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Troubleshooting magnetic stirrer digestion method for detection of *Trichinella* spp. in fox muscle samples

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Magnetic stirrer digestion

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International Commission on Trichinellosis: Recommendations for quality assurance in digestion testing programs for *Trichinella*

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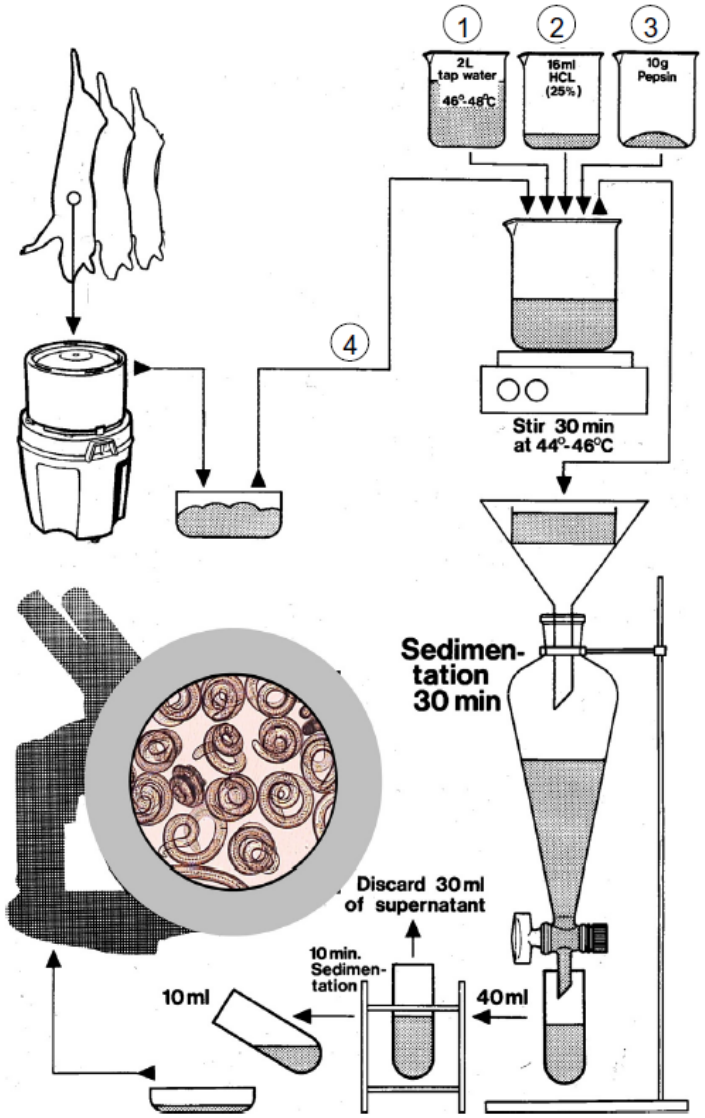

Fig. 1. Diagram of a magnetic stirrer method for pooled sample digestion (steps labelled as 1–4 indicate the required sequential order for preparing the digest).

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Recent experiences with *Trichinella* digestion at SSI

February 2023 – Sled dog

Pernille Klein-Ipsen, DVM
University of Copenhagen

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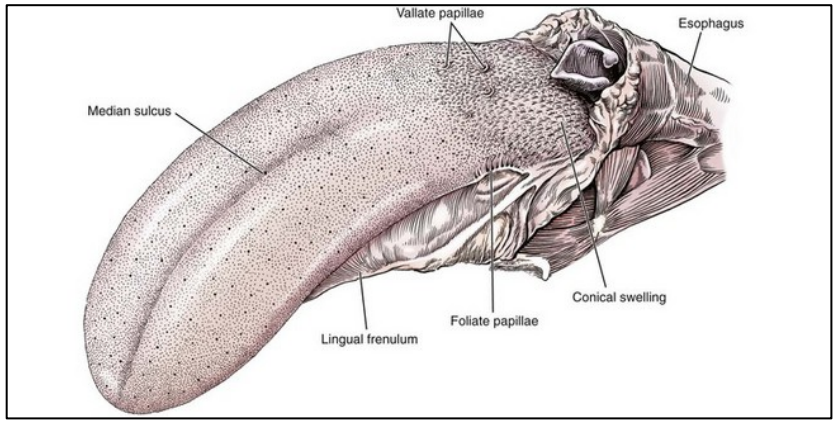
Endoparasites in Greenland Sled Dogs

- Magnetic stirrer digestion of sled dog tongue

Sled dog in Ittoqqortoormiit, September 2022.



https://veteriankey.com/soft-tissues-of-the-oral-cavity/

February 2023 – Sled dog

Endoparasites in Greenland Sled Dogs

- Magnetic stirrer digestion of sled dog tongue

Rebecca Berg, DVM, PhD
Parasitology research and preparedness
Statens Serum Institut

February 2023 – Sled dog

Endoparasites in Greenland Sled Dogs

- Magnetic stirrer digestion of sled dog tongue
- Positive – 316 larvae (Unpublished)

March 2023 - *Trichinella* digestion PT

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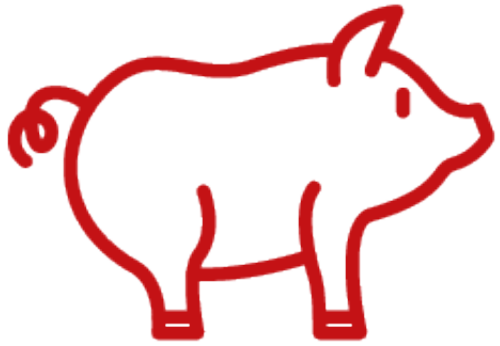
- Magnetic stirrer digestion of chopped pork meat (100g)
- Positive result

Meat	Weight(g)	Code	Spiked larvae	Recovered larvae	Delta
Pork	100g	7405	0	0	0
Pork	100g	6267	0	0	0
Pork	100g	5834	5	5	0



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Digestion of fox muscle (surveillance)

June 2023 – Surveillance of infection in foxes

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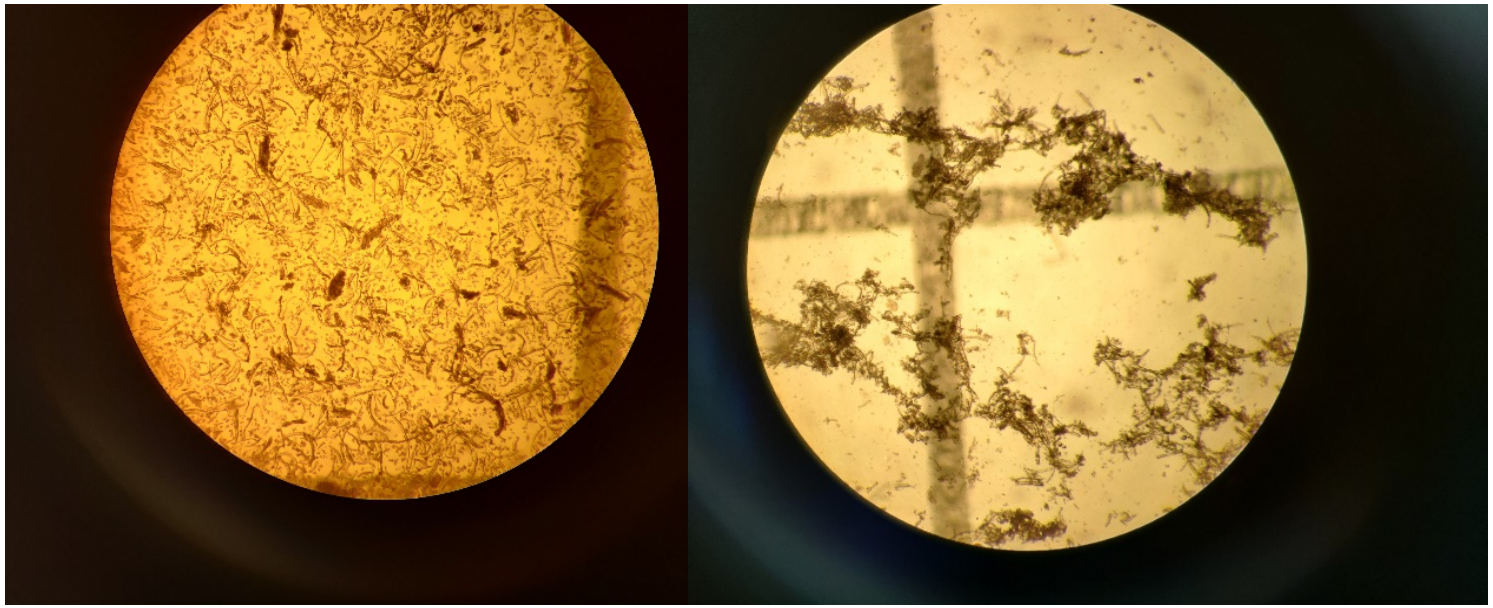
Red foxes collected in Denmark

- SSI and the University of Copenhagen (Danish Veterinary Consortium – DK-VET)

- Magnetic stirrer digestion of lower front leg muscle
- Result:



Pathology watch, University of Copenhagen

Troubleshooting

- Factors with influence
  - Meat
    - ~~Animal species, age etc.~~
    - ~~Storage~~
    - ~~Preparation for digestion~~
  - Temperature (~~room~~ / sample / digestion mix)
  - Pepsin
    - Lot
    - Liquid or powder
    - pH
    - Activity (units or NF)
    - Concentration
  - HCl
    - Lot
    - pH
    - Concentration
  - Digestion time
  - Sedimentation time
  - Personel
  - Digestion fluid:meat ratio

pH measurements of pepsin, HCl and digestion fluid

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- Pepsin: 4,5
- HCl: 0,5
- Mixed digestion fluid:

Time	0	8	15	23	31	33	38	42	46	49
Temperature (Celcius)	25	30	35	40	44	45	46	47	48	49
pH	1,2	1,5	1,5	1,3	1,5	1,5	1,5	1,3	1,5	1,5

- Guidelines from International Commission on Trichinellosis: *pH = 1–2 (max. pepsin activity pH = 1.5–1.6).*

Testing different pepsin lots

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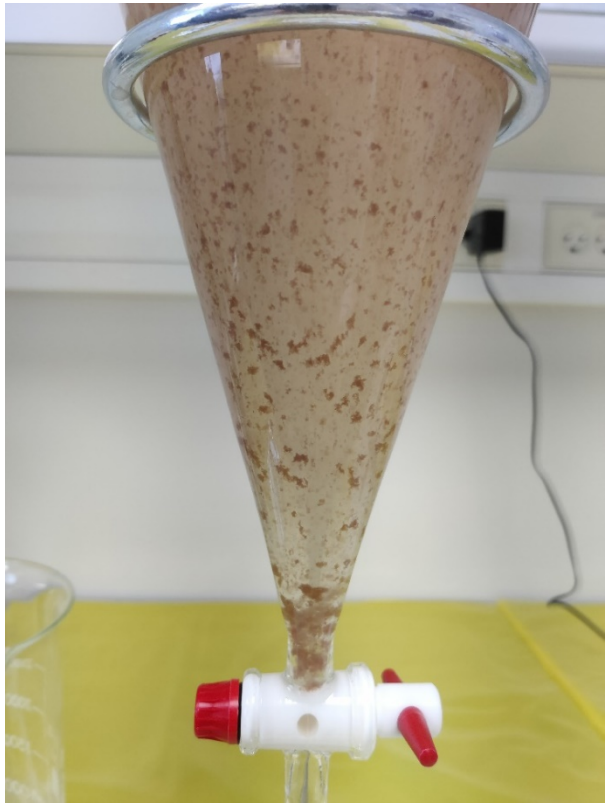
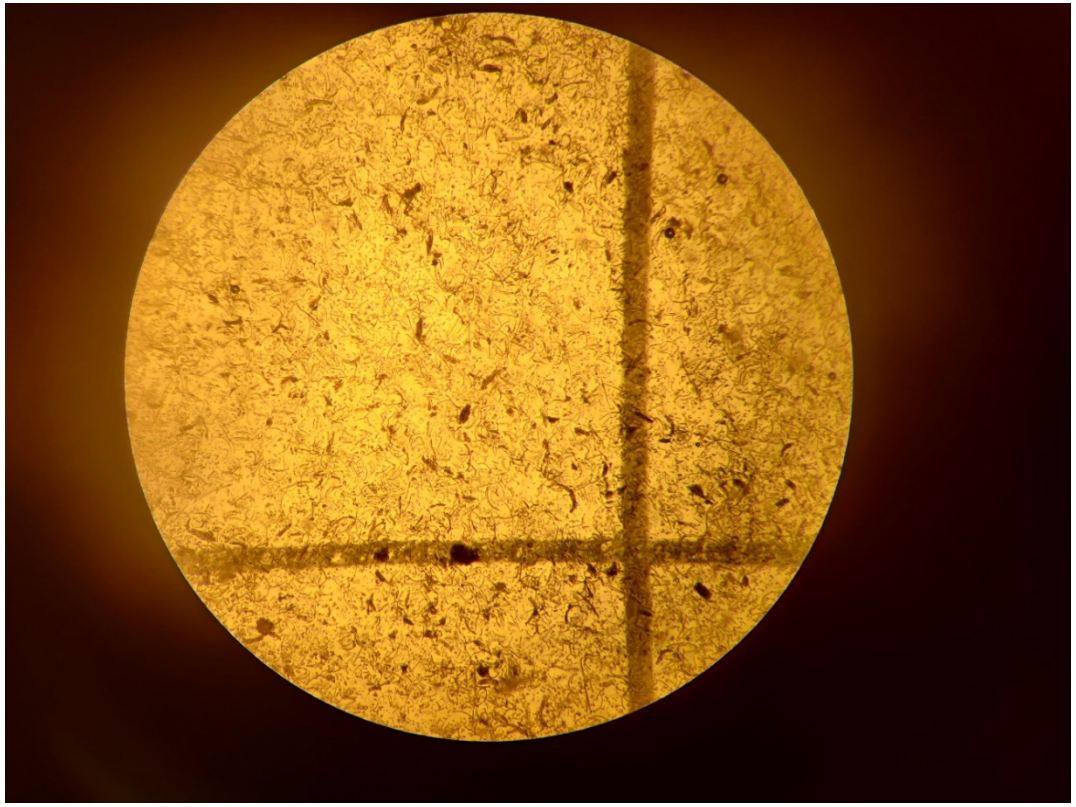
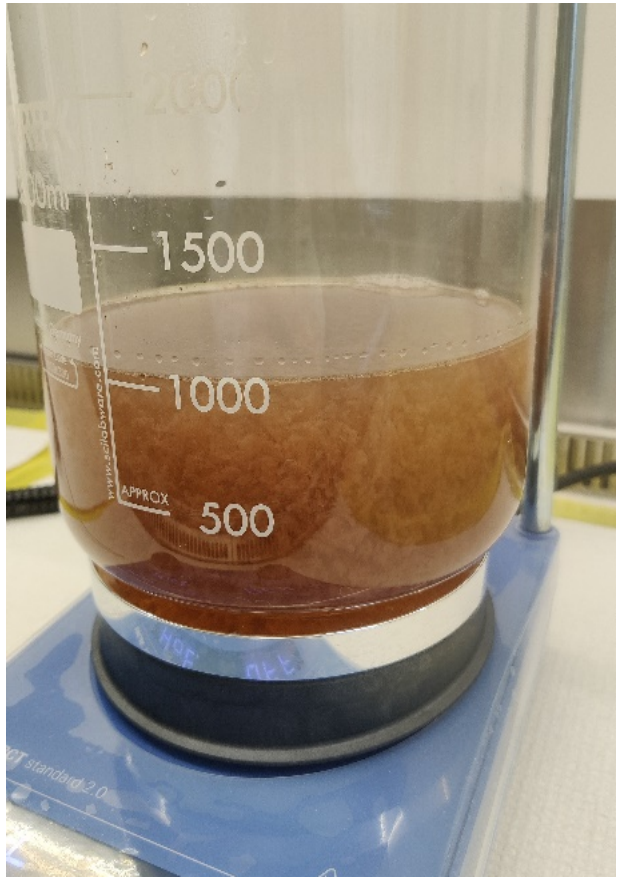
- Two digestion setups with pork (two different pepsin lots)
  - Result - Slight difference between lots (bias?)
- Digestion of thigh muscle from red fox with different pepsin lot
  - Result – same problem!

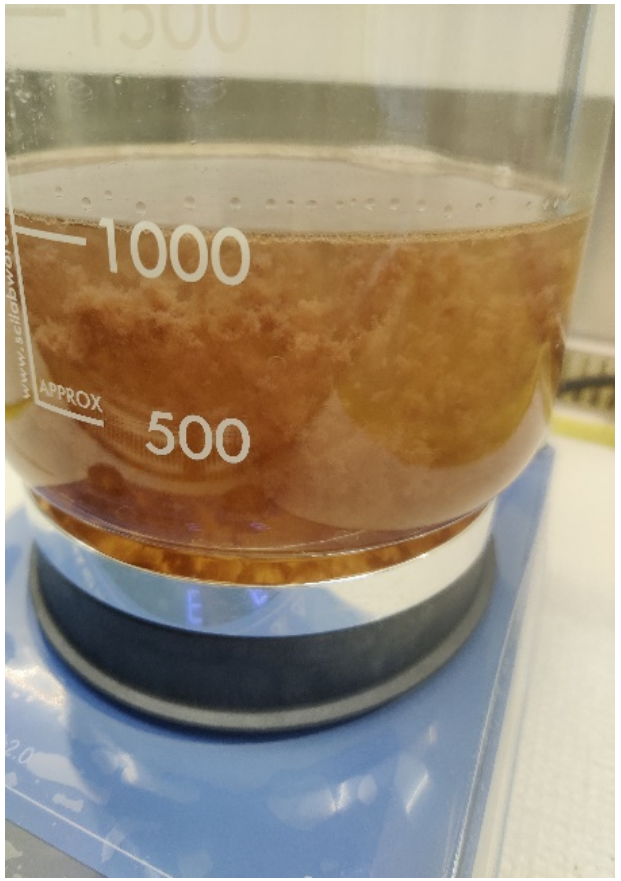


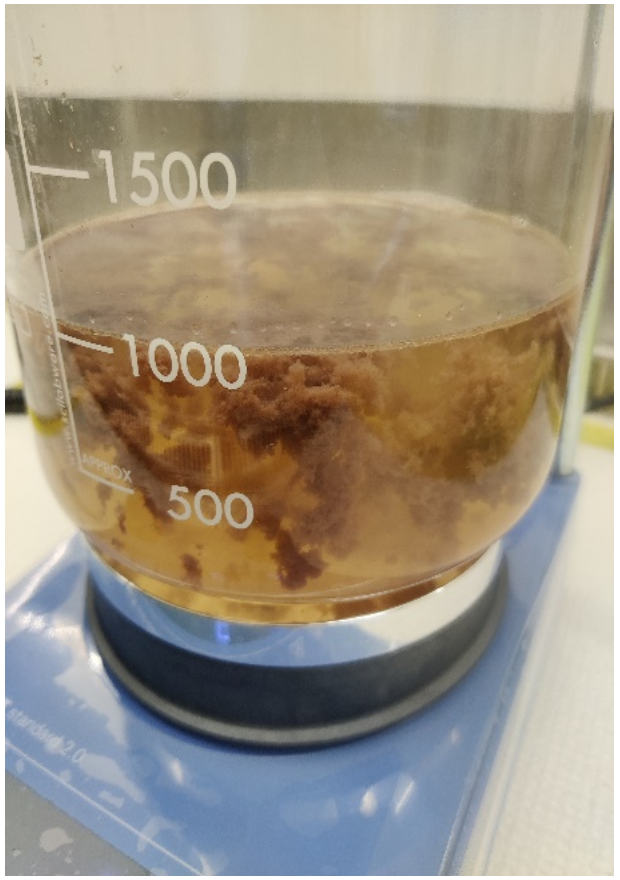


Testing extended digestion time

- Digestion of thigh muscle from red fox – 2 hour digestion
  - Result – same problem!
  - pH after digestion – 2,2







Testing increased HCl concentration (1%)

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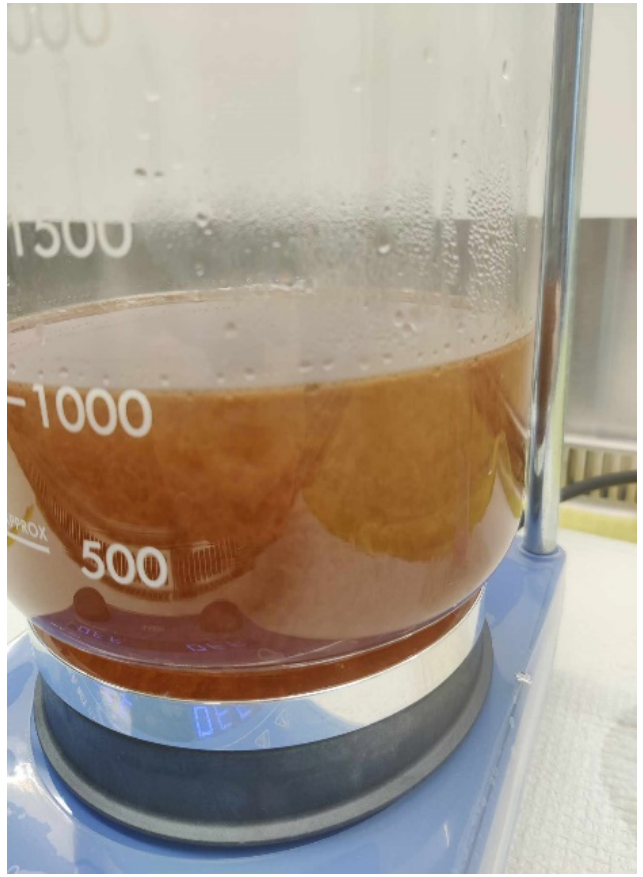
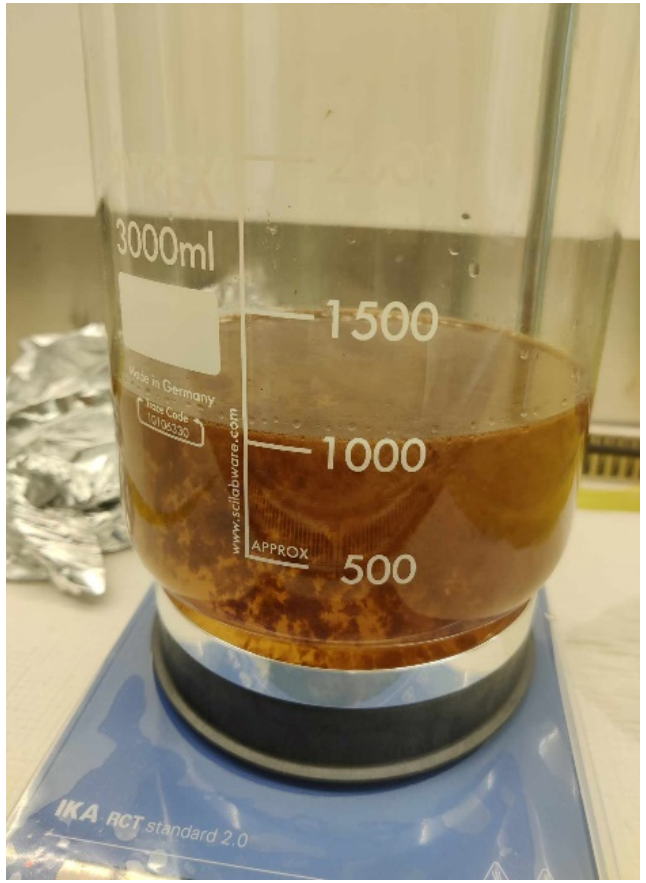
- Digestion of thigh muscle from red fox – 2+1 hour digestion and 1% HCl
  - Unfortunately no pH measurements
  - Result – same problem (Slightly more smooth mix, but after 3 hours of digestion!)



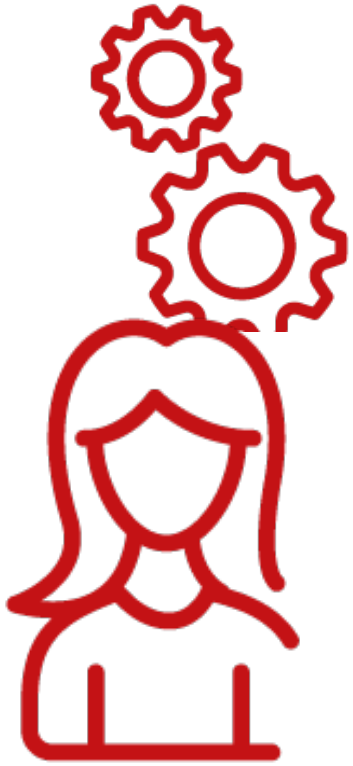


Testing increased pepsin concentration

- Digestion of thigh muscle from red fox – 2 hours with 3% + 1 hour digestion (6%)
  - pH when meat added + during and after digestion : 2,2
  - Result – same problem

Troubleshooting

- Factors with influence (varying importance)
  - Meat
    - ~~Animal species, age etc.~~
    - ~~Storage~~
    - ~~Preparation for digestion~~
  - Temperature (~~room / sample~~ / digestion mix)
  - Pepsin
    - ~~Lot~~
    - Liquid or powder
    - ~~pH~~
    - Activity (units or NF)
    - ~~Concentration~~
  - HCl
    - Lot
    - ~~pH~~
    - ~~Concentration~~
  - ~~Digestion time~~
  - Sedimentation time
  - Personel
  - Digestion fluid:meat ratio ←



Addition of buffer?

Acknowledgements

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Questions?
Suggestions?
Corrections?

https://www.mammal.org.uk/species-hub/full-species-hub/discover-mammals/species-fox/