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Assessment of new phytase products through an in-vitro digestion model alongside a broiler chicken trial

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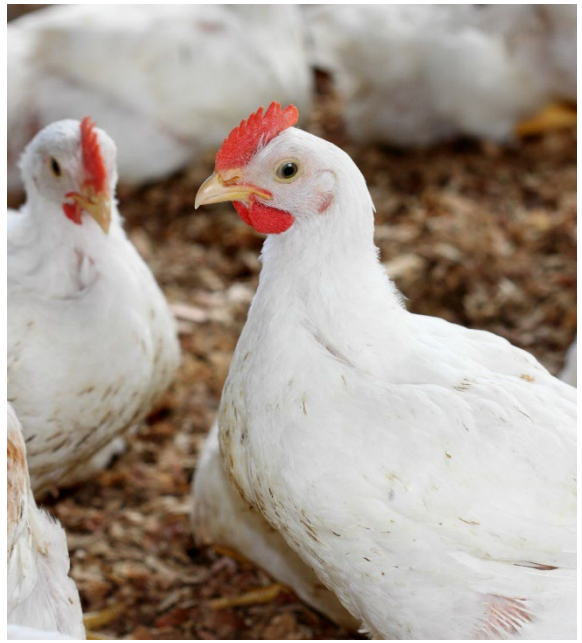
Context

- Phytases are key enzymes in animal production
- New generation phytases are now available
- Authorized products vary according to the geographical area
- Their nutritional value needs evaluation for optimal inclusion



- The objective was to evaluate new phytase products through an *in-vivo* trial and an *in-vitro* model and to compare these two approaches

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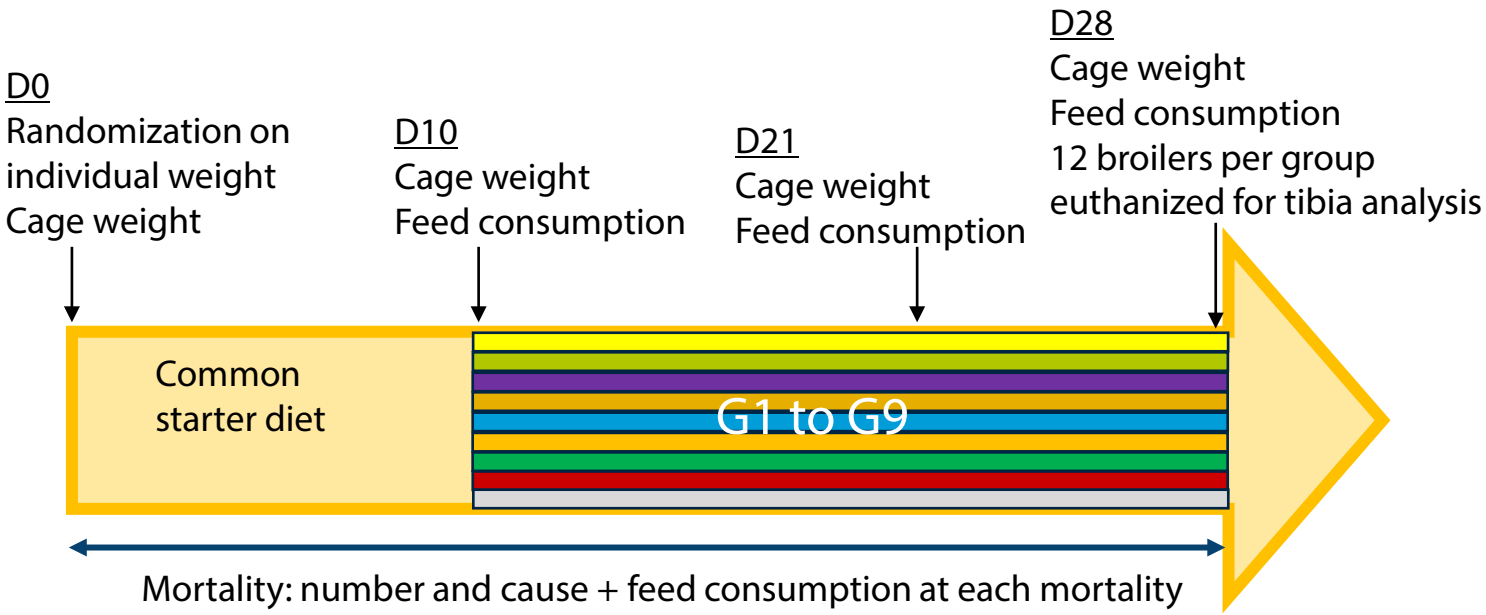
In-vivo screening

- Ross 308 male
- 4 broiler chickens per cage
- Randomization on initial individual body weight
- Diets: - A common starter diet from 0 to 10 days
  - Grower diets from 10 to 28 days :
    - 3 groups with increasing level of aP: 0.15, 0.30 and 0.45 % - 8 replicates
    - 6 groups with phytase (A to F) at 1.5 * recommended dose - 12 replicates



In-vivo phytase screening

Starter diet

INGREDIENTS (%)	
Wheat	33.34
Corn	24
SBM	30.2
Wheat bran	2.0
Sunflower meal	2.0
DDGS	3.0
Soybean oil	1.7
Na Bica	0.2
Dicalcium phosphate	1.8
Limestone	0.6
Salt	0.2
Premix and AA	1.25
NUTRIENTS (%)	
AME (kcal/kg)	2700
CP	21.5
Total P	0.74
Available P	0.45
Calcium	1.0
Lys dig	1.15

Phytase diets formulated by substituting a portion of wheat in the aP 0.15 diet by phytase mix

Grower diets

INGREDIENTS (%)	aP 0.15	aP 0.30	aP 0.45
Wheat	38.0	38.0	37.8
Corn	25.9	26.4	26.2
SBM	20.7	21.3	21.7
Wheat bran	4.1	2.9	2.0
Sunflower meal	3.5	3.0	3.0
DDGS	3.0	3.0	3.0
Soybean oil	2.5	2.5	2.7
Na Bica	0.2	0.2	0.2
Dicalcium phosphate	-	0.9	1.8
Limestone	0.9	0.7	0.4
Salt	0.2	0.2	0.2
Premix and AA	1.02	1.02	1.01
NUTRIENTS (%)			
AME (kcal/kg)	2800	2800	2800
CP	19.0	19.0	19.0
Phytic acid	0.28	0.27	0.26
Total P	0.42	0.57	0.72
Available P	0.15	0.30	0.45
Calcium	0.60	0.75	0.90
Lys dig	1.0	1.0	1.0

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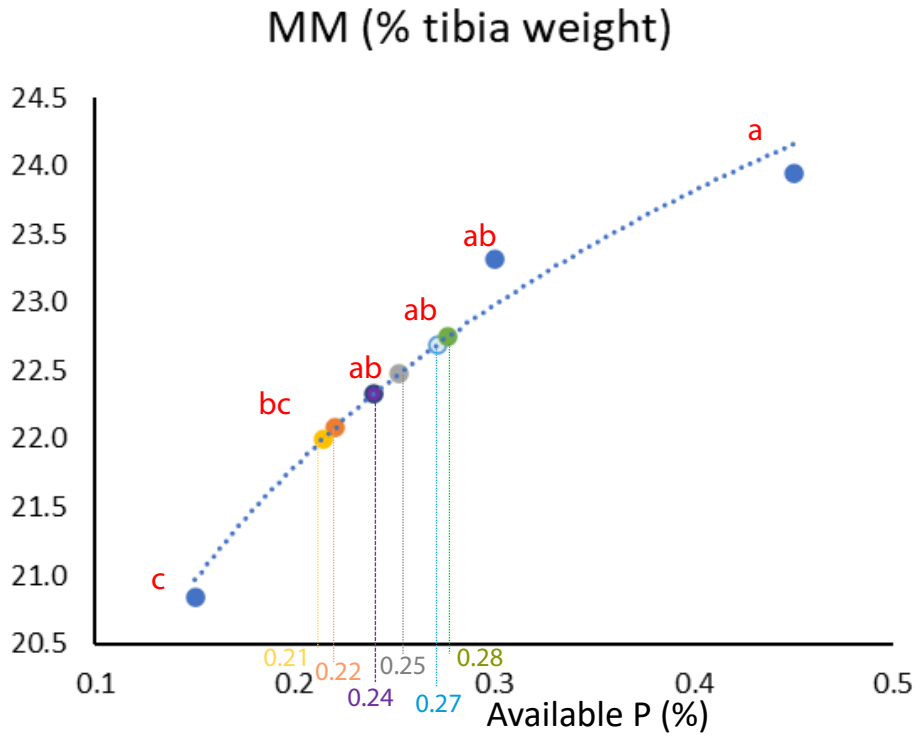
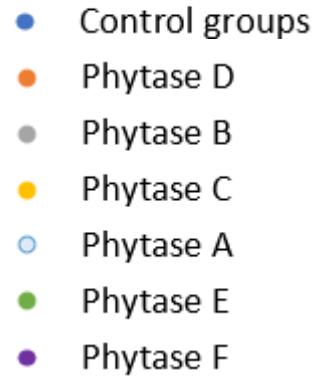
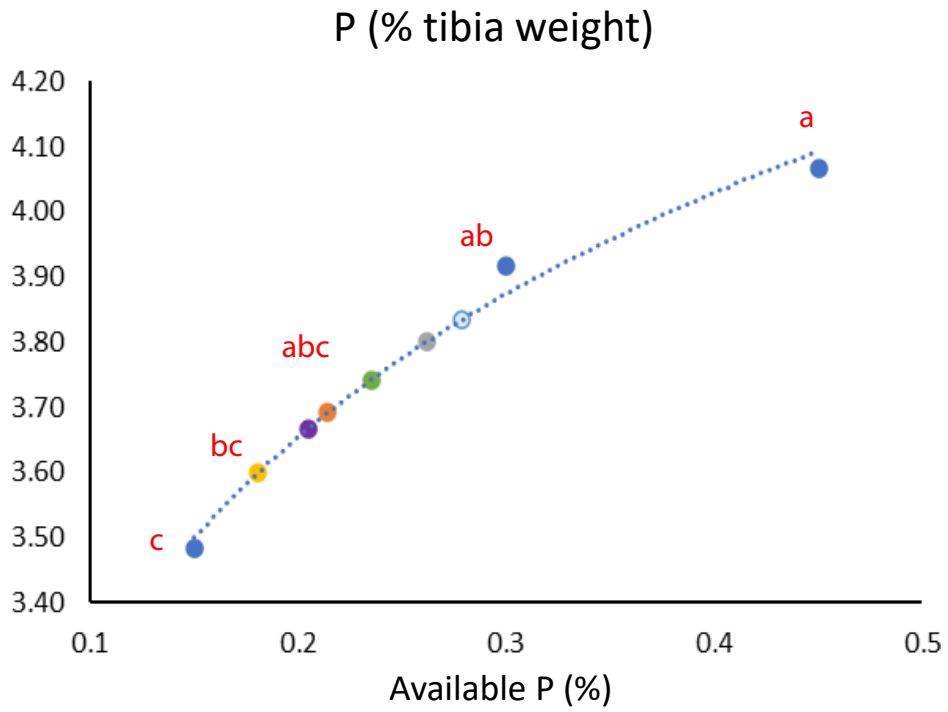
In-vivo screening - Results







aP equivalent (%)	Phytase A	Phytase B	Phytase C	Phytase D	Phytase E	Phytase F
Analyzed phytase dose (U/kg)	1235	1485	1770	934	1650	2301
Tibia MM (% tibia)	0.12	0.10	0.06	0.07	0.13	0.09

In-vitro screening

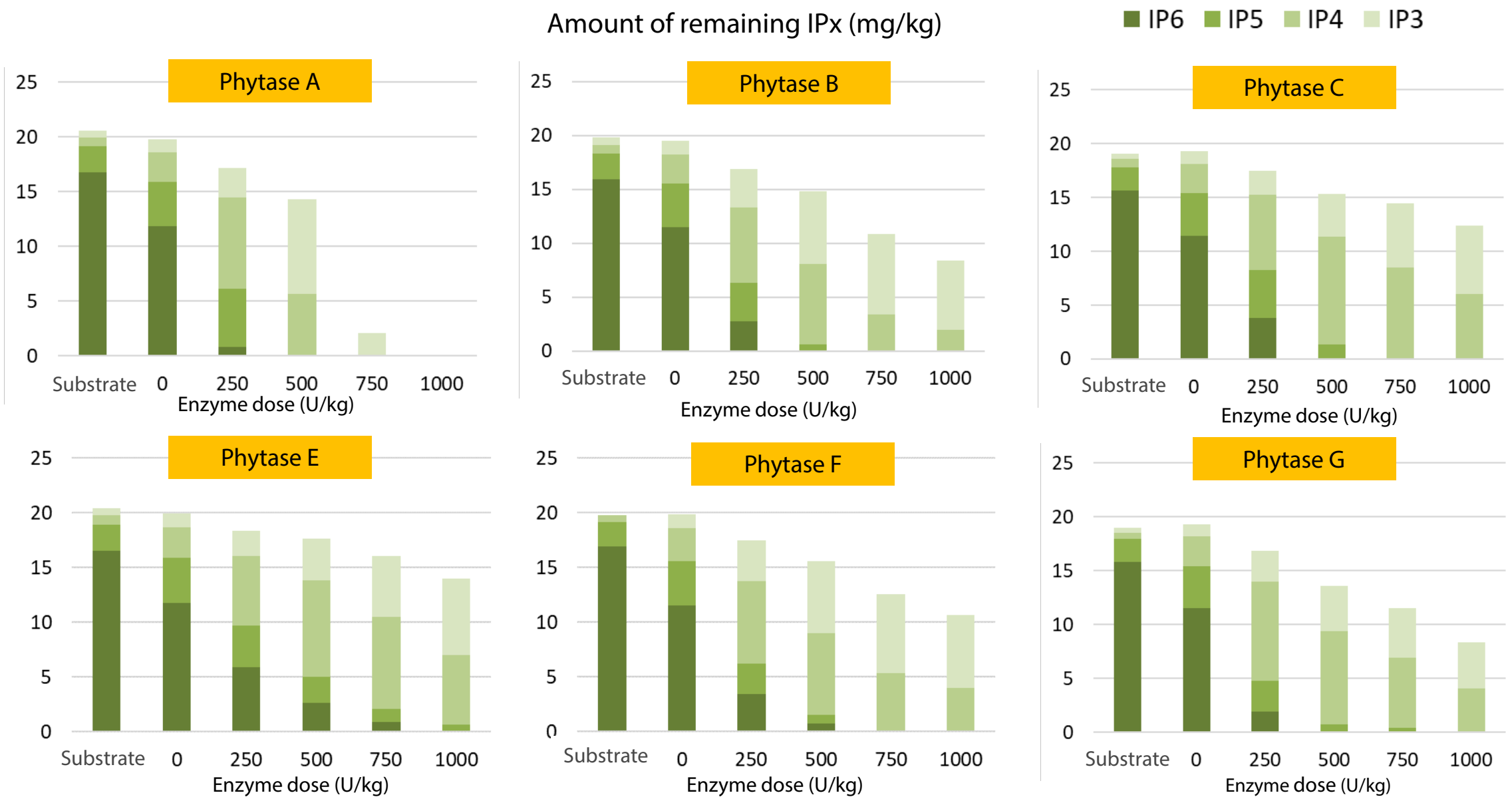
3-STEP DIGESTION SIMULATION MODEL (Rivière *et al.*, 2021)

- Extraction of the phytase and phytase activity measured
- 5 doses tested for each phytase: 0, 250, 500, 750 and 1000 U/kg - in duplicate
- Action on a blend of ingredients: 30% corn, 30% wheat, 25% soybean meal, 15% sunflower meal

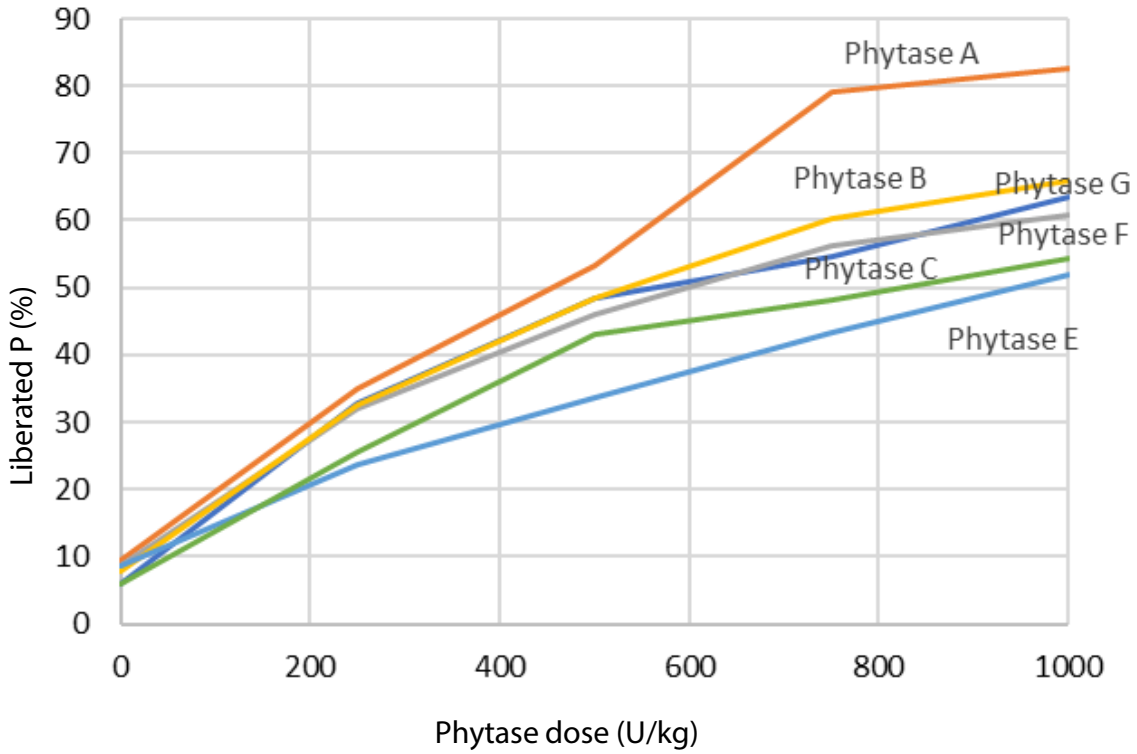
    1. Humidification with phytases in a pH 5 buffer – 40 °C
    2. Addition of pepsin and HCL – 40 °C
    3. Addition of pancreatin and $NaHCO_3$ – 40 °C

- Inositol phosphates (IP) 6, 5, 4 and 3 measured in the residual substrate – liberated P calculated

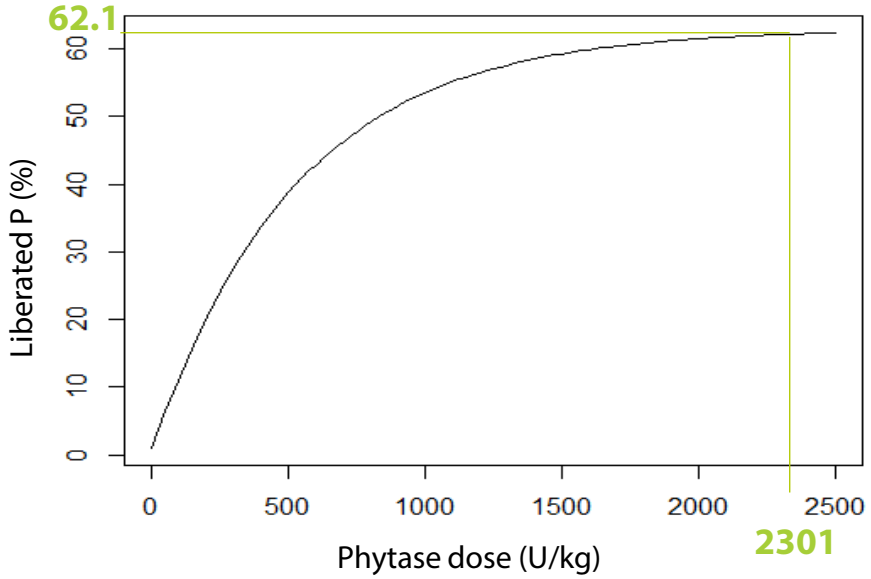
In-vitro screening - Results



In-vitro phytase screening



Orskov model : liberated P (%)= a + b * (1 – e $^{-c * dose}$)



Ex. for phytase F - equation: 0.96 + 62.01 * (1 – exp (- 0.001883 * x))

Calculation of the P liberated (%) using

- P released (%) in the in-vitro model = 62.1 %
- Phytic acid content in the in-vivo grower diets (0.28 %) = 0.17

In-vivo / in-vitro phytase screening

IN VITRO

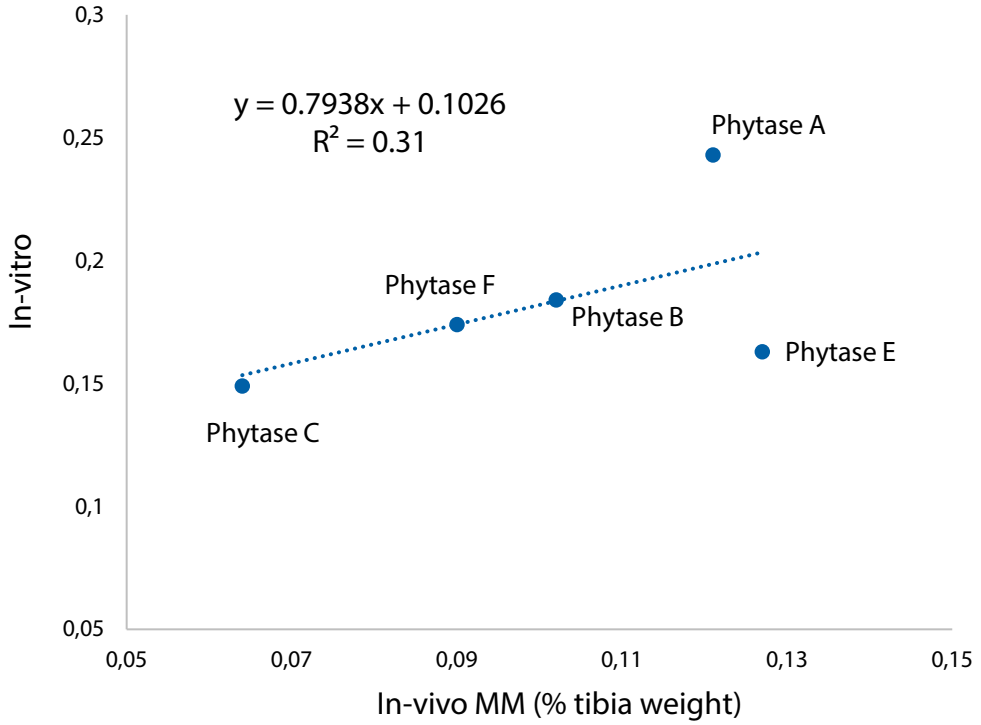
	Phytase A	Phyase B	Phytase C	Phytase E	Phytase F
Phytase dose (U/kg)	1235	1485	1770	1650	2301
liberated P (%)	0.24	0.18	0.15	0.16	0.17

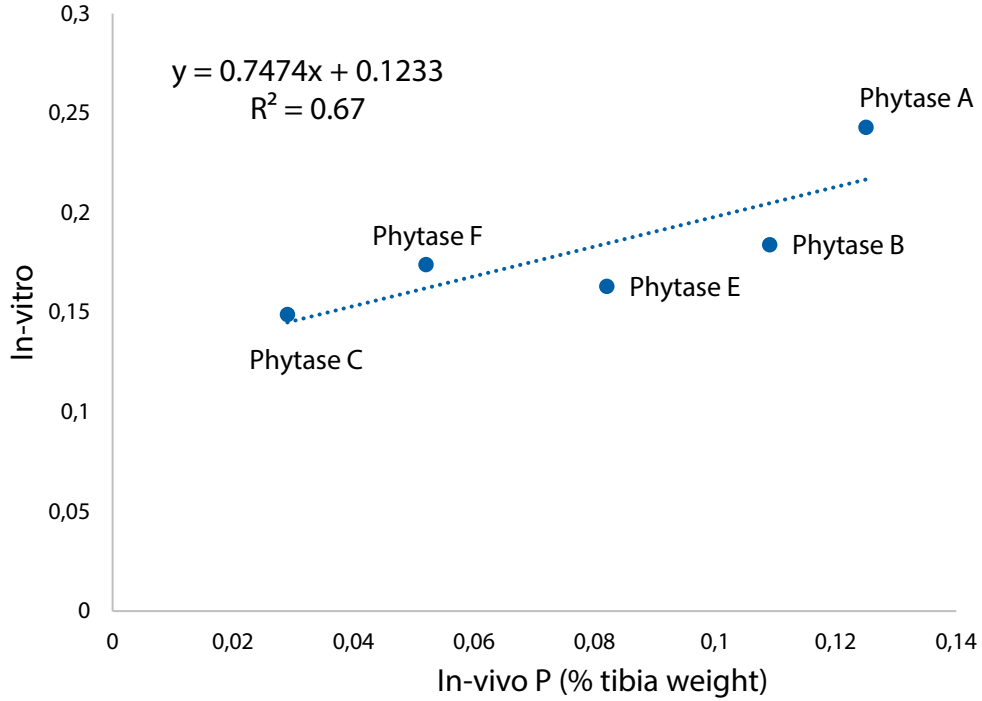
IN VIVO

aP equivalent (%)	Phytase A	Phytase B	Phytase C	Phytase D	Phytase E	Phytase F
Analyzed phytase dose (U/kg)	1235	1485	1770	934	1650	2301
ADG	0.41	0.41	0.40	0.31	1.17	1.11
FCR	0.23	0.16	0.31	0.34	1.28	0.88
Tibia MM (% tibia)	0.12	0.10	0.06	0.07	0.13	0.09
P (% TW)	0.13	0.11	0.03	0.06	0.09	0.05

In-vivo / in-vitro phytase screening

Phytase P equivalent





Conclusion

- Hierarchy between phytases varies with the response criteria *in-vivo*
- Interesting correlation between *in-vitro* and *in-vivo* results linked to "phosphorus" response
- The in-vitro model is a valuable assessment tool for phosphorus release and valorization. Particularly interesting to evaluate many products
- Zootechnical response necessary to evaluate the extra-phosphoric effect

In-vivo

- 👍 Evaluation of extra phosphoric effects
- 👍👎 Individual and inter-assay variation
- 👎 Costly
- 👎 Labor intensive
- 👎 Time consuming

In-vitro

- 👎 Single evaluation criteria
- 👍 Standardized
- 👍 Less expansive
- 👍 Shorter
- 👍 3 R

Thank you for your attention

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