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3 **Fermented liquid feed for pigs: a review**
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Abstract

Since the announcement of the ban on the use of antibiotics as antimicrobial growth promoters in the feed of pigs in 2006 the investigation towards alternative feed additives has augmented considerably. Although fermented liquid feed is not an additive, but a feeding strategy, the experimental work examining its possible advantages also saw a rise. The use of fermented liquid feed (FLF) has two main advantages, namely the simultaneous provision of feed and water which may result in an alleviation of the transition from the sow milk to solid feed and may also reduce the time spent to find both sources of nutrients. Secondly, offering FLF with a low pH may strengthen the potential of the stomach as first line of defense against possible pathogenic infections. Because of these two advantages FLF is often stated as an ideal feed for weaned piglets, the results obtained so far are rather variable, but in general show a better body weight gain and worse feed/gain ratio for the piglets. However, for growing-finishing pigs on average a better feed/gain ratio is found compared to pigs fed dry feed. This better performance is mostly associated with a less harmful microbiota and a better gut morphology. This review provides an overview of the current knowledge of FLF for pigs, dealing with the FLF itself and its effect on the gastro-intestinal tract and animal performance.

Keywords: fermented liquid feed, pig, gastro-intestinal tract, microbiota

1. Introduction

77
78
79 The use of microbial fermentation to conserve or improve food is not new. For thousands of
80 years fermented foods and beverages contributed significantly to the diet of humans and animals.
81 Even in the Bible there are references to the use of yoghurt (e.g. Genesis 18:8). Most fermented
82 foods owe their origin to the fact that processes used in their production are inhibitory to many
83 microorganisms. As a result, fermented products generally have a longer shelf life than their
84 original substrate (Adams and Mitchell, 2002). This inhibitory action is also one of the positive
85 things associated with the use of fermented liquid feed (FLF) in pigs.

86 The interest in fermentation of feed for improving the performance of piglets and pigs
87 had a surge after the announcement of the planned ban in the EU on the use of antibiotics as
88 antimicrobial growth promoter (AMGP) in the feed of pigs. In three recent reviews the potential
89 of FLF, as an alternative for the use of growth-promoting antibiotics in the piglet feed, is
90 discussed (Brooks, 2008; Niba et al. 2009; Plumed-Ferrer and Von Wright, 2009). In this

91 literature review the current knowledge about the use of fermented liquid feed in pigs will be
92 given. Although the use of co-products in FLF is also of importance, this subject will only be
93 briefly discussed (for review see Scholten et al. 1999b).

94

95 **2. Definition of fermented liquid feed**

96

97 It is important to define liquid feeding and differentiate it from other feeding systems. Liquid
98 feeding involves the use of a diet prepared either from a mixture of liquid food industry by-
99 products and conventional dry materials, or from dry raw materials mixed with water. A liquid
100 feed should not be confused with wet/dry feeder systems where water and feed are kept separate
101 up to the point of delivery to the pig (Brooks et al. 2003a).

102 By definition FLF is feed mixed with water, at a ratio of 1:1.5 to 1:4 and fermented for a
103 period long enough to reach steady state conditions (Chae, 2000; Brooks, 2003; Brooks et al.
104 2003a). It is usually prepared by spontaneous fermentation (spontaneous fermented liquid feed;
105 SFLF) or inclusion of the feed mixture with a culture of lactic acid bacteria (LAB) as inocula
106 (controlled or inoculated fermented liquid feed; IFLF). If there is almost no time between mixing
107 and feeding or the period for fermentation is too short to reach steady state conditions, the term
108 liquid feed (LF) or non-fermented liquid feed (NFLF) is used (Canibe and Jensen, 2003; Plumed-
109 Ferrer and Von Wright, 2009; Brooks, 2008).

110

111 **3. Desired properties of FLF**

112

113 From the moment that feed and water are mixed, there is a possibility that fermentation will
114 begin. According to Canibe and Jensen (2003), the initial phase of fermentation is characterized
115 by low levels of LAB, yeasts, and lactic acid, high pH, and, importantly, a blooming of
116 enterobacteria. This phase is followed by a second phase, in which a steady state is reached, and

117 which is characterised by high levels of lactic acid bacteria, yeasts, and lactic acid, low pH, and
118 low enterobacteria counts.

119 Recently Brooks (2008) further subdivided the initial phase in a phase 1 in which the
120 high pH allows the proliferation of coliform bacteria and a phase 2 in which growth and
121 fermentation by LAB inhibits pathogenic and spoilage organisms by the production of organic
122 acids (particularly lactic acid), hydrogen peroxide and bacteriocins, as well as by lowering the
123 pH and redox potential. In phase 3, the steady state phase, the LAB population and pH stabilizes,
124 but over time, the yeast concentration of the feed can continue to increase. These phases are
125 represented in Figure 1.

126 Van Winsen *et al.* (2000) pointed out that lactic acid concentration is the main
127 responsible for the antimicrobial effect of FLF. Hence, strains to be used as inoculant for FLF
128 production should have a high capacity for lactic acid production and should be active against
129 enteric pathogens. According to Van Winsen *et al.* (2001) the desirable characteristics for FLF
130 include (1) a pH below 4.5, (2) LAB concentration above $9 \log_{10}$ CFU/ml, (3) lactic acid
131 concentration above 150 mmol/l and (4) acetic acid and ethanol concentration below 40 and 0.8
132 mmol/l, respectively. Beal *et al.* (2002) stated that to prevent the growth of *Salmonella* spp.,
133 liquid feed needs to contain at least 75 mmol/l lactic acid. Beal *et al.* (2002) and Brooks *et al.*
134 (2003b) stated that to reduce the concentration of enterobacteria, the concentration of lactic acid
135 should be higher than 100 mmol/l. This concentration of lactic acid can have a beneficial effect
136 on feed intake, daily gain and F/G ratio (Roth and Kirchgessner, 1998). The D-configuration of
137 lactic acid seems to be equally metabolized, although at a slower rate, as the L-lactic acid (Everts
138 *et al.* 2000).

139 Acetic acid can cause a loss of palatability and although Van Winsen *et al.* (2001) set the
140 limit at 40 mmol/l, other authors indicated that a concentration of acetic acid above 30 mmol/l
141 could already negatively affect the palatability of the FLF (Brooks 2003, 2008; Brooks *et al.*

142 2003b). Not only yeasts produce acetic acid, but also heterofermentative LAB can produce acetic
143 acid to a high degree and cause loss of palatability of the feed.

144 When the fermentation is dominated by yeasts, this can have either a negative or a
145 positive effect dependent on the strains present. A drawback can be the production of ‘off-
146 flavours’ and ethanol, which might diminish the palatability as well as dry matter (DM) and
147 energy content of the feed (Jensen and Mikkelsen, 1998). In fact, Jensen and Mikkelsen (1998)
148 indicated that carbon dioxide loss can be as high as 3.1 % DM. In contrast, these authors also
149 pointed out that the presence of yeasts in the FLF feed might benefit the health status of the
150 gastro-intestinal tract (GIT) of the pigs. Yeasts have the ability of binding enterobacteria to their
151 surface, thereby blocking the binding of these bacteria to the gut epithelium (Mul and Perry,
152 1994). Jensen and Mikkelsen (1998) found an inverse relationship between the concentration of
153 yeast and enterobacteria in the GIT of pigs. Therefore, high concentrations of yeasts in the FLF
154 may be beneficial. However, the population diversity of yeasts present in FLF is very high and
155 deserves further investigation (Olstorpe et al. 2008).

156

157 **4. Possible advantages and disadvantages of FLF**

158

159 One of the main advantages, besides the enteropathogen reducing effect, of the use of FLF is the
160 fact that it is a cost effective feeding strategy to replace AMGP. Probiotics, organic acids and
161 yeast cell walls, often suggested as alternatives to the use of AMGP, are relatively high priced.
162 Combined with the relatively high concentrations in which those products are needed in the feed
163 to have a significant effect, makes their use financially questionable in practical pig production.
164 The use of FLF may solve this problem, because it is characterised by high numbers of LAB and
165 yeast, a low pH and high concentration of lactic acid (Jensen and Mikkelsen, 1998).

166 Also the fact that co-products from the human food industry (Scholten et al. 1999a, b,
167 2001a, b) can be used, may contribute to the reduction in feed cost (Brooks et al. 2003a). Liquid

168 co-products of many sorts are used for pig feeding around the world. In European pig nutrition,
169 recycling of liquid co-products from human food industries has increased in particular, the starch
170 and sugar rich by-products: liquid wheat starch, potato steam peel and cheese whey. In 1996,
171 about 300,000 tonnes of products of the dairy industry were used in pig farms in The
172 Netherlands (Scholten et al. 1999b). Detailed data such as in The Netherlands are not available
173 from other countries, but using information from trade sources it could be estimated at least 30%
174 of all pigs in the EU are fed liquid diets and a majority of these incorporate at least some dairy
175 by-products (Table 1; EFSA, 2006).

176 Another benefit associated with feeding diets in a liquid form is the fact that piglets are
177 provided with water and feed simultaneously (Brooks et al. 2001; Brooks and Tsourgiannis,
178 2003; Russell et al. 1996; Brooks et al. 2003a; Brooks, 2008). In this way, the piglets do not need
179 separate trainings for feeding and drinking (Partridge and Gill, 1993; Russell et al. 1996). Barber
180 (1992) indicated that while some pigs in a group may find a drinker within a few minutes of
181 entering a pen, other pigs may take more than 24 h, long enough to induce symptoms of
182 dehydration. Providing the food in a liquid form most often results in a higher feed intake after
183 weaning (Russell et al. 1996). Also the feed digestion can be improved when presenting the food
184 in a liquid form (Gill et al. 1987; Barber et al. 1991). Barber et al. (1991) demonstrated that there
185 was a linear improvement in both DM and energy digestibility as the feed to water ratio
186 increased from 1:2 to 1:4. Jensen and Mikkelsen (1998) also concluded that feeding the diets as
187 LF or FLF improves growth performances and DM intake, but that the feed/gain ratio (F/G) was
188 only improved in growing-finishing pigs fed LF compared to DF.

189 Although a reduction in loss as dust during handling and feeding may occur (Forbes and
190 Walker, 1968), and improve the pigs environment and health due to the reduction of dust in the
191 atmosphere (Kneale, 1972; Brooks et al. 2003a), other authors have however observed a higher
192 feed wastage. Russell et al. (1996) pointed out that the trough design was one of the possible

193 reasons for the lesser performance of the piglets on FLF in their trial. More feed wastage was
 194 noted by FLF fed piglets compared to dry fed piglets. After changing the trough design, the
 195 piglets fed FLF performed better, but still worse than the piglets on the dry feed. A higher feed
 196 wastage by piglets on FLF was also seen by Plumed-Ferrer and Von Wright (2009).

197 According to Brooks *et al.* (2003a) and Brooks (1999) other possible advantages linked
 198 to the use of liquid feed are:

- 199 • Improved intakes at high ambient temperatures;
- 200 • Improved materials handling (system can act both as a feed mixing and distribution
 201 system);
- 202 • Increased accuracy of rationing (computer control brings a degree of accuracy to the
 203 system that it is difficult to emulate with dry feeding systems), also when using co-
 204 products with different properties (DM-content) from batch to batch;
- 205 • Reduction in nitrogen loading through the easy adoption of “step” and “phase feeding”;
- 206 • Reduction in phosphate loading through activation of endogenous phytase in cereal
 207 grains and/or the addition of exogenous enzymes to diets. This was also investigated by
 208 Carlson and Poulsen (2003) and Nitrayová *et al.* (2009);
- 209 • Improved enzyme activity by a possible reduction in particle size of the feed used, which
 210 in turn reduces separation of materials in the trough and ensures the pig receiving a more
 211 homogenous diet. However, attention should be paid not to use too small particle sizes, as
 212 this is a predisposing factor for the development of gastric ulcers.

213
 214 A very actual research issue nowadays is reduction of enteropathogens, such as
 215 *Salmonella* spp. and *E. coli*, in the pig production. FLF, by excluding pathogens from the feed,
 216 seems to be an excellent feeding strategy to achieve this. From surveillance studies it was clear
 217 that liquid feeding had a positive effect on gut health and reduced the incidence of *Salmonella*
 218 spp. (Tielen *et al.* 1997; Lo Fo Wong *et al.* 2004). However, Van der Wolf *et al.* (1999) showed
 219 that when trough feeding was applied, in which the feed and water are left to soak for several
 220 hours, the risk on *Salmonella* infections was increased. They hypothesised that this soaking
 221 period allowed the proliferation of enteropathogens. This proliferation was indeed seen in the
 222 NFLF in the study of Canibe and Jensen (2003). According to Van der Wolf *et al.* (1999, 2001)
 223 this was not the case when complete diets with fermented co-products were fed and this feeding
 224 strategy lowered the prevalence of *Salmonella* in finishing pig units. Feeding FLF also does not

225 always give a reduction in the incidence of diarrhoea, as could be seen in the challenge
226 experiment performed by Amezcua et al. (2007) with *Escherichia coli* (*E. coli* O149:K91:F4).

227 Another aspect, that can regain interest nowadays, is the fact that feeding LF or FLF
228 rather than dry feed can change the microbial conversion of tryptophan in the hind gut toward
229 indole at the expense of skatole (boar taint) resulting in a lower skatole and a higher indole
230 deposition in the back fat of fattening pigs and thus reduce boar taint (Kjeldsen, 1993). However,
231 Hansen et al. (2000) found that giving FLF to pigs had no effects on skatole concentration in
232 caecum, colon, blood and backfat and boar odour attributes, whereas administration of FLF plus
233 50 mg/kg of zinc bacitracin for the last week before slaughter decreased the corresponding
234 skatole concentrations and the typical boar odours (pig and manure odour). However, meat from
235 the two groups of pigs given FLF, either with or without zinc bacitracin, was significantly worse
236 in terms of the scores for three flavour attributes (pig flavour, rancid flavour and total off-
237 flavour) compared with meat from pigs given dry food.

238 Liquid feeding of diets is sometimes associated with the development of diseases like
239 haemorrhagic bowel syndrome, gastrointestinal tympany, gastric torsion or gastric ulcers
240 (Brooks, 2008; Plumed-Ferrer et al. 2005). A direct link is not easily found, because these
241 diseases are mostly caused by a multifactorial condition. In the experiment of Plumed-Ferrer et
242 al. (2005) the incidence of gastric ulceration was remarkably low, although two main
243 predisposing risk factors, feeding FLF and feeding more than three times a day, were present.

244 The fermentation process can cause a loss of essential nutrients from the feed, such as
245 vitamins and amino acids (AA), especially the free synthetic AA added to the feed (Canibe and
246 Jensen, 2003; Niven et al. 2006). For example, the production of biogenic amines, such as
247 cadaverine from L-lysine, can occur (Pedersen, 2001; Pedersen et al. 2002a, b; Brooks et al.
248 2003b; Niven et al. 2006; Canibe et al. 2007b, c, 2008). Biogenic amine formation causes an
249 irreversible loss of AA for the pig, because it is caused by a decarboxylation rather than a

250 desamination (Dierick et al. 1986a, b). This is why some authors advocate the fermentation of
251 only the grain fraction instead of the complete feed (Pedersen et al. 2002c; Scholten et al. 2002;
252 Brooks et al. 2003b; Moran et al. 2006; Brooks, 2008; Canibe et al. 2007a; Canibe and Jensen,
253 2007).

254 Pigs often eat more DM when fed LF or FLF than when fed dry diets, so when
255 formulating diets to be fed as FLF, care should be taken to formulate them on the basis of
256 realistic estimations of DM intake per day. Otherwise the piglets will consume too much of some
257 nutrients. Like proteins, which can depress feed utilisation and ultimately depress DM intake
258 (Brooks et al. 2001) or cause protein-induced diarrhoea (Brooks, 2008). Brooks (2008) also
259 pointed out that the fermentation of nutritionally balanced liquid feed will improve performance
260 only if it increases feed intake or improves gut health. If the intake would be unaffected, it could
261 be that the biochemical changes produced by fermentation will produce a diet that is less
262 balanced.

263

264 **5. Fermentation variables**

265

266 The use of LF was already advocated by Henderson in 1814, but it was Smith (1976) who first
267 pointed to the fact that when feed was soaked in water for a certain length of time, prior to
268 feeding, that lactic acid bacteria and yeast naturally present on grains proliferate and produce
269 lactic acid, acetic acid and ethanol and then reduce the pH of the feed. Generally, the LAB
270 dominate fermentations in liquid feed. However, at low operating temperatures and particularly
271 when co-products from brewing and ethanol production are used, yeasts can dominate the
272 fermentations (Brooks et al. 2003b).

273 Fermentation can be influenced by several factors. In Figure 2 the most important factors
274 playing a role in this are given. The LAB naturally present on the different feed components or
275 LAB added to the feed determine, together with the fermentation parameters, the degree of acid

276 production (primarily lactic acid). The faster this production, the faster the drop in pH and the
277 faster pathogenic bacteria, such as *Salmonella* spp. or *E. coli*, are reduced. Also strains
278 originating from the air can influence the fermentation profile. An acid environment does not
279 exclude the possible growth of yeasts and fungi present on the feed or in the air. These can
280 produce “off-flavours” and taints that make the feed less palatable (Moran, 2001; Brooks et al.
281 2003b). In the past few years some studies also investigated the population diversity of LAB or
282 yeasts in FLF and its changes (Plumed-Ferrer et al. 2004; Pedersen et al. 2004; Canibe et al.
283 2007a; Olstorpe et al. 2008) and they found a wide range of variation in the populations of the
284 feeds. Other parameters such as environmental temperature or fermentation temperature, interval
285 between and degree of backslopping (partial replacement of FLF by fresh LF in continuous
286 fermentation) and the actual feed:water ratio of the FLF, can have an effect on the actual
287 fermentation characteristics of the FLF (Choct et al. 2004a).

288

289 ***5.1. Spontaneous versus inoculated fermentations***

290

291 In the FLF the fermentation may happen spontaneously or it may be induced. The induced
292 fermentation can be achieved either by backslopping or inoculation of the feed with a LAB strain
293 (Plumed-Ferrer and Von Wright, 2009). Beal et al. (2005) pointed out, that spontaneous
294 fermentation is not a reliable system to obtain a safe and palatable final product. They fermented
295 56 samples of wheat and 44 of barley. Only a few spontaneous fermentations reached the
296 threshold of 100 mmol/l lactic acid needed to eliminate *Salmonella* spp. (20% after 72 h
297 fermentation at 30°C). Other studies have shown as well that uncontrolled/spontaneous
298 fermentations result in higher concentrations of both acetic acid and biogenic amines, which
299 adversely affect the palatability and intake of liquid diets (Brooks et al. 2003b; Niven et al.
300 2006).

301 Brooks et al. (2003b) stated that to benefit from the use of FLF, fermentation needs to be
302 controlled. In this view, spontaneous fermentations seem to be too variable. This problem can be
303 diminished by the inoculation of the liquid feed with LAB that produce high concentrations of
304 lactic acid rapidly (Jensen and Mikkelsen, 1998; Brooks et al. 2003b). In the literature there are
305 already many LAB species used to produce FLF. *Lactobacillus plantarum* is often used to
306 inoculate liquid feed to produce FLF (Van Winsen et al. 2000; Demečková et al. 2002; Heres et
307 al. 2003; Plumed-Ferrer et al. 2005; Niven et al. 2006; Missotten et al. 2007). Also, *Pediococcus*
308 *spp.* are often used to inoculate liquid feed (Geary et al. 1999; Beal et al. 2002; Niven et al. 2006;
309 Missotten et al. 2007). In fact, combinations of these two strains have been used to produce FLF
310 (Jensen and Mikkelsen, 1998; Moran, 2001; Moran et al. 2006). An overview of all LAB species
311 found in literature to produce FLF are given in Table 2.

312 In a recent study, Olstorpe et al. (2008) demonstrated that the species composition in
313 fermented liquid feed may vary considerably. They showed that *Pediococcus pentosaceus* was
314 the dominant population at the beginning of a spontaneous fermentation, but that after 3 days of
315 continuous fermentation the *Lactobacillus plantarum* population had become the dominant
316 population. This was also observed in inoculated FLF, where the LAB strain used to inoculate
317 the feed did not stay the dominant LAB strain in the FLF (Geary et al. 1999; Missotten, 2010).
318 To maintain the possible probiotic effect of the added strain, the use of batch fermentation may
319 thus be more suitable (Brooks, 2008).

320

321 **5.2 Complete feed or grain fraction**

322

323 Fermenting the whole feed is the easiest way to produce FLF, but it can bring along some
324 problems, like the use of essential nutrients from the feed by the microorganisms (Brooks et al.
325 2003b; Brooks, 2008). To eliminate this possibility it is often suggested to only ferment the grain
326 fraction. This grain fraction may then be used to make a range of diets, so that “step” or “phase

327 feeding” can be implemented using the same fermented grain. A grain is also a more consistent
328 product to ferment, compared to a complete feed, consisting out of multiple nutrients (Brooks et
329 al. 2003b). However, Beal et al. (2005) reported that spontaneous fermentation of grain is often
330 not sufficient to produce FLF with desirable characteristics. So inoculation with a LAB strain is
331 necessary, particularly considering that fermentation will have to produce more lactic acid to
332 compensate for the dilution and buffering effects of the other feed components when the
333 complete feed is produced (Brooks, 2008).

334

335

336 ***5.3. Continuous or batch fermentations***

337

338 *5.3.1. Continuous fermentations*

339

340 For FLF the term continuous fermentation is actually not the exact term to be used, because the
341 fermentation is not continuously renewed with a fresh feed mixture, but at regular time intervals
342 (usually coinciding with feeding times). This is known as ‘backslopping’ (Salovaara, 1998). In
343 practice, backslopping can involve the retention of a specified quantity of previously fermented
344 product in the tank and mixing in fresh substrate or the addition of a portion of the fermented
345 product to a fresh tank of substrate to act as an inoculum (Nout et al. 1989). In this way, a
346 portion of a previous successful fermentation is retained as an inoculum for the next batch
347 (Moran et al. 2006). This allows for the gradual selection of LAB and an accelerated
348 fermentation (Nout et al. 1989), although Brooks (2008) also pointed out the possibility that this
349 may result in the development of a microflora dominated by yeasts, which may compromise the
350 palatability and nutritional value of the feed. As mentioned before however, high concentrations
351 of yeasts in the FLF may be beneficial as well.

352 In their study, Jensen and Mikkelsen (1998) stated that a fermentation period of 8 hours
353 would be most practical for the farmers, since they feed their pigs three times a day. They
354 investigated whether 8 hours would suffice to ferment a new mix in the fermentation vessel, with
355 an as low as possible residue from the previous fermentation. They concluded that to ensure an
356 efficient fermentation, half of the previous feed has to remain in the tank between fermentations,
357 although it took 3-5 days to reach steady state conditions.

358 Plumed-Ferrer et al. (2005) showed that maintaining 25% in the tank to inoculate the
359 fresh added LF to the tank (backslopping at 8:00 a.m. and 14:00 p.m. every day), was sufficient
360 to maintain a proper fermentation. Moran et al. (2006) found that there was no advantage in
361 keeping more than 20% of the fermented wheat (backslopped after 24 h) when performing
362 fermentation. So although a retention of 50% is mostly used, it seems that a lower proportion can
363 be used with 20% being the lowest percentage still ensuring the exclusion of coliforms.

364 When using pipeline-feeding systems, a spontaneous fermentation of the LF occurs in the
365 pipes, and residues of feed in the pipes can thus be seen as a special form of backslopping (Royer
366 et al. 2004a, 2004b, 2005). Hansen and Mortensen (1989) pointed out that it was detrimental to
367 sterilize pipeline-feeding systems as this removed the LAB and increased the pH of the feed by
368 1.5-2.0 units. This allowed coliform bacteria to proliferate, causing outbreaks of diarrhoea, until
369 the LAB re-established themselves after 3 to 5 days and lowered the pH again. This growth of
370 *Enterobacteriaceae* can also occur when the fresh feed mix is added to the fermentation vessel
371 when applying backslopping, because of the dilution effect the pH can go above the growth
372 inhibiting level of 4.5 (Brooks, 2008).

373

374 5.3.2. Batch fermentation

375

376 This type of fermentation, where the feed and water mixture is fermented in a vessel without
377 replacements of a part of the FLF, is most often used in *in vitro* experiments. Sometimes batch

378 fermentations of the feed or the grain fraction are used to produce FLF for *in vivo* experiments
379 (Scholten et al. 2002), because they are easier to control and if undesirable fermentation occurs it
380 is only within one batch (Brooks, 2003; Brooks et al. 2003b). Recently Brooks (2008) also
381 suggested that batch fermentations may be more suitable for preserving the probiotic effect of
382 added strains than the backslopping technique.

383

384 **5.4. Temperature**

385
386 Jensen and Mikkelsen (1998) investigated whether fermentations at different temperatures would
387 give a different result. This was the case, but they found that fermenting the feed at temperatures
388 above 20°C did not give major advantages to the FLF compared with the FLF produced at 20°C
389 (Figure 3). At 20°C the coliform count was just above the detection limit of 3 log₁₀ CFU/g FLF.
390 They did stress the fact that the temperature needs to be at least 20°C if the required pH at
391 feeding has to be lower than 4.5. Enteric pathogens, such as *E. coli* and *Salmonella* spp., do not
392 tolerate pH values below 4.5 (Merrell and Camilli, 2002)

393 Later Beal et al. (2002) investigated the effect of fermentation on the exclusion of
394 *Salmonella* Typhimurium. They found that the reduction time was much shorter at 30°C versus
395 20°C. So although the minimal temperature for getting optimal FLF is a temperature of 20°C, a
396 temperature of 30°C is preferable since it allows a fast production of lactic acid and a fast
397 exclusion of enteropathogens (Brooks, 2003).

398 It should also be avoided with backslopping to add cold water to the system. For example
399 adding water immediately from the tap (5-7°C) will cold-shock the system. This could cause the
400 induction of cold-shock protein formation in enteropathogens, which can protect them and allow
401 them to persist longer in the feed (Brooks et al. 2001, Beal et al. 2002). Furthermore it disturbs
402 the growth of the LAB and it allows the yeasts to become dominant (Brooks et al. 2001).

403 Not only the temperature of the fermentation vessel is important, also the environmental
404 temperature is of importance. Niba et al. (2009) stated that FLF may contribute considerably to
405 the African agriculture, especially in semi-arid and hot areas. In such areas, ambient
406 temperatures ($\pm 30^{\circ}\text{C}$) could support efficient lactic acid fermentation of feeds.

407

408 ***5.5. Feed:water ratio***

409

410 By definition, the feed to water ratio of LF or FLF can fluctuate between 1:1.5 and 1:4. Barber et
411 al. (1991) demonstrated that there was a linear improvement in both DM and energy digestibility
412 as the feed to water ratio increased from 1:2 to 1:4, while Gill (1989) demonstrated that there
413 was an improvement of the F/G ratio as the feed to water ratio increased from 1:2 to 1:3.5. The
414 same was observed by Hurst (2002) and Hurst et al. (2008) for a feed to water ratio from 1:1.5 to
415 1:3. Although the intake of solid food by newly weaned pigs is generally low, the results
416 obtained by Russell et al. (1996) demonstrated that the dry matter feed intake of the newly
417 weaned pig can be increased by providing it with FLF, even though that this implied a
418 consumption of a greater volume of material. When piglets are offered FLF with different DM
419 percentages (14.5 – 25.5%), they maintain their DM intake in the lowest DM group by
420 increasing their total volumetric intake. The dietary DM concentration also had no effect on
421 weight gain or F/G ratio (Geary et al. 1996). All these studies support the theory that the pig will
422 limit intake of water not originating from LF or FLF, e.g. from nipple drinkers, to maximise feed
423 intake (Yang et al. 1981). Hence the total volumetric intake will be comparable when the same
424 diet is fed in liquid or dry form (Geary et al. 1996).

425 Although, a wide range of ratios can be used, it becomes clear from the overview given
426 in Plumed-Ferrer and Von Wright (2009) and Niba et al. (2009) that the slurry is most of the
427 time given to pigs in a feed to water ratio between 1:2 and 1:3. In batch fermentations in the lab
428 sometimes higher ratio's are being used for experimental purposes.

429

430 **6. Effect on GIT health**

431

432 The concept of gut health is complex and, at present, an ill-defined notion. Conway (1994)
433 proposed that there are three major components of ‘gut health’, namely the diet, the intestine and
434 its commensal microbiota. The intestine is an important compartment, being a major site of
435 digestion, nutrient absorption and hydro-mineral exchange homeostasis, harbouring a complex
436 microbiota and a highly evolved mucosal immune system (Lallès et al. 2007). Post-weaning GIT
437 disorders in pigs result not only from alterations in GIT architecture and function but also from
438 major changes in the adapting enteric microbiota (Konstantinov et al . 2004) and immune
439 responses (Stokes et al. 2004; Baily et al. 2005). Undoubtedly all these aspects of GIT
440 physiology, microbiology and immunology contribute interactively to the so-called gut health
441 balance (Lallès et al. 2007). In this part the effect of feeding FLF on GIT pH, microbial
442 population and GIT morpho-histology will be discussed.

443

444

445

446 **6.1. Effect on GIT pH**

447

448 A lowering of the gastric pH in piglets fed FLF compared to DF or LF piglets is often seen
449 (Mikkelsen and Jensen, 1998; Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003). The pH in
450 the small intestine of FLF fed piglets is however often higher than in piglets fed DF or LF
451 (Mikkelsen and Jensen, 1998 and 2000; Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003).
452 This may be related to an increased secretion of pancreatic juice, stimulated by the low pH and
453 high lactic acid concentrations in the FLF (Jensen and Mikkelsen, 1998; Plumed-Ferrer and Von

454 Wright, 2009). In Table 3 the results obtained in the study of Canibe and Jensen (2003) are given
455 as a good example of pH in the different GIT segments when pigs are fed FLF, LF or DF.

456 Radecki et al. (1988) suggested that the improved growth of pigs fed on diets containing
457 organic acids may be related to a lower gastric pH, allowing a better proteolytic activity in the
458 stomach and inhibiting the growth of undesirable pathogenic bacteria. This can also be the case
459 when feeding FLF. The stomach is indeed an important barrier against pathogens (Lallès et al.
460 2007) and lowering the pH may improve this barrier and prevent coliform scours (Easter, 1993),
461 especially in newly weaned piglets which are often not capable of producing enough gastric acid
462 (HCl) themselves yet (Partridge and Gill, 1993).

463

464 **6.2. Effect on GIT microbial population**

465

466 The use of FLF can influence the microbial population in the GIT. Canibe and Jensen (2003)
467 found no differences among the different types of feeding in the number of LAB grown at 37°C
468 from the distal small intestine (Table 4). At an incubation temperature of 20°C however, the
469 proportion of LAB able to grow was significantly higher in pigs fed FLF compared to the DF or
470 LF fed piglets. This indicated that the LAB population in the GIT of the two groups was
471 different. They also found that the proportion of yeast was, almost in all GIT compartments,
472 significantly higher in the FLF fed group (Table 4). In contrast, all segments of the FLF fed
473 piglets showed less coliform bacteria (Table 4). This was also apparent in the study of Moran
474 (2001) in which he found that the ratio of LAB to coliform bacteria in the lower gut of the pigs
475 weaned with FLF was shifted in favour of the LAB, while in piglets fed dry feed, this ratio was
476 shifted in favour of the coliforms.

477 Demečková et al. (2002) showed that it is possible through feeding the sows FLF that the
478 bacterial gut population of their offspring can be influenced. The newborn pig usually has a
479 sterile gut at birth and acquires its characteristic flora through contact with its mother and its

480 surroundings. The most significant contributor of bacteria to the piglet's surroundings is the sow.
481 Demečková et al. (2002) reasoned that if the gut microflora of the sow could be manipulated this
482 would impact on the development of the piglet's gut microflora. The piglets from sows fed FLF
483 indeed showed lower coliform counts in their faeces compared to piglets from sows fed NFLF or
484 dry diets. Also the LAB population was augmented in the faeces of the piglets from sows fed
485 FLF compared to the other piglets.

486 Feeding FLF to pigs reduced the microbial activity (ATP concentration) in the GIT,
487 significantly in the stomach and small intestine, of piglets compared to piglets fed NFLF (Jensen
488 and Mikkelsen, 1998). This is also seen when feeding AMGP (Jensen, 1988). In Canibe and
489 Jensen (2003) this reduction in ATP concentration in the GIT was not found, although the
490 microbial population from the caecum and colon of the piglets fed with FLF showed a reduced
491 fermentation capacity (Højberg et al. 2003).

492

493 **6.3. Effect on GIT morpho-histology**

494

495 The transition from liquid milk to solid feed is often accompanied with a reduced feed intake and
496 growth check (Brooks and Tsourgiannis, 2003). This results in shortening of villi and crypt
497 deepening in the small intestine, which is generally associated with a reduced digestive capacity
498 (Pluske et al. 1997; Montagne et al. 2007). Liquid feed, either fresh or fermented, is stated to
499 help overcome this reduced feed intake (Scholten et al. 1999b). Pluske et al. (1997) and Vente-
500 Spreeuwenberg and Beynen (2003) stated that it is mainly due to a higher nutrient intake that
501 villus architecture can be maintained. So it would seem that feeding the diet in a liquid form is
502 positive because it reduces the transition gap from milk to the weaner diet (Russell et al. 1996;
503 Brooks et al. 2001).

504 Most studies conducted until now included a comparison between DF and LF (Deprez et
505 al. 1987; da Silva et al. 2001; Hurst, 2002). In most studies, an improvement is seen in the

506 morphology of the pigs fed with LF. Moran (2001) however included also FLF in his experiment
507 (Table 5). From these results an improvement in villus length/crypt depth ratio could not be seen
508 for the piglets fed LF or FLF, as the villus length was reduced compared to feeding DF. Moran
509 (2001) however found a correlation between the dry matter intake and mucosal integrity.

510 Scholten et al. (2002) found that pigs fed diets containing 45% fermented liquid grain
511 (wheat) had improved villus architecture compared to piglets fed a fresh liquid diet (without
512 fermented grain). In the first section of the small intestine, pigs had significantly greater villus
513 length and a greater villus/crypt ratio. Bruininx et al. (2010) found that the supernatants of diets
514 containing either prefermented cereals or their fermentation end-products (organic acids and
515 ethanol) clearly modulated cellular growth and metabolism of enterocyte-like Caco-2 cells,
516 although these effects were not observed in the *in vivo* characteristics (villus length, crypt depth
517 and brush-border enzyme activities) measured in a section of the jejunum of weanling pigs. In the
518 first two conducted experiments comparing piglets fed FLF with piglets fed DF Missotten (2010)
519 found comparable results to those of Moran (2001). In these experiments it was observed that the
520 piglets fed FLF took up proportional more water and less DM from the FLF because of a fast
521 sedimentation of the solids in the FLF. In a third experiment however, this sedimentation was
522 hindered by adding sepiolite (SPLF[®], Grupo Tolsa S.A., Spain) to the feed. In this third
523 experiment the piglets fed FLF had larger villi than the piglets fed the dry feed in which the same
524 LAB strain, used to inoculate the FLF, was present.

525

526 **7. Growth promotion**

527

528 According to the review of Jensen and Mikkelsen (1998) several investigations have shown that
529 feeding either LF or FLF improves feed intake and growth performance of pigs. In piglets

530 however this is not accompanied by a better F/G ratio, while for slaughter pigs the F/G was
531 improved on average by 6.9 % (Table 6; Jensen and Mikkelsen, 1998).

532 Plumed-Ferrer and Von Wright (2009) also gave an overview of *in vivo* trials performed
533 and their effect on the pigs. However, focus in that review was put on fermentation parameters
534 and not all performance data were given and some studies discussed are not that relevant for
535 comparing dry feeding (DF) to LF or FLF. For example, Vente-Spreuwenberg et al. (2003)
536 compared three liquid diets with different carbohydrate sources or the study of Kim et al. (2001)
537 was an experiment comparing a pelleted feed versus a milk formula for early weaned piglets. In
538 Table 7 and 8 all the relevant performance data found in the literature, for piglets and growing-
539 finishing pigs respectively are presented. Challenge experiments like Amezcua et al. (2007) and
540 Canibe et al. (2008) were excluded as they are less representative for normal commercial
541 situations.

542 In Table 9, additional studies that did not fit into the structure of Tables 7 and 8 are
543 presented. It should be noted however, that differences measured for the types of feeding may be
544 due to different levels of wastage rather than different efficiencies of feed utilisation. This
545 wastage does not allow to calculate the F/G ratio with the actual feed consumption (Brooks et al.
546 2003a). Russell et al. (1996) found that this wastage was partly responsible for the lesser
547 performance of the piglets on FLF. This lower F/G ratio was ameliorated by altering the through
548 design, but not completely eliminated. In the three experiments conducted by Missotten (2010)
549 the F/G ratio of the piglets was improved by reducing the sedimentation of the solids in the feed
550 slurry. In the third experiment, where sepiolite was used to reduce this sedimentation, the piglets
551 fed FLF showed a better F/G ratio than the control (dry) fed group (1.33 vs 1.52 respectively).

552 For slaughter pigs, from 20 kg to slaughter, Smith (1976) also performed a test
553 comparing LF to FLF, but no advantage was observed in offering the LF fermented compared to
554 offering it fresh. The study of Scholten et al. (2002) is included in Table 7, but it has to be

555 stressed that these piglets were restricted fed and that their performance was very poor. The
556 objective of that study was not to determine the growth performance, but the effect on the GIT
557 morphology. Most trials however used *ad libitum* feeding.

558 Since the review of Jensen and Mikkelsen (1998) more feeding trials with FLF have been
559 performed as can be seen in Tables 7 to 9, but the conclusion made by these authors is still valid.
560 These authors concluded that feeding LF or FLF results in an improved feed intake and growth
561 performance, but that the F/G ratio was on average not improved for piglets, while for growing-
562 finishing pigs on average a better F/G ratio is found compared to pigs fed DF. Recently however,
563 Missotten (2010) showed that the F/G ratio can be improved by feeding piglets with FLF in
564 which the sedimentation of the solids is controlled.

565

566 **8. Conclusion**

567

568 Although care has to be taken that undesirable fermentation does not occur during FLF
569 production, its utilisation in pig feeding seems to ameliorate growth performance, provided that
570 sedimentation of the solids in the liquid slurry is hindered. Preventing this mal-fermentation is
571 usually performed by adding LAB strains to the feed under controlled fermentation parameters.
572 Until now not one strain has been found that also remains the dominant strain in the FLF. So
573 further investigation is warranted in this field, because spontaneous fermentations are not reliable
574 and inoculation is needed to initiate a consistent fermentation process. The application of
575 fermenting only the grain fraction of the feed can also be applied. This prevents some problems
576 with fermenting complete diets (e.g. loss of essential nutrients), but other problems such as the
577 need for an even higher lactic acid production arise. Furthermore, the use of batch or
578 backslopping fermentation is still under debate. The choice of the type of fermentation will
579 largely depend on the discovery of a good inoculant for producing FLF.

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- 927

928 Table 1. Pig numbers and estimated market shares of liquid feeding (dairy and other products) in
 929 some European countries (EFSA, 2006)

Country	Pig numbers x 10 ⁶	The share of liquid feeding	
		All pig categories % fed	Sows % fed
Iceland	-	70	0
Denmark	13.4	60	30
Finland	1.4	60	20
Switzerland	-	50-60	30
The Netherlands	11.1	50	15
Ireland	1.8	40-50	20
Italy	8.9	40	5-10
Sweden	1.9	30	10
France	15.3	30	5-10
Austria	3.1	30	5-10
Germany	26.3	30	3-5
Norway	-	25-30	2
UK	4.8	20	10
Belgium	6.3	10	2
Greece	0.994	10	2
Spain	25.4	1	0
Portugal	2.348	0	0
EU-25	151.6	30	

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931

932 Table 2. Lactic acid bacteria strains used in studies, related to pigs, for producing fermented
 933 liquid feed either for *in vitro* or for *in vivo* experiments

Strain(s)	Experiment type	Study
<i>Lactobacillus plantarum</i>	<i>In vivo</i>	Van Winsen et al. (2001); Van Winsen et al. (2002)
<i>Lactobacillus plantarum</i>	<i>In vitro</i>	Van Winsen et al. (2000); Christensen et al. (2007); Moran (2001)
<i>Lactobacillus plantarum</i> PC-81-11-02	<i>In vivo</i>	Moran (2001); Demečková et al. (2002)
<i>Lactobacillus plantarum</i> REB1-Rif ^R	<i>In vivo</i>	Plumed-Ferrer et al. 2005
<i>Lactobacillus plantarum</i> VTT E-78076	<i>In vitro and In vivo</i>	Canibe et al. (2001); Canibe et al. (2008)
<i>Lactobacillus plantarum</i> LQ80	<i>In vitro</i>	Kobashi et al. (2008)
<i>Lactobacillus plantarum</i> 23E13, 98L11 and 2P22	<i>In vivo</i> (whey)	Amezcuca et al. (2007)
<i>Lactobacillus plantarum</i> + <i>Pediococcus</i> spp.	<i>In vitro</i>	Jensen and Mikkelsen (1998); Moran (2001; with <i>Pediococcus pentosaceus</i>) and Moran et al. (2006)
<i>Lactobacillus plantarum</i> CNCM I-840 + <i>Streptococcus infantarius</i> CNCM I-841 (Adjulact Pro [®])	<i>In vitro</i>	Missotten et al. (2007)
<i>Pediococcus acidilactici</i> CNCM MA 18/5M (Bactocell [®])	<i>In vitro</i>	Niven et al. (2006); Missotten et al. (2007)
<i>Pediococcus acidilactici</i>	<i>In vitro and In vivo</i>	Moran (2001); Geary et al. (1999)
<i>Pediococcus pentosaceus</i>	<i>In vitro</i>	Moran (2001); Beal et al. (2002)
<i>Lactobacillus bavaricus</i> + <i>Pediococcus acidilactici</i> + <i>Streptococcus bavaricus</i>	<i>In vitro</i>	Jensen and Mikkelsen (1998)
<i>Lactobacillus alimentarius</i>	<i>In vitro</i>	Jensen and Mikkelsen (1998)
<i>Lactobacillus lactis</i> subsp. <i>cremoris</i> 303	<i>In vivo</i>	Lawlor et al. (2002)
<i>Lactobacillus rhamnosus</i> LC 705 + <i>Propionibacterium freudenreichii</i> spp. <i>shermanii</i> JS (Bioprofit [®])	<i>In vitro</i>	Canibe et al. (2001)
<i>Lactobacillus brevis</i>	<i>In vitro</i>	Moran (2001)
<i>Lactobacillus amylophilus</i>	<i>In vitro</i>	Moran (2001)
<i>Lactobacillus curvatus</i>	<i>In vitro</i>	Moran (2001)

935 Table 3. The pH along the gastrointestinal tract of pigs fed either dry feed (DF), non fermented
 936 liquid feed (NFLF) and fermented liquid feed (FLF; feed to water ratio 1:2.5, backslopping with
 937 50% retention at 20°C) (n = 5; Canibe and Jensen, 2003)

Segment	Diet			P-value
	DF	LF	FLF	
Stomach	4.4 ^a	4.6 ^a	4.0 ^b	0.003
Proximal small intestine	5.9	5.8	5.7	0.48
Mid small intestine	6.0 ^a	5.8 ^b	6.1 ^a	0.008
Distal small intestine	6.4 ^a	5.7 ^b	6.1 ^{ab}	0.02
Caecum	5.7	5.5	5.7	0.17
Proximal colon	5.9	5.8	5.8	0.72
Mid colon	6.1	6.0	6.1	0.54
Distal colon	6.4 ^{ab}	6.2 ^a	6.5 ^b	0.04

938 ^{a,b}: means within rows with a different superscript differ significantly ($p < 0.05$)

939

940

941 Table 4. Microbial counts [\log_{10} CFU/g sample] along the gastrointestinal tract of pigs fed either
 942 dry feed (DF), non fermented liquid feed (NFLF) and fermented liquid feed (FLF; feed to water
 943 ratio 1:2.5, backslopping with 50% retention at 20°C) (n = 5; Canibe and Jensen, 2003)

Segment	Diet			P-value
	DF	NFLF	FLF	
LAB (20°C)				
Stomach	<5.4(3) ^a	7.9 ^b	9.0 ^c	<0.001
Distal small intestine	<6.3(5) ^a	<6.5(3) ^a	7.2 ^b	0.01
Caecum	<6.0(5)	<6.2(2)	<6.6(2)	0.21
Mid colon	<6.1(5)	<6.3(3)	<6.3(4)	0.34
LAB (37°C)				
Stomach	8.8	8.7	8.9	0.35
Distal small intestine	8.2	8.6	8.4	0.41
Caecum	8.7 ^{ab}	9.0 ^a	8.3 ^b	0.04
Mid colon	9.2 ^a	9.2 ^a	8.5 ^b	0.01
Enterobacteria				
Stomach	3.8 ^a	5.7 ^b	<3.2(4) ^c	<0.001
Distal small intestine	5.5 ^a	6.6 ^b	<4.1(3) ^c	<0.001
Caecum	5.9 ^a	6.3 ^a	5.0 ^b	0.02
Mid colon	6.2 ^a	6.6 ^a	4.7 ^b	0.004
Yeasts (20°C)				
Stomach	<3.4(2) ^a	3.7 ^a	5.4 ^b	0.001
Distal small intestine	<3.4(3) ^a	3.9 ^b	7.0 ^c	<0.001
Caecum	<3.2(2)	<3.3(1)	<5.1(1)	0.07
Mid colon	<3.2(3) ^a	<3.3(1) ^a	<4.6(1) ^b	0.03
Yeasts (37°C)				
Stomach	<3.3(4) ^a	<3.6(2) ^a	4.2 ^b	0.03
Distal small intestine	<4.0(3)	3.6	4.5	0.08
Caecum	<3.9(2)	<3.4(3)	<3.6(3)	0.59
Mid colon	<3.7(3)	<3.3(4)	<3.4(2)	0.69

944 Values in brackets indicate the number of samples with values below detection levels. The approximate detection
 945 levels (\log_{10} cfu/g) were as follows: stomach: LAB, 5; enterobacteria, 3; yeasts, 3. Small intestine, caecum
 946 and colon: LAB, 6; enterobacteria, 4; yeasts, 3. "<" indicates that some observation from which the mean
 947 was calculated had values below detection levels. When no colonies were detected, the detection limit was
 948 applied to make the calculations. Therefore some values are lower than actually reported

949 ^{a,b,c}: means within rows with a different superscript differ significantly ($p < 0.05$)

950 Table 5. Mean villus length (V) and crypt depth (C) en V/C ratio in different sections of the small intestine of piglets killed 14 days post-
 951 weaning. Piglets were either fed dry feed (DF), non fermented liquid feed (NFLF), fermented liquid feed (FLF) or suckled further. The liquid
 952 feeds were supplied every hour (1), every 4 hours (4) or every 12 hours (12) (adapted from Moran, 2001)

	Section of intestine sampled*	DF	Suckled	NFLF(1)	NFLF(4)	NFLF(12)	FLF(1)	FLF(4)	FLF(12)
V [μm]	0.25	655 ^a	646 ^a	593 ^{ab}	621 ^{ab}	486 ^b	538 ^{ab}	565 ^{ab}	430 ^b
	0.50	650 ^a	596 ^{abc}	605 ^{ab}	570 ^{abc}	452 ^{ab}	598 ^{abc}	560 ^{abc}	487 ^{bc}
	0.75	578 ^a	515 ^{ab}	557 ^a	576 ^a	458 ^{ab}	518 ^{ab}	531 ^{ab}	406 ^b
C [μm]	0.25	244 ^b	306 ^a	251 ^{ab}	261 ^{ab}	268 ^{ab}	234 ^b	268 ^{ab}	230 ^b
	0.50	260 ^{ab}	308 ^a	262 ^{ab}	283 ^{ab}	250 ^b	249 ^b	290 ^{ab}	233 ^b
	0.75	249	282	238	245	241	240	280	243
V/C	0.25	2.68	2.11	2.36	2.37	1.81	2.29	2.10	1.87
	0.50	2.50	1.94	2.31	2.01	1.81	2.40	1.93	2.10
	0.75	2.32	1.83	2.34	2.35	1.90	2.15	1.90	1.67

953 * The small intestine was extended to its full length by cutting the mesentery. Sections were removed proportionately at 0.25, 0.50 and 0.75 intervals along the intestine.
 954 ^{a,b,c}:within rows, means with a different superscript differ significantly ($p < 0.05$) (on V/C ratio no statistics were performed)
 955

956 Table 6. Difference (% change relative to dry feed) in daily body weight gain (BWG) and
 957 feed/gain ratio (F/G) in experiments conducted to compare liquid vs dry feeding (Jensen and
 958 Mikkelsen, 1998)

	No. of trials [*]	Daily BWG		F/G ratio	
		Av ± StDev	Min; Max	Av ± StDev	Min; Max
Piglets					
LF vs DF	10	12.3 ± 9.4	(-7.5; 34.2)	-4.1 ± 11.8	(-32.6; 10.1)
FLF vs DF	4	22.3 ± 13.2	(9.2; 43.8)	-10.9 ± 19.7	(-44.3; 5.8)
FLF vs LF	3	13.4 ± 7.1	(5.7; 22.9)	-1.4 ± 2.4	(-4.8; 0.6)
Slaughter pigs					
LF vs DF	9	4.4 ± 5.4	(-2.6; 15.0)	6.9 ± 3.5	(1.9; 12.7)

959 ^{*}: according to the studies indicated in Tables 7 and 8 with [§]
 960 DF: dry feed; LF: liquid feed; FLF: fermented liquid feed
 961

962 Table 7. Effect of dry feeding (DF), liquid feeding (LF) or fermented liquid feeding (FLF) on growth performance of weaned piglets

Study	No of pigs/treatment	Weight range (kg)	Feeding strategy			Daily body weight gain [g]			Feed/gain ratio [kg/kg]		
			DF	LF	FLF	DF	LF	FLF	DF	LF	FLF
Kornegay et al. (1981) [§]	156	9-26	ad lib [§]	ad lib		430	400		2.40	2.54	
Kornegay et al. (1981) [§]	126	7-22	ad lib	ad lib		380	380		1.85	1.93	
Kornegay et al. (1981) [§]	186	8-21	ad lib	ad lib		360	380		1.77	1.84	
Nielsen et al. (1983) [§]	92	9-16				153 ^a	179 ^a	220 ^b	2.07 ^a	1.86 ^b	1.95 ^b
Nielsen et al. (1983) [§]	190	8-20				305	315	333	1.69	1.68	1.69
Van de Pas et al. (1989)	1754	8-23	ad lib	ad lib		446	417		1.61	1.72	
Van de Pas et al. (1989)	2138	8-24	ad lib	ad lib		427	431		1.54	1.68	
Van de Pas et al. (1989)	594	8-24	ad lib	ad lib		451	439		1.65	1.78	
Danish Pig federation (1991) [§]	520	7-32				438 ^a	480 ^b				
Danish Pig federation (1991) [§]	320	7-40				461 ^a	527 ^b				
Hansen and Jørgensen (1992) [§]	170	7-10				146 ^a	196 ^b		1.75	1.69	
Hansen and Jørgensen (1992) [§]	360	6-9				142 ^a	171 ^b		1.53 ^a	2.03 ^b	
Partridge et al. (1992) [§]	20	6-12				281 ^a	312 ^b		1.12	1.12	
Russell et al. (1996) [§]	24	3-7 weeks	ad lib		s/ad lib [§]	343 ^a		428 ^b	1.31 ^a		1.89 ^b
Russell et al. (1996) [§]	48	3-7 weeks	ad lib		s/ad lib	397 ^a		454 ^b	1.37 ^a		1.44 ^b
Mikkelsen and Jensen (1997) [§]	8	8-10					260	290		1.16	1.16
Mikkelsen and Jensen (1998)	10	8-20					468	440		1.11	1.14
Geary et al. (1999)	24	7 kg and then fed for 28 days		ad lib	i/ad lib [§]		474	496		1.15	1.11
Pedersen (2001): trial 1	300	9-24.8	ad lib	acidif. lact. acid (pH 4)	s/ad lib	387 ^a	535 ^b	392 ^a	2.08 ^a	1.71 ^b	2.08 ^a
Lawlor et al. (2002) (+AMGP)	192	8.4-16.8	ad lib	ad lib		338	286		1.13	1.68	
		4-8 weeks		(res ad lib in week 1) [§]							
Lawlor et al. (2002) (+AMGP)	150	7.8-17.8	ad lib	ad lib (res ad lib in week 1)		391	352		1.17	1.56	
		4-8 weeks									
Lawlor et al. (2002) (+AMGP)	112	7.7-19	ad lib	1: fresh		408	1: 416		1.16	1: 1.32	
		4-8 weeks		2: acidif. lact. acid (pH 4) [§]			2: 433			2: 1.27	
				ad lib (res in week 1)							

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964 Table 7 (continued). Effect of dry feeding (DF), liquid feeding (LF) or fermented liquid feeding (FLF) on growth performance of weaned piglets

Study	No of pigs/treatment	Weight range (kg)	Feeding strategy			Daily body weight gain [g]			Feed/gain ratio [kg/kg]		
			DF	LF	FLF	DF	LF	FLF	DF	LF	FLF
Lawlor et al. (2002) (-AMGP)	112	8-17.8 4-8 weeks	ad lib	acidif. lact. acid (pH 4) ad lib (res ad lib in w1)	i/ad lib (res ad lib in w1)	361	389	347	1.13	1.34	1.36
Scholten et al. (2002)	20 first 4 d 10 last 4 d	8 kg fed for 8 days	res		res 45% ferm wheat [§]	10		43			
Choct et al. (2004b) roller mill	12	8.35 kg fed for 21 days	medium fine ad lib		medium fine ad lib	365		365	1.64 ^a		0.98 ^c
						414		389	1.37 ^b	0.92 ^c	
Choct et al. (2004b) hammer mill	12	9 kg fed for 21 days	medium fine ad lib		medium fine ad lib	541		471	1.16		0.82
						486		495	1.17	0.89	
Pedersen et al (2005)	16	9.1-26	ad lib	1: fresh 2: with WWDG* res		547 ^a	1: 453 ^b 2: 433 ^b		1.34 ^a	1: 1.24 ^b 2: 1.26 ^b	
Canibe et al. (2007a)	40 at start and 32 after day 14	8 kg fed for 42 days	ad lib		res 1: ferm grain 2: s/FLF	399 ^a		1: 312 ^b 2: 282 ^b	1.39		1: 1.55 2: 1.56
Missotten (2010)	6	7.4 fed for 8 days	ad lib		ad lib	133		46	1.62		3.66
Missotten (2010)	15	7.9-13.8	ad lib		ad lib	254		183	1.57		1.85
Missotten (2010)	15	6.3-11.9	1: ad lib 2: ad lib + probiotic		ad lib	1: 184 ^a 2: 185 ^a		253 ^b	1: 1.52 ^a 2: 1.48 ^a		1.33 ^b

965 ^{a,b,c}: means within the same study with different superscripts are significantly different at $p < 0.05$

966 * WWDG: wet wheat distillers grain

967 [§] ad lib: *ad libitum* fed; res: restrictedly fed; s/:spontaneous fermentation; i/: inoculated fermentation; acidif: acidified; lact. acid: lactic acid; ferm: fermented

968 [§]: study used in review of Jensen and Mikkelsen (1998)

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972 Table 8. Effect of dry feeding (DF), liquid feeding (LF) or fermented liquid feeding (FLF) on growth performance of growing-finishing pigs

Study	No of pigs/treatment	Weight range (kg)	Feeding strategy			Daily weight gain [g]			Feed/gain ratio [kg/kg]		
			DF	LF	FLF	DF	LF	FLF	DF	LF	FLF
Forbes and Walker (1968) [§]	62	38-slaught	Res [§]	res		700	705		3.14	2.91	
Forbes and Walker (1968) [§]	36	35-slaught	res	res		764	777		3.34	3.07	
Forbes and Walker (1968) [§]	36	33-slaught	res	res		659	673		3.46	3.05	
Kneale (1972) [§]	30	25-slaught	res	ad lib [§]		665 ^a	765 ^b		3.38 ^a	2.95 ^b	
Smith (1976) [§]	6	25-slaught	res	res		629	635		3.14	3.08	
Smith (1976) [§]	64	20-slaught	res	res		552	574		3.50	3.32	
Nielsen and Madsen (1978) [§]	64	21-slaught	ad lib	ad lib		604	604		3.01	2.86	
Patterson (1989) [§]	48	34-slaught	res	res		758	738		2.92	2.85	
Smed (1994) [§]	1500	31-slaught	ad lib	ad lib		655 ^a	735 ^b		2.96	2.74	
Scholten et al. (1997)		25-112		ad lib	Ad lib with co-products		749	768		2.69	2.58
da Silva et al. (2002)	16	6.7-90	ad lib	ad lib		755	720		2.73	2.77	
Hurst (2002)	G: 65 [§] B: 68 [§]	42-86 41-87	ad lib	ad lib		G: 794 ^a B: 831 ^a	G: 895 ^b B: 973 ^b				
Hurst et al. (2008)	33	47-84.1	ad lib	ad lib		892	963		2.58	2.53	
Hurst (2002)	18 to 10 at slaughter	9.55-85.1	ad lib	ad lib		641 ^a	796 ^b		1.72	1.87	
Pedersen et al. (2002c)	1403	25-120		ad lib	ad lib ferm grain [§]		924	957		2.61 ^a	2.46 ^b
Meat and Livestock Commission (in Brooks et al. 2003a)		34-103	ad lib	ad lib		754 ^a	796 ^b		2.53 ^a	2.20 ^b	
Canibe and Jensen (2003)	20	31-101	res	res	res	961 ^{ab}	995 ^a	931 ^b	Similar: between 2.11 - 2.15		
Xuang Dung et al. (2005)	5	27.4-80	res	res	res	552 ^a	1: 541 ^a 2: 601 ^b	598 ^b	3.79 ^a	1: 3.76 ^a 2: 3.41 ^{ab}	3.09 ^b

973 ^{a,b}: means within the same study with different superscripts are significantly different at $p < 0.05$

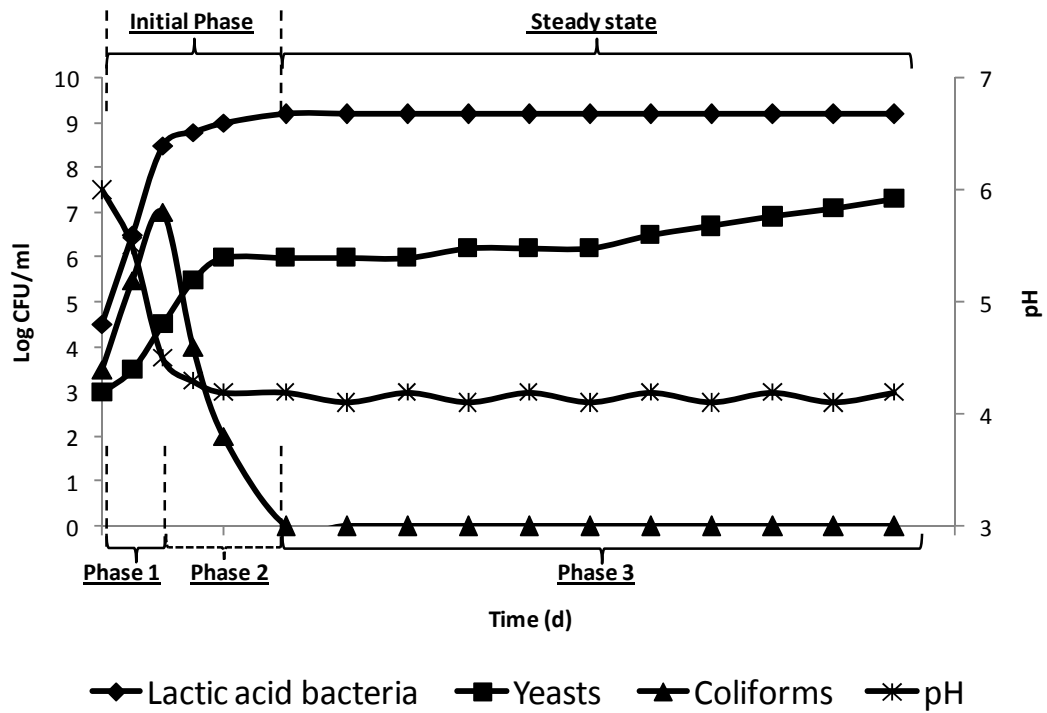
974 [§] ad lib: *ad libitum* fed; res: restrictedly fed; acidif: acidified; lact. acid: lactic acid; ferm: fermented; G: gilts; B: boars

975 [§]: study used in review of Jensen and Mikkelsen (1998)

976 Table 9. Effect of dry feeding (DF), liquid feeding (LF) or fermented liquid feeding
 977 (FLF) on growth performance of piglets or growing-finsihing pigs reported in studies
 978 not included in Table 7 and 8

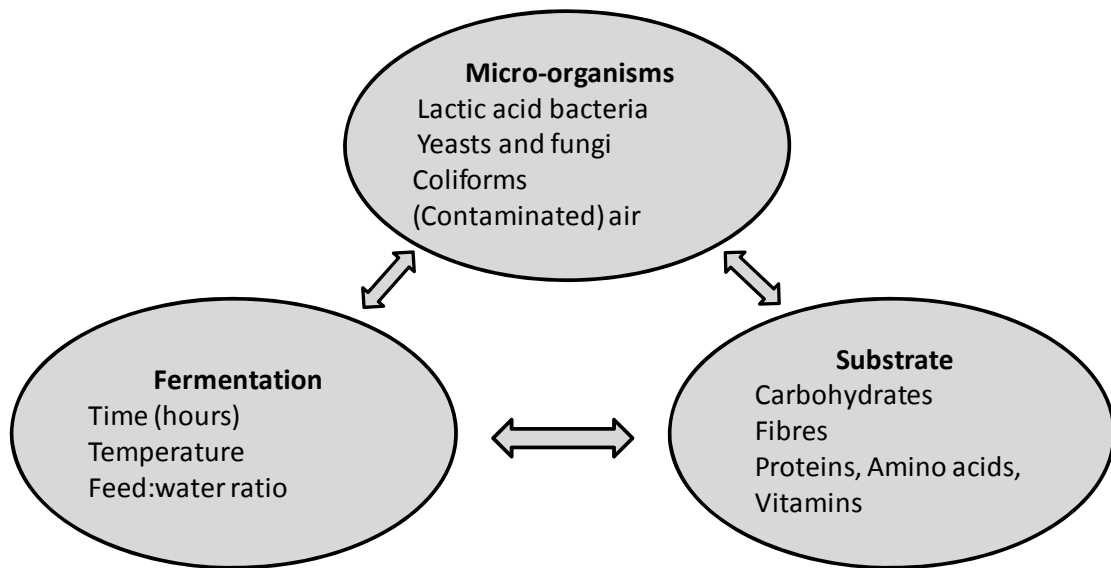
Study	Experimental groups	No of pigs/treatment	performance		
			Daily weight gain [g]	Feed/gain ratio [kg/kg]	
Braude (1971)	Dry pellets	-	617 ^a	3.31 ^a	
	Wet pellets	-	618 ^a	3.29 ^a	
	Wet meal	-	633 ^b	3.21 ^b	
Kim (1999)*	DF	-	774 ^a	2.28	
	LF	-	745 ^b	2.27	
Kim (1999)**	DF	-	763 ^a	2.24	
	LF	-	747 ^b	2.26	
Chae (2000) Growing-finishing pigs	DF	-	797 ^a	3.38 ^a	
	LF	-	862 ^b	3.32 ^a	
55-110 kg BW ^s	LF between 55-90 and DF between 90-110	-	852 ^b	3.16 ^b	
Pedersen (2001): trial 2	DF	291	452	2.11	
9.8-26.7 kg BW	Partly FLF	288	404	1.80	
	Fully FLF	291	363	1.96	
Hurst et al. (2008) 47.2-86 kg BW	Dry pellets	16	962 ^a	2.09 ^a	
	Soaked pellets 1:1.5	16	1041 ^{ab}	1.94 ^b	
	restrictedly fed	Soaked pellets 1:3	16	1051 ^b	1.87 ^{bc}
		Soaked pellets 1:3 (acidified with lactic acid pH 4)	16	1091 ^b	1.79 ^c
Han et al. (2006) Initial BW 5.7 kg Fed <i>ad libitum</i>	DF for 40 days	24	459 ^a	1.55 ^a	
	LF for 10 days +	24	482 ^b	1.56 ^{ab}	
	DF for 30 days				
	LF for 20 days +	24	475 ^{ab}	1.60 ^b	
	DF for 20 days				

979 Notes: *From weaning at 14 d of age until about day 157 (first 14 d after weaning fed in conventional
 980 nursery).
 981 **From weaning at 14 d of age until about day 157 (first 14 d after weaning fed in special
 982 nursery).
 983 ^s BW: Body weight
 984 ^{a,b}: means within the same column and study with different superscripts are significantly different at
 985 $p < 0.05$
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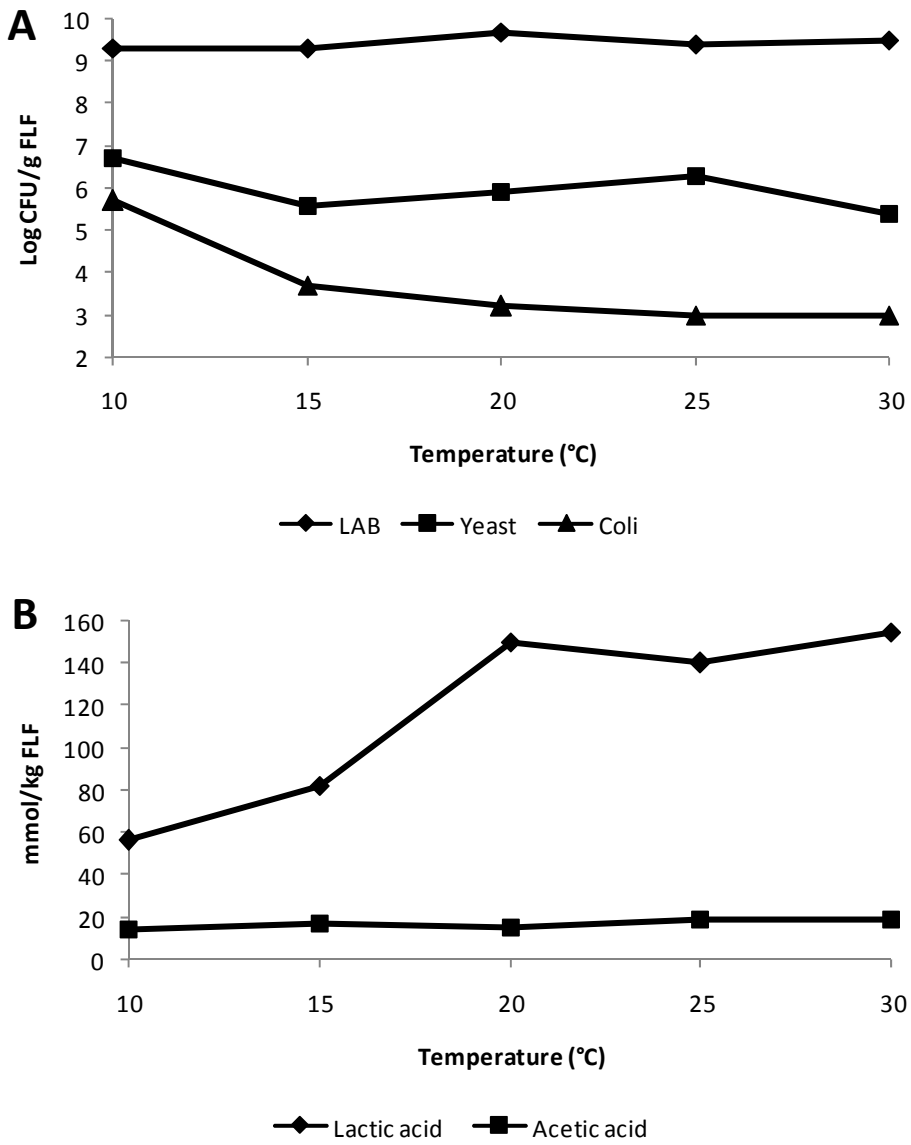
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Figure 1. Schematic representation of the microbial population and pH during the different phases in the fermentation of FLF according to Brooks (2008; phases 1, 2 and 3) and according to Canibe and Jensen (2003; initial and steady state)



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Figure 2. Interactions in the fermented liquid feed between the present micro-organisms, fermentation parameters and substrate quantity and quality affects the final end product, adapted from Niba et al. (2009)



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Figure 3. The effect of fermentation temperature on various bacterial populations [log₁₀ CFU/g FLF] (A) and concentration of organic acids [mmol/kg FLF] (B) in FLF in steady state (n = 3). Redrafted after Jensen and Mikkelsen (1998)

