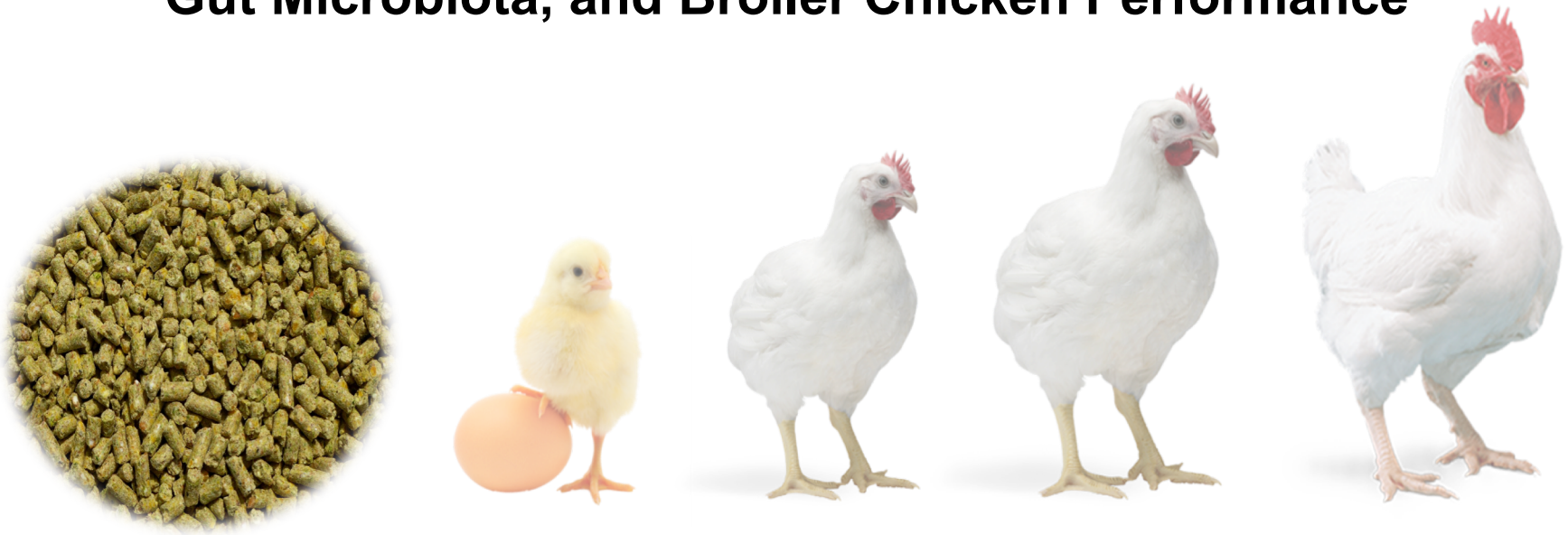
“Application of Glycoside Hydrolase Family 43 (GH43) in Broiler Feed: A Potential Approach to Improve Digestibility, Gut Microbiota, and Broiler Chicken Performance”



Presented by **Mr. Virak Sorn** **ID: 60110700502**

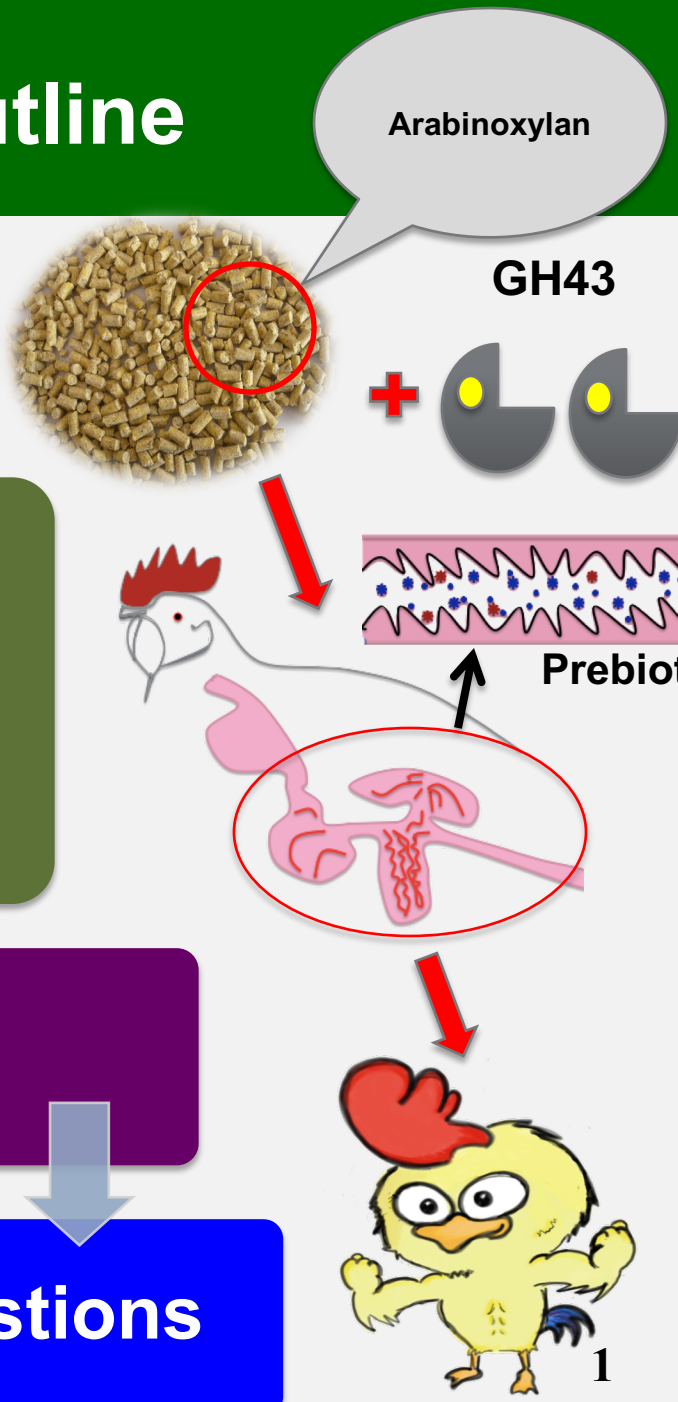
Presentation Outline

Introduction

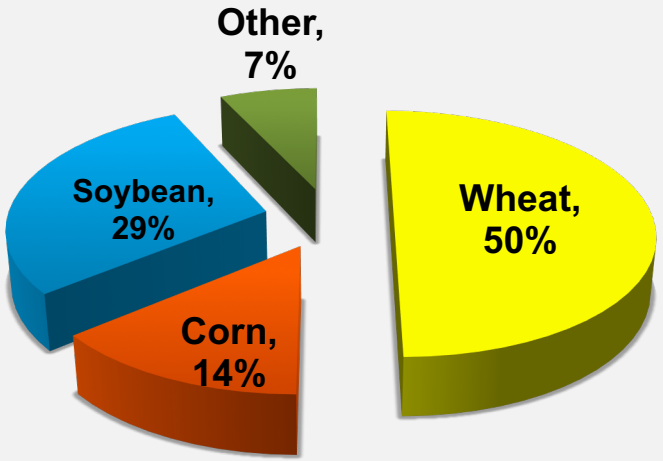
- Problem statement
- Prebiotics
- Structure of xylan
- Glycoside hydrolases family 43 (GH43)

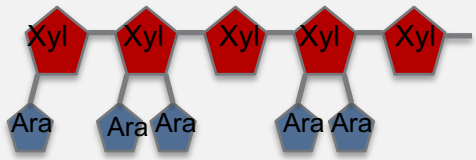
Functions of GH43

Conclusion and Suggestions



Chicken Feed Composition





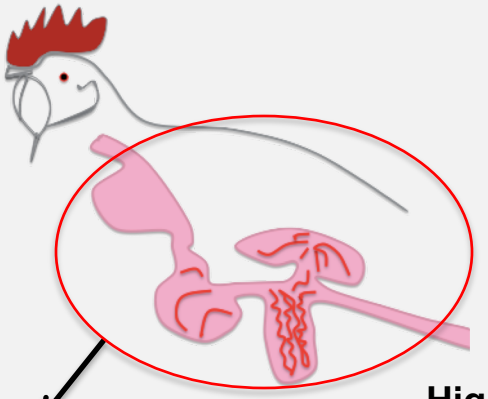
Arabinoxylans

Starch

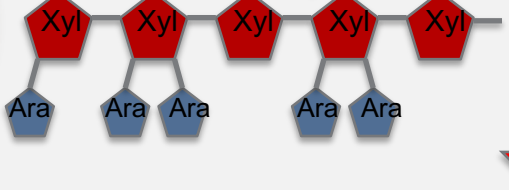
Ref: Lakna, 2017 and Sinha et al., 2012

Problem Statement

Feed



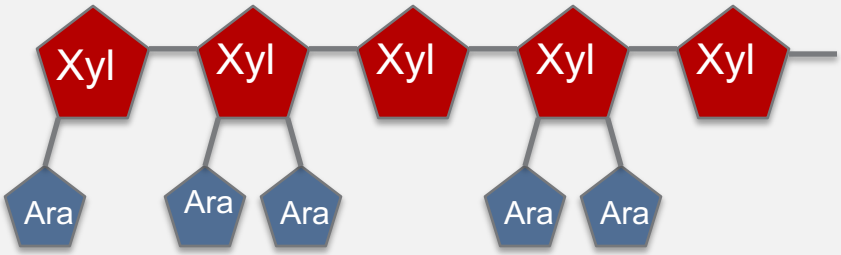
High viscosity



Unhappy
Sick

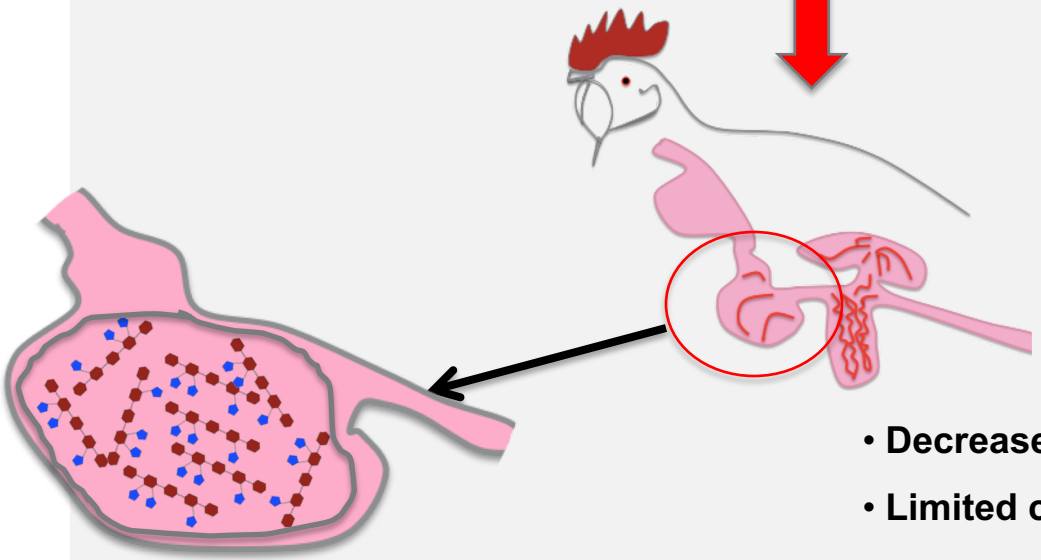
Arabinoxylan (high viscosity) course of poor growth performance

Problem Statement (Cont.)



Arabinoxylan

Absorb water

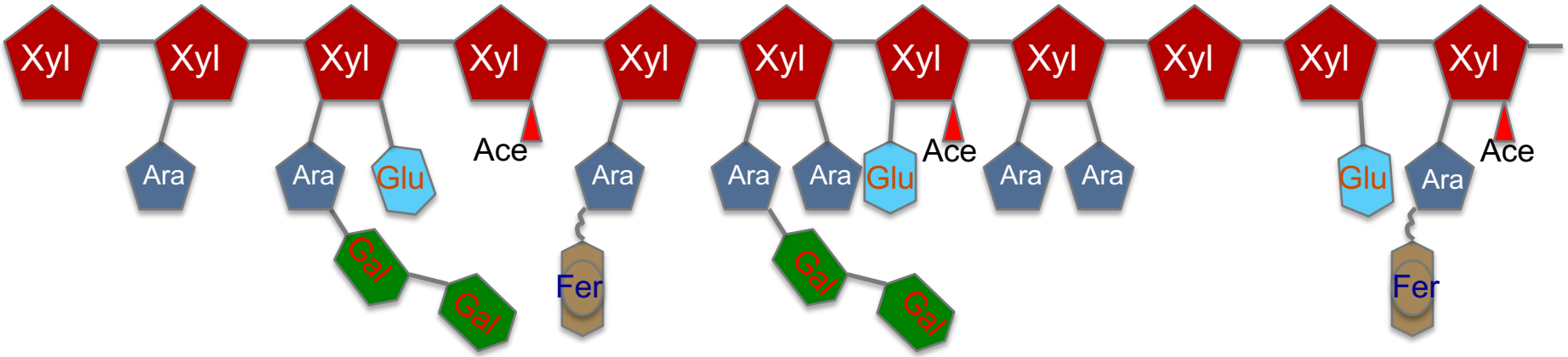


Swelling → **High viscosity** →

- **Decreased transit rate**
- **Limited contact between digestive enzyme and substrate**
- **Increased bacterial fermentation in small intestine**

Poor growth:
Low weigh, low feed intake and high FCR

Complex Structure of Xylan



Xylose/Xyl

Galactose/Gal

Glucuronic acid/Glu

Ferulic acid ester/Fer

Arabinose/Ara

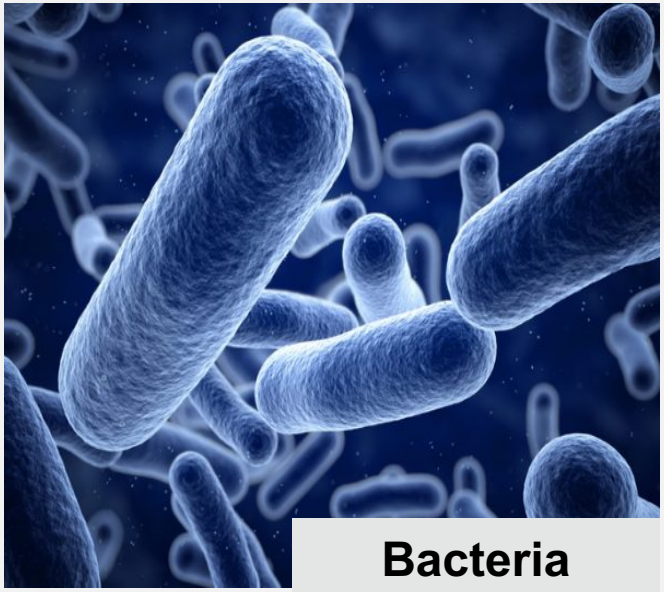
Acetic acid ester/Ace

Catalytic cleavage

Mechlke *et al.*, 2017

What is Glycoside Hydrolase Family 43?

- **Glycoside hydrolase family 43 (GH43) is a group of enzymes that hydrolyze the glycosidic bonds of hemicelluloses.**



Bacteria

Fungi

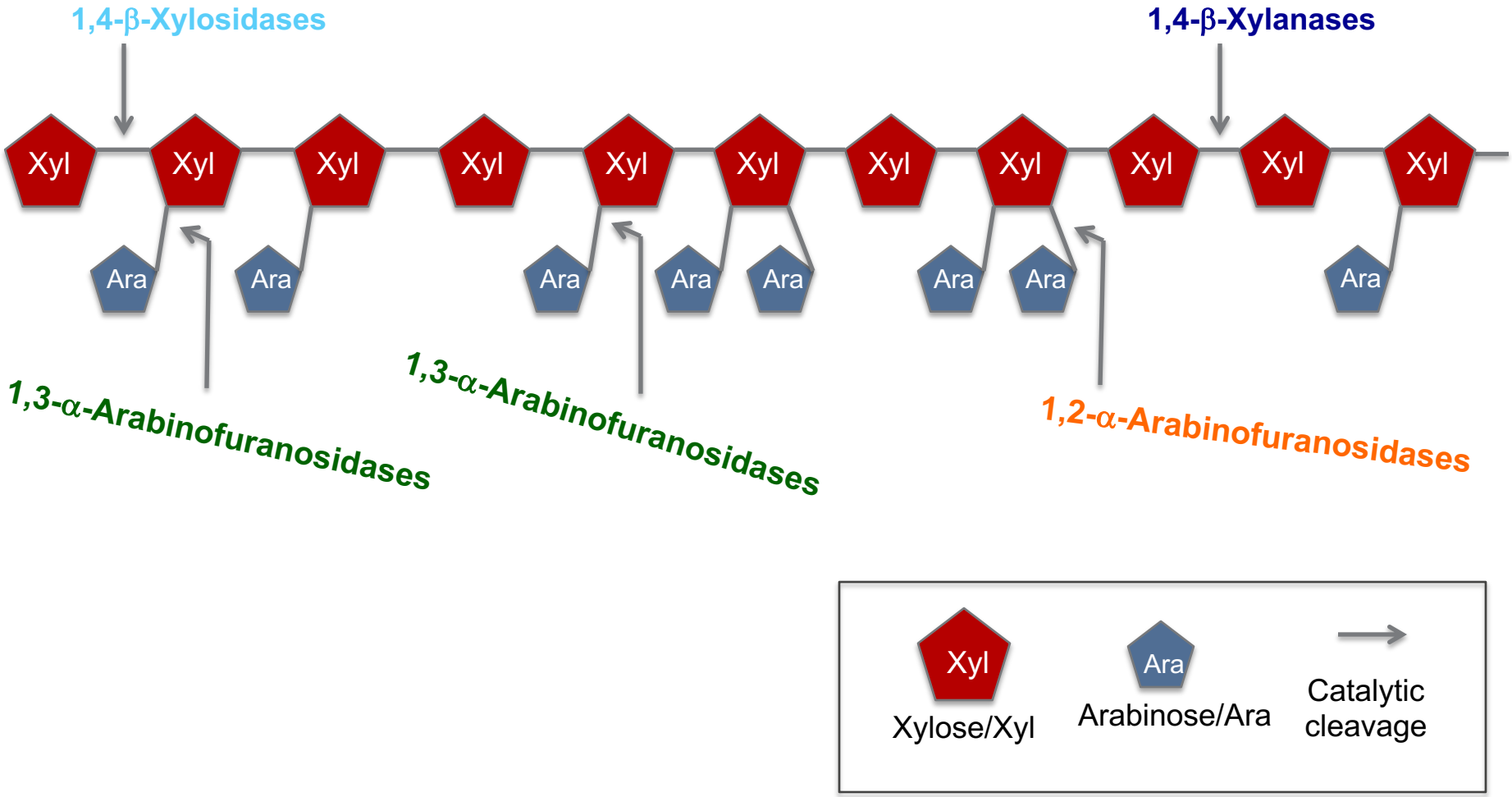
- **We can get GH43 from bacteria and fungi, most of GH43 got from bacteria (de Camargo, *et al*., 2018).**

Barnes, 2017; Oman, 2017; Sierra, 2016

Properties of GH43 from Bacteria and Fungi

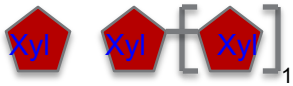
Bacteria and fungi	Enzymes	pH	Tem-	Reference
Geobacillus stearothermophilus	β-xylosidase	**5**	**40 °C**	**Shallom *et al.*, 2005**
Holotrichia parallela	β-xylosidase	3-9 **(6)**	**40 °C**	**Sheng *et al.*, 2014**
Herbinix hemiceellulosilytica	β-xylosidase and arabinofuranosidase	5.5-6.5 **(6)** 5-7 **(6)**	48-64 **(55) °C** 40-55 **(50) °C**	**Mechelke *et al.*, 2017**
Enterobacter sp.	β-xylosidase	5.5-7 **(6)**	**50 °C**	**Campos *et al.*, 2014**
Halothermothrix orenii	Arabinofuranosidase, xylanase & arabinanase	**6.5**	45-68 **(60) °C**	**Hassan *et al.*, 2015**
Clostridium thermocellum B8	Arabinofuranosidase	**5-6**	**50 °C**	**de Camargo *et al.*, 2018**
Humicola insolens	β-xylosidase	5-10 **(6.5-7)**	50-60 **(50) °C**	**Yang *et al.*, 2014**
Trichoderma reesei	Xylosidase and α-arabinofuranosidases	5-9 **(7.5)**	20-70 **(55) °C**	**Matsuzawa *et al.*, 2015**

Wheat Arabinoxylan

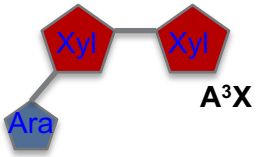


Mechlke *et al.*, 2017

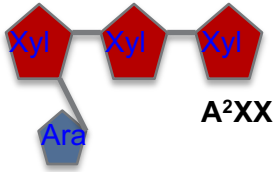
Hydrolysis Products from Arabinoxylan Degradation



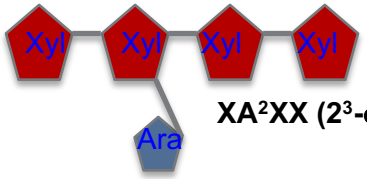
X, X_2-X_5 (Xylose, Xylobiose, -triose, -tetraose, -pentose)



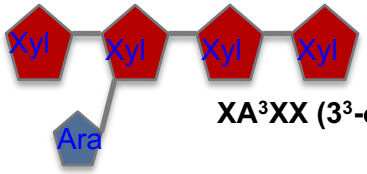
A^3X (3^2-α-L-Arabinofuranosyl-xylobiose)



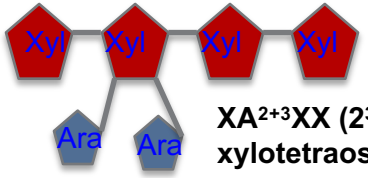
A^2XX (2^3-α-L-Arabinofuranosyl-xylotriose)



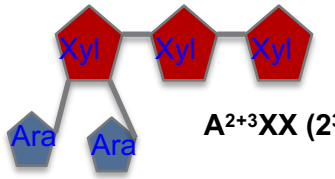
XA^2XX (2^3-α-L-Arabinofuranosyl-xylotetraose)



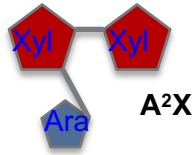
XA^3XX (3^3-α-L-Arabinofuranosyl-xylotetraose)



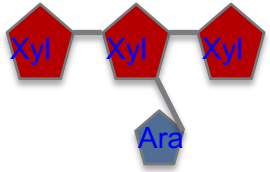
$XA^{2+3}XX$ (2^3, 3^3-di-α-L-Arabinofuranosyl-xylotetraose)



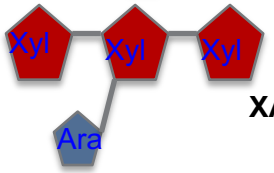
$A^{2+3}XX$ (2^3, 3^3-di-α-L-Arabinofuranosyl-xylotriose)



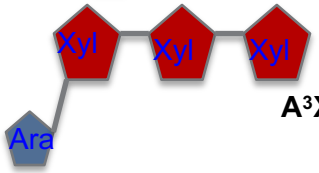
A^2X (2^2-α-L-Arabinofuranosyl-xylobiose)



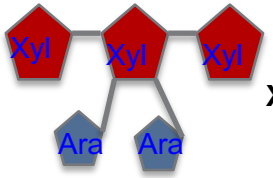
XA^2X (2^2-α-L-Arabinofuranosyl-xylotriose)



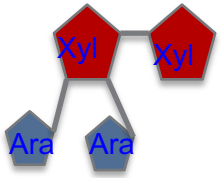
XA^3X (3^2-α-L-Arabinofuranosyl-xylotriose)



A^3XX (3^3-α-L-Arabinofuranosyl-xylotriose)



$XA^{2+3}X$ (2^2, 3^3-α-L-Arabinofuranosyl-xylotriose)



$A^{2+3}X$ (2^2, 3^2-α-L-Arabinofuranosyl-xylobiose)

Mechlke *et al.,* 2017, Lei et al., 2016

Prebiotics

- Prebiotic is a non-viable food component that confers health benefit on the host associated with modulation of microbiota.

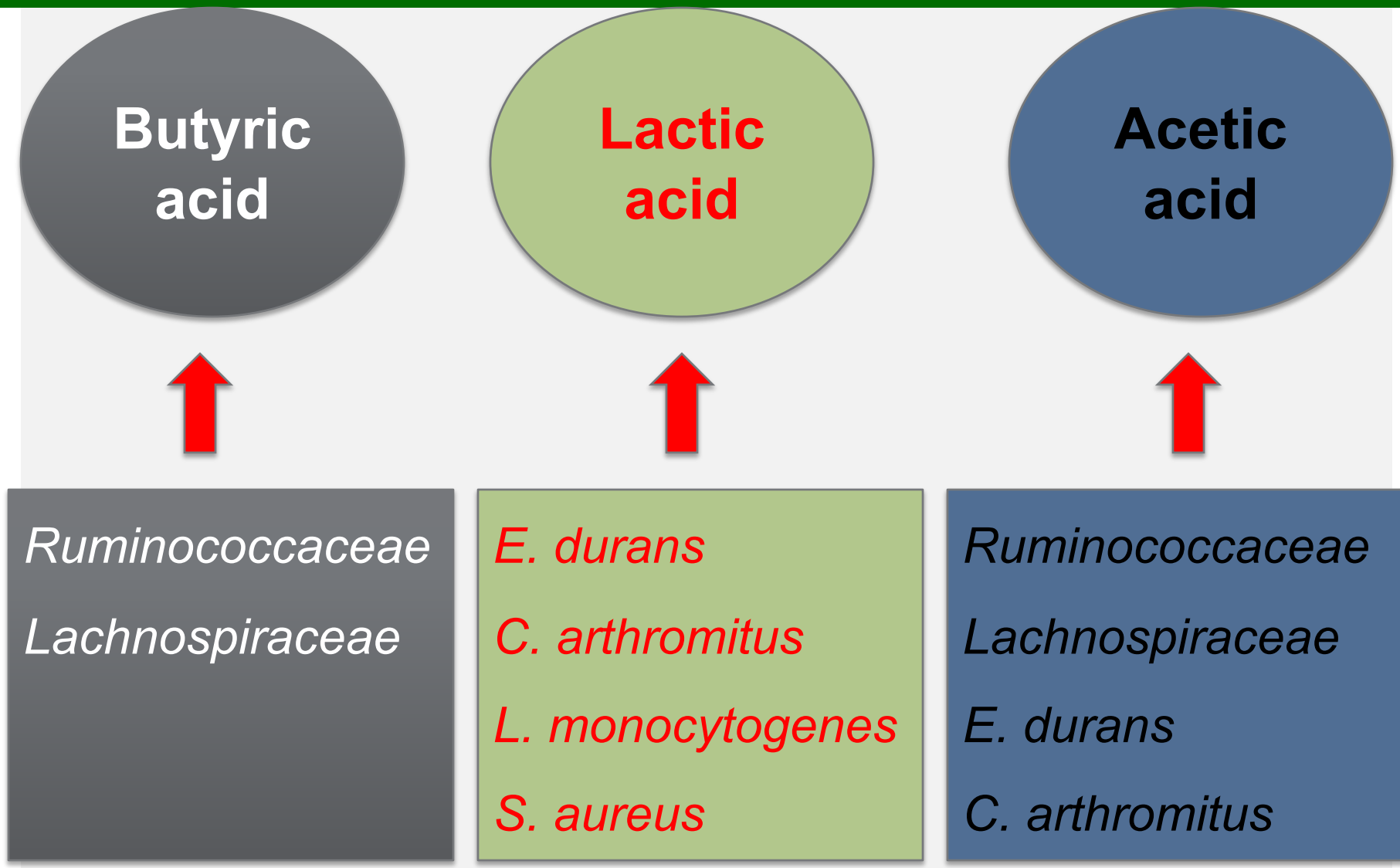
Hydrolysis products of arabinoxylan

Type of Prebiotics:

- Xylooligosaccharides (XOS)
- Arabinoxylooligosaccharides (AXOS)
- Fructooligosaccharides (FOS)
- Galactooligosaccharides (GOS)
- Inulin

Ref: Yacoubi et al., 2017

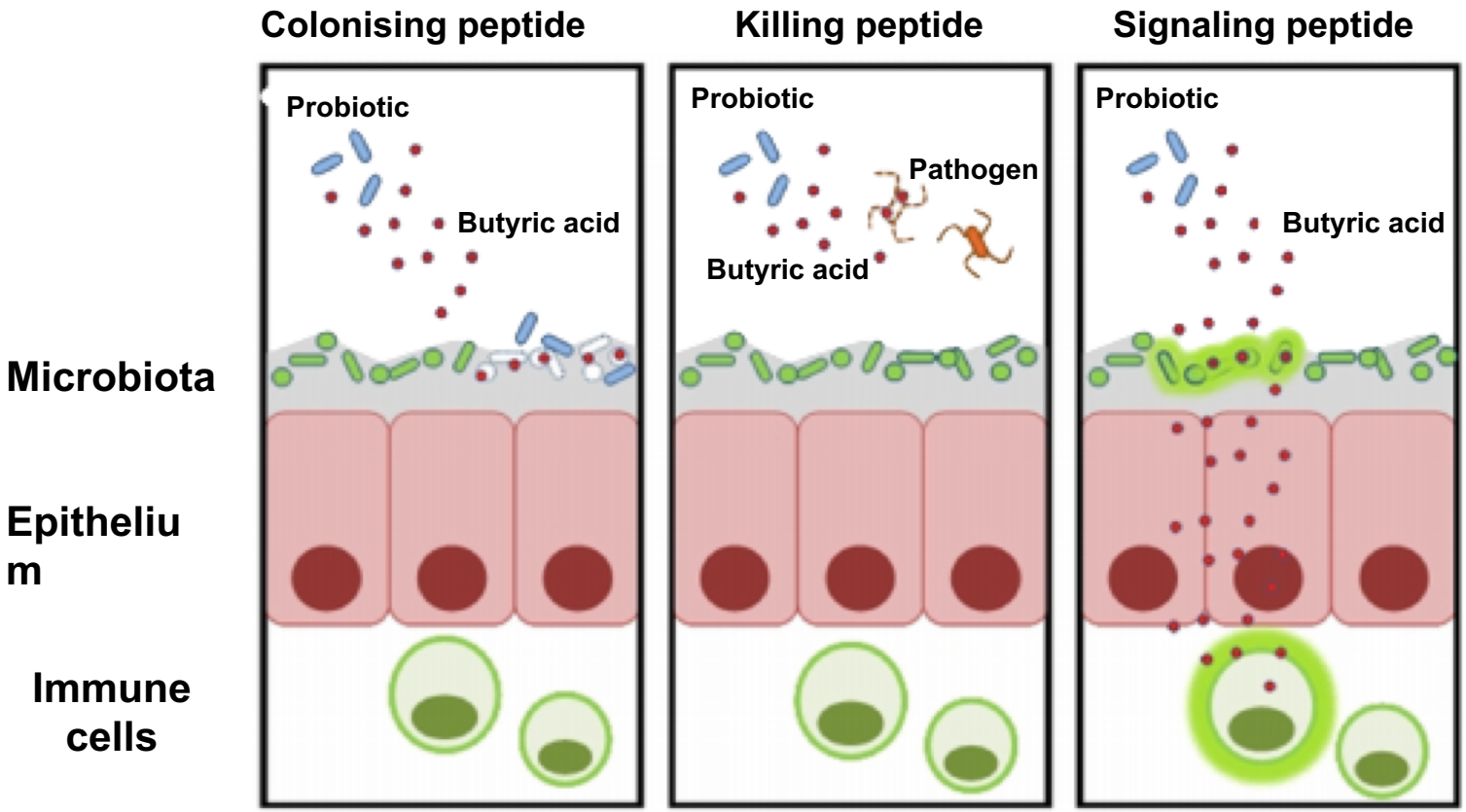
Short-Chain Fatty Acid (SCFA)



Ref: Yacoubi et al., 2017

Immune System

Immune system is the body's defense against infectious organisms and other invaders.



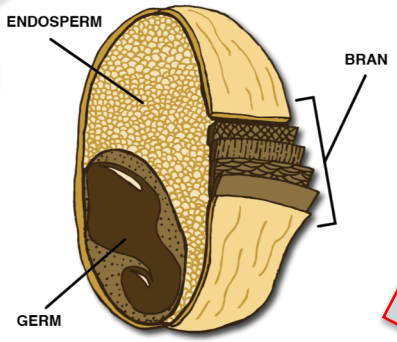
Ramkrishna *et al.,* 2015; Samanta et al., 2015; Dobson et al., 2012

Objective

To review, make better understanding about the:

- **Mechanism of GH43 in broiler feed**
- **Important of GH43 to improve digestibility**
- **Gut microbiota**
- **Broiler chicken performance by decreased viscosity of digesta.**

Hypothesis



Cereals

GH43

AXOS
XOS

Hydrolysis

Low Viscosity

Digestibility

Arabinose
Xylose

Prebiotics

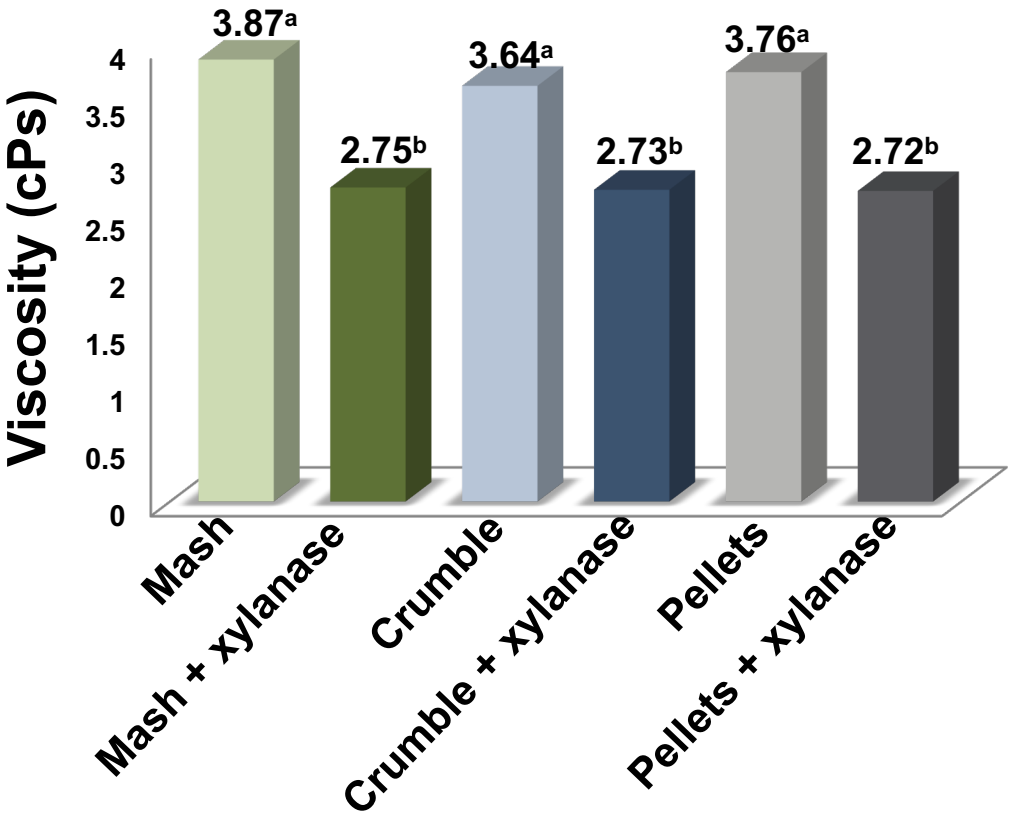
SCFA

Gut Microbiota

Increase Probiotics

Hosseini and Afshar, 2016; Zhang et al., 2014; Yacoubi et al., 2017; Lie et al., 2016

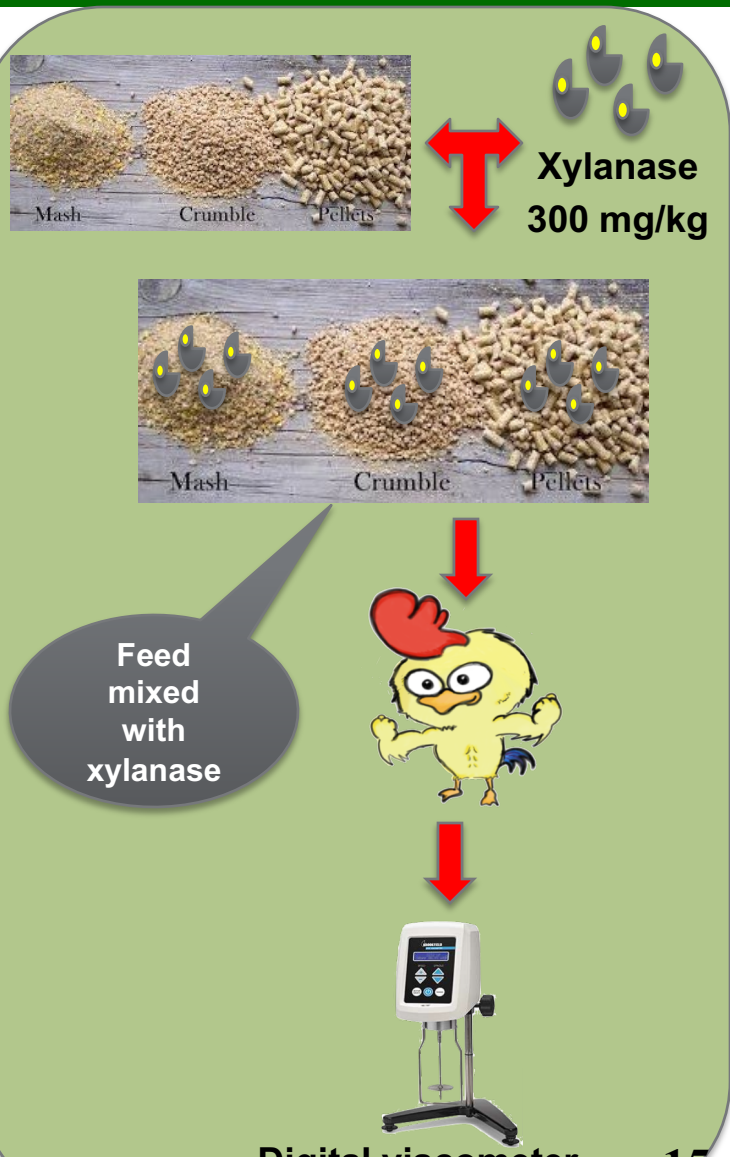
Effects of Xylanase Enzyme on Viscosity of Jejunal Digesta of Broilers, (42 Days of Age)



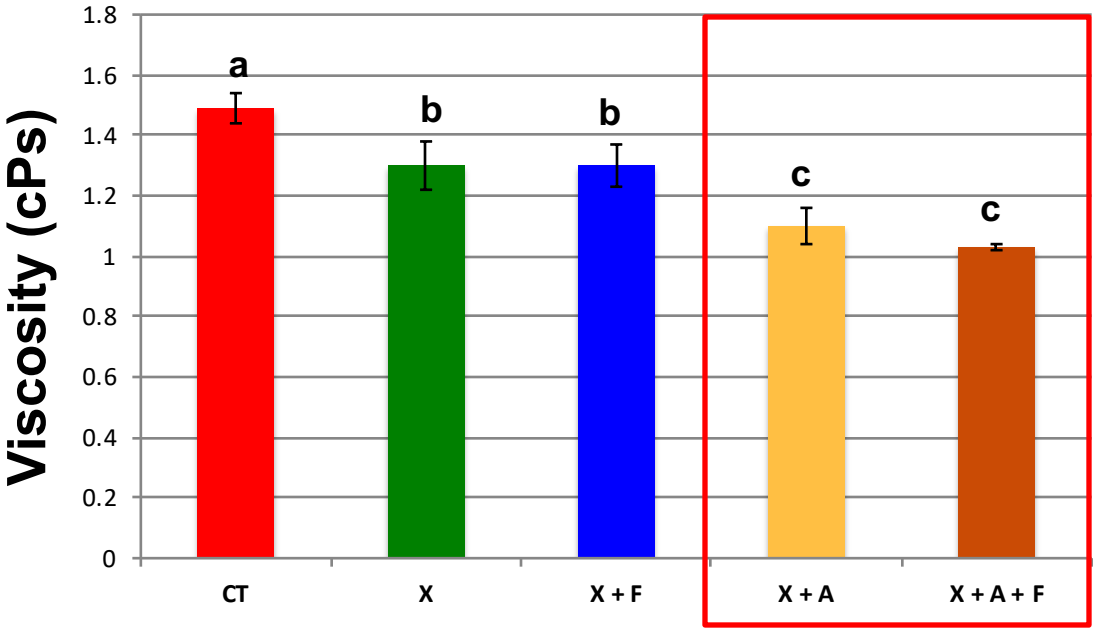
Control = high viscosity

Xylanase = low viscosity

Hosseini and Afshar, 2016



Effects of Enzymes on Gut Viscosity of Broilers on Day 36



CT = Control;

X = Xylanase;

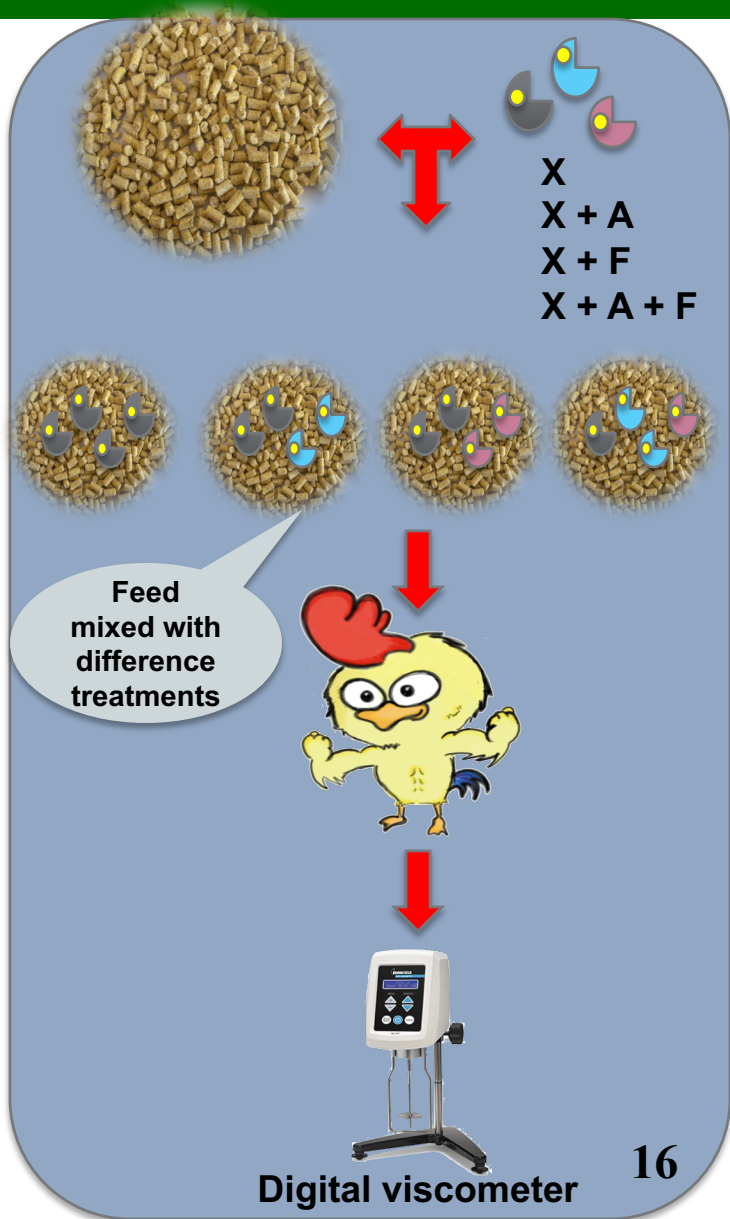
A = Arabinofuranosidase (GH43);

F = Feruloyl esterase

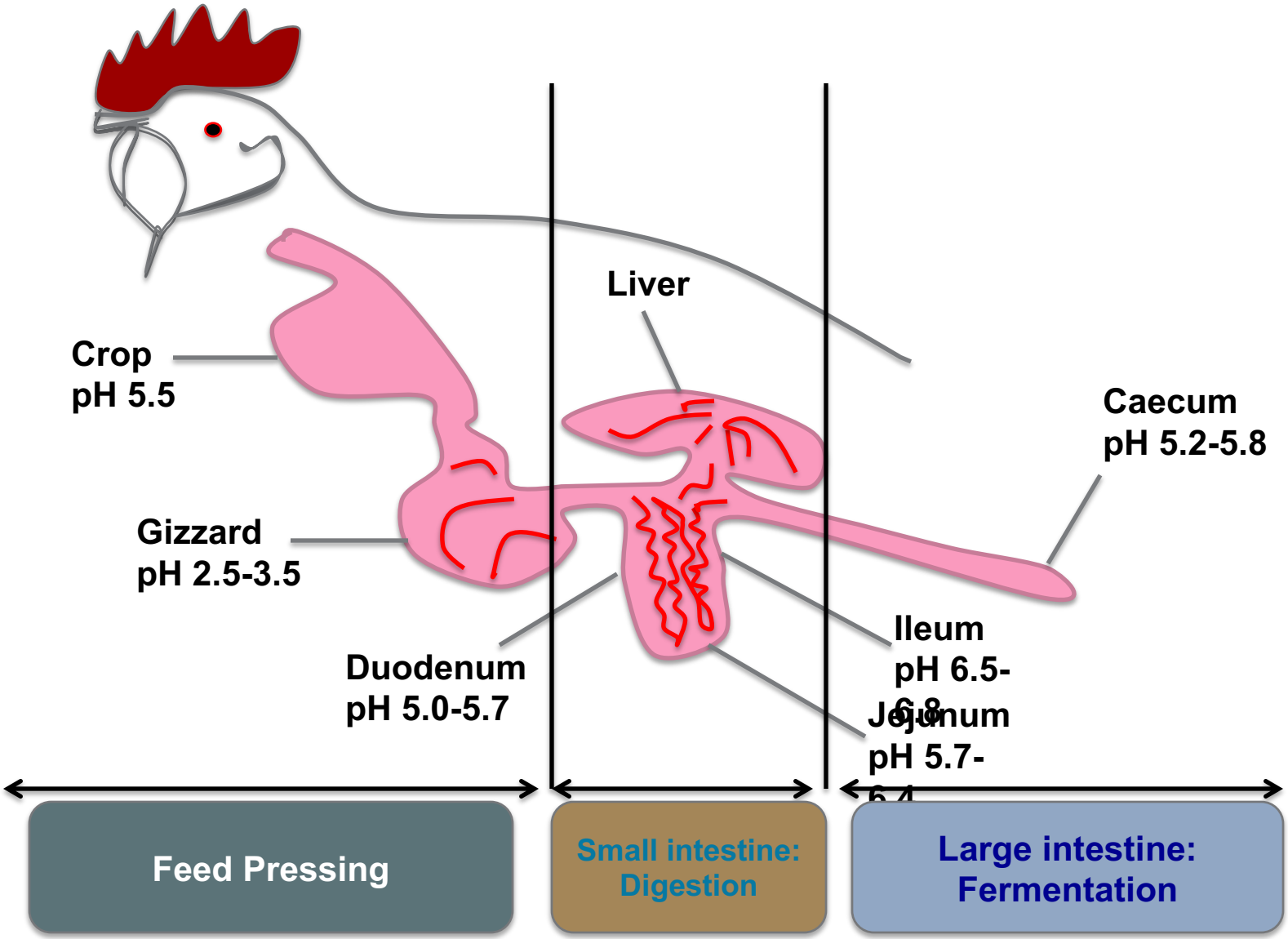
Control = high viscosity

X + A and X + A + F = lowest viscosity

Lei *et al.*, 2016



Physiology of Broiler



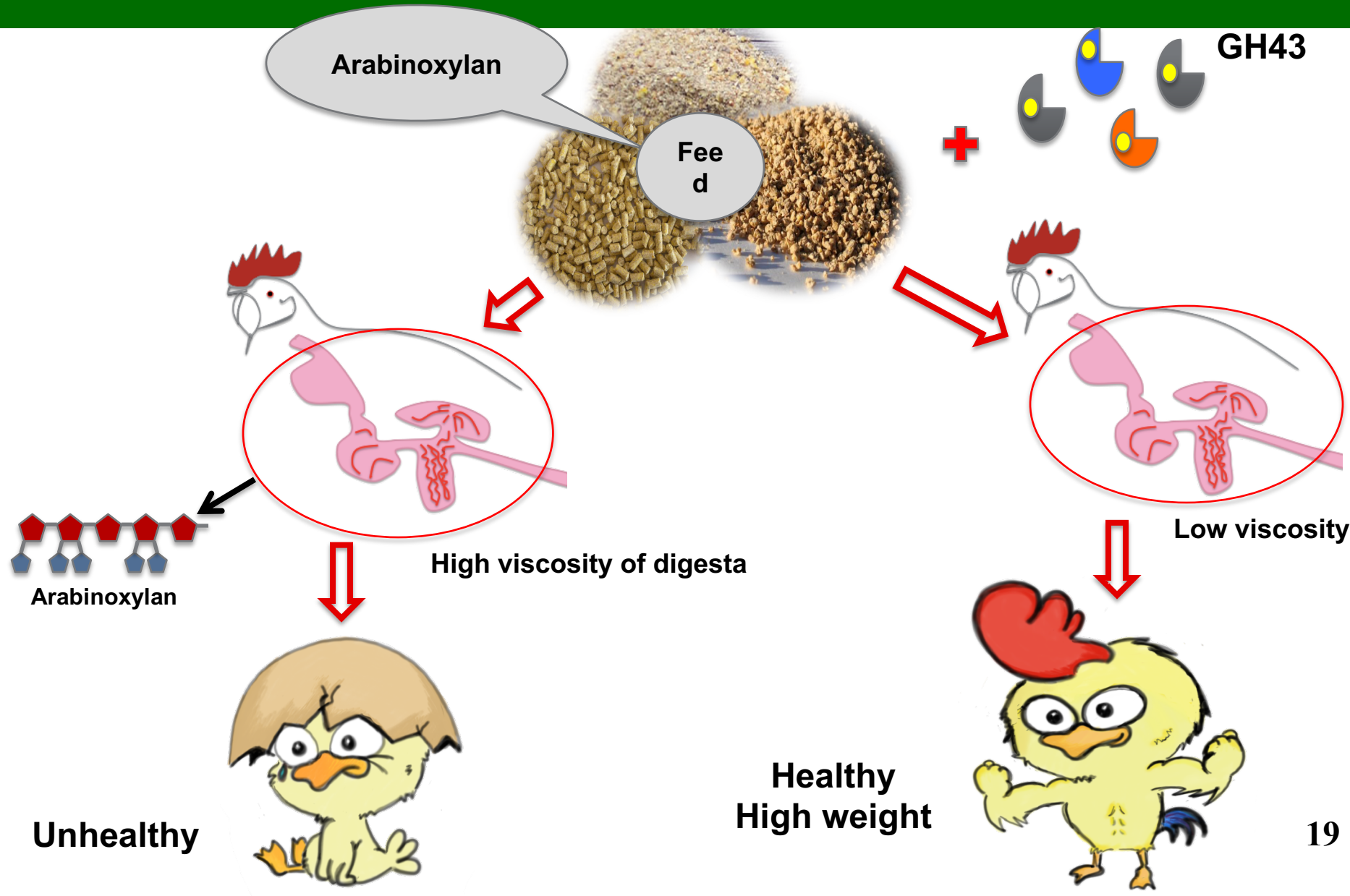
Effects of Xylanase on Free Sugars (mg/g as Received) in Gastrointestinal Tract of Broilers

Items	Crop (pH 5.5)		Gizzard (pH 2.5-3.5)		Duodenum (pH 5-6)		Jejunum (pH 6.5-7)		Ileum (pH 7-7.5)		Caecum (pH 8)	
	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase
Arabinose	0.37^{f}	$\mathbf{0.63^{ef}}$	0.42^{f}	$\mathbf{0.85^{de}}$	0.65^{ef}	$\mathbf{1.18^{bc}}$	1.08^{cd}	$\mathbf{1.49^{b}}$	1.37^{b}	$\mathbf{2.71^{a}}$	0.58^{ef}	$\mathbf{1.18^{bc}}$
Xylose	0.42^{f}	$\mathbf{0.72^{f}}$	0.55^{f}	$\mathbf{1.19^{de}}$	1.01^{e}	$\mathbf{1.54^{cd}}$	1.44^{cd}	$\mathbf{1.93^{b}}$	1.66^{bc}	$\mathbf{2.94^{a}}$	0.58^{f}	$\mathbf{1.54^{cd}}$
Glucose	2.63^{d}	$\mathbf{2.64^{d}}$	2.71^{d}	$\mathbf{2.74^{d}}$	22.95^{b}	$\mathbf{23.37^{b}}$	50.81^{a}	$\mathbf{51.80^{a}}$	10.07^{c}	$\mathbf{12.10^{c}}$	1.21^{e}	$\mathbf{1.29^{e}}$

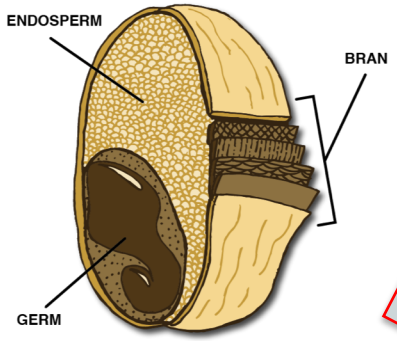
Means within a raw with no common superscripts differ significantly.

Zhang *et al.*, 2014

Function of GH43: Digestibility System



Hypothesis: Gut Microbiota



Cereals

GH43

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Increase Probiotics

Hosseini and Afshar, 2016; Zhang et al., 2014; Yacoubi et al., 2017; Lie et al., 2016

Effects of Enzyme on the Abundance (%) of the Taxonomic Groups Within the Ileal Sample (14 Days of Age)

Cluster	Cluster	WC	WE
Ileum	Enterococcaceae	$1.28^{b} \pm 0.19$	$6.08^{a} \pm 0.54$
	Enterococcus durans	$1.26^{b} \pm 0.17$	$5.06^{a} \pm 0.38$
	Clostridiaceae	$0.41^{b} \pm 0.02$	$0.54^{a} \pm 0.01$
	Candidatus arthromitus	$0.40^{b} \pm 0.02$	$0.53^{a} \pm 0.02$

WE = have high amount of probiotics than the control

WC = Wheat Control;

WE= Wheat + Enzymes

Yacoubi *et al.*, 2017

Effects of Enzyme on the Abundance (%) of the Taxonomic Groups Within the Cecal Sample (14 Days of Age)

Cluster	Cluster	WC	WE
Cecum	**Lachnospiraceae**	**46.30^{b} ± 0.31**	**51.63^{a} ± 0.36**
	Lachnoclostridium bacterium	**3.91^{b} ± 0.05**	**4.72^{a} ± 0.03**
	Lachnoclostridium sp.	**0.42^{b} ± 0.02**	**1.11^{a} ± 0.03**
	Blautia sp.	**0.94^{b} ± 0.03**	**1.06^{a} ± 0.01**
	Coprococcus sp.	**3.35^{b} ± 0.07**	**8.21^{a} ± 0.12**
	Fusicatenibacter sp.	**0.44^{b} ± 0.002**	**1.42^{a} ± 0.005**
	Tyzzerella sp.	**0.28^{b} ± 0.004**	**0.36^{a} ± 0.002**
	Ruminococcaceae	**9.46^{b} ± 0.42**	**11.91^{a} ± 0.19**
	Ruminiclostridium sp.	**1.08^{b} ± 0.08**	**3.14^{a} ± 0.12**
	Ruminococcus gauvreuii	**0.12^{b} ± 0.001**	**0.41^{a} ± 0.002**

Yacoubi *et al.*, 2017

Effects of Enzymes on SCFA, Lactic, Acetic and Butyric Acids Concentrations (μM) in the Ileal and Cecal (14 Days of Age)

	Parameters	WC	WE
Ileal	Total SCFA	21.1 ± 2.3	21.4 ± 1.9
	Acetate	1.6 ± 0.4	1.8 ± 0.3
	Lactate	19.5 ± 2.2	19.7 ± 2.0
	Butyrate	< 0.1	< 0.1
Cecum	Total SCFA	39.4^{b} ± 2.5	65.6^{a} ± 5.6
	Acetate	30.6^{b} ± 2.1	49.7^{a} ± 4.9
	Lactate	2.5^{b} ± 0.3	3.7^{a} ± 0.5
	Butyrate	5.3^{b} ± 0.6	10.2^{a} ± 1.0

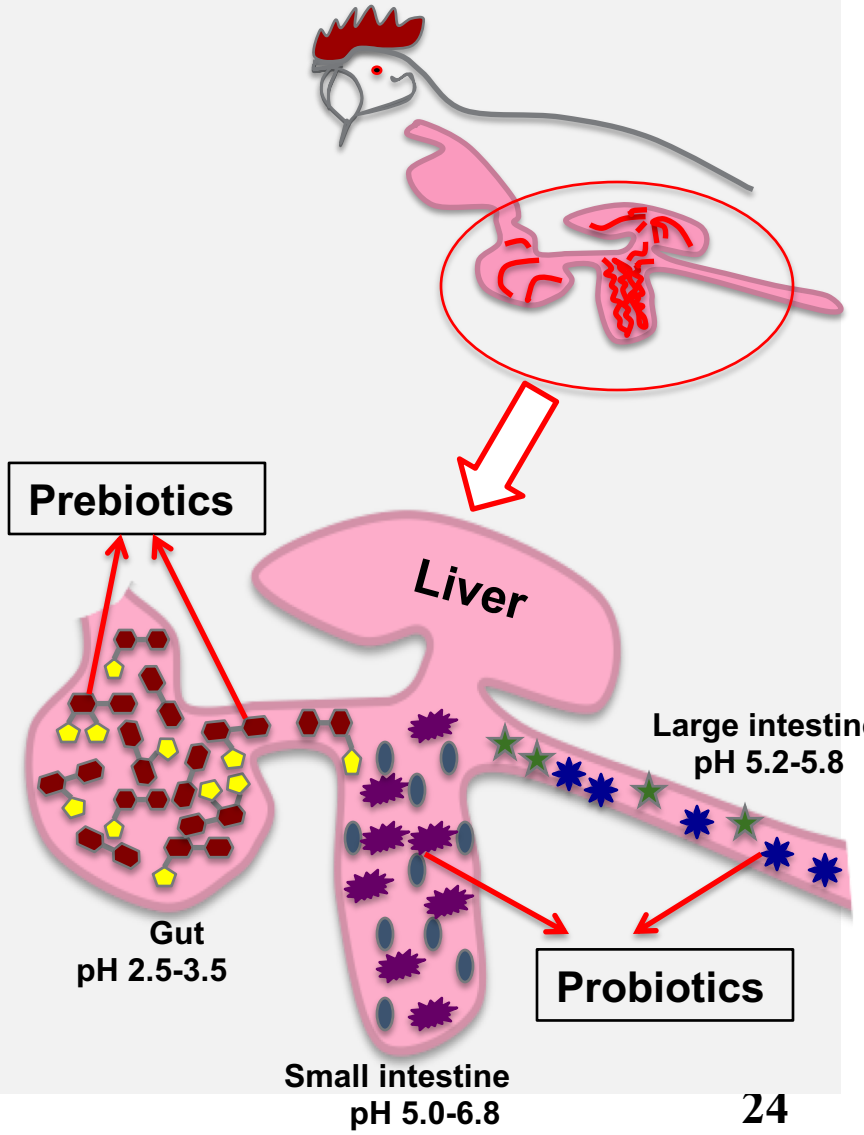
WE = have high amount of SCFA, lactic, butyric and acetic acids

WC = Wheat Control;
WE= Wheat + Enzymes

Yacoubi *et al.*, 2017

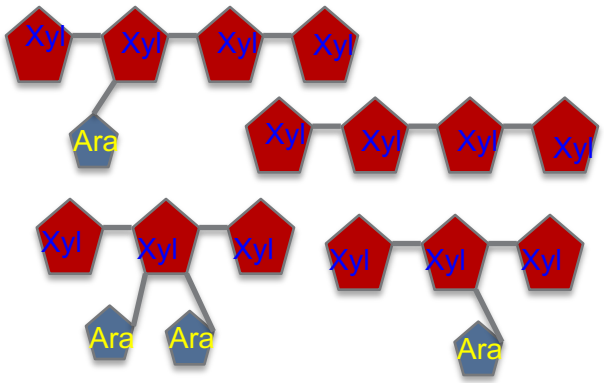
Probiotics in Gastrointestinal Tract of Broilers

Ileum
Candidatus arthromitus
Enterococcus durans
Ceca
Ruminococcaceae families
Lachnospiraceae families



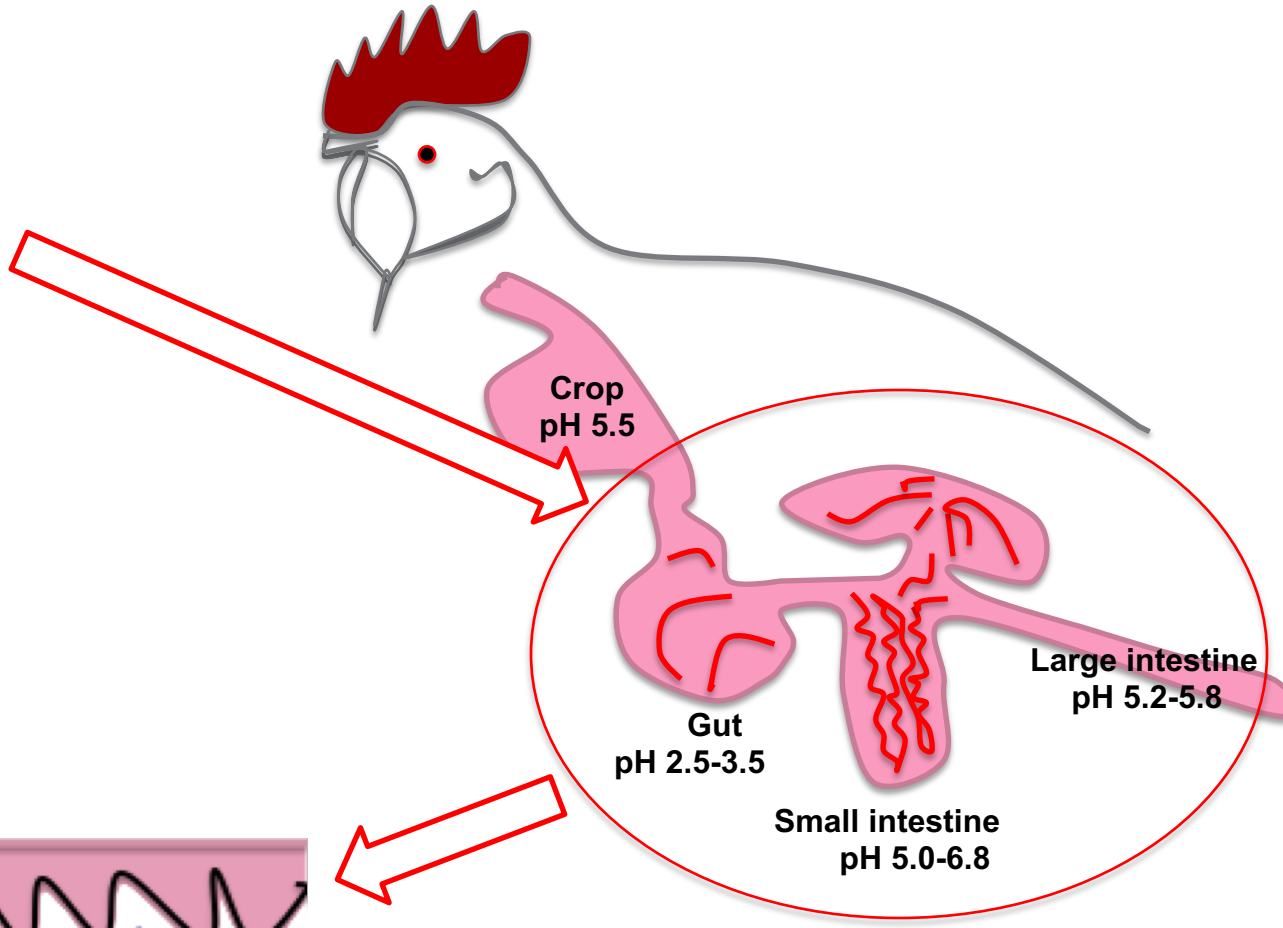
Ref: Yacoubi et al., 2017

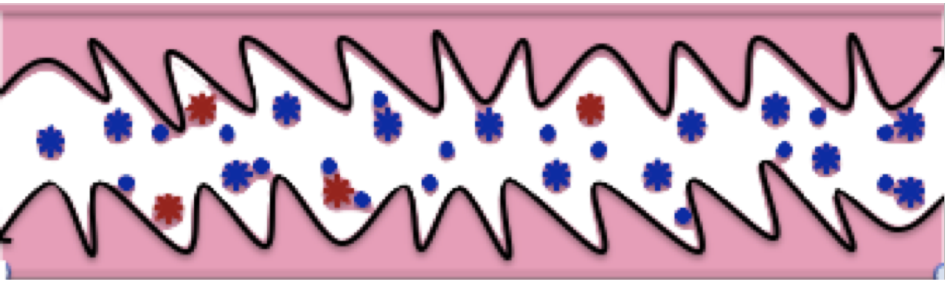
Gut Microbiota (Cont.)



Xylan-oligomers:

AXOS and XOS





Prebiotics

Improved balance of gut microbiota

Effects of Xylanase on Growth Performance of Broilers

Items	Control	Xylanase	SEM	p-value
Initial BW (g/bird)	122.3	**122.5**	0.74	0.982
7 to 21 days				
BW gain (g/bird)	498.9	**528.4**	4.91	0.029
Feed intake (g/bird)	798.2	**803.2**	12.95	0.953
FCR	1.60	**1.52**	0.03	0.048

SEM, standard error of the mean;

BW, body weight;

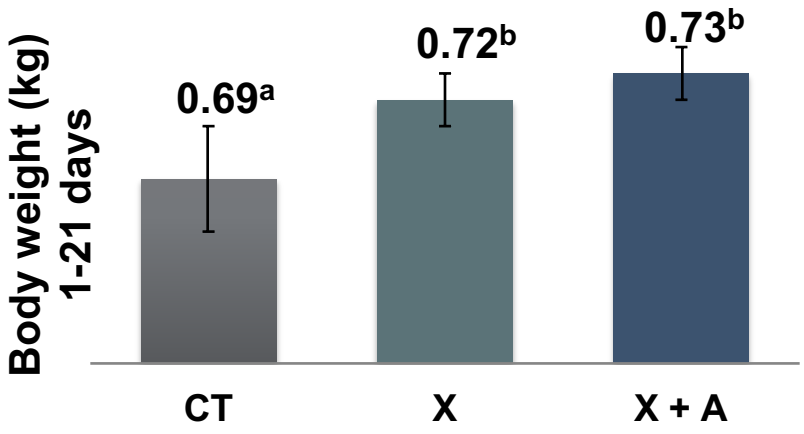
FCR, feed conversion ratio

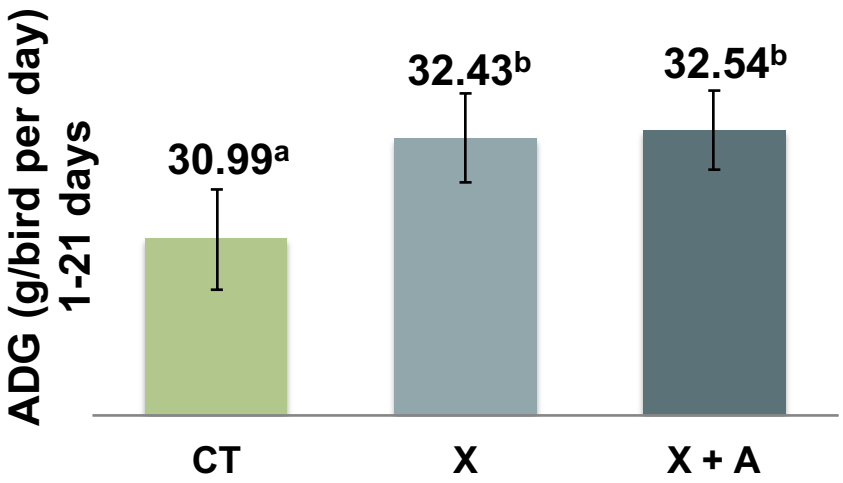
Increased BW by 5.8%

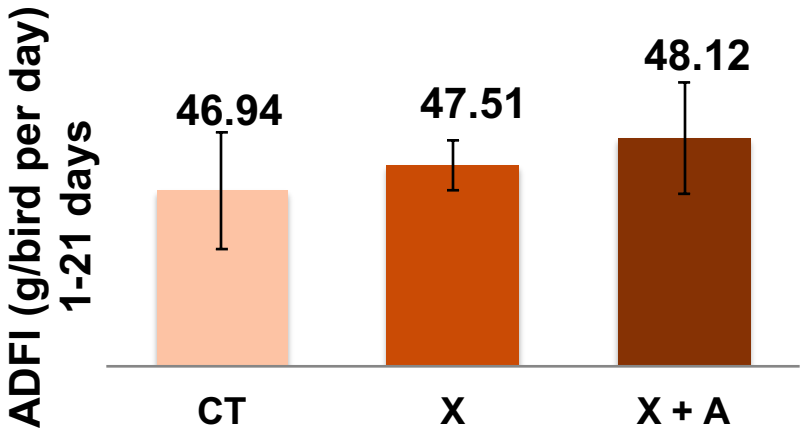
Improved FCR by 5.3%

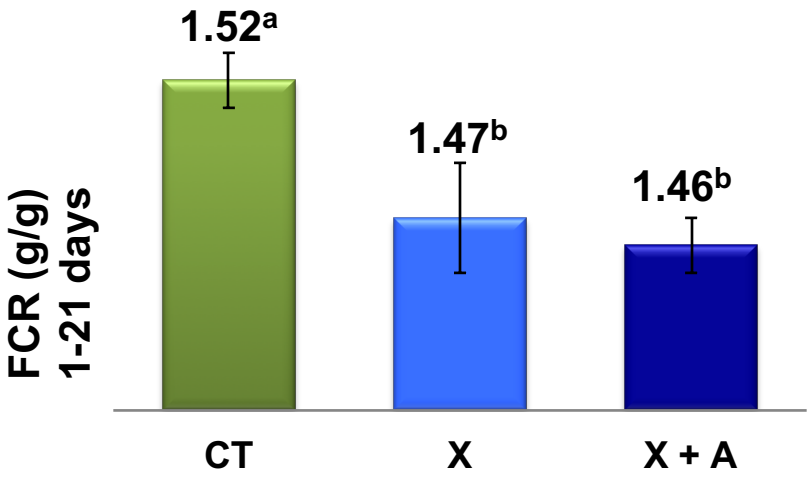
Zhang *et al.*, 2014

Effects of Enzymes on Growth Performance of Broilers





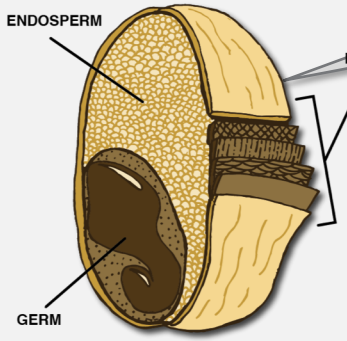




CT = Control; X = Xylanase; A = Arabinofuranosidase;

Lei *et al.*, 2016

Conclusion and Suggestion



Arabino -xylan

Cereals

Feed

GH43

AXOS and XOS

Low viscosity

High weight

- Body Weight
- Feed Conversion Ratio

Prebiotics for gut microbiota

Improved balance of gut microbiota

Healthy Chicken

Conclusion and Suggestion (cont.)

- Decreased viscosity of digesta system
- Multi-functional to releases (XOS, AXOS, Arabinose and xylose)
- Increasing broiler growth performance
- Important for probiotics in small and large intestine
- Improved immune system stimulation

Zhang *et al.,* 2014; Hosseini and Afshar, 2016

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All Friends in Biochemical Technology Division

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THANK YOU FOR YOUR ATTENTIONS!

QUESTIONS AND ANSWERS

Enzymes for the Research Study

Items	Microorganism	City
Feruloyl esterase	*Clustridium thermocellum*	Megazyme, Ireland
Xylanase	*Neocallimastix patriciarum* *Aspergillus niger*	Asiapac, China Hangzhou, China
Arabinofuranosidase	*Bacillus pumilus*	RCBBE, China

Research Center of Biotechnology and Biomass Energy

Lei *et al.,* 2016; Zhang et al., 2014

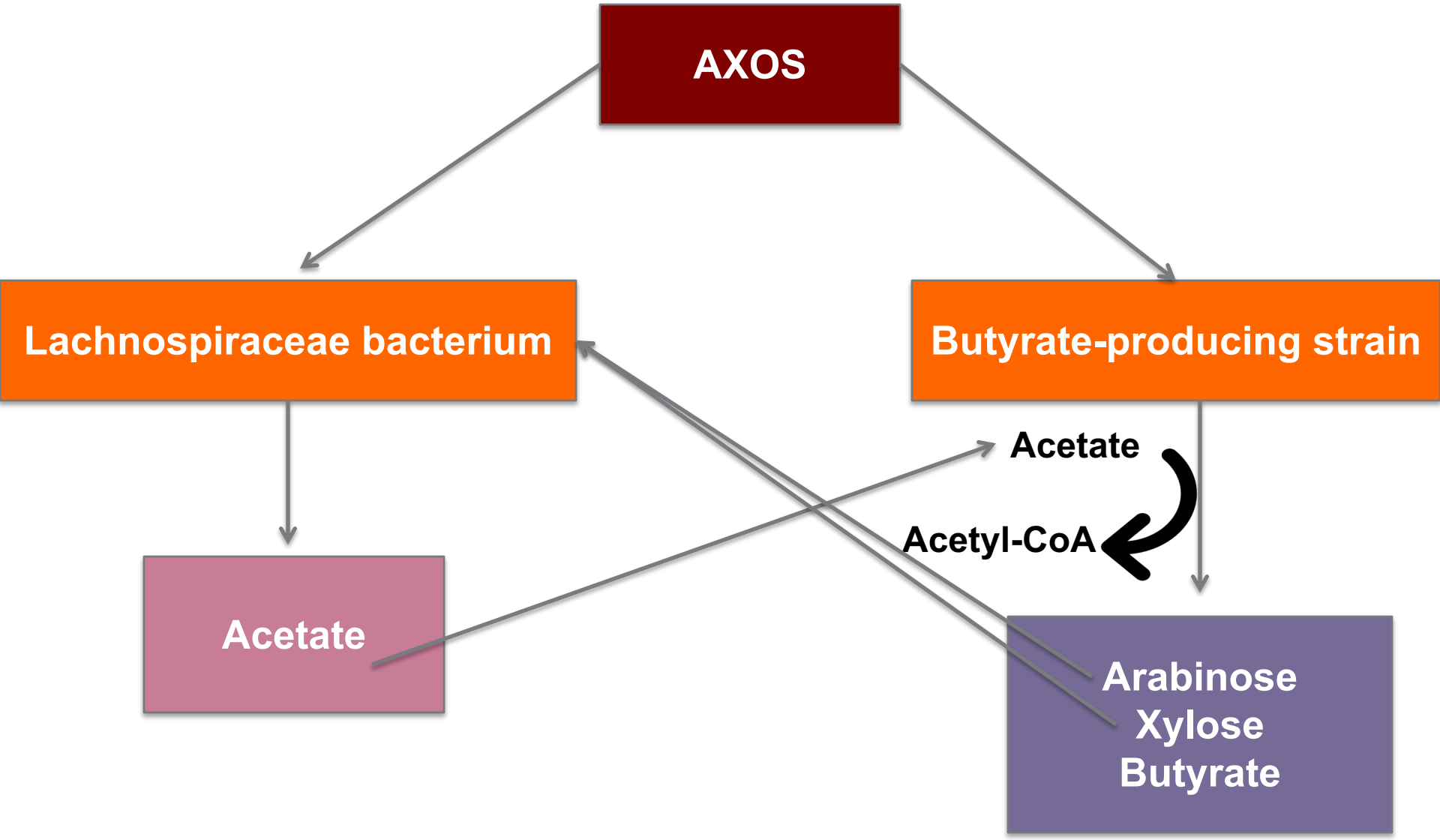
How Probiotics Use Prebiotics

- The beneficial microflora (Ruminococcaceae, Lachnospiraceae) fermented AXOS and XOS into the production of SCFA viz:
  - Acetic acid
  - Propionic acid
  - Butyric acid

- (Ruminococcaceae, Lachnospiraceae) secreted α-arabinofuranosidase and β-xylosidase for growth and multiplication.

Samanta *et al.*, 2014

Cross-feeding of AXOS



Ref: Riviere et al., 2016

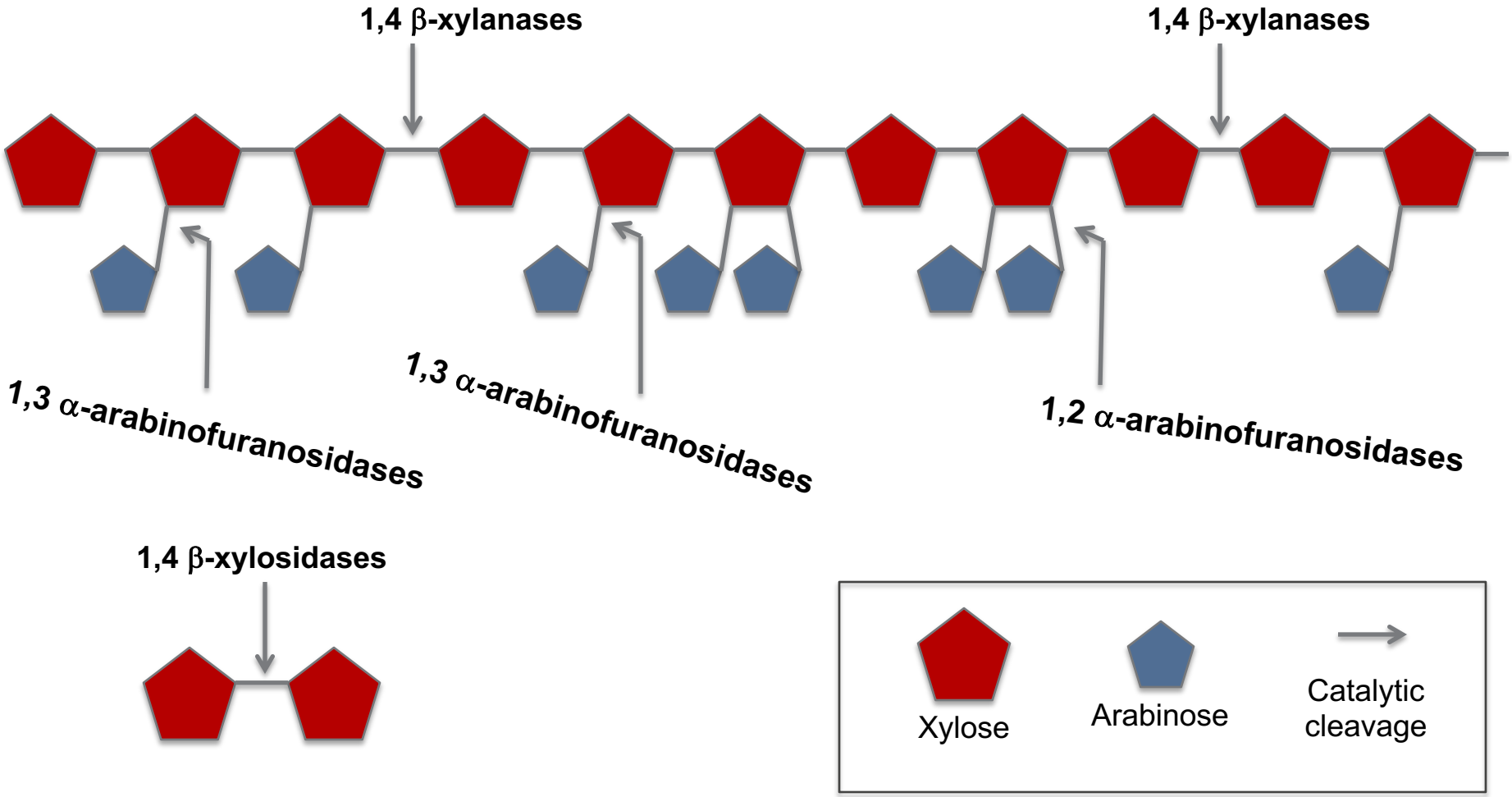
Feed Conversion Ratio

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Average daily gain}}$$

Average Daily Gain (ADG)

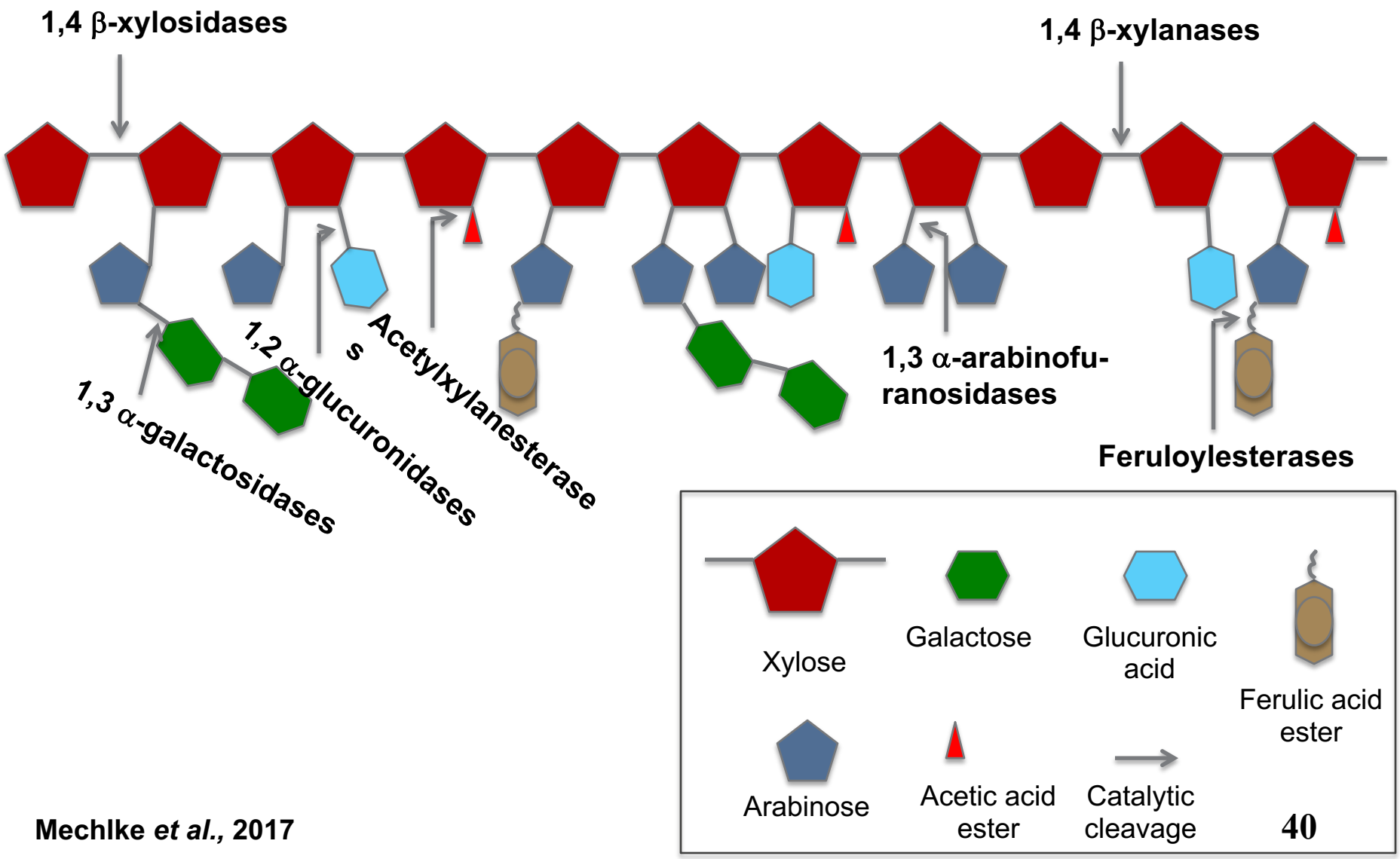
$$ADG = \frac{\text{Finish weight} - \text{start weight}}{\text{Age (days)}}$$

Enzyme Hydrolysis System



Mechlke *et al.*, 2017

Highly Branched Corn Fiber Glucuronoarabinoxylan



Mechlke *et al.*, 2017

Compositions of basal diets ingredients (%)

Ingredients (%)	Grower (0–21 d)	Finisher (22–42 d)
Wheat (12.5% CP)	50.55	51.00
Corn	14.00	19.32
Soybean meal (48% CP)	29.00	22.00
Soybean oil	2.50	4.00
Other	3.95	3.68

Hosseini and Afshar, 2016

Compositions of basal diets ingredients (%) and nutrient levels of the basal diet to broilers

Ingredients (%)	Starter (d 7–21)
Wheat	60.0
Corn	7.5
Soybean meal	28.0
Other	4.5

Nutrient levels (%)	Starter (d 7–21)
ME (MJ/kg)	12.2
CP	21.6
Calcium	1.0
Soluble NSP	1.9
Insoluble NSP	8.36
Other	2.31

Hosseini and Afshar, 2016

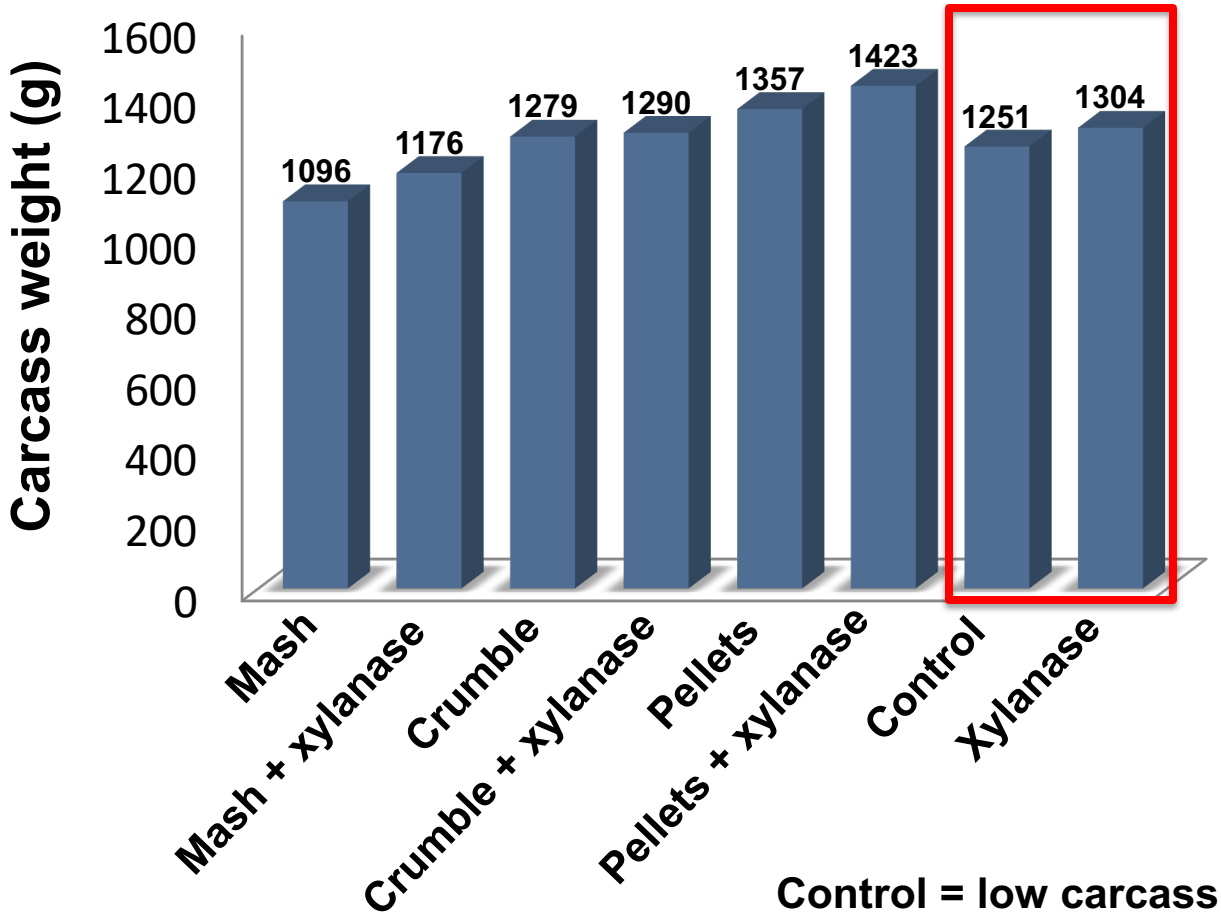
Effects of Xylanase on Oligosaccharide (mg/g as Received) in Gastrointestinal Tract of Broilers

Items	Crop (pH 5.5)		Gizzard (pH 2.5-3.5)		Duodenum (pH 5-6)		Jejunum (pH 6.5-7)		Ileum (pH 7-7.5)		Caecum (pH 8)	
	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase	Control	Xylanase
Isomatose	0.15^{c}	**1.17^{c}**	0.13^{c}	**0.10^{c}**	1.79^{a}	**1.80^{a}**	2.14^{a}	**2.22^{a}**	1.13^{b}	**0.90^{b}**	0.36^{c}	**0.32^{c}**
Panose	0.07^{d}	**0.10^{d}**	0.09^{d}	**0.15^{d}**	1.37^{b}	**1.22^{b}**	2.41^{a}	**2.15^{a}**	1.55^{b}	**1.43^{b}**	0.62^{c}	**0.60^{c}**
Isomaltriose	0.14^{d}	**0.20^{d}**	0.18^{d}	**0.17^{d}**	0.42^{c}	**0.48^{c}**	1.14^{ab}	**1.25^{a}**	0.90^{b}	**1.25^{a}**	0.29^{d}	**0.36^{cd}**
1-Kestose	0.15^{e}	**0.18^{e}**	0.13^{e}	**0.14^{e}**	0.37^{cd}	**0.52^{bc}**	0.57^{ab}	**0.65^{a}**	0.32^{d}	**0.40^{cd}**	0.07^{e}	**0.06^{e}**

Means within a raw with no common superscripts differ significantly.

Zhang *et al.*, 2014

Effects of Xylanase Enzyme on Carcass Weight (CW) of Broilers, (42 Days of Age)



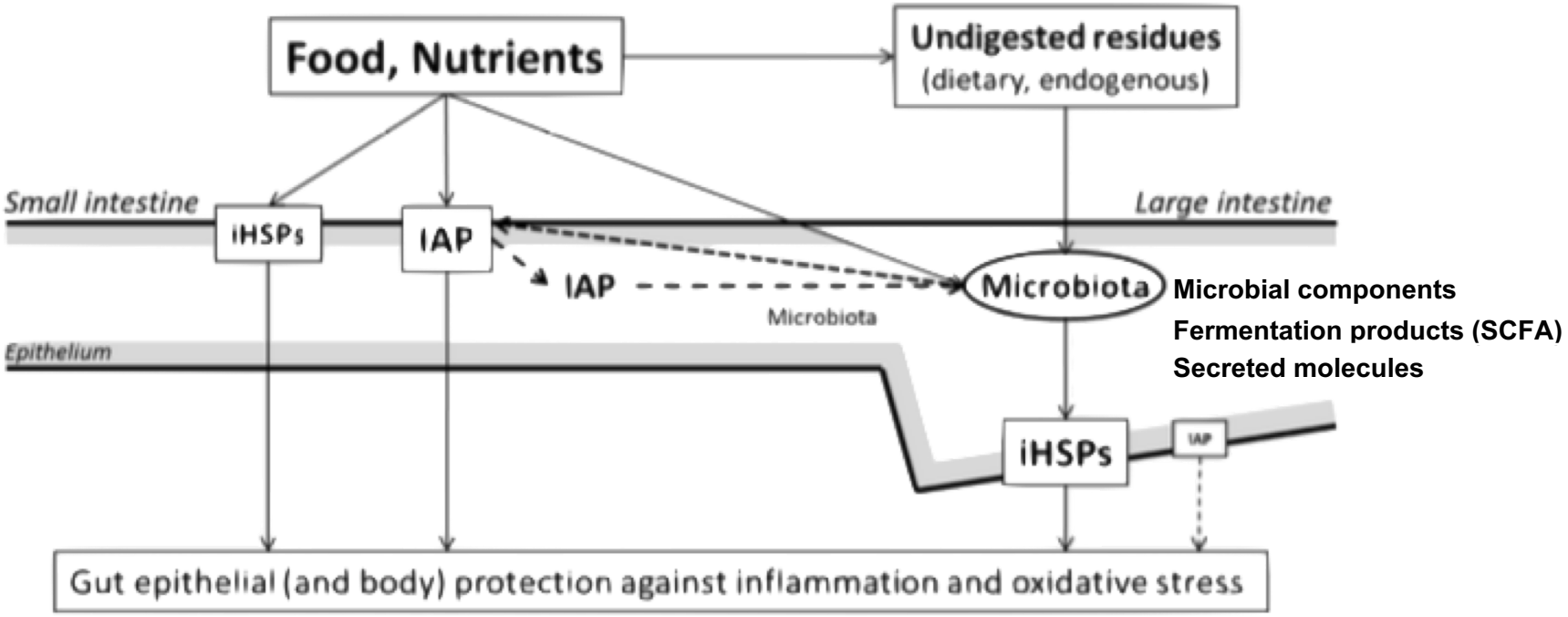
Chicken's carcass

Control = low carcass weight

Xylanase = high carcass weight

Hosseini and Afshar, 2016

Anti-inflammation



Hosseini and Afshar, 2016