Taking Advantage of *in vivo* Hydrolysis in Animal Feeds for Phosphorus Adjustment

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Introduction

- Phosphorus (P) content is “quality characteristic” towards **animal feeds** are optimized
- **Phytic acid** (inositol phosphate, IP) accounts for up to 90% of P-content in grain[1] and is considered as **antinutrient** (binds essential mineral cations)
- During germination phytic acid is hydrolyzed by **intrinsic enzymes** and the liberated phosphate is further utilized by the plant
- Monogastric animals show insufficient enzymatic hydrolysis and the subsequent input into the environment through **fertilization/ manure** results in **eutrophication**

Aim

- Process for the **P-adjustment in animal feed** on the **basis of rye bran** (1.5 Mt/a market volume in Germany[2])

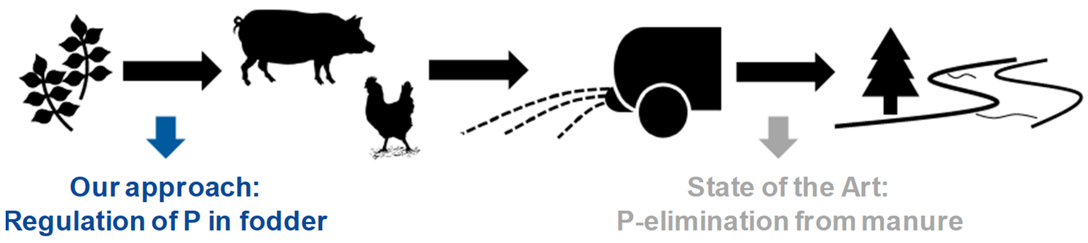


Fig. 1: Schematic illustration of the project idea: Phosphorus-adapted fodder to reduce the phosphorus content in the resulting manure.

- Determination of the **efficiency of intrinsic enzymes hydrolyzing phytic acid *in vivo*** (consortium of enzymes)
- Hydrolysis as function of **t, T, pH** and **bran to water ratio %(w/v)**

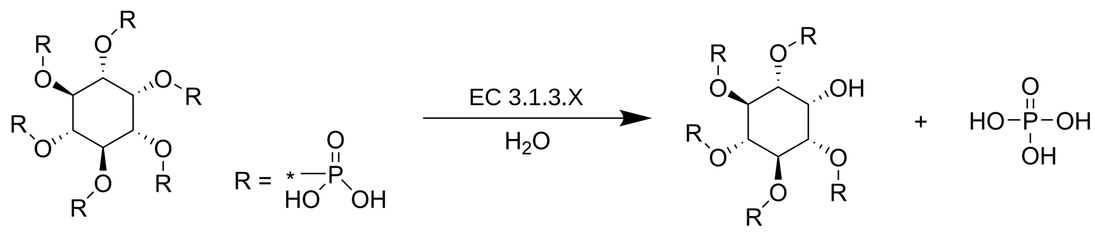
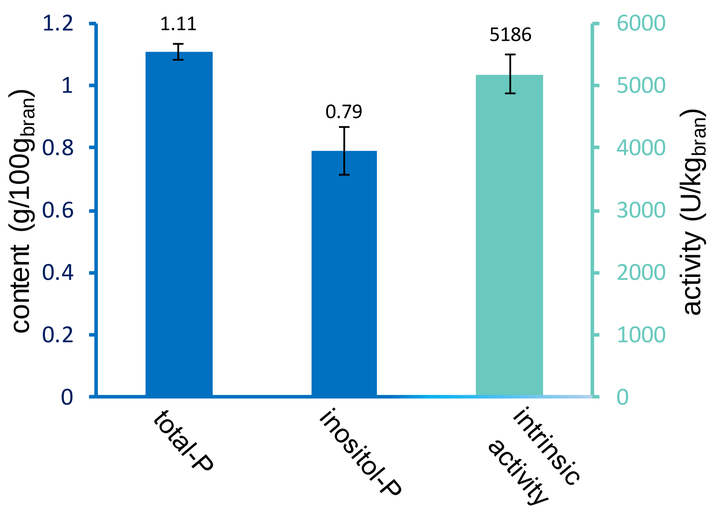


Fig. 2: Reaction scheme for the hydrolysis of phytic acid using phosphoric monoester hydrolases (phytases)

Analytics and Statistics

- Quantification of **total Phosphorus** by dry ashing, IP_3 - IP_6 **concentrations** by HPLC[3] and **intrinsic enzyme activity** by molybdenum blue reaction[4]



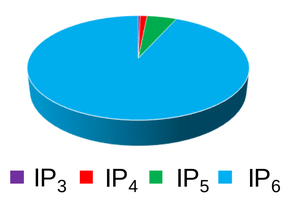


Fig. 3: Total-P, IP and intrinsic enzyme activity in rye bran (left). Distribution of differently phosphorylated inositols in rye bran (right). Data collected over a period of 20 weeks. Different storage conditions (-20 °C, 4 °C, room temperature) showed no influence on total-P, inositol-P and intrinsic activity.

- **Design of Experiments** (**DoE**): Temperature hard-to-change, split plot design with I optimization, prediction by polynomial regression

Results: Intrinsic Enzyme Activity

- **Maximum** of 5565 U/kg_{bran} (42.3 °C, pH 5.33, 43 min)

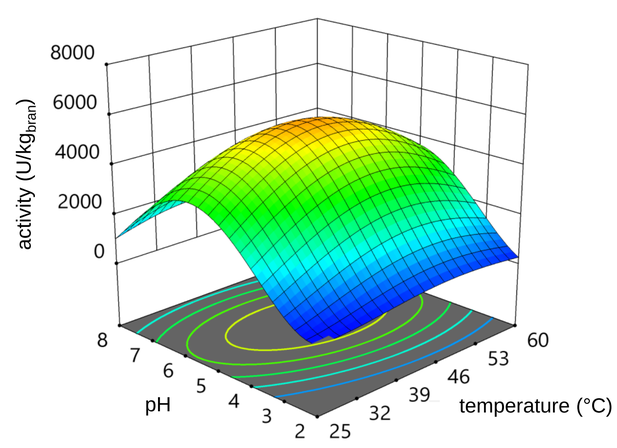


Fig. 4: Enzyme activity as function of temperature and pH. Reaction conditions: 0.1 - 0.2 g bran in 50 ml reaction solution (1.5 mM phytic acid, 0.25 M potassium acetate). Temperature: 25 - 60 °C, pH 2 - 8. Design Expert® 12 (Stat-Ease, Inc.).

Results: IP-Degradation

- Model predicts 100% degradation
- Experiments show **79% degradation** (33 °C, pH 5.5, 14.1%(w/v), 6 h)

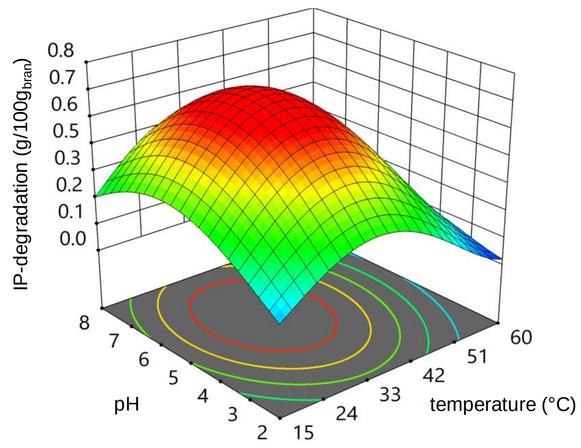


Fig. 5: Inositolphosphate-degradation as function of temperature and pH. Conditions: 1.5 g ground bran incubated at certain temperature, pH, water content and time. Orbitally shaken at 120 rpm. Termination by adding 0.5 M HCl. Extraction of IP by IEX (aminopropyl). Design Expert® 12 (Stat-Ease, Inc.).

Comparison of Results

- Product inhibition, substrate limitation and lower reaction rates (IP_5-IP_3) are not considered by the model

Tab. 1: Comparison of data sets including the validation point

	maximum intrinsic activity	maximum IP-degradation	validation point
°C	**42.8**	33.0 (**40.0**)[b]	42.3
pH	5.3	5.5	5.0
$\%(w_{bran}/v_{H2O})$	0.4[c]	14.1	19.4
min	60	360	195
U/kg_{bran}	**5565**	-	716[a] (**4653**)[b]

[a]Activity calculated from the IP-degradation obtained at the validation point. [b]Value calculated from the respective data set for 30 min reaction time. [c]Fixed value for the activity assay.

Conclusion

- Results suggest that mainly **acid histidine phosphatases** are involved[5]
- **Majority** of the inositol phosphate is initially **degraded** within **30 min** at a rate of **4653 U/kg_{bran}**
- Utilizing intrinsic enzymes can **reduce the use of conventional phytases** in the feed sector

References:

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