Eur J Nutr 41 [Suppl 1] (2002) : I/26 – I/31
DOI 10.1007/s00394-002-1104-5

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Effects of lactulose on the intestinal microflora of periparturient sows and their piglets

Summary The periparturient period of animals (and humans) is very stressful and influenced by the microecosystem of the gastrointestinal tract (GIT). Performance and productivity of animal husbandry depend on the health of animal mothers and their offspring. We investigated the influence of prebiotic amounts of lactulose in sows and their piglets. Two experimental trial sows received daily 30 ml lactulose, 71 field trial sows received daily 45 ml lactulose during their periparturient period (10 days before until 10 days after parturison). The weaners of trial sows received 15 ml lactulose per 1 kg baby food 10 days before and 10 days after weaning.

The effect of lactulose was recorded by performance parameters like number of piglet born alive, losses until weaning, body mass of piglets, daily weight gain of weaners until 35 days after weaning. The effect of lactulose on GIT microflora was estimated by bacterial counts of faeces of sows (total aerobic bacteria, Gram-negative bacteria, Clostridium (C.) perfringens). In order to show a previously unknown effect of lactulose we investigated the levels of antibodies to phospholipase C (PLC) of C. perfringens in plasma of experimental sows and in colostral and ripe milk of field sows. Lactulose influenced the performance parameters of sows in a non-significant way. In case of weaners we recorded significant daily weight gains. Lactulose significantly influenced total aerobic bacterial counts, C. perfringens counts in faeces of sows 20 days after parturison. Under experimental conditions it was shown that trial sows and their piglets had higher IgG-antibody levels to C. perfringens PLCs than the control animals. Similar results were found under field conditions. Trial sows had significant higher IgG-anti LPS (J5) antibodies in milk 10 days after birth.

Key words lactulose – periparturient sows – gastrointestinal microecosystem – bacterial counts – phospholipases C of Clostridium perfringens – LPS – antibodies

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Background

Animals (and humans) live their whole life in a close relationship with their intestinal microflora. This consists of viruses, bacteria, fungi, yeasts, and protozoas. The predominant bacteria are anaerobic, particularly in the lower ileum, caecum and colon. The effect of microbes upon the host may be considered in three ways: physical, chemical and biological [1–3]. The ontogenic origin of host-microbe association in humans and in animals is a random inoculum, depending upon the particular environment of the parturition. This begins with the microflora of the vagina, faeces, environment and the colostral milk of the mother [1, 4, 5]. Within three or four days the colostrum-fed newborn will have standard strains of bacteria, e. g. Bifidobacterium spp., Lactobacillus spp., E. coli-strains, yeast spp. After weaning the microflora of adults will be developed. The intimacy of the microbes to the host leads to coevolution of the pair

and the host directs the evolution and the genetic change of the microorganisms within [1, 5].

But the bacteria are also able to influence the host. Already Dubos and Schaedler [6] found that Lactobacilli protect the host from lethal doses of endotoxin. They also found that mild stress of general nature would lower the intestinal Lactobacilli and increase the Gram-negative flora [6]. These results were confirmed by Lisko et al. [7]. They investigated faeces of Russian cosmonauts and found reduced Bifidobacteria and Lactobacilli levels and increased aerobes, pathogenic Enterobacteria and Proteus spp. after the flight. In animal husbandry the most stressful times of life are the peripartal period, weaning from the mothers, moving, changing and crowding of animals as well as changing of food. Under these conditions the microflora is influenced by lack of water, too much and unknown feed, toxic substances like mycotoxins, heavy metals, stressful environmental conditions like nonphysiological pH, osmotic and oxidative stress. Many of the bacteria in the gastrointestinal tract (GIT) are able to respond to these stressful situations with the expression of proteins [8–13]. An important group of these proteins are phospholipases C (PLC), phosphodiesterases with special target sites at different phospholipid molecules. Some of them are toxic for humans and animals. They interact with membrane phospholipids (phosphatidylcholine, phosphatidylinositol, phosphatidylethanol-amine, phosphatidylserine, sphingomyelin) and were isolated from a wide variety of Gram-positive and Gram-negative bacteria. Bacterial PLCs are also able to cut the phosphatidylinositolphosphate anchor molecules of the membrane-bound alcalic phosphatase [14]. In the GIT they are produced by Bacillus cereus, Clostridium (C.) perfringens, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa and others. These enzymes are involved in the osmoprotection of bacteria. In most cases the PLCs are phosphate-regulated. The osmoprotectants choline, betaine, and dimethylglycine can also induce the PLC production [10]. In addition, they are produced under conditions of high salt concentrations to protect the salt responsible enzymes of the microorganisms. Lower salt tolerances are induced by disaccharides, e. g. saccharose and trehalose [15]. Other ways of self-protection are extracellular polymeric substances, e. g. gel-like, highly hydrated biofilms, glycocalyxes, capsules, S-layers, alginates etc. [16]. These are also well-known virulence factors of bacteria [17].

Aim of the study

The objective of our studies was to investigate the effect of the well-known prebioticum lactulose [20–23] on the microflora of the GIT especially on total aerobic bacterial counts, on counts of Gram-negative aerobic bacteria and on C. perfringens counts of periparturient sows and their piglets. Another aim was to analyse antibody levels to stress-induced bacterial products like PLCs of C. perfringens after lactulose treatment.

Material and methods

Sites, animal and experimental design

Studies were done under experimental conditions with only 2 trial sows and 2 control sows at the Institute of Applied Agriculture and Ecology of the University of Rostock and under field condition with 71 trial sows and 68 control sows at a pig farm (PIC-breed) in Brandenburg, Germany. The piglets of the trial sows were supplemented with 15 ml lactulose in 1 kg of piglet food from 10 days before until 10 days after weaning. Healthy sows and their piglets which were born healthy and had a body mass of at least 1000 g fulfilled the inclusion criteria. The piglets were weaned 21 days after birth and were kept until 31 days of life in the field trial. The following data were recorded: sows – number of the litter, length of pregnancy, body weight of sows 10 days before birth and at weaning. Piglets – number of born piglets, number of alive born piglets and weaned piglets, litter masses, average number and mass (kg) of sold piglets per sow.

Lactulose was given to sows once daily from ten days before until 10 days after birth. Under experimental conditions the trial sows received 30 ml of lactulose; under field conditions trial sows received 45 ml of lactulose. Control sows did not receive any placebo. The field trail was carried out from May to December 2000. Control examinations of animals were performed twice daily recording new born piglets, dead and ill piglets, illnesses of the sows. All ill animals were treated as usual by a veterinarian.

Collecting of specimens

Faeces of sows were collected 10 days before birth, one day, 10 days and 20 days after birth. Milk samples of sows were taken one day, 10 days and 20 days after birth. All specimens were frozen until investigation in the laboratory. Under experimental conditions blood specimens of sows were collected 10 days before, one day, 10 days, 19 days and 21 days after birth. Blood specimens of piglets were also collected at birth, 10 days, 19 days, and 21 days after birth.

Bacteriological investigations

One gram of faeces was diluted in 10 ml sterile phosphate buffered saline solution (pH 7.4), vortexed and di-

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luted to 10^{-6}. Each dilution step was twice plated on nutrient agar plates (SIFIN Berlin), McConkey agar plates (SIFIN Berlin) and 5% sheep blood agar plates with 200 µg neomycin/ml and 100 µg polymyxin/ml. The sheep blood agar plates were anaerobically cultivated.

Immunological investigations

Milk samples of the sows were investigated for C-reactive protein (CRP), IgG- and IgM-anti-phospholipase C of C. perfringens (PLC) and IgG-anti-LPS (E. coli J5). The CRP and antigen-specific immunoglobulins were determined via enzyme immunoassay (EIA). In all the EIA microtiter plates (96 wells, flat bottom), Dulbecco-phosphate buffered saline (pH 7.4) with 0.1% (v/v) tween 20 (Sigma, Taufenkirchen, Germany) as wash buffer, a constant test volume of 100 µl/well and 3,3',5,5'-tetramethylbenzidin as substrate were used. The CRP was detected with polyclonal antibodies from rabbit anti-CRP, as capture and with peroxidase-labelled as detection antibody (DAKO, Hamburg, Germany). The standard was the purified protein after affinity chromatography and anion-exchange chromatography. For the determination of the antigen-specific immunoglobulins, purified phospholipase C from Clostridium perfringens (Fluka-Sigma-Aldrich, Taufenkirchen, Germany) and LPS from E. coli J5 (Sigma, Taufenkirchen, Germany) were used as antigen for the coating of the microtiter plates. Standard was a pooled serum. The bound antigen-specific immunoglobulins were detected with species-specific, peroxidase-labelled secondary antibodies (anti-IgG from Dianova, Hamburg, Germany and anti-IgM from Bethyl, Montgomery, Texas, USA). The levels of IgG-anti-PLC were shown as measured value of the optical density (o. d.).

Statistical analysis

The software for the statistical analysis was Sigma Stat (SPSS Science, Erkrath, Germany).

Results

Experimental trial

The most important results of this trial were significant differences in litter mass, numbers of dead born and still born piglets and litter weight 28 days after birth (Table 1). Interestingly, the level or the increase of IgG antibodies against PLC of C. perfringens were significantly different between control and trial piglets (Fig. 1). These differences were also seen in blood specimens of their mothers (Fig. 2).

Table 1 Influence of lactulose SOLVAY on important performance parameters of sows and their piglets (experimental trial)

Parameter	Trial sows		Control sows	
	1	2	1	2
Alive born piglets (n)	11	12	6	12
Dead born piglets (n)	0	0	3	1
Litter mass (kg)	15.84	17.3	12.45	13.93
Weaned piglets 28 days (n)	7	9	5	6
Weaned piglets 28 days (kg)	50.4	47.4	26.0	34.0
Losses of piglets (%)	36.4	25.0	11.96	58.3

1 control pigs; *2* pigs receiving lactulose SOLVAY

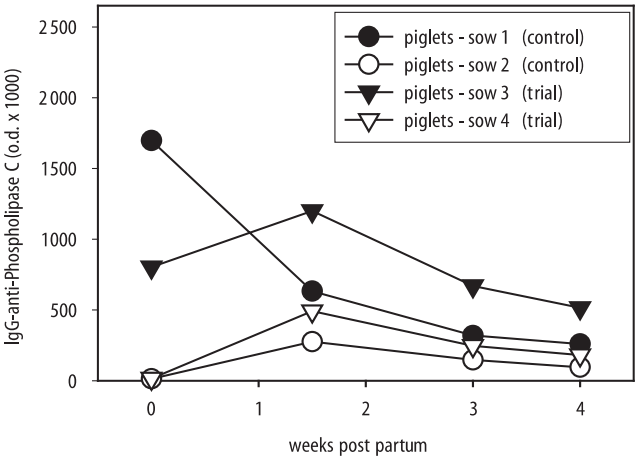

Fig. 1 IgG-anti-phospholipase C (C. perfringens) in blood from piglets of sows with and without lactulose application

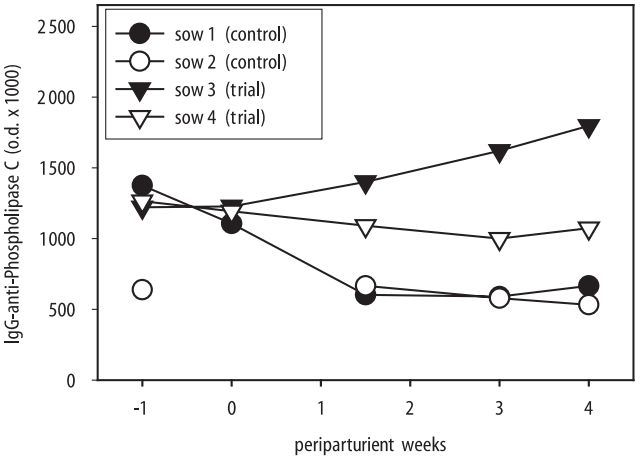

Fig. 2 IgG-anti-phospholipase C (C. perfringens) in blood from sows with and without lactulose application

Field trial

Table 2 shows the results of the performance of control and trial sows. Interestingly, the length of pregnancy was significantly shortened in trial sows than in control sows. We could also show that the weaned piglets of the trial group were heavier than those of the control group. The daily weight gain of weaners 10 days and 35 days after weaning was significantly different between trial and control piglets. The losses of piglets and weaners were reduced by 3.2 %.

In our bacteriological investigation of sows' faeces we found a significant difference in total bacterial counts and in C. perfringens counts 20 days after birth. The results are shown in Figs. 3 and 4. Gram-negative bacteria were reduced one day after birth (Fig. 5). The investigations of immunological parameters of colostral and ripe milk showed that the CRP levels in milk were not different in trial and control sows.

IgG-antibodies to C. perfringens PLC decreased from birth to 10 days after birth in the trial group but remained in trial sows at a higher but not significantly different level in comparison to control sows. IgM-antibodies to C. perfringens PLC were higher in control sows in comparison to trial sows. The results are shown in Fig. 6. IgG-anti-LPS (E. coli, J5, Rc mutant) were significantly higher in milk of trial sows 10 days after birth (Fig. 7).

Table 2 Influence of lactulose SOLVAY on important performance parameters per sows (field trial)

Parameter	Trial group (n = 71)		Control group (n = 68)	
	0	s	0	s
Duration of pregnancy	114[a]	1.1	115.5[b]	1.3
Alive born piglets (n)	10.06	3.62	9.74	3.82
Dead born piglets (n)	1.54	1.90	1.32	2.31
Litter mass (kg)	17.70	5.23	16.94	4.75
Weaned piglets 21 days (n)	8.06	1.98	7.72	2.01
Weaned piglets 21 days (kg)	40.62	12.8	38.90	3.2
Losses of piglets (%)	19.9		21.9	
Daily weight gain Weaners 35 d (g)	387[a]	90	375[b]	83
Losses of weaners (%)	3		4.6	

Significant differences a/b

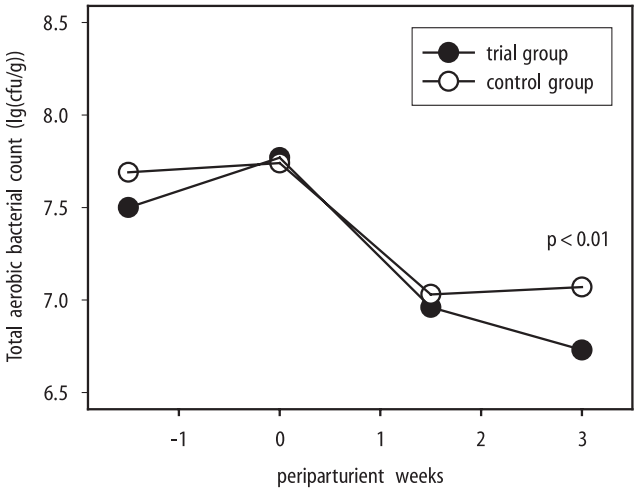

Fig. 3 Total aerobic bacterial count in faeces of sows with and without lactulose application

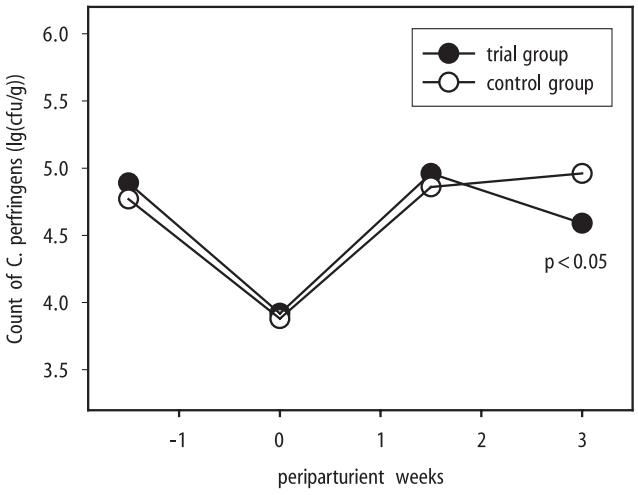

Fig. 4 Count of C. perfringens in faeces of sows with and without lactulose application

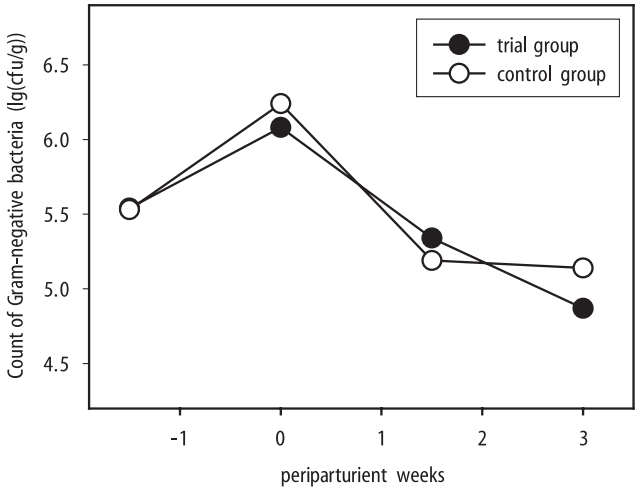

Fig. 5 Count of Gram-negative bacteria in faeces of sows with and without lactulose application

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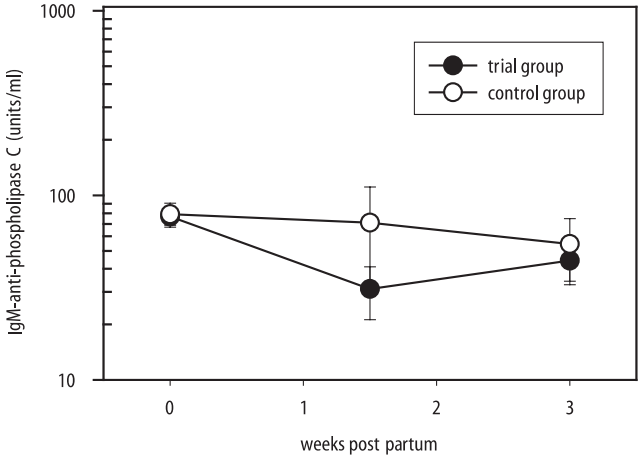

Fig. 6 IgM-anti-phospholipase C (C. perfringens) in milk of sows with and without lactulose application

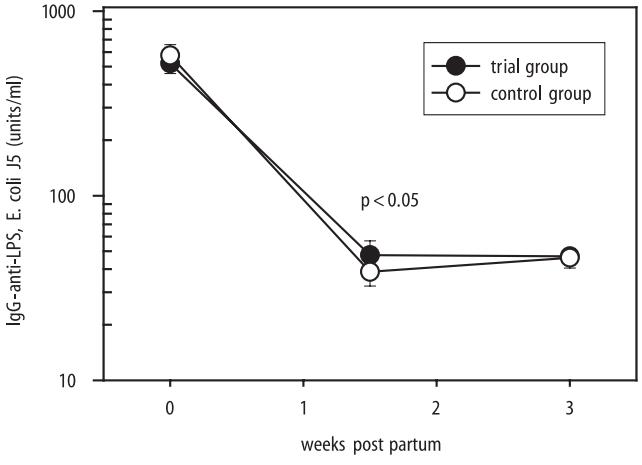

Fig. 7 IgG-anti-LPS (E. coli J5, Rc mutant) in milk of sows with and without lactulose application

Discussion

Fuller and Gibson [4] divided the microorganisms of the intestines into groups with health-promoting functions and those with a harmful and pathogenic effect. Numerous scientists investigated the health-promoting effect of prebiotics like indigestible sugars, e. g. fructooligosaccharides, inulins and lactulose [3, 18]. The positive effects of lactulose on colonic metabolism is well known [20–23]. Greve et al. [23] and Liehr and Heine [24] reported about the inhibitory effect of lactulose on TNF α-production of monocytes stimulated by endotoxin.

With our results we could show like others [3] that lactulose influences bacterial microorganisms in faeces. Interestingly, we found significant effects in total aerobic bacterial counts and in C. perfringens counts 10 days after finishing the lactulose supplementation (Figs. 3 and 4). C. perfringens counts were highly reduced at parturison in both groups because of drastic reduction of food. The results of Gram-negative bacteria were different (Fig. 5). At parturison we found a significant difference between the two groups. At day 20 after birth these results were not significant.

With our results concerning antibodies to C. perfringens PLC we think we have found a new mechanism of lactulose. We could show that the sows developed high antibody levels to C. perfringens PLCs and provide their piglets with these antibodies (IgG) by colostral milk. Thus, PLCs of bacteria and antibodies against them must have an importance for animals. We believe that antibodies to PLCs of bacteria protect the host from their effects in all kinds of phospholipids occurring in the GIT. The periparturient supplementation of sows with lactulose influenced the utilization of PLC-antibodies by PLCs. Recently, we could also show that in vitro cultivation of C. perfringens with lactulose inhibited the expression of PLC (unpublished). This means that lactulose prevents C. perfringens from environmental stress like nonphysiological pH, lack of water, energy stress, and final products.

PLCs were not produced or at a lower level. We only show the lactulose effect on one bacterium species. As written above, other Gram-positive and Gram-negative bacteria of the GIT have the same self-protection system to defence environmental stress. We think that lactulose supplementation protects bacteria from this environmental stress [10, 25]. PLCs also cut glycosylphosphatidylinositol anchor molecules. The alkaline phosphatase (AP) is a membrane-bound ectoenzyme present in many organs, including the intestine. Poelstra et al. [26] reported about detoxification of bacterial endotoxin by AP. This function of AP is abolished by phosphatidylinositol cutting PLCs [10, 8]. In our investigations we could not find a significant difference between AP activity in faeces of either animal group (data not shown). On the other hand we demonstrated a significant difference in IgG-anti LPS (J5)-antibodies of milk (Fig. 7) 10 days after birth. We explain this with a lower utilisation of those antibodies.

Our results show that the lactulose supplementation in periparturient sows leads to better results of sows and their piglets. Especially the significant reduction of the length of pregnancy in the trial group, reduced losses of their piglets and weaners, significant daily weight gains of weaners produce evidence of usefulness of lactulose in periparturient sows. Considering the results of the field trial we suggest a higher daily dose of lactulose. We think that about 80 ml per day and sow are necessary to show more significant differences, e. g. in IgG antibodies to C. perfringens PLC, performance parameters like losses of piglets, numbers of alive born piglets, and numbers of weaned piglets.

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