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Published in:
International Journal of Food Microbiology

DOI:
[10.1016/j.ijfoodmicro.2006.04.022](https://doi.org/10.1016/j.ijfoodmicro.2006.04.022)

Publication date:
2006

Document version
Early version, also known as pre-print

Citation for published version (APA):
Lei, V., Friis, H., & Michaelsen, K. F. (2006). Spontaneously fermented millet product as a natural probiotic treatment for diarrhoea in young children: an intervention study in Northern Ghana. *International Journal of Food Microbiology*, 110(3), 246-253. <https://doi.org/10.1016/j.ijfoodmicro.2006.04.022>

1 **Spontaneously fermented millet product as a natural probiotic**
2 **treatment for diarrhoea in young children: An intervention study**
3 **in Northern Ghana**

4

5 **Vicki Lei^a, Henrik Friis^b and Kim Fleischer Michaelsen^c**

6 ^aThe Royal Veterinary and Agricultural University, Dept. of Food Science, Food
7 Microbiology, Rolighedsvej 30, 1958 Frederiksberg C, Denmark, ^bUniversity of Copenhagen,
8 Dept. of Epidemiology, Institute of Public Health, Blegdamsvej 3, 2200 Copenhagen N,
9 Denmark and ^cThe Royal Veterinary and Agricultural University, Dept. of Human Nutrition,
10 Rolighedsvej 30, 1958 Frederiksberg C, Denmark

11

12 Corresponding author:

13 Vicki Lei

14 The Royal Veterinary and Agricultural University

15 Dept. of Food Science

16 Food Microbiology

17 Rolighedsvej 30

18 1958 Frederiksberg C

19 Denmark

20

21 Phone: +45 35 28 33 48

22 Fax: +45 35 28 32 14

23 E-mail: vil@kvl.dk

24

25 **Abstract**

26 Indigenous lactic acid fermented foods may have potential as probiotic
27 treatment for diarrhoea, due to high levels of lactic acid bacteria. In this study the effect of a
28 millet drink, spontaneously fermented by lactic acid bacteria, as a therapeutic agent among
29 Ghanaian children with diarrhoea, was assessed. Children below 5 years of age coming to
30 Northern Ghana health clinics for treatment of diarrhoea were randomised to two groups.
31 Children of both groups received treatment for diarrhoea given at the local clinic, whereas the
32 intervention group in addition received up to 300 ml fermented millet drink (KSW) daily for 5
33 days after enrolment. The clinical outcome of diarrhoea and reported well-being were
34 registered every day for the 5-day intervention and again 14 days after diagnosis. Among 184
35 children (mean age 17.4, standard deviation 11.3 months) included, no effects of the
36 intervention were found with respect to stool frequency, stool consistency and duration of
37 diarrhoea. However, KSW was associated with greater reported well-being 14 days after the
38 start of the intervention ($p=0.02$). The fact that no effect of KSW on diarrhoea was observed
39 could be, because many children had a mild form of diarrhoea, and many were treated with
40 antibiotics. Either this could have affected the lactic acid bacteria, or the lactic acid bacteria in
41 KSW had no probiotic effects. It is speculated that the effect after two weeks could be due to
42 a preventing effect of KSW on antibiotic-associated diarrhoea which could help reducing
43 persistent diarrhoea.

44

45 *Keywords:* Probiotics, African fermented millet, acute diarrhoea, intervention study

46

47 **1. Introduction**

48 Diarrhoea is a major cause of morbidity and mortality among young children in
49 the developing world. It is estimated that, worldwide, around 1.5 million children die each
50 year due to diarrhoea, the vast majority of these in the developing countries (Victora et al.,
51 2000). In Africa, children below 5 years of age have a median of five episodes of diarrhoea
52 per year with a minimum of 1.6 and a maximum of 9.9 episodes (Kirkwood, 1991). Hence,
53 interventions that prevent, shorten or alleviate the diarrhoea period would be very beneficial.

54 Studies, both from industrialised (e.g. Isolauri et al., 1991; Guarino et al., 1997;
55 Shornikova et al., 1997a,b,c; Guandalini et al., 2000; Simakachorn et al., 2000; Rosenfeldt et
56 al., 2002a,b) and developing countries (Pant et al., 1996) have shown that oral treatment with
57 defined probiotic cultures significantly shortens the duration of diarrhoea in children, as also
58 reviewed in the meta-analyses of Szajewska and Mrukowicz (2001), Cremonini et al. (2002),
59 D'Souza et al. (2002), Huang et al. (2002), Van Niel et al. (2002) and Hawrelak et al. (2005).
60 Indigenous fermented foods have, however, not been sufficiently tested as a probiotic
61 treatment of diarrhoea. To our knowledge, only two studies (Darling et al., 1995; Yartey et
62 al., 1995) have tested the effect of traditionally fermented food on diarrhoea. Darling et al.
63 (1995) tested three maize porridges, i.e. conventional, amylase-digested and fermented, and
64 amylase-digested. Yartey et al. (1995) used fermented maize-based rehydration solution and
65 compared the effectiveness to unfermented maize-based rehydration solution and ordinary
66 oral rehydration solution (ORS). None of the studies found significant variation between the
67 different treatments with respect to stool frequency and duration of diarrhoea. However, it
68 was found by Lorri and Svanberg (1994) that a group of young children living in one village
69 in Tanzania who consumed lactic-fermented cereal gruels regularly, had a 40% lower
70 frequency of diarrhoea over a nine-month period than similar children living in a nearby
71 village not using fermented gruels.

72 *In vitro* studies of indigenous fermented cereals indicate that the fermentation
73 process induces anti-diarrhoeal functions (Mensah et al, 1988; Nout et al., 1989; Mensah et
74 al., 1990; Mensah et al., 1991; Odugbemi et al., 1991; Kingamkono et al., 1998; Kimmons et
75 al., 1999; Tetteh et al., 2004). In addition, indigenous fermented foods have been proven
76 effective in preventing diarrhoea and in obtaining improved nutritional health (Armar-
77 Klemesu et al., 1991; Svanberg, 1992; Kingamkono et al., 1999; Oberhelman et al., 1999;
78 Saran et al. 2002). It is important to notice that, for the developing countries to benefit, there
79 is a need for effective, acceptable, cheap and easily accessible products. Expensive products
80 for the treatment are unsustainable, which means that using preparations of defined probiotic
81 starter cultures may not be a possibility.

82 Fermentation of foods is traditionally carried out daily in Africa and other
83 developing countries, and a vast number of different spontaneously fermented food and drink
84 products are used. The immediate benefits of these fermented foods are the keeping qualities
85 and the increased nutritional value (e.g. Ezeji and Ojimekwe, 1993; Antony et al., 1998a,b;
86 Mugula and Lyimo, 1999, Ouoba et al., 2003). These products have a high content of bacteria
87 with a large bio-diversity (Hayford et al., 1999; ben Omar et al., 2000; Escalante et al., 2001;
88 Paludan-Müller et al., 2002; Muyanja et al., 2003), and there is a possibility that some of
89 these could be probiotic. *Koko* is a millet gruel produced in Northern Ghana and
90 spontaneously fermented by lactic acid bacteria, mainly *Weissella confusa* and *Lactobacillus*
91 *fermentum* (Lei and Jakobsen, 2004). The final product of *koko* is boiled, but a fermented and
92 un-boiled part of the product has by tradition been used for adults and children with upset
93 stomachs and as a refreshing drink during fastening periods instead of water (Lei and
94 Jakobsen, 2004). This specific part of the product, called *koko sour water* (KSW), contains
95 live lactic acid bacteria in levels of 10^8 colony forming units/ml and has a pH (standard
96 deviation; SD) of 3.6 (0.2). The lactic acid bacteria isolates from KSW showed low levels of

97 antimicrobial activity towards *Listeria innocua*, but no activity towards the bacteriocin-
98 sensitive *Lactobacillus sakei*. In addition, growth of all lactic acid bacteria isolates was
99 unaffected by the presence of 0.3% (v/v) oxgall bile and the isolates were able to survive, but
100 were not able to grow in growth medium adjusted to pH 2.5 (Lei and Jakobsen, 2004). It is
101 possible that this product could have a potential as a probiotic product, and this paper reports
102 on the ability of KSW to alleviate diarrhoea in children below the age of five in Northern
103 Ghana.

104

105 **2. Methods and materials**

106

107 *Study areas*

108 The study was conducted in two districts, A and B, in Northern Ghana. District
109 A comprised three health clinics in three villages, Nyankpala, Pong-Tamale and Savelugu,
110 situated 20 to 30 km from Tamale, the capital city of the Northern Region (Fig. 1). District B
111 comprised another three health clinics in three villages situated in Northwest Ghana called
112 Tuna, Sawla and Kalba (Fig. 1). District A and B are located approx. 250 km apart. District
113 A, located close to the regional capital, had a good infrastructure, whereas the infrastructure in
114 District B was considered bad. District B was considered poorer than District A, and with
115 people having to travel further to reach a health clinic than people in District A. The climate
116 of the two districts during the study was hot, with 28-33°C during daytime and humid, with
117 heavy rain showers daily. Clinical records obtained from the health clinics from 1998 to 2001
118 showed a peak of diarrhoea incidences in June and July during the rainy season. *Koko*
119 produced from millet was well known in the two districts, however, to use KSW as a drink
120 was not common practice.

121

122 *Study design and objectives*

123 The study was a controlled randomised intervention study conducted during the
124 late rainy season in Northern Ghana in August and September 2001 among weaned or partly
125 weaned children below the age of five years with diarrhoea. Diarrhoea was defined as three or
126 more watery stools in 24 hrs (WHO, 1990). Only children, whose parents or guardians had
127 consented to participate and were living within a distance which made a daily follow-up visit
128 by the field assistants possible, were included in the study. The objectives were to investigate
129 whether KSW given to children with diarrhoea was capable of shortening the duration of
130 diarrhoea and reduce the prevalence of complications. Randomisation was carried out after
131 prescription of medical treatment. The numbers were randomised by blocks of four (2 KSW
132 patients for each 2 control patients). The parents or guardian were asked to pick a number
133 from an envelope with the 4 numbers. The children randomised to KSW, were given a daily
134 supply of 300 ml KSW. There was no interference with the medical treatment, in that all
135 children were treated for diarrhoea as prescribed by the individual health clinics. Thus, all
136 children were treated for their illness, as they normally would have been. Overall, the medical
137 treatment for acute diarrhoea included malaria treatment and often antibiotics like
138 metronidazol, co-trimoxazole, amoxicillin and chloramphenicol, oral sodium-glucose
139 rehydration (ORS), advice on feeding and admission to hospital in severe cases. Children
140 retained at the health clinic for observation were categorised as ‘in-patients’. These children
141 were treated until they were considered well enough to return home. All other patients were
142 categorised as ‘out-patients’, as they were sent home after consultation at the health clinics.

143

144 *Koko and koko sour water production*

145 *Koko and koko sour water* production is described in detail by Lei and Jakobsen
146 (2004). In brief, pearl millet (*Pennisetum glaucum*) is steeped overnight and wet-milled

147 together with spices such as ginger, chilli pepper, black pepper, and cloves. Addition of water
148 to the flour makes a thick slurry, which is then sieved and left to ferment and sediment for 2-3
149 h. The fermented top-layer (called *koko sour water*; KSW) is then decanted to a pot and
150 boiled for 1-2 h. After boiling, the thicker sediment from the fermentation is added until the
151 desired consistency is achieved. For the present intervention study, a special production of the
152 KSW was carried out, in that KSW was produced purely from millet and contained no spices.
153 Each of the six health clinics had a local *koko* production site attached for daily supply of
154 KSW. The pH of KSW was measured daily from all production sites using colour-fixed
155 indicator sticks (Baker-pHIX pH 3.6-6.1, J.T. Baker, Phillipsburg, USA) whilst daily
156 microbiological investigations of the product from the production sites in District A were
157 carried out locally at the laboratory established in Tamale (Lei and Jakobsen, 2004). The
158 criteria for satisfactory and complete fermentation of KSW were pH 3.6 according to Lei and
159 Jakobsen (2004). If the pH on any day during the intervention study was higher than 3.6, as
160 registered from the indicator sticks, and hence creating an environment possible for survival
161 of pathogenic microorganisms, the KSW was not used. In the previous study by Lei and
162 Jakobsen (2004), KSW was found on average to contain 10^8 colony-forming units per ml of
163 lactic acid bacteria, which was dominated by *Weissella confusa* and *Lactobacillus fermentum*.
164 The keeping qualities of the KSW had been studied prior to the intervention study, showing a
165 minimum of 72 hour long keeping quality of the product at ambient temperature based upon
166 freshness and sensory quality (unpublished results).

167

168 *Recruitment and training of field assistants*

169 Field assistants were nursing staff attached to the health clinics, recruited by the
170 head of the clinics and trained for the study in a two-day workshop prior to the study.
171 Training included lectures on children's diarrhoea, group discussions, small working group

172 sessions and testing of the questionnaires described below. The field assistants were all able
173 to speak the local language as well as English.

174

175 *Observations schedule*

176 On admission to the health clinic, the children were examined clinically,
177 diagnosed and treated medically as indicated above. If a child fulfilled the inclusion criteria,
178 the parents or guardian were asked to participate in the study. After obtained consent, field
179 assistants interviewed the parents or guardian of the enrolled children about the age of the
180 child, the address of the parents or guardian in addition to questions about the family
181 structure. The medical treatment given by the health clinic and/or by the parents or guardian
182 was registered. The degree of dehydration and sickness was estimated by the categories none,
183 some and severe. The length and the nude weight of the child were measured. The length was
184 measured by locally produced infantometers to the nearest cm, and the weighing was carried
185 out by using the MP25 Infant Weighing Pack (CMS Weighing Equipment Ltd., London, UK).
186 Each field assistant was equipped with a weighing scale and each health clinic was equipped
187 with an infantometer. The participating children were daily visited by field assistants in their
188 homes for the first 5 days after diagnosis and again on the 14th day after diagnosis. During the
189 home visits the field assistants monitored the treatment initiated by the clinics and followed
190 the condition of the children by filling in a standardised daily questionnaire. This
191 questionnaire contained the same questions to be answered for the first 5 days, detailing the
192 health of the child during the past 24 hrs. The questions asked were on number of stools,
193 consistency of the stool, blood in the stool, vomiting, the overall health, and the food and
194 drink intake. In addition, the child was weighed daily. For the consistency of the stool, each
195 field assistant was carrying a picture of line drawings depicting stool consistency with given
196 numeric values (Young et al. 1998) for the mothers to identify the daily stool consistency of

197 the child. The children randomised to the KSW group were given the first KSW at enrolment
198 at the health clinic. Field assistants subsequently brought fresh KSW daily to the children's
199 home during the next 4 days after diagnosis. The 300 ml KSW was administered in 500 ml
200 clear plastic containers with volumetric graduations for every 50 ml. The children were
201 offered to drink as much as possible of the 300 ml over the 24 h before the next visit of the
202 field assistant. The intake of the KSW water was recorded at the subsequent visit. On the 14th
203 day after enrolment, the children of both groups were visited for a follow-up with a special
204 questionnaire. This follow-up questionnaire contained questions on the general well-being of
205 the child, i.e. behaving normally and following the normal eating and drinking pattern. It was
206 registered whether the children had had diarrhoea or had been otherwise sick since the last
207 visit. In addition, the weight of the children was recorded. It was emphasised that the same
208 field assistant observed the same child during the study period.

209

210 *Socio-economic characteristics of the participating households*

211 The population, from which the participants were recruited, was relatively poor.
212 The larger majority of the population in the northern region of Ghana are Muslim. Most of the
213 families are farmers with little or no education, producing food crops for own consumption as
214 well as for trade at the local markets. The dietary intake of the families varies from season to
215 season, with food supplies most scarce during the rainy season from June to September. Daily
216 food in this period mainly consists of cereal products like maize, sorghum and millet.
217 Occasionally, the daily meals include fish or chicken, and rarely beef. Furthermore, the
218 Northern Region has a large production of groundnuts.

219

220 *Statistical analyses*

221 The anthropometric module of Epi Info™ for DOS was used to calculate the Z
222 scores of respectively weight for age (WAZ), weight for height (WHZ) and height for age
223 (HAZ). SPSS version 10.0 for Windows was used to perform the data analysis. The
224 distributions of data were assessed using normal probability plots. For normally distributed
225 data, mean and standard deviation (SD) or 95% confidence interval (95% CI) were given and
226 t-tests used to test for differences between groups. For data that were not normally distributed,
227 median and interquartile range were given and the Mann-Whitney test used to compare
228 groups. The Chi-square test was used to test for differences in proportions between groups.
229 Multiple linear and logistic regression analyses were used to assess and control for
230 confounding. The level of significance used was 0.05.

231

232 *Ethical considerations*

233 The study protocol was approved by the Central Scientific-Ethical Committee of
234 Denmark (624-01-0020) and the Ministry of Health, Northern Region, Ghana, with whom the
235 study was carried out in collaboration with. Informed consent from the parents or guardian of
236 the enrolled children was obtained.

237

238 **3. Results**

239 Among the 190 children with diarrhoea recruited for the study, the median
240 (interquartile range; IQR) age was 13 months (9; 24), the mean weight (SD) was 8.5 kg (1.9)
241 and the mean height (SD) 76.6 cm (8.6). The Z-score of weight-for-age (mean (SD)) was -1.8
242 (1.1), the height-for-age Z was -0.7 (1.5) and the weight-for-height Z was -1.7 (1.0). The
243 children had had diarrhoea for a median (IQR) of 48 h (24; 96) prior to enrolment. Based on
244 clinical assessment, 46 (24.2%) of the 190 children were judged dehydrated at enrolment, and

245 on examination, 172 (90.5%) were found to have malaria parasitaemia, according to the
246 medical examination at the clinic.

247 Of the enrolled children, 179 (94.2%) were the youngest in the family. The
248 median (IQR) number of siblings to the enrolled child was 2 (1; 4). Of the 138 (72.6%)
249 children with reported previous diarrhoea, the mean (SD) number of episodes the previous 12
250 months was 2.7 (1.8). In total, 136 (71.6%) of the children were treated with antibiotics at the
251 day of enrolment, of which metronidazol was given to 72 (52.9%), co-trimoxazole to 57
252 (41.9%), amoxicillin to 20 (14.7%) and chloramphenicol to 6 (4.4%). Thus, 19 (14%) were
253 treated with two antibiotics. Furthermore, 174 (91.6%) were treated for malaria using
254 chloroquine.

255 The median (IQR) age for the females was 12 months (9; 20) and 15 months (9;
256 24) for the males (P=0.5). The average (95% CI) weight-for-age Z for the females was -1.7 (-
257 2.0; -1.5), and -1.9 (-2.1; -1.7) for the males (P=0.65).

258

259 *Baseline comparison*

260 As seen in Table 1, the simple randomization resulted in baseline equivalence
261 between the two groups for most background variables. However, the median age was slightly
262 higher and the mean weight-for-age Z score lower in the control group compared to the KSW
263 group. This was also the case for the mean weight-for-height Z score and height-for-age Z
264 score. In addition, the treatment frequency for malaria was higher in the KSW group.

265

266 *Intervention*

267 Of the 190 children recruited, 184 children (96.8%) completed the study. Of the
268 six children lost to follow-up, three were from the KSW group and three from the control
269 group. Three children in the control group died during or after the study, while there was no

270 death in the KSW group (P=0.21). One child died between the 5th and 14th day of the study,
271 the other two within two weeks after end of study.

272 The children in the KSW group consumed on average (SD) 711 ml (307) over
273 the 5 intervention days. Table 2 shows the average (SD) intake for KSW and ORS per day for
274 the children in the KSW group and the average intake of ORS in the control group. Very little
275 KSW was consumed the first 24 hours after enrolment. Of ORS the KSW group on average
276 (95% CI) drank 193 ml (70; 318) less than the control group (P=0.02).

277 For the intervention there were no significant differences between children
278 randomly allocated to the KSW group or the control group with respect to stool frequency,
279 stool consistency, duration of diarrhoea and whether the children were defined as cured
280 during the five days after commencement of treatment (Table 3).

281 Similarly, there were no differences in any of the outcome between the KSW
282 and control group in subgroups of the children based on sex, district or antibiotic treatment
283 (results not shown).

284

285 *Day 14 follow-up*

286 Fourteen days after the start of the treatment 88 (91%) of the children in the
287 KSW group was regarded as being well (i.e. behaving normally and having normal eating and
288 drink pattern) by their parents or guardian, compared to 73 (78%) in the control group
289 (P=0.02). The proportion of children with diarrhoea between day 5 and the day 14 follow-up
290 was 13 (13.5%) in the KSW group and 19 (20%) in the control group, but this difference was
291 not significant (P=0.31). In addition, the number of children that had been somehow sick
292 between day 5 and the day 14 follow-up was 19 (20%) in the KSW group and 27 (29%) in the
293 control group. This difference was also not significant (P=0.25).

294

295 *Overall*

296 Controlling for baseline differences in age, WAZ, and malaria treatment, and the
297 differences in the ORS intake during treatment, using multivariable linear or logistic
298 regression analysis, did not change the estimate effect of KSW on weight, WAZ or cure
299 (results not shown).

300

301 **4. Discussion**

302 Overall, no effects were found of KSW on stool frequency, stool consistency
303 and duration of diarrhoea during the five days after commencement of KSW treatment.
304 However, a moderate but yet significant difference showed that the parents of the KSW group
305 perceived the children to be better than the children not having received KSW.

306 The present study did not interfere with the medical treatment given to the
307 patients. Nearly three quarters of the patients were treated with antibiotics, of which many
308 were broad-spectrum antibiotics. The level of susceptibility of defined lactic acid bacteria
309 cultures to antibiotics seems to be species-dependent (Danielsen and Wind, 2003), however
310 great variations can be found from strain to strain all dependent on the environment from
311 which the lactic acid bacteria have been isolated (Charteris et al., 1998; Charteris et al., 2001;
312 Temmerman et al., 2002). Tests of susceptibility to antibiotics have to our knowledge not
313 been carried out on indigenous lactic acid bacteria isolates from spontaneous fermentations. It
314 is therefore not known whether the lack of effect of the KSW was due to susceptibility of the
315 lactic acid bacteria in the product to the antibiotics given to the children.

316 Probiotics are known to have a better effect the earlier from onset of diarrhoea
317 the treatment is commenced (Rautanen et al., 1998; Rosenfeldt et al., 2002a,b). That any cure
318 of diarrhoea by KSW was not shown, might be due to the fact that the children from both
319 groups came to the clinic two days after onset of diarrhoea and most were mildly sick and

320 quickly cured. There is a possibility that the predominant lactic acid bacteria of KSW did not
321 possess probiotic properties effective against diarrhoea. *Weissella confusa* and *Lactobacillus*
322 *fermentum* was found to dominate KSW (Lei and Jakobsen, 2004). Representative isolates
323 showed no pronounced antimicrobial effects towards *Lactobacillus sakei* and *Listeria*
324 *innocua*, they were, however, moderately acid and bile tolerant (Lei and Jakobsen, 2004). The
325 importance of using documented probiotic cultures compared to traditional starter cultures is
326 shown in studies by Pedone et al. (2000) and Olukoya et al. (1994). The use of specific
327 probiotic cultures, documented to affect diarrhoea as starter culture for KSW may cure or
328 alleviate diarrhoea in children.

329 A tendency of a positive long term effect of KSW was shown in the present
330 study. In addition, studies showing a preventive effect of fermented cereals (Lorri &
331 Svanberg, 1994) and of probiotics on diarrhoea in developing countries have been carried out
332 (Armar-Klemesu et al., 1991; Kingamkono et al., 1999; Oberhelman et al., 1999; Chandra,
333 2002). Furthermore, an overall improvement in health and thriving in poor and
334 undernourished children receiving probiotics was shown by Saran et al. (2002).

335 Studies indicate that defined probiotic cultures are capable of reducing
336 antibiotic-associated diarrhoea (Vanderhoof et al., 1999; Bergogne-Bérézin, 2000; Cremonini
337 et al., 2002; D'Souza et al., 2002; Hawrelak et., 2005). Antibiotic-associated diarrhoea
338 commence after approx. 5-7 days after onset of antibiotic treatment (Turck et al., 2003; Yapar
339 et al., 2005). It is possible that the positive effect seen at the 14-day follow-up could be
340 caused by the lactic acid bacteria from the KSW preventing and/or treating any antibiotic-
341 associated diarrhoea as well as improving health and well-being of the children. However,
342 since the study was not blinded it is possible that the long term effect of KSW seen could be
343 due to bias from the group receiving the KSW.

344 Any effect in reducing diarrhoea by the use of spontaneously fermented cereal
345 foods is yet to be proven. Darling et al. (1995) found no significant effect of a fermented
346 maize-sorghum porridge compared to a non-fermented on duration of diarrhoea, frequency of
347 stools or vomiting. Yartey et al. (1995) investigated fermented and un-fermented maize gruel
348 as a substitute for conventional ORS and found no significant effect of stool output, stool
349 frequency and duration of diarrhoea between the three combinations. Neither of these two
350 studies reported on antibiotic treatment *per se*. There are, however, some indications that
351 fermented foods could possess probiotic potential. Willumsen et al. (1997) show that an
352 amylase digested and fermented porridge was more effective than conventional porridge in
353 the treatment of acute diarrhoea, with respect to repair of mucosal damage. Kingamkono et al.
354 (1999) investigated a lactic acid fermented maize gruel compared to a non-fermented maize
355 gruel on the prevalence of faecal enteric bacteria such as *Campylobacter*, enterohaemorrhagic
356 *Escherichia coli* (EHEC:O157), enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* and
357 *Shigella* in faecal swabs of young children and found a significant lower prevalence of these
358 bacteria in the group receiving the fermented product. Furthermore, the *in vitro* ability of
359 fermented food to reduce the presence of pathogenic bacteria is pronounced (Mensah et al,
360 1988; Nout et al., 1989; Mensah et al., 1990; Mensah et al., 1991; Odugbemi et al., 1991;
361 Svanberg et al., 1992; Kingamkono et al., 1998; Kimmons et al., 1999; Tetteh et al., 2004).

362 There is convincing evidence that certain probiotic strains are effective in
363 preventing and treating acute diarrhoea. A possible explanation for the often found
364 inconsistency of results may be the use of different species and subspecies of cultures with
365 different abilities to adapt to the human intestinal tract, and with varying abilities to induce
366 immune responses. The prospect of being able to use a locally produced product as a probiotic
367 treatment is immense. Because of the improved keeping qualities and the increased nutritional
368 value, it is likely that traditionally fermented foods have an important role in preventing acute

369 diarrhoea. With the low cost and the widespread availability in some populations with high
370 prevalence of acute diarrhoea, the effects of traditional fermented foods need to be further
371 investigated.

372

373 **Acknowledgements**

374 The project was funded by The Danish Council for Development Research (no.
375 90963) which is highly appreciated. Special appreciation goes to the people in the two study
376 areas in Northern Ghana. A special thanks to the Ministry of Health, Tamale branch (Ghana),
377 the health clinics involved in the study, supervised by Margaret Appiah, Sylvester Kpiebaya,
378 Rose Ayirety, George Tambro, George Sobiitey, James S. Kousie and the field assistants.
379 Finally, the authors are grateful to Anne Hvid Karsten for technical assistance.

380

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622 **Table 1.** Baseline characteristics^a of 190 children below 5 years with diarrhoea randomised to
 623 koko sour water (KSW) or no KSW for additional treatment (control).

	KSW	Control
	(n=97)	(n=93)
Males (%)	65	61
Age (months)^b	12 (8;21)	15 (11;24)
Weight (kg)^b	8.5 (8.1;8.9)	8.6 (8.2;9.0)
Height (cm)^c	75.5 (73.6;77.2)	77.8 (76.0;79.6)
Weight for age (Z score)^c	-1.6 (-1.8;-1.4)	-2.1 (-2.3;-1.9)
Weight for height (Z score)^c	-1.5 (-1.7;-1.3)	-1.8 (-2.0;-1.6)
Height for age (Z score)^c	-0.6 (-0.9;-0.3)	-0.9 (-1.2;-0.6)
Breastfed (%)	79	71
Duration of diarrhoea (h)^c	52.9 (47.8;58.1)	52.7 (47.2;58.1)
Dehydration (%)^d	25.0	24.2
Mildly sick (%)^d	68.0	69.9
In-patients (%)	16.5	15.1
Treated with antibiotics (%)	67.0	76.3
Treated for malaria (%)	96.9	86.9
Number of stools last 24 hours^c	4.4 (4.0;4.7)	4.4 (4.1;4.7)
Episodes of diarrhoea last year^c	1.7 (1.3;2.1)	2.2 (1.9;2.6)

624 ^a SPSS version 10.0 for Windows was used to perform the data analysis. The distributions of
 625 data were assessed using normal probability plots. For normally distributed data, mean and
 626 standard deviation (SD) or 95% confidence interval (95% CI) were given and t-tests used to
 627 test for differences between groups. For data that were not normally distributed, median and
 628 interquartile range were given and the Mann-Whitney test used to compare groups.

629 ^b Median (25;75 interquartile range)

630 ^c Mean (95% confidence interval)

631 ^d Based on clinical assessment

632 **Table 2.** Average (Standard deviation; SD) intake in ml of koko sour water (KSW) and oral
 633 rehydration solution (ORS) per day during the intervention of the children in respectively the
 634 KSW group and the group receiving no KSW for additional treatment (control).

	Day 1		Day 2		Day 3		Day 4		Day 5		
	KSW	control	KSW	control	KSW	control	KSW	control	KSW	control	Total
K	29	-	148	-	173	-	182	-	202	-	711
S	(67)		(85)		(81)		(80)		(78)		(307)
W											
O	32	72	125	158	52	107	23	37	6	43	240
R	(86)	(260)	(201)	(196)	(141)	(158)	(102)	(89)	(31)	(128)	(378)
S											404
											(434)

635

636 **Table 3.** The effect of koko sour water (KSW) or no additional treatment (control) for all 190
 637 children^a on stool frequency, stool consistency, duration of diarrhoea, weight difference and
 638 whether the children were defined as cured. Data shown for selected days of the 5 day
 639 intervention.

	KSW (n = 97)	Control (n = 93)	P value
Stool frequency*			
Day 1 (day of enrolment)^b	4 (3;6)	4 (3;5)	0.99
Day 2^b	2 (1;3)	2 (1;3)	0.46
Day 5^b	2 (1;3)	2 (1;3)	0.78
Stool consistency**			
Day 1 (day of enrolment)^b	2 (1.9;2.4)	2 (1.7;3)	0.71
Day 2^b	3 (2;3)	3 (2;3.3)	0.42
Day 5^b	4 (3;5)	4 (3;5)	0.60
Weight difference (kg)^b			
From day 1 to day 3	0.0 (0.0;0.1)	0.0 (0.0;0.1)	0.23
From day 1 to day 4	0.1 (0.0;0.2)	0.1 (0.0;0.3)	0.26
From day 1 to day 14	0.2 (0.0;0.4)	0.2 (0.1;0.5)	0.19
Children defined as cured (%)			
Day 2	60.0	56.0	0.69
Day 5	77.7	84.3	0.34

640 ^a SPSS version 10.0 for Windows was used to perform the data analysis. The distributions of
 641 data were assessed using normal probability plots. For normally distributed data, mean and
 642 standard deviation (SD) or 95% confidence interval (95% CI) were given and t-tests used to

643 test for differences between groups. For data that were not normally distributed, median and
644 interquartile range were given and the Mann-Whitney test used to compare groups.

645 ^b Median (25;75 interquartile range)

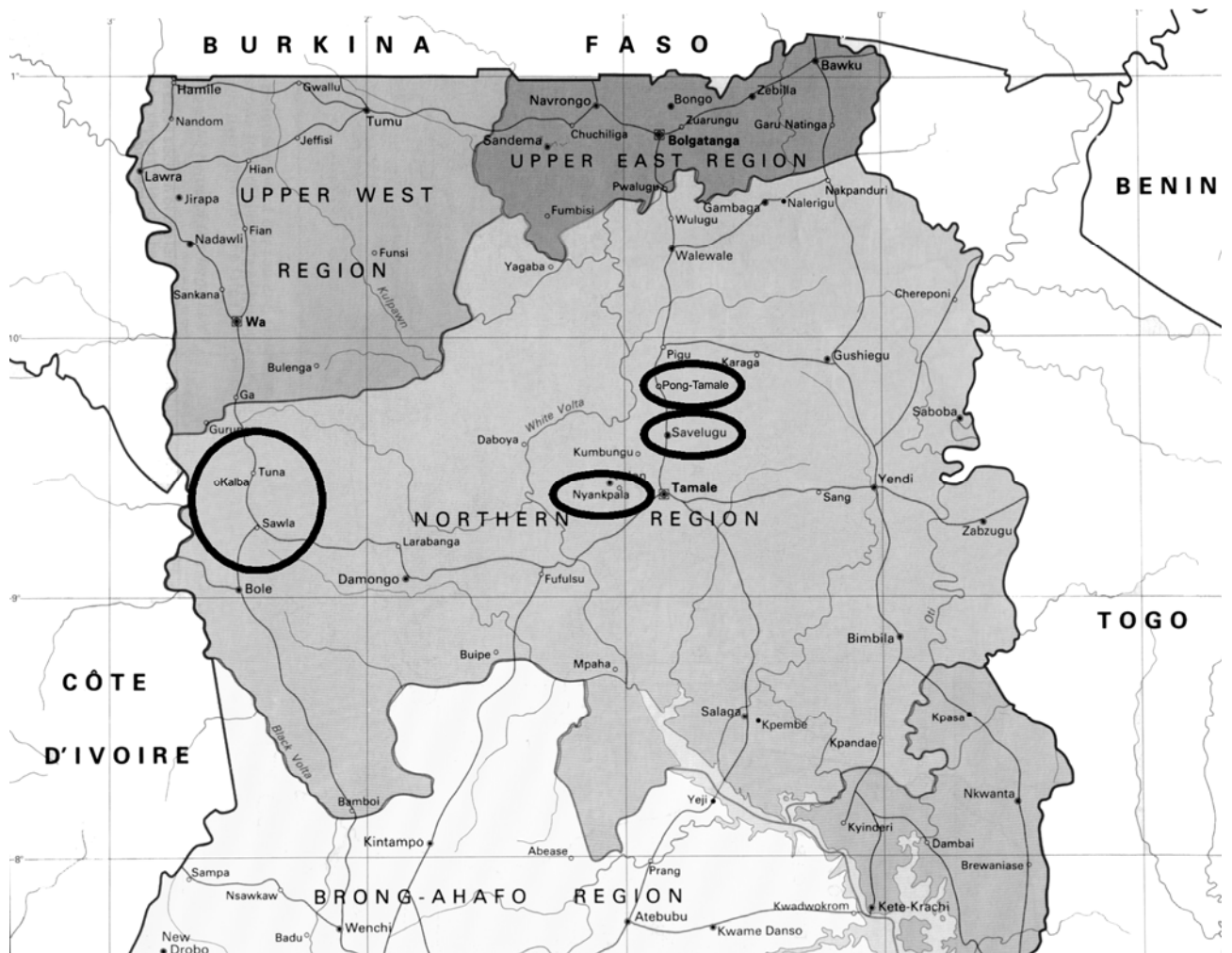
646 * Stool frequency: More than 3 stools in 24 hours were defined as diarrhoea (WHO, 1990)

647 ** Stool consistency: 1=very watery, 2=water ring with formed particles, 3=liquid, creamy,
648 4=loose soft, 5=soft formed, 6=normal formed (1, 2, and 3 are defined as diarrhoea) (Young
649 et al. 1998)

650 **Fig. 1 (Lei et al.)**

651 The koko sour warter (KSW) intervention study was conducted in two districts, A and B, in
652 Northern Ghana. District A comprised three health clinics in three villages, Nyankpala, Pong-
653 Tamale and Savelugu, situated 20 to 30 km from Tamale, the capital city of the Northern
654 Region. District B comprised another three health clinics in three villages situated in
655 Northwest Ghana called Tuna, Sawla and Kalba. District A and B are located approx. 250 km
656 apart.

657



658

659 Fig. 1 (Lei et al.)

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