The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses

Einfluss des Zusatzes von organischen Säuren und ätherischen Ölen zum Futter auf die mikrobiologische Qualität von Hühnerschlachtkörpern

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Introduction

Food poisoning cases have been increasing worldwide during last decades. Especially, *Campylobacter* spp. and *Salmonella* spp. have caused sporadic cases of food-borne illness, therefore have become an important international public health and economic issue, and are often associated with consumption of poultry (Rodrige et al., 1990; Duguid and North, 1991; Jones, 2001).

Elimination or reduction of pathogens from the food supply is a difficult task. Approaches from “farm to fork” will likely be required to impact the incidence of food-borne illness associated with these pathogens. Elimination of these organisms from broilers and other poultry before they reach the processing plant will improve the chances of producing processed carcasses free from these organisms (Stern et al., 2001). Even when a few birds arrive at the plant colonized or externally contaminated with pathogens such as these, other birds, and ultimately other carcasses, may become contaminated as well (Mead et al., 1994, The National Advisory Committee On Microbiological Criteria For Foods, 1994).

With increasing concerns about antibiotic resistance, the ban on subtherapeutic antibiotic usage in Europe, there is increasing interest in finding alternatives to antibiotics for poultry production. The intestinal microbiota, epithelium and immune system provide resistance to enteric pathogens. Inhibition of pathogens by the intestinal microbiota has been called bacterial antagonism, bacterial interference, barrier effect, colonisation resistance, and competitive exclusion. Mechanisms by which the indigenous intestinal bacteria inhibit pathogens include competition for colonisation sites, competition for nutrients, production of toxic compounds, or stimulation of the immune system.

Herbs and species have been used for centuries to provide favourable flavours. They also have antimicrobial activity (Tassou et al., 1995; Ultee et al., 1998; Lambert et al., 2001). Antibacterial activity of herbs and species is mainly caused by essential oil components such as carvacrol, α-terpineol, terpinen-4-ol, eugenol, linalool, (-)-thujone, (cis+trans) citral, nerol, geraniol, and menthone (Dorman and Deans, 2000). The essential oils are hydrophobic and their primary site of toxicity is the membrane. They accumulate in the lipid bilayer according to a partition coefficient that is specific for the compound applied, leading to disruption of the membrane structure and function. Essential oils show the bactericidal effect due to their major phenolic components provoking damage in bacterial envelope, which plays a fundamental role in the life of bacteria. However, a critical concentration of the essential oils is needed to cause leakage of cellular constituents (Juven et al., 1994; Siskema et al., 1994; Mendoza-Yepes et al., 1997). Concentrations ranging from 0.05 to 1% were determined to observe the bactericidal effect (Dickens et al., 2000).

In the food animal industry, organic acids were originally added to animal feeds to serve as fungistats (Paster, 1979; Dixon and Hamilton, 1981), but in the past 30 yr, formic and propionic acids and various combinations have also been examined for potential bactericidal activity in feeds and feed ingredients contaminated with food-borne pathogens, particularly *Salmonella* spp. (Khan and Katamay, 1969; Vanderwal, 1979; Williams, 1981; Hinton and Linton, 1988; Humphrey and Lanning, 1988; Iat et al., 1990; Mian and Shottes, 1992; Bercheri and Barrow, 1996). In general, potential bacterial targets of biocidal compounds include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and function (Davidson, 2001).

The aim of this study presented here is to use of organic acids and essential oils in broiler feed to reduce level of microorganisms and contamination on the carcasses.

Material and Method

Birds

In this study, a total of 800 Ross 308 broiler chickens were used. Broiler chicks were kept in different pens (1.5x 2.5 m) based on the diet regimes for 42 days. Birds were divided into 4 groups: a basal diet (control) group, the basal diet supplemented with essential oil, organic acid combination, and essential oil+organic acid groups (200 birds for each group; 50 birds x 4 pens) as given at Table 1. For microbiological analyses, randomly chosen 20 birds from each group were used. Broiler chickens used were mixed sex and kept in deep litter.

Ingredients of the diets were shown in Table 1. Diets were fed *ad libitum* and birds had free access to drinking water.

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Birds were slaughtered on 42nd day of age. Slaughtering was carried out under commercial conditions in a pilot plant. The equipment and plant were disinfected after slaughtering each group. Samples were taken immediately after air chilling.

**Microbiological Analyses**

10 g of neck-skin from each carcass was trimmed and homogenized in 90 ml peptone water1 using stomacher2 for 2 min. The homogenates were then serially diluted in 0.1% peptone water and plated out on Plate Count Agar (PCA)3, Violet Red Bile Glucose Agar (VRBG)4, Violet Red Bile Lactose Agar (VRBA)5 and Baird Parker Agar Base6 with Egg Yolk Tellurite Emulsion7 for the enumeration of Total Aerobic Mesophilic Bacteria (TVC), Enterobacteriaceae, Coliforms and Staphylococci/Micrococci, respectively. PCA plates were incubated at 30°C for 2-3 days, VRBA, VRBG and Baird Parker plates were incubated at 37°C for 1 day. Incidence of Salmonella spp. was determined by a 2-step enrichment procedure. Neck skin samples were aseptically trimmed to 25 g and homogenised for 2 min in 225 ml buffered peptone water8 using a stomacher. Following overnight incubation at 37°C, 0.1 ml buffered peptone water from each sample was inoculated in duplicate into tubes containing 10 ml of Rappaport-Vassiliadis broth9 (RV) and incubated at 42°C for 2 days as previously recommended (ANONYMOUS, 1989; CLOAK et al., 1999). Brilliant green agar10 (BGA) plates were inoculated from each of RV tubes.

### Table 1. The Ingredients of diets given to broiler chickens

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Broiler Feeding Diet (0-21st days)</th>
<th>Broiler Feeding Diets (22-42nd days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Diet (Control)</td>
<td>Essential Oil</td>
</tr>
<tr>
<td></td>
<td>Basal Diet (Control)</td>
<td>Essential Oil</td>
</tr>
<tr>
<td>Corn</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>34.5</td>
<td>34.5</td>
</tr>
<tr>
<td>Fish Meal</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Di Calcium Phosphate</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Trace Vitamin Premix1</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Trace Mineral Premix1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>DL-Methionin</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Coccidiostat (Cygro)2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Essential Oil 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Organic Acid Combination4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Essential Oil+Organic Acid 5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Calculated Chemical analyses, %

| Crude protein | 23.11 | 19.35 |
| Calcium | 1.18 | 0.92 |
| Total Phosphor | 0.79 | 0.66 |
| Methionin | 0.51 | 0.35 |
| Lysine | 1.47 | 1.06 |
| Metabolisable energy, KCal/Kg | 3101 | 3241 |

1: For each kg of the diet; vitamin A 12000 IU; vitamin E 35.0 mg; vitamin K2 5.0 mg; vitamin B1 3.0 mg; vitamin B2 7.0 mg; vitamin B6 5.0 mg; vitamin B12 0.015 mg; Calcium D-Pentotenat 10.0 mg; Folic acid 1.0 mg; D-Biotin 0.045 mg; Choline chloride 125.0 mg; vitamin C 50.0 mg; Mn 80 mg; Fe 60.0 mg; Cu 5 mg; Co 0.2 mg; Se 0.15 mg.

2: For each kg of cygro; maduramycin Amonium 500 ppm

3: Origanum onites, 15 g/kg.

4: Lactic acid 200 g/kg; Formic acid 250 g/kg, and Propionic acid, 80 g/kg.

5: Origanum onites, 15 g/kg Lactic acid; 150 g/kg Formic acid; 180 g/kg, and Propionic acid 60 g/kg.

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1Oxoid CM9, Oxoid LTD, Basingstoke, England
2Interscience, 30.ch du bois des Arpents 78860 ST Nom La Breteche, France.
3Oxoid CM325, Oxoid LTD, Basingstoke, England.
4Oxoid CM 485, Oxoid LTD, Basingstoke, England.
5Oxoid CM107, Oxoid LTD, Basingstoke, England.
6Oxoid CM509, Oxoid LTD, Basingstoke, England.
7Oxoid CM669, Oxoid LTD, Basingstoke, England.
8Oxoid CM263, Oxoid LTD, Basingstoke, England.
9Merc 5406, Merck kGaA 64271 Darmstadt, Germany.
10Merc 3785, Merck kGaA 64271 Darmstadt, Germany.
broths after 24-48 h and incubated for 18-24 h at 37°C as previously described (De Smedt et al., 1991; De Zutter et al., 1991). Suspected colonies were confirmed biochemically by inoculating into Triple Sugar Iron Agar (TSI)1 and Lysine Iron Agar (LIA)2 slopes incubated at 37°C for 24 h, and final confirmation carried out using specific Salmonella O and H agglutinating anti sera3.

Statistical Analyses

The analysis of data gathered from trials was carried out by using conducting Duncan’s test in SPSS computer program (SPSS, 2003).

Results

The results showed that supplementation of organic acid combination and/or Origanum onites reduced the numbers of bacteria investigated. These reductions were significant in most of the cases. TVC obtained from essential oil supplemented group was significantly lower than those of obtained from the other groups. No significances were observed in the numbers of coliforms and Enterobacteriaceae among groups. The numbers of Staphylococci/Microccci were significantly lower for the organic acid or essential oil supplemented group, but not for combination of both treatments and control (Table 2).

The incidence of Salmonella spp. on the carcasses was significantly lower in organic acid supplemented group, and essential oil and organic acid combination supplemented group than essential oil supplemented group and control group. While 0.82 of the control group birds were Salmonella spp. positive, only 0.20 of birds fed organic acid combination were positive for Salmonella spp. (Table 3). However, it should be stressed that the initial Salmonella spp. load of broilers at the start point of feeding has not been examined. Therefore these reductions observed in the numbers of Salmonella spp. contaminated birds are not exact reduction rates but are expressions of a trend. A number of studies have reported that the inclusion of organic acids in feeds may influence the intestinal microflora (Voigt et al., 1981; Voigt et al., 1982). Various acids including formic, acetic, propionic and lactic have been added to swine feed resulting in significant reductions in Enterobacteriaceae in the feed (Van De Wal, 1980). In addition the number of pigs infected with Salmonella was reduced. Another organic acid, propionic acid and its salts are commonly used at low levels in poultry diet growth (Tassou et al., 1995; Ultee et al., 1998; Teesedre and Waterhouse, 2000; Lambert et al., 2001) and to reduce the bacterial load of carcasses (Izat et al., 1989; Izat et al., 1990).

This study showed that organic acid combination reduced the levels of bacteria investigated significantly (p<0.05) on the neck skin of carcasses in most of the cases (Table 2). These reductions, although seemed to be very low but significant, may have serious impacts on industry. The aim of this study was to extent the shelf-life of carcasses and to reduce the pathogen numbers as much as possible. According to Ayres, (1960) meat spoilage, i.e. smell and slime formation is apparent when the total numbers on the skin reach 107 to 108 cfu cm-2.

Therefore under the same conditions, meat with even 1 log higher initial load than the other spoils earlier and causes economical losses.

It was also shown that organic acid combination reduced the number of Salmonella spp. positive samples. While 0.82 of control group birds were Salmonella spp. positive, only 0.20 of birds fed organic acid combination were positive for Salmonella spp. (Table 3). However, it should be stressed that the initial Salmonella spp. load of broilers at the start point of feeding has not been examined. Therefore these reductions observed in the numbers of Salmonella spp. contaminated birds are not exact reduction rates but are expressions of a trend. A number of studies have reported that the inclusion of organic acids in feeds may influence the intestinal microflora (Voigt et al., 1981; Voigt et al., 1982). Various acids including formic, acetic, propionic and lactic have been added to swine feed resulting in significant reductions in Enterobacteriaceae in the feed (Van De Wal, 1980). In addition the number of pigs infected with Salmonella was reduced. Another organic acid, propionic acid and its salts are commonly used at low levels in poultry diet growth (Tassou et al., 1995; Ultee et al., 1998; Teesedre and Waterhouse, 2000; Lambert et al., 2001) and to reduce the bacterial load of carcasses (Izat et al., 1989; Izat et al., 1990).

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Discussion

Elimination of pathogen organisms from broilers and other poultry before they reach the processing plant will improve the chances of producing processed carcasses free from these microorganisms (Stern et al., 2001). Therefore feed supplementation of some materials, such as organic acids and essential oils have been used to inhibit bacterial growth (Tassou et al., 1995; Ultee et al., 1998; Teesedre and Waterhouse, 2000; Lambert et al., 2001) and to reduce the bacterial load of carcasses (Izat et al., 1989; Izat et al., 1990).

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Table 2. Numbers of bacteria investigated on the neck skin of broiler carcasses (log cfu g⁻¹) fed by various feed supplementations

<table>
<thead>
<tr>
<th>Groups</th>
<th>TVC</th>
<th>Coliforms</th>
<th>Enterobacteriaceae</th>
<th>Staphylococci/Micrococi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.90a</td>
<td>4.09 ± 0.13</td>
<td>4.07 ± 0.18</td>
<td>5.24 ± 0.10</td>
</tr>
<tr>
<td>Organic acid Combination</td>
<td>5.58b</td>
<td>3.58 ± 0.08</td>
<td>3.58 ± 0.12</td>
<td>4.51b ± 0.08</td>
</tr>
<tr>
<td>Essential Oil</td>
<td>5.07c</td>
<td>3.57 ± 0.17</td>
<td>3.71 ± 0.16</td>
<td>3.45c ± 0.14</td>
</tr>
<tr>
<td>Essential Oil+Organic Acid Combination</td>
<td>6.22a</td>
<td>3.85 ± 0.16</td>
<td>3.77 ± 0.19</td>
<td>5.23a ± 0.11</td>
</tr>
</tbody>
</table>

a, b, c: Mean values within a column with no common superscript differ significantly.

Table 3. Incidence of Salmonella spp. contaminated birds fed by various feed supplementations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Salmonella spp., %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>Organic acid Combination</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>Essential oil</td>
<td>0.44 ± 0.12</td>
</tr>
<tr>
<td>Essential oil and organic acid combo</td>
<td>0.33b ± 0.11</td>
</tr>
</tbody>
</table>

a, b, c: Mean values within a column with no common superscript differ significantly.
to inhibit mould growth (Paster, 1979; Dixon and Hamilton, 1981). IZAT et al. (1989) showed that feeding propionic acid (53.5% propionic acid, 9.5% ammonium hydroxide, 11.5% 1.2-propanediol, and 25.5% water) combination altered intestinal microflora and reduced the numbers of coliforms and E. coli in the small intestines and on the carcasses. They reported that although the reductions observed on the carcasses were not statistically significant, reductions in the numbers of bacteria monitored in the small intestines were significant. Although the same organic acid formulation was not used, our results agreed with the results of IZAT et al. (1989) that no significant reductions in the numbers of coliforms and Enterobacteriaceae were observed. They also showed that although no statistical differences were observed in the Salmonella levels in the intestines by feeding propionic acid combination, the post chill carcasses had significantly lower amount of Salmonella on the carcasses, which was also in agreement with our results. In contrast another study showed that increasing amount of dietary lactic acid and fumaric acid (0.25, 0.5, 1.0 and 2.0%) did not offer protection from caecal Salmonella. In this study, the carcasses were divided into four groups as control, organic acid supplemented group, essential oil supplemented group, and essential oil supplemented group. At the end of a-42 day of growth period, for microbiological analyses 20 birds from each group were randomly chosen. Then chickens were slaughtered and microbiological analyses were carried out on the neck skin of the carcasses. In addition, the incidence of Salmonella spp. on the carcasses were considered, although it was not significant, essential oil supplementation also reduced the incidence of Salmonella spp. on the carcasses. Whilst the highest incidence was found to be on the control group carcasses with 0.82, essential oil supplemented group had 0.44 Salmonella spp. positive samples (Table 3).

Combination of both treatments gave relatively lower reductions in the numbers of microorganisms investigated; in some cases no reduction was observed. No significant differences (p<0.05) between control group and essential oil + organic acid supplemented group for the bacteria investigated were observed (Table 2). Although a synergic effect of two agents was expected, the results did not support this aim, and probably at the micro-molecular level using both agents together reduced the individual efficiency of each agent. In addition the occurrence of true synergy depends on using a suitable combining dose (Lambert and Lambert, 2003). Therefore in order to demonstrate the true synergy, essential oils and organic acids should be combined in a suitable dose. This might be the reason for not observing the synergetic effect in the present study. Incidence of Salmonella spp. in the group supplemented with both agents was 0.33 which was between organic acid combinations supplemented group 0.20 and essential oil 0.44 supplemented groups (Table 3).

All over the results showed that supplementation of organic acids and essential oils or both had some reducing effects on the bacterial load of broiler carcasses. These reductions seemed to be higher in essential oil supplemented group in most of the cases. All supplantations caused remarkable reducing effect on the numbers of coliforms. This might be due to the higher extent than those organic acid combinations affected in the intestines on essential oil supplemented group. At the end of study it was reported by IZAT et al. (1990) that inclusion of 0.4% and 0.8% LUPRO SIL NC (Propionic acid combination) decreased the numbers of coliforms and E. coli in the small intestine without any effect on intestinal pH and when periodic dosage of Luprosil NC (0.4%) was added to the diet, the number of Salmonella typhimurium on post chill carcasses also reduced.

The essential oil supplementation also resulted in lower microbial load on the carcasses. The reductions observed on the carcasses were varied. Reductions in the numbers of coliforms and Enterobacteriaceae were not significant, as it was in organic acid combination. However, TVC and the numbers of Staphylococci/Micrococci were significantly (p<0.05) lower in essential oil supplemented group (Table 2). In addition, significant differences (p<0.05) were found between organic acid and essential oil supplemented groups in the TVC, and in the numbers of Staphylococci/Micrococci (Table 2). It seemed that essential oil affected TVC and the numbers of Staphylococci/Micrococci in a higher extent than those organic acid combinations affected. Several researchers showed inhibitory effect of Origanum onite-essential oils on bacteria. Sæde, (2003) reported that Oreganum onites L. hydrosols showed greatest inhibitory effect against Escherichia coli ATCC 25922, E. coli O157:H7 ATCC 33150, Staphylococcus aureus ATCC 2392 and Yersinia enterocolitica ATCC 1501. Baydar et al., (2004) also showed that O. onites had inhibitory effect on Aeromonas hydrophila ATCC 7965, Bacillus amylovorans ATCC 3842, B. brevis FMC 3, B. cereus FMC 19, B. subtilis IMG 22, Corynebacterium xerosis UC 9165, Enterococcus faecalis ATCC 15753, Escherichia coli DM, Klebsiella pneumoniae FMC 5, Listeria monocytogenes Scott A, Micrococcus luteus LA 2971, Mycobacterium smegmatis RUT, Proteus vulgaris FMC 1, Staphylococcus aureus Cowan 1, Yersinia enterocolitica EU.

When the incidence of Salmonella spp. considered, although it was not significant, essential oil supplementation also reduced the incidence of Salmonella spp. on the carcasses. Whilst the highest incidence was found to be on the control group carcasses with 0.82, essential oil supplemented group had 0.44 Salmonella spp. positive samples (Table 3).

Summary

This study was conducted to determine the effects of organic acid combination (for each kg of the diet, 200 mg lactic acid, 250 mg formic acid, and 80 mg propionic acid) or essential oil (for each kg of the diet 15 mg Origanum onite) supplementation on the microbiological quality of chicken carcasses. In this study, a total of 800 broiler chickens were divided into four groups as control, organic acid supplemented group, essential oil supplemented group, and essential oil and organic acid supplemented group. At the end of a-42 day of growth period, for microbiological analyses 20 birds from each group were randomly chosen. Then chickens were slaughtered and microbiological analyses, including determination of Total Viable Count (TVC), the numbers of coliforms, Enterobacteriaceae and Staphylococci/ Micrococci were carried out on the neck skin of carcasses. In addition, the incidence of Salmonella spp. on the carcasses for each group was determined.

Results showed that significant reductions were observed in the numbers of bacteria investigated for both supplemented groups. The Total Viable Count observed (TVC) on the neck skin was 5.90 log cfu g⁻¹ on the control group, however it was 5.07 log cfu g⁻¹ for essential oil sup-
Zusammenfassung

Einfluss des Zusatzes von organischen Säuren und ätherischen Ölen zum Futtern auf die mikrobiologische Qualität von Hühnerschlachtkörpern

In der vorliegenden Studie wurde der Einfluss des Zusatzes einer Kombination verschiedener organischer Säuren (200 g/kg Milchsäure, 250 g/kg Ameisensäure, 80 g/kg Propionsäure) sowie eines ätherischen Öls (15 g/kg Origanum onites) zum Futtern auf die mikrobiologische Qualität von Hühnerschlachtkörpern untersucht. Es wurden insgesamt 800 Broiler verwandt, die auf vier Behandlungen aufgeteilt wurden: (1) Kontrolle, (2) organischer Säuren, (3) ätherisches Öl, (4) organischer Säuren und ätherisches Öl. Am Ende der 42-tägigen Mastperiode wurden je 20 Broiler zufällig aus jeder Behandlung für die mikrobiologischen Untersuchungen ausgewählt. Die Tiere wurden geschlachtet und folgende Keime in der Halshaut bestimmt: Gesamtzahl lebender Keime (TVC) sowie Anzahlen an coliformen Keimen, Enterobakterien und Staphylokokken/Mikrokokken. Zusätzlich wurde die Häufigkeit an Salmonella spp. auf jedem Schlachtkörper bestimmt.

Der Einsatz von organischen Säuren und dem ätherischen Öl führte zu einer signifikanten Verminderung der Bakterienzahlen. Die Gesamtanzahl lebender Keime (TVC) auf der Halshaut betrug für die Kontrolle 5,90 log cfu/g, für das ätherische Öl 5,07 log cfu/g und für die organischen Säuren 5,58 log cfu/g. Die Häufigkeit an Salmonellen spp. am Schlachtkörper war ebenfalls beim Einsatz der organischen Säuren (0,20) oder des ätherischen Öls (0,44) geringer als in der Kontrolle (0,82). Die Ergebnisse unterstreichen, dass organische Säuren und ätherische Öle geeignet sind, die mikrobielle Belastung von Geflügelschlachtkörpern zu reduzieren. Hierdurch kann eine Lebensmittelvergiftung und ein früher Fleischverderb vermieden werden.

Stichworte

Broiler, Fütterung, organische Säuren, ätherische Öle, mikrobielle Qualität, Schlachtkörper

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