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Effects of dietary glycerol on glycerol kinase gene expression and gut microbiota in growing piglets

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Overview

- Alternative pig nutrient sources. What is the need?
- Glycerol as an alternative feedstuff-perspectives
- Usage limitations and the role of glycerol kinase (GK)
- Objectives of the present work
- Experimental design and traits of glycerol
- Results
- Conclusions
- Implications

- Pig feeding with **cereal**-based diets (↑ energy content)
- The turn towards biofuels globally → increased price of **high energy feedstuffs:**

  a. reduced land availability (compete biofuel crops)

  b. increasing use of cereals (rich in starch) or sugar beet and molasses (rich in sugars) in bioethanol production

- Second generation technologies (from perennial crops) are limited
- Importance of evaluating the use of alternative nutrient sources for pigs

- Glycerol (biodiesel by-product) is a promising alternative nutrient source:

  - **high energy** content (14.4 MJ DE/kg)

  - sweet taste → high **palatability**

  - **readily absorbed** (>90%)

  - **does not compete** other feedstuffs

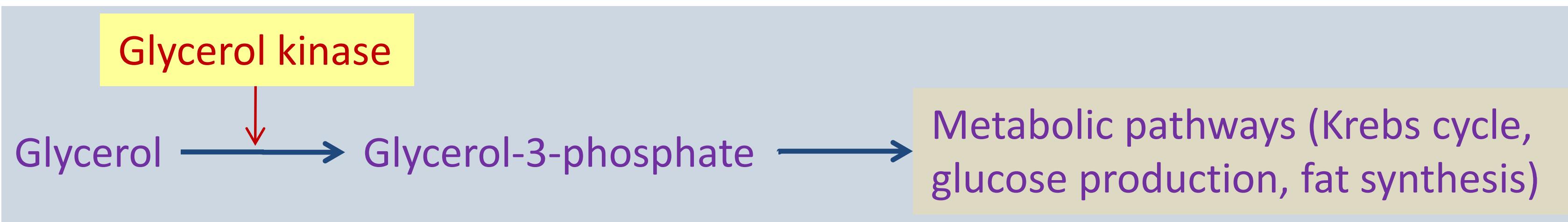
  - significant **lowering** of glycerol **prices** is expected

  - **enormous surplus** will become available (100 kg of fats or oils contain 10 kg glycerol + 90 kg fatty acids)

- Limitations of dietary glycerol addition??

Introduction

- Current limitations are defined by the association of high dietary glycerol levels with low animal performance (body weight gain etc.)
- Role of glycerol kinase (GK)??



Adapted from Bartelt & Schneider, 2002

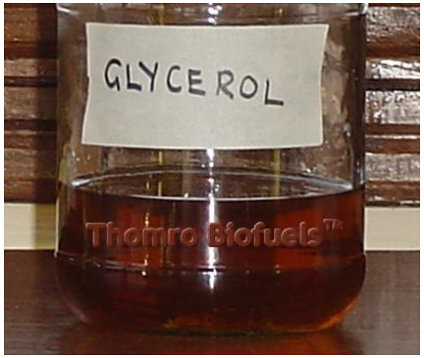
- The role of GK may supply with more precise info about dietary addition levels and glycerol utilization
- Additionally, there are no data on the effect of glycerol on gut microbiota → overall nutrient digestibility

Objectives

To study the effects of adding crude glycerol at 7.5 and 15.0 % (at the expense of maize mainly) in piglet diets, on:

- Glycerol kinase gene expression in liver tissue homogenates

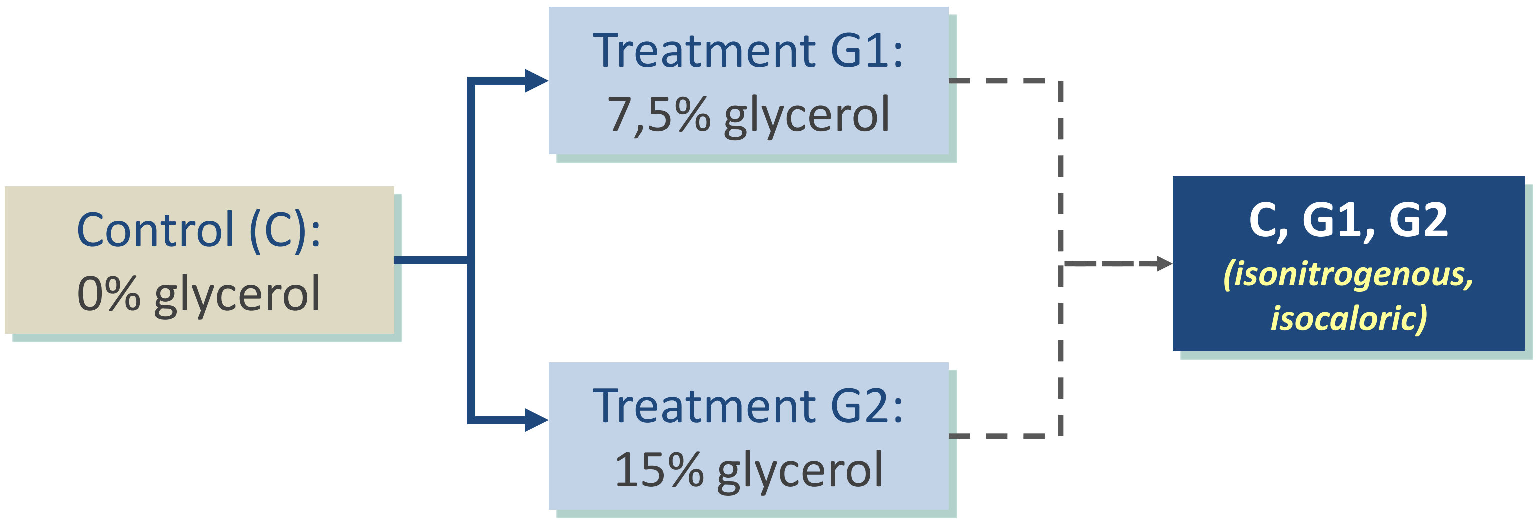
- Piglet performance traits (feed intake, average daily gain, FCR) and



- Selected constituents of gut microbiota monitored at the ileal and caecal level

Materials and Methods

- 18 weaned Large White × Pietrain piglets (aged 30 d, average BW= 8 kg)
- individually kept in metabolism crates for 42 days
- allotted into 3 treatments Control (C), G1 and G2



Materials and Methods

Main ingredients and chemical composition of diets and crude glycerol (%)

	Treatment			
	C	G1	G2	
Maize	61.0	47.8	38.5	
Soybean meal	30.0	31.0	32.9	
Wheat bran	4.9	10.0	10.0	
Crude glycerol	-	7.5	15.0	Glycerol
Dry matter	89.4	90.0	90.7	97.7
Digestible energy (MJ/kg)	13.5	13.5	13.6	14.4
Crude protein	19.5	19.5	19.5	-
Ether extract	3.0	2.7	2.4	0.5
Ash	6.3	6.1	6.1	5.4
Na	0.2	0.2	0.3	2.1

Materials and Methods

- Feed intake, weight gain and FCR were determined weekly
- At the end of the growing period (72 days of age) pigs were euthanatized

    - liver was blast frozen for further RNA isolation and GK gene expression analysis (calculated as GK/β-actin ratio)

    - ileum and caecum were blast frozen and subsequently assessed for selected constituents of microbiota composition (expressed as log CFU/g wet digesta)

- Linear and quadratic effects of dietary glycerol were studied by using polynomial contrasts (SPSS v.17.0)

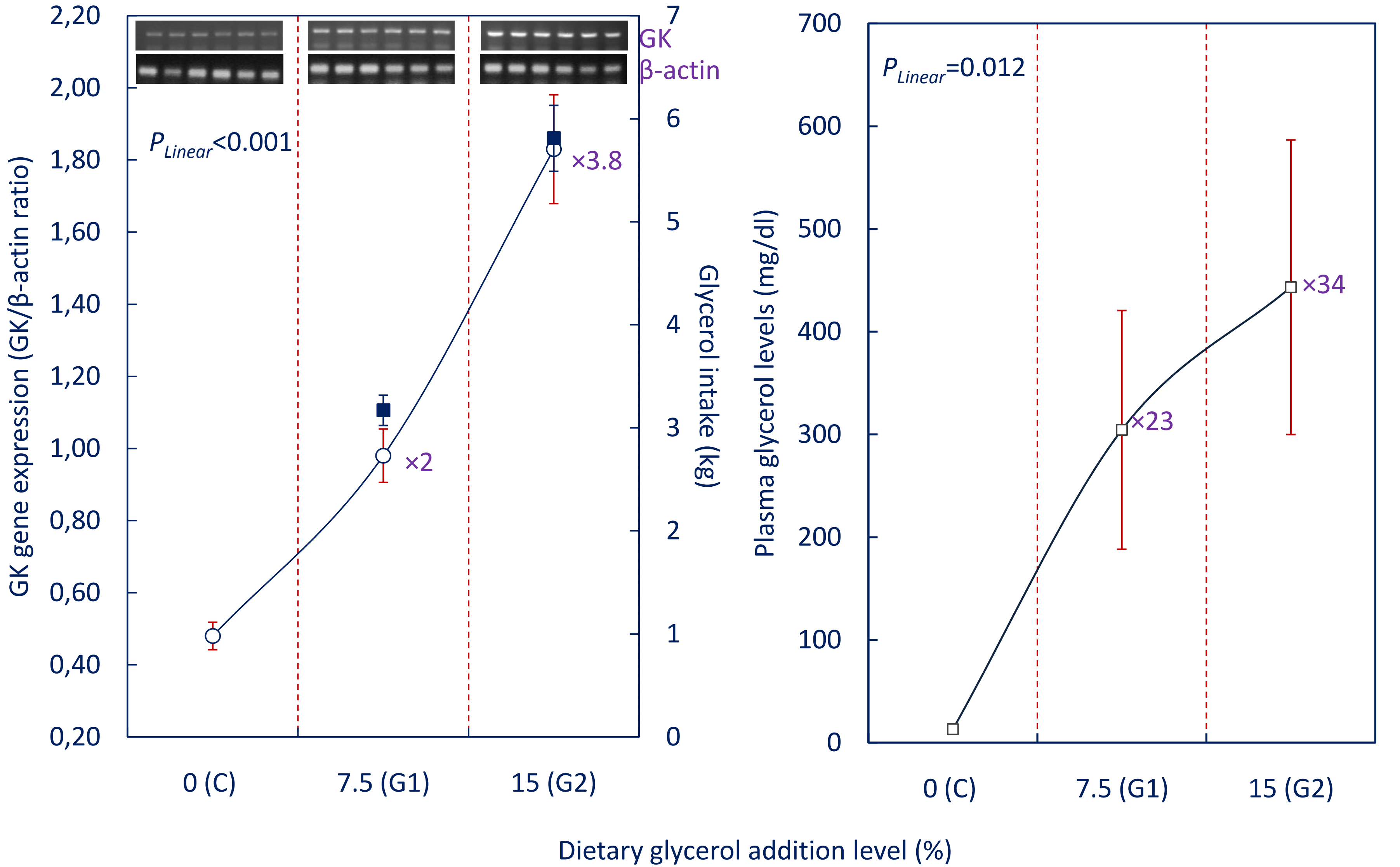
Results

Feed intake, body weight (BW) gain and feed conversion ratio (FCR) for the whole growing period (30-72 days of age)

	Glycerol addition level (%)			SEM[1]	P_{Linear}[2]	$P_{Quadratic}$[2]
(treatment)	0 (C)	7.5 (G1)	15.0 (G2)			
Feed intake (kg)	40.1	42.3	38.7	3.73	0.719	0.379
		+5.5%	-4.6%			
BW gain (kg)	22.8	25.8	23.6	2.04	0.694	0.163
		+10%	+4%			
FCR (kg/kg)	1.77	1.64	1.63	0.071	0.075	0.335
		-7%	-8%			

[1] *SEM, standard error of means*
[2] *P-values of polynomial contrasts*

GK gene expression and blood plasma glycerol levels at the end of the growing period (72 days of age)



Results

Gut microbiota composition (log CFU/g wet digesta) at the end of the growing period (72 days of age)

- **No effects** of dietary glycerol addition on **ileal** microbiota
- However, in **caecum**:

	Glycerol addition level (%)			SEM[1]	P_{Linear}[2]	$P_{Quadratic}$[2]
(treatment)	0 (C)	7.5 (G1)	15.0 (G2)			
Clostridium	7.29	7.17	6.54	0.341	0.043	0.405
Lactobacillus	7.15	6.76	6.41	0.336	0.044	0.912
Gram+ cocci	5.40	5.22	4.49	0.491	0.082	0.525

[1] *SEM, standard error of means*

[2] *P-values of polynomial contrasts*

Conclusions

- Increasing dietary glycerol levels up to 15% did not have any negative impact on performances and health status
  - reconfirmation with higher *n* of piglets
- High absorption of dietary glycerol
  - high variation in plasma levels (due to feeding time)
- Increasing GK gene expression with dietary glycerol
  - increased capacity to activate glycerol for utilization
- No adverse impact on gut microbiota
  - further investigation

Implications to be aware of…

- Methanol residues (<0.5%) → toxicity

  - limiting factor for dietary levels

- High viscosity (<18ºC) → problems during mixing

  - water addition for easy handling

- High sodium (Na) or potassium (K) content

  - Na/K ratio maintenance

- Other residues (e.g. FA) → final product quality

  - amounts depend on biodiesel process efficiency
  - types depend on the oil or fat used

Acknowledgements

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Thank you for your attention

Results

Additional GLYCEROL DATA

	Glycerol addition level (%)			**G2/G1 ratio**
(treatment)	0 (C)	7.5 (G1)	15.0 (G2)	**2.00**
Glycerol intake (kg)		3.17	5.81	**1.83**
Plasma glycerol (mg/dl)		304	443	**1.46**
GK expression		0.99	1.82	**1.84**