The role of added feed enzymes in promoting gut health in poultry

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Outline

The links between enzymes and gut health

Production of pre-biotics in-situ

Digestion of feed substrates and its relationship with gut health

Summary and implications

The links between enzymes and gut health

Mechanisms of action of carbohydrases and proteases in broiler diets

Xylanase; endo-glucanase

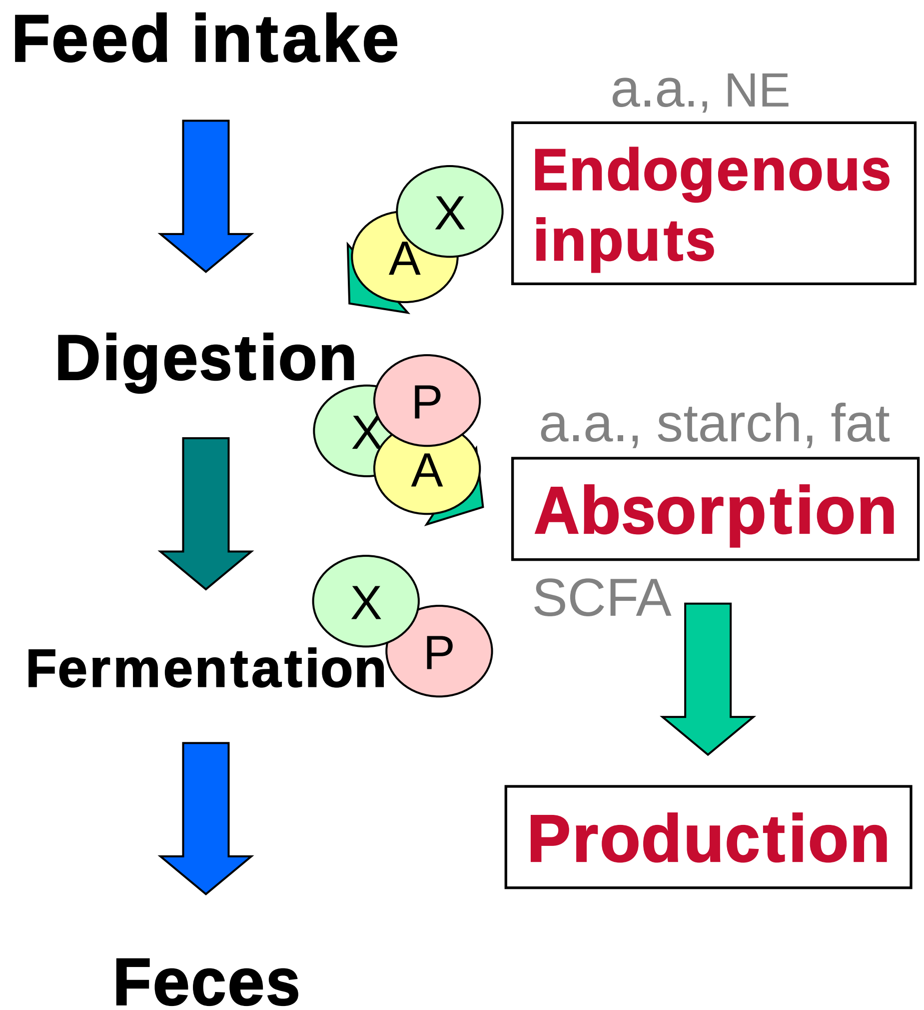
- Reduced viscosity (Choct, 1999)
- Improved access to cell contents (Cowieson, 2005)
- Prebiotic effects (Fernandez et al., 2000)
- Possible reduction of endogenous inputs (Satchithanandam et al., 1990)

Amylase

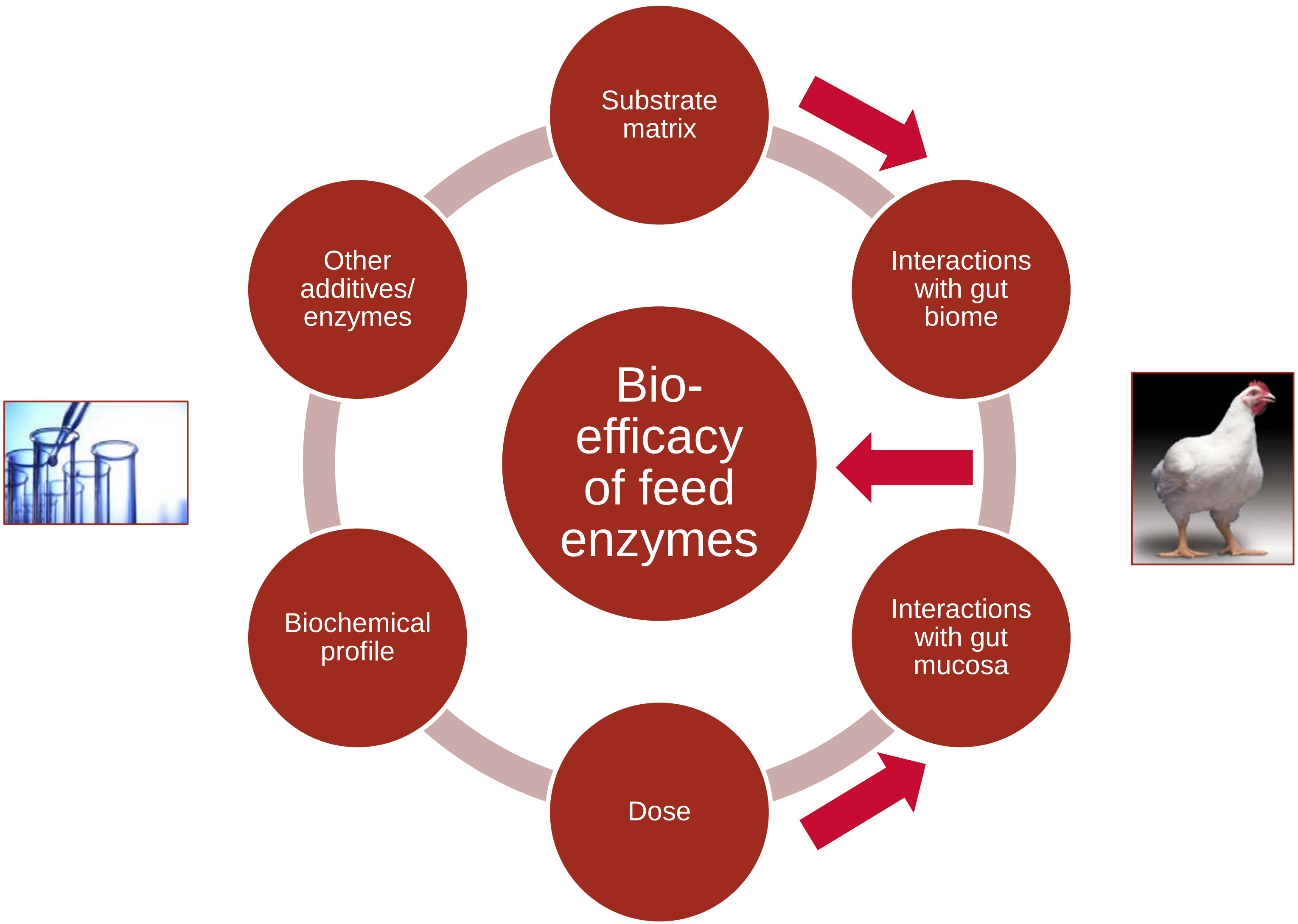
- Down regulation of pancreatic amylase (Jiang et al., 2008)
- Augmentation of pancreatic amylase activity in young animals (Gracia et al., 2003)
- Improvement of digestion of resistant starch in corn and corn by products (Sharma et al., 2010)

Protease

- Hydrolysis of dietary protein and increased protein solubility (Caine et al., 1998)
- Disruption of protein-starch interactions in corn (Mc Allister et al., 1993; Belles et al., 2000)
- Disruption of protein-fiber interactions (Colombatto et al, 2009)

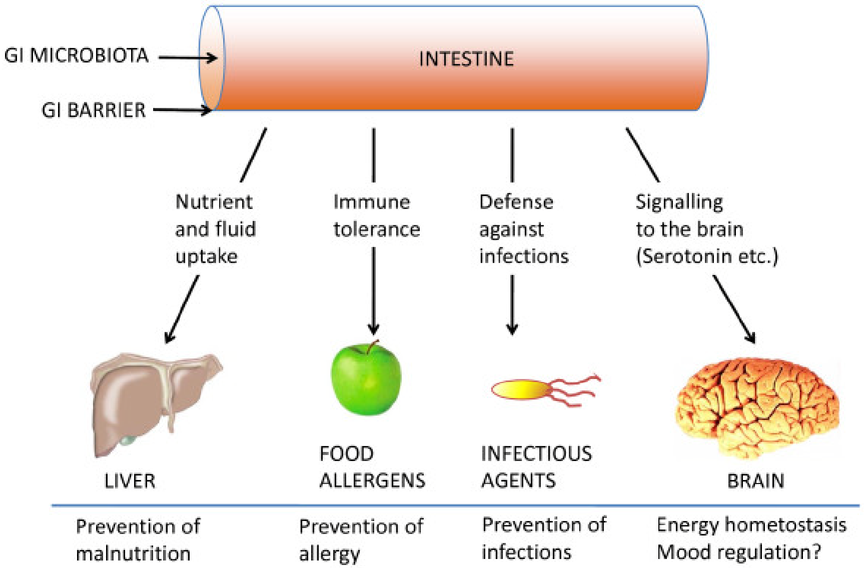


Bio-efficacy of feed enzymes is affected by complex interactions in the gut system



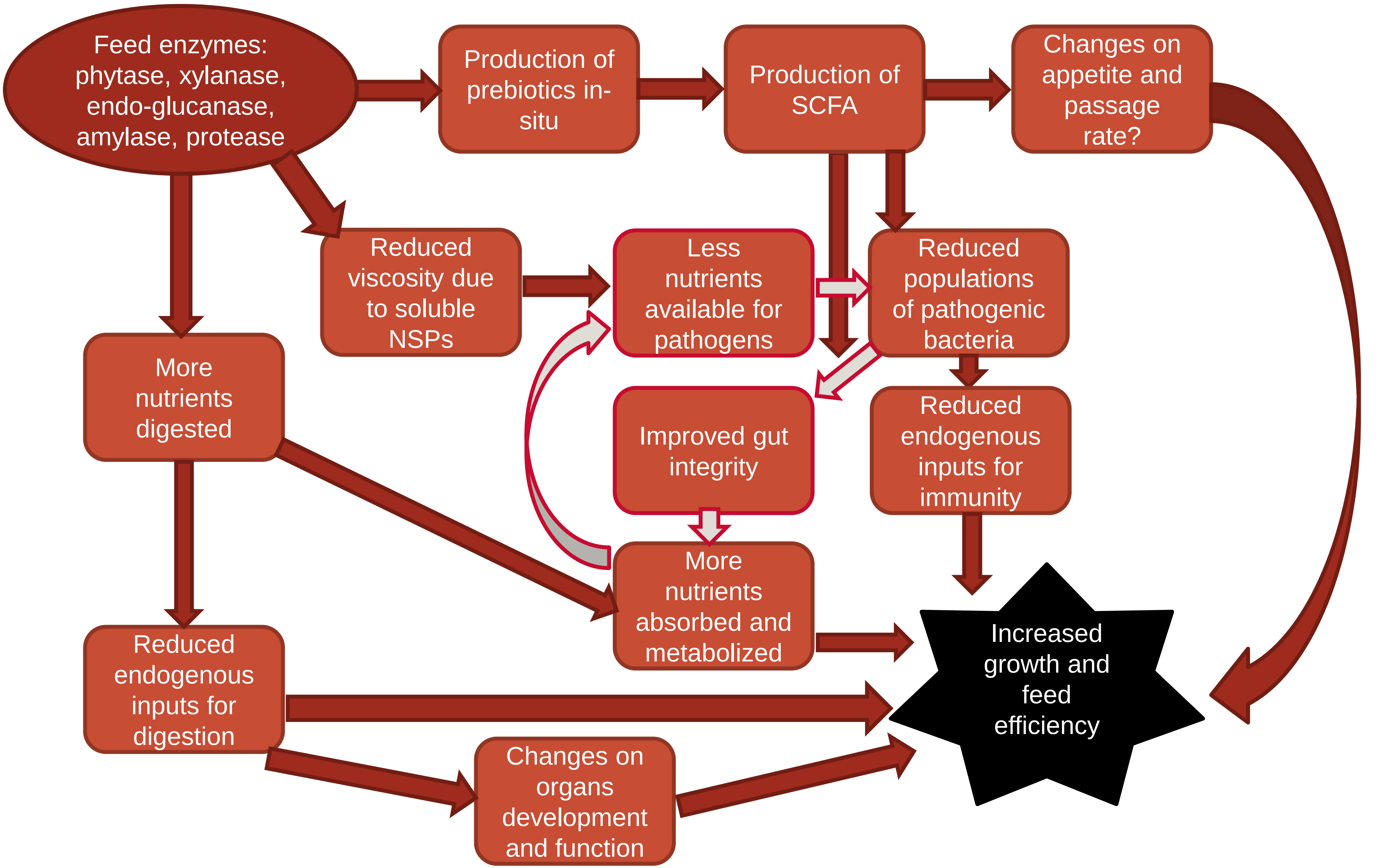
Gut health

Maintenance of optimal intestinal structure and function to enable the expression of the full genetic potential for growth and yield and to fully utilize the dietary nutrients (modified from Hoerr, 2010)



(taken from Bischoff, 2011)

Possible links between feed enzymes and gut health



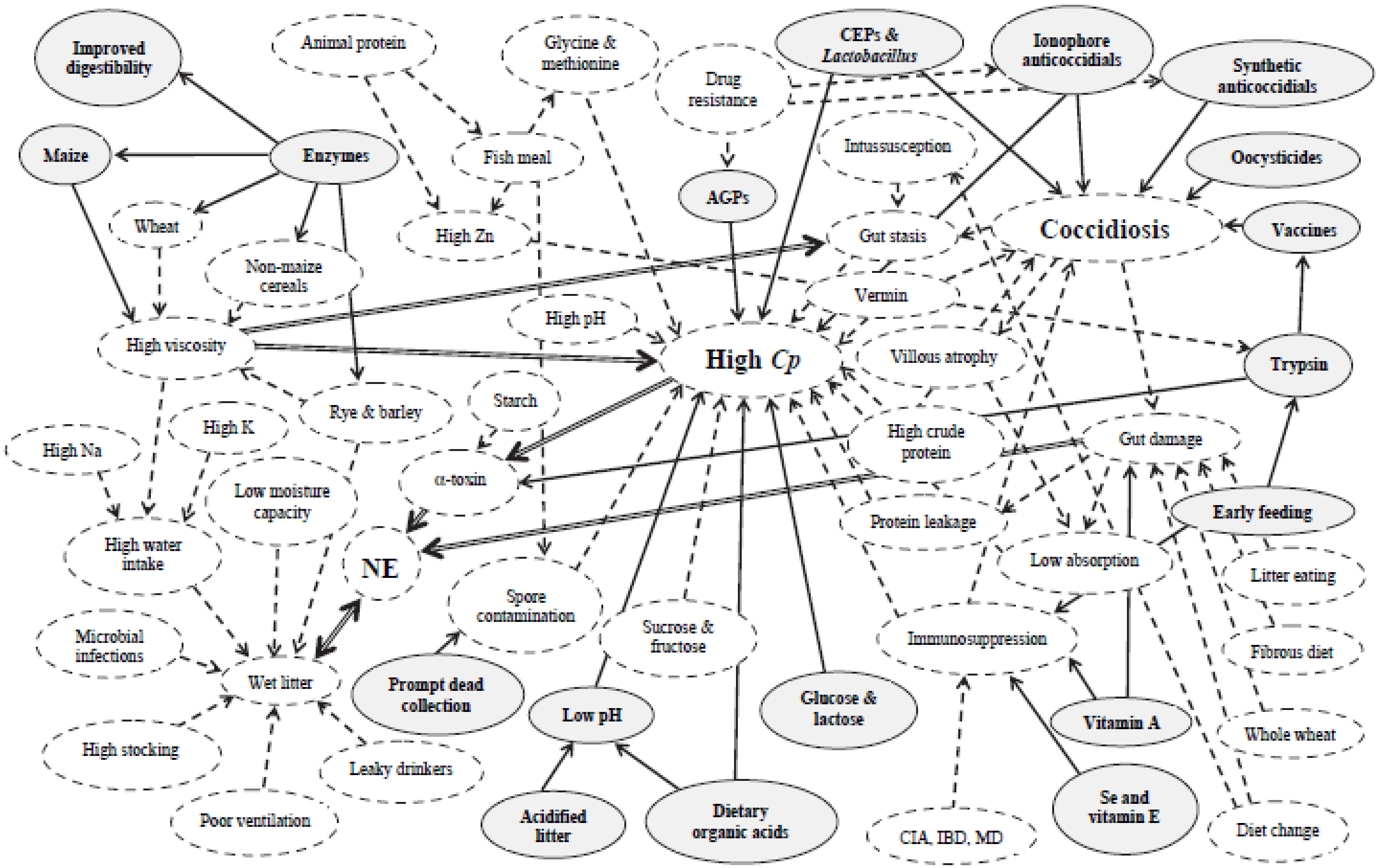
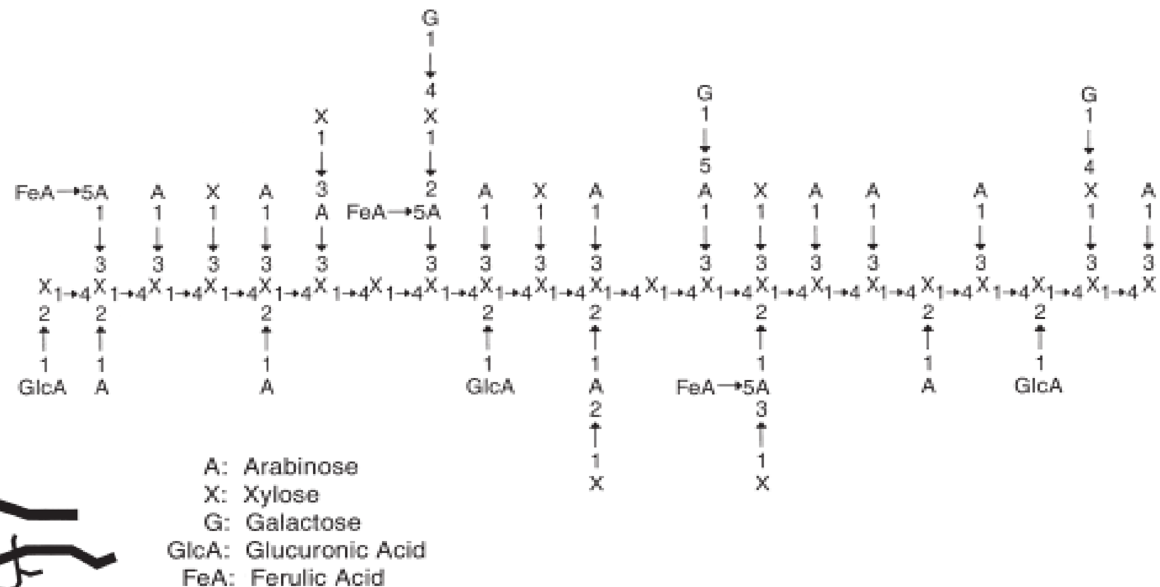


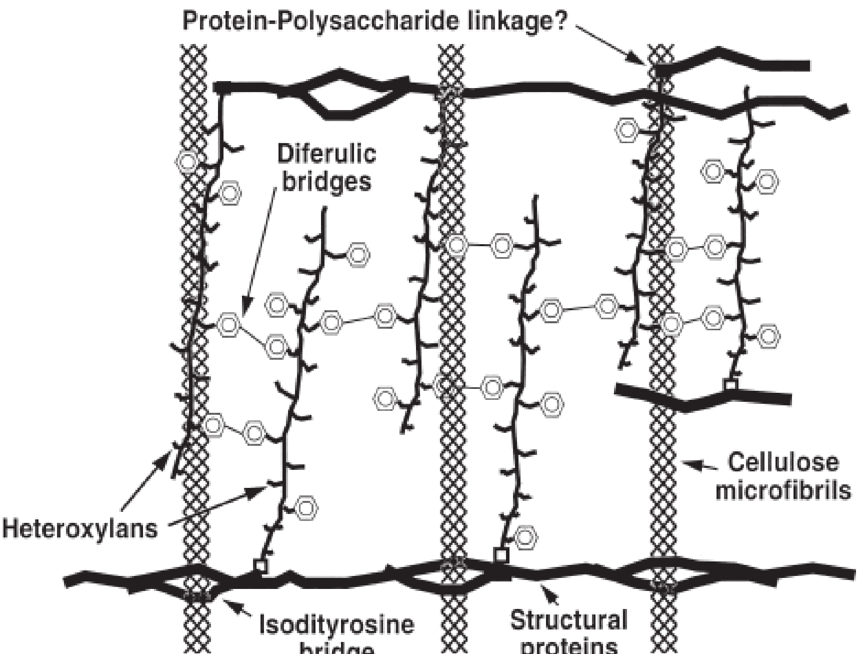
Figure 2. *The intercurrent coccidiosis-NE syndrome: a network of potentially important pathophysiological, medicinal, nutritional and husbandry factors. Those with solid-line arrows and ellipses are beneficial in controlling disease, those with dashed-line arrows and ellipses impart high disease risk. Major high-risk relationships are shown by double-line arrows. AGP, antibiotic growth promoter; CIA, chick infectious anaemia; CEP, competitive exclusion product; Cp,* Clostridium perfringens; *IBD, infectious bursal disease; MD, Marek's disease; NE, necrotic enteritis.*

Williams, 2005

Production of pre-biotics in-situ

Arabinoxylans from cereals are structurally complex





	Diferulic acids/ xylose units	Arabinose/ xylose
Wheat	1/217	
Corn	1/41	

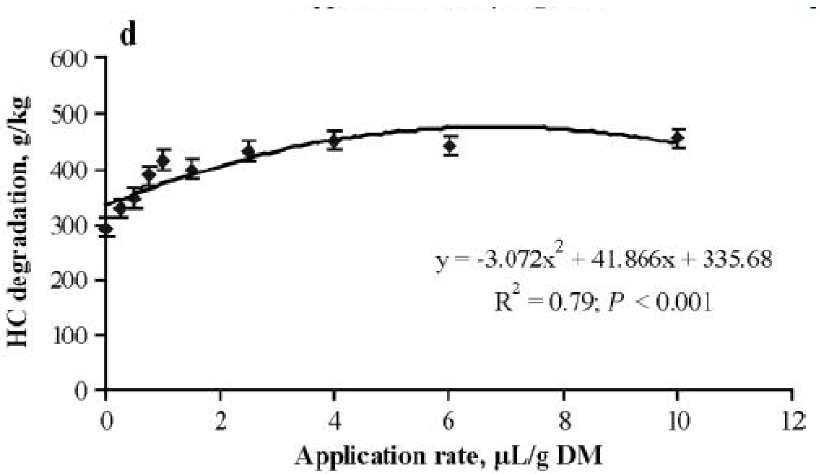
(Bunzel et al., 2001)

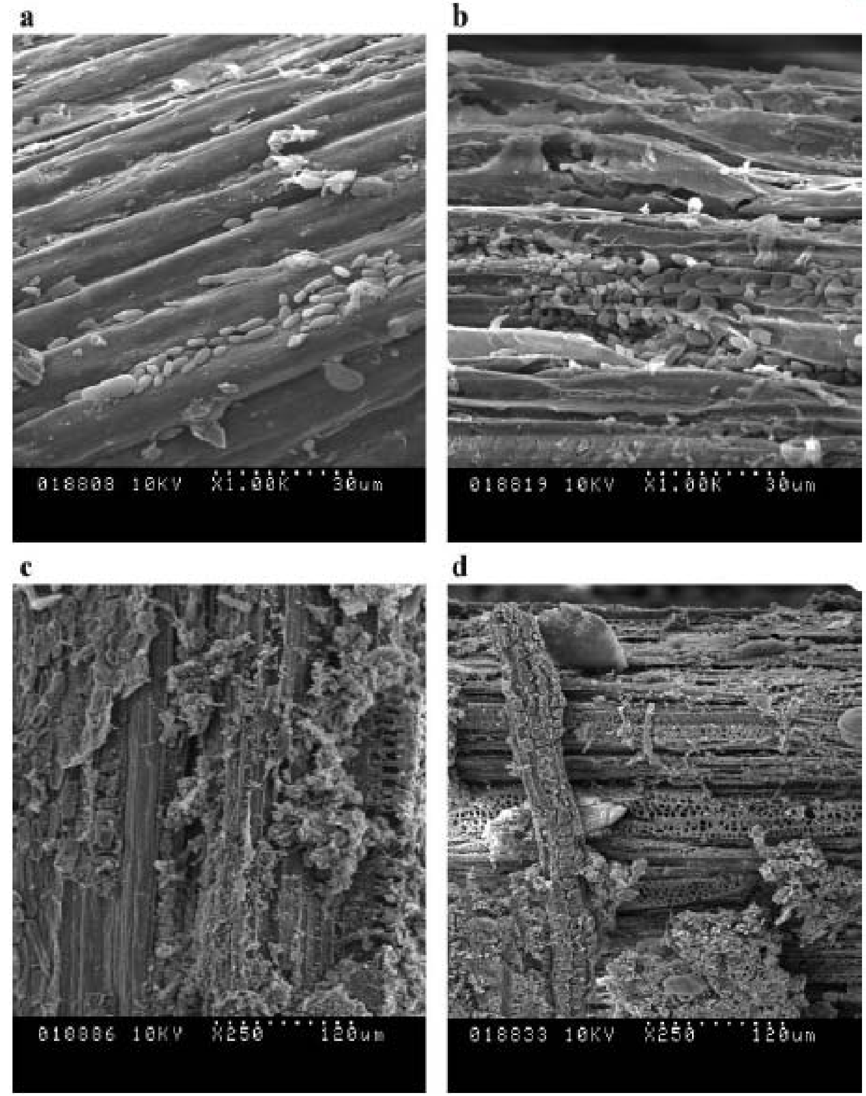
Digestion of fiber was affected by proteases in a rumen model (Colombatto and Beauchemin, 2009)

Serine protease tested in digestion of alfalfa in rumen batch model

Protease increased in vitro disappearance of DM, NDF, hemicellulose, and protein

Protease may have "*acted by removing structural proteins in the cell wall, allowing ruminal microbes to gain faster access to digestible substrates*"

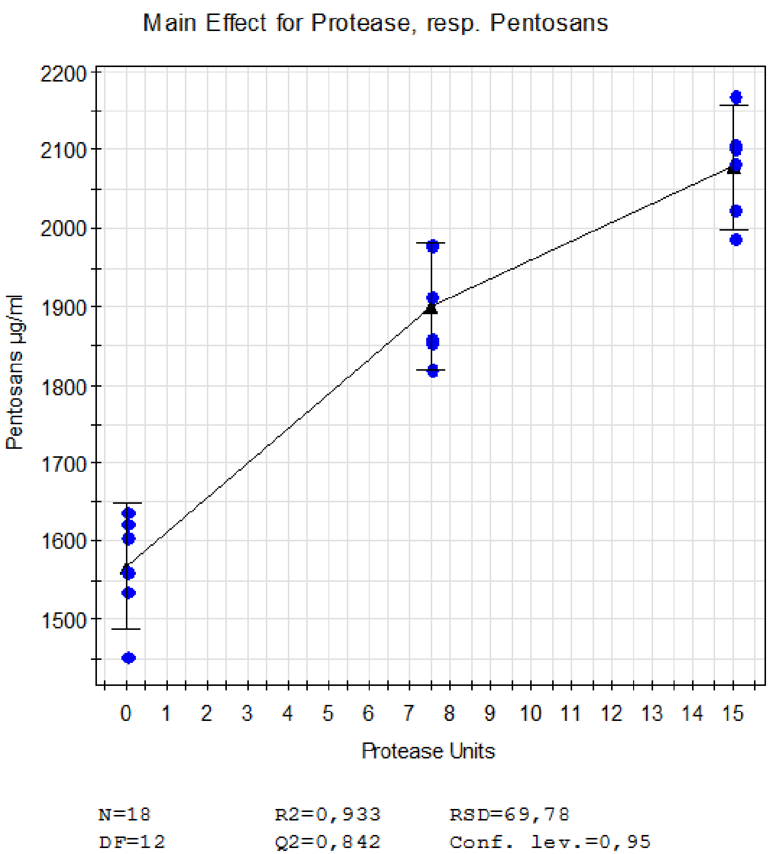


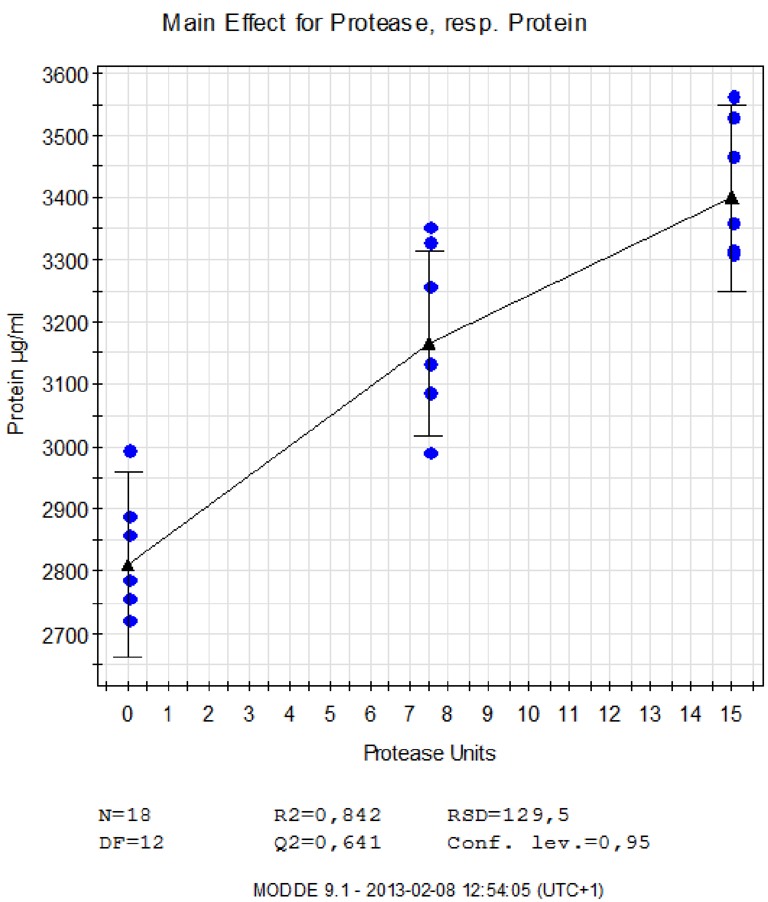


From Colombatto and Beauchemin, 2009

Figure 4. Scanning electron microscopy images of alfalfa hay samples, untreated (a) or enzyme-treated (b; Protex 6L, Genencor Int., Rochester, NY) at 0 h, or untreated (c) or enzyme treated (d) at 18 h postincubation with ruminal fluid in vitro.

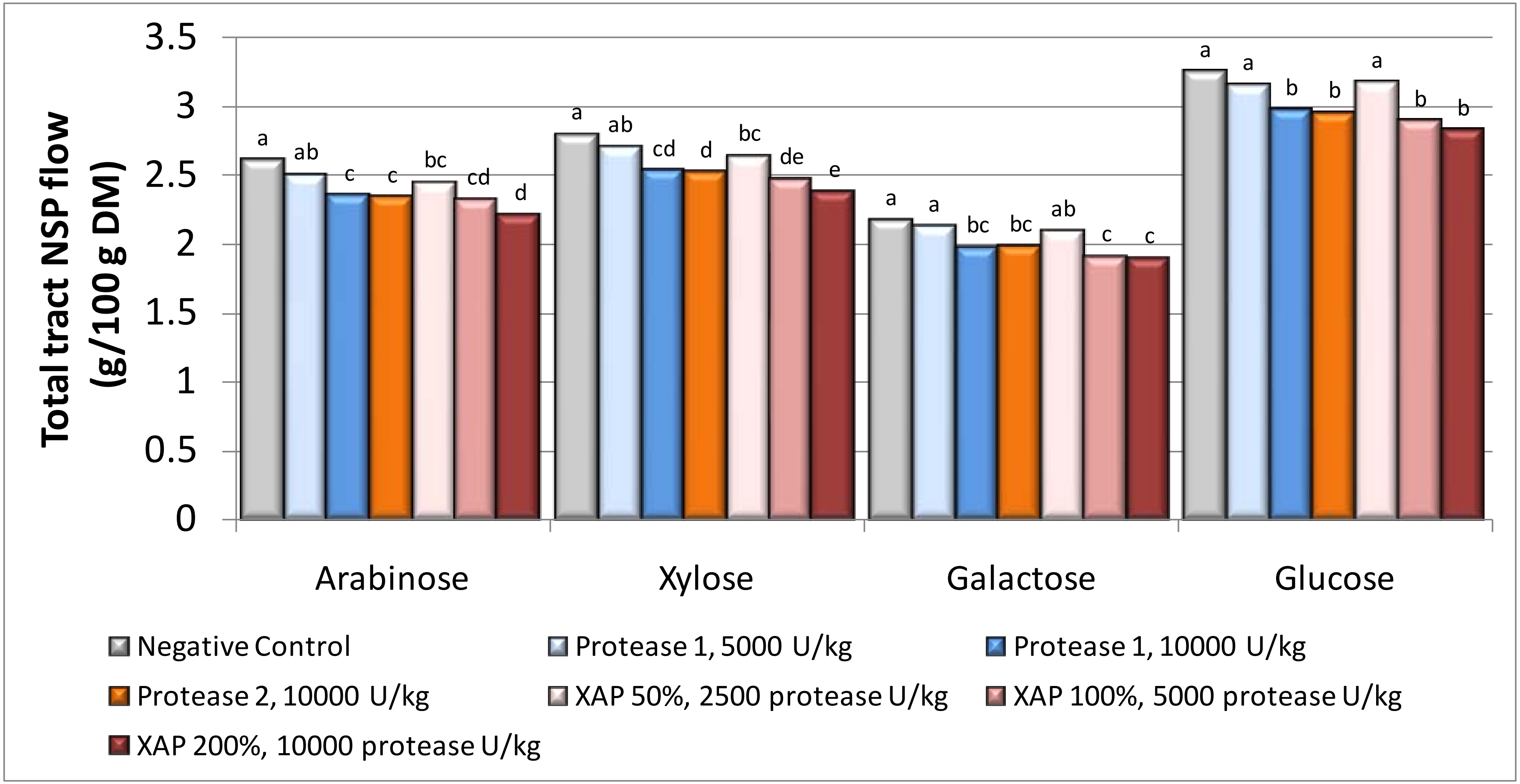
Solubilisation of pentosans and protein from corn-DDGS due to protease on top of xylanase are correlated



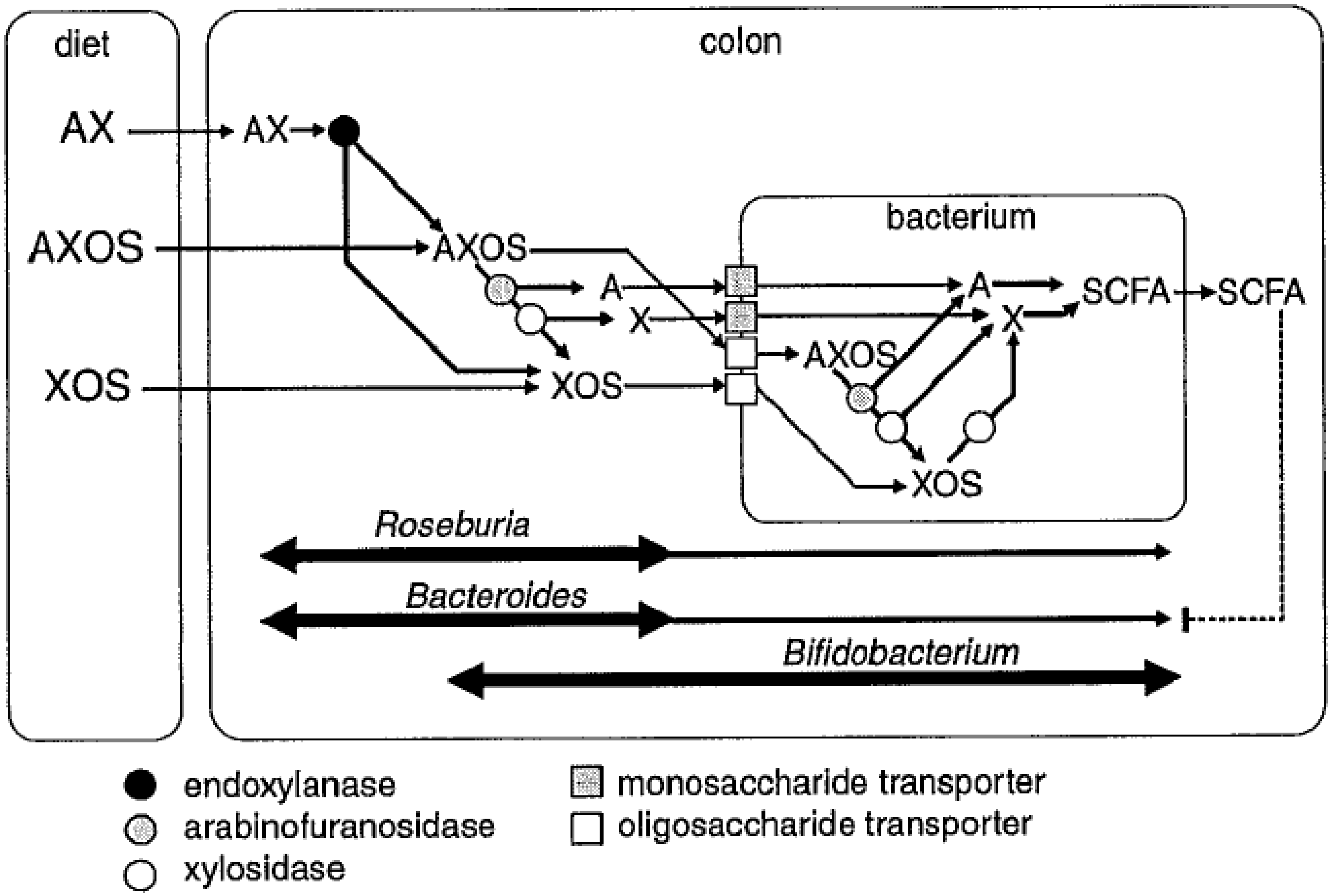


Pedersen et al., unpublished

Carbohydrases had an additive effect on arabinose and xylose flow on top of proteases



Prebiotic effects of cereal derived arabino-xylo-oligosaccharides have been well studied in humans and rats



Broekaert et al., 2011

Health-related effects of cereal derived AXOS in humans

Increased faecal acetate, propionate and butyrate (Grasten et al., 2003)

Normalization of stool moisture and consistency (Okazaki et al., 1990)

Reduced initial lesions of colon cancer development in rats (Hsu et al., 2004)

Attenuation of increases in

- serum cholesterol, triglycerides under adverse dietary conditions (Lopez et al., 1999)
- glucose levels in diabetes II subjects (Liu et al., 2004)

Feruloylated AXOS have anti-oxidant properties (Ou et al., 2007)

Effects of cereal derived AXOS in chickens

- Increased caecal Bifidobacteria levels (Courtin et al., 2008a, 2008b)
- Reduction of *Salmonella* in caeca, cloaca and spleen (Eeckhaut et al., 2008)

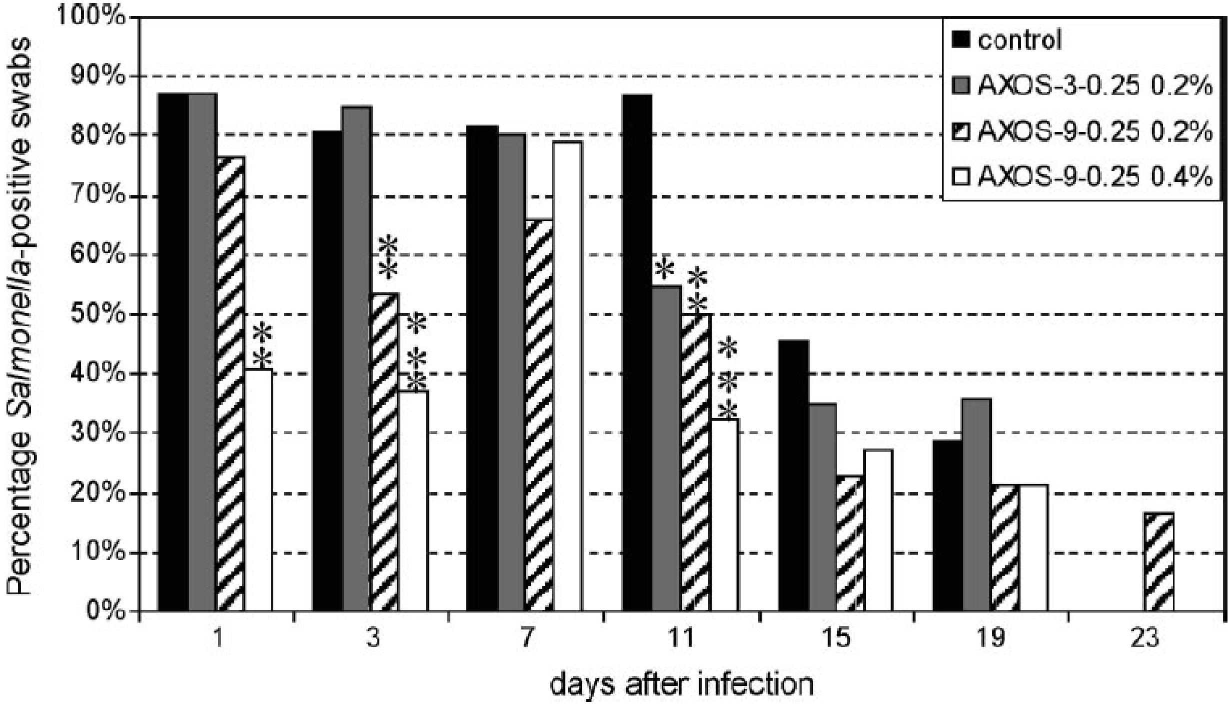


Figure 1. Effects of 0.2% arabinoxylooligosaccharide (AXOS)-3-0.25, 0.2% AXOS-9-0.25, or 0.4% AXOS-9-0.25 in the diet on the percentage of *Salmonella*-positive cloacal swabs at different times after inoculation with *Salmonella* Enteritidis, measured after direct plating of the swabs. For a given time, the values marked with a symbol above the bars are significantly different from the control group according to the Cochran-Q test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Eeckhaut et al., 2008

Increased production of SCFA due to xylanases have been reported

- Choct et al. reported increased SCFA production in the caeca of broilers fed wheat based diets supplemented with xylanase from *Trichoderma ressei* and protease from *B. subtilis*

Table 2. *Effect of enzyme on bird performance, apparent metabolisable energy (AME), digesta viscosity, starch digestion along the intestinal tract, and production of volatile fatty acids in the ileum and caeca (means ± SD)*

Variable	Control		Enzyme		Significance
Food intake (g/bird d)	126	18	125	13	NS
Growth (g/bird d)	64·2	10·7	65·2	10·1	NS
Food conversion (g food/g gain)	2·102	0·175	1·933	0·182	NS
AME (MJ/kg DM)	13·7	0·9	14·5	0·3	***
Duodenal viscosity (cP)	2·9	0·9	1·7	0·2	***
Jejunal viscosity (cP)	4·6	2·0	2·3	0·6	***
Ileal viscosity (cP)	14·0	8·4	3·9	0·9	***
Duodenal starch digestibility (%)	28·5	10·6	30·9	14·0	NS
Jejunal starch digestibility (%)	73·2	8·2	78·8	7·9	*
Ileal starch digestibility (%)	94·9	7·7	98·4	1·5	*
Ileal VFA (mmol)	9·2	5·9	6·2	2·3	*
Caecal VFA (mmol)	340	235	519	295	*

*** $P<0·001$; ** $P<0·01$; * $P<0·05$.

Choct et al. 1999

Not all xylanases have the same effects on production of SCFAs

Factors affecting fibre degradation and SCFA production in vivo:

- Specificity
- Small versus large intestine activity
- Pepsin resistance
- Age of birds
- Diet
- Health status

Table 8. Ileal and caecal volatile fatty acid (VFA) production (mmol/bird) in broiler chickens fed on diets containing normal-metabolisable energy (ME) wheat and low-ME wheat with or without enzyme supplementation†

(Mean values and standard deviations)

		Ileal VFA production		Caecal VFA production	
Wheat	Enzyme	Mean	SD	Mean	SD
Normal-ME	Control	12·6[bc]	4·5	788·6[a]	279·8
Normal-ME	Xylanase A	11·1[bc]	3·6	599·5[a]	131·6
Normal-ME	Xylanase B	11·5[bc]	3·7	748·6[a]	236·1
Normal-ME	Xylanase C	12·4[bc]	4·8	513·4[a]	187·4
Low-ME	Control	26·3[a]	8·0	670·1[a]	167·7
Low-ME	Xylanase A	12·3[bc]	7·9	697·0[a]	276·4
Low-ME	Xylanase B	19·5[ab]	8·8	799·7[a]	313·8
Low-ME	Xylanase C	10·1[c]	4·5	561·0[a]	270·1
Probability of greater *F* value in ANOVA					
Wheat		*		NS	
Enzyme		*		NS	
Wheat × enzyme		*		NS	

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

*$P<0·05$.

† For details of diets and procedures, see Table 1 and p. 54.

Choct et al. 2004

Positive effects of xylanases have been reported in chickens challenged with pathogens

- Necrotic enteritis (*C. perfringens*)
  - Reduced endotoxin concentrations in plasma, and apoptosis index in ileum post-challenge (Liu, 2012)
  - Increased expression of nutrient transporters (SGLT1, PepT1, and L-FABP) in the jejunum of challenged broilers (Guo et al., 2014)

- Salmonella
  - Reduced horizontal transmission with S. Heidelberg (Amerah et al., 2012)

- Campylobacter
  - Reduced counts in caeca of chickens fed wheat based diets (Fernandez et al., 2000)

Production of prebiotics in-situ

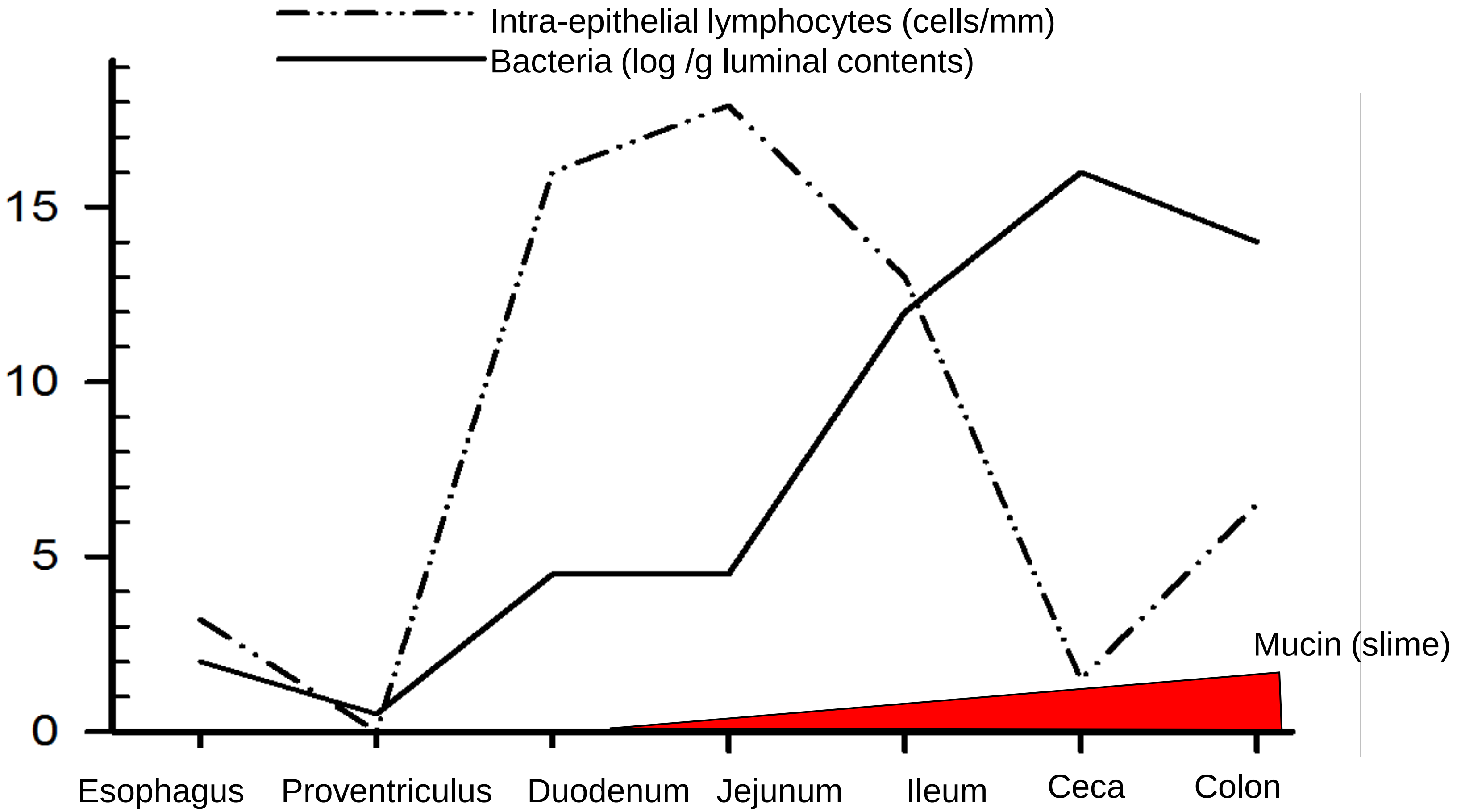
Cereal arabinoxylo-oligosaccharides have been attributed prebiotic effects in humans and chickens

Xylanases can solubilise arabinoxylans and produce oligosaccharides in the bird's gastro intestinal tract, with increased production of SCFAs

Prebiotic effects of xylanase depend on enzyme characteristics and other factors affecting gut microbial communities

Digestion of feed substrates and its relationship with gut health

Small intestine balances conflicting objectives: absorbing nutrients and defending host from pathogens



Intra-epithelial lymphocytes (IEL) in chickens are natural killer (NK), B and T cells (Klasing, 2005).

Proliferation of pathogenic *C. perfringens* was influenced by cereal type only in digested samples (in-vitro simulation)

Table 2. *C. perfringens proliferation in various digested diets incubated at 40°C (first trial)*

	Corn-based diet ($n = 6$)	Barley-based diet ($n = 7$)	Wheat-based diet ($n = 7$)	TG medium plus pancreatin and pepsin ($n = 7$)	TG medium ($n = 3$)
Median ($\times 10^8$ CFU/ml)	3.78^A	5.90^B	5.80^B	1.70^C	1.02^C
First quartile ($\times 10^8$ CFU/ml)	3.41	4.90	5.25	1.45	0.89
Third quartile ($\times 10^8$ CFU/ml)	4.06	7.95	6.90	2.40	1.20

^{A,B,C} Median values with different superscript letters are statistically different ($P < 0.05$).

Table 3. *C. perfringens proliferation in various non-digested diets incubated at 40°C (third trial)*

	Corn-based diet ($n = 6$)	Barley-based diet ($n = 6$)	Wheat-based diet ($n = 6$)	TG medium ($n = 5$)
Median ($\times 10^8$ CFU/ml)	5.60^A	6.01^A	6.05^A	1.75^B
First quartile ($\times 10^8$ CFU/ml)	5.40	5.75	4.86	1.02
Third quartile ($\times 10^8$ CFU/ml)	6.28	6.44	6.63	2.80

^{A,B} Median values with different superscript letters are statistically different ($P < 0.05$).

Annette et al., 2002

High undigested protein levels might be associated to increased susceptibility to Necrotic Enteritis

TABLE 5. Mean *Clostridium perfringens* populations[1] in the ileum and cecum and body weight of birds on d 28 of experiment 2

Protein source	Protein level (g/kg)	Body weight (g)	Ileum (cfu/g)	Cecum (cfu/g)
Fishmeal	230 g/kg	1,064*	3.93*†	4.57*†
Fishmeal	400 g/kg	1,125*	6.98*†	7.55*†
Soy protein concentrate	230 g/kg	794*	1.69*†	3.25*†
Soy protein concentrate	400 g/kg	689*	5.28*†	6.36*†
SEM		29	0.29	0.31
Effects		*P*-value		
Protein source		< 0.01	< 0.01	< 0.01
Protein level		0.37	< 0.01	< 0.01
Source × level		< 0.01	0.33	0.83

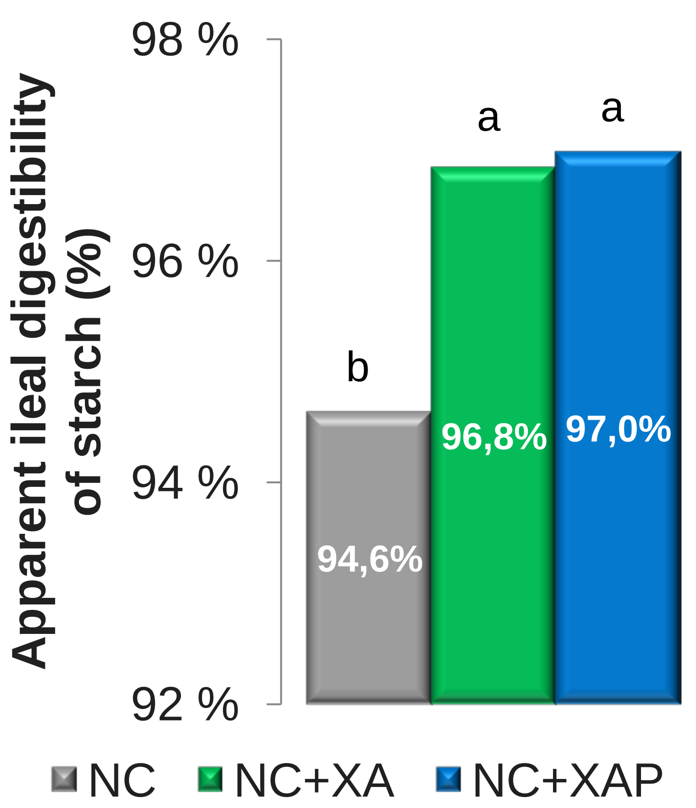
[1]Means are $\log_{10}$ colony-forming units counted on blood agar containing 100 mg of neomycin/L.

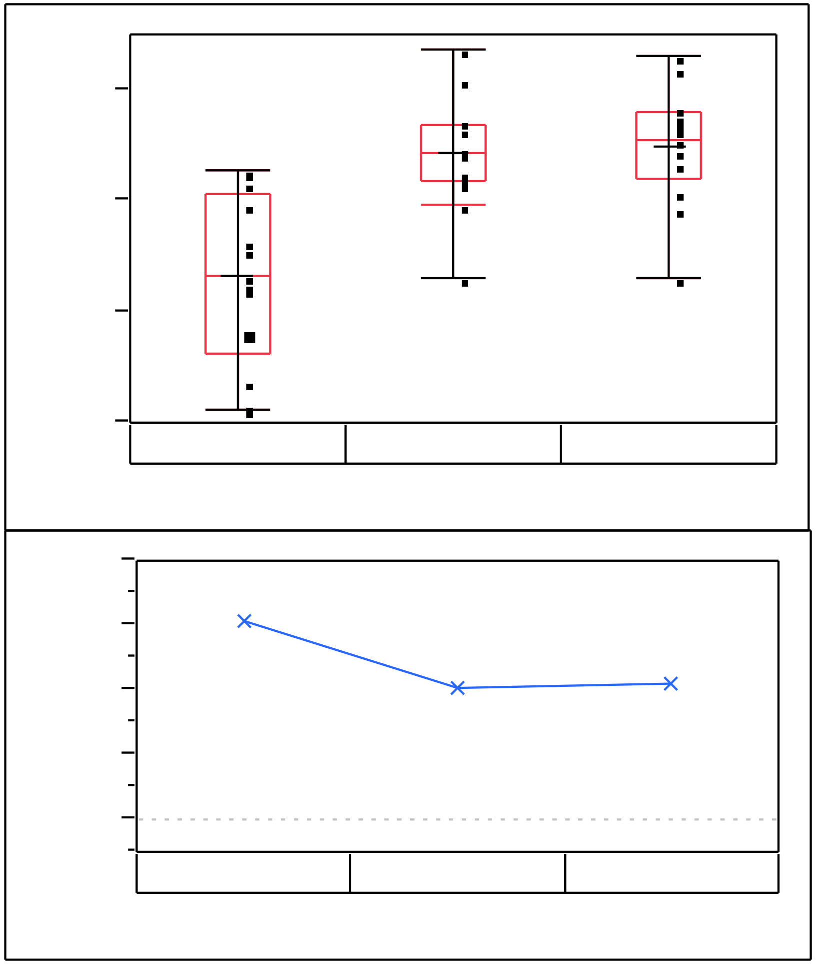
*Protein sources at the same protein concentration are significantly different ($P < 0.05$).

†Crude protein level within the same protein source are significantly different ($P < 0.05$).

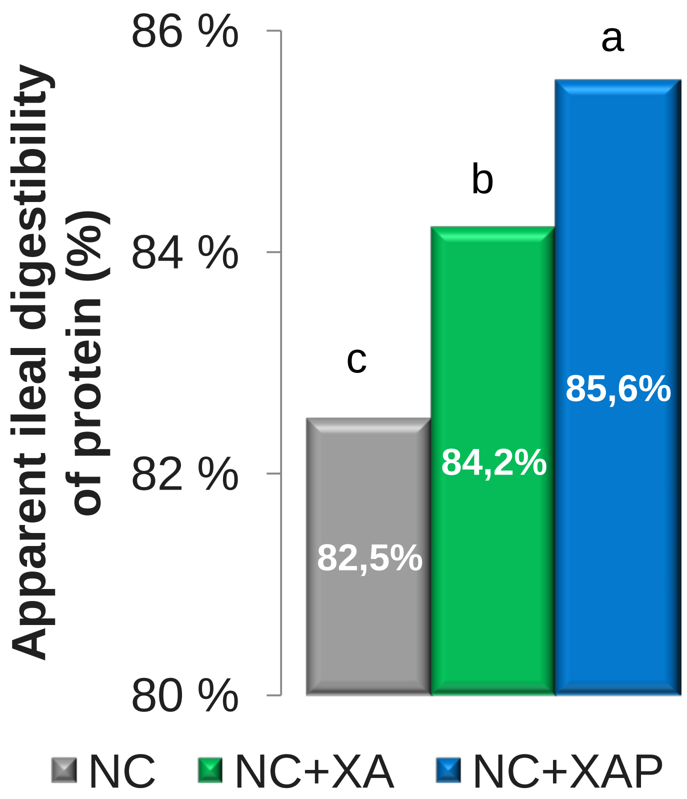
Drew et al., 2004

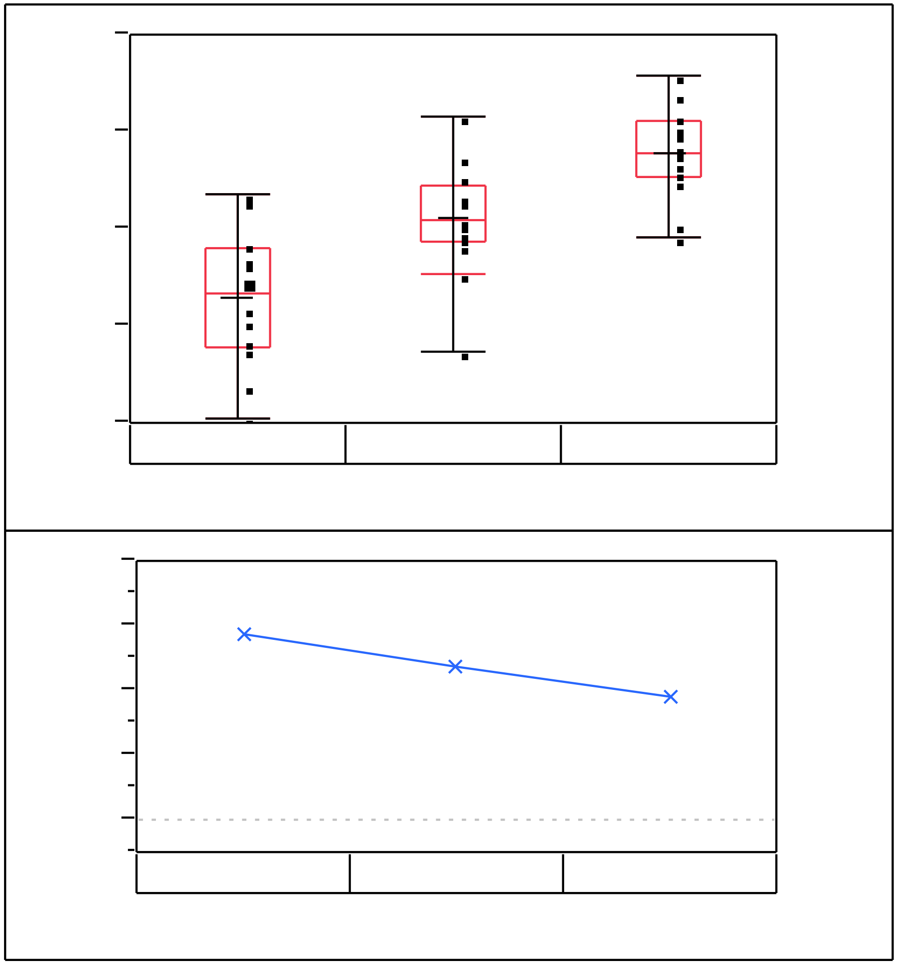
In 13 broiler trials, ileal digestibility of starch was increased by Xylanase + Amylase and Protease enzymes





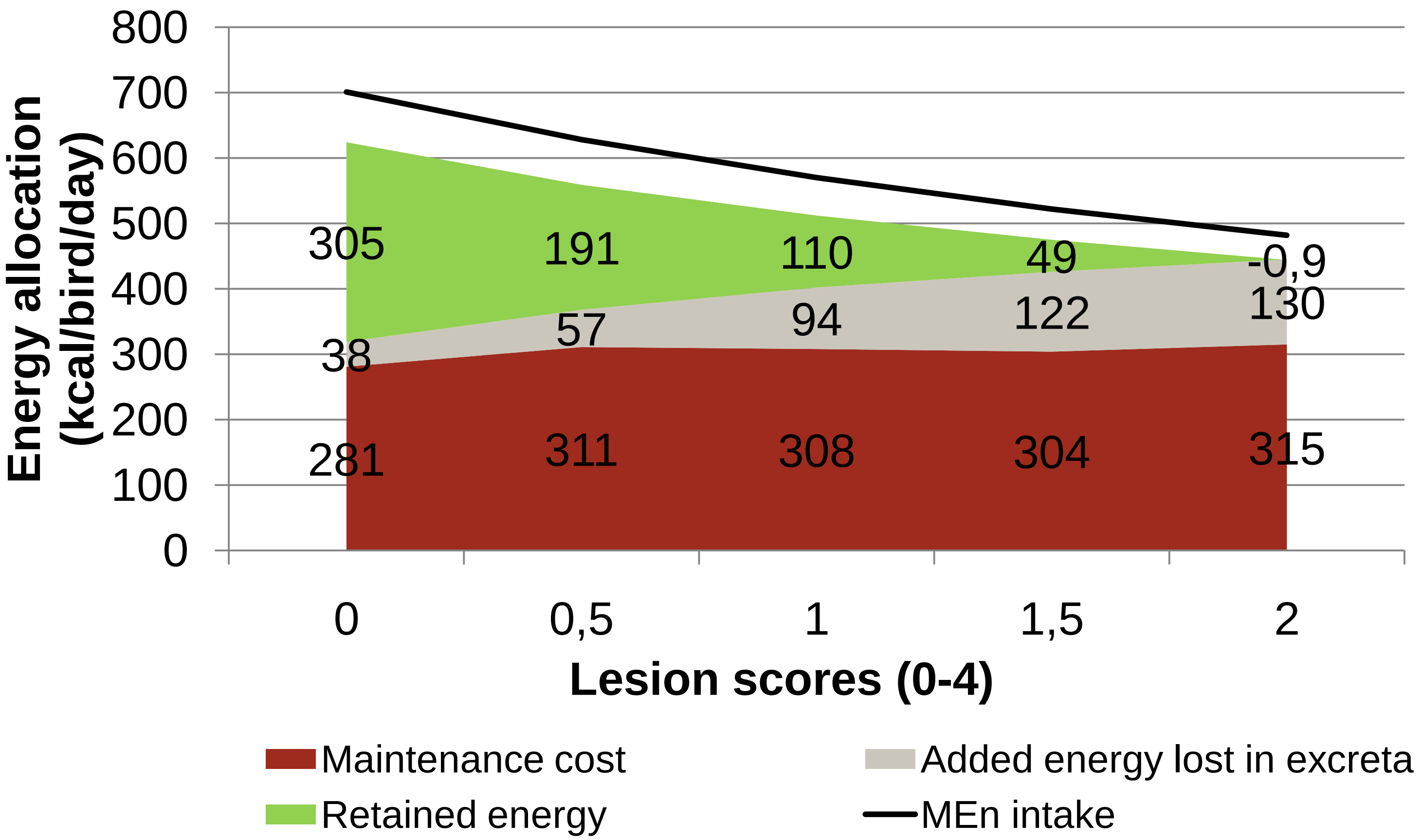
In 13 broiler trials, ileal digestibility of crude protein was increased by Xylanase + Amylase and Protease enzymes





Mal-absorption plays a significant role in economic losses due to sub-clinical enteric disease

Energy partitioning of 42-48 d old broilers challenged with oocysts of three Eimeria species



Teeter et al. 2011; Broussard et al., 2008

Bacillus DFMs and enzymes in a challenge situation

Cobb x Cobb males

8 pens/trm; 50 birds/pen

Necrotic Enteritis challenge model, mild mortality (~10-15%)

- Coccivac B at 0 d
- Reused litter
- A field strain of *C. Perfringens* in feed at 20, 21 and 22 d

Mortality, lesion scores, performance

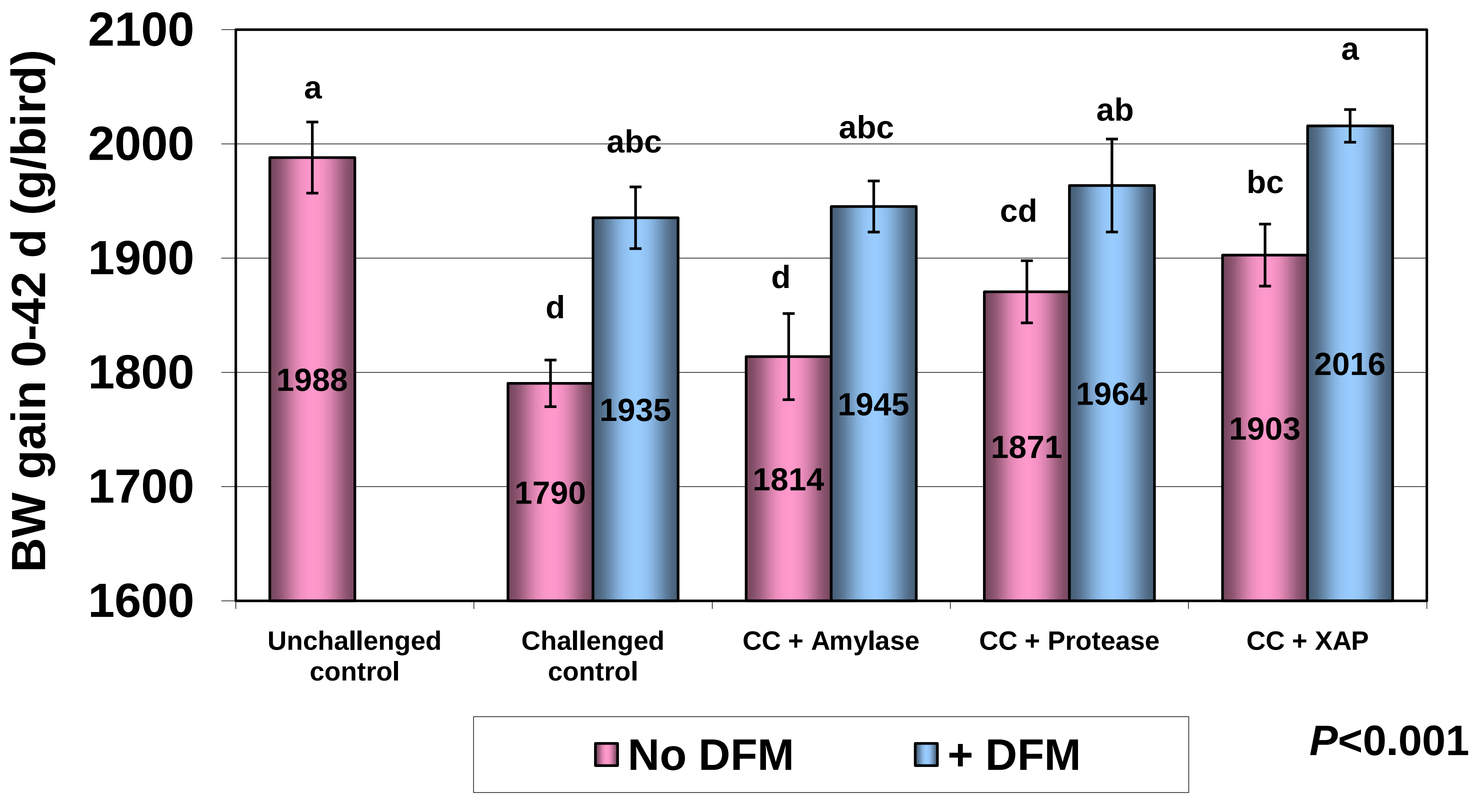
Corn/SBM/DDGS based diets, 500 FTU/kg of *E. coli* phytase in the background

Southern Research Centre, GA; Mathis et al., 2013

Treatments

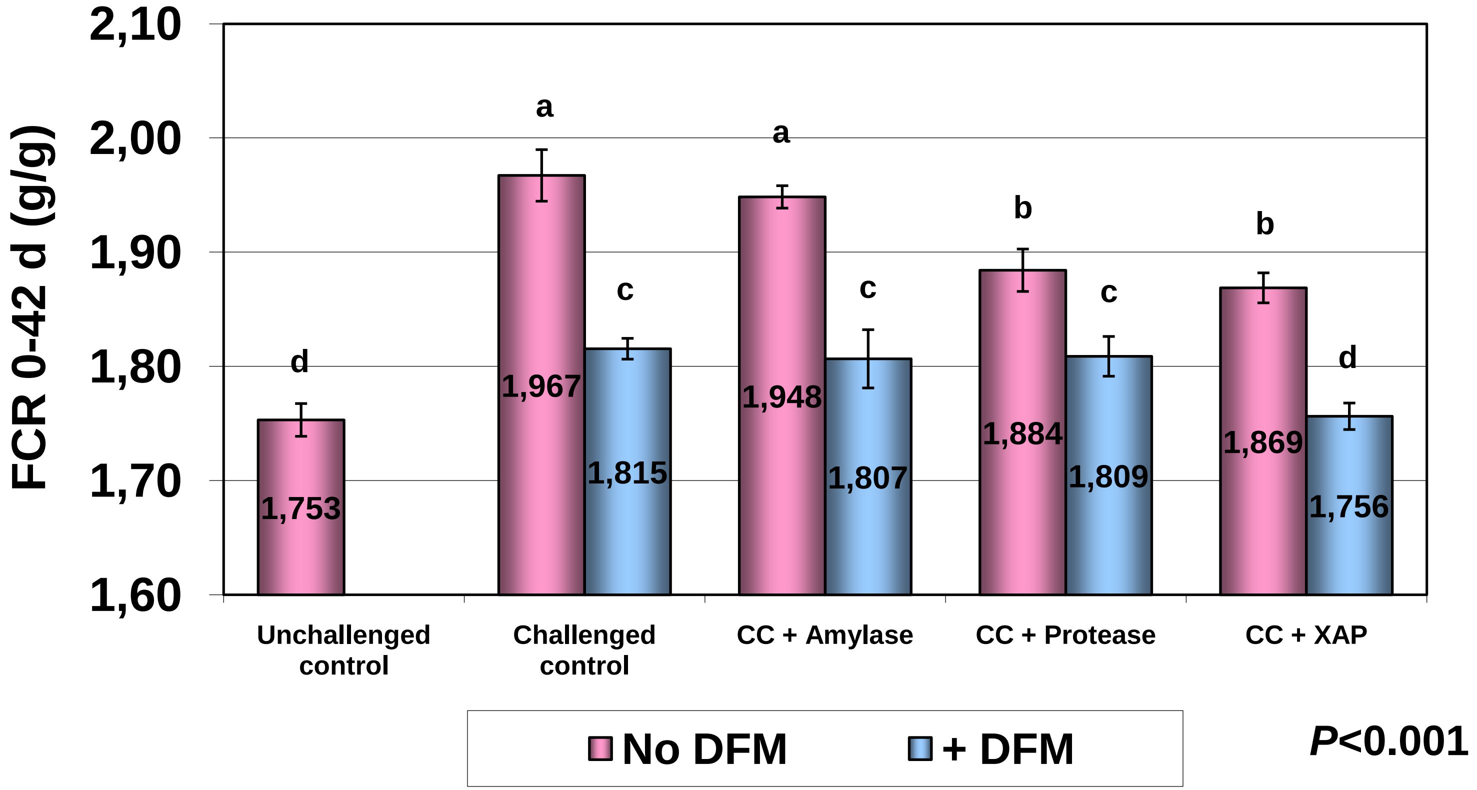
1. Unchallenged Control
2. Challenged Control (CC)
3. CC + A = Amylase from *B. licheniformis* (200 U/kg)
4. CC + P = Protease from *B. subtilis* (5,000 U/kg)
5. CC + XAP = AP + xylanase from *T. ressei* (2,000 U/kg)
6. CC + DFM (3 strains *Bacillus subtilis*; 7.5 x 10^4 CFU/g)
7. CC + DFM + A
8. CC + DFM + P
9. CC + DFM + XAP

42-day body weight gain was affected by DFMs and enzymes



CC = Challenged Control; birds were challenged with C. perfringens at 20, 21 and 22 d
DFM is a combination of 3 *Bacillus subtilis* strains; XAP is xylanase, amylase, and protease
a, b: means without a common letter differ at P<0.05

Bacillus DFM and XAP reduced 42-day FCR to the level of the unchallenged control



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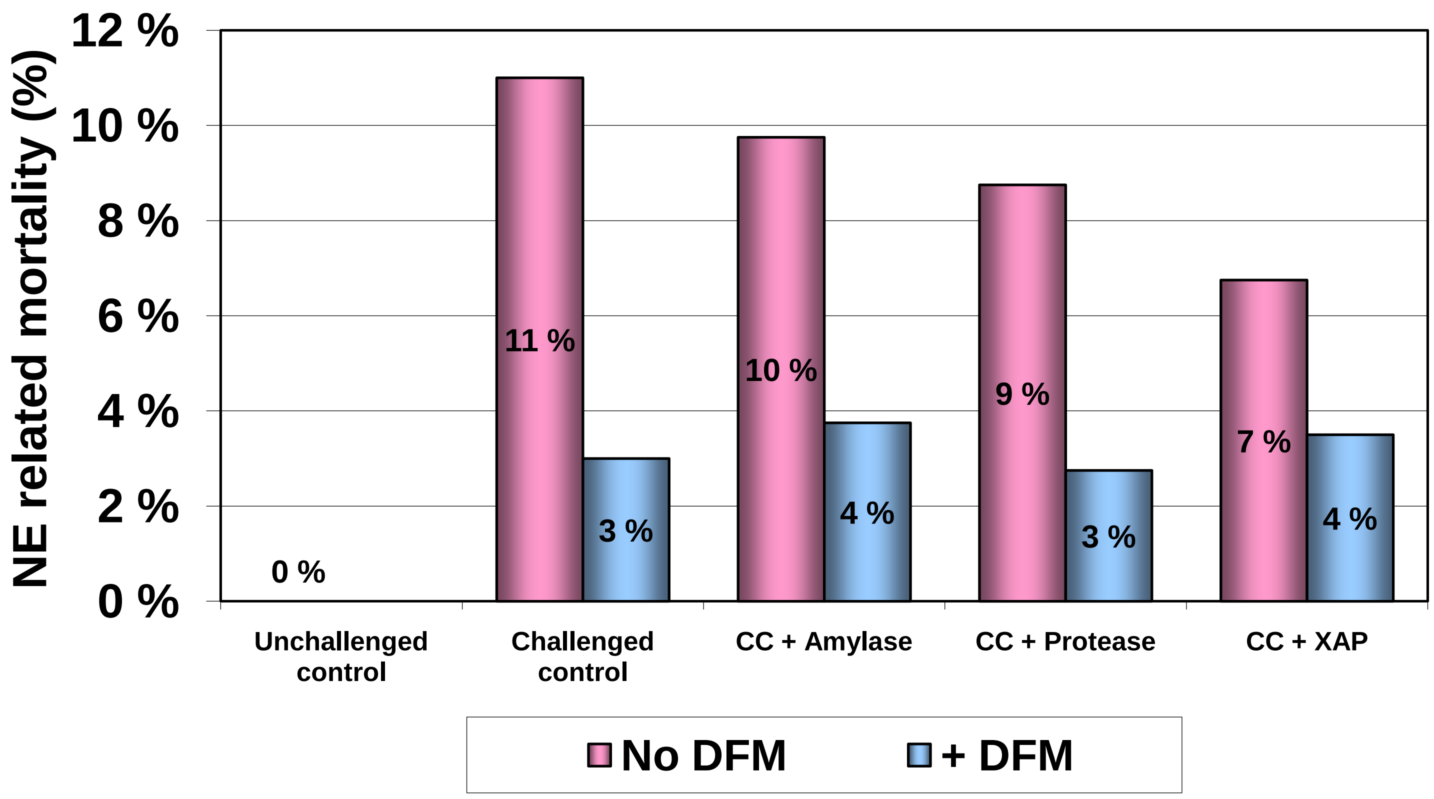
Mortality related to NE



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Digestion of feed substrates and its relationship with gut health

Increased absorption of nutrients in the small intestine, in particular readily available N and C sources, due to enzymes can contribute to maintain gut health in chickens

Conversely, clinical episodes of enteric disease causing mal absorption can reduce the potential of exogenous enzymes to increase nutrient utilization

Summary and implications

- Exogenous enzymes targeting feed arabinoxylans have the capacity to create prebiotics in situ
  - Increase SCFA production
  - Reduce negative effects of Necrotic enteritis
  - Reduce *Salmonella* and *Campylobacter* prevalence

- Reduction of undigested substrates in the small intestine can play a role in reducing nutrient availability for the growth of pathogens

- Exogenous enzymes are only one of the various tools to manage intestinal health in commercial poultry

Thank you!

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