In vitro prediction of calcium and phosphorus availability using marine sourced calcium and limestone in monogastric diets

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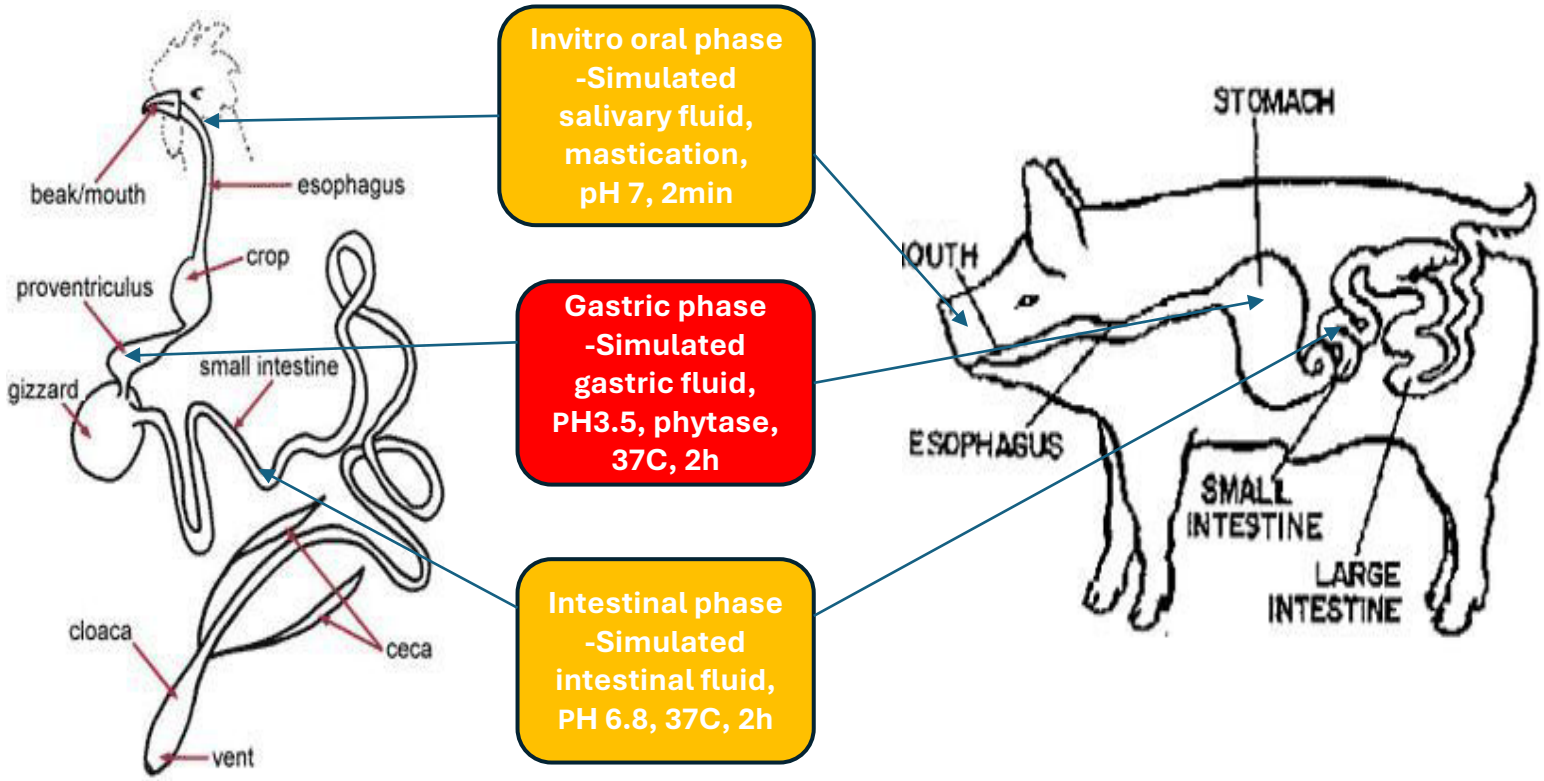
Mineral availability in monogastrics

- Availability of mineral is limited in monogastric nutrition (Tofuko A et. al., 2022 & M. Marounek et.al., 2008).
- Phytate- plant storage form of phosphorus and a known chelator of minerals (C.L. Walk et.al., 2012).
- Calcium is a main mineral that interacts with phytate due to its abundance in diets.
- Phytase promote release of phytate phosphorus and reduces antinutritional dietary interaction
- But does the source or nature of calcium matter?

Simulation of the monogastric digestive system

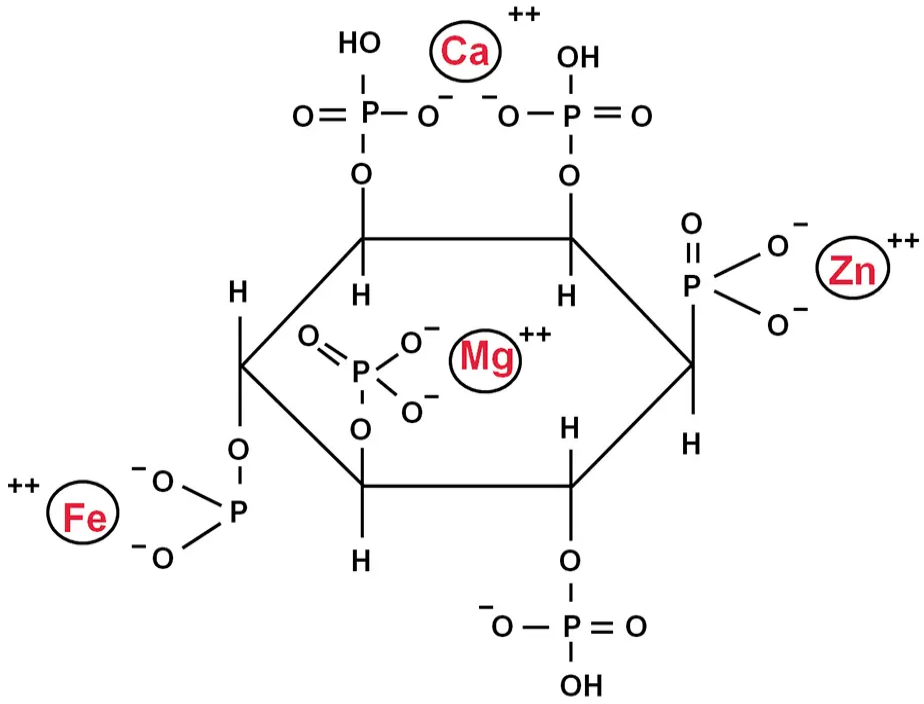


- Internationally accepted Peer reviewed INFOGEST protocol (Brodkorb, A. et.al., 2019)
- Incorporate elements that's relevant to digestion *in vivo.* Thus, physiologically relevant method

Identifying interactions in diets with calcium

2mM Phytic acid (Dietary reference for Corn and Soybean meal+30mM calcium (0.96%Ca in Feed)

Reference: Angel and Applegate 2000

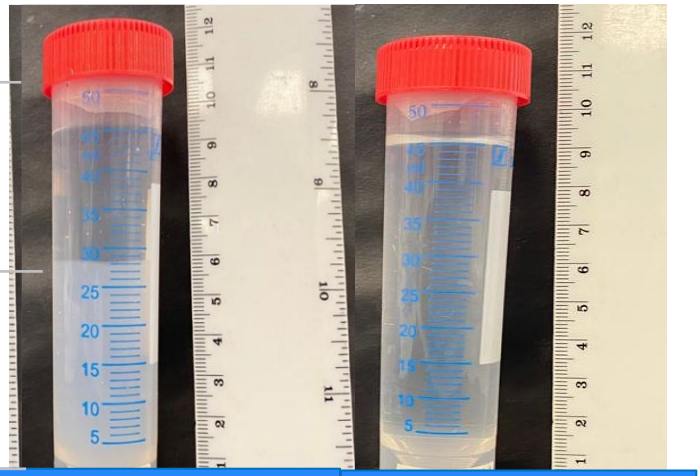


Phytate as chelator of minerals

No Phytase

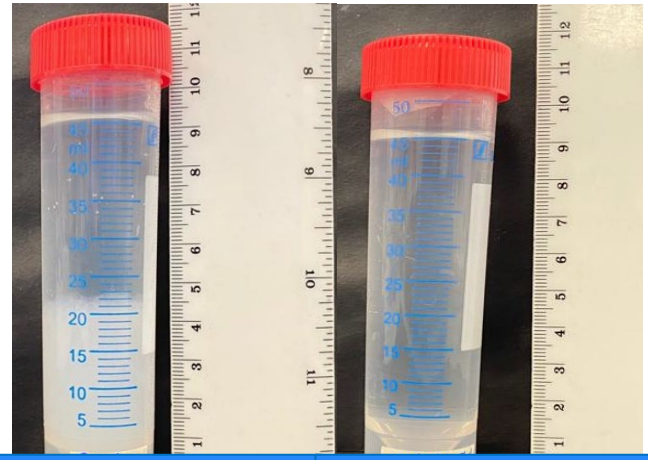
Unbounded Ca & P

Ca-Phytate complex



Positive control +Ca | Negative control -Ca

Phytase



Positive control +Ca | Negative control-Ca

Phytase decreases the interaction but does not eliminate it

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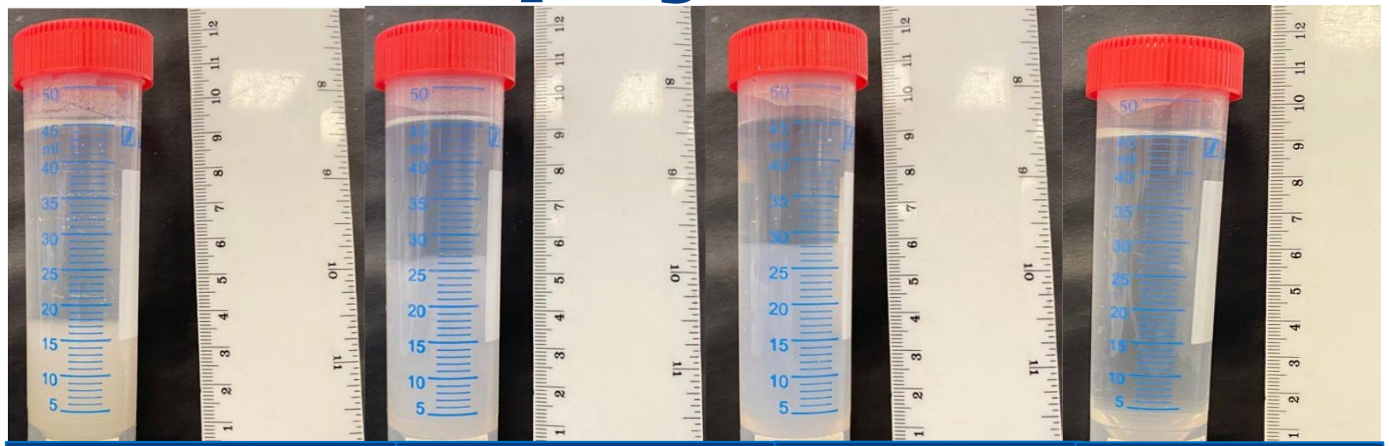
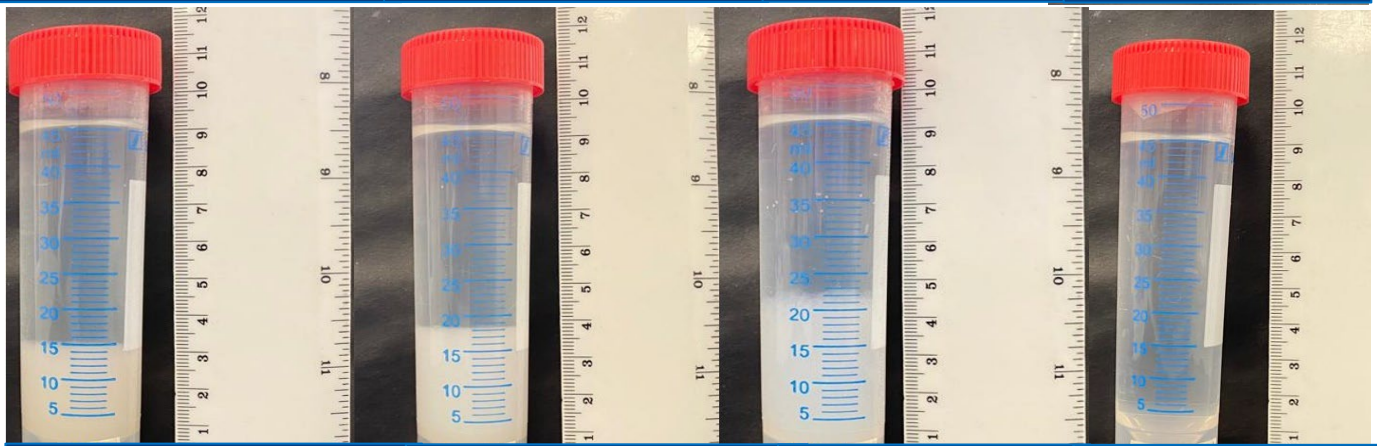
Does calcium source matter for calcium and phytate interaction

No Phytase



Marine mineral complex | Feed grade Limestone | +Ve Ctrl +Ca | -Ve Ctrl -Ca

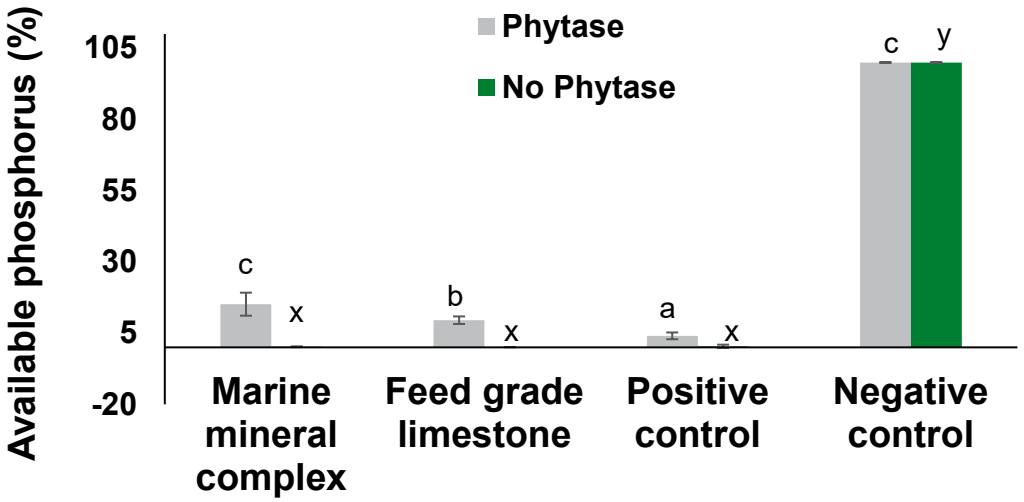
Phytase

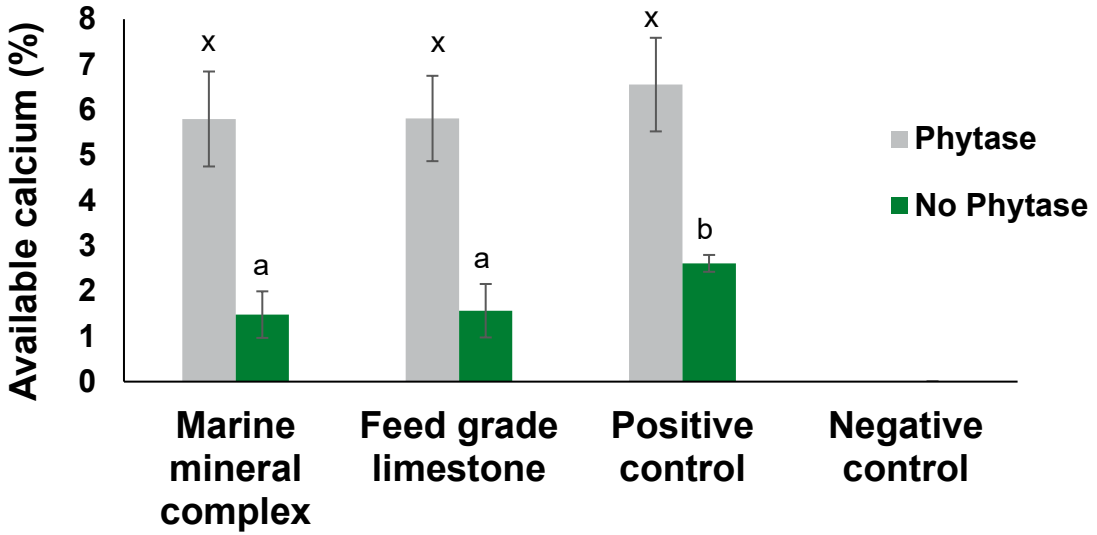


Marine mineral complex | Feed grade Limestone | +Ve Ctrl +Ca | -Ve Ctrl -Ca

- Three (3) sources of calcium with equal concentration of phytic acid was used.
- No precipitation in the negative control due to lack of calcium.

Calcium sources and phytase inclusion affects Calcium – phosphorus interaction





	Soluble Phosphorus	Soluble Calcium
Calcium source	<0.001	<0.001
Enzyme (-/+)	<0.001	<0.001
Ca source Vs Enzyme	<0.001	<0.001

- Enzyme inclusion also affect phosphorus and calcium release
- Marine mineral complex improved release of phytate phosphorus compared to feed grade limestone.
- While maintaining calcium level as Limestone

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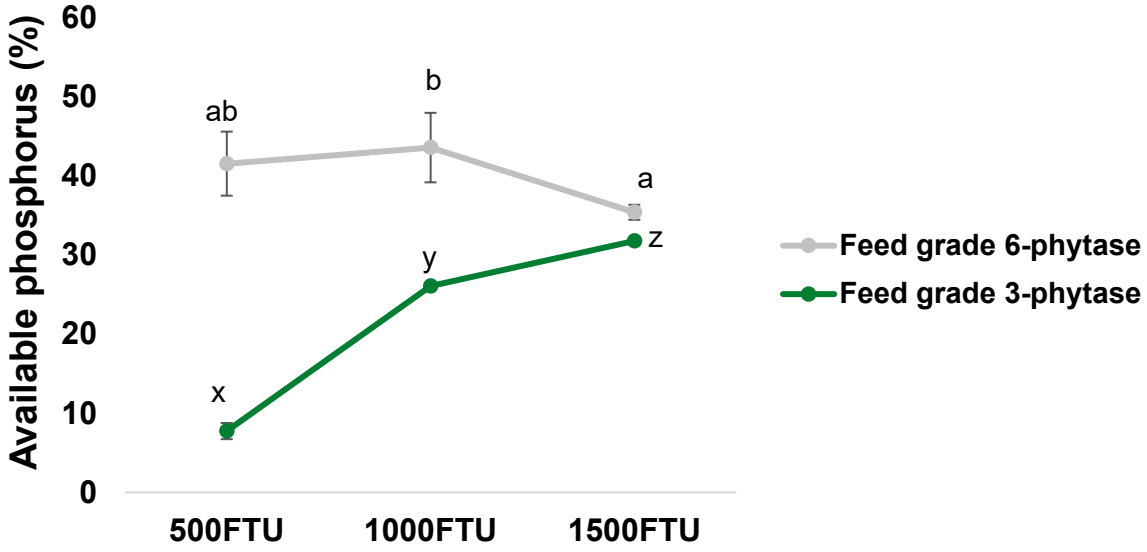
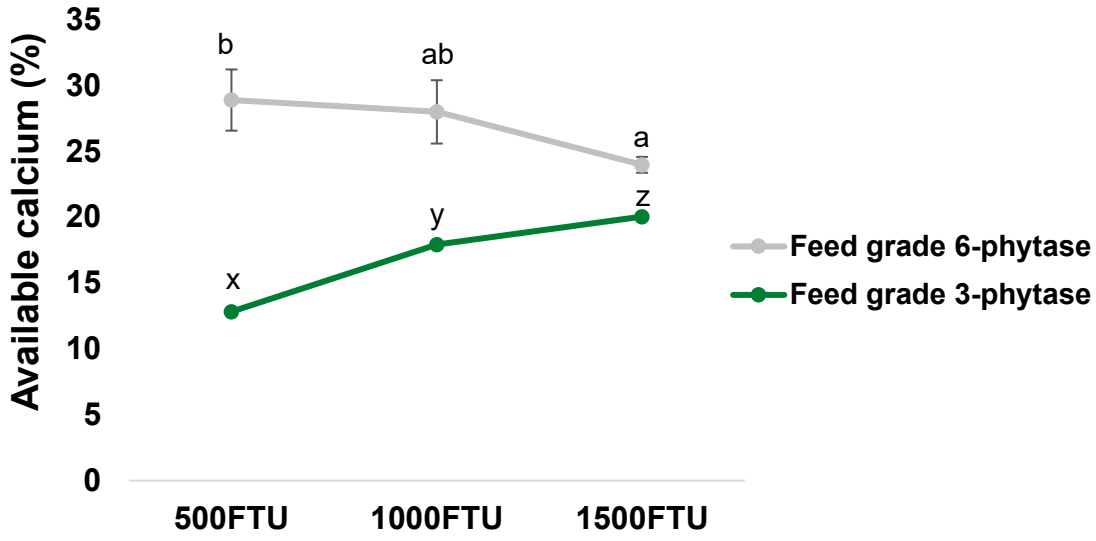
Type of phytase and inclusion rate affects Ca and P release

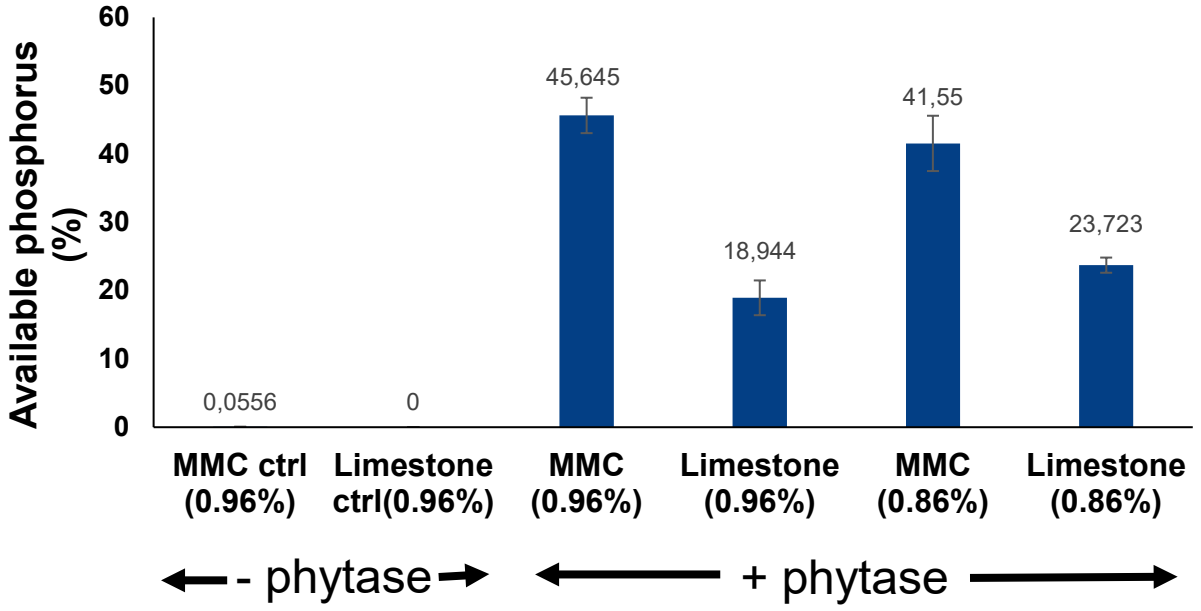




- **Calcium(0.96%) and 2mM phytic acid** (Angel and Applegate 2000)
- **Type of commercial phytase affects phytate phosphorus and calcium release.**
- **Inclusion rate of phytase also affects calcium – phosphorus availability.**
- **Feed grade 6-phytase showed superior activity over 3-phytase**

Calcium source and concentration impact on Ca-P interaction

A



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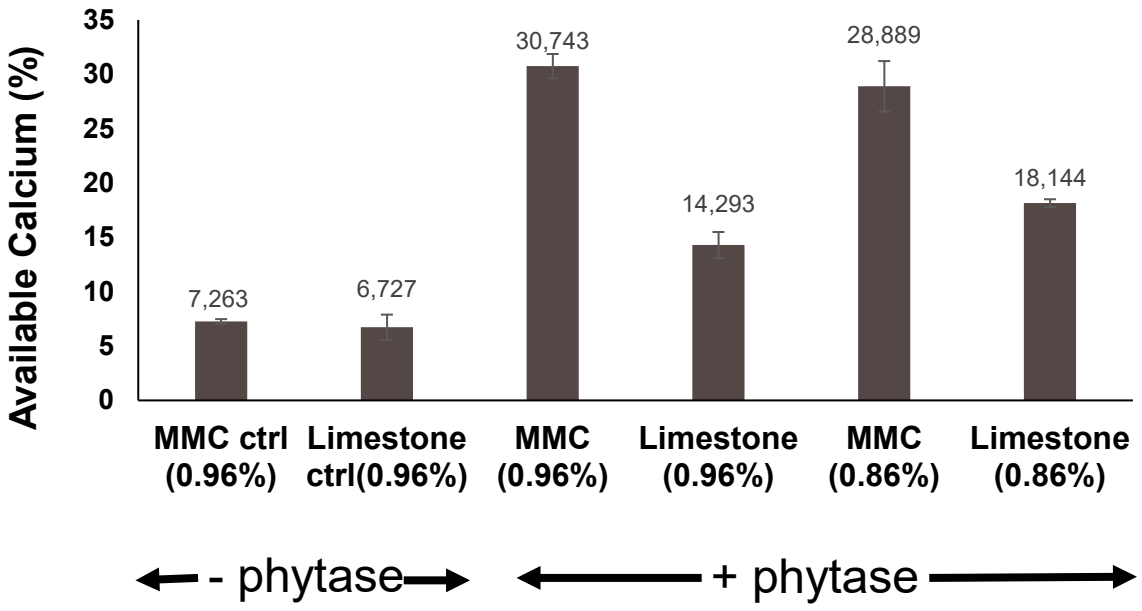

- Marine mineral complex improved phytate phosphorus release at similar concentration as Feed grade limestone
- More soluble calcium at lower calcium dose from MMC
- Reduced calcium phosphorus interaction translate to more phosphorus.

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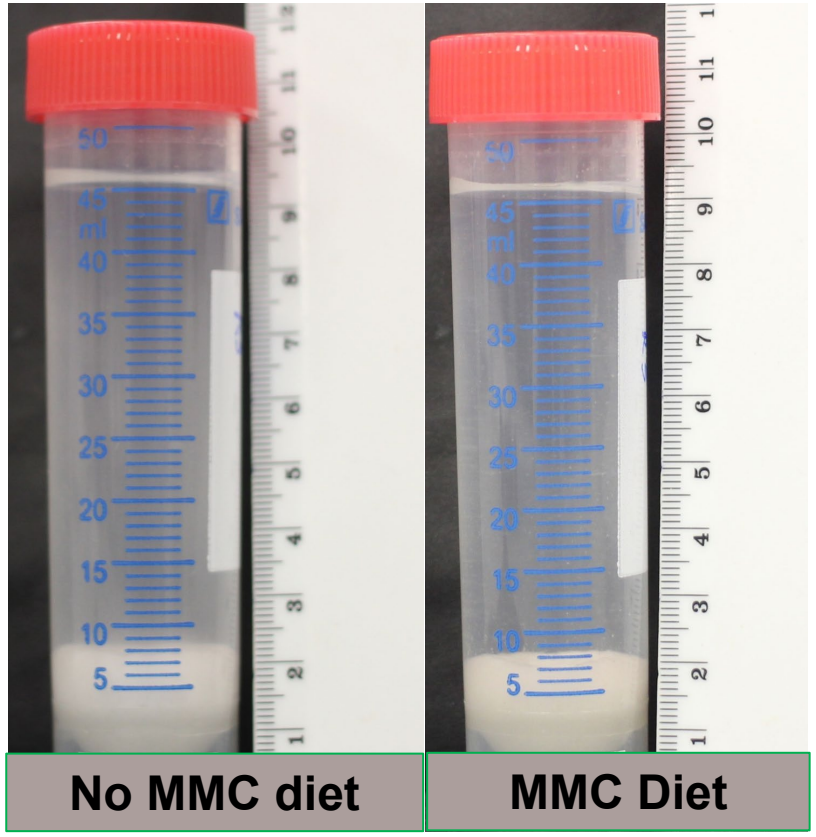
Supplementing feed grade limestone diet with Marine mineral complex(MMC)

	Swine growers' diet	
	No MMC diet	MMC diet
Phytic acid	2mM	2mM
MCP	0.45%	0.45%
Feed grade limestone	0.91%	0.4%
MMC	-	0.4%
Phytase(FTU)	1500	1500
Added Ca	0.46%	0.38%



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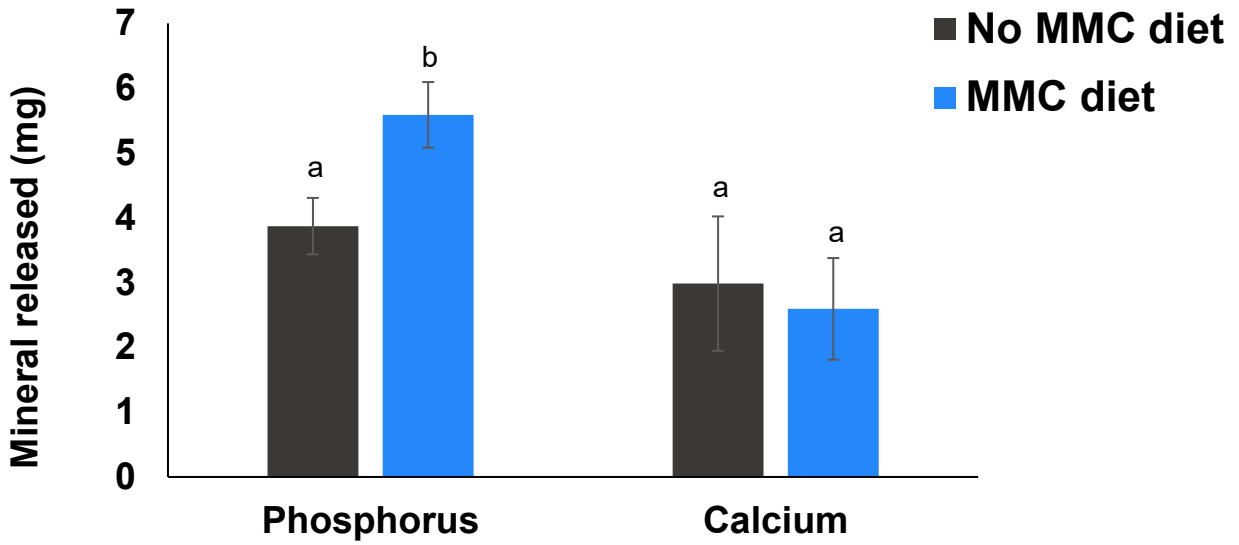
Reduced calcium from Marine mineral complex improves release of Phytate-P



- Supplementation of feed grade limestone with marine mineral complex,
  - improved phytate phosphorus release
  - Increased phosphorus may allow reduction of MCP.

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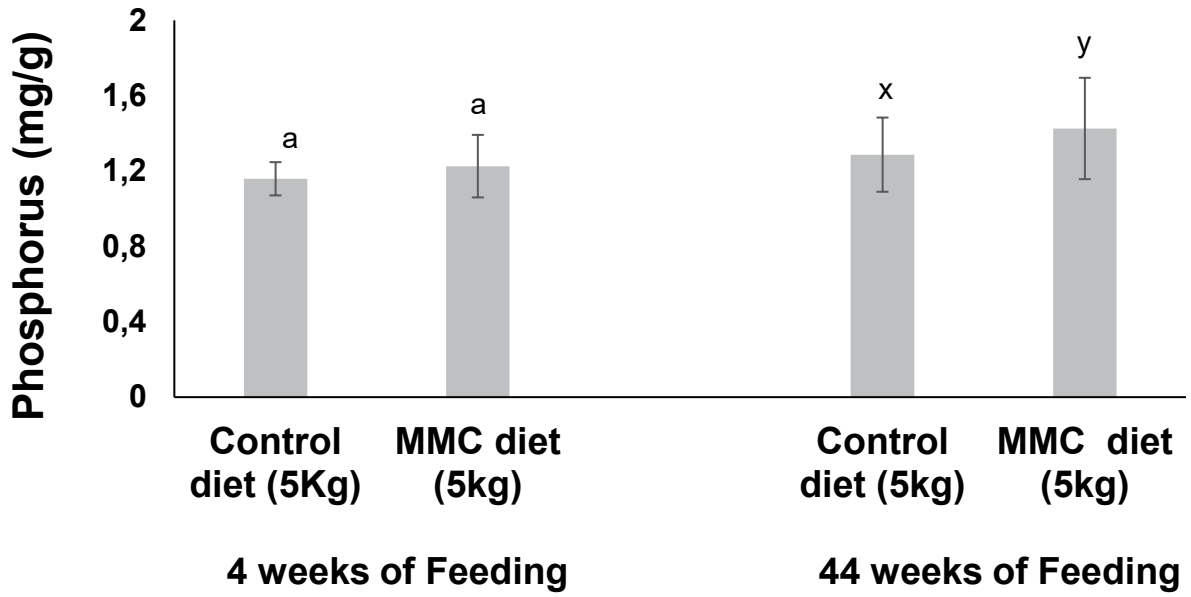
In vivo proof of concept trial



- Significant increase in phosphorus content of eggshell from laying hens fed MMC diet.
- Reduced interaction with phytate leading to more phosphorus for shell mineralisation

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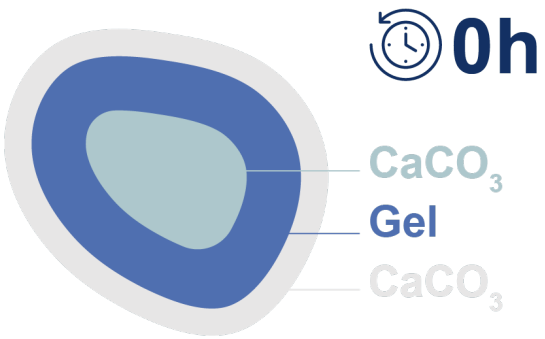
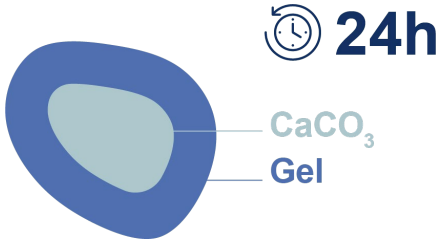
How it works | removing interactions

Removing interactions with marine polysaccharide gel

- MMC has a unique structure with an outer calcified layer, a polysaccharide hydrogel and inner calcified core.
- There are also minerals trapped within the polysaccharide hydrogel.
- This structure is vital for the removal of the interaction between calcium, phosphorous and phytate.
- The hydrogel is present throughout the material, also when it is ground down

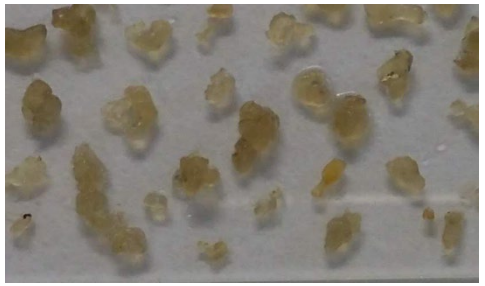


Original material



Outer calcified layer removed, and calcium left in the carbohydrate hydrogel layer.

Inner calcified core removed only polysaccharide hydrogel layer left.



Decalcification was achieved by EDTA-4Na, with refreshing the EDTA-4Na every 24hrs

Conclusion

- Overall, the source and nature of calcium supplement affects mineral availability.
- Using Marine mineral complex allows more efficient release of phytate phosphorus
- Marine mineral complex may allow safe reduction of MCP
- Marine mineral complex could be a more sustainable alternative to limestone.