Use of pure xylanases in pig and poultry nutrition

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Introduction

The fundamental principles of using NSP-degrading enzymes have been extensively discussed in numerous published papers. This presentation introduces a recently developed pure xylanase and provides information about some new discoveries concerning the mode of action of NSP-hydrolysing enzymes.

New insights into the mode of action of NSP-degrading enzymes

Research into the mode of action of NSP-degrading enzymes has recently focused increasingly on the effect of enzymes on the gut microflora and the morphology of the gastrointestinal tract. Both these areas of activity are closely related to the most frequently cited enzyme effects, namely viscosity reduction and breakdown of the cage effect. They could be described as secondary effects and have been the subject of extensive studies in the context of dissertations at the Free University of Berlin. A few preliminary results were presented by Prof. Simon at our last conference two years ago (SIMON, 1997). The most important conclusions were:

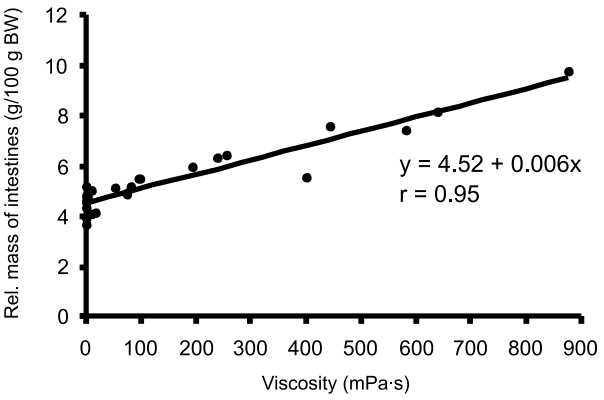
- The number of luminal and mucosa-associated enterobacteria and gram-positive cocci is reduced by xylanase supplementation of a wheat ration.
- The number of gut-wall-associated lactobacilli is higher in animals fed xylanase-supplemented diets.
- A proven correlation exists between the viscosity of the chyme and the relative gut mass of broilers, which theoretically suggests a lower energy maintenance requirement.

The last point deserves closer consideration. Reduced chyme viscosity as achieved through the use of suitable xylanases leads to a lower relative gut mass. As the tissue of the digestive tract has a particularly high and energy-intensive protein turnover, changes in relative gut mass can affect heat production and energy requirement.

Figure 1 shows the relationship between viscosity and relative gut mass. It is important to bear in mind that the actual viscosity values measured in the small intestine after feeding conventional modern wheat rations are generally below 25 mPa·s, in most cases even below 10 mPa·s (Figure 1). A close, statistically significant correlation between the two parameters can only be demonstrated by using extreme rations which lead to very high viscosity values. On the basis of the calculation reported by SIMON (1998 and 2000) a reduction in viscosity from, say, 20 mPa·s to 5 mPa·s, which would be conceivable under practical conditions, would reduce the relative gut mass by about 0.1 g. For a 1000 g broiler this would be equivalent to an energy saving in the daily maintenance requirement of 1.3 kJ or 0.2 %. This morphological enzyme effect does exist, but accounts for a relatively small proportion of the overall performance response.

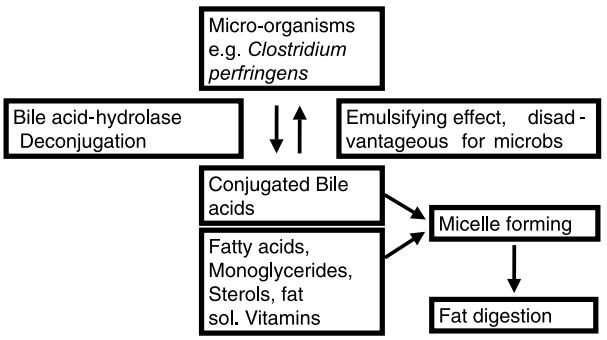
In parallel to the morphological investigations, further studies on the significance of the gut microflora have been conducted. In addition to the difficult direct determination of individual groups of bacteria and their distribution in the intestinal tract, a few auxiliary parameters have been employed. For example, some enzyme activities produced by microorganisms of the digestive tract have been studied.

Figure 1: Relation of jejunum digesta viscosity and relative mass of intestines of broiler chickens (after SIMON, 1998)



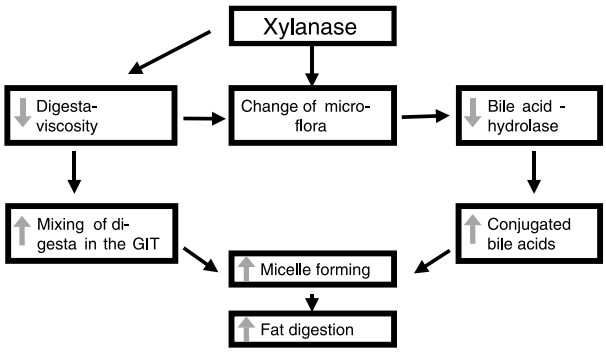
One of the selected parameters was the bile acid hydrolase activity (Figure 2). In their conjugated form, i.e. bound to taurine or glycine, bile acids emulsify fats in the digestive tract. Together with fatty acids, monoglycerides and fat-soluble vitamins they form particles known as micelles, a necessary precursor for effective fat absorption. But the emulsifying effect of the conjugated bile acids is harmful to the microbes of the digestive tract, i.e. they have bactericidal action. Some microorganisms such as *Clostridium perfringens* seem to have developed their own defence mechanism. They produce bile acid hydrolases which lead to deconjugation of the bile acids, thereby considerably weakening their emulsifying action (COLE and FULLER, 1984). In this way the microorganisms interfere with micelle formation and hence fat absorption.

Figure 2: Negative influence of some micro-organisms on fat digestibility



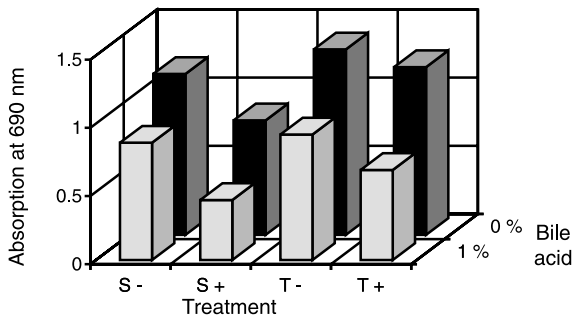
The interactions between xylanase supplementation, viscosity and fat digestion have been adequately researched. It is known that enzyme supplements, for example by altering substrate availability in the small intestine, modify the microflora (Figure 3). The objective of the studies presented below was to examine whether xylanase supplementation reduces the production of bile acid hydrolases by altering the microbe population in the gut. This might result in more conjugated bile acids becoming available and would ultimately explain the marked beneficial effect of xylanases on fat digestibility.

Figure 3: Relation of xylanase and fat digestion (adapted from HÜBENER et al., 1999)



In a trial with broilers a pentosan-rich wheat/rye diet was fed containing soya oil or tallow as fat source (Figure 4). Both diets were administered with and without supplementation of the xylanase ZY68. The broilers were killed on day 13, jejunal digesta samples collected under anaerobic conditions and inoculated into microtitre plates containing a complete medium. In a parallel set of experiments, 1 % bile acid (taurocholate) was added to the complete medium. This is approximately equivalent to the physiological concentration of conjugated bile acids in the digestive tract. In the groups with tallow as the fat source greater microbial growth was observed. Xylanase supplementation of the diet generally resulted in lower microbial growth. The bile acid supplement reduced the bacterial density. This was to be expected since conjugated bile acids can have bactericidal action, as mentioned earlier. This antibacterial activity of the bile acid was relatively stronger in the xylanase groups, i.e. the population of bile acid hydrolase-forming organisms was reduced in the broilers fed the enzyme-supplemented diet. They were thus better equipped for optimal fat digestion.

Figure 4: Microbial growth in complete medium - Jejunum digesta, day 13 (adapted from HÜBENER et al., 1999)



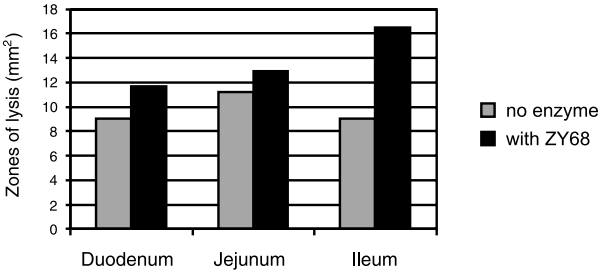
➔ addition of bile acids to the medium reduces microbial growth
➔ addition of xylanase reduces microbial growth

This finding that bile acid-deconjugating microorganisms are suppressed was confirmed in previous studies by HÜBENER et al. (1998, 1999). In these experiments a reduction in bile acid hydrolase activity and a slight increase in lipase activity after xylanase administration were also observed directly.

A further interesting study by the same research team (HÜBENER et al., 1998) was concerned with the production of carbohydrate-degrading enzymes by intestinal microorganisms. By measuring these enzyme activities in the gut it is possible to draw inferences about the microbial population and its metabolic activity.

A broiler trial was carried out to investigate the effect of xylanase supplementation on intestinal β-glucanase production. Two groups received a pentosan-rich diet, either with or without ZY68 supplementation. The broilers were killed at 4 weeks old and the bacterial β-glucanase activity determined in different sections of the small intestine. Figure 5 shows that supplementation with the xylanase ZY68 caused a marked increase in β-glucanase activity in the anterior sections of the digestive tract. This means that xylanase supplementation modified the gut environment in a way that was beneficial for glucanase-producing gut microorganisms. This finding is highly relevant if we consider that β-glucan degradation by microorganisms is only worthwhile in conjunction with other NSP-degrading enzymes (i.e. in the presence of complete enzyme systems). This means that increased β-glucanase production by gut microorganisms should generally be associated with an increased production of further carbohydrases, which is likely to promote the desired partial hydrolysis of NSPs as a whole.

Figure 5: Influence of xylanase addition to the feed on bacterial β-glucanase activity in the small intestine (adapted from HÜBENER et al., 1998)



➔Xylanase addition increases bacterial β-Glucanase activity and hence the NSP (β-glucan) degradation in the GIT.

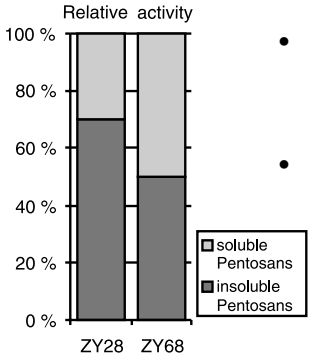
Properties of the xylanase ZY68

The new xylanase ZY68 has been approved since July 1999. It is produced by recombinant DNA technology. The gene coding for xylanase production was transferred from the strain Thermomyces lanuginosus to an Aspergillus production strain. ZY68 is characterised by further improvements in substrate specificity, pH profile and heat stability.

Figure 6 shows a schematic representation of substrate specificity. While the xylanase in ZY28 still attacks primarily insoluble pentosans, i.e. the focus is clearly on eliminating the cage effect, ZY68 has a more balanced effect on insoluble and soluble arabinoxylans. This ensures that any soluble pentosans formed during the degradation of inso-

luble pentosans are immediately hydrolysed further to molecule fragments that are no longer viscosity-forming. This is reflected in the high viscosity-reducing activity of ZY68, which is evident both in vitro, i.e. after incubation of feed samples, and in vivo, i.e. by measuring digesta samples. We know that a powerful viscosity-reducing effect of an enzyme under certain circumstances can substantially enhance performance responses. In addition it is a necessary prerequisite to prevent viscosity-induced wet litter.

Figure 6: Substrate specificity of ZY28 and ZY68 (schematic representation)



- **ZY28**: Focus is on degradation of insoluble pentosans (eliminating of the cage effect)
- **ZY68**: More balanced effect on insoluble and soluble pentosans (eliminating of the cage effect and effective viscosity reduction)

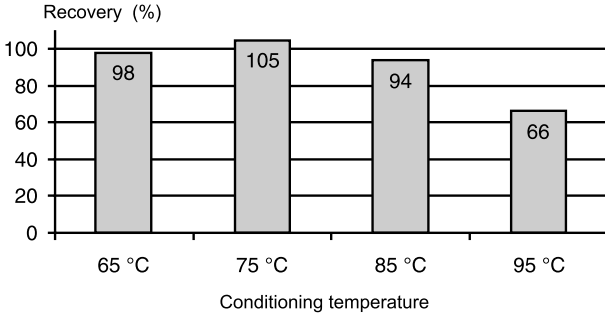
Enzymes work within a defined pH range. According to current knowledge, an optimal effect in the animal requires high activity especially in the anterior gut sections, i.e. the area of low pH levels. It is also desirable that the activity remains high further along the small intestine because here, too, effective absorption can take place. ZY68 is characterised by a broad pH spectrum. At least 60 % of the maximum activity is reached in the region from pH 4.5 to 7.

The heat stability of an enzyme is determined by its "intrinsic stability", i.e. the stability of the molecular structure, and by the formulation of the product, i.e. the extent to which it is bound to a carrier and any coating. Both factors have been optimised in the product ZY68. The xylanase from Thermomyces lanuginosus has excellent intrinsic molecular stability and the proven coating procedure provides additional mechanical protection.

The intrinsic molecular stability can be measured in vitro. This is done by incubating enzymes in a buffer solution with a defined pH and measuring the residual activity at different times. ZY68 was completely recovered after incubation for 30 min at 60 °C. These experiments do not allow any direct inferences to be drawn about the degree of pelleting stability, for example at 60 °C. This is because the in vitro measurements constitute a long-term heat treatment, which does not actually occur in pelleting. Moreover, in vitro tests are performed in liquid medium whereas feed has a low moisture content and the enzyme is additionally stabilised by the presence of a substrate. Yet this in vitro test does provide a suitable auxiliary parameter for comparing enzyme stability. It is also a way of demonstrating the superiority of ZY68 over other xylanase products.

Figure 7 shows the result of a pelleting trial at the Danish Biotechnical Institute in Kolding. ZY68-containing feed samples were pelleted under standardised conditions at different conditioning temperatures and the determined recovery rate compared with the activity content in the meal-type feed. At temperatures up to 85 °C the xylanase was recovered almost completely. At a conditioning temperature of 95 °C, followed by brief exposure to an even higher temperature in the press, analysis showed an activity loss of about one-third.

Figure 7: Pelleting stability of ZY68 (Danish Biotechnical Institute, 1997)

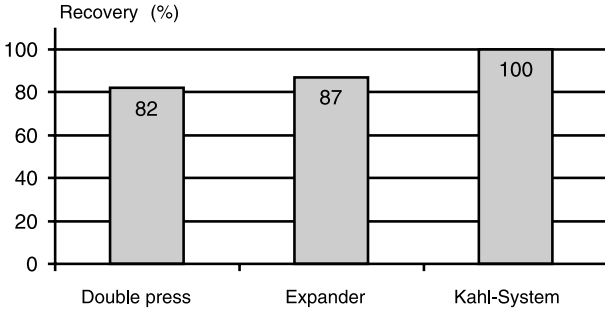


Feed: Standard poultry feed with ZY68 (1000 g/t)

Parameters of pelleting machine: „Simon Heesen" pilot pelleting system, 300 kg/h, steam 2 kg/cm^2 for 20-30 seconds, die with 3 mm bore and 35 mm pelleting length

This high stability of ZY68 determined under controlled conditions has since been corroborated by commercial data. In Figure 8 results have been compiled from feed mills working with different production technologies (double presses, expanders, Kahl compactors). All these procedures constitute greater thermal stress than normal pelleting, yet recovery rates were very good to satisfactory throughout.

Figure 8: Stability of ZY68 - results from field tests carried out in Germany and The Netherlands (1999)



Feed: Poultry and pig feeds with ZY68 (200 - 300 g/t)

The benefits of ZY68 are summarised below. ZY68 has

- balanced activity on insoluble and soluble pentosans,
- a broad pH spectrum and
- excellent heat stability.

ZY68 has minimal secondary activities. A pure xylanase will produce excellent performance responses with virtually all conventional ration types used in western Europe and is therefore sufficient on its own. This is justified by the following facts:

- Supplementation of further enzyme activities such as proteases, α-amylases and β-glucanases brought no additional effects.
- Pentosans are quantitatively the largest NSP fraction in all cereal varieties.

One exception are extremely barley-rich broiler rations. Here the additional use of a suitable endo-β-glucanase is necessary. But these ration types are only relevant in some markets. As a general principle, the development of customised monocomponent enzymes with specific, desired properties is the right approach. Novo Nordisk led the way with the provision of a pure xylanase. Pure β-glucanases and others may follow. In the future it will be possible to combine products in accordance with the proposed application. Under current conditions a versatile product which caters for the major production objectives must be considered the optimal solution. ZY68 meets this requirement.

Animal trial results

Presented below are several recent balance and growth trials in which ZY68 was tested in various livestock categories and ration types.

Table 1 gives an example of trials comparing ZY28 with the new xylanase ZY68. 540 male broilers were assigned to three treatments, each with six replicates, and fed from 1 to 42 days of age. A pelleted, wheat-rich broiler ration was used which contained either no enzyme, 400 units of xylanase from ZY28 or 200 units of xylanase from ZY68. Both enzyme supplements significantly improved feed conversion. In the case of ZY68 a statistically significant increase in liveweight gains of 4 % was also recorded. This trial clearly demonstrates the superiority of the xylanase from ZY68. Based on xylanase activity measured in FXU, the efficacy of ZY68 in this trial was twice as high as that of ZY28. This performance ratio was confirmed in further broiler trials. Balance studies to determine metabolisable energy (RIJKSTATION VOOR KLEINVEE-TEELT, 1998b) in which 400 g/tonne ZY28 was compared with ZY68 at increasing dose rates from 100 to 800 g/tonne, revealed an activity equivalence of 200 g ZY68 to 400 g ZY28.

Table 1: Efficacy of ZY68 and ZY28 in broiler fattening (RIJKSSTATION VOOR KLEINVEETEELT (B), 1998a)

ZY28 FXU/kg	ZY68 FXU(w)/kg	Live weight (g/animal)	relative	Feed conv. ratio (1:)	relative
0	0	2271^{b}	100	1.82^{a}	100
400	0	2321^{ab}	102	1.75^{b}	96
0	200	2354^{a}	104	1.76^{b}	97

Animals: 540 male broilers, 3 groups, 6 replicates, 30 chicks each 1st – 42nd day

Feed: ad libitum, pelletized, 50 % wheat starter/ fattener with 23.6/22.6 % CP, 12.0/12.1 MJ AME/kg

Rearing: floor pens, straw litter

In a further trial (Table 2) factors other than growth parameters, such as ileal digestibility, viscosity of the digesta and content of metabolisable energy were also determined. 108 male broilers were assigned to two treatment groups, one control and one test group with dietary ZY68 supplementation. Fattening parameters were recorded to 24 days of age. Then some of the birds were killed and ileal digestibility was determined by marker technology and the viscosity of the ileal contents measured. Further broilers were used for determination of metabolisable energy in the feed. As can be seen from table 2, ZY68 considerably increased fattening performance to 24 days. Growth and feed conversion were improved by 9 and 10 % respectively. Viscosity was reduced from just under 10 mPa·s to 2 mPa·s, again confirming the potent viscosity-reducing effect of ZY68. In most cases the parameters of ileal nutrient digestibility and metabolisable energy were also significantly improved by xylanase supplementation. A further group not featured here received 300 g ZY68/tonne of feed. This higher dosage caused no significant further improvement in any of the studied parameters.

Table 2: Influence of ZY68 on growth and digestibility parameters of broiler chickens (Research Centre Foulum (DK), 1998)

ZY68	(g/t)	0	200	ZY68	(g/t)	0	200
gain	(g/bird)	530^{b}	578^{a}	viscosity	(mPa·s)	9.38^{a}	2.00^{b}
relat.	(%)	100	109	CP dig.	(%)	82.4^{a}	85.4^{a}
FCR	(1:)	1.58^{a}	1.43^{b}	fat. dig	(%)	58.8^{b}	64.0^{a}
relat.	(%)	100	90	AME_N	MJ/kg	13.44^{b}	13.82^{a}

Animals: 108 male broilers, 2 groups, growth test for 24 days followed by measurements of ileal digestibility and AME_N

Feed: ad libitum, mash, 76 % wheat 22.6 % CP, 13.44 MJ AME_N/kg

Rearing: floor pens and cages, respectively

Further fattening trials, some using up to 30 % barley, up to 60 % triticale or even rye components along with wheat, confirm the beneficial effect of ZY68 on fattening performance and litter quality. Positive results are also available from fattening trials with turkeys.

An extensive piglet rearing trial was conducted at the Free University of Berlin (Table 3). 192 piglets assigned to two groups and housed in double pens were used. The feed was produced from a pentosan-rich batch of wheat and contained neither growth promoters nor acids or probiotics. The diet of the test group was supplemented with 400 g ZY68/tonne. The piglets received their appropriate feed variant in the creep from as early as 15 days of age. The feed intake of the enzyme group was found to be higher throughout the pre-weaning period. Table 3 shows the rearing result for the first six weeks post weaning. The liveweight gain of the enzyme group was 13 % higher than that of the control. This was primarily due to increased feed consumption by the treated animals. The feed conversion ratio was therefore improved only marginally by 2 %. It was also observed during the daily inspections that enzyme supplementation had a favourable effect on scouring. Both the incidence and severity of diarrhoea were reduced. This observation was confirmed in further piglet trials.

A further piglet trial underlines the versatility of ZY68 (Table 4). As well as wheat, the feed used here also contained barley and triticale components. 45 piglets were divided into three groups and housed in single pens. ZY68 was administered at dose rates of only 300 and 400 g/tonne. Daily gains were again improved substantially, while the effect on feed conversion efficiency was less marked. The higher dosage of 400 g/tonne brought no additional improvement in performance compared with 300 g/tonne.

Table 3: Efficiency of ZY68 in piglets (SIMON et al., 1998)

ZY68	Daily gain		Daily feed intake		Feed conv. ratio	
(g/t)	(g)	relative	(g)	relative	(1:)	relative
0	527[b]	100	879[b]	100	1.67	100
400	595[a]	113	978[a]	111	1.64	98

Animals: 192 piglets, 2 groups, 48 replicates, 2 piglets each, 29th – 70th day

Feed: ad libitum, pelletized, type 1/2 with 53/66 % wheat 23/20 % CP, 13.9/13.6 MJ AME/kg

Rearing: double boxes

Table 4: Efficiency of ZY68 in piglets (BOLDUAN, 1998)

ZY68	Daily gain		Feed conv. ratio	
(g/t)	(g)	relative	(1:)	relative
0	344	100	1.83	100
300	373	108	1.77	97
400	365	106	1.80	99

Animals: 45 piglets, 3 groups, 15 replicates 8-19 kg of live weight

Feed: ad libitum, mash, 37 % wheat, 33 % barley, 8 % triticale 17.7 % CP, 13.3 MJ AME/kg

Rearing: single boxes

Further trials, some run in the field and featuring rations containing other cereal components, confirmed the benefits of ZY68 in the piglet sector. The tests, conducted with different dose rates, resulted in a recommended inclusion rate of 200-400 g/tonne of complete feed.

Studies show that performance enhancements through NSP enzymes can also be expected in fattening pigs. These are usually slightly lower than in piglets or broilers, but still sufficient to make enzyme use a viable commercial proposition. Recent digestibility studies with ZY28 show that, as in poultry, the observed performance enhancements may be due to improvements in nutrient utilisation. In the studies (DRESCHEL, 1999) eight fattening pigs were used for determination of faecal digestibility and six adult ileorectostomised minipigs for determination of precaecal digestibility. Table 5 shows that the digestibility of crude fat in particular was improved both at the precaecal and the faecal level. The possible reasons for this were discussed earlier in the section on the mode of action of xylanases.

Table 5: Effect of ZY28 on nutrient digestibility in fattening pigs (DRESCHEL et al., 1998)

nutrient	praec. digestibility (%)			faecal digestibility (%)		
ZY28 (g/T)	0	300	Diff.	0	300	Diff.
Organic matter	74.4	76.4	+ 2.0	82.8[b]	85.2[a]	+ 2.4
Crude protein	68.4[b]	76.8[a]	+ 8.4	77.4	79.9	+ 2.5
Crude fibre	22.9	22.4	- 0.5	40.1	42.5	+ 2.4
Crude fat	65.0[b]	78.5[a]	+ 13.5	18.1	29.3	+ 11.2

Animals: 8 fattening pigs and 6 adult ileorectostomised minipigs

Feed: barley 26 %, triticale 17 %, rye 14 %, wheat 10 %, mash, 18 % CP, 13.4 MJ AME/kg

Rearing: digestibility cages

The precaecal digestibility of crude protein was also significantly increased. Analysis of the amino acids lysine, methionine, cystine and threonine, which are not featured here, revealed statistically significant improvements by between 4 and 9 percentage points. Similar studies in fattening pigs are currently also being conducted with ZY68.

Conclusions

- New insights have been gained concerning the effect of xylanases on the intestinal microflora. ZY68 led to reduced bile acid hydrolase activity and increased bacterial β-glucanase production.
- ZY68 has advantages in terms of its activity on insoluble and soluble pentosans, the pH spectrum and temperature stability.
- ZY68 has been extensively tested in pig and poultry nutrition. The results of these trials demonstrate the excellent efficacy of this product.

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