



## Pancreatic duct ligated minipigs - model to study effects / impact of exocrine pancreas on digestion of different nutrients

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## Exocrine pancreatic insufficiency

B  
A  
C  
K  
G  
R  
O  
U  
N  
D

### Exocrine pancreatic insufficiency (EPI)

- Well known disease in humans and animals
- Etiology:
  - Genetics (for example german shepherd)
  - Cystic fibrosis
  - Alcoholism (main factor in humans)
  - Trauma
  - Cancer (causing obstruction of the pancreatic duct)
  - Autoinflammatory genesis
  - Morbus Crohn....

ESVCN, Wien, Resident class

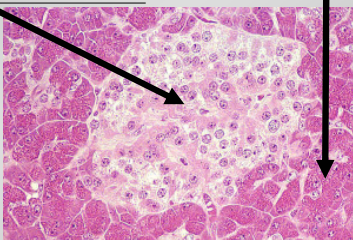


## Pancreas

B  
A  
C  
K  
G  
R  
O  
U  
N  
D

### Pancreas: histological view

Langerhans cells  
endocrine part of the pancreas      exocrine part of the pancreas



## Therapy of exocrine pancreatic Insufficiency

B  
A  
C  
K  
G  
R  
O  
U  
N  
D

### Therapy of EPI I:

- **Enzyme supplementation**  
(in cases of acute pancreatitis for pain management !)
- Multienzyme-preparations (mainly of porcine origin)
- Monoenzymes (proteases, amylases, lipases !)
  - Microbial origin
    - » Bacterial
    - » Fungal

ESVCN, Wien, Resident class



## Pancreas

B  
A  
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D

### Impact of exocrine pancreas on digestion:

- Secretion of **lipase, amylase** and **protease**
  - adaptation on changes of diet's composition
- High reserve capacity
  - clinical symptoms if > 90 % of pancreatic capacity is lost
- Neutralisation of pH in chyme
  - Buffering of chyme by Na-bicarbonate-secretion
- Antimicrobial effect



## Therapy of exocrine pancreatic Insufficiency

B  
A  
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D

### Therapy of EPI:

#### **Substitution of missing digestive enzymes**

Up to date mainly multienzyme-products of **porcine origin**

#### Now trend for **microbial products**:

- + defined factors of production
- + risk for infection / zoonosis ↓
- + specific modification of enzymes is possible
- + combination of most effective monoenzymes



## Therapy of EPI II:

### – Special dietetics

- Application of high digestible nutrients
- Extracorporeal (pre)digestion (esp. in dogs) using
  - Pancreatic enzyme preparations
  - Raw pancreatic tissue



Minipigs used as model for „normal pigs“ and humans  
(pig as well known model species for investigations on questions of digestion processes in humans)

Ligation of the ductus pancreaticus accessorius:

**Induction of a chronic exocrine pancreatic insufficiency (EPI)** without provoking an endocrine pancreatic insufficiency

For practical reasons important- pigs normally have only one pancreatic duct (rats > 10 !)

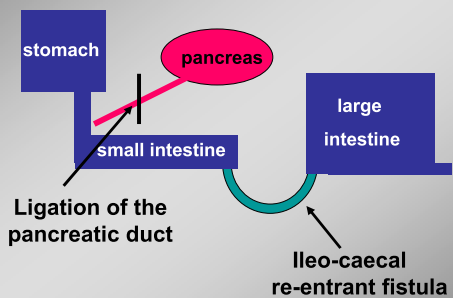
Ileocaecal re-entrant fistula:

**Quantification of precaecal digestion processes** (among others to test the efficacy of substituted enzymes)



## The project

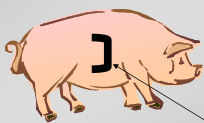
- Cooperation with a pharmaceutical company, producing pancreatic enzymes for substitution therapy (human)
- Model of the pancreatic duct ligated and fistulated minipig is established since 1996 (TABELING)
  - ⇒ Efficiency of different enzymes and enzyme preparations ?
  - ⇒ Impact of exocrine pancreas on digestion processes ?



Interestingly less problems with animals with pancreatic duct ligation and re-entrant fistula !



## The pancreatic duct ligated and fistulated minipig



Ileo-caecal re-entrant fistula  
⇒ quantitative collection of chyme



### Animals:

- Göttinger Minipigs (female)
- Adult, maintenance
- body weight: 30–40 kg
- ileo-caecal re-entrant fistula



### Control pigs (C)

⇒ intact pancreas

### Pancreatic duct ligated pigs (PL)

⇒ chronic exocrine pancreatic insufficiency (tested for EPI regularly)



### Aims of the project:

- Evaluation of EPI impact on digestibility and absorption of nutrients (prc. / total tract)
- Evaluation of enzymes for substitution therapie (multienzyme preparations / monoenzymes)
  - to give advice for nutrition of patients with EPI
  - to develop new -more efficient- enzyme products and galenic preparations



### Important results of studies of the project

(TABELING 1998; FASSMANN 2001; HELDT 2001; MANDISCHER 2002; FUENTE-DEGE 2003; KAMMLOTT 2003; KARTHOFF 2004; BECKER 2005; ZANTZ 2006)

⇒ total loss of pancreatic enzymes causes:  
(values of healthy control pigs; C = 100)

	Prececal (%)	Total (%)
Chyme- / Feces (dm) ↑↑↑	230 - 340	180 - 420
Digestion of crude fat ↓↓↓	19.7 - 45.2	10.4 - 33.0
Digestion of crude protein ↓↓↓	32.3 - 49.9	56.2 - 73.6
Digestion of starch ↓	63.7 - 90.0	97 - 99.8

⇒ for estimation of cfa-digestion feces collection is suitable

⇒ for estimation of cp and starch digestion determination of prc. digestibility rates is necessary !



### Therapy of exocrine pancreatic insufficiency

#### Proof of efficiency of digestive enzymes for substitution therapy:

- in vitro (correlation with in vivo studies is not always sufficient ..)
- in vivo (up to now „golden standard“)

#### Determination of digestibility of nutrients in animals with experimentally induced EPI

- ⇒ good transferability of results on humans
- determination of prc. digestibility rates of starch and cp;
  - determination of total tract digestibility rates of cfa)



### Effect of diet composition on prececal digestibility (%) of starch in PL-pigs

Starch content of the diet (% in DM)	Fat content of the diet (% in DM)	Digestibility rate of starch	Author
60.0	9.01	61.9	TABELING (1998)
56.2	2.21	63.5	MANDISCHER (2002)

24.1	30.1	92.5	FASSMANN (2001)
26.6	32.2	87.7	TABELING (1998)
27.1	33.5	75.6	FUENTE-DEGE (2003)



### Model: pancreatic duct ligated fistulated minipigs

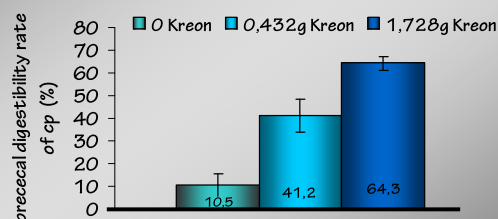
⇒ Digestibility trials with different diets (often orientating on human nutrition)

- Control pigs  
(„normal values“ of healthy individuum)
- Pancreatic duct ligated minipigs
  - without enzymes  
(effect of loss of exocrine pancreatic function)
  - + enzymes



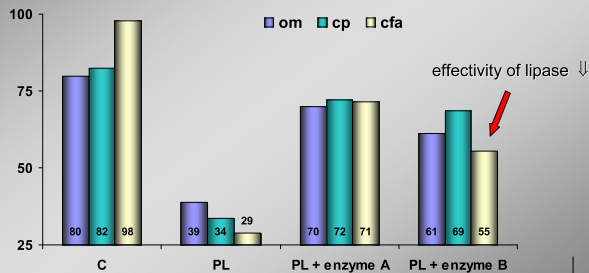
### Rough benchmark about amounts of enzymes necessary for therapy

**Rough benchmark: Amounts of enzymes per meal necessary to improve digestibility in pancreatic duct ligated minipigs**





**Example: Effect of different enzyme preparations  
(used in same dosage) on prececal digestibility rates**



**LPS (µg/g) in ileal chyme  
Control vs. PL**

Starch (% in DM)	Fat (% in DM)	LPS Control	LPS PL	Author
60.0	9.01	-----	91.7	TABELING (1998)
56.2	2.21	15.7	120	MANDISCHER (2002)

24.1	30.1	12.6 a	576 b	FASSMANN (2001)
26.6	32.2	29.0 a	634 b	TABELING (1998)
27.3	34.2	7.10 a	202 b	HELDT (2001)



**But there are some other  
interesting effects of EPI  
(beside markedly reduction of digestibility).....**



**LPS (µg/g) in feces  
Control vs. PL**

Starch (% in DM)	Fat (% in DM)	LPS Control	LPS PL	Author
60.0	9.01	-----	1008	TABELING (1998)
56.2	2.21	15.4 a	193 b	MANDISCHER (2002)

24.1	30.1	39.8	418	FASSMANN (2001)
26.6	32.2	58,7 a	1353 b	TABELING (1998)
27.3	34.2	17.2 a	102 b	HELDT (2001)

**BACTERIAL OVERGROWTH !**

Direct effect of EPI missing antimicrobial pancreatic juice

Indirekt effect of EPI ⇒ nutrients available in the gut lumen ↑



**Further effects of EPI**

- Increasing number of bacteria in chyme and feces  
“bacterial overgrowth” (well known in humans with EPI)


Method we used to test this effect:  
⇒ LPS-content in chyme and feces

LPS (lipopolysaccharides) are component part of cell wall of gram-negative bacteria; therefore it can be used as an indirect marker



**Effect of EPI on endogenous N-losses**

estimated after feeding an almost N-free diet  
(rich in starch !)




# Endogenous N-losses, estimated by feeding an almost N-free diet

Chyme collection: mean of day 5 & 7  
Feces collection: mean of day 6 & 7

	Control	PL	Relativ (C=100)
Amount of chyme (g dm/24 h)	56.6 ± 9.82	336 ± 124	593
Amount of feces (g dm/24 h)	37.6 ± 10.1	42.6 ± 20.1	113
Ileal N-Flux (g/24 h)	1.02 ± 0.42	2.64 ± 1.09	259
Faecal N-excretion (g/24 h)	0.51 ± 0.25	1.21 ± 0.48	237

⇒ Significant effects of pancreatic duct ligation on

- amount of chyme
- ileal N-flux
- faecal N-excretion




# Effect of EPI on alpha-tocopherol level in serum

## Further effects of EPI

Maldigestion and malabsorption of crude fat:  
⇒ uptake of fat soluble vitamins ↓

Does the enzyme supplementation (increasing cfa digestibility) affect uptake of fat soluble vitamins ?

Is it necessary to give “extra” vitamin supplements to (enzyme supplemented) EPI patients (as it is recommended) ?




# Endogenous N-losses, estimated by feeding an almost N-free diet

Chyme collection: mean of day 5 & 7  
Feces collection: mean of day 6 & 7

	Control	PL	Relativ (C=100)
Prc. endogenous N-losses (mg/kg bw <sup>0.75</sup> / day)	74.0 ± 26.4	224 ± 89.3	303
Total endogenous N-losses (mg/kg bw <sup>0.75</sup> / day)	36.8 ± 16.0	86.4 ± 32.0	234

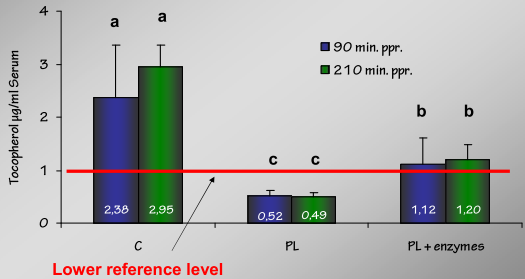
⇒ endogenous N-losses are much higher in PL !

this should be taken into account not only for experimental studies on crude protein digestibility but also for therapy of EPI patients




# Alpha-Tocopherol concentrations in serum

## Effect of EPI and enzyme supplementation on alpha-tocopherol level in serum



Lower reference level



# Endogenous N-losses; comparing results with other study (WÜNSCHE et al. 1987)

Chyme collection: mean of day 5 & 7  
Feces collection: mean of day 6 & 7


	WÜNSCHE et al. (1987)	C	PL
Prececale endogenous N-losses (g/kg dm-intake)	2.22	2.14	5.54
Total tract endogenous N-losses (g/kg dm-intake)	1.36	1.07	2.54

⇒ Results of control pigs are comparable to those of other studies in pigs (WÜNSCHE et al. 1987; DE LANGE et al. 1989 u.a.)

⇒ C & PL: endogenous N-losses (prc.) > endogenous N-losses (total tract)

⇒ PL: much higher endogenous N-losses (prc. and total tract)

Higher endogenous losses are supposed to be an effect of very large mass of chyme (as a result of maldigestion and malabsorption)  
In further studies the effect of enzyme supplementation (amylase !) to the almost N-free diet will be tested



# Insufficiency of exocrine pancreas causes:

## Direct effects:

- digestibility of nutrients (↓ - ↓↓ ↓↓)
- antimicrobial effect
- buffering (pH in chyme ↑; results not shown here)

## Indirect effects:

- high flux of chyme ⇒ secondary effects (N-losses)
- high nutrient density in lumen (bacterial overgrowth)
  - meteorism, energetic losses
- acidity of gastric content (missing/lower intensity of feed back mechanism)





## EPI - for Vets mainly important as a disease of dogs



... and often as a disease in german shepherds



## Enzyme supplementation in dogs:

How to do it properly with maximum effect for the dog and lowest costs for the owner...

- feed **several meals per day**
- **feed wet feed** (or add water to dry feed till it is soaked)
- **add enzymes** (mix it under the feed), open capsules etc.
- let the enzymes work (**time !**)
  - ca. 24 h at 6°C (fridge)
  - ca. 6 h at room temperature
- amount of enzymes:  
as much as necessary, less as possible (costs !), try it !



## Symptoms of EPI in dogs

- malnutrition (body weight ↓)
- adaphagia (often uptake of “unusual” feed)
- large amounts of fatty feces (stetorrhea)
- meteorism and flatulence
- dull coat (absorption of essential fatty acids ↓)



## Enzyme supplementation in dogs:

What's about the **diet** ? Low in fat ? Rich in fat ?

If enzyme supplementation works properly, high fat diets are positive, because of high energy density (most dogs suffer from malnutrition)

“Fat tolerance” is individual - just try what works best !

**Addition of essential fatty acids and fat soluble vitamins** is recommended - even enzyme supplementation increases absorption of those vitamins



## Enzyme supplementation in dogs:

### A) Application of pancreatic enzyme powder with the meal

- ⇒ suboptimal effects (gastric acidity and passage rate differ from those in humans)
- ⇒ high doses necessary (costs !)
- ⇒ often still symptoms (steathorrhoe)

### B) Extracorporal pre-digestion

Cave: Enzymes need for effective work:

- **Humidity**
- **Time**
- **Temperature** (not to high ⇒ denaturation)



There is no (only slight) effect of enzyme supplementation if enzymes are given on dry feed !!!