Advances in the use of enzymes and probiotics in poultry nutrition

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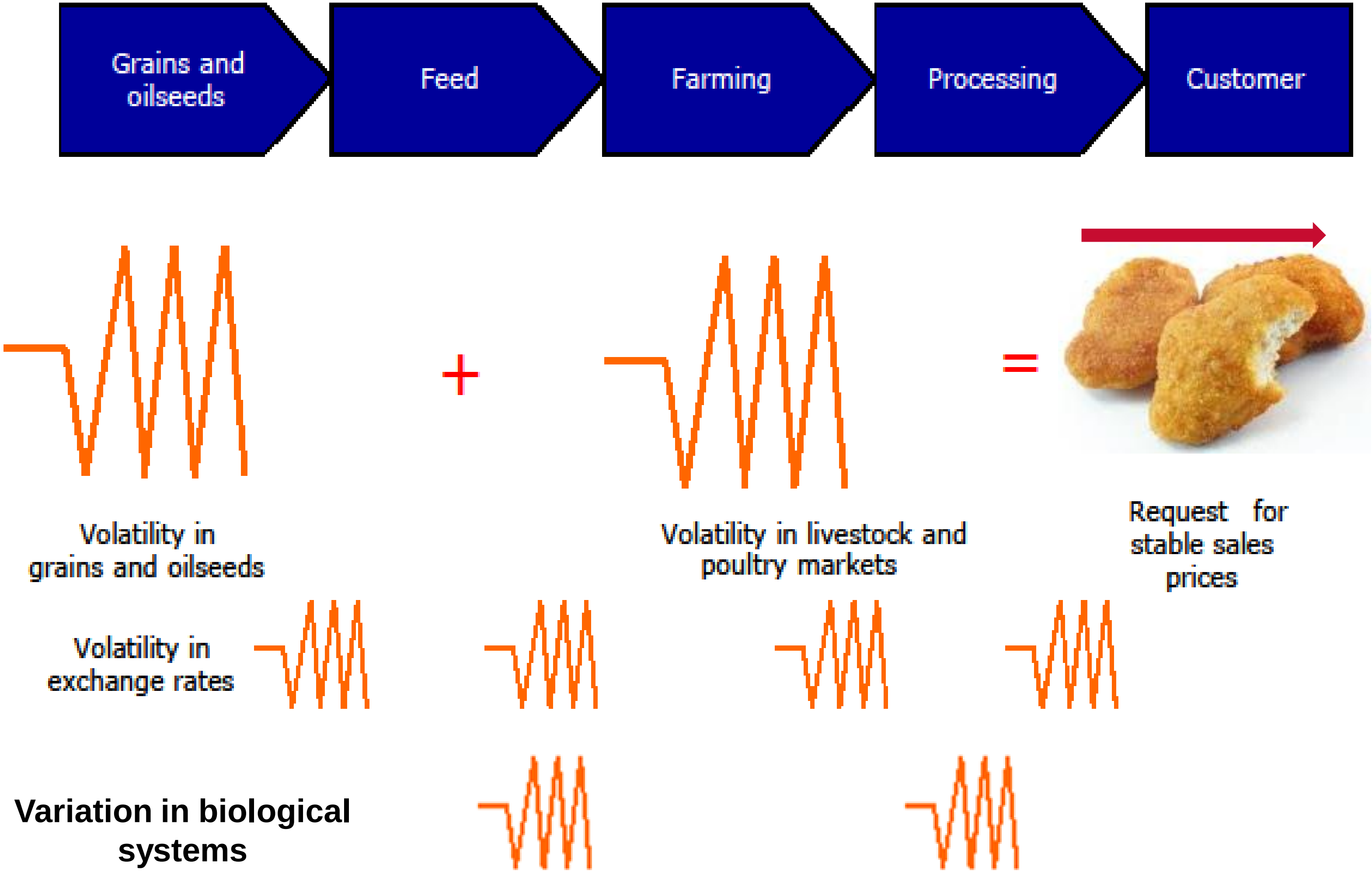
Outline

- Variability in poultry production
- The role of enzymes and DFMs
- Recent studies:
    - Nutrient digestibility
    - Animal performance
    - Enteric challenge
- Mechanisms of action

Dealing with volatility is a key factor determining the success (survival) of companies in animal agriculture



Grains and oilseeds

Feed

Farming

Processing

Customer

+

=

Volatility in grains and oilseeds

Volatility in livestock and poultry markets

Request for stable sales prices

Volatility in exchange rates

Variation in biological systems

Ingredient Nutrient digestibility

Poultry performance

Market intelligence

Information exchange

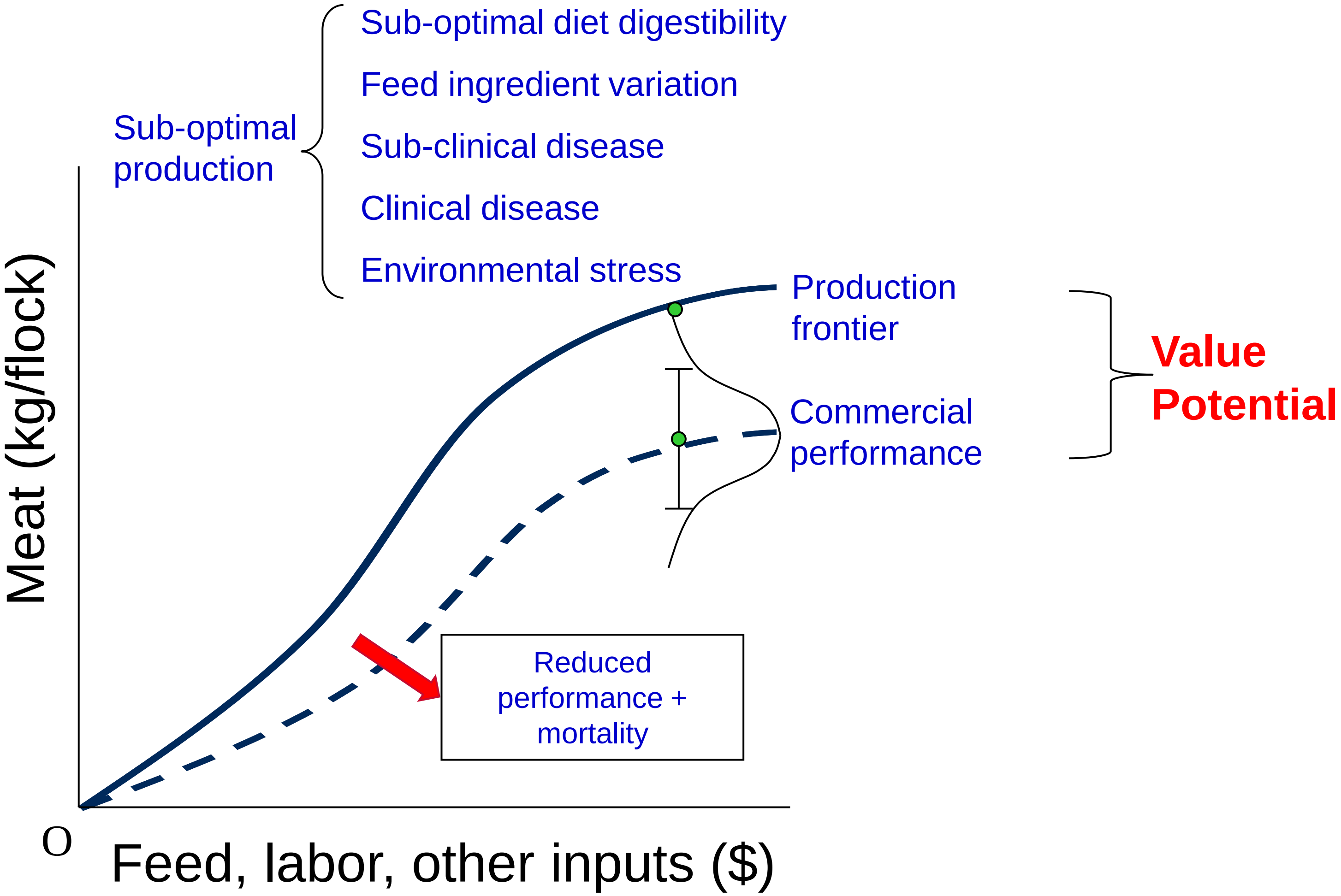
Market power

Hedging/price management

Risk management

Rabobank, 2011

There is a gap between genetic potential and commercial performance in poultry



Sub-optimal production

- Sub-optimal diet digestibility
- Feed ingredient variation
- Sub-clinical disease
- Clinical disease
- Environmental stress

Production frontier

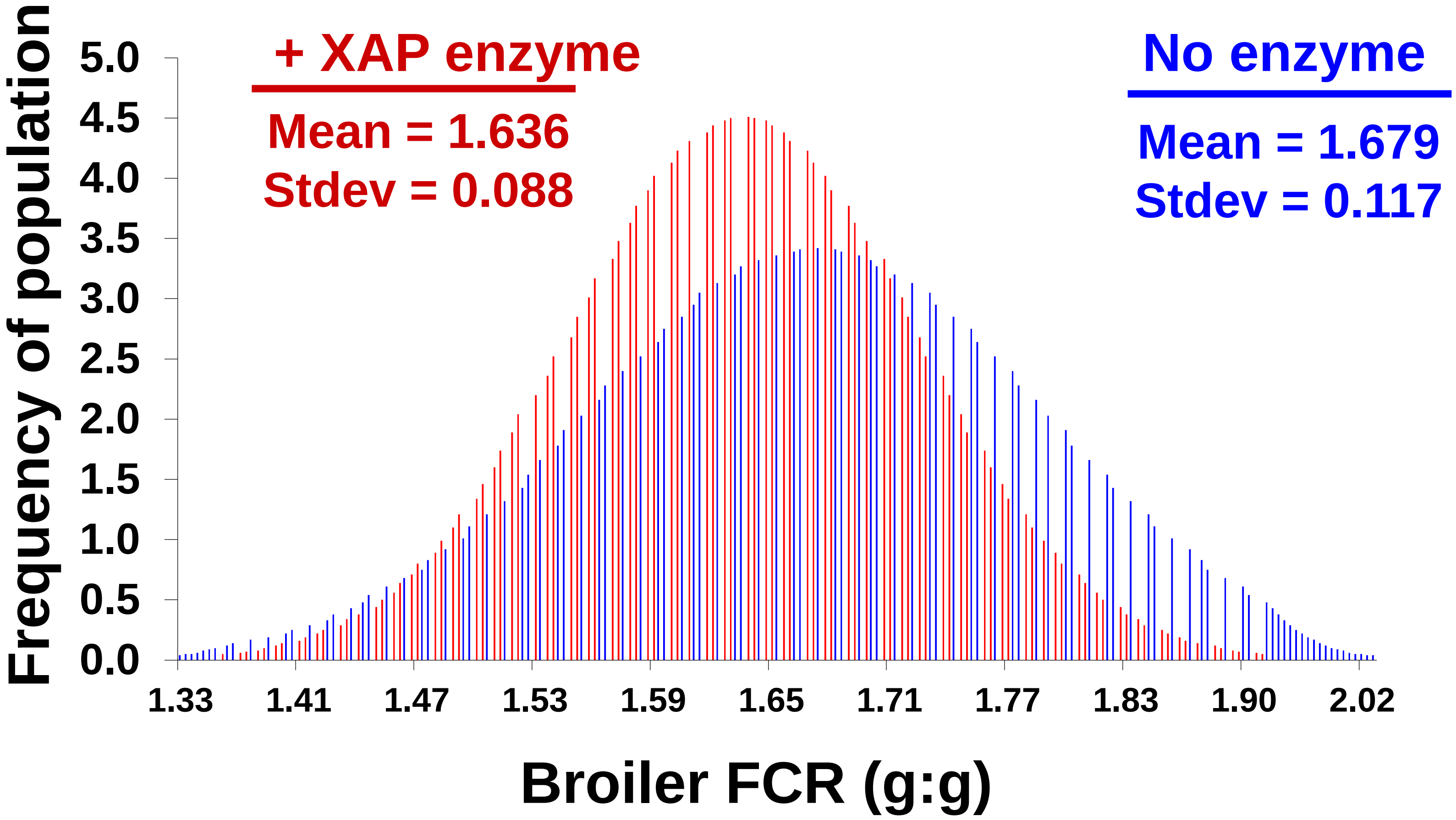
Commercial performance

Value Potential

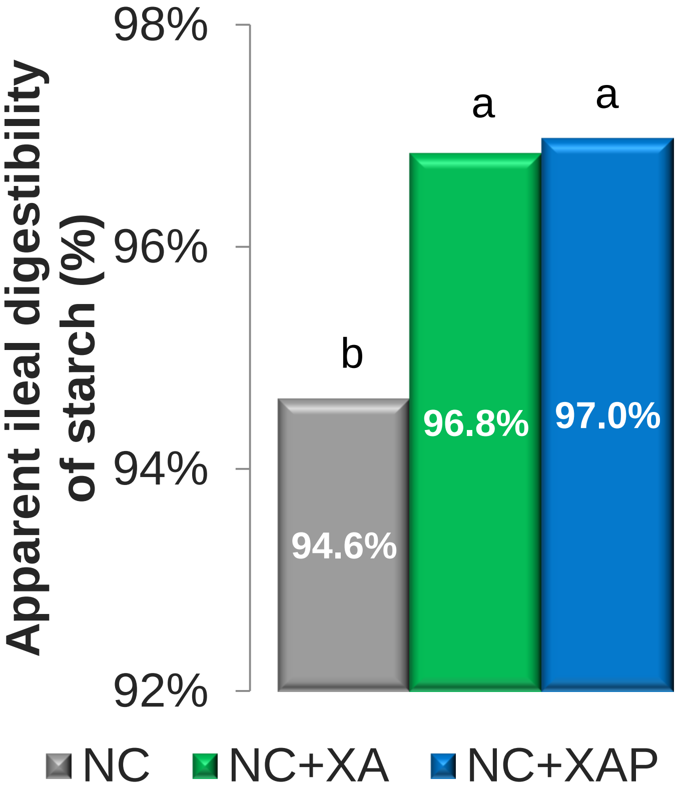
Reduced performance + mortality

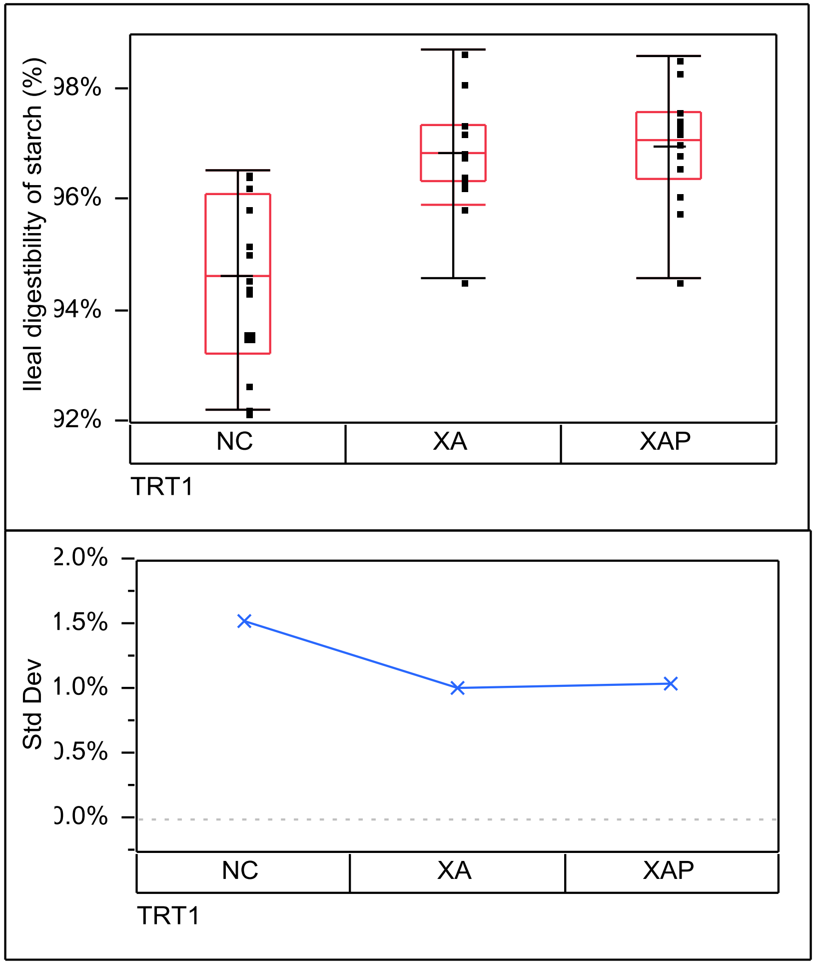
THE ROLE OF ENZYMES AND DFMs IN POULTRY NUTRITION

Enzyme addition to diets with 26 different corn sources reduced the variation in broiler FCR and improved the mean response

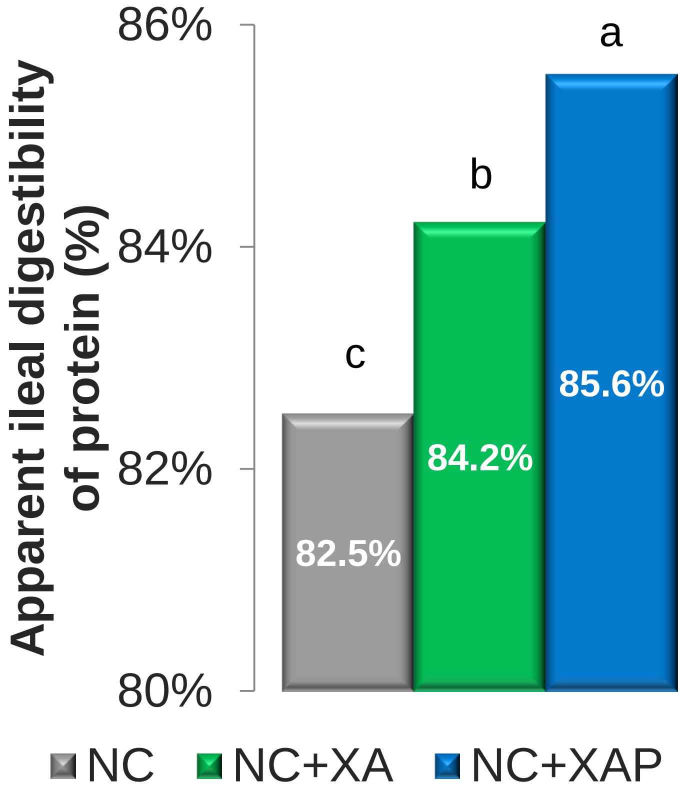


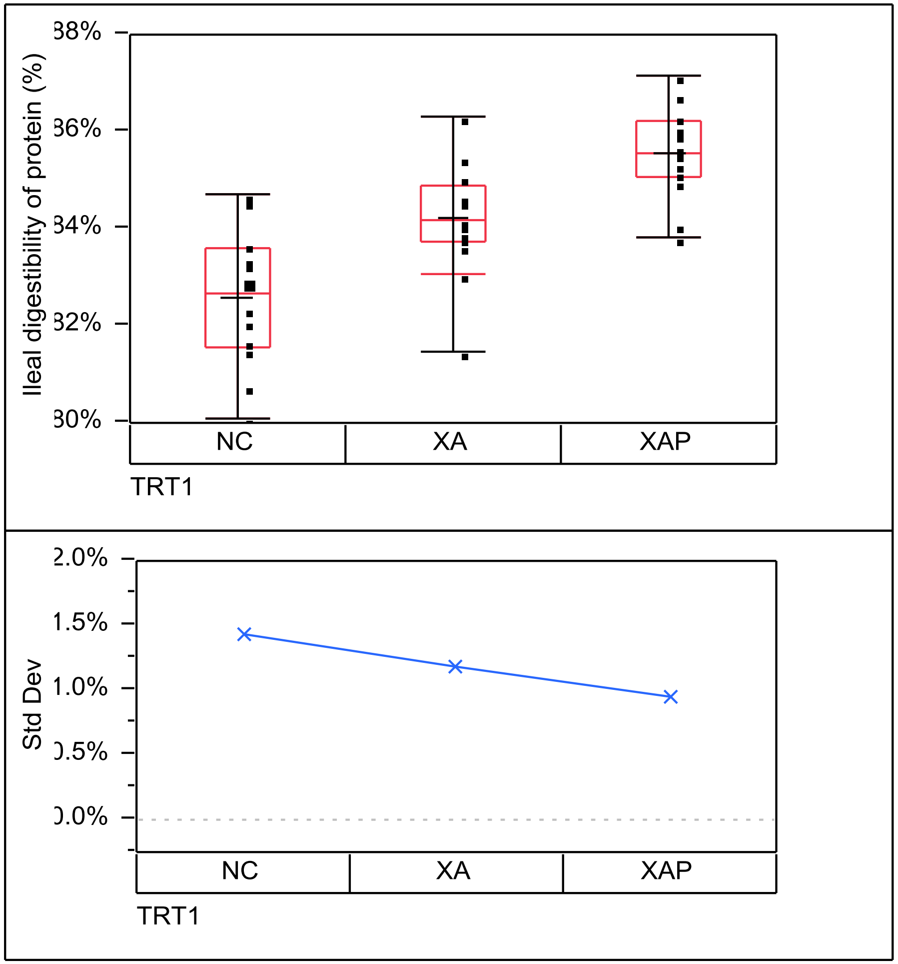
In 13 broiler trials, ileal digestibility of starch was increased by Xylanase + Amylase and Protease enzymes





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



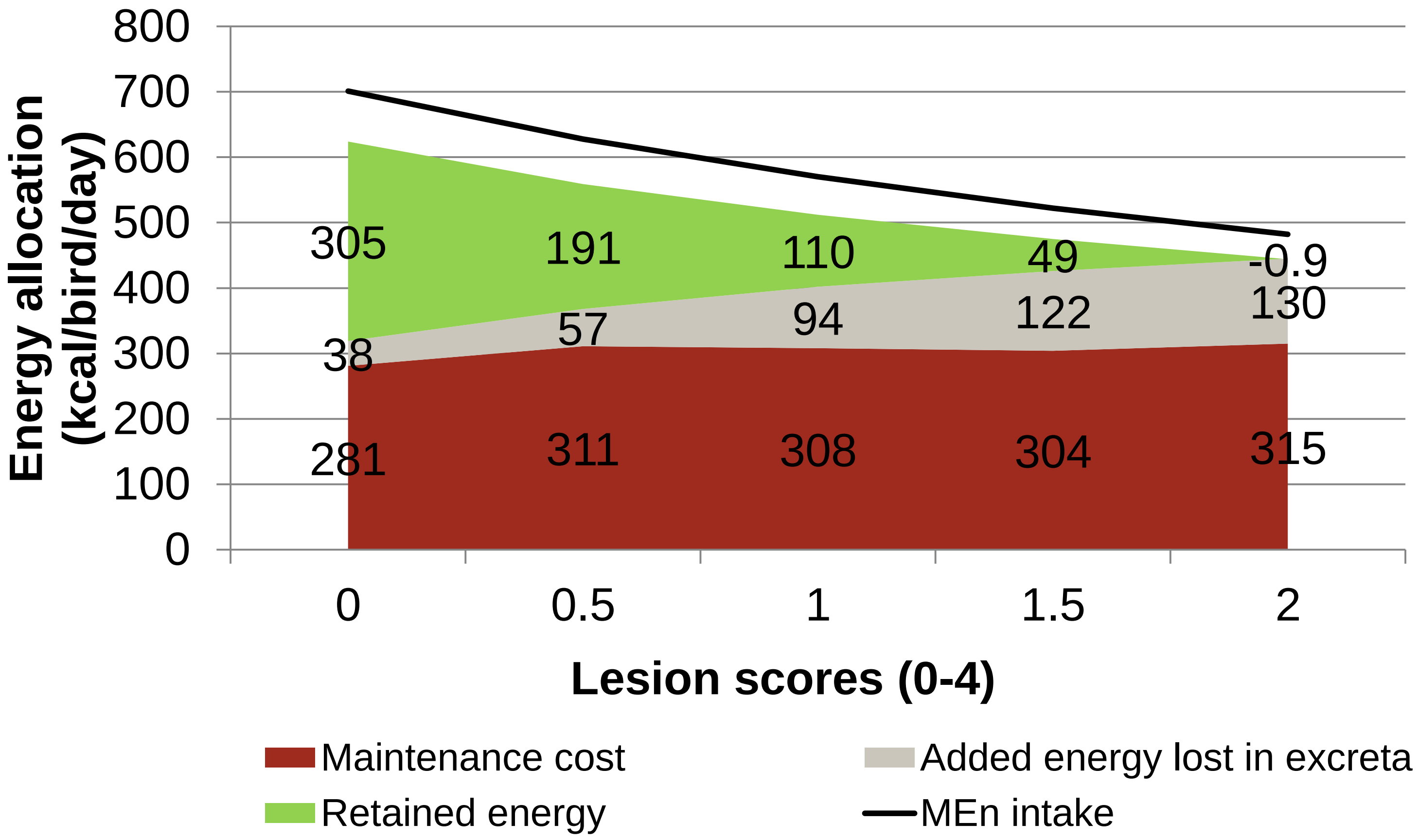


Enzymes might affect gut health through changes in the available substrate and direct effect in the mucosa

- Xylanases have been shown to have pre-biotic effects in poultry (Fernandez, 2000) and other species through selective stimulation of beneficial bacteria and production of short-chain fatty acids (SCFA) (Broekaert et al., 2011)
- Increased undigested protein appears to be a predisposing factor for dysbacteriosis related to necrotic enteritis (Dahiya et al., 2007)
- Protease has been shown to improve performance of chickens challenged with *Eimeria spp.* (Peek et al, 2009)

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

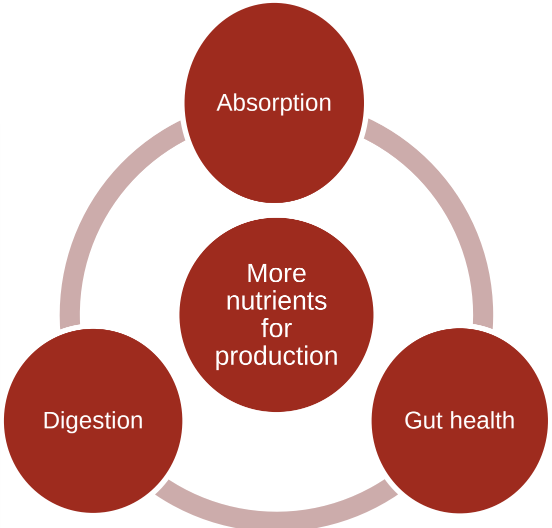
Energy partitioning of 42-48 d old broilers challenged with oocysts of three Eimeria species



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

Enzymes and DFMs in poultry nutrition

The advantage of enzymes	The advantage of DFMs
Hydrolyze substrate • Specific • Quick • pH dependent Activity can be standardized Functionality can be designed	Live organisms • Metabolism in-situ • Reproduce • Adapt to substrate in the gut Modulate microbial populations Modulate immunity



Absorption

More nutrients for production

Digestion

Gut health

TRIAL 1
DFMs AND ENZYMES
NUTRIENT DIGESTIBILITY

Materials and Methods (1/2)

384 Ross-308 broilers were placed in wired floor cages (8 birds, 6 treatments, 6 replicate cages/treatment)

Day –old chicks were administered a live *Eimeria* vaccine (Immucox, Pacificvet)

A 3x2 factorial arrangement was used

- Enzyme: 1) no enzyme, 2) xylanase from *T. reesei* (X; 2000 U/kg) and amylase from *B. licheniformis* (A; 200 U/kg), or 3) XA plus protease from *B. subtilis* (XAP; 5000 U/kg)
- DFM : 1) no DFM, or 2) a combination of spores from three defined strains of *B. subtilis* (DFM; 7.5 x 10^4 CFU/g)

500 FTU of *E. coli* phytase / kg feed was used in the background

Data were analysed with Proc Mixed (SAS) by age

Materials and Methods (2/2)

Four chickens per cage were selected at 11 d and 21 d for collection of ileal digesta samples

TiO_2 (0.30%) was used as inert marker

Digesta from the lower ileum were expressed by gentle flushing with distilled water, pooled by cage, frozen and lyophilised

- 11 d samples: IDE, protein
- 21 d samples: IDE, protein, starch, fat; NSPs, resistant oligo-saccharides (Englyst Carbohydrates, UK)

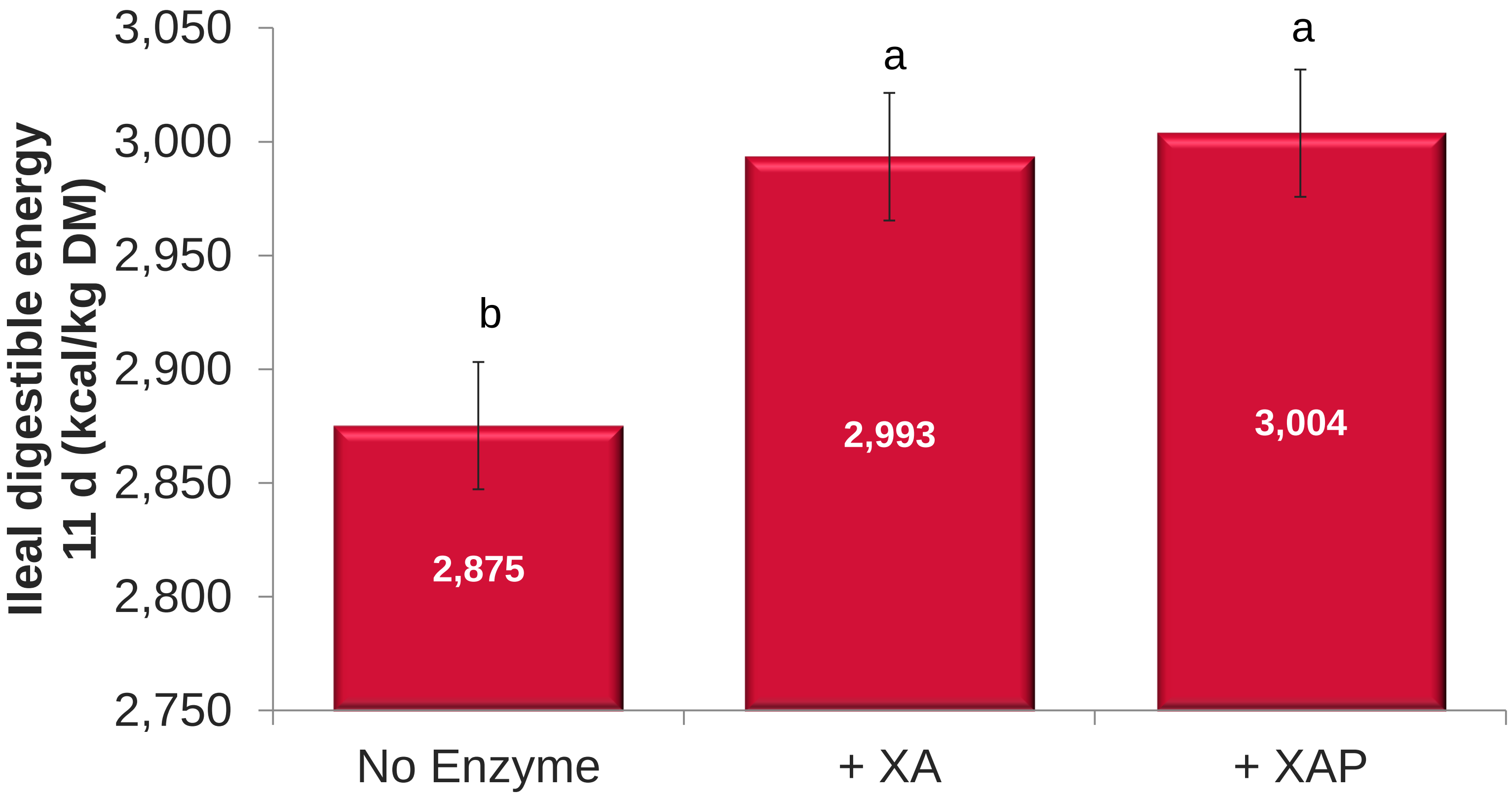
Feed intake and total excreta output were measured over four consecutive days (from day 17 to 20) for the determination of AME_n

Experimental diets

INGREDIENTS %	Starter (0-21 d)
Corn	46.22
Wheat Middlings	6.73
Corn DDGS	7.00
Soy Bean Meal 48%CP	32.81
DFM / Enzyme Premix	0.30
An/Veg Fat Blend	3.00
L-Lysine HCl	0.27
DL-methionine	0.30
L-threonine	0.11
Inert Marker (TiO_2)	0.30
Salt	0.34
Limestone	1.12
Dicalcium Phosphate	1.20
Poultry Premix	0.30

SPECIFICATIONS	Starter (0-21 d)
DM %	89.1
ME POULTRY (KCAL/KG)	2950
PROTEIN %	23.0
CRUDE FAT %	6.3
CALCIUM %	0.85
AVAILABLE P %	0.38
NA %	0.18
DLYS %	1.21
DMETH %	0.62
DM+C %	0.86
DTHREO %	0.76
DTRYP %	0.21
STARCH %	32.19
NSP %	11.46

Apparent ileal digestible energy at 11 d



SEM=28 kcal

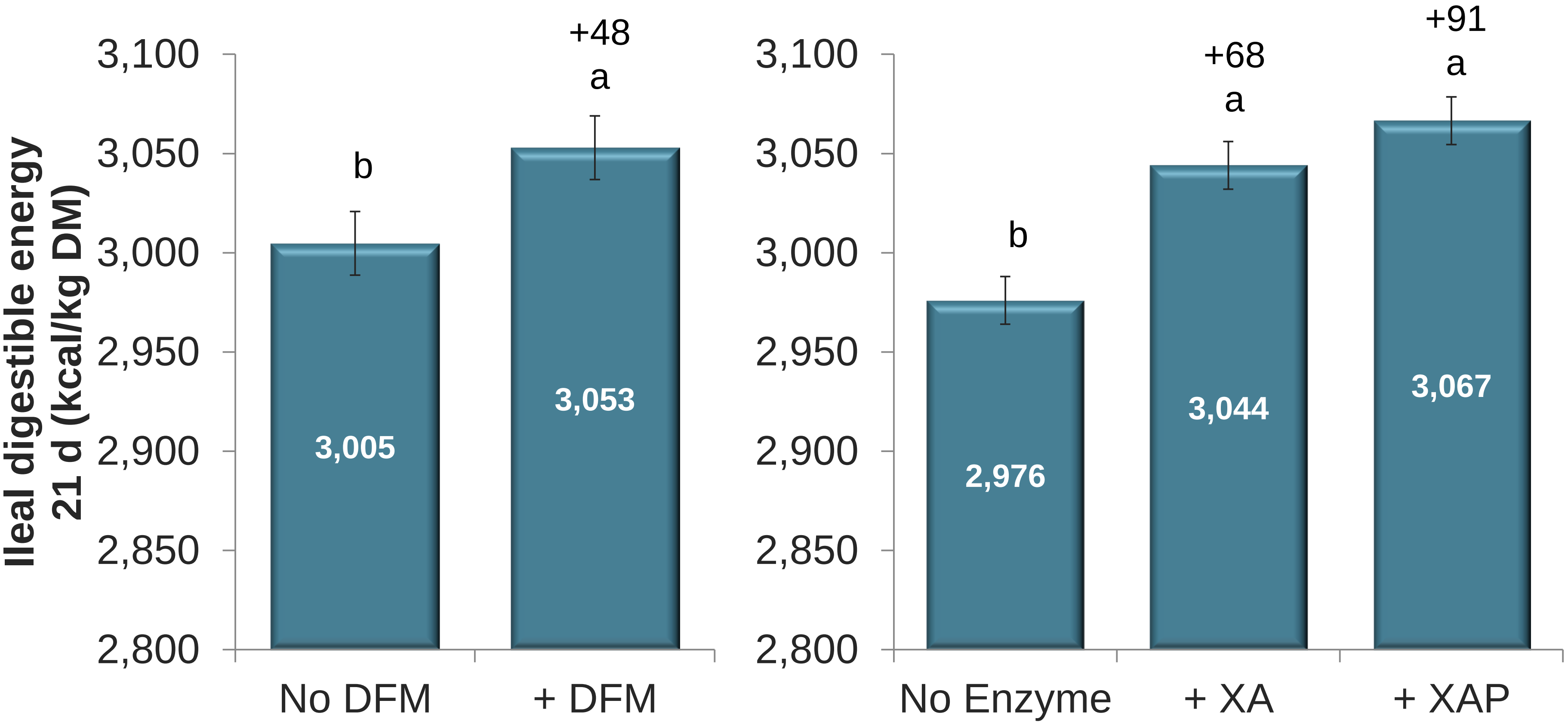
Means with different letters differ at *P*<0.05

500 FTU/kg of phytase was present in the background

XA=Xylanase + Amylase; XAP=XA + Protease

Enzyme	*P*=0.005
DFM	*P*=0.15
Enzyme x DFM	*P*=0.66

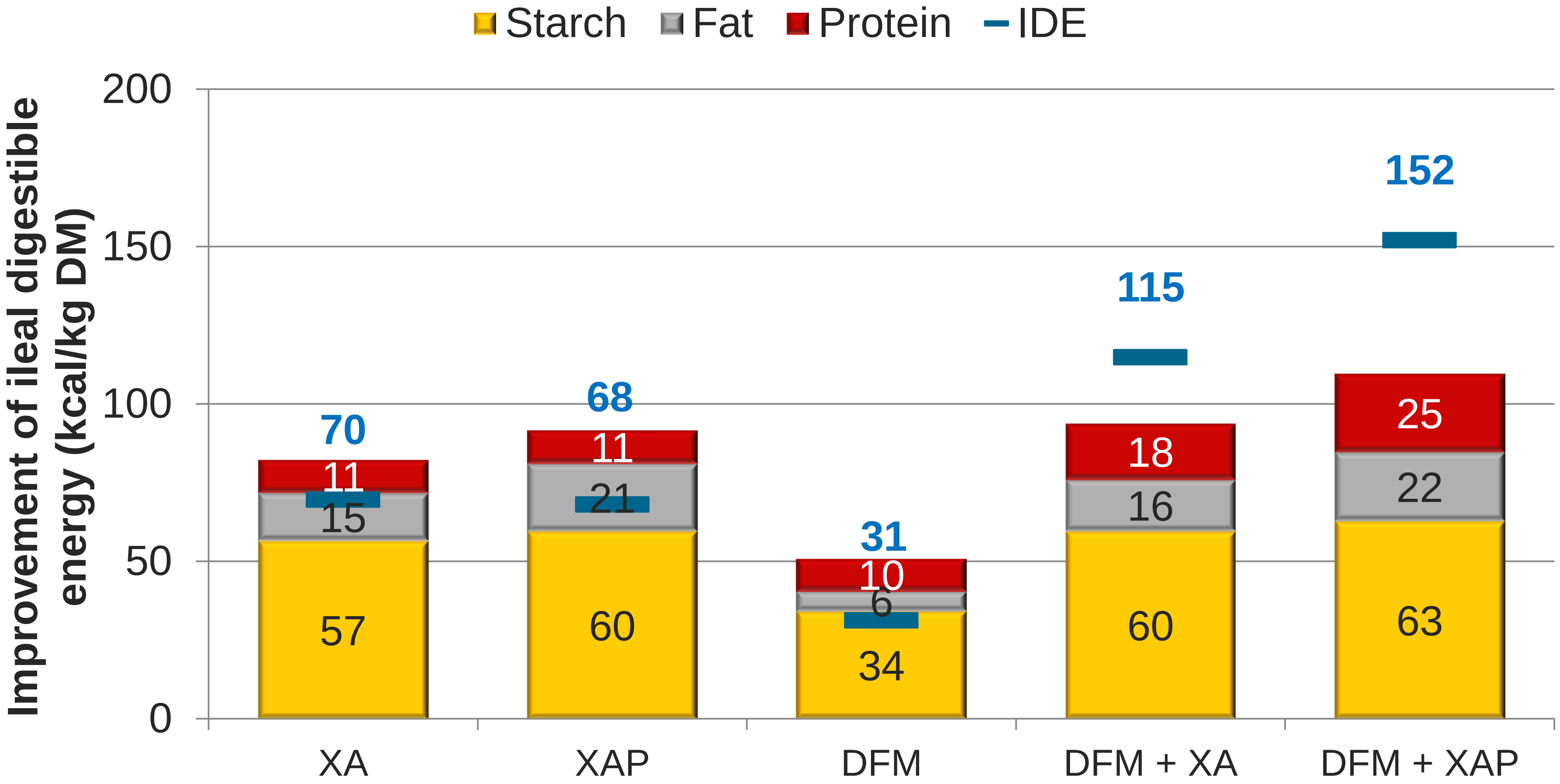
DFM and XAP enzymes exhibited independent effects on AME$_n$



Means with different letters differ at *P*<0.05
500 FTU/kg of phytase was present in the background
XA=Xylanase + Amylase; XAP=XA + Protease

Enzyme	*P*=<0.001
DFM	*P*=0.001
Enzyme x DFM	*P*=0.78

Improvements on apparent ileal energy digestibility at 21 d were not always explained by digestibility of starch, fat, and protein

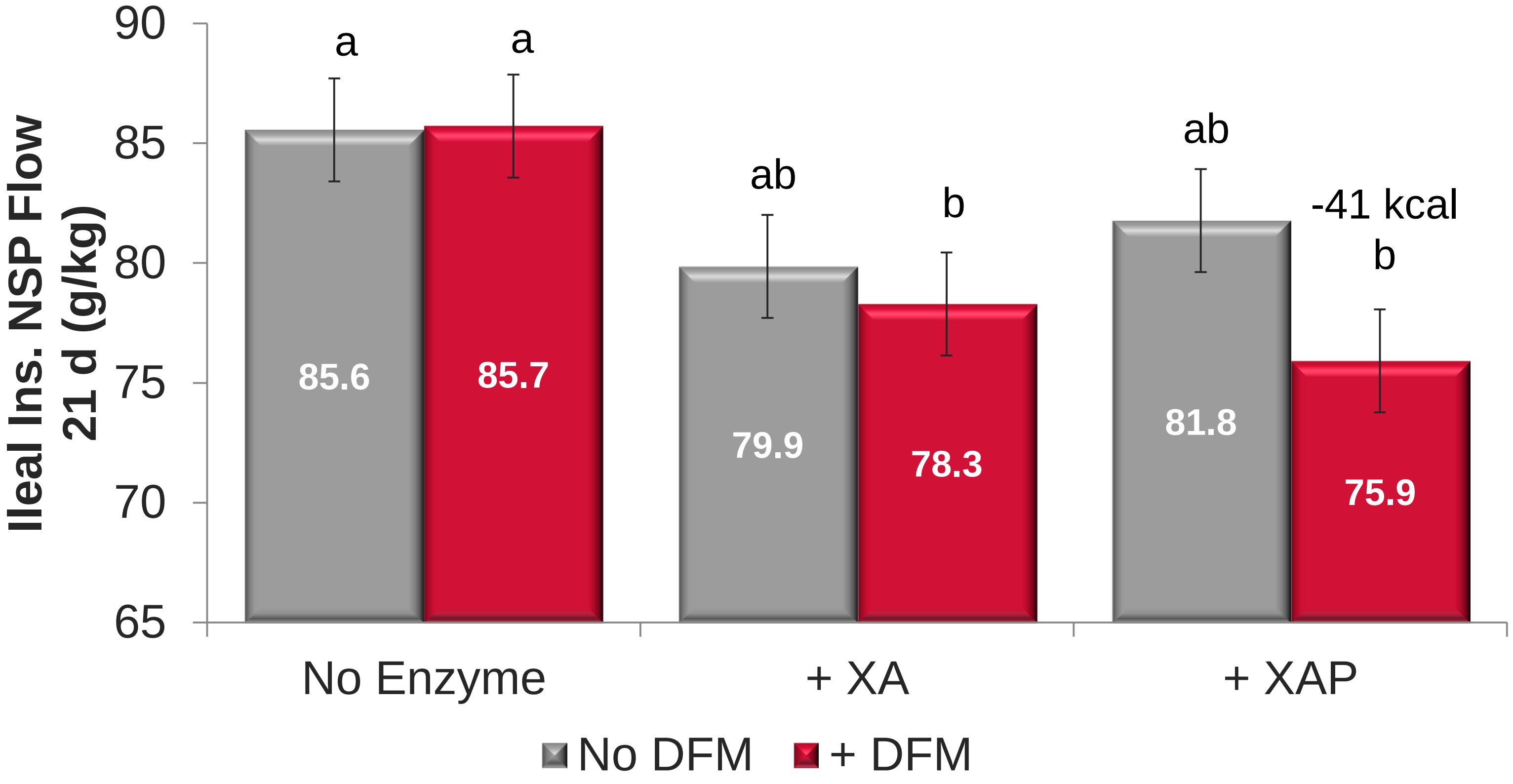


500 FTU/kg of phytase was present in the background
XA=Xylanase + Amylase; XAP=XA + Protease
Assumed gross energy content: **starch: 4.2 kcal/g; protein: 5.5 kcal/g; fat: 9.4 kcal/g**.

IDE

Enzyme	*P*=0.002
DFM	*P*=0.014
Enzyme x DFM	*P*=0.56

Combining a specific *Bacillus* DFM with XAP enzymes decreased insoluble and total NSP flow compared to control



Means with different letters differ at $P<0.05$
500 FTU/kg of phytase was present in the background
XA=Xylanase + Amylase; XAP=XA + Protease

Enzyme	*P*=0.003
DFM	*P*=0.18
Enzyme x DFM	*P*=0.005

Trial 1 - Conclusions

- Enzymes increased ileal digestible energy at 11 d
- Both DFMs and enzymes increased ileal digestible energy and AMEn compared to control at 21 d
- DFM and enzyme effects on AMEn at 21 d appeared to be additive
- An interaction between DFMs and enzymes was present on the ileal flow of insoluble NSPs, which explains part of the differences in energy digestibility due to DFMs and enzymes

TRIAL 2
DFMs AND ENZYMES
ANIMAL PERFORMANCE

Materials and Methods

- Growth performance trial to 42 d (6 reps, 22 birds/rep)
- Ross 308 broilers (1 day old)
- Oral administration of live *Eimeria* vaccine (Immucox) on day 1
- Control diet contained 500 FTU/kg of *E. coli* phytase (Phyzyme XP; Danisco Animal Nutrition)
- Body weight and feed intake were recorded on d 1, 21, 28, 35 and 42

Trial Design: 3 × 2 Factorial

Treatments	Enzymes	DFM
1. NC	No	No
2. NC + XA	Xylanase[1] + amylase[2]	No
3. NC + XAP	Xylanase + amylase + protease[3]	No
4. NC + DFM	No	*Bacillus subtilis*[4]
5. NC + DFM + XA	Xylanase + amylase	*Bacillus subtilis*
6. NC + DFM + XAP	Xylanase + amylase + protease	*Bacillus subtilis*

[1] Xylanase from *T. reesei* (2000 U/kg)
[2] Amylase from *B. licheniformis* (200 U)
[3] Protease from *B. subtilis* (5000 U)
[4] Combination of spores from 3 strains of *Bacillus subtilis* (7.5×10^4 cfu/g)

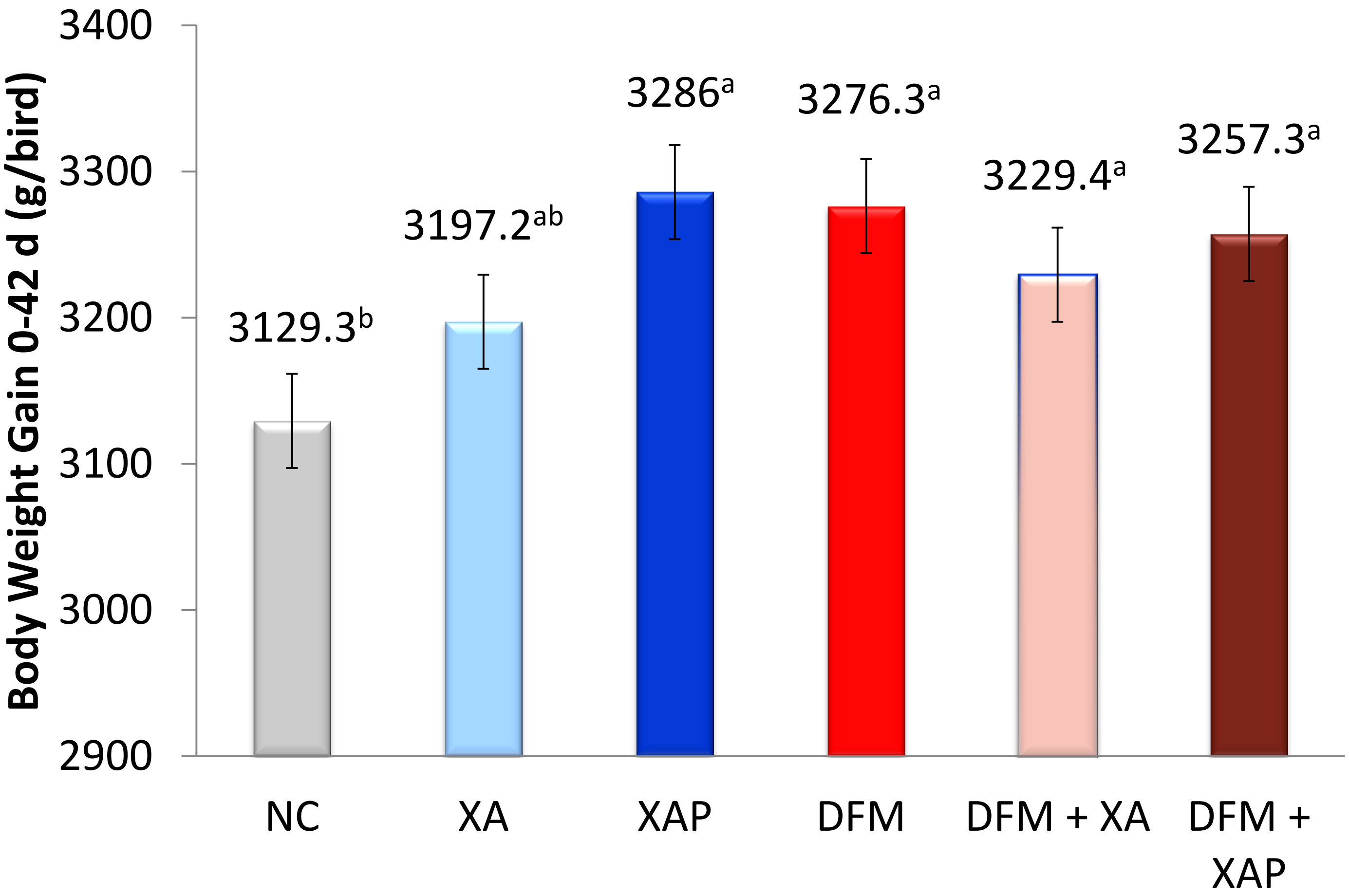
- Data were analysed by ANOVA using the Mixed Procedure of SAS

Diet Formulation

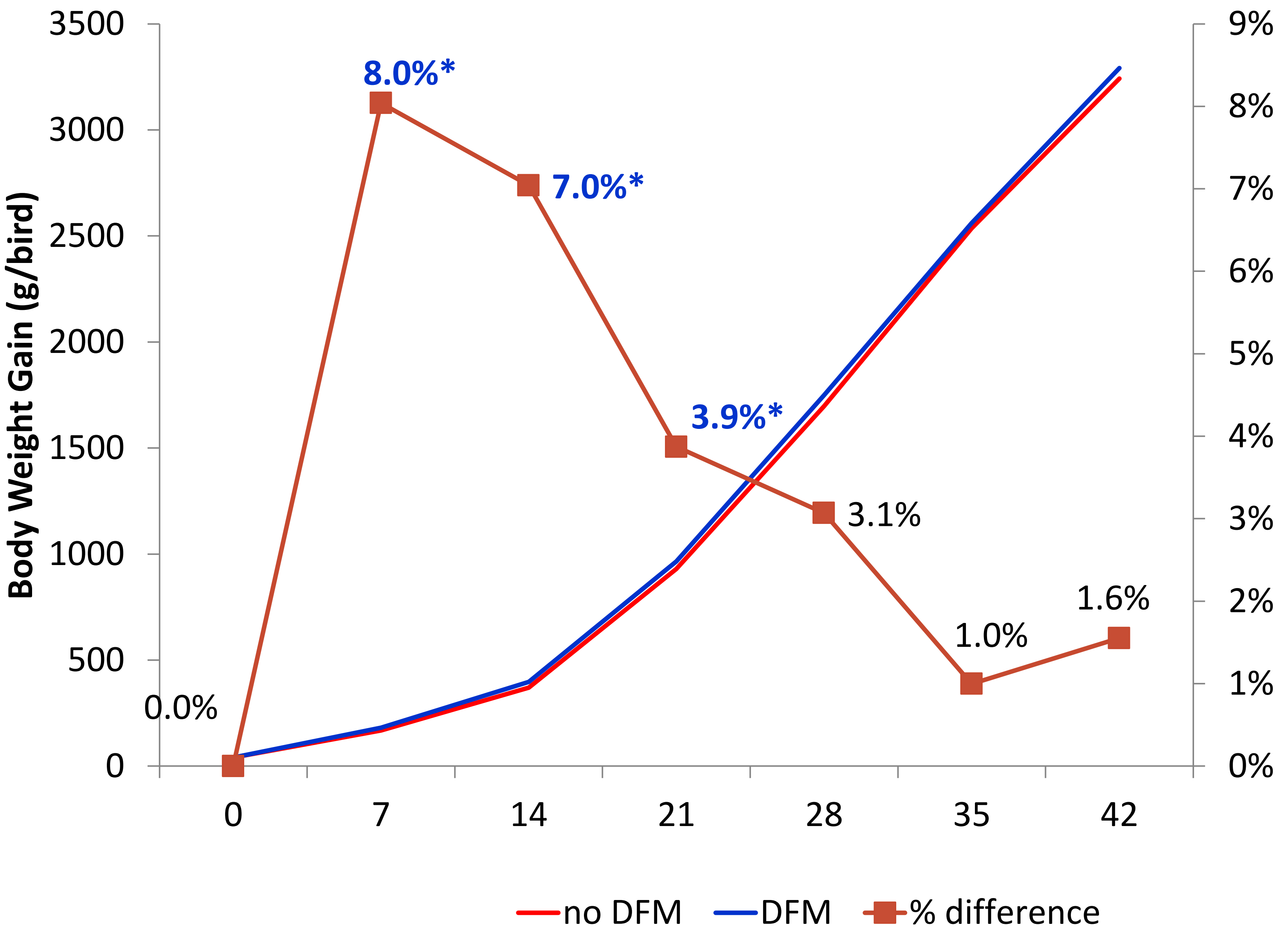
INGREDIENTS %	Starter (0-21 d)	Finisher (21-42 d)
Corn	46.22	46.73
Wheat Middlings	6.73	10.00
Corn DDGS	7.00	7.00
Soy Bean Meal 48%CP	32.81	26.19
Wheat/Enzyme Blend	0.30	0.30
Animal/Veg Fat Blend	3.00	5.75
L-Lysine HCl	0.27	0.30
DL-Methionine	0.30	0.28
L-Threonine	0.11	0.12
Inert Marker (TiO_2)	0.30	0.00
Salt	0.34	0.37
Limestone	1.12	1.14
Dicalcium Phosphate	1.20	1.22
Poultry Premix	0.30	0.30

INGREDIENTS	Starter (0-21 d)	Finisher (21-42 d)
DM %	89.1	89.3
ME POULTRY (KCAL/KG)	2950	3100
PROTEIN %	23.0	20.4
CRUDE FAT %	6.3	9.0
CALCIUM %	0.85	0.85
AVAILABLE P %	0.38	0.38
NA %	0.18	0.19
DLYS %	1.21	1.07
DMETH %	0.62	0.57
DM+C %	0.86	0.78
DTHREO %	0.76	0.68
DTRYP %	0.21	0.18
STARCH %	32.19	33.00
NSP %	11.46	11.34
INSOLUBLE ARABINOXYLANS %	4.51	4.81
SOLUBLE ARABINOXYLANS %	0.62	0.62

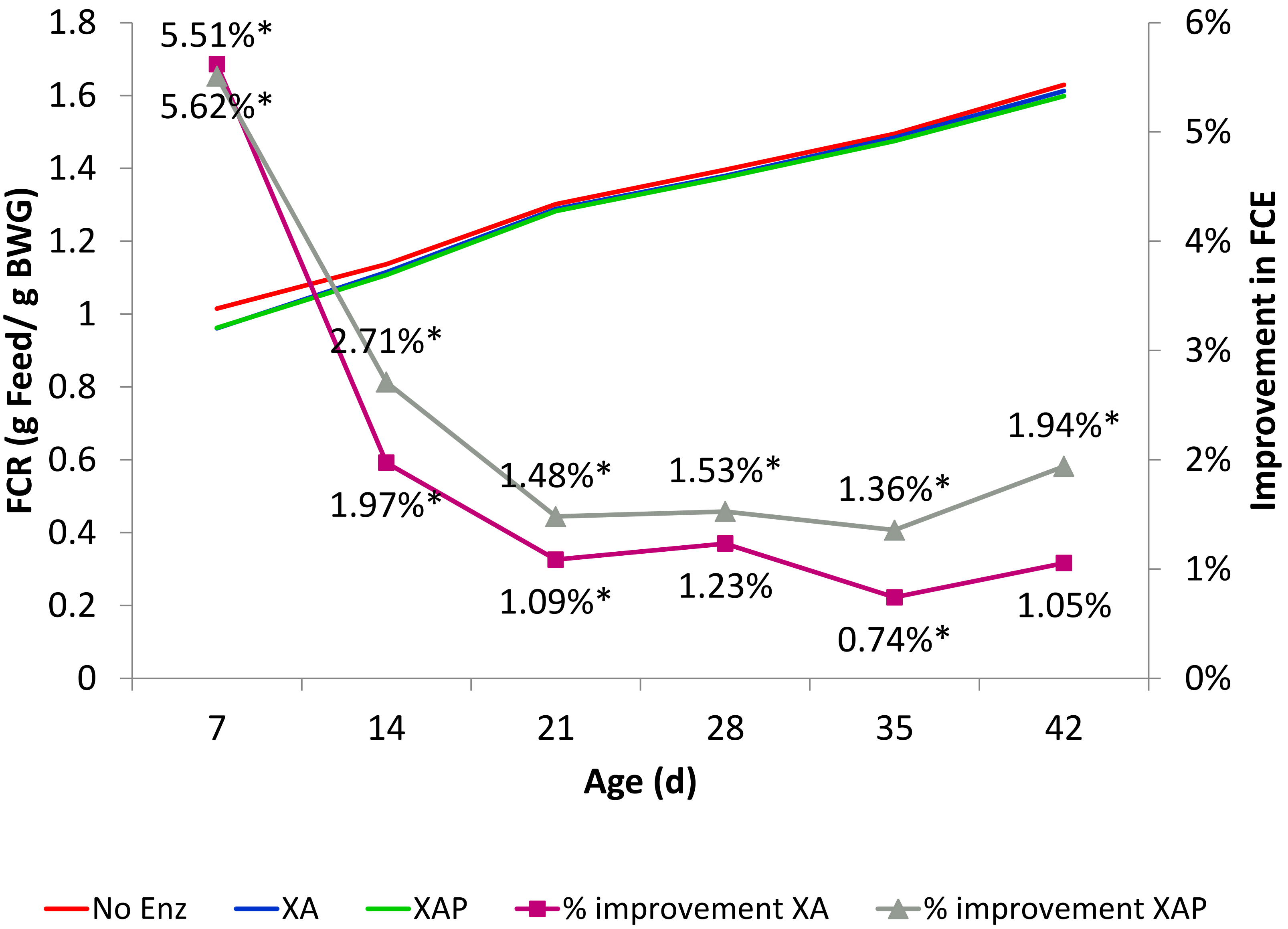
All treatments containing DFM and XAP alone increased BW gain compared to the NC



Enzyme × DFM $^{ab}P < 0.05$
Enzymes; $P = 0.09$
DFMs; $P = 0.07$

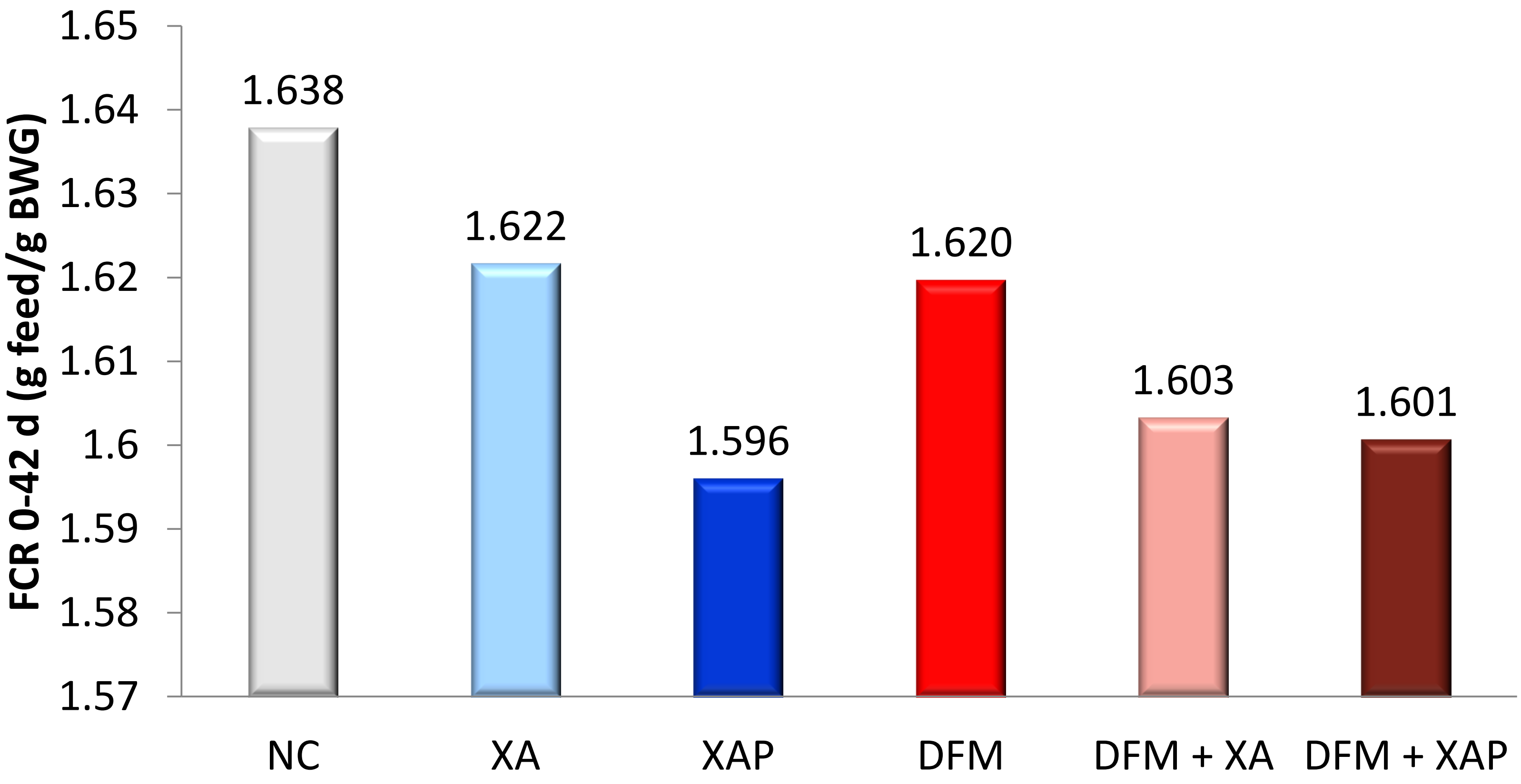

Body Weight Gain (g/bird)
3500
3000
2500
2000
1500
1000
500
0
9%
8%
7%
6%
5%
4%
3%
2%
1%
0%
0
7
14
21
28
35
42
0.0%
8.0%*
7.0%*
3.9%*
3.1%
1.0%
1.6%
no DFM
DFM
% difference

*$P < 0.05$



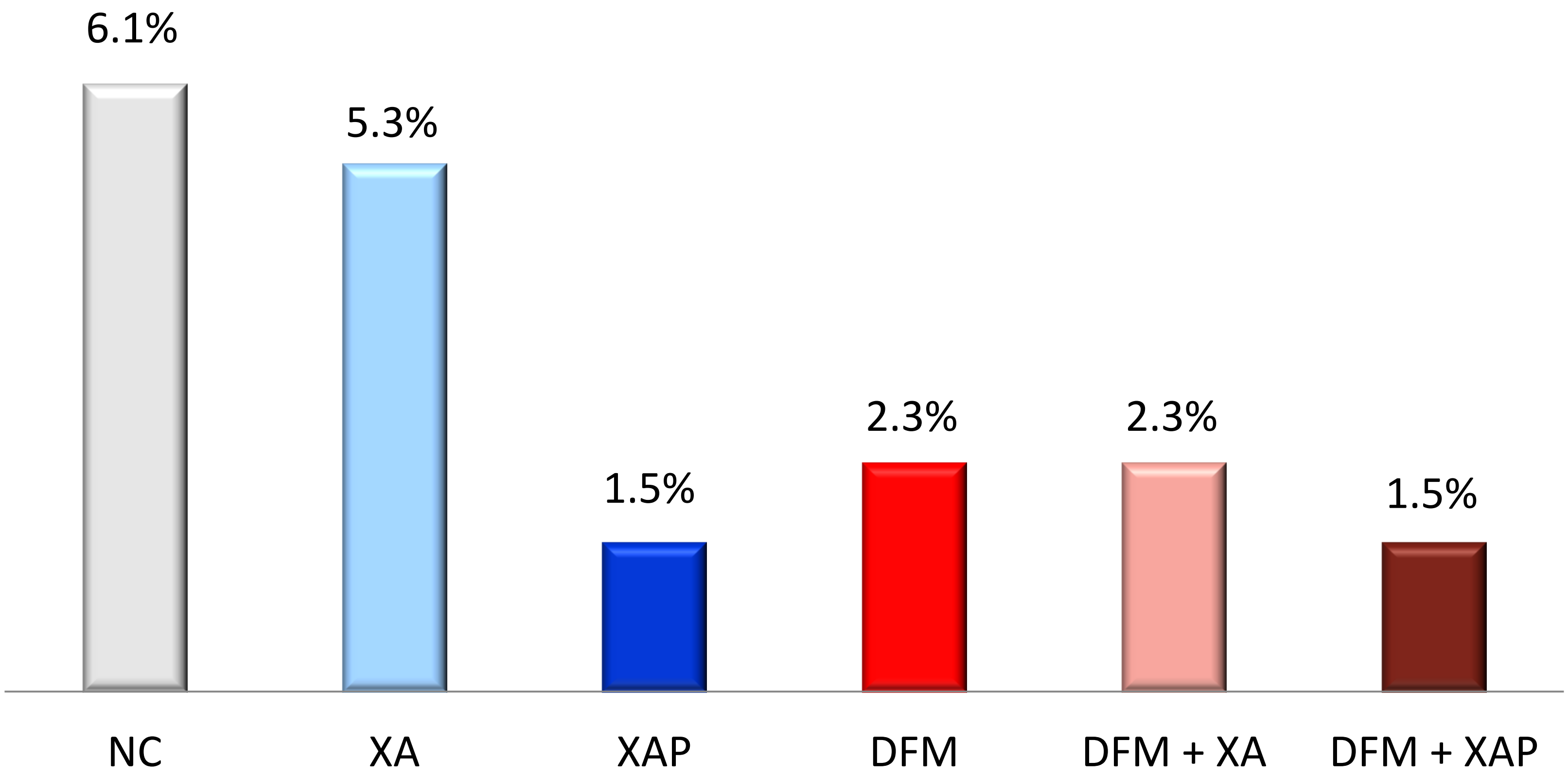
Enzyme × DFM; *P* = 0.28, Enzymes; *P* < 0.05, DFMs; *P* = 0.54

The main effect of enzymes was on reducing FCR



Enzyme × DFM; $P = 0.28$
Enzymes; $P < 0.05$
DFMs; $P = 0.54$

Mortality; d 1 to 42



Trial 2 - Conclusions

- Feeding DFMs and enzymes alone or in combination increased feed intake and body weight gain

- DFMs were more effective at enhancing feed intake and weight gain during the first 3-4 weeks of production

- Enzymes, particularly XAP reduced FCR through the study

- Enzymes and DFMs may have complementary effects on growth performance

TRIAL 3
DFMs AND ENZYMES
ENTERIC CHALLENGE MODEL

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

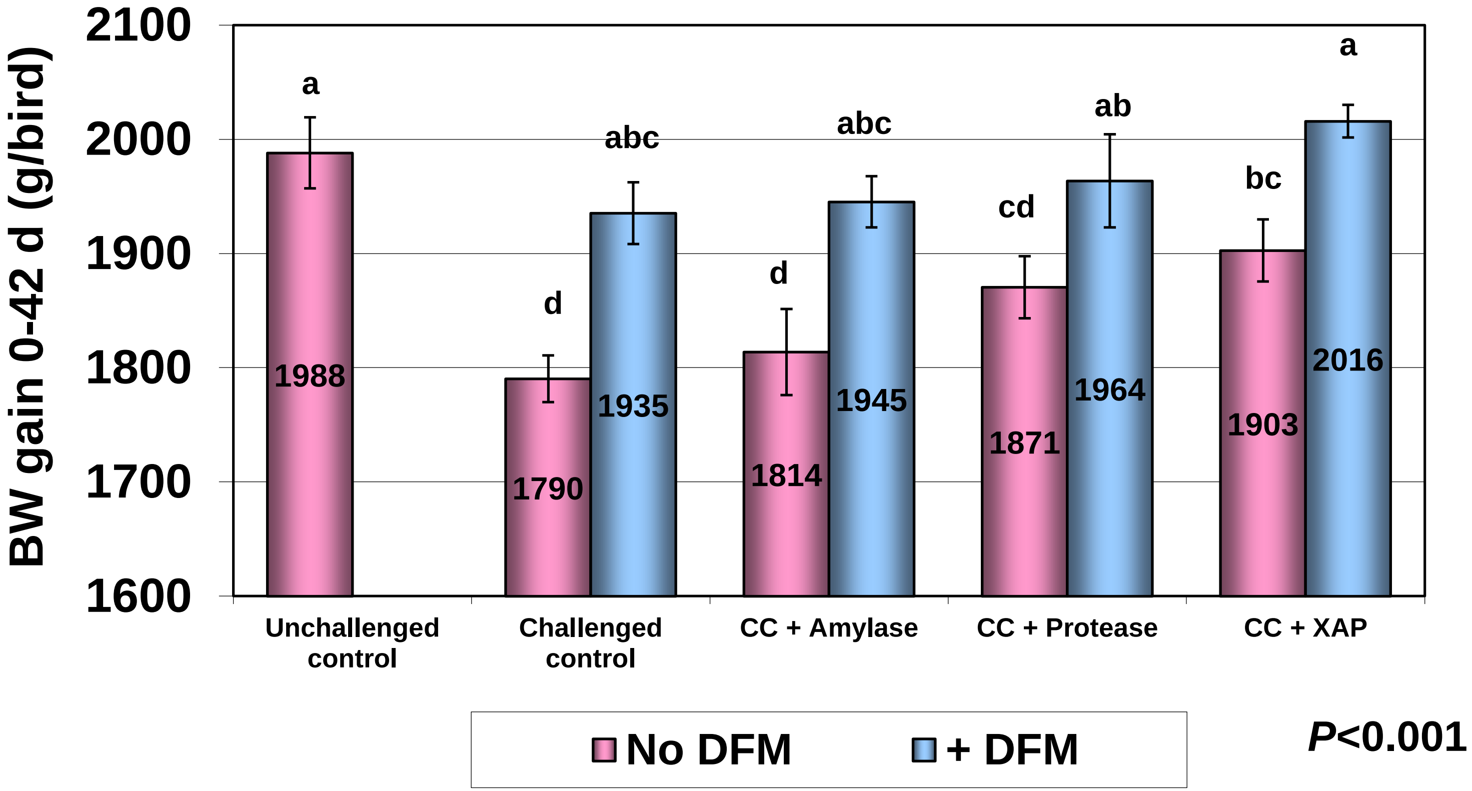
Corn/SBM/DDGS based diets, 500 FTU/kg of *E. coli* phytase in the background

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

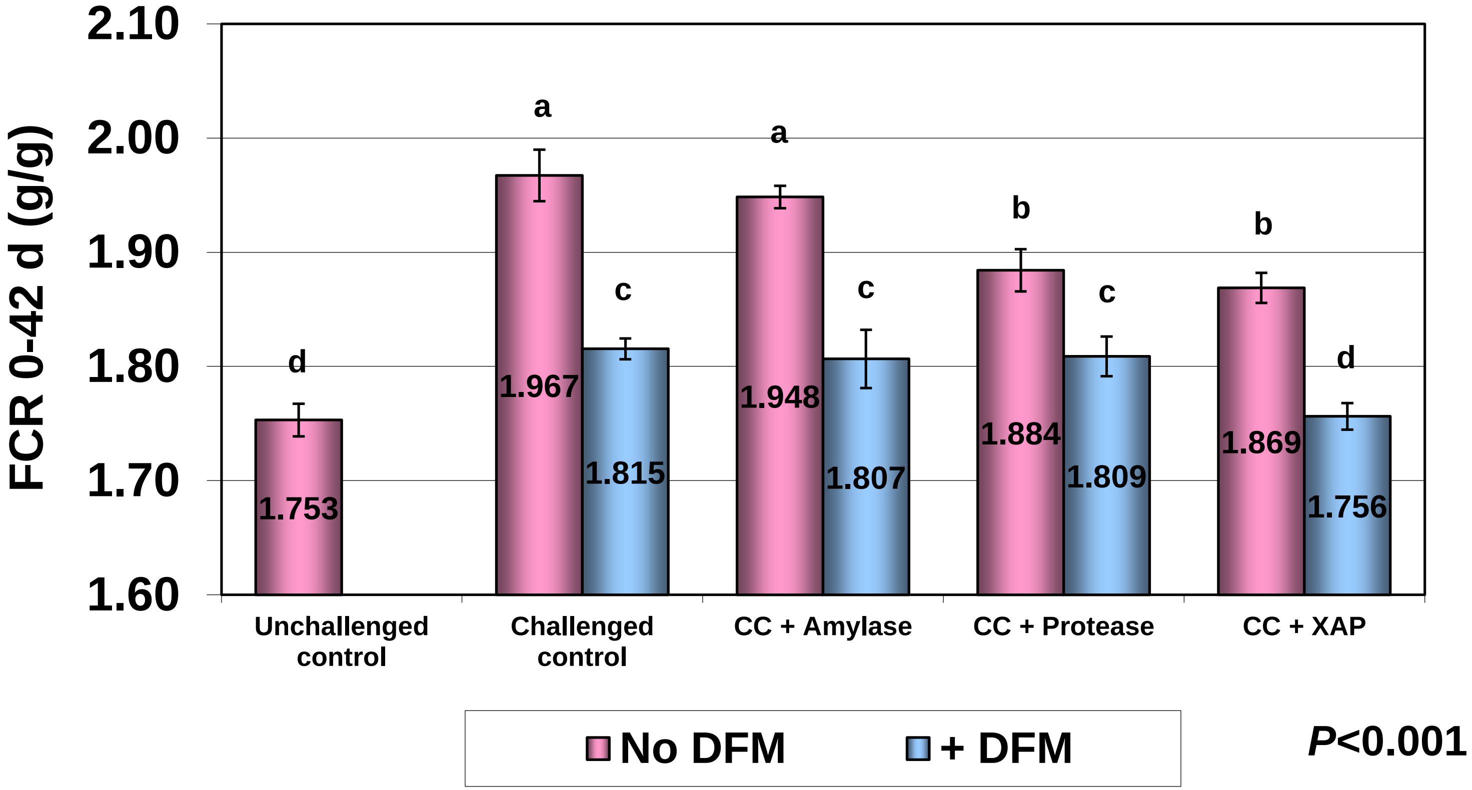
42-day body weight gain was affected by DFMs and enzymes



***P*<0.001**

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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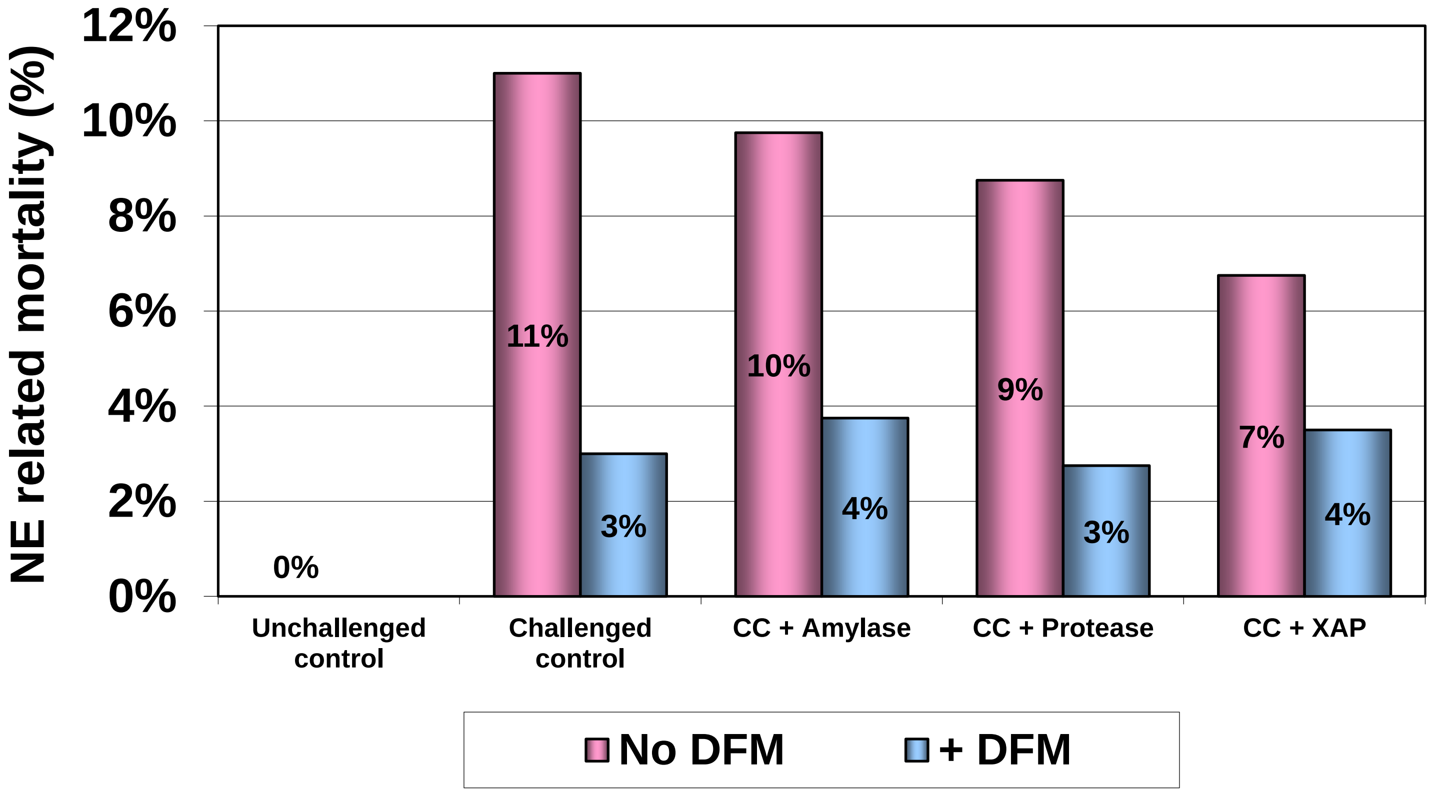
DFM is a combination of 3 *Bacillus subtilis* strains; XAP is xylanase, amylase, and protease
a, b: means without a common letter differ at $P<0.05$

Mortality related to NE



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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

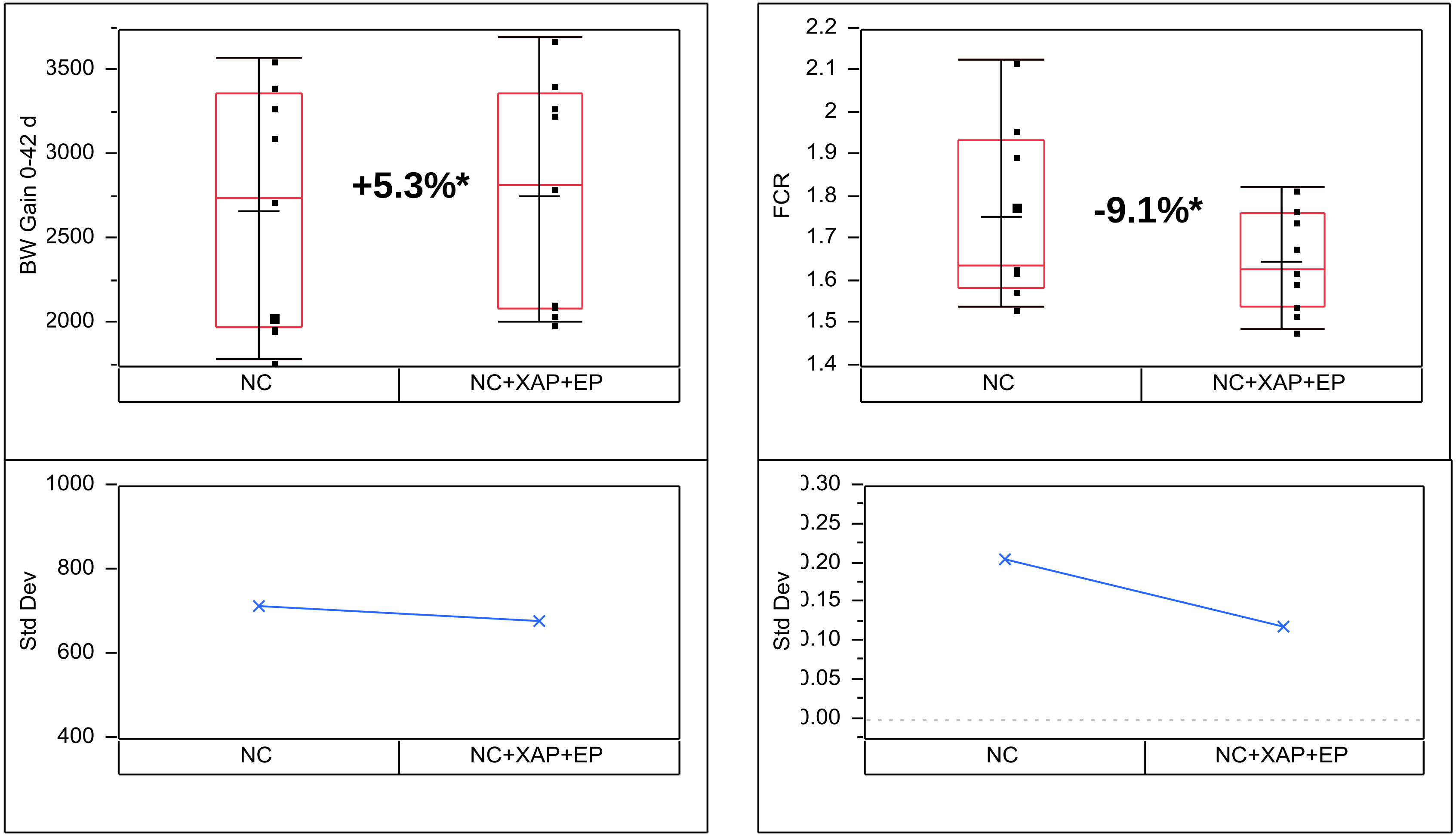
Trial 3 - Conclusions

- Effects of DFMs on growth and efficiency were notable under enteric challenge conditions

- Effects of enzymes were enhanced by the presence of the DFM, particularly on FCR

- Generally, the best performance results were obtained with combinations of XAP enzymes and the DFM

A reduction in variation of FCR due to XAP+DFMs was evident in 9 broiler chicken trials

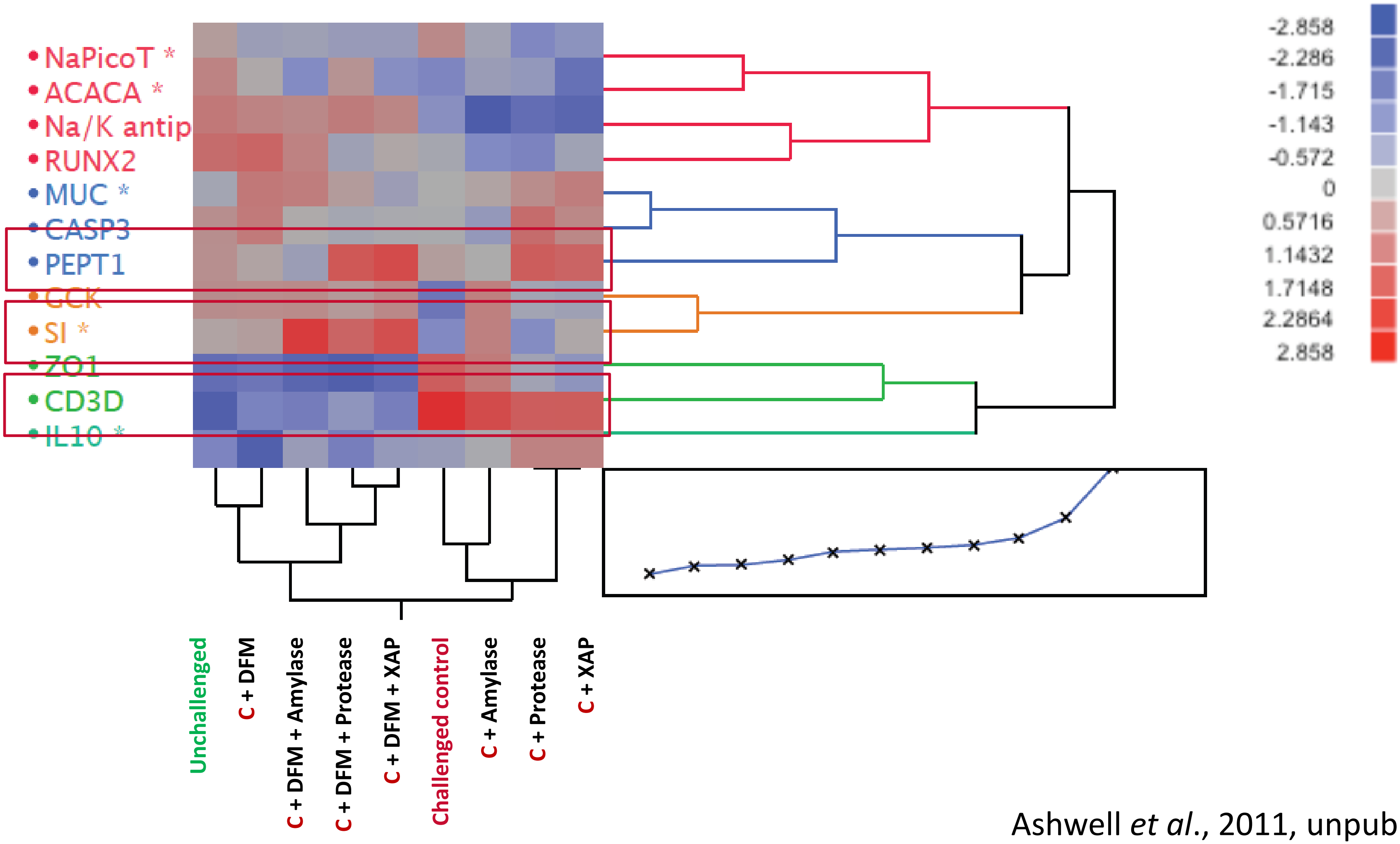


3 broiler chicken trials with no challenge, 4 trial with live *Eimeria* vaccination and 2 trials with NE challenge were analyzed

* Treatments significantly differ at *P*<0.05

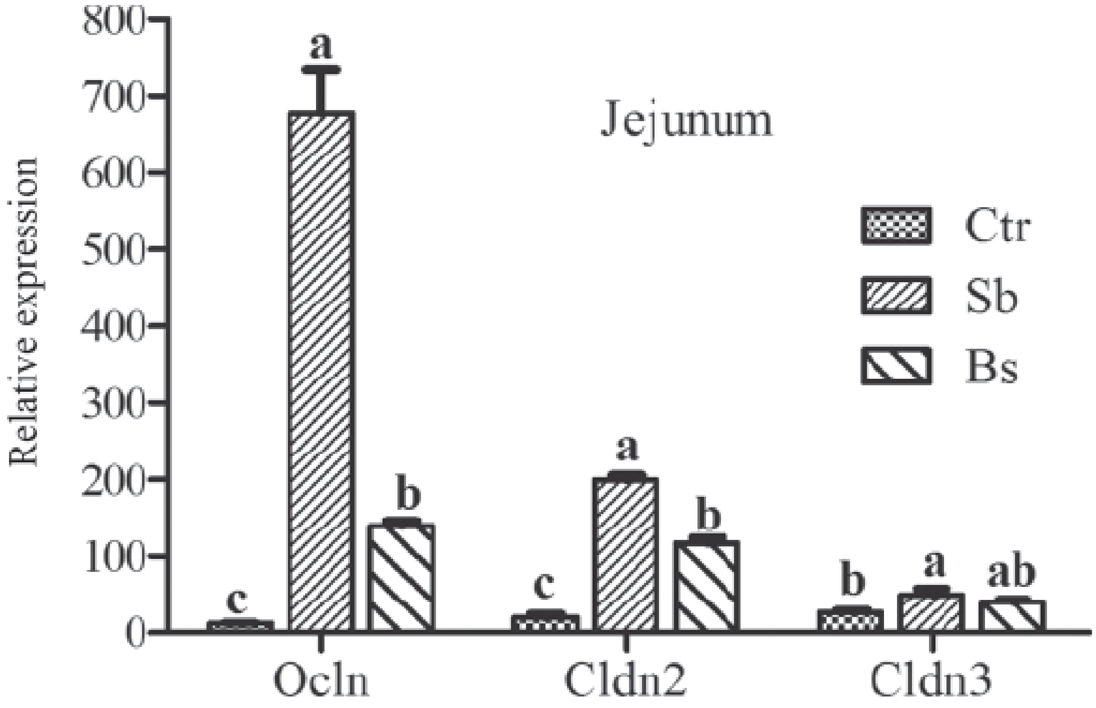
MODE OF ACTION

Gene expression patterns in the jejunum of challenged broilers fed DFM + enzymes clustered distinctively



Ashwell *et al.*, 2011, unpublished

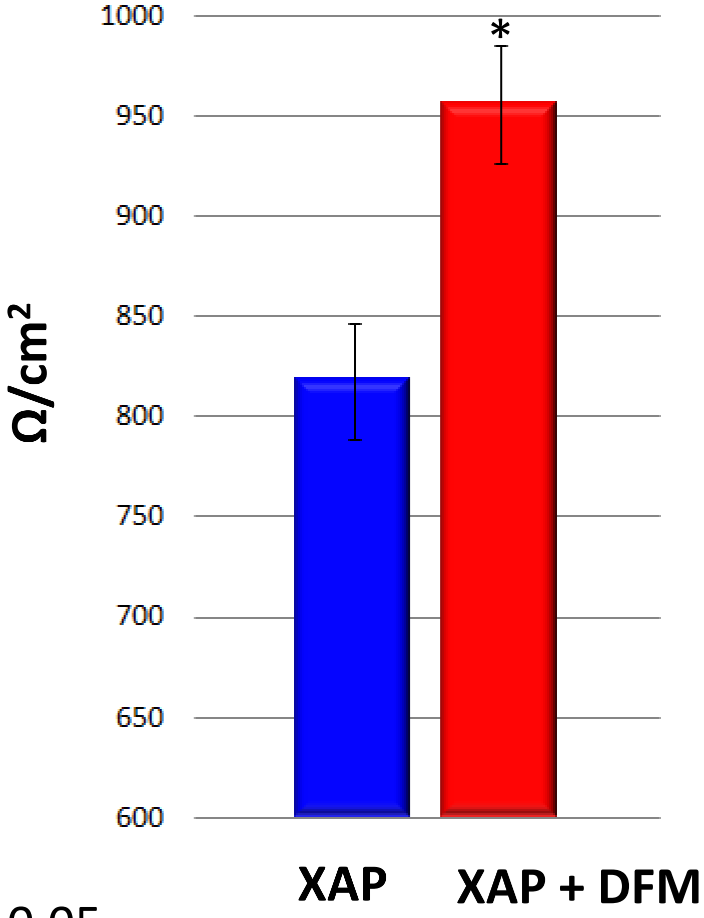
DFMs are reported to accelerate intestinal barrier maturation



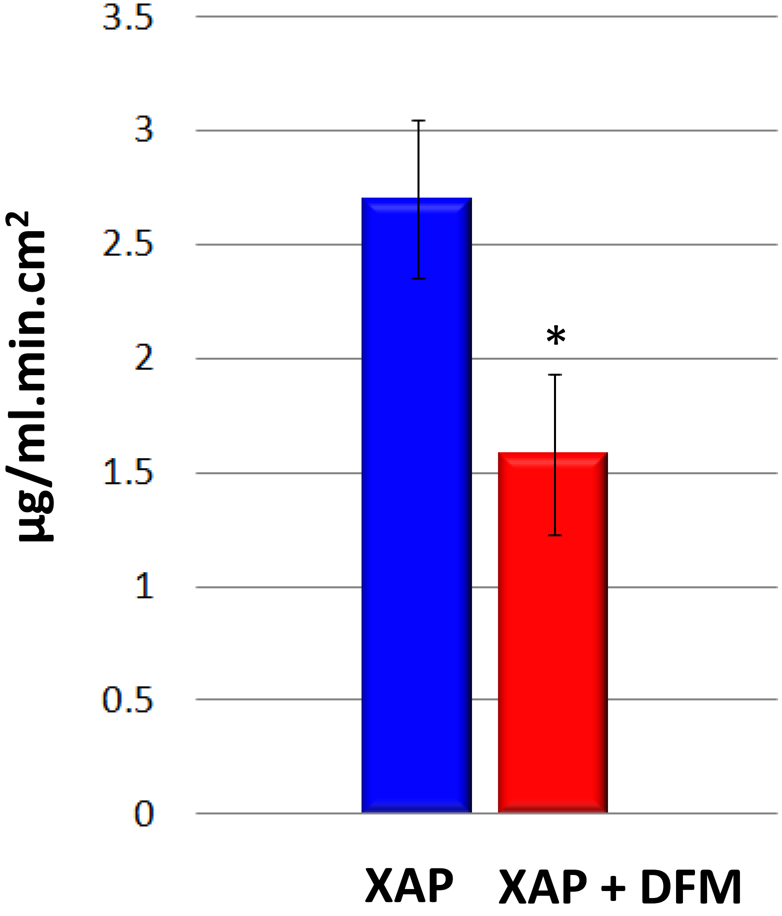
Bacillus and *saccharomyces* increased gene expression of the tight junction proteins claudin 2 and 3 and occludin in the jejunum of broilers. ^{abc} $P<0.05$ (Rajput *et al.*, 2013)

DFM & Enzymes enhanced intestinal integrity in the colon of first cycle laying hens

Trans-epithelial Electrical Resistance (TER)



Apparent Permeability Coefficient

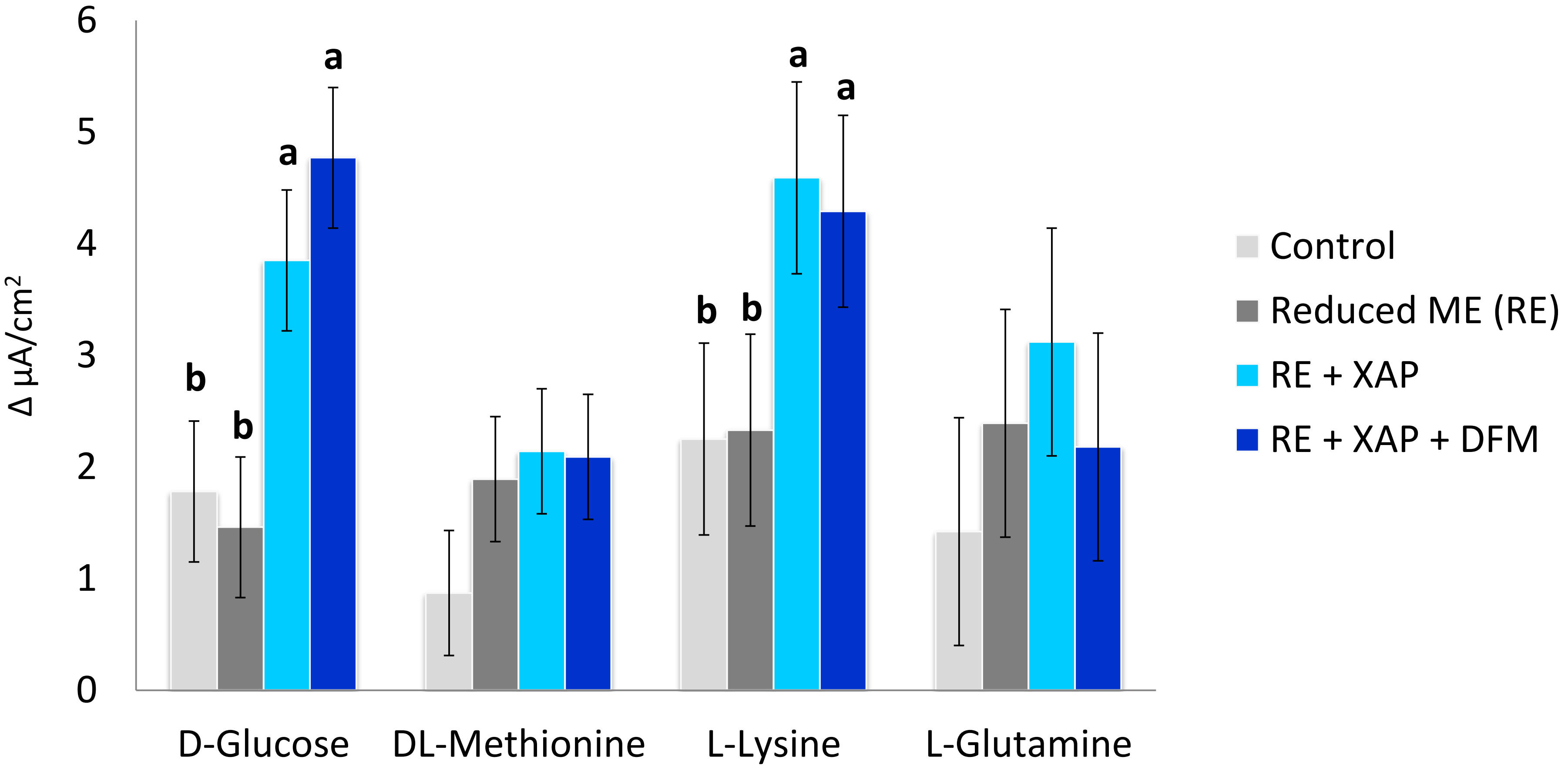


*$P < 0.05$

XAP = xylanase, amylase, protease
DFM = 3 strains of *Bacillus subtilis*

Murugesan *et al.*, 2013

Enzymes increased ileal nutrient flux in laying hens fed a reduced energy diet



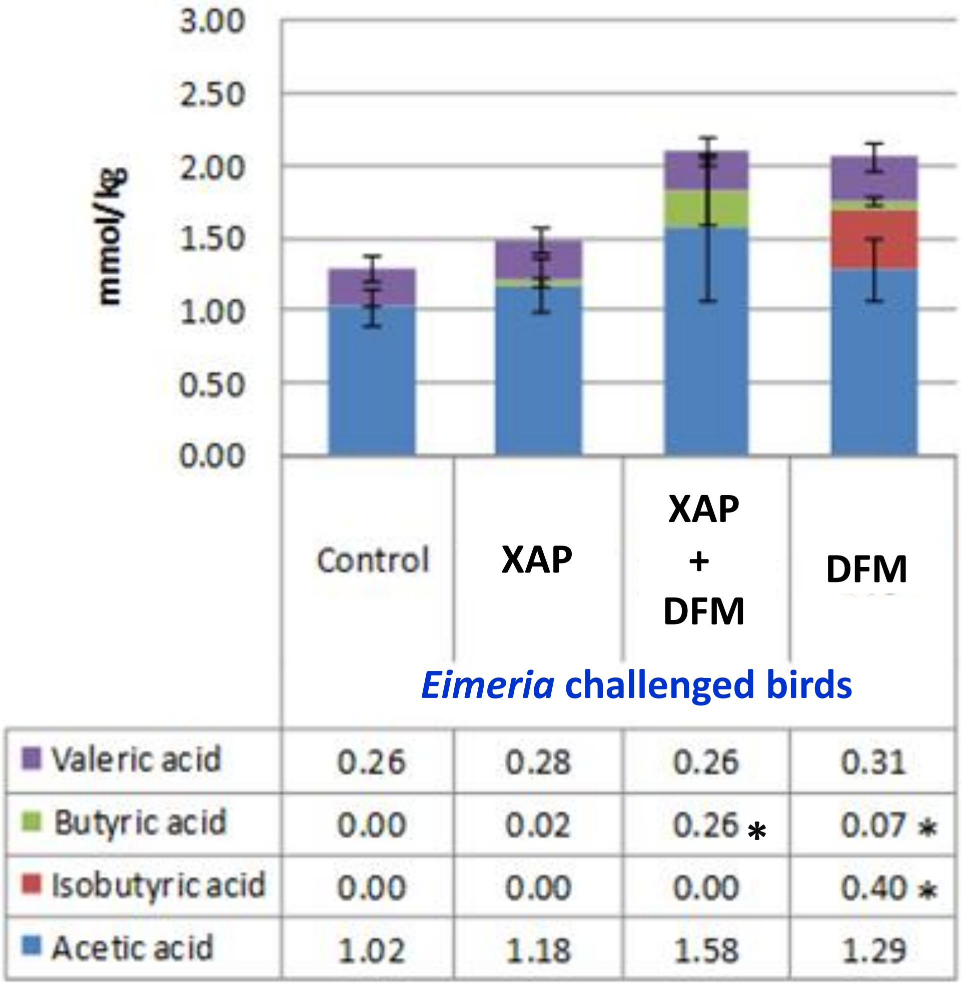
^{ab} $P < 0.05$

XAP: xylanase, amylase, protease

DFM: 3 strains of *Bacillus subtilis*

Murugesan *et al.*, 2013

Altering available substrates affected SCFA production in the ileum broilers challenged with *Eimeria maxima*



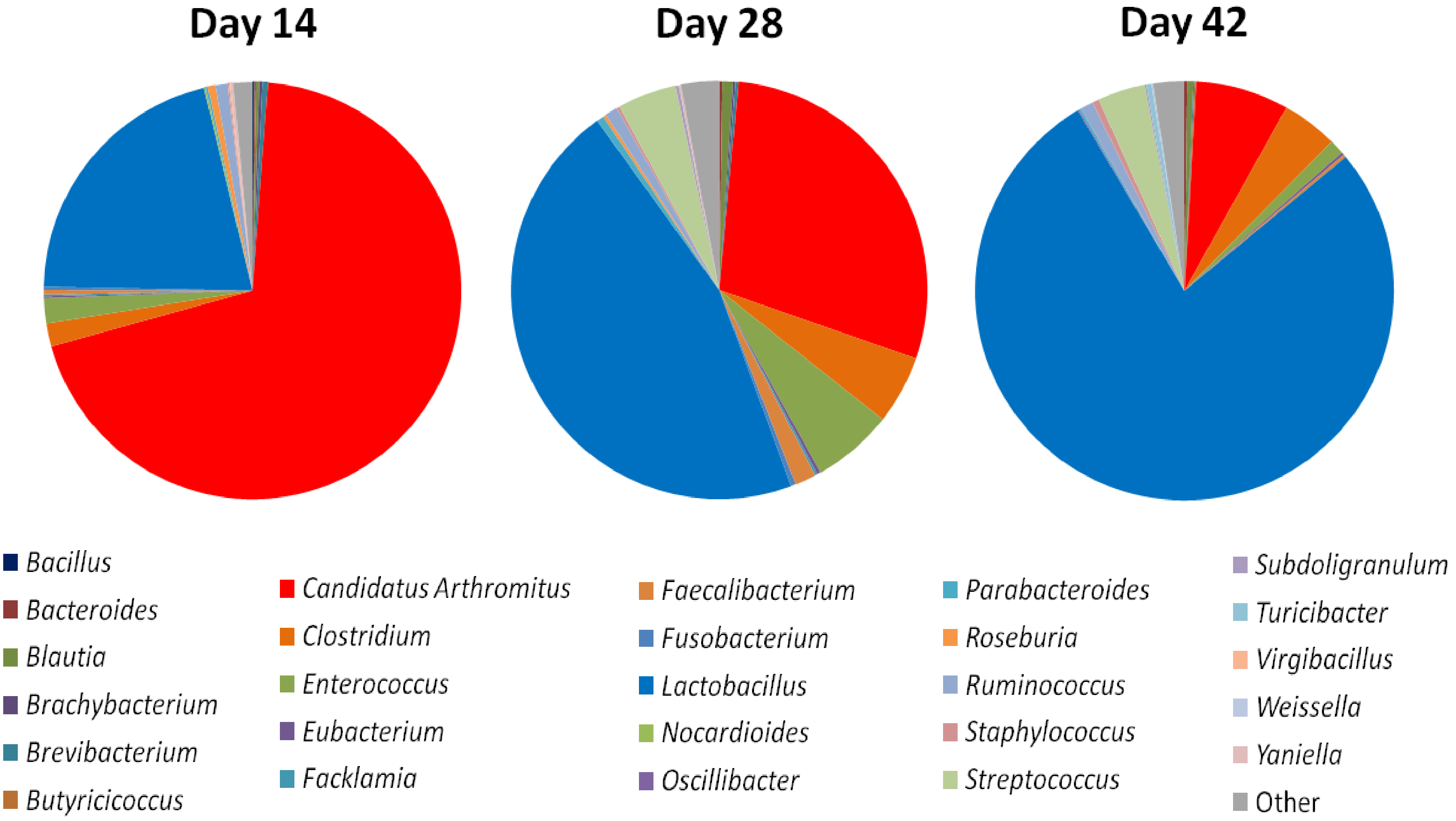
	Control	XAP	XAP + DFM	DFM
🟪 Valeric acid	0.26	0.28	0.26	0.31
🟩 Butyric acid	0.00	0.02	0.26 *	0.07 *
🟥 Isobutyric acid	0.00	0.00	0.00	0.40 *
🟦 Acetic acid	1.02	1.18	1.58	1.29

* $P < 0.05$

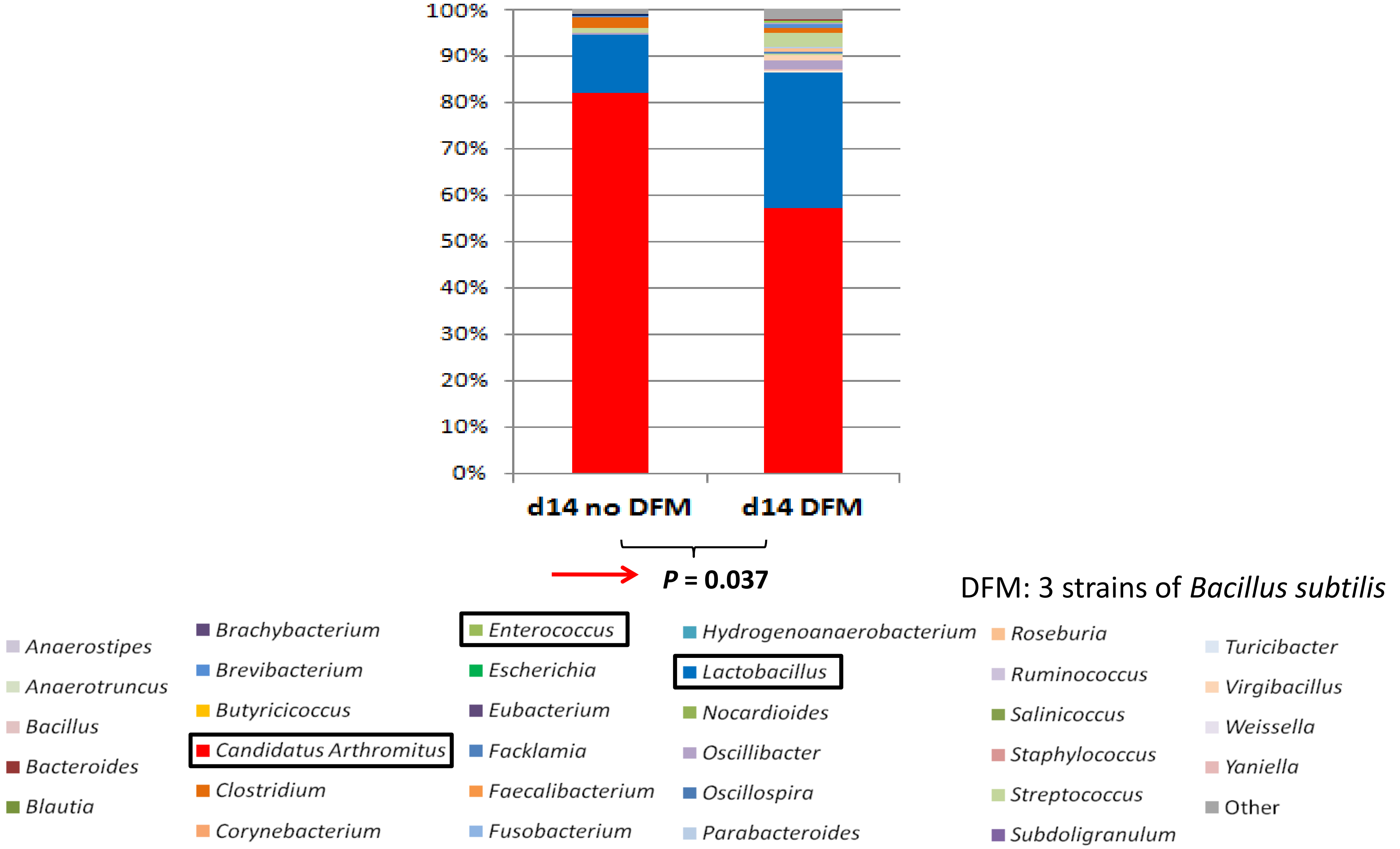
- XAP and DFMs increased the amount of butyric vs control
- DFMs alone increase the amount of isobutyric and butyric acid vs control
- DFMs increase total SCFA production in the ileum

Danisco Animal Nutrition, 2012, unpublished

In a mature healthy chicken gut, *Lactobacillus* represents the major genera



DFMs appear to promote a more ‘mature’ gut microbiota early



P = 0.037

DFM: 3 strains of *Bacillus subtilis*

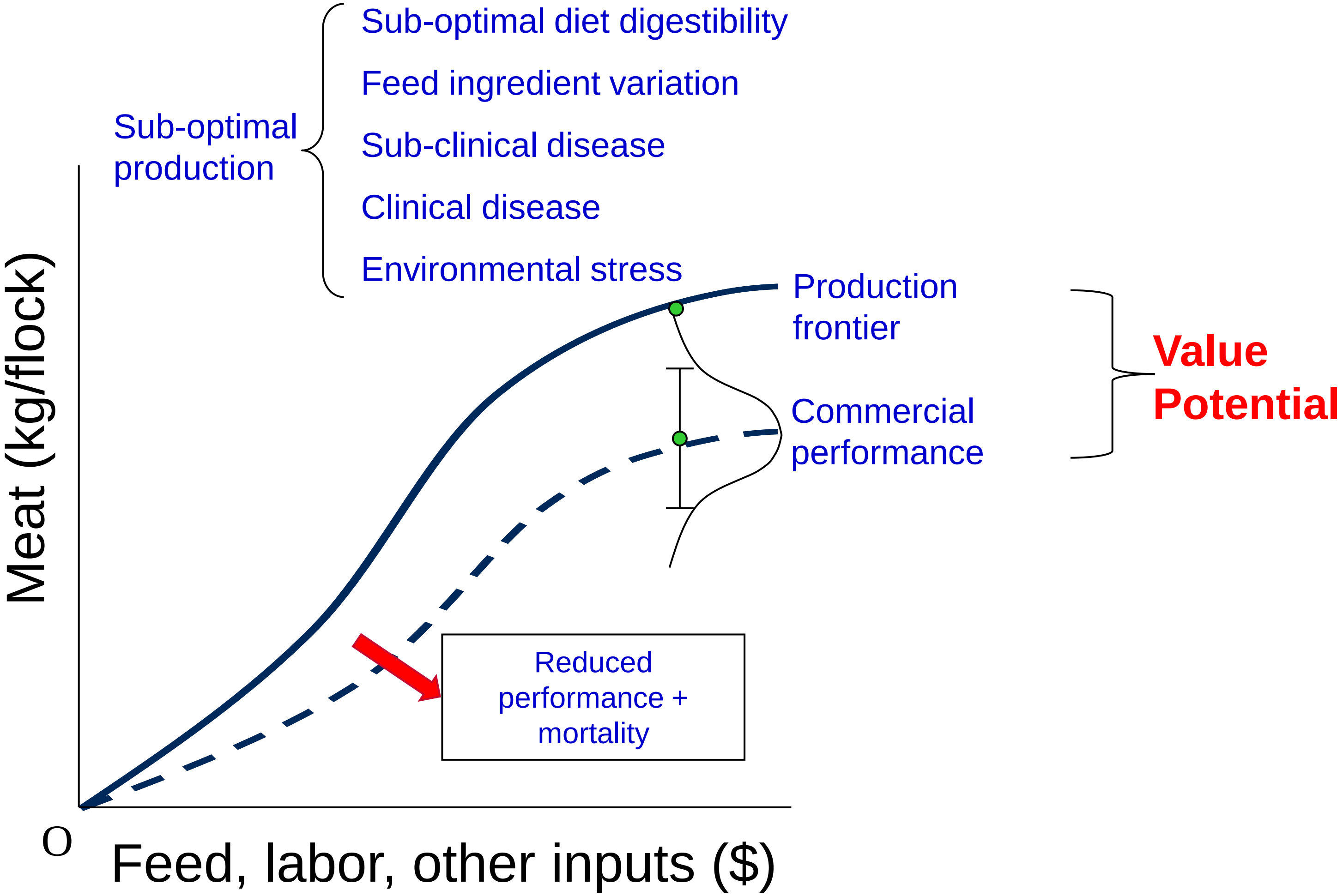
Enzymes can only offer part of the solution to improve digestibility and performance in poultry

- More nutrients available for absorption & metabolism
  - enhanced nutrient digestibility
  - reduced endogenous inputs from digestion
  - less nutrient available for pathogens

DFM can provide an adequate environment for enzymes

- Improved intestinal health
  - enhance intestinal barrier function
  - modulation of the microbial community structure
  - modulation of the immune response

Complementary nutrition and gut health solutions will enable producers to optimize the potential productivity of poultry



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