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EFFECT OF ANAEROBIC STORAGE, PROCESSING AND β-GLUCAN CONTENT OF HIGH MOISTURE BARLEY ON ITS NUTRIENT VALUE FOR GROWING SWINE

by

ERIKA MARIKO WELTZIEN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECT OF ANAEROBIC STORAGE, PROCESSING AND β-GLUCAN CONTENT OF HIGH MOISTURE BARLEY ON ITS NUTRIENT VALUE FOR GROWING SWINE submitted by ERIKA MARIKO WELTZIEN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

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Date... February 21, 1986

ABSTRACT

Two studies were undertaken at The University of Alberta to determine the feeding value of high moisture barley (HMB) for growing swine. The HMB containing approximately 25% moisture and a dry barley (approximately 13% moisture) were both harvested from the same 54-acre field . On a dry matter basis, the HMB yielded 19.6% more than did the dry barley.

In the first experiment, 56 crossbred gilts and 24 crossbred barrows of an average initial weight of 28 kg were randomly assigned to diets based on either anaerobically stored HMB that had been hydrated to a moisture level of 28.8% or dry barley. The pigs were allowed feed and water ad libitum and taken off test at 55 kg. Pigs fed the HMB-based diet consumed significantly more feed ($P<0.05$), on a dry matter basis, than did pigs fed the dry barley-based diet. Average daily gain (ADG) and feed conversion efficiency (FCE) were not significantly affected by diet ($P>0.05$). The average daily feed intake (ADFI) was significantly higher ($P<0.05$) for barrows than for gilts but there were no significant differences ($P>0.05$) in ADG or FCE among the sexes.

In the second experiment, eight ileally cannulated crossbred barrows (57.6 kg bodyweight) were allocated to diets based on ground or rolled HMB and ground or rolled dry barley, using a 4 x 4 Latin square design. Pigs were fed on an equal dry matter basis and fecal, ileal, and urinary samples were collected. The β-glucan contents of the HMB- and dry barley-based diets were similar (5%) on a dry matter basis.

There were no significant differences among the four treatment groups in the apparent ileal digestibilities of the dry matter, crude protein, gross energy, starch, or β-glucan components of the diets. The average ileal digestibility coefficient for β-glucan of the four diets was 79.6% (range: 76 to 82.2%), suggesting that this component is readily digested by growing pigs. The ileal digestiblity coefficients for neutral detergent fibre (NDF) and phosphorus were, however, lower ($P<0.05$) for the ground dry barley-based diet than for the other diets. The apparent ileal digestibilities of lysine, methionine, isoleucine, alanine, valine, and aspartic acid

were significantly higher ($P<0.05$) for the ground HMB-based diet than for the dry barley-based diets. Fecal digestibility coefficients for crude protein, NDF, starch, and β-glucan were higher ($P<0.05$) for the ground HMB diet than for the rolled dry barley diet. For both the HMB and dry barley diets, grinding resulted in a significant ($P<0.05$) improvement in the apparent fecal lysine digestibility as compared to rolling.

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I. INTRODUCTION

Barley is the primary cereal grain used in Western Canada as a source of energy in swine feeds. Although the digestible energy of barley as compared to that of wheat or corn is lower for swine, economics as well as the environmental conditions favour the production and use of barley in Western Canada. Because of the unpredictable and usually short growing season, it is often necessary to harvest grain before it has dried in the field. Grain harvested when it contains enough moisture (at least 20%) to promote fermentation when ensiled is referred to as high moisture grain (Hunt et al. 1960).

Advantages that have been claimed for the use of high moisture barley (HMB) include the following:

1. Earlier harvest reduces dependency upon the weather.
2. Harvesting of HMB results in a higher dry matter yield as compared to dry barley (Krall 1972; Marx 1981).
3. Grain quality is maintained since the possiblity of damage to the grains due to the heat of drying is eliminated.
4. HMB is more palatable than dry barley (Krall 1972), possibly because of its lower dust content (Erichsen and Martin 1970).
5. Fermentation of HMB during anaerobic storage increases the availability of phosphorus in swine diets (Trotter and Allee 1979) and improves feed consumption, average daily gain (ADG), and feed conversion efficiency (FCE) of poultry by altering the composition of the β-glucan fraction (Hesselman et al. 1981).

There are a number of alternative methods of preservation of HMB and these, together with method of processing, may significantly influence the performance of swine fed HMB-based diets. A further important consideration is the effect of anaerobic storage on the β-glucan component of barley on the performance of swine.

II. HIGH MOISTURE BARLEY

One of the major economic advantages of HMB is the increase in dry matter yield which is associated with earlier harvesting. Yield increases of 1.4 to 17% have been observed for HMB as compared to dry barley (Krall 1972; Marx 1981). The potential dry matter yield of barley is at a maximum if harvested when the moisture content of the kernel is reduced to approximately 42% (Harlan 1920, as cited by Krall 1972).

A. Methods of Preservation of High Moisture Grain

The high moisture levels render HMB extremely susceptible to rapid deterioration by molds and bacteria unless preventative measures are taken to avoid this. The conventional preservation method of drying has become less popular in recent years, due to increased fuel prices. This has stimulated, or at least sustained, an interest in alternative methods of preservation such as chemical treatment or anaerobic storage.

Chemical Preservatives

HMB can be chemically preserved by treatment with organic acids, alkalis, or sulfur dioxide gas.

Organic Acids

Organic acids such as propionic, acetic, and butyric acids, are the main chemical preservatives used to treat high moisture grains. These acids inhibit seed respiration and fungal growth and thus prevent heating and subsequent deterioration of the grain (Jones et al. 1970; Young et al. 1970). They offer an alternative to artificial drying, allowing HMB to be stored aerobically without deterioration, and do not adversely affect the feeding value of the grains (Jones et al. 1974). Organic acid treated HMB has generally been reported to have a feeding value for pigs equal to that of dry barley on a dry matter basis (Cole et al. 1970; Livingstone et al. 1971; Perez-Aleman et al. 1971; Bowland and Corbett 1973; Cole et al. 1975; Cole et al. 1980).

Although propionic acid (or a combination of propionic with other organic acids) is the most effective (Goering and Gordon 1973) and most frequently used organic acid in the preservation of high moisture grain, it is fungistatic, not fungicidal; therefore, tolerant fungi may grow if sufficient acid is not applied evenly to the grain (Lord et al. 1982). Safety precautions must be used when treating grain with organic acids in order to avoid inhalation of fumes or contact of the acid with the skin or eyes (Jones et al. 1974). A serious disadvantage of using such grain is that it corrodes metal equipment (Forsyth 1975). In addition, lower vitamin E and carotene levels have been observed in acid treated high moisture corn than in non-treated high moisture corn (Lynch et al. 1975).

Alkalis

Sodium hydroxide and ammonia are alkalis that have been successfully used in the prevention of microbial growth in high moisture grain (Berger et al. 1981; Laksesvela 1981) and have been shown to increase fibre digestibility in ruminants (Orskov et al. 1980). However, since alkalis are capable of destroying amino acids or of reducing their availability (Bjarnason et al. 1976), the treatment of grain with alkali significantly lowers its protein digestibility for pigs and rats and is therefore not recommended (Pringle et al. 1983).

Sulphur Dioxide Gas

Although the use of sulfur dioxide gas as a preservative for high moisture grains has shown some promise (Mathison et al. 1985), it has been suggested that its fungicidal properties may be inferior to its bactericidal properties and that it may induce thiamin deficiency and cause a lowered nutritional value (Gibson and Kennelly 1985). In addition, sulfur dioxide gas is extremely corrosive to storage equipment and may produce odour and flavour problems when fed to livestock (Vidal and Jayaraman 1982). Thus, it cannot be recommended as an alternative to other methods of preservation of moist grain at the present time.

Anaerobic Storage of High Moisture Grain

Hyde and Burrell (1982) described the principle behind airtight storage of grain as the provision of an oxygen-free atmosphere in order to kill or inactivate harmful organisms including insects, microorganisms and molds before they can cause serious damage to the grain. Such organisms respire aerobically using oxygen to break down carbohydrates to carbon dioxide and water. Milner et al. (1947), as cited by Hyde and Burrell (1982), reported that the respiration of grain, and thus the production of carbon dioxide, increased in grain containing greater than 15% moisture. Some organisms, including certain yeasts and bacteria, can respire anaerobically and cause fermentation of carbohydrates to produce lactic or acetic acids or alcohol but considerably less heat is released than with aerobic respiration. Thus, when dealing with high moisture grain, it is imperative that the silo be completely airtight in order to prevent the proliferation of insects and to limit the growth of aerobic organisms that could cause deterioration and overheating of the grain. Anaerobic storage may be carried out in pits or sealed tower silos that are often glass lined.

Since HMB tends to heat shortly after removal from the silo due to microbial activity (Jones et al. 1970; Young et al. 1970), it cannot be left in the feeders in large amounts for feeding over an extended period of time as can dry barley. Instead, it must be mixed on a daily basis and fed in amounts that can be consumed within a day (Jones et al. 1974). It has been reported that the ADG and FCE of pigs fed HMB that was allowed to become moldy were significantly poorer than those of pigs fed dried, unmolded barley (Livingstone and Livingston 1970; Livingstone et al. 1971).

B. Changes in Grain During Anaerobic Storage

Certain changes occur to a much greater extent in moist grain during airtight storage than in dry grain. The appearance of the grain does not change much but the anaerobic fermentation process that occurs at moisture levels above 16% results in a sour odour and a bitter taste to the grain (Hyde and Oxley 1960). Virtually no changes occur in the chemical

composition of low moisture grains during anaerobic storage, but at higher moisture contents, there is an increase in the level of reducing sugars and a corresponding decrease in nonreducing sugars (Shvetsova and Sosedov 1958, as cited by Hyde and Burrell 1982). Protein, nitrogen, starch, and fat contents are not significantly affected by anaerobic storage of HMB (Hyde and Burrell 1982).

The availability of phosphorus has been reported to increase during storage through the action of phytase on phytin, the organic form of phosphorus present in grain (Greaves and Hirst 1925, as cited by Pomeranz 1982; Trotter and Allee 1979).

Vitamin E levels have also been reported to decrease during storage of high moisture corn, although selenium content does not appear to be affected (Young et al. 1975).

A claim that has been made to support the argument that HMB is a superior feed to dry barley is that fermentation during anaerobic storage increases the digestibility of β-glucans (Hesselman et al. 1981). These substances have been implicated in the lowered feeding value of barley and will be discussed in detail later.

C. Feeding Value of HMB for Swine

Despite the fact that there has been widespread interest in the use of high moisture grains, most of the research has dealt with high moisture corn or sorghum, acid preserved high moisture grain, or storage systems that were not oxygen limiting. In addition, much of the research has focused on the feeding of HMB to dairy and beef cattle, rather than to swine. It has been suggested that the chief advantage of HMB for cattle lies not in improved animal performance but in its high palatability (Krall 1972).

Jamieson et al. (1967) reported that, in swine diets, the digestible energy of ground or rolled HMB was the same as that of ground or rolled dry barley. Forbes and coworkers (1963; 1964; 1965), as cited by Livingstone and Livingston (1970), and Erichsen and Martin (1970) reported similar growth rates and FCE for pigs fed dry barley or HMB (22.5% to 33.8% moisture) that had been stored in airtight steel containers. Cole et al. (1970) also reported no

differences in the digestibility coefficients for dry matter, nitrogen or gross energy between pigs fed HMB and dried barley diets. Pringle et al. (1983) compared diets based on HMB stored in an airtight silo, acid-preserved HMB, sodium hydroxide-preserved HMB, and air-dried barley. There were no significant differences in the dry matter or protein digestibilities between the anaerobically stored or acid-preserved HMB diets but the neutral detergent fibre (NDF) digestibility was reduced in the dried barley diet relative to the other diets. Protein digestiblity was severely decreased in the alkali-treated HMB diet.

In contrast to these findings, Livingstone and Livingston (1970) reported that, on a dry matter basis, the use of anaerobically stored moist barley had an adverse effect on the growth rate and FCE of pigs. This can be explained by the fact that fungal and bacterial growth was observed and, as a result, fermentation and deterioration of the diets was sometimes allowed to occur prior to feeding. Thus, proper storage of moist grain is essential if growth rates and feed conversion efficiencies similar to those typical of swine fed dry barley diets are to be achieved.

D. Effect of Particle Size - Grinding versus Rolling

An important consideration in the feeding of HMB is the optimum method of processing. It has traditionally been suggested that the nutritive value of grains may be improved by physically breaking the grain kernels so as to expose the starch and proteins to digestive enzymes (Morrison 1956). The conventional method of processing barley has been to grind it in a hammer mill before its inclusion in pig diets. However, hammer milling becomes very difficult with barleys containing more than 22% moisture and rolling is sometimes carried out as a more convenient alternative (Cole et al. 1970; English et al. 1973). Varied results have been obtained in studies comparing the performance of pigs fed hammer milled barley with that of pigs fed rolled barley.

Lawrence (1970) compared the feeding of whole, ground, rolled, and coarsely crimped dry barley to pigs and observed that the overall digestibilities of the whole or crimped barley diets were significantly poorer and that the rate of passage of these diets through the gut was

faster than for the other diets. In the same study, there were no significant differences in growth rate and FCE among pigs fed rolled barley or barley that had been ground through 1.56, 4.68, or 9.36 mm screens. However, the barley that was ground the most finely had the greatest retention time in the alimentary tract and had the highest digestibility coefficients for ether extract and crude fibre fractions of the diet. These findings agree with those of Beames and Ngwira (1978), Simonsson (1978), and Simonsson and Bjorklund (1978). Owsley et al. (1981) also reported that increasing the fineness of the grind of barley significantly improved the apparent ileal digestibility coefficients for dry matter, starch, gross energy, and nitrogen components of the diet. On the other hand, Cole et al. (1970) reported that pigs given rolled moist barley, whether chemically or anaerobically preserved, required 11% more feed on a dry matter basis per unit of gain than those fed milled barley. However, the authors indicated that difficulty in obtaining an even distribution of the chromic oxide marker in the rolled barley diets may have resulted in a bias against the digestibility of the rolled barley diets. Livingstone et al. (1971) also reported reduced nitrogen digestibility for pigs fed rolled versus ground anaerobically stored HMB, acid-treated HMB, or dry barley. Young (1970) observed that the rolling or grinding of either dry or high moisture corn had no significant influence on feed consumption or average daily gain of pigs. Cole et al. (1975) reported a significant interaction between storage method and feed preparation. In their study, pigs fed propionic acid-treated barley grew more quickly when the barley was ground rather than rolled whereas no significant difference in growth rate was observed among pigs fed ground or rolled dry barley.

The incidence of gastric lesions in swine has been observed to increase with fineness of the grind of barley (Simonsson and Bjorklund 1978). Lower dry matter contents and acidic pH in the stomach ingesta, possibly due to the faster rate of passage of very finely ground particles through the stomach were also noted. Simonsson and Bjorklund (1978) suggested that the cause of the lesions was probably not directly due to the lowered gastric pH but to the increased fluidity of the ingesta which comes more into contact with the unprotected mucous membranes, thus exposing it to a greater acidic effect than when coarser diets are fed.

Livingstone and Livingston (1970) reported that grinding or rolling had no significant effect on the performance of pigs fed either HMB or dry barley diets. Lawrence et al. (1980) also observed no significant difference in the performance of pigs fed rolled, finely ground, or coarsely ground dry barley. They observed improved performance with decreased particle size of moist barley diets, and suggested that an interaction may exist between moisture content and the optimium degree of fineness of grinding.

The failure of researchers to reach a consensus as to the influence of method of processing barley on the performance of pigs may be due to a number of factors. One reason is that the term "rolled" has been used to describe anything from barley that has been almost ground to barley that is merely cracked (Cole et al. 1975). In addition, genetic and environmental differences resulting in differences in chemical and physical properties of the barleys may also be responsible (Lawrence et al. 1980).

III. BARLEY β-GLUCANS

Extensive research has been conducted for many years to determine the nutritive value of barley and possible methods for its improvement. Jakobsen et al. (1960), as cited by Herstad and McNab (1975), reported low digestibility coefficients for nutrients of barley as compared to corn or wheat in experiments with poultry. Reduced ADG, lowered FCE, and an increased incidence of sticky droppings usually occur in poultry fed barley-based diets (Burnett 1966; White et al. 1981). Lowered performance has also been demonstrated for swine fed barley-based diets as compared to corn- or wheat-based diets (Larsen and Oldfield 1961).

The inferior performance of poultry and swine fed barley-based diets has been linked to the presence in barley of high levels of a compound identified as β-glucan (Rickes et al. 1962; Burnett 1966; Petersen and Sauter 1968; Hesselman and Thomke 1982). β-glucans comprise approximately 4 to 10.7% of the total dry matter content of barley (Anderson et al. 1978; Prentice et al. 1980; Martin and Bamforth 1981; Bourne et al. 1982; Henry 1984). It has been observed that a high percentage of β-glucans in the diet results in an increased viscosity of both the digesta in the small intestine and the feces (sticky droppings) of poultry (Burnett 1966; White et al. 1981). White et al.(1983) postulated that the greater viscosity caused an impairment of nutrient absorption in the small intestine and could potentially reduce the nutritive value of barley. While a number of studies have examined the adverse effects of dietary β-glucan on the performance of poultry, there is little information available concerning the effects of these polysaccharides on the digestibility of dietary components for swine.

Prerequisites to such an investigation are: 1. an understanding of the physical and chemical properties of β-glucan and of the enzymes capable of degrading this component of barley, and 2. the selection of an appropriate method of analysis of β-glucan.

A. Structure and Distribution of Barley β-Glucans

β-glucans are polysaccharides that are located primarily in the endosperm cell walls of barley (Fincher 1975; Forrest and Wainwright 1977b; Wainwright and Buckee 1977; Martin and Bamforth 1981). They include all compounds of two or more glucose molecules linearly joined together by β-glucosidic linkages (Bourne and Pierce 1972; Thompson and LaBerge 1977). Barley β-glucan chains contain a mixture of randomly arranged β-1,3- and β-1,4-linkages which occur in approximately a 3:7 ratio (Clarke and Stone 1963; Bourne and Pierce 1972; Fleming and Kawakami 1977). Approximately 50% of the endosperm cell wall β-glucans can be removed by water at 65°C without destroying the cell wall structure (Forrest and Wainwright 1977a,b). These water soluble β-glucans give rise to very viscous solutions when solubilized (Aastrup 1979a) and are sometimes referred to as "gums" (Preece and Mackenzie 1952). The water-insoluble β-glucans are thus responsible for retaining the structural integrity of the endosperm cell walls (Forrest and Wainwright 1977a,b).

β-glucans, especially mixed-linkage β-glucans (1,3-1,4-β-glucans), account for approximately 75% of the endosperm cell walls of barley (Fincher 1975; Forrest and Wainwright 1977a,b; Wainwright and Buckee 1977). Palmer (1975) suggested that the endosperm cell wall of barley is composed of three layers: an inner layer of β-glucan, a middle layer of protein, and an outside layer of β-glucan and pentosan. The β-glucans are firmly linked to peptide sequences which account for 3 to 5 percent of total cell wall content (Thompson and Laberge 1981), forming polymers of very high molecular weight (4×10^7 daltons). This protein material forms an integral part of the β-glucan structure of the endosperm cell wall (Fleming and Kawakami 1977; Forrest and Wainwright 1977a). The rest of the endosperm cell wall consists mainly of pentosans containing arabinose and xylose but these do not appear to be covalently bound to β-glucans (Forrest and Wainwright 1977a).

By forming such a large proportion of the endosperm cell wall, β-glucans play an important structural role in the endosperm and a lesser one in the aleurone layer where arabinoxylan is the major polysaccharide (Coles 1979). This role changes as the barley matures,

however. The endosperm cell walls provide structural support as the barley grains develop, but at maturity, when the endosperm cells are filled with starch and protein, structural rigidity of the wall becomes less important (Thompson and LaBerge 1977). Since the endosperm cell walls of barley, unlike those in other grains, completely enclose the cell, they form a physical barrier to proteolytic and amylolytic enzymes that would otherwise hydrolyze the endosperm reserves (Morgan and Gothard 1977; Thompson and LaBerge 1977; Allison et al. 1978). Thus, the endosperm cell walls must be degraded, at least partially, before hydrolytic enzymes can act on the cell contents or food reserves (i.e. starch and protein) of the endosperm (Fincher 1975).

B. Endo-β-Glucanases

Barley β-glucans are not homogeneous in nature; molecular weights of 2×10^5 to 4×10^5 have been reported (Bourne and Pierce 1970; Ducroo and Delecourt 1972; Prentice et al. 1980) and the proportion of β-1,3-linkages to β-1,4-linkages is variable (Fleming and Kawakami 1977). Thus, a spectrum of enzymes is required for complete degradation of β-glucan to glucose (Bathgate and Dalgliesh 1975). Enzymes capable of ultimately degrading β-glucans into simple sugars of low viscosity may be described as either endo- or exo-β-glucanases (Bourne and Pierce 1972). Barley contains both types of β-glucanases but it is the former group that is of greater importance in lowering the viscosity of β-glucans in solution (Scott 1972a). The endo-β-glucanases can further be divided into three groups: endo-β-1,4-glucanase, endo-β-1,3-glucanase, and endo-β-1,3-1,4-glucanase, each specific for the cleavage of a particular type of glucosidic linkage (Manners and Marshall 1969; Ballance et al. 1976). Endo-β-1,3-glucanase is specific for substrates such as laminarin which contain only β-1,3-glucosidic linkages; it is activated by calcium ions and inhibited by EDTA and citrate-phosphate buffer (Manners and Marshall 1969). Endo-β-1,4-glucanase is capable of hydrolyzing substrates containing β-1,4-glucosidic linkages such as carboxymethylcellulose and barley β-glucan but has little effect on insoluble cellulose or laminarin and endo-β-1,3-1,4-glucanase hydrolyzes only β-glucans containing both β-1,3- or β-1,4-linkages

(Manners and Marshall 1969; Manners and Wilson 1976).

A series of enzymatic reactions is involved in the degradation of β-glucans in the endosperm cell wall (Wainwright and Buckee 1977). It has been shown that the initial step is catalyzed by an acidic carboxypeptidase identified as β-glucan solubilase which releases β-glucan into solution (Bamforth et al. 1979). The mode of action of this enzyme for the freeing of β-glucan thus entails splitting of the peptide linkages that are bound to the β-glucan in the endosperm cell wall matrix (Bamforth et al. 1979). This enzyme is produced in the aleurone layer and is released at the onset of germination before endo-β-glucanase, α-amylase, or protease (Bamforth 1981). In addition to solubilizing the β-glucan from the endosperm cell walls, endo-β-glucan solubilase may also be involved in the initial catabolism of the high-molecular weight β-glucans into smaller β-glucan subunits (Martin and Bamforth 1981). The β-glucan subunits released by endo-β-glucan solubilase are of high viscosity and become the substrate for endo-β-1,3-glucanase to give a product which can subsequently be degraded by endo-β-1,4-glucanase and endo-β-1,3-1,4-glucanase (Bamforth 1981).

C. Factors Influencing β-Glucan Content of Barley

Barley endosperm cell walls are unique amongst those of the common cereals in that they contain such a high percentage (75%) of β-glucan (Fincher 1975; Forrest and Wainwright 1977a). Rye grass cell walls, which are composed of approximately 40% β-glucan, contain the next largest proportion of β-glucan (Burke et al. 1974) and oats have been reported to have a β-glucan content similar to that of rye (Clarke and Stone 1963; Wood et al. 1977; Anderson et al. 1978). While wheat endosperm cell walls contain approximately 7.5% β-glucan (Mares and Stone 1973), rice, corn, and triticale contain little β-glucan (Anderson et al. 1978).

Total β-glucan content in barley is influenced by both environmental and varietal factors (Willingham et al. 1960; Bourne et al. 1976; Aastrup 1979b; Morgan and Riggs 1981).

Varietal Factors

Some varieties of barley consistently contain more β-glucan than others whether they are grown under similar or different conditions and locations. For example, Coles (1979) reported that Triumph, a variety of barley, had a lower β-glucan content than Minerva under all conditions tested. Bamforth and Martin (1981) studied the β-glucan content of eight cultivars of barley grown in several locations and observed that a varietal rather than an environmental effect was largely responsible for the wide range of β-glucan values (6.13 to 10.7%) obtained. Other researchers (Bourne and Pierce 1972; Prentice et al. 1980; Bourne et al. 1982; Hesselman and Thomke 1982) have shown similar varietal differences in β-glucan content. Molina-Cano and Conde (1982) calculated that genetic factors accounted for 89% of the variation in β-glucan content over geographical locations and 71.5% of the variation over location and sowing date. Differences in the β-glucan content of barley cultivars observed by other researchers (Anderson et al. 1978; Bourne et al. 1982; Morgan et al. 1983) also suggested that this trait may be controlled to a large extent by genetic factors rather than environmental factors.

Environmental Factors

The environmental factors that can influence the β-glucan content of barley are: climate, soil moisture, fertilization, and sowing date. Bourne and Pierce (1972) reported that five varieties of barley were ranked the same in β-glucan content in each of five locations but that the β-glucan content varied considerably from location to location. Hesselman and Thomke (1982) also observed varietal differences to be less important than climatic factors or stage of ripeness of the grains in influencing the β-glucan content of grain.

Climatic conditions have been shown to greatly affect β-glucan content in barley. Hot, sunny and dry conditions leading to soil moisture stress during the ripening period have been reported to cause an increase in β-glucan levels, whereas cool moist conditions tend to lower them (Aastrup 1979b; Coles 1979; Hesselman and Thomke 1982). Aastrup (1979b) observed a

decrease in the amount of soluble and total β-glucans in rain-treated grains. Since there was negligible endo-β-glucanase activity in rain-treated grains and no real difference in β-glucan structure between the rain-treated and control grains, it seemed unlikely that precipitation brought about β-glucan degradation. Coles (1979) suggested that this phenomenon could be due to lower production of β-glucans or a modification of the β-glucan structure which renders it inaccessible to endogenous β-glucanase.

Ewertson (1977), as cited by Hesselman and Thomke (1982), reported an increase in β-glucan content with increased nitrogen fertilization up to a level of 80 kg of nitrogen per hectare.

The results of Molina-Cano and Conde (1982) indicated that, of all the environmental factors, sowing date had the greatest influence on β-glucan content. They observed that the later the sowing date, the higher the gum content.

D. Methods of β-Glucan Analysis

Numerous methods of determining the β-glucan content of barley have been developed over the years. However, differences between the methods, especially in the extraction and fractionation procedures, along with variations in the fractions of β-glucan measured, have resulted in a wide range in the estimates of the total β-glucan content of barley.

Early Procedures

Most of the earlier procedures involved solubilization of the β-glucan from barley with water, after treatment with 80 to 85% ethanol under reflux, to inactivate endogenous β-glucanases and amylases (Preece and Mackenzie 1952; Bourne and Pierce 1970; Fleming et al. 1974; Fleming and Kawakami 1977). Aqueous extracts of barley, however, contained starch and arabinoxylan as well as β-glucan (Preece and Mackenzie 1952). The amount of β-glucan obtained was also dependent on the temperature of extraction (Fleming and Kawakami 1977; Wood et al. 1978). Significantly more material was extracted at 80°C than at 45°C and it

contained a lower proportion of protein and a higher proportion of starch. Papain (Bass and Meredith 1955; Sparrow and Meredith 1969) and α-amylase (Bourne and Pierce 1970), or both papain and α-amylase (Scott 1972b) were used with water to extract β-glucan from raw barley. These enzymes may have been contaminated with β-glucanase enzymes, however. All these methods involved precipitation of the β-glucan for subsequent measurement by weight or by glucose estimation following acid hydrolysis (Anderson et al. 1978).

Viscosity Determination of β-Glucan

Greenberg and Whitmore (1974) developed a method whereby the viscosity of an acid extract of barley could be used to estimate the β-glucan content. Morgan and Gothard (1977) developed a rapid viscometric technique which related the falling time of a ball in a precision bore tube filled with barley extract to the β-glucan content of barley. Aastrup (1979a) demonstrated a close relationship ($R^2=0.99$) between the viscosity of an acid extract of barley and the soluble β-glucan content but not the insoluble β-glucan content. Other workers (Smith et al. 1980; Morgan et al. 1983) showed that the viscosity was attributable mainly to β-glucan. However, Anderson et al. (1978) reported that viscosity measurements were not always well correlated with β-glucan content. The viscometric technique gave lower values for β-glucan content than enzymic methods in most cases. Although the viscosity of barley is mainly due to water-soluble β-glucans, the presence of any other viscous material would influence the interpretation of a viscometric method. Bathgate (1977) speculated that proteins and starch, extracted with the β-glucan, could also contribute to the viscosity of a barley extract. The usually lower values for β-glucan content that are obtained using viscosity tests may be partly explained by the fact that viscosities of most extracts start to decrease after three hours incubation, thus indicating enzymic or acidic hydrolysis (Bathgate 1977). Viscosity measurements have also been shown to increase with barley nitrogen content (Morgan 1977) and to vary with the molecular weight of the β-glucan extracted (Bourne and Pierce 1972; Fleming et al. 1974; Martin and Bamforth 1981). Thus, the value of viscosity tests appears to

be limited to a rapid screening method for ranking barley on the basis of its β-glucan content.

Fluorometric Procedure

A rapid, fluorometric screening procedure for the determination of barley β-glucans using the fluorescent dye Calcofluor has also been developed (Jensen and Aastrup 1981). Calcofluor is specific for β-glucosidic linkages and quantitatively binds to β-glucans (Aastrup and Erdal 1980). It is a rapid reproducible method; however, the correlation between the values derived for β-glucan content from enzymatic methods and those derived from the fluotometric method was not as good for malt and barley samples as it was for beer samples (Jensen and Aastrup 1981). This may be due to the fact that Calcofluor also stains non-β-glucan compounds such as the β-glucosidic linkages of cellulose (Jensen and Aastrup 1981).

Infra-Red Reflectance

Allison et al. (1978) calibrated a near infra-red reflectance instrument to estimate the acid-soluble β-glucan content of barley. It is a very rapid (approximately one minute per sample) and simple test designed for screening large numbers of barley samples. Its values correlate well ($r=0.87$) with direct estimates of β-glucan although the correlation is lower at elevated β-glucan levels (Allison et al. 1978).

Difference Method

Another method used for the determination of β-glucan in barley is the difference method in which β-glucan content is calculated as the difference in yield between the glucose released from complete acid hydrolysis of a barley extract by glucose oxidase and the glucose released by amyloglucosidase from α-glucans such as starch (Fleming et al. 1974; Fleming and Kawakami 1977; Wood et al. 1977). This method, however, is subject to large errors when large amounts of starch are present, as is the case for barley or when the acid hydrolysis or amyloglucosidase degradation do not go to completion (Forrest and Wainwright 1977b; Martin

and Bamforth 1981). Bendelow (1975) reported that another source of error was the presence of β-glucanase activity in amyloglucosidase preparations.

Enzymatic Methods

More recent methods for β-glucan analysis of barley rely on direct estimation of the extracted β-glucan. Purified β-glucanase of either bacterial or fungal origin is used to degrade the β-glucan into glucose units which are then enzymically measured to determine total β-glucan content (Forrest and Wainwright 1977b; Anderson et al. 1978; Prentice et al. 1980; Martin and Bamforth 1981). The main difference between these methods is that they utilize various methods to extract β-glucan from the barley grain for subsequent enzymic measurement. Forrest and Wainwright (1977a) and Martin and Bamforth (1981) used hydrazine to extract barley β-glucan and to destroy endogenous enzymes. Hydrazinolysis extracts all the β-glucan by degrading the peptide linkages which bind the β-glucan to the endosperm cell wall (Forrest and Wainwright 1977a).

Although the extraction and fractionation methods as well as the methods of determining the fractionated product vary in these procedures, they all require purified enzyme preparations that are exclusively specific for β-glucan. Since pure β-glucanase is not yet commercially available, extensive purification procedures of crude proteolytic or amylolytic enzyme preparations which contain β-glucanase activity must be undertaken to ensure that they are free of amylase and other polysaccharide-degrading enzymes in order to prevent degradation of starch in the extracts and thus give a deceptively high β-glucan estimation. Enzyme purification involves methods such as ion exchange chromatography, ultrafiltration, gel filtration, and heat inactivation (Bourne et al. 1982).

Thus, enzymatic methods, although currently the most reliable, are extremely labour-intensive, time-consuming, and unsuitable for screening large numbers of barley samples. A simple, rapid, and reliable method of analysis is needed to predict the feeding and malting qualities of barley, if barleys with low levels of β-glucan and high levels of

β-glucanases are to be selected. Until such a method is developed, rapid methods such as the viscosity or infra-red reflectance methods will have to suffice for screening purposes and direct enzymatic methods will have to be used to substantiate or verify samples of particular interest.

E. Nutritional Effects of β-Glucans in the Diet

β-glucans have long been implicated as an important factor in the lowered feeding value of barley as compared to corn diets. Burnett (1966) connected the presence of sticky droppings and poor performance of chicks fed "low-enzyme" barleys with β-glucan. He concluded that this component of barley gave rise to fairly stable viscous conditions in the small intestine and was thus responsible for the sticky nature of the droppings as well as impaired nutrient uptake. The production of sticky droppings along with increased water consumption by poultry fed barley diets commonly occur in poultry fed indigestible hydrocolloids (Gohl et al. 1978).

Other compounds that give viscous solutions in water have been tested to verify that the lowered feeding value of barley is due, at least in part, to the presence of β-glucans. Burnett (1966) reported that the addition of methylethylcellulose, hydroxypropylmethylcellulose, gum arabic, and pectin to the diets of chicks impaired feed conversion efficiencies markedly. Addition of pectinase to the pectin-supplemented diets significantly improved chick performance. Kratzer et al. (1967) fed a variety of natural and synthetic hydrocolloids to chicks and noted a marked reduction in growth. Gohl and Gohl (1977), as cited by Lance (1984), showed similar responses to pectin, gum arabic, and barley β-glucan in rat feeding trials due to delayed passage of digesta. White et al. (1981) noted an increase in the viscosity of the intestinal contents in chicks fed a corn-based diet supplemented with barley β-glucan.

There is a paucity of information, however, concerning the effects of barley β-glucan on swine performance. Froseth et al. (1981) observed that growing pigs performed better when fed diets based on certain barley cultivars than on others and suggested that this was due to high β-glucan and (or) crude fibre contents in these barley cultivars. Thomke et al. (1980) also

attributed the reduced growth and lowered digestibility of organic matter, crude protein, and gross energy for starter pigs fed barley-based diets to the presence of 1,3-1,4-β-glucans. Honeyfield et al. (1983) suggested that the high β-glucan levels present in barley lowered the digestibility coefficient for energy and thus the available metabolic energy of the grain for growing and finishing pigs. Davies and Radcliffe (1984) compared two barley varieties, one which was reported by Anderson et al. (1978) to contain 30% more β-glucan than the other and observed no significant difference in performance of growing pigs fed diets based on these barleys.

F. Methods to Improve the Feeding Value of Barley

Water Treatment

Numerous attempts have been made over the last thirty years to improve the feeding of barley for monogastrics. Fry et al. (1957), as cited by Petersen and Sauter (1968), were the first to demonstrate that water treatment increased the feeding value of pearled barley. The positive effect of water treatment on the feeding quality of barley has since then been confirmed by many workers (Willingham et al. 1959, 1960; Potter et al. 1965; Gohl et al. 1978).

Gohl (1977), as cited by Hesselman and Thomke (1982), reported that treatment of high viscosity barley with distilled water resulted in the liberation of carbohydrates of low molecular weight with a simultaneous decrease in viscosity. Since this decrease in viscosity occurred between pH 3.8 and pH 6.5, it was presumed to be linked to enzymatic activity. It is likely that prolonged moist conditions may favour the activity or increased production of endogenous enzymes in the barley and thus solubilization of β-glucans into smaller units which have lower viscosities in solution (Gohl 1977, as cited by Hesselman and Thomke 1982). Hesselman and Thomke (1982) suggested that the lowered viscosity they observed in barley harvested under wet conditions was due to enzymatic degradation of β-glucans similar to that

thought to occur in water treated barley.

Anaerobic Storage

Hesselman et al. (1981) observed that the performance of broilers fed diets based on HMB (55% and 70% dry matter) that was anaerobically stored prior to drying was superior to that of broilers fed diets based on the same HMB dried immediately after harvest. These authors reported that the β-glucan content of the HMB was reduced during anaerobic storage and that no significant difference was obtained between the performance of broilers fed diets based on anaerobically stored HMB diets and that of broilers fed β-glucanase supplemented dried barley diets. Hesselman et al. (1981) speculated that the beneficial effect of anaerobic storage and β-glucanase supplementation of barley for broilers was due to endogenous enzymatic changes to the structure of β-glucans. The effect of anaerobically induced changes to the β-glucan fraction of barley on the performance of swine has yet to be determined.

Enzyme Supplementation of Diets

Since water or heat treatment of barley would be impractical in most feeding systems, the more direct approach of enzyme supplementation of barley diets has been studied, especially in poultry diets. Most experiments involving enzyme supplementation of barley-based diets utilized crude enzyme preparations of bacterial, fungal, or malt origin. Many researchers (Jensen et al. 1957; Willingham et al. 1959, 1960; Arscott and Rose 1960) obtained a growth response in chicks fed enzyme-supplemented barley based diets that was similar to that observed with water treatment of barley. Petersen and Sauter (1968) tested the supplementation of various industrial enzyme preparations containing amylase, protease, lipase, or cellulase to barley diets and reported a growth response in chicks of 10 to 15% that did not appear to be related to any specific enzyme.

The crude enzyme preparations used in these experiments typically contained high levels of α-amylase activity which was thought to be largely responsible for the improved feeding

value of barley. Cunningham and Brisson (1957), however, observed that supplemental pancreatic or malt amylases had no effect on piglet growth rate and survival rates or on the digestibility of raw or cooked starch by baby pigs. They suggested that orally ingested amylases are destroyed by unfavourable pH conditions in the stomach and that starch may require the action of other enzymes or activators for its complete breakdown to glucose. Although Willingham et al. (1959) detected the presence of α-amylase in several crude enzyme preparations of various origins that were successful in improving growth rate and feed utilization by chicks fed barley-based diets, crystalline α-amylase, when added to these diets, was completely ineffective in improving the growth rate of chicks. Since pH inactivation of α-amylase in crude preparations did not alter chick growth rate, enzymes other than α-amylase were possibly responsible for the improvement in feeding value (Willingham et al. 1959).

β-glucanase, an active component of many of the crude enzyme preparations, was associated with the positive response in chicks fed barley-based diets (Rickes et al. 1962). Moscatelli et al. (1961) reported that β-glucanase from *Bacillus subtilis* was a very specific and rapidly acting enzyme with an optimum temperature of 50°C and an optimum pH of 6.5 to 6.6 at 45°C. In addition, they observed no loss of activity after 24 hours at pH 2 and pH 10 at room temperature (20 to 22°C). β-glucanase isolated from *Bacillus subtilis* is active from pH 5.9 to 7.7 with an optimum pH of 6.5 to 6.8 (Ducroo and Delecourt 1972; Huber and Nevins 1977). β-glucanases are very thermolabile (Bourne et al. 1976; Gohl et al. 1978) and are rapidly inactivated at temperatures above 60°C (Ducroo and Delecourt 1972; Erdal and Gjertsen 1971; Scott 1972b).

Rickes et al. (1962) reported that after extensive purification of a β-glucanase obtained from a *Bacillus subtilis* the preparation proved to be extremely potent, not only *in vitro* but also *in vivo*, and significantly improved growth rate of chicks when fed at a level of 10 μg/kg of a barley-based diet. This provided additional evidence that the relatively high levels of β-glucan in barley were at least partially responsible for the growth depression observed when barley replaced corn in chick diets.

Another potent source of β-glucanase activity that has been used to improve the feeding value of barley is *Trichoderma viride*, a cellulase degrading fungus (White 1981; White et al. 1983). The addition of this fungal enzyme lowered the intestinal fluid viscosity of chicks fed barley diets as well as that of chicks fed β-glucan added to a corn-soybean meal diet. Gohl et al. (1978) reported that the addition of β-glucanase to autoclaved barley diets caused rapid hydrolysis of β-glucan and thus improved the performance of chicks. The dry matter content of the excreta has also been shown to increase as a result of β-glucanase supplementation (Gohl et al. 1978; Hesselman et al. 1982). Mannion (1981) studied the influence of bacterial and fungal enzyme preparations known to contain endo-β-glucanase activity on the feeding value of barley for broiler chickens. Body weight gain and feed consumption were increased by 12 to 25% and 3 to 21%, respectively. The metabolizable energy of the diets was improved by 1.53 MJ per kg of dry matter. Hesselman et al. (1982) reported that increasing the levels of β-glucanase supplementation of diets based on barley harvested at 55% and 70% dry matter increased live weight of chicks at three weeks by 10 to 26%, improved FCE by 4.9 to 11%, and reduced the incidence of sticky droppings. These results agree with those of Thomke (1972), Hesselman et al. (1981), and De Silva et al. (1983).

Age, at least in the case of poultry, seems to be an important determinant of the degree of enzyme response, with younger birds generally showing a much greater response than older birds (Petersen and Sauter 1968). Mannion (1981) observed an increase in growth of chicks due to enzyme supplementation only up to two and three weeks of age. Gohl et al. (1978) observed that wet and sticky droppings are a much greater problem in younger birds. Berg (1961) was unable to increase the growth of eight-week old pullets fed enzyme-supplemented barley diets and like other researchers (Arscott and Rose 1960; Petersen and Sauter 1968), failed to obtain an improvement in egg production or feed efficiency in laying hens from enzyme-supplemented barley-based diets. Thus, enzyme supplementation of barley-based diets may not be cost-effective for older birds since the benefit decreases with age.

Until fairly recently, little work had been done in the area of β-glucanase supplementation of swine diets. Newman et al. (1980) and Newman and Pepper (1984) supplemented barley diets of growing pigs with bacterial diastase, another source of β-glucanase activity. They observed a small improvement in growth rate and FCE in pigs fed a covered barley cultivar supplemented with diastase and no response in pigs fed a hulless waxy barley cultivar. Without diastase supplementation, the pigs fed the hulless waxy cultivar performed better than those fed the covered barley cultivar. These authors also observed a slight increase in carcass yield and loin eye area of carcasses from pigs fed both barleys supplemented with diastase. Newman et al. (1982) compared the feeding value of two hulless barley isotypes of different viscosities for growing pigs with and without the addition of bacterial diastase. The diastase treatment resulted in a higher rate of gain and better FCE in pigs fed the higher viscosity cultivar and significantly improved the digestible energy and the digestible protein of both cultivars. Nevertheless, the reported improvements in performance of swine fed β-glucanase supplemented diets have been much smaller than for poultry.

Although there is much evidence that dietary enzyme supplementation improves the feeding value of barley, there is not much data concerning the effect on the digestibility of specific nutrients. Leong et al. (1958) and Potter et al. (1965) reported increases in the metabolizable energy of barley of 23.8% and 18%, respectively, in response to enzyme supplementation. Potter et al. (1965) attributed the increase to an improved digestibility of protein and fat and an apparent increased digestiblity of nitrogen-free extract. Burnett (1966) also suggested that the presence of highly viscous material in the intestine could lead to lowered protein and carbohydrate digestibilities.

Growth response to enzyme supplementation was influenced by both the variety of barley (Daghir and Rottensten 1966; Herstad and McNab 1975) and the environmental conditions in which the barley was grown (Willingham et al. 1960). Willingham et al. (1960) reported that barleys grown in arid areas respond to a greater extent to dietary enzyme supplementation than barleys grown in humid areas. Even though it has been shown that the

failure to get a response to water treatment or enzyme supplementation of barleys in some studies was due to varietal differences, it could not be attributed solely to environmental factors since different results were sometimes obtained for barleys grown in the same area (Willingham et al. 1959).

It has been speculated that the beneficial effect of water-treatment, enzyme supplementation, or anaerobic storage of barley on the performance of poultry or swine is due, at least in part, to the action of enzymes of endogenous or exogenous origin on the β-glucan component of the grain. However, there is little evidence concerning the digestibility of barley β-glucan on swine diets. Anaerobic storage of HMB may combine the benefits of water treatment and endogenous enzyme activity on the β-glucan fraction.

The objectives of the studies described herein were to investigate the effect of anaerobically strored HMB on the performance of swine and on the digestibility of dietary β-glucan and other dietary components.

IV. EXPERIMENT 1

A. OBJECTIVE

The objective of this experiment was to compare the performance of pigs fed a diet based on anaerobically stored high moisture barley (HMB) with that of pigs fed a dry barley-based diet.

B. MATERIALS AND METHODS

Fifty-six crossbred (Yorkshire x Landrace) gilts and twenty-four crossbred (Yorkshire x Landrace) barrows of an average initial liveweight of 28 kg were allocated on the basis of initial weight and sex to one of two dietary treatments (Table IV.1). Both diets were formulated on an as fed basis to meet or exceed the nutrient requirement recommendations of the National Academy of Sciences-National Research Council (NAS-NRC 1979) for growing swine.

The HMB and the dry barley were of the same variety (Klondike) and were grown in the same field under the same conditions. The 54-acre field was divided into four equal rectangular areas which were assigned to either high moisture or dry harvesting. Sections one and three were harvested at a moisture content of approximately 25% and sections two and four were harvested at a moisture content of approximately 13%. The dry barley was stored in granaries while the HMB was hydrated to a moisture level of 28.8% and anerobically stored in a Harvestore®.

The HMB was ground daily in a hammer mill and then mixed with the remaining dietary ingredients to ensure that the pigs were fed fresh feed daily. The dry barley diet was fed once weekly because a preliminary trial indicated no significant difference in feed intake of pigs fed this diet once daily versus once weekly. Both diets were fed ad libitum and water was available at all times from water bowls. The uneaten portion of the HMB ration was weighed daily and discarded. Feed consumption of the dry barley diet was determined weekly as were the

Table IV.1 Formulation and chemical composition of experimental diets.

	High moisture barley	Dry barley
Ingredients, % dry matter basis		
High moisture barley	80.4	—
Dry barley	—	80.4
Soybean meal	15.2	15.2
Iodized salt	0.6	0.6
Ground limestone	1.35	1.35
Dicalcium phosphate	1.35	1.35
Vitamin-mineral premix*	1.1	1.1
Total	100.0	100.0
Chemical analysis†		
Dry matter, %	74.2	88.6
Crude protein, %	18.2	18.8
Gross energy, MJ/kg	18.8	18.4
Ether extract, %	3.6	3.4
Neutral detergent fibre, %	22.8	23.0
Starch, %	53.5	54.1
β-glucan, %	4.9	5.2
Phosphorus, %	0.75	0.79
Calcium, %‡	0.83	0.83
Ash, %	5.9	6.6

*Supplied the following per kilogram of air dry diet: 60 mg zinc; 6 mg manganese; 75 mg iron; 6 mg copper; 0.1 mg selenium; 250 mg choline; 2500 IU vitamin A; 250 IU vitamin D; 11 IU vitamin E; 6 mg riboflavin; 22.5 mg niacin; 12.5 mg calcium pantothenate; and 15 μg vitamin B_{12}.

†Dry matter basis.

‡Calculated values.

pig weights. The pigs were housed in groups of four of the same sex in solid concrete floored, 1.5 x 4.6 m pens, with straw bedding. The environmental temperature was maintained at 22 to 23°C throughout the experimental period. Pigs were taken off test when they reached an average of 55 kg body weight.

Diets were analyzed for dry matter, crude protein, ether extract, and ash contents according to the methods of the Association of Official Analytical Chemists (AOAC 1980). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, U.S.A.). Following starch hydrolysis in an α-amylase solution for 24 h (McQueen and Nicholson 1979), diets were analyzed for neutral detergent fibre (NDF) content (Van Soest and Wine 1967). Starch content of the diets was determined by acid hydrolysis (AOAC 1980) and subsequent glucose analysis (Oser 1965). Diets were analyzed for β-glucan content as described in experiment 2.

Statistical Analysis

The pen was considered the experimental unit. Performance data were analyzed by least squares analysis of variance of unequal numbers (Harvey 1960). Performance means were adjusted by covariate analysis to the mean initial pig weight of 28 kg (Steel and Torrie 1980).

C. RESULTS

Fresh weight yields of the HMB and dry barley were 1921 kg/acre and 1398 kg/acre, respectively. On a dry matter basis, the HMB yielded 19.6% more than did the dry barley (Table IV.2).

Two pigs failed to complete the test, one due to pneumonia and the other due to lameness. While there was no significant difference in initial weight among treatments, there was considerable within-treatment variation in initial weight. The effect of initial weight was found to be significant in the case of average daily feed intake (ADFI) and average daily gain (ADG); therefore, it was removed by covariate analysis.

Table IV.2 Yields of dry and high moisture barley.

	Dry	HMB†	HMB as % of dry barley
Fresh weight yield (kg/acre)	1398	1921	137
Yield on dry matter basis (kg/acre)	1210	1448	120
% dry matter	86.7	75.4	87.0

† High moisture barley.

Pigs fed the HMB-based diet consumed significantly ($P<0.05$) more feed, on a dry matter basis, than did pigs fed the dry barley-based diet (Table IV.3). However, ADG and feed conversion efficiency (FCE) were not significantly affected by diet ($P>0.05$).

The ADFI was significantly higher ($P<0.05$) for barrows than for gilts but there were no significant differences ($P>0.05$) in ADG or FCE among the sexes. There was no significant ($P>0.05$) sex by diet interaction effect on performance of the pigs.

D. DISCUSSION

It has long been realized that the dry matter yield of barley peaks several weeks before it is normally harvested (Brenchley 1912, as cited by Flippot and Pelletier 1980). Harlan (1920), as cited by Krall (1972), reported that barley kernels reached physiological maturity when translocation of plant materials to the kernel had ceased and the moisture content dropped below 42%. The higher dry matter yield (19.6%) observed in the present experiment for HMB as compared to dry barley is slightly greater than previous reports of yield increases for HMB. Krall (1972) reported yield increases for HMB that ranged from 1.4 to 16.9% on irrigated land and from -5.9 to 17.3% on unirrigated land. Similar yield increases in favour of HMB were reported by Marx (1981). Mathison (1981), however reported that at least 5 to 15% of the dry matter in HMB can be lost during ensiling due to the production of ethanol, lactic acid, and volatile fatty acids.

The results of this experiment suggest that pigs fed diets based on anaerobically stored HMB perform as well as pigs fed diets based on dry barley. These observations are in agreement with those of other researchers (Forbes 1965, as cited by Livingstone and Livingston 1970; Erichsen and Martin 1970). The crude protein, gross energy, ether extract, and ash content of the two diets were similar, suggesting that differences in proximate analysis of the diets were not responsible for the increased ADFI of pigs fed the HMB-based diet. The daily grinding, mixing, and feeding of the HMB diet, along with daily removal of uneaten feed, may have been responsible for the higher feed intake of pigs fed this diet. Another possibility is that

Table IV.3 Effect of diet and sex on the performance of growing pigs - least square means†.

	Diet			Sex		
	High moisture barley	Dry barley	Significance	Barrows	Gilts	Significance
No. of pigs	39	39		23	55	
Initial weight, kg	27.5	29.0		28.8	27.7	
Final weight, kg	55.0	54.9		54.7	55.1	
Average daily feed intake, kg	2.05 (0.03)‡	1.91 (0.03)	*	2.04 (0.04)	1.92 (0.03)	*
Average daily gain, kg	0.76 (0.01)	0.73 (0.01)	NS	0.75 (0.01)	0.73 (0.01)	NS
Feed conversion efficiency	2.71 (0.04)	2.63 (0.04)	NS	2.72 (0.05)	2.61 (0.03)	NS

†Means adjusted to a common on-test pig weight of 28 kg.
‡Values in parentheses represent standard error of the mean.
*Means are significantly different ($P<0.05$); NS, no significant difference ($P>0.05$).

fermentation of the HMB during anaerobic storage may have increased the palatability of the grain. This is consistent with the suggestion by Krall (1972) that the benefit of HMB as a feed for cattle was in the increased palatability and not in improved gains or FCE which were small. In the present study, the observed increase in ADFI of pigs fed the HMB-based diet did not result in a significant improvement in either ADG or FCE; however, there was a tendency for ADG to be improved. The greater ($P<0.05$) feed intake of barrows versus gilts observed in the present experiment concurs with previously reported differences in feed intakes of barrows and gilts under ad libitum feeding conditions (Walstra et al. 1977, as cited by Fuller 1985).

V. EXPERIMENT 2

A. OBJECTIVES

The objectives of this experiment were:

1. To compare the digestibility of ground or rolled high moisture barley (HMB)-based diets with that of ground or rolled dry barley-based diets fed to grower pigs.
2. To determine the influence, if any, of method of processing (grinding versus rolling) on the digestibility of high moisture or dry barley-based diets fed to grower pigs.
3. To investigate the possibility that fermentation of HMB during anaerobic storage increases the digestibility of β-glucans.

B. MATERIALS AND METHODS

Digestibility Trial

Eight crossbred barrows were fitted with T-piece cannulae at the distal end of the ileum, approximately 5 cm from the ileocaecal junction, by an adaptation of the method of Sauer et al. 1983. The pigs weighed an average of 57.6 kg at the start and 79.0 kg at the end of the experiment. They were individually confined in stainless steel metabolism crates that permitted separate collection of urine and feces. The environmental temperature was maintained at 22 to 23°C throughout the seven week trial.

A 4 x 4 Latin square design was used to allocate the pigs to ground or rolled HMB and ground or rolled dry barley diets (Table V.1). The diets were formulated on an as fed basis to meet or exceed the nutrient requirements of growing swine, as outlined by the National Academy of Sciences-National Research Council (NAS-NRC 1979).

The high moisture and dry barleys were obtained from the source described in Experiment 1. The HMB was stored in a Harvestore® and ground in a hammermill or rolled daily and then mixed with concentrate to ensure that the pigs were fed fresh feed each day.

Table V.1 Formulation and chemical composition of experimental diets based on dry barley or high moisture barley (HMB).

	Ground dry barley	Rolled dry barley	Ground HMB	Rolled HMB
Ingredients, % dry matter basis				
High moisture barley	—	—	80.4	80.4
Dry barley	80.4	80.4		
Soybean meal	15.2	15.2	15.2	15.2
Iodized salt	0.6	0.6	0.6	0.6
Ground limestone	1.35	1.35	1.35	1.35
Dicalcium phosphate	1.35	1.35	1.35	1.35
Vitamin-mineral premix*	1.1	1.1	1.1	1.1
Total	100.0	100.0	100.0	100.0
Chemical analysis†				
Dry matter, %	87.9	87.9	74.5	74.5
Crude protein, %	18.4	18.3	18.5	18.2
Gross energy, MJ/kg	18.1	18.2	18.4	18.5
Ether extract, %	3.6	3.4	3.9	3.8
Neutral detergent fibre, %	22.7	21.7	25.0	24.6
Starch, %	53.0	54.5	51.7	51.7
β-glucan, %	5.1	5.1	5.0	5.0
Phosphorus, %	0.75	0.79	0.72	0.71
Calcium, %‡	0.83	0.83	0.83	0.83
Ash, %	7.0	6.1	6.7	6.3
Dysprosium, ppm	5.3	5.3	5.2	5.2

*Supplied the following per kilogram of air dry diet: 60 mg zinc; 6 mg manganese; 75 mg iron; 6 mg copper; 0.1 mg selenium; 250 mg choline; 2500 IU vitamin A; 250 IU vitamin D; 11 IU vitamin E; 6 mg riboflavin; 22.5 mg niacin; 12.5 mg calcium pantothenate; and 15 μg vitamin B_{12}.

†Dry matter basis.

‡Calculated values.

For each treatment, the pigs were allowed a six day adjustment period, followed by a six day collection period. The pigs were fed equal amounts twice daily, at 0800 h and 1600 h. The HMB diets contained the same level of barley, on a dry matter basis, as the dry barley diet. Each pig received 1856 g of the HMB diets or 1600 g of the dry barley diets per day for the first test period. This was increased to 2324 g and 2000 g per day of the HMB and dry barley diets, respectively, for the remainder of the trial. Water was available ad libitum.

A five day total collection of urine and feces was carried out from days 7 to 11 of each test period for each pig. Ileal digesta were collected for a total of 12 hours: 0800-1030 h, 1230 to 1430 h, and 1630 to 1830 h on day 11 and from 1030 to 1230 h, 1430 to 1630 h, and from 1830 to 2030 h on day 12 of each test period. Urine was filtered through glass wool and a mesh screen in order to prevent contamination from feces and feed and was collected in polyethylene containers to which 100 mL of 1% HCl were added to prevent nitrogen loss. One percent of the urine collected on each day of the five day period was kept for subsequent analysis. Feed, fecal, and ileal samples were frozen at -20°C, freeze-dried, and then ground in a Wiley mill equipped with a 0.4 mm mesh screen and thoroughly mixed. Fecal, ileal, and urine samples for each pig were pooled within each period and stored for analysis at 4°C.

Dry matter, ash, crude protein, and phosphorus contents of feed, fecal, and ileal samples were determined according to the methods of the Association of Official Analytical Chemists (AOAC 1980). Following starch hydrolysis in an α-amylase solution for 24 hours (McQueen and Nicholson 1979) samples were analyzed for neutral detergent fibre (NDF) content (Van Soest and Wine 1967). Hydrolysis of the samples in 4N HCl was carried out prior to ether extract determination (AOAC 1980) as well as before glucose measurement for starch determination (Oser 1965). An adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, U.S.A.) was used to determine gross energy. Amino acid contents were determined in duplicate following acid hydrolysis in 6N HCl for 24 hours (Blackburn 1968) using a Beckman 121MB amino acid analyzer. Dysprosium chloride ($DyCl_3.6H_2O$) was incorporated as an inert marker into each diet at a concentration of 5.2 ppm. Feed, fecal, and ileal samples were analyzed for

dysprosium concentration according to the instrumental neutron activation analysis method (Kennelly et al. 1980).

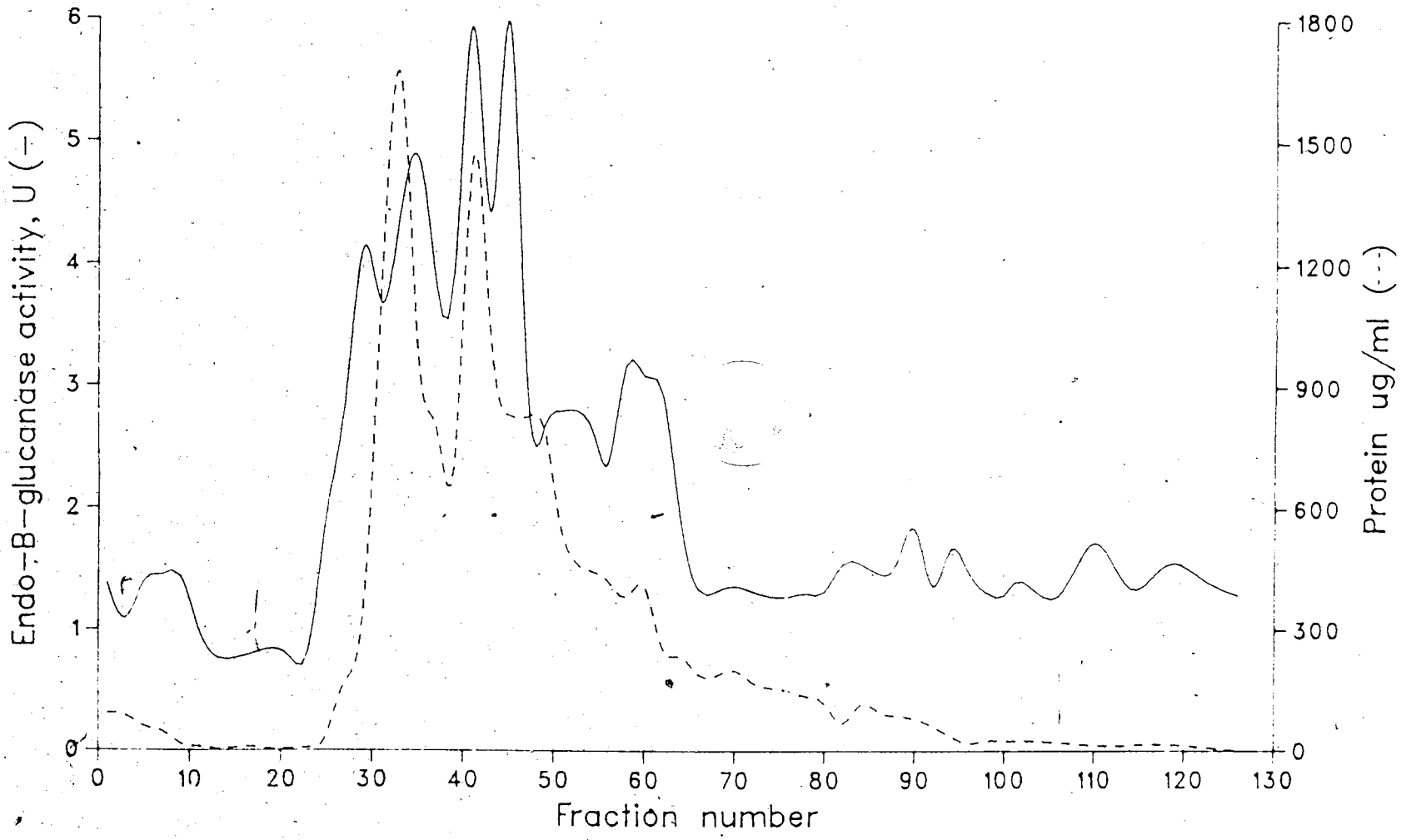
Endo-1,3-1,4-β-Glucanase Purification

The enzyme source, Novo Cereflo 200L, was a generous gift of Van Waters and Rogers (Lachine, Quebec). This crude preparation contained an endo-1,3-1,4-β-glucanase which was derived from *Bacillus subtilis*. The Novo Cereflo 200L was stored at 4°C and all purification steps were carried out at this temperature.

Following ammonium sulphate precipitation of protein, the crude enzyme preparation was centrifuged (Lance 1984; Scopes 1982) and resuspended in 820 mL of 0.2 mM citrate-20 mM phosphate buffer, pH 8.5 (Sober 1968). The resuspended enzyme was washed with approximately 2.5 L of 0.2 mM citrate-20mM phosphate buffer (pH 8.5) in a recirculating ultrafiltration flow unit (Millipore Ltd., 3610 Nashua Drive, Missisauga, Ontario) driven by a peristaltic pump. A Millipore Pellicon Cassette system was used with a PTGC 000 01 membrane (10,000 mw exclusion), a PSSP 00F 11 filtrate screen (10^6 mw), and a PSSP 000 00 retentate screen. Ultrafiltration was continued until the retentate was concentrated to a final volume of 500 mL.

A column (4.5 cm x 13 cm) of diethylaminoethyl (DEAE) cellulose (Whatman DE 32, Whatman Co., Clifton, New Jersey, 07014) was equilibrated with 0.2 mM citrate-20 mM phosphate buffer, pH 8.5. The ultrafiltered enzyme preparation was applied to the column and washed with 4 L of the equilibrating buffer. The enzyme was eluted with a 0-1 M NaCl linear gradient (Lance 1984). Fifteen mL fractions were collected by an automatic fraction collector. These fractions were analyzed for protein (Bradford 1976) and endo-β-glucanase activity (Figure V.1) (Bendelow 1963; Lance 1984). A unit of activity (U) was defined as the amount of enzyme that produced 1 μmole of glucose equivalents per minute at 40°C from substrate in 50 mM maleate buffer, pH 6.5. Those fractions containing the highest enzyme activity (fractions 25 to 64) were combined, equilibrated during ultrafiltration with 0.2 mM citrate-20

Figure V.1 Ion exchange chromatogram of a partially purified *Bacillus subtilis* endo-β-glucanase preparation on diethylaminoethyl cellulose (Whatman DE 32). 500 mL of the ultrafiltered enzyme preparation were applied to a column (4.5 cm x 13 cm) equilibrated with 0.2 mM citrate-20 mM phosphate buffer, pH 8.5. Fifteen-mL fractions were eluted with a 0-1 M NaCl linear gradient and analyzed for protein (---) and endo-β-glucanase activity (--).


Endo-B-glucanase activity, U (—)
Protein ug/ml (---)
Fraction number
0
1
2
3
4
5
6
300
600
900
1200
1500
1800
0
10
20
30
40
50
60
70
80
90
100
110
120
130

mM phosphate buffer (pH 6.5) containing 0.15 M NaCl, and concentrated to a volume of 75 mL. A column (4.5 cm x 53 cm) was packed with Bio-Gel P-30 (100-200 mesh) polyacrylamide gel (Bio-Rad Laboratories, Richmond California 94804, U.S.A.) and equilibrated with 0.2 mM citrate-20 mM phosphate buffer, pH 6.5, containing 0.15 M NaCl (Lance 1984). Sodium azide, to a final concentration of 0.02% (w/v) was added to preclude microbial growth. Blue dextran 2000 (2 x 10^6 mw) and bromphenol blue (670 mw) were used to measure void volume and elutant volume of the column, respectively. Fifty mL of the concentrated enzyme solution were applied to the column and 15 mL fractions were collected by an automatic fraction collector and analyzed for protein and endo-β-glucanase activity as before. Fractions containing the highest enzyme activity were pooled.

Although the purification procedures described removed most of the contaminating enzymes, 2.06 U of α-amylase activity per mg of protein remained in the gel-filtered enzyme. This residual acitivity was removed by two consecutive precipitations with starch in ethanol (Schwimmer and Balls 1949; Henry 1984).

Aliquots of the bulked Bio-Gel fractions were analyzed for endo-1,3-1,4-β-glucanase activity by the neocuproine hydrochloride procedure for determining reducing sugars (Dygert et al. 1965). A 0.5% (w/v) solution of purified barley β-glucan (84.7% dry matter) (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) in a 0.05 M maleate buffer (pH 6.5) containing 0.005 M sodium azide was used as a substrate for the enzyme and 100 μl samples were removed from the incubation medium at 5-minute intervals for glucose determination (Lance 1984). Glucose was used as a standard.

Starch, carboxymethyl cellulose, and reduced laminarin, each in 0.05 M maleate buffer, pH 6.5 containing 0.005 M sodium azide, were used as substrates to test for activity of α-amylase, endo-1,4-β-glucanase, and endo-1,3-β-glucanase, respectively (Lance 1984). Since activities of these potentially contaminating enzymes were not detectable after 20 minutes, incubations were carried out at 40°C for 24 hours. The neocuproine hydrochloride procedure was used to measure the reducing sugars produced by hydrolysis of these substrates.

β-Glucan Analysis

For the determination of β-glucan content, samples (50 mg) of diets and freeze-dried digesta were washed with 80% (w/v) ethanol to inactivate endogenous enzymes; β-glucan was solubilized by autoclaving at 121°C and 18 psi for 1 hour; β-glucans were hydrolyzed by the addition of 3.5 units (U) of purified endo-1,3-1,4-β-glucanase; and the resulting oligosaccharides were hydrolyzed with 0.5 M H_2SO_4 (Lance 1984). Glucose content of the neutralized hydrolysates was determined using the glucose oxidase-peroxidase (GOD-PAP) kit of Boehringer Mannheim (Cat. No. 166391). To calculate the β-glucan recovery factor, freeze dried digesta samples (50 mg) were spiked with purified barley β-glucan (20 mg) and analyzed using this procedure. The recovery factor was also determined for purified barley β-glucan alone. A factor of 0.9 was included in the calculation to convert glucose into β-glucan equivalents to account for water of hydrolysis (Bourne et al. 1982).

Statistical Analysis

Data were analyzed by analysis of variance of a Latin square design (Steel and Torrie 1980). Means for significant treatment differences were compared using the Student-Newman-Keuls multiple range test (Steel and Torrie 1980). Sufficient ileal digesta for chemical analysis could not be collected from two of the pigs in the third period; therefore, adjustments were made for unequal numbers of observations per mean.

C. RESULTS

β-Glucanase Purification

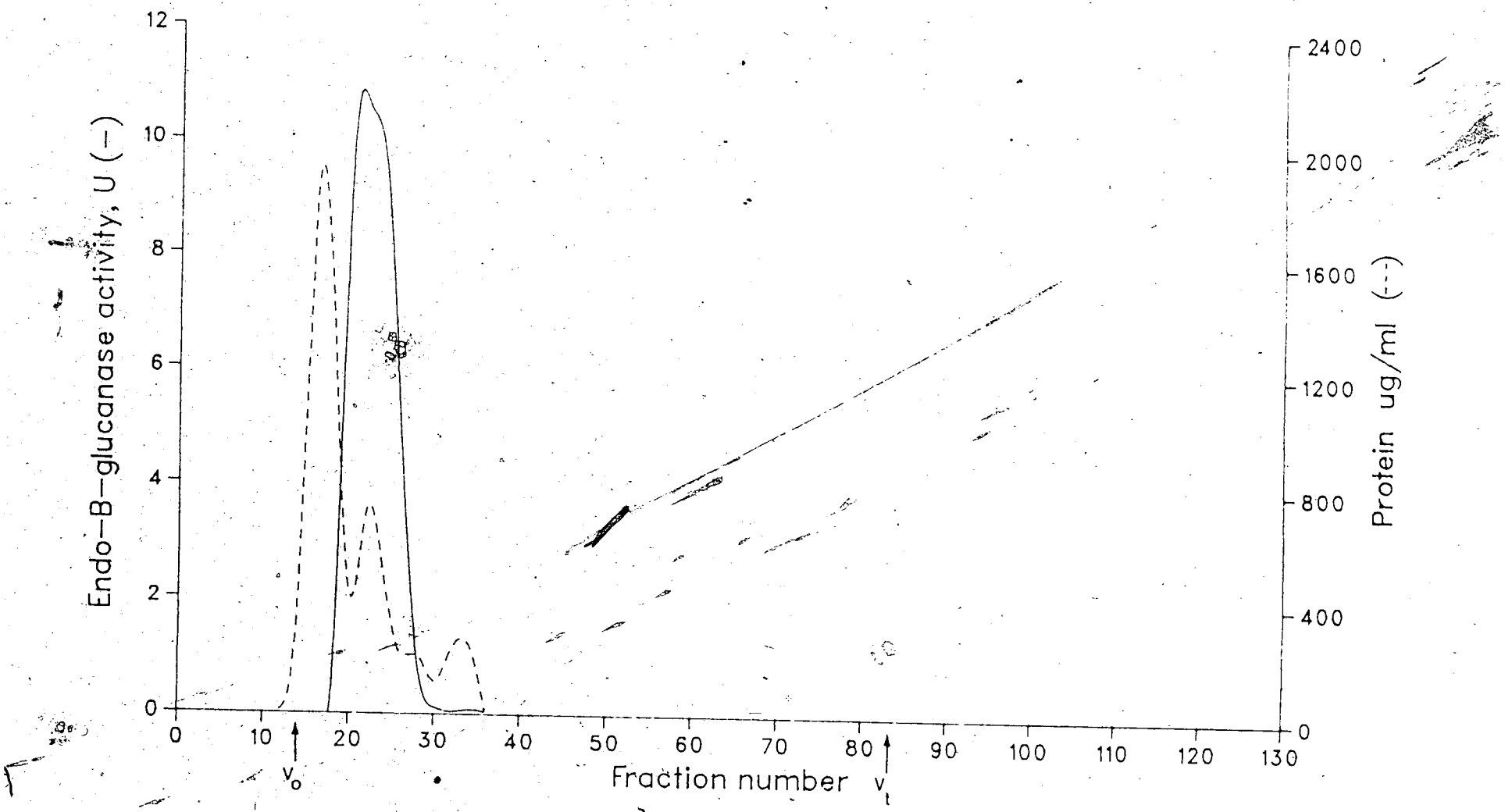
The ammonium sulphate precipitation, ultrafiltration, ion exchange chromatography, and gel filtration steps resulted in an endo-β-glucanase specific activity of 415 U per mg of protein (Table V.2). The endo-β-glucanase was eluted from the Bio-Gel column as a single peak and fractions 20 to 26 were pooled (Figure V.2).

Table V.2 Purification of endo-β-1,3-1,4-glucanase from a *Bacillus subtilis* enzyme preparation.

Enzyme Preparation	Volume (ml)	Protein (mg)	Specific Activity (units†/mg. protein)			
			Endo-β-1,3-1,4 glucanase	Endo-β-1,4-glucanase	Endo-β-1,3 glucanase	α-amylase
Crude *Bacillus subtilis* preparation	1000	8704	66	0.004	0.0	215
Pooled Bio-Gel column fractions	105	45.8	415	0.0	0.0	2.06
Final purified enzyme preparation	70	13	400	0.0	0.0	0.0

†1 unit of activity (U) produces 1 μmole of glucose equivalents per minute at 40°C from substrate in 50 mM maleate buffer, pH 6.5.

Figure V.2 Gel chromatogram of purified *Bacillus subtilis* endo-β-glucanase on Bio-Gel P-30 (100-200 mesh). Fifty mL of the concentrated, ultrafiltered enzyme preparation were applied to a column (4.5 cm x 53 cm) equilibrated with 0.2 mM citrate-20 mM phosphate buffer, pH 6.5, containing 0.15 mM NaCl and 0.02% (w/v) sodium azide. Fifteen-mL fractions were eluted with the equilibrating buffer and analyzed for protein (···) and endo-β-glucanase activity ( ). Void volume (Vo) and total volume (Vt) of the column were determined using blue dextran 2000 (2 x 10^6 mw) and bromphenol blue (670 mw), respectively.


Endo-B-glucanase activity, U (-)
Protein ug/ml (---)
Fraction number
V_o
V_t
0
2
4
6
8
10
12
0
400
800
1200
1600
2000
2400
0
10
20
30
40
50
60
70
80
90
100
110
120
130

When incubated with carboxymethylcellulose for 24 hours, the crude enzyme preparation was found to contain a very small amount of endo-1,4-β-glucanase (0.004 U) but this was removed during the purification procedure. Both the crude and the purified enzyme preparations were free of any endo-1,3-β-glucanase activity. There remained, however, some amylase contamination of the elutant from the Bio-Gel column. All traces of amylase activity were removed by two consecutive precipitations with starch in ethanol but endo-1,3-1,4-β-glucanase activity was not reduced by this procedure. The final endo-1,3-1,4-β-glucanase specific activity was found to be 400 U at 40°C and pH 6.5 using 6.7 mg β-glucan/ml as substrate.

Digestibility

Mean recovery factors of 89.9% and 91.8% were obtained for the spiked ileal samples and the purified barley β-glucan, respectively; the standard error of the mean for recovery factors was 0.67%. The dietary β-glucan values obtained in this experiment (5%) were within the ranges reported previously for barley (Anderson et al. 1978; Prentice et al. 1980; Martin and Bamforth 1981).

There were no significant ($P>0.05$) differences among the four treatments in the apparent ileal digestibilities of the dry matter, crude protein, gross energy, ether extract, starch, or β-glucan components of the diets (Table V.3). The ileal digestibility coefficients for NDF and phosphorus, were, however, lower ($P<0.05$) for the ground dry barley-based diet than for the other diets. The apparent ileal digestibilities of methionine and alanine were significantly higher ($P<0.05$) for the HMB-based diets than for the dry barley-based diets (Table V.4). The apparent ileal digestibilities of lysine, isoleucine, valine, and aspartic acid were significantly higher ($P<0.05$) for the ground HMB diet than for either of the dry barley diets. There was a trend for improvement of the ileal digestibility coefficients for these amino acids for the rolled HMB as compared to either of the dry barley diets but this improvement was not statistically significant ($P>0.05$). The fecal digestibility coefficient for NDF was higher ($P<0.05$) for the

Table V.3 The effect of moisture content and method of processing of barley on the apparent ileal digestibilities of diet components for growing pigs.

	Ground dry barley	Rolled dry barley	Ground HMB†	Rolled HMB	SEM‡
Dry matter, %	61.7	65.8	66.1	64.6	1.29
Crude protein, %	71.3	71.4	74.2	71.1	1.07
Gross energy, %	63.6	67.5	67.7	66.6	1.32
Ether extract, %	66.0	68.0	67.1	67.6	1.75
Neutral detergent fibre, %	40.7b	46.8a	49.8a	54.0a	2.01
Phosphorus, %	23.2b	36.2a	38.3a	32.6a	3.02
Starch, %	74.8	78.7	79.2	74.8	1.48
β-glucan, %	76.0	81.6	78.7	82.2	1.98

†High moisture barley.
‡Standard error of the mean.
a,b Means in the same row with different letters are significantly different ($P<0.05$).

Table V.4 The apparent ileal digestibilities of amino acids of ground or rolled dry and high moisture barley for growing pigs.

	Ground dry barley	Rolled dry barley	Ground HMB†	Rolled HMB	SEM‡
Indispensable amino acids, %					
Arginine	81.9	80.6	83.9	81.8	0.78
Histidine	80.9	80.7	82.7	81.0	0.63
Isoleucine	74.3b	74.2b	79.0a	77.3ab	0.86
Leucine	75.4	75.4	79.2	77.0	0.95
Lysine	74.0b	74.4b	78.6a	75.7ab	1.01
Methionine	71.5b	71.4b	77.6a	76.3a	1.28
Phenylalanine	71.4	71.7	75.7	73.7	1.05
Threonine	69.7	68.7	74.6	71.8	1.31
Valine	72.3c	73.3bc	77.5a	76.6ab	1.05
Dispensable amino acids, %					
Alanine	68.1b	67.3b	74.6a	72.2a	1.23
Aspartic acid	71.3b	70.4b	76.0a	73.8ab	1.14
Glutamic acid	80.7	80.0	82.5	79.8	0.85
Glycine	62.3	61.9	66.9	65.3	1.56
Proline	72.7	70.0	75.6	74.2	1.53
Serine	71.7	70.5	75.6	72.2	1.22
Tyrosine	62.5	61.5	67.4	63.1	1.50

†High moisture barley.
‡Standard error of the mean.
a,b,c Means in the same row with different letters are significantly different ($P<0.05$).

HMB diets than for the dry barley diets (Table V.5). This was the only clearcut difference between the fecal digestibility coefficients of the HMB diets and those of the dry barley diets. The other differences are best expressed between the methods of processing of the barleys. The ground HMB diet had higher ($P<0.05$) crude protein, starch, and β-glucan fecal digestibility coefficients than the rolled dry barley diet. A similar difference ($P<0.05$) was observed between the fecal ether extract digestibilities of the ground and rolled dry barley diets. However, the rolled dry barley diet had higher ($P<0.05$) dry matter, gross energy, starch, and fecal β-glucan digestibilities than had the ground dry barley diet. No significant difference ($P>0.05$) was observed in the digestibility of fecal phosphorus among the four diets.

For both HMB and dry barley diets, grinding resulted in a significant ($P<0.05$) improvement in the apparent fecal lysine digestibility as compared to rolling. However, within each processing method (grinding versus rolling), the HMB diet had a significantly higher ($P<0.05$) apparent fecal lysine digestibility than the dry barley diets (Table V.6). The fecal threonine and arginine digestibilities of ground barleys were also higher ($P<0.05$) than for the rolled barleys. This relationship was also noted for histidine, glutamic acid, and proline between the ground HMB and rolled HMB diets ($P<0.05$). In addition, the ground HMB diet was observed to have significantly higher ($P<0.05$) lysine, methionine, alanine, isoleucine, leucine, glycine, serine, and valine fecal digestibilities than the other diets.

Apparent fecal digestibilities were significantly ($P<0.01$) higher (3 to 9 percentage points) than apparent ileal digestibilities for glutamic acid, glycine, proline, and serine but not for aspartic acid, alanine, and tyrosine. In the case of the indispensable amino acids, the apparent fecal digestibilities were significantly higher ($P<0.01$) than the apparent ileal digestibilities for arginine and histidine only.

Significant differences ($P>0.05$)in the excretion, retention, absorption and apparent digestibility of nitrogen were not observed among treatments (Table V.7).

Table V.5 The effect of moisture content and method of processing of barley on the apparent fecal digestibilities of diet components for growing pigs.

	Ground dry barley	Rolled dry barley	Ground HMB†	Rolled HMB	SEM‡
Dry matter, %	71.4b	74.4a	73.4ab	71.7b	0.60
Crude protein, %	73.9ab	74.2ab	75.8a	72.3b	0.53
Gross energy, %	70.9b	73.7a	72.3ab	70.8b	0.60
Ether extract, %	44.8a	39.4b	44.0ab	43.3ab	1.26
Neutral detergent fibre, %	38.7b	42.2b	46.8a	46.6a	1.21
Phosphorus, %	35.4	38.0	37.8	35.4	1.70
Starch, %	86.4b	89.9a	88.5a	86.1b	0.57
β-glucan, %	87.8b	92.5a	91.2a	86.2b	0.79

†High moisture barley.
‡Standard error of the mean.
a,b,c Means in the same row with different letters are significantly different ($P<0.05$).

Table V.6 The apparent fecal digestibilities of amino acids of ground or rolled dry and high moisture barleys for growing pigs.

	Ground dry barley	Rolled dry barley	Ground HMB†	Rolled HMB	SEM‡
Indispensable amino acids, %					
Arginine	85.7a	83.3b	86.7a	82.4b	0.46
Histidine	86.0a	85.1ab	86.2a	84.0b	0.35
Isoleucine	74.2b	72.7b	77.0a	72.8b	0.58
Leucine	77.2b	75.5b	78.9a	75.4b	0.50
Lysine	72.7b	70.4c	74.5a	72.1b	0.47
Methionine	63.6b	60.5b	68.0a	62.3b	1.17
Phenylalanine	74.6	73.7	76.5	74.0	0.67
Threonine	73.8a	70.1b	75.6a	71.9b	0.59
Valine	75.0b	75.3b	78.2a	76.2b	0.59
Dispensable amino acids, %					
Alanine	66.9b	64.5c	70.8a	68.3b	0.77
Aspartic acid	74.1b	71.8c	75.8a	72.7bc	0.58
Glutamic acid	84.9a	84.4a	85.4a	81.9b	0.47
Glycine	69.9b	69.4b	72.5a	69.8b	0.69
Proline	82.5ab	82.9ab	83.8a	81.2b	0.50
Serine	77.4b	76.6b	79.4a	76.3b	0.47
Tyrosine	61.0a	55.9b	65.6a	64.1a	1.28

†High moisture barley.
‡Standard error of the mean.
a,b,c Means in the same row with different letters are significantly different ($P<0.05$).

Table V.7 The effect of moisture content and method of processing of barley on the nitrogen balance of growing pigs.

	Ground dry barley	Rolled dry barley	Ground HMB†	Rolled HMB	SEM‡
Nitrogen (g/day):					
Intake	49.2	48.9	48.7	47.9	
Fecal output	9.4	9.8	9.5	10.8	0.30
Urinary output	19.1	16.8	16.5	11.9	1.84
Retained	20.6	22.4	22.7	25.1	1.91
Absorbed	39.7	39.2	39.2	37.1	0.29
Apparent nitrogen digestibility (%)	80.9	80.1	80.5	77.3	0.65

†High moisture barley
‡Standard error of the mean: no significant treatment differences ($P>0.05$)

D. DISCUSSION

There were no significant differences ($P>0.05$) in the apparent ileal digestibility coefficients for dry matter, crude protein, gross energy, ether extract, or starch between the HMB and dry barley diets. Previous studies have indicated that fecal digestibility coefficients for dry matter, crude protein, and gross energy were not significantly influenced by the moisture content of barley (Cole et al. 1970; Pringle et al. 1983).

Anaerobic storage significantly improved ($P<0.05$) the ileal digestibility coefficients for phosphorus and NDF for the ground barley diets but not for the rolled barley diets. It has been reported (Greaves and Hirst 1925, as cited by Pomeranz 1982; Trotter and Allee 1979) that the availability of phosphorus in barley increases during anaerobic storage. The ileal digestibility for phosphorus was significantly higher ($P<0.05$) for the ground HMB diet than for the ground dry barley diet; therefore, anaerobic storage may have caused an increase in the availability of phosphorus. However, the ileal digestibility coefficients for phosphorus were similar for the rolled barley treatments. No significant differences ($P>0.05$) were observed in the fecal digestibility coefficients for phosphorus among the four treatments. It should be noted that since much of the dietary phosphorus was supplied by dicalcium phosphate which is highly digestible by the pig, it would be difficult to detect differences in phosphorus digestibility attributable to the barley component of the diets.

Pringle et al. (1983) reported a 6 percentage point improvement in the fecal digestibility coefficient for NDF for anaerobically stored ground HMB as compared to ground dried barley while the results of this experiment indicated a 9.1 percentage point increase in the ileal digestibility coefficient for this component. Also observed were percentage point increases of 4.4 to 8.0 in the apparent fecal digestibility coefficients of NDF for the ground HMB and rolled HMB as compared to the ground dry and rolled dry barley diets, respectively. NDF is a measure of the total plant cell wall content and includes lignin, cellulose, and hemicellulose (Van Soest and Wine 1967). It has been suggested that fermentation during storage of HMB renders the β-glucan, a major component of the endosperm cell wall, more susceptible to

degradation (Hesselman et al. 1981). Analysis of the diets indicated that the β-glucan content of barley which had been harvested from the same field was not affected by anaerobic storage. This indicates that substantial hydrolysis of β-glucan to glucose did not take place, although incomplete degradation to oligosaccharides may have occurred during storage. This would not have been detected by the analytical procedure used, however.

The NDF contents of the barley diets used in this experiment ranged from 22 to 25% on a dry matter basis. The β-glucan content of these diets was approximately 5% of the dry matter, or approximately 20 to 23% of the NDF content. The ileal digestibility coefficients for NDF were no higher than 54%; however, those of the β-glucan component were as high as 82%. This suggests that barley β-glucan is as highly digestible as starch in the growing pig and that any improvement in the nutritive value of barley during anaerobic storage is due to an improved digestibility of NDF constituents other than β-glucan. These observations are supported by the lack of a significant treatment effect on the ileal digestibility coefficients for starch. Barley β-glucan comprises 75% of the endosperm cell wall and surrounds the starch granules within the endosperm. Because of the high ileal digestibility coefficients for β-glucan which were similar among the four diets, it is unlikely that protection of starch by this cell wall constituent significantly influenced the degradability of starch.

The presence of high levels of β-glucan in barley has been cited as a reason for the inferior performance observed in poultry (Burnett 1966; Hesselman and Thomke 1982) and swine (Honeyfield et al. 1983) fed barley-based diets as compared to corn- or wheat-based diets. However, β-glucanase supplementation of barley-based diets has resulted in only a 6% improvement in ADG in pigs (Newman and Pepper 1984) while improvements of up to 25% have been observed in the ADG of chicks (Mannion 1981; White et al. 1983). The failure to obtain a comparable improvement in the ADG of growing pigs in response to the addition of β-glucanase to the diet as with poultry may be due to the ability of pigs to degrade β-glucan. High levels of β-glucan in barley can lead to very viscous conditions in the alimentary tract of the chick. Increased viscosity of the intestinal contents may be a problem of greater concern in

chicks than in other animals such as pigs since poultry have a lower water content as well as low enzyme activity in the upper part of their digestive tract (Sturkie 1975). In fact, it has been suggested (Munck 1972) that while increased intestinal viscosity due to the feeding of barley-based diets may cause problems in chicks, it may have a beneficial effect in starter pigs by preventing scours. Another possibility for the less dramatic response in pigs fed β-glucanase supplemented barley diets may be that the enzyme is inactivated by the stomach pH of the pig which is much more acidic (pH 2 to 4) than the crop pH of 4.4 to 6.7 in the chicken. The discrepancy between fecal and ileal digestibility coefficients for β-glucan indicates a considerable degree of hindgut fermentation of β-glucan.

One possible explanation for the high digestibility of β-glucan in this experiment was the existence of a heat-stable inhibitor of endo-β-glucanase in the digesta. Such an inhibitor could cause an underestimation of β-glucan content of ileal and fecal samples. This possibility was investigated by spiking samples of digesta with purified barley β-glucan. The recovery factors for β-glucan were similar for the spiked digesta samples and for the purified β-glucan substrate alone. Thus, it is unlikely that heat-stable inhibitors of endo-β-glucanase were present in the digesta.

The digestibility coefficients for dietary nutrients for pigs have traditionally been determined through fecal collection and analysis methods (Schneider and Flatt 1975). However, Zebrowska (1973), as cited by Low (1985), demonstrated that most of the nitrogen from protein and free amino acids infused into the large intestine was not absorbed by the pig for protein synthesis. The amino acids instead undergo modification by the microflora in the hindgut of the pig and most of the absorbed nitrogen is excreted in the urine as ammonia, amines, or amides (Tanksley, Jr. and Knabe 1984). Since the large intestine does not play a role in amino acid absorption by the pig, one would expect a more accurate estimation of amino acid availability by recovery of the amino acids from the ileal digesta than from the feces (Hodgdon et al. 1977; Sauer et al. 1981). Sauer et al. (1981) noted that ileal digestibility measurements give a more sensitive indication of amino acid availabilities than fecal

digestibility measurements since they are not confounded with the effects of hindgut fermentation. The observed tendency for fecal digestibility coefficients of dispensable amino acids to be overestimated relative to ileal digestibility coefficients in this study is in agreement with previous reports (Sauer et al. 1981; Owsley et al. 1981). Of the indispensable amino acids, only the digestibilities of arginine and histidine were overestimated ($P<0.01$) by fecal analysis. In contrast, Sauer et al. (1981) observed an overestimation of fecal versus ileal digestibility coefficients for all indispensable amino acids with the exception of arginine, lysine, and methionine. No clear explanation can be offered for the discrepancies between these reports but it should be noted that while the diets of Sauer et al. (1981) contained approximately 11% crude protein on a dry matter basis, the diets used in the present study contained approximately 18% crude protein on a dry matter basis. Modification of amino acid profiles due to hindgut fermentation may be influenced by the quantity of amino acids present in the digesta. A probable reason for the lower level of significant differences in the ileal digestibility values is that there were missing data due to the difficulty in obtaining sufficient ileal digesta from two of the pigs during the fourth period. One of these pigs was fed the rolled HMB diet; collection of ileal samples from swine fed the rolled HMB-based diet proved difficult because the apparently large particle size of the digesta impaired digesta flow through the cannula. Thus, ileal samples collected from these animals were typically of smaller volume than those collected from pigs fed the other three diets. Although not all of the differences were statistically significant ($P<0.05$), there was a definite trend for improved ileal amino acid digestibilities for pigs fed the HMB diets as compared to those fed the dry barley diets, particularly for the ground HMB diet.

Of particular interest are the ileal digestibility coefficients for lysine, threonine, and methionine, the three most limiting amino acids in barley. The ileal digestibility coefficient for lysine was the highest for pigs fed the ground HMB diet while that of the rolled HMB diet was intermediate between the ileal digestibility coefficients of the ground HMB and dry barley diets. The ileal digestibility coefficients for methionine were also highest for the HMB diets. Although

not significant, there was a trend for improved ileal digestibilities of threonine for the HMB diets. The fecal digestibility coefficients were also highest ($P<0.05$) for all the amino acids measured, except for phenylalanine, for pigs fed the ground HMB diets. However, the improvements in the apparent ileal digestibility coefficients of some of the essential amino acids that were observed for the ground HMB diet were not reflected by improvements in apparent nitrogen digestibility or biological value.

While there was a consistent trend ($P<0.05$) for the ground HMB diet to have improved ileal digestibility coefficients for lysine, isoleucine, valine, and aspartic acid, digestibility coefficients for the rolled HMB diet tended to be intermediate between those of the ground HMB and dry barley diets. This trend was not significant ($P>0.05$) but was observed in the apparent ileal digestibilities of most of the other amino acids studied. Thus, it seems that anaerobic storage of HMB may improve the digestibility of many of the amino acids but that the magnitude of the improvement is dependent upon the method of processing. These observations are consistent with those of Sauer et al. (1977) who observed that increased fineness of grinding improved apparent ileal amino acid availabilities. It appears that grinding, as a method of processing HMB, is preferable to rolling and that anaerobic storage generally improves the digestibility of HMB as compared to dry barley.

VI. CONCLUSION

The results of the present studies suggest that harvesting of barley at a moisture content of 25% results in up to a 20% improvement in dry matter yield as compared to harvesting at a grain moisture content of 13%. This is attributed mainly to a reduction in grain losses during harvesting.

Pigs fed a HMB-based diet consumed significantly ($P<0.05$) more feed on a dry matter basis than did pigs fed a dry barley-based diet. FCE was not significantly affected by diet ($P>0.05$) but there was a trend towards an improvement in ADG of growing swine fed a diet based on HMB. The daily grinding, mixing, and feeding of HMB, the daily removal of uneaten feed, and the lower dust level associated with this diet may have contributed to the observed increased feed intake of the pigs. In addition, fermentation during anaerobic storage may have increased the palatability of the grain.

Moisture content of dietary barley had no significant effect ($P>0.05$) on the apparent ileal digestibilities of the dry matter, crude protein, gross energy, ether extract, or starch components of the barley-based diets. Anaerobic storage significantly improved the ileal digestibility coefficients for phosphorus and NDF for the ground barley diets but not for the rolled barley diets. However, there were no significant differences ($P>0.05$) among treatments in the ileal or fecal digestibilities of the β-glucan fraction. Because the ileal digestibility coefficients for NDF were no higher than 54% while those of the β-glucan component were as high as 82%, it appears that the observed improvement in the digestibility of the NDF component of anaerobically stored HMB for swine is due to an improved digestiblity of NDF constituents other than β-glucan. There was a consistent trend for the ground HMB diet to have improved digestibility coefficients for lysine, isoleucine, valine, and aspartic acid relative to the dry barley diets but digestiblity coefficients for the rolled HMB diet tended to be intermediate between the two. Thus, grinding as a method of processing HMB is preferable to rolling and anaerobic storage generally improves the ileal digestibility of NDF and phosphorus components of HMB as compared to dry barley.

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VIII. APPENDICES

Appendix 1. Amino acid (AA) contents† of experimental diets (Experiment 1) and amino acid requirements for 35 to 60 kg pigs.

	High moisture barley	Dry barley	NAS-NRC (1979) requirements
Indispensable AA, %			
Arginine	0.99	0.92	0.20
Histidine	0.42	0.38	0.18
Isoleucine	0.74	0.64	0.49
Leucine	1.33	1.18	0.58
Lysine	0.89	0.80	0.68
Methionine	0.23	0.20	—
Phenylalanine	0.86	0.77	—
Threonine	0.69	0.60	0.43
Valine	0.97	0.84	0.49
Dispensable AA, %			
Alanine	0.80	0.68	
Aspartic acid	1.47	1.31	
Glutamic acid	3.68	3.35	
Glycine	0.76	0.66	
Proline	1.42	1.27	
Serine	0.85	0.76	
Tyrosine	0.44	0.40	
AA recovery as nitrogen, %	86.03	83.25	

†Dry matter basis.

Appendix 2. Amino acid (AA) contents† of experimental diets (Experiment 2).

	Ground dry barley	Rolled dry barley	Ground HMB‡	Rolled HMB
Indispensable AA, %				
Arginine	1.05	1.01	1.04	1.02
Histidine	0.43	0.44	0.44	0.44
Isoleucine	0.71	0.73	0.77	0.76
Leucine	1.23	1.24	1.36	1.34
Lysine	0.90	0.90	0.93	0.90
Methionine	0.22	0.24	0.24	0.24
Phenylalanine	0.84	0.87	0.88	0.89
Threonine	0.71	0.69	0.74	0.72
Valine	0.90	0.97	1.00	1.00
Dispensable AA, %				
Alanine	0.76	0.77	0.83	0.81
Aspartic acid	1.47	1.45	1.52	1.50
Glutamic acid	3.67	3.70	3.69	3.65
Glycine	0.73	0.74	0.76	0.75
Proline	1.40	1.40	1.44	1.47
Serine	0.84	0.84	0.87	0.85
Tyrosine	0.45	0.43	0.49	0.51
AA recovery as nitrogen, %	85.73	86.50	87.56	87.14

†Dry matter basis.
‡High moisture barley.