UNIVERSITY OF COPENHAGEN

Spontaneously fermented millet product as a natural probiotic treatment for diarrhoea in young children

Lei, Vicki; Friis, Henrik; Michaelsen, Kim Fleischer

Published in:
International Journal of Food Microbiology

DOI:
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

Download date: 17. sep.. 2019

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

Vicki Lei[a], Henrik Friis[b] and Kim Fleischer Michaelsen[c]

[a]The Royal Veterinary and Agricultural University, Dept. of Food Science, Food Microbiology, Rolighedsvej 30, 1958 Frederiksberg C, Denmark, [b]University of Copenhagen, Dept. of Epidemiology, Institute of Public Health, Blegdamsvej 3, 2200 Copenhagen N, Denmark and [c]The Royal Veterinary and Agricultural University, Dept. of Human Nutrition, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

Corresponding author:

Vicki Lei

The Royal Veterinary and Agricultural University

Dept. of Food Science

Food Microbiology

Rolighedsvej 30

1958 Frederiksberg C

Denmark

Phone: +45 35 28 33 48

Fax: +45 35 28 32 14

E-mail: vil@kvl.dk

Abstract

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

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

1. Introduction

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

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

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

Fermentation of foods is traditionally carried out daily in Africa and other developing countries, and a vast number of different spontaneously fermented food and drink products are used. The immediate benefits of these fermented foods are the keeping qualities and the increased nutritional value (e.g. Ezeji and Ojimelukwe, 1993; Antony et al., 1998a,b; Mugula and Lyimo, 1999, Ouoba et al., 2003). These products have a high content of bacteria with a large bio-diversity (Hayford et al., 1999; ben Omar et al., 2000; Escalante et al., 2001; Paludan-Müller et al., 2002; Muyanja et al., 2003), and there is a possibility that some of these could be probiotic. *Koko* is a millet gruel produced in Northern Ghana and spontaneously fermented by lactic acid bacteria, mainly *Weissella confusa* and *Lactobacillus fermentum* (Lei and Jakobsen, 2004). The final product of *koko* is boiled, but a fermented and un-boiled part of the product has by tradition been used for adults and children with upset stomachs and as a refreshing drink during fastening periods instead of water (Lei and Jakobsen, 2004). This specific part of the product, called *koko sour water* (KSW), contains live lactic acid bacteria in levels of 10^8 colony forming units/ml and has a pH (standard deviation; SD) of 3.6 (0.2). The lactic acid bacteria isolates from KSW showed low levels of antimicrobial activity towards *Listeria innocua*, but no activity towards the bacteriocin-sensitive *Lactobacillus sakei*. In addition, growth of all lactic acid bacteria isolates was unaffected by the presence of 0.3% (v/v) oxgall bile and the isolates were able to survive, but were not able to grow in growth medium adjusted to pH 2.5 (Lei and Jakobsen, 2004). It is possible that this product could have a potential as a probiotic product, and this paper reports on the ability of KSW to alleviate diarrhoea in children below the age of five in Northern Ghana.

2. Methods and materials

Study areas

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

Study design and objectives

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

Koko and koko sour water production

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

Recruitment and training of field assistants

Field assistants were nursing staff attached to the health clinics, recruited by the head of the clinics and trained for the study in a two-day workshop prior to the study. Training included lectures on children’s diarrhoea, group discussions, small working group sessions and testing of the questionnaires described below. The field assistants were all able to speak the local language as well as English.

Observations schedule

On admission to the health clinic, the children were examined clinically, diagnosed and treated medically as indicated above. If a child fulfilled the inclusion criteria, the parents or guardian were asked to participate in the study. After obtained consent, field assistants interviewed the parents or guardian of the enrolled children about the age of the child, the address of the parents or guardian in addition to questions about the family structure. The medical treatment given by the health clinic and/or by the parents or guardian was registered. The degree of dehydration and sickness was estimated by the categories none, some and severe. The length and the nude weight of the child were measured. The length was measured by locally produced infantometers to the nearest cm, and the weighing was carried out by using the MP25 Infant Weighing Pack (CMS Weighing Equipment Ltd., London, UK). Each field assistant was equipped with a weighing scale and each health clinic was equipped with an infantometer. The participating children were daily visited by field assistants in their homes for the first 5 days after diagnosis and again on the 14^{th} day after diagnosis. During the home visits the field assistants monitored the treatment initiated by the clinics and followed the condition of the children by filling in a standardised daily questionnaire. This questionnaire contained the same questions to be answered for the first 5 days, detailing the health of the child during the past 24 hrs. The questions asked were on number of stools, consistency of the stool, blood in the stool, vomiting, the overall health, and the food and drink intake. In addition, the child was weighed daily. For the consistency of the stool, each field assistant was carrying a picture of line drawings depicting stool consistency with given numeric values (Young et al. 1998) for the mothers to identify the daily stool consistency of the child. The children randomised to the KSW group were given the first KSW at enrolment at the health clinic. Field assistants subsequently brought fresh KSW daily to the children's home during the next 4 days after diagnosis. The 300 ml KSW was administered in 500 ml clear plastic containers with volumetric graduations for every 50 ml. The children were offered to drink as much as possible of the 300 ml over the 24 h before the next visit of the field assistant. The intake of the KSW water was recorded at the subsequent visit. On the 14th day after enrolment, the children of both groups were visited for a follow-up with a special questionnaire. This follow-up questionnaire contained questions on the general well-being of the child, i.e. behaving normally and following the normal eating and drinking pattern. It was registered whether the children had had diarrhoea or had been otherwise sick since the last visit. In addition, the weight of the children was recorded. It was emphasised that the same field assistant observed the same child during the study period.

Socio-economic characteristics of the participating households

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

Statistical analyses

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

Ethical considerations

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

3. Results

Among the 190 children with diarrhoea recruited for the study, the median (interquartile range; IQR) age was 13 months (9; 24), the mean weight (SD) was 8.5 kg (1.9) and the mean height (SD) 76.6 cm (8.6). The Z-score of weight-for-age (mean (SD)) was –1.8 (1.1), the height-for-age Z was –0.7 (1.5) and the weight-for-height Z was –1.7 (1.0). The children had had diarrhoea for a median (IQR) of 48 h (24; 96) prior to enrolment. Based on clinical assessment, 46 (24.2%) of the 190 children were judged dehydrated at enrolment, and on examination, 172 (90.5%) were found to have malaria parasitaemia, according to the medical examination at the clinic.

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

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

Baseline comparison

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

Intervention

Of the 190 children recruited, 184 children (96.8%) completed the study. Of the six children lost to follow-up, three were from the KSW group and three from the control group. Three children in the control group died during or after the study, while there was no death in the KSW group (P=0.21). One child died between the 5th and 14th day of the study, the other two within two weeks after end of study.

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

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

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

Day 14 follow-up

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

Overall

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

4. Discussion

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

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

Probiotics are known to have a better effect the earlier from onset of diarrhoea the treatment is commenced (Rautanen et al., 1998; Rosenfeldt et al., 2002a,b). That any cure of diarrhoea by KSW was not shown, might be due to the fact that the children from both groups came to the clinic two days after onset of diarrhoea and most were mildly sick and quickly cured. There is a possibility that the predominant lactic acid bacteria of KSW did not possess probiotic properties effective against diarrhoea. *Weissella confusa* and *Lactobacillus fermentum* was found to dominate KSW (Lei and Jakobsen, 2004). Representative isolates showed no pronounced antimicrobial effects towards *Lactobacillus sakei* and *Listeria innocua*, they were, however, moderately acid and bile tolerant (Lei and Jakobsen, 2004). The importance of using documented probiotic cultures compared to traditional starter cultures is shown in studies by Pedone et al. (2000) and Olukoya et al. (1994). The use of specific probiotic cultures, documented to affect diarrhoea as starter culture for KSW may cure or alleviate diarrhoea in children.

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

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

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

There is convincing evidence that certain probiotic strains are effective in preventing and treating acute diarrhoea. A possible explanation for the often found inconsistency of results may be the use of different species and subspecies of cultures with different abilities to adapt to the human intestinal tract, and with varying abilities to induce immune responses. The prospect of being able to use a locally produced product as a probiotic treatment is immense. Because of the improved keeping qualities and the increased nutritional value, it is likely that traditionally fermented foods have an important role in preventing acute diarrhoea. With the low cost and the widespread availability in some populations with high prevalence of acute diarrhoea, the effects of traditional fermented foods need to be further investigated.

Acknowledgements

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

References

Antony, U., Moses, L.G. and Chandra, T.S., 1998. Inhibition of *Salmonella typhimurium* and *Escherichia coli* by fermented flour of finger millet (*Eleusine coracana*). World Journal of Micribiology & Biotechnology 14, 883-886.

Armar-Klemesu, M.A., Wheeler, E.F., Brakohiapa, L.A. and Yamamoto, S., 1991. Infant feeding and growth in rural Ghana: Is the use of traditional fermented porridge a case for early supplementation? Journal of Tropical Pediatrics 37, 111-115.

ben Omar, N., Ampe, F., Raimbault, M., Guyot, J.P. and Tailliez, P., 2000. Molecular diversity of lactic acid bacteria from cassava sour starch (Colombia). Systematic and Applied Microbiology 23, 285-291.

Bergogne-Bérézin, E., 2000. Treatment and prevention of antibiotic associated diarrhea. International Journal of Antimicrobial Agents 16, 521-526.

Chandra, R.K., 2002. Effect of *Lactobacillus* on the incidence and severity of acute rotavirus diarrhoea in infants. A prospective placebo-controlled double-blind study. Nutrition Research 22, 65-69.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K., 1998. Antibiotic susceptibility of potentially probiotic Lactobacillus species. Journal of Food Protection 61, 1636-1643.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K., 2001. Gradient diffusion antibiotic susceptibility testing of potentially probiotic lactobacilli. Journal of Food Protection 64, 2007-2014.

Cremonini, F., Di Caro, S., Nista, E.C., Bartolozzi, F., Capelli, G., Gasbarrini, G. and Gasbarrini, A., 2002. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Alimentary Pharmacology & Therapeutics 16, 1461-1467.

D'Souza, A.L., Rajkumar, C., Cooke , J. and Bulpitt, C.J., 2002. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. British Medical Journal 324, 1361-1364.

Danielsen, M. and Wind, A., 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. International Journal of Food Microbiology 82, 1-11.

Darling, J.C., Kitundu, J.A., Kingamkono, R.R., Msengi, A.E., Mduma, B., Sullivan, K.R. and Tomkins, A.M., 1995. Improved energy intakes using amylase-digested weaning foods in Tanzanian children with acute diarrhea. Journal of Pediatric Gastroenterology and Nutrition 21, 73-81.

Escalante, A., Wacher, C. and Farres, A., 2001. Lactic acid bacterial diversity in the traditional Mexican fermented dough pozol as determined by 16S rDNA sequence analysis. International Journal of Food Microbiology 64, 21-31.

Ezeji, C. and Ojimelukwe, P.C., 1993. Effect of fermentation on the nutritional quality and functional- properties of infant food formulations prepared from bammarra-groundnut, fluted-pumpkin and millet seeds. Plant Foods for Human Nutrition 44, 267-276.

Guandalini, S., Pensabene, L., Abu Zikiri, M., Dias, J.A., Casali, L.G., Hoekstra, H., Kolacek, S., Massar, K., Micelic-Turk, D., Papadopoulou, A., de Sousa, J.S., Sandhu, B., Szajewska, H. and Weizman, Z., 2000. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: A multicenter European trial. Journal of Pediatric Gastroenterology and Nutrition 30, 54-60.

Guarino, A., Canani, R.B., Spagnuolo, M.I., Albano, F. and DiBenedetto, L., 1997. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. Journal of Pediatric Gastroenterology and Nutrition 25, 516-519.

Hayford, A.E., Petersen, A., Vogensen, F.K. and Jakobsen, M., 1999. Use of conserved randomly amplified polymorphic DNA (RAPD) fragments and RAPD pattern for characterization of *Lactobacillus fermentum* in Ghanaian fermented dough. Applied and Environmental Microbiology 65, 3213-3221.

Hawrelak, J.A., Whitten, D.L. and Myers, S.P., 2005. Is *Lactobacillus rhamnosus* GG effective in preventing the onset of antibiotic-associated diarrhoea: A systematic review. Digestion 72, 51-56.

Huang, J.S., Bousvaros, A., Lee, J.W., Diaz, A. and Davidson, E.J., 2002. Efficacy of probiotic use in acute diarrhea in children - A meta-analysis. Digestive Diseases and Sciences 47, 2625-2634.

Isolauri, E., Kaila, M., Mykkanen, H., LING, W.H. and Salminen, S., 1991. A human *Lactobacillus* strain (*Lactobacillus Casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 88, 90-97.

Kimmons, J.E., Brown, K.H., Lartey, A., Collison, E., Mensah, P.P.A. and Dewey, K.G., 1999. The effects of fermentation and or vacuum flask storage on the presence of coliforms in complementary foods prepared for Ghanaian children. International Journal of Food Sciences and Nutrition 50, 195-201.

Kingamkono, R.R., Sjogren, E. and Svanberg, U., 1998. Inhibition of enterotoxin production by, and growth of enteropathogens in a lactic acid-fermenting cereal gruel. World Journal of Microbiology & Biotechnology 14, 661-667.

Kingamkono, R., Sjogren, E. and Svanberg, U., 1999. Enteropathogenic bacteria in faecal swabs of young children fed on lactic acid-fermented cereal gruels. Epidemiology and Infection 122, 23-32.

Kirkwood, B.R., 1991. Diarrhoea. In: Feachem, R.G. and Jamison, D.T. (eds). Disease and mortality in sub-Saharan Africa. Oxford University Press, 134-157.

Lei, V and Jakobsen, M., 2004. Microbiological characterization and probiotic potential of *koko* and *koko sour water*, African spontaneously fermented millet porridge and drink. Journal of Applied Microbiology 96, 384-397.

Lorri, W and Svanberg, U., 1994. Lower prevalence of diarrhoea in young children fed lactic-acid fermented cereal gruels. Food and Nutrition Bulletin 15, 57-63.

Mensah, P.P.A., Tomkins, A.M., Drasar, B.S. and Harrison, T.J., 1988. Effect of fermentation of Ghanaian maize dough on the survival and proliferation of four strains of *Shigella flexneri*. Transactions of the Royal Society of Tropical Medicine and Hygiene 82, 635-636.

Mensah, P.A.A., Tomkins, A.M., Drasar, B.S. and Harrison, T.J., 1990. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. Lancet, 336, 140-143.

Mensah, P., Tomkins, A.M., Drasar, B.S. and Harrison, T.J., 1991. Antimicrobial effect of fermented Ghanaian maize dough. Journal of Applied Bacteriology 70, 203-210.

Mugula, J.K. and Lyimo, M., 1999. Evaluation of the nutritional quality and acceptability of fingermillet-based tempe as potential weaning foods in Tanzania. International Journal of Food Sciences and Nutrition 50, 275-282.

Muyanja, C.M.B.K., Narvhus, J.A., Treimo, J. and Langsrud, T., 2003. Isolation, characterisation and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. International Journal of Food Microbiology 80, 201-210.

Nout, M.J.R., Rombouts, F.M. and Havelaar, A., 1989. Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. International Journal of Food Microbiology 8, 351-61.

Oberhelman, R.A., Gilman, E.H., Sheen, P., Taylor, D.N., Black, R.E., Cabrera, L., Lescano, A.G., Meza, R. and Madico, G., 1999. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. Journal of Pediatrics 134, 15-20.

Odugbemi, T., Odujinrin, O.M.T., Akitoye, C.O., Oyerinde, J.P.O., and Esumeh, F.I., 1991. Study on the pH of *ogi*, Nigerian fermented weaning food, and its effect on enteropathogenic *Escherichia coli*, *Salmonella typhi* and *Salmonella paratyphi*. Journal of Tropical Medicine and Hygiene 94, 219-223.

Olukoya, D.K., Ebigwei, S.I., Olasupo, N.A. and Ogunjimi, A.A., 1994. Production of dogik –an improved ogi (Nigerian fermented weaning food) with potentials for use in diarrhea control. Journal of Tropical Pediatrics 40, 108-113.

Ouoba, L.I.I. Rechinger, K.B., Barkholt, V., Diawara, B., Traore, A.S. and Jakobsen, M., 2003. Degradation of proteins during the fermentation of African locust bean (Parkia biglobosa) by strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. Journal of Applied Microbiology 94, 396-402.

Paludan-Muller, C., Madsen, M., Sophanodora, P., Gram, L. and Moller, P.L., 2002. Fermentation and microflora of plaa-som, a Thai fermented fish product prepared with different salt concentrations. International Journal of Food Microbiology 73, 61-70.

Pant, A.R., Graham, S.M., Allen, S.J., Harikul, S., Sabchareon, A., Guevas, L. and Hart, C.A., 1996. *Lactobacillus GG* and acute diarrhoea in young children in the tropics. Journal of Tropical Pediatrics 42, 162-165.

Pedone, C.A., Arnaud, C.C., Postaire, E.R., Bouley, C.F. and Reinert, P., 2000. Multicentric study of the effect of milk fermented by *Lactobacillus casei* on the incidence of diarrhoea. International Journal of Clinical Practice 54, 568-571.

Rautanen, T., Isolauri, E., Salo, E. and Vesikari, T., 1998. Management of acute diarrhoea with low osmolarity oral rehydration solutions and *Lactobacillus* strain GG. Archives of Disease In Childhood 79, 157-160.

Rosenfeldt, V., Michaelsen, K.F., Jakobsen, M., Larsen, C.N., Moller, P.L., Pedersen, P., Tvede, M., Weyrehter, H., Valerius, N.H. and Paerregaard, A., 2002a. Effect of probiotic *Lactobacillus* strains in young children hospitalized with acute diarrhea. Pediatric Infectious Disease Journal 21, 411-416.

Rosenfeldt, V., Michaelsen, K.F., Jakobsen, M., Larsen, C.N., Moller, P.L., Tvede, M., Weyrehter, H., Valerius, N.H. and Paerregaard, A., 2002b. Effect of probiotic *Lactobacillus* strains on acute diarrhea in a cohort of nonhospitalized children attending day-care centers. Pediatric Infectious Disease Journal 21, 417-419.

Saran, S., Gopslsn, S. and Krishna T.P., 2002. Use of fermented foods to combat stunting and failure to thrive. Nutrition 18, 393-396.

Shornikova, A.V., Casas, I.A., Isolauri, E., Mykkanen, H. and Vesikari, T., 1997a. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. Journal of Pediatric Gastroenterology and Nutrition 24, 399-404.

Shornikova, A.V., Isolauri, E., Burkanova, L., Lukonikova, S. and Vesikari, T., 1997b. A trial in the Karelian Republic of oral rehydration and *Lactobacillus* GG for treatment of acute diarrhoea. Acta Paediatrica 86, 460-465.

Shornikova, A.V., Casas, I.A., Mykkanen, H., Salo, E. and Vesikari, T., 1997c. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatric Infectious Disease Journal 16, 1103-1107.

Simakachorn, N., Pichaipat, V., Rithipornpaisam, P., Kongkaew, C., Tongpradit, P. and Varavithya, W., 2000. Clinical evaluation of the addition of lyophilized, heat-killed *Lactobacillus acidophilus* LB to oral rehydration therapy in the treatment of acute diarrhoea in children. Journal of Pediatric Gastroenterology and Nutrition 30, 68-72.

Svandberg, U., Sjogren, E., Lorri, W., Svennerholm, A.M. and Kaijser, B, 1992. Inhibited growth of common enteropathogenic bacteria in latic-fermented cereal gruels. World Journal of Microbiology & Biotechnology 8, 601-606.

Svanberg, U., 1992. Fermentation of cereals – Traditional household technology with improved nutritional potential for young children. IRD Currents 2, 21-24.

Szajewska, H. and Mrukowicz, J.Z., 2001. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: A systematic review of published randomized, double-blind, placebo-controlled trails. Journal of Pediatric and Gastroenterology and Nutrition 33, S17-25.

Temmerman, R., Pot, B., Huys, G. and Swings, J., 2002. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. International Journal of Food Microbiology 81, 1-10.

Tetteh, G.L., Sefa-Dedeh, S.K., Phillips, R.D. and Beuchat, L.R., 2004. Survival and growth of acid-adapted and unadapted *Shigella flexneri* in a traditional fermented Ghanaian weaning food as affected by fortification with cowpea. International Journal of Food Microbiology 90, 189-195.

Turck, D., Bernet, J.P., Marx, J., Kempf, H., Giard, P., Walbaum, O., Lacombe, A., Rembert, F., Toursel, F., Bernasconi, P., Gottrand, F., McFarland, L.V. and Bloch, K., 2003. Incidence and risk factor of oral antibiotic-associated diarrhea in an outpatient pediatric population. Journal of Pediatric Gastroenterology and Nutrition 37, 22-26.

Van Niel, C.W., Feudtner, C., Garrison, M.M. and Christakis, D.A., 2002. *Lactobacillus* therapy for acute infectious diarrhea in children: a meta-analysis. Pediatrics 109, 678-684.

Vanderhoof, J.A., Whitney, D.B., Antonson, D.L., Hanner, T.L., Lupo, J.V. and Young, R.J., 1999. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. Journal of Pediatrics 135, 564-568.

Victora, C.G., Bryce, J., Fontaine, O. and Monasch, R., 2000. Reducing deaths from diarrhoea through oral rehydration therapy. Bulletin of the World Health Organization 78, 1246-1255.

WHO, 1990. World Health Organization. Diarrhoeal diseases control programme. The treatment and prevention of acute diarrhoea. WHO/CDD/SER/80.2 Rev 2, 8-45.

Willumsen, J.F., Darling, J.C., Kitunda, J.A., Kingamkono,R.R., Msengi, A.E., Mduma, B., Sullivan, K.R. and Tomkins, A.M., 1997. Dietary management of acute diarrhoea in children: Effect of fermented and amylase-digested weaning foods on intestinal permeability. Journal of Pediatric Gastroenterology and Nutrition 24, 235-241.

Yapar, N., Sener, A., Karaca, B., Yucesoy, M., Tarakei, H., Cakir, N. and Yuce, A., 2005. Antibiotic-associated diarrhea in a Turkish outpatient population: investigation of 288 cases. Journal of Chemotherapy 17, 77-81.

Yartey, J., Nkrumah, F., Hori, H., Harrison, K. and Armar, D., 1995. Clinical-trial of fermented maize-based oral rehydration solution in the management of acute diarrhea in children. Annals of Tropical Paediatrics 15, 61-68.

Young, R.J, Beerman, L.E., and Vanderhoof, J.E., 1998. Increasing oral fluids in chronic constipation in children. Gastroenterology Nursing 21, 156-161.

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

	KSW (n=97)	**Control (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)

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

[b] Median (25;75 interquartile range)

[c] Mean (95% confidence interval)

[d] Based on clinical assessment

Table 2. Average (Standard deviation; SD) intake in ml of koko sour water (KSW) and oral rehydration solution (ORS) per day during the intervention of the children in respectively the 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**
KSW	29 (67)	-	148 (85)	-	173 (81)	-	182 (80)	-	202 (78)	-	711 (307)
ORS	32 (86)	72 (260)	125 (201)	158 (196)	52 (141)	107 (158)	23 (102)	37 (89)	6 (31)	43 (128)	240 (378) 404 (434)

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

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

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

[b] Median (25;75 interquartile range)

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

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

Fig. 1 (Lei et al.)

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

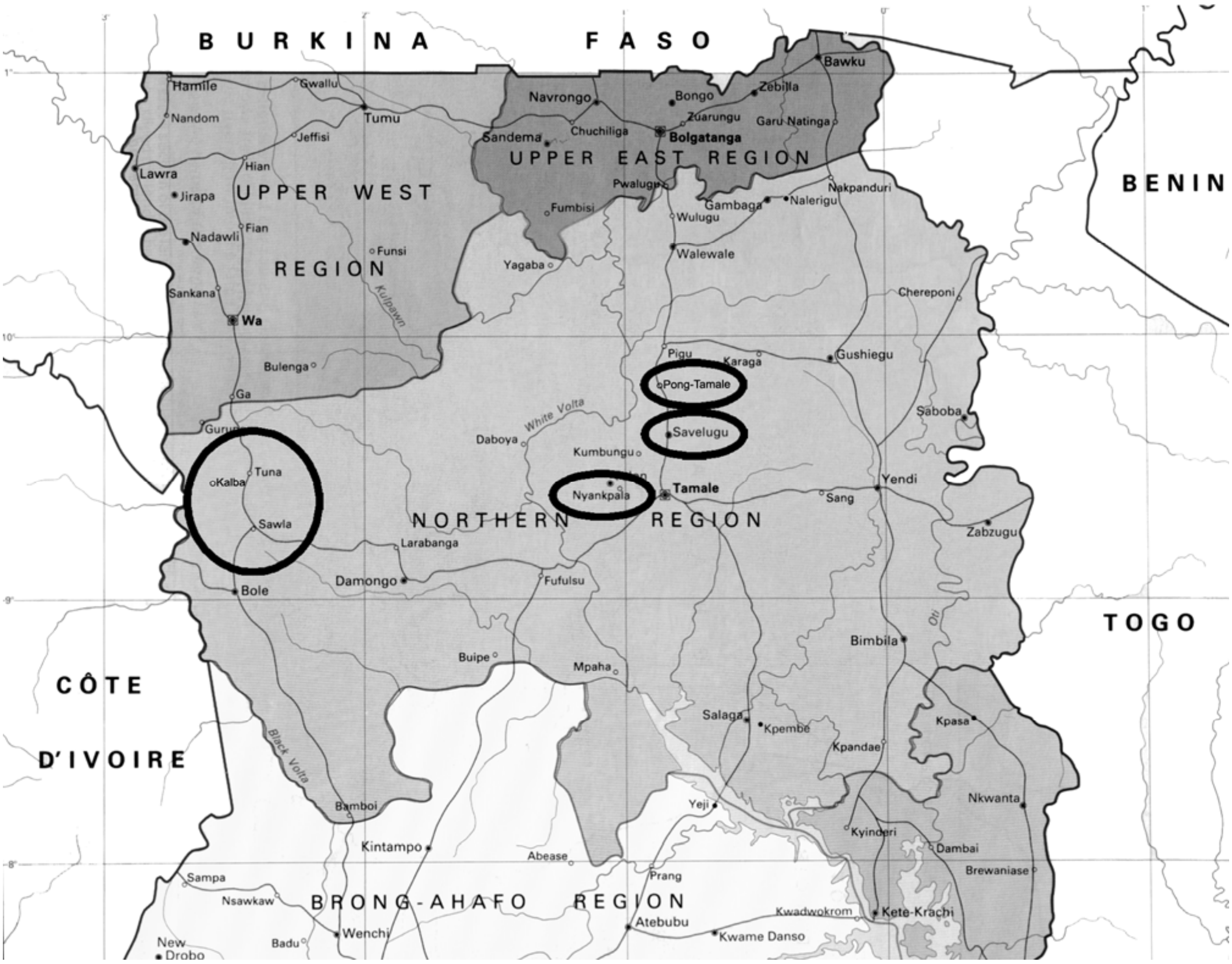

Fig. 1 (Lei et al.)