# New concept for the provision of essential limiting amino acids

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Danish researchers set out to develop healthy bacteria that can produce essential amino acids in the pig's intestines. This will reduce the feed price, reduce loss of nitrogen to the environment and create healthier pigs.

An exciting new project has just received €1 million in public research funds by the Danish National Advanced Technology Foundation. The project, which will run over the next three years, is led by the global bioscience company Chr. Hansen in cooperation with scientists in animal physiology at The Faculty of Agricultural Sciences at Foulum, Aarhus University, Denmark's second largest university. The project aims at developing special Bacillus subtilis strains to reduce the protein content in pigs' feed.

As protein contains substantial amounts of nitrogen this will help reduce the loss of nitrogen from pigs by more than 10% – calculated to correspond to the loss of nitrogen caused by two million slaughter pigs.

The objective of this project is to develop several Bacillus subtilis strains each of them able to overproduce one specific essential amino acid in an amount relevant for the pig industry. The development will be carried out by classical strain improvement of a Bacillus subtilis which was previously developed to grow in conditions corresponding to the intestinal environment.

## 60% nitrogen loss

Supplying pigs with essential amino acids to meet their requirements and at the same time reduce the level of dietary crude protein is a challenge. Many protein sources used today have a low protein value because the content of essential amino acids deviates from the requirements of the animals.

This problem results in a surplus of dietary crude protein leading to both ineffective growth of the pigs and heavy environmental nitrogen load. A typical pig feed ration in Europe thus results in a nitrogen loss of 60% of the ingested nitrogen.

Additionally, high dietary crude protein content increases the risk of diarrhoea and thus increases the use of antibiotics.

A higher provision of available limiting essential amino acids would result in reduced nitrogen loss to the environment, improved production economy, healthier pigs and reduced antibiotic use. This project aims to fulfil this objective.

## 'Good' and 'bad' bacteria

When we talk about bacteria, we typically think of pathogen bacteria as E. coli or clostridia causing different diseases.

However the pig's gut contains billions of bacteria and some of these are important for the health of the animal, stimulating the immune system and fighting pathogens.

Probiotic additives, i.e. Bacillus subtilis, contain such healthy bacteria to improve growth and health.

There are many different types or strains of Bacillus subtilis with different characteristics. In this research project Bacillus subtilis strains with the specific ability to excrete amino acids in the intestinal environment will be developed.

## Healthy bacteria

Bacillus subtilis has been used in the pig industry for more than 10 years due to the probiotic effect including improved intestinal health and growth performance.

Selected non-GMO strains of Bacillus subtilis have been found to overproduce and excrete specific amino acids.

Thus, Kisumi et al. (1971) measured increased arginine excretion by Bacillus subtilis mutants from 0.1g/l in the wildtype strain to 4.3g/l in the mutant strain.

Previous development activities at Chr. Hansen A/S have already selected a special new strain of Bacillus subtilis which is able to produce and excrete 20 x more valine than the wild-type strain.

## Cost efficient feed rations

It was calculated that formulation of a grower/finisher diet replacing some of the soybean meal with cheaper protein sources (sunflower meal and rapeseed cake) and addition of the new Bacillus product will

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| Feedstuff in feed ration (%) | Diet 1 Original diet | Diet 2 New diet with Bacillus added |
|---|---|---|
| Barley | 30.0 | 28.2 |
| Wheat | 50.0 | 50.0 |
| Toasted soy bean meal (protein source) | 14.5 | 4.4 |
| Sunflower meal (protein source) | - | 8.0 |
| Rape seed cake (protein source) | - | 4.0 |
| Fat | 2.0 | 2.0 |
| Crude protein (g/kg) | 156 | 150 |
| Bacillus product added supplying limiting amino acids | - | x |
| Reduction in feed price €/100kg including Bacillus product | - | 0.87 |

***Table 1. Feed optimisation for grower/finisher pigs with reduced protein content by adding limiting amino acids by the new Bacillus product (histidine, isoleucine, valine and tryptophan). Lysine, methionine and threonine are added as synthetic amino acids in both rations. Only feedstuff composition is shown.***

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result in a reduction of crude protein content from 156 to 150g/kg corresponding to up to 10% reduced N excretion under Danish feeding conditions (Table 1).

The price for this feed ration is expected to decrease with €0.87/100kg.

The nitrogen reduction per produced pig is anticipated to be higher in most other pig producing countries.

It was thus calculated that feed optimisations of a grower diet in the USA with a reduction in soybean meal and the addition of the Bacillus based product supplying the limiting amino acid will result in more than 25% reduced N-loss and a reduced feed price of 4%.

## Synthetic amino acids

The surplus of protein in the feed rations is today – partly – solved by adding few synthetic amino acids to the feed ration in order to reduce the dietary protein content.

However, typical feed rations in both Europe and the USA still contain excess protein. This is due to the use of cheap and low quality protein sources in feed formulations and high prices or unavailability of synthetic essential amino acids, as only a few of the essential amino acids are produced synthetically and thus are commercially available.

This project targets amino acids that are either high priced or not commercially available today.

The new Bacillus strains will be offered at a reasonable price – in fact the swine producer will be able to reduce the feed price by as much as 4%, as the feed ration can be composed differently.

Supplying amino acids through a probiotic as Bacillus will give additional value by both adding amino acids and supplying the well known effects from probiotics as improved health and growth performance to the animal.

This added value is not given by eventually competing new upcoming and economically reasonable synthetic amino acids. Also the fact that Bacillus is added to the feed as thermo stabile spores gives the Bacillus strain advantages compared to synthetic amino acids that often have to be sprayed on the feed after pelleting.

The idea of supplying essential amino acids through a non-GMO probiotic is novel and a patent application has been filed.

## Environmental effects

The high nitrogen leakage and run-off from intensive agriculture result in high nutrient levels in water often followed by severe damage to the ecosystems.

The EU framework directive was initiated several years ago with the aim to reduce N leaching (Water Plan for the Aquatic Environment III (VMP III) in Denmark).

Denmark is one of the countries with difficulties to fulfil these objectives and a new action plan for the aquatic environment was initiated at the beginning of 2010.

Nitrogen leaching has to be reduced with a minimum of 13% in 2015 compared to 2003 (VMP III).

## Complete project package

This high risk project includes a full package from development and characterisation of Bacillus strains, proof of concept, first in simple in vitro systems followed by tests and model descriptions in the pig carried out by Aarhus University, to upscaling of the strain for production, product launch and business development.

Upon completion of the project in 2013 the product will be ready for marketing in the USA and for the registration process in Europe. In summary, the project will aim at solutions that will:

- Reduce feed cost due to inclusion of cheaper feedstuffs and lower protein content (approximately 4%).
- Deliver a solution so that the N-excretions from pig production can be reduced (approximately 10-25%, depending on pig producing country).
- Improve health by reduced risk of diarrhoea due to reduced protein content. ■