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Rapport 62

Effect of several plant products on prevention of *E. coli* adhesion in the gastrointestinal tract of weaned piglets

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WAGENINGEN UR

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In Nederland vindt het meeste onderzoek voor biologische landbouw en voeding plaats in voornamelijk door het ministerie van LNV gefinancierde onderzoekprogramma's. Aansturing hiervan gebeurt door Bioconnect, het kennisnetwerk voor de Biologische Landbouw en Voeding in Nederland (www.bioconnect.nl). Hoofduitvoerders van het onderzoek zijn de instituten van Wageningen UR en het Louis Bolk Instituut. Zij werken in de cluster Biologische Landbouw (LNV gefinancierde onderzoeksprogramma's) nauw samen. Dit rapport is binnen deze context tot stand gekomen.
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Abstract
A challenge experiment showed that there was a substantial difference in effect of several plant products in the diet on response of *E. coli* infected pigs

Key words
Piglets, *E. coli*, anti-adhesion, plant products, Safewastes

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Samenvatting
Een challengeproef met biggen liet zien dat enkele plantenstoffen een celgetal-verlagend effect hadden op *E. coli* in het maagdarmkanaal van biggen

Trefwoorden:
Biggen, *E. coli*, anti-adhesie, plantenstoffen, Safewastes

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Effect of several plant products on prevention of *E. coli* adhesion in the gastrointestinal tract of weaned piglets

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Preface

This research was initiated by the EU project Safewastes ‘Evaluating physiological and environmental consequences of using organic wastes after technological processing in diets for livestock’. This is a project within the sixth framework programme of the EU. The partners contributing to this project come from Austria, Belgium, Germany, Greece, Italy, Poland and The Netherlands.
The Dutch Commodity Board for Feeds and the Dutch ministry of Agriculture, Nature and Food Quality financially contributed to the experiment described in this report. As some of the products may be interesting for patenting, they are reported under code (SW7 and SW11).

A.W. Jongbloed
(Project leader)

Samenvatting

Er is een challengeproef uitgevoerd met zes verschillende voeders. Voor elk voer zijn 12 individueel gehuisveste biggen ingezet. De volgende voeders werden gebruikt: 1) Basisvoer (BD; negatieve controle); 2) BD + Gistproduct (positieve controle, 2,5 g/kg voer); 3) BD + Product SW7 (25 g/kg voer); 4) BD + Product SW11 (25 g/kg voer) 5) BD + Sesamzaadschilfers (25 g/kg voer); 6) BD + Tijm/Carvacrol (*Origanum spp.*) preparaat met een concentratie van 8%; 1,0 g/kg voer). De keuze van de onderzochte producten in de eerste vijf behandelingen was gebaseerd op de resultaten van een *in vitro* onderzoek van Becker et al. (2006), en omvatte een range van de *in vitro* adhesiecapaciteit voor *E. coli* K88 van hoog naar laag. De Tijm/Carvacrol behandeling werd uitgevoerd op verzoek van het Nederlandse ministerie van Landbouw, Natuur en Voedselkwaliteit.

De voeders waren gepelleteerd en werden ad libitum verstrekt van dag 2 na spenen (op een leeftijd van circa 28 dagen) tot het eind van het experiment op dag 22 na het spenen. Op dag 7 heeft men de biggen oraal geïnoculeerd met ETEC (*E. coli* O149 K91+ K88ac+ (F4ac)). De voeropname en de faecale consistentiescore zijn elke dag geregistreerd, het levend gewicht werd bij de start, op dag 6, 13 en 22 gemeten. Van dag 8 tot dag 19 werd de faecale uitscheiding van *E. coli* bepaald. Op dag 21 werden bloedmonsters genomen. Op dag 22 werden de biggen opgeofferd en werd schraapsel van de mucuslaag van de dunne darm bemonsterd voor bepaling van de receptorstatus van de biggen voor *E. coli*. Daarnaast werden chymusmonsters uit het coecum genomen voor bepaling van de bacteriële samenstelling. De voeders werden geanalyseerd op Weende analysecomponenten, mineralen, zetmeel en suikers. Daarnaast is het basisvoer en de experimentele producten geanalyseerd op de oplosbare suikers en op de suikers in het Neutral Detergent Residue.
Analyseresultaten van de voeders lieten zien dat de chemische samenstelling vrijwel gelijk was voor wat betreft Weende analysecomponenten en mineralen, maar de samenstelling van de specifieke suikers in de experimentele producten was duidelijk verschillend.
Eén dier werd uit de proef genomen op dag 13 als gevolg van slechte conditie. Vier van de 72 biggen bleken receptornegatief te zijn voor *E. coli* K88, die volgens groot toeval allemaal in de behandeling met sesamzaadschilfers waren. Daarom werd de statistische analyse uitgevoerd zonder deze receptornegatieve biggen. De inoculatie van de dieren met *E. coli* was succesvol. Na inoculatie met *E. coli* was er en aanmerkelijke daling in voeropname bij alle behandelingen. Deze daling in voeropname was verschillend tussen de behandelingen (negatieve controle had de grootste daling en de positieve controle de minste). De daling in voeropname was min of meer parallel aan de *E. coli* uitscheiding in de faeces, wat is geëvalueerd met een longitudinale analyse. Er waren ook significante verschillen in de feces consistentiescore (negatieve controle het slechtst en de positieve controle het best). Bacterietellingen in de caecale monsters lieten geen verschillen tussen de behandelingen zien in die van de aerobe, anaerobe, Enterococci, Lactobacilli en Coliforme bacteriën. Ook waren er geen verschillen in de diverse bloedparameters tussen de behandelingen. Conclusie: de bevindingen van het *in vitro* onderzoek naar de bindingscapaciteit van plantaardige componenten voor *E. coli* zijn voor een groot deel bevestigd in het *in vivo* model met de biggen. Het Gistproduct en de Sesamzaadschilfers hebben een celgetal-verlagende werking op *E. coli* in het maagdarmkanaal van biggen evenals het Tijm/Carvacrol product.
De biggen waren niet van biologische oorsprong en werden niet onder biologische houderijomstandigheden gehouden. De vraag kan gesteld worden of het effect van de *E. coli* infectie bij de verschillende proefvoeders anders zou zijn dan verkregen met de conventionele biggen in deze proef. Er is geen enkele aanwijzing dat het effect van de proefbehandelingen niet zou gelden voor biologisch gehouden biggen.

Summary

A challenge experiment was carried out which comprised six treatment groups each consisting of 12 individually-housed piglets. Each group received one of the following pelleted diets: 1) Basal Diet (BD; Negative control); 2) BD + Yeast product (Positive control, 2.5 g/kg diet); 3) BD + Product SW 7 (25 g/kg diet); 4) BD + Product SW 11 (25 g/kg diet) 5) BD + Sesame seed expeller (25 g/kg diet); 6) BD + Thyme/Carvacrol (*Origanum spp.*; 1.0 g/kg diet). The choice of the products used in the first five treatment groups was based on the results of an *in vitro* study by Becker et al. (2006), and comprised a range of the *in vitro* adhesion capacity to *E. coli* K88+ from high to low. The Thyme/Carvacrol treatment was carried out on request of the Dutch Ministry of Agriculture, Nature and Food Quality.

The experimental diets were given ad libitum from day 2 until the end of the experiment at day 22 post-weaning. At day 7, the piglets were inoculated orally with ETEC (*E. coli* O149 K91+ K88ac+ (F4ac)). Daily feed intake and faecal consistency score were registered all days, body weight was measured at start, days 6, 13 and 22. From day 8 to day 19 the faecal shedding of *E. coli* was determined. At d22 piglets were sacrificed and scrapings of the small intestine mucus layer were sampled and assessed for the receptor status of *E. coli*. In addition, chyme samples were taken from the caecum for assessing bacterial composition. Furthermore, blood chemistry was assessed at d21. Diets were analysed for proximate nutrients, minerals, starch and reducing sugars. In addition, the basal diet and experimental products were analysed for soluble sugars and sugars in the Neutral Detergent Residue.

Results showed that the experimental diets were quite similar in their chemical composition for both proximate analyses and minerals, but analyses on specific sugars in the experimental products gave substantially different results. One animal was withdrawn from the experiment at d13 due to bad condition. Four piglets out of 72 showed to be receptor negative for *E. coli* K88, which by chance were only in one treatment group. Therefore, statistical analysis was performed without these receptor-negative piglets. The inoculation of the animals was successful. After inoculation with *E. coli,* there was a substantial drop in feed intake for all treatments. This drop in feed intake was different among the treatments (Negative control most decrease, Positive control least decrease). This was more or less parallel with *E. coli* shedding in the faeces, which was evaluated with a longitudinal analysis. There were also significant differences in faecal score (Negative control worst and Positive control best). Bacterial evaluation of the caecal samples showed no differences between treatments in bacterial counts of total aerobes, anaerobes, Enterococci, Lactobacilli and coliform bacteria. Also, blood chemistry results showed no differences among treatments. It is concluded that the *in vitro* findings on the binding capacity of the plant materials to *E. coli* were confirmed to a large extent in the *in vivo* model with the piglets. The Yeast product and the Sesame seed expeller have a cell count-reducing effect on *E. coli* in the gut of piglets as well as the Thyme/Carvacrol product.

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1 Introduction

New safe and effective additives have been investigated since the EU enacted the ban on in-feed antibiotics (Stein, 2007). The research activity, however, is still expanding because of the controversial results emerged in several tested products and the seeking of new alternatives.
In the present study, different plant materials were tested in weaning piglets with the aim to match the purpose of the EU-Project Safewastes to test these products to enhance health of livestock. In the same context, our group has tested *in vitro* several plant materials to evaluate the binding capacity of these products for gastrointestinal bacteria isolated in pigs, poultry, veal calves and man. The *in vitro* study revealed promising results for some materials comparable with commercial products (Becker et al., 2006; 2007). Despite these positive effects, the data have to be confirmed in *in vivo* trials. In this experiment this was tested in piglets.
The tested products were four different plant materials and one yeast product. The proposed hypothesis is that the carbohydrates of these products can function as alternative adhesion matrices for *E. coli* K88, inhibiting bacterial adhesion to the gastrointestinal receptors. In order to examine the efficacy of four different plant materials in diets for piglets against enterotoxigenic *E. coli* (ETEC), a challenge experiment with weaning piglets was carried out.
The objectives of this study in ETEC challenged weaning piglets were to determine if 1) the positive effect obtained in an *in vitro* experiment of three plant materials (Product SW 7, Product SW 11 and sesame seed expeller) and the yeast product to bind ETEC is confirmed in the *in vivo* model on ETEC faecal shedding; 2) the addition of several other plant materials to a piglet diet has an effect on reducing ETEC faecal shedding after ETEC oral infection; 3) addition of a thymol/carvacrol formulation has an effect on reducing *E. coli* K88 faecal shedding after ETEC oral infection.
First, a literature review is presented on the use of herbs in animal diets to improve animal health, after which the results of the animal experiment are described.

2 Literature review

2.1 General

For thousands of years, herbs and spices have provided distinctive flavouring properties to foods and many have been proven as potent antimicrobial agents. The use of these substances was very common in both western and eastern culture. Some of the most common plant products known for their antimicrobial properties belong to the genus *Allium* as garlic, onion and leek; others are thyme, oregano, marjoram, basil and cumin. The natural plant antimicrobials are found in barks, roots, stems, leaves, flowers and fruits of many plants.
In recent years, *in vitro* studies demonstrated the antibacterial activity of some of these products (Sen et al., 1998; Friedman et al., 2002) and more recently, the supplementation of plant products to pig diets has been proposed in order to counteract intestinal disorders, especially in the weaning period. However, the effects on growth performance and gut health are not so consistent, which may depend on the plant product tested.
One of the main issues concerning plant products is their characterization (Cowan, 1999). Plant products contain several different active compounds in different concentrations, and in case of processing, their composition is largely affected by the method of extraction (solvent and extraction conditions) and the niche of the plant used related to plant variety and age, climatic conditions and geographic origin. Characterization is important for scientific as well as for legal purposes.
All characteristics affecting plant product composition affect at the same time their biological effects. Hence, for scientific purposes, it is better to work with pure active substances or with accurately controlled blends.
With regard to legislation, traceability, and thus characterization, it is one of the main prerequisites to register additives. It is, therefore important to accurately assess all the different compounds that the product contains.

2.2 Effect of plant products on microbiota

Plant products have been used intensively in the past for medical and food preservation purposes. The use is mostly motivated by their antimicrobial activity (Didry et al., 1994). This aspect has been studied in several *in vitro* studies with promising results. The application in modern animal husbandry is rather recent and there is too limited information available about the actual possibilities of these products. However, the legislative requirements and the great interest to this sector are motivating the appearance of the first studies using plant products in vivo (Evans and Martin, 2000; Manzanilla, 2006).
It is difficult to define the antimicrobial or different active compounds present in a plant product. Usually, antimicrobial substances in plant products have very different chemical structures, with high occurrence of phenolic rings, mostly hydrophobic and some of them with similar structure to important molecules from bacterial metabolism such as receptors or enzyme substrates (Cowan, 1999). It also known that many of these substances are secondary metabolites that plants use against predators, or with different functions such as pigmentation, aromatizing or flavouring.
Table 1, adapted from Cowan (1999), presents the principal chemical structures producing antimicrobial activity in plant products and the mechanism of action referenced so far. Some of these effects need to be better investigated while some of them are more studied such as the hydroxyl group (-OH) present in phenolic compounds. The importance of this group on antimicrobial activity is well known (Cowan, 1999) and any variation in its position inside the molecule, as it happens between carvacrol and thymol, produces marked differences in antimicrobial power (Dorman and Deans, 2000). Components with phenolic structures, such as carvacrol and thymol are highly active against tested bacteria despite their low capacity to dissolve in water. The importance of the hydroxyl group in the phenolic ring was confirmed in terms of activity when carvacrol is compared to its methyl ester. The high activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenolic nucleus, which is known to enhance the antimicrobial activity of phenols. The introduction of alkylation has been proposed to alter the distribution ratio between the aqueous and non-aqueous phases, including bacterial phase, by reducing the surface tension or altering the species selectivity.
It was suggested that plant products act via two main mechanisms of action. The first is related to the general hydrophobicity of plant products, which facilitates their adhesion to the bacterial surface inducing unstabilization (Tsuchiya et al., 1996). The second mechanism is the inactivation of different molecules of the bacteria (such as enzymes or receptors) by their adhesion to specific sites (Sharon and Ofek, 1986).

Table 1 Chemical structures implicated in antimicrobial effect of PP and related mechanism of action (Cowan, 1999)

Class	Subclass	Mechanism of action
Phenol compounds	Simple phenols and phenolic acids	Enzyme inactivation (1)
		Membrane un-stabilizers (2)
	Quinones	Irreversible adhesion to adhesins,
	Flavonoids, flavones and flavonols	membrane polypeptides and enzymes that become inactive (3)
	Tannins	1,2,3 and metal chelators
	Coumarins	Interact with eukaryote DNA
Terpenoids		Membrane un-stabilizers
Alkaloids		Insertion in cellular wall or in DNA structures
Lectines and polypeptides		Block viral fusion and adsorption
		Di-sulphur bridges formation
Poliacetilens		???

Some authors suggested a higher efficacy of plant products against gram negative organisms but others did not find any difference between gram positive and negative bacteria and, sometimes even the opposite effect was proposed. Actually, it is possible that some plant products have specific actions and other plant products have diverse effects due to the different nature and composition. In fact, as it happens for antibiotics, the chemical structure will determine the mode of action and hence a possible selective effect of the plant product. For instance, alkalic chains plus a phenol group seem to perform better activity against gram negative bacteria, due to the characteristic of their cellular wall. In any case, the specific effect of some plant products could be interesting in therapeutic or preventive applications like with antibiotics.
The effect of plant products on different bacterial species has been determined in various *in vitro* studies using spectrophotometric measurements or agar plate inhibition rings. Many of these studies have explored the real antimicrobial power of classic herbal products or spices (Dorman and Deans, 2000; Friedman et al., 2002).
From the latter studies we can draw some conclusions. First of all, different bacteria have different sensitivity to different plant products. It can be also observed how some compounds as α-terpinen are highly effective against a very interesting target as Salmonella but not against the other micro-organisms.

Table 2 Antibacterial activity of different essential oils against 25 different bacteria tested (Lis-Balchin, 2003)

Essential oil	Antibacterial activity on 25 bacteria
Peppermint	15-22
Petitgrain	21
Pine niddle	19
Rosewood	24
Rosemary	21
Sage, Dalmatian	16
Tea tree	24
Thyme	14-25
Verbena	18

Moreover some plant products show very different results than their main components as in the case with thyme and thymol. These variations are due to synergisms or interferences with other substances present in the plant products (Table 2).
In the application of plant products *in vivo* it is important to consider the dosage. Compared to antibiotics, the *in vitro* dose of PP to obtain similar effect is normally 10- to 100-fold higher (Karaman et al., 2001). So far, we know that complex media as the chyme in the digestive tract could affect the *in vitro* effective dosage.

2.3 Receptor analogs as anti-adhesive agents

There is evidence that receptor analogs as agents for anti-adhesion therapy would be practical primarily against pathogens that bind to animal cells via carbohydrate-specific adhesins (i.e. lectins). In this case the receptor analogs are saccharides that are structurally similar to those of the glycoprotein and glycolipid receptors for the adhesins and, therefore, act by competitive inhibition. Less than three decades ago mannose was first shown to be a receptor for enterobacteria (Ofek et al., 1977). Since then, the sugar speciations of many bacteria have been determined, leading to the development of receptor-like carbohydrates, which inhibit the adhesion of pathogens to host cells and tissues (Ofek et al., 2003; Table 3). The concentration of the carbohydrates required for effective inhibition of adhesion *in vitro* are usually in the millimolar range, because the affinity of the saccharides for the bacterial lectins is low. It can be increased several orders of magnitude by covalently linking a hydrophobic residue such as phenyl or methyl umbelliferyl to the saccharide (Firon et al., 1987). Affinity can be similarly increased by attaching many copies of the saccharide to a suitable carrier, yielding multivalent adhesin inhibitors, as demonstrated for type 1 fimbriated *Escherichia coli* (Lindhorst et al., 1998).

Table 3 Carbohydrates preventing bacterial colonization and/or infection *in vivo* (modified from Ofek et al., 2003b)

Organism	Animal, site of action	Inhibitor
C. jejuni	Mouse intestine	Milk oligosaccharides
E. coli, type 1 fimbriated	Mouse GIT	Mannose
E. coli, P fimbriated	Mouse urinary tract	Globotetraose (Galα1,4Gal)-containing GP*
E. coli K99	Calf GIT	glycopeptides
H. pylori	Piglet GIT	Sialyl-3'-LacNAc
Shigella flexneri, type 1 fimbriated	Guinea pig eye	Mannose
S. pneumoniae	Rabbit and rat lungs	Sialyl-3'-Galβ(1$\rightarrow$4)LacNAc
S. sobrinus	Rat oral cavity	Oxidized α1,6 glucan
S. pyogenes	Mouse pharynx	Hyaluronan

*Gp: glycoprotein found in dove and pigeon egg white

The feasibility of using saccharides to protect against experimental infections by bacteria expressing adhesive lectins was first demonstrated more than two decades ago. Administration of methyl α-mannoside together with *E. coli* expressing the mannose-specific type 1 fimbrial lectin into the bladders of mice reduced the extent of bladder colonization by uropathogenic *E. coli* by about two thirds compared to animals that had received the bacteria alone or with methyl α-glucoside, a sugar that does not inhibit the mannose-specific bacterial lectin. Subsequently many studies have confirmed the ability of saccharides to prevent experimental infections caused by different pathogenic bacteria in a variety of animals (Table 4).

Table 4 Anti-adhesin activity of plant constituents (modified from Ofek et al., 2003)

Plant	Constituent	Bacterium affected
Azadirachta indica (neem stick)	ND	*S. sanguis*
Camillia sinensis (green tea)	(-) epicatechin gallate, (-) gallocathechin gallate	*P. gingivalis*
Oolong tea	polyphenol	*S. mutans; S. sobranus*
Gilanthus nivalis (snowdrop)	Mannose-sensitive lectin	*E. coli*
Gloipeltis furcata and *Gigartina teldi* (seaweeds)	Sulphated polysaccharides	*S. sobrinus*
Hop bracht	Polyphenols (36-40 kDa)	*S. mutans*
Melaphis chinensis	gallotannin	*S. sanguis*
Persea americana (avocado)	tannins	*S. mutans*
Legume storage protein	glycoprotein	*E. coli*

ND: not determined

2.4 Dietary inhibitors of adhesion

Some of the most efficient anti-adhesion agents identified so far are present in feedstuffs. Feedstuffs containing either a mixture of inhibitors or an inhibitor with a broad spectrum of activity could be especially effective. While it may be possible to find suitable inhibitors for particular pathogens, it is unlikely that it will be possible to match every individual or group of pathogens with specific diets that contain complementary adhesin inhibitors (Ofek et al., 2003). However, caution should be used, because some dietary components may also be bactericidal and selective pressures imposed by such compounds are undesirable and should be avoided. Human milk and plant-derived constituents are rich in oligosaccharides and related compounds to which many bacteria bind.

Because of their ready availability, plant materials possessing anti-adhesion activities are attractive candidates for antibacterial agents. There is, however a relative paucity of information regarding the anti-adhesive properties of most plant materials. Although plant lectins are well represented in the diets, and many of these lectins are very characteristic, their application to anti-adhesion therapy is very limited. Theorically, these lecitins could interact with animal cell surface saccharides to block adhesion mediated by lectin-carrying bacteria and they may enhance clearance of bacteria from the host (Slifkin and Doyle, 1990).
Feed lectins may have deleterious effects as well. They may bind to mucosal cells and thus function as receptors for bacterial glycans and enhance bacterial adhesion to the tissue. Moreover, some dietary lectins may reach the GI tract in a functional form and may similarly enhance the binding of the bacteria to the different parts of the intestine.
A practical advantage in the search of dietary plant extracts for agents to use in the therapy of bacterial infections is that clinical trials are probably easier to perform, mainly because toxicity is usually not as much of an issue. Among the plant extract listed in Table 4, those obtained from *Vaccinium macrocarpon* (cranberry) are the most thoroughly studied with respect to their anti-adhesion activity *in vitro* and, so far, are the only ones that showed to be effective in *in vivo* trials. The initial *in vitro* experiments on the effects of cranberry extracts on bacterial adhesion were stimulated by the long known anecdotal evidence on the beneficial effects of cranberry juice consumption in therapy of urinary tract infections.
Several lines of evidence implicate two different cranberry constituents as active anti-adhesive agents. One of these is a high molecular mass (> 15 kDa) material and the other is a protoanthocyanin. The high molecular mass material is devoid of proteins and carbohydrates and behaves in some respects like tannin (Ofek et al., 1996). It inhibited the adhesion to animal cells of uropathogenic *E. coli* including P fimbriae-, S fimbriae- and non-fimbrial adhesin I (NFA-I)-expressing strains, but did not act on the adhesins of diarrhoeal *E. coli* (enterotoxigenic *E. coli*), nor did it act on the type 1 fimbrial lectin. In addition it inhibited the co aggregation of Gram-negative pairs of oral bacterial more often than it inhibited co aggregations between Gram positive bacteria. Extracts containing protoanthocyanins in their condensed form inhibited adhesion of P fimbriated *E. coli* to erythrocytes.

Table 5 Anti-adhesion effects of juices or extracts from Vaccinium spp. (cranberry). From Ofek et al., 2003 (modified)

Bacterium	Disease	Adhesion assay	Effect
E. coli	UTI and pyelonephritis	HA, UroEp.	Inhibition
E. coli	Diarrhea	HA	No inhibition
E. coli	Meningitis	HA	Inhibition
Oral bacteria	Dental decay periodontitis	Co aggregation and buccal epithelial adhesion	Inhibition
H. pylori	Gastric ulcer	Human gastric mucus, TC cells	Inhibition

I or NI: inhibition or no inhibition of adhesion by either cranberry juice or by a high molecular mass
TC: tissue culture cells; HA: hemagglutination assay; UroEp.: uroepithelial cells

2.5 Conclusion

The literature review shows that many plant products have shown to exert antibacterial effects *in vitro*. This may be related to phenolic compounds which are present in many essential oils. Also several plant products may have anti-adhesion properties which are related to oligosaccharides and related compounds. In this respect the effect of cranberry on bacterial adhesion has been most widely studied in man. Most studies with plant products have been carried out with laboratory animals on preventing bacterial colonization and/or infection with pathogenic bacteria. This needs to be confirmed in studies with pigs who receive a much more complicated diet than laboratory animals.

3 In vivo experiment on piglets

3.1 Material and methods

3.1.1 Experimental treatments

The experiment comprised six treatment groups each consisting of 12 individually housed piglets. Each group received one of the following diets: 1) Basal Diet (BD; Negative control); 2) BD + Yeast product; 3) BD + Product SW 7; 4) BD + Product SW 11; 5) BD + Sesame seed expeller; 6) BD + Thyme/Carvacrol (*Origanum spp.*) (Table 6). The choice of the products used in the first five treatment groups was based on the results of the *in vivo* study (Becker et al., 2006), and comprised a range of the *in vitro* adhesion capacity from high to low. Treatment 6 was carried out on request of the Research Group on Biological Farming of the Dutch Ministry of Agriculture, Nature and Food Quality.

The experimental diets were given ad libitum from day 2 until the end of the experiment at day 22 post-weaning. At day 7, all the piglets were inoculated orally with ETEC (*E. coli* O149 K91+ K88ac+ (F4ac)).

Table 6 Experimental treatments

Group	Treatment
I	Negative control treatment, basal diet (BD)
II	BD + Yeast Product
III	BD + Product SW 7
IV	BD + Product SW 11
V	BD + Sesame seed expeller (organically grown)
VI	BD + Thyme/Carvacrol (*Origanum spp*)

3.1.2 Animals and Housing

The experiment was conducted in the Experimental Unit of the Animal Science Group of Wageningen UR, at Lelystad. The experimental protocol was approved by the Ethical Committee of the Animal Sciences Group. A total of 78 piglets (72 piglets for the experiment + 6 spare piglets) from a commercial herd were used (Tempo × (Finnish Landrace × Great Yorkshire$_{\text{sow line}}$). The piglets originated from 13 litters (per litter 3 castrates and 3 gilts).The breeding sows were not vaccinated against ETEC. The piglets were weaned at 4 to 5 weeks of age, 7.0 ± 0.18 kg of BW, and were housed in an experimental unit equipped with automatic heating and thermostatically controlled ventilation.

Picture 1 Overview of the experimental unit

On the weaning day, based on weight, litter and gender, the 78 piglets were selected and transported to the experimental unit and allocated individually into 72 pens (6 pens with 2 piglets). On day 6, in the pens with 2 piglets, those littermates were sacrificed that had the worst ADFI and ADG. Each of the 6 treatment groups consisted of 12 replicates (pens) with 1 pig. Pen size was 53.5 × 85 cm and equipped with a Tenderfoot floor, which was approximately 25 cm above the concrete floor. Each pen had a feed bin and a water nipple.

Picture 2 View of the pen

The room temperature at weaning was kept at 24 °C and was lowered to 22 °C after 3 days. Temperature (minimum/maximum) and relative humidity were registered daily. During the experiment, faeces were removed from the pen once a day and the use of water to clean the pen was omitted to reduce the risk of microbial cross contamination among piglets.

3.1.3 Treatments, Diets and Feeding

Six different diets, with no antibiotics, organic acids, Zn or Cu above physiological requirements were formulated: 1) Basal Diet (BD; Negative control); 2) BD + Yeast product; 3) BD + Product SW 7; 4) BD + Product SW 11; 5) BD + Sesame seed expeller; 6) BD + Thymol/Carvacrol (*Origanum spp*.). The thymol/carvacrol preparation was in a formulation with 80 g essential oil/kg. Details of ingredient composition and calculated nutrients content of the diets are given in Table 7. These diets were formulated based on CVB (2005) and provided all required nutrients for this category of pigs. The experimental products were milled over a 1 mm sieve. It was necessary to add a flavour to the diets in order to mask possible difference in taste. An amount of corn starch was used to exchange the amount of the products under investigation. The basal diet was mixed as one batch (composition diet 1 except maize starch) at Research Diet Services at Wijk bij Duurstede. Thereafter, the diets 2-6 were mixed after addition of 2.5 g/kg of yeast product for diet 2; 25 g/kg of Product SW 7 for diet 3; 25 g/kg of Product SW 11 for diet 4; 25 g/kg of Sesame seed expeller for diet 5; 1.0 g/kg of Thyme/Carvacrol formulation for diet 6. The diets were pelleted without steam (pellet diameter 3.0 mm).

Animals had *ad libitum* access to feed and fresh drinking water. The first four days Colistin (60 mg Colistin sulphate/litre of water) was added to the drinking water to improve successful inoculation of ETEC.

Table 7 Formulation and estimated nutrient contents of the basal diet (g/kg, as-fed)

Ingredients	Amount
Wheat	400.0
Barley	298.0
Soybean meal	150.0

[a] The premix supplied per kg diet: Cu 20 mg; Mn 30 mg; Zn 65 mg; Fe 150 mg; Se 0.3 mg; I 0.5 mg; Co 0.15 mg; choline chloride 150 mg; Vit. A 10,000 IE; Vit. D3 2,000 IE; Vit. E 20 IE; Vit. K3 1.5 mg; Vit. B1 1.0 mg; Vit. B2 4.0 mg; Vit. B12 20 µg; Vit. B6 1.5 mg; Folic acid 0.2 mg; Pantothenic acid 15 mg; Niacin 25 mg; Biotin 25 µg; Antioxidant 50 mg; Aroma 200 mg

3.1.4 Observations and Sample Collection

Individual BW was registered on the day of weaning (d 1), on d 6, d 13 and on the slaughter day (d 22). Individual feed intake was registered daily. Diet samples were analyzed before the experiment started to evaluate the presence of ETEC.

Health status of the pigs was monitored twice daily throughout the experiment. Severity of diarrhoea was characterized using the faecal consistency score (FCS) by De Cuypere et al. (1990). Faecal score (0, normal; 1, soft faeces; 2, mild diarrhoea; 3, severe diarrhoea) was performed twice daily by two trained people who scored apart from each other, with no prior knowledge of dietary treatments allocation was used to ascribe the diarrhoea score of pigs. Faecal score was not done on d 21.

On d 7, the piglets were inoculated orally twice (at 9.00 h and 15.00 h) with a suspension of 5 x 10^9 cfu *E.coli* O149 K91$^+$ K88ac+ (F4ac) (strain 1000; CIDC, Lelystad). The challenge took place via inserting 5 ml of bacterial suspension with a concentration of 10^9 cfu/ml by means of a syringe into the mouth of the piglets.

Rectal samples were collected on d 2 and d 5 to evaluate the presence of ETEC in the piglets possibly originated from the farm where the piglets were born. Fresh faecal samples were collected daily from all piglets after the challenge for 12 consecutive days (d 8-19) to count ETEC. If after two consecutive days no ETEC was counted, no further faeces were collected.

At the end of the experiment (d 22), the piglets were euthanized via application (0.4 ml/kg of BW) of sodium pentobarbital (Nembutal, Ceva Sante Animale) via the ear vein. The pigs were opened immediately from sternum to pubis and the whole gastrointestinal tract was removed and scrapings of the small intestine mucus layer were sampled and assessed for the presence of specific adhesion spots for ETEC (Geenen et al., 2005). In addition, chyme samples were taken from the caecum for assessing bacterial composition.

Blood samples from the anterior vena cava were collected in tubes containing EDTA on d 6, d 9 and d 12 post-weaning to perform a DNA-test in order to evaluate pig's susceptibility to ETEC F4ab/ac. On d 21 blood samples were collected to assess white blood cells, haemoglobin, lymphocytes, mono- and granulocytes.

3.1.5 Analytical Procedures

After grinding, the test products were analysed for contents of dry matter, crude ash, crude protein, total crude fat and crude fibre. The composite feed samples taken at feed production were analysed for dry matter, ash, nitrogen, crude fat, crude fibre, starch and sugars, and minerals in duplicate. Dry matter, ash, nitrogen, crude fat and crude fibre contents were determined after air-drying using AOAC procedures (1984). Starch content was determined enzymatically according to the amyloglucosidase/hexokinase method (NEN 3574). Sugars were determined with a modified Luff-Schoorl method. The Ca, P, Mg, Na, K, Cu, Fe and Zn contents were determined using the inductively coupled plasma atomic emission spectrometry (ICP-AES). Sugars in the test products were determined by HPLC and after preparing the NDR fraction of the test products, the sugars in NDR were analysed by HPLC by the method described by De Jonge et al. (1992). All these analyses were performed by the Chemical and Endocrinological laboratorium of ASG at Lelystad.

Faecal samples per piglet were analyzed for ETEC on Columbia blood agar base plates containing 50 µg/ml Streptomycin, 25 µg/ml Tetracyclin, 50 µg/ml Vancomycin and 5% sheep blood. The plates were incubated during 18-24 h at 37°C. ETEC colonies were counted and expressed as ^{10} log-values cfu/g in the original samples. In cases where no *E. coli* were isolated, the ^{10} log-values was considered to be equal to zero.

The four faecal consistency scores per day were averaged before entering the statistical analysis.

3.1.6 Statistical analyses

Response parameters, except for bacterial count in faeces, were analysed using ANOVA (Analysis of Variance) with Genstat according to the following statistical models:

$$Y_{ij} = \mu + Litter_i + Diet_j + Error_{ij}$$

Where:

Y	= Response parameter
μ	= General mean
Litter	= Effect of litter as block factor (i = 1.2)
Diet	= Effect of experimental diet (j = 6)
Error	= Error term

For each response parameter the *P*-value of the model and the least significant difference (LSD) with $P=0.05$, were calculated. Effects with $P \leq 0.05$ were considered as statistically significant.

Also, a model was run in which the effect of sex and the interaction between sex and diet could be demonstrated.

$$Y_{ij} = \mu + Sex_i + Diet_j + Interaction_{ij} + Error_{ij}$$

Where:

Y	= Response parameter
μ	= General mean
Sex	= Effect of sex (i = 1.2)
Diet	= Effect of experimental diet (j = 6)
Interaction	= Interaction between effect of sex and diet
Error	= Error term

In evaluating bacterial counts in faeces, we used a longitudinal analysis with a Wood curve, which describes the counts in the course of time (Diggle et al., 2002). In this approach, there are three parameters: 1. the intercept, 2. the course of counts in the beginning after inoculation which is an inclining slope, and 3. the course of counts in the second part of the experiment, which is a declining slope. In this approach the coefficients are estimated per pen (piglet). As no faecal count was scored just before inoculation, we took the measurement on the day after inoculation as zero point which was assumed to be equal for all pigs. Therefore, only the second and third parameter are presented.

4 Results

4.1 Chemical analyses of the experimental products and diets

Results of the chemical analyses in the experimental diets are listed in Table 8.

Table 8 Chemical analyses of the complete experimental diets

		Treatments					
	Dimension	Neg. C	Yeast	SW7	SW11	Sesame	Oregano
Dry matter	g/kg fresh	889.4	889.6	889.7	891.2	892.2	884.5
Ash	g/kg fresh	57.3	56.0	57.4	57.0	59.3	56.9
Nitrogen	g/kg fresh	33.7	33.2	33.8	34.2	34.7	33.3
Crude fat	g/kg fresh	29.7	28.7	30.6	30.1	34.3	29.4
Crude fibre	g/kg fresh	29.3	28.1	33.3	34.9	30.5	30.3
Starch	g/kg fresh	414	395	380	373	373	392
Sugar	g/kg fresh	37.0	35.6	35.8	44.6	37.2	36.4
Calcium	g/kg fresh	10.6	10.5	10.3	10.7	10.9	10.4
Magnesium	g/kg fresh	1.5	1.4	1.5	1.6	1.6	1.4
Phosphorus	g/kg fresh	6.6	6.2	6.5	6.7	6.6	6.3
Sodium	g/kg fresh	1.2	1.1	1.2	1.2	1.2	1.2
Potassium	g/kg fresh	6.6	6.3	7.2	7.0	6.7	6.4
Copper	mg/kg fresh	17	21	16	19	19	20
Zinc	mg/kg fresh	60	60	57	57	60	58
Iron	mg/kg fresh	329	284	287	289	287	299

Table 8 shows that the experimental diets were quite similar in their chemical composition for both proximate analyses and minerals. Analyses on specific sugars in the Negative control diet 1 and in the experimental products are listed in Table 9.

Table 9 Chemical analyses of the free sugars in the Negative control diet and some experimental products and the sugars in the NDR fraction

	Fraction	Dimension	Neg. C	Yeast	SW7	SW11	Sesame
Dry matter		g/kg fresh	889.4				
Sucrose	soluble	g/kg fresh	28.8	6.2	43	30	14.3
Glucose	soluble	g/kg fresh	5.3	0.5	94	2.1	20.4
Xylose	soluble	g/kg fresh	-	0	0	-	9.2
Fructose	soluble	g/kg fresh	1.9	0	179	3	17.3
NDR		g/kg fresh	94	112	189	328	104
Glucose	NDR	g/kg NDR	243	230	377	327	238
Xylose [a]	NDR	g/kg NDR	263	317	100	191	low
Mannose [a]	NDR	g/kg NDR	low	low	low	low	391
Xylose+Mannose	NDR	g/kg NDR	263	317	100	191	391
Arabinose	NDR	g/kg NDR	110	174	19	39	82

[a] Xylose and mannose could hardly be distinguished on the chromatogram (at 10.21 and 10.45 minutes, respectively)

Results in Table 9 show large differences in concentrations of free sugars between products SW7 and SW11; in the latter low concentrations were detected of glucose and fructose, while these were high in SW7. Concentrations of free sugars were low in the yeast product. Concentrations of the sugars xylose and mannose were hardly distinguishable in the ND residue. As may be expected, the concentrations of xylose + mannose were high in the yeast product, but also the concentration of mannose was high in sesame seed expeller.

4.2 Animal performance

All the piglets were in good health condition when the inoculation with *E. coli* was applied. However, one or two days after this inoculation, many pigs showed soft faeces or mild diarrhoea. Piglet 1315 of group SW7 showed severe diarrhoea and a slight body weight decrease (0.6 kg) in the week after the *E. coli* inoculation, but a decrease of 2.0 kg in BW in the third week. As performance of this piglet in the last week showed to be an outlier, the results of this animal were removed from d 13 onwards. No medical interventions or treatments were performed and no piglet died during the study.
The initial body weight of the piglets was on average 7.0 kg and the average final weight was 13.2 kg. In Table 10, the body weight, average daily gain, feed intake and feed conversion ratio of the piglets receiving the treatments of the most important periods presented; more details are given in Appendix 1. Statistical analysis showed no significant sex effect or interaction of sex x diet, and therefore, these factors were omitted from the model.

Table 10 Body weight, feed intake (FI; g/d), average daily gain (ADG; g/day) and feed conversion ratio (FCR) of the treatment groups

Days post-weaning	Perfor-mance	Treatment							*P*-value
		Neg. C	Yeast	SW7	SW11	Sesame	Oregano	LSD	Treatment
Day 6 - 13	ADG	89	173	146	123	133	172	67.8	0.42
Day 13 - 22	ADG	601 ^{ab}	585 ^{a}	579 ^{a}	610 ^{ab}	631 ^{ab}	659 ^{b}	63.7	0.06
Day 6-13	FI	324	355	336	321	333	351	52.9	0.66
Day 13 - 22	FI	708 ^{abc}	703 ^{abc}	697 ^{ab}	686 ^{a}	745 ^{bc}	753 ^{b}	53.9	0.02
Day 13 - 22	FCR	1.18	1.21	1.22	1.13	1.20	1.14	0.08	0.14

For both the pre- and post-challenge period, the differences in body weight, average daily gain, feed intake and feed conversion ratio, among dietary treatments, were not significantly different (P>0.05), although from 13-22 d daily growth rate approached significance (P=0.06). The BW was comparable among the groups at both 1 and 2 weeks (d 22) after the *E. coli* challenge. ADG was low during the first week of the study and was on average 78 g (13 piglets lost BW), and only 139 g in the second week (9 piglets lost BW) (Figure 1). During d 6-12, ADG was numerically lowest in the Negative control group (89 g/d) followed by SW11 (123 g/d) compared to the groups Sesame, SW7, Oregano and Yeast (133, 146, 172 and 173 g/d, respectively) (P>0.05). During the last phase (d 13-22) the Oregano group showed numerically highest ADG (659 g/d) and ADG of groups SW7 and Yeast were lowest (579 and 585 g/d, respectively) (P=0.06). The LSD for growth rate is relatively high due to the large variation, and growth rate is, therefore, in this experiment not a very accurate response parameter.
Over the period d 1-6 and 6-13 after piglets' arrival, the average daily feed intake among treatments was comparable (P>0.05; Figure 2). During the period d 13-22 feed intake was significantly different among treatments (P=0.02). Average feed intake in this period in the Oregano group (753 g/d) and Sesame (745 g/d) was higher compared to the SW11 group (697 g/d, P<0.05).
The feed conversion ratio from 13-22 d post-weaning was not significantly different among treatments (P=0.14). FCR of piglets in groups SW11 and Oregano were most favourable (1.13 and 1.14, respectively).

Figure 1 Average daily gain of the piglets in the three periods (treatment 1=negative control, 2=yeast product, 3=SW7, 4=SW11, 5=sesame and 6= oregano)

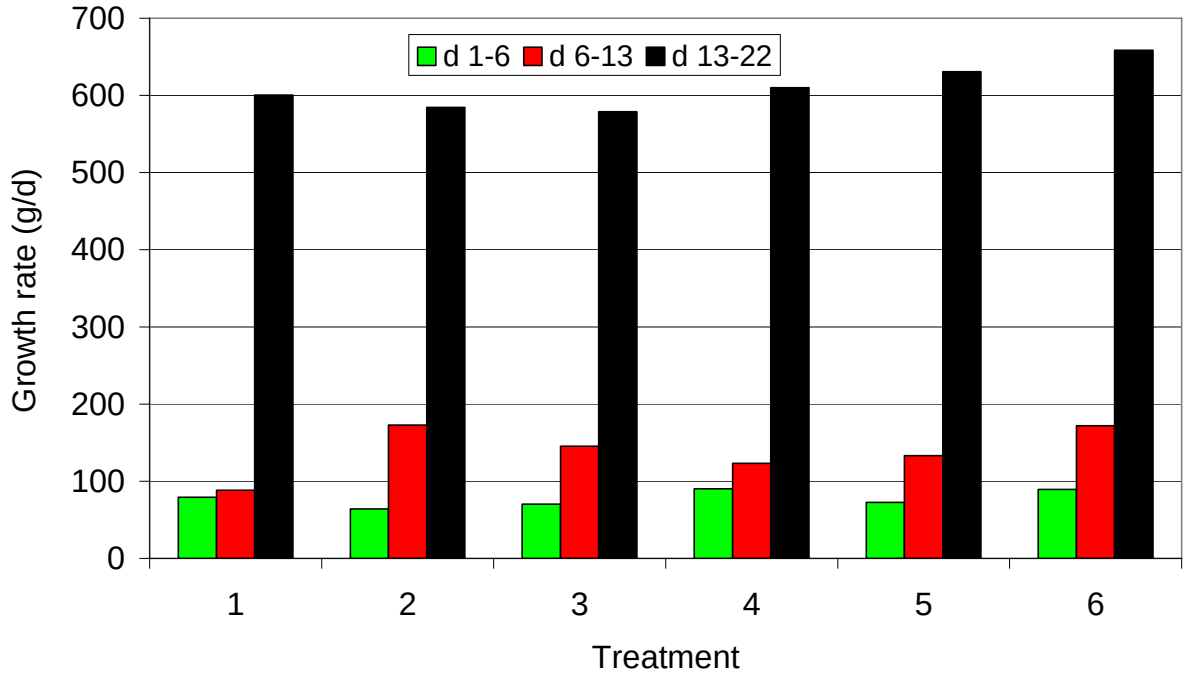
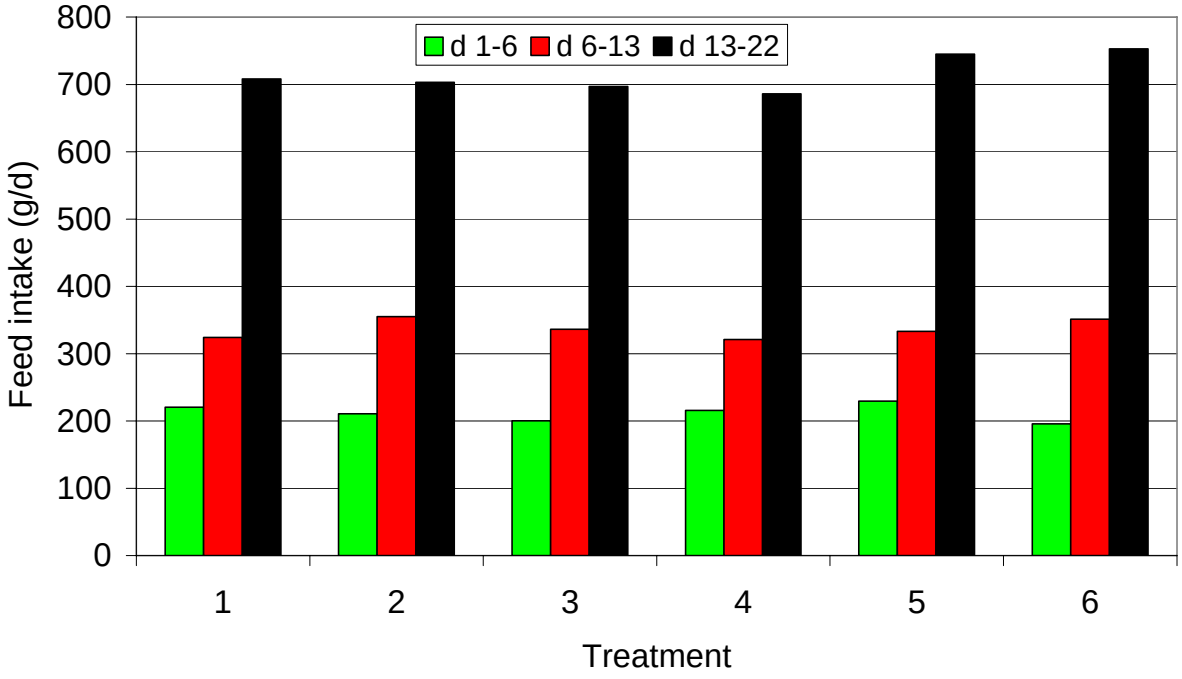


Figure 2 Average daily feed intake of the piglets in the three periods (treatment 1=negative control, 2=yeast product, 3=SW7, 4=SW11, 5=sesameand 6=oregano)



4.3 Faecal consistency

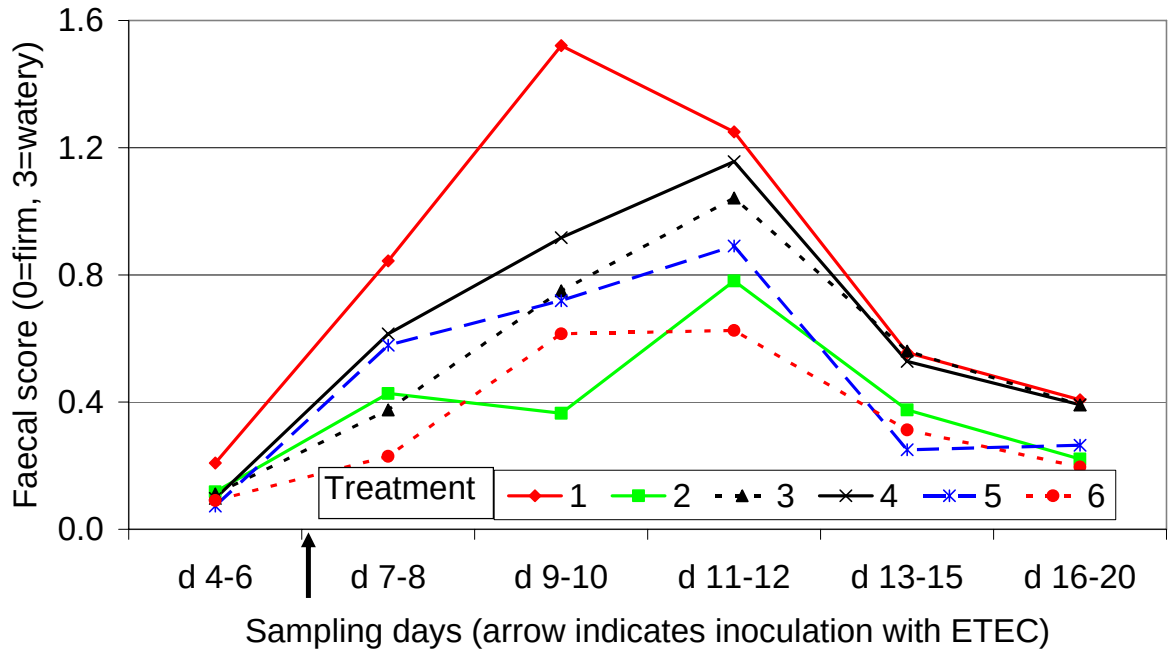
In Table 11, the average faecal consistency score is presented in the three periods from d 4 to d 22 (Figure 3). No difference was observed among dietary treatments in the two days before the *E. coli* challenge. During the first week after inoculation, the groups Yeast and Oregano revealed a significantly lower faecal consistency score compared to the Negative control group ($P<0.05$). From d 13 onwards, the faecal consistency score decreased in all treatments. In this last phase, the Negative control, SW7 and SW11, showed a higher faeces score compared to the groups Sesame, Oregano and Yeast ($P<0.05$).

Table 11 Faeces consistency score* of the experimental treatments[1]

Days post-weaning	Treatment							*P*value
	Neg. C	Yeast	SW7	SW11	Sesame	Oregano	LSD	Treatment
Day 4 - 6	0.20	0.13	0.11	0.10	0.07	0.09	0.11	0.24
Day 6 - 13	1.13^{c}	0.48^{a}	0.72^{ab}	0.84^{bc}	0.65^{ab}	0.47^{a}	0.29	<0.01
Day 13 - 21	0.44^{c}	0.25^{ab}	0.42^{bc}	0.44^{c}	0.23^{a}	0.22^{a}	0.18	0.03

*Score with 0 = normal; 1 = pasty; 2 = thin faeces; 3 = severe watery diarrhoea
[1]Means without sharing a common superscript letter differ (P< 0.05)

Figure 3 Faecal score of piglets on the different treatments during several days (treatment 1=negative control, 2=yeast product, 3=SW7, 4=SW11, 5=sesame and 6= oregano)



4.4 Faecal shedding of *E. coli*

In Table 12, the results of the analysis of *E. coli* O149K91F4 K88ac in faeces are presented, while the counts of total *E. coli* are listed in Appendix 2. Faecal samples taken before the challenge (day 2 and 5) were negative for *E. coli*. However, in our experiment it appeared that four piglets were receptor negative for *E. coli*. Although the chance is very low, it appeared that all four piglets got the Sesame treatment, despite the fact that they were from different litters. Therefore, in the model the counts were corrected for the receptor status of the piglets by deleting these results.

Table 12 Slopes of curves representing the increase and decrease of *E. coli* counts on the six treatments during 12 days

	Treatment						*P*	SED
Slope	Neg. C	Yeast	SW7	SW11	Sesame	Oregano		
Increasing	0.000	-1.180	1.251	1.917	-0.230	-0.705	0.064	1.203
Decreasing	0.000	0.134	-0.517	-0.562	-0.146	0.195	0.105	0.364

It should be mentioned that the increasing and decreasing slopes can not be evaluated completely separately from each other. If an increasing slope is higher than the decreasing slope is most likely to be also more negative. In the course of time after the *E. coli* inoculation, in all the experimental groups, first the number of *E. coli* in the faeces less or more increased, and thereafter it gradually decreased with different patterns among the treatments. Table 4.5 shows that compared to treatment 1, the treatments II, V and VI had a lower increase in *E. coli*, while treatments III and IV had a higher increase. In this evaluation sex had no effect.

4.5 Bacterial counts in caecal chyme

Results of bacterial counts in caecal chyme of the piglets receiving the different diets are presented in Table 13.

Table 13 Bacterial counts in caecal chyme of piglets on the six treatments (^{10} log-values cfu/g)

Analysis of the counts showed that there were no statistically significant differences in bacterial counts among the different treatments. Therefore, it was omitted to further analyse samples from ileum for bacterial counts.

	Treatment						*P*	LSD
	Neg. C	Yeast	SW7	SW11	Sesame	Oregano		
Aerobic	5.74	5.61	5.57	5.87	5.77	5.77	0.84	0.66
Anaerobic	6.76	6.85	6.61	6.97	7.33	7.18	0.30	0.58
Enterococci	2.80	2.31	2.80	2.43	2.78	2.19	0.63	1.07
Lactobacilli	6.60	6.26	6.22	6.42	7.06	6.57	0.14	0.51

4.6 Measurements in blood

Table 14 shows the results of some blood measurements of the piglets at d21 of experiment.

Table 14 Results of some blood measurements of the piglets at d21 of experiment

	Treatment						*P*	LSD
	Neg. C	Yeast	SW7	SW11	Sesame	Oregano		
White blood cells (g/l)	18.25	17.43	17.30	18.51	18.41	17.96	0.99	3.73
Hb (mmol/l)	6.16	6.19	6.64	6.33	6.56	6.59	0.27	0.53
Lymphocytes (%)	54.3	56.0	55.0	54.2	52.5	56.4	0.94	6.30
Mono+granulocytes (%)	45.7	44.0	45.0	45.8	47.5	43.6	0.94	6.30

Table 14 shows that there are no significant differences between the dietary treatments on the blood parameters measured.

5 Discussion

5.1 Chemical analyses

Chemical analyses of the complete diets show that the different diets were almost identical in their chemical composition, which means that manufacturing of the diets was as planned. Additional analyses of the experimental products revealed that in product SW 7 the concentrations of soluble glucose and fructose were much higher than in the other products tested. It is a pity that the peaks of the chromatograms of xylose and mannose of the NDF residue were so close that it was impossible to discriminate them from each other. When xylose and mannose concentrations are added together then the yeast product and sesame seed expeller (317 and 391 g/kg NDF, respectively) have much higher concentrations than the other products tested (100 and 191 g/kg NDF, respectively, for SW 7 and SW 11). There were also large differences in the concentration of arabinose (yeast product and sesam seed expeller 174 and 82 g/kg NDF, respectively, while the concentration of arabinose was low in SW 7 and SW 11 (19 and 39 g/kg NDF, respectively).

Table 15 Offered amount of carbohydrates present in NDF residue (g/kg diet)
[a] Chromatograms of xylose and mannose hardly to distinguish

When the amounts of carbohydrates present in NDF residue offered are calculated then there are hardly differences in amounts per kg of diet offered, which is due to the low inclusion levels. Therefore, it seems likely that the specific chemical and/or physical structure of the products are responsible for the effect exerted. It may be considered what the effect of the products is at higher incorporation levels in the diet.

5.2 Animal performance, faecal score and *E. coli* shedding

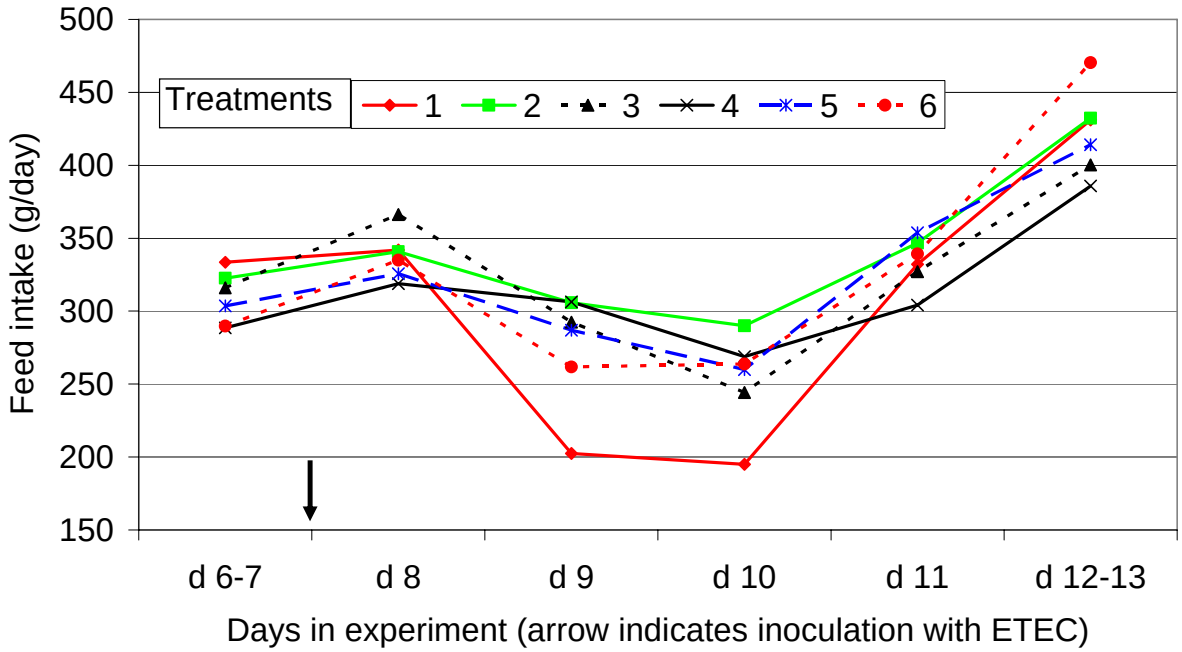
The present study was designed to evaluate the effect of plant materials to reduce *E. coli* faecal shedding after *E. coli* oral infection in weaning pigs. The infection was successful as can be concluded from Table 15 and Figure 4, in which the course of feed intake from d 6 to d 13 is shown. This figure shows that for all treatments there was a depression in feed intake on d 9 and d 10. This is characteristic for infection studies with *E. coli* on piglets. The depression in feed intake was highest on the Negative control diet and least on diets SW11, Yeast and Sesame. This may mean that piglets on the Negative control and SW7 suffered more from the infection with *E. coli* than on the Yeast, SW11 and Sesame diets. Feed intake restored quickly and on d 11 it was almost equal with that one on d 8.

Table 5.1 Difference in daily feed intake compared to d 8 of the same treatment (g)

Treatment	d 9	d 10	d 11	d 12
Negative control diet	-139	-147	-10	+67
Yeast product	-35	-51	+6	+60
SW7	-74	-122	-39	+29
SW11	-13	-50	-15	+36
Sesame seed expeller	-39	-66	+28	-1
Carvacrol/thymol product	-73	-71	+4	+76

	Glucose
	Xylose [a]
	Mannose [a]
	Xylose+Mannose
	Arabinose
	Xylose+Mannose+Arabinose

Figure 4 Course of feed intake just before and after the challenge with *E. coli* (treatment 1=negative control, 2=yeast product, 3=SW7, 4=SW11, 5=sesame and 6= oregano)

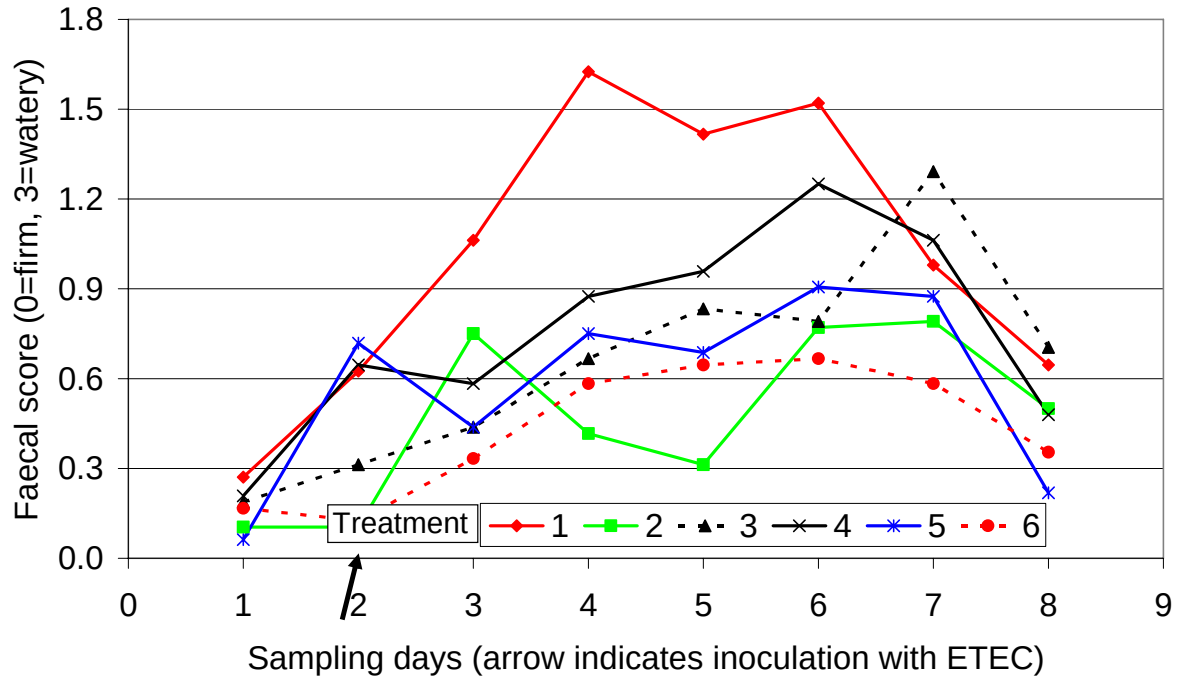


All the diets were supplemented with a flavour in order to mask possible difference in taste. It is possible, however, that the thymol/carvacrol aroma was still perceived by the piglets despite the flavouring product added to the diets. Comparable feed intake to thymol/carvacrol was observed in sesame fed piglets.

Growth rate reflected to a large extent the feed intake, and was below 100 g/d in the first week. In the week after inoculation with *E. coli*, growth rate showed the largest differences among treatments but the LSD was also high, and no significant differences could be demonstrated (P=0.13).

The faecal consistency score was markedly affected by the dietary treatments after the *E. coli* challenge (Figure 5). There were quite irregular patterns between the treatments: the Negative control, the Sesame and Oregano groups had more or less a smooth pattern, while the SW7 and SW11 diets had the highest faecal score at 4 or 5 days after inoculation. The groups fed the yeast product and the Oregano product showed more firm faeces than the other treatments. Piglets fed sesame seed expeller and SW7 showed an intermediate consistency score of faeces, while the Negative control group showed the worst faecal consistency score. Manzanilla et al. (2004) noted also a reduction of diarrhoea persistency after a plant extract mixture (with carvacrol) supplementation.

Figure 5 Course of faecal score of the piglets (treatment 1=negative control, 2=yeast product, 3=SW7, 4=SW11, 5=sesame and 6= oregano)



The results reported in Table 12 indicate that yeast product (treatment II) had the least increase in slope (increase in *E. coli* K88 faecal shedding) followed by the Oregano and the Sesame groups. The products SW11 had the highest increase in slope followed by SW7, which were even higher than the Negative control treatment. These results corroborate to a large extent the previous findings *in vitro* on the adhesion ability of these products to *E. coli*.

The present data revealed the ability of a yeast product, a thymol/carvacrol product and to a lesser extent of a sesame seed expeller to reduce faecal *E. coli* shedding. Yeast preparations were previously supplemented in the diets of weaning piglets aimed to evaluate their effect on piglet immune functions and gut health status (Mathew et al., 1998; Van Heughten et al., 2003; Davis et al., 2004; Rozeboom et al., 2005). Mathew et al. (1998) showed no effect of yeast in reducing intestinal *E. coli* count. Similarly, Van Heughten et al. (2003) failed to elicit any effect of yeast on faecal coliforms count of weaning pigs. However, it should be mentioned that the effect may also depend on *E. coli* strain and its fimbriae. The previous data are not in agreement with the results of the current study. In this regard, it is important to consider that our study was conducted after *E. coli* challenge, therefore, it is possible that yeast is more effective in case of acute *E. coli* infection than current farm microbial concentrations. The positive effect of the yeast product in our study should be due to the ability of some mannose oligosaccharides of the yeast wall to function as receptors for enterobacteria (Ofek et al., 1977) and therefore inhibiting bacterial adhesion to the GI mucosa. The thymol/carvacrol product showed similarly a positive effect in reducing *E coli* K88 faecal shedding. Recently some authors reported the results of these additives on intestinal equilibrium of weaned pigs (Manzanilla et al., 2004, 2006; Nofrarias et al., 2006). Manzanilla et al. (2004) did not find any effect of a mixture of carvacrol and other plant extracts on intestinal enterobacteria count of the pigs. The different results of this trial compared to our experiment is probably due to the composition of the supplemented product. In fact, we used a commercial thymol/carvacrol preparation while Manzanilla et al. used a mixture of carvacrol, cinnamaldehyde and capsicum oleoresin. Also, more or less common feed ingredients may contain properties that improve gut health. Jansman et al. (2004) showed a slight improved gut health when linseed or linseed expeller were included in diets of weaned piglets.

The effects of intestinal receptor status on the sensitivity of the response towards an *E. coli* challenge were shown by Geenen (2005). Receptor positive animals, in fact, were shown to be significantly more sensitive than receptor negative animals. This could also be concluded in our experiment where the receptor negative pigs had slightly better scores compared to the receptor positive pigs receiving the Sesame treatment. Comparing the results of feed intake and faecal score with the piglets in the same treatment group, they were almost similar. However, when analysing *E. coli* shedding, it was found that only on d 1 faecal shedding of the four receptor negative was substantially lower than of the 8 receptor positive piglets in this treatment group (2.91 vs. 6.33,

respectively). The receptor negative piglets may bias the results, and therefore, the results of these piglets were deleted from the data files before statistical analyses were performed.
The new finding of the present study was the effect of sesame seed expeller in possible reducing the faecal *E. coli* shedding. To our best knowledge, in fact, no author supplemented the diet of young pigs with sesame seed expeller to evaluate the gut health status. Piglets fed sesame seed expeller, in the present study, revealed faecal *E. coli* values almost comparable to the yeast fed pigs and generally lower than the other experimental groups during all the post-challenge period. The efficacy of sesame seed expeller to bind *E. coli* was already demonstrated *in vitro* in our lab. This property of the sesame seed expeller shown *in vitro* could explain its positive effect to reduce the faecal *E coli* shedding in our piglets. A lower efficacy than sesame to counteract *E. coli* was observed in both the SW 7 and SW 11, although SW 7 resulted in lower *E. coli* shedding than SW 11. These groups in fact revealed values comparable to the Negative control group and generally higher than the other treatments.
Comparison of the different treatments in response to different response criteria is given in Table 16.

Table 16 Ranking order of effects (1= best; 6 = worst)

Treatment	*In vitro*	Feed intake d 6 - 13	*E. coli* in faeces; increasing slope	Faecal score d 7 - 13	Average *in vivo*
Negative control diet	5	6	4	6	5.3
Yeast product	1	1.5	1	1.5	1.3
SW7	2	3	5	4	4.0
SW11	4	5	6	5	5.3
Sesame seed expeller	3	4	3	3	3.3
Carvacrol/thymol product	-	1.5	2	1.5	1.7

In general, the *in vitro* findings on the binding capacity of the plant materials to *E. coli* were confirmed to a large extent in the *in vivo* model of the piglets. The yeast product (treatment 2) was the most effective both *in vitro* and *in vivo*, but the high response *in vitro* of SW 7 (treatment 3), was partly observed in the animals. In addition, SW 11 performed slightly better than the Negative control *in vitro*, while no difference was observed in the *in vivo* model. This is not surprising because SW 11 was only slightly better *in vitro* than the control. The sesame seed expeller showed a higher *E. coli* shedding than the yeast product. When taking also into account the response parameters feed intake from d 6-13 and the faecal score, then we almost get the same ranking order as the *in vitro* ranking order.
In conclusion, the results of the present experiment showed clear evidence of the efficacy of the yeast product, sesame seed expeller and to a lesser extent SW 7 to decrease *E. coli* K88 faecal shedding as the result of reduced *E. coli* K88 gastrointestinal colonisation after *E. coli* K88 oral challenge. The *in vitro* model seems to be a reasonably reliable method to evaluate the efficacy of plant materials in inhibiting *E. coli* gastrointestinal proliferation. The thymol/carvacrol preparations revealed an evidence of the properties of these substances to control *E. coli* proliferation and improve faecal consistency.

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Appendices

Appendix 1 Body weight (BW, kg), feed intake (FI, g/day), average daily gain (ADG, g/day) and feed conversion ratio (FCR) of the treatment groups

Days post-weaning	Item	Treatment						LSD	*P*-value Treatment
		Neg. C	Yeast	SW7	SW11	Sesame	Oregano		
Day 1	BW	7.00	6.85	6.81	7.00	7.04	7.05	0.50	0.91
Day 6	BW	7.40	7.17	7.16	7.45	7.40	7.50	0.50	0.61
Day 13	BW	8.02	8.38	8.23	8.31	8.33	8.70	0.66	0.39
Day 22	BW	12.8	13.1	12.9	13.2	13.4	14.0	1.03	0.14
Day 1 - 6	ADG	79	64	70	90	73	89	46.6	0.77
Day 6 - 13	ADG	89	173	146	123	133	172	67.8	0.42
Day 13 - 22	ADG	601 [ab]	585 [a]	579 [a]	610 [ab]	631 [ab]	659 [b]	63.7	0.06
Day 1 - 6	FI	220	211	200	216	229	196	29.1	0.19
Day 6-13	FI	324	355	336	321	333	351	52.9	0.66
Day 13 - 22	FI	708 [abc]	703 [abc]	697 [ab]	686 [a]	745 [bc]	753 [b]	53.9	0.02
Day 13 - 22	FCR	1.18	1.21	1.22	1.13	1.20	1.14	0.08	0.14

Appendix 2 Numbers of *E. coli* O149K91F4 K88ac (^{10}log-value cfu/g) in faeces in the post-inoculation period (treatment 1=, 2=, 3=, 4=, 5= and 6=)

Treatment	d8	d9	d10	d11	d12	d13	d14	d15
Negative control	4.83	4.39	4.96	3.28	3.09	2.76	0.95	1.28
Yeast product	4.43	2.36	1.66	0.93	1.28	2.29	1.59	2.42
SW7	5.73	2.93	3.98	4.08	4.79	2.64	1.29	2.04
SW11	5.71	4.34	5.32	5.07	4.98	4.99	2.35	1.40
Sesame	6.33	3.51	3.00	2.63	1.99	3.32	2.70	0.00
Oregano	5.12	3.21	2.97	2.43	2.89	3.36	1.47	2.25