

1. Arbejdstitel for projektet

Identification of biological marker(s) for differentiating meat of high and uniform tenderness

2. PhD uddannelse

Forskeruddannelsesprogram

Food Science

Evt. samarbejdsinstitution

Hovedvejleder
(navn/grad/titel/institut)

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3. Baggrunden for projektet

Tenderness is the most important factor for determination of the eating quality of fresh meat. Muscle growth, and consequently daily gain, is the difference between the rate of muscle protein synthesis and the rate of muscle protein degradation. Studies indicate that the variation in the rate of protein degradation at the time of slaughter explains more than 50% of the variation of meat tenderization. The link between the tenderization and the rate of muscle protein degradation *in vivo* is believed to be the proteolytic enzymes, the calpains. The calpain system comprises μ - and mM-calcium-dependent calpains and the muscle specific calpain, p94, and their inhibitor, calpastatin. The contribution of the individual components of the calpains to the rate of muscle protein degradation is not completely clear. Factors determining muscle protein degradation comprise feeding level and composition, genotype etc. Consequently, it is important to understand the influence of the calpain system on the rate of protein degradation in order to regulate and develop markers for tenderness. Use of markers for tenderness can thus differentiate meat according to this quality trait and therefore cause high and uniform tenderness of fresh meat products.

An other aspect of muscle growth is satellite cell proliferation. During muscle growth the number of myonuclei or the content of DNA increases despite the myonuclei are mitotically inactive. This is due to the satellite cells residing between the sarcolemma and the myofiber basement membrane. These cells can divide and fuse with an existing fibre and thereby add more DNA to the fibre. This will support the rate of protein synthesis and degradation. The fusion process requires proteolytic activity and some studies indicate that the mM Ca-dependent calpain is involved in this process and may therefore indirectly increase protein turnover and consequently meat tenderness.

4. Formålet med projektet (hypotese)

We hypothesize that the calpains are involved in regulating the rate of muscle protein degradation. μ M-calpain is mainly involved in regulating the rate of muscle protein degradation directly, while mM Calpain is indirectly involved via regulating the fusion of satellite cells with the muscle fibres. The objectives are to study the role of the calpain system in relation to muscle protein degradation and fusion of satellite cells. Results from such studies may lead to the development of markers which can predict the tenderness of fresh meat as it will be verified *in vivo* experiments.

5. Den planlagte forskning

Phase 1:

- Objectives: to study the role of the calpain system in relation to muscle protein degradation and fusion of satellite cells:
 - Experimental work and methods: Because it is impossible to measure protein degradation *in vivo*, the experiments in phase 1 will be conducted on muscle cell cultures (satellite cells) from either pigs or cattle. The influence of the individual calpains on muscle protein degradation and fusion will be examined by the use of chemical inhibition and by use of antisense and siRNA (corresponding to gene knockout) towards components in the calpain system. Expression of the calpains will be measured at the gene level by RT-PCR, at protein level by WLB and at the activity level by zymography. Moreover we will use proteomics to characterize the muscle protein profile. Muscle protein degradation rate will be measured by release of previously incorporated ³H-tyrosin or release of methylhistidine to the medium. Variation in muscle protein degradation rate will be varied by addition of anabolic or catabolic hormones or growth factors.

Phase 2:

- Objectives: to select and verify marker(s) to predict muscle protein degradation and meat tenderness:
 - Experimental work and methods: Following identification of marker(s) for muscle protein degradation rate in cell culture and meat tenderness, the use of the marker(s) will be examined *in vivo* by selecting slaughter animals, which vary considerably with regard to tenderness. Chops from these animals will be sampled and assayed for tenderness and the marker(s).

Research environment

The PhD student will be associated to the research unit "Muscle Biology and Meat Quality" at ARK. This group has experience with all the methods that are to be used in this PhD education (see above).

Ph.D. education

The student will follow relevant PhD courses at the Faculty of Agricultural Sciences or at other relevant providers. It is intended that the student will spend part of the education period at a foreign university or research institute.

Results will be presented and discussed at regular meetings with supervisors, and that the results form the basis for a minimum of 4 publications in relevant, peer-reviewed journals.