

Factors affecting response of wheat based diets to enzyme supplementation

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Summary

There have been many reports on the use of xylanases in wheat based diets in the past 12 months, but there is apparently still a lack of agreement on how and why these products work. This paper discusses some of the factors which affect not only the magnitude but also the direction of the response to enzyme addition to wheat based diets. It is important that such factors are considered when attempting to interpret whether a particular study supports or detracts from a particular hypothetical mechanism of action.

Introduction

In the last year alone, over 20 scientific articles have been published examining the effect of xylanase- or β -glucanase-based enzymes on the nutritive value of wheat- or barley-based diets for broiler chickens, respectively. The majority of the reports indicate benefits in performance when these preparations were used, but the responses are by no means consistent across all studies. The highly variable nature of the data in the literature, particularly with respect to wheat, no doubt contributes to the lack of agreement on the mechanism of enzyme action (Cowan *et al.* 1993; Bedford, 1995a, 1996; Smits and Annison, 1996). The apparent lack of consistency in response to enzyme supplementation is the focal point of this paper, which attempts to draw some general conclusions from the mass of published data.

Reasons for Variable Responses to Enzyme Supplementation

Wheat as a feed ingredient

The widespread commercial use of enzymes in wheat-based diets is in itself testament to their efficacy. However, the underlying reason for their use in the first

place is that the wheat samples used in broiler feed manufacture vary considerably and in many instances significantly depress performance (Rogel *et al.* 1987; Classen *et al.* 1995). Reviewing such data and that provided by others (Longstaff and McNab, 1986; Annison, 1990; Wiseman and Inbarr, 1990) clearly demonstrates that classical proximate analysis has failed to consistently identify those factors which determine wheat quality. Starch content, bushel weight, thousand kernel weight and some baking qualities such as Hagberg falling number have not been shown to have a repeatable, significant effect (Rogel *et al.* 1987; Wiseman and Inbarr, 1990; Classen *et al.* 1995). Recent understanding of the physiological responses to feeding diets rich in rye and wheat (Bedford and Classen 1992), in addition to isolation and re-feeding studies (Fengler and Marquardt, 1988; Choct and Annison, 1992) identified the soluble, viscous arabinoxylans as being a likely candidate for the limitations in feeding rye and wheat. Some authors suggest that lower intestinal viscosities (<10 cP) do not pose a problem for performance (Cowan *et al.* 1993), but the methodology used did not allow such a statement to be made. Even when intestinal viscosities well below 10cP are encountered, in well controlled experiments the relationship between Food Conversion Ratio and viscosity is very good, with an R^2 of 0.94 ($p < 0.01$, Bedford, 1995b), and even suggests that the gain in performance to be made with each cP fall is linearly related. Recently, in vitro methods for predicting the intestinal viscosity of a given diet have been described which also allow for a rapid identification of viscous and non-viscous wheats (Bedford and Classen, 1993). Recent work by Dusel (1997, unpublished) and McCracken *et al.* (personal communication) indicates that the viscosity and therefore the feeding value of the grain are very much dependent upon the variety and the environment in which the grain was grown. Whilst feed manufacturers are highly unlikely to be using pure varieties at the point of feed manufacture, variability in

the viscosity of the sample received at the mill will depend upon the blend of varieties. Prior knowledge of the varieties grown in the area from which the mill receives its wheat, and their extract viscosity will allow some estimate in the likely maximum variation in viscosity of the mixed samples received at the mill. This may in future allow for titration of enzyme activity against viscosity of wheat, particularly if rapid NIR (Near Infrared Spectroscopy) techniques can be used to identify viscous and non-viscous samples.

Even if variety is fixed, the relationship between grain inclusion rate and intestinal viscosity is exponential (Bedford and Classen, 1992). The response of a wheat based diet to xylanase addition must therefore take into account inclusion level of the target grain. Allen *et al.* (1996a) confirmed this point, demonstrating that increasing the inclusion rate of wheat significantly increased the ME:gain and subsequent FCR, and thus influenced the subsequent response to enzymes.

The application of heat and moisture to the diet during the processing of pelleting or expanding is well known to increase the subsequent intestinal viscosity of the bird, which in itself will influence the scale of response observed to addition of enzyme (Teitge *et al.* 1991; Bedford and Morgan 1996). Over-processing may mask any enzyme response once the thermal stability of the enzyme or in fact other heat sensitive nutrients is exceeded (Bedford, 1997).

Variability in enzymes

The xylanases used in wheat based diets around the world come principally from three source organisms: *Aspergillus*, *Trichoderma* and *Humicola* spp. These organisms differ substantially in their requirements for growth, their production efficiencies and most importantly, characteristics of the xylanase produced. Identification of an enzyme as a xylanase and denomination of its activity in international units tends to imply that all are equally effective. Such is the difference in pH optima, substrate preference, temperature optimum and interfering factors, however, that the conditions of the xylanase assay used for each organism differ substantially, making direct comparison of international units impossible. Substrate source has a major effect, for example, on determined activity of xylanases from different sources as shown in Table 1.

What is particularly striking is that all enzymes shown in Table 1 come from the same organism. Differences between xylanases from different source organisms may be expected to be even greater.

Such effects are not confined to the test-tube, but are evident in effects on the bird. Many published trials suggest that the benefit of *Humicola* xylanases for example, is less dependent upon its viscosity reducing potential than those from *Trichoderma* (Cowan *et al.* 1993). Moreover, the two major xylanases from *Trichoderma longibrachiatum*, pI 5 and pI 9, differ substantially in this respect also (Bedford 1997, unpublished data; see Figure 1).

Therefore, the observation of a particular response to enzyme supplementation in one experiment but not in another needs to take into account not only the source organism of the xylanase, but also the predominant isozyme in the mixture used. It is not uncommon to observe diametrically opposed responses to xylanases of different origin. Some of our data for example show intestinal viscosity being increased by an *Aspergillus* xylanase preparation (whilst at the same time performance was improved slightly) compared with the usual decrease observed with *Trichoderma* xylanase preparations.

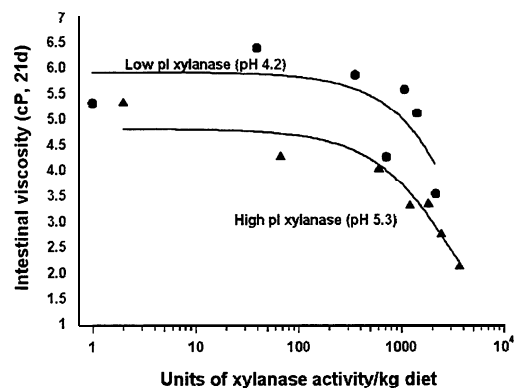


Figure 1 Effect of low and high pI xylanase on intestinal viscosity.

Table 1 Comparison of specific activities and Km of xylanases A, B and C from *Streptomyces lividans* on two different xylan substrates (Kluepfel *et al.* 1992)

	Oat Spelt Xylan		Birchwood Xylan	
	Km	Vmax	Km	Vmax
Xylanase A	2.6	5570	1.1	616
Xylanase B	3.7	1960	1.8	921
Xylanase C	4.2	650	4.1	3022

Variability in secondary diet constituents – fat

Whilst xylanases are added to wheat based diets to address the problems caused by arabinoxylans, the subsequent scale of response is dependent upon many non-wheat related dietary ingredients, in particular, the source and inclusion level of fat. As *digesta* viscosity increases, the digestion of all fats is impaired, the effect being more dramatic for saturated compared with unsaturated fats (Table 2).

It is likely that this difference is due to a greater dependence upon emulsification of the saturated compared with unsaturated fat sources for efficient absorption. Pasquier *et al.* (1996) clearly demonstrated that emulsification of **triolein/phospholipid/cholesterol** and subsequent rate of lipolysis was very much dependent upon solution viscosity. This observation was independent of gum type used to generate the range in viscosities (gum **arabic**, pectins and guar gums). In fact, increasing solution viscosity from 1 to 4 cp reduced the percent of emulsified triglycerides from 80% to 35% and cut emulsion surface area down by 75%.

These data suggest that even very low intestinal viscosities (<5 cp) will have a large effect on the digestibility of diets rich in fat (Pasquier *et al.* 1996), particularly if the fat source relies heavily on external forces (*i.e.* peristalsis) for emulsification, for example, diets containing saturated fat (e.g. tallow). The higher the fat content of the ration, the greater the strain on the emulsification systems and hence the greater the response to viscosity reduction.

Further, as a result of these effects on fat digestion, it has been determined that absorption of vitamin A and E into the liver is significantly influenced by fat type

and enzyme addition (Table 3). In the trial described in Table 2, the rye plus tallow diet in the absence of enzyme treatment had to be withdrawn at 28 days of age due to the prevalence of rickets (evidently vitamin D absorption was limited due to poor fat digestibility), which has also been observed in birds fed rye (Campbell *et al.* 1983).

In Vitamin A or E marginal diets this would result in a larger than expected response based on improved energy and protein digestibility alone. In many trials, it has been demonstrated that the performance of birds fed viscous diets can be significantly improved by addition of bile salts and emulsifiers. Such observations confirm the relevance of fat digestion as a limiting factor in such diets, and further, that the presence of these compounds will mitigate enzyme response in some circumstances. The benefits **enzymes** confer on the absorption of fat soluble compounds has also been noted in some in house trials, in which the absorption of pigments has been significantly increased by use of enzymes in wheat-based and barley-based diets. The value of such an observation may extend beyond simple pigmentation savings since some pigments are implicated as potentiators of the immune system (Haq *et al.* 1996).

Variability in environmental challenge

The response to **xylanases** is most often driven by changes in intestinal microfloral populations. Whilst intestinal viscosity is deemed a good indicator of the potential problems that can occur when a particular wheat is fed, the absolute response to enzyme addition is very much dependent upon the bacterial challenge present during the study. Circumstantial evidence for such a statement comes from the fact that in floor pen

Table 2 Effect of fat source on growth and fat digestibility in birds fed rye-based diets (Danicke *et al.* 1995).

Diet	Weight at 28 days	Fat digestibility %
Soybean Oil, No enzyme	1128 ^b	74.1 ^b
Soybean Oil, Plus enzyme	1254 ^a	93.1 ^a
Tallow, No enzyme	145 ^d	23.1 ^d
Tallow, Plus enzyme	1059 ^c	50.2 ^c

Table 3 Effect of fat type and xylanase supplementation on liver vitamin A and E content (Danicke *et al.* 1997, in press).

Diet	Vitamin A (mg/kg liver)	Vitamin E (mg/kg liver)
Soybean Oil, No enzyme	3.150 ± 1.108	0.109 ± 0.044
Soybean Oil, Plus enzyme	6.026 ± 1.409	0.210 ± 0.121
Tallow, No enzyme	2.250 ± 0.952	0.102 ± 0.029
Tallow, Plus enzyme	3.904 ± 1.227	0.125 ± 0.045

trials, the majority of the response to **enzymes** is in the grower/finisher stage, 22-42 days of age (Bedford, 1995b). In cage trials, where the microbial challenge from the litter is perhaps not as great during the finisher phase, the response is greatest in the starter period (Bedford and Morgan 1996).

It is clear that enzymes alter microbial populations, as indicated by microscopic examination and metabolic determinants such as ATP and VFA analyses. Enzyme addition to wheat-based diets leads to reductions in ileal microbial populations, possibly through enhanced nutrient retrieval by the bird, **effectively** limiting substrate availability in the intestinal lumen. At the same time an increase in caecal fermentation has been noted, probably due to provision of small oligomeric NSP as readily fermentable substrate (Bedford and Morgan, 1996; Choct *et al.* 1996; Smits, 1996). Several researchers have indicated that, particularly in **rye-based** diets, the negative effects of viscous grains can be overcome through the use of antibiotics (Fernandez *et al.* 1973; Vrangas and Wenk, 1993). Demonstration of a synergy between a mixture of monensin and avoparcin with a **xylanase** in wheat-based diets again points to a microbial interaction with response to **xylanases** (Allen *et al.* 1996b).

Evidence presented by Smits (1996) indicated that fat digestion was impaired to a **far** greater degree in conventional compared with germ **free** rats. These data add to the body of evidence which implicates microbes as the cause of bile salt deconjugation (Feighner and Dashkevicz 1988), and fat saturation, both activities being counter to good emulsification. Thus, the scale of the enzyme response will be dependent upon the microbial loading in the animals on test. This particular parameter is very rarely reported in the **wheat/xylanase** literature. Moreover it is impossible to standardise. No

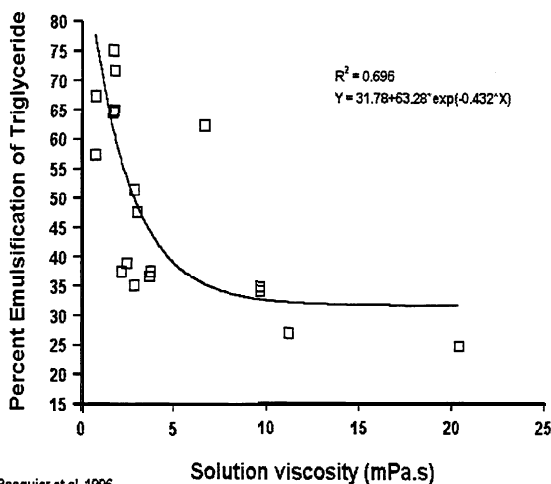
doubt there is considerable variation between experiments in microfloral populations and species dominance, which only contributes to the problems in correct interpretation of results when they are contrary to expectations.

Performance of birds **after** coccidial challenge when fed wheat-compared with maize-based diets is evidently poorer, but addition of a **xylanase** to the wheat diet partially overcomes the growth depressing effects of the challenge (Morgan *et al.* 1995). Such information suggests that the positive response to enzyme addition to a wheat-based diet would depend in part upon the coccidial challenge in the trial, again an aspect which is rarely reported in the literature.

One interpretation of the above is that the immune function of the bird may be enhanced in the presence of a viscosity reducing enzyme. Such data suggest that the performance of birds may well be significantly enhanced on addition of an **enzyme** when an immune challenge is holding back bird performance. **Performance** of the bird can be maintained to some degree by vitamin fortification of the ration. The fat soluble vitamins are evidently absorbed with greater efficiency in the presence of enzymes than in their absence. Thus variability in response to enzymes may be dependent in some part upon relative immune status of the animal due to supply of vitamins.

Breed and age of bird

Often overlooked or assumed to be of little significance, our own experience has been that some breeds present higher intestinal viscosity than others on the same diet and thus tend to respond to a lesser extent. Breeds need to be taken into account, therefore, in interpretation of data relating to addition of xylanases to wheat-based diets. (Table 4).



Pasquier *et al.* 1996

Figure 2 Influence of solution viscosity on triglyceride emulsification.

Table 4 Influence of breed of broiler on performance and intestinal viscosity when fed diets containing triticale and xylanase.

	Ross	Cobb
FCR (8-37 days)	1.964 ^b	2.049 ^a
Intestinal viscosity (cP) 21d	5.50 ^a	4.88 ^b

Data presented are main effects from a factorial design experiment where 0, 50% or 100% of wheat in ration was substituted with triticale and fed either with or without xylanase (*Trichoderma longibrachiatum* derived xylanase).

Age of bird (Petersen et al. 1993) also influences intestinal viscosity, with viscosity declining with age. This needs to be taken in context with the relative microbial loading however, since a given intestinal viscosity may have far more deleterious effects on performance in a mature, bacterially rich, small intestine, compared with a relatively sterile young bird.

More recent evidence suggests that even within a strain, individual bird differences, especially when fed low-ME (high viscosity) wheats, can be very large indeed (Hughes and Choct, 1997). Despite the fact that birds were offered identical diets, the individual bird variation in terms of wheat AME, (Low, Average and High being 9.6, 12.2 and 14.7 MJ/kg DM, respectively) and FCR (1.77, 1.96 and 2.07 respectively) was not explained by intestinal viscosity, but was correlated with ileal starch digestibility. This does not cast doubt on the relevance of intestinal viscosity, but rather, as the authors suggest, supports the view that the response to viscosity reduction by use of xylanases may well rely on the interaction with many other factors such as transit time and microbial proliferation in each individual bird.

Conclusion

The principal factor controlling the magnitude of response to enzymes in wheat based diets is intestinal viscosity. Experience and fundamental information relating to enzymes that do not reduce viscosity effectively (principally *Humicola* spp and some *Aspergillus*) are limited, and mechanisms of action are probably somewhat different. Figure 3 shows factors influencing the relative response of wheat-based diets to Xylanase supplementation. In short, many factors influence intestinal viscosity, the relevance of which is very much dependent upon the environment presented to the bird. The fact that intestinal viscosity per se has been shown to influence many parameters of the intestinal tract clearly indicates that failure properly to control this variable could lead to problems in performance.

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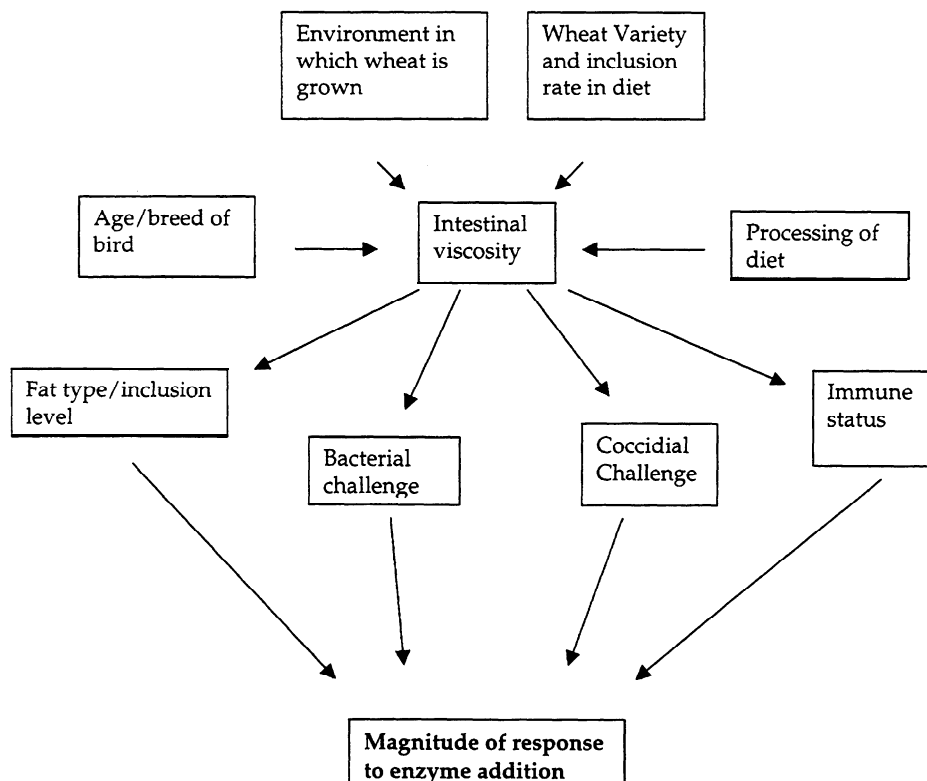


Figure 3 Factors which play a role in determining the relative scale of response to use of a xylanase in a wheat-based diet.

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