Muramidase inclusion reduces gut inflammation in weaned piglets, especially in high protein diets

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Weaning is an abrupt 1-day event which brings challenges

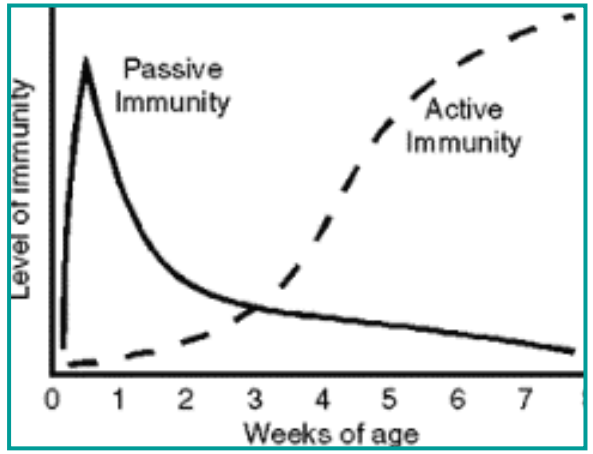
Resulting in post-weaning diarrhea and growth rate

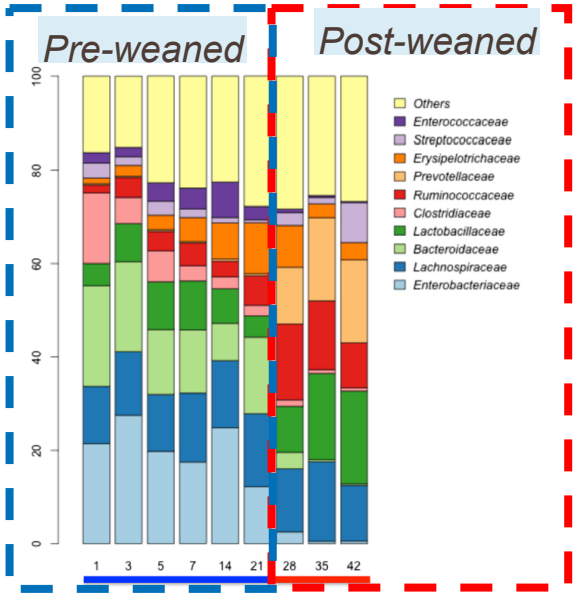
Abrupt transition in diet (milk to solid)→ **digestive stress**

Immune system immature → hyper-reactive and prone to inflammation

Big changes on gut **microbial community** creates instability and opportunity for pathogens

Environmental stress, lower feed intake and reduced growth rate





Frese et al. 2015

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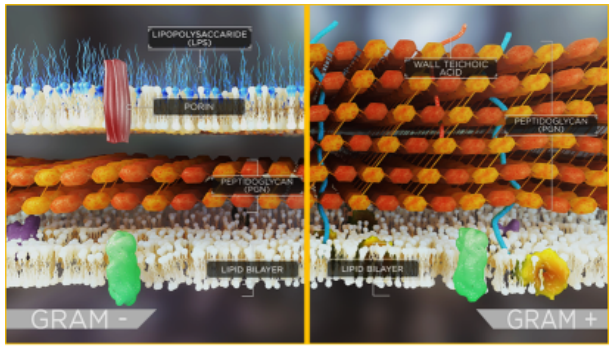
Understanding the dynamics of gut microbial community

Peptidoglycans accumulation in the gut may deteriorate as consequences of weaning stress

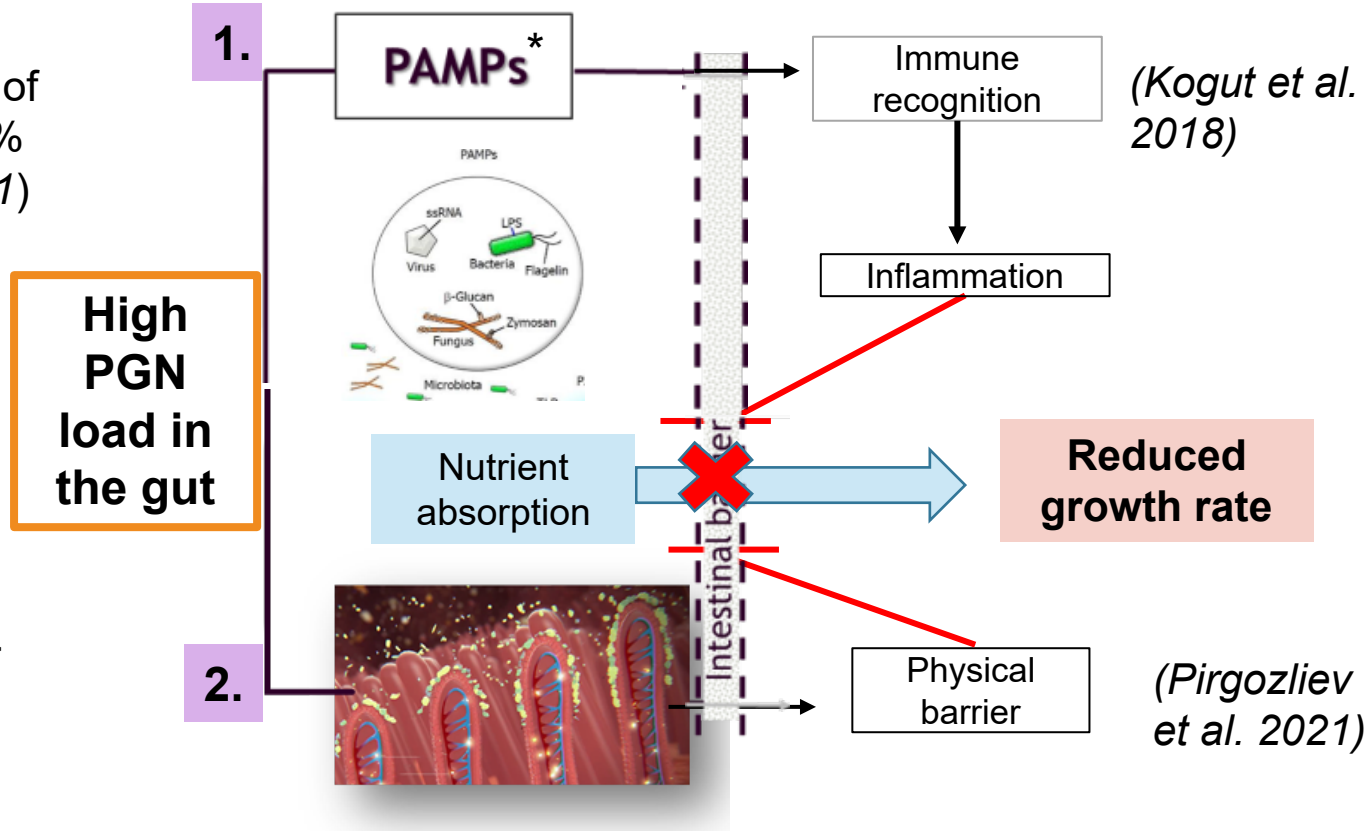
- Naturally **gut bacterial turnover** occurred **releasing bacterial cell wall fragments such as LPS or PGN** in the gastrointestinal tract (*Wheeler et al. 2014*)

- **Peptidoglycans (PGN)** are the major component of bacterial cell walls mainly in Gram+ bacteria-> 50% Bacterial cell wall recycling (*Reith and Mayer, 2011*)



- **Factors** promoting **higher bacterial turnover (**=> PGN accumulation in the gut**):**
  - Husbandry practices (e.g. age of weaning, sanitary conditions, animal density, etc)
  - Pathogen load
  - Nutritional challenges (e.g. high protein content; NSP levels; Mycotoxins: etc)
  - Environmental stress (e.g. temperature)

Hypothesis of the impact of PGN accumulation in the gut



*Pathogen –associated molecular patterns

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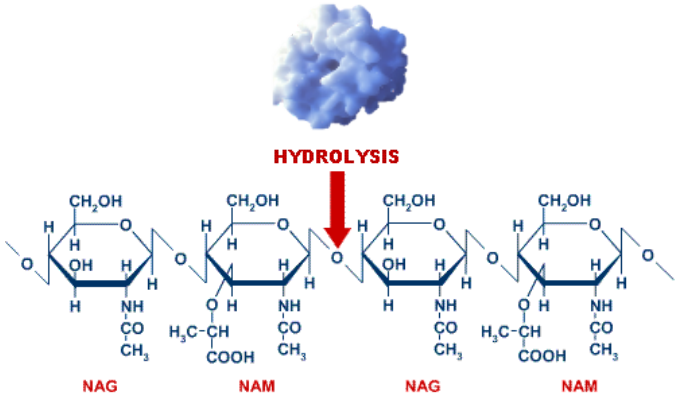
Hydrolysis of peptidoglycans has multiple positive effects on the host

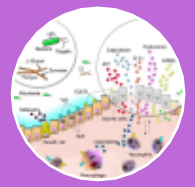
- Glycosyl hydrolytic **enzymes** that target PGN from bacterial cell wall releasing sugars and AA (*Vollmer et al., 2008*).

- **Types of muramidases** used as feed additives depending on the origin:
  - ***Hen egg white lysozyme****:* antimicrobial potential
  - ***Microbial muramidase***: lack of antimicrobial potency in MIC assay *(Litchenberg et al. 2017)* but mode of action still unclear

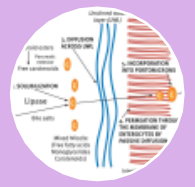


- **Previous *in vivo* studies** using **microbial muramidase:**

Increases **growth performance** of piglets (*Schliffka et al. 2019*) and chickens (Sais et al., 2020; Pirgozliev et al., 2021; Goes et al., 2022) ; and **reduced diarrhea** incidence (*Planchenault et al. 2022*)



Reduces gut inflammation *in vitro* and in broiler chickens (Frederiksen et al. 2021; *Wang et al. 2021; Amer et al. 2023*)



Improves **nutrients digestibility** and **absorption** in broiler chickens (*Goodarzi Boroojeni et al., 2019; Sais et al., 2020; Pirgozliev et al., 2021; Goes et al., 2022; Perez-Calvo et al. 2023*)

But no information available to explain the positive effect of microbial muramidase on weaned piglets

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The **objective** of this study was to evaluate the **effect of the dietary muramidase inclusion** during 2 weeks post-weaning on different **gastrointestinal functionality** parameters using a high protein diet as a challenge **in early weaned piglets**

Material and methods

Experimental design

- Trial run in DSM experimental facilities (France)
- **21d weaned piglets**
- Average **initial BW = 6.1 ± 0.6kg**
- Experimental length: **2 weeks post-weaning**
- **Challenge model:** High protein diet (>4 points)

- **Treatments** (16 piglets/treatment):

	CP level (%)	Muramidase inclusion*
LP	16.7	No
LP+		Yes
HP	21.0	No
HP+		Yes

*Muramidase inclusion 100 LSU(F)/kg feed = 1000 g/MT (Balancius ™)

Ingredients, %	LP	HP
Corn	56.60	32.60
Barley	10.00	20.00
Soybean meal 48%	23.00	35.00
Soy concentrate	-	3.00
Soybean oil	3.00	3.00
Dicalcium phosphate	2.00	1.80
Calcium Carbonate	0.50	0.50
Salt	0.50	0.50
Vitamin-mineral Premix [1]	3.00	3.00
L-Met	0.30	0.20
L-Thr	0.30	0.10
L-Lys	0.80	0.30
Analysed composition		
DM, %	89.40	89.41
CP, %	**16.68**	**21.01**
Fat, %	6.46	5.81
Fibre, %	2.23	2.69
ME (MJ/kg)	13.45	13.68

[1] Vitamin-mineral premix provided per kilogram of diet: Vitamin A: 15'000 I.U.; Vitamin E: 100 mg.; Vitamin K: 20.0 mg; Vitamin C: 100 mg; Vitamin B1: 3.00 mg; Vitamin B2: 10.00 mg; Vitamin B6: 6.00 mg; Vitamin B12: 0.04 mg; Pantothenic acid: 25.0 mg; Folic acid: 1.50 mg; Biotin 0.2 mg; Choline: 326 mg; Mn: 60.0 mg; Fe: 200 mg; Cu: 160 mg; Zn: 100 mg; I: 2.0 mg; Se: 0.4 mg; Lys: 2 848 mg; Met: 513 mg; Thr: 1 354 mg; Trp: 296 mg; L-Val 196 m g.

Material and methods

Sampling and parameters evaluated at the end of the experiment (d14)

Farm assessment:

- Growth performance
- Diarrhea: fecal DM

Nutrient absorption:

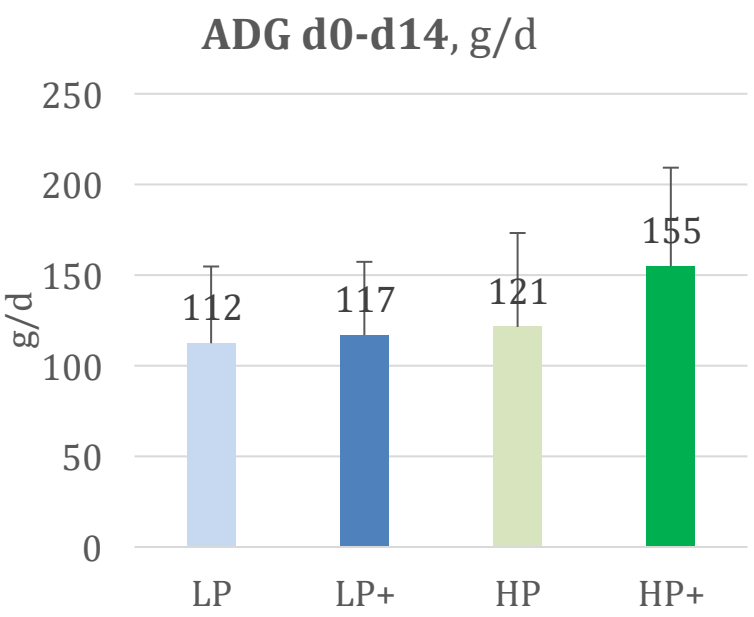
- Plasma urea nitrogen (PUN, analyzed by COBAS)
- Plasmatic vitamins A and E (HPLC)

Gut inflammation measured at different levels:

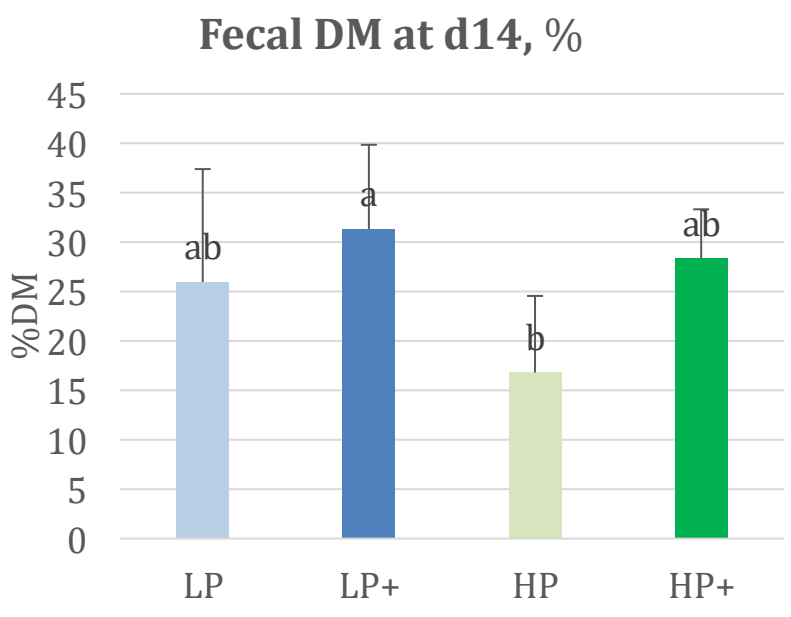
- Fecal lipocalin-2 (NGAL; ELISA assay ABCAM ab207924 kit)
- Gene expression from mesenteric lymph nodes (MLN), ileal Peyer patches (PP) and ileal lamina propria (ILP) (PCR array; 42 genes)

Statistical analyses by two-way ANOVA of JMP® software, protein level and muramidase inclusion as main variables

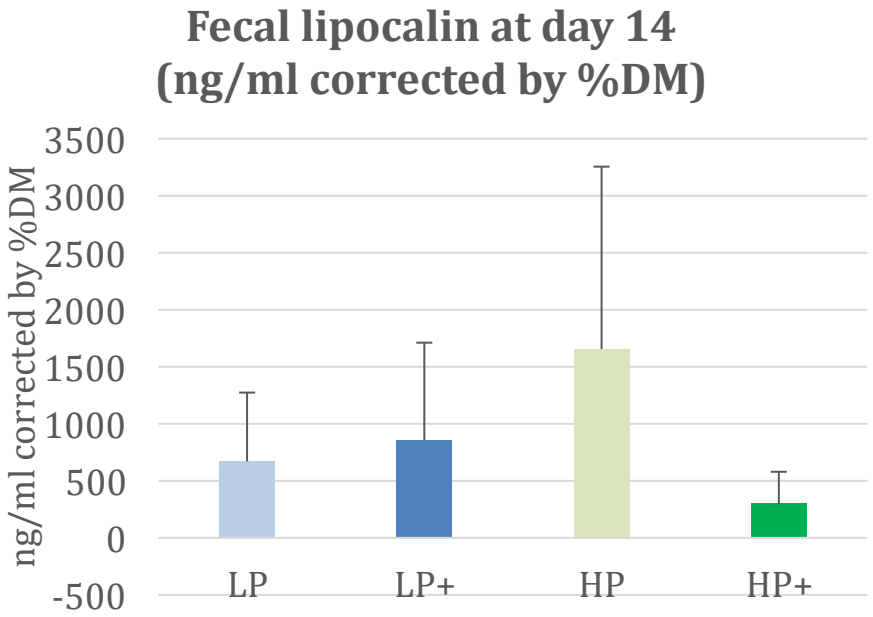
Muramidase reduces diarrhea and gut inflammation, specially under challenge conditions (HP diet) promoting piglets' growth by 30g/d



CP: P = **0.05**
MUR: P = 0.11
CP x MUR: P= 0.23



CP: P = 0.06
MUR: **P = 0.01**
CP x MUR: P= 0.33



CP: P = 0.56
MUR: P = 0.12
CP x MUR: **P= 0.04**

Non invasive sampling (N= 16)
Day 14 = 35 days old piglets

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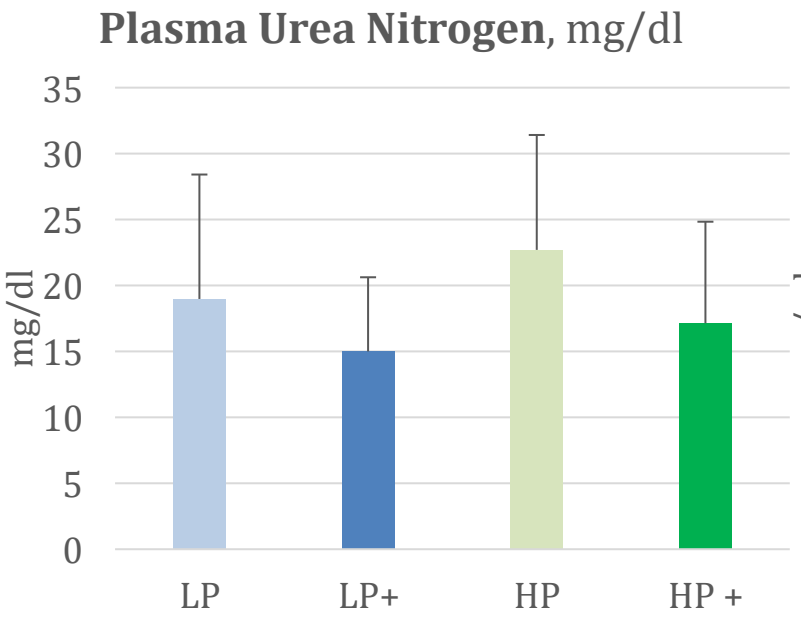
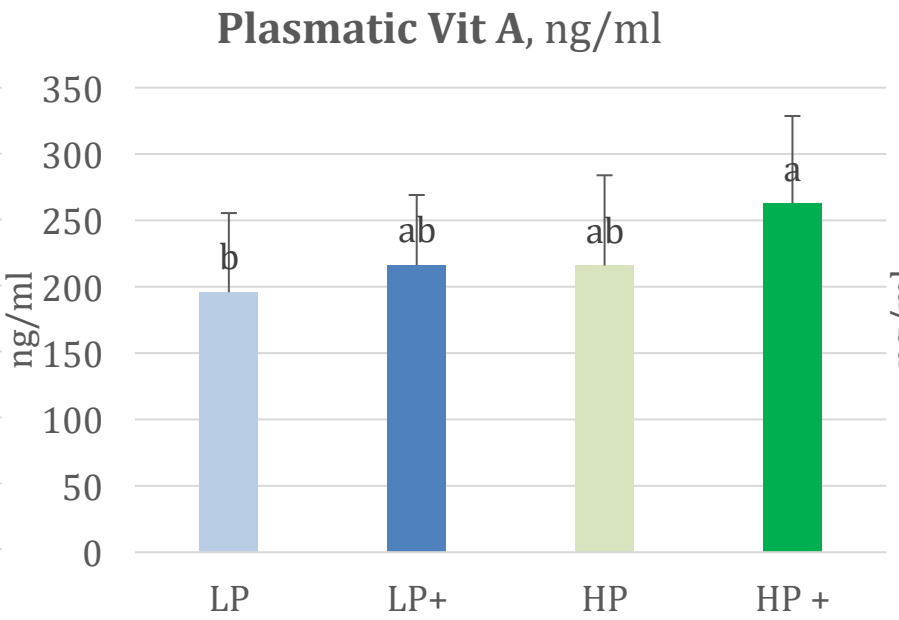
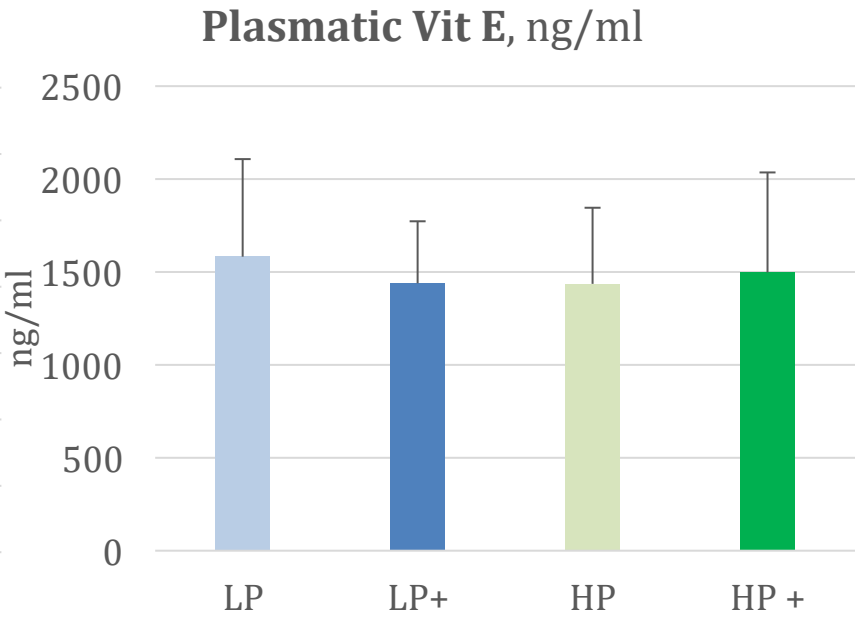
Muramidase improves nitrogen utilization and vitamin A absorption



CP: P = 0.15
MUR: **P = 0.02**
CP x MUR: P= 0.70



CP: **P = 0.04**
MUR: **P = 0.03**
CP x MUR: P= 0.40



CP: P = 0.70
MUR: P = 0.72
CP x MUR: P= 0.38

Plasmatic Vitamin A in piglets is more stable between 12 and 147 days of age compared to **vitamin E** which decreases continuously from 19 to 40 days of age (Buchet et al. 2017)

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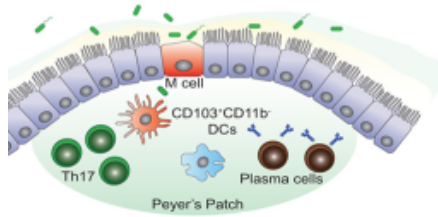
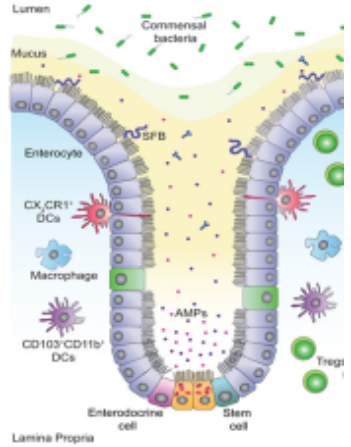
Muramidase modulates the immune response at ileum

The **immune effect of CP** was only detected in the lamina propria

Peyer Patches		Fold change/ LP					P values	
Immune response	Gene	LP	LP+	HP	HP+	CP	MUR	CP x MUR
Anti-inflammatory	**IL10RA**	1	-1.32	1.09	-1.66	0.96	<0.05	0.65
Pro-inflammatory	**IL27**	1	-2.08	-1.07	-1.53	0.46	<0.05	0.09
	TNFSF14	1	-1.92	1.25	-1.68	0.06	<0.05	0.48



Lamima Propia		Fold change/ LP					P values	
Immune response	Gene	LP	LP+	HP	HP+	CP	MUR	CP x MUR
Anti-inflammatory	**IL10**	1	-1.10	1.23	-2.21	0.59	0.02	0.24
	IL10RA	1	-1.28	-1.24	-2.22	0.05	0.05	0.99
Pro-inflammatory	**IL17B**	1	1.75	3.45	-1.86	0.63	0.07	<0.01
	IL27	1	-1.05	-1.72	-2.33	<0.01	0.45	0.77
	CCL2	1	-2.03	1.12	-1.66	0.81	0.02	0.94
	CSF3	1	-1.32	-1.95	-1.77	<0.01	0.44	0.35
	CXCL12	1	-1.68	-1.01	-3.38	0.27	0.01	0.52



Over the 42 genes evaluated, only are presented those which showed fold change +/- 1.5 and P>0.05

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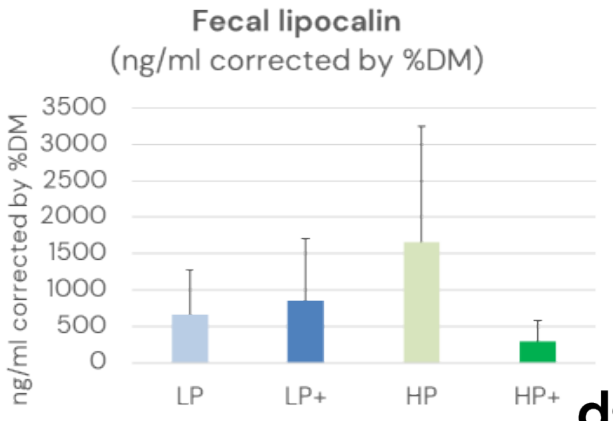
Muramidase reduces inflammation at mesenteric lymph nodes (key mediators of the adaptative immune response)

High protein diet downregulated genes encoding **anti-inflammatory** cytokines and **upregulated** genes encoding **pro-inflammatory** cytokines

Mesenteric lymph nodes		Fold change/ LP				P values		
Immune response	Gene	LP	LP+	HP	HP+	CP	MUR	CP x MUR
Anti-inflammatory	**IL12B**	1	1.21	-1.14	-1.79	0.01 ↓	0.36	0.21
Pro-inflammatory	**IL27**	1	-1.49	-1.18	-1.48	0.20	0.02 ↓	0.26
	IL17B	1^{b}	2.38^{ab}	2.72^{a}	-1.69^{b}	0.52	0.89	0.02
	CCL2	1	-1.20	2.18	2.05	0.02 ↑	0.87	0.69
	CXCL12	1	-1.90	1.99	1.92	<0.01 ↑	0.24	0.43
	CXCL9	1	-2.83	3.71	1.91	0.05 ↑	0.04 ↓	0.16

The interaction detected for the gene encoding the **pro-inflammatory IL17B** with a **downregulation in MUR and HP diets,** but not in low protein diets, are in agreement with the results observed in the **fecal lipocalin** (inflammation biomarker)



CP: P = 0.56
MUR: P = 0.12
CP x MUR: **P= 0.04**

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Conclusion

Dietary **muramidase** supplementation for 2 weeks post-weaning **reduced gut inflammation measured** in MLN and feces, **improved nitrogen utilization and Vit A absorption,** resulting in **lower post-weaning diarrhea in piglets,** especially in HP diets

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