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Keeping under control a liquid feed fermentation process for pigs: A reality scale pilot based study

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ABSTRACT

An original and fully automated liquid feeding pilot has been designed and implemented to monitor and optimize the fermentation process of liquid feed for pigs at a pre-industrial scale. The installation was designed and instrumented to continuously record the temperature, pH and redox potential (E_h) during the fermentation course of wheat flour based feed mixed with water in a 1:2.5 (w:w) ratio. Single and multiple batches experiments were carried-out with feed inoculation achieved by leftover or with a selected culture of lactic acid producing bacteria (LAB). Physicochemical and microbiological characteristics of the fermentation process which include lactic and acetic acids and ethanol concentrations, enumerations of lactic acid producing bacteria, yeasts, total coliforms and *Escherichia coli*, were monitored and analyzed as a function of the main feed control factors: incubation time, operating temperature, feed time schedule and percentage of leftover. From batch experiments, it was observed that increasing the operating temperature from 15 to 30 °C, accelerates the rate of fermentation by reducing about 5–6-folds the process latency and the duration to reach a pH value of 4.0 which is considered as optimal to achieve biosafety. Nevertheless, this does not prevent the blooming of coliforms as their counts increases from 4 to 6 $\log_{10}$ CFU/mL within 24 h. In opposite, multiple batches are proved to be effective in both accelerating the fermentation rates and reducing the survival of Coliform bacteria in fermented liquid feed (FLF). Feed fermented at 25 °C during 24-h cycles with a 22% leftover ensures the prominence of LAB strains over yeasts with a population level that stabilizes at around 9 $\log_{10}$ CFU/mL (*vs.* 7 $\log_{10}$ CFU/mL for single batches), a lactic acid production up to 35 g/kg dry matter (DM) and a pH value between 5 and 3.5 throughout the period. Concomitantly, total Coliforms number decreases from 7.5 to 2.2 $\log_{10}$ CFU/mL within 72 h whereas *E. coli* became undetectable beyond 48 h. Addition of a starter culture (*Pediococcus acidilactici*, Bactocell®) at 9 $\log_{10}$ CFU/kg DM at the initial stage of FLF production reduces 25–35 times the total coliforms and *E. coli* counts. No significant differences in the amounts of organic compounds produced by the microflora as compared to the control FLF after 80 h nor in the microbial levels are observed. It is concluded that sequences of fermentation cycles allows, in a given temperature range, establishing a positive, robust, microbial ecosystem.

Abbreviations: FLF, fermented liquid feed; LAB, lactic acid bacteria; DM, dry matter; CFU, colony forming unit.

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1. Introduction

Feeding pigs with fermented liquid feed (FLF) is a strategy that has gained worldwide interest in swine nutrition but especially in Europe since 2006 when EU legislation (EC 1831/2003) has banned the use of Antibiotics Growth Promoters (AGPs) in the herds. The benefits of FLF over non-FLF or dry mash diets, are documented in several studies which include (*i*) a reduction in feeding costs with recycling of co-products from the food industries (Scholten et al., 1999), (*ii*) an improvement of gut health with reduced pathogens in the GI-tract and feces (Russel et al., 1996; Jensen and Mikkelsen, 1998; Demečková et al., 2002; Canibe and Jensen, 2003) and (*iii*) an increase of animals growth performance through better intake or feed digestion (Jensen and Mikkelsen, 1998; Kim et al., 2001; Brooks et al., 2003; Dung et al., 2005).

Fermented feeds are spontaneously produced by the endogenous microflora hold on the dry cereals that is mainly composed of lactic acid producing bacteria (LAB) and yeasts. Orientated fermentations are also carried-out either by using part of a previous FLF as inoculum (process known as leftover) or by addition of a specific starter culture. In particular, addition of LAB strains with potential probiotic properties in pigs have been investigated as microbial inoculants (Geary et al., 1999; Missotten et al., 2007, 2009).

Regardless of the type of fermentation, FLF should promote the development of large LAB populations ($\approx$9 $\log_{10}$ CFU/mL), quickly achieve low pH values (below 4.5) and high lactic acid concentrations (>100 mmol/L) (Jensen and Mikkelsen, 1998; Van winsen et al., 2001a; Brooks, 2003). However, an adequate control of this multi-dimensional process is required to produce feeds with optimal and reliable quality. Indeed, under inappropriate or uncontrolled fermentation conditions, yeasts can get the upper hand in the liquid feed and/or enteropathogens may proliferate, which both impair the diet's hygienic quality and nutritional value. This is well exemplified by the reported detrimental consequences of high levels of ethanol, or acetic acid on feed palatability, on dry matter (DM) losses and reduced energy content (Jensen and Mikkelsen, 1998; Brooks et al., 2001, 2003). Another potential drawback is the occurrence of microbial amino acids decarboxylation, especially free lysine, producing toxic biogenic amines, mainly cadaverine (Niven et al., 2006; Canibe and Jensen, 2010).

The operating parameters that can markedly influence the fermentations patterns, *i.e.* the time of incubation, the operating temperature, the feed time schedule and percentage of leftover, have been mostly investigated in bioreactors at a laboratory-scale (Jensen and Mikkelsen, 1998; Brooks et al., 2003; Plumed-Ferrer et al., 2005; Moran et al., 2006; Niba et al., 2009; Canibe and Jensen, 2012). It was thus necessary to acquire thorough knowledge of liquid feed fermentation under larger-scale conditions in order to improve existing on-farm FLF systems.

Therefore, an original liquid feeding pilot has been set-up at a pre-industrial scale to continuously monitor the processes taking place during these fermentations. Considering the fermentation process as a multi-variables dynamic process including obviously time, biology and physicochemistry, the objective of the present study was to determine the limits within which it was possible to initiate and maintain a microbial ecosystem which ensures a robust and rapid steady-state of FLF.

Single batch and multiple batches were both performed in this study. Single batch experiments aims at characterizing the initial steps of FLF and mimicking the fermentation process which occurs on farm where water push is performed between two feed deliveries. The rationale of this is that microbial "initial conditions" are determinant in the rate, extent and safety of the feed. Hence, single batch experiments were performed by incubating the feed till the pH level has reached a plateau value. In opposite, multiple batches experiments were performed to assess the possibility to establish a "positive" stable microbial ecosystem and validate its ability to reduce pathogens development in the feed especially Coliforms and *E. coli*. The approach takes into account competitions between microbial populations which highly rely on the metabolites released and their subsequent physicochemical bulk modifications. Equal attention was paid to rates and extents of the physicochemical phenomena occurring during the process.

2. Materials and methods

2.1. Liquid feeding pilot

A reality scale pilot was purposely conceived and implemented at the Department of Biochemical Engineering of the University of South Brittany in the framework of the VALORIAL knowledge cluster "ProBioMàs" research program dedicated to pigs liquid feed quality that was developed in close collaboration with animal nutrition companies (Lallemand S.A.S., ACEMO S.A. and InVivo). It is based on two independent liquid feed producing lines each including one fermentation and one mixing tank linked up to a 25 m long delivery pipe. Equipments are made of stainless steel except the delivery pipelines which are of PVC. The fermentation vessels (70 L utile volume) are kept at a desired temperature (between 15 and 45 $\pm$ 1 °C) by circulating water through the double envelop. The vessels are placed on load cells to monitor weight and equipped with a sampling window and probes (pHD-S sc, Hach Lange, Netherlands) that continuously record pH, temperature and REDOX potential of the content. The dry components (*i.e.* cereal meals) are filled through the top of the tank from three short augers (3 m^3) and holding bins. A small hopper accurately weighs small amounts of the additives (*i.e.* culture starter) from 50 up to 800 g $\pm$ 2%. Cold water can be added into the tank from a 1 m^3 buffer tank. The ingredients are mixed together with a central mixer fitted with three blades specially designed to keep all particles in homogenous suspension throughout the whole mixing and fermentation times without resulting in significant oxygenation. Once the fermentation is completed, the liquid feed is transferred to the mixing tank, similar to the fermentation vessel, and stirred until distribution. Feed delivery *via* the pipeline network can be achieved either by pushing the feed with fresh water or with the next ration. Between