July 27-30, 2015

104th Annual Meeting of the Poultry Science Association

Louisville, Kentucky

Presented at:

"From Egg to Plate – The Influence of Gut Health" Symposium

Role of nutrition in gut health

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Higher purpose!

1/3 > 1/7 > 0/9

1960 2010 2050

|—— Achieved ——| |—— Dream ——|

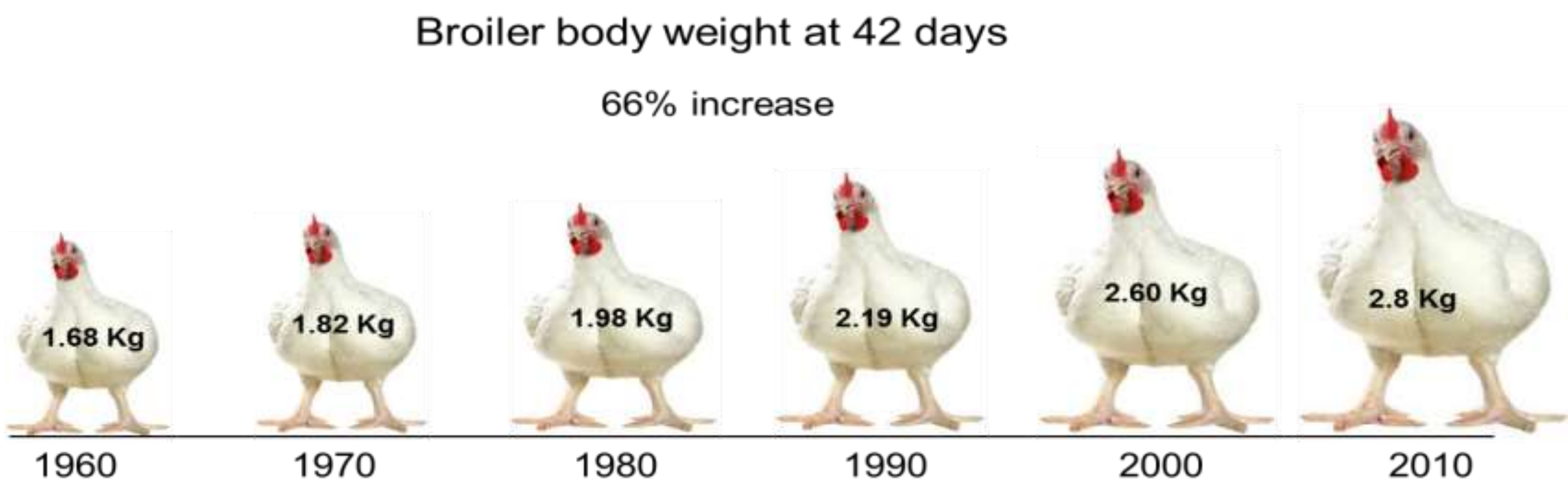
Food on plate for extra 4B people

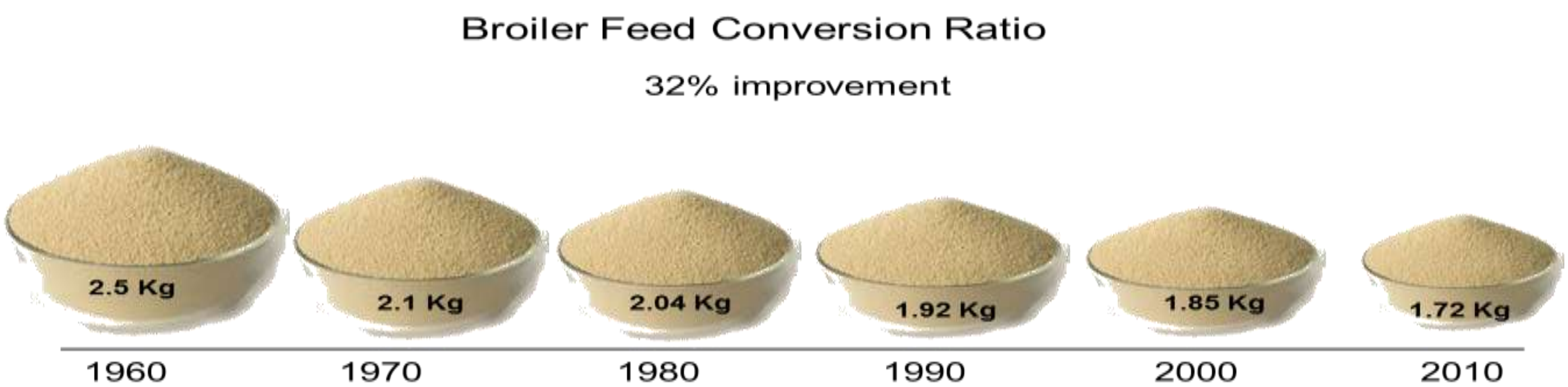
Extra 3B still to feed

Annual meat production will need to rise by over 200 million tonnes or 74%

Approximates Based on 2011 Hunger report, www.bread.org, FAO, 2009

How animal production contributed to this achievement?





Based on Rauw 1998 & Ross recommendations

How animal production contributed to this achievement?

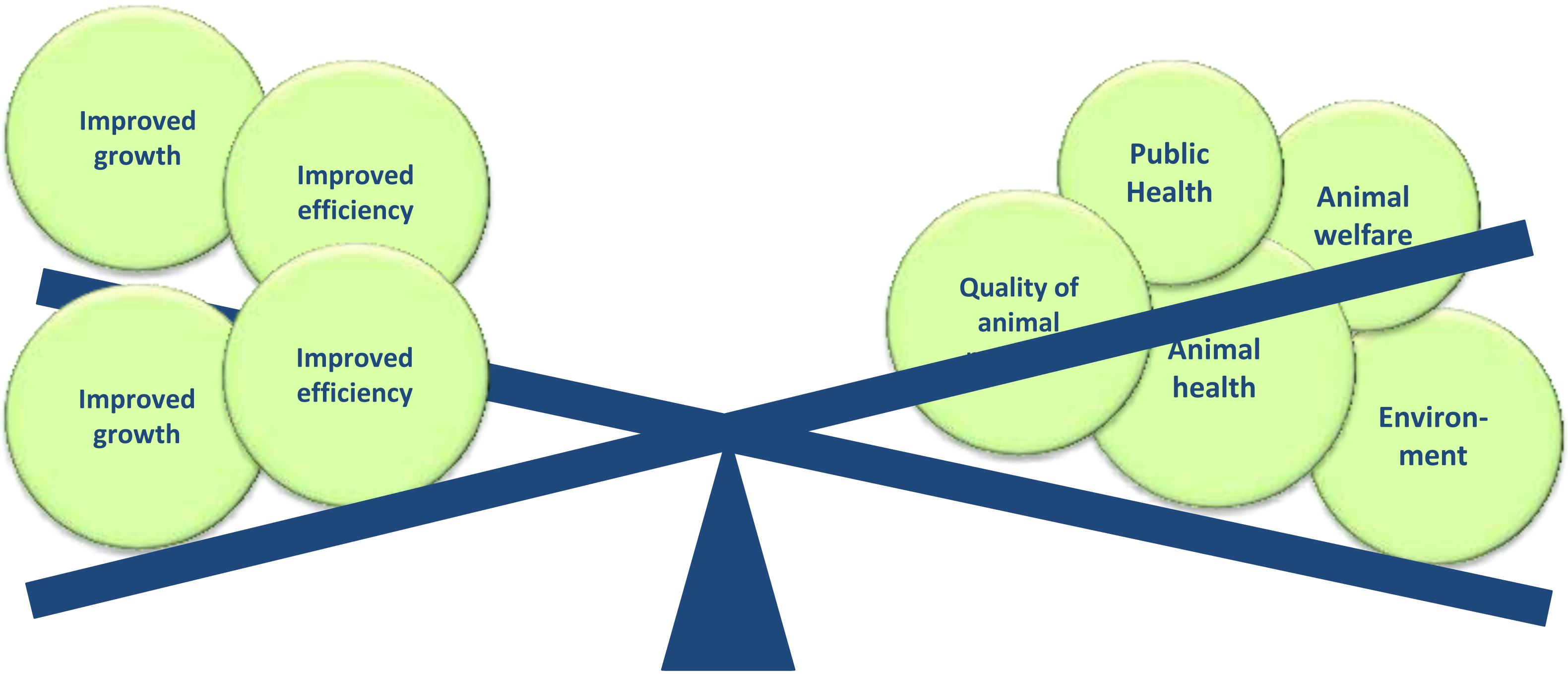
Changes in global human population, pig and poultry inventories, and production and international trade of pig and poultry meat between 1996 and 2005.

	1996	2005	Annual growth (%)
Human population	5,762	6,451	1.1
Inventory			
Pigs (million)	859	963	1.1
Poultry (million)	14,949	18,428	2.1
Production			
Pig meat (thousand tons)	79,375	103,226	2.6
Poultry meat (thousand tons)	56,408	81,856	3.7
International trade			
Pig meat (thousand tons)	6,398	9,557	4.0
Poultry meat (thousand tons)	5,359	9,234	5.3

Source: FAOSTAT

Otte *et al*., 2007

Then why 'dream' is still distant?



Improvements in Animal production efficiencies in past 50 years were built on the backbone of using AGPs to support performance under intensive production conditions

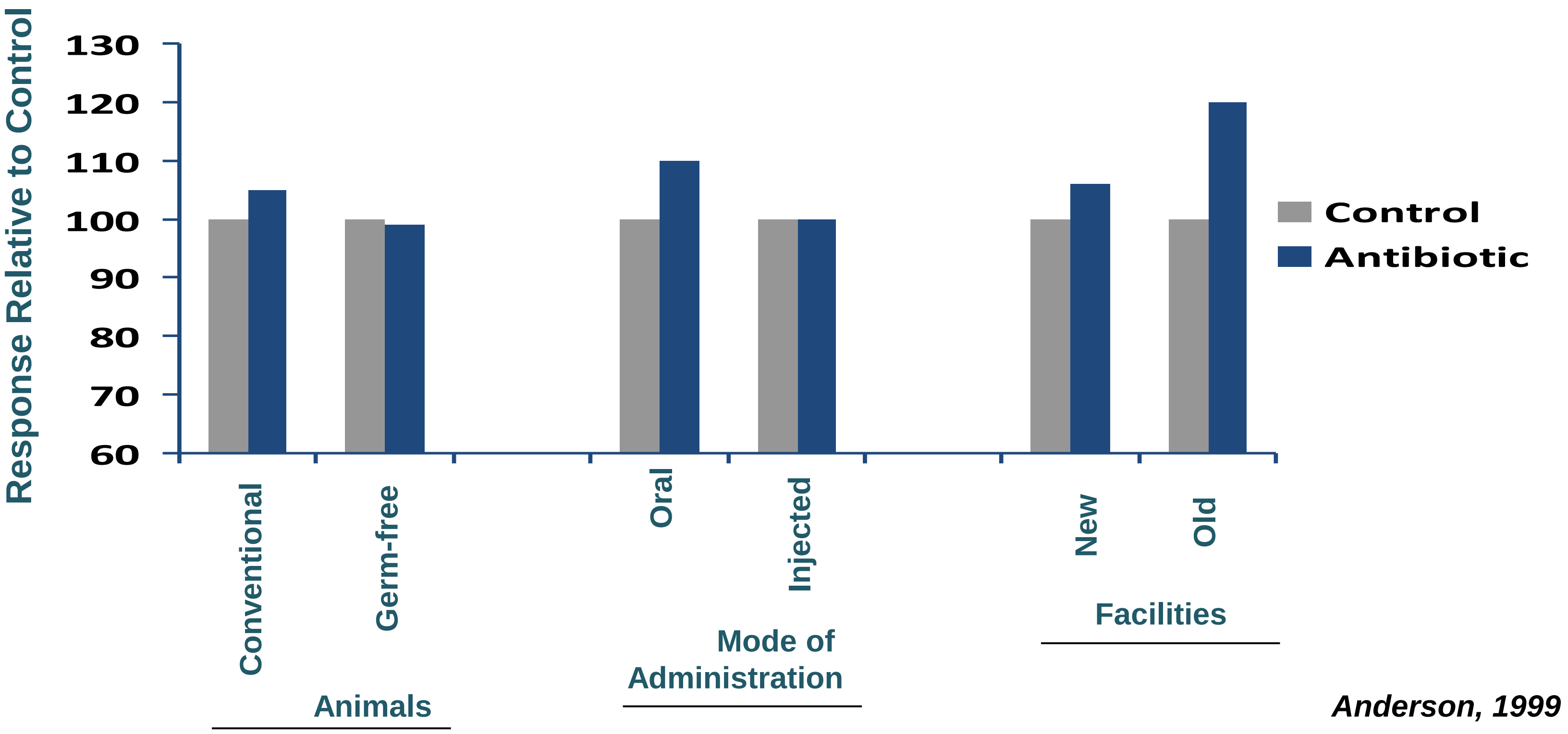
Most of these issues are gut health related

Bad news is AGPs are going away, we must think beyond

Today’s Research Topic:

Strategies to Support gut health during transition to post AGP era

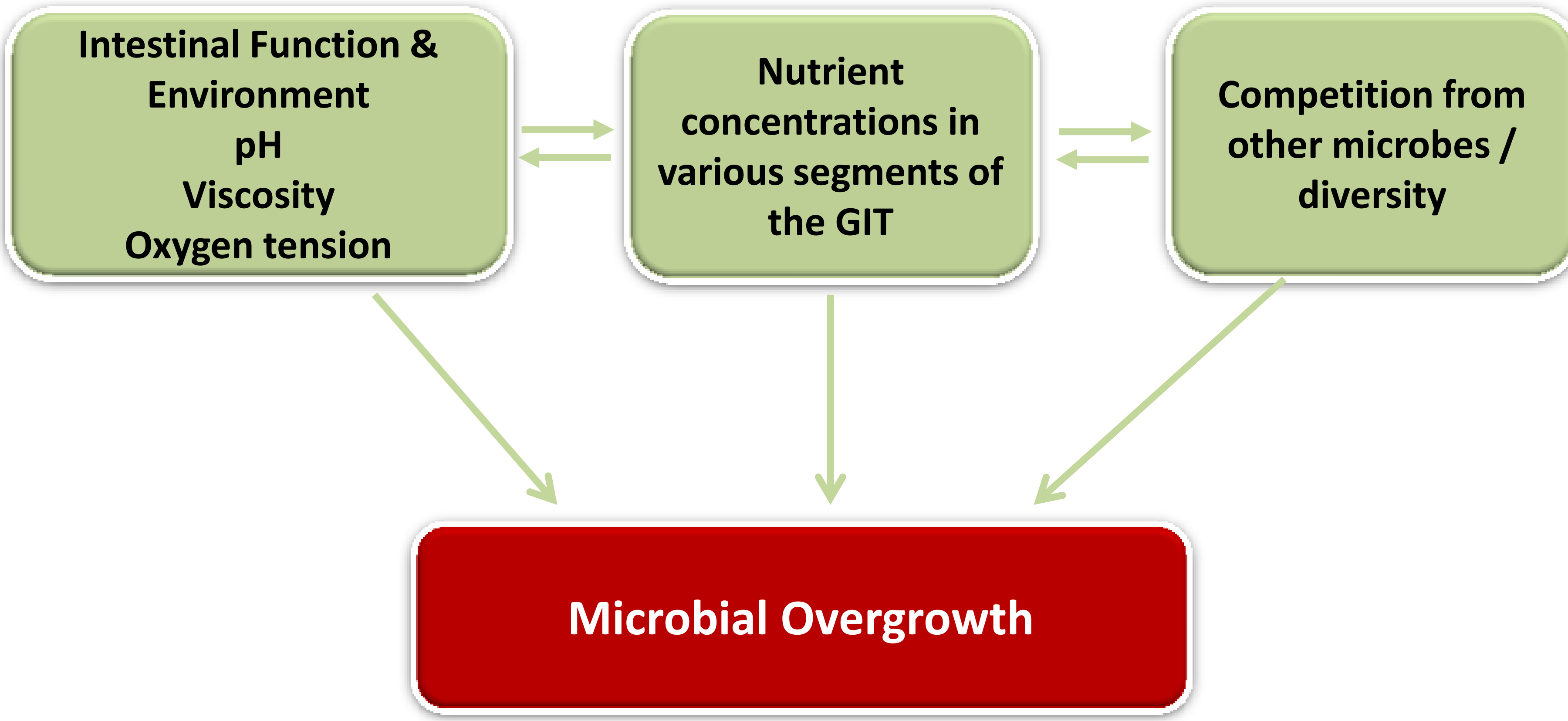
When, how and why AMGPs work?



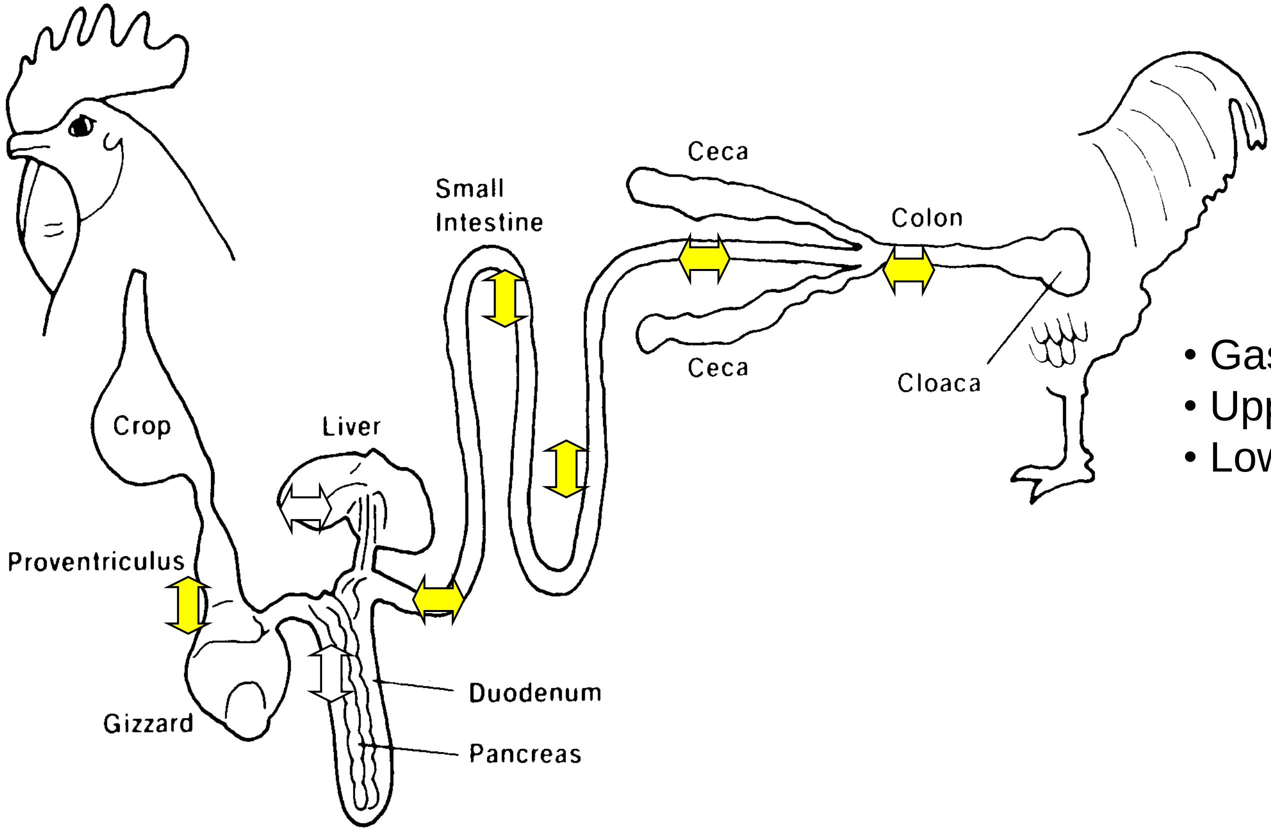
- AMGPs work more effectively in the **gut**

- AMGPs work more effectively under **Stress / Challenge / High microbial loads in environment**

Some of the factors affecting microbial load / diversity



Gizzard development & Reverse Peristalsis are Critical Component to Intestinal Health



- Gastric reflux
- Upper intestinal reflux
- Lower intestinal reflux

- Peristalsis and reverse peristalsis determine the extent of digestion
- Gizzard is the gastrointestinal "pacemaker" (Duke, 1994)

Adapted from J.Brake, 2008

Symptoms of Poor Gut Motility

- Proventricular hypertropy and gizzard atrophy
- Gizzard / Proventriculus act as a conduit rather than grinding organ
- Increased feather and litter picking
- Poor protein and fat digestion
- Reduced feed conversion
- Increased susceptibility to enteric pathogens / microbial overgrowth
- Poor water and electrolyte reabsorption = wet litter
- Increased mortality rate / disease

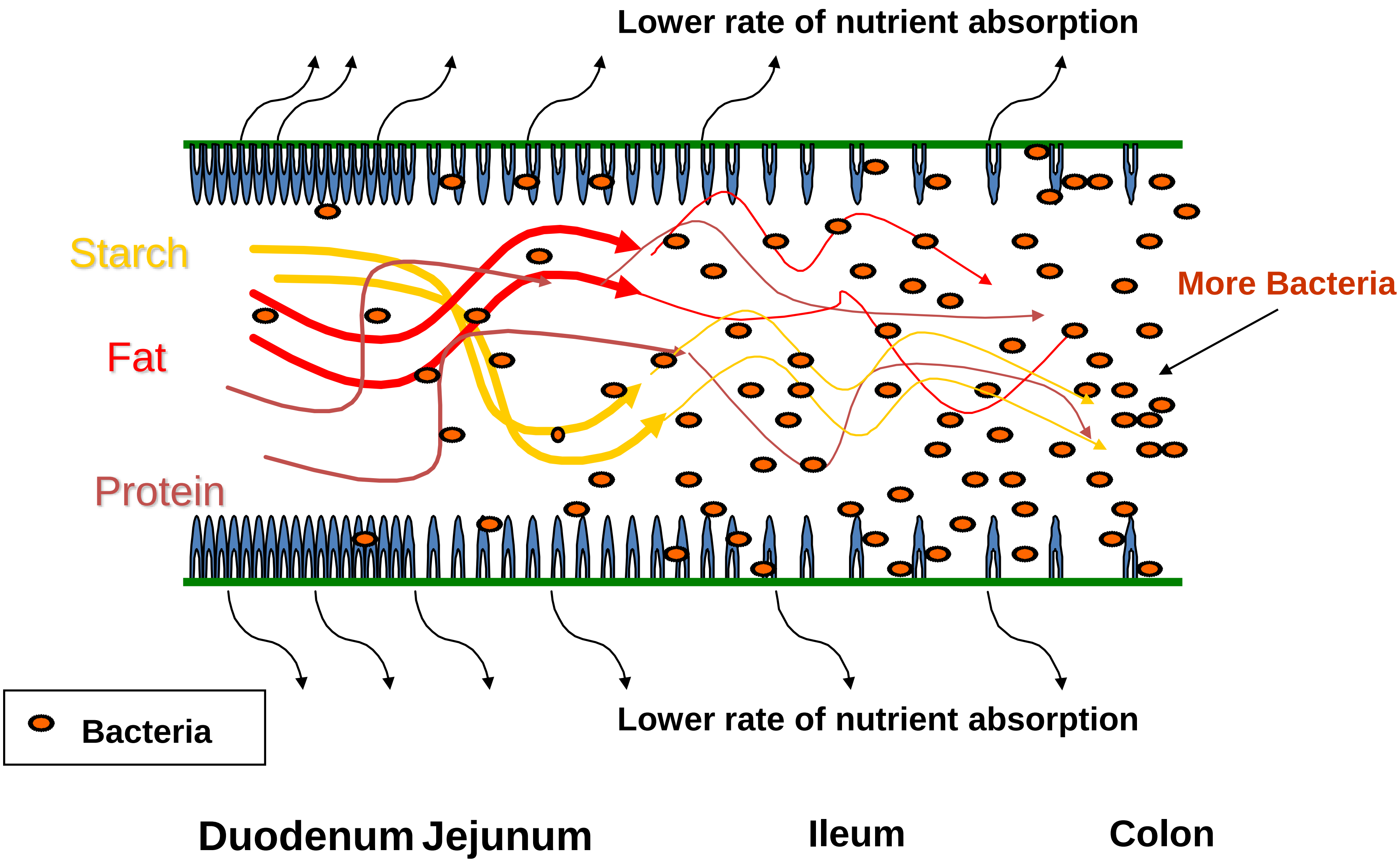
J.Brake, 2008

Importance of Particle Size / Structure in feed

Average particle size (micron)	Nitrogen retention @ 7 days (%)	Fecal AME_n (kcal/g)
561	50.2^c	2.78^b
783	56.6^b	2.79^b
997	58.6^a	2.84^a

Krabbe, 2000

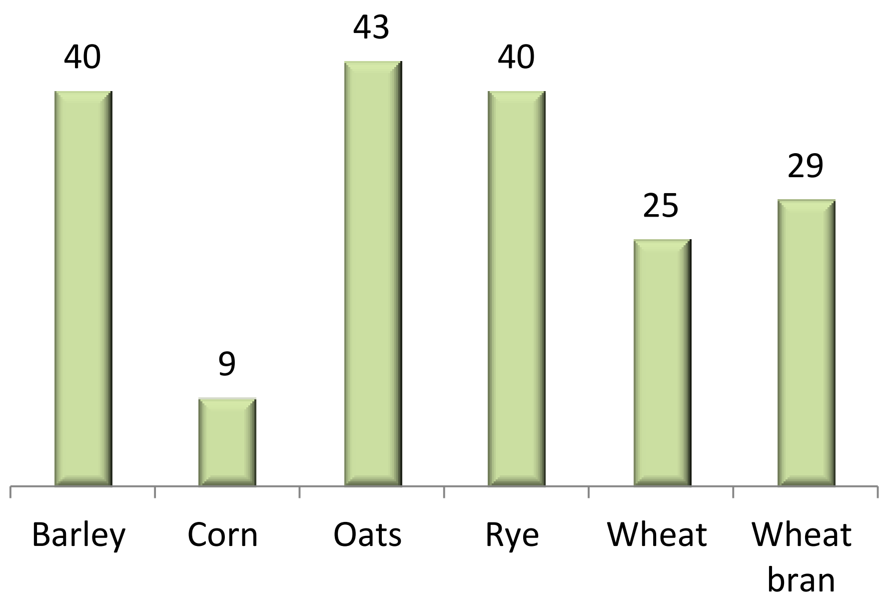
Level of nutrient digestion has a large impact on populations of ileal bacteria



NSP contents in diet and resulting impact on undigested nutrients in lumen of intestine and oxygen tension contribute further to the problem

Animal diets contain Cereals

unconventional cheap ingredients with high NSP contents

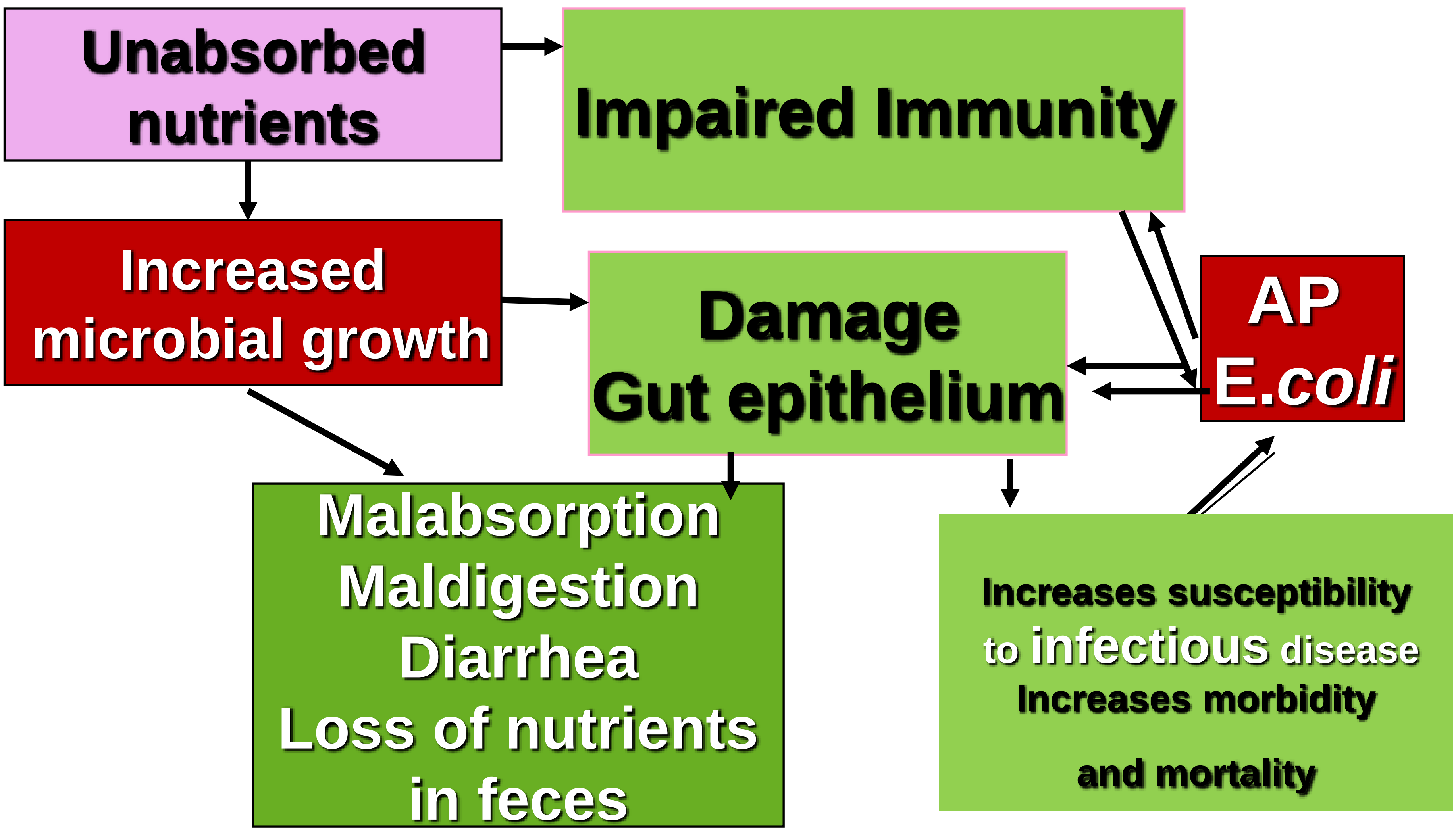


Concentration of soluble NSPs g/Kg DM

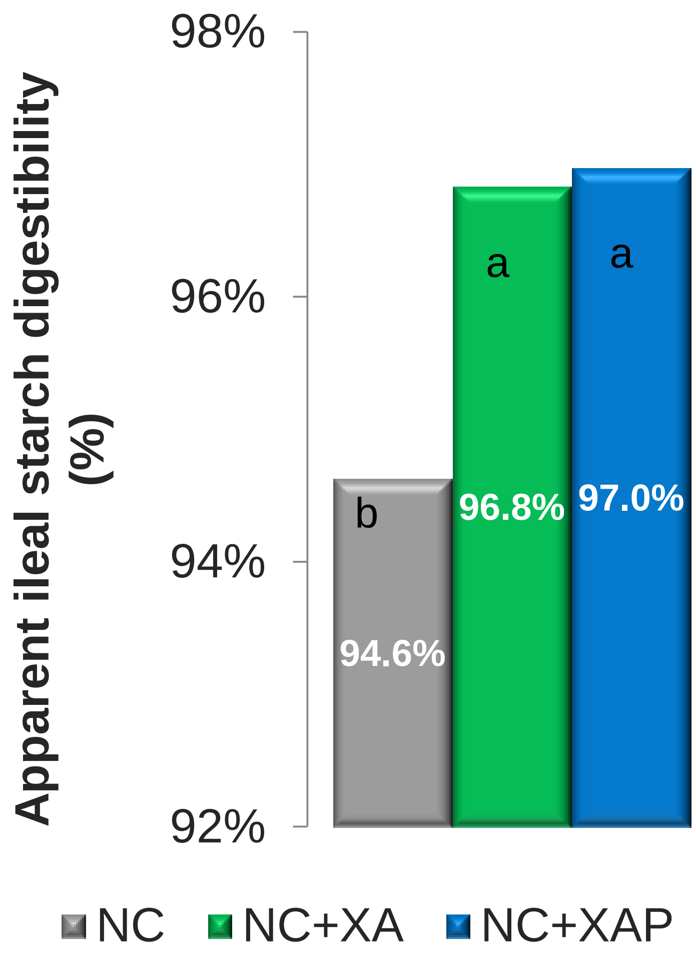
Soluble NSP levels are high

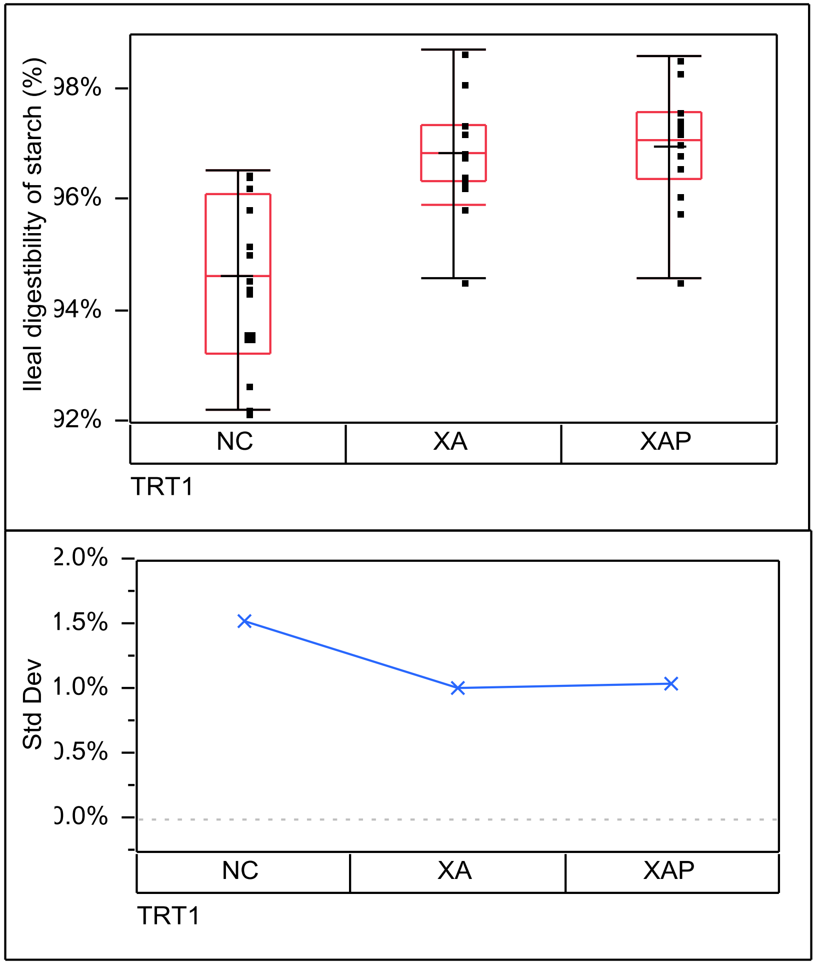
Bach Knudsen, Anim. Feed Sci. Technol. 67:319-338

Further Impacts of reduced nutrient digestion / more substrate for pathogen growth



Feed Enzymes are commonly used to increase nutrient digestibility and reduce feed cost

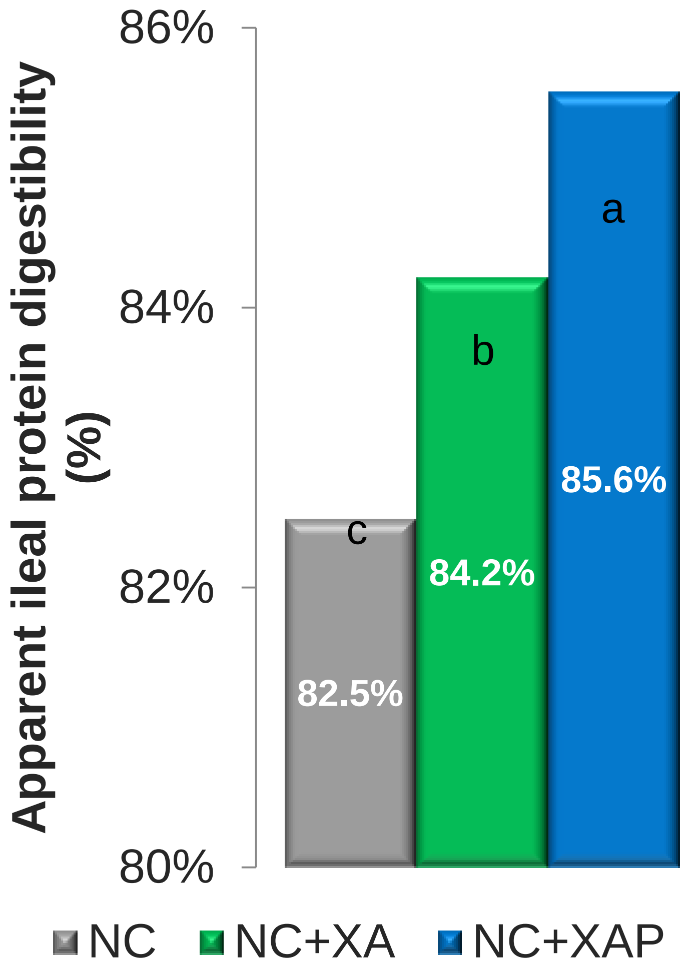


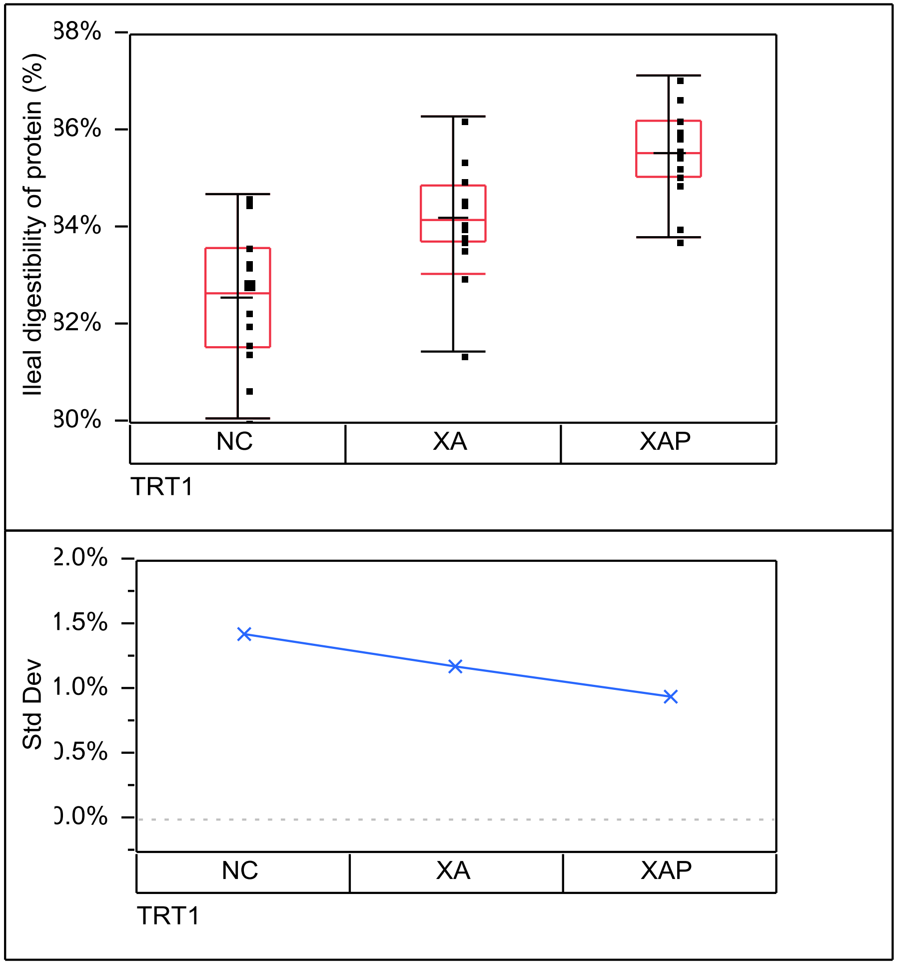


Enzyme source: XA=Xylanase+Amylase, XAP=Xylanase, Amylase & Protease

Romero et al. 2011

Feed Enzymes are commonly used to increase nutrient digestibility and reduce feed cost



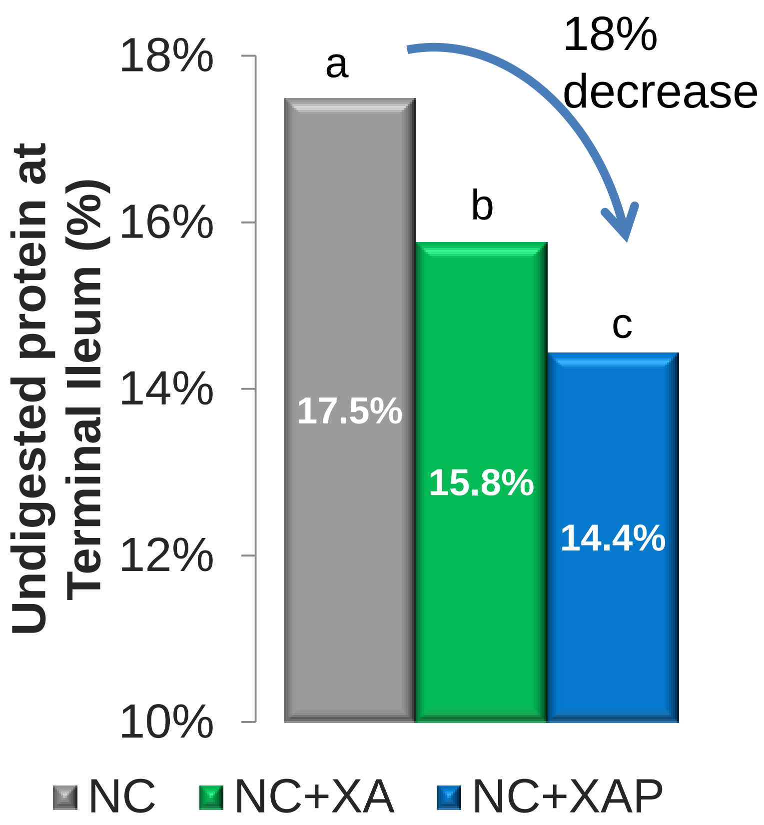


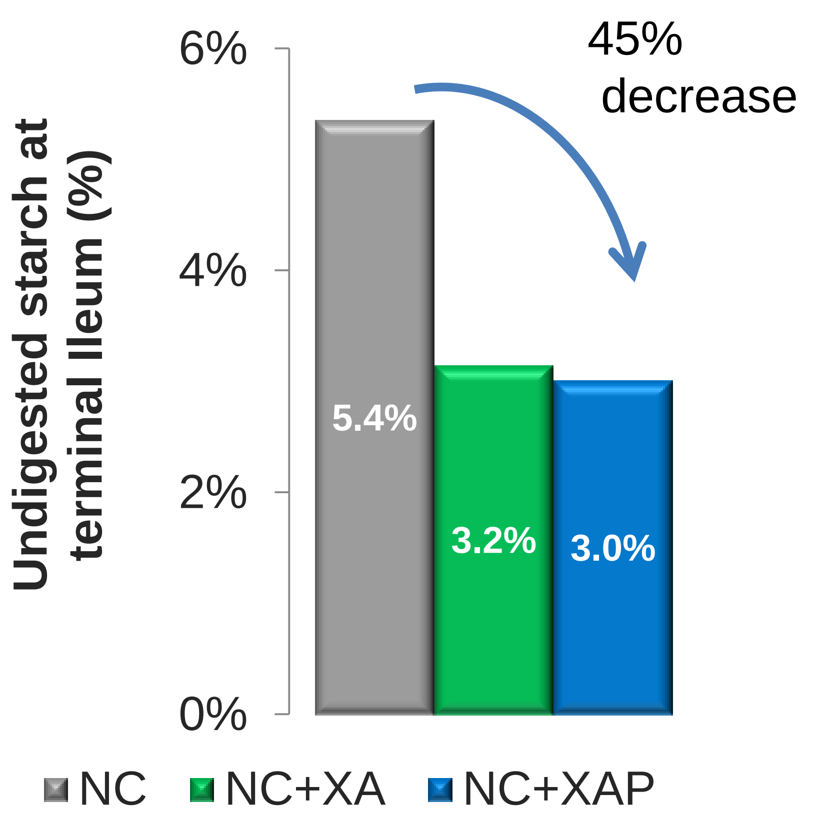
Average of 13 broiler ileal digestibility trials, corn-soy diets, +/- DDGs

Enzyme source: XA=Xylanase+Amylase, XAP=Xylanase, Amylase & Protease

Romero et al. 2011

Feed Enzymes are commonly used to increase nutrient digestibility and reduce feed cost… but also undigested substrates for gut microbes



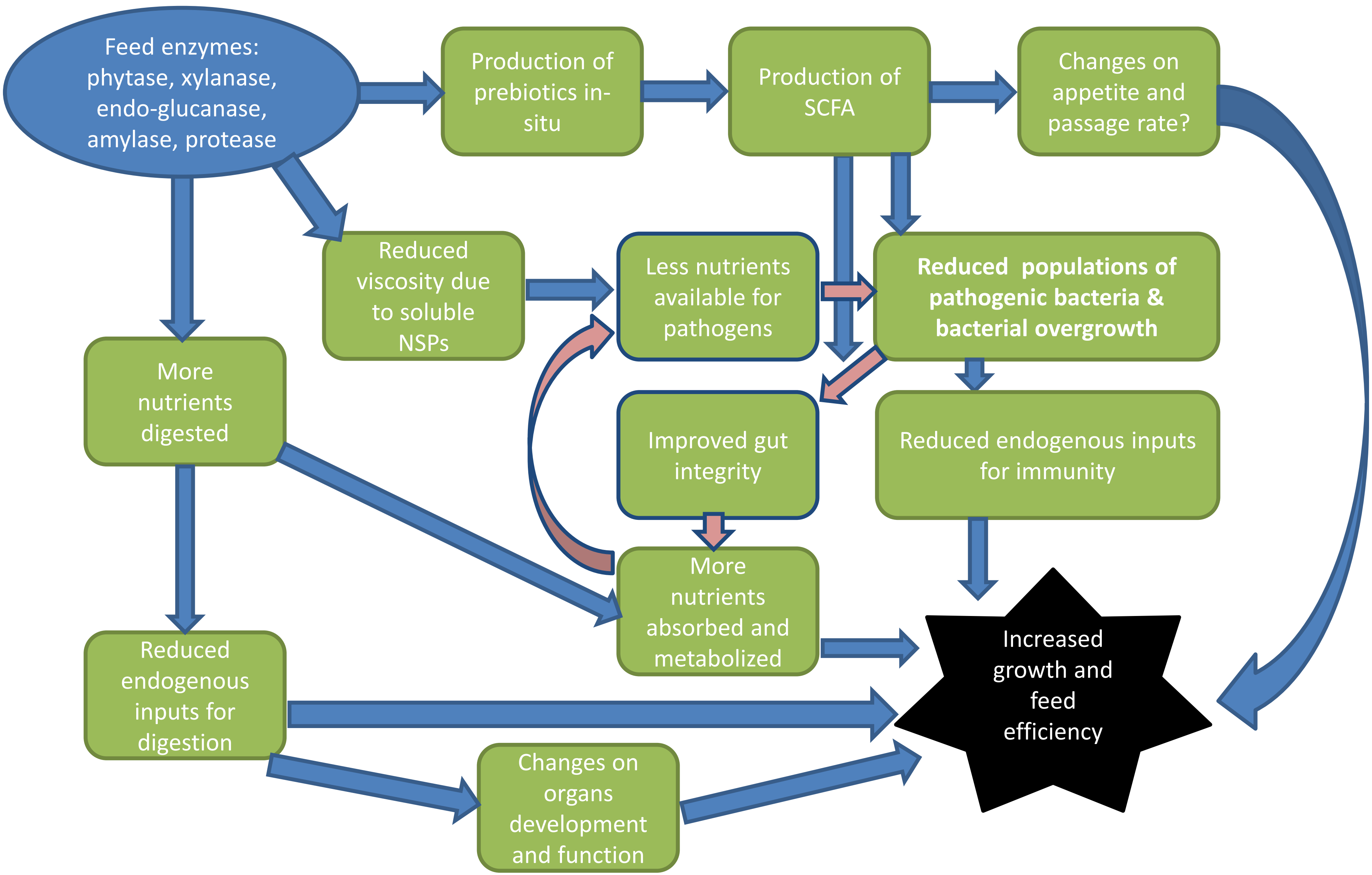


Average of 13 broiler ileal digestibility trials, corn-soy diets, +/- DDGs

Enzyme source: XA=Xylanase+Amylase, XAP=Xylanase, Amylase & Protease

Romero et al. 2011

Enzymes are part of the solution to reducing microbial overgrowth



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

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

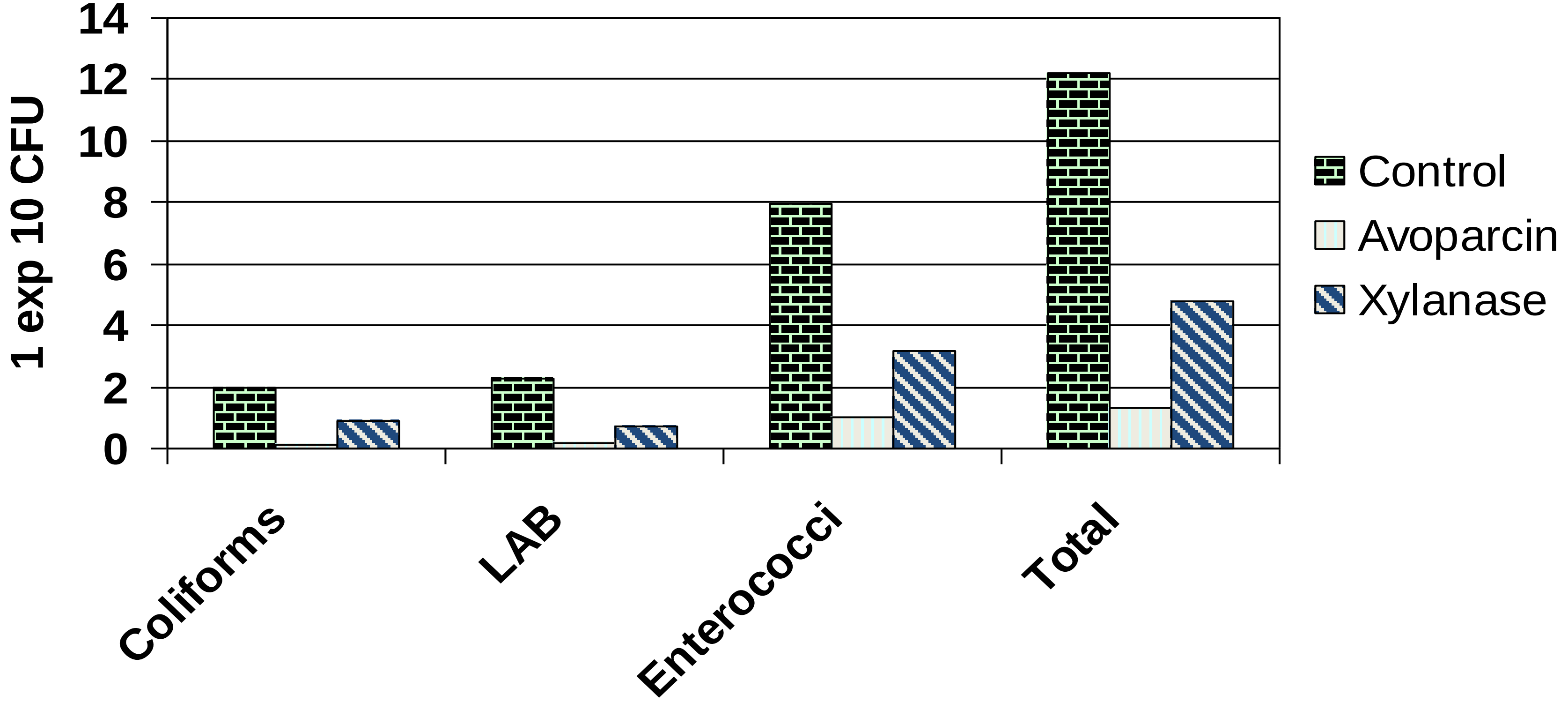
	Corn-based diet (n = 6)	Barley-based diet (n = 7)	Wheat-based diet (n = 7)
Median ($\times 10^8$ CFU/ml)	3.78^{A}	5.90^{B}	5.80^{B}
First quartile ($\times 10^8$ CFU/ml)	3.41	4.90	5.25
Third quartile ($\times 10^8$ CFU/ml)	4.06	7.95	6.90

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

Annette et al., 2002

Small intestinal bacterial populations are directly controlled by growth promoters (in-feed antibiotics)

Enzymes reduce substrate availability & luminal viscocity having similar effects



Apajalahti and Bedford1998 Western Nutr. Conf.

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.

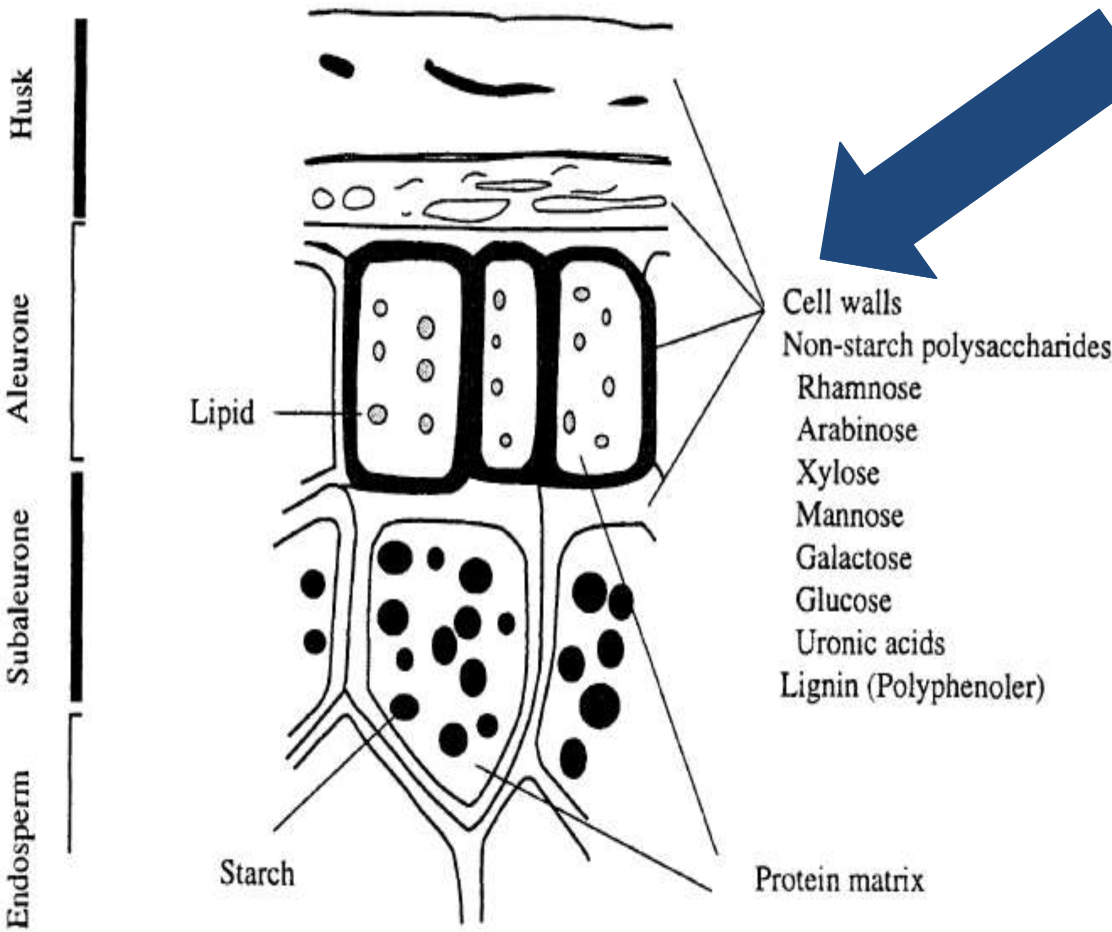
*Protein sources at the same protein concentration are significantly different ($P < 0.05$).

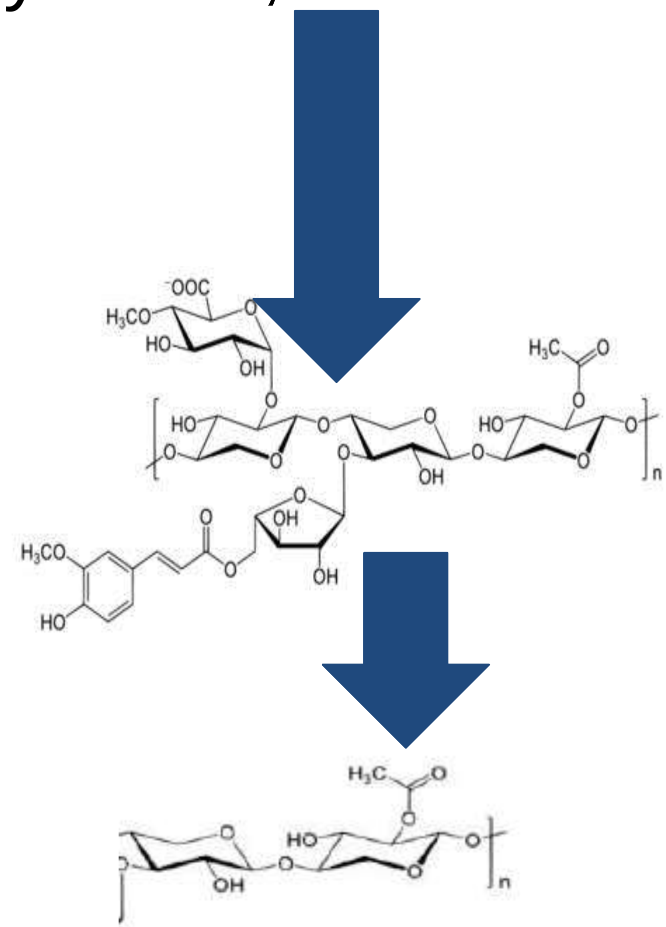
†Crude protein level within the same protein source are significantly different ($P < 0.05$).

Drew et al., 2004

Fiber degrading enzymes can also release short chain oligosaccharides (NSP-HP) from depolymerization of cell wall non-starch polysaccharides (NSP)

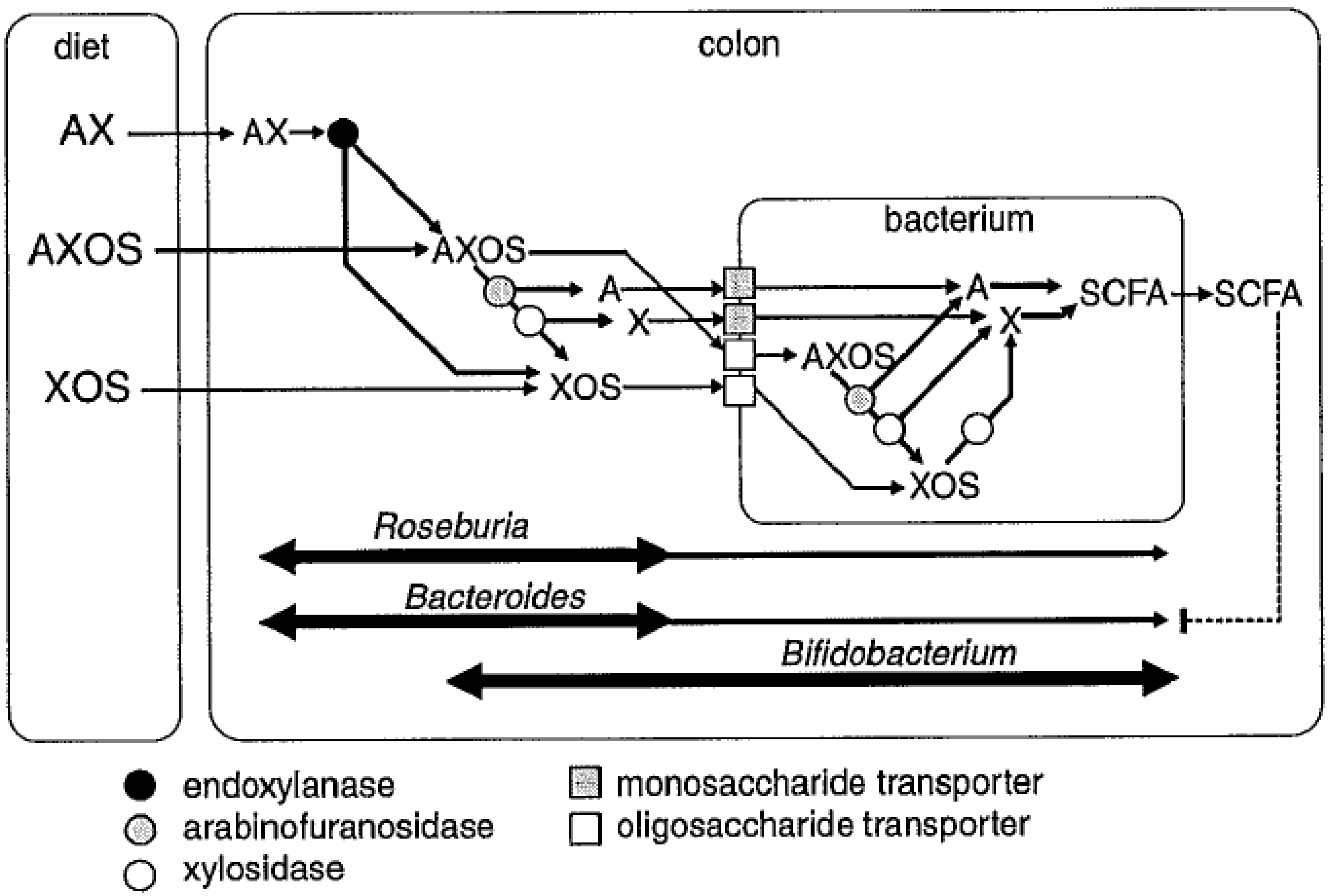
Xylanase, Cellulases





Bach Knudsen 2001 Anim. Feed Sci. Technol. 90:3-20

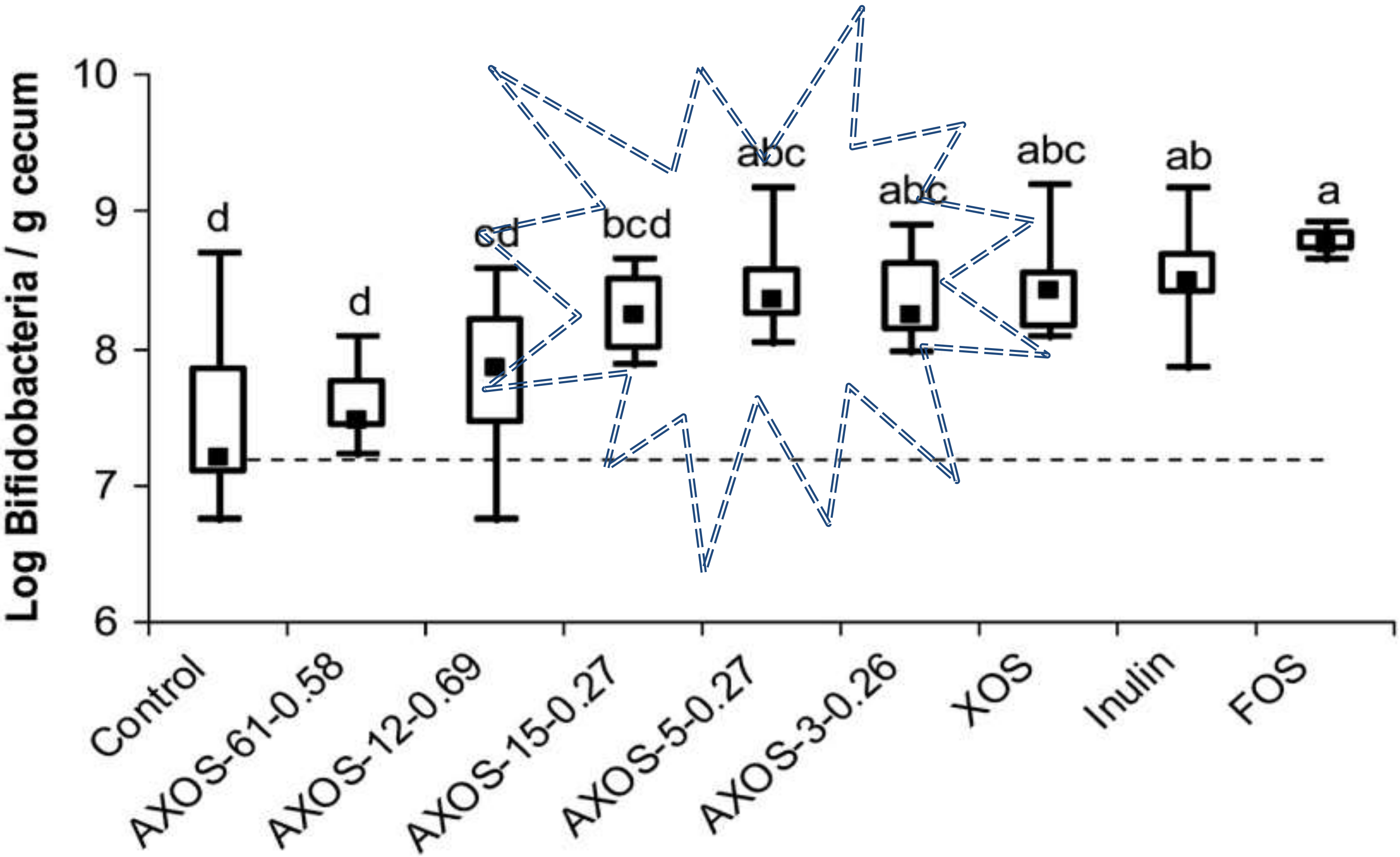
Arabino-xylo-oligosaccharides are pre-biotics & facilitate increased VFA production



Broekaert et al., 2011

Further support for prebiotic effects of Xylo Oigosachharides on *Bifidobacter* populations

Effect of different oligosaccharides on the concentration of bifidobacteria in the cecum of rats fed diets containing structurally different wheat-derived AXOS for 14 d.



Van Craeyveld V et al. J. Nutr. 2008;138:2348-2355

Effects of cereal derived AXOS in chickens

- Reduced FCR and increased caecal Bifidobacteria levels (Courtin et al., 2008a, 2008b); DP=15, 0.25-0.5% AXOS
- Reduced *Salmonella* in caeca, cloaca and spleen (Eeckhaut et al., 2008)

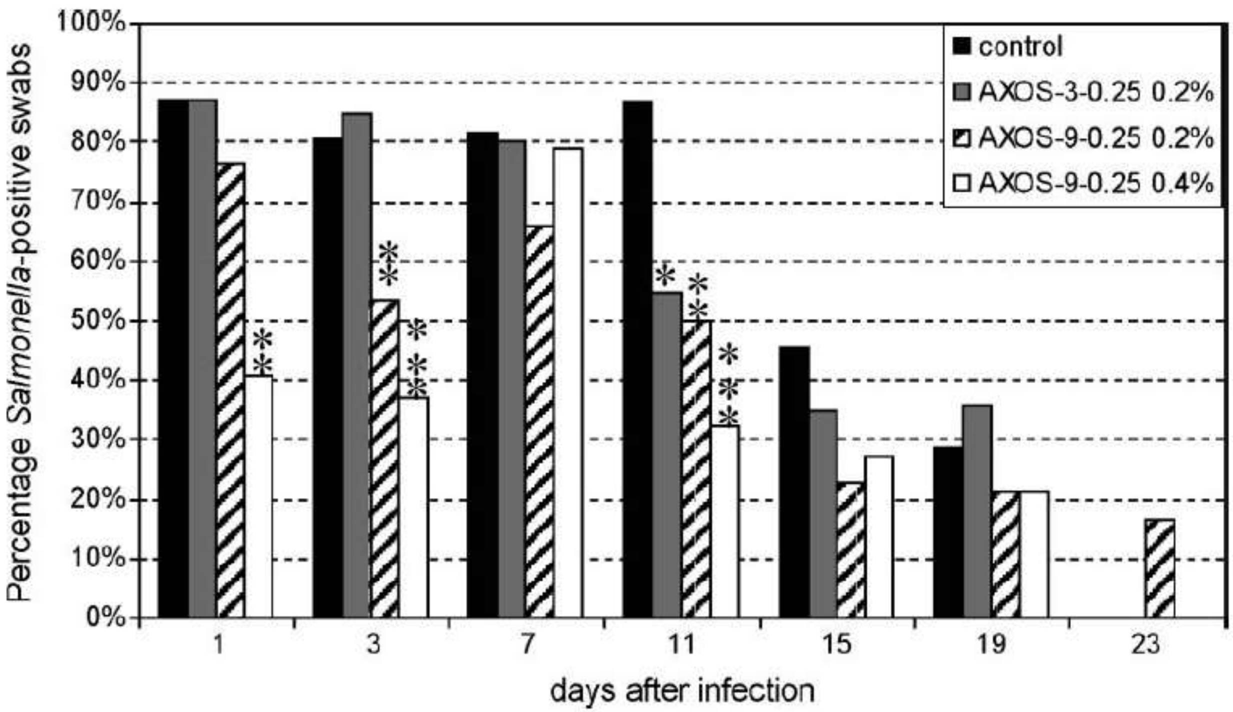
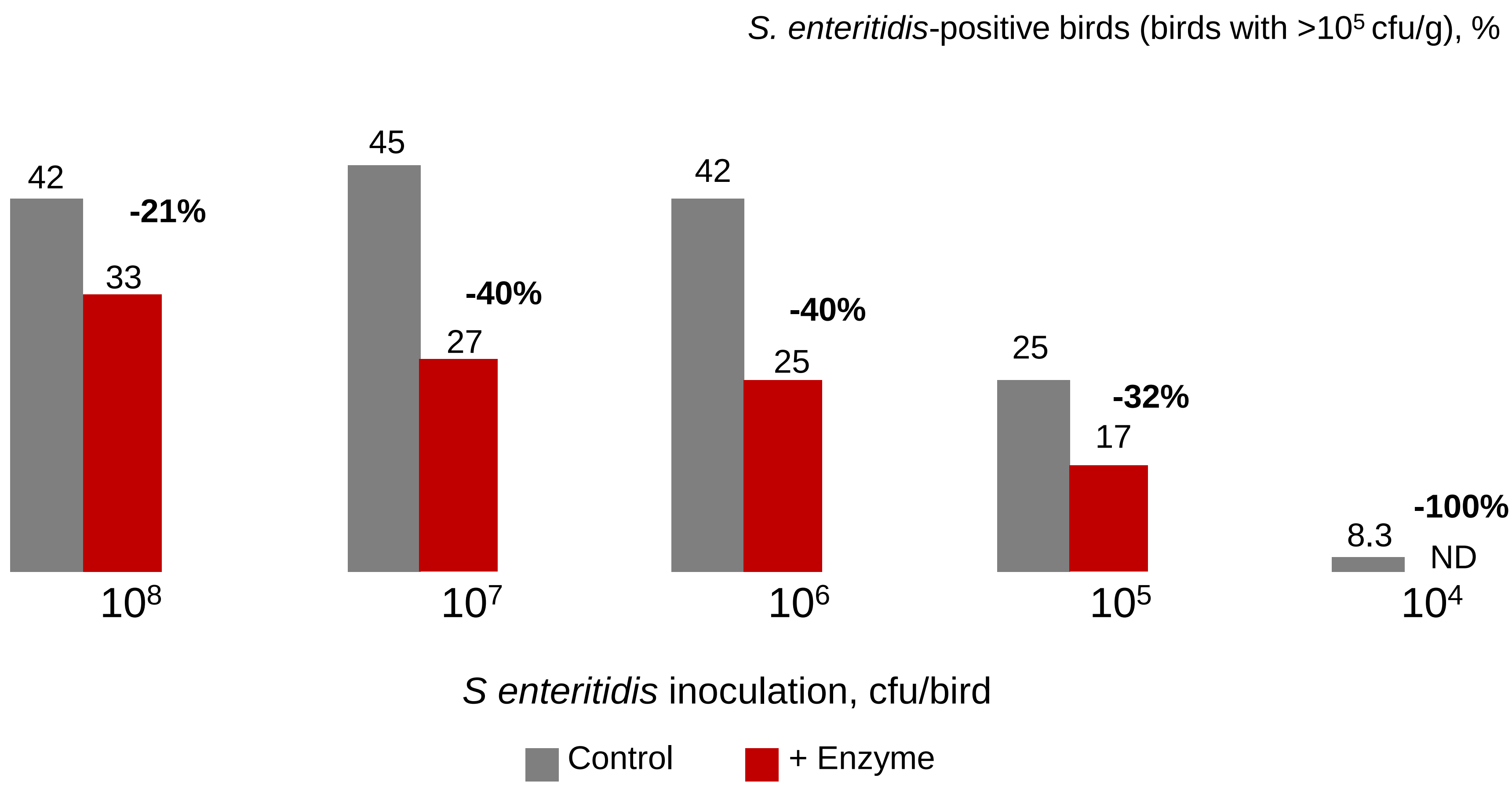


Figure 1. Effects of 0.2% arabinoxylooligosaccharide (AXOS)-3-0.25, 0.2% AXOS-9-0.25, or 0.4% AXOS-9-0.25 in the diet on the percentage of *Salmonella*-positive cloacal swabs at different times after inoculation with *Salmonella* Enteritidis, measured after direct plating of the swabs. For a given time, 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

Enzymes and Salmonella challenge



Enzyme source: combination XAP

Study done at Bristol University, UK

Phytase and gut microbiota?

Phytase:

Increase: Digestibility of Ca, P and minerals

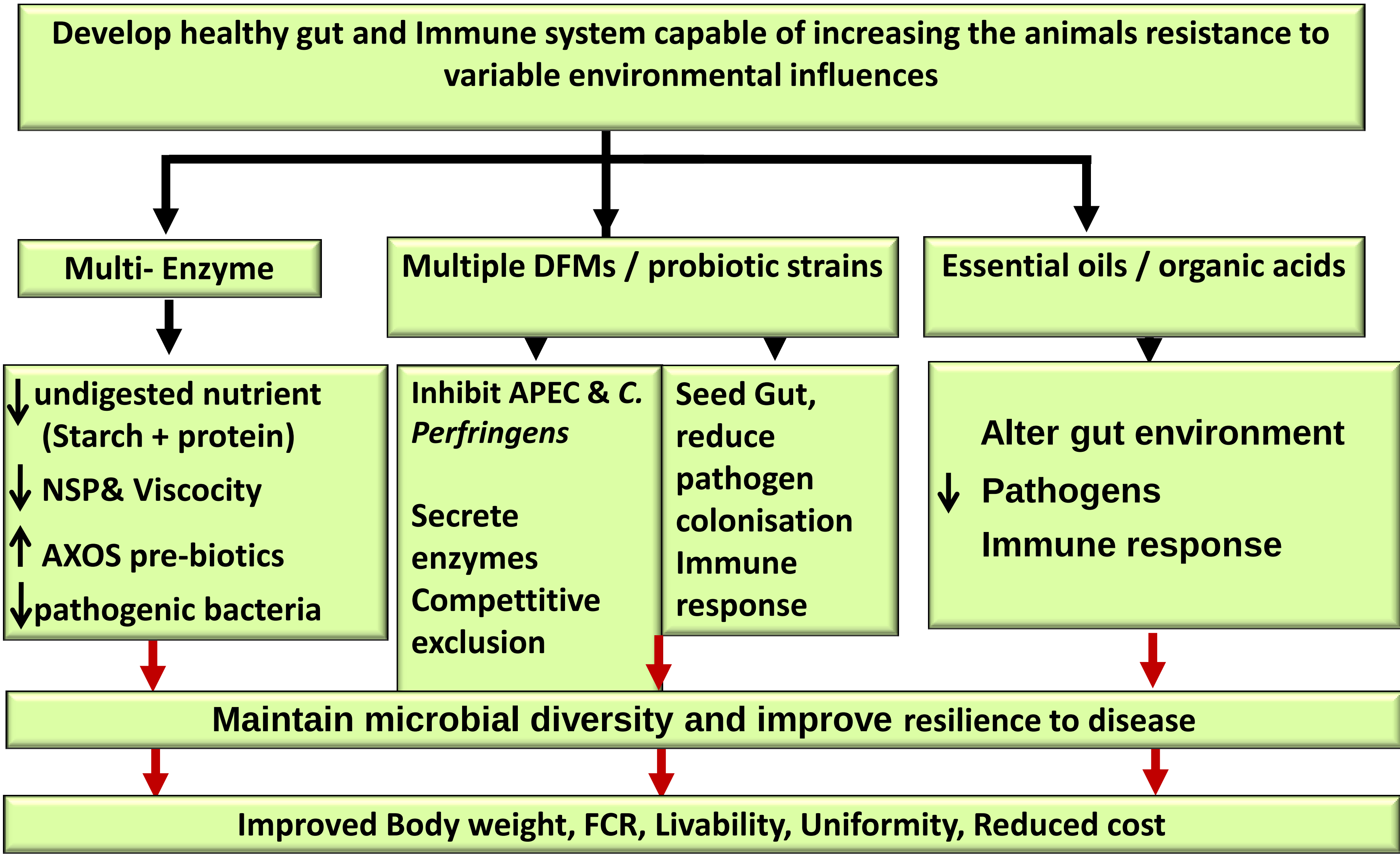
Reduce: Intestinal mucin production and endogenous losses

All influence nutrient supply and the intestinal environment which will alter the selection pressures on bacterial species.

Adeola and Cowieson, 2011 JAS 89: 3189

- Increased ileal numbers of the strict **anaerobes clusters of Clostridium** group without changing total bacterial numbers (Metzler-Zebeli et al., 2010; J. Anim. Sci. 88, 147–)
- Altered gut microbiota in piglets; pronounced effects in the small intestines bolstering ***Bifidobacteria, Clostridium numbers*** *(Wang and Lei, 2011.* J. Anim. Sci. 89 (E-Suppl.1):187.
- Increased ceca **acetate** in broilers *(*Smulikowska et al., 2010; J. Anim. Physiol. Anim. Nutr. 94:15)

Nutrition in a Post-AGP Era requires a multi-factorial approach



Direct Fed Microbials (DFM's) / Probiotics

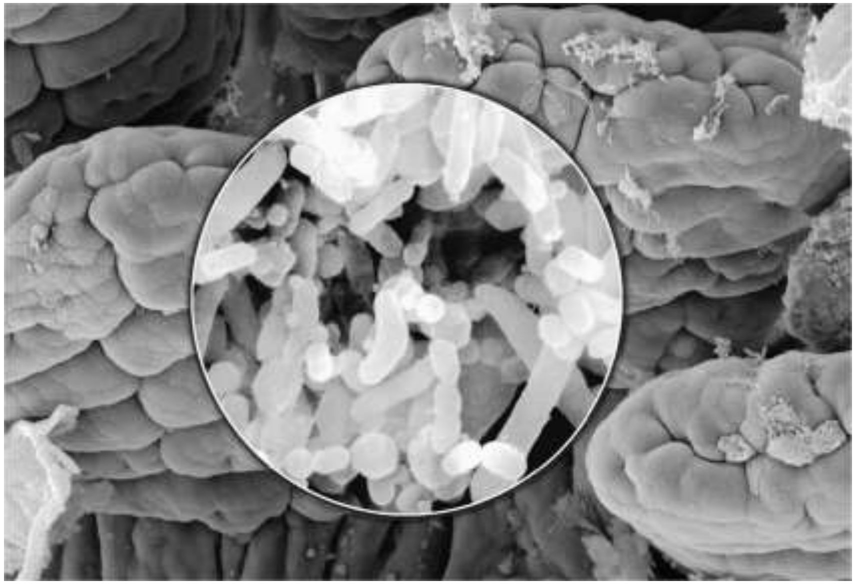
Immune mediators, organic acids, growth factors....

- substances produced during bacterial metabolism that influence digestion and other biological functions.

Bacteriocins (AAFCO 36.14) ...

- "A peptide produced by some strains of bacteria which inhibits the growth of, or kills, other bacteria."

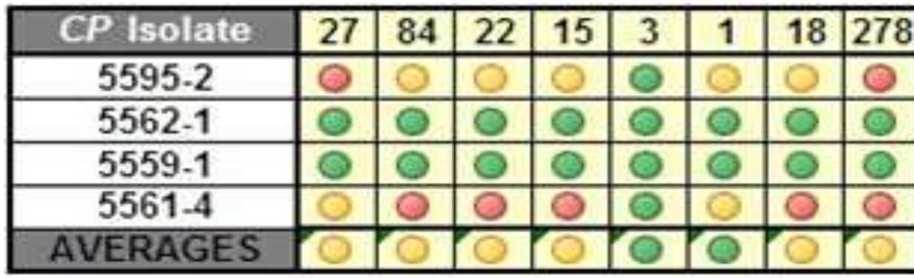
- Saunders Comprehensive Veterinary Dictionary, 3 ed. © 2007

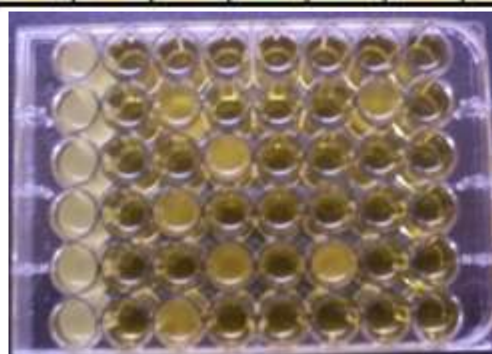


Potential mode of action of probiotics/DFMs in animal nutrition

1. Specific strains of Bacillus are able to inhibit APEC and CP

- Bacteriocin producing strains:





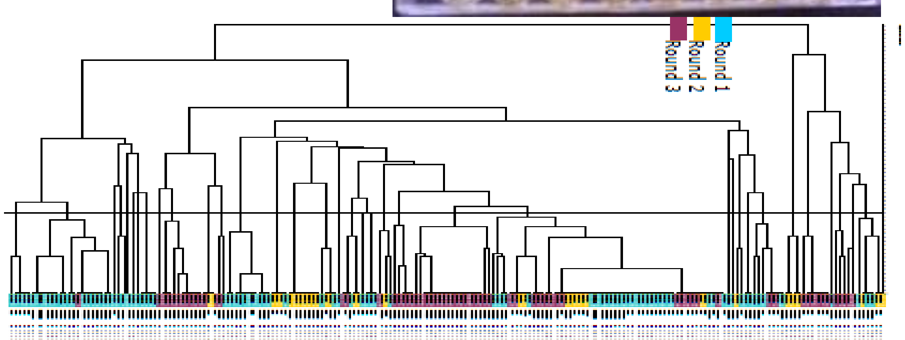
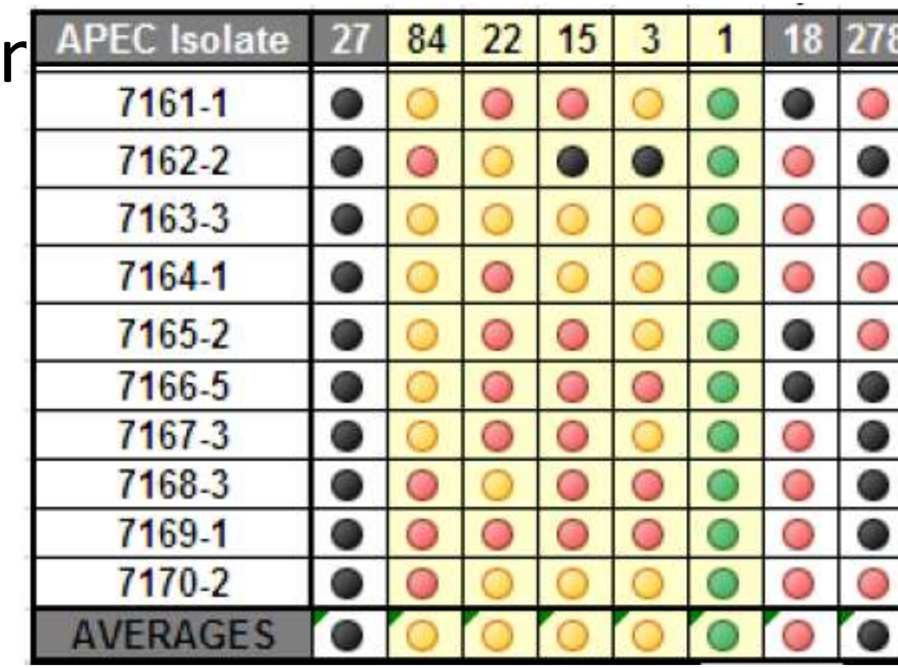
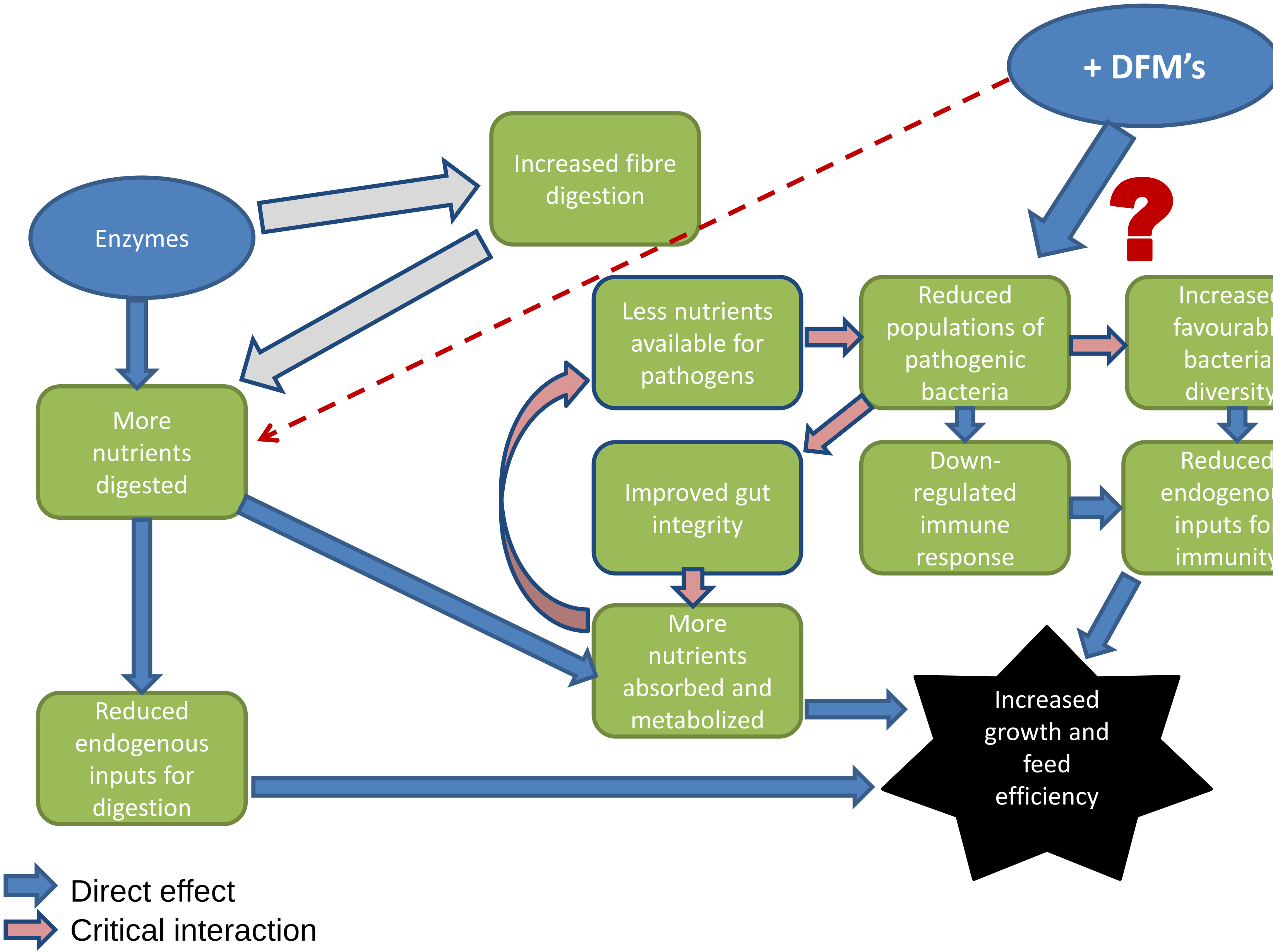


Figure 2. Diversity of Avain Pathogenic E. coli (APEC)



DuPont data

What happens when we add DFM's to Enzymes?



Example of what a Multiple solution can achieve

- Necrotic Enteritis challenge model
  - Coccivac at 0 d
  - Reused litter
  - Finely ground corn
  - A field strain of *C. Perfringens* in feed at 19, 20 and 21 d
- Mild mortality (~10-15%)
- Mortality, lesion scores, performance
- Samples for microbial profiling, microarrays
- Corn/SBM/DDGS based diets, 500 u/kg of phytase

Taken from Australia HUB

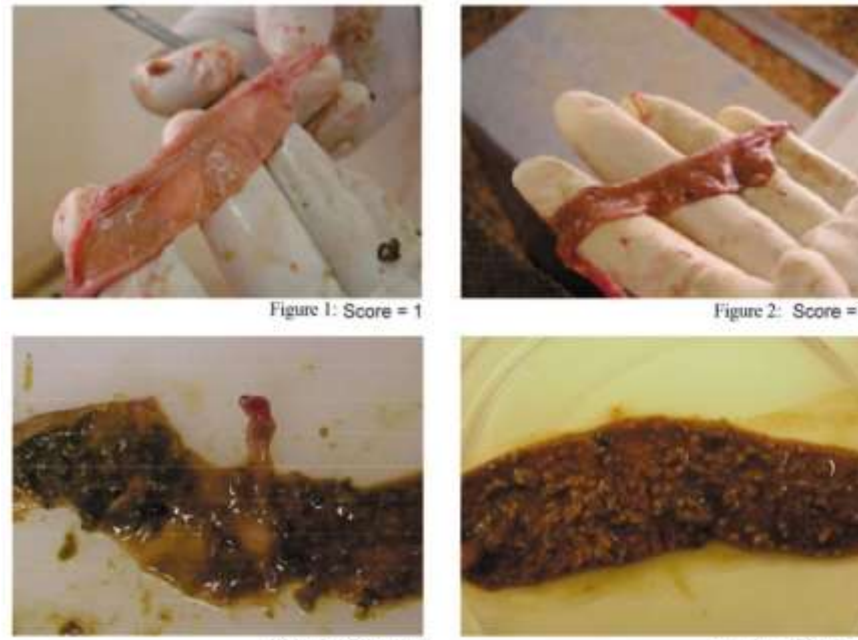


Figure 1: Score = 1
Figure 2: Score = 2
Figure 3: Score = 3
Figure 4: Score = 4

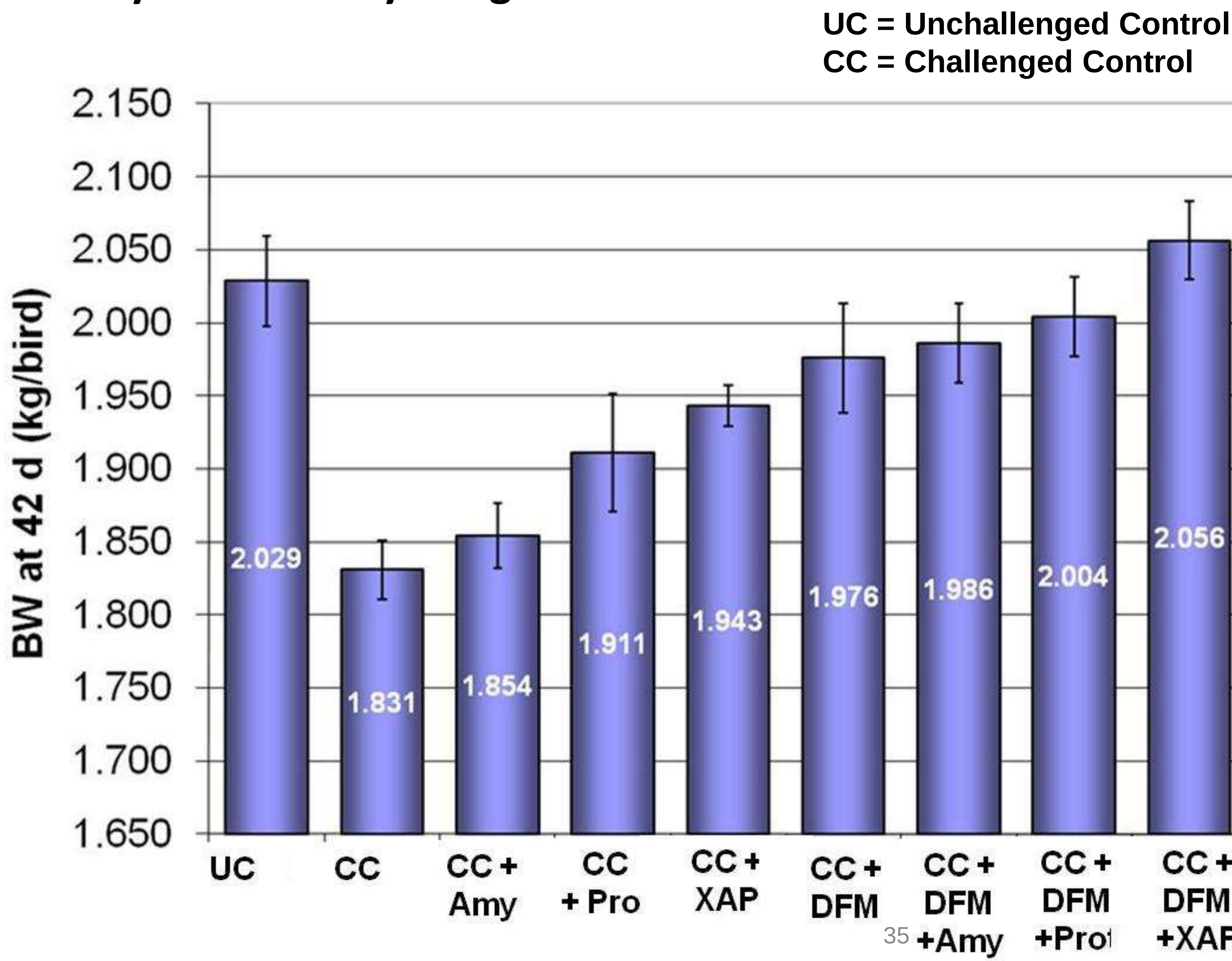
Southern Poultry system:
0 = healthy
1 = thin-walled or friable
2 = focal necrosis or ulceration
3 = large patches of necrosis

Treatments

1. Negative Control – Unchallenged, untreated control
2. Positive Control – Challenged, untreated control
3. LAT - Amylase
4. P3000 - Protease
5. XAP Enzyme
6. DFM (3-strain bacillus)
7. DFM+ LAT Amylase
8. DFM + P3000 protease
9. DFM + XAP enzyme

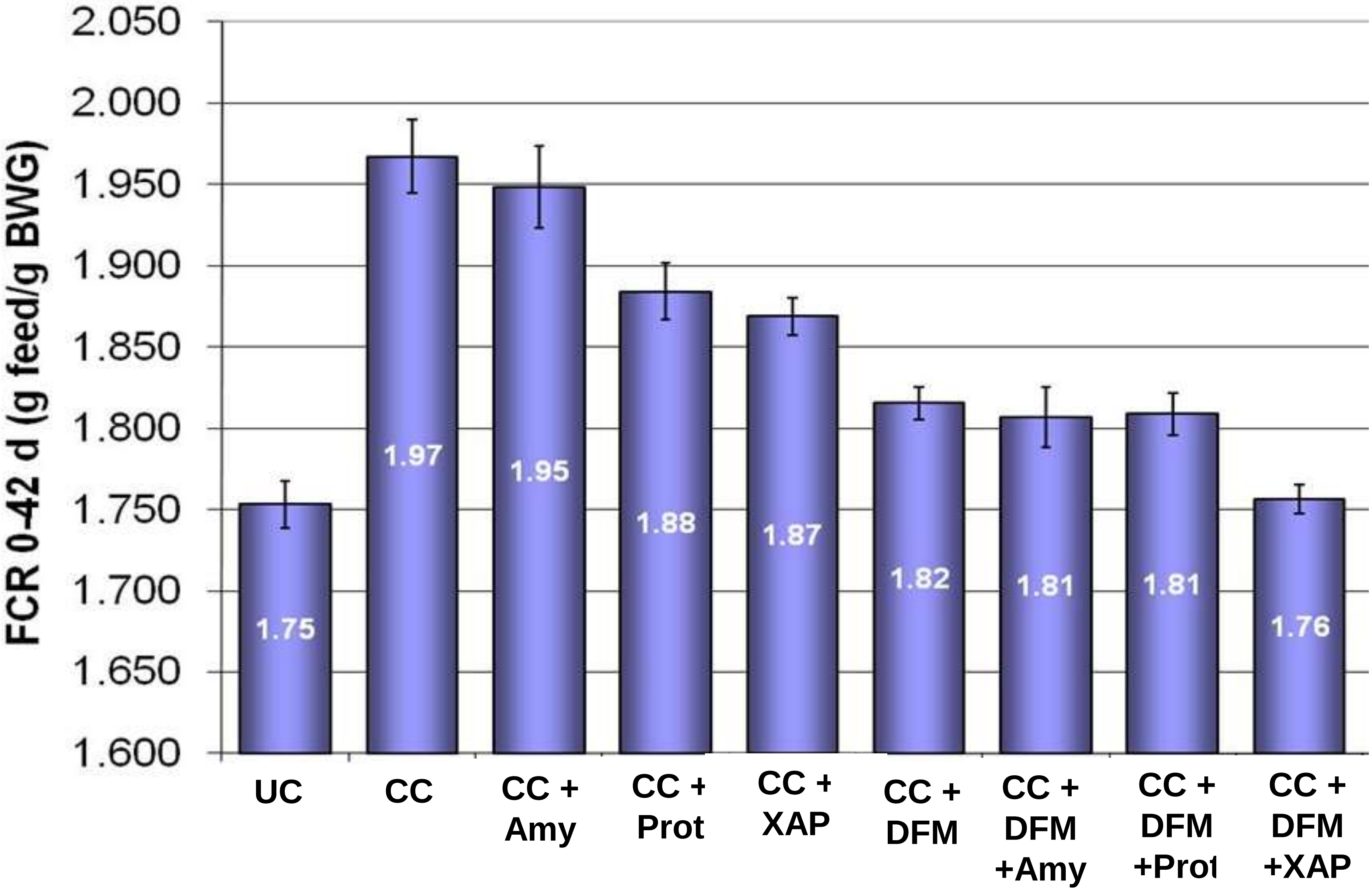
Corn-Soy diets with some animal protein

42-day Broiler Body Weight



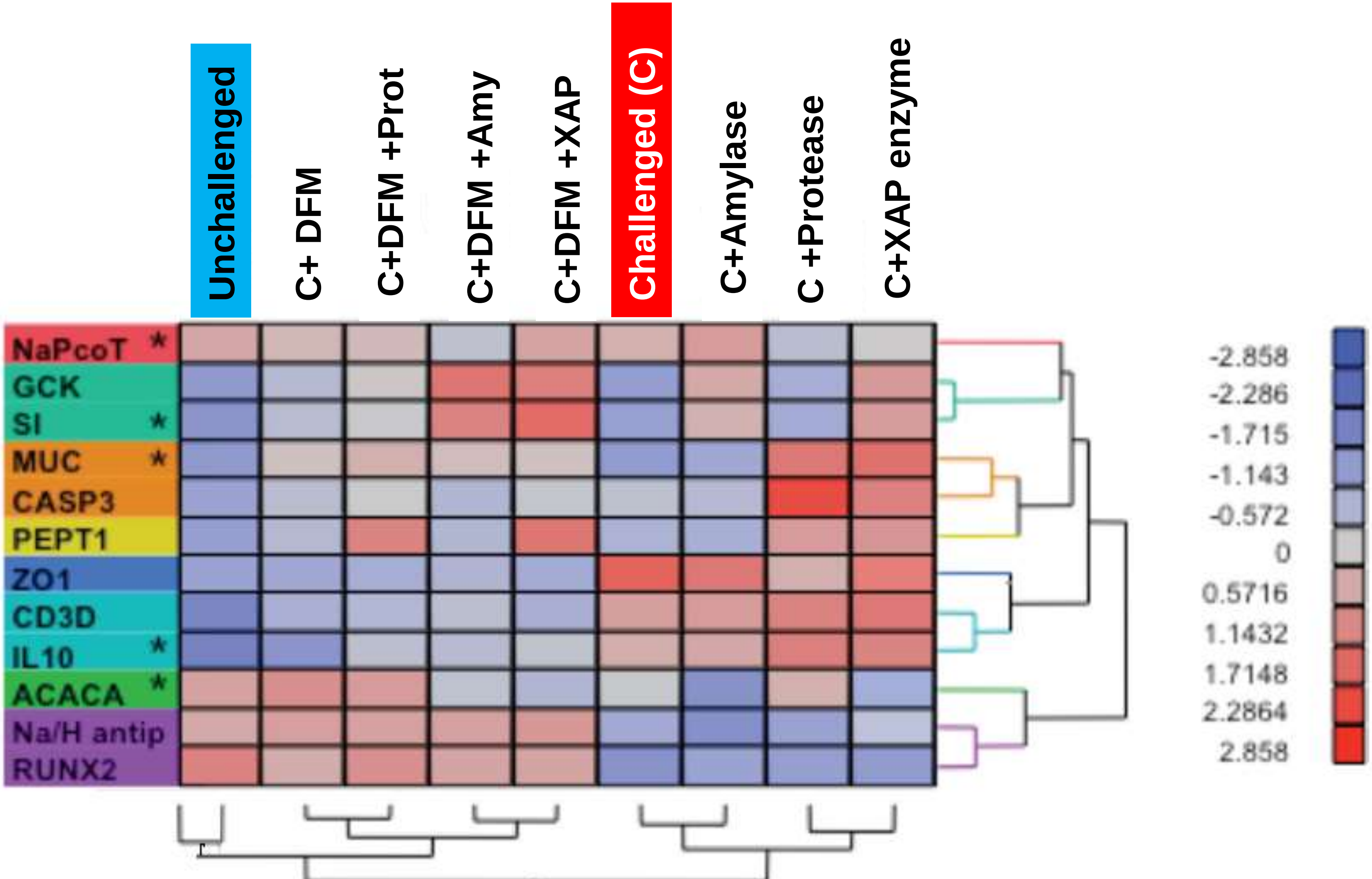
42-day Broiler FCR

UC = Unchallenged Control
CC = Challenged Control



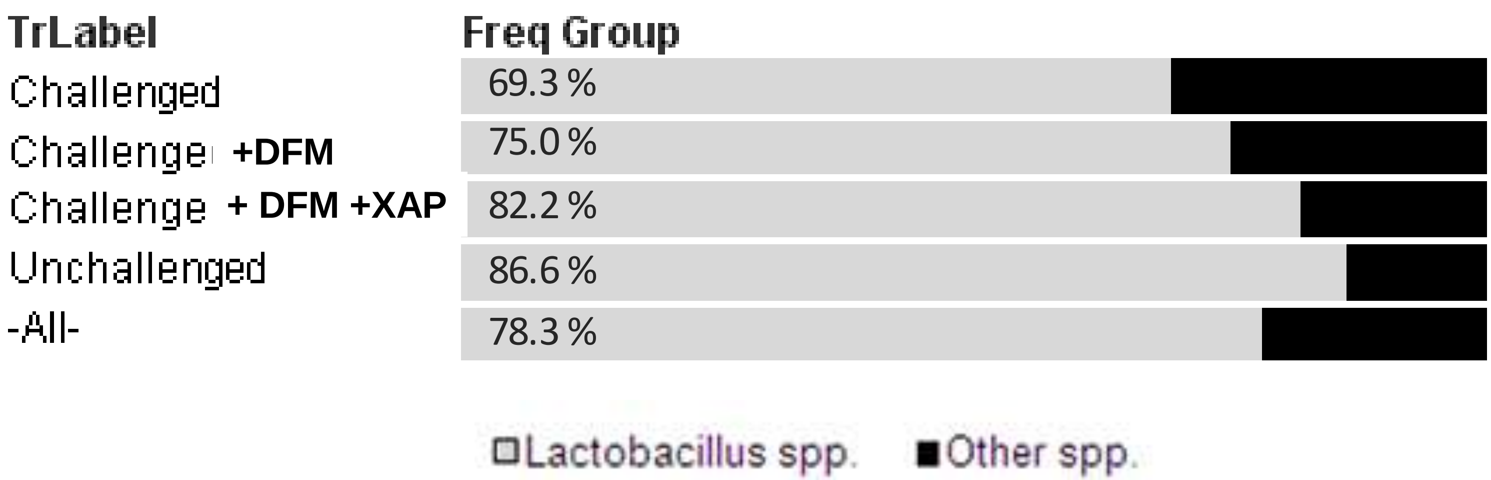
Why do we get the added Performance from Combining Enzymes+DFMs?

Gene expression of ileal mucosa



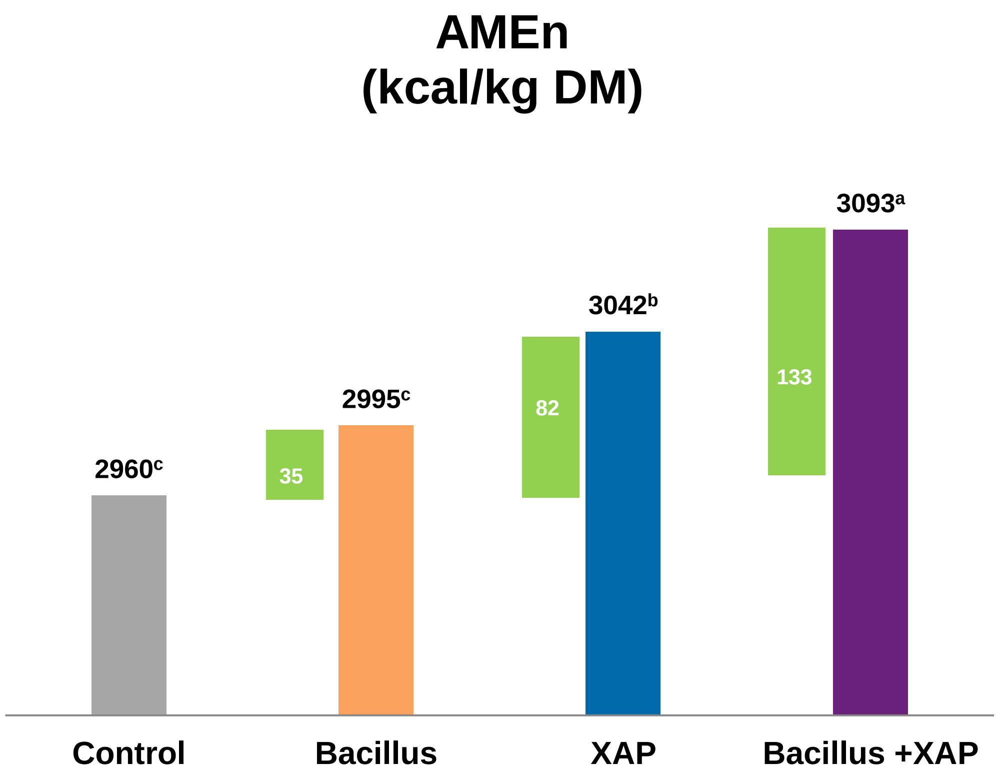
Gene		
NaPcoT	Na-Pi cotransported IIb	Rate limiting transporter for Pi
GCK	Glucokinase	Initial step in glucose metabolism
SI	Sucrase isomaltase	Galactose metabolism
MUC	Mucin	Intestinal integrity/protection
CASP3	Caspase 3	Apoptosis
PEPT1	Oligopeptide transporter 1	Nutrient transport
ZO1	Tight unction protein 1	Tight junction formation, intestinal integrity
CD3C	T-cell antigen CD3	T-cell marker
IL10	Interlukin 10	Infalmmation
ACACA	acetyl-CoA carboxylase A	Fatty acid biosynthesis
Na/H antip	Na/H antiporter	pH regulation
PEPT1	Oligopeptide transporter 1	Nutrient transport

Changing undigested nutrients alters proportion of *Lactobacillus* in 21 d broilers, after challenge



Relative proportion of *Lactobacillus* spp. at 21d in jejunum in broiler chickens.
ChiSq. <0.0001

Enzymes also show synergy with other DFMs on AMEn

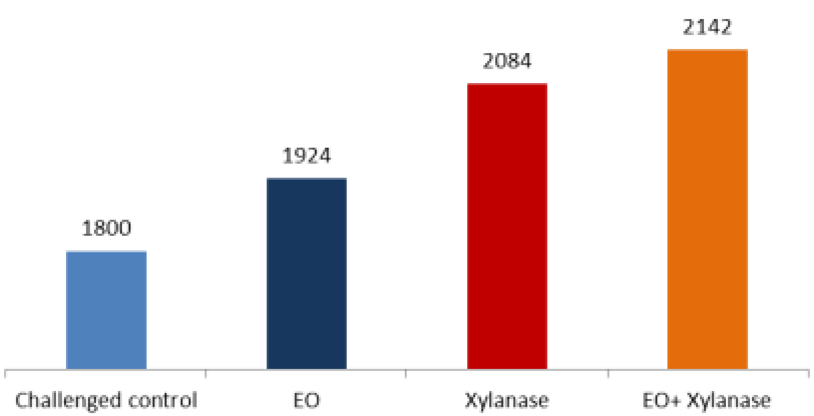


Animal: Broilers
Enzyme: XAP; Probiotic: 3 Bacillus strains

Romero *et al.*, 2013

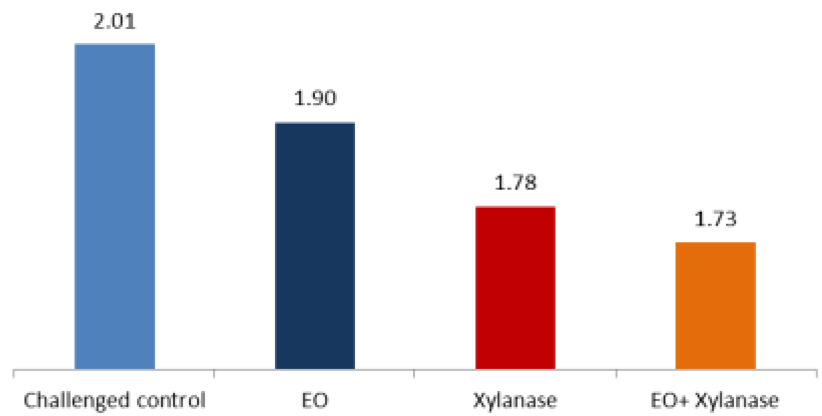
Enzymes show synergy with other feed additives-Essential Oils

Weight gain (g)

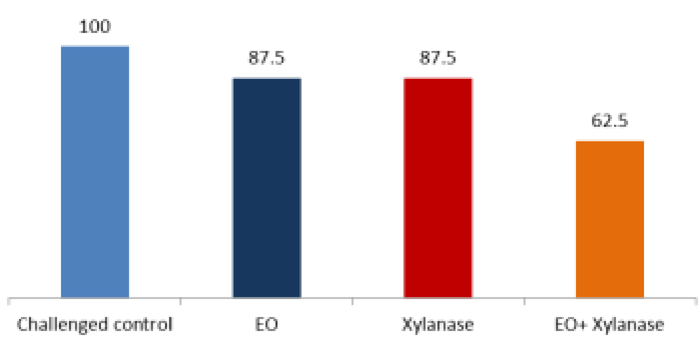


FCR (g/g)

P <0.05



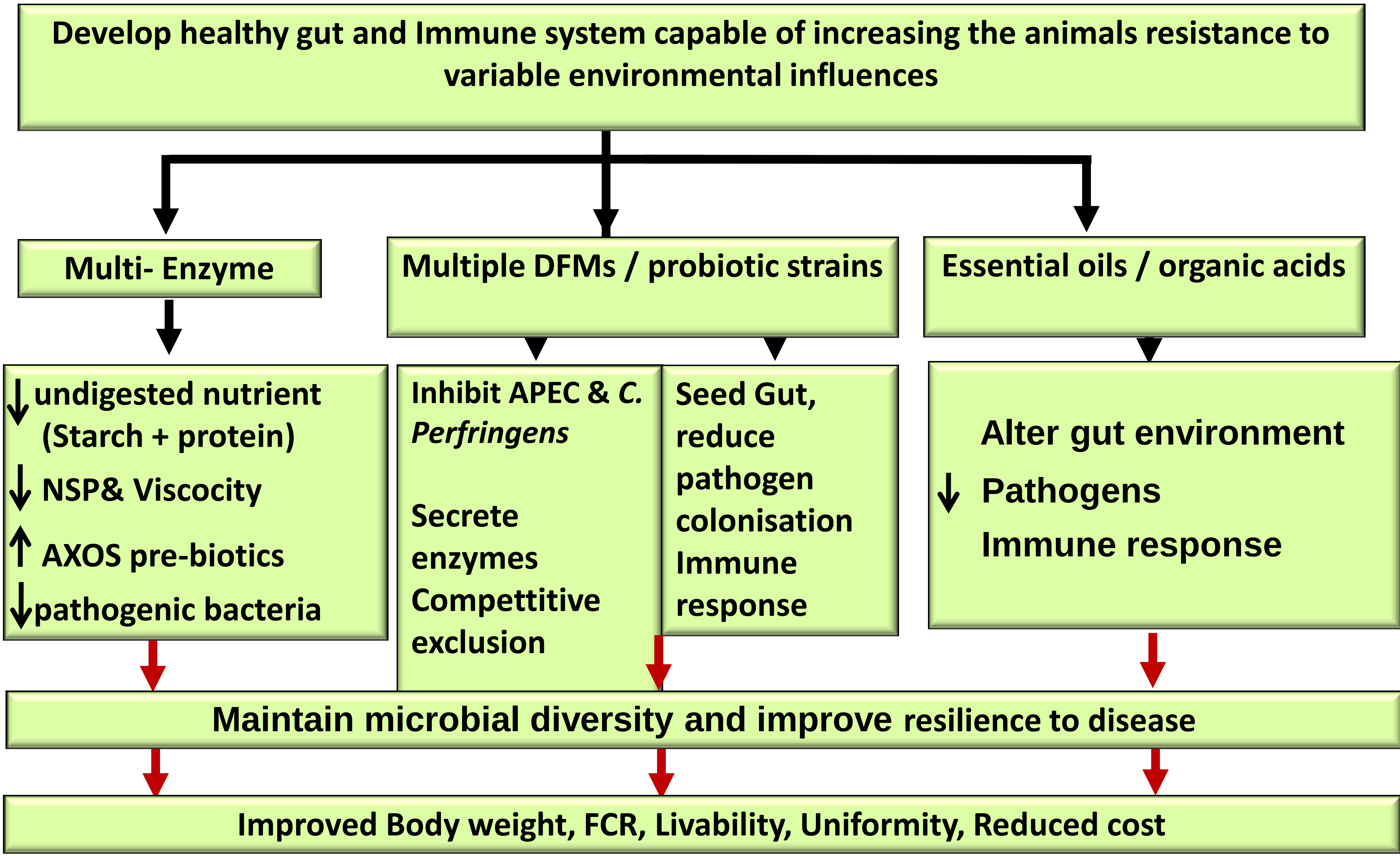
Positive drag swab samples D14 (%)



Amerah *et al*., 2012

Challenge: Salmonella Heidelberg d1

An optimum solution to replace AGP's requires a multi-factorial approach



Thanks to:

Elijah Kiarie
Luis Romero
Ahmed Amerah

Thank you for your attention