Effect of acid addition to pig liquid feed on its microbial and nutritional characteristics☆

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Abstract

An in vitro study was performed to describe the effect of addition of acid products to liquid feed on the course of fermentation. A standard grower diet with added extra lysine, methionine, and threonine was formulated. Three experimental treatments were prepared: the grower diet, ‘Control’; the grower diet + 4.8 g solid acidifier Boliflor® FA 2300S/kg liquid feed, ‘FA2300S’; and the grower diet + 2.0 g formic acid/kg liquid feed, ‘Formic’. Feed and water (1 to 2.75) were incubated in bioreactors with a volume of 1 l at 20 °C. A sample was taken after 0, 6, 24, and 48 h of incubation. After 48, 55, 72, 79, and 96 h of incubation, 90% of the mixture was removed and replaced with fresh feed and water. A sample was taken at 96, 102, and 108 h of incubation. Enterobacteriaceae counts were highest in the ‘Control’ diet and lowest in the ‘Formic’ diet at all sampling times. From 96 to 108 h of incubation, ∼5–19% total lysine, threonine, and methionine, and ∼26–42% free lysine, threonine, and methionine disappeared. Addition of 2.0 g formic acid or 4.8 g Boliflor® FA 2300S per kg pig liquid feed impeded a blooming of Enterobacteriaceae during the first hours of fermentation but had no effect on amino acid degradation. A disappearance of, mainly, free amino acids occurred during fermentation of liquid feed as prepared in the present study.

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1. Introduction

The use of liquid feed in European pig production is widespread and the interest for it, due to its reducing effect on Enterobacteriaceae in the gastrointestinal tract, has increased during the last years as a consequence of the total ban of antibiotic growth promoters in the European Union. Successful fermentations of liquid feed can be obtained by mixing fresh feed and water with material from a previous successful fermentation, which acts as inoculum for the new mixture (Jensen and Mikkelsen, 1998). However, during the initial phase of fermentation high levels of Enterobacteriaceae proliferate in the liquid feed due to the still relatively high pH and low acidity of the mixture at this stage.

Feeding fermented liquid feed (FLF) to pigs improves their gastrointestinal health compared to dry feed and/or non-FLF (van Winsen et al., 2001; Canibe and Jensen, 2003). However, more variable results on

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the effect of FLF on growth performance of pigs have been observed (Russell et al., 1996; Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003). Disappearance of amino acids and production of high levels of off-flavours, like acetic acid and biogenic amines, during fermentation of liquid feed have been suggested as important contributors to the negative results observed on growth performance (Brooks et al., 2001; Pedersen, 2001).

The aim of the present in vitro study was to investigate the effect of adding acid-containing products to liquid feed of suboptimal microbial quality on microbial and nutritional characteristics of the mixture.

2. Materials and methods

A standard grower diet with added (g/kg) 2.0 lysine, 2.5 methionine, and 2.0 threonine was formulated and used as mash. Three experimental treatments were prepared: 1) the grower diet with added amino acids, ‘Control’; 2) the grower diet with added amino acids +4.8 g Boliflor® FA 2300S (a solid feed acidifier containing 251 g formic acid/kg, 151 g ammonium formate/kg, 25 g potassium sorbate/kg, and 543 g diatomaceous earth carrier/kg, Kemira GrowHow, Finland)/kg liquid feed, ‘FA2300S’; and 3) the grower diet with added amino acids +2.0 g formic acid/kg liquid feed, ‘Formic’. The ‘FA2300S’ diet was formulated to contain approximately 2 g acids and salts/kg liquid feed in order to compare it with the ‘Formic’ diet, containing 2 g formic acid/kg liquid feed. Feed and water were mixed in the ratio of 1 to 2.75 (w/w) and incubated in bioreactors with a volume of 1 l at 20 °C. Duplicate determination of each treatment was carried out. The samples were incubated during 48 h, during which no feed or water was removed or added. A sample was taken after 0, 6, 24, and 48 h of incubation. After 48 h of incubation, 90% of the mixture was removed and replaced with fresh feed and water, and the same procedure was followed at 55, 72, 79, and 96 h of incubation. A sample was taken at 96, 102, and 108 h of incubation. The first 48 h of incubation simulate the dynamics of a ‘new-established’ FLF system and the last 12 h of incubation simulate the dynamics of a FLF system already running, that is, in steady state.

Analyses on concentration of lactic acid and short chain fatty acids (SCFA), total nitrogen and amino acid, and enumeration of lactic acid bacteria, Enterobacteriaceae and yeasts were carried out basically as described by Canibe and Jensen (2003), except that the incubation time for lactic acid bacteria and yeasts was three days.

3. Results

The number of lactic acid bacteria in the ‘Control’ diet increased from <3.0 log cfu/g sample to 9.5 log cfu/g during the initial 48 h of incubation (Fig. 1). The counts in the ‘FA2300S’ and ‘Formic’ diets showed a very small increase during the same period. From 96 to 108 h of incubation, the counts of lactic acid bacteria increased in all diets, with the highest levels being counted in the ‘Control’ diet and the lowest in the ‘Formic’ diet. Enterobacteriaceae counts in the ‘Control’ diet increased ~100 fold during the first 24 h of incubation, and decreased somewhat at time 48 h. The Enterobacteriaceae counts in the ‘FA2300S’ and ‘Formic’ diets decreased with time of incubation during the first 48 h. From 96 to 108 h of incubation, the Enterobacteriaceae counts decreased in all three diets, the counts being lowest in the ‘Formic’ diet. The yeast counts were low during the whole incubation period in all diets (between <3.0 and 5.4 log cfu/g) (data not shown).

Formic acid was not detected in the ‘Control’ diet at any sampling time, and the levels in the ‘Formic’ and ‘FA2300S’ diets were rather constant during the whole
incubation period (between 33.3 and 44.2 mmol/kg). Lactic acid was detected at the highest concentration in the 'Control' diet, whereas the 'Formic' diet did not contain detectable amounts of this acid at any sampling time (Fig. 2). Whereas the concentration of acetic acid in the 'FA2300S' and 'Formic' diets was kept at low levels during the first 48 h of incubation, the levels in the 'Control' diet increased. The same pattern was observed from 96 to 108 h of incubation.

From 96 to 108 h of incubation, ~5–9% total lysine, ~12–13% total threonine, and ~17–19% total methionine (in g/kg crude protein) were degraded in all diets. The decrease in the concentration (g/kg crude protein) of free amino acids from 96 to 108 h of incubation was ~26–34% for free lysine, ~31–38% for free threonine, and ~31–42% for free methionine.

The concentration of tyramine, putrescine, cadaverine and histamine in the samples taken after 108 h of incubation was below detection levels (10 mg/kg DM) (data not shown).

4. Discussion

In the 'Control' diet, it was the low pH and high lactic acid level that killed the Enterobacteriaceae (van Winsen et al., 2000), and therefore the time needed for lactic acid bacteria to proliferate and produce high amounts of lactic acid corresponded to the interval measured before Enterobacteriaceae levels started to decrease. In the 'FA2300S' and 'Formic' diets, it was the acid added that had the bactericidal effect on Enterobacteriaceae (Canibe et al., 2001), and because the acid was present at high concentrations already from the beginning of the incubation, the levels of Enterobacteriaceae started decreasing also from the beginning of incubation of liquid feed.

Decarboxylation of amino acids in FLF, which results in biogenic amines, can have two negative effects for the animals fed the FLF: there is a loss of available free amino acids to the host, and the production of biogenic amines can impair palatability of the FLF and thereby impair feed intake, as has been suggested for cadaverine (biogenic amine produced from lysine) (Brooks et al., 2001). In the present study, disappearance of amino acids was measured but, unexpectedly, biogenic amines were not detected, what is difficult to explain. However, increasing levels of cadaverine with time of fermentation of liquid feed have been observed (Niven et al., 2006). Recently, Niven et al. (2006) concluded that the loss of lysine from FLF is due to the metabolism of lysine by E. coli. The acid-containing products used in the current study reduced the number of Enterobacteriaceae in the FLF, but the data suggested that reduction of the number of these bacteria to the extent reached in this study is not effective avoiding or attenuating amino acid disappearance in FLF.

5. Conclusion

Addition of 2.0 g formic acid/kg liquid feed or 4.8 g Boliflor® FA 2300S/kg liquid feed to pig liquid feed are strategies that impede the proliferation of Enterobacteriaceae during the first hours of fermentation. However, they do not attenuate the disappearance of amino acids in fermented liquid feed. Further studies are needed to identify the microorganisms involved in the degradation of amino acids in fermented liquid feed, so...
that procedures to prepare fermented liquid feed of good nutritional quality while keeping its beneficial microbial characteristics can be defined.

References


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