A combination of direct fed microbials and xylanase, amylase and protease enzymes improves nutrients digestibility, gut health, performance and welfare of broilers

22 October 2015 AMENA, Mexico

Y. Dersjant-Li, A. Awati, C. Evans and K. Gibbs

Danisco Animal Nutrition/DuPont

Outline of the presentation

- **Introduction: synergy between enzymes and DFM**
- Nutrients digestibility
- Immune response under challenge
- Compatible with AGPs
- Animal welfare and economic benefit

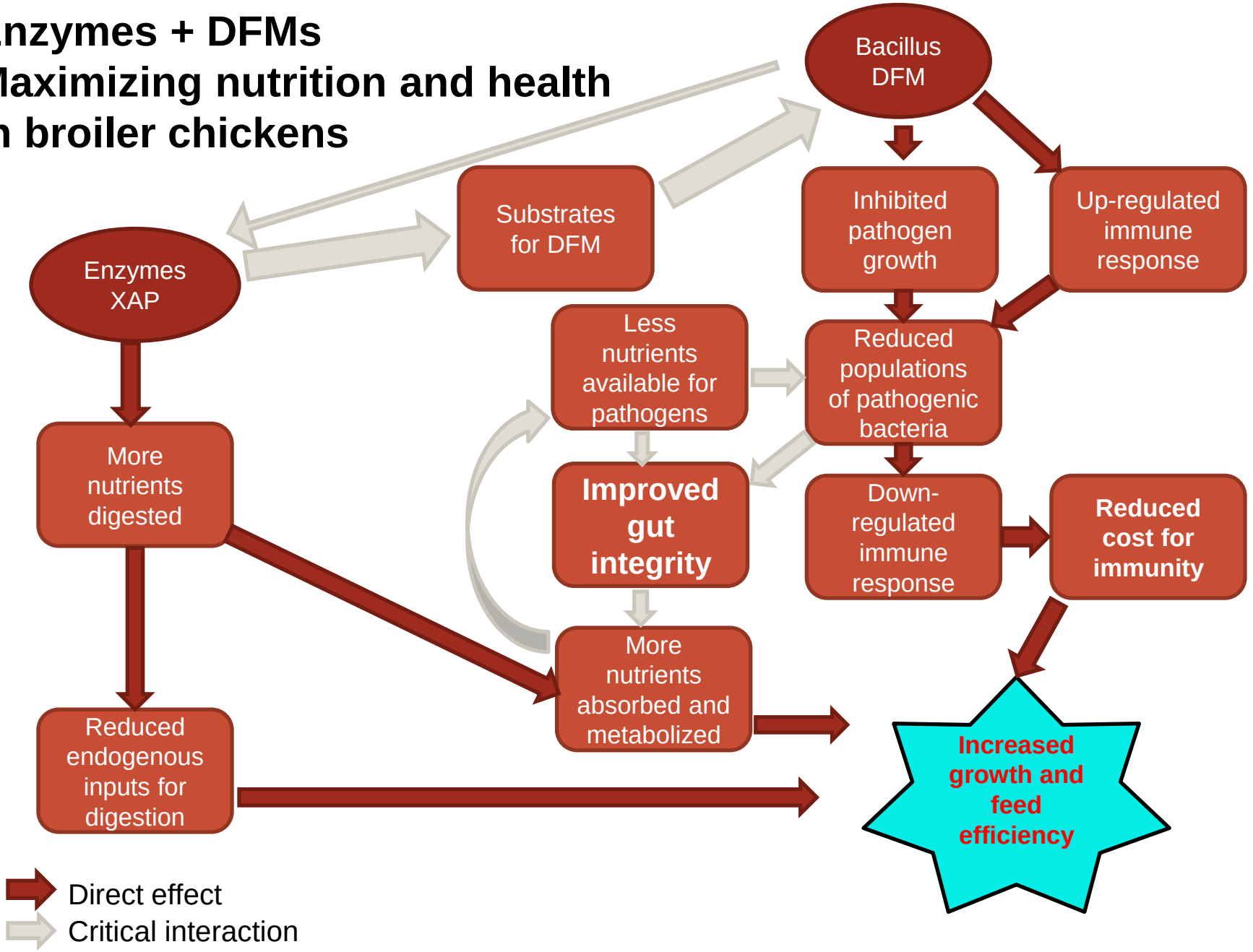
Key words (abbreviations)

Mixed enzymes:

XAP = **X**ylanase (an endo-xylanase from Trichoderma reesei)
Amylase (alpha-amylase from *Bacillus licheniformis*)
Protease (serine protease from *B. subtilis*)

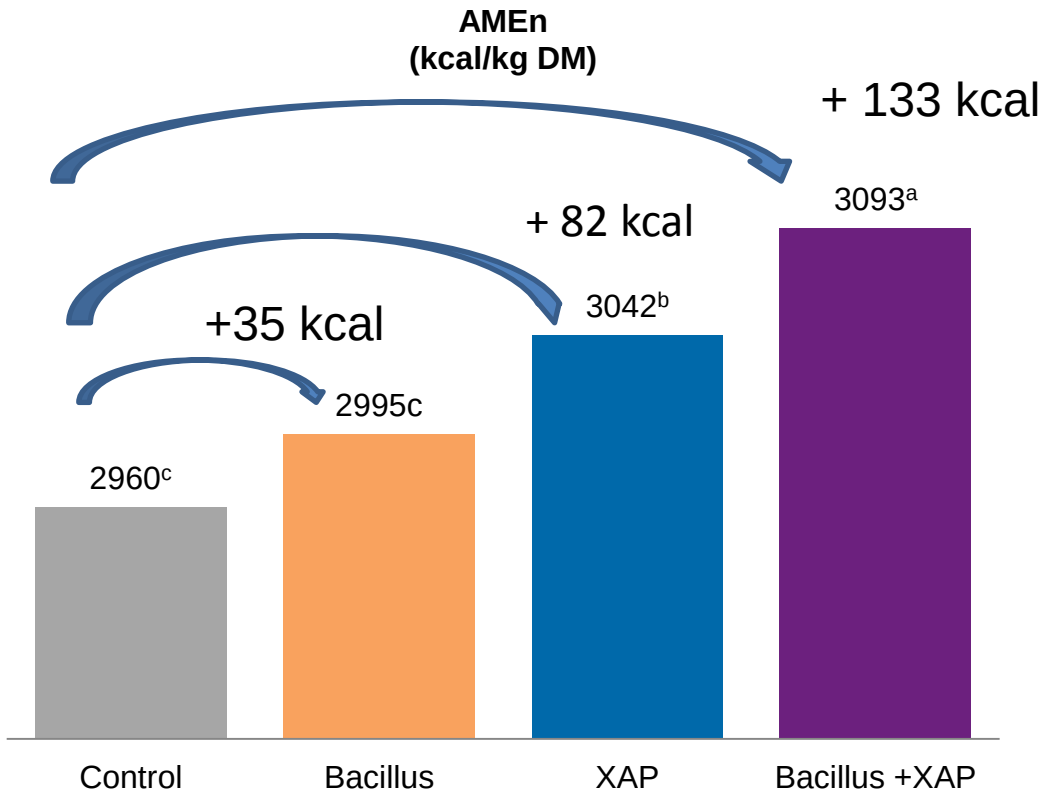
DFM = **D**irect **F**ed **M**icrobials (probiotics):
a combination of spores from three *Bacillus* strains

Enzymes + DFMs
Maximizing nutrition and health in broiler chickens



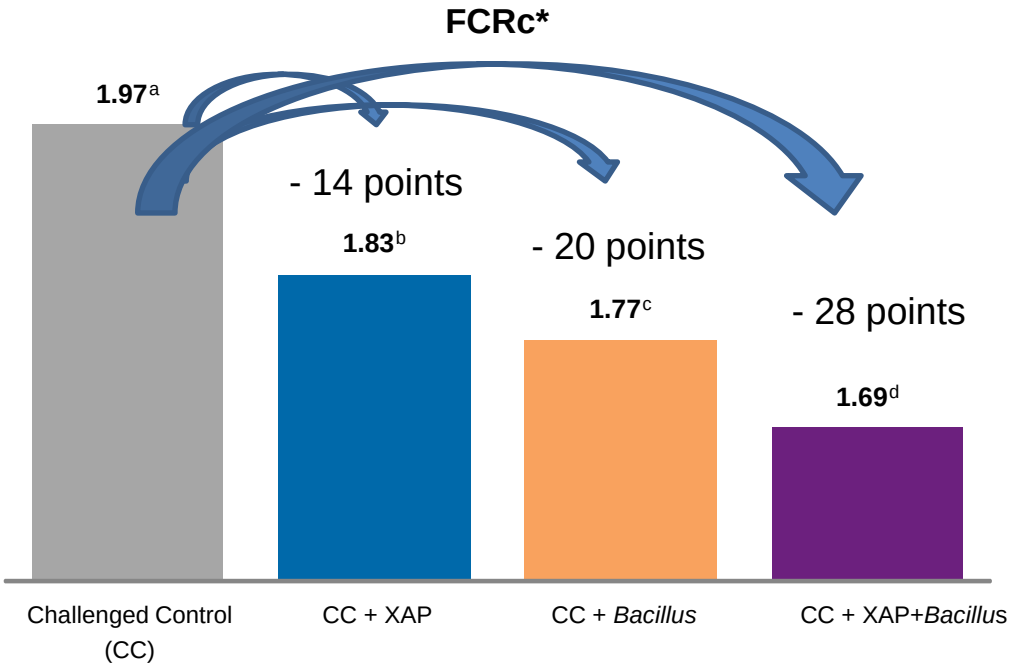
Synergies between enzymes and DFM

- **Corn/SBM/corn DDGs**
- **Ross 308 male broiler**
- **6 replicate pens**
- **22 birds/pen**
- **DFM: 3 *Bacillus* strains**
- **XAP: xylanase, amylase, protease**
- **21d digestibility study**



Romero *et al*., Animal Feed Science and Technology *181 (2013) 35– 44*

Additive effect of enzymes and DFM



[abc] *Values without a common superscript are significantly different (P<0.05)*
XAP= xylanase, amylase and protease
Bacillus = 3 strains combination

- Cobb x Cobb 500 male broilers;
- Corn/SBM, 10% corn DDGs
- Challenge: NE (necrotic enteritis) induced by a broth culture of *Clostridium perfringens* during day 20-22

* FCRc is body weight corrected FCR, corrected 3 points for every 100g difference in BW versus challenged control

Mathis et al., 2013 Poultry Science Association, 102nd Annual Meeting, San Diego, CA, USA

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- Introduction: synergy between enzymes and DFM
- **Nutrients digestibility**
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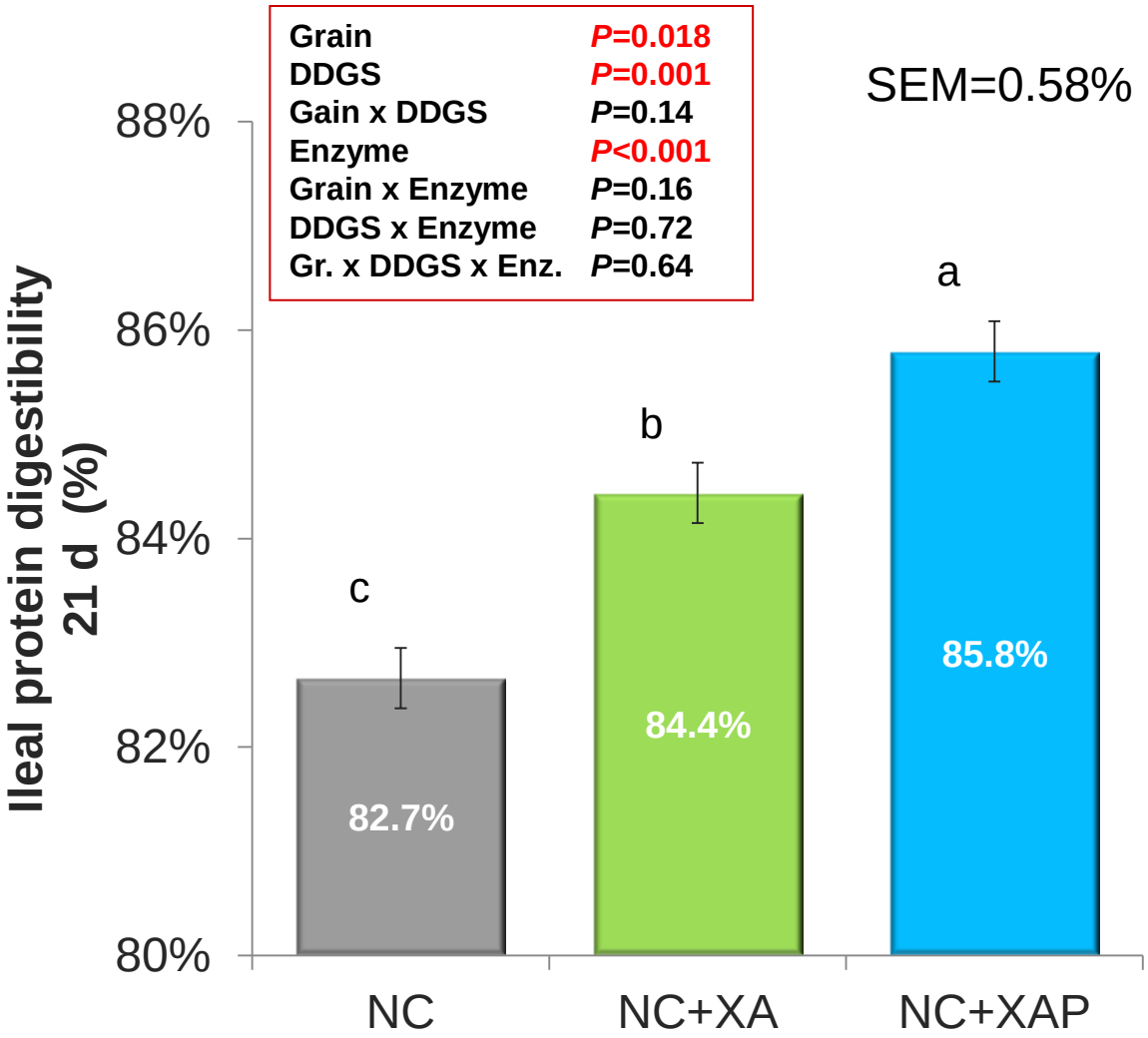
- Animal welfare

Protease on top of xylanase and amylase further improved protein digestibility, with no interactions at 21 d

- 6 reps / treatment; 6 or 4 male broilers / cage

- 2 x 2 x 3 factorial design:
  - Base grains:
    - Corn/SBM (CS)
    - Wheat/SBM (WS)
  - DDGS-canola:
    - no;
    - 10-14% DDGS-5% canola meal (Mixed diet)
  - Enzyme treatments:
    - Control;
    - Xylanase/Amylase (XA);
    - XA + Protease (XAP)

- 500 FTU/kg of *E. coli* phytase in the background



Romero *et al*., 2014 Poultry Science 93 :2501–2513

Enzymes and DFM combination improved energy utilization more effectively in **high fiber diets**

2 x 4 factorial design:

Low fiber: Corn/SBM (2.6% CF)

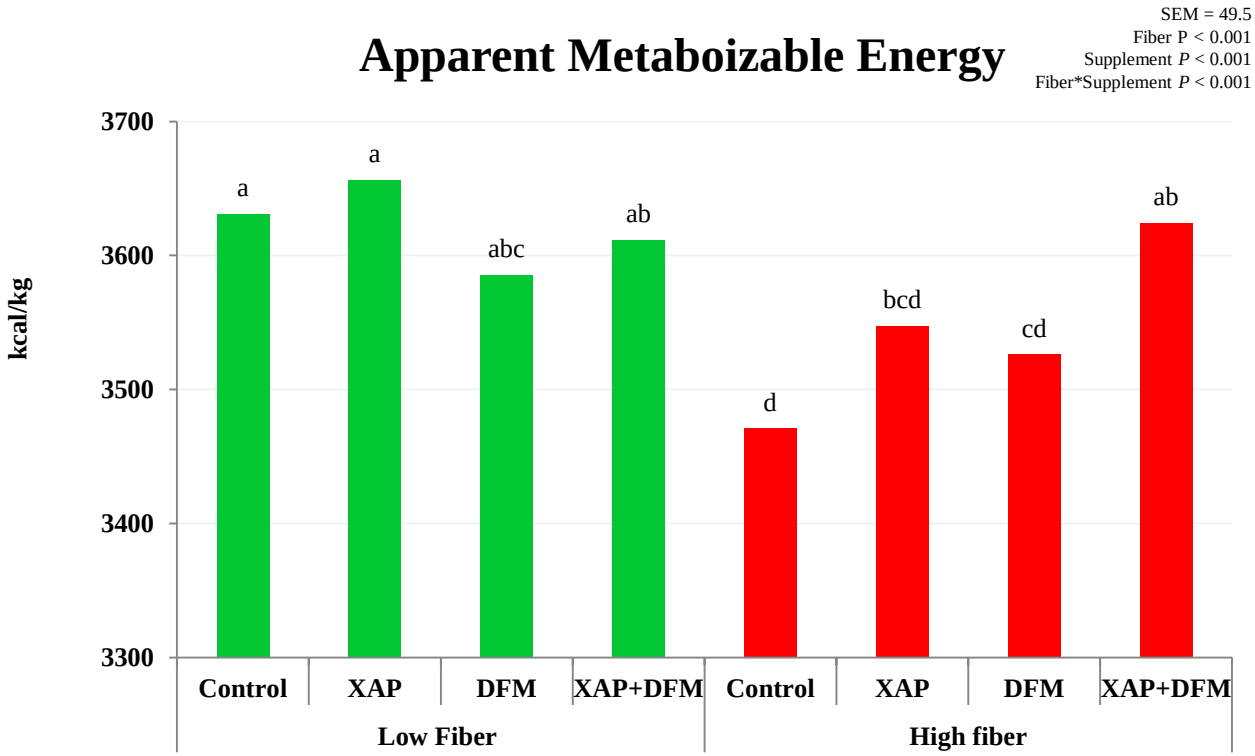
High fiber: wheat middlings + DDGS (3.4% CF)

- NC
- XAP: xylanase, amylase, protease
- DFM: 3 strains of *Bacillus* spp. (75000CFU/g)
- XAP+DFM

• 21d digestibility study

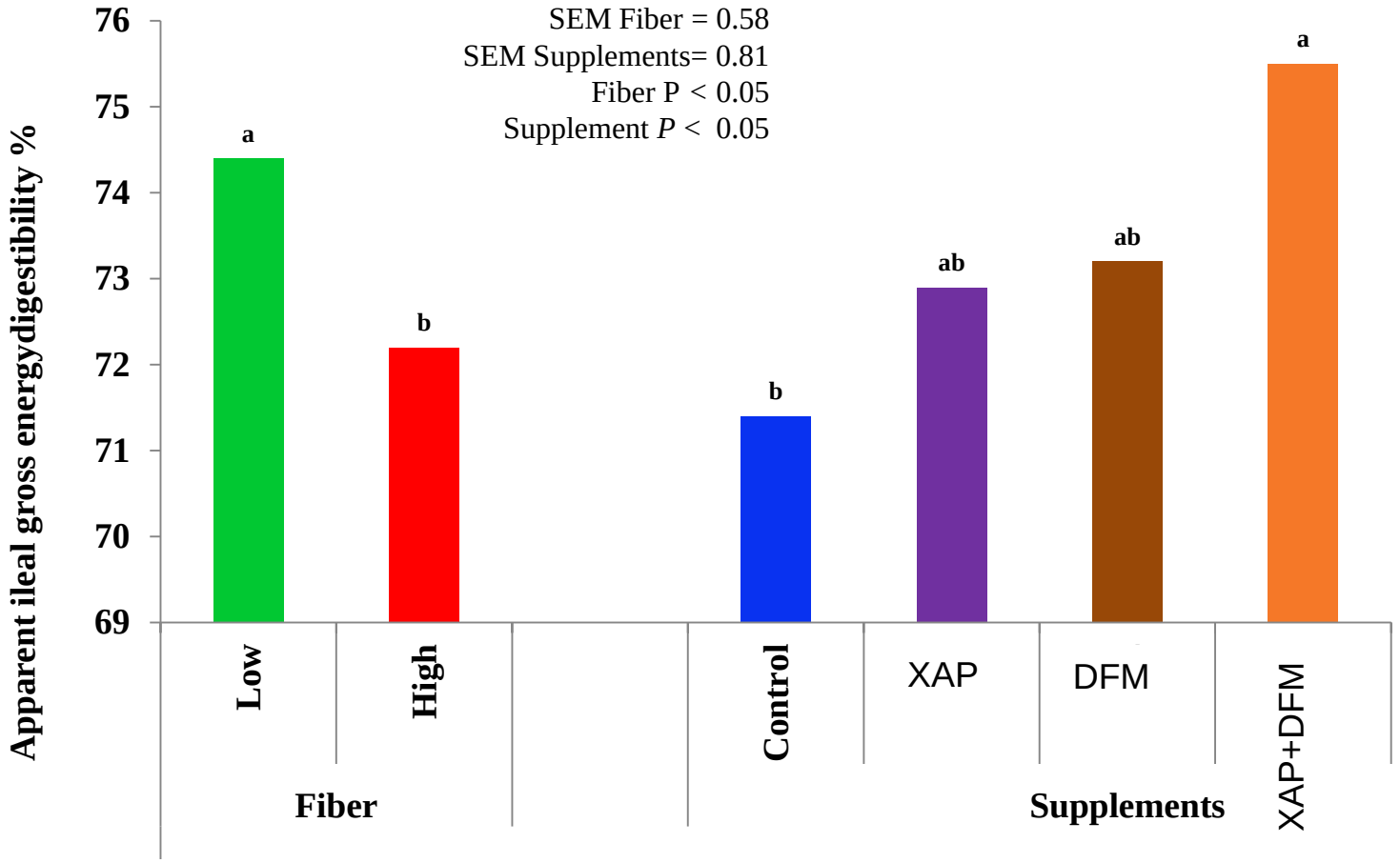
• Cobb 500 broilers

• 8 replicate (x 6 birds/cage)



Singh *et al*. Poult. Sci. 94 (E-Suppl. 1): 9 (2015)

Apparent ileal gross energy digestibility



- High fiber reduced ileal energy digestibility
- XAP+DFM improved ileal energy digestibility vs control (P<0.05)

Singh *et al*. (2015)

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- **Immune response under challenge**
- Compatible with AGPs
- Animal welfare

202 Effect of multi-enzymes and DFM combination on performance, intestinal histology and immune response of broilers with or without a coccidia challenge. Yueming Dersjant-Li*[1], Ajay Awati[1], Kirsty Kemmett[1], and Kirk C. Klasing[2], *[1]Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK, [2]Department of Animal Science, University of California Davis,, Davis, CA.*

PSA, 2015

Background:

- Coccidiosis and necrotic enteritis (NE) are two enteric poultry diseases
- NE affects up to 40% of commercial broiler flocks and cost approximately 5¢ per broiler in the United States (McDevitt et al., 2006)
- Avian coccidiosis, caused by the intracellular parasite *Eimeria,* was estimated to contribute to annual loss of more than $3 billion worldwide (Williams, 1999)

Functionality of the gastro-intestinal tract (GIT)

- Maintaining gut “health” requires ≈ 20% of dietary energy

- ↑ maintenance requirement of GIT “health” and barrier capacity

↓

reduced production

- GIT responds to many “challenges” through changes to:
  - Innate immune responsiveness (including inflammation and acute phase response)
  - Other responses

Challenge model:

- High fiber diet

+

- A mild coccidial challenge:

at day 5 challenged birds received 6-fold Advent® coccidiosis vaccine

↓

- Induced a necrotic enteritis-like syndrome

(Klasing et al., 2002, Journal of Nutrition 132, 2274-2282)

Basal diet composition

Ingredients	g/kg
Corn	363.2
Wheat	**199.6**
Rye	**80**
Wheat middlings	**37.3**
Soybean meal	209.8
Poultry meal	80
L-lysine HCl	2.70
DL-methionine	2.40
L-threonine	0.56
Salt	2.80
Isoleucine	0.06
Limestone	10
DCP	3.1
Choline chloride	0.62
Phytase and cornstartch	1.0
Vitamins and trace minerals premix	7.0
Calculated nutrient composition	
Crude protein (%)	22.4
ME kcal.kg^{-1}	2910
Lysine (%)	1.30
Digestible lysine (%)	1.19
Methionine (%)	0.58
Methionine + cysteine (%)	0.97
Fat (%)	3.29

Trial design

4 Treatments with 8 replications (6 birds/pen), 2 x 2 factorial arrangement:

Un-challenge		Challenge	
Control (UC)	**UC + XAP*+DFM****	**Control (CC)**	**CC + XAP+DFM**

* XAP: xylanase, amylase, protease

** DFM: 3 strains of *Bacillus* spp. (75000CFU/g)

Animals: Cobb 500, 0-21d raised on built up litter

Feeding: *ad lib* in mash form

Statistics: 2 x 2 factorial analysis on main effect and interactions; comparison of treatment means

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* The enzymes consisted of an endo-xylanase from *Trichoderma reesei*, alpha-amylase from *Bacillus licheniformis* and serine protease from *B. subtilis* (XAP). The DFM contained a combination of spores from three strains of *Bacillus spp.*

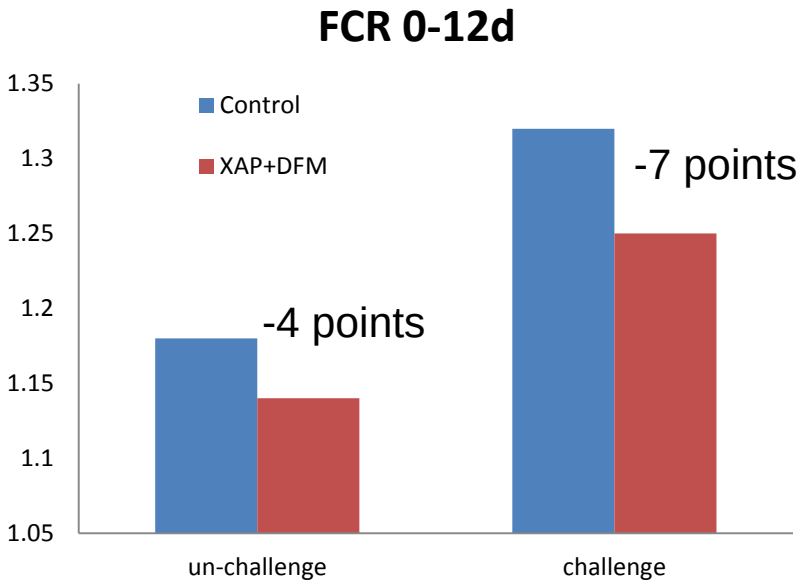
Performance 0-12 day

Un-challenge:

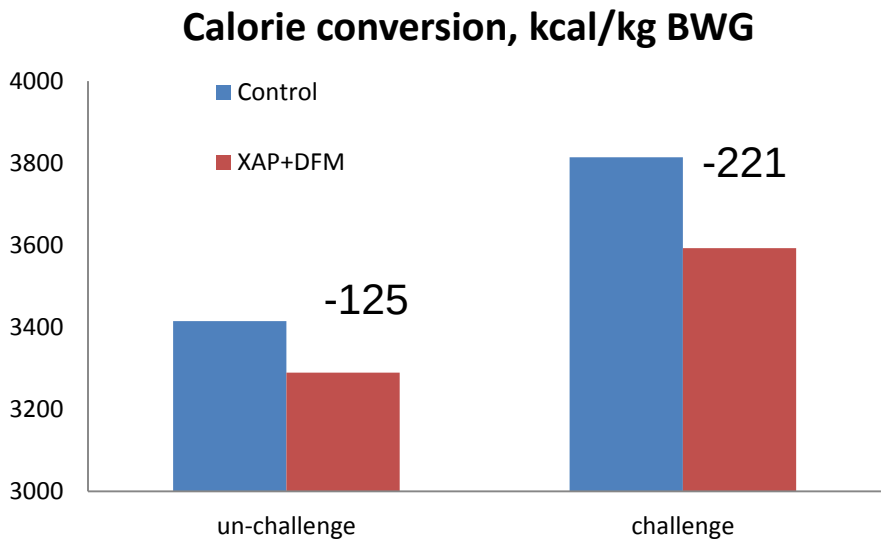
- XAP+DFM improved 3.4% BWG
- Reduced 3.5% FCR
- Used125 kcal less energy per kg BWG

Challenge:

- XAP+DFM improved 8% BWG
- Reduced 5.8% FCR
- Used 221kcal less energy per kg BWG



SEM = 0.018; P<0.01



SEM = 51; P<0.01

Performance: 0-21 day

1-21d	Gain g/b/d	FI g/b/d	FCR	Cal conversion (kcal/kg BWG)
UC	34.1 ab	44.4 a	1.30 b	3754 c
UC + XAP+DFM	35.2 a	43.4 a	1.24 c	3562 d
CC	32.0 c	44.9 a	1.40 a	4051 a
CC+ XAP + DFM	33.1 bc	44.2 a	1.33 b	3852 b
SEM	0.51	0.79	0.012	33.3
P Treatment	0.04	0.31	<0.0001	<0.0001
P Challenge	0.0003	0.40	<0.0001	<0.0001

[a, b]: different superscript in a column indicates significant difference at $P < 0.05$;

UC: un-challenged control; CC: challenged control;

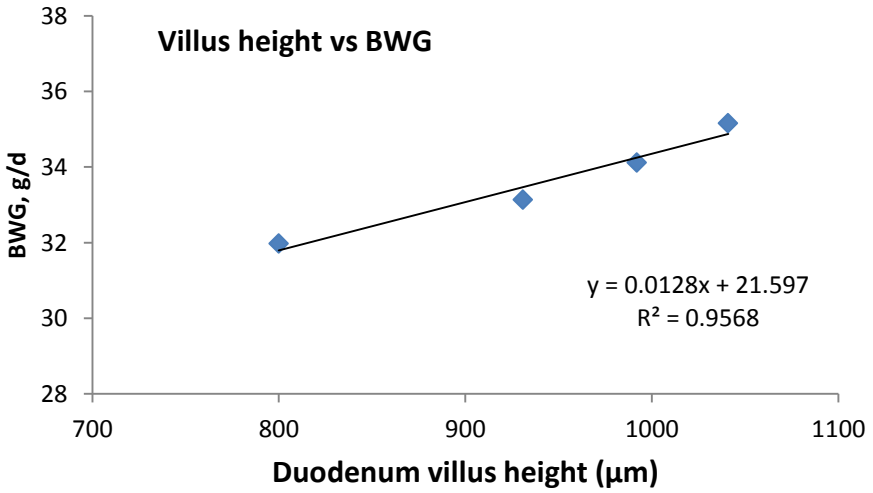
- **CC + XAP+DFM maintained performance comparable to UC**

Intestinal morphology (duodenum) day 12

	Villus height (µm)		IE lymphocytes (number)		Lamina Propria (µm)	
UC	**992**	**ab**	**42.9**	**b**	**19.3**	**b**
UC + XAP+DFM	**1041**	**a**	**42.9**	**b**	**21.1**	**ab**
CC	**800**	**b**	**61**	**a**	**27.8**	**a**
CC+ XAP + DFM	**931**	**ab**	**50.5**	**ab**	**20.8**	**ab**
SEM	68.8		3.88		2.63	
P Treatment	0.20		0.20		0.34	
P Challenge	0.037		0.002		0.13	

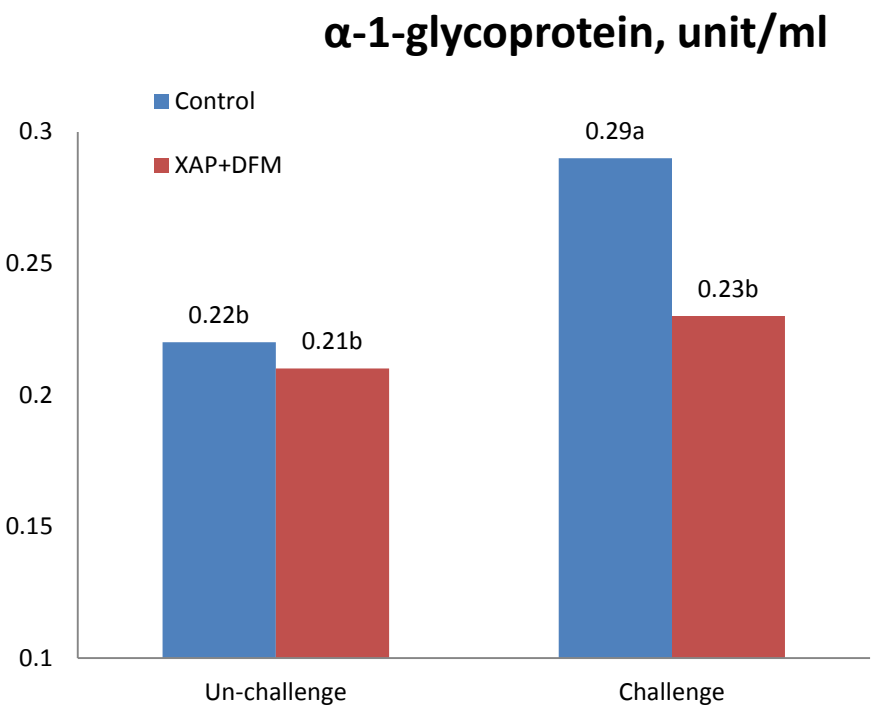
[a, b]: $P < 0.05$;
UC: un-challenged control;
CC: challenged control;

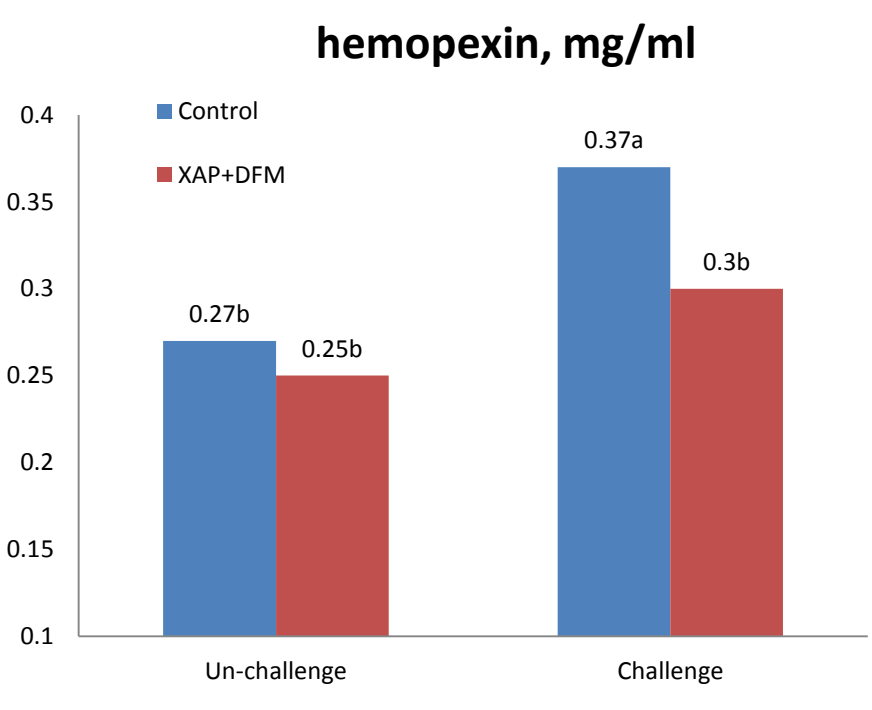
- **CC+ XAP+DFM maintained VH, IE (intraepithelial) lymphocytes and Lamina Propria level comparable to UC**



Plasma acute phase proteins (APP) day 12

7 days post challenge





SEM = 0.013; P < 0.05

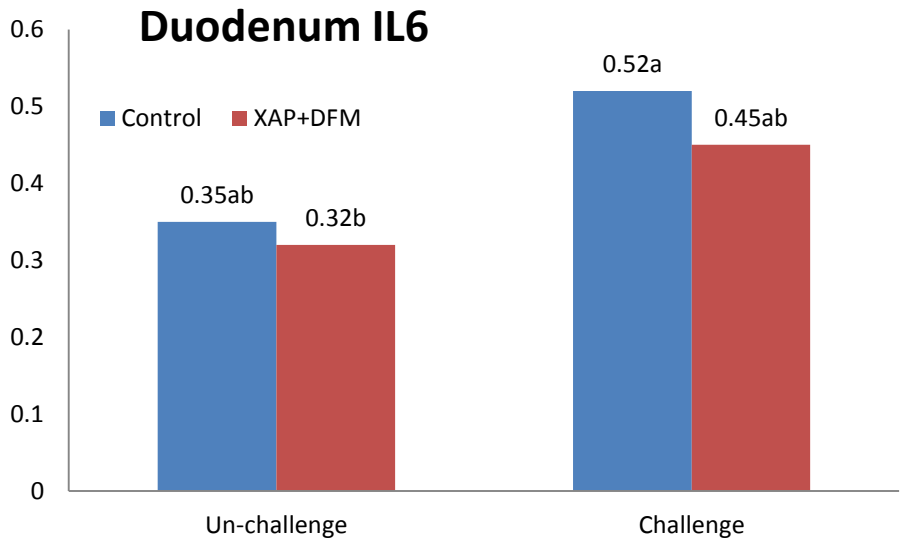
SEM = 0.025; P < 0.05

- **Challenged control (CC) increased APP at day 12, 7 days post challenge, indicating inflammatory response**
- **CC + XAP+DFM reduced APP to the level comparable to unchallenged control**

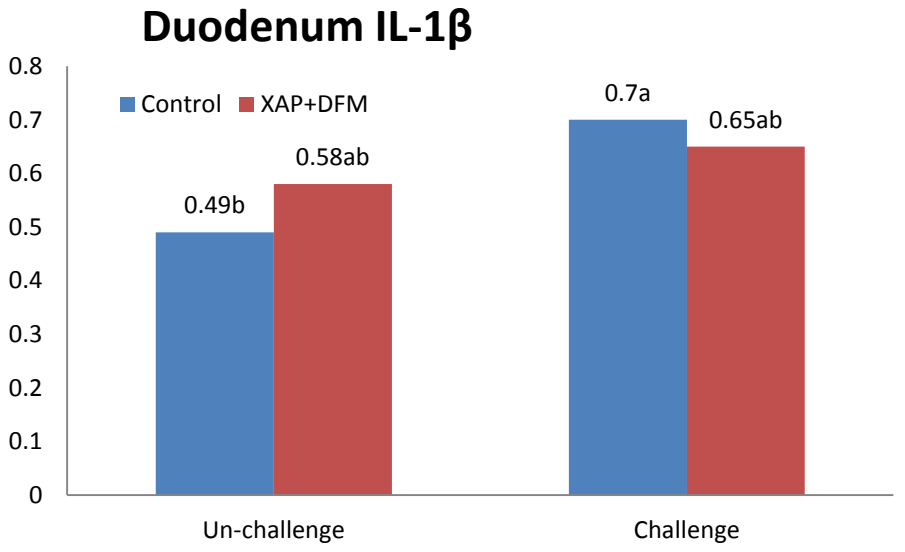
Intestinal mucosal cytokines, day 12

7 days post challenge

- IL-6 and IL-1β are the pro-inflammatory cytokines
- CC increased IL-6 and IL-1β at 7 days post challenge
- XAP+DFM limited the level of inflammation
- Reduced the negative effects of inflammation on performance/gut function
- Which was correlated with reduced heterophil in circulation



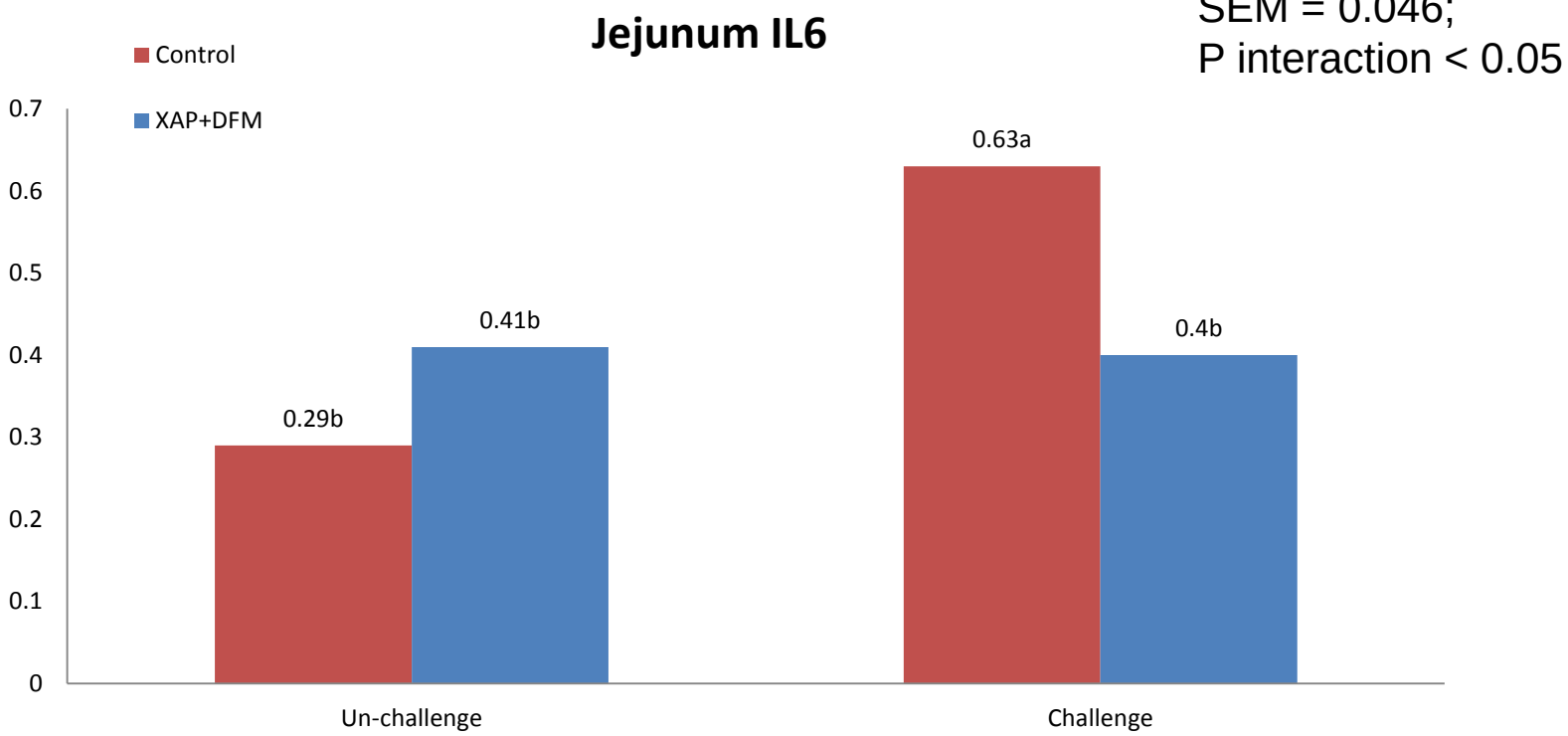
SEM = 0.059; P < 0.05



SEM = 0.059; P < 0.05

Intestinal mucosal cytokines, day 21

16 days post challenge

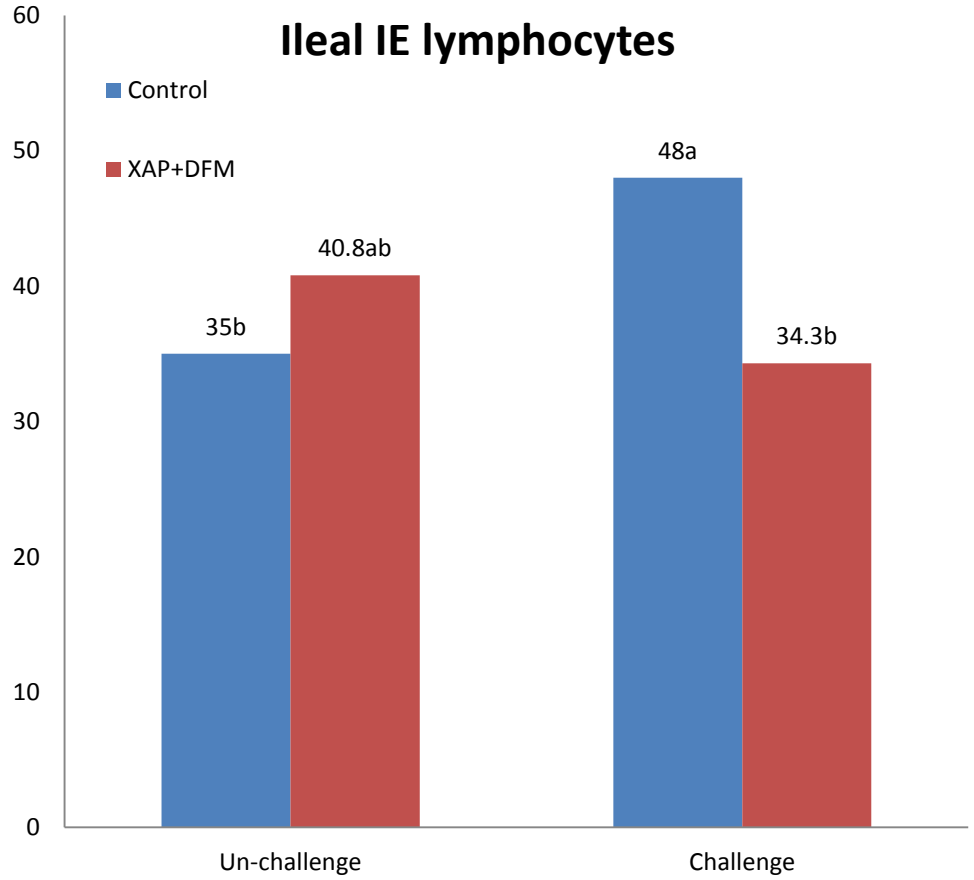


- **At 16 days post challenge, inflammatory response still observed in CC in jejunum, indicating a prolonged effect**
- **CC+ XAP+DFM significantly reduced jejunum IL6 vs CC**
- **CC+XAP+DFM limited the prolonged inflammatory response, promoting the restoration of homeostasis and gut balance**

Challenge X Treatment Interaction

day 12:

- Test x challenge interaction was found for ileal intraepithelial lymphocytes (IEL)
- CC + XAP+DFM significantly reduced IEL vs CC
- UC + XAP+DFM numerically increased IEL
- Indicating reduced inflammatory response under challenge



SEM = 3.48; P interaction < 0.05

Summary

- **Xylanase, amylase and protease + *Bacillus* strain improved feed efficiency under both non-challenged and moderate NE-like challenged situation**
- **XAP + *Bacillus* strain was more effective under challenge (higher improvement at day 12 , similar improvement at day 21)**
- **Challenge induced inflammatory responses: increased IL6, APP, lymphocytes, reduced Villus Height and increased crypt depth**
- **Addition of XAP + *Bacillus* strain reduced inflammatory response, maintained gut integrity under challenge**
- **Reduced energy cost for immune response under challenge and improved energy efficiency**

Outline of the presentation

- Introduction: synergy between enzymes and DFM
- Nutrients digestibility
- Immune response under challenge
- **Compatible with antibiotic growth promoters (AGPs)**
- Animal welfare


China to ban antibiotics as growth promoters
Estimated 400,000 tons of antibiotics produced annually
Release Date: 2011-09-13
China's Ministry of Agriculture has announced a forthcoming ban on antibiotics as growth promoters in animal feed.
BBC
NEWS
Sign in
News
Sport
Weather
Home
Video
World
UK
Business
Tech
Science
Magazine
McDonald's to cut use of antibiotics in chicken
5 March 2015
Business
BIG MOVE: CHICAGO SCHOOLS TO BUY ANTIBIOTIC-FREE CHICKEN
ANALYSIS | Health Canada's quiet move to end use of antibiotics to fatten up animals
Still loopholes as Ottawa takes tiny step to curb antibiotic use in livestock
By Kelly Crowe, CBC News
Posted: Jul 09, 2014 11:23 AM ET
Last Updated:
CNN Money
Business
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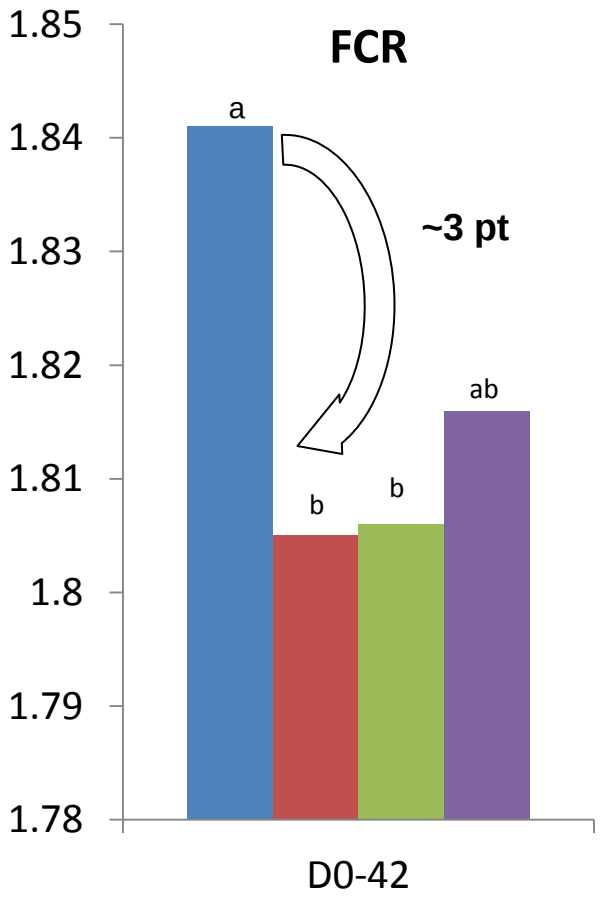
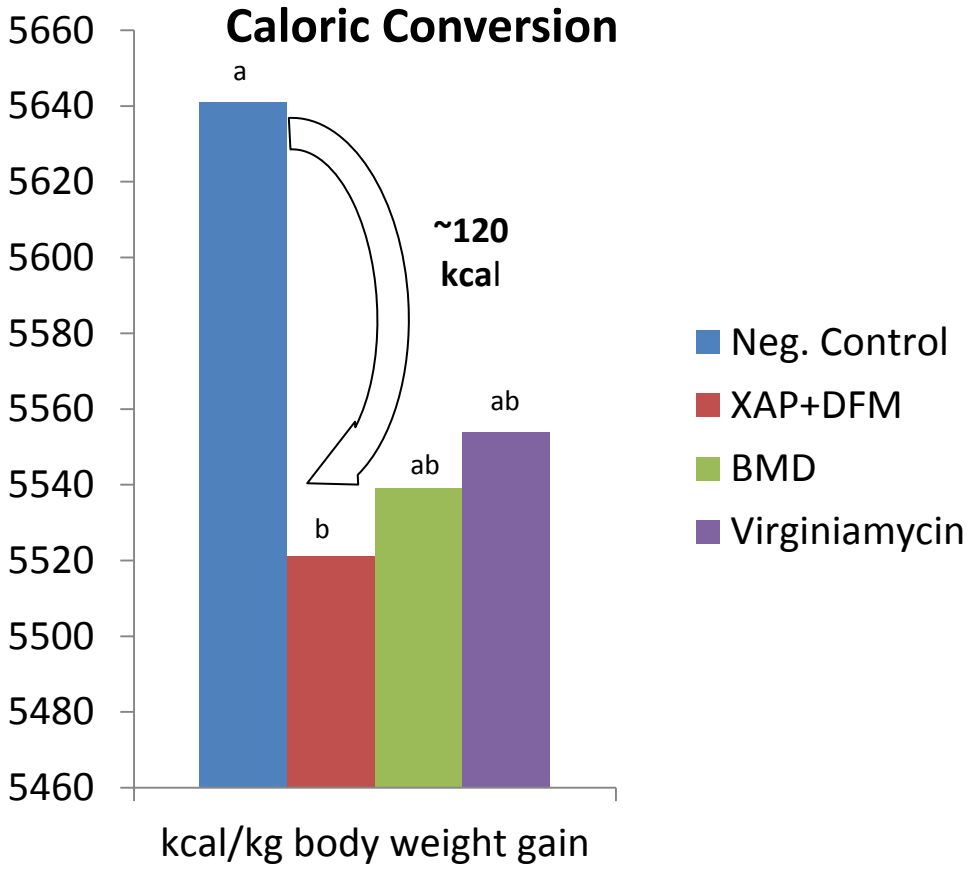
Tyson to phase out antibiotics in chicken
Perdue Farms eliminates antibiotic use
www.latimes.com/.../la-fi-perdue-antibiotics-201
Sep 3, 2014 - The company eliminated antibiotic use to
2007. Perdue said 95% of its chickens are now free of so-called

XAP+DFM as alternative to AGPs





Flores *et al*., 2014, Poult. Sci. 93(E-Suppl. 1): 19

XAP+ DFM in broiler diets with/without AGPs

Trial design:

- Cobb 500 male broilers, 9 replications (40 birds/pen) using built up litter
- 6 treatments, 1-42 days
  - Control 1 (C1)
  - C1 + XAP+ DFM (xylanase, amylase and protease and *Bacillus* DFM)
  - Control 2 (C2 =C1+BMD (Bacitracin Methylene Disalicylate, 55g/ton of feed))
  - C2 + XAP+ DFM
  - Control 3 (C3 = C1+ virginiamycin, 22g/tonne of feed)
  - C3 + XAP+ DFM

Diets:

- Control diet based on corn/soybean meal and 10% wheat containing 500 FTU/kg phytase
- Fed *ad-libitum* in pelleted form in three phases: 1-10 (crumble); 11-21 and 22-42 days

Flores et al., 2015Poult. Sci. 94 (E-Suppl. 1): 8

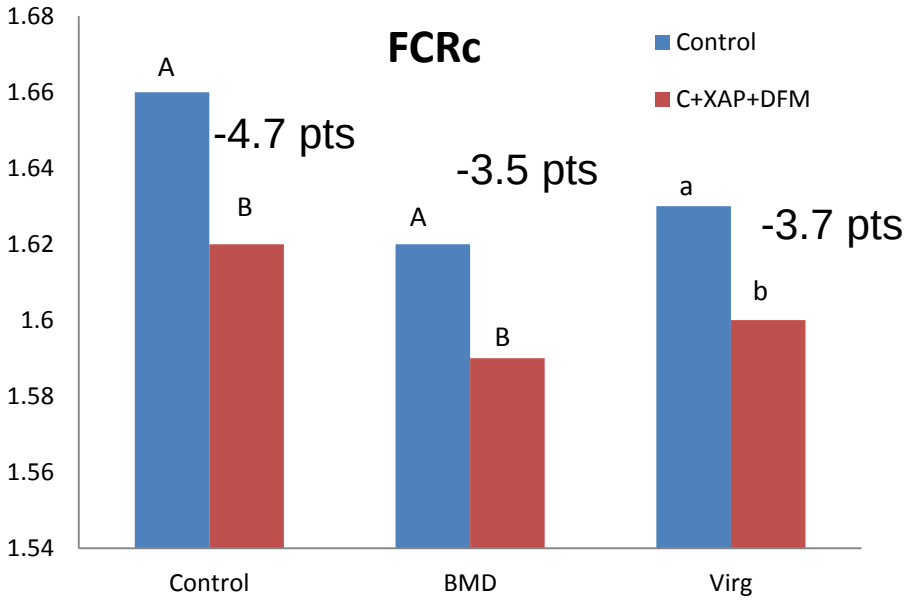
Feed composition, %

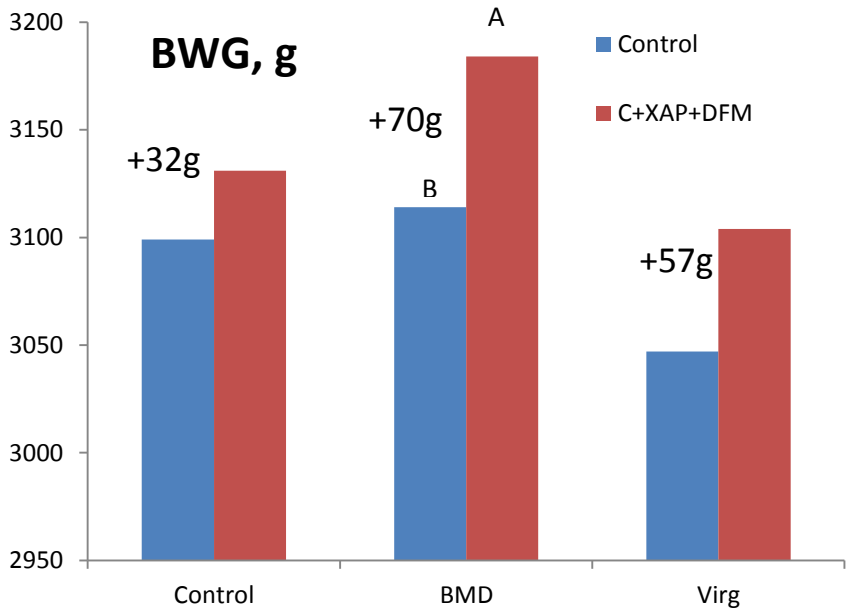
	Starter	Grower	Finisher
Corn	**47.85**	**52.88**	**60.03**
Soybean Meal	**31.00**	**24.78**	**17.99**
DL - Methionine	0.31	0.27	0.20
Lysine HCL	0.29	0.25	0.21
L - Threonine	0.10	0.08	0.05
Fat, Blended	0.61	1.75	1.49
Wheat	10.00	10.00	10.00
Limestone	1.35	0.89	0.68
Monocalcium Phosphate	0.65	0.33	--
Salt	0.34	0.28	0.10
Sodium Bicarbonate	--	0.03	0.28
Trace Minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
Choline	0.10	0.10	0.10
Coban 90[3]	0.05	0.05	0.05
LO - DDGS	5.00	5.00	5.00
MBM	2.04	3.00	3.52
Phytase[4]	**0.01**	**0.01**	**0.01**
Analyzed Nutrient %[5]	**Starter**	**Grower**	**Finisher**
Moisture	12.98	12.25	12.49
Dry Matter	87.02	87.75	87.51
Crude Protein	21.7	19.7	18.9
Crude Fat	4.60	4.86	4.98
Fiber	3.4	3.6	3.1
Ash	4.88	4.68	4.47

[1] All diets included phytase at 500 FTU/kg feed

[2] Test materials provide 2000U xylanase, 200U amylase and 4000U protease/kg feed and 75000cfu/g feed of 3 *Bacillus strains*

Comparison of treatment means





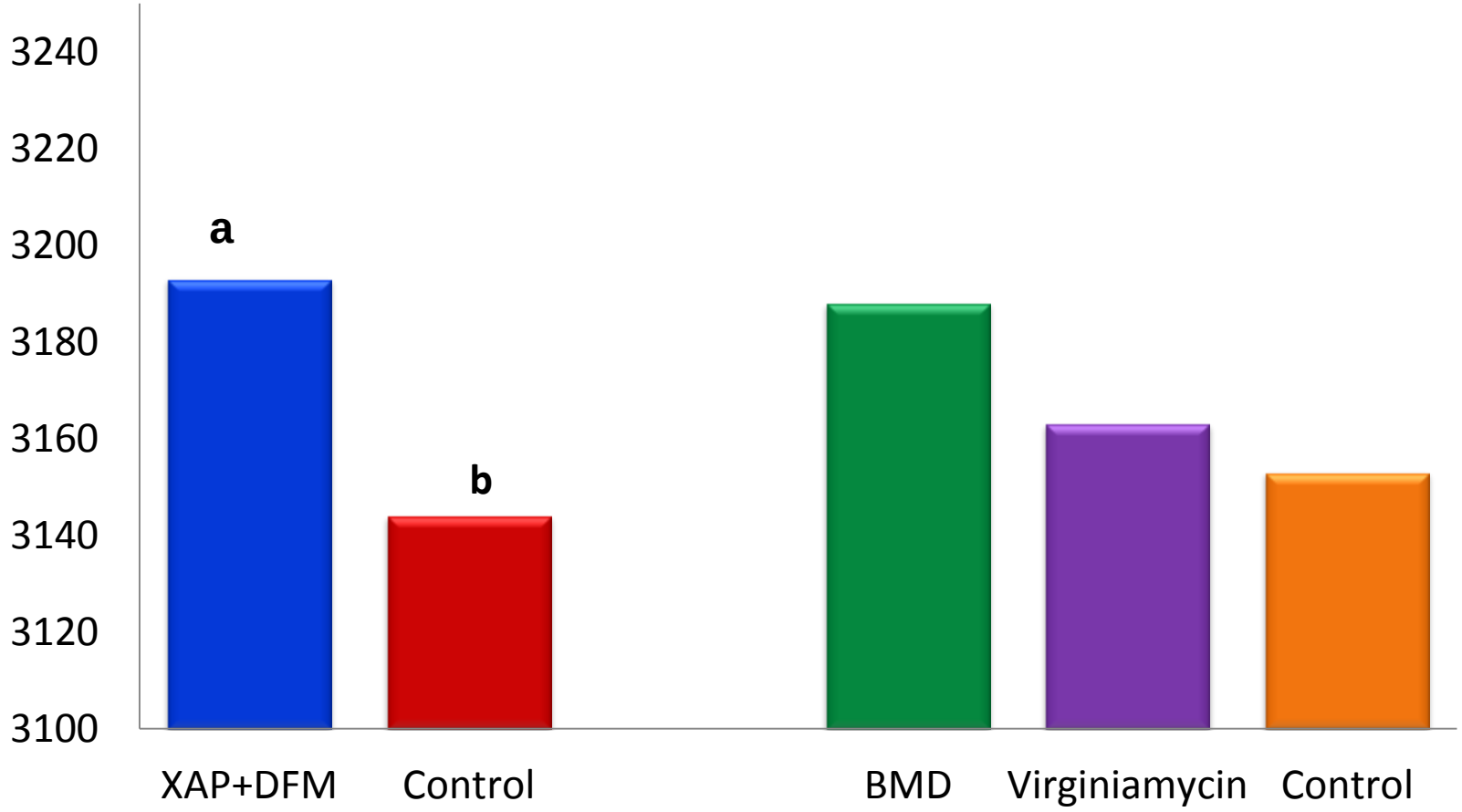
^{AB} = P<0.10; ^{ab} = P<0.05 (paired comparison)

FCRc: Body weight corrected FCR, 3 points correction per 100g BW vs C1

Pair comparison, P values				
	BWG, g	FCR	FCRc	ADFI, g
C1 vs XAP+DFM	0.49	0.05	0.07	0.26
BMD vs BMD+ XAP+DFM	0.08	0.18	0.09	0.99
Virg vs Virg + XAP+DFM	0.15	0.02	0.02	0.86

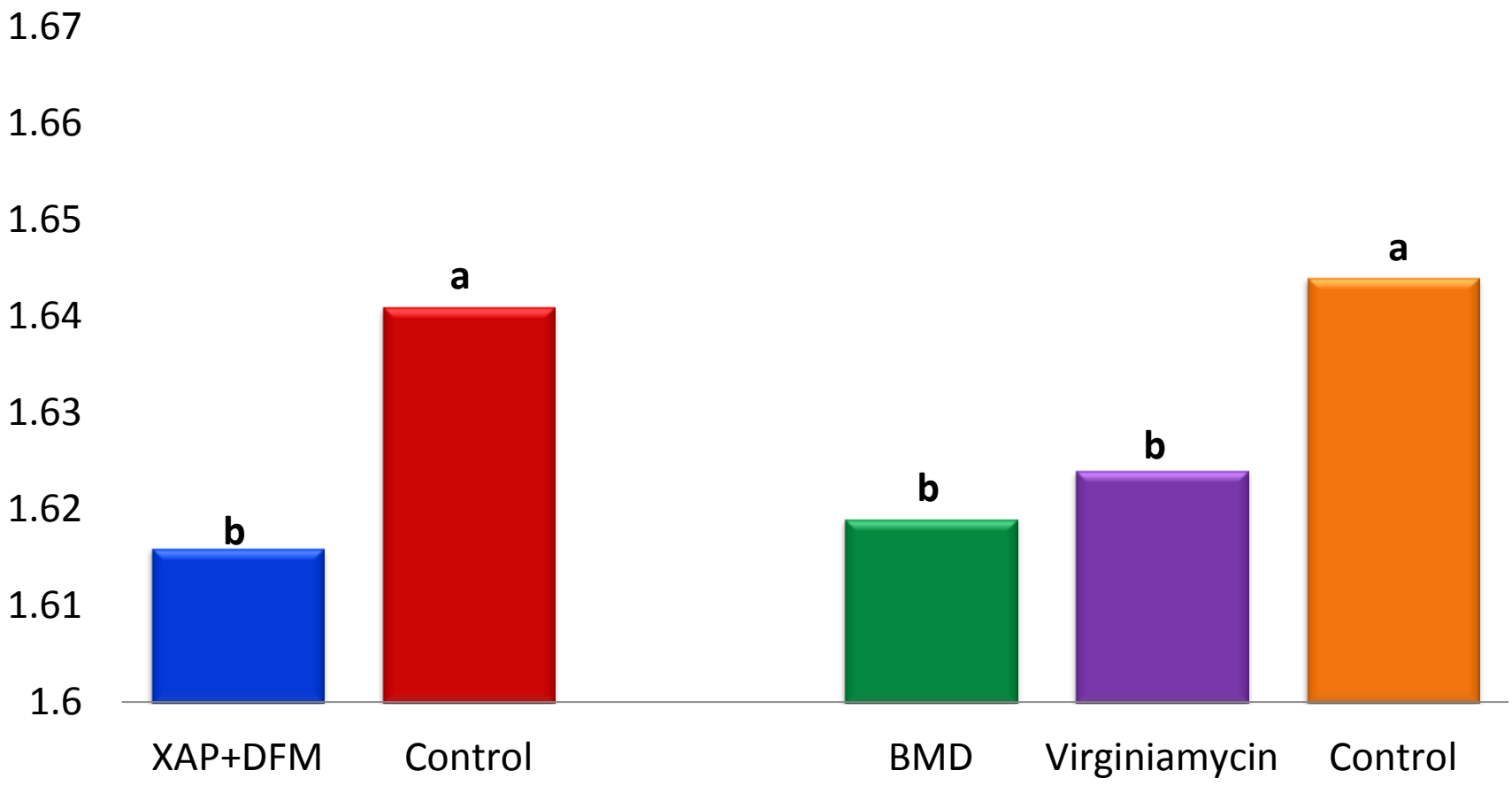
Virg: virginiamycin

Main Effects: Body Weight (g) – Day 42



[ab] Superscripts indicate significant differences between treatments (P< 0.05)

Main Effects: Feed Conversion Ratio – Day 0-42



[ab] Superscripts indicate significant differences between treatments

Summary

- XAP + *Bacillus* DFM significantly improved BW, average daily gain, and reduced FCR compared to the control
- Significant reduction in FCR with AGP inclusion compared to non-medicated treatment
- XAP+ *Bacillus* DFM can be used as alternative to AGPs or added on top of AGPs

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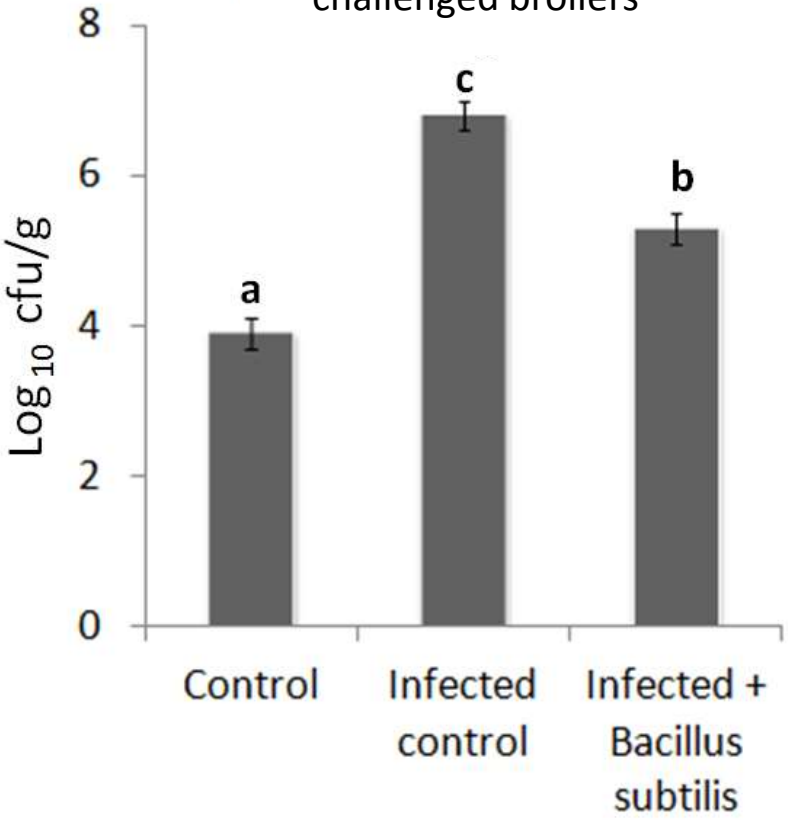
- Nutrients digestibility
- Immune response under challenge
- Compatible with AGPs
- **Animal welfare and economic benefit**

XAP+DFMs reduced pathogenic bacterial counts

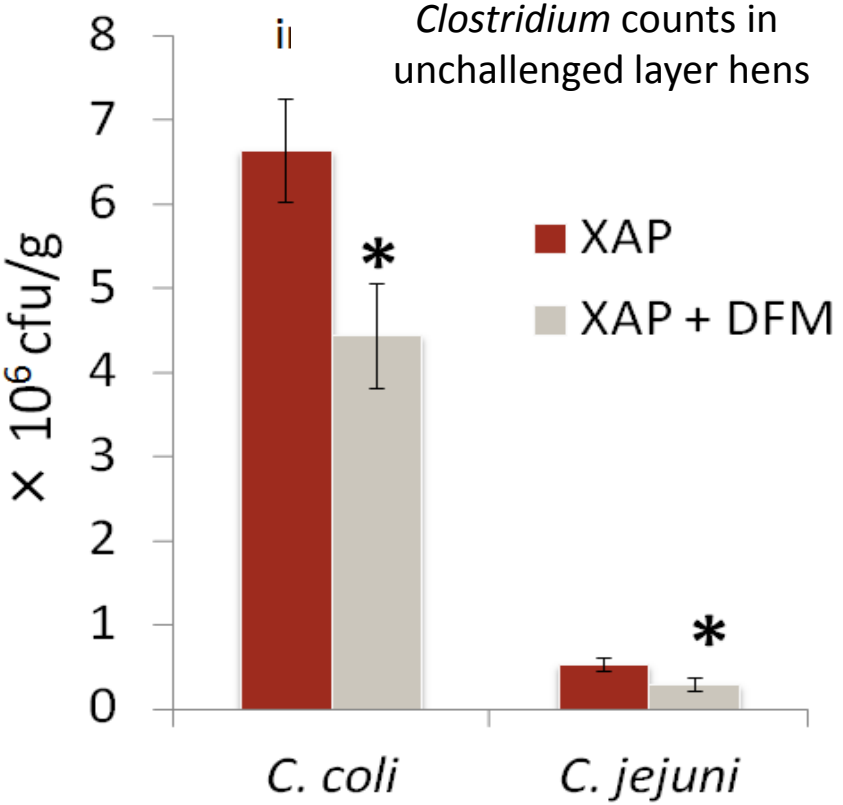
Clostridium perfringens counts in challenged broilers



[abc] $P < 0.05$

Jayaraman *et al.*, 2013

Clostridium counts in unchallenged layer hens



*$P < 0.05$

XAP: xylanase, amylase, protease

DFM: 3 strains of *Bacillus subtilis*

Murugesan *et al.*, 2013

Effect of XAP and *Bacillus* DFM combination on animal performance under commercial settings

- Trial in NL
- Male broiler Ross 308
- Commercial diet (wheat/corn/SBM)
- Containing Salinomycin
- 5 replications/tr
- 700 broilers/rep
- Treatments:
  - Control (500 FTU/kg phytase)
  - C + *Bacillus* + XAP

	Control	DFM+XAP	P
Day 0-21			
Body weight gain (g)	781	774	0.41
Feed intake (g)	1102.9	1071.3	0.07
FCR	**1.41^{a}**	**1.38^{b}**	**0.015**
Calorie conversion	**4149.1^{a}**	**4066.7^{b}**	**0.015**
Day 0-42			
Body weight gain (g)	2328^{b}	2433^{a}	0.04
Feed intake (g)	4063^{b}	4166^{a}	0.003
FCR	**1.75**	**1.71**	**0.22**
Calorie conversion	**5302.6**	**5205.3**	**0.144**
Mort, %	**4.11**	**3.71**	**0.6**
Production Efficiency Factor	296	315	

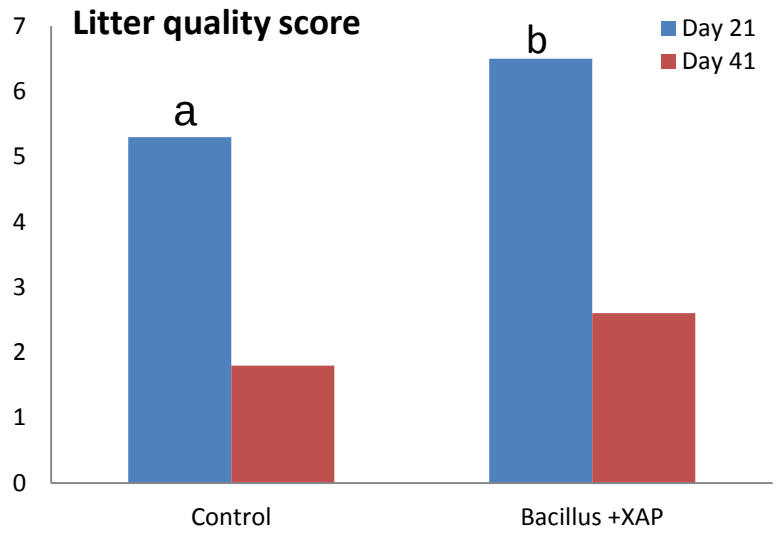
Calorie conversion: kcal /kg BWG

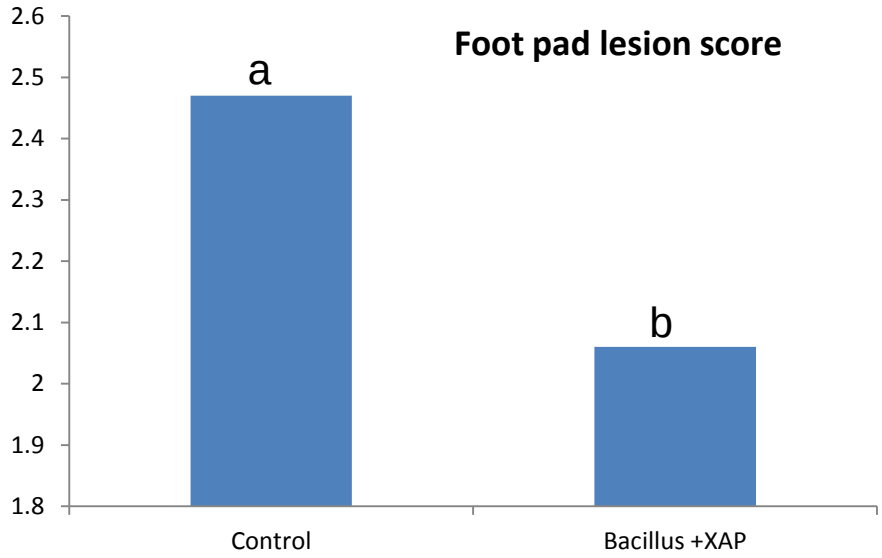
XAP+DFM resulted in 100 kcal saving per kg BWG, indicating economic benefits

Effect of XAP and *Bacillus* DFM combination on animal welfare parameters under commercial settings – trial in NL

Litter score 0-10:
0: wet;
10: dry & friable

Day 42





Dersjant-Li *et al*., 2015, J. Appl. Poult. Res. 00:1–11

Take home message

- Maintaining ‘gut health’ is important for production efficiency
- Xylanase, amylase and protease enzymes and *Bacillus* DFM can help to maintain ‘gut integrity’
- The XAP + *Bacillus* DFM combination is more effective than used individualy, espacilly under challenge
- Using XAP+ *Bacillus* DFM can result in economic benefit in broilers production

Thank you for your time

Questions

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