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ZOOTECHNICAL FEED ADDITIVES: ENZYMES, PROBIOTICS AND PREBIOTICS – MODE OF ACTION

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SUMMARY

In practical use, two functional groups of zootechnical additives are of prime importance, i.e. digestibility enhancers and gut flora stabilizers. Within the first group, mainly enzymes having specificities for partial hydrolysis of cereal non-starch-polysaccharides (NSP-enzymes) and to release phosphorus from phytic acid (phytases) are authorised for the use as feed additives.

Both NSP-enzymes, xylanases and β-glucanases, are capable to reduce digesta viscosity and to eliminate partially or completely the antrinutritive effects of NSP. Thus, the main scope of the use of NSP-enzymes is to eliminate the deleterious effects of antinutitive substances in chickens and piglets.

Addition of microbial phytases to feed of poultry and pigs is highly effective in improving phosphorus availability from plant material. The main scope of the use of phytases is the increase of phosphorus digestibility through action on the target feed material. Furthermore, by using phytases as feed additive, it is possible to reduce the phosphorus pollution into the environment considerably.

Selected micro - organisms (probiotics) are authorised as feed additives acting as “gut flora stabilizer”. Although the majority of studies on the effectiveness of probiotics in piglets point towards improved animal performance, these effects are rarely significant and not consistent. However, when the incidence of post weaning diarrhoea was recorded, in almost all studies, including different probiotic strains, as significant reduction was observed. Therefore, probiotics act primary as safety factors in a production system without antibiotic growth promoters rather than to replace antibiotics in terms of

growth promoters. Any of the probiotic organisms are also authorized as additive in poultry feed.

Prebiotics are defined as selectively fermented feed ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health. However, the effectiveness and the mode of action of prebiotics in farm animals are not well documented.

According to the Regulation (EC) No 1831/2003, zootechnical feed additives are one of five categories of additives, *i.e.* (a) technological additives, (b) sensory additives, (c) nutritional additives, (d) zootechnical additives and (e) coccidiostats and histomonostats. The category of zootechnical feed additives includes "any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment". The same regulation defines also the functional groups within the category of zootechnical feed additives:

- Digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet, through action on target materials.
- Gut flora stabilisers: micro-organisms or other chemically defined substances which, when fed to animals, have a positive effect on the gut flora.
- Substances which favourably affect the environment.
- Other zootechnical additives.

In practical use the digestibility enhancers and gut flora stabiliser are of prime importance. Within the first group are mainly enzymes authorised for the use as feed additives having specificities for partial hydrolysis of cereal non-starch-polysaccharides (NSP-enzymes) and enzymes capable to release phosphorus from phytic acid (phytases).

ENZYMES AS FEED ADDITIVES (DIGESTIBILITY ENHANCERS)

1. Aims for the use of enzymes as feed additives

At present more than sixty enzyme preparations are authorised in the European Union for the use as feed additives. Since the animals produce digestive enzymes with a high hydrolytic capacity for most nutrients, and in addition the micro-organisms of the gastrointestinal tract express a large scale of hydrolytic enzymes, the aims for the use of enzymes as feed additives should be inquired first. One aim is the reduction or elimination of antinutritive effects of feed constituents. Such an antinutritive effect might be the increase of digesta viscosity in the intestines by specific fractions of non-starch-polysaccharides (NSP). Since the animals do not produce enzymes capable to hydrolyse NSP and in some animal categories the intestinal microbial activities are not adequate, this antinutritive effect can be reduced or eliminated by addition of specific microbial endo-enzymes catalysing a partial hydrolysis of the NSP. It means, partial depolymerisation eliminates already the antinutritive property and no further degradation of the substrates is necessary.

Another aim of enzyme addition to the diet is the degradation and utilisation of feed constituents not degradable by digestive enzymes of the host. In this case a degradation of the target substrate is requires at a degree which results in products that can be absorbed by the animal. An example for this application is the use of phytases in order to improve phosphorus digestibility in monogastric animals. At the same time, the addition of phytases has the potential to reduce phosphorus pollution into the environment and affects therefore favourably the environment, which is a further scope for zootechnical feed additives.

Finally, some enzyme preparations contain amylase or protease activities. That would act as a quantitative completion of digestive enzymes produced by the animals. This type of application seems not to be justified, especially not as zootechnical feed additives which are designed to act in healthy animals. Therefore, NSP-hydrolysing enzymes and phytases are of prime practical importance.

2. NSP-hydrolysing enzymes

The target substrates of these enzymes in use as feed additives are arabinoxylans (pentosans) and 1,3-1,4-β-glucans. Both occur in cereal grains, while pentosans are high in rye, triticale and in some batches of wheat, 1, 3-1, 4-β-glucans are high in barley (Table 1).

Soluble fractions of both NSP produce high viscosities in the intestinal contents of animals with low microbial activity in the intestines, which is specially the case in chicks and to a smaller extent in piglets. Elevated viscosity in the intestinal tract may be the reason for impaired performance and nutrient digestibility combined in severe cases with diarrhoea. Thus, many microbial β-glucanases and xylanases are authorised for the use as single enzyme preparations or in combined enzyme preparations. The effectiveness of enzyme addition depends on the cereals used in the ration, the animal species and the age of the animals. Most pronounced effects were observed in broiler and turkey chicks and are less pronounced and reproducible in piglets. With regard to the cereal basis the effectiveness of the specific enzymes (xylanasas and β-glucanases, respectively) follows the ranking: rye > barley > triticale > wheat > maize(?). Furthermore, within the same cereal the NSP content may vary considerably depending on the variety and the environmental conditions during the plant development. For wheat Dusel et al. (1997) have demonstrated that an increasing content of soluble pentosans enhanced the extract viscosity of wheat as well as the digesta viscosity in broiler chicken, while the content in metabolisable energy decreased (Table 2). Due to this fact it is understandable that the effectiveness of xylanase addition may differ from batch to batch of the same cereal (Table 3).

Studies on the mode of action of NSP-enzymes have shown that complex mechanisms are involved in the overall effect (Figure 1), including acceleration of digesta passage rate (Dänicke et al. 1997), improvements of the digestibility of nutrients (Dänicke et al. 2000a), morphological modifications of the intestinal mucosa (Viveros et al. 1994), modifications of the protein turnover and proportion of endogenous nitrogen at the terminal ileum (Dänicke et al. 2000b) and modifications of the composition and metabolic activity of the microbial population in the gastro-intestinal tract (Dänicke et al. 1999, Hübener et al. 2002, Hirsch et al. 2006).

3. Phytase

In concentrate feed of plant origin approximately 70 per cent of total phosphorus is bound in the form of phytic acid and phytate, respectively. Phytic acid is a hexaphosphoric acid ester of inositol. It occurs in feedstuffs mainly as the salt of phytic acid, which is called phytate and result from reactions with mainly divalent cations (Figure 2). Phosphorus bound in this form can only be utilised by the animal if specific enzymes (phytases) are available in the gastro-intestinal tract, capable of hydrolysing the ester bound and releasing the residues of phosphoric acid from the inositol ring. However, the organisms of the animals do not produce these enzymes (at least not in a significant quantity). Sources of phytases are microorganisms and to some extent plant material. Therefore, the availability of phosphorus from phytate is restricted to a high degree in monogastric animals, amounting in some diet formulations to 30 per cent or less. The addition of microbial phytases to feed of poultry and pigs is an efficient way to improve the digestibility of phosphorus and those minerals and trace elements bound as phytate (see Figure 2). The efficiency is especially high if feedstuffs with low phytase activity are used to produce a compound feed (Figure 3).

Furthermore, it has to be considered, that indigenous phytases of plants are rather heat sensitive (Simon and Igbasan 2002) and may be inactivated during heat drying and heat treatment during feed processing at a high degree.

The conventional way to meet the phosphorus requirement of the animals is to supply phosphorus in a highly available form, e.g. monocalcium phosphate or dicalcium phosphate. Using this strategy for supply with available phosphorus, the phosphorus naturally contained in feedstuffs is utilised only marginally, the major portion remains undigested and is excreted via faeces. In regions with intense animal production phosphorus pollution into the environment is a serious ecological problem. Therefore, the availability of phytases became an important tool in reducing phosphorus excretion by monogastric farm animals.

In experimentes with diets formulated with feedstuffs containing almost no phytase activity a reduction of phosphorus excretion to 44 per cent was possible in piglets (Pallauf and Rimbach 1997). This reduction will not be achieved under most feed formulations in the EU, but a reduction by 30 per cent seems to be a realistic figure in practice. Furthermore, the potential of phytases of both fungal and bacterial origin in improving P-utilization in broiler chickens was demonstrated (Elkhalil et al. 2007).

At present, seven phytase preparations are authorised as feed additives in the EU. Five of them are of fungal origin. All of them have a pH optimum in the acidic range and due to that they are probably only able to act in the stomach. In addition, other properties of these enzymes are not yet optimal for their application. Therefore, many efforts are made to develop new phytases with improved thermostability (stability during feed processing), with broader pH optima (to be active in several segments of the digestive tract), with better proteolytic stability (delayed proteolytic inactivation during the passage trough the digestive tract) and with improved hydrolytic kinetics (higher degree of hydrolysis of inositolphoshates).

Alternative future strategies for improving the supply of available phosphorus may be contributions of plant breeding (reduced phytate content of plants, selection for high phytase activity, and expression of optimised phytases in genetically modified plants).

4. Conclusions (Digestibility enhancers)

- Xylanases and β-glucanases have the potential to hydrolyse cereal NSP partially.
- The beneficial effects of NSP-hydrolysing enzymes for the animals are mainly based on the reduction of digesta viscosity and improved nutrient digestibility.
- The effectiveness of NSP-hydrolysing enzymes depends on the cereal, the NSP-contents and their solubility, the animal species and age. The effects are most pronounced in chicken, but are also documented in piglets.
- Phytases improve effectively the availability of phosphorus in monogastric animals and to a smaller extent of calcium from feedstuffs of plant origin.
- The use of phytases as feed additive in poultry and pig feed contributes effectively to reduce the environmental pollution with phosphorus due to animal production.
- Specific plant breeding techniques, including expression of specific enzymes in genetically modified plants, might be future alternatives to enzyme production by biotechnology using micro-organisms.

GUT FLORA STABILIZER

1. Micro-organisms as feed additives (probiotics)

Since the ban of antibiotics as feed additives (growth promoters) in the EU in January 2006, selected micro-organisms come to the fore as feed additives acting as "gut flora stabiliser". However, the concept of beneficial effects of oral intake of micro-organisms on health and longevity in men is almost one hundred years old and goes back to Elie Mechnikoff. In animal nutrition some micro-organisms are in use as feed additives for more the 20 year and they are defined as probiotics according to Fuller (1989) as viable micro-organisms which after sufficient oral intake lead to beneficial effects for the host animal because of an improvement of the intestinal microbial balance. Actually 26 preparations containing specified strains of bacteria or yeast are authorised in the EU as feed additives (Table 4). From this table it becomes obvious, that bacteria of the genus Lactobacillus, which are most effective as additives in human nutrition (Sanders 2000), play only a marginal role as additives in animal nutrition. The main reason for that is the bad stability of lactobacilli as desiccated vegetative cells during storage or during feed processing, e.g. pelleting. Most stable under such conditions are bacterial spores like spores of bacteria of the genus Bacillus. The stability of other bacteria not forming spores can be improved by specific coating or confection processes. The recommended dosage of micro-organism is usually in the range 10^8 to 10^9 colony forming units per kg of feed. Most probiotics are authorised for young animals (piglets, chicken and calves). Out of 15 probiotic preparations authorized for the use in poultry 14 are authorized for broilers, two for turkeys for fattening and three for layers.

The effectiveness of probiotics for farm animals is generally expressed in terms of performance parameters like body weight gain and feed conversion ratio. Figure 4 gives a summary of such data from 22 published trials. The majority of the studies on fattening broilers and turkeys revealed improvements in performance parameters. However, in some trials no or adverse effects were observed and positive effects were rarely significant. Probabely this kind of literature evaluation does not reflect the real situation since the tendency and possibility of publishing experimental results of no or adverse effects are less developed than in the opposite case. However, with this type of feed additives a great variability of the response of the animal has to be expected because the effects are mediated by modifying the microbial community in the gastrointestinal tract and the microbial environment and intestinal status may be very different. Therefore, the range between no and significant effect seems to be normal. Our own experiences with feeding trials with probiotics are in line with the above described situation. In one experiment we did not see any effects in broilers, in another study with turkey significant positive effects of probiotics were observed, In all other studies with broilers and turkeys trends towards improved gain and feed conversion ratio were observed in the range of 1 to 3 per cent (Männer et al. 2002). In these experiments E. faecium, Bacillus cereus and S. cerevisiae preparations have been used. The situation is similar concerning the effects in piglets. Although the majority of the experiments point to improved weight gain of piglets receiving the probiotics, there were also some adverse effects documented. Furthermore, only less than half of the reported positive effects were significant, compared to control animals. The situation is even less clear, when feed conversion is chosen as a parameter for performance.

The mode of action of probiotics is best studied in piglets but the principals should be also valid for poultry. When incidence of post weaning diarrhoea was recorded in almost all studies including different probiotic strains, a significant reduction was observed in animals receiving the additive. Studies on the mode of action of the probiotic bacterial strains Enterococcus faecium NCIMB 10415 and Bacillus cereus var. toyoi in piglets have shown that both can be found within a short period in all segments of the intestinal tract (Macha et al. 2004) and reduced significantly the incidence of post weaning diarrhoea (Figure 5) (Taras et al. 2006).

Both probiotics modified the intestinal microbiota, combined with a reduced identification frequency of various *E. coli* sero-pathovars (Scharek et al. 2005). Furthermore, it was shown that transport kinetics of the intestinal mucosa (Lodemann et al. 2006) and the immune status (Scharek et al. 2005) were involved in the response of the animals to the probiotics.

Substantial modifications in the intestinal bacterial population due to probiotic additives were also found in poultry. For instance in an experiment with turkeys receiving a commercial type diet the effect of a E. faecium probiotic strain on lactobacilli in the intestine was studied at 7, 14, 21 and 28 days of age (Vahjen et al. 2002). When digesta samples were analysed for lactic acid bacteria the same CFU were detected for control animals and animals receiving E. faecium. However, analysing the same samples with a 16S RNA probe specific for the genus Lactobacillus, an enhanced metabolic activity of lactobacilli was indicated in digesta of animals receiving the probiotic. The latter observation agrees well with the increased concentration of lactic acid in ileal digesta in probiotic treated animals (Figure 6). Since in the same animals the pH measured in the gizzard was significantly reduced, it was concluded that the E. faecium strain may be already active in the crop.

Therefore, it may be concluded that probiotics act primarily as safety factors in a production system without antibiotic growth promoters rather than acting as an effective replacement of antibiotics in terms of growth promoters.

2. The prebiotic concept

The prebiotic concept originates also from human nutrition. Prebiotics are defined as selectively fermented feed ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health (Gibson et al. 2004). Frequently studied substances with regard to these effects are e.g. inulin, fructooligosaccahrides or mannanoligosaccharides among many other substances. Actually, no such substances are authorized as feed additives in the EU, but they are frequently discussed as alternatives to antibiotic growth promoters and are expected to act like "gut flora modifiers". However, the effectiveness and the mode of action of prebiotics in farm animals are not well documented.

Inulin and fructooligosaccharides are discussed as "bifidogenic factors" by supporting the population of bifidobacteria in the intestinal tract which is believed to be beneficial for the host. However, this effect seems not to be of significance e.g. in piglets, because bifidobacteria represent only a very small proportion of the intestinal microbiota of piglets. Using the fluorescence in situ hybridisation technique Loh et al. (2006) were even unable to identify bifidobacteria in intestinal contents in more than half of piglets in a study on the effects of inulin.

More research is required in this field with the target species of farm animals. Furthermore, it has to be clarified weather or not this type of substances has to be classified as feed additives.

3. Conclusions (Gut flora stabilizer)

- Probiotics (viable micro-organisms) are feed additives with a potential to improve farm animals performance (rarely significant) and to reduce effectively incidence of diarrhoea (studied in piglets).
- Probiotic micro-organisms can be found within a short period in all segments of the intestinal tract of piglets (shown for an *Enterococcus faecium* and a *Bacillus cereus* strain).
- Probiotics modify the microbiota in the intestinal tract. Evidently transport kinetics of the intestinal mucosa and the immune status are also involved in the animals' response to probiotics.
- The effectiveness and the mode of action of prebiotics in farm animals are not well documented yet.
- Within a production system without antibiotic growth promoters, probiotics may act as a safety factor. Due to the mode of action they cannot replace antibiotics solely or in terms of growth promoters.

Table 1. Content of 1,3-1,4-β-glucans and pentosans in cereals g/kg (adapted from Jeroch et al. 2008)

Cereal	β-Glucans		Pentosans	
	Total	Soluble	Total	Soluble
Barley	26-66	**24-50**	31-77	5-10
Rye	18-47	-	66-122	**19-45**
Triticale	7-36	-	46-140	6-21
Wheat	3-11	-	35-75	5-23
Maize	~1	-	33-68	4-10

Table 2. Variation of pentosan content, in vitro extract viscosity, ileal digesta viscosity (in broilers) and apparent metabolisable energy (AME) – content of wheat varieties (Dusel et al. 1997)

Variety	Soluble pentosans (% of DM)	Viscosity (mPas·s)		AME_n 1) (MJ/kg DM)
		Wheat	Ileal digesta	
Ibis	0.76	1.3	13.1	14.37^{b}
Bussard	1.14	1.4	14.1	14.58^{b}
Aron	1.29	1.7	27.5	14.45^{b}
Zentos	1.43	2.2	20.6	14.06^{a}
Alidos	1.71	3.5	78.0	14.01^{a}

*Values in a column not sharing a common superscript are significantly different at $P < 0.05$

Table 3. Effect of addition of commercial xylanases on the content of metabolisable energy of two wheat varieties, measured in broilers (Dusel et al.1998.)

Wheat variety	Enzyme addition	AMEn (MJ/kg DM) 1)
Ibis	Non	14.8 ± 0.24^{b}
	Xylanase A	14.9 ± 0.19^{b}
	Xylanase B	14.7 ± 0.17^{b}
Alidos	Non	14.0 ± 0.39^{a}
	Xylanase A	$14,7 \pm 0.28^{b}$
	Xylynyse B	14.6 ± 0.14^{b}

*Values in a column not sharing a common superscript are significantly different at $P < 0.05$.

Table 4. Authorised micro-organisms[1)] for the use as feed additives in the EU

Containing one strain	**n**	**Combination of strains**	**n**
Enterococcus faecium	6	Enterococcus faecium (two strains)	2
Bacillus subtilis	2	Enterococcus faecium + Lactobacillus rhamnosus	1
Bacillus cereus	1	Bacillus licheniformis + Bacillus subtilis	1
Pediococcus acidilactici	1	Lactobacillus casei + Enterococcus faecium	1
Lactobacillus farciminis	1	Streptococcus infantarius + Lactobacillus plantarum	1
Lactobacillus acidophilus[2)]	2		
Saccharomyces cerevisiae	5		
Klyveromyces marxianus	2		
Total	20		6

[1)] Defined strains of indicated species.

[2)] Authorised for cats/dogs and layers, respectively.

Figure 1. Model for the mode of action of non-starch-polysaccharides (NSP) and of NSP hydrolysing enzymes (E).

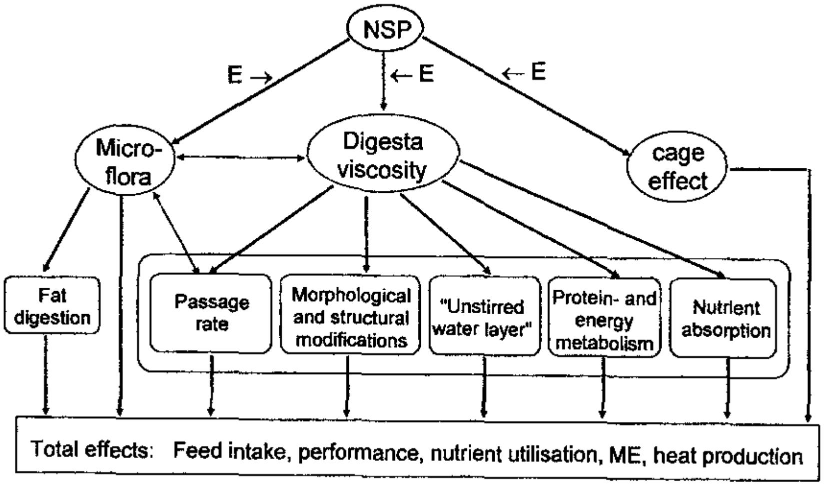


Figure 2. Structure of phytic acid/phytate (myo-inositol 1,2,3,4,5,6-hexakisdihydrogen phosphate).

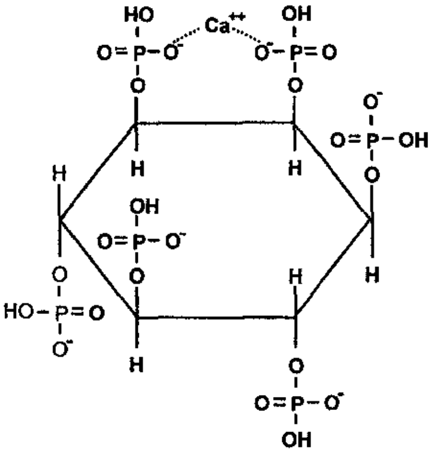


Figure 3. Phytase activity (units per kg) in various feedstuffs of plant origin (according to Eeckhout and De Paepe, 1994).

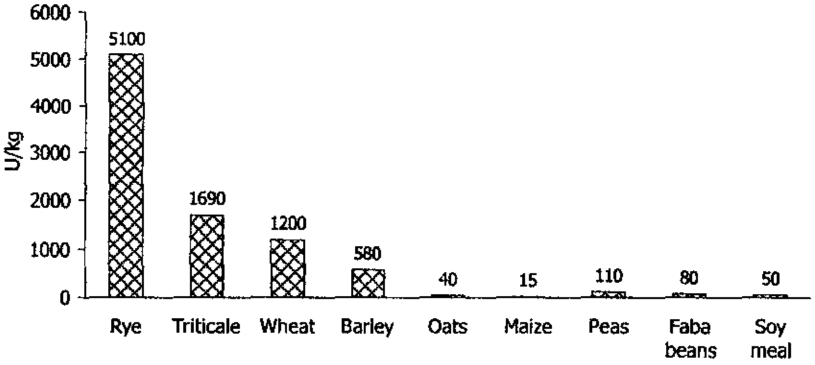


Figure 4. Relative effects of probiotics broilers and turkeys on daily weight gain and feed conversion ratio (Data expressed as per cent of control animals). The evaluated data originate from 22 experiments, published in peer reviewed journals.

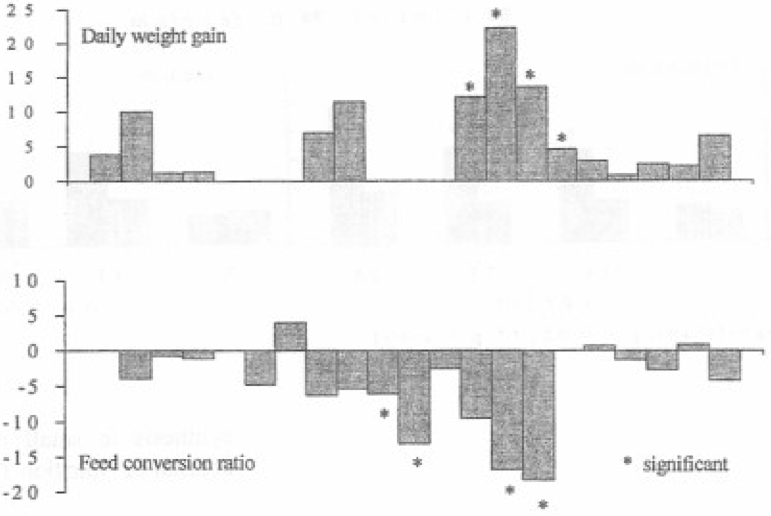


Figure 5. Effects of the probiotics *Enterococcus faecium* NCIMB 10415 and Bacillus cereus var. *toyoi* on the incidence of diarrhoea of weaned piglets during 28 to 56 days of age (Taras et al. 2005, 2006).

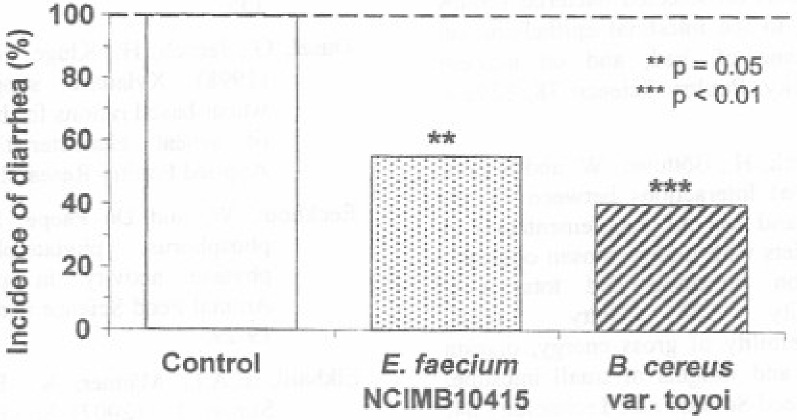
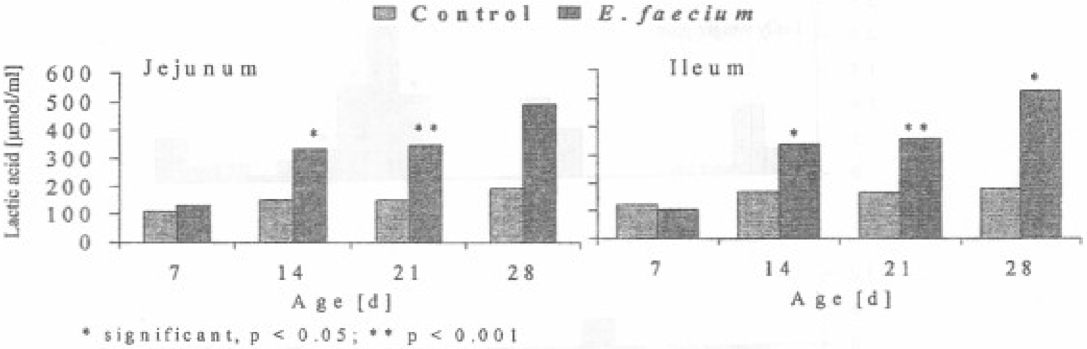


Figure 6. Effect of a probiotic E. faecium preparation on the lactic acid content in small intestine digesta of turkey.



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