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in

Molina-Cano J.L. (ed.), Brufau J. (ed.).
New trends in barley quality for malting and feeding

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 20

1991
pages 55-62

To cite this article / Pour citer cet article

Chesson A. **Effects of supplementary enzymes in barley diets.** In : Molina-Cano J.L. (ed.), Brufau J. (ed.). *New trends in barley quality for malting and feeding.* Zaragoza : CIHEAM, 1991. p. 55-62 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 20)

Effects of supplementary enzymes in barley diets

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SUMMARY - The addition of ß-glucanase to barley-based diets for poultry is widely recognised as beneficial and producers in countries in which barley and oats are the major domestic grains are already making use of this technology on a practical scale. Although there is a suggestion that the value of ß-glucanase addition declines with the age of the bird, the nutritive value of treated barley invariably is enhanced when fed to broiler chicks and any problems associated with sticky droppings eradicated. Generally the performance of birds fed enzyme supplemented barley-based diets can match those fed wheat-based diets. The mechanism underlying the observed productive benefit is not fully understood but probably relates to the release of nutrients trapped within intact endosperm cells. Extending the application of feed enzymes to oats and rye, which exhibit many of the problems associated with barley when fed to poultry, also seems a practical proposition. Addition of amylolytic enzymes to augment a capacity to digest starch has no effect in poultry but may benefit the (very) early weaned piglet. However any advantage conferred by enzyme addition to weaner (starter) diets for pigs seems independent of the source of cereal starch and does not selectively advantage barley. The addition of cell wall (fibre) degrading enzymes to cereal-based diets for growing pigs appears to be of marginal benefit. The response shown is small and inconsistent and does not appear to be greater in cereals, like barley, with a high fibre content. This situation is likely to change as improved enzyme products appear on the market.

RESUME - "Effects de l'addition d'enzymes pour les régimes à base d'orge". L'addition de ß-glucanase aux régimes à base d'orge destinés aux volailles est largement considérée comme avantageuse et les producteurs des pays où l'orge et l'avoine sont les principales productions utilisent d'ores et déjà cette technique à l'échelle pratique. bien que certains suggèrent que l'avantage d'ajouter de la ß-glucanase diminue avec l'âge du poulet, la valeur nutritionnelle de l'orge est toujours augmentée par ce traitement en ce qui concerne l'alimentation de poulets de chair, et tous les problèmes de souillures par les fientes sont ainsi supprimés. En général les performances des poulets alimentés à base de régimes d'orge supplémenté avec des enzymes sont équivalentes à celles des régimes à base de blé. Le mécanisme responsable de l'augmentation de production observée n'est pas complètement compris mais est probablement lié à la libération de nutriments retenus à l'intérieur de la cellule intacte de l'endosperme. L'application des enzymes à l'avoine et au seigle, qui présentent à peu près les mêmes problèmes que l'orge, lorsqu'ils sont utilisés pour l'alimentation des volailles, semble également avoir un bien-fondé pratique. L'addition d'enzymes amylolytiques pour augmenter la capacité à digérer l'amidon n'a pas d'effet sur les volailles mais peut être avantageuse pour les jeunes cochons sevrés très tôt. Cependant les avantages conférés par l'addition d'enzymes aux régimes pour cochonnets après le sevrage semblent indèpendants de la source d'amidon céréalier et l'orge n'est pas plus avantageux que les autres. L'addition d'enzymes de dégradation de la paroi végétale aux régimes à base de céréales pour les porcins à l'engraissement ne semble présenter qu'un intèrêt marginal. La réponse observée est faible et divergente et ne semble pas plus forte pour les céréales qui, comme l'orge, ont une haute teneur en fibre. Cette situation pourrait changer ave l'apparition sur le marché de meilleurs produits enzymatiques.

Introduction

Barley, although widely used as a component of pig and ruminant rations, is recognised as being inferior to either wheat or maize as an ingredient in poultry diets. Two factors in particular militate against the feeding of barley to poultry. Firstly, because it has a relatively high fibre content which is considered to be undigested by all species of poultry, barley is classed as a low energy cereal (Table 1). However this property alone does not preclude its use. Probably the overriding factor responsible for the unpopularity of barley in poultry diets is its association with the condition known as "sticky droppings". Gel-forming polysaccharides leached from the fibre component of barley and some other cereal grains may result in the excretion of sticky droppings which increases the water-holding capacity of the litter on which broilers are reared. This can cause hock problems and breast damage in the bird and results in products of reduced quality. Similarly in laying birds kept on raised wire floors, sticky droppings tend to adhere to the mesh and to mark the eggs as they roll away reducing their market value.

The addition of enzymes able to improve the nutritive value of barley-based diets for poultry and to alleviate the problems caused by sticky droppings is one of the

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Table 1. Feed characteristics of cereal grains fed to pigs and poultry (Givens and Moss, 1990; Allen, 1981).

Cereal grain	NDF[1] content (g/kg ODM[2])	Metabolisable energy content	
		Poultry-TME_N[3] (MJ/kg ODM)	Pigs (MJ/kg DM[4])
Maize	116.9	16.1	15.1
Wheat	123.7	15.5	16.0
Triticale	118.5	14.4	–
Barley	200.9	14.5	13.5
Oats	309.8	14.8	12.4
Rye	357.1	–	12.8

[1] NDF = neutral detergent fibre which approximates to cell wall content
[2] ODM = organic dry matter
[3] TME_N = true metabolizable energy by correction of Nitrogen retention
[4] DM = dry matter

earliest recorded applications of enzyme technology to the feed industry. Since it is also the application which has proved to be the most consistently successful, it is invariably the first example cited by those marking enzyme products in support of the concept of supplementary enzymes and their place in feed formulation.

Enzyme addition to barley-based poultry diets

Improvement in the nutritive value of barley fed to chickens by enzyme supplementation using a crude amylolytic preparation was first reported by Jensen *et al.* (1957). This and other experiments made around the same time using similar fungal enzyme preparations (Fry *et al.*, 1958; Berg, 1959) showed that enzyme incorporation significantly improved growth rate and feed conversion efficiency and reduced the incidence of sticky droppings. Interpretation of the results obtained, however, proved difficult since the response to supplementation did not seem to relate to the starch content of the barley but did depend on the variety, date of harvest and growing conditions (Laerdal *et al.*, 1959; Willingham *et al.*, 1960). This problem was further compounded when attempts to amplify the response to enzyme supplementation by the use of more highly purified bacterial amylase failed to produce any response at all (Willingham *et al.*, 1959; Anderson *et al.*, 1961). The problem was finally resolved by Moscatelli *et al.* (1961) and Rickes *et al.* (1962) who were able to confirm that the changes in nutritive value were due, in large part at least, to a secondary activity present in the crude enzyme preparations and not to amylase as initially believed. This secondary activity was identified as a ß-glucanase and its substrate as the mixed-linked glucan which forms the major part of the barley endosperm cell wall (Fincher and Stone, 1986; Henry, 1987; Table 2).

Effect of ß-glucanase addition

The fact that ß-glucanase addition increases the nutritive value of barley fed to poultry is beyond doubt although there is limited evidence of an age effect which suggests that ß-glucanase addition is of value only in younger birds (Elwinger and Saterby, 1987; Al-Bustany and Elwinger, 1988). Numerous studies made since the 1960's have confirmed that enzyme addition to barley-based diets increases the metabolisable energy (ME) content and improves food intake and performance when fed to broiler chicks. Results from a representative range of more recent studies of this type are shown in Table 3. More extensive summaries of experiments made to measure the response of poultry to supplementation of diets with enzymes are provided in the reviews of Chesson (1987) and Dierick (1989).

Despite the wealth of data confirming the overall increase in performance, the manner in which enzyme addition achieves its effect remains unclear. Perhaps the simplest explanation is that ß-glucans which form the bulk of the barley endosperm walls act to restrict access to nutrients found in the endosperm. The common, but not universal, observation that the digestibility values for fat, starch and nitrogen and, consequently, ME are increased by enzyme addition support this view (Hersted and McNab, 1975; Mannion, 1981; Classen *et al.*, 1985; Broz and Frigg, 1986a; Hesselman and Aman, 1986; Newman and Newman, 1987; Rotter *et al.*, 1989a). What remains uncertain is whether this restriction is a result of protection by intact endosperm walls which have escaped

Table 2. Polysaccharide composition of endosperm and aleurone cell walls of barley and wheat. Values are calculated from the monosaccharide data of Fincher and Stone (1986) and expressed as a percentage of total carbohydrate.

Polysaccharide	Barley		Wheat	
	Endosperm	Aleurone	Endosperm	Aleurone
Mixed-linked glucan	75	26	20	29
Cellulose	2	2	4	2
Arabinoxylan	20	67	70	65

Table 3. Response by poultry to enzyme supplementation of barley- and oat-based diets.

Enzyme preparation and application rate (g/kg feed)	Age of birds[1] (d)	Response to supplementation (%)		Reference
		LWG[2]	FCE[3]	
Barley-based diets				
ß-Glucanase 0.05-0.5 g (ex *Aspergillus niger*, Finizyme, Culter)	8-25	1.6-10.5	0-4.5	Broz and Frigg, 1986a
ß-Glucanase 0.5-2.0 g (Avizyme, Culter)	8-25	6-16.6	3.8-9.1	
Cellulase 0.5 g (ex *Irpex lacteus,* Driselase, Kyowa Hakko Kogyo Co.)	8-25	4.0	2.1	Broz and Frigg, 1986b
Cellulase 0.1 g (ex *Trichoderma viride,* Onozuka-35, Yakult Honsha Co.)	8-25	7.5	2.1	
ß-Glucanase 0.5 g (Glucanase GV-P, Grinsted Products)	1-19	29-27.6	14-18	Hesselman and Aman, 1986
ß-Glucanase 0.5 g (Glucanase GV-P, Grinsted Products)	1-21	2.1-4.7	0.6-1.8	Elwinger and Saterby, 1987
ß-Glucanase 0.5 g (Enzyme Development Corporation)	4-25	3.4-11.3	6.7-10.1	Newman and Newman, 1987
ß-Glucanase 0.8 g (Glucanase GV-P, Grinsted Products)	Hens 20-80 weeks	0	0	Al Bustany and Elwinger, 1988
ß-Glucanase 0.2 g (ex *Bacillus subtilis* Zymo-best, Premier Malt Co.)	Turkeys 1-28	3.6 4-12 weeks,	4.7 no change	Stevens *et al.*, 1988
Cellulase 0.06 g (ex *T. viride,* Miles Laboratories Inc.)	7-21	2-41	2.7-20.3	Rotter *et al.*, 1989b
Cellulase 0.12-0.5 g (Celluclast, Novo A/S)	7-21	53.0	19.1	Rotter *et al.*, 1989c
Cellulase 0.12-0.5 g (Cellulase Tu, Miles Laboratories Inc.)	7-12	62.3	28.8	
Oat-based diets				
ß-Glucanase 0.5-2.0 g (Avizyme, Culter)	8-25	18.9-24.3	8.0-10.7	Broz and Frigg, 1986a
Cellulase 0.5 g (ex *Irpex lacteus,* Driselase, Kyowa Hakko Kogyo Co.)	8-25	7.9 9.2	2.2 2.7	Broz and Frigg, 1986b
Cellulase 0.1 g (ex *T. viride,* Onozuka-35, Yakult Honsha Co.)	8-25			
ß-Glucanase 0.5 g (Glucanase GV-P, Grinsted Products)	1-21	2.0-4.5	1.3-7.2	Elwinger and Saterby, 1987
ß-Glucanase 0.23-2.3 g (ex *B. subtilis,* Zymo-best, Premier Malt Co.)	0-42	68-70	24-26	Campbell *et al.*, 1987
ß-Glucanase 0.08-0.7 g (Glucanase GV-P, Grinsted Products)	1-22	-4.0	2.0	Pettersson *et al.*,1987

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[1] Broiler chicks unless otherwise stated.

[2] LWG, percentage improvement in liveweight gain compared to untreated control diets.

[3] FCE, percentage improvement in feed conversion efficiency compared to untreated control diets.

disruption during feed processing or whether release of nutrients is slowed by the presence of gels formed by soluble glucan leached from the wall. The increased viscosity of digesta caused by the presence of ß-glucan gels in the gut lumen also is claimed to have negative effects on food intake and the rate of absorption of potential nutrients across the gut mucosa (White *et al.*, 1983; Campbell *et al.*, 1984). Certainly viscosity data obtained *in vitro* often closely reflect those anti-nutritional properties of barleys which can be reduced or eradicated by enzyme addition (Rotter *et al.*, 1989b, 1990). However, for all practical purposes, attempting to distinguish between hydrolysis of a soluble glucan leading to viscosity reduction and insoluble-glucan leading to increased disruption of the endosperm wall is probably futile. Both depend on the action of a single enzyme, both can be achieved with limited bond cleavage and both effects probably contribute to the observed improvements in nutrient digestibility and nitrogen retention. Only the reduction in the incidence of sticky droppings can be unambiguously ascribed to the destruction of ß-glucan gels.

Enzyme addition to other cereal-based diets for poultry

The successful treatment of barley-based diets suggested that other cereal-based diets with poor nutritional characteristics might also benefit by enzyme addition. Oats were an obvious target since their endosperm cell walls also contain high levels of mixed-linked glucan which, like barley ß-glucan, is partially water soluble (Henry, 1985, 1987; Aman and Graham, 1987; Table 4) and which can, when incorporated into broiler diets, give rise to the similar problems. As might be expected addition of ß-glucanase appears to improve the nutritional properties of oats fed to broiler chickens (Broz and Frigg, 1986a; Campbell *et al.*, 1987; Table 3) although exceptions have been noted (Pettersson *et al.*, 1987).

It is now widely accepted that the poor performance of rye-fed birds is a consequence of the leaching out of soluble pentosan (Table 4), which in a manner similar to barley glucan, increases the viscosity of digesta and impedes the release/uptake of nutrients. Treatment with enzyme preparations able to degrade pentosans (predominantly endo-ß-xylanase) reduces the incidence of sticky droppings of rye-fed broilers and improves litter quality (Pettersson and Aman, 1988, 1989). However these authors suggest that viscosity reduction was not the major factor causing the improvements in live-weight gain (15-27%) and food conversion efficiency (5-10%) observed. While both soluble and insoluble pentosan was attacked by the added enzyme, there was a net increase in the amount of soluble pentosan present for much of the feeding period produced by the release of additional pentosan during cell wall degradation. Aman and his co-workers concluded that cell wall degradation, rather than viscosity reduction, was the paramount effect leading to the nutritional benefits conferred by enzyme addition to rye. They further argued that this observation offered support for their previous suggestion (Hesselman and Aman, 1986) that a comparable effect occurred when ß-glucanase was added to barley-based diets.

Table 4. Total and water-soluble mixed-linked glucan and pentosan content of some cereal grains (Henry, 1985; Aman and Graham, 1987; Pettersson and Aman, 1989).

Cereal	% Dry matter			
	Mixed-linked glucan		Pentosan	
	Water-soluble	Total	Water-soluble	Total
Barley	2.7	4.4	0.2	5.7
Oat	2.3	3.3	0.4	7.7
Rye	0.7	1.9	2.6	8.5
Wheat	0.7	0.7	1.2	6.6

Addition of single or multiple enzyme activities

Endosperm walls of barley and oats are unique in being largely composed of a single homopolysaccharide, a mixed-linked glucan of relatively simple structure (Table 2). Single enzymes (ß-glucanases) able to attack the 1,4 or 1,3 linkages found in barley mixed-linked glucans can rapidly destroy the integrity of the polymer *in situ* causing substantial disruption of the endosperm cell wall. The integrity of walls from the other cell types found in barley (e.g. the aleurone) or those found in other cereal grains which possess a far lower mixed-linked glucan content remain unaffected by treatment with ß-glucanase alone. Work in this laboratory (Murison *et al.*, 1989) has shown that enzyme preparations containing cellulolytic and xylanolytic activities are required to maximise the release of protein from aleurone cells of wheat and barley (Table 5). If cell wall disruption leading to the release of nutrients not otherwise available to the bird is the major mode of action of added enzymes, then it would seem advantageous to provide ß-glucanase activity as part of a general cell wall degrading enzyme preparation. This would ensure maximum release of the higher quality protein found in aleurone cells. The broader range of activities present would also convert more of the non-starch polysaccharide (fibre) content of barley to monosaccharides capable of being absorbed and making a contribution to the birds nutrition. However it should be stressed that "saccharification" *per se* is not now seen as major reason for the addition of enzymes to pig and poultry diets. One reason for this is the growing recognition that not all sugars available for release from plant cell walls have the same metabolic worth and that release of some sugars may be positively deleterious (Longstaff *et al.*, 1988).

Table 5. Release of protein from isolated aleurone cells by commercial ß-glucanase and "cellulase" preparations.

Enzyme[1]	Incubation time (h)	Protein released (mg protein/g washed cell preparation)
Glucanase[2]	6	14.4
	24	16.7
"Cellulase"	6	20.2
	24	22.4

[1] The concentration of the two enzyme preparations were adjusted to the same ß-(1,4)-glucanase activity

[2] Although marketed as a ß-glucanase, the preparation did show some cellulolytic activity.

Enzyme addition to barley-based pig diets

Concern that the soluble glucan thought to contribute to the relatively poor performance of broilers fed barley-based diets might also depress performance when fed to pigs has not proved justified. Although ß-glucanase addition to hullness barley, known to have a higher soluble glucan content than the hulled varieties, significantly improved dry matter and protein digestibility in 20 kg pigs, this was not reflected in values for average daily weight gain or food conversion efficiency (Thacker *et al.*, 1988). Similarly, glucanase supplementation marginally increased the apparent digestibility of starch measured at the terminal ileum in 80 kg pigs but did not affect crude protein digestibility or overall performance (Graham *et al.*, 1989).

Gel-forming polysaccharides leached from cereal cell walls may have anti-nutritional effects in the pig. However these effects are evidently small and, when destroyed by enzyme addition, no significant productive benefit accrues. The major reason for this difference between pigs and poultry is the far greater capacity for microbial fermentation found in the pig. Addition of enzymes active against NSP (e.g. ß-glucanase) to poultry diets will result in a net increase in polysaccharide hydrolysis, while in the pig the net extent of hydrolysis remains essentially the same and only the site of hydrolysis is changed. Any increase in starch digestibility produced by enzyme addition to poultry diets, for example, is a net gain to the bird and will be reflected as an increase in TME_N. A similar increase in starch digestibility measured at the terminal ileum of the pig, as detected by Graham *et al.* (1989), represents a much smaller net gain in energy, since starch escaping the foregut is available as a substrate for microbial fermentation in the hindgut. Enzyme addition shifts the site of hydrolysis from the hindgut to the foregut and the nutritional benefit gained is the increased metabolic worth of glucose absorbed directly rather than as the VFA equivalent produced by microbial fermentation.

The factors which lead to the selection of barley as the major energy source in pig diets are those normally considered during least cost formulation. The option of enzyme supplementation is not currently included in the least cost exercise, partly because of lack of information and partly because the response to enzyme addition is thought to be small and independent of the type of cereal fed. There are some sound reasons for this view. Research leading to enzyme addition to pig diets has tended to focus on two distinctly different strategies in recent years. The first, supplementing the young animals own amylolytic (and proteolytic) capacity during the period immediately following weaning, is clearly independent of the source of the cereal starch provided. The second, addition of cell wall degrading activities to diets for growing pigs to promote nutrient release and saccharification of cell wall polysaccharides, might be thought to favour cereal sources such as barley with a high fibre content. However this would only be the case if saccharification, rather than release of additional nutrients, made the greater contribution to any productive benefit achieved. This has yet to be demonstrated.

Augmenting the digestive capacity of the young pig

Physiological studies on the effect of diet, weaning and age on pancreatic development offer some support for the view that augmenting the young pigs own amylolytic and, possibly, proteolytic capacity could be beneficial during the crucial period of weaning onto cereal-based starter diets when growth may be checked and the animal may succumb to disease (Lindemann *et al.*, 1986; Owsley *et al.*, 1986). Immediately following weaning the output of amylase falls dramatically, virtually back to the level detectable at birth. Production of proteolytic enzymes also appears to be checked but the effect is not so acute as that shown by pancreatic amylase. However, enzyme production recovers fairly rapidly, which suggests that supplementation would only be of benefit for a relatively short period of, perhaps, 14-21 days.

There have been few reported attempts designed specifically to exploit this theoretical niche for enzyme addition and most data is in the form of manufacturers information. A retrospective examination of the scientific data does tend to support the view that addition of amylase and protease to diets (usually barley-based) of weaned animals (3-7 weeks, 4-16 kg liveweight) does increase liveweight gain and feed conversion efficiency

(Collier and Hardy, 1986; Inborr and Ogle, 1988), an effect not shown when similar enzymes are added to the diets of older pigs (Casteels and Buysse, 1976; Cromwell *et al.*, 1988). There is also some evidence that enzyme treatment/pretreatment of a barley-based feed reduced the incidence of diarrhoea in piglets (Inborr and Ogle, 1988).

Manufacturers data tends to be unequivocal and to show substantial benefits of supplementation with enzyme preparations containing amylolytic and other activities. However the tendency in such trials to use very early weaned pigs (21 days), whose pancreatic size and capacity would be much reduced compared to piglets weaned between 7 and 21 days later, may act to magnify the response obtained (Inborr and Ogle, 1988; Inborr 1990). Weaning at this very young age is generally contrary to current advice which suggests weaning at around six weeks of age to allow better development of the piglets immune system. Attempts to augment existing enzyme activities of animals of this age may be less advantageous.

Polysaccharidase addition to cereal-based diets for older pigs

Interest continues in the application of cell wall degrading activities, usually collectively and incorrectly referred to as "cellulase" enzyme preparations, to diets for older pigs. A steady stream of reports from Eastern Europe regularly indicate improvements in intake and food conversion efficiency following enzyme addition. However many of these reports are in the form of abstracts and contain insufficient detail to allow an evaluation of the results obtained. This is important since trials made in Western Europe and the U.S.A. with apparently similar enzyme products have not produced the same definitive results. At present, although enzyme addition appears to be marginally beneficial, the response shown is small and inconsistent and does not appear to favour cereals with a high fibre content.

Technically the hydrolysis of plant cell walls (fibre), which consist of a complex and interconnected water-insoluble polymer matrix and requires the simultaneous action of numerous different enzyme activities, is difficult even under ideal conditions. This problem is compounded when hydrolysis has to occur under the environmental conditions found in the digestive tract and within a time span imposed by the host. Present enzyme formulations, although improving, are not wholly suited to this task and it is not surprising that results have been less favourable than initially anticipated. While there are real possibilities for the design and production of enzyme formulations better suited to cell wall hydrolysis *in vivo*, these will not be available in the near future. In the meantime, limitations imposed on the efficacy of current enzyme products can only be overcome by pre-treatment. This is likely to be a viable proposition only when heat is available at no extra cost to the process (applicable in the case of some by-products of the distillery, brewing and starch-extraction industries) and when water is not a limiting factor. Compensation for lower temperature can be provided by longer holding times, but adequate levels of moisture are an absolute requirement to support hydrolysis. Wet (pipe) feeding systems used in some larger pig enterprises for growing animals could, even at ambient temperatures, provide suitable conditions for enzyme hydrolysis. This option seems to have been little explored.

Enzyme supplemented barley as a feed ingredient

As Rotter *et al.* (1989a) pointed out, barley provides an excellent cereal source for poultry and pig production. The protein content is higher than maize and the amino-acid balance better than wheat. Although there is reluctance amongst feed manufacturers to reduce the energy density of broiler diets by inclusion of barley, in theory this should not pose a problem since the chicken will compensate by manipulating food intake. It is now generally accepted that the performance of birds on enzyme supplemented barley-based diets can match those fed wheat-based diets and that enzyme supplemented barley-based diets can be more cost effective than maize-soyabean. Countries in which barley and oats are the major domestic grain have been the first to take advantage of the opportunity offered by enzyme supplementation to increase the use of barley in poultry diets (Pettersson and Aman, 1989). There seems no reason why enzyme supplemented barley should not appear in least cost solutions elsewhere and challenge the monopoly currently held by wheat and maize.

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