

BARLEY β -GLUCAN: AN ANTINUTRITIONAL FACTOR IN POULTRY FEEDING

J. M. McNAB

Institute of Animal Physiology and Genetics Research, Edinburgh Research Station,
Roslin, Midlothian EH25 9PS

AND R. R. SMITHARD

Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne,
Newcastle upon Tyne NE1 7RU

CONTENTS

INTRODUCTION	45
STRUCTURE OF β -GLUCANS	46
MEASUREMENT OF β -GLUCANS	47
BARLEY AS A DIETARY INGREDIENT FOR POULTRY	48
TREATMENT TO IMPROVE THE NUTRITIVE VALUE OF BARLEY	49
Irradiation	49
Effects of water treatment	50
Effects of enzyme treatments.	51
MODE OF ACTION OF β -GLUCAN AND β -GLUCANASE	54
CONCLUSIONS	56
REFERENCES	57

INTRODUCTION

Barley is the essential starting material for the production of beer and malt whisky and is an important component of farm animal diets in many European countries, particularly those in the north. In Scandinavia, barley and oats are virtually the only cereal grains grown for feeding to animals. Because its fibre content is relatively high (about 50-60 g/kg) and is poorly digested by birds, barley is generally classed as a low energy cereal (Herstad, 1987). However, the overriding feature which makes barley unpopular as a constituent of poultry diets is the presence in the grain of a polysaccharide known as mixed-linked β -glucan, often abbreviated to β -glucan. The mixed-linked (1 \rightarrow 3) (1 \rightarrow 4)- β -glucans occur together with arabinoxylans and mannose-containing polysaccharides in cereal cell walls. In barley it constitutes about 70% of the starchy endosperm walls and about 25% of isolated aleurone cell walls (Åman & Graham, 1987). Although cellulose and mixed-linked β -glucan are both polymers of β -D-glucose residues, the mixed-linked β -glucan differs from the highly regular β -(1 \rightarrow 4) structure of cellulose in having both 4-O-linked β -D-glucopyranosyl units (~ 70%) and 3-O-linked β -D-glucopyranosyl units (~ 30%) (Parrish *et al.* 1960; Perlin & Suzuki, 1962; Luchsinger *et al.* 1965; Clarke & Stone, 1966; Fleming & Manners, 1966; Woodward *et al.* 1983a; Aspinall & Carpenter, 1984).

The low digestibility of mixed-linked β -glucan by poultry and its propensity to form gels in aqueous media are believed to be responsible for the excretion of sticky droppings which

reduce the water holding capacity of the litter on which broilers are reared. This can cause hock problems and damage to the breasts of birds, thereby reducing the quality and market value of the meat products. In laying birds kept on raised wire floors the droppings adhere to the mesh and mark the eggs as they roll away, also lowering their quality and marketability (Herstad, 1987). Cereal mixed-linked β -glucan is of significance in two other areas. In brewing barley mixed-linked β -glucan is considered to influence the rate at which cereal modifies during malting and to interfere with filtration and reduce the clarity of the beer (Henry, 1987). In human nutrition mixed-linked β -glucan is believed to be involved in the beneficial effects that barley and oat products are claimed to exert on plasma cholesterol (Davidson *et al.* 1991), and oat products on post-prandial glycaemia (Braaten *et al.* 1991).

STRUCTURE OF β -GLUCANS

The polymers which constitute cereal mixed-linked β -glucan are composed of β -D-glucopyranose units joined by (1 \rightarrow 3) or (1 \rightarrow 4) β -glycosidic bonds. In mixed-link β -glucan the pattern of the β -1,3 and β -1,4 linkages follows no set order, but neither does the occurrence of the two types of bonds appear completely random (Staudte *et al.* 1983). Selective enzymolysis of mixed-linked β -glucan with cellulase (EC 3.2.1.4) or with laminarinase (EC 3.2.1.39) indicates that most (85–95%) of the polymer is composed of two main structural units, (1 \rightarrow 3)-linked cellotriosyl and (1 \rightarrow 3)-linked cellotetraosyl units (Parrish *et al.* 1960; Woodward *et al.* 1983a). Methylation analysis suggests that a small proportion (5–15%) of the polymer may comprise longer cellulose-like sequences (4–11 units) of (1 \rightarrow 4)-linked glucose residues (Woodward *et al.* 1983a; Wood *et al.* 1991). Whilst some evidence for the presence of stretches of contiguous β -(1 \rightarrow 3)-linked glucose units has been presented (Bathgate *et al.* 1974; Fleming & Kawakami, 1977), carbon-13 nuclear magnetic resonance of barley β -glucan (White *et al.* 1983; Wood *et al.* 1991) and methylation of oligosaccharides produced by the hydrolysis of the β -glucan with lichenase (EC 3.2.1.73) (Edney *et al.* 1991) have both failed to confirm the existence of multiple sequences of β -(1 \rightarrow 3)-linked residues. ^{13}C -NMR has also confirmed the absence of α -(1 \rightarrow 4) bonds (White *et al.* 1983). HPLC of lichenase hydrolysis fragments and ^{13}C -NMR of the β -glucans both indicate that there is a small difference between the structures of the mixed-linked β -glucans from barley and oats; in oat β -glucan one third is composed of β -(1 \rightarrow 3)-linked cellotriosyl units but in barley β -glucan these account for only one quarter of the polymer (Wood *et al.* 1991). Using sedimentation ultracentrifugation, Woodward *et al.* (1983b) determined the weight average and number average molecular weights for barley water-soluble β -glucan to be 290000 and 210000 respectively. However White *et al.* (1983) found, on the basis of reducing sugar analysis, that β -glucan isolated from the intestinal contents of chicks had an average molecular weight of 47000; the elution volume from Bio-Rad P-60 which they reported was equivalent to that of a globular protein of at least 60000 Daltons. The weight average (71900) and number average molecular masses (49000) of β -glucan from oat aleurone has been determined following fractionation by preparative size exclusion chromatography (Varum *et al.* 1991).

The presence of both (1 \rightarrow 3) and (1 \rightarrow 4) bonds in the β -glucan chains causes irregularities which are absent from cellulose. In the β -(chair) form of glucopyranose (Figure 1, II), the building unit of both mixed-linked β -glucans (Figure 1, III) and cellulose (Figure 1, IV), all the hydroxyl groups and the hydroxymethyl group have equatorial conformations; this contrasts with the α -form (Figure 1, I) in which the hydroxyl group attached to carbon 1 is in the axial orientation. In cellulose the regularity of the polymer chains and the equatorial orientation of the hydroxyl groups facilitate extensive hydrogen bond cross-

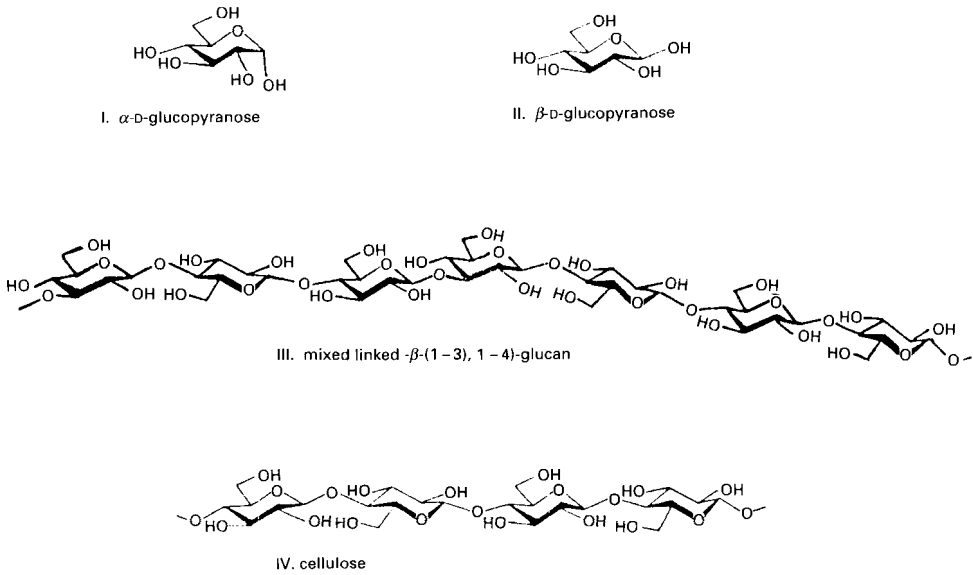


Fig. 1. Conformational formulae of α -D-glucopyranose, β -D-glucopyranose, mixed-linked β -(1 \rightarrow 3), (1 \rightarrow 4)-glucan and cellulose (adapted from Åman & Graham, 1987).

linking which confers crystallinity and resistance to solubilization in water. In the more water-soluble mixed-linked β -glucans such extensive interchain cross-linking is stereochemically not possible and these polymers readily form viscous gels. The difference between soluble and the insoluble mixed-linked β -glucan does not appear to have been elucidated but it has been suggested that the presence in the latter of long-sequences (up to 10) of β -(1 \rightarrow 4)-linked glucose units may permit close intermolecular association of part of the mixed-linked β -glucan with other cell wall polysaccharides such as cellulose or arabinoxylans (Åman & Graham, 1987).

MEASUREMENT OF β -GLUCANS

The application of analytical methods which eliminate the need for extraction and which are specific to the (1 \rightarrow 3), (1 \rightarrow 4)- β -glucans has facilitated research and development in plant breeding in the role of the (1 \rightarrow 3), (1 \rightarrow 4)- β -glucans in human and animal nutrition. Methods of analysis which depend on the extraction of the β -glucans have been described (Gill *et al.* 1982) but although extractions of β -glucans with hot perchloric acid (Ahluwalia & Ellis, 1984) and hydrazine (Martin & Bamforth, 1981) have been reported to be complete, doubt remains whether all the relevant material has been completely extracted and whether degradation products formed during the extraction procedure from polysaccharides other than β -glucans might interfere with the final value.

The use of endo- β -1,3-1,4-glucanase (*EC* 3.2.1.73) which has no activity on either β -1,3-glucans or β -1,4-glucans to achieve selectivity currently provides the highest degree of analytical specificity (Anderson *et al.* 1978). Because this enzyme may be used directly, on milled grain, the constraints imposed by extraction do not apply. However, the purity of the enzyme must be assured; contamination of the β -glucanase by, for example, bacterial amylase from which it is frequently isolated can lead to the overestimation of the β -glucan content. The oligosaccharides released by specific hydrolysis with (1 \rightarrow 3), (1 \rightarrow 4)- β -glucanase have been measured as glucose following either acid hydrolysis (Anderson *et al.*

1978) or hydrolysis with glucosidase (McCleary & Glennie-Holmes, 1985). The oligosaccharides have also been estimated without further hydrolysis as reducing sugars, using *p*-hydroxybenzoic acid hydrazide as the chromophore (Henry, 1984), but because the oligosaccharides are an uncharacterized mixture this method needs careful calibration against an appropriate β -glucan standard. The dependence of these methods on glucose or reducing sugar as the final analyte makes it essential to minimize interference from reducing sugars in the sample either by extraction with aqueous ethanol, before introducing the β -glucanase, or by adjusting results by means of a blank determination. Hydrogenation of reducing sugars present in the sample to alditols with sodium borohydride prior to specific (1 \rightarrow 3), (1 \rightarrow 4)- β -glucanase hydrolysis has also been used to reduce interference to very low levels (Henry & Blakeney, 1986, 1988).

The ability of β -glucans to form gels has been used in their estimation by viscometry of aqueous extracts (Burnett, 1966). Dependence upon extraction, the potential contribution from other constituents of the grain and the influence of molecular weight distributions on solution viscosity (White *et al.* 1981) all place severe limits on the reliability of this method. A further limitation is that it can be applied only to that portion (25–45%) of the β -glucan which is water soluble. Nevertheless viscometry may be relevant in interpreting the effects of β -glucans on digestion.

A system has been developed for determining β -glucan concentrations in large numbers of samples by specific binding of the fluorochrome, Calcofluor, to the β -glucan (Aastrup & Jørgensen, 1988). Calcofluor preferentially precipitates barley β -glucan from aqueous solutions containing protein, pentosan and starch (Wood & Fulcher, 1978); it has a weak affinity for polysaccharides containing pentoses and galactose but does form a complex with cellulose. When applied to suspensions prepared from barley or malt results from the fluorimetric technique correlated moderately well ($r^2 = 0.86$ for barley, 0.95 for malt) with assays made by precipitation of β -glucan with ammonium sulphate (Jensen & Aastrup, 1981). When Calcofluor binding was applied, using flow injection analysis, to solutions of β -glucans extracted from barley and malt with perchloric acid, correlations with values obtained by enzymic assay were appreciably higher ($r^2 = 0.95$ for barley, 0.97 for malt; Jørgensen & Aastrup, 1988).

BARLEY AS A DIETARY INGREDIENT FOR POULTRY

Barley has long been considered inferior to either maize or wheat as an ingredient in poultry diets. Its effect on production has been most clearly evident in the rates of growth of young birds. In experiments where maize directly replaced barley on a weight basis, the mean 8-week weight of birds fed on the maize-based diet (1160 g) was 10% higher than that of birds fed on the barley-based diet (982 g); this was accompanied by an 11% advantage in gain:feed ratio for the maize diet (Berg, 1959). Speculation at that time ascribed the low nutritive value of barley to the indigestible fibre of the hull, and plant breeders developed a variety which loses its hull during harvesting. However, it was subsequently shown that there was little difference in feeding value between the hull-less barley and conventional barley varieties (Anderson *et al.* 1961). In part of a series of 28 experiments, Anderson *et al.* (1961) subsequently showed that the mean 4-week weight gain of broiler chicks was reduced by 19% when hull-less barley replaced maize in their diets but little or no difference could be detected between the performances of birds fed on diets containing hull-less and conventional hulled barley. Although the performances of the birds used in these experiments compare unfavourably with those achieved by strains of birds and diets used in current commercial practice, they do clearly indicate the extent to which barley is at a

disadvantage. With mature hens the extent to which dietary barley affects the efficiency of egg production seems to be greatly influenced by the type of cage management; group-caged birds fed on a maize-based diet required 15% less food per dozen eggs than birds fed on a barley-based diet, but the difference was only about 2% when the birds were kept in individual cages (Berg, 1959). Another concern in practical poultry production has been the incidence of larger volumes of wetter droppings when barley replaced other cereals. It was frequently noted that birds fed on barley-based diets tended to drink more water than those fed on maize-containing diets (Willingham *et al.* 1959; Arscott & Rose, 1960); for example, Berg (1959) found that birds fed on a barley diet consumed more (12–28%) water than those fed with a maize diet. The production of sticky droppings was another problem almost always encountered with birds fed on diets containing barley. Hesselman *et al.* (1981) found that the incidence (22–52%) of sticky droppings clinging to the down of 4-day old chicks fed on a barley diet was 7% higher than that among chicks fed on a commercial diet containing no barley.

There appeared to be little difference in the nutritive values of several different varieties of barley which were grown in the same area of the USA (Willingham *et al.* 1959) but evidence from both the USA (Willingham *et al.* 1960*a*) and Sweden (Gohl & Thomke, 1976) suggests that the geographical area of production can affect the nutritional properties of barleys. Whilst differences in their β -glucan contents or their endogenous β -glucanase activities seem obvious explanations for these effects, it should be noted that in the Swedish experiment there were also substantial differences in the tannin and crude protein contents of the different samples of barley which were studied. However, Coon *et al.* (1979) have reported that there is only a very weak correlation ($r = -0.041$) between chick weight gain and the tannin content of the different varieties of barley used in the diets. Their finding that supplementation of the diets with threonine or lysine had a profound effect on the ranking of the diets based on different barley varieties introduced two other variables into the factors determining the nutritive value of barley.

TREATMENT TO IMPROVE THE NUTRITIVE VALUE OF BARLEY

Because of its agronomic importance in many parts of the world, frequent attempts have been made to explain the shortcomings of barley and to identify ways of improving its nutritive value for chickens. Both before and since the recognition of β -glucan in the grain as a factor in the poor performance of chicks fed on barley-based diets, many experiments involving treatment of barley have been carried out. These treatments can be classed under the following three headings: irradiation, water treatment and subsequent drying, enzyme addition.

These experiments have identified some processes which are able to improve the status of barley as an ingredient in poultry diets and some have clearly implicated β -glucan as one of the factors which affect its nutritional value. Nevertheless it remains unclear whether the removal or destruction of β -glucan alone would make barley equal to wheat as a source of protein and energy for poultry.

Irradiation

Gamma irradiation is the most recent and least studied of the measures which have been used in attempts to ameliorate the adverse effects of barley on poultry nutrition. Although gamma irradiation has received most attention as a means of decontaminating or sterilizing foodstuffs, it has also been reported to improve the feeding value of barley and its hull-less counterpart for chicks (Classen *et al.* 1985; Campbell *et al.* 1986). These effects have been interpreted in terms of depolymerization of β -glucan. The likelihood that this treatment will

lead to either fruitful scientific insights or commercially successful or acceptable processes must be viewed with some doubt.

Effects of water treatment

Despite reports that the growth of chicks was either depressed (Hamm, 1958) or unaffected (Newman *et al.* 1985) when water-treated barley replaced the untreated cereal in their diets, there is fairly convincing evidence from many other sources that soaking barley in water improves its nutritive value to the young chick. Although water treatment has been under investigation for many years (Fry *et al.* 1957) it is still not certain which components of the barley are affected by the water. Adams & Naber (1969) found that soaking barley grains in water for between 16 and 24 h and drying them before incorporating them in a diet elicited an 8% increase in 4-week chick weight. However, similar improvements in the nutritive value of equivalent maize- and wheat-based diets brought about by this type of treatment suggest that it is not a change in the β -glucan of barley alone which is responsible for the effect of soaking in water. The fact that the improvements in the nutritive value of barley and wheat elicited by treatment with either water or hydrochloric acid (0.1 mol/l) in these experiments were similar (Adams & Naber, 1969) renders unlikely the suggestions that bacterial enzyme activity (Thomas *et al.* 1961) or synthesis of antibiotic (Willingham *et al.* 1960*b*) during the soaking are responsible for the observed improvement. Improvements in the 3-week weights of chicks fed on barley-based diets brought about by wetting ground barley of different cultivars with an equal weight of water (40°) followed by oven-drying (70°) were prodigious (35–67%); the live weights achieved with the water-treated barley of all the cultivars exceeded that gained with a corresponding maize diet (Willingham *et al.* 1959). These improvements greatly exceeded the 12% increase reported by Anderson *et al.* (1961) for a similar diet based on hull-less barley.

Whilst there is a considerable body of data detailing the improvement in the nutritive value to young growing birds of barley that has been subjected to water treatment, the evidence for a corresponding improvement in the performance of mature birds is less convincing. Berg (1959) did report that hens fed on diets containing barley which had been soaked in water required about 2% less food to produce a dozen eggs than those which had been fed on diets containing untreated barley but no statistical significance was indicated for these results.

The improvements in nutritive value gained from treating barley with water did not appear to be variety dependent or influenced by proximate composition (Willingham *et al.* 1959, 1960*a*) but grain grown in the western United States responded more than that from the mid-west. It has generally been assumed that the warm, humid conditions prevailing in the east and mid-west during maturation of the crop induced the same changes as water treatment. There may also be differences in endogenous β -glucanase activity between different barley cultivars and between barleys grown at different locations. Novacek & Petersen (1967) showed that the husk, pericarp, germ, aleurone and endosperm all responded slightly to soaking but the improvement with unfractionated barley was greater than could be accounted for by the sum of the improvements observed in the various anatomical components. Because the way in which the endosperm was isolated influenced the extent of that fraction's response, the greatest effect being observed with intact endosperm, the effect caused by soaking was attributed to the rupture of the cell walls. It is possible that water treatment may disrupt the protective barrier of the β -glucan and release the contents, mainly starch and protein, to the digestive processes (Hesselman & Åman, 1985). This would be consistent with the increased availability of energy which appears to be a characteristic feature of water-treated barley (Table 1). Gohl (1977) has

Table 1. *Metabolizable energy (MJ/kg) of barley, untreated, water-treated and supplemented with enzymes, in diets of poultry*

Barley	Metabolizable energy			References
	Untreated	Water-treated	Enzyme supplemented	
Pearled	12.6	15.9	15.6	Leong <i>et al.</i> 1958, 1962
Pacific Northwest	13.1	17.2	15.0	Leong <i>et al.</i> 1962
Western USA	10.8	13.2	12.8	Potter <i>et al.</i> 1965
British untreated	11.5	—	11.8	Herstad & McNab, 1975
British autoclaved	11.2	—	11.8	Herstad & McNab, 1975
Ingrid	11.8–12.6	—	—	Gohl & Thomke, 1976
Hulled	14.5	—	—	Classen <i>et al.</i> 1985
Hull-less	15.2	—	—	Classen <i>et al.</i> 1985
Bedford	12.2	—	12.2	Rotter <i>et al.</i> 1990
Bonanza	12.5	—	13.1	Rotter <i>et al.</i> 1990
Scout	11.1	—	13.9	Rotter <i>et al.</i> 1990
Harrington	13.0	—	13.3	Rotter <i>et al.</i> 1990

presented evidence to indicate that stimulation of endogenous β -glucanase and its subsequent action on β -glucan, as happens during malting (Bamforth, 1982), is one of the factors contributing to the increase in nutritive value of barley as a result of water treatment. Beneficial alterations to the structure of the starch (Fry *et al.* 1957, 1958; Potter *et al.* 1965) have also been proposed as contributory factors. Lawrence (1976) suggested that soaking improves the nutritive value of barley to the pig by converting damaged starch into the more digestible maltodextrins. Allowing barley grains to germinate before drying, grinding and incorporating into diets did not elicit any growth response in broiler chickens (Adams & Naber, 1969). The demonstration by Potter *et al.* (1965) that the increase in metabolizable energy of water-treated barley could be accounted for by significant increases in digestible crude protein and crude fat, and that there were only minor increases in digestibility of nitrogen-free extract casts some doubt on the suggestion that changes in starch are significant factors in the improvement induced in barley by water treatment.

Unlike supplementation with β -glucanase or other enzymes, interest in water treatment to improve the nutritive value of barley has died. This is probably because giving foods as a slurry or wet mash does not accord with normal broiler husbandry practices and because drying the wet grain or meal, which would be needed to allow water-treated barley to fit in with dry feeding, would incur prohibitive energy costs.

Effects of enzyme treatments

Improvements in the performance of poultry fed on diets containing barley to which enzymes had been added were first reported more than 35 years ago (Jensen *et al.* 1957). It was shown that adding between 1.0 and 4.5 g/kg of a very crude enzyme preparation consisting of takadiastase (*EC* 3.4.23.6) and clarase to diets containing 635 g pearled barley/kg increased the weight attained by broilers at 4 weeks of age by just over 11%. Corresponding improvements in food conversion efficiency were also noted. In what was a very popular topic of research at that time, similar observations were made by many others (Wharton *et al.* 1958; Willingham *et al.* 1958, 1959; Arscott & Rose, 1960; Anderson *et al.* 1961; Rose & Arscott, 1962). Whilst these studies provided necessary verification of the phenomenon their limited empiricism contributed little, if anything, to an understanding of the aetiology of the problem or the basis of the observed improvements. The common

Table 2. *Effect of adding crude enzyme preparations to barley-based diets on weight gain and gain/feed ratio of chickens*

Increase (%) in		
Weight gain	Gain/feed ratio	References
10.2-18.5	5-15	Arcscott & Rose (1960)
3.8-31.5	0-12.1	Willingham <i>et al.</i> (1959)
0-26.2	0-19.2	Willingham <i>et al.</i> (1959)
-3.9-26.3	0-11.0	Willingham <i>et al.</i> (1959)
7.0-14.4	3.4-11.7	Anderson <i>et al.</i> (1961)

feature of all these studies was the improvement that was conferred on the performance of broilers fed on diets based on barley or pearled barley by the addition of crude fungal or bacterial enzymes (Table 2). The inclusion of malted barley (25-100 g/kg) was also shown to induce similar effects (Laerdal *et al.* 1959; Willingham *et al.* 1959; Anderson *et al.* 1961; Rose & Arcscott, 1962). The other two unwelcome features associated with barley-based diets—high water consumption by birds and the production of sticky droppings—were also invariably alleviated by supplementation of the diets with the enzyme preparations. Although only small increases in excreta dry matter content have been noted (288 → 302 g/kg), they have been accompanied by significant improvements in visual scoring (1.6 → 2.5; on a scale where 0 = clean, 4 = very dirty) of cage cleanliness (Hesselman *et al.* 1981). The usual route of administration of the enzyme preparations has been their inclusion in the diet. When enzyme supplementation of the diet was compared with inclusion of enzyme in drinking water, the dietary route gave superior improvements in performance but no measurement was made of enzyme intake via the drinking water (Hesselman *et al.* 1981).

Despite the fairly substantial research effort directed at improving the nutritive value of barley-based diets, results were often equivocal and clear recommendations, other than avoidance of barley in poultry diets, were rarely made. Enzyme preparations rich in α -amylase (EC 3.2.1.1) activity were effective in some experiments (Fry *et al.* 1957; Herstad & McNab, 1975; Moss *et al.* 1977; Mannion, 1981) but proved ineffective in others (Willingham *et al.* 1959; Anderson *et al.* 1961). Poor definition of the principal enzyme and the presence of enzymes of unknown activity would explain some of these apparently conflicting findings. Other variables which are now known to have contributed to differences between the results obtained in different laboratories are the variety of the barley, the area in which it was grown, the climate during the growing season and its ripeness (Laerdal *et al.* 1959; Willingham *et al.* 1960a; Hesselman & Aman, 1986). It is also worth noting that effects of treatment on metabolizable energy observed in more recent experiments tend to be smaller than those reported in early work (Table 1). It is likely that this reflects developments in the techniques for measuring metabolizable energy. Two of the major factors in these developments which are likely to have a bearing on the results are improved precision of the measurement of metabolizable energy and the increased use of mature birds in the determinations.

In each study, except that of Newman *et al.* (1985) who used waxy barley, where comparison has been made between water treatment of barley and supplementation of barley-based diets with enzyme, the improvement brought about by the more prolonged treatment with water followed by drying before using in the diet has been superior to that of enzyme supplementation.

Supplementation of barley-based diets with a crude fungal enzyme (Leong *et al.* 1958, 1962) achieved a substantial increase in metabolizable energy (Table 1). Similar increases have been reported by a number of others (Stutz *et al.* 1961; Potter *et al.* 1965; Morimoto *et al.* 1966; Herstad & McNab, 1975; Mannion, 1981; Hijikuro, 1983; Broz & Frigg, 1986). Potter *et al.* (1965) ascribed the improvement (18%) in metabolizable energy to big increases in the digestibilities of the protein (45 \rightarrow 75%), fat (0 \rightarrow 76%) and nitrogen-free extract (75 \rightarrow 81%) of the barley. Enhanced retention of dietary nitrogen has also been reported (Herstad & McNab, 1975; Mannion, 1981). Rotter *et al.* (1990) found that different varieties of barley incorporated into semipurified diets gave different responses in metabolizable energy ranging from 0 to 25% but in each case there was an increase in the apparent protein digestibility of the diet. Broz & Frigg (1986) reported modest changes in fat digestibility (72.5 \rightarrow 74.5%) and in nitrogen retention (48.4 \rightarrow 51.5%) and Classen *et al.* (1985) reported small increases in the absorption of fat (65.7 \rightarrow 77.6%) and starch (84.1 \rightarrow 88.8%).

Fermentation of waxy barley with *Rhizopus oligosporus*, the organism used to produce the Indonesian fermented soyabean food, tempeh, gave bigger increases in liveweight gain and gain/feed ratio than either water treatment or supplementation of barley-based diets with β -glucanase (Newman *et al.* 1985).

Study of the effects of enzyme supplementation of poultry diets was moved substantially forward by the isolation of β -glucanase from a crude bacterial amylase prepared from *Bacillus subtilis* (Moscatelli *et al.* 1961; Rickes *et al.* 1962). The purified enzyme was shown to hydrolyse barley β -glucan *in vitro* and, when added to diets containing barley, produced a pronounced growth response in chicks. This established for the first time the relationship between β -glucanase activity and the growth of chicks fed on diets containing barley. The involvement of β -glucan in the poor performance of barley was confirmed by Burnett (1966) who showed that the conditions in the chicks' intestines encourage the formation of a viscous solution. He suggested that digestion and absorption of protein could be reduced although a mechanism was neither proposed nor tested.

A resurgence of interest in feeding barley to poultry, arising in part from the readier availability of enzyme preparations rich in β -glucanase, has augmented understanding of the effects of β -glucan on the digestive processes but most of the studies have been directed towards finding how best to counteract them. Many of these experiments have endorsed the findings of increased weight gain and gain/feed ratio (Table 3) that were achieved in earlier studies with crude enzyme preparations of poorly specified activities (Gohl *et al.* 1978; Hesselman *et al.* 1981, 1982; Mannion, 1981; Campbell *et al.* 1984; Hesselman 1984; Classen *et al.* 1985; Broz & Frigg, 1986; Hesselman & Åman, 1986; Elwinger & Säterby, 1986; Petterson *et al.* 1990; Salih *et al.* 1991). Many of the studies have also shown that food intake is increased by the addition of enzymes rich in β -glucanase to barley-based diets. Enzyme preparations, exhibiting β -glucanase activity, have been isolated from a number of bacterial and fungal sources, including *Bacillus amyloliquefaciens*, *B. subtilis* and *Trichoderma viride*, and used to ameliorate the adverse effects of barley on chick growth. The actions of these enzymes are also believed to be the cause of increased excreta dry matter concentration and the decreased viscosity and stickiness of faeces from birds fed on barley-based diets (White *et al.* 1981; Broz & Frigg, 1986; Elwinger & Säterby, 1986; Hesselman & Åman, 1986; Jeroch *et al.* 1991).

The addition of enzyme preparations which include β -glucanase activity undoubtedly enhances the nutritive value of barley-based diets for chickens. However, there are two issues having a bearing on the practical value of supplementing diets with enzymes in commercial broiler production which have not yet been investigated adequately. One is the effect of treatments applied to diets during feed compounding, which could both disrupt the

Table 3. *Effect of adding β -glucanase to barley-based diets on feed intake, weight gain and gain/feed ratio of broiler chickens*

Increases (%) in			References
Feed intake	Weight gain	Gain/feed	
—	4.4	5.6	Gohl <i>et al.</i> (1978)
—	35.3–38.1	15.9	Gohl <i>et al.</i> (1978)
—	10.5	13.2	Suga <i>et al.</i> (1978)
24.4–32.4	35.1–42.6	8.0–15.5	Hesselman <i>et al.</i> (1981)
9.6–16.1	17.1–21.0	5.5–10.1	Hesselman <i>et al.</i> (1981)
20.2–22.6	22.9–26.9	2.1–5.5	Mannion (1981)
1.1–14.0	19.7–26.0	10.1–19.3	Mannion (1981)
—	2.0–6.0	1.11–6.2	Rexen (1981)
5.2–13.6	13.1–22.5	6.2–10.4	White <i>et al.</i> (1981)
15.5–16.8	24.9–29.3	4.1–11.0	Hesselman <i>et al.</i> (1982)
13.5–13.8	20.2–23.5	5.8–8.6	Hesselman <i>et al.</i> (1982)
—	3.5–17.6	0–10.9	Hijikuro (1983)
5.9–16.1	10.1–20.7	3.0–6.1	Campbell <i>et al.</i> (1984)
—	10.2	4.4	Classen <i>et al.</i> (1985)
—	4.8	9.6	Newman <i>et al.</i> (1985)
—	6.4–16.6	3.5–7.9	Broz & Frigg (1986)
10.0–17.7	5.7–25.5	14.6–19.0	Hesselman & Åman (1986)
11.8–19.5	0.7–27.6	21.4–37.5	Hesselman & Åman (1986)
3.4–8.9	7.0–17.8	4.4–8.6	Elwinger & Säterby (1986)
0–12	2–41	3–20	Rotter <i>et al.</i> (1989)
113–119	199–223	40.0–47.2	Pettersson <i>et al.</i> (1990)

physical structure of the food and affect the stability of the supplementary enzymes; the other is the effect which the pH changes and the endogenous enzymes of the digestive tract might have on supplementary enzymes as the food passes through the avian gut. More than 30 years ago Arscott & Rose (1960) showed that pelleting alone achieved as big an increase in gain/feed ratio, in broiler chickens fed on barley-based diets, as enzyme supplementation and it produced an even bigger increase in liveweight gain. Broz (1989), using enzyme preparations which have been developed more recently, has also demonstrated that pelleting and enzyme supplementation produce similar benefits. A degree of resistance of supplementary enzymes to denaturation by the temperatures and shearing forces to which feeds are subjected has been inferred from growth performance experiments. For example, McCracken *et al.* (1993) have presented results which suggest that supplementary enzymes remain effective as a means of enhancing the performance of broiler chicks fed on barley-based diets, even after both pasteurization and pelleting. However, it is still not known, from direct assay of enzyme activities, what proportion of the added enzymes remains active after feed compounding, nor are the proportions of supplementary enzymes remaining active in the different sections of the avian gut known. It would appear that the major obstacle to progress in this area lies in the difficulty of reliably measuring the enzyme activities within the matrix of diets and digesta.

MODE OF ACTION OF β -GLUCAN AND β -GLUCANASE

Despite the many poultry feeding experiments it is still not clear precisely what effects β -glucan has on digestion. There has been no lack of theories to explain the consequences of β -glucan consumption but experiments set up to test proposed models have sometimes produced equivocal results. A popular, simple and credible hypothesis is that β -glucan, by

virtue of its location, and perhaps its role in the grain, hampers the release of the nutrients contained in the endosperm to the digestive processes of the gastrointestinal tract of the bird. Explicit demonstration that this happens is not available but observations that digestibility of fat, nitrogen and starch (Classen *et al.* 1985; Broz & Frigg, 1986; Hesselman & Aman, 1986) are increased when β -glucanase is added to barley-based diets could be explained in terms of such an interaction. Interestingly in this context, intact fragments of undigested endosperm were frequently found in the excreta of chicks fed on a diet containing hull-less barley. In contrast however, the small increases (6–10%) in slaughter weights of birds when a β -glucanase preparation from *B. amyloliquefaciens* was added to barley-based diets were not accompanied by any change in the digestibilities of the major nutrients (Jeroch *et al.* 1990); the improved performance in that experiment was ascribed solely to increased food intake. Improved digestibility coefficients would also explain the higher metabolizable energy values observed when enzymes are added to chick diets containing barley (Potter *et al.* 1965; Herstad & McNab, 1975; Mannion, 1981; Hijikuro, 1983; Broz & Frigg, 1986; Rotter *et al.* 1990).

Whether the formation of β -glucan gels in the intestine exert their adverse effect on growth through food intake depression or through impaired efficiency of digestion is unresolved. Destruction of the gelling properties of barley β -glucan by addition of dietary β -glucanase is believed to increase the rate of passage of digesta through the chicken intestine and thereby result in increased food consumption (Hesselman *et al.* 1982; Campbell *et al.* 1984; Broz & Frigg, 1986). Salih *et al.* (1991) found that, up to an age of 6 weeks but especially in very young chicks, increases in weight gains brought about by addition of β -glucanase to barley-based diets were accompanied by reductions in transit times by as much as 17% and in digesta viscosity of 42%; on the other hand fat digestibility at most was increased from 0.532 to 0.763. In animals with simple stomachs the presence of viscous gel in the stomach or in both the stomach and intestine slowed gastric emptying and small intestinal transit (Meyer *et al.* 1988, Meyer & Doty, 1988) but whether a similar mechanism might apply in the quite different digestive tract of the chicken remains a matter for conjecture. It has been suggested that the formation of a viscous solution in the intestine impedes the mixing of the gut contents and consequently influences transport of nutrients at the surface of the mucosa (White *et al.* 1983). The concept of an unstirred liquid layer between the surface of the mucosa, tangential to the apices, and the digesta is used by physiologists to explain absorption in kinetic terms (Johnson & Gee, 1981; Davenport, 1983). Viscous polymers slow the absorption of glucose from gut sections *in vitro* (Elsenhans *et al.* 1980, 1981; Johnson & Gee, 1981) and from the human small intestine *in vivo* (Flourie *et al.* 1984). It is not known whether the effective thickness of this unstirred layer is affected by the viscosity of the intestinal contents, but it seems reasonable to speculate that any increase in the viscosity of the digesta will be likely to slow down the migration of nutrients and allow their accumulation in the contents of the intestine. Meyer *et al.* (1988) have postulated that the presence of viscous gels in the guts of simple-stomached animals lowers plasma glucose by slowing both the rates of gastric emptying and glucose absorption in the small intestine; the slow absorption allows accumulation of glucose in the digesta where it results in increased stimulation of intestinal sensors with consequent increased feedback inhibition of the stomach. No comparable studies have been made with birds. However a build up of nutrients in the intestine might be expected to favour an increased population of microflora and may offer an explanation for the higher concentrations of microorganisms observed in the guts of chickens fed on diets containing barley (Elwinger & Säterby, 1986).

The improvement in bird performance achieved by supplementation of barley-based diets with β -glucanase has been associated with a reduction in digesta viscosity resulting

from β -glucan hydrolysis (White *et al.* 1981). Digesta viscosity from birds fed on a diet based on hull-less barley supplemented with β -glucanase was between 0.58 and 0.82 times that of birds fed on the same diet without added enzyme (Salih *et al.* 1991). However appealing the simple notion may be that viscosity is the prime determinant of the poor performance of birds fed on diets containing barley, it fails to explain the decline in the adverse effect as the birds grow older. In the experiment by Salih *et al.* (1991) although birds fed on the hull-less barley-based diet were still 9.4% lighter at 8 weeks of age than those fed on the same diet supplemented with β -glucanase, between 6 and 8 weeks there was no difference in weight gain. In that trial the advantage in weight gain conferred by the enzyme treatment declined from 75% up to 2 weeks, to 26% at 4 weeks and 6% at 6 weeks. Up to 2 weeks of age enzyme supplementation did elicit a big improvement (28%) in gain/food ratio but after that the effect was absent. Furthermore, even while the gap in performance between those birds which did and those which did not receive enzyme was closing there remained a significant difference between the viscosity of their digesta. The results of this experiment, taken as a whole, suggest that digestibility is affected only in quite young birds but that the principal mechanism in older birds operates through lower food intake associated with the impaired flow of digesta through the gut.

As birds fed on the unsupplemented barley-based diet aged the changes in digesta viscosity and passage rate indicated either an adaptation or change in the ability of the bird to cope with the presence of β -glucan in the diet (Salih *et al.* 1991). *Streptococcus faecium* declined rather erratically as a proportion of the microbial population in the duodenum of Single Comb White Leghorn cockerels aged to 16 weeks. Salih *et al.* (1991) suggest that intestinal microflora, including *S. faecium* which predominate in the young bird may deconjugate bile acids (Coates *et al.* 1981; Campbell *et al.* 1983*a, b*) and lead to malabsorption of fat. The improvement in fat digestion as the birds grow older may be a consequence of the change in microbial population. It is also possible that the change in intestinal microflora includes the proliferation of bacteria capable of degrading β -glucans. In the piglet the population of lactobacilli capable of degrading β -glucans appears to be quite volatile (Jonsson & Hemmingsson, 1991) but it remains to be determined whether or not β -glucan degrading bacteria play a part in the adaptation of birds to barley-based diets.

CONCLUSIONS

An abundance of experiments has shown that barley containing β -glucan in diets of young chickens impairs growth and can lead to management problems arising from the production of sticky droppings. Many of them have shown that the deleterious effects of barley are ameliorated either by treatment of the barley with water or by supplementation of the barley-based diets with enzyme preparations containing β -glucanase activity. Adverse effects on the digestion of nutrients and on the passage of digesta through the gut have been implicated in the aetiology of the problems associated with barley. However, the unequivocal demonstration that digestibilities or availabilities of the nutrients in barley-based diets have been enhanced by treatments designed to destroy β -glucan is lacking. Despite a considerable weight of literature on the subject, comparatively few experiments have substantially improved our understanding of the phenomenon. Although reports of experiments on the antinutritive effects of pentosans isolated from other cereals are now beginning to be published (Choct & Annison, 1992*a, b*), as yet no experiment has reported directly the effects of β -glucan which has been isolated from barley and added to a poultry diet, nor have direct *in vivo* studies on the effects of β -glucan on nutrient uptake across the gut of birds been undertaken. Perhaps the current lively interest in the biochemistry and

molecular biology of glucan- and xylan-degrading enzymes will provide the impetus for a more fundamental examination of the roles of the substrates of these enzymes in poultry nutrition.

REFERENCES

- Astrup, S. & Jørgensen, K. G. (1988). Application of the Calcofluor flow injection analysis method for determination of β -glucan in barley, malt, wort, and beer. *Journal of the American Society of Brewing Chemists* **46**, 76–81.
- Adams, O. L. & Naber, E. C. (1969). Effect of physical and chemical treatment of grains on growth of and feed utilization by the chick. 1. The effect of water and acid treatments of corn, wheat, barley and expanded or germinated grains on chick performance. *Poultry Science* **48**, 853–858.
- Ahluwalia, B. & Ellis, E. E. (1984). A rapid and simple method for the determination of starch and β -glucan in barley and malt. *Journal of the Institute of Brewing* **90**, 254–259.
- Åman, P. & Graham, H. (1987). Mixed-linked β -(1 \rightarrow 3), (1 \rightarrow < 4)-D-glucans in the cell walls of barley and oats – chemistry and nutrition. *Scandinavian Journal of Gastroenterology* **22** (Suppl. 129), 42–51.
- Anderson, J. O., Dobson, D. C. & Wagstaff, R. K. (1961). Studies on the value of hullless barley in chick diets and means of increasing this value. *Poultry Science* **40**, 1571–1584.
- Anderson, M. A., Cook, J. A. & Stone, B. A. (1978). Enzymatic determination of 1,3:1,4- β -glucans in barley grain and other cereals. *Journal of the Institute of Brewing* **84**, 233–239.
- Arcott, G. H. & Rose, R. J. (1960). Use of barley in high-efficiency broiler rations. 4. Influence of amyolytic enzymes on efficiency of utilization, water consumption and litter condition. *Poultry Science* **39**, 93–95.
- Aspinall, G. O. & Carpenter, R. C. (1984). Structural investigations on the non-starchy polysaccharides of oat bran. *Carbohydrate Polymers* **4**, 271–282.
- Bamforth, C. W. (1982). Barley β -glucans—their role in malting and brewing. *Brewers Digest* **57**, 22–27, 35.
- Bathgate, G. N., Palmer, G. H. & Wilson, G. (1974). Action of endo- β -1,3-glucanase on barley and malt β -glucans. *Journal of the Institute of Brewing* **80**, 278–285.
- Berg, L. R. (1959). Enzyme supplementation of barley diets for laying hens. *Poultry Science* **38**, 1132–1139.
- Braaten, J. T., Wood, P. J., Scott, F. W., Riedel, K. D., Poste, L. M. & Collins, M. W. (1991). Oat gum lowers glucose and insulin after an oral glucose load. *American Journal of Clinical Nutrition* **53**, 1425–1430.
- Broz, J. (1989). Evaluation of *Trichoderma viride* enzyme complex as a feed additive for broilers. *Proceedings of the 7th European Symposium on Poultry Nutrition*, pp. 237–238 [J. Brufau, editor]. Lloret de Mar: World's Poultry Science Association.
- Broz, J. & Frigg, M. (1986). Effects of beta-glucanase on the feeding value of broiler diets based on barley or oats. *Archiv für Geflügelkunde* **50**, 41–47.
- Burnett, G. S. (1966). Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. *British Poultry Science* **7**, 55–75.
- Campbell, G. L., Campbell, L. D. & Classen, H. L. (1983*b*). Utilization of rye by chickens: effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. *British Poultry Science* **24**, 191–203.
- Campbell, G. L., Classen, H. L. & Ballance, G. M. (1986). Gamma irradiation treatment of cereal grains for chick diets. *Journal of Nutrition* **116**, 560–569.
- Campbell, G. L., Classen, H. L. & Goldsmith, K. A. (1983*a*). Effect of fat retention on the rachitogenic effect of rye fed to broiler chicks. *Poultry Science* **62**, 2218–2223.
- Campbell, G. L., Classen, H. L. & Salmon, R. E. (1984). Enzyme supplementation of barley diets for broilers. *Feedstuffs* **56** (19), 26–27.
- Choct, M. & Annisson, G. (1992*a*). The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* **67**, 123–132.
- Choct, M. & Annisson, G. (1992*b*). Anti-nutritive effect of wheat pentosans in broiler chickens: role of viscosity and gut microflora. *British Poultry Science* **33**, 821–834.
- Clarke, A. E. & Stone, B. A. (1966). Enzymic hydrolysis of barley and other β -glucans by a β -(1 \rightarrow 4)-glucan hydrolase. *Biochemical Journal* **99**, 582–588.
- Classen, H. L., Campbell, G. L., Rossnagel, B. G., Bhatti, R. & Reichert, R. D. (1985). Studies on the use of hullless barley in chick diets: deleterious effects and methods of alleviation. *Canadian Journal of Animal Science* **65**, 725–733.
- Coates, M. E., Cole, C. B., Fuller, R., Houghton, S. B. & Yokota, H. (1981). The gut microflora and the uptake of glucose from the small intestine of the chick. *British Poultry Science* **22**, 289–294.
- Coon, C. N., Shepler, R., McFarland, D. & Nordheim, J. (1979). The nutritional evaluation of barley selections and cultivars from Washington State. *Poultry Science* **58**, 913–918.
- Davenport, H. W. (1983). *Physiology of the Digestive Tract: an introductory text*, 5th edn. Chicago, IL: Year Book Medical Publishers.
- Davidson, M. H., Dugan, L. D., Burns, J. H., Bova, J., Story, K. & Drennan, K. B. (1991). The hypocholesterolemic effect of β -glucan in oatmeal and oat bran: a dose-controlled study. *Journal of the American Medical Association* **265**, 1833–1839.

- Edney, M. J., Marchylo, B. A. & MacGregor, A. W. (1991). Structure of total barley beta-glucan. *Journal of the Institute of Brewing* **97**, 39–44.
- Elsenhans, B., Süfke, U., Blume, R. & Caspary, W. F. (1980). The influence of carbohydrate gelling agents on rat intestinal transport of monosaccharides and neutral amino acids *in vitro*. *Clinical Science* **59**, 373–380.
- Elsenhans, B., Süfke, U., Blume, R. & Caspary, W. F. (1981). *In vitro* inhibition of rat intestinal surface hydrolysis of disaccharides and dipeptides by guaran. *Digestion* **21**, 98–103.
- Elwinger, K. & Säterby, B. (1986). Comparison of β -glucanase (BG) and virginiamycin (VM) in broiler diets containing barley and oats. In *European Poultry Conference*, Paris, pp. 249–253 [M. Larbier, editor]. Paris: World's Poultry Science Association.
- Fleming, M. & Kawakami, K. (1977). Studies of the fine structure of β -D-glucans of barleys extracted at different temperatures. *Carbohydrate Research* **57**, 15–23.
- Fleming, M. & Manners, D. J. (1966). A comparison of the fine-structure of lichenin and barley glucan. *Biochemical Journal* **100**, 4P–5P.
- Flourie, B., Vidon, N., Florent, C. H. & Bernier, J. J. (1984). Effect of pectin on jejunal glucose absorption and unstirred layer thickness in normal man. *Gut* **25**, 936–941.
- Fry, R. E., Allred, J. B., Jensen, L. S. & McGinnis, J. (1957). Influence of water-treatment on nutritional value of barley. *Proceedings of the Society for Experimental Biology and Medicine* **95**, 249–251.
- Fry, R. E., Allred, J. B., Jensen, L. S. & McGinnis, J. (1958). Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poult. *Poultry Science* **37**, 372–375.
- Gill, A. A., Morgan, A. G. & Smith, D. B. (1982). Total β -glucan content of some barley cultivars. *Journal of the Institute of Brewing* **88**, 317–319.
- Gohl, B. (1977). *Effects of Hydrocolloids on Productive Value and Feeding Characteristics of Barley*. Ph.D. Thesis, University of Uppsala.
- Gohl, B., Aldén, S., Elwinger, K. & Thomke, S. (1978). Influence of β -glucanase on feeding value of barley for poultry and moisture content of excreta. *British Poultry Science* **19**, 41–47.
- Gohl, B. & Thomke, S. (1976). Digestibility coefficients and metabolizable energy of barley diets for layers as influenced by geographical area of production. *Poultry Science* **55**, 2369–2374.
- Hamm, D. (1958). Pelleting, soaking, and adding enzymes to feeds. In *Proceedings of the Arkansas Formula Feed Conference*. Fayetteville, AR: University of Arkansas.
- Henry, R. J. (1984). A simplified enzymic method for the determination of (1 \rightarrow 3) (1 \rightarrow 4)- β -glucans in barley. *Journal of the Institute of Brewing* **90**, 178–180.
- Henry, R. J. (1987). Pentosan and (1 \rightarrow 3), (1 \rightarrow 4)- β -glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. *Journal of Cereal Science* **6**, 253–258.
- Henry, R. J. & Blakeney, A. B. (1986). Determination of total β -glucan in malt. *Journal of the Institute of Brewing* **92**, 354–356.
- Henry, R. J. & Blakeney, A. B. (1988). Evaluation of a general method for the measurement of (1 \rightarrow 3), (1 \rightarrow 4)- β -glucans. *Journal of the Science of Food and Agriculture* **44**, 75–87.
- Herstad, O. (1987). Cereal with higher fibre content (barley, oats, millet). *Proceedings of the 6th European Symposium on Poultry Nutrition*, pp. A15–A25 [H. Vogt, editor]. Königslutter: World's Poultry Science Association.
- Herstad, O. & McNab, J. M. (1975). The effect of heat treatment and enzyme supplementation on the nutritive value of barley for broiler chicks. *British Poultry Science* **16**, 1–8.
- Hesselman, K. (1984). Beta-glucanase supplementation to barley based diets for broiler chickens. *Proceedings of the XVII World's Poultry Science Congress, Helsinki*, pp. 389–391.
- Hesselman, K. & Åman, P. (1985). A note on microscopy studies on water- and β -glucanase-treated barley. *Swedish Journal of Agricultural Research* **15**, 139–143.
- Hesselman, K. & Åman, P. (1986). The effect of β -glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- or high-viscosity. *Animal Feed Science and Technology* **15**, 83–93.
- Hesselman, K., Elwinger, K., Nilsson, M. & Thomke, S. (1981). The effect of β -glucanase supplementation, stage of ripeness, and storage treatment of barley in diets fed to broiler chickens. *Poultry Science* **60**, 2664–2671.
- Hesselman, K., Elwinger, K. & Thomke, S. (1982). Influence of increasing levels of β -glucanase on the productive value of barley diets for broiler chickens. *Animal Feed Science and Technology* **7**, 351–358.
- Hijikuro, S. (1983). Improvement of feeding value of barley by enzyme supplementation. *Japan Agricultural Research Quarterly* **17**, 55–58.
- Jensen, L. S., Fry, R. E., Allred, J. B. & McGinnis, J. (1957). Improvement in the nutritional value of barley for chicks by enzyme supplementation. *Poultry Science* **36**, 919–921.
- Jensen, S. Å. & Aastrup, S. (1981). A fluorimetric method for measuring 1,3:1,4- β -glucan in beer, wort, malt and barley by use of Calcofluor. *Carlsberg Research Communications* **46**, 87–95.
- Jeroch, H., Helander, E., Schlöffel, H.-J., Engerer, K.-H., Pingel, H. & Gebhardt, G. (1991). [Examination of the effectiveness of an enzyme preparation "Avizyme®" containing β -glucanase in broiler fattening rations based on barley.] *Archiv für Geflügelkunde* **55**, 22–25.
- Jeroch, H., Jackisch, B., Aboud, M., Weber, K. & Gebhardt, G. (1990). [Results of further evaluation of an enzyme preparation containing β -glucanase, Prowiko B, in broiler fattening rations based on barley.] *Archiv für Tierernährung* **40**, 317–327.

- Johnson, I. T. & Gee, J. M. (1981). Effect of gel-forming gums on the intestinal unstirred layer and sugar transport *in vitro*. *Gut* **22**, 398–403.
- Jonsson, E. & Hemmingsson, S. (1991). Establishment in the piglet gut of lactobacilli capable of degrading mixed-linked β -glucans. *Journal of Applied Bacteriology* **70**, 512–516.
- Jørgensen, K. G. & Aastrup, S. (1988). Quantification of high molecular weight (1 \rightarrow 3) (1 \rightarrow 4)- β -D-glucan using Calcofluor complex formation and flow injection analysis. II. Determination of total β -glucan content of barley and malt. *Carlsberg Research Communications* **53**, 287–296.
- Laerdal, O. A., Bird, H. R., Sunde, M. L. & Phillips, P. H. (1959). Improvement in nutritional value of some barleys by the addition of malt or enzyme supplements. *Poultry Science* **38**, 1221.
- Lawrence, T. L. J. (1976). Some effects of processing on the nutritive value of feedstuffs for growing pigs. *Proceedings of the Nutrition Society* **35**, 237–243.
- Leong, K. C., Jensen, L. S. & McGinnis, J. (1958). Effect of water treatment and fungal enzyme addition on metabolizable energy of pearled barley. *Poultry Science* **37**, 1220.
- Leong, K. C., Jensen, L. S. & McGinnis, J. (1962). Effect of water treatment and enzyme supplementation on the metabolizable energy of barley. *Poultry Science* **41**, 36–39.
- Luchsinger, W. W., Chen, S.-C. & Richards, A. W. (1965). Mechanism of action of malt beta-glucanases. 9. The structure of barley beta-D-glucan and the specificity of A₁₁-endo-beta-glucanase. *Archives of Biochemistry and Biophysics* **112**, 531–536.
- McCleary, B. V. & Glennie-Holmes, M. (1985). Enzymic quantification of (1 \rightarrow 3) (1 \rightarrow 4)- β -D-glucan in barley and malt. *Journal of the Institute of Brewing* **91**, 285–295.
- McCracken, K. J., Urquhart, R. & Bedford, M. R. (1993). Effect of heat treatment and enzyme supplementation of barley-based diets on performance of broiler chicks. *Proceedings of the Nutrition Society* (In the press).
- Mannion, P. F. (1981). Enzyme supplementation of barley based diets for broiler chickens. *Australian Journal of Experimental Agriculture and Animal Husbandry* **21**, 296–302.
- Martin, H. L. & Bamforth, C. W. (1981). An enzymic method for the measurement of total and water-soluble β -glucan in barley. *Journal of the Institute of Brewing* **87**, 88–91.
- Meyer, J. H. & Doty, J. E. (1988). GI transit and absorption of solid food: multiple effects of guar. *American Journal of Clinical Nutrition* **48**, 267–273.
- Meyer, J. H., Gu, Y. G., Jehn, D. & Taylor, I. L. (1988). Intra-gastric vs in-traintestinal viscous polymers and glucose tolerance after liquid meals of glucose. *American Journal of Clinical Nutrition* **48**, 260–266.
- Morimoto, H., Kubota, D. & Akai, N. (1966). [Effect of cellulase on barley ration. 1. Feeding of layers and determination of metabolizable energy.] *Japanese Poultry Science* **3**, 167–170.
- Moscatelli, E. A., Ham, E. A. & Rickes, E. L. (1961). Enzymatic properties of a β -glucanase from *Bacillus subtilis*. *Journal of Biological Chemistry* **236**, 2858–2862.
- Moss, B. R., Beeckler, A. F., Newman, C. W. & El-Negoumy, A. M. (1977). Enzyme supplementation of broiler rations. *Poultry Science* **56**, 1741.
- Newman, R. K., Newman, C. W. & Eslick, R. F. (1985). Effect of fungal fermentation and other treatments on nutritional value of waxy barley fed to chicks. *Poultry Science* **64**, 1514–1518.
- Novacek, E. J. & Petersen, C. F. (1967). Metabolizable energy of the anatomical parts and other fractions of western barley and the effect of enzymes and water treatment. *Poultry Science* **46**, 1008–1015.
- Parrish, F. W., Perlin, A. S. & Reese, E. T. (1960). Selective enzymolysis of poly- β -D-glucans, and the structure of the polymers. *Canadian Journal of Chemistry* **38**, 2094–2104.
- Perlin, A. S. & Suzuki, S. (1962). The structure of lichenin: selective enzymolysis studies. *Canadian Journal of Chemistry* **40**, 50–56.
- Pettersson, D., Graham, H. & Åman, P. (1990). Enzyme supplementation of broiler chicken diets based on cereals with endosperm cell walls rich in arabinoxylans or mixed-linked β -glucans. *Animal Production* **51**, 201–207.
- Potter, L. M., Stutz, M. W. & Matterson, L. D. (1965). Metabolizable energy and digestibility coefficients of barley for chicks as influenced by water treatment or by presence of fungal enzyme. *Poultry Science* **44**, 565–573.
- Rexen, B. (1981). Use of enzymes for improvement of feed. *Animal Feed Science and Technology* **6**, 105–114.
- Rickes, E. L., Ham, E. A., Moscatelli, E. A. & Ott, W. H. (1962). The isolation and biological properties of a β -glucanase from *B. subtilis*. *Archives of Biochemistry and Biophysics* **96**, 371–375.
- Rose, R. J. & Arscott, G. H. (1962). Use of barley in high-efficiency broiler rations. 5. Further studies on the use of enzymes, soaking and pelleting barley for chicks. *Poultry Science* **41**, 124–130.
- Rotter, B. A., Friesen, O. D., Guenter, W. & Marquardt, R. R. (1990). Influence of enzyme supplementation on the bioavailable energy of barley. *Poultry Science* **69**, 1174–1181.
- Rotter, B. A., Marquardt, R. R., Guenter, W., Biliaderis, C. & Newman, C. W. (1989). In vitro viscosity measurements of barley extracts as predictors of growth responses in chicks fed barley-based diets supplemented with a fungal enzyme preparation. *Canadian Journal of Animal Science* **69**, 431–439.
- Salih, M. E., Classen, H. L. & Campbell, G. L. (1991). Response of chickens fed on hull-less barley to dietary β -glucanase at different ages. *Animal Feed Science and Technology* **33**, 139–149.
- Staudte, R. G., Woodward, J. R., Fincher, G. B. & Stone, B. A. (1983). Water-soluble (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucans from barley (*Hordeum vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residues. *Carbohydrate Polymers* **3**, 299–312.
- Stutz, M. W., Matterson, L. D. & Potter, L. M. (1961). Metabolizable energy of barley for chicks as influenced by water treatment or by presence of fungal enzyme. *Poultry Science* **40**, 1462.

- Suga, Y., Kawai, M., Noguchi, S., Shimura, G. & Samejima, H. (1978). Application of cellulolytic and plant tissue macerating enzyme of *Irpex lacteus* Fr. as feed additive enzyme. *Agricultural and Biological Chemistry* **42**, 347–350.
- Thomas, J. M., Jensen, L. S. & McGinnis, J. (1961). Interference with the nutritional improvement of water-treated barley by antibiotics. *Poultry Science* **40**, 1204–1208.
- Varum, K. M., Martinsen, A. & Smidsrod, O. (1991). Fractionation and viscometric characterisation of a (1 → 3), (1 → 4)- β -glucan from oat, and universal calibration of a high-performance size-exclusion chromatographic system by the use of fractionated β -glucans, alginates and pullulans. *Food Hydrocolloids* **5**, 363–374.
- Wharton, F. D., Classen, L. J. & Fritz, J. C. (1958). Influence of amylases on growth of chicks and poults. *Federation Proceedings* **17**, 497.
- White, W. B., Bird, H. R., Sunde, M. L., Marlett, J. A., Prentice, N. A. & Burger, W. C. (1983). Viscosity of β -D-glucan as a factor in the enzymatic improvement of barley for chicks. *Poultry Science* **62**, 853–862.
- White, W. B., Bird, H. R., Sunde, M. L., Prentice, N., Burger, W. C. & Marlett, J. A. (1981). The viscosity interaction of barley beta-glucan with *Trichoderma viride* cellulase in the chick intestine. *Poultry Science* **60**, 1043–1048.
- Willingham, H. E., Jensen, L. S. & McGinnis, J. (1958). Studies on the role of enzyme supplements and water treatment on the nutritional value of barley. *Poultry Science* **37**, 1253.
- Willingham, H. E., Jensen, L. S. & McGinnis, J. (1959). Studies on the role of enzyme supplements and water treatment for improving the nutritional value of barley. *Poultry Science* **38**, 539–544.
- Willingham, H. E., Leong, K. C., Jensen, L. S. & McGinnis, J. (1960*a*). Influence of geographical area of production on response of different barley samples to enzyme supplements or water treatment. *Poultry Science* **39**, 103–108.
- Willingham, H. E., McGinnis, J., Nelson, F. & Jensen, L. S. (1960*b*). Relation of superiority of water-treated barley over enzyme supplements to antibiotics. *Poultry Science* **39**, 1307.
- Wood, P. J., & Fulcher, R. G. (1978). Interaction of some dyes with cereal β -glucans. *Cereal Chemistry* **55**, 952–966.
- Wood, P. J., Weisz, J. & Blackwell, B. A. (1991). Molecular characterization of cereal β -D-glucans. Structural analysis of oat β -D-glucan and rapid structural evaluation of β -D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. *Cereal Chemistry* **68**, 31–39.
- Woodward, J. R., Fincher, G. B. & Stone, B. A. (1983*a*). Water-soluble (1 → 3), (1 → 4)- β -D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydrate Polymers* **3**, 207–225.
- Woodward, J. R., Phillips, D. R. & Fincher, G. B. (1983*b*). Water-soluble (1 → 3), (1 → 4)- β -D-glucans from barley (*Hordeum vulgare*) endosperm. I. Physicochemical properties. *Carbohydrate Polymers* **3**, 143–156.