# Effects of antimicrobial feed additives on gut microbiology and blood parameters of weaned piglets

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# Einfluss von antimikrobiell wirksamen Futterzusatzstoffen auf die Mikrobiologie des Verdauungstrakts und Blutparameter von Absetzferkeln

## 1 Introduction

As a result of antibiotic resistance in human pathogenic bacteria, alternatives for Antibiotic Growth Promoters (AGP) have been developed in recent years. Herbs and botanicals can be seen as such an alternative (WENK, 2003). Essential oils derived from different plants have been tested over the years to identify their effects on animal performance. Essential oil extracts from Oregano, with its chemical main component carvacrol, has been used as feed additive and was investigated in numerous feeding trials where it showed a positive influence on piglets performance (e.g. WALD et

**Zusammenfassung**

In einem Fütterungsversuch mit 120 Absetzferkeln (8 kg Anfangsgewicht) wurden 2 Futterzusatzstoffe (ätherische Öle aus Oregano, Anis und Zitrusfruchtschalen bzw. Avilamycin) mit einer unbehandelten Kontrollgruppe verglichen. Nach 50 Tagen Verfütterung verbesserten die ätherischen Öle numerisch den Gewichtszuwachs, Futterverzehr und die Futterverwertung (+5 %, +3 % und –1 %), während Avilamycin geringere Effekte aufwies (+1 %, –1 % und –2 %). Am Versuchstag 22 wurden von den 3 Behandlungen jeweils 12 repräsentative Tiere geschlachtet und Blutproben sowie Chymusproben aus dem Ileum, Caecum und Colon entnommen. Die beiden Futterzusatzstoffe hatten keinen Einfluss auf das Blutbild, reduzierten jedoch die Gehalte an anaeroben und aeroben Keimen, flüchtigen Fettsäuren und Ammoniak im Chymus des Ileums, Caecums und Colons, sowie die Gehalte an biogenen Aminen im Caecum. Die pH-Werte und die Gehalte an Trockenmasse (T) in den untersuchten Darmabschnitten blieben unverändert mit Ausnahme eines erhöhten T-Gehalts im Colon bei Verfütterung ätherischer Öle. Diese Befunde weisen darauf hin, dass die eingesetzten ätherischen Öle ähnlich dem Avilamycin eine antimikrobielle Aktivität *in vivo* aufweisen.

**Schlagworte:** Ferkel, phytogene Futterzusatzstoffe, ätherische Öle, Oregano, antimikrobielle Leistungsförderer, Avilamycin.

**Summary**

In a feeding trial involving 120 weaned piglets (8kg initial body weight) two feed additives (essential oils blend derived from oregano, anise and citrus peels vs. Avilamycin) were compared with a negative control group. After 50 days of feeding essential oils improved numerically weight gain, feed intake and feed to gain ratio (+5 %, +3 % and –1 %), whereas Avilamycin effects were less pronounced (+1 %, –1 % and –2 %). At experimental day 22 twelve representative animals from each treatment group were slaughtered and blood samples as well as chyme samples from ileum, caecum, and colon were retrieved. Both feed additives did not affect blood analysis but reduced contents of anaerobic and aerobic germs, volatile fatty acids, and ammonia in chyme of ileum, caecum and colon as well as contents of biogenic amines in caecal chyme. The pH value and dry matter (DM) contents in chyme of the respective intestinal segments remained unchanged except for an increased colonic DM content due to essential oils. These results indicate that essential oils tested in the present study exert an antimicrobial activity *in vivo* similar to Avilamycin.

**Key words:** Piglets, phytogenic feed additive, essential oils, oregano, antimicrobial growth promoter, avilamycin.

al., 2001; MOLNAR and BILKEI, 2005). According to RODEHUTSCORD and KLUTH (2002), the reason for this positive influence could be the antimicrobial activity of essential oils, which has been observed in vitro (SIVROPOULOU et al., 1996; SI et al., 2006).

In the present study, the effect of addition of a blend of essential oils derived from oregano, anise and citrus peels to a weaner piglet diet was compared to the antibiotic growth promoter Avilamycin. Thereby, the main focus of the experiment was to investigate the mode of action of these feed additives on gut microbiology in young pigs. Moreover, effects of the essential oil blend or Avilamycin on blood parameters and growth performance were investigated.

## 2 Material and Methods

### 2.1 Experimental design, housing and feeding

A total of 120 (65 male and 55 female) newly weaned piglets (crossbred of Large White × Pietrain, 28 days of age, mean body weight of 8.2 ± 2.3kg) were used to study the effects of two feed additives (essential oils and Avilamycin) on gut parameters and performance. Piglets were distributed among 40 blocks containing 3 animals according to genetics (litter), sex and initial live weight. The animals of each block were allotted to three treatments: (1) feed with no additions of potentially growth enhancing agents like antibiotics, organic acids, probiotics, or excessive contents of Zinc and Copper (*negative control group*), (2) feed supplied with an essential oil blend (*essential oils group*), and (3) feed supplied with the antimicrobial feed additive Avilamycin (*Avilamycin group*).

Over the time course of the experiment, the animals were fed 3 different types of diet formulations, a starter diet (day 1 to 7), a grower I diet (day 8 to 22) and a grower II diet (day 23 to day 50). The composition of these diet formulations is given in Table 1.

Table 1: Composition of basal diets
Tabelle 1: Zusammensetzung der Basalrationen

| | Feed ingredients (%) | | |
|---|---|---|---|
| | **Starter diet** | **Grower I diet** | **Grower II diet** |
| Maize (pressure cooked) | 36.9 | 2 | — |
| Wheat (pressure cooked) | 20.0 | — | — |
| Barley | — | 40.0 | 26.4 |
| Maize | — | 35.0 | 50.0 |
| Lactose | 9.0 | 2.0 | — |
| Dextrose | 5.0 | — | — |
| Soya protein (concentrate) | 6.5 | 4.5 | — |
| Soya HP | — | — | 18.3 |
| Sweet whey powder | 6.0 | 4.5 | 0.2 |
| Potato protein | 5.0 | 4.0 | — |
| Fish meal 65 % | 5.0 | 4.0 | — |
| Soya oil | 0.52 | 0.45 | 0.52 |
| Beet – Vinasse | 3.0 | — | — |
| L-Lysin | 0.45 | 0.50 | 0.55 |
| DL-Methionin | 0.20 | 0.16 | 0.16 |
| Tryptophan | 0.09 | 0.08 | 0.05 |
| Threonin | 0.14 | 0.14 | 0.20 |
| Diatomaceous earth | 0.50 | 0.50 | 0.50 |
| Vitamin and Trace elements Premix | 1.7 | 2.0 | 3.1 |
| *Analyzed Contents* | | | |
| Dry matter (g/kg) | 919 | 897 | 908 |
| Crude protein (g/kg DM) | 203 | 195 | 192 |
| Crude fat (g/kg DM) | 67 | 47 | 34 |
| Starch (g/kg DM) | 450 | 489 | 529 |
| Sugar (g/kg DM) | 39 | 46 | 46 |
| Crude fibre (g/kg DM) | 26 | 33 | 34 |
| Crude ash (g/kg DM) | 62 | 64 | 58 |
| Calculated energy contents (MJ ME/kg)*) | 15.2 | 14.8 | 14.9 |

*) GfE 1987

For the essential oils group, the botanical product Biomin® P.E.P. 1000 (Biomin GmbH, Austria) was used. This product is permitted in the EC to be fed as feed additive to piglets (Reg. (EC) No 1831/2003). It contains essential oils derived from Oregano *(Origanum vulgare)*, Anise *(Pimpinella anisum)* and Citrus peels *(Citrus sinensis).* Chicory (*Cichorium intybus)* powder acts as carrier substance. As main active ingredients the product contains the phenolic monoterpenes Carvacrol, Thymol, Anethol and the cyclic monoterpene – hydrocarbon Limonen. One kg of the product contains 20 g of the essential oils blend. The product was added at amounts of 2 g per kg of finished starter and grower I diets (corresponding to 40 ppm essential oils), and 1 g per kg of finished grower II diet, respectively (corresponding to 20 ppm essential oils). Analysis of carvacrol (the major compound of the essential oil blend) by the Institute of Applied Botany and Pharmacognosy, University of Veterinary Science, Vienna, using the SPME GC technique (ARTHUR and PAWLISZYN, 1990) revealed 9.5 g of the chemical component per kg of the phytogenic feed additive. For the starter, grower I and grower II diets, the respective carvacrol contents were 23.1, 15.1 and 8.5 mg per kg of finished feed while other feeds contained no or only traces of carvacrol.

For the Avilamycin group, Maxus100 (Elanco Animal Health LTD) was used. It is based on the active ingredient Avilamycin (Tetracycline) which was permitted for use as

AGP in EC during time course of the experiment (year 2005). Maxus 100 contains 10 % Avilamycin. The product was added to the starter, grower I and grower II diet at amounts of 0.4 g per kg (corresponding to 40 ppm Avilamycin). Analysis of Avilamycin by AGES (Austrian Agency for Health and Food Safety) using agar diffusion method (Eli Lilly and company, Indianapolis, USA) revealed 42.0, 44.5 and 44.5 mg per kg of finished starter, grower I and grower II diet while for other diets the respective contents were below limit of detection or quantification (< 5 mg per kg).

The entire study comprised 3 consecutive experimental replicates processing 10, 15 and 15 blocks of animals. For all experimental replicates, piglets were housed in the same air-conditioned room equipped with 3 pens with identical construction (slatted floor, heated lying area, a height adjustable nipple drinker, a cup drinker and a round feeding hopper). The three treatments (negative control, essential oils, Avilamycin) were allotted to each one of the 3 pens. Feed and water was offered *ad libitum* to the piglets throughout the experiment.

On 22$^{nd}$ day of each experimental replicate, 4 blocks of animals (2 female and 2 male castrated) with body weights ranging most close to the median of the entire pens were sacrificed in order to obtain samples of gut contents and tissues for further investigations (mean body weight of sacrificed animals: 13.7±1.7kg). The remaining animals were maintained in their pens and were fed until day 50 to observe zootechnical performance over the entire production period.

## 2.2 Sample collection and analysis

*Live weight and feed consumption*

Animals were weighed individually at the start of the trial and on trial days 21 and 50. Feed intake of pens was evaluated daily during the first 21 trial days (representing all animals), and then at day 22, 29, 36, 43 and 50 (representing animals not sacrificed). Feed to gain ratio was calculated for days 0 to 21 (all animals) and days 0 to 50 (animals not sacrificed) from respective pen means of growth data and feed intake.

*Feed samples*

Feed samples of all diets were collected at the beginning of each new feeding period and were analyzed for their contents of dry matter, crude protein, crude fat, crude fibre, starch, sugar and crude ash according to standard methods (NAUMANN and BASSLER, 1997).

*Sampling and analysis of chyme and blood*

The 12 selected blocks of animals were professionally slaughtered in the farms own processing plant after morning feeding. During bleeding, blood was collected (25 ml EDTA tubes; 0.5 ml EDTA per 25 ml whole blood) and frozen at –80 °C.

From each animal about 100g of chyme from terminal ileum (at least 15 cm before end of ileum), caecum and colon (at the flexura centralis) were collected, immediately analyzed for pH value (Testo 206, pH meter, Testo AG, Germany), separated into several 10 ml plastic bottles and stored at –80 °C.

All chyme samples were analysed microbiologically by plate-count technique. One gram of chyme sample was homogenized in (1/4 strength) Ringer's solution and decimally diluted. Appropriate dilutions were spread plated on selective media and incubated for analysis of aerobic/anaerobic total colony count (plate-count agar, 30 °C for 72 h, aerobic/anaerobic conditions), lactobacilli (MRS agar, 37 °C, 72 h, anaerobic), lactococci (M17 agar, 37 °C, anaerobic), bifidobacteria (Wilkins-Chalgren agar modified by the addition of acetic acid (1 ml/L) and mupirocin (100 mg/L), 72 h, anaerobic), enterococci (kanamycin-esculin-azide agar, 37 °C, 48 h), clostridia (DRCM agar, 37 °C, 72 h, anaerobic) and enterobacteria (VRB agar, 37 °C, 24 h, overlay technique).

For volatile fatty acid (VFA) analysis exactly 500 mg chyme were mixed with 4 ml aqua destilate, 2 ml internal standard (oenanthic acid, 0.1 mg/ml) and 300 µl azidiol. This mixture was acidified with 50 µl 6n HCl. After 2 h incubation the samples were centrifuged at 9000g for 10 minutes. After centrifugation the liquid phase was used for gas chromatographic analysis, which was made on a Carlo-Erba 5000 gas-chromatographer (direct injection method) according to Restek applications note # 591155B (Restek corporation, Belleforte, USA).

Biogenic amines were analyzed with HPLC according to the method described by MAYER et al. (2006).

Ammonia contents of chyme samples were analyzed photometrically with an enzymatic test kit (Boehringer/R-Biopharm, Germany).

Frozen blood samples were sent to a commercial veterinary laboratory (Vetmed Labor, Ludwigsburg, Germany) and a blood screening was made. A small blood analysis (leucocytes, erythrocytes, hemoglobin, hematocrit, mean cell volume (MCV), hemoglobin per erythrocyte (HBE), mean corpuscular hemoglobin concentration (MCHC), thrombocytes) and a differential blood analysis (basophil

granulocytes, eosinophil granulocytes, neutrophil granulocytes, lymphocytes, monocytes) was conducted with an automated procedure.

### 2.3 Statistics

One animal of trial group 3 (Avilamycin) had to be excluded from statistical analysis because of irregular growth data.

The data was submitted to two-way analysis of variance with the dietary treatment (1, 2, 3) and animal block (1, 2, 3 … 40) as factor levels. For feed intake and feed conversion ratio the pen was used as statistical factor instead of animal block. The following tables present the mean values of the animal groups and the pooled standard error (S.E.) as retrieved from the analysis of variance. The mean values were tested by a multiple comparison procedure (Student-Newman-Keuls test). Significant differences among means ($p < 0.05$) are marked by superscript letters $^{a,b,c}$. Values showing a tendency to differ ($p < 0.1$) are marked by superscript letters in parenthesis $^{(a),\ (ab),\ (b)}$. Additionally, the means of the essential oils group and the Avilamycin group were compared via a linear contrast with the means of the negative control group. Significant differences ($p < 0.05$) are marked with an asterisk$^{*}$.

## 3 Results

*Live weight and feed consumption*

As shown in Table 2, animals supplied with essential oils showed a numerically higher weight gain, feed intake and slightly reduced feed to gain ratios compared to the negative control (for the 50 day period: +5 %, +3 % and –1 %). For the Avilamycin group, the numerical differences were smaller and less consistent (+1 %, –1 % and –2 %). However, none of these figures reached statistical evidence.

*Chyme analysis*

Microbial investigations showed that colony counts were influenced by both feed additives (Table 3). Compared to the negative control, both feed additives decreased ($p < 0.05$) anaerobic germ count in the ileal chyme and the same tendency was found for aerobic bacteria. In caecum, counts of aerobic and anaerobic bacteria, lactococci and clostridia were lower ($p < 0.05$) in the essential oil group than in the Avialmycin group, whereas negative control had intermediate counts. In caecum, contents of bifidobacteria were decreased in the Avilamycin group ($p < 0.05$) compared to the other groups and counts of clostridia in chyme of essential oil group was lower ($p < 0.05$) compared to the negative control.

Contents of volatile fatty acids (VFA) in the ileal chyme were not systematically influenced by either feed additives (Table 4). On the contrary, in ceacum both feed additives decreased ($p < 0.05$) acetic acid concentration and the same tendency was observed in colonic chyme. Essential oils and Avilamycin in the diets led to numerically decreased contents of summarized VFA in caecum (–9 %; –12 %) and colon (–10 %; –10 %).

Biogenic amine concentrations in ileal and colonic chyme were not influenced by either feed additive (Table 5). In ceacum, essential oils and Avilamycin led to decreased ($p < 0.05$) contents of Methylamin, Isopropylamin and Sper-

Table 2: Zootechnical performance
Tabelle 2: Zootechnische Leistungen

| | Neg. control | Ess. Oils | Avilamycin | S.E. |
|---|---|---|---|---|
| *Body weight (kg)* | | | | |
| Day 0 | 8.2 | 8.2 | 8.3 | 0.5 |
| Day 21 | 12.8 | 13.1 | 13.1 | 1.2 |
| Day 50 | 29.7 | 30.8 | 30.1 | 3.7 |
| *Weight gain (g/day)* | | | | |
| Day 0 to 21 | 219 | 232 | 228 | 57 |
| Day 0 to 50 | 434 | 455 | 438 | 73 |
| *Feed intake (g/day)* | | | | |
| Day 0 to 21 | 342 | 355 | 348 | 44 |
| Day 0 to 50 | 777 | 802 | 766 | 85 |
| *Feed/gain (g/g)* | | | | |
| Day 0 to 21 | 1.56 | 1.53 | 1.52 | 0.04 |
| Day 0 to 50 | 1.78 | 1.76 | 1.74 | 0.04 |

Table 3: Colony counts in the chyme of ileum, caecum and colon
Tabelle 3: Keimzahlen im Ileum-, Caecum- und Colon-Chymus

| | Neg. control | Ess. Oils | Avilamycin | S.E. |
|---|---|---|---|---|
| *Colony counts in the chyme of ileum (log[CFU][1])per g fresh matter)* | | | | |
| Aerobic bacteria, sum (12/12/12)[2)] | 4.49 (a) | 3.83 (b) | 3.76 (b) | 0.77 |
| Anaerobic bacteria, sum (12/12/12) | 4.60 a | 3.96 b | 3.81 b | 0.74 |
| Lactobacilli (10/9/9) | 4.93 | 4.79 | 4.58 | 0.83 |
| Lactococci (10/10/11) | 4.43 | 4.09 | 4.28 | 0.73 |
| Bifidobacteria (10/6/11) | 4.43 | 3.80 * | 3.96 | 0.63 |
| Enterococci (12/9/12) | 3.53 | 3.44 | 3.68 | 0.45 |
| Clostridia (12/12/12) | 4.18 | 3.95 | 4.47 | 0.77 |
| Enterobacteria (1/0/0) | 2.30 | — | — | — |
| *Colony counts in the chyme of caecum (log[CFU][1])per g fresh matter)* | | | | |
| Aerobic bacteria, sum (12/12/12) | 4.80 ab | 4.46 b | 5.01 a | 0.49 |
| Anaerobic bacteria, sum (12/12/12) | 4.86 ab | 4.53 b | 5.14 a | 0.48 |
| Lactobacilli (10/9/9) | 5.89 | 5.57 | 5.59 | 0.67 |
| Lactococci (10/10/11) | 5.04 ab | 4.61 b | 5.47 a | 0.78 |
| Bifidobacteria (10/6/11) | 5.96 (a) | 5.32 (ab) | 5.19 (b) | 0.66 |
| Enterococci (12/9/12) | 4.10 | 3.74 | 3.91 | 0.48 |
| Clostridia (12/12/12) | 5.43 a | 4.99 b | 5.33 a | 0.43 |
| Enterobacteria (1/0/0) | — | — | — | — |
| *Colony counts in the chyme of colon (log[CFU][1])per g fresh matter)* | | | | |
| Aerobic bacteria, sum (12/12/12) | 5.33 | 5.48 | 5.48 | 0.49 |
| Anaerobic bacteria , sum (12/12/12) | 5.60 | 5.53 | 5.55 | 0.33 |
| Lactobacilli (10/9/9) | 6.53 | 6.78 | 6.66 | 0.54 |
| Lactococci (10/10/11) | 5.29 | 5.51 | 6.01 | 0.93 |
| Bifidobacteria (10/6/11) | 6.60 a | 6.49 a | 5.78 b | 0.48 |
| Enterococci (12/9/12) | 4.49 (b) | 4.55 (b) | 5.12 (a) | 0.66 |
| Clostridia (12/12/12) | 6.32 a | 5.81 b | 6.07 ab | 0.47 |
| Enterobacteria (1/0/0) | 2.30 | | — | — |

1 Log(10) of colony forming units (CFU)
2 (xx/yy/zz) = number of samples with detectable colony counts (xx: Neg. Contr, yy: Ess. Oils, zz: Avilamycin)
a b Means without similar superscripts differ significantly ($p < 0.05$)
(a) (b) Means without similar superscripts show tendency to differ significantly ($p < 0.10$)
* Means are statistically different from respective negative control level ($p < 0.05$)

midin. Concentrations of Agmatin, Dimethylamin and Tyramin in chyme of the three investigated gut areas were below the detection limit.

Dry matter (DM) contents of ileal and caecal chyme were similar for all treatments, but DM of colonic chyme was increased ($p < 0.05$) in the essential oil group compared to other groups (Table 6). Ammonia contents of chyme were numerically decreased in the essential oils group (ileum: –18 %; ceacum –24 %) and in the Avilamycin group (ileum: –7 %; caecum: –17 %; colon: –17 %). However, these differences were statistically not significant. pH of chyme was not influenced by treatment.

*Blood analysis*

Blood analysis did not differ between the 3 trial groups. The following average contents of blood parameters were found in the blood samples: leukocytes 17 g/L; hematocrit 37 %; lymphocytes 51 %; hemoglobin 10 g/dL; erythrocytes 6T/L; HBE 17 p/g; MCV 60 fl; MCHC 28 g/dL; neutrophil granulocytes 44 %; thrombocytes 320 g/L.

## 4 Discussion

This study aimed to investigate gut parameters thought to be influenced by feed additives with antimicrobial activity like AGP (Antibiotic Growth Promoters), organic acids or phytobiotic substances. Antibiotic activity of feed additives may lead to less growth of undesired microbes in the intestine, less exposure to microbial products stressing the organism, and a higher absorption of nutrients, all of these factors resulting in an improved growth performance of piglets kept under common housing conditions (GREIFE and BERSCHAUER, 1988). Following this paradigm it was

Table 4: Contents of volatile fatty acids in the chyme of ileum, caecum and colon
Tabelle 4: Flüchtige Fettsäuren (VFA) im Chymus von Ileum, Caecum und Colon

| | Neg. control | Ess. Oils | Avilamycin | S.E. |
|---|---|---|---|---|
| *VFA in chyme of ileum (mmol/kg fresh matter)* | | | | |
| Acetic acid | 10.3 | 8.8 | 10.0 | 0.2 |
| Propionic acid | 1.36 | 1.93 | 1.65 | 0.02 |
| Lactic acid | 0.06 | 0.05 | 0.05 | 0.01 |
| Butyric acid | 0.76 | 0.79 | 0.95 | 0.01 |
| Valearic acid | 0.32 | 0.36 | 0.24 | 0.00 |
| Capric acid | 0.11 | 0.10 | 0.09 | 0.00 |
| Sum of VFA | 12.9 | 12.1 | 12.9 | 0.20 |
| *VFA in chyme of caecum (mmol/kg fresh matter)* | | | | |
| Acetic acid | 59.6 [a] | 49.5 [b] | 50.4 [b] | 9.7 |
| Propionic acid | 44.0 | 41.9 | 36.7 * | 8.7 |
| Lactic acid | 0.000 | 0.001 | 0.001 | 0.001 |
| Butyric acid | 21.8 | 21.1 | 22.7 | 6.7 |
| Valearic acid | 8.21 | 7.94 | 7.22 | 3.51 |
| Capric acid | 0.26 | 0.26 | 0.24 | 0.12 |
| Sum of VFA | 134 | 121 | 117 | 29 |
| *VFA in chyme of colon (mmol/kg fresh matter)* | | | | |
| Acetic acid | 56.6 [(a)] | 50.3 [(b)] | 52.3 [(ab)] | 6.7 |
| Propionic acid | 44.8 | 41.1 | 38.9 | 7.8 |
| Lactic acid | 0.000 | 0.000 | 0.001 | 0.001 |
| Butyric acid | 23.7 | 21.1 | 22.5 | 5.0 |
| Valearic acid | 9.89 | 9.15 | 7.78 | 3.05 |
| Capric acid | 0.58 | 0.48 | 0.41 | 0.36 |
| Sum of VFA | 136 | 122 | 122 | 23 |

[a b] Means without similar superscripts differ significantly ($p < 0.05$)
[(a) (b)] Means without similar superscripts show tendency to differ significantly ($p < 0.10$)
* Means are statistically different from respective negative control level ($p < 0.05$)

not the primary ambition of this study to get performance data. It was more important to take a view at parameters of gut physiology associated with microbial activity, which may possibly explain the growth promoting effect of essential oils. Therefore we investigated a blend of essential oils derived from oregano, anise and citrus peels in comparison to a well-known AGP (Avilamycin).

*In vitro* trials showed that numerous essential oils or their main active compounds exert antimicrobial activity. Especially oregano and anis essential oils are well known for such an antimicrobial activity against various bacterial strains (SIVROPOULOU et al., 1996; HAMMER et al., 1999; WENK, 2003; SI et al., 2006). Similarly, NAMKUNG et al. (2004) report that a herbal extract containing oregano and thyme appears to reduce proliferation of coliformic bacteria.

The main active compound of the essential oils blend used in the present study was the phenolic substance carvacrol. This lipophilic substance was found to be a very efficient antimicrobial compound *in vitro* (BEN ARFA et al., 2006). Similarly, SI et al. (2006) report carvacrol to have a high antimicrobial potential against pathogenic bacteria in swine intestinal tract. The inhibitory action of aromatic compounds like carvacrol is related to the hydrophobicity and on their partition in the cytoplasmic microbial membranes (LANCIOTTI et al., 2003). Such lipophilic compounds posses a high affinity for cell membranes and they affect lipid ordering and the bilayer stability resulting in a membrane integrity decrease.

In the present study essential oils as well as Avilamycin decreased contents of aerobic and anaerobic bacteria in the ileal chyme. This is in accordance to the antimicrobial activity of the both feed additives. Moreover, it seemed that antimicrobial activity of the essential oils product led to lower contents of aerobic and anaerobic bacteria, lactococci and clostridia in the caecum. Surprisingly the feed antibiotic (Avilamycin) had no effect on that microbiota in this gut area. The reductive effect of the essential oils product on clostridia was also found in the colon whereas Avilamycin led to decreased contents of bifidobacteria. But in another study testing oregano essential oils as feed additives to piglets no influence on gut microflora could be observed (GÖSSLING, 2001). It may therefore be hypothesized that

Table 5: Contents of biogenic amines in chyme of ileum, caecum and colon
Tabelle 5: Biogene Amine im Chymus von Ileum, Caecum und Colon

| | Neg. control | Ess. Oils | Avilamycin | S.E. |
|---|---|---|---|---|
| *Biogenic amines in chyme of ileum (mg/kg fresh matter)* | | | | |
| Colamin (12/12/12)[1)] | 7.1 | 7.6 | 6.8 | 4.1 |
| Methylamin (6/9/7) | 6.9 | 6.6 | 6.7 | 2.5 |
| Histamin (3/0/2) | 16.3 | — | 16.8 | — |
| Pyrrolidin (12/12/12) | 45.2 | 43.8 | 36.3 | 20.1 |
| Isopropylamin (1/2/0) | 2.4 | 3.4 | — | — |
| Putrescin (12/12/12) | 14.0 | 8.8 | 11.6 | 17.0 |
| Cadaverin (12/12/12) | 41 | 32 | 43 | 36 |
| Spermidin (12/12/12) | 9.7 | 10.8 | 9.8 | 6.7 |
| Spermin (11/8/8) | 10.4 | 14.4 | 11.4 | 3.6 |
| *Biogenic amines in chyme of caecum (mg/kg fresh matter)* | | | | |
| Colamin (12/12/12) | 4.4 | 4.0 | 3.9 | 2.4 |
| Methylamin (12/11/9) | 8.7 [a] | 6.4 [b] | 5.5 [b] | 2.2 |
| Histamin (6/7/5) | 17.4 | 14.5 | 16.4 | 7.4 |
| Pyrrolidin (12/12/12) | 17.4 | 11.7 | 13.3 | 11.0 |
| Isopropylamin (7/3/4) | 4.1 [a] | 1.4 [b] | 2.1 [b] | 0.8 |
| Putrescin (12/12/12) | 61.5 | 64.5 | 43.6 | 41.9 |
| Cadaverin (12/12/12) | 106 | 90 | 104 | 66 |
| Spermidin (12/12/12) | 33.3 [a] | 21.8 [b] | 18.9 [b] | 10.0 |
| Spermin (9/8/6) | 12.2 | 14.1 | 10.6 | 4.6 |
| *Biogenic amines in chyme of colon (mg/kg fresh matter)* | | | | |
| Colamin (12/12/12) | 6.6 | 5.5 | 5.4 | 3.6 |
| Methylamin (10/11/11) | 10.2 | 6.3 | 6.3 | 5.3 |
| Histamin (5/6/3) | 20.1 | 15.0 | 19.1 | 9.8 |
| Pyrrolidin (12/12/12) | 18.2 | 17.3 | 14.2 | 10.9 |
| Isopropylamin (7/7/5) | 6.6 | 6.9 | 6.5 | 7.1 |
| Putrescin (12/12/12) | 60.8 | 58.2 | 39.0 | 44.7 |
| Cadaverin (12/12/12) | 154 | 141 | 134 | 110 |
| Spermidin (12/12/12) | 27.2 | 41.0 | 24.4 | 19.9 |
| Spermin (6/6/6) | 12.0 | 11.0 | 13.8 | 6.47 |

[1] (xx/yy/zz)= number of samples with detectable biogenic amines (xx: Neg. Contr; yy: Ess. Oils; zz: Avilamycin)
[a b] Means within row without similar superscripts differ significantly ($p < 0.05$)
[*] Means within row are statistically different from respective control level ($p < 0.05$)

Table 6: Dry matter, ammonia contents and pH values in chyme of ileum, caecum and colon
Tabelle 6: Trockenmasse, Ammoniakgehalte und pH-Werte im Chymus von Ileum, Caecum und Colon

| | Neg. control | Ess. Oils | Avilamycin | S.E. |
|---|---|---|---|---|
| *Ileum* | | | | |
| Dry matter(%) | 12.9 | 12.3 | 11.9 | 3.1 |
| Ammonia (mg/kg fresh matter) | 58 | 47 | 54 | 19 |
| pH value | 6.5 | 6.5 | 6.3 | 0.3 |
| *Caecum* | | | | |
| Dry matter(%) | 15.7 | 13.5 | 13.8 | 4.4 |
| Ammonia (mg/kg fresh matter) | 137 | 104 | 113 | 67 |
| pH value | 5.7 | 5.5 | 5.8 | 0.3 |
| *Colon* | | | | |
| Dry matter(%) | 17.3 [b] | 22.0 [a] | 18.4 [b] | 4.1 |
| Ammonia (mg/kg fresh matter) | 283 | 294 | 247 | 146 |
| pH value | 5.7 | 5.7 | 5.7 | 0.2 |

[a b] Means within row without similar superscripts differ significantly ($p < 0.05$)

sensitivity of microbial colony counts based on plate-count techniques is limited when used as parameter of antimicrobial activity of essential oils or APG *in vivo*. Nevertheless, the present study clearly demonstrates that essential oils may decrease bacterial contents in the investigated gut areas, especially in the terminal ileum.

The volatile fatty acids (VFA) are produced in the gastrointestinal tract by microbial fermentation of carbohydrates and endogenous substrates (IMOTO and NAMIOKA, 1978; BERGMAN, 1990). In the present study, VFA contents of chyme were generally in accordance with literature (MÖLLER, 2001). The essential oils additive and Avilamycin decreased contents of acetic acid in the caecum ($p < 0.05$) and the colon ($p < 0.1$), and, numerically the sum of all investigated VFA. MÖLLER (2001) tested an oregano essential oil in weaner piglets and reports that contents of VFA ($C_2 - C_5$) in the gut were numerically decreased in the small intestine. MANZANILLA et al. (2004) showed that the proportion of acetate to butyrate was increased in the caecum and colon of early weaned pigs when adding an oregano based essential oil product to the piglets' diets. In total, essential oils clearly change microbial VFA production in the piglets' intestine. According to the similarity of the effects on VFA observed in the present study for both feed additives, the reduced production of VFA due to the essential oils may be interpreted as a result of an antimicrobial activity of this feed additive.

Biogenic amines in chyme are produced by intestinal microbes via decarboxylation of amino acids. This can stress digestion and may have negative effects on performance. Moreover, the formation of biogenic amines has toxicological impacts in the gut. These negative effects can be overcome by feeding nutritional antibacterials (DIERICK et al., 1986). Also the use of organic acids as feed additives is known to reduce the intestinal load of biogenic amines (ECKEL et al., 1992). In the present study there was no statistically significant influence of either feed additive on production of biogenic amines in the ileum and colon but there were lower contents of some biogenic amines in the caecum chyme. Moreover, summarized contents of all investigated biogenic amines were numerically lower in both groups with feed additives. In total, the influence of the tested essential oils on production of biogenic amines in the three investigated gut sequences was very similar to the investigated AGP (Avilamycin). Given that biogenic amines are products of the gut microflora, in analogy to argumentation of reduced VFA concentrations these results give further rise to the existence of an antimicrobial activity of essential oils *in vivo*.

Ammonia contents and pH values in chyme were not influenced by one of the both feed supplements tested in the present study. But it is interesting that in the group fed essential oils, ammonia contents were numerically lower except in the colon. Ammonia in the gastrointestinal tract is often considered as putrefactive product released by bacteria like *E. coli* (SHIM et al., 2005). Therefore, decreased ammonia contents are a sign for lower bacterial activity. Consequently the numerical reduction of Ammonia concentration in the essential oil group is in accordance to the concept of an antimicrobial activity of the essential oil blend *in vivo*.

Blood analysis ranged within normal values without being changed by Avilamycin or essential oils, respectively. This is in accordance to data of CHO et al. (2006) indicating that essential oils do not directly affect blood analysis. In total, normal blood analysis values indicated an overall proper health status of animals independent of the intestinal effects of the feed additives tested in the present study.

The changes in chyme parameters observed in the present study give rise to the hypothesis that the mode of action of the essential oil blend is similar to that of the feed antibiotic Avilamycin. Bacterial activity in the gut was reduced by both additives as bacterial counts as well as contents of volatile fatty acids, biogenic amines, and ammonia were reduced. This may explain the positive effects of essential oils on piglets' performance reported in literature. RODEHUTSCORD and KLUTH (2002) summarized the effects of numerous essential oils on weaner piglets and mentioned that growth rate and feed to gain ratio can be improved on average by 2 % in each case. WALD et al. (2001) reported even higher growth rates and feed to gain ratios by 7 % due to oregano essential oils in piglet diets. More recent data from MOLNAR and BILKEI (2005) confirm the growth promoting effect of oregano feed supplement in piglets. Furthermore, WENK (2003) mentions that herbs, spices and essential oils can stimulate feed intake of animals. In the present study, animals supplied with the essential oils additive tended to consume more feed. Daily weight gains were numerically highest in the essential oils group and feed to gain ratios were slightly decreased by both feed additives. In another study testing the same feed additive as in the present experiment, the growth promoting effect of the essential oils revealed to be statistically significant (STEINER et al., 2006).

In summary, we conclude that antimicrobial activity of essential oils used as feed additives to weaner piglets is one of the major mode of action explaining the well established

growth promoting effect of these substances. Further evidence of this hypothesis is provided by more detailed investigations on nutrient digestibility (ZITTERL-EGLSEER et al. 2007) as well as on histological and molecular biological parameters of gut tissues (KROISMAYR et al. 2007).

## 5 Acknowledgement

The authors whish to thank Biomin GmbH, Herzogenburg, Austria, for supporting this study.

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Eingelangt am 23. Februar 2007
Angenommen am 29. Juli 2008