The water soluble fraction has often been referred to as the barley "gums",

Table 3. Pentosan and/or β -glucan content and viscosity measurements of various cereal grains.

Ref. ¹	Cereal Grain ²	β-glucan			Pentosan			Viscosity
		Sol.	Insol.	Tot.	Sol.	Insol.	Tot.	
(%)								cP
1	B(Leth)	-	-	5.8	-	-	-	-
	B(Wpg)	-	-	2.8	-	-	-	-
2	O	-	-	3.32	-	-	-	-
3	B	2.90	2.11	5.01	-	-	-	-
	O	.68	1.82	2.50	-	-	-	-
	W	.09	.25	.34	-	-	-	-
	R	.01	1.92	1.93	-	-	-	-
	T	.00	.34	.34	-	-	-	-
4	B	-	-	3.0	-	-	-	-
5	B(Tri.)	.67	3.77	4.44	-	-	-	7.84
	B(Zep.)	1.45	4.11	5.55	-	-	-	58.3
	B(Min.)	2.34	4.02	6.36	-	-	-	632
6	B	1.62	5.07	6.69	-	-	-	-
7	B(M)	-	-	4.5-8.2	-	-	-	-
	B(F)	-	-	5.1-7.2	-	-	-	-
	W	-	-	1.4	-	-	-	-
	O	-	-	5.7	-	-	-	-
	R	-	-	2.4	-	-	-	-
	T	-	-	1.2	-	-	-	-
8	R	-	-	-	2.1	7.7	9.8	-
9	B	-	-	5.1	-	-	-	-
10	W	-	-	.52-1.00	-	-	-	-
11	B(Sca.)	2.4	2.0	4.4	-	-	-	-
	B(Mon.)	2.6	2.6	5.2	-	-	-	-
	O	2.5	.64	3.2	-	-	-	-
12	W(Win.)	-	-	-	6.03	.68	6.71	-
13	W(Spr.)	-	-	.72-1.00	-	-	5.5-6.8	-
	W(Win.)	-	-	.74-1.00	-	-	5.3-6.3	-
14	R	-	-	-	2.08	3.33	5.41	2.13 ³
	W	-	-	-	.44	3.84	4.28	1.13
	T	-	-	-	.56	5.52	6.10	1.20
15	B(M)	-	-	3.55	-	-	-	-
16	O	-	-	3.2-6.3	-	-	-	-

¹ Reference list: 1) Bendelow, 1975; 2) Wood et al. 1977; 3) Anderson et al. 1978; 4) Wood et al. 1978; 5) Aastrup, 1979a; 6) Martin and Bamforth, 1980; 7) Prentice et al. 1980; 8) Antoniou et al. 1981; 9) Prentice and Faber, 1981; 10) Beresford and Stone, 1983; 11) Aman and Graham, 1987; 12) Hashimoto et al. 1987; 13) Aman, 1988; 14) Fengler and Marquardt, 1988a; 15) Henry, 1988; 16) Welch and Lloyd, 1989.

² Cereal grain: B(Leth) = barley (growing location: Lethbridge, Alta.); B(Wpg) = barley (growing location: Winnipeg, Man.); O = oats; B = barley; W = wheat; R = rye; T = triticale; B(Tri.) = barley (CV: Triumph); B(Zep.) = barley (CV: Zephyr); B(Min.) = barley (CV: Minerva); B(M) = barley (malting); B(F) = barley (feed); B(Sca.) = Scandinavian barley; B(Mon.) = Montana barley; W(Win.) = winter wheat; W(Spr.) = spring wheat.

³ The viscosity measurements given for this reference are relative to water which is given a viscosity value of 1.00.

while the the insoluble portion has been termed "hemicelluloses" (Preece and MacKenzie, 1952b; Schuster et al. 1967; Morrall and Briggs, 1978). According to Aman and Graham (1987), 54% of the mixed-linked β -glucan in barley was soluble compared to 80% in oats. As seen in Table 3, the percentage of soluble β -glucan for a particular cereal grain varies widely between references. This may be due in part to the extraction methodology used. The data of Aastrup (1979a) suggest that the percentage of soluble β -glucan increases as total β -glucan content increases.

Aspinall and Telfer (1954) isolated laevorotatory glucosans from barley grain and concluded that they contained unbranched chains containing approximately equal proportions of 1-3 and 1-4 linkages. According to Bacic and Stone (1981), the β -glucans occurring in barley and oat endosperm are unbranched polysaccharides containing (1-4) and (1-3)- β -linked glucopyranosyl units in a ratio of between 2 and 3 to 1. Woodward et al. (1983b) found (1-3) and (1-4) linkages in a ratio of 28:72 for Clipper barley. Approximately 90% (w/w) of barley β -glucan is comprised of cellotriosyl and cellotetraosyl residues separated by single (1-3)- β -linkages with the remainder consisting of up to ten or more adjacent (1-4)-linkages (Woodward et al., 1983b). The insertion of irregularly spaced (1-3)-linkages in the barley β -glucan results in an irregular molecule which is not prone to intermolecular association thus explaining the molecule's solubility in water (Woodward et al., 1983a).

When situated in the cell walls, the β -glucans are covalently bound to protein forming large molecules of approximately 10^7 dalton (Forrest and Wainwright, 1977). Estimates of the molecular weight of oat β -glucan have ranged from $3-6 \times 10^4$ (Acker et al., 1955; Podrazky, 1964) to 1.6×10^5 (Smith and Montgomery, 1959). Molecular weight values ranging from 2×10^4 to 4×10^7 have been obtained for barley β -glucan, however, the data is difficult to interpret due to the variations in starting materials and methodology used.

White et al. (1983) demonstrated an increase in intestinal viscosity in chicks fed barley β -glucan compared to wheat-fed chicks. Gohl et al.

(1978) observed that the viscosity of a barley diet was inversely related to excreta dry matter content. According to Forrest and Wainwright (1977), the intrinsic viscosity of a particular β -glucan is proportional to its molecular weight. The extract viscosity of barley grain is closely related ($R^2=0.99$, S.E.E.=0.19) to the content of soluble β -glucan (Aastrup, 1979a) (Table 3).

Preece and Hobkirk (1955), observed marked decreases in the viscosity of solutions of laevorotatory glucosan when treated with enzyme. Physical and chemical examination of the components of water extracts from water- and enzyme-treated, low- and high-viscosity barleys showed that (1-3,1-4)- β -glucan had been degraded to a low molecular form by the enzyme with a concomitant reduction in viscosity (Hesselman and Aman, 1986).

In a comparison of the non-starch polysaccharides in various cereal grains, Preece and Hobkirk (1953) found rye to be exceptionally rich in high molecular weight pentosan material with very little β -glucans. Wheat also contained high levels of the pentosans, however, the level of glucans was more diffuse in the wheat than in the rye grain. Table 3 shows the pentosan content of rye (5.4-9.8%), wheat (4.3-6.8%) and triticale (6.1%). According to Preece and Hobkirk (1953), wheat gum was found to be located primarily in the endosperm and barley husk only contained small amounts of gum.

Antoniou *et al.* (1981) found that supplementing wheat-based diets with rye pentosans resulted in substantial depressions in chick growth performance. These authors concluded that the antinutritional activity of pentosans may be attributed to their ability to form highly viscous solutions effectively reducing the efficiency of nutrient digestion and absorption (Antoniou and Marquardt, 1981). Fengler and Marquardt (1988a) isolated and purified the highly viscous pentosan-rich factor in rye grain and found it to contain 79% pentoses with a xylose-to-arabinose ratio of 62 to 38 with traces of minerals and protein and 10% glucose-containing carbohydrates.

The pentosans imbibe considerable amounts of water to form solutions

of high viscosity (Udy, 1956; Medcalf et al. 1968). Ward and Marquardt (1987) concluded that the water insoluble pentosans which become highly hydrated without passing into solution were the major antigrowth factor since they are present at higher levels than the soluble pentosans in rye grain (Puma rye (DM basis): 1.9% water-soluble and 6.5% water-insoluble pentosans).

The monomeric units most frequently found in cereal pentosans are the pentose sugars D-xylose and L-arabinose (D'Appolonia, 1973). According to Perlin (1951), cereal grain pentosans consist of a straight chain of anhydro-D-xylopyranosyl units linked β (1-4) to which are attached anhydro L-arabinofuranosyl units at the 2 or 3 positions of individual anhydro xylose units. The arabinosyl residues appear to play an important role in determining the shape of the molecule. The water soluble pentosans from wheat also contain a small amount of hexose sugar and protein.

Ward and Marquardt (1987) examined the effects of various milling fractions of rye grain and found the antinutritive factor of rye to be mainly associated with the endosperm of the grain. Andrewartha et al. (1979) observed that a water-soluble arabinoxylan from wheat flour exists in solution as a fully extended rod with an axial ratio (length to width) of approximately 140. According to Preece and Hobkirk (1955), naturally occurring enzymes in wheat may attack the internal linkages of the pentosan molecule causing a rapid decrease in viscosity with a slow release of reducing groups.

Effect of Geographical Location and Growing Conditions on Beta-glucan Levels

Several studies have been conducted to determine what effect, if any, geographical location and growing conditions have on the β -glucan content of cereal grains. Bendelow (1975) observed a positive relationship of geographic location and environmental moisture levels on barley β -glucan content. For example, barley (CV Conquest) grown in Winnipeg, Manitoba

(high moisture conditions), Melfort, Saskatchewan (medium moisture conditions) and Lethbridge, Alberta (low moisture conditions) contained 1.9, 2.6, and 3.5% β -glucans by weight, respectively. Similarly, Aman and Graham (1987) analyzed the β -glucan content of barley cultivars grown in Scandinavia and Montana and found that the barleys grown in Montana had a higher β -glucan content, which they attributed to a shorter dry growing season compared to that present in Scandinavia. Aastrup (1979b) found increasing amounts of precipitation during the growing season resulted in decreased acid flour extract viscosity simultaneous to decreases in soluble and total β -glucan content and suggested that this was due to lower β -glucan production in the grain kernel. No differences in β -glucan structure were observed between treatments. The percentage of husk relative to the total kernel weight increases with increased moisture during the period of growth. Therefore the total quantity of β -glucan decreases as most is located in the endosperm.

In contrast to the above findings, some data do not show an effect of moisture conditions during growing on the β -glucan content of barley. For example, Anderson et al. (1978) did not find differences in the β -glucan content of barley cultivars grown near Winnipeg and Lethbridge, which represent extremes in moisture conditions. However, these results could be affected by the relative moisture levels at Winnipeg or Lethbridge in that particular growing season. If Winnipeg experienced an especially dry growing season or Lethbridge an exceptionally wet growing season, the predicted environmental effect on β -glucan content would be masked. Similarly, Gill et al. (1982) did not find a growing location effect on β -glucan content of barley grown in two locations in the United Kingdom likely having similar environmental conditions. This same explanation may account for the data of Aalto et al. (1988) who found negligible differences in the fibre content of barley grown in various locations within Finland.

Physiological Effects of Non-Starch Polysaccharides

Gastrointestinal Rate of Passage

According to Tuckey et al. (1958), rate of passage influences the nutrition of the chick by: 1) determining the length of time during which nutrients may be absorbed, 2) determining the intestinal microbial population and 3) limiting daily feed intake. These authors found that the addition of oat hulls to a corn-soy diet resulted in a numerical but not statistically significant increase in rate of passage. The oat hulls however were pulverized and Tuckey et al. (1958) suggested that form of fibre may be more important than the level of fibre. Gohl and Gohl (1977) investigated the effects of a number of hydrocolloids including pectin, guar gum and barley β -glucans on the transit time and stool weight in rats. For all thickening agents, fecal dry matter decreased as the level of inclusion increased and barley β -glucans and pectin were observed to have a retarding effect on digesta transit in rats. According to these authors, the effects of hydrocolloids on digesta transport is probably not purely physical since there is a delay after the rats were returned to normal feed before fecal dry matter and digesta transit times are similar to the control group. Hydrocolloids which reduce transit time may help stabilize intestinal flora. Rainbird and Low (1986) found that the supplementation of a control diet with pectin and guar gum or wheat bran increased the mean time for the gastric half emptying time of digesta and nitrogen.

Intestinal Unstirred Water Layer

Several studies have been carried out to determine the effect of non-starch polysaccharides on the unstirred water layer in the small intestine (Johnson and Gee, 1981; Flourie et al. 1984). There is no clear division between the intestinal contents and the unstirred water layer but rather a region of diminishing stirring becoming nearly immobile at the mucosal surface. Johnson and Gee (1981) studied the effects of guar gum and Na-carboxymethyl cellulose (CMC) on *in vitro* glucose transport using everted sacs of rat jejunum. The presence of either gel-forming polysaccharide was associated with a significant decrease in glucose transport and test sacs incubated for 15 minutes with guar gum had a mucosal unstirred water layer nearly 50% thicker than control sacs incubated in guar free buffer. Subsequent stirring of the guar containing medium resulted in glucose transport similar to the guar free control. These authors attributed this increase in glucose transport to a reduction in the thickness of the unstirred water layer covering the intestinal villi. It is also possible that the diffusion of solutes (nutrients) in media containing viscous gums such as β -glucans or pentosans through the unstirred water layer may be obstructed by the polysaccharide polymers themselves. Flourie et al. (1984) found an thickening of the jejunal apparent unstirred water layer in humans when pectin was added to the perfusion solution. In this study, a close relationship between the log of the unstirred water layer thickness and pectin concentration ($r=.96$, $P<.001$) was observed.

Improvement of Cereal Grain Nutritive Value

Introduction

Since the early 1950's, much research has been carried out in an effort to improve the nutritive quality of the traditional low energy cereal grains such as barley, rye and oats. The following discussions will focus on gamma irradiation, antibiotic supplementation, water treatment and enzyme supplementation as methods utilized to improve the nutritional value of these grains.

Gamma Irradiation

Gamma irradiation of diets based on the low ME cereals (barley, oats and rye) has resulted in increased nutritional value and concurrent improvement in the growth performance of chicks fed rye- (MacAuliffe *et al.* 1979; Patel *et al.* 1980; Campbell *et al.* 1983a, b), barley- (Classen *et al.* 1985; Campbell *et al.* 1986a) and oat-based diets (Campbell *et al.* 1986a, 1987) (Table 4). The ranges of improvements in chick body weight gain and feed conversion efficiency observed with gamma irradiation were for barley (4-20% and -6-3%), hulless barley (6-44% and 4-21%), oats (including oat groats and hulless oats) (45-63% and 24-38%) and rye (33-75% and 14-30%), respectively, compared with the non-irradiated controls (Table 4). The data tabulated in Table 4 also exemplify the positive effects of gamma irradiation in terms of fat retention and bone ash percentages. Barley diets subjected to gamma irradiation showed minimal response in terms of fat retention and bone ash (4-5% and 0-2%, respectively) while hulless barley, oats (including oat groats and hulless oats) and rye diets showed improvements of 9-41% and 0-3%, 13-351% and 19-220% and 8-29%, respectively, compared to the non-irradiated controls. The wide variation in response to gamma irradiation for a particular cereal may be due to a variety of factors including dietary inclusion

Table 4. Effect of gamma irradiation on the nutritive value of various cereal grains¹.

Reference	Dose (Mrad)	Cereal grain	BW ²	G/F ³	Fat ⁴ Ret.	Bone ash
MacAuliffe <i>et al.</i> 1979	10	Corn	82	-	-	106
		Rye	137	-	-	111
Patel <i>et al.</i> 1980	10	Rye	163	119	-	-
Campbell <i>et al.</i> 1983b	2	Rye	133	114	119	109
	6		175	130	124	111
	10		166	125	122	108
Campbell <i>et al.</i> 1983a	4	Rye	171	-	220	129
		Wheat	103	-	110	101
Classen <i>et al.</i> 1985	10	Barley (H) ⁵	120	104	111	103
Campbell <i>et al.</i> 1986a	6	Wheat	99	105	109	99
		Triticale	94	95	110	98
		Barley	104	103	105	100
		Barley (H) ⁵	106	107	109	100
		Oats	145	124	113	101
		Oats (H) ⁵	159	129	233	105
	6	Barley	94	94	104	102
		Barley (H) ⁵	144	121	141	101
Campbell <i>et al.</i> 1987	3	Wild oat	158	132	-	-
	6	(groats)	161	138	351	102
	9		163	134	-	-

¹ All values given are relative to non-irradiated controls where the control is given a value of 100.

² BW = Body weight.

³ G/F = Gain to Feed ratio.

⁴ Fat ret. = Fat retention.

⁵ (H) = hulless varieties of either barley or oats.

rate, bird age and breed, and grain cultivar. As seen in Table 4, chicks fed diets based on the traditional high energy cereals (wheat and corn) showed minimal response to gamma irradiation for all of the parameters observed. The magnitude of improvements seen with gamma irradiation is greatest for cereals of initially low nutritional value.

Gamma irradiation is effective in increasing the nutritive value of barley, oats and rye since it decreases the viscosity caused by the high molecular weight β -glucans and pentosans (Campbell et al. 1983b; Classen et al. 1985; Campbell et al. 1986a, 1987). Gel permeation chromatography confirmed the reduction in molecular weight by irradiation since the elution of β -glucan from irradiated grain was substantially retarded suggesting a reduction in the molecular weight by random cleavage of the polymers of β -glucan and pentosans (Bhatty and MacGregor, 1988). Other studies have reported increased levels of reducing sugars in irradiated barley or rye (Campbell et al. 1983a; Classen et al. 1985). These results imply that the decreased viscosity must be due to a depolymerization process rather than a shift from water soluble to insoluble β -glucans. This will decrease digesta viscosity in the gastrointestinal tract and thereby allow more efficient nutrient digestion and absorption.

Antibiotic Supplementation

Many studies have been carried out to determine the effects of antibiotic supplementation on the nutritional quality and accompanying chick growth performance of low ME cereal grains such as barley, oats and rye (Table 5). These studies have employed a wide variety of antibiotic supplements including penicillin, oleandomycin, bacitracin, streptomycin, terramycin and virginiamycin with penicillin being the antibiotic most commonly utilized. According to Table 5, the range in improvement of body weight gain and feed conversion efficiency for birds fed antibiotic

Table 5. Effect of antibiotic supplementation on the nutritive value of various cereal grains¹.

Ref. ²	Antibiotic	Cereal grain	BW ³	FC ⁴	G/F ⁵	Nutrient ret. ⁶		
						N	Fat	DM
1	Oleandomycin	Corn	109	-	104	-	-	-
		Barley	109	-	102	-	-	-
2	Mixture ⁷	T. rye	109	-	103	-	-	-
		W. rye	101	-	102	-	-	-
3	Penicillin	Wheat	107	-	104	-	-	-
		Rye	127	-	166	-	-	-
	Bacitracin Terramycin	Rye	112	-	102	-	-	-
		Rye	118	-	104	-	-	-
4	Chlortetracycline	Rye	125	-	-	-	-	-
	Penicillin	Rye	151	-	-	-	-	-
	Bacitracin	Rye	149	-	-	-	-	-
5	Bacitracin	Corn	106	101	-	-	-	-
		Wheat	114	110	-	-	-	-
6	Penicillin	Wheat	109	-	105	-	-	-
		Corn	104	-	106	-	-	-
		Rye	134	-	104	-	-	-
7	Penicillin	Rye	107	101	107	-	-	-
	Penicillin	Rye	146	120	122	-	-	-
8	Penicillin	Rye (MM) ⁸	158	124	127	141	-	107
		Rye (FM)	117	106	110	105	-	102
		Wheat (MM)	112	103	109	107	-	100
		Wheat (FM)	108	105	103	110	-	102
9	Penicillin	Rye	191	119	161	-	128	104
		Wheat	112	103	109	-	108	100
10	Penicillin	Rye	124	-	111	-	-	-
		Wheat	106	-	103	-	-	-
11	Penicillin	Rye	149	114	131	139	164	109
		Wheat	103	100	103	113	114	106
12	Penicillin	Rye	119	107	110	-	109	-
		Wheat	100	98	100	-	102	-
13	Lincomycin	Barley	117	-	101	-	114	-
14	Bacitracin	Rye	102	101	102	93	-	-
15	Virginiamycin	Corn	103	-	104	-	-	-
16	Penicillin + Streptomycin	Oat groats	94	-	108	-	134	-
17	Avoparcin	Mix ⁹	110	102	108	-	-	-
	Virginiamycin		111	102	108	-	-	-
	Bacitracin		106	103	105	-	-	-

Table 5. Continued.

Ref. ²	Antibiotic	Cereal grain	BW ³	FC ⁴	G/F ⁵	Nutrient ret. ⁶		
						N	Fat	DM
18	Penicillin	Rye	-	-	-	-	128	109

¹ All values given are relative to a nonsupplemented control which is assigned a value of 100.

² Reference: 1) Moran and McGinnis, 1968; 2) Moran et al. 1969; 3) MacAuliffe and McGinnis, 1971; 4) Graber et al. 1974; 5) Johnston and Arscott, 1974; 6) Patel and McGinnis, 1976; 7) Misir and Marquardt, 1978a; 8) Misir and Marquardt, 1978b; 9) Marquardt et al. 1979; 10) Patel et al. 1980; 11) Antoniou and Marquardt, 1982a; 12) Antoniou and Marquardt, 1982b; 13) Classen et al. 1985; 14) Broz, 1987b; 15) Fethiere and Miles, 1987; 16) Campbell et al. 1987; 17) Lund, 1987; 18) Fengler et al. 1988.

³ BW = body weight.

⁴ FC = feed consumption.

⁵ G/F = gain to feed ratio.

⁶ Nutrient retention: N = nitrogen, DM = dry matter.

⁷ Mixture of antibiotics: penicillin, chlortetracycline, tylosine and oleandomycin.

⁸ Rye (or wheat) plus either meat meal (MM) or fish meal (FM).

⁹ Mixture of cereal grains: wheat, oats, barley and corn.

supplemented diets were for wheat (1-14% and 3-9%), corn (3-9% and 4%), barley (9-17% and 1-2%) and rye (1-91% and 2-61%), compared to their nonsupplemented counterparts. The data shown in Table 5 clearly depict the differential response to antibiotic supplementation between the various cereal grains tested. In general, the low ME cereals (barley, rye) respond to antibiotic supplementation with greater magnitude than the high ME cereals (corn, wheat). The same trends are evident in the nutrient retention data seen in Table 5. The ranges in improvement of N, fat and DM retention observed with antibiotic supplementation of wheat- and rye-based diets are 7-13%, 2-14% and 0-6%, and -7-41%, 9-64% and 2-9%, respectively.

Johnston and Arscott (1974) reported that chicks fed diets supplemented with Zn bacitracin had significantly reduced ($P < .05$) intestinal weights compared to nonsupplemented birds and proposed that the antibiotic may alleviate low levels of disease and thereby increase the ease of absorption of nutrients since less effort is required by the intestinal wall to defend itself from harmful organisms. MacAuliffe and McGinnis (1971) reported that since rye-based diets responded favourably to antibiotic supplementation, rye must contain a component (possibly pentosans) which stimulates the growth of a harmful microflora in the chick intestinal tract. The data of Antoniou and Marquardt (1982b) showed that although rye fed chicks showed improved performance with supplemental penicillin, the birds still did not perform as well as wheat fed chicks, suggesting that besides harmful microflora, another factor such as viscosity caused by high molecular weight polysaccharides affects nutrient digestion and absorption.

Although antibiotic supplementation improves the nutritional quality of barley, oats and rye, it has little or no effect on the incidence of sticky feces (Patel and McGinnis, 1976, Wagner and Thomas, 1977, Patel et al. 1980, Fengler et al. 1988). Wagner and Thomas (1977) suggested that antibiotics partially alleviate the problems with feeding rye but that sticky feces indicate another contributor to the problem. Fengler et al.

(1988) suggested that penicillin may reduce the microbial population in the lower gut and thus reduce their competitiveness for nutrients ordinarily used by the chick.

Water Treatment

Water treatment (soaking) of barley and rye grain has been shown to increase their nutritional value for young broiler chicks (Table 6). According to these data, the ranges of improvement of body weight and feed conversion efficiency of chicks fed diets containing water treated grain are for barley, 3-63% and 0-40% and for rye grain, -14-71% and -10-116%, respectively. The wide range in response for a particular cereal grain may be attributed to a variety of factors including grain cultivar, length of soaking time, dietary inclusion rate of grain and age of birds utilized to assess the effectiveness of water treatment. The average improvements in body weight and feed conversion efficiency of chicks fed diets containing water treated barley or rye grain are 30% and 19%, and 24% and 25%, respectively.

The magnitude of response to water treatment of barley is somewhat dependant on the cultivar and growing conditions, with the relative response to water treatment being greater for grain grown under low moisture conditions as compared to that grown under high moisture conditions (Willingham et al. 1960, Reference 4; Table 6). Differences in response could not be explained by differences in proximate analysis.

Fry et al. (1957) proposed that water treatment somehow altered a carbohydrate component of barley by permitting the action of certain endogenous enzymes thereby increasing its energy availability. The data of Willingham et al. (1959) showed that water treatment improved the nutritional quality of nontreated barley but not autoclaved barley with respect to chick growth performance suggesting that the increased nutritive value of water treated barley must be due to endogenous enzymes.

Table 6. Effect of water treatment on the nutritive value of various cereal grains.¹

Reference ²	Cereal grain	BW ³	FC ⁴	G/F ⁵	Fat dig.
1	Barley (pearled)	137	-	134	-
	Barley (pearled)	122	-	129	-
2	Barley	116	-	112	-
	Barley	163	124	124	-
3	Barley	131	-	131	-
	Barley	120	-	121	-
4	Barley (N. York)	123	-	106	-
	Barley (Oregon)	164	-	120	-
	Barley (N. York)	127	-	113	-
	Barley (Oregon)	153	-	126	-
5	Barley	134	-	116	-
	Barley	136	-	140	-
6	Barley	114	-	106	-
7	Rye	121	-	120	-
	Rye	136	-	126	-
	Rye	125	-	116	-
8	Rye	102	103	98	-
	Rye	106	96	110	-
	Rye	132	111	122	-
9	Rye	122	97	126	145
10	Rye	171	81	216	-
11	Barley (regular)	103	-	101	-
	Barley (pearled)	103	-	100	-
12	Rye (20 hours) ⁶	84	93	90	118
	Rye (40 hours)	111	98	112	139
	Rye (60 hours)	121	101	120	143
	Rye	155	112	138	129

¹ All values given are relative to a nonsupplemented control which is assigned a value of 100.

² References: 1) Fry et al. 1957; 2) Willingham et al. 1959; 3) Thomas et al. 1960; 4) Willingham et al. 1960; 5) Thomas et al. 1961; 6) Rose and Arscott, 1962; 7) Fernandez et al. 1973; 8) Misir and Marquardt, 1978d; 9) Antoniou and Marquardt, 1982a;

Table 6. Continued.¹

10) Boros et al. 1985; 11) Newman et al. 1985; 12) Ward and Marquardt, 1988.

³ BW = body weight.

⁴ FC = feed consumption.

⁵ G/F = gain to feed ratio.

⁶ Length of time the rye was soaked with water.

Thomas *et al.* (1960) found grain microbial counts were increased substantially when barley was water treated and that chick growth was positively correlated with the microbial counts. Autoclaving the water treated barley did not decrease its nutritive value which would infer that the changes responsible for improvement were effected prior to feeding and were not due to the presence of enzymes produced during treatment which later act in the gastrointestinal tract.

Willingham (1964b) noted increased levels of antibiotic and enzyme activities in water treated barley and postulated improved nutritive value of barley by improved energy utilization and/or additional energy release. Potter *et al.* (1965) showed the AME_n of water treated barley was increased 21.8% over untreated barley. These authors attributed the increased bioavailable energy to increased nutrient digestibility since the digestibilities of protein, fat and N-free extract were increased from 45, 0 and 75%, respectively, for untreated barley to 88, 84 and 78%, respectively, for water-treated barley. Similar improvements in AME_n have been reported by Leong *et al.* (1962).

Several studies have shown that the addition of water extract of rye to a wheat or corn based diet results in bird growth depression which suggests that the water extract contains some if not all of the antinutritive properties of rye (Misir and Marquardt, 1978d; Fernandez *et al.* 1973; Boros *et al.* 1985). Misir and Marquardt (1978d) found chicks fed wheat diets supplemented with the lyophilized water extract of rye showed increased incidence of beak impaction, vent blockage and excreta wetness compared to nonsupplemented wheat, however the growth depression was not as great as expected. This suggests that not all of the growth depressing factor was removed by water extraction. Although water treatment improved the nutritional value of rye, chick growth performance was still lower than in wheat fed birds (Antoniou and Marquardt, 1982a). These authors noticed that there was an association between the performance of chicks fed rye soaked for different time periods and the corresponding reductions in the viscosity of rye extracts suggesting that

the high viscosity of rye may be at least partially responsible for its poor nutritional value. Ward and Marquardt (1988), in a study with Leghorn chicks, found that water treatment had a pronounced effect on rye flour compared to bran in terms of fat absorption. The water extract of rye flour, but not rye bran, was viscous compared to that of water, suggesting the presence of viscous water soluble compounds which are more concentrated in the rye flour. Ward and Marquardt (1988) suggested that water soaking of rye is effective as it results in the destruction of the antinutritive factor by endogenous and/or exogenous (microbial) enzymes.

Enzyme Supplementation

The use of enzyme supplementation as a means of improving the nutritional value of cereal grains such as barley, oats and rye for poultry has been extensively examined since the mid 1950's. Comprehensive summaries of this field of research are given in Tables 7 and 8. Although a majority of the research has utilized broiler chickens as the experimental unit, a number of studies have examined the effects of enzyme supplemented cereal grains for Leghorn chickens and/or commercial turkeys.

Studies dating back to the late 1950's have shown the positive effects of enzyme supplementation on the growth performance of broiler chickens. A majority of the early research utilized barley as the test grain. Since that time, however, many studies have been carried out to evaluate the relative benefits of enzyme supplementation on a wide variety of poultry feed ingredients including oats, rye, wheat, hulless barley, maize, distillers-spent grain and others.

Several experiments have also examined how barley stage of ripeness (early yellow ripeness vs. combine ripeness) affects the efficacy of an enzyme supplement. Hesselman et al. (1981) found that the relative effect of enzyme supplementation (β -glucanase) was greater for early yellow ripe barley compared to combine ripe barley (Table 7: Ref. 18). Several

Table 7. The effect of enzyme supplementation on the growth and/or productive performance of broiler and Leghorn chickens and turkeys fed corn-, wheat-, barley-, oats- or rye-based diets.

Author	Species ¹	Age (days)	Enzyme	Test Grain	Inclusion Rate (%)	Body wt. ²	Feed Cons.	Gain/Feed
1 ³	BC	28	Bacterial amylase	B ⁴	63.5	26.7	-	6.3
	BC	14	Fungal amylase	B	63.5	33.3	6.8	11.2
2	BC	21	Not specified	B	64.9	11.0	-	6.3
3	BC	14	Fungal amylase	B	63.5	28.6	-	17.4
4	BC	21	Crude fungal enzyme	B	63.5	15.2	-	3.5
5	BC	28	Fungal enzyme concentrate	PB	63.5	34.3	-	20.0
6	BC	20	Fungal enzyme	B	63.5	7.9	-	15.2
7	BC	56	Amylolytic supplement	B	61.1	20.7	-	8.8
8	T	28	Bact. amylase (<i>B. subtilis</i>)	C	45.0	1.0	-	-1.4
	T	28	Bact. amylase (<i>B. subtilis</i>)	B	45.0	41.3	-	18.6
9	BC	28	Fungal enzyme supplement	B	50.0	13.6	9.4	14.2
10	BC	28	Bacterial & Plant origin	B	63.7	5.8	-	0.0
11	T	140	Crude enzyme (<i>B. subtilis</i>)	C	69.5-86.0	-5.2	-	11.8
	T	140	Crude enzyme (<i>B. subtilis</i>)	B	69.5-86.0	4.1	-	0.0
12	BC	28	Bact. amylase (<i>B. subtilis</i>)	C	67.0	0.9	-	1.9
	BC	28	Bact. amylase (<i>B. subtilis</i>)	B	67.0	13.7	-	4.1
	T	21	Bact. amylase (<i>B. subtilis</i>)	C	45.0	1.9	-	1.5
	T	21	Bact. amylase (<i>B. subtilis</i>)	B	45.0	15.0	-	3.2
13	BC	21	Bact. amylase (<i>B. subtilis</i>)	C	64.0	2.1	-	-2.0
	BC	21	Bact. amylase (<i>B. subtilis</i>)	R	64.0	14.5	-	8.8
14	BC	21	Bacterial amylase	B	73.0	3.0	-	4.0
15	BC	20	β -glucanase	B (MV)	70.0	4.4	-	5.6
	BC	20	β -glucanase	B (HV)	70.0	38.0	-	15.9
16	BC	13	Pectic enzyme	C	62.5	0.2	-	1.8
	BC	13	Pectic enzyme	R	62.5	30.6	-	13.6
17	SCWL	18	Culture filtrate (<i>T. viride</i>)	C	61.5	8.7	-	-
	SCWL	18	Culture filtrate (<i>T. viride</i>)	W	75.0	15.4	-	-
	SCWL	18	Culture filtrate (<i>T. viride</i>)	B	73.5	30.3	-	-
	SCWL	18	Culture filtrate (<i>T. viride</i>)	O	74.5	35.9	-	-
	SCWL	18	Culture filtrate (<i>T. viride</i>)	R	73.5	34.9	-	-
18	BC	21	β -glucanase	B (EYR)	65.0	42.6	32.4	11.5
	BC	21	β -glucanase	B (CR)	65.0	20.0	16.1	5.5
19	BC	28	Bact. amylase (<i>B. subtilis</i>)	B	68.2	22.8	20.2	2.2
	BC	28	Fungal amylase (<i>Rhizopus</i>)	B	68.2	25.1	22.6	2.1
20	BC	21	<i>T. viride</i> culture filtrate	B	73.6	18.7	9.1	8.3
21	BC	21	β -glucanase	B (CR)	65.0	11.3	7.5	3.3
22	BC	28	β -glucanase (plant & bact.)	B	73.1	-	16.1	3.6
23	BC	21	Cellulase (<i>T. viride</i>)	HB	60.0	10.1	-	4.3
24	BC	13	Cellulase (<i>T. viride</i>)	W	57.0	6.6	0.0	6.2
	BC	13	Cellulase (<i>T. viride</i>)	R	57.0	19.6	-1.9	22.2
25	BC	21	β -glucanase	B	51.2	5.9	-	6.7
	BC	21	β -glucanase	B (H)	55.7	8.5	-	10.1
26	BC	21	β -glucanase	B (P)	47.6	4.9	-	9.5
27	BC	25	Cellulase (<i>T. viride</i>)	M	61.5	0.0	0.0	0.0
	BC	25	Cellulase (<i>T. viride</i>)	W	68.0	-2.3	-4.8	2.3
	BC	25	Cellulase (<i>T. viride</i>)	B	64.0	7.4	4.9	2.2
	BC	25	Cellulase (<i>T. viride</i>)	O	66.0	9.2	5.7	2.7
	BC	25	Cellulase (<i>T. viride</i>)	R	65.0	25.7	15.5	8.4
28	BC	25	β -glucanase	M	61.5	-0.3	-	0.6
	BC	25	β -glucanase	B	64.4	6.0	-	3.8
	BC	25	β -glucanase	O	61.5	18.9	-	8.0
29	BC	14	β -glucanase	B (HV)	65.0	22.8	17.7	13.2
	BC	14	β -glucanase	B (LV)	65.0	25.2	14.7	36.6
30	T	42	β -glucanase (<i>B. subtilis</i>)	W	?	-2.2	-	0.3
	T	42	β -glucanase (<i>B. subtilis</i>)	B	?	6.2	-	7.8
	T	42	β -glucanase (<i>B. subtilis</i>)	HB	?	1.7	-	6.7
31	BC	25	Pectinase (<i>A. niger</i>)	R	60.0	9.9	2.2	7.6
	BC	25	Cellulase (<i>T. viride</i>)	R	60.0	10.9	1.5	9.2
32	BC	21	β -glucanase (<i>B. subtilis</i>)	WOG	58.0	69.7	-	-19.3
33	BC	21	β -glucanase	B	65.0	2.9	2.8	0.6
	BC	21	β -glucanase	O	65.0	2.6	1.2	1.3
	BC	21	β -glucanase	DO	65.0	8.1	0.0	7.6
34	BC	28	β -glucanase	B(DSG)	20.0	8.9	1.8	7.1
	BC	28	β -glucanase	DB(DSG)	20.0	-0.9	2.7	-3.6

Table 7 Continued. The effect of enzyme supplementation on the growth and/or productive performance of broiler and Leghorn chickens and turkeys fed corn-, wheat-, barley-, oats- or rye-based diets.

Author	Species ¹	Age (days)	Enzyme	Test Grain	Inclusion Rate (%)	Body wt. ²	Feed Cons.	Gain/Feed
35	BC	22	β -glucanase	O	72.9	-1.1	0.0	-0.6
	BC	22	β -glucanase	DO	66.0	-5.6	-7.2	1.4
36	T	28	β -glucanase (<i>B. subtilis</i>)	W	?	-1.0	-	-0.9
	T	28	β -glucanase (<i>B. subtilis</i>)	B	?	10.3	-	12.8
	T	28	β -glucanase (<i>B. subtilis</i>)	HB	?	33.0	-	23.3
37	BC	29	Cellulolytic enz. (<i>T. viride</i>)	T	30.0	10.4	3.7	5.8
	BC	29	Cellulolytic enz. (<i>T. viride</i>)	R	30.0	2.8	-2.5	5.8
38	BC	21	Crude enzyme (<i>A. niger</i>)	W/C	28.9/28.9	-3.8	-	1.9
	BC	21	Crude enzyme (<i>A. niger</i>)	B	52.3	12.3	-	5.5
39	BC	34	β -glucanase/pentosanase	W	57.0	2.3	2.2	0.6
	BC	34	β -glucanase/pentosanase	R	57.0	33.9	25.7	5.9
	BC	34	β -glucanase/pentosanase	T	57.0	3.0	0.7	2.2
40	SCWL	21	Cellulase (<i>T. viride</i>)	B	65.5	62.3	21.8	33.0
41	T	84	β -glucanase	HB	44.6-57.8	1.3	0.5	1.3
42	BC	21	Crude enzyme (<i>A. niger</i>)	B (LV)	58.2	16.6	-	3.6
	BC	21	Crude enzyme (<i>A. niger</i>)	B (HV)	58.2	54.7	-	17.9
43	BC	21	Pentosanase (<i>A. niger</i>)	R	58.1	52.2	-	19.9
44	SCWL	17	Cellulase (<i>T. viride</i>)	W	59.9	5.5	0.7	4.4
	SCWL	17	Cellulase (<i>T. viride</i>)	R	59.9	15.5	-0.7	16.6

¹ Species: BC = Broiler chickens; T = Turkeys; SCWL = Single Comb White Leghorn chickens.

² Performance parameters (weight gain, feed consumption, gain/feed) are given as a percentage change (+/-) compared to nonsupplemented diets.

³ Reference list: 1) Willingham *et al.*, 1959; 2) Laerdal *et al.*, 1960; 3) Thomas *et al.*, 1960; 4) Willingham *et al.*, 1960; 5) Leong *et al.*, 1961; 6) Thomas *et al.*, 1961; 7) Rose and Arscott, 1962; 8) Moran and McGinnis, 1965; 9) Potter *et al.*, 1965; 10) Dagher and Rottensten, 1966; 11) Moran and McGinnis, 1966; 12) Moran and McGinnis, 1968; 13) Moran *et al.*, 1969; 14) Herstad and McNab, 1975; 15) Gohl *et al.*, 1978; 16) Patel *et al.*, 1980; 17) Quershi *et al.*, 1980; 18) Hesselman *et al.*, 1981; 19) Mannion, 1981; 20) White *et al.*, 1981; 21) Hesselman *et al.*, 1982; 22) Campbell *et al.*, 1984; 23) Classen *et al.*, 1985; 24) Marquardt and Fengler, 1985; 25) Newman and Newman, 1985b; 26) Newman *et al.*, 1985; 27) Broz and Frigg, 1986a; 28) Broz and Frigg, 1986b; 29) Hesselman and Aman, 1986; 30) Salmon *et al.*, 1986; 31) Broz, 1987; 32) Campbell *et al.*, 1987; 33) Elwinger and Saterby, 1987; 34) Pettersson and Aman, 1987; 35) Pettersson *et al.*, 1987; 36) Salmon, 1987; 37) Scholtessyk and Knorr, 1987; 38) Classen *et al.*, 1988; 39) Pettersson and Aman, 1988; 40) Rotter *et al.*, 1989; 41) Stevens *et al.*, 1989; 42) Campbell *et al.*, 1989; 43) Grootwassink *et al.*, 1989; 44) Pawlik *et al.*, 1990.

⁴ Test grain: B = barley; WOG = wild oat groats; PB = pearled barley; C = corn; R = rye; W = wheat; HB = hullless barley; B (MV) = barley (medium viscosity); B (HV) = barley (high viscosity); O = oats; B (EYR) = barley (early yellow ripeness); B (CR) = barley (combine ripeness); B (LV) = barley (low viscosity); M = maize; B(DSG) = barley (distillers-spent-grains); DB(DSG) = dehulled barley (distillers-spent-grains); DO = dehulled oats; T = triticale; W/C = wheat/corn.

Table 8. The effect of enzyme supplementation on the nutrient utilization of corn-, wheat-, barley-, oats- or rye-based diets fed to broiler and Leghorn chickens and turkeys.¹

Author	Species ³	Age (days)	Enzyme	Test Grain ⁴	ME	DM	CP	Fat	Starch	Bone Ash
1 ²	BC	7	Crude fungal enzyme	PB	23.8	-	-	-	-	-
	SCWL	7	Crude fungal enzyme	B	14.5	-	-	-	-	-
2	BC	14	Fungal enzyme supplement	B	18.2	-	66.6	100	-	-
3	BC	14	Bacterial amylolytic enz.	B	35.3	-	-	-	-	-
4	SCWL	21	Bacterial amylase	C	-2.1	-	-	-	-	-
	SCWL	21	Bacterial amylase	R (T)	6.4	-	-	-	-	-
	SCWL	21	Bacterial amylase	R (W)	2.9	-	-	-	-	-
5	BC	15	α -amylase	B	3.2	4.0	4.1	-	-	-
	BC	15	Diastase	B	1.3	2.5	5.0	-	-	-
6	BC	28	Amylase (<i>B. subtilis</i>)	B	8.3*	-	8.6	-	-	-
	BC	28	Amylase (<i>Rhizopus</i>)	B	5.8*	-	0.9	-	-	-
7	BC	14	Pectic enzyme	R	-	-	-	-	-	-0.2
8	BC	21	Cellulase (<i>I. viride</i>)	HB	-	-	-	18.1	5.6	0.7
9	BC	15	β -glucanase (<i>A. niger</i>)	B	1.7*	2.0	6.5	2.8	-	-
10	BC	18	Pectinase (<i>A. niger</i>)	R	6.0*	-	4.1	-	-	-
	BC	18	Cellulase (<i>T. viride</i>)	R	7.4*	-	9.9	-	-	-
11	BC	21	β -glucanase (<i>B. subtilis</i>)	WOG	-	-	-	109.7	-	7.1
12	SCWL	Adult	Protease (<i>B. subtilis</i>)	CM	12.8	-	-	-	-	-
	SCWL	Adult	α -amylase (<i>B. subtilis</i>)	CM	0.4	-	-	-	-	-
13	BC	28	β -glucanase	B (DSG)	2.9*	-	1.4	2.4	-	-
	BC	28	β -glucanase	DB (DSG)	0.0*	-	1.4	1.2	-	-
14	BC	24	Cellulolytic enzyme	T	7.2*	-	3.1	3.2	0.3	-
	BC	24	Cellulolytic enzyme	R	2.0*	-	17.8	1.1	-0.3	-
15	BC	21	β -glucanase(bact. & plant)	W	-	-	-	-6.2	0.0	-
	BC	21	β -glucanase(bact. & plant)	B	-	-	-	4.8	0.6	-
	BC	21	β -glucanase(bact. & plant)	HB	-	-	-	44.8	7.5	-
	BC	21	β -glucanase(bact. & plant)	OG	-	-	-	61.6	1.0	-
16	T	14	β -glucanase	HB	-	-	-	-	-	1.4
17	SCWL	7	Cellulase (<i>I. viride</i>)	W	-	1.6	-	-1.4	-	-
	SCWL	7	Cellulase (<i>T. viride</i>)	R	-	8.9	-	19.3	-	-
18	SCWL	12	Cellulase (<i>T. viride</i>)	R	-	4.2	-	20.9	-	-

¹ All values are presented as a percentage change in nutrient utilization (digestion/absorption) with enzyme supplementation compared to the nonsupplemented diets. ME = metabolizable energy (ME values marked * indicate dietary ME values, otherwise for test grain only); DM = dry matter; CP = crude protein.

² Reference list: 1) Leong *et al.* 1962; 2) Potter *et al.* 1965; 3) Novacek and Petersen, 1967; 4) Moran *et al.* 1969; 5) Herstad and McNab, 1975; 6) Mannion, 1981; 7) Grammer *et al.* 1982; 8) Classen *et al.* 1985; 9) Broz and Frigg, 1986a; 10) Broz, 1987; 11) Campbell *et al.* 1987; 12) Beltranena *et al.* 1987; 13) Petterssen *et al.* 1987; 14) Scholtyssek and Knorr, 1987; 15) Edney *et al.* 1988; 16) Stevens *et al.* 1988; 17) Fengler *et al.* 1988; 18) Pawlik *et al.* 1990

³ Species: BC = broiler chicken; SCWL = single comb white leghorn; T = turkey.

⁴ Test grain: PB = pearled barley; B = barley; C corn; R (T) = rye (*Tetrapetkus*); R (W) = rye (Western); R = rye; HB = hullless barley; WOG = wild oat groats; CM = canola meal; B (DSG) = barley (distillers-spent-grain); HB (DSG) = hullless barley (distillers-spent-grain); T = triticale; W = wheat; OG = oat groats.

studies also report that the growth performance improvements observed with enzyme supplementation are more pronounced in high viscosity barley varieties compared to their low viscosity counterparts (Table 7: Ref. 15, 29, 42). According to data in Table 7, the ranges in percentage improvements observed with enzyme supplementation were for weight gain (-6.2 to 69.7%), feed consumption (-7.2 to 32.4%) and gain to feed ratio (-19.3 to 36.6%). The average improvements seen with enzyme supplementation for weight gain, feed consumption and gain to feed ratio are 14.9, 6.9 and 7.0%, respectively. The nature of the enzyme supplements in the tables are widely varied and includes β -glucanase, amylases, pectic enzyme, cellulase and pentosanase and are of bacterial or fungal origin.

A number of studies which report the effects of enzyme supplementation on bioavailable energy (ME) and the digestibilities of dry matter, crude protein, fat and starch are summarized in Table 8. For the studies shown, the range of percentage improvements observed were for ME (-2.1 to 35.3%) and for the digestibilities of dry matter (1.6 to 8.9%), crude protein (1.4 to 66.6%), fat (-6.2 to 109.7%) and starch (-0.3 to 7.5%). The average percentage improvements for ME and the digestibilities of dry matter, crude protein, fat and starch are 7.9, 3.9, 10.8, 25.4 and 2.1%, respectively. The majority of studies evaluating the effects of enzyme supplementation on growth performance and/or nutrient utilization have utilized young chickens or turkeys (0 to 4 weeks of age) as the test animal.

The relative effect of enzyme supplementation is dependent on the age of the bird as younger birds respond to enzyme supplementation in a more dramatic manner than mature birds. Numerous studies have exemplified the positive effects of enzyme supplementation on the growth performance of young broiler chickens (Thomas *et al.*, 1961, 1960; Laerdal *et al.*, 1960; Rose and Arscott, 1962; Petersson and Aman, 1988; Rotter *et al.*, 1989c). Generally, the positive effects of enzyme supplementation on growth performance are primarily seen from 0 - 3 weeks in broiler chickens (Reese *et al.*, 1983; Elwinger and Saterby, 1987; Classen *et al.*, 1988a)

and 0 - 4 weeks in turkeys (Salmon, 1987; Salmon *et al.*, 1986; Stevens *et al.*, 1988). Birds older than this generally show little or no response to enzyme supplementation with respect to growth performance (weight gain, feed consumption, feed efficiency). A number of studies involving laying hens (Berg, 1959, 1960; Bustany and Elwinger, 1988) and turkey breeder hens (Harper *et al.* 1982) have demonstrated that enzyme supplementation of barley-based diets had a marginal effect on rate of lay, feed efficiency or egg weight. Bustany and Elwinger (1988) reported that laying hens utilized barley- or wheat-based diets equally well, due to a more mature digestive system which can adapt to barley-based diets containing high β -glucan levels.

Several early studies reported the positive effects of enzyme supplementation on young broiler chickens fed barley-based diets with respect to weight gain ($P < .05$) but not feed efficiency ($P > .05$) (Daghir and Rottensten, 1966; Moran and McGinnis, 1968). Daghir and Rottensten (1966) found that weight gain was highly positively correlated ($R = .88$) with feed consumption indicating that improvements in body weight gain were mainly due to increased feed consumption rather than changes in the ME of the barley due to enzyme supplementation. Similarly, Hesselman *et al.* (1981) reported significant ($P < .01$) increases in body weight gain and feed consumption when barley-based broiler diets were enzyme supplemented. These researchers concluded that the increase in feed consumption was not due to a palatability factor since adding β -glucanase to the water source instead of the feed yielded similar results with respect to chick growth performance. Campbell *et al.* (1984) attribute a portion of the improvement in growth to an increase in feed intake as a result of increased rate of passage.

Research has shown that feeding young chicks or poults diets which have a relatively high content of β -glucans from barley and oats or pentosans from rye exhibit very sticky and wet droppings without enzyme treatment (Campbell *et al.*, 1987). Fadel *et al.* (1987) found that the addition of β -glucanase to barley-based diets generally resulted in a

decrease in excreta fats and an increase in excreta dry matter. These authors suggested that dietary β -glucans may be sequestering dietary fat and bile acid resulting in increased excreta fat levels. A number of studies have shown that enzyme supplementation of diets based on rye, oats, and barley results in increased dry matter content of excreta accompanied by improved litter condition (Moran and McGinnis, 1968; Gohl *et al.*, 1978; Salmon, 1987; Quereshi *et al.*, 1980; Hesselman *et al.*, 1981; White *et al.*, 1981; Hesselman *et al.*, 1982; Fadel *et al.*, 1987).

Moran and McGinnis (1965) studied the effects of a bacterial amylase (*Bacillus subtilis*) on barley- and corn-based turkey starter diets (0-4 weeks). The enzyme supplement dramatically improved 4 week weight gain and feed conversion efficiency of barley but not corn fed poults. They suggested that the β -glucans in barley support the establishment of "undesirable" microflora which are eliminated by the enzyme through alteration or modification of the intestinal substrate. The enzyme supplement had little effect on the corn-based diets since corn does not contain appreciable levels of β -glucans. Moran and McGinnis (1966) concluded that barley contains a fraction which supports unfavourable intestinal microbial growth and/or fails to support the growth of favourable microorganisms suggesting that the enzyme supplement has very little effect on older birds since their gut microflora is not influenced by diet to as great an extent as in younger birds. According to Campbell *et al.* (1986a), polysaccharide gums (β -glucans and pentosans) result in increased gastrointestinal tract microbial activity. These gums decrease digesta rate of passage. Consequently, microorganisms are not translated down the gastrointestinal tract resulting in microbial overgrowth.

Leong *et al.* (1961) studied the effect of enzyme supplementation on a pearled barley based diet for broiler chickens and found improvements of 34 and 20% for weight gain and gain/feed respectively compared to the nonsupplemented control diet. Pearling barley involves the removal of the outer fibrous hull of the barley kernel leaving a product with a relatively low fibre content. Leong *et al.* (1961) found that this pearled

barley still showed a substantial response to enzyme supplementation and concluded that it was not fibre *per se* which is the factor determining the efficacy of an enzyme supplement. Burnett (1966) studied the effect of a bacterial enzyme supplement on the viscosity of the water extract of the small intestine contents of broiler chickens fed barley-based diets and found that the enzyme supplement decreased the viscosity of the intestinal contents. Since the enzyme supplement gave similar results with a β -glucan solution, Burnett (1966) concluded that the enzyme responsible for lowering the viscosity was endo- β -glucanase. According to deSilva *et al.* (1983), the treatment of barley endosperm by β -glucanase results in the breakdown of β -glucans which are major components of the endosperm cell walls surrounding the starch granules in the barley kernel.

Burnett (1966) also concluded that the poor nutritional values of certain barley varieties is related to the relative levels of β -glucan, which result in stable highly viscous conditions in the small intestine, thus preventing nutrient absorption. White *et al.* (1983) fed broiler chicks a corn/soybean meal diet containing 2% Hydroxyethyl Cellulose (HEC), a complex polysaccharide which causes depressed growth performance and increased viscosity of intestinal contents and found that supplementing this diet with cellulase (*Trichoderma reesei*) which hydrolyzes the HEC, eliminated the adverse effects of the HEC and decreased the intestinal contents viscosity. These authors postulated that the improvements seen in β -glucanase supplemented barley were due to a hydrolysis of the β -glucan and decrease in the viscosity of intestinal contents. The data of Classen *et al.* (1985) indicate linear depressions in body weight, tibia ash, fat and starch absorption as hullless barley is substituted for wheat in broiler starter diets. However, enzyme supplementation (cellulase) resulted in improvements ($P < .05$) in all measured parameters. These authors noted that the viscosity of a β -glucan solution was decreased by cellulase incubation and suggested that the increase in growth performance with enzyme supplementation may be due to a decrease in viscosity (hydrolysis of β -glucan) as indicated by an

increase in reducing sugar levels.

Several studies have examined various barley varieties and found endogenous β -glucanase levels tended to be inversely related to barley viscosity measurements (Campbell et al., 1984, 1989). Viscosity measurements which were higher for hulless than hulled barley were correlated with chick performance. Hulless barley yielded inferior growth performance compared to the hulled varieties. Willingham et al. (1960) studied the effect of enzyme supplementation on barley varieties grown in various geographical locations within the U.S.A. These authors found the barleys grown in the Western U.S.A. showed more response to enzyme supplementation than varieties grown in the Eastern U.S.A. These results suggest that the geographical location has an effect on the component within barley which is affected by the enzyme supplement. Broiler chickens fed low viscosity barley had superior growth rates compared to those fed high viscosity barley. These differences were however eliminated by supplementing the diets with β -glucanase (Newman and Newman, 1987; Edney et al. 1989). Gohl et al. (1978) concluded that the relative effectiveness of β -glucanase supplement is dependent on the viscosity of the barley.

According to Marquardt and Fengler (1985), the poor utilization of rye-based diets by young broiler chickens is due to water soluble and highly viscous pentosans which are not digestible but also affect the digestibility of other nutrients. Marquardt and Fengler (1985) added graded levels of pentosans to a wheat-based diet and reported depressed fat and DM retention values which were improved by the addition of an enzyme supplement (extract of *Trichoderma viride*). According to Grootwassink et al. (1989), enzymic hydrolysis of rye pentosans using the pentosanase component of *Aspergillus niger* results in a rapid decrease in extract viscosity with minimal free sugar release (arabinose and xylose). These authors concluded that the viscosity of rye-based diets due to pentosans is the reason for poor growth rates common in nonsupplemented rye-based broiler chicken diets.

Aastrup (1979a) found the viscosity of the acid flour extract of barley was closely related ($r^2=.99$, $SE=.19$) to the soluble β -glucan content of barley and concluded that the component within barley which causes high viscosity in the small intestine is the β -glucan. Aastrup (1979a) also measured the β -glucan as a percentage of total carbohydrate in low and high viscosity barley varieties and found levels of 13-15 and 40%, respectively. White et al. (1981) fed barley-based diets supplemented with a culture filtrate (*Trichoderma viride*) to young broiler chickens and found a dramatic decrease in intestinal content viscosity along with improved growth performance ($P<0.05$) compared to the nonsupplemented control. These authors suggested that the hydrolysis of β -glucans is not the direct cause for improved growth performance from a nutritional standpoint, but rather the indirect cause since the hydrolysis of β -glucans results in decreased intestinal content viscosity compared to the nonsupplemented diets.

Grammer et al. (1982) noted that supplementation of rye based diets with a pectic enzyme resulted in improved body weights ($P<.05$) while also reducing the rachitic effects of rye grain for the young broiler chick. These authors did not attribute the improved nutritional performance of rye with enzyme supplementation to a decrease in intestinal content viscosity.

Numerous researchers have reported the positive effects of enzyme supplementation in increasing the bioavailable energy of rye- (Moran et al., 1964; Scholtyssek and Knorr, 1987), oats- (Broz and Frigg, 1986a) and barley-based broiler diets (Stutz and Matterson, 1961; Leong et al., 1962; Potter et al., 1965; Willingham and Earle, 1964; Broz and Frigg, 1986a). Leong et al. (1962) studied the effects of fungal enzyme supplement on the ME of regular and pearled barley and found 23.8 and 14.5% respective improvements compared to the nonsupplemented control grains. These authors concluded that the portion of barley affected by the fungal enzyme supplement to yield the increased ME is concentrated in the inner part of the barley seed and that pearling (removal of 35% of the

outer part of the seed) produces a product containing a higher proportion of the fraction acted on by the enzyme supplement. Moran and McGinnis (1965) also suggested that the improvements seen in growth performance of the barley fed poults is energy related since the growth increment obtained with enzyme supplements is lost by the addition of energy (corn oil) to the diet.

Hesselman and Aman (1985) examined the effects of enzyme supplementation on starch disappearance in the various sections of the broiler chicken gastrointestinal tract. They found 96-98 and 85-88% starch disappearance at the end of the small intestine for enzyme supplemented and nonsupplemented birds, respectively. For the nonsupplemented birds, there is another 10% disappearance in the caeca and colon (starch fermentation), however, there is no evidence that these breakdown products are utilized to any degree. A more anterior breakdown (disappearance) of starch is probably more efficient utilization since microbial action on starch in the lower gut is reduced. A more anterior disappearance also explains the increased feed intake seen with enzyme supplemented barley diets since starch is a major component of cereal based diets. In subsequent research, Hesselman and Aman (1986) fed barley based diets to broilers (0-19 day old) and found that those diets supplemented with β -glucanase resulted in birds which not only had better growth rate but also had approximately 10% shorter intestines than their nonsupplemented counterparts. The β -glucanase supplementation resulted in increased nitrogen digestion and β -glucan degradation anterior to the ileocecal junction.

Early authors (Stutz and Matterson, 1961; Potter et al., 1965), speculated that the improved performance was due to greater utilization of the fat, nitrogen and nitrogen-free extract in the barley presumably because of the breakdown of the cellular structure resulting in improved energy utilization (Willingham and Earle, 1964). These hypotheses have since been substantiated with further studies where enzyme supplementation was shown to improve DM retention (Broz and Frigg, 1986a; Fengler et al.

1988), organic matter digestion (Broz and Frigg, 1986a), starch digestion (Hesselman and Aman, 1985; Edney et al., 1989), fat absorption (Scholtyssek and Knorr, 1987; Edney et al., 1989; Fengler et al., 1988; Pawlik et al., 1990) and nitrogen absorption (Broz and Frigg, 1986a; Scholtyssek and Knorr, 1987; Edney et al., 1989). The studies of Pawlik et al. (1990) demonstrated correlation coefficients between the aqueous extract of the diet and excreta viscosity and fat retention of .67 ($P < .0001$) and .83 ($P < .0001$), respectively. Both rye- and wheat-based diets responded to enzyme supplementation, however, the response was more dramatic for the rye-based diets.

Summary

Traditional chicken broiler diets have not utilized barley, oats or rye grain to any great extent since high dietary levels of these grains have consistently resulted in poor bird growth performance compared with wheat- or corn-based diets. Previous research has demonstrated the presence of highly viscous and water-soluble β -glucans in barley and oats and pentosans in rye which increase digesta viscosity and thereby impede nutrient digestion and absorption in the young broiler chick. The relative β -glucan (barley, oats) and pentosan (rye) levels are affected by genetic factors and growing conditions. A number of methods including gamma irradiation, antibiotic supplementation, water treatment and enzyme supplementation have been shown to increase the nutritive value of barley, oats and rye through increased utilization of ME, protein and lipid.

MANUSCRIPT I: THE EFFECT OF ENZYME SUPPLEMENTATION,
FAT SOURCE AND METHOD OF DETERMINATION ON THE APPARENT METABOLIZABLE
ENERGY OF HULLESS BARLEY FOR THE YOUNG BROILER CHICK

ABSTRACT

The effect of enzyme supplementation on the nitrogen-corrected apparent metabolizable energy (AME_n) of hulless barley and apparent protein digestibility (APD) of hulless barley-based diets was examined utilizing two methods of determination. Experiment 1 employed a chick bioassay in which the energy balance of a diet containing material of known ME (sucrose) was compared to that of a diet in which hulless barley was substituted for sucrose. Enzyme supplementation increased ($P < .05$) the AME_n and APD of barley diets containing either corn oil or tallow as a dietary fat source by 20 and 4.3% and 33 and 8.6%, respectively. Fat source did not influence ($P > .05$) the enzyme effect. The growth data supported the AME_n and APD results as enzyme supplementation positively affected weight gain and feed conversion efficiency in all barley diets. In Experiment 2, hulless barley was substituted for an entire semi-practical diet and the energy balance of the diets was compared. Enzyme supplementation increased the AME_n of hulless barley 4.8%, while the APD of barley diets containing 30 and 60% hulless barley increased 1.7 and 3.8%, respectively. Increasing dietary barley levels resulted in depressed growth performance compared to the control diet. Enzyme supplementation improved feed conversion efficiency ($P < .05$), but not weight gain ($P > .05$). These results suggest that the hydrolysis of β -glucans increases the bioavailable energy and protein digestibility of barley for young broiler chicks. The extent of AME_n improvement observed with enzyme supplementation of barley is dependent on the chick bioassay utilized in the AME_n determination.

KEY WORDS: bioassay, hulless barley, apparent metabolizable energy, protein digestibility, enzyme supplementation, fat source

INTRODUCTION

Traditionally, barley has not been extensively utilized as a chicken broiler feedstuff due to its high fibre and associated low metabolizable energy (ME) content (NRC, 1984). The use of barley in broiler diets has consistently been associated with poor growth performance and unmanageable litter conditions compared with corn- or wheat-based rations. As a result, hulless barley varieties containing lower fibre levels have been developed in an attempt to increase the ME of barley (Rossnagel *et al.*, 1981). Although the hull does contribute to a lower ME content, the primary growth depressing factors in barley are the highly viscous, water soluble β -glucans (Burnett, 1966; White *et al.*, 1981; Hesselman and Aman, 1986) located in the endosperm cell walls. The relative levels of β -glucans in barley are influenced by genetic factors and environmental growing conditions (Willingham *et al.*, 1960; Aastrup, 1979b; Bourne and Wheeler, 1984). Partial hydrolysis of the β -glucans by β -glucanase leads to a dramatic decrease in viscosity with concurrent improvements in bird growth performance (Hesselman and Aman, 1986; Edney *et al.*, 1989; Rotter *et al.*, 1989b). The effects of enzyme supplementation on the growth performance of broilers fed barley-based diets have been extensively documented (Gohl *et al.*, 1978; Broz and Frigg, 1986a,b; Classen *et al.*, 1988a; Rotter *et al.*, 1989b,c). There are, however, very few reports on the ME of enzyme supplemented barley (Leong *et al.*, 1962; Potter *et al.*, 1965; Mannion, 1981).

Leong *et al.* (1962) reported 23.7 and 14.5% respective increases in the ME of pearled and regular barley when supplemented with a crude fungal amylase. The greater response observed with pearled barley suggests that the primary site of enzyme action is in the inner portions of the kernel (endosperm) where the majority of β -glucans are located (Preece and Mackenzie, 1952a). Potter *et al.* (1965) observed similar responses to an uncharacterized fungal enzyme supplement and attributed the increase in the ME of the barley to increased digestibilities of protein, fat and

nitrogen-free extract. Increases in the ME of barley-based diets have also been attained with using α -amylase preparations (bacterial and fungal origins) (Herstad and McNab, 1975; Mannion, 1981). Previous research has shown substantial improvements in the bioavailable energy of barley grain with enzyme supplementation. Depending on relative cereal grain costs (wheat/corn vs. barley), this is a factor to consider when formulating chicken broiler diets.

The ME of a feedstuff may be determined using a wide variety of methods including prediction equations and balance trials using both young chicks (Hill and Anderson, 1958; Scott et al., 1982) and mature birds (Sibbald and Slinger, 1963; Farrell, 1978; Campbell et al., 1986b; Sibbald, 1986). Two types of chick bioassays have been used to determine the ME of feedstuffs. Hill and Anderson (1958) developed a bioassay in which the test feedstuff is substituted for glucose and the energy balance of the two diets are compared. In the second method, originally developed by Potter and Matterson (1960), the energy balance of a semi-practical diet supplemented with the test feedstuff is compared to the energy balance of the diet by itself. A variation of this method was developed and extensively tested by Sibbald and Slinger (1963). Several studies have shown the ME of cereal grains to be somewhat dependent on the age of bird used in the ME bioassay with higher ME values observed with mature birds (Coates et al., 1977; Farrell et al., 1983). The method used in the determination of ME of a feedstuff may also influence the ME value obtained (Miller, 1974; Sibbald, 1975; Pesti, 1984; Pesti et al., 1986).

The objectives of this research were: 1) to determine the effect of enzyme supplementation on the AME_n of hulless barley as determined by two methods (Scott et al., 1982; Sibbald and Slinger, 1963); and 2). to determine the effect of fat source on the relative response to enzyme supplementation.

MATERIALS AND METHODS

General Procedures for AME Studies. Although utilizing different methods of AME_a determination, Experiments 1 and 2 were carried out simultaneously in the same room and under the same management procedures. Four hundred and fifty day-old vaccinated (Marek's) male Arbor Acre broiler chicks were obtained from a local commercial hatchery. From Day 1 to 7 the birds were housed in electrically heated Jamesway chick batteries¹ and fed a commercial chick starter crumblized diet containing 20% crude protein. On Day 7, the birds were individually weighed and placed into narrow weight classes. Birds of relatively high or low weights were discarded. Four birds were randomly assigned to each of 84 pens such that all pens had similar initial weights. Each treatment consisted of 6 replicates (pens). From Day 7 to 17 the birds were fed the experimental diets (mash form) containing .3% chromic oxide as an analytical marker. The cereal grain studied was hulless barley (Cultivar Scout). This variety was previously shown to contain a high level of β -glucans and have a high extract viscosity (Rotter *et al.*, 1989c). The enzyme preparation (dry form) used in both experiments was crude cellulase (*Trichoderma viride*)². Its activity as declared by the manufacturer was 23,880 cellulase units per g. Pen weights were recorded on Day 7 and 14. Prior to each weighing, the birds were fasted for 4 h to achieve consistent gut fill between all birds. During the balance trial, chicks were housed in Petersime chick batteries³ and had 24 h access to light, feed and water. Feed consumption was recorded during Day 7 through 14 so that a feed to gain ratio could be calculated for each replicate. Excreta were collected from Day 14 to 17. Excreta samples were freeze dried, equilibrated to atmospheric environmental conditions for 24 h and ground in a small coffee grinder

¹ Jamesway Manufacturing Co., Ft. Atkinson, WI 53538.

² Cellulase TV concentrate, Miles Laboratories Inc., Elkhart, IN 46515

³ Petersime Incubator Co., Gettysburg, OH 45328.

prior to laboratory analysis. Diet samples were also finely ground prior to laboratory analysis.

Experiment 1 ($AME_n(H)$). Semi-purified diets were formulated to contain 62.25% sucrose as a reference ingredient (Scott et al., 1982) (Table 9). The sucrose was then replaced by Scout barley with (.04 g enzyme/kg diet) or without enzyme supplementation with either 4% corn oil or tallow as the dietary fat source. The AME_n values derived using this method of determination will hereafter be referred to as $AME_n(H)$.

Experiment 2 ($AME_n(S)$). A basal semi-practical diet was formulated as outlined by Sibbald and Slinger (1963) (Table 10). The entire diet (basal and fat source) was substituted by Scout barley to make up either 30 or 60% of the diet. Substitution of the basal diet by barley did not affect vitamin and mineral levels since these components were added to the complete diet at a rate of 3 g/100 g diet. All diets were fed with (.04 g enzyme/kg diet) or without enzyme supplementation. The AME_n values derived using this method of determination will hereafter be referred to as $AME_n(S)$.

Chemical Analyses. Feed and excreta samples were analyzed for gross energy using a Parr adiabatic oxygen bomb calorimeter⁴ equipped with a digital thermometer. Nitrogen content was determined using the Kjeldahl procedure (No. 7.021) as described in Association of Official Analytical Chemists (AOAC, 1984) and chromic oxide was analyzed according to Williams et al. (1962). Excreta samples were analyzed for uric acid content (Marquardt, 1983) and the apparent protein digestibility (APD) calculated as described by Rotter et al. (1989a).

Calculations and Statistical Analyses. The AME_n of Scout barley was calculated as outlined by Hill et al. (1960) (Experiment 1) or by the use of simultaneous equations as outlined by Sibbald and Slinger (1963) (Experiment 2). In both cases, a correction for N-retention using a value of 36.5 kJ (8.73 kcal) per g N retained was used. The ME value used for

⁴ Parr Instrument Co., Moline, IL, 61265.

Table 9. Composition of semi-purified experimental diets (Experiment 1)

Ingredients	Diets			
	1	2	3	4
	(g/kg) ¹			
Sucrose	625.3	625.3	-	-
Scout barley	-	-	625.3	625.3
Soybean protein concentrate ²	250.0	250.0	250.0	250.0
Tallow	-	40.0	-	40.0
Corn oil	40.0	-	40.0	-
D,L-Methionine	6.0	6.0	6.0	6.0
Glycine	4.0	4.0	4.0	4.0
Vitamin/Mineral mixes ^{3,4}	74.8	74.8	74.8	74.8
Calculated composition				
ME (kcal/kg)	3528	3489	2878	2838
Crude protein (%)	21.8	21.8	30.1	30.1
(determined, Nx6.25, %)	(22.3)	(22.6)	(31.7)	(31.8)

¹ Diets 5 through 8 respectively were Diets 1 through 4 supplemented with enzyme (Cellulase Tv concentrate., Miles Lab., Elkhart, IN, 46515).

² Soybean protein concentrate (US Biochem. Corp.), 84.1% CP.

³ Vitamin mix provided per kg diet: Vit. A, 4500 IU; Vit. D, 4500 IU; Vit. E, 50 IU; menadione, 1.5 mg; thiamin, 15 mg; riboflavin, 15 mg; nicotinic acid, 50 mg; folic acid, 6 mg; pyridoxine, 6 mg; biotin, 0.6 mg; Vit. B₁₂, 20 µg; choline chloride, 2 g; Ca pantothenate, 20 mg; (after Scott et al. 1982).

⁴ Mineral mix provided per kg diet: CaHPO₄·2H₂O, 17.25 g; CaCO₃, 12.33 g; KH₂PO₄, 8.33 g; KCl, 0.83 g; NaCl, 5.0 g; MnSO₄·H₂O, 0.29 g; FeSO₄·7H₂O, 0.42 g; MgSO₄, 2.5 g; KIO₃, 1.67 mg; CuSO₄·5H₂O, 25 mg; ZnCO₃, 0.13 g; CoCl₂, 1.42 mg; NaMoO₄·2H₂O, 6.92 mg; Na₂SeO₃, 0.17 mg. (after Scott et al. 1982).

Table 10. Composition of semi-practical experimental diets (Experiment 2).

Ingredients	Diets		
	1	2	3
	(g/kg) ¹		
Basal diet ²	950	665	380
Scout barley	-	300	600
Tallow	50	35	20
Other ingredients ³	30	30	30
Calculated composition			
ME (kcal/kg)	3002	2875	2747
Crude protein (%)	29.4	23.5	18.9
(determined, Nx6.25, %)	(31.7)	(26.7)	(22.0)

¹ Diets 4 through 6 were respectively Diets 1 through 3 supplemented with enzyme (Cellulase Tv concentrate, Miles Lab., Elkhart, IN, 46515).

² Basal diet composition (g/kg): wheat (CV. Katepwa), 160; yellow corn, 160; meat meal (46.6% CP), 50; soybean meal (45.7% CP), 500; dried whey, 40; dehydrated alfalfa meal, 40; tallow, 50.

³ Includes the vitamin and mineral mixes which were added to the remaining ingredients at 3 g/100 g diet. The vitamin and mineral mixes provided per kg diet: Vit A, 48.6 mg; Vit. D, 48.6 mg; menadione, 24.4 mg; Ca pantothenate, 6.1 mg; choline chloride, 96.9 mg; riboflavin, 4.1 mg; niacin, 1.2 mg; Vit B₁₂, 48.6 mg; D,L-methionine, 48.6 mg; limestone, 0.96 g; dicalcium phosphate, 1.00 g; iodized salt, 0.23 g; MnO₂, 12.8 mg; ZnO, 6.4 mg; Cr sesquioxide, 3.2 g. (after Sibbald and Slinger, 1963).

sucrose was 15.40 MJ/kg DM (3.68 kcal/g DM) (NRC, 1984).

Data were analyzed using the general linear models (GLM) procedure of the Statistical Analysis Systems package (SAS, 1984). A completely randomized design having a 2 x 2 x 2 (diet x enzyme x fat, Experiment 1) or a 3 x 2 (diet x enzyme, Experiment 2) factorial arrangement of treatments was used.

RESULTS AND DISCUSSION

The growth performance data for Experiment 1 are shown in Table 11. Feed consumption was not affected significantly by diet, enzyme or fat source. The diet x fat interaction was significant for weight gain ($P=.03$) but not feed consumption or feed to gain ratio ($P>.05$). Fat source had a minimal effect on weight gain for barley-based diets while there was an 18 g decrease in body weight when tallow was substituted for corn oil in the sucrose-based diets. Diet type (sucrose versus barley) did not have an effect on feed to gain ratio ($P>.05$) but approached significance for weight gain ($P=.06$). The diet x enzyme interaction was highly significant ($P<.01$) for weight gain and feed to gain ratio as a positive enzyme effect was seen in barley- and not sucrose-based diets. Enzyme supplementation improved weight gain and feed conversion efficiency by 12 and 9%, respectively, for barley diets containing corn oil and 12 and 11%, respectively, for the same diets containing tallow. Fat source had a significant ($P<.01$) effect on feed to gain ratios, since diets containing corn oil yielded superior performance compared to tallow containing diets.

The growth data for Experiment 2 are shown in Table 12. Feed consumption was not affected ($P>.05$) by diet, enzyme or an interaction of these factors. Weight gain and feed to gain ratio were adversely affected ($P<.01$) by increasing levels of dietary barley. Classen et al. (1985) observed a linear decrease in body weight with increasing dietary levels of hulless barley. Enzyme supplementation improved feed to gain ratio

Table 11. Effect of enzyme supplementation on feed consumption, weight gain and feed to gain ratio of birds fed semi-purified diets containing corn oil or tallow (Experiment 1).

Diets ¹	Enzyme	Feed Consumption		Weight Gain		Feed to gain ratio	
		-	+	-	+	-	+
		(g)		(g)		(g/g)	
S + CO		205	192	162	151	1.27	1.27
S + TA		187	187	146	146	1.30	1.29
B + CO		194	199	151	169	1.29	1.17
B + TA		201	201	147	164	1.37	1.22
SEM		4.8		5.0		.02	
ANOVA, probabilities							
Source of variation							
	Diet (D)	.1		.06		.3	
	Enzyme (E)	.4		.5		.009	
	Fat (F)	.5		.1		.0002	
	D X E	.2		.002		.0002	
	D X F	.03		.4		.2	
	E X F	.6		.5		.5	
	D X E X F	.2		.4		.8	

¹ S + CO (Sucrose + Corn oil); S + TA (Sucrose + Tallow);
B + CO (Barley + Corn oil); B + TA (Barley + Tallow).

Table 12. Effect of enzyme supplementation on feed consumption, weight gain and feed to gain ratio of birds fed semi-practical diets (Experiment 2).

Diets	Enzyme	Feed Consumption		Weight Gain		Feed to gain ratio	
		-	+	-	+	-	+
		(g)		(g)		(g/g)	
0 ¹		240	230	175	161	1.38	1.43
30		245	245	161	171	1.52	1.43
60		236	234	131	139	1.82	1.69
SEM		5.7		4.6		.03	
ANOVA, probabilities							
Sources of variation							
	Diet (D)	.2		.0001		.0001	
	Enzyme (E)	.4		.6		.02	
	D X E	.6		.03		.008	

¹ Percentage of diet made up by barley.

($P < .05$) but did not affect weight gain ($P > .05$). Enzyme supplementation increased body weight in the 30 and 60% barley diets by 6 and 7%, respectively. The significant ($P < .05$) diet x enzyme interactions for weight gain and feed to gain ratio is primarily due to the lack of response to enzyme in the basal diet. For reasons unknown to the author, this diet showed a negative response to enzyme supplementation for all parameters studied.

In Experiment 1, AME_n and APD data reflected growth performance trends (Table 13). Diet type (sucrose- vs. barley-based) had a significant effect ($P < .01$) on dietary $AME_n(H)$ and APD values as the substitution of sucrose by barley resulted in depressions of both parameters. The enzyme effect was also significant ($P < .01$) for both $AME_n(H)$ and APD. The presence of a significant ($P < .01$) diet by enzyme interaction suggests that the enzyme exerted a much greater effect in the barley- compared to sucrose-based diets. These results were expected since the substrate for the enzyme (β -glucanase) is β -glucans which are a component of barley and not sucrose. The enzyme by fat interaction was not significant ($P > .05$) for $AME_n(H)$ or APD indicating the response obtained with enzyme was not influenced by fat type. Fat type (corn oil vs. tallow) affected dietary $AME_n(H)$ ($P < .05$) but not APD ($P > .05$) (Table 13). Enzyme supplementation of barley-based diets containing corn oil or tallow resulted in 20 and 33% increases ($P < .05$) in $AME_n(H)$, respectively. The significant ($P = .02$) three way interaction however, could indicate that enzyme treatment may have been more effective in barley-based diets that contained tallow compared to those that contained corn oil. This may, in part, be attributed to an effect of enzyme treatment on fat absorption. Other studies have shown that highly unsaturated fats such as corn oil have much higher apparent absorbability than tallow in the young chick (Fedde et al., 1960; Carew et al., 1972; Kussaibati et al., 1982). Carew et al., (1972) found maximum absorption of corn oil by one week old chicks, whereas tallow reached maximum absorbability at a later age. Kussaibati et al. (1982) showed the addition of bile salts significantly increased ($P < .05$) the digestibility

Table 13. Effect of enzyme supplementation on the apparent protein digestibility (APD) and available energy (AME_n) of semi-purified diets containing corn oil (CO) or tallow (TA) (Experiment 1)

		APD		AME _n of complete diet		AME _n of grain only	
Diets ¹	Enzyme	-	+	-	+	-	+
		(%)		(MJ/kg)		(MJ/kg)	
S + CO		85.9	86.9	15.14	15.10	-	-
S + TA		86.5	85.0	15.09	14.73	-	-
B + CO		81.4	84.9	12.56	13.92	11.33	13.53
B + TA		79.0	85.8	12.09	13.97	10.67	14.20
SEM		.46		.17			
ANOVA, probabilities							
Sources of variation							
	Diet (D)	.001		.0001			
	Enzyme (E)	.0001		.0001			
	Fat (F)	.2		.02			
	D X E	.0001		.0001			
	D X F	.9		1.00			
	E X F	.7		.6			
	D X E X F	.009		.02			

¹ S + CO (Sucrose + Corn oil); S + TA (Sucrose + Tallow);
B + CO (Barley + Corn oil); B + TA (Barley + Tallow).

of diets containing animal fat but not maize oil, suggesting that bile acid levels in the young chick are not adequate for maximum tallow absorption. In studies using an enzyme supplement of bacterial and plant origin, Dagher and Rottensten (1966) concluded that at least a portion of the positive influence of enzymes on growth performance must be due to an increase in the ME of the ration. The increases in AME_n due to enzyme addition in this study is in agreement with previous findings. Leong et al. (1962) reported 24 and 15% increases in the ME of pearled and regular barley respectively with the addition of a crude fungal enzyme preparation. Potter et al. (1965) found a fungal enzyme supplement increased the ME of barley by 18.2% and attributed the increase in bioavailable energy to significantly ($P < .05$) increased digestibilities of protein, fat and nitrogen-free extract.

Diet type (sucrose versus barley) had a highly significant effect on APD ($P < .01$) (Table 13). As seen in the sucrose based-diets (Table 13), the protein source (soybean concentrate) was highly digestible and not affected by enzyme supplementation. The substitution of sucrose by barley resulted in a 9% increase in the crude protein content of the diet, however the APD decreased. The β -glucans in the Scout barley diets have a negative effect on the utilization of the entire diet with respect to AME_n and APD. Previous studies have demonstrated the nutrient digestibility and growth depressing effects of viscous and indigestible polysaccharides such as β -glucans and other gums for young growing rats (Harmuth-Hoene and Schwerdtfeger, 1979) and chickens (Burnett, 1966). Potter et al. (1965) found the percent digestible protein in barley was almost doubled by the addition of a fungal enzyme preparation. The data of Hesselman and Aman (1986) showed that β -glucanase supplementation of barley did result in increased starch degradation and nitrogen digestion in 19 day old broiler chickens. The $AME_n(S)$ and APD results (Experiment 2) showed similar trends to the growth data (Table 14). Increasing levels of dietary barley did not have a significant effect ($P > .05$) on AME_n whereas APD was depressed ($P < .05$) as the level of barley increased from 0 to 60%.

The enzyme effect was not significant ($P > .05$) for APD and approached significance ($P = .06$) for $AME_n(S)$. In the 60% barley diets, enzyme supplementation resulted in respective increases of 3.8 and 3.0% for APD and $AME_n(S)$. This relatively small response to enzyme may suggest that other factors than the β -glucans in barley may be limiting the bird's response to enzyme supplementation. The diet x enzyme interaction was not significant ($P > .05$) for either parameter studied indicating that the enzyme effect was not dependent on the dietary inclusion rate of hulless barley.

The increases in AME_n , APD and growth performance cannot be attributed to increased utilization of β -glucans. Complete degradation of the β -glucans would only increase dietary glucose by 3% which does not explain the substantial increases in chick growth performance seen in earlier studies (Willingham *et al.*, 1959; Herstad and McNab, 1975; Hesselman *et al.*, 1982; White *et al.*, 1983; Broz and Frigg, 1986a; Hesselman and Aman, 1986; Edney *et al.*, 1989; Rotter *et al.*, 1989c). According to Burnett (1966) and White *et al.* (1983), the improvements observed upon the addition of enzyme (β -glucanase) to barley-based diets are due a decrease in intestinal content viscosity resulting in improved nutrient digestion and absorption.

Two methods of AME_n determination were used in the present studies. Experiment 1 ($AME_n(H)$) employed substitution of a material of known AME_n (Scott *et al.*, 1982) while Experiment 2 ($AME_n(S)$) involved the substitution of an entire semi-practical basal diet (Sibbald and Slinger, 1963). Since these experiments were performed simultaneously under identical experimental conditions, a comparison of the two methods is possible. The barley $AME_n(S)$ results of Experiment 2 may be compared with the tallow containing diets in Experiment 1. The $AME_n(S)$ values for non-supplemented barley were 9.6% higher than the $AME_n(H)$ values (11.69 and 10.67 MJ/kg DM, respectively) (Tables 3, 4). This is in agreement with Schang *et al.* (1982) who found $AME_n(S)$ values tended to be higher than $AME_n(H)$ for all cereal grains, but this was only significant for barley ($P < .05$). This

Table 14. Effect of enzyme supplementation on the apparent protein digestibility (APD) and available energy (AME_n) of semi-practical diets (Experiment 2).

		APD		AME _n of complete diet		AME _n of grain only	
Diets	Enzyme	-	+	-	+	-	+
		(%)		(MJ/kg)		(MJ/kg)	
	0 ¹	83.8	82.5	11.61	11.70	-	-
	30	80.2	81.6	11.55	11.84	11.69	12.25
	60	78.8	81.8	11.73	12.08	-	-
	SEM	1.1		.06			
ANOVA, probabilities							
Sources of variation							
	Diet (D)	.04		.2			
	Enzyme (E)	.3		.05			
	D X E	.2		.7			

¹ Percentage of diet made up by barley.

trend is similar to that observed by Pesti et al. (1986) where the $AME_n(S)$ value of poultry by-product meal was 7.9% higher than the $AMEn(H)$ value. However, an earlier study showed no differences between the two methods for the AME_n of maize (Pesti, 1984). For enzyme supplemented barley, the differences between the two methods were substantially larger for reasons discussed earlier.

In summary, enzyme supplementation increased the bioavailable energy (AME_n) of barley and APD of barley-based diets for young broiler chicks. These improvements were also reflected in the weight gain and feed to gain ratios of chicks fed enzyme supplemented barley-based diets. Diets containing corn oil as the dietary fat source yielded superior chick performance compared with those containing tallow. The relative response to enzyme supplementation was more pronounced when tallow was the dietary fat source. Method of determination affected the AME_n values of hulless barley for broiler chicks. For nonsupplemented barley, the $AMEn(S)$ value was higher than the $AMEn(H)$ value however, this trend was reversed when the barley was enzyme supplemented.

MANUSCRIPT II: THE EFFECT OF ENZYME SUPPLEMENTATION ON
THE APPARENT METABOLIZABLE ENERGY AND NUTRIENT DIGESTIBILITIES
OF WHEAT, BARLEY, OATS AND RYE FOR THE YOUNG BROILER CHICK

ABSTRACT

The influence of enzyme supplementation on the bioavailable energy (AME_n) and apparent digestibilities of lipid (ALD) and protein (APD) was examined for diets containing either wheat, hulled or hullless barley, naked oats, or spring rye fed to young broiler chicks. Dietary AME_n , APD, and ALD values were depressed ($P < .01$) for all test grains (except hulled Bedford barley) as inclusion rate of the grain replacing wheat increased. The antinutritives, β -glucans (barley, oats) and pentosans (rye) had the most pronounced effect on ALD. The decreases in ALD were 43, 77 and 67% for chicks fed diets containing 70% Scout barley (hullless), Terra oats, and Gazelle rye, respectively, compared with those fed the control wheat diet. Enzyme supplementation increased ($P < .01$) AME_n , APD, and ALD for all test cereals. The corresponding increases in the AME_n of the enzyme supplemented diets containing 70% HY320 wheat, Bedford barley, Scout barley, Terra oats, and Gazelle rye diets were 4, 7, 42, 33, and 14%, respectively, compared to their nonsupplemented counterparts. Enzyme treatment also improved ($P < .01$) weight gains and feed conversion efficiencies of chicks fed diets containing each of the cereals. Overall, the results demonstrate that the nutritive value of cereal grains such as wheat, barley, oats, and rye can be improved by the addition of crude fungal extracts to the diet of young chicks.

KEY WORDS: enzyme supplementation, protein digestibility, lipid digestibility, bioavailable energy, cereal grains

INTRODUCTION

High energy and protein diets have been utilized to compensate for the young broiler chick's limited feed intake capacity and to maximize growth during the starter phase. Consequently, the primary dietary energy sources in commercial broiler diets have been the traditional high energy cereals, corn (maize) and wheat. Barley, rye, and oats which contain lower levels of bioavailable energy have received very limited use in broiler chicken diets. High dietary concentrations of these grains have consistently resulted in poor chick performance and unmanageable litter conditions caused by sticky droppings.

Conventional barley and oat cultivars contain high fibre levels coupled with low ME levels compared with corn or wheat (Aman and Hesselman, 1984). Hullless barley and naked oats cultivars have been selected in an effort to decrease the fibre level, and thereby increase the level of bioavailable energy. However, Burnett (1966) demonstrated that the highly viscous water-soluble β -glucans in barley and oats and not the fibre *per se* are the primary growth depressing factors limiting the bioavailable energy of these cereal grains. Their content is influenced by genetic factors and environmental growing conditions (Willingham *et al.*, 1960; Aastrup, 1979b). Henry (1985) found the mean water-soluble β -glucan content of barley, oat and wheat varieties to be 2.89, 2.13, and 0.67%, respectively. Barley β -glucans increase digesta viscosity and consequently decrease the absorption of nitrogen and carbohydrate (Burnett, 1966; Hesselman and Aman, 1986).

Rye grain does not support acceptable chick growth performance although it contains low β -glucan concentrations. Fernandez *et al.* (1973) reported that birds fed water-extracted rye had significantly improved growth performance compared with untreated rye alone, and concluded that the growth-depressing factor in rye was a water-soluble compound. Antoniou *et al.* (1981) found raw rye contained 10% total pentosans (arabinose and xylose) which, when added to wheat-based diets at levels

found in similar rye-based diets, resulted in severe growth depressions. Recently, Bedford et al. (1991) determined that the intestinal viscosity of chicks fed rye-based diets was highly correlated with high molecular weight carbohydrates in gut contents. An addition of pentosanase reduced concentrations of these complexes.

Growth performance of chicks fed barley, oats, or rye grain has been improved through the use of dietary enzyme supplements. Partial hydrolysis of the β -glucans in barley and oats and pentosans in rye results in a dramatic decrease in extract viscosity and improvement in chick performance (Broz and Frigg, 1986a,b; Hesselman and Aman, 1986; Broz, 1987a, b; Elwinger and Saterby, 1989; Fengler et al., 1988; Although the effects of enzymes on growth performance are well documented, there are few reports on the ME of enzyme-supplemented wheat, barley, oats, and rye.

Leong et al. (1962) reported 23.7 and 14.5% increases in the ME of pearled and regular barley, respectively, when supplemented with a fungal enzyme preparation. Potter et al. (1965) observed similar responses to an uncharacterized fungal enzyme supplement and attributed the increased barley ME to increased digestibilities of protein, fat and N-free extract. Moran et al. (1969) showed 6.4 and 2.9% increases in the ME of Tetrapetkus and Western rye, respectively, when supplemented with a fungal (*Aspergillus* sp.) enzyme preparation. Similarly, Broz (1987b) found the ME of a 60% rye diet increased 6.0 and 7.4% when supplemented with pectinase (*Aspergillus niger*) and cellulase complex (*Trichoderma viride*), respectively.

The objectives of the present research were: 1) to determine the effect of enzyme supplementation and level of dietary inclusion on the dietary AME_n of four cereal grains (hulled and hullless barley, spring rye, naked oats and wheat) and 2) to determine the effect of enzyme supplementation on the apparent protein (APD) and lipid (ALD) digestibilities of broiler diets containing these grains at two inclusion levels.

MATERIALS AND METHODS

Six hundred 1-day-old male Arbor Acres broiler chicks that had been vaccinated for Marek's disease were obtained from a local commercial hatchery. From Days 1 to 7, the birds were housed in electrically heated Jamesway chick batteries¹ and fed a commercial chick starter crumbled diet containing 20% crude protein. On Day 7, the birds were individually weighed and placed into narrow weight classes. Birds having relatively high or low body weights were discarded. Groups of four birds were then assigned randomly to each of 84 pens such that all pens had similar initial weights. Each treatment was randomly assigned to six pens. From Days 7 to 17, the birds were fed the experimental diets (mash form) that contained .3% chromic oxide as an analytical marker. During the balance trial, the birds were housed in Petersime chick batteries² equipped with raised wire floors. Birds had 24 h access to light, feed, and water throughout the entire experiment. Pen weights were recorded on Days 7 and 14. Prior to each weighing, the birds were fasted for 4 h to ensure a consistent gut fill among all birds. Feed consumption for each pen was recorded during Days 7 to 14 in order to calculate feed:gain ratios. Excreta were collected on plastic lined trays from Days 14 to 17. The lyophilized excreta samples were equilibrated with atmospheric moisture conditions for 24 h, and then finely ground in a small coffee grinder prior to laboratory analysis. Diet samples were also finely ground prior to laboratory analysis.

The AME_n chick bioassay used in the present experiment was similar to the procedure outlined by Sibbald and Slinger (1963) with several modifications. The modified basal diet was formulated to contain 70% wheat (cultivar HY320, 13.28% crude protein, N x 6.25) as a reference material (Table 15). The test diets substituted each of the four test

¹ Jamesway Manufacturing Co., Ft. Atkinson, WI 53538.

² Petersime Incubator Co., Gettysburg, OH 45328.

Table 15. Composition of experimental diets.

Ingredient	Diets ¹		
	1	2	3
	(g/kg diet)		
Test cereal ²	0	350	700
Wheat ³	700	350	0
Soybean protein concentrate ⁴	160	160	160
Tallow	50	50	50
Corn oil	10	10	10
Mineral premix ⁵	55	55	55
Vitamin premix ⁶	25	25	25

¹ Diet 1 was used as a negative control for all cereal grains tested. All diets were fed with and without crude enzyme preparation (Cellulase Tv conc., Miles Laboratories Inc., Elkhart, IN 46515).

² Test cereals included: Hulled barley (Bedford), 12.77% CP, N x 6.25; Hulless barley (Scout), 13.29% CP, N x 6.25; Spring rye (Gazelle), 14.27% CP, N x 6.25; Naked oats (Terra), 14.59% CP, N x 6.25.

³ Semi-dwarf variety (HY320), 13.28% CP, N x 6.25.

⁴ Soybean protein concentrate, 84.1% CP (US Biochem. Corp., Cleveland, OH 14128).

⁵ Mineral mix provided per kg diet: ground limestone, 15.41 g; dicalcium phosphate, 18.33 g; potassium carbonate, 1.02 g; iodized salt, 2.5 g; MnO, 64.2 mg; ZnO, 31.4 mg; CuSO₄, 9.92 mg; sodium selenite, 0.32 g; lysine, 0.40 g; D,L-methionine, 5.05 g; chromic oxide, 3.0 g.

⁶ Vitamin mix provided per kg diet: vitamin A, 15000 IU; vitamin D, 2000 IU; vitamin E, 100 IU, menadione, 5.0 mg; riboflavin, 0.763 mg; calcium pantothenate, 5.37 mg; niacin, 21.7 mg; vitamin B₁₂, 10.35 µg; choline chloride, 2.0 g; biotin, 1.65 µg; folic acid, 0.383 mg; thiamin, 0.53 mg; pyridoxine, 1.47 mg.

cereals (hulled barley, cultivar Bedford, 12.77% crude protein, N x 6.25; hulless barley, cultivar Scout, 13.29% crude protein, N x 6.25; spring rye, cultivar Gazelle, 14.27% crude protein, N x 6.25; naked oats, cultivar Terra, 14.59% crude protein, N x 6.25) at 35% increments for wheat to make up 35 or 70% of the total diet. The remaining 30% of the diet, the protein-energy (PE) supplement, consisted of a supplemental protein source (soybean concentrate)³, energy source (tallow and corn oil, and vitamin and mineral premixes. The PE supplement was formulated to meet or exceed National Research Council (NRC, 1984) nutrient requirements for all cereal grains tested in the present experiment (Table 16).

All diets, including the basal wheat diet, were fed without and with enzyme supplement (.04 g/kg diet). The enzyme preparation (dry form) used was crude cellulase (*Trichoderma viride*)⁴. Its activity as determined by the manufacturer was 23,880 cellulase units per gram.

Chemical Analyses Feed and excreta samples were analyzed for gross energy using a Paar adiabatic oxygen bomb calorimeter⁵ equipped with a digital thermometer. Nitrogen content was determined using the Kjeldahl procedure (Procedure 7.021) as described in Association of Official Analytical Chemists (AOAC, 1984) and chromic oxide analyzed according to Williams et al. (1962). Total lipid content of feed and excreta samples were determined as outlined by Marchello et al. (1973). Excreta samples were analyzed for uric acid content using a spectrophotometric method (Marquardt, 1983). The APD and ALD were calculated as described by Rotter et al. (1989a).

Calculations and Statistical Analyses The dietary AME_n values were calculated as outlined by Hill et al. (1960) with a correction for N retention using a value of 36.5 kJ (8.73 kcal) per g N retained. Data were analyzed using the General Linear Models (GLM) procedure from SAS

³ 84.1% crude protein, US Biochem. Corp., Cleveland, OH 14128. used a

⁴ Cellulase TV concentrate, Miles Laboratories Inc., Elkhart, IN 46515.

⁵ Paar Instrument Co., Moline, IL 61265.

Table 16. Calculated composition of experimental diets.

Diet	ME ¹	CP ²	Ca	P ³	Lys	Met
	(MJ/kg)	(%)				
70 W + 0 T ⁴	12.77	23.2 (24.2) ⁵	1.02	.48	1.22	.77
Scout 35 W + 35 T	12.53	23.2 (24.7)	1.01	.50	1.22	.77
O W + 70 T	12.30	23.2 (25.0)	1.00	.51	1.22	.76
Bedford 35 W + 35 T	12.53	23.0 (24.3)	1.01	.50	1.22	.77
O W + 70 T	12.30	22.9 (24.1)	1.00	.51	1.22	.76
Terra 35 W + 35 T	12.40	23.6 (24.9)	1.08	.58	1.29	.78
O W + 70 T	12.03	24.1 (25.2)	1.15	.67	1.49	.79
Gazelle 35 W + 35 T	12.51	23.5 (24.5)	1.02	.48	1.23	.77
O W + 70 T	12.25	23.9 (25.1)	1.02	.46	1.23	.76

¹ Values calculated according to NRC (1984).

² Crude protein

³ Available phosphorus

⁴ W=wheat; T=test cereal

⁵ Determined crude protein, N x 6.25, %

software (SAS Institute, 1986). The AME_n bioassay for each test cereal completely randomized design with a 2 x 3 (enzyme by cereal level) factorial arrangement of treatments. Orthogonal contrasts were utilized to compare means for supplemented and unsupplemented cereals (Snedecor and Cochran, 1980).

RESULTS

The test diets containing 35% wheat and 35% of the test cereals (except rye) but no supplemental enzyme supported weight gains in chicks that were the same ($P>.05$) as that obtained with the control wheat diet (Table 17). The test rye diet (35%), however, yielded growth that was only 75% ($P<.05$) of that obtained with the control wheat diet. The corresponding feed to gain ratio was not affected ($P>.05$) when 35% Scout barley was substituted into the control diet, was slightly decreased ($P<.05$) 6% (1.44 versus 1.53 g) by substitution of 35% Bedford barley or 35% Terra oats for the wheat, and was markedly ($P<.01$) increased (18%; 1.80 versus 1.53 g:g) when 35% rye was substituted for wheat. Feed consumption was decreased in all cases when barley, rye or oats was substituted for wheat and the depression was most severe at total (70%) replacement of wheat. The complete substitution of the test cereal (70%) for the wheat (0%) yielded weight gains, feed intakes, feed:gain values that were inferior to the control wheat diet (0%) and the diets containing 35% of the test grain. An exception was the feed:gain ratio of chicks fed the Bedford barley diet which was better than that obtained for the chicks fed the control wheat diet (1.46 versus 1.53 g:g).

Enzyme supplementation improved ($P<.05$) weight gain and feed:gain ratios of all chicks fed only one cereal (Table 17). The respective improvements in weight gain and feed:gain ratios for chicks fed the diets containing 70% of only one cereal were; 2.9% and 9.2% for wheat, 49% and 24.4% for Scout barley, 12.5% and 4.1% for Bedford barley, 29.6% and 20.6%

Table 17. Effect of enzyme supplementation and level of dietary inclusion of cereals in the diet on weight gain, feed consumption, and feed to gain ratios of chicks.

Dietary Cereal Inclusion Rate (%)	Enzyme	Barley				Rye		Oats	
		Scout		Bedford		Gazelle		Terra	
		-	+	-	+	-	+	-	+
Weight gain (g)									
0 ¹		139	143	139	143	139	143	139	143
35		138	138	138	143	105	135	137	143
70		100	149	120	135	81	105	100	146
SEM		3.5		4.2		3.5		3.6	
Source of variation		Probabilities							
Diet (D)		.0001		.006		.0001		.0001	
Enzyme (E)		.0001		.03		.0001		.0001	
D X E		.0001		.3		.002		.0001	
Feed Consumption (g)									
0		213	199	213	199	213	199	213	199
35		205	189	198	200	188	202	197	192
70		175	197	174	190	171	178	159	197
SEM		5.1		5.9		5.9		5.3	
Source of variation		Probabilities							
Diet (D)		.003		.0013		.0001		.0003	
Enzyme (E)		.5		.7		.6		.2	
D X E		.0013		.07		.08		.0004	
Feed to Gain Ratio (g/g)									
0		1.53	1.39	1.53	1.39	1.53	1.39	1.53	1.39
35		1.50	1.37	1.44	1.41	1.80	1.50	1.44	1.35
70		1.77	1.32	1.46	1.40	2.14	1.70	1.61	1.36
SEM		.03		.02		.05		.02	
Source of variation		Probabilities							
Diet (D)		.04		.5		.0001		.1	
Enzyme (E)		.0001		.02		.0001		.0002	
D X E		.002		.4		.07		.2	

1 Replacement for HY320 wheat, (0=70% wheat)

for rye, and 46% and 15.5% for oats. The interaction between diet and enzyme treatment ($P < .002$) for weight gain of chicks that consumed Scout barley, rye or oats indicates that the response to enzyme was greater for test cereals than for control wheat and Bedford barley. In the instance of feed intake, enzyme supplementation tended to increase feed intake of chicks fed the 70% cereal test diets but decreased intake of the wheat diet. With respect to feed:gain ratios, the diet by enzyme interaction was significant ($P < .05$) for Scout barley only.

The AME_n (Table 18), APD (Table 19), and ALD (Table 20) results reflect those trends seen in the growth data. Complete substitution of wheat by a unsupplemented test cereal resulted in significant depressions ($P < .01$) in dietary AME_n for all test cereals. Compared with the unsupplemented wheat control, the relative decreases in dietary AME_n were 28, 6, 32, and 24% for Scout and Bedford barley, Gazelle rye, and Terra oats, respectively. The depression in AME_n values with increasing dietary concentrations of test cereal was primarily linear for Scout and Bedford barley and Terra oats (95.5, 97.2, and 96.0% of the sum of squares, respectively). For the unsupplemented rye, the quadratic contrast was also significant ($P < .01$; 17.2% of the sum of squares). There was a 30% decrease in dietary AME_n when the content of rye in the diet was increased from 0 to 35% rye, whereas increasing rye to 70% of the diet only resulted in a further 2% reduction in dietary AME_n . Enzyme supplementation at the 70% inclusion rate, improved ($P < .05$) the dietary AME_n by 4.1, 42, 7, 14, and 33% for HY320 wheat, Scout and Bedford barley, Gazelle rye, and Terra oats, respectively. The response to enzyme for Bedford barley and Gazelle rye, was primarily linear ($P < .05$) as seen in the contrasts (83.7 and 96.2% of sum of squares, respectively).

Similar results were obtained for APD: increasing concentrations of unsupplemented test cereal resulted in significant ($P < .01$) and primarily linear decreases in dietary APD for all cereals with the exception of Bedford barley ($P > .05$) (Table 19). In the case of Scout barley and Terra oats, the quadratic contrasts were also significant ($P < .05$) (9 and 18% of

Table 18. Apparent metabolizable energy (AME_a) in young broiler chicks when fed enzyme supplemented diets containing different concentrations of Scout barley, Bedford barley, Gazelle rye and Terra oats.

Apparent Metabolizable Energy (N-corrected)									
Dietary Cereal Inclusion Rate	Enzyme	Barley				Rye		Oats	
		Scout		Bedford		Gazelle		Terra	
		-	+	-	+	-	+	-	+
(%)		(MJ/kg diet)							
0 ¹		14.14	14.72	14.14	14.72	14.14	14.72	14.14	14.72
35		13.24	14.86	13.86	14.66	9.84	13.48	12.91	14.94
70		10.23	14.49	13.29	14.16	9.62	10.96	10.78	14.32
SEM		.28		.10		.37		.25	
Source of variation		Probabilities							
Diet (D)		.0001		.0002		.0001		.0001	
Enzyme (E)		.0001		.0001		.0001		.0001	
D X E		.0001		.6		.0001		.0001	
Contrasts									
Linear (-E)		.0001 (95.8) ²		.003 (97.2)		.0001 (86.5)		.0001 (96.0)	
Quadratic (-E)		.1 (4.2)		.7 (1.3)		.0004 (17.2)		.06 (17.5)	
Linear (+E)		.2 (37.0)		.002 (83.7)		.0001 (96.2)		.014 (50.9)	
Quadratic (+E)		.1 (63.0)		.1 (16.3)		.1 (3.8)		.012 (54.9)	

¹ Replacement for HY320 wheat, (0=70% wheat)

² Percent of sum of squares.

Table 19. Apparent protein digestibility (APD) in young broiler chicks when fed enzyme supplemented diets containing different concentrations of Scout barley, Bedford barley, Gazelle rye and Terra oats.

Apparent Protein Digestibility (APD)									
Dietary Cereal Inclusion Rate	Enzyme	Barley				Rye		Oats	
		Scout		Bedford		Gazelle		Terra	
		-	+	-	+	-	+	-	+
(%)						(%)			
0 ¹		85.1	88.0	85.1	88.0	85.1	88.0	85.1	88.0
35		81.6	88.7	83.7	88.3	71.1	85.5	82.9	88.3
70		66.8	86.5	83.0	87.0	67.5	74.6	68.4	84.2
SEM		1.3		.4		1.4		1.2	
Source of variation ————— Probabilities —————									
Diet (D)		.0001		.1		.0001		.0001	
Enzyme (E)		.0001		.0001		.0001		.0001	
D X E		.0001		.5		.0009		.0001	
Contrasts									
Linear (-E)		.0001 (91.3) ²		.1 (97.1)		.0001 (96.8)		.0001 (82.5)	
Quadratic (-E)		.03 (8.7)		.7 (4.9)		.1 (5.3)		.002 (17.5)	
Linear (+E)		.07 (42.0)		.2 (54.2)		.0001 (88.6)		.0001 (71.2)	
Quadratic (+E)		.04 (58.0)		.2 (45.8)		.03 (11.4)		.002 (15.2)	

¹ Replacement for HY320 wheat, (0=70% wheat)

² Percent of sum of squares.

Table 20. Apparent lipid digestibility (ALD) in young broiler chicks when fed enzyme supplemented diets containing different concentrations of Scout barley, Bedford barley, Gazelle rye and Terra oats.

Apparent Lipid Digestibility (ALD)									
Dietary Cereal Inclusion Rate		Barley				Rye		Oats	
		Scout		Bedford		Gazelle		Terra	
	Enzyme	-	+	-	+	-	+	-	+
(%)		(%)							
0 ¹		76.1	79.3	76.1	79.3	76.1	79.3	76.1	79.3
35		63.6	81.1	73.4	82.7	32.0	68.2	52.0	75.1
70		43.2	79.9	75.2	85.0	24.9	48.3	17.4	51.0
SEM		.02		.10		.02		.02	
Source of variation		Probabilities							
Diet (D)		.0001		.3		.0001		.0001	
Enzyme (E)		.0001		.0001		.0001		.0001	
D X E		.0001		.1		.0001		.0001	
Contrasts									
Linear (-E)		.0001 (97.9) ²		.8 (12.0)		.0001 (88.8)		.0001 (98.8)	
Quadratic (-E)		.4 (2.1)		.4 (91.3)		.0001 (14.6)		.2 (1.2)	
Linear (+E)		.8 (9.5)		.001 (99.0)		.0001 (97.4)		.0001 (88.5)	
Quadratic (+E)		.4 (90.5)		.7 (1.0)		.3 (2.6)		.002 (15.2)	

¹ Replacement for HY320 wheat, (0=70% wheat)

² Percent of sum of squares.

sum of squares, respectively). In these cereals, a small depression in APD was observed when the level of inclusion of the test cereal was increased from 0 to 35% of the diet while further increasing the dietary inclusion rate to 70% resulted in a major depression in APD. Enzyme supplementation improved APD in the 70% test diets by 3.4, 29, 5, 11, and 23%, respectively, for HY320 wheat, Scout and Bedford barley, Gazelle rye, and Terra oats. Enzyme supplementation of the test cereals, increased APD of all diets with the exception of those containing rye to a level near or equal to that seen in the unsupplemented wheat control diet. Diets containing rye did not respond to enzyme supplementation to the same extent as the other cereals examined in the present study.

Dietary inclusion level did not affect the ALD of Bedford barley ($P > .05$); however the ALD of the other test cereals were affected negatively ($P < .05$) by increasing inclusion rates (Table 20). The substitution of the test cereal at the 70% inclusion level for the control wheat decreased ALD by 43, 67, and 77%, respectively, for Scout barley, Gazelle rye, and Terra oats. The quadratic contrast was significant ($P < .01$) for Gazelle rye only as the majority of the ALD depression occurred when rye was increased from 35 to 70% of the diet. The enzyme effect on ALD was highly significant ($P < .01$) for all four test cereals. The ALD of the 70% test diets was increased by 4.2, 85, 13, 94, and 193% for HY320 wheat, Scout and Bedford barley, Gazelle rye, and Terra oats, respectively. For Scout and Bedford barley, enzyme supplementation increased the dietary ALD to a level greater or equal to that seen for the unsupplemented control wheat diet.

DISCUSSION

With the exception of Bedford barley, increasing dietary concentrations of test grain resulted in depressions in growth performance and dietary nutritive values (AME_n , APD, and ALD) for all grains studied.

Observations of this nature have been made previously by other researchers studying rye (Moran *et al.* 1970; Misir and Marquardt 1978a; Boros *et al.* 1985), hulless barley (Classen *et al.* 1985) and naked oats (Cave, 1984). In the present studies, enzyme supplementation had a significant ($P < .05$) effect on cereal grain nutritive value and subsequent chick growth performance which further supports previous literature findings (Broz and Frigg, 1986a,b; Hesselman and Aman, 1986; Campbell *et al.*, 1989; Edney *et al.*, 1989; Grootwassink *et al.*, 1989; Rotter *et al.*, 1989c).

The relative effect of enzyme varied widely between the four test cereals. The differential effects of enzyme supplementation on Scout (hulless) and Bedford (hulled) barley are in agreement with previous studies. Rotter *et al.* (1989c) showed that the magnitude of response to enzyme supplementation is greater for high viscosity barleys such as Scout than for low viscosity barleys such as Bedford. In their study, enzyme supplementation resulted in weight gain and feed:gain ratio improvements of 2.0 and 2.7%, respectively, for Bedford barley and 30 and 12.7%, respectively, for Scout barley. Leong *et al.* (1962) observed 23.8 and 14.5% increases in the ME of pearled and regular barley, respectively, when supplemented with a crude fungal enzyme preparation. The differential response of pearled and regular barley to enzyme occurs because pearling results in grain where the inner portion (endosperm) of the seed contributes a larger percentage of the seed weight. The data of Herstad and McNab (1975) showed that supplementation of a barley-based diet with a bacterial α -amylase resulted in a 6.5% increase in the AME_n of one barley variety while another showed no response. The lack of AME_n response to enzyme supplementation in that study may be partially attributed to the fact that these barley cultivars were grown under high moisture conditions in the U.K. and thus may have contained relatively lower β -glucan levels than Western Canadian barleys or the enzyme preparation may not have contained an active β -glucanase. Previously, Willingham *et al.* (1959) observed that the response to a fungal amylase supplementation varied widely between different barley cultivars.

Previous studies have shown the effects of enzyme supplementation on the AME_n of rye- (Moran et al., 1969; Broz, 1987a,b) and barley-based diets (Broz and Frigg, 1986a,b). Improvements in AME_n in the enzyme supplemented diets followed trends seen in the ALD and APD values.

The APD and ALD were affected greatly by the cultivar and dietary inclusion rate of test cereal grain. The significant quadratic contrast ($P < .05$) for Scout barley and Terra oats occurred because the major portion of the APD depression occurred between the 35 and 70% inclusion levels (Table 5). Perhaps, at the 35% level, the oats or hulless barley did not contribute sufficient amounts of β -glucan to substantially increase digesta viscosity and consequently depress nutrient digestion and absorption. In an early study, Burnett (1966) suggested that a decrease in digesta viscosity mediated by enzyme supplementation would create an environment more conducive to optimum digestion of crude protein and carbohydrates. Subsequent studies have reported improvements in N retention when diets were supplemented with cellulolytic enzyme preparations (Broz, 1987a; Scholtyssek and Knorr, 1987).

Enzyme supplementation of diets containing Gazelle rye, improved APD by 20% in the 35% diet but only by 11% in the 70% rye diet. This observation was not unexpected. There are several possible reasons for this. The growth performance results showed that birds consuming enzyme supplemented 70% rye diet can not increase feed consumption, suggesting a physical limitation. As demonstrated by Bedford et al. (1991), it is possible that the viscous pentosans accumulate in the gut and the hydrolytic capacity of the enzyme becomes limiting. The fact that the enzyme is still partially effective was reflected in an improved feed:gain ratio for the 70% rye diet (2.14 versus 1.70 g:g), but the feed utilization is much worse than for the other test cereals. The level of enzyme used in this study (.04 g/kg diet) may be adequate for the barley and oat but not rye diets, particularly at high concentrations of rye.

These results would suggest that the response to enzyme of Scout barley and oats which both contain a high concentration of a water-soluble

and highly viscous β -glucans (Hesselman and Aman, 1985) is different from that of rye which contains high concentration of a water-soluble and highly viscous pentosan (Antoniou et al., 1981). The crude enzyme preparation used in this study contains both β -glucanase- and pentosanase-like activities which presumably hydrolyse the respective substrate and thereby reduce the viscosity of these compounds. It is not surprising therefore, that the response to enzyme treatment of Scout barley and oats are similar and that a somewhat different response was obtained with rye grain as the specificity and activities of the two types of enzymes may be very different. An enzyme with xylanase or pentosanase activity is required for rye and wheat and a β -glucanase or cellulase for oats and barley. Previous studies have shown the viscosity of the water extract of rye was reduced dramatically by Cellulase Tv; the same enzyme preparation used in the present studies (Fengler et al., 1988). However, Antoniou and Marquardt (1981) found that although both the water soluble and insoluble pentosans depress growth, they suggested insoluble pentosans are the major antinutritive factor because of their higher concentration in rye grain. It is possible that the enzyme preparation used for rye containing diets has to be more target specific for high pentosanase activity or added at much higher concentrations than in the present study.

The highly significant ($P < .01$) diet by enzyme interaction for APD for Scout barley, Terra oats, and Gazelle rye was caused by the much greater response to enzyme treatment in birds fed the high concentration (70%) of test grain compared to those fed the lower concentration (0 and 35%). This interaction however was not significant ($P > .05$) for Bedford barley, presumably because of its lower content of the highly viscous water-soluble β -glucans (Rotter et al., 1989b).

Apparent lipid digestibilities showed similar trends to that seen with APD except that the inclusion level effects were more pronounced (Table 6). Similar depressions in fat absorption have been observed in earlier studies with rye (Antoniou et al., 1981) and hullless barley (Classen et al., 1985). Enzyme supplementation completely eliminated the

depression of ALD seen in the unsupplemented Scout barley diets. Classen et al. (1985) observed an 18.1% increase ($P < .05$) in fat absorption when a Scout barley diet was supplemented with a cellulase preparation isolated from *T. viride*. Similar to the present studies, Edney et al. (1989) observed the differential effect of a β -glucanase supplement on fat absorption of several cereal grains. Enzyme supplementation significantly ($P < .01$) increased fat absorption in diets containing hulless (Scout) barley and oat groats but not hulled barley. The ALD of enzyme supplemented Bedford barley diets increased as the level of barley increased from 0 to 70% (Table 4). The reason for this is not known, as barley and wheat have similar ether extract values and fatty acid compositions (NRC, 1984).

The positive effect of enzyme supplementation on the wheat control diet in terms of AME_n , APD and ALD is of some interest. Creek et al. (1962) suggested the presence of a thermolabile growth inhibitor in raw wheat germ that depresses fat and protein utilization. Mollah et al. (1983) found the AME_n and starch digestion of low ME Australian wheats to be significantly correlated ($r = .91$, $P < .01$) and most variable in birds fed wheat diets compared to rice and sorghum. Thus, enzyme supplementation in the present studies may have increased the wheat AME_n through enhanced starch digestion and reduced variability among birds. However, Rogel et al. (1987) found no evidence to suggest that viscous polysaccharides in wheat were inhibiting its digestion. Fengler et al. (1988) previously also demonstrated that enzyme treatment of wheat-based diets produced beneficial responses and thus, depending on relative costs and benefits, it may be advantageous to supplement wheat-based broiler starter diets with an enzyme preparation.

In summary, AME_n , APD, and ALD values of diets containing Bedford barley, Scout barley, Terra oats, and Gazelle rye were significantly affected by level of inclusion and enzyme supplementation. The present data suggested that diets containing 70% enzyme supplemented hulled barley, hulless barley, or naked oats can support chick growth performance

that is equal or superior to a conventional wheat-based diet. The enzyme supplement may have increased the AME_n, APD, and ALD by decreasing digesta viscosity and thereby enhancing nutrient digestion and absorption.

MANUSCRIPT III: THE EFFECTS OF ENZYME SUPPLEMENTATION
ON THE NUTRITIVE VALUE OF RYE GRAIN (*Secale cereale*)
FOR THE YOUNG BROILER CHICK

ABSTRACT

Two dose-response experiments utilizing differing enzyme concentrations were carried out to determine the level of a crude enzyme preparation (*Trichoderma viride* cellulase) required to obtain maximum growth performance in young broiler chicks fed a 60% rye diet. Enzyme concentrations utilized in these studies ranged from 0.05 - 12.8 g enzyme/kg diet. Enzyme supplementation at the 3.2 g/kg diet level yielded increases in feed consumption and weight gain of 71 and 193%, respectively, and the feed to gain ratio was improved by 43% compared with that for the unsupplemented rye control diet. As no further growth response was obtained by increasing the enzyme concentration, the enzyme level utilized in a subsequent study was 3.2 g/kg diet. A balance study using 2-wk-old broiler chicks was carried out to determine the effect of enzyme supplementation on the bioavailable energy (AME_n) and apparent protein digestibility (APD) of a 60% wheat diet replaced by rye in 10% increments. In the 60% rye diets, the enzyme addition increased the dietary AME_n and APD by 23 and 12%, respectively, compared to the unsupplemented diet. The AME_n and APD of the 60% wheat control diet were increased 9.7 and 4.6%, respectively, by enzyme addition. The data suggest that the AME_n of rye grain is increased by enzyme supplementation, however alternate methodology will have to be employed to quantitatively determine the extent of these improvements.

KEY WORDS: rye, pentosans, enzyme supplementation, apparent metabolizable energy, protein digestibility

INTRODUCTION

The growth-depressing effects of rye grain (*Secale cereale*) on broiler chickens are well documented. Halpin et al. (1936) observed that birds fed a 30% rye diet to 24 wks of age weighed 75% as much as birds fed a 30% corn diet and exhibited sticky feces if the diet contained more than 15% rye. Subsequent studies have demonstrated that bird growth performance is compromised by increasing the dietary rye component of the diet.

It is generally believed that the antinutritive factors in rye are the highly viscous pentosans (Antoniou and Marquardt, 1981; Ward and Marquardt, 1987). Fernandez et al. (1973) concluded that the water extract of rye contained the antinutritive factor because birds fed water-extracted rye exhibited similar growth rates and feed conversion efficiency to corn-fed birds. The data of Fengler and Marquardt (1988b) demonstrated that the addition of 1.3% water-soluble pentosans to a wheat-based diet had the same growth depressing effect as a 56% rye diet in terms of chick performance, and concluded that the pentosans must be the major antinutritive factor in rye. Antoniou and Marquardt (1981) supplemented wheat-based diets with levels of water-soluble (1.4%) and insoluble (3.4%) pentosans similar to those found in a rye-based diet and found that the water-insoluble pentosans inhibited growth to a greater degree than the water-soluble pentosans. These authors concluded that although both classes of pentosans depress growth, the insoluble pentosans are the major antinutritive factor due to their higher concentration in rye.

Enzyme supplementation decreases viscosity of intestinal contents and improves nutrient digestibility and absorption in broiler chickens fed diets containing rye (Patel et al., 1980; GrootWassink et al., 1989). Fengler and Marquardt (1988a) showed that the addition of *Trichoderma viride* cellulase to either a pentosan solution or a water extract of rye decreased the viscosity of the solution to that of water. The addition of

Trichoderma viride cellulase to rye-based broiler diets resulted in increased fat and dry matter retention and decreased excreta viscosity (Fengler et al., 1988).

Although the effect of enzyme supplementation on growth has been extensively studied, there are very few reports in the literature demonstrating the effects of enzyme supplementation on the bioavailable energy of rye. In early studies, Moran et al. (1969) reported that the ME of Western and Tetrapetkus rye grain was increased 6.4 and 2.9%, respectively, when supplemented with a crude fermentation product of *Aspergillus* sp. origin. Later studies have also exemplified the increases in bioavailable energy seen when rye-containing diets are supplemented with a pectinase (*Aspergillus niger*) or cellulase complex (*Trichoderma viride*) (Broz, 1987b), or a cellulolytic enzyme preparation (Scholtyssek and Knorr, 1987). Recently, Pettersson and Aman (1989) reported that the ME of a 30.5% rye diet was increased significantly ($P < 0.05$) by supplementation with a pentosanase preparation with concurrent improvements ($P < 0.05$) in the ileal digestibilities of organic matter, crude protein and starch. The objectives of the present research were to: 1). determine the enzyme concentration required to obtain optimum growth performance in young broiler chicks; 2). determine the effect of enzyme supplementation on the bioavailable energy of diets containing varied levels of rye grain.

MATERIALS AND METHODS

Enzyme Dose Response Studies

Two preliminary enzyme dose response studies were carried out to determine the level of crude enzyme preparation required to obtain a maximum growth response in chicks fed a 60% rye diet. The enzyme supplement used in these studies was a crude fungal cellulase preparation (dry form) from *Trichoderma viride* (Tv Cellulase)¹. Its activity as

¹ Miles Laboratories Inc., Elkhart, IN 46515.

determined by the manufacturer was 23,880 cellulase units per gram. In Experiment 4, 450 1-day-old vaccinated (Marek's) male, Arbor Acres, broiler chicks obtained from a local commercial hatchery were individually weighed and placed into narrow weight classes. Birds of relatively low or high body weights were discarded. Four birds were then assigned to each of 70 pens such that all pens had similar initial weights. The birds had 24 h access to feed, water and light, and were housed in Petersime chick batteries² equipped with raised wire floors. The birds were fed the experimental diets from Day 1 to 14. The basal diet was formulated to meet or exceed National Research Council (NRC, 1984) nutrient requirements (Table 21). Diets were supplemented with enzyme at seven levels: 0.0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 g/kg diet. Each treatment was randomly assigned to 10 pens. Pen weights were determined on Days 1, 7 and 14. Prior to the 7- and 14-day weighings, the birds were starved for 4 h to ensure a consistent gut fill between all birds. Feed consumption was recorded for Weeks 1 and 2 in order to calculate weekly and overall feed to gain ratios.

The results of Experiment 4 showed a significant growth response between the .8 and 1.6 g/kg enzyme supplementation, therefore a second dose-response study (Experiment 5) was carried out using higher concentrations of enzyme: 0.0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 g/kg diet. Each diet was fed to 40 birds in 10 replicates (pens) of 4 birds per pen. Experimental procedures and the basal diet were identical to those employed in Experiment 4 with a few exceptions. In Experiment 5, the birds were housed in electrically heated Jamesway chick batteries³ and fed a commercial chick starter crumblized diet containing 20% crude protein for Days 1 to 3. On Day 3, birds were individually weighed and assigned to pens as described in Experiment 4. Experimental diets were fed from Days 3 to 14. Pen weights were determined at 3, 10 and 14 days of age. Feed consumption was recorded from 3 to 10 and from 11 to 14 days.

2 Petersime Incubator Co., Gettysburg, OH 45328.

Table 21. Composition and calculated analysis of experimental diets (Experiments 4 and 5).

Ingredients	Amount (g/kg)
Rye grain ¹	600.0
Soybean meal ²	260.0
Tallow	50.0
Corn oil	15.0
Vitamin premix ³	25.0
Mineral premix ⁴	50.0
Total	1000.0
Calculated composition	
Metabolizable energy (kcal/kg)	2796
Crude protein (%)	22.44
(Determined, Nx6.25,%)	(22.16)
Lysine (%)	1.22
Methionine (%)	0.63
Calcium (%)	1.00
Available phosphorus (%)	0.41

¹ Spring variety (CV Gazelle), 15.50% crude protein (determined).

² 48.2% crude protein (determined).

³ Vitamin premix provided per kg of diet: vit. A, 15000 IU; vit. D, 2000 IU; vit. E, 100 IU; menadione, 4.0 mg; riboflavin, 3.7 mg; Ca pantothenate, 20 mg; niacin, 23 mg; vit. B₁₂, 14 µg; choline chloride, 1.7 g; biotin, 105 g; pyridoxine, 1.6 mg.

⁴ Mineral premix provided per kg of diet: ground limestone, 14.8 g; Ca biphosphate, 15.5 g; salt (iodized), 2.5; MnO, 23.4 mg; ZnO, 13.5 mg; FeSO₄·H₂O, 134 mg; lysine, 1.2 g; d,l-methionine, 3.4 g.

Rye Grain AME_n Study

Four hundred and fifty 1-day-old vaccinated (Marek's) male, Arbor Acres broiler chicks were obtained from a commercial hatchery. From Days 1 to 7, the birds were housed in Jamesway chick batteries³ and fed a commercial chick starter crumbled diet containing 20% crude protein. On Day 7, birds were individually weighed and placed into narrow weight classes as described above. Groups of four birds were then randomly assigned to each of 84 pens in Petersime batteries such that all pens had a similar initial weight. From Day 7 to 17, the birds were fed the experimental diets that were in a mash form and contained .3% chromic oxide as an analytical marker. Each treatment contained six replicates (pens) with four birds per replicate. Birds had 24 h access to light, feed and water throughout the experiment. Pen weights were recorded on Days 7 and 14. Prior to each weighing, the birds were starved for 4 h to ensure consistent gut fill between all birds. Feed consumption was recorded during Day 7 through 14 so that a feed to gain ratio could be calculated for each replicate (pen) of birds. Excreta were collected on plastic lined trays from Day 14 to 17. Excreta samples were lyophilized, equilibrated with atmospheric conditions for 24 h and then finely ground in a small coffee grinder prior to laboratory analysis. Diet samples were also finely ground prior to laboratory analysis.

The AME_n chick bioassay utilized a practical control diet containing 60% wheat (Cultivar HY320, 14.2% crude protein, N x 6.25) as a reference material. The test diets substituted rye (Cultivar Gazelle, 13.7% crude protein, N x 6.25) at 10% increments for wheat up to 100% substitution (Table 22). The remaining 40% of the diet, termed the PE (protein-energy) supplement, consisted of a protein source (soybean meal), energy source (tallow and corn oil) and vitamin and mineral premixes (Table 22). The PE supplement was formulated such that diet nutrient levels met or exceeded NRC (1984) requirements for all combinations of wheat and rye used. All

³ Jamesway Manufacturing Co., Ft. Atkinson, WI 53538.

Table 22. Composition and calculated analysis of experimental diets (Experiment 6).

Ingredients	Diets (g/kg) ¹						
	1	2	3	4	5	6	7
Wheat ²	600	500	400	300	200	100	-
Rye ³	-	100	200	300	400	500	600
Other ⁴	400	400	400	400	400	400	400
Calculated composition							
ME (kcal/kg)	2879	2862	2845	2827	2810	2792	2775
CP (%)	21.0	21.0	20.9	20.9	20.8	20.8	20.7
(Det., Nx6.25%)	(19.8)	(19.8)	(20.0)	(20.6)	(20.5)	(20.3)	(21.0)
Lysine (%)	1.22	1.22	1.22	1.22	1.23	1.23	1.23
Methionine (%)	0.64	0.63	0.63	0.63	0.63	0.63	0.62
Calcium (%)	1.00	1.00	1.01	1.01	1.01	1.01	1.01
Available phosphorus (%)	0.47	0.47	0.47	0.47	0.46	0.46	0.46

¹ Diets 8 through 14 were respectively Diets 1 through 7 supplemented with crude enzyme preparation (Cellulase Tv concentrate, Miles Laboratories Inc., Elkhart, IN 46515).

² Semi-dwarf variety (CV HY320), 14.22% crude protein (determined).

³ Spring variety (CV Gazelle), 13.72% crude protein (determined).

⁴ Other ingredients provided per kg of diet: soybean meal (44.53% crude protein; determined), 270 g; tallow, 54.5 g; corn oil, 5.5 g; vitamin premix, 25 g; mineral premix, 45 g.

The vitamin mix provided per kg of diet: vit. A, 15000 IU; vit. D, 2000 IU; vit. E, 100 IU; menadione, 5 mg; riboflavin, 3.7 mg; Ca pantothenate, 6.2 mg; niacin, 23 mg; vit. B₁₂, 14 µg; choline chloride, 1.6 g; biotin, 103 g; pyridoxine, 1.6 mg; lysine, 1.0 g; d,l-methionine, 3.2 g. The mineral mix provided per kg diet: ground limestone, 13.5 g; Ca biphosphate, 18.0 g; salt (iodized), 2.5 g; MnO, 49 mg; ZnO, 12.4 mg; FeSO₄·H₂O, 150 mg; CuSO₄, 1.4 mg; Na selenite, 15 mg; Cr oxide, 3 g.

diets including the basal wheat diet were fed without and with enzyme supplement (3.2 g/kg diet).

Chemical Analyses

Nitrogen content of feed (Experiments 4, 5 and 6) and excreta samples (Experiment 6) was determined using the Kjeldahl procedure (Procedure 7.021) as described in Association of Official Analytical Chemists (1984). For the AME_n study, feed and excreta samples were analyzed for gross energy using a Parr adiabatic oxygen bomb calorimeter equipped with a Parr 1720 Automatic Controller⁴. Chromic oxide content was analyzed according to Williams et al. (1962). In addition, excreta samples were analyzed for uric acid content (Marquardt, 1983) and the apparent protein digestibility was calculated as outlined by Rotter et al. (1989a).

Calculations and Statistical Analyses

The dietary AME_n values were calculated as described by Hill et al. (1960) with a correction for N retention using a value of 36.5 kJ (8.73 kcal) per gram of N retained.

Data were analyzed using the General Linear Model (GLM) procedures of Statistical Analysis Systems (SAS, 1984). One-way analysis of variance (enzyme level) and comparison of least square means was performed on the data from Experiments 4 and 5. In Experiment 5, a nonlinear regression line was fitted to the data to determine the level of enzyme required to obtain a maximum growth response. Experiment 6 used a completely randomized design with a 2 x 7 (enzyme x rye level) factorial arrangement of treatments. In Experiment 6, orthogonal contrasts were used to compare means (Snedecor & Cochran, 1980).

4 Parr Instrument Co., Moline, IL 61265.

RESULTS AND DISCUSSION

Since the results of Weeks 1 and 2 for Experiment 4 showed similar trends, only the overall data are presented (Table 23). Enzyme supplementation significantly improved ($P<.01$) chick growth performance with respect to weight gain, feed consumption and feed to gain ratio when compared with the nonsupplemented rye control. The lowest enzyme concentration (0.05 g/kg diet) improved feed consumption and weight gain 43 and 120%, respectively, while the feed to gain ratio was improved by 35% compared to the unsupplemented control. Increasing enzyme concentrations yielded further improvements in all performance parameters recorded. The highest enzyme concentration (1.6 g/kg diet) resulted in 86, 256, and 48% improvements in feed consumption, weight gain, and feed to gain ratio, respectively. However there was a significant improvement in weight gain ($P<.05$) when enzyme concentration was increased from .8 to 1.6 g/kg diet, the maximal effect of enzyme may not have been achieved at the 1.6 g/kg diet supplementation. These results are in contrast to those of Fengler *et al.* (1988), who found that increasing Tv cellulase levels from .01 to 1.0 g/kg diet in a 59% rye diet did not affect nutrient (dry matter and fat) retentions ($P<.05$). Previous studies have demonstrated that relative fat retention values are a good measure of the nutritional value of rye-based diets (Marquardt *et al.*, 1979; Antoniou *et al.*, 1981; Antoniou and Marquardt, 1982b). The reason for this was not established, however Fengler *et al.* (1988) utilized Single Comb White Leghorn chicks which have a much lower growth potential than the commercial broiler chicks used in the present studies.

The results of Experiment 5 are given in Table 24. A comparison of the least square means showed that maximum growth performance was probably reached at 3.2 g enzyme/kg diet as there were no significant improvements obtained by further increasing the enzyme concentration up to 12.8 g/kg diet. Over the entire experiment, enzyme supplementation (3.2 g/kg diet) increased feed consumption and weight gain by 71 and 193%, respectively,

Table 23. Effect of level of enzyme supplementation on feed consumption, weight gain and feed to gain ratio of broiler chicks fed a 60% rye diet (Experiment 1).

Enzyme level	Feed Consumption		Weight Gain		Feed to Gain Ratio	
(g/kg diet)	(g)		(g)		(g/g)	
0.0	132a	(100) ¹	39a	(100)	3.38a	(100)
0.05	189bc	(143)	86b	(220)	2.19bc	(65)
0.1	179b	(136)	85b	(216)	2.14bc	(63)
0.2	201bcd	(153)	99bc	(251)	2.05bc	(61)
0.4	213cd	(162)	109c	(278)	1.96bcd	(58)
0.8	220de	(167)	117c	(298)	1.90cd	(56)
1.6	245e	(186)	140d	(356)	1.76d	(52)
SEM	6.2		4.4		0.06	

Source of variation (probabilities)

Enzyme	0.0001	0.0001	0.0001
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a-e Means within a column with different letters are different ($P < .05$).

¹ Relative values where the unsupplemented rye is assigned a value of 100.

Table 24. Effect of level of enzyme supplementation on feed consumption, weight gain and feed to gain ratio of broiler chicks fed a 60% rye diet (Experiment 5).

Enzyme level	Feed Consumption		Weight Gain		Feed to Gain Ratio	
(g/kg diet)	(g)		(g)		(g/g)	
0.0	175a	(100) ¹	57a	(100)	3.11a	(100)
0.1	235b	(134)	107b	(187)	2.19b	(70)
0.2	250bc	(142)	119b	(207)	2.11bc	(68)
0.4	237b	(135)	113b	(197)	2.11bc	(68)
0.8	262bcd	(149)	134c	(234)	1.96bcd	(63)
1.6	285cde	(162)	151cd	(262)	1.91cd	(61)
3.2	299de	(171)	168de	(293)	1.78d	(57)
6.4	299de	(171)	170de	(296)	1.77d	(57)
12.8	309e	(176)	172e	(300)	1.80d	(58)
SEM	8.1		5.7		0.05	

Source of variation (probabilities)

Enzyme	0.0001	0.0001	0.0001
--------	--------	--------	--------

a-e Means within a column with different letters are different ($P < .05$).

¹ Relative values where the unsupplemented rye is assigned a value of 100.

while the feed to gain ratio improved 43% compared to the unsupplemented rye control diet.

A nonlinear regression line using overall values was fitted to the weight gain and feed consumption data to determine the enzyme concentration required to obtain 95% of the maximum growth response. These values were 1.89 and 2.51 g/kg diet, respectively, for weight gain and feed consumption (Table 25). Therefore, to allow for a margin of error, the enzyme concentration used in Experiment 6 was 3.2 g/kg diet.

The chick growth data for the AME_a study are shown in Table 26. The main effects for enzyme and rye concentration, as well as their interaction were highly significant ($P < .01$) for all growth parameters. Feed consumption and weight gain of birds fed the unsupplemented diets mainly decreased in a linear manner (98.7 and 98.9% of sum of squares, respectively) with a concurrent linear increase in the feed to gain ratio (95.4% of sum of squares) as the dietary level of rye increased from 0 to 60%.

The level of rye (CV Gazelle) at all concentrations adversely affected growth and feed conversion efficiency (Table 26). Similar depressions in growth performance have been observed in other studies when increasing increments of rye were substituted for wheat (Patel and McGinnis, 1976) or corn (Moran et al., 1970). Moran et al. (1970) found that dietary rye levels of less than 25% did not adversely affect broiler growth performance. Although the enzyme effect was large ($P < .01$), increasing the concentration of enzyme-supplemented rye to 60% still resulted in depressions of all growth parameters recorded compared to the results obtained with wheat. Patel et al. (1980) demonstrated that supplementation of a rye-based diet with pectic enzyme improved weight gain and feed conversion efficiency by 31 and 14%, respectively. However, this level of bird performance was still substantially lower than that seen in corn-fed birds. In the present study, substitution of enzyme supplemented rye for unsupplemented wheat decreased feed consumption and

Table 25. Parameter values for the nonlinear regression of weight gain and feed consumption ratio data (Experiment 2).

	Stage	A	Parameter B	C	Enz. level required ¹
Weight Gain ²	1	62.683 (1.405) ³	0.566 (0.041)	2.982 (0.587)	0.57
	2	103.729 (2.824)	0.526 (0.034)	1.216 (0.259)	1.88
	1 + 2	167.608 (3.977)	0.526 (0.032)	1.468 (0.286)	1.89
Feed Consumption	1	124.369 (1.756)	0.344 (0.031)	5.436 (1.177)	0.53
	2	173.120 (3.787)	0.353 (0.029)	1.180 (0.320)	2.71
	1 + 2	299.987 (5.408)	0.327 (0.026)	1.523 (0.366)	2.51

¹ Enzyme concentration (g/kg diet) required to achieve 95 percent of maximum performance calculated using the non-linear regression parameters.

² The non-linear regression equation used for weight gain and feed consumption is: $Y = A + B(1 - e(-C * X))$ where Y = weight gain or feed consumption; A, B and C = non-linear regression parameters and X = enzyme level (g/kg diet).

³ Standard error.

Table 26. Effect of enzyme supplementation and level of dietary inclusion on feed consumption, weight gain and feed to gain ratio of broiler chicks fed diets containing different proportions of rye and wheat (Experiment 6).

Rye Level	Feed Consumption		Weight Gain		Feed to Gain Ratio		
	Enzyme	-	+	-	+	-	+
(%)		(g)		(g)		(g/g)	
0 ¹		236	233	128	139	1.86	1.67
10		229	229	120	136	1.90	1.69
20		199	233	96	127	2.08	1.84
30		192	223	85	124	2.26	1.80
40		173	228	66	129	2.64	1.77
50		158	225	57	125	2.76	1.80
60		138	213	45	112	3.13	1.91
SEM		5.6		3.8		0.06	

Source of variation (probabilities)

Diet (D)	0.0001	0.0001	0.0001
Enzyme (E)	0.0001	0.0001	0.0001
D X E	0.0001	0.0001	0.0001

Contrasts

Linear (-E)	0.0001 (98.7) ²	0.0001 (98.9)	0.0001 (95.4)
Quadratic (-E)	0.8 (0.0)	0.3 (.2)	0.015 (2.7)
Cubic (-E)	0.9 (0.0)	0.2 (.3)	0.4 (0.2)
Linear (+E)	0.03 (66.5)	0.0001 (76.8)	0.0001 (65.1)
Quadratic (+E)	0.4 (8.9)	0.8 (0.2)	0.7 (0.3)
Cubic (+E)	0.5 (6.5)	0.07 (12.2)	0.03 (14.2)

¹ 60% wheat.

² Percent of sum of squares.

weight gain values by 10 and 12%, respectively, and the feed to gain ratio increased 3%.

Numerous studies have demonstrated the improvements in chick growth performance when rye based diets are supplemented with pectinase (Patel et al., 1980; Broz, 1987b), cellulase (Broz, 1987a; Scholtyssek and Knorr, 1987) and pentosanase (Pettersson and Aman, 1988; 1989; GrootWassink et al., 1989) enzyme preparations. In the present study, enzyme supplementation of the 60% rye diet resulted in a 39% improvement in the feed to gain ratio. Feed consumption and weight gain increased 54 and 148%, respectively. The improvements in growth performance seen with enzyme supplementation may be attributed to improved digestion and absorption of N (Scholtyssek and Knorr, 1987; Broz, 1987b; Pettersson and Aman, 1989), dry matter (Fengler et al., 1988), fat (Fengler et al., 1988; Pettersson and Aman, 1989) and N-free extract (Scholtyssek and Knorr, 1987). Enzyme supplementation also appeared to affect the wheat-based diet with improvements in weight gain and feed to gain ratio being 8.6 and 11.3%, respectively. Similar results have been reported previously (Pawlik et al., 1990).

The results of the dietary AME_n and apparent protein digestibility (APD) data are shown in Table 27. The main effects of enzyme supplementation and rye level were highly significant ($P < .01$) for both the AME_n and APD. For both unsupplemented and enzyme-supplemented diets, the AME_n and APD values decreased as the dietary level of rye increased, however this depression was much more pronounced for the unsupplemented diets. These results suggest that although the enzyme supplement has a positive effect in terms of nutrient utilization, the antinutritive effects of rye are not entirely overcome by the addition of the enzyme preparation used in the present studies. It is also possible that pentosans are not the only antinutritive factor in rye. The enzyme by rye level interaction approached significance ($P = .09$) for AME_n and was significant ($P < .05$) for APD as the enzyme effect increased with increasing dietary concentrations of rye. At the 60% rye level, enzyme

Table 27. Effect of enzyme supplementation and level of dietary inclusion on the apparent metabolizable energy (AME_n) and apparent protein digestibility (APD) of diets containing different proportions of rye and wheat fed to young broiler chicks (Exp. 3).

Rye Level	Enzyme	AME _n		APD	
		-	+	-	+
<hr/>					
(%)		(MJ/kg DM)		(%)	
0 ¹		12.41	13.61	82.4	86.2
10		12.03	13.28	81.8	85.5
20		11.69	12.62	79.4	85.5
30		11.61	12.49	79.3	84.6
40		10.59	12.23	76.1	83.1
50		10.34	12.07	72.8	83.1
60		9.86	12.16	73.3	82.2
SEM		0.25		1.0	
Source of variation (probabilities)					
	Diet (D)	0.0001		0.0001	
	Enzyme (E)	0.0001		0.0001	
	D X E	0.09		0.013	
Contrasts					
	Linear (-E)	0.0001 (96.2) ²		0.0001 (93.5)	
	Quadratic (-E)	0.48 (0.8)		0.60 (0.4)	
	Linear (+E)	0.0001 (87.9)		0.0002 (92.9)	
	Quadratic (+E)	0.03 (11.3)		0.78 (0.4)	

¹ 60% wheat.

² Percent of sum of squares.

supplementation increased the dietary AME_n and APD values by 23 and 12%, respectively, compared to the unsupplemented diet whereas the corresponding improvements in the 60% wheat control diet were 10 and 5%, respectively.

In conclusion, enzyme supplementation has a significant ($P < .05$) effect on the AME_n and APD values of rye-based diets. These changes are accompanied by concurrent improvements ($P < .05$) in chick growth performance as evidenced by weight gain, feed consumption and feed to gain ratio values.

GENERAL DISCUSSION

The present data demonstrate the significant positive effects of dietary enzyme supplementation (Tv Cellulase) on the growth performance (body weight gain and feed conversion efficiency) of young broiler chicks fed wheat-, barley- (hulled and hulless), oats- and rye-based diets. Observations of this nature have been reported previously (Willingham *et al.*, 1958a; Herstad and McNab, 1975; Patel *et al.*, 1980; Hesselman *et al.*, 1982; White *et al.*, 1983; Broz and Frigg, 1986a,b; Hesselman and Aman, 1986; Broz, 1987a,b; Scholtyssek and Knorr, 1987; Pettersson and Aman, 1988; Campbell *et al.*, 1989; Edney *et al.*, 1989; Grootwassink *et al.*, 1989; Rotter *et al.*, 1989c). The enhanced growth performance was accompanied by similar improvements in dietary nutrient utilization (AME_n, APD and ALD). The substantial improvements in AME_n, APD, ALD and growth performance with dietary enzyme supplementation cannot be attributed to increased utilization of β -glucans. Complete degradation of the β -glucans would only increase dietary glucose content by 3% which does not account for the substantial increases in chick growth performance seen in previously published data as well as the present research. According to Burnett (1966) and White *et al.* (1983), the growth performance improvements observed with the addition of enzyme (β -glucanase) to barley-based broiler diets are due a decrease in intestinal content viscosity, resulting in improved nutrient utilization. Burnett (1966) suggested that a decrease in digesta viscosity mediated by enzyme supplementation would create an environment more conducive to optimum digestion of crude protein and carbohydrates. Similarly, previous research has suggested that enzyme supplementation of diets based on these cereal grains (barley, oats, rye) results in enhanced digestion and absorption of nitrogen (Scholtyssek and

Knorr, 1987; Broz, 1987a,b; Pettersson and Aman, 1989), dry matter (Fengler *et al.*, 1988), fat (Fengler *et al.*, 1988; Pettersson and Aman, 1989) and nitrogen free extract (Scholtyssek and Knorr, 1987) compared to their nonsupplemented counterparts. The data of Harmuth-Hoene and Schwerdtfeger (1979) and Burnett (1966) have demonstrated the nutrient digestibility and growth depressing effects of viscous and indigestible polysaccharides such as β -glucans and other gums for young growing rats and chickens.

The increases in dietary AME_n due to enzyme addition in the present studies is in agreement with previous findings. Leong *et al.* (1962) reported 24 and 15% increases in the ME of pearled and regular barley respectively with the addition of a crude fungal enzyme preparation. Potter *et al.* (1965) found a fungal enzyme supplement increased the ME of barley by 18.2% and attributed the increase in bioavailable energy to significantly ($P < .05$) increased digestibilities of protein, fat and nitrogen-free extract.

The present data has demonstrated the positive effects of enzyme supplementation on apparent protein digestibility (APD) and also demonstrated that APD values were dramatically affected by the cultivar and dietary inclusion rate of test cereal grain. For all test grains with the exception of Bedford barley (hulled), increasing inclusion rates resulted in a depression of APD with Gazelle rye showing the most pronounced depression in APD. Previous studies have shown that enzyme supplementation of barley resulted in increased starch degradation and nitrogen digestion (Hesselman and Aman, 1986; Broz, 1987a; Scholtyssek and Knorr, 1987).

In the present research, apparent lipid digestibilities (ALD) showed similar trends to that seen with APD except that the inclusion level effects were more pronounced. Depressions in fat absorption have been observed in earlier studies with rye (Antonioni *et al.*, 1981) and hulless barley (Classen *et al.*, 1985). The present data indicate that although the enzyme supplement has a highly significant effect on ALD, the 70%

enzyme supplemented Gazelle rye and Terra oats based diets do not exhibit ALD values similar to those seen in the nonsupplemented control diet. Perhaps the enzyme concentration may be limiting in these diets. Conversely, enzyme supplementation in the 70% Scout barley diet completely eliminated the negative effects of the β -glucans. Similarly, Classen et al. (1985) found enzyme supplementation completely eliminated the depression of ALD seen in the nonsupplemented Scout barley diets.

The relative effect of enzyme supplementation varied widely between the test cereals (barley, hulless barley, rye and oats). The present data showed a definite differential response to enzyme supplementation and dietary inclusion rate between the two barley cultivars examined (Bedford (hulled); Scout (hulless)). For each parameter observed, the Scout barley exhibited greater depressions as inclusion rates increased but then also responded to enzyme supplementation to a greater degree than the hulled Bedford barley. The differential response presumably occurs since the hulless barley contains higher levels of β -glucans than its hulled counterpart. Similar effects have been reported previously for lipid absorption (Edney et al. 1989), ME (Leong et al. 1962) and chick performance (Rotter et al, 1989b).

The consistent positive response in the wheat control diets to enzyme supplementation in the current studies with respect to AME_n , APD and ALD is an interesting observation since wheat has traditionally been regarded as a high quality cereal grain which is relatively digestible for the young broiler chick. This data indicates that enzyme supplementation of wheat based diets may be a viable option and deserves consideration on a commercial basis. Creek et al. (1962) suggested the presence of a thermolabile growth inhibitor in raw wheat germ which depresses fat and protein utilization. Mollah et al. (1983) found the AME_n and starch digestion of low ME Australian wheats to be significantly correlated ($r=.91$, $P<.01$) and most variable in birds fed wheat diets compared to rice and sorghum. Thus, enzyme supplementation in the present studies may increase the wheat AME_n through enhanced starch digestion and reduced

variability among birds. To the contrary, Rogel et al. (1987) found no evidence to suggest that viscous polysaccharides in wheat were inhibiting its digestion.

The results of Experiment 1 showed that fat type (corn oil vs. tallow) affected dietary AME_n ($P < .05$) as diets containing corn oil had higher AME_n values than their tallow containing counterparts. However, enzyme supplementation eliminated the differential effects between the corn oil- and tallow-containing diets with the barley in the tallow-containing diets actually exhibiting greater AME_n values than the barley in the corn oil-containing diets. The results without enzyme supplementation were expected since Carew et al. (1972) reported maximum absorption of corn oil by one-week-old chicks, whereas tallow maximum absorbability was delayed to an older age. Kussaibati et al. (1982) showed the addition of bile salts significantly increased ($P < .05$) the digestibility of diets containing animal fat but not maize oil, suggesting that bile acid levels in the young chick are not adequate for maximum tallow absorption.

Experiments 1 and 2 (Manuscript 1) attempted to determine the effect of enzyme supplementation on the bioavailable energy (AME_n) of hulless barley grain using two methodologies. Experiment 1 ($AME_n(H)$) employed substitution of a material of known AME_n (Scott et al., 1982) while Experiment 2 ($AME_n(S)$) involved the substitution of an entire semi-practical basal diet (Sibbald and Slinger, 1963). The $AME_n(S)$ values for non-supplemented hulless barley were 9.6% higher than the $AME_n(H)$ values (11.69 and 10.67 MJ/kg DM, respectively). Similarly, Schang et al. (1982) showed $AME_n(S)$ values tended to be higher than $AME_n(H)$ for all cereal grains, but this was only significant for barley ($P < .05$). Pesti et al. (1986) reported the $AME_n(S)$ value of poultry by-product meal was 7.9% higher than the $AME_n(H)$ value. However, an earlier study showed no differences between the two methods for the AME_n of maize (Pesti, 1984). The absence of a response to enzyme supplementation in Experiment 2 ($AME_n(S)$) suggests that the diet contained (or lacked) a factor which

limited bird growth performance and thereby masked any possible enzyme effect. Therefore, an accurate comparison of the two methodologies was difficult, if not impossible.

Experiments 4 and 5 were conducted to determine the enzyme concentration (Tv Cellulase) required to achieve maximum growth response in broiler diets containing 60% rye. Nonlinear regression analysis concluded that the level of enzyme required was substantially greater than that utilized in Experiments 1, 2 and 3 (3.2 vs. .04 g enzyme/kg diet). These results were expected since the enzyme preparation (Tv Cellulase) contained more β -glucanase and less pentosanase activities while the rye grain contains a relatively high level of pentosans compared with β -glucans. An enzyme preparation containing more pentosanase activity would be more suited to the pentosan substrate in rye and would likely be required in lesser concentrations than the enzyme employed in the present studies to achieve the same results. These results are in contrast to those of Fengler et al. (1988) who found that increasing Tv cellulase levels from .01 to 1.0 g/kg diet in a 59% rye diet only marginally decreased diet and excreta viscosities while nutrient (dry matter and fat) retentions were not affected ($P > .05$). The total amount and nature of the rye pentosans in these two studies may have differed since Fengler et al. (1988) utilized a different cultivar of rye (CV Puma).

One of the objectives of the present study was to calculate the AME_n of rye grain with and without enzyme supplementation for each dietary inclusion level of rye (Manuscript 3). Literature AME_n values were utilized to calculate the energy contribution of the protein/energy (PE) supplement and the energy values of the various dietary components were assumed to be additive in nature. However, it has been shown in previous research that the addition of rye pentosans to wheat based diets has an adverse affect on the nutritive value of the total diet (Fengler and Marquardt, 1988b; Antoniou and Marquardt, 1981; Antoniou et al., 1981). Fengler and Marquardt (1988b) added graded levels of rye pentosans to a wheat-based diet and demonstrated increasing depressions in nutrient

digestion and accompanying chick growth performance, however the depression did not appear to be linear. Since the effect of graded dietary pentosans levels does not appear to be linear, it is difficult to determine quantitatively their effect on the nutritive value and bioavailable energy of other dietary components. Therefore, AME_n values for each level of rye with and without enzyme could not be calculated.

SUMMARY AND CONCLUSIONS

The present research has demonstrated the positive effects of enzyme supplementation on wheat-, barley- (hulled and hullless), naked oats- and rye-based diets with respect to bioavailable energy (AME_n) and the apparent protein (APD) and lipid (ALD) digestibilities. These improvements were also reflected in the growth performance of enzyme supplemented chicks compared to their nonsupplemented counterparts. The present data suggest that diets containing enzyme supplemented 70% hulled barley, hullless barley, or naked oats can support chick growth performance which is equal or superior to a conventional wheat-based diet. Dietary fat source (corn oil vs. tallow) affected both the dietary nutritive value and growth performance of barley fed chicks. The birds fed corn oil as a dietary energy source had superior growth rates compared to those fed tallow. These data indicate that the corn oil (vegetable source) is more digestible than the tallow (animal source) in the young broiler chick as seen in previous research. The enzyme supplement serves to increase the bioavailable energy and apparent digestibilities of lipid and protein by decreasing digesta viscosity and thereby enhancing nutrient digestion and absorption and chick performance.

The following conclusions may be drawn from the present data:

1. The extent of AME_n improvement observed with enzyme supplementation of barley is dependent on the chick bioassay utilized in the AME_n determination.
2. Chick growth performance and dietary nutrient utilization are adversely affected by substituting increasing concentrations of barley

(hulled and hulless), naked oats or spring rye into a wheat-based control diet.

3. Enzyme supplementation of barley- (hulled and hulless), naked oats- or spring rye-based broiler diets results in improved nutrient utilization (AME_n , APD and ALD) and chick growth performance. For all test grains with the exception of rye, enzyme supplementation results in chick growth performance equal or superior to that seen in the nonsupplemented wheat control diet.

4. There is a differential response to enzyme supplementation between hulled and hulless barley. While the hulless barley (CV Scout) depresses chick growth to a greater degree than the hulled barley (CV Bedford) when substituted into the wheat control diet, it also shows a response to enzyme supplementation of a greater magnitude than the hulled barley.

5. Wheat-based control diets showed a relatively small but consistent response to enzyme supplementation.

6. The enzyme concentration required to achieve maximum growth response in a 60% rye diet is considerably greater than that required for the barley- or oats-based diets.

7. Even at relatively high enzyme concentrations, the rye-based diets did not support chick growth rates similar to those seen in the wheat-based control diets suggesting that a factor other than enzyme concentration is limiting chick growth.

8. Due to the highly viscous nature of rye, it is impossible to determine the AME_n of rye without enzyme supplementation using methodology currently employed by the industry.

Further research is suggested to:

1. develop the methodology necessary to determine the AME_n of a cereal grain such as rye, oats or barley for the young broiler chicken.
2. determine the effect of dietary inclusion rate on the AME_n , APD and ALD of rye, oats and barley grain.
3. establish optimum conditions (enzyme specificity, concentration and level of grain in diet) for enzyme supplementation of rye-based diets for young broiler chicks.
4. determine what application enzyme supplementation may have in wheat-based broiler diets with respect to relative costs versus benefits.

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