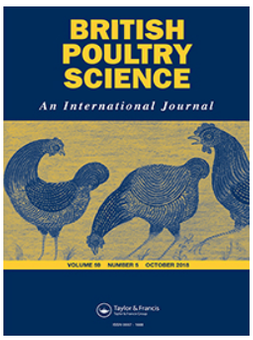

British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

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To cite this article: Michael R. Bedford (2018) The evolution and application of enzymes in the animal feed industry: the role of data interpretation, British Poultry Science, 59:5, 486-493, DOI: 10.1080/00071668.2018.1484074

To link to this article: https://doi.org/10.1080/00071668.2018.1484074

Published online: 27 Jul 2018.

BRITISH POULTRY SCIENCE
2018, VOL. 59, NO. 5, 486–493
https://doi.org/10.1080/00071668.2018.1484074

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GORDON MEMORIAL LECTURE

The evolution and application of enzymes in the animal feed industry: the role of data interpretation

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ABSTRACT

1. Enzymes have been used commercially for nearly 40 years and save significant costs through sparing of expensive nutrients but the mechanism by which this is achieved is still debated.
2. The research focused on non-starch polysaccharidase (NSPase) enzymes is used as an example of where greater progress could have been made if the details of the work had been described more fully and the analysis of the data generated had been broader in scope and more critical.
3. Lack of standardisation of the details presented in the materials and methods has been identified as a significant barrier to meaningful retrospective analysis and thus limits advances in the understanding of the mode of action of these enzymes.
4. The identity of the enzyme employed and its activity is often lacking, and more importantly the purity is rarely disclosed. Contaminant activities which are neither listed nor assayed could play a significant role in the responses observed.
5. The dose optimum of most enzymes is often considerably higher than that employed in most studies. Thus studies claiming synergy between two 'activities' should ensure that the response is not related to each enzyme simply augmenting the dose of just one activity in the finished feed. This is a common problem, and coupled with the lack of factorial experiments to justify the presence of each enzyme in a multi-enzyme product, it is not surprising that there is still debate as to whether single or multi-enzymes are best suited poultry rations.
6. The three proposed mechanisms for NSPases (viscosity, cell wall and prebiotic) are discussed, and along with their strengths and weaknesses it is suggested that a re-evaluation of each is needed. Viscosity may have to be re-evaluated as being a function not only of the cereal being fed, but of the age of the animal as well. The cell wall theory as described is poorly modelled *in vitro* and hence the validity of these data is questioned. The prebiotic theory may need significant modification as it appears that the quantities of oligomers produced are insufficient to generate the additional volatile fatty acids (VFA)'s reported. It is likely that all three mechanisms play a role in the responses observed, but the prebiotic mechanism probably plays by far the most important part in low viscosity diets.
7. Future research would be improved if it considered all potential mechanisms when designing a trial. Significant failings are apparent as a result of adherence to tenets in explanation of the results. Most importantly, it should be emphasised that a hypothesis is there to be tested, not defended.

ARTICLE HISTORY
Received 1 May 2018
Accepted 10 May 2018

KEYWORDS
Cell wall theory; feed enzymes; mechanism of action; NSPase; prebiotic; viscosity

Introduction

The use of enzymes in animal feed was first reported in 1925 (Hervey 1925). Feeding a 'fungal enzymic material' to female leghorns for 20 weeks resulted in a 22% increase in final body weight. Since the diet consisted solely of cereals, cereal by-products and liquid skim milk, and the 'enzyme' was added at 50 g/kg, the growth response noted could just have easily have been due to the supplemental 'enzyme' providing limiting amino acids, vitamins, minerals or perhaps antibiotics as much as it could have been due to the 'enzyme' activity implicated. Nevertheless, this pioneering work is of interest and is still relevant today because it is clear, with hindsight, that the 'active component (s)' implicated in the response and the actual mode of action were not necessarily one and the same. With the possible exception of phytases, this problem is still alive today when any feed enzyme, enzyme mixture or cocktail is employed. It is probably fair to say that in most experiments, the precise reason for the response observed is not known or fully understood and consequently the activity(ies) responsible are not unequivocally identified. This seems a bold statement as the use of feed enzymes is commonplace in the industry, but in truth this state of affairs is based on empirical rather than mechanistic research. Systematic reviews of the literature have shown that feed enzymes deliver a beneficial performance response more often than not, and the scale of response has been associated with several nutrient, ingredient and environmental factors (Rosen 2001, 2002a, 2002b, 2003). The lack of consensus with regard to how NSPases work, why the responses are variable and how and when phytase may spare energy or amino acids does not detract from the commercial benefit realised on use of these products. Despite almost 40 years of research, the advances in the understanding of the mode of action of commercial feed enzymes have not

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This article is the text of the 2018 Gordon Memorial Lecture, given at the Spring Meeting of WPSA UK Branch held at Croke Park, Dublin 9–11 April 2018.

progressed very much, whereas the 'amount of action' they deliver has been understood to a large degree almost from the outset of their use. This paper will focus on the development of the fibre-degrading enzymes (NSPases) in monogastric nutrition and attempt to explain why we have not advanced as far in our understanding of their mode of action as perhaps we should/could have. As will become apparent, failings in experimental design and reporting of experimental details have contributed to this predicament.

Mode vs amount of action

This phrase was first coined by the late Dr Gordon Rosen who made the point that the amount of action that a product may deliver can be measured, but the mechanism by which this happens can only be inferred. In many respects, we are not too distant from where Hervey was in 1925; the product works but we are not certain of why. Hervey (1925) made the statement that it was the enzymic complex that was responsible, simply because it was known to these authors that this activity was present. An application of hindsight allows us to broaden the list of potential reasons as to why the product functioned, none of which relies on the enzymatic activity of the product. The principal cause of this conundrum has been the lack of definitive tests which would allow us to either reject or accept a hypothetical mode of action.

NSPases – the early years

Mistaken identity

Work in the USA in the mid-to-late 1950s had demonstrated that the performance of barley-fed chickens could be improved by soaking the cereal in water prior to feeding (Fry et al. 1957). The fact that water treatment and germination of barley liberated maltose lead the researchers to focus on starch digestion and hence attention was focused on administration of exogenous amylases (Jensen 1957). Several subsequent studies showed that the performance of a barley-based diet could be equilibrated with that of a maize-based diet on inclusion of the *Aspergillus*-derived amylase product. Further work ensued with many laboratories reporting similar beneficial effects when barley-based diets were supplemented with bacterial or fungal derived amylases. It was not until work with highly purified amylases failed to deliver the responses that had previously been observed that the roles of both amylase and starch digestion were questioned (Willingham et al. 1959). Subsequent work demonstrated that indeed the target substrate was more likely to be soluble, viscous β 1-3,1-4 glucan (Burnett 1966) and that the 'amylases' used up to that point had probably contained significant amounts of β 1-3,1-4 glucanase. It is well recognised today that barley, especially the pearled barley used in the studies conducted in the 1950s, impairs digestion through increasing intestinal viscosity and this responds markedly to the addition of β 1-3,1-4 glucanases (Campbell and Bedford 1992; Hesselman et al. 1982). It was simply fortuitous that the 'successful' enzymes which were used and identified as amylases actually contained many other activities, the β 1-3,1-4 glucanase, which was responsible for the benefit, being attributed to the amylase. Even today, the possibility of a mistaken allocation of an economic value to a given enzyme activity to another is a real problem for many commercial products, especially those that contain multiple activities. Even relatively pure, mono-component enzymes often have ancillary enzyme activities which are produced by the host organism, which in some circumstances may be significant with regard to the outcome observed. This has implications when attempts are made to justify the value of a particular mixture of activities as discussed below.

Dosage and mixtures

The relationship between the dose of an enzyme and the response observed is most often log-linear (Zhang et al. 2000, 1996; Rosen 2001, 2002a, 2002b). This is consistently overlooked in studies where dosage of an enzyme is tested and the data are analysed using quadratic models. Use of such models, particularly in a dose-response trial that does not employ log increments in dosage, is bound to result in an underestimation of the optimum biological dose. An artificial example of this is shown in Figures 1 and 2, where the first figure shows a quadratic fit to 5 doses of an enzyme and the second shows the influence of inclusion of a 6th log increment in dose. Such an example highlights the problems that can occur as a result of fitting an inappropriate model and moreover identifies a significant problem issuing from such work, namely the suggestion that a quadratic model is acceptable.

Use of quadratic dose models is especially problematic when it comes to justification of a combination of enzyme activities. For example, when two enzyme products are tested factorially, e.g. an amylase and a β 1-3,1-4 glucanase, it is often the case that only one dose of each is tested in a

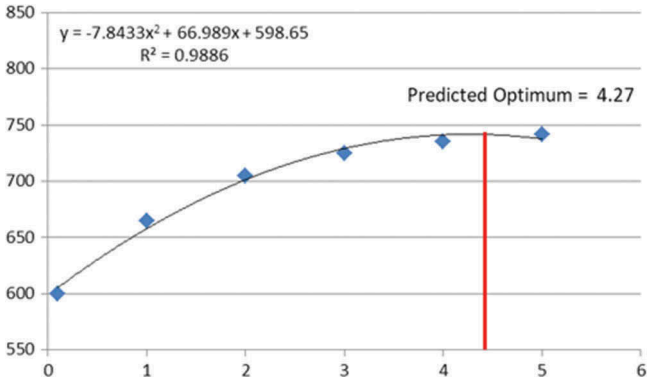

Figure 1. Fitting of artificial weight gain data showing a quadratic fit to linear increments in dose of enzyme.

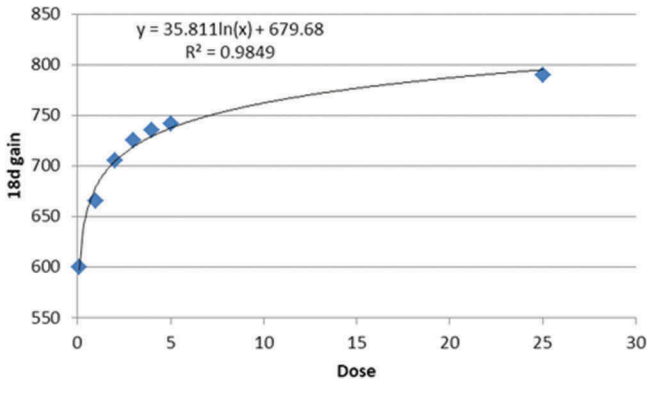

Figure 2. Influence of inclusion of an extra dose set at a log increment on the same data displayed in Figure 1.

2 × 2 factorial. If the combination of two such activities is shown to be of greater benefit than each on its own, then the common misapprehension is that this signifies a synergy. However, such an interpretation is clearly open to challenge. If, for example, the amylase has a contaminant β 1-3,1-4 glucanase activity and the β 1-3,1-4 glucanase is dosed sub-optimally, the combination of the two may result in additional benefit simply because the β 1-3,1-4 glucanase activity is delivered at a dose which is closer to the optimum. This is eminently plausible given the log-linear relationship between dose and response as described above. Unless each activity is dosed at increasing levels such that it is clear that the highest dose employed of each individual activity is at the 'discernible' optimum, then synergy via their combination cannot be confirmed. A recent review expands on these significant errors in the literature and goes further to suggest that the data supporting the presence of each activity in many 'multi-enzymes' are, in many cases, inadequate to justify the presence and dose of each activity (Masey O'Neill et al. 2014b).

Analysis

Linked to the above, it is clear that the NSPase literature is not particularly consistent in reporting activities of the main enzyme activity, let alone the ancillary activities. Indeed, Rosen noted that 28% of papers related to feed enzyme use failed to report the dosage of enzyme employed in each treatment (Rosen 2002a). The failure to accurately report enzyme assay results and enzyme classes employed markedly limits the ability to interpret the literature. For example, the xylanase class of enzyme has been reported as a glycanase, hemicellulose or pentosanase, and coupled with this issue is the frequent failure to report the conditions of the assay employed, both of which clearly obfuscate interpretation. Even when papers do report enzyme activities, far fewer report the activity determined in feed after manufacture (which may involve thermal losses if the diet is pelleted) (Rosen 2002a). If the feed is analysed, it is almost exclusively analysed for the activity of interest to the authors, with no measurements of activities which may or may not be known to be present in the products or of potential value to the animal. As has previously been suggested, standardised assays measuring phytase, xylanase, B 1-3, 1-4 glucanase, mannanase and cellulase as a minimum (Masey O'Neill et al. 2014b) should be reported to enable much more informed *post hoc* analysis. Moreover, the production host and source organism for any GM products should always be reported so that the relevance of the assay results is enhanced. A family 10 xylanase, for example, may give different responses than a family 11 in the animal; thus, knowledge of the identity of the enzyme investigated is of significant value for interpretation of the results. Knowledge of the host organism also gives some indication of what ancillary activities may be produced in the background and hence 'contaminate' the target enzyme. Until such a regime of consistency in reporting is implemented, the rate of progress in understanding of NSPase activity will be slow.

Current understandings on mode of action and limitations in the data

For NSPases, there are three principal modes of action that are suggested, namely:

(1) Viscosity reduction
(2) Cell wall destruction
(3) Generation of prebiotics

It is not the intention of this paper to provide a comprehensive review of each mechanism, for such information the reader is referred to other reviews (Simon 1998; Masey O'Neill et al. 2014b; Aftab and Bedford 2018). Rather the goal is to highlight the strengths and weaknesses of the data supporting each mechanism and to conclude with regard to the likely contribution of each mechanism to the final response and suggest that there are data that are still missing.

Viscosity reduction

As mentioned earlier, the viscous b-glucans present in barley were first implicated as the reason for its poor nutritive value by Burnett (1966). Subsequent work showed that feeding B 1-3, 1-4 glucanases reduced intestinal viscosity of barley-fed chickens markedly, particularly young broilers, and also reduced faecal moisture, improved nutrient digestibility and improved growth rate and efficiency (Classen 1996; Almirall et al. 1995; Hesselman et al. 1982). Viscosity was also implicated in rye (Bedford and Classen 1992; Teitge et al. 1991) and to a lesser extent in wheat (Choct et al. 1999; Hughes and Zviedrans 1999; Steenfeldt et al. 1998) based rations as well with the mode of action being simply that a more viscous intestinal content results in more inefficient mixing of digesta and movement of solutes, with a resultant depression in nutrient digestibility (Bedford 2000).

Questions remaining

Viscosity

Is viscosity reduction per se the reason for the response to added NSPases or is there some additional mechanism to consider? Furthermore, is increasing viscosity simply invoking larger stresses on the animal through the same mechanism(s) or do alternate/additional mechanisms come into force once a threshold is passed? It is clear that feeding viscous diets and reducing viscosity with enzymes which target the soluble, viscous carbohydrate result in improved performance (Bedford and Classen 1992; Barrier-Guillot et al. 1995; Burnett 1966). It has also been shown that if the viscous arabinoxylans are extracted from wheat and fed to broilers at 35 g/kg, viscosity of the intestinal digesta was increased and concomitantly performance markedly depressed (Choct and Annison 1992), but this effect was lost if the arabinoxylans were depolymerised (and thus no longer viscous) with a xylanase prior to feeding. If a fermentable viscous pectin (Langhout et al. 1999) or non-fermentable viscous carboxylmethylcellulose (Smits et al. 1997, 1998) is fed to broilers, then intestinal viscosity is elevated and performance suffers. Thus, the hypothesis that increased intestinal viscosity depresses nutrient digestibility and subsequently performance seems to be well supported. However, there does seem to be some equivocation regarding the role of the microbiome in implementing the effects of increased viscosity. Although high-viscosity diets do increase intestinal viscosity in germ-free chicks, performance and nutrient digestibility do not suffer (Schutte and

Langhout 1999; Langhout et al. 2000). This suggests that intestinal viscosity per se is not necessarily always detrimental to the animal. Furthermore, feeding antibiotics seems to mitigate the negative effects of highly viscous diets in some studies (Moran and McGinnis 1965; MacAuliffe and James 1971) but not all (Choct and Annison 1992; Moran and McGinnis 1966), with the choice and dose of antibiotic (MacAuliffe and James 1971) and age of the animal (Moran and McGinnis 1966) perhaps playing a role in determining the success of the antibiotic strategy. Higher doses of more effective antibiotics seem to be required in young birds fed rye-based diets (MacAuliffe and James 1971), which tend to elicit much higher intestinal viscosities (Bedford 1996). In some cases perhaps viscosity is so overwhelming that digestion is compromised to the point that feed passage is slowed, digestion almost halted and conditions of the small intestine are adequately anaerobic and sufficient undigested protein and starch is present to fuel a significant bacterial overgrowth. Indeed, such conditions were noted in the work of Choct et al. (Choct et al. 1999). This overgrowth is as much the reason for depressed performance as is the reduced nutrient density of the ration and in such cases large quantities of the right antibiotic can be beneficial and negate the detrimental effects of viscosity. Under less extreme circumstances, the bird can cope by secreting more enzymes and eating more feed to compensate for the marginal reduction in diet digestibility. Examining this hypothesis leads to several questions which need addressing with regard to the viscosity theory.

a. Intestinal viscosity in the broiler seems to increase with age to 21–28 d of age and then reduces significantly thereafter (Fischer 2003). This may explain the success seen with antibiotic strategies in younger poults (1–4 weeks) fed barley-based diets (Moran and McGinnis 1965), which could not be repeated in older (8–20 weeks) birds (Moran and McGinnis 1966). Thus, the involvement of a 'malevolent' microbiome may be limited to younger birds when diet viscosity peaks and drives increased ileal fermentation. It is interesting to note that the explanation for the increment in viscosity up to 21–28 d of age is a result of development of a microbiome that is capable of partially degrading insoluble arabinoxylan, and in doing so producing soluble, viscous arabinoxylans which are not depolymerised until the microbiome has adapted further to utilise such carbohydrates. If this is the case then it is interesting to speculate that the success of the antibiotic strategy employed by MacAuliffe and McGuinness (MacAuliffe and James 1971) may have been due to direct inhibition of those bacteria that would have solubilised insoluble xylan to create viscous, soluble material in the 21–28 d old bird. Feeding a fermentable viscous carbohydrate after this critical age may be less detrimental than before due to the ability of the adapted microbiome to reduce the viscosity *in situ*, thereby reducing the quantities of undigested starch and protein and thus improving performance. Feeding a non-fermentable carbohydrate will likely not result in such an age-related reduction in viscosity and thus the negative effects persist beyond the adaptive age as noted by Smits (Smits et al. 1997).
b. In older birds, presumably the microbiome has developed to the point where it can digest and utilise the some of the viscous carbohydrates. Under such circumstances the growth response to a 'de-polymerising' enzyme is likely less attributable to the additional, but more marginal, reduction in viscosity compared with the younger bird. The responses that are noted may, however, be more related to the production of fermentable oligosaccharides from the remaining soluble viscous carbohydrate (CHO). Thus, viscosity in older birds may simply be an indicator of the amount of fermentable oligosaccharides that *could* be generated on use of the correct enzyme. Indeed, increased fermentation of xylans in the caeca of xylanase supplemented birds has recently been noted in older but not younger birds, suggesting that there is indeed an adaption of the microbiome over time, enabling it to utilise xylan as a significant fuel for production of VFAs (Lee et al. 2017a).
c. It is possible that the effects of intestinal viscosity change from simply marginally reducing digestibility to significantly delaying feed passage with much more serious consequences as noted above. A re-analysis of the data provided by Bedford and Classen (1992) showed that feed intake actually increased with increasing intestinal viscosity up to 20 mPas, beyond which intake was depressed markedly with each further increment in viscosity. The suggestion is that below 20mPas, the animal perceives the reduction in dietary nutrient density and compensates by eating more, maintaining gain but compromising feed conversion ratio (FCR). Beyond 20 mPas the intestine can no longer move the digesta rapidly and in effect the digestive system backs up and intake and thus gain is reduced and FCR increases markedly. At such a point, the microbiome involvement in growth depression likely begins to play a larger role. Age of the animal will play a role here as will fermentability of the viscous carbohydrate as noted in (a) above. If this analysis is correct, then the influence of viscosity and the response to a viscosity-reducing enzyme will be dependent upon the viscosity of the control-fed animals. If it is greater than 20 mPas, the response to a reduction in viscosity would be an increase in intake coupled with a large improvement in FCR and gain and a change in ileal and caecal microbiome populations. If it is less than 20 mPas, then the effect of reducing viscosity would be to reduce intake, improve FCR and not influence gain dramatically. There would likely be little change in the ileal microbiome but perhaps a change in the caecal microbiome as more fermentable CHO enter the caeca.

The three questions posed above, relating to the effect of the age of the animal, the relative fermentability of the viscous carbohydrate (generally B 1-3,1-4 glucan is much more readily fermented than highly substituted arabinoxylan) and the absolute viscosity level generated by the diet, could, if understood more fully, enable the implementation of more effective strategies of use of feed enzymes and perhaps feed ingredients. Indeed, a fundamental question is raised here; is intestinal viscosity a consequence of the

interaction of the diet with the microbiome and not simply dependent upon the diet itself? If so, any intervention which alters the microbiome responsible for producing viscous material from the insoluble NSP and/or reducing the size of the soluble, viscous NSP will change the viscosity and hence the impact on the performance of the animal.

Cell wall hypothesis

Since the cell walls encapsulating the starch and protein in the endosperm of the common cereals used in animal feed are made of materials that cannot be digested by the animal itself, it seems self-evident that any enzyme capable of puncturing these cell walls should expedite the digestion of the contents, especially if the cells were intact. Microscopic analysis of feed and digesta shows that intact cells are clearly present and that the use of cell wall degrading enzymes has clearly resulted in greater disruption of cell walls by the time the digesta had reached the proximal small intestine (Bedford and Autio 1996). Thus, it seems plausible that the use of such enzymes does lead to the degradation of endosperm cell walls, hence enhancing diet digestibility by enabling endogenous proteases and amylases more rapid access to the previously encapsulated protein and starch. *In vitro* incubation of feed raw materials with the relevant enzymes has shown such destruction of the cell walls taking place (Ravn et al. 2017), albeit to a lesser extent than seen *in vivo*, but nevertheless this does seem to support the concept as a working hypothesis. As a consequence, there has been a considerable drive to look for combinations of enzymes which more rapidly and completely degrade the complex structure of the endosperm cell walls. If this mechanism is relevant, then it seems evident that the enzyme complex employed should contain all activities necessary to completely break through the cell wall and accomplish such a feat within the time constraints dictated by the intestinal tract.

However, scrutiny of the *in vitro* work available reveals that whenever commercial doses of feed enzymes were used in the simulation, there was negligible destruction of cell walls (Morgan et al. 1995; Tervila-Wilo et al. 1996), whereas those *in vitro* studies where cell wall degradation was noted used dosages that were 10–15 (Tervila-Wilo et al. 1996; Parkkonen et al. 1997), 25 (Le et al. 2013) and up 50 (Ravn et al. 2017) times higher than that used commercially. Moreover, the *in vivo* data have shown destruction of cell walls as early as the jejunum (Bedford and Autio 1996) which questions the validity of the long incubation times often employed *in vitro*. Furthermore, the proposition that a complex enzyme mixture is required to degrade the complex cell wall structures in vegetable ingredients (Meng et al. 2005), which seems plausible from a stoichiometric viewpoint, is severely challenged by the fact that the *in vivo* data where significant cell wall destruction was noted were generated using a relatively pure, mono-component xylanase (Bedford and Autio 1996). As a couple of recent reviews have commented (Masey O'Neill et al. 2014b; Aftab and Bedford 2018), the definitive work to prove the presence and dose of the enzymes in almost all multi-carbohydrase is indeed at the optimum and that they are truly synergistic is yet to be published. Thus, the questions remaining regarding the cell wall hypothesis are still:

(1) Is it relevant – i.e. are the added enzymes really depolymerising intact cell walls *in vivo?* Or is a marginal increase in permeability all that is needed?
(2) If so how many activities are needed to optimise this response *in vivo?*
(3) If not, what is the mechanism by which the animal is clearly able to break open cell walls more effectively in the presence of an NSPase?

Prebiotic hypothesis

The third proposed mechanism of action for NSPases has always been treated as the poor cousin and given very little consideration, but ironically it may turn out to be far more important than first envisaged and could explain the apparent cell wall destruction noted *in vivo*, at least in more mature animals. Work with rats had suggested that increased large intestinal fermentation was correlated with increased cell proliferation in the large intestine and secretion of enteroglucagon and Peptide YY (PYY) (Goodlad et al. 1987), and that these hormones had a role to play in delaying gastric emptying, small intestinal motility and growth rate and enzyme secretion. Resistant starch had a similar effect, the increment in PYY and Glucagon-like peptide-1 (GLP-1) concentrations in the blood correlating with butyrate and propionate concentrations in the large intestine (Zhou et al. 2012) of rats. Thus, increased large intestinal fermentation could be effective in moderating stomach emptying, enabling more efficient grinding and acid degradation of cereal cell walls. In this manner, stimulation of large intestinal fermentation would be expected to improve digestion of all of the diet, not just the cereal components. It was proposed in 1995 that xylanases may affect the microbial activity in the GI tract of poultry through provision of fermentable oligosaccharides and low molecular weight polysaccharides, or prebiotics, as a result of limited hydrolysis of the soluble and insoluble arabinoxylans in cereals (Morgan et al. 1995). It was suggested at the time that this would improve host performance largely through energy provision but given the potential feedback on the gizzard it may also enhance protein digestibility. There is clear evidence that feeding NSPases influences the microbiome of both the ileum and caecum (Bedford and Apajalahti 2001; Gonzalez-Ortiz et al. 2016; McCracken et al. 2006) and results in increased caecal fermentation (Masey O'Neill et al. 2014a), levels of butyrate in particular responding to NSPase inclusion (Choct et al. 1999; Gonzalez-Ortiz et al. 2016). The mode of action seems to be at least partly through the provision of oligosaccharides since feeding isolated and relatively pure arabino-xylo-oligosaccharides to broilers results in similar performance outcomes compared with the NSPase itself (Courtin et al. 2008). Thus, the hypothesis suggests that the benefit of an NSPase is that it quantitatively produces fermentable prebiotics which provide an energy source for saccharolytic bacteria in the caeca. This benefits the host in two ways; as a result of increased energy recovery from the diet (in terms of VFAs) and as a result of enterohormone responses to the elevated butyrate levels (Furness et al. 2013), the gizzard grinds more efficiently and the diet is more effectively digested. The consequence of the latter benefit is that the small intestinal digesta contains endosperm cell walls which appear to be more completely degraded. This helps to

explain the anomaly between the *in vitro* and *in vivo* data as described above in the cell wall hypothesis section. The questions that remain for this hypothesis are as follows:

(1) The oligosaccharide work that has been published has used doses ranging from 25 g to 20 kg per tonne of feed and found in many cases, even at the lowest level of inclusion, significant performance responses (Courtin et al. 2008; Suo et al. 2015; Eeckhaut et al. 2008; Morgan et al. 2017). The low levels in particular do not provide enough substrate to generate a meaningful amount of VFA; thus, the classical prebiotic route has to be questioned.
(2) In chickens, it appears that feeding a xylanase over an extended period of time is required to elevate plasma PYY levels and increase the weight and digesta content of the caeca (Lee et al. 2017b), which suggests that some degree of adaptation is required. Some studies have shown that performance benefits in low viscosity wheat- and triticale-based diets are not seen until the bird has reached 21 d of age (Mendes et al. 2013). If the enzyme were to generate significant quantities of XOS which are quantitatively fermented, then there should not be a delay in the response to the inclusion of the enzyme.

An alternate hypothesis is that the xylanase, for example, is not producing a prebiotic per se but a signalling molecule which stimulates bacterial species that could degrade xylan to produce xylanases. This builds up into the quantitative degradation of fermentable xylan resulting in the increase in VFAs noted. It is the fermentation of dietary fibre per se rather than the oligosaccharides generated by the enzyme that provides the additional energy and feedback to the gizzard which the oligosaccharides generated by the xylanase itself could never achieve on their own. Recent work has demonstrated that the caeca of very young chicks (11 d) contain a lot of soluble sugars but low concentrations of VFAs, suggesting a naïve and poorly populated caeca (Lee et al. 2017a). As the bird ages, however, the sugar concentrations decrease and VFA concentrations increase, signalling an increasingly functional caeca. Most interesting was the fact that as the birds fed the xylanase aged, they had proportionately less soluble xylose and arabinose in the caeca, coupled with greater quantities of acetic and butyric acid. This suggests that feeding a xylanase results in an adaptive change in the microbiome of the enzyme-supplemented birds which gives them a greater capacity to hydrolyse xylan. Indeed, a recent study (Bedford and Apajalahti, 2018) has demonstrated that the caecal microbiome from chickens feed a xylanase for 35 d had a far greater ability to ferment xylose, XOS, AXOS and wheat bran than their control counterparts. This suggests that there is some degree of 'training' or evolution of the microbiome towards one that is able to degrade fibre sources that otherwise would be ignored.

Thus, the prebiotic hypothesis likely needs to adapt to consider a process where the NSPases are not quantitatively producing fermentable sugars, but rather sending signals to the microbiome to develop its fibre degrading capacity. Identification and optimisation of these signalling molecules is clearly a field that should attract interest.

One final point, the enzyme, in producing small amounts of oligomers, will degrade both soluble and insoluble fibre. Small breaks in the insoluble fibre increase the amorphous nature of the fibre which is known to reduce lag time for attachment of fibrolytic bacteria (Wang et al. 2001). Thus, NSPases may well be improving fibre digestibility by modification of the fibre itself as well as modification of the microbiome's fibre degrading capacity.

Conclusions

It is clear that hypothesis and theories are formed and become incredibly difficult to change, even when mounting evidence contradicts the tenet. Explanations to devalue challenging data are often easier, and less controversial, to produce than a completely new hypothesis. Whilst it is never wise to 'throw the baby out with the bathwater', it often pays to question the tenets that exist. Often theories rely on several assumptions which are not validated. Thus, it is important to continuously question whether the data have been generated in an acceptable and repeatable way, make sense and fit the hypothesis. If they do not, then alternative or even additional mechanisms should be considered rather than simply fitting the data to the current theory.

Disclosure statement

No potential conflict of interest was reported by the author.

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