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Development of a model for anemia in minipigs

Regulatory Toxicology

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Starting point

- Piglets are born with a very small iron depot.
- Unless they live under conditions where they can forage in the soil, they need to be provided with an iron subsidy shortly after birth.
- Furthermore, a constant iron supply via the diet is required.
- This makes the pig an excellent anemia model as the uptake of iron can be controlled.

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Background

Development of an anemia model in domestic pigs was attempted.

Challenge:

Not all piglets developed anemia within the given period (6 weeks of age).

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Background – domestic piglets

- Providing a 75 mg/kg Fe supplement and 50 ppm Fe in the diet resulted in a mean change in the hemoglobin (Hb) level from 5.4 (variation 4.6-7) at 5 weeks of age to 4.5 mmol/L (variation 3.9-4.7) at 7 weeks of age.

- Providing no Fe supplement and 100 ppm Fe in diet resulted in a change in hemoglobin (Hb) level from 3.9 (variation 2.7-4.7) at 5 weeks of age to 4.0 mmol/L (variation 3.5-4.5) at 7 weeks of age.

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Background – domestic piglets

Challenge:

- The body weight range of the domestic piglets varied a lot.
- The weight difference between the largest and smallest piglet was up to 5 kg at 7 weeks of age.

This is a challenge when, in the actual study, the animals were dose with a fixed dose in capsules and not according to body weight.

Background for setting up an anemic model in minipigs

- To evaluate the effect **of low iron diet (50 ppm iron)** in combination with 2 different concentrations of iron (30 mg/kg and 60 mg/kg) administered parenterally after birth in juvenile minipigs.

- The goal was to **evaluate the development of a defined anemia** (Hb ≥3 ≤5 mmol/l) in otherwise healthy animals up to 8 weeks of age and evaluate if there was any difference in the development of anemia between males and females.

- Note: *Caring of non-anemic piglets will include an iron supplement of 100 mg/kg and a diet containing app. 160 ppm iron*

Anemic model in Göttingen minipigs -endpoints

Evaluation of:

- Hb levels
- Body weight gain
- TIBC (Total iron capacity binding) *
- Fe (Serum iron)

*TIBC was measured as an alternative to measuring Transferrin. Measuring TIBC is faster and cheaper

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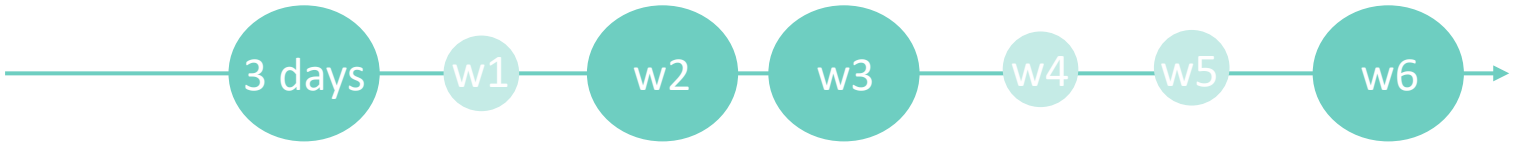
Study outline

- The piglets from **4 sows** were used
- Each group consisted of **5 males** and **5 females**



- All animals received a parenteral iron supplementation within **3 days of birth**.
  The animals in group 1 received 30 mg/kg and the animals in group 2 received 60 mg/kg.
- From the animals were approximately **2 weeks old**, they were offered a low content iron diet (approximately 50 ppm) from SDS (Special diet services).
- From the animals were **3 weeks old** blood samples for Haemoglobin, Serum Iron and Total iron binding capacity were collected weekly.
- Body weights were recorded twice weekly.
- At **6 weeks of age** the animals were dosed with capsules size 4 (app. 14x5 mm).

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Results

Haemoglobin (mmol/l) - 30 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
1	3.9	3.5	3	3.1	2.8
2	4.3	3.5	3	3	3.1
3	4.8	4	3.4	3.6	3.6
4	4.1	3.4	2.5	2.4	2.3
5	4	3.2	2.5	2.1	
Mean (M)	**4.22**	**3.52**	**2.88**	**2.84**	**2.95**
6	4.5	3.8	3.3	3.7	4
7	4.9	4.5	3.7	4.2	4.2
8	3.4	3.2	3.1	3.4	3.6
9	3.2	3.2	2.3	2.9	2.7
10	5.1	4	3.4	3.5	2.9
Mean (F)	**4.22**	**3.74**	**3.16**	**3.54**	**3.48**

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Results

Haemoglobin (mmol/l) - 60 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
11	4.6	4	3.9	3.6	4.2
12	5.3	4.2	3.8	3.8	3.5
13	6.1	5.9	3.5	5.2	5.3
14	5.5	3.9	3.7	4	4.2
15	4.9	4.2	5.1	3.4	3.2
Mean (M)	**5.28**	**4.44**	**4**	**4**	**4.08**
16	6.5	5.5	4.6	5.6	
17	6.1	5.6	4.8	5	5
18	5	3.9	3.2	3.7	4.3
19	5.1	4.6	4	3.9	2.8
20	4.1	3.1	2.5	2.7	2.5
Mean (F)	**5.36**	**4.54**	**3.82**	**4.18**	**3.65**

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Results
Body weights (kg) - 30 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
1	3.1	3.8	4.1	5	6.3
2	2.6	3.4	4	5.1	6.3
3	1.7	2.4	3.5	4.1	5
4	2.9	3.5	4	4.9	6
5	2.1	2.6	3.1	4.1	5.3
Mean (M)	**2.48**	**3.14**	**3.74**	**4.64**	**5.78**
6	2	3	3.5	4.3	5.3
7	3.7	5.1	6.7	7.4	8.3
8	2.5	3.4	4	4.6	5.4
9	2.4	3.1	3.6	4.4	5.3
10	1.9	2.6	3	3.7	4.9
Mean (F)	**2.63**	**3.44**	**4.16**	**4.88**	**5.84**

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Results
Body weights (kg) - 60 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
11	2.6	3.2	3.6	4.8	5.7
12	1.3	1.8	2.2	2.9	3.9
13	2.8	3.8	5.2	5.7	6.7
14	2.3	3.2	3.9	4.7	5.6
15	2.2	2.8	3.1	4	5.1
Mean (M)	**2.24**	**2.96**	**3.6**	**4.42**	**5.4**
16	2.9	3.8	4.8	5.4	6.5
17	1.5	2.1	3.2	3.9	4.7
18	2.6	3.7	4.4	5.2	5.9
19	2.1	2.7	2.8	3.7	4.8
20	2.2	3	3.3	4.1	5.1
Mean (F)	**2.26**	**3.06**	**3.7**	**4.46**	**5.40**

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Results

Serum Fe (µmol/l) - 30 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
1	2.8	2	3.2	3.8	2.2
2	2.4	2	3.6	3.4	3.6
3	3.6	2.8	4.8	4.8	4.6
4	2	2	7	4.6	3.8
5	1.8	1.8	3	6	5
Mean (M)	**2.52**	**2.12**	**4.32**	**4.52**	**3.84**
6	2.8	3	4.8	4.4	3.8
7	3.2	2.6	4.8	3.6	3.2
8	5.8	2.4	5.8	3.6	3.8
9	6.4	2.8	3.4	4.4	2.8
10	2.4	2.2	4.4	10.2	4.8
Mean (F)	**4.12**	**2.6**	**4.64**	**5.24**	**3.68**

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Results

Serum Fe (µmol/l) - 60 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
11	3.4	2.2	3.4	7.6	5.4
12	3	2.6	3.6	6.2	5
13	7	3.8	9.6	4.2	5.4
14	3.4	2	5.6	3.6	4.2
15	2.8	2.4	3	6	3.8
Mean (M)	**3.92**	**2.6**	**5.04**	**5.52**	**4.76**
16	5	3	6	4.6	4.2
17	18.6	4.8	5.4	4.6	3.8
18	2.8	2	4.6	2.6	2.6
19	3.2	2.6	3	4.4	4.8
20	2.2	2	2.4	3.8	3
Mean (F)	**6.36**	**2.88**	**4.28**	**4**	**3.68**

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Results

TIBC (µmol/l) - 30 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
1	152.4	161.2	168.2	146	163.8
2	146.2	149.4	171.2	160.2	172.2
3	131.8	165	193.2	176	163.6
4	130.4	139.6	155.8	162	171.6
5	165.2	168.4	183	152	170.8
Mean (M)	**145.2**	**156.72**	**174.28**	**159.24**	**168.4**
6	138.4	164	199.6	167.2	178
7	160.6	176.6	178.4	104	171.8
8	158	162	161	153	150
9	144.8	161.2	152	157	169
10	125	147.2	164	156	168.8
Mean (F)	**145.36**	**162.2**	**171**	**147.44**	**167.52**

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Results

TIBC (µmol/l) - 60 mg/kg

Animal No.	3-weeks of age	4-weeks of age	5-weeks of age	6-weeks of age	7-weeks of age
11	144	135.2	140.2	131.4	148
12	137.6	141	164.6	148.2	160.2
13	125	146.4	156.6	167	161.8
14	131.4	154.2	153.6	145.6	147.8
15	146.8	147	151.8	137.4	154.8
Mean (M)	**136.96**	**144.76**	**153.36**	**145.92**	**154.52**
16	134.2	147.8	171.4	170.6	177.4
17		121.4	141.8	150	162.8
18	135.2	148.6	136.6	129	136
19	138.6	149	154	150.4	173.6
20	155.4	157.8	149.4	139.4	168
Mean (F)	**140.85**	**144.92**	**150.64**	**147.88**	**163.56**

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Conclusion

- The minipigs develop an aneamia within the defined range (Hb ≥3 ≤5 mmol/l) .
- No differences were seen between males and females.
- Group 1 (30 mg/kg) developed an anaemia in the low end of the range.
- Group 2 (60 mg/kg) developed an anaemia in the midst of the range.
- No differences were seen in the body weights.
- No clear differences in the serum Fe values were observed between the groups.
- A tendency for higher TIBC was observed in group 1 (30 mg/kg). However, TIBC was in both case way above the iron availabe for binding.
- The minipigs could be dosed with capsules size 4.

Initial study design for the client

- During the validation study one animal in Group 1 receiving 30 mg/kg Fe was observed with reduced appetite and subdued behavior on a single day in week 7. The animal was found dead the following morning.
- The hemoglobin level measured the week before was 2.1 mmol/l.
- At necropsy an enlarged spleen was observed.
- These findings indicted that the death of the animal was most likely caused by the anemia.
- Based on this the client initiated their first trial providing the piglets 60 mg/kg Fe

Refinement/altering of study design

- Several trials have been conducted since with different formulations.
- A lowering of the iron supplement has been introduced and different iron contents in the diet have been tried out to optimize.
- TIBC and Serum Fe have over time been taken out of the study design as it did not add additional value when evaluating the clints formulations.
- In contrast not only Hb is measured but all red blood cell parameters.
- Study specific: The increase in Hb levels and the iron utilization is evaluated for the different formulations.

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Take home message

The main challenge in setting up an anemic diet model in juvenile minipigs is:

- That the staff is used to caring for, working with and handling non-anemic piglets.
- That the sow and piglets are housed in pens where separating the piglets and the sow during feeding is easy so that the piglets do not get access to the sows' diet.

Thank you!

Contact us

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