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Effect of thyme, oregano and their major active components on performance and intestinal microbial populations of broilers

A dissertation submitted in partial fulfillment

of the requirements for the degree of

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by

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Qena, Egypt

IN MEMORY OF MY LATE BELOVED MOTHER

Effect of thyme, oregano and their major active components on performance and intestinal microbial populations of broilers.

The aim of the present study implied an evaluation of the potential of different increasing levels of thymol, carvacrol, a mixture of thymol and carvacrol, as well as thyme and oregano as feed additives in broiler diets, in order to observe their influence on feed intake, performance, digestibility, carcass traits and gastro-intestinal microflora. Thymol, carvacrol or their combination were offered at 0, 100, 200, 300, 400 or 500 mg/kg. while thyme or oregano were offered at 0, 10, 15, 20, 25 or 30 g/kg diet to 120 unsexed chickens in each trial from 4-42 days of age. Different levels of thymol revealed quadratic effects on body weight gain (BWG) and feed intake (FI), as well as linear improvements on feed conversion ratio (FCR) and nitrogen-corrected apparent metabolizable energy (AME_N) contents. Thymol addition also exhibited a linear increase of *Lactobacillus spp.* colony forming units (CFU) in the crop just as a quadratic increase in total viable count. Various levels of carvacrol revealed linear and quadratic effects for BWG, FI and FCR. Highest improvements for AME_N were observed when the maximal level of carvacrol was supplemented. Moreover, significant increases for total viable count were observed in this trial. The mixture of thymol and carvacrol revealed linear and quadratic improvements for BWG, FI and FCR, as well as an increase in carcass weight. The addition of thyme at different levels displayed a quadratic effect for BWG and a linear response for FCR among thyme treatments. Moreover, the treatment exhibited a significant improvement in AME_N concentrations. Quadratic effects among thyme treatments were observed for carcass weight, dressing percentage, liver weight, giblets percentage and intestinal *Lactobacillus* CFU. Increasing levels of oregano revealed quadratic responses in BWG, FI and FCR, as well as linear and quadratic effects on carcass weight and a linear decrease in abdominal fat. Furthermore, oregano supplementation improved AME_N concentrations. No clear effects were observed for intestinal bacterial counts of broilers. All treatments exhibited highest improvements when medium concentrations were supplemented to diets. In conclusion it can be stated that thyme and oregano and their active components thymol and carvacrol can be used as effective feed additives to improve performance and gut health of broiler chickens. However, future research is needed to determine the optimal dietary inclusion level and the exact mode of action of the examined plants and active components.

Einfluss von Thymian und Oregano sowie ihrer wesentlichen Wirkstoffe auf Leistung und intestinale mikrobielle Besiedelung von Broilern

Das Ziel dieser Arbeit war, den Einfluss von Thymol, Carvacrol, einer Mischung von Thymol und Carvacrol, sowie von Thymian und Oregano auf das Wachstum, die Futter- und Energieaufnahmen sowie Schlachtleistung und Keimzahlen in Kropf, Dünndarm und Dickdarm von Masthühnern zu prüfen. Dazu wurden fünf Versuche durchgeführt. In jedem Versuch standen 120 Küken zur Verfügung, die auf 6 Versuchsrationen aufgeteilt wurden und vom 4. bis um 42. Tag die jeweilige Ration erhielten. Thymol, Carvacrol oder deren Kombination wurden in Konzentrationen von 0, 100, 200, 300, 400 oder 500 mg/kg angebotenen., während Thymian oder Oregano in Konzentrationen von 0, 10, 15, 20, 25 oder 30 g/kg dem Futter zugefügt wurden. Die Thymolkonzentration hatte quadratische Effekte auf die mittleren täglichen Zunahmen (TZ) und Futteraufnahmen und bewirkte lineare Verbesserungen des Futteraufwandes (FCR) und der Aufnahme an stickstoffkorrigierter scheinbarer umsetzbarer Energie (AME_N). Zudem konnte ein linearer Anstieg der koloniebildenden Einheiten von *Lactobacillus spp.* beobachtet werden und auch eine quadratische Wirkung auf die Gesamtkeimzahlen. Die Ergänzung der Rationen mit verschiedenen Mengen Carvacrol führte zu positiven linearen und quadratischen Effekten auf die TZ, Futteraufnahmen und den Futteraufwand. Zusätzlich konnte eine Erhöhung der AME_N-Aufnahme sowie eine Erhöhung der Gesamtkeimzahl bei höchster Carvacrol-Ergänzung erzielt werden. Die Thymol-Carvacrol-Mischung führte zu linearen und quadratischen Effekten auf die TZ, Futteraufnahmen und den Futteraufwand und bewirkte einen linearen Anstieg in Schlachtgewicht, Ausschlachtung und abdominalem Fett. Die Zugabe von verschiedenen Mengen an Thymian erzielte einen quadratischen Effekt auf die TZ und einen lineare Effekt auf den Futteraufwand und die AME_N-Aufnahmen. Des Weiteren konnten quadratische Effekte beim Schlachtgewicht, Lebergewicht und den koloniebildenden Einheiten von *Lactobacillus spp.* in Darm beobachtet werden. Bei der Ergänzung des Futters mit unterschiedlichen Mengen von Oregano zeigten sich quadratische Effekte bei TZ, Futteraufnahme und Futteraufwand. Lineare und quadratische Auswirkungen auf das Schlachtgewicht, sowie eine lineare Abnahme des abdominalen Fettes konnten ebenso beobachtet werden. Der Zusatz von Oregano zeigte keinen klaren Auswirkungen auf die intestinalen Keimzahlen von Masthähnchen. Bei allen Versuchen wurden beste Auswirkungen auf die untersuchten Leistungsparameter festgestellt, wenn mittlere Konzentrationen der entsprechenden Futterzusätze ergänzt wurden. Als Folgerung kann abgeleitet werden dass sich Thymian, Oregano, sowie deren Wirkstoffe Thymol und

Carvacrol als effektive Futterzusatzstoffe eignen um Mastleistungen und Darmgesundheit bei Masthähnchen positiv zu beeinflussen. Allerdings besteht weiterhin Forschungsbedarf um die optimale Dosierung und Wirkungsweise von Kräutern und deren Wirkstoffen präzieser zu definieren.

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ABBREVIATIONS

AME_N ……....	Nitrogen-corrected apparent metabolizable energy
ADF……	Acid detergent fibre expressed inclusive residual ash
*a*NDF……..…….	Neutral detergent fibre assayed with a heat stable amylase and expressed inclusive residual ash.
AOAC…….	Association of Official Analytical Chemists
ATP…….	Adenosine triphosphate
B. cereus……..	*Bacillus cereus*
BW……..	Body weight
C. perfringens…….	*Clostridium perfringens*
Ca……..……..	*Calcium*
CFU…….	Colony forming units
CP ……..….	Crude protein
DM……...	Dry matter
E. coli……...……	*Escherichia coli*
EO……	Essential oils
EU……	European Union
FCR…….	Feed conversion ratio
FI…….	Feed intake
GE……	Gross energy
GfE……...	Gesellschaft für Ernährungsphysiologie.
L. monocytogenes……	*Lesteria monocytogenes*
MIC……..	Minimum inhibitory concentrations
N……...	Nitrogen
n.a…….	Not analysed
ND……	Not detected
NS…….	Not significant
S. aureus……...……	*Staphylococcus aureus*
SEM…….	Standard error of the means

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1. GENERAL INTRODUCTION

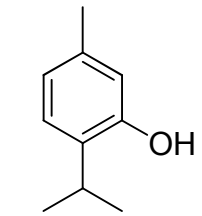
1 GENERAL INTRODUCTION

Natural feed additives of plant origin are believed to be safer, healthier and less regarded as chemical hazards than synthetic additives. Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency. Moreover, active components of herbs may improve digestion and stimulate the immune function in broilers (Ghazalah and Ali, 2008). Great amounts of these active components can also be found in essential oils (EO) of the associated plant or herb. Essential oils are mainly extracted by steam distillation from diverse plant material. Hence, the chemical composition and concentration of active components varies greatly dependent on their source.

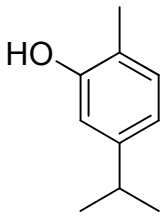
Since the EU banned most of the antibiotic growth promoters in broiler nutrition (Anonymous, 2003) due to cross and multiple resistance (Neu, 1992), much research has been conducted to explore the use of plants, plant extracts and EO as effective substitutes. In some studies the ability of EO to be used as alternative growth promoters has already been proven and thus started to play a decisive role in nutrition of poultry.

Thymol and its isomer carvacrol, components derived from thyme and oregano plants (Figure 1), are classified as monoterpene phenols and have already proven their antimicrobial effect *in vitro* (Ouwehand et al., 2010). This effect is mainly due to the lipophilic character of the active principles, which permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations of essential oils also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998).

Nevertheless, there is only limited data on *in vivo* effects of thyme, oregano, thymol and carvacrol. Therefore, the objective of the present study implied an evaluation of the potential of increasing levels of thyme and oregano and of thymol, carvacrol and their combination as feed additives in broiler diets, in order to observe their influence on feed intake, performance, carcass traits and intestinal microflora.



Thymol ($C_{10}H_{14}O$)



Carvacrol ($C_{10}H_{14}O$)

Figure 1. Chemical structures of thymol and carvacrol

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2 SCOPE OF THE THESIS

2 SCOPE OF THE THESIS

The scope of this thesis was to examine the effects of thymol, carvacrol or their mixture as principle components in thyme and oregano and effects of thyme or oregano leaves supplemented to broiler diets in increasing levels. This thesis was conducted in order to:

1. Give an overview of some selected herbal ingredients on their mode of action regarding their effects on antimicrobial activity and performance of broilers.
2. Determine the supplementation of thymol, carvacrol or their mixture concerning growth performance, nutrient digestibility, carcass variables and gastro-intestinal bacterial population of broilers.
3. Determine the supplementation of thyme or oregano leaves concerning growth performance, nutrient digestibility, carcass variables and gastro-intestinal bacterial population of broilers.
4. Determine the optimal dietary inclusion level and mode of action of these herbal ingredients.
5. Achieve the optimal growth performance and disease resistance in broiler production.

Review of literature of herbs and essential oils, and their active components including chemical compositions, mode of action, broiler performance are presented in chapter 3. Effects of supplementation of thymol, carvacrol and their mixture including three different trials each with 6 inclusion levels (0, 100, 200, 300, 400 and 500 mg/kg) are presented in chapter 4. Supplementation of thyme and oregano including two trials each with 6 inclusion levels (0, 10, 15, 20, 25 and 30 g/kg) are presented in chapter 5.

3 Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review

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Abstract

Since the EU banned antibiotic growth promoters in broiler nutrition, much research has been conducted to explore the use of possible effective substitutes. One possibility is the application of herbs or their essential oils (EO). Numerous *in vitro* studies have already confirmed the antibacterial actions of these feed additives. Consequently, several *in vivo* studies were performed to confirm their beneficial qualities. Performance variables that were dominantly observed and analyzed are feed intake body weight gain and feed conversion ratio. Most of the trials showed slight positive effects, however significant results were rare. Since there are almost unlimited possibilities concerning dosage and combinations of herbs and EO there is still more research needed. A lack of standardization leads to difficulties when it comes to comparing results. One major factor in this concern is the varieties of active components. Chemical composition depends significantly on variables like plant species, physical and chemical soil condition, harvest period, stage of maturity at harvest, technology of drying, duration of storage and extraction process. Therefore, it is necessary to define and declare the composition of the feed additive used in experiments. Generally, it can be stated that herbs and EO have the potential to be considered as an alternative to antibiotic growth promoters in broiler nutrition. Nevertheless, there is still further research under more standardized condition needed to evaluate the right dosage and combination as well as the exact mechanism of actions.

1. Introduction

Natural feed additives of plant origin, also referred to as phytogenic substances are healthier, less regarded as chemical hazards and generally regarded as safe (GRAS; Burdock and Carabin, 2004). Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight (BW) gain, higher production rates and improved feed efficiency. Moreover, active components of herbs may improve digestion and stimulate the immune function in broilers (Ghazalah and Ali, 2008). Great amounts of these active components can also be found in essential oils (EO) of the associated plant or herb. Essential oils are mainly extracted by steam distillation from diverse plant material. Hence, the chemical composition and concentration of active components varies greatly dependent on their source.

Since the EU banned most of the antibiotic growth promoters in broiler nutrition (Anonymous, 2003) due to cross and multiple resistance (Neu, 1992), much research has been conducted to explore the use of plants, plant extracts and EO as effective substitutes. In some studies the ability of EO to be used as alternative growth promoters has already been proven and thus started to play a decisive role in nutrition of poultry. Anyhow, only limited research is available, which handicaps full comprehension of physiological responses. Until active components and their mode of action are identified in poultry, standardization will be imprecise (Applegate et al., 2010).

Recently, Brenes and Roura (2010) published a review which gives a brief summary of main effects of EO in poultry nutrition. However, this illustration did mainly focus on the mode of action of EO, rather than going into details that concern performance characteristics of broilers. Likewise, Windisch et al. (2008, 2009) contributed reviews on phytogenic feed additives to young piglets and poultry. It was concluded that the respective additives act mainly through a combination of different modes such as antioxidantive and antimicrobial action as well as effects on gut tissue. Nevertheless, and despite these solid overviews, the studies comprised mainly plant extracts or commercial products, consisting of different active components which were not clearly defined.

Therefore, the purpose of this study is to give an overview on and definition of phytogenic feed additives such as herbs, their EO, chemical composition and mode of action, as well as on the use of these ingredients in broiler diets with particular attention paid to feed intake and performance characteristics.

2. Chemical components of essential oils of selected herbs

Herbs are commonly used for flavour, colour, aroma and preservation of food or beverages. Another option implies preparing extracts such as EO - also called volatile or ethereal oils. Essential oils are aromatic oily liquids obtained from plant material such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots (Burt, 2004). Burt (2004) also mentions that EO can be obtained by a variety of laboratory methods, whereas the method of steam distillation is most commonly used for commercial production. Numerous publications have presented data on the composition of EO. Herbal plants contain between 0.1 and 30 g/kg EO (European Pharmacopoeia, 2004). Essential oils are secondary metabolites that are highly enriched in compounds based on isoprene structures that are called terpenes (Cowan, 1999). To date, there are more than 3,000 chemical compounds that have been isolated from EO. Monoterpenes, made by coupling of two isoprene units, constitute 90% of the essential oil molecules which allows for a wide variety of structures (Bakkali et al., 2008).

The chemical composition of an EO defines its mode of action as well as its attributes. Differences between, or within, EO depend significantly on several variables, such as plant species, physical and chemical soil conditions, harvest time, degree of plant maturity, technology of drying, duration of storage and extraction process (Burt, 2004; Bakali et al., 2008). Data about the chemical composition of EO taken from different literature also suffers from this indisposition. This can also be due to lacks of standardisation implying differences in analytical methods such as gas chromatography or mass spectrometry. Varying results lead to difficulties in using EO, since optimal dosage and mixture are yet to be identified. Thus, it is meaningful to define active chemical components in EO of some selected herbs, which are commonly used as feed additives in broiler diets. Analytical monographs can be found in European Pharmacopoeia (2004) and can be used as reference values in order to ensure good quality of the used additive.

3. *In vitro* antimicrobial properties of essential oils

Almost all EO exhibit antimicrobial action, some more strongly than others. These actions, known in folk medicine since ancient times, are still of use today. Phenols, alcohols, ketones and aldehydes are mainly associated with the antibacterial actions of EO, although the exact mechanism of actions has not been studied in great detail (Lambert et al., 2001). It is commonly known that the antimicrobial action of EO depends on the lipophilic character of their components. The components permeate the cell membranes and mitochondria of the microorganisms and inhibt, among others, the membrane bound electron flow and therewith the

energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations of EO also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998). Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, an extensive loss of cell contents or critical molecules and ions will lead to death (Burt, 2004).

Naturally, there is great economic interest in EO due to their antimicrobial action. For this reason, there are several publications that deal with antimicrobial actions of EO. A concern is that it is difficult to compare results of these studies if different testing methods were used. Testing of antimicrobial activity can be classified as diffusion, dilution or bioautographic methods. The outcome of a test can be affected by factors such as the volume of the inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature (Rios et al., 1988).

Table 1 presents minimum inhibitory concentrations (MIC) of EO and some of their components tested *in vitro* against selected bacteria. Applied tests were either agar diffusion method using a filter paper disc or a dilution method using agar or liquid broth cultures.

Numerous studies have examined this action. Preuss et al. (2005) compared different EO, including oregano, fenugreek, sage and cinnamon concerning their effect on *Staphylococcus aureus*. Oregano EO and its component carvacrol were found to be most potent and proved bactericidal. Similar findings were observed when 13 EO sources and their components, including oregano, rosemary, thyme, thymol and carvacrol, were tested against 11 different food bourne pathogens. While *Escherichia coli* and *Clostridium perfringens* were sensitive to most of the EO tested, also at lower concentrations, *Streptocouccus epidermis* and *Salmonella* serovars were only sensitive at high concentrations (Ouwehand et al., 2010). Hammer et al. (1999), who did a relatively large study, investigated the antimicrobial effect of 52 EO. Oregano and thyme EO exhibited lowest MIC against *E. coli*, *S. typhimurium* and *S. aureus.* However, six EO, including sage failed to inhibt any organisms, even at the highest concentration.

In turn much research has been conducted to understand and explain this effect. Mainly, principle components of the apparent most powerful EO sources, oregano and thyme have been examined and tested against different bacteria. When thymol and carvacrol were tested against the two pathogens *Pseudomonas aeruginosa* and *S. aureus* it was assumed that an impairment of the cell membrane causes leakage of potassium and phosphate ions and dissipates pH of those bacteria (Lambert et al., 2001). Ultee et al. (2002) discovered that carvacrol and *p*-cymene

caused an expansion of the liposomal membrane of *Bacillus cereus*. They hypothesized that carvacrol destabilizes the cytoplasmic membrane and also acts as a proton exchanger, subsequently reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool will finally lead to cell death. Moreover, the exact action on the ATP pool of both carvacrol and thymol, were examined by Helander et al. (1998). They suggested that both agents decreased the intracellular ATP pool of *E. coli* and also increased extracellular ATP, indicating destructive action on the cytoplasmic membrane.

Another noticeable aspect is that Gram-positive bacteria seem to be more susceptible to the actions of EO than Gram-negative bacteria, which was demonstrated by Smith-Palmer et al. (1998) who investigated 21 plant EO against five important food-bourne pathogens. It is not exactly known why there is a difference in sensivity, but it may be related to the outer membrane of Gram-negative bacteria which configures the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (Nikaido, 2003).

Further studies on the mode of action of certain EO, their combinations and mixing of their active components at various levels against pathogenic and spoilage microorganisms are needed in order to explain and to expand the knowledge on usage of such natural additives.

Table 1: Minimum inhibitory concentrations (µl/ml) of selected essential oils (EO) and of their active components tested *in vitro* against Gram-positive and Gram-negative bacteria (adapted from BURT, 2004)

EO or component	*Escherichia coli*	*Salmonella typhimurium*	*Bacillus cereus*	*Staphylococcus aureus*	*Listeria monocytogenes*
Anise	>1	n.a.[1]	n.a.	>1	>1
Cinnamon	0.5	n.a.	n.a.	0.4	0.3
Clove	0.4-2.5	>20	n.a.	0.4-2.5	0.3
Marjoram	>1	n.a.	n.a.	0.5	0.2
Oregano	0.5-1.2	1.2	n.a.	0.5-1.2	n.a.
Rosemary	4.5-10	>20	0.2	0.4-10	0.2
Thyme	0.45-1.25	0.45-20	n.a.	0.2-2.5	0.16-0.45
Carvacrol	0.23-5	0.23-0.25	0.19-0.9	0.18-0.45	0.38-5
Thymol	0.23-0.45	0.06	0.45	0.14-0.23	0.45
Eugenol	1	0.5	n.a.	n.a.	>1.0
α-Terpineol	0.45-0.9	0.23	0.9	0.9	>0.9
References	Farag et al., 1989; Chaibi et al., 1997; Smith-Palmer et al.,1998; Cosentino et al., 1999; Hammer et al., 1999; Burt and Reinders, 2003.				

[1] n.a., not analysed.

4. Influence of herbs and essential oils on feed intake

Feed intake can be influenced by a large number of factors. Selection of food depends on visual appearance, temperature, viscosity, saliva production, nutritive value of feed, toxicity of feed components, particle size and social interaction (Blair, 2008).

Several studies confirmed the positive influence of feeding EO (Table 2) or herbs on feed intake (Table 3). Cross et al. (2007) compared 5 culinary herbs and their EO in a study with 165 female broilers. They found that the average feed intake was reduced for birds which were fed 10 g/kg of oregano herb, while birds which were fed 1g/kg oregano EO consumed comparable amounts of feed as the control birds. However, rosemary EO supplementation resulted in a higher feed intake than the supplementation of rosemary EO. Significant improvements on feed intake were also discovered when 100 or 200 mg/kg of thyme EO (Bölükbasi et al., 2006) or 300, 500 or 700 mg/kg of oregano EO were added to the diet (Calislar et al., 2009; Kirkpinar et al., 2010). On the other hand, Cross et al. (2003) discovered a decrease of feed consumption from day 8 till 14 when relatively high amounts (5 g/kg) of thyme EO were supplemented. However, this effect disappeared in the later experimental period. The authors suggested that this may be due to the strong taste of thyme EO that might have been unpalatable for the young chicks, who, in later days adapted to the flavour. Table 4 presents effects of different active components of EO on feed intake and performance variables. Carvacrol and its isomer thymol, which are active components of thyme and oregano EO are mainly responsible for the characteristic flavouring. In order to assess their individual effects Lee et al. (2003b) added 200 mg/kg of each thymol and carvacrol to a broiler diet. Results revealed that carvacrol lowered feed intake which again may be due to surpressed appetite of the birds. In later studies Lee et al. (2004a, b) added 100 mg/kg of thymol or cinnemaldehyde to diets which were characterized by its growth depressing effects either due to the addition of carboxymethyl cellulose or induced through the substitution of maize grain to a rye grain-based diet. In both experiments cinnamaldehyde counteracted the negative effect of the growth depressing diets, possibly through its appetite stimulating effect. Cinnamaldehyde, the main principle of cinnamon, is responsible for the characteristic odour and is known for its established position as an irreplaceable flavour in the food industry.

Various studies have been performed with mixtures of EO (Table 5). RepaXol™, for example, is a commercial product and an encapsulated blend of EO. It includes oregano, thyme, cinnamon and capsaicin EO and is encapsulated with a double coating which is supposed to ensure better energy and nutrient utilisation. Zhang et al. (2005) observed a reduced feed intake after adding 200 or 300 mg/kg of RepaXol™ over a period of 42 days. CRINA® Poultry is a commercial

product containing 29% active ingredients, including thymol. The product is supposed to stimulate the secretion of endogenous digestive enzymes and balance the gut microbial ecosystem and thus improve growth performance. However, CRINA® Poultry did not have any impact on feed intake when a diet containg 100 mg/kg of the product was fed (Lee et al., 2003a). Likewise, Jang et al. (2007) did not observe any significant effects on feed intake when feeding CRINA® Poultry at levels of 25 or 50 mg/kg. Ulfah (2006) noticed an increased average daily feed intake after feeding a diet containing 18 mg/kg of each oregano, cinnamon, eucalyptus and thyme EO. It was assumed that the EO assisted in returning the microbial population in the gastrointestinal tract to more balanced levels and thus improved overall feed intake. Similarly, Ertas et al. (2005) noticed an increase in feed intake after three weeks when supplementing anise, oregano and clove EO mixture at 200 and 400 mg/kg to the broiler diet. Active ingredients like carvacrol, thymol, anetole and eugenol may be responsible for the appetizing effect of the respective EO mixture and are consequently responsible for the increased feed intake. However, this effect disappeared when the whole treatment duration (35 days) was observed.

5. Influence on body weight gain

Just like feed intake, BW gain also depends on several factors like genotype, housing, hygienic conditions, management, feeding system and diet attributes. Essential oils have been shown to increase BW gain by their ability to destroy pathogen microorganisms in the digestive system and consequently increasing the production of digestive enzymes which improve utlilisation of digestive products (Hernández et al., 2004).

Several studies confirmed the positive influence of herbs (Table 3) and their EO (Table 2) on BW gain. Significant improvements have been observed when 400 mg/kg of anise EO were added to the diet (Simsek et al., 2007). Çiftçi et al. (2005) observed an improvement of approximately 15% when BW gain was compared to the control group. Likewise, Cross et al. (2007) reported a significant improvement in BW gain when supplementing 1 g/kg EO of thyme. When 10 g/kg of the corresponding herb was fed, it was noticed that thyme herb did not achieve the same positive results as its EO. Nevertheless, another study showed that an addition of 5 g/kg thyme herb improved BW gain by approximately 6% when compared to the corresponding control group (Toghyani et al., 2010). Similarly, Mohamed and Abbas (2009) observed an increase of BW gain by 6% when adding 1 g/kg of fennel. However, negative impacts on BW gain have also been reported. A dosage of 5 g/kg thyme EO caused a substantial decrease in BW gain approaching almost a level of significance (Cross et al., 2003).

Addition of 100 mg/kg thymol, the major component of thyme EO, did not show any effects on BW gain when compared to the control treatment. Lee et al. (2003a) concluded that this was due to the good environmental conditions, as well as the composition of the basal diet. In a later study, they wanted to override this effect by feeding a rye-based diet in order to evaluate whether the growth depressing effect of rye could be reduced. Eventually, the addition of thymol showed no effects on BW gain (Table 4). However, the addition of 100 mg/kg cinnamaldehyde tended to show improvements when compared to the rye control diet (Lee et al., 2004a).

There are also some studies of commercial and non-commercial blends or mixtures of EO (Table 5). Since active components of EO are known for their synergistic effect and some for their suppressing effects, it is important to evaluate the optimal configuration of EO composition. Tiihonen et al. (2010) added a mixture of 15 mg/kg thymol and 5 mg/kg cinnamaldehyde to a diet over a period of 42 days. They found that BW gain was improved by approximately 5% and concluded that this might be due to beneficial changes in the intestinal microflora. Another EO mix derived from oregano, anise and clove increased BW gain significantly by approximately 16% compared to the control group. The mixture was tested at different levels, whereas 200 mg/kg of the composition gave best results after 5 weeks of trial (Ertas et al., 2005). CRINA® Poultry added at 25 mg/kg improved total BW gain slightly, yet not significantly (Jang et al., 2007). However, an inclusion of 100 or 200 mg/kg CRINA® Poultry resulted in a decrease of BW gain over a growing period of 25 days (Abildgaard et al., 2010). RepaXol™ a commercial product containing EO from oregano, thyme, cinnamon and capsaicin showed no effect on BW gain when fed at 50-100 mg/kg (Zhang et al., 2005). Unfortunately, the exact amounts of comprised EO and their active components in the above mentioned commercial products were not stated. Even though most of the blends shown to have positive effects on performance, they are rather hard to compare to other studies. In order to achieve best results in BW gain when feeding EO, it is highly important to know the exact composition and formulation of the feed additive used.

6. Influence on feed conversion ratio

The feed conversion ratio (FCR) describes the relation of feed intake and BW gain. More precisely, it is the animal's overall efficiency in converting feed mass into body mass over a specific period of time.

Significant improvements of FCR have been observed in several studies (Tables 2 and 3). An inclusion level of 400 mg/kg of anise EO enhanced FCR by approximately 12% (Çiftçi et al.,

2005), while 100 and 200 mg/kg of thyme EO changed FCR beneficially as well (Bölükbasi et al., 2006). Likewise, fennel seeds at the dose of 2 and 3 g/kg improved FCR by almost 16% (Mohammed and Abbas, 2009). Nevertheless, Toghyani et al. (2010) discovered that thyme herb at an inclusion level of 10 g/kg downgraded FCR by approximately 4%. Carvacrol, the major component of oregano EO led to a significantly improved FCR when added at a level of 200 mg/kg over a period of 28 days (Table 4). Surprisingly, this improvement has been observed even though feed intake had been surpressed and BW gain was lowered. Accordingly, the improved FCR could be due to an increased efficiency of energy and nutrient utilisation or altered carcass composition, although this aspect has not been analysed in the course of the experiment (Lee et al., 2003b).

Blends of EO are quite common as feed additives in broiler nutrition (Table 5). The effect of several EO may combine and intensify the positive action on broiler performance. Ertas et al. (2005) observed a significant improvement after feeding 200 mg/kg of a mixture containing EO from oregano, clove and anise. The FCR was improved by approximately 12% compared to the control group and 8% when compared to the group which has been fed an antibiotic treatment. Similarly, the inclusion of a blend that contained oregano, thyme, cinnamon and eucalyptus EO tended to improve FCR by 5% compared to the control treatment (Ulfah, 2006). However, several commercial EO blends were included in trials but only two studies confirmed a significant improvement of FCR. The addition of 100 mg/kg of RepaXol® optimized FCR by 4% (Zhang et al., 2005), while the supplementation of 100 mg/kg of XTRACT™ enhanced FCR equally by 4% (Jamroz et al., 2005).

Studies on EO mixtures showed that the positive action of EO is superior to the effect of a single EO. The synergistic effect of EO or combined active components, however, needs to be studied in more detail and under standardized conditions.

Table 2: Effect of different essential oils (EO) on feed intake, body weight (BW) gain and feed conversion ratio (FCR)

No. of Birds	Source of EO	mg/kg	Feed intake[1]	BW gain[1]	FCR[1]	Reference
200	Anise	100	NS[2] (0)	NS (+2)	NS (-2)	Çiftçi et al. (2005)
200	Anise	100	n.a.[3]	NS (+2)	n.a.	Simsek et al. (2007)
200	Anise	200	NS (0)	NS (+2)	NS (-2)	Çiftçi et al. (2005)
200	Anise	200	n.a.	NS (+2)	n.a.	Simsek et al. (2007)
200	Anise	400	NS (0)	p<0.01 (+15)	p<0.05 (-12)	Çiftçi et al. (2005)
200	Anise	400	n.a.	p<0.05 (+14)	n.a.	Simsek et al. (2007)
200	Thyme	100	p<0.05 (+3)	NS (0)	p<0.01 (+2)	Bölükbasi et al. (2006)
105	Thyme	120	NS (-2)	NS (+2)	NS (-5)	Tekeli et al. (2006)
200	Thyme	200	p<0.05 (+4)	NS (0)	p<0.01 (+1)	Bölükbasi et al. (2006)
165	Thyme	1000	NS (-10)	P<0.05 (+13)	NS (+2)	Cross et al. (2007)
480	Thyme	1000	NS (-2)	NS (0)	NS (-1)	Cross et al. (2003)
480	Thyme	3000	NS (-2)	NS (0)	NS (-1)	Cross et al. (2003)
480	Thyme	5000	NS (0)	NS (-7)	NS (+7)	Cross et al. (2003)
105	Oregano	120	NS (+2)	NS (+4)	NS (-2)	Tekeli et al. (2006)
720	Oregano	150	NS (-6)	NS (-2)	NS (-4)	Basmacioglu et al. (2004)
1,200	Oregano	200	NS (0)	NS (+1)	NS (-1)	Baretto et al. (2008)
720	Oregano	300	NS (-3)	NS (+1)	NS (-2)	Basmacioglu et al. (2004)
165	Oregano	1000	NS (-10)	NS (-6)	NS (+1)	Cross et al. (2007)
720	Rosemary	150	NS (0)	NS (+1)	NS (-2)	Basmacioglu et al. (2004)
720	Rosemary	300	NS (-1)	NS (+1)	NS (-4)	Basmacioglu et al. (2004)
165	Rosemary	1000	p<0.05 (-16)	NS (-9)	NS (+8)	Cross et al. (2007)
105	Clove	120	NS (0)	NS (+4)	NS (-5)	Tekeli et al. (2006)
1,200	Clove	200	NS (0)	NS (-1)	NS (0)	Baretto et al. (2008)
1,200	Cinnamon	200	NS (+1)	NS (+1)	NS (0)	Baretto et al. (2008)
165	Majoram	1000	NS (+4)	NS (+5)	NS (+1)	Cross et al. (2007)

1 Numbers in parentheses indicate percentage of change in comparison to control group.

[2] NS, not significant (P>0.05).

[3] n.a., not analysed.

Table 3: Effects of different herbs on feed intake, body weight (BW) gain and feed conversion ratio (FCR)

No. of Birds	Duration (days)	Supplement	g/kg	Feed intake[1]	BW gain[1]	FCR[1]	Reference
165	28	Majoram	10	NS[2] (+1)	NS (+4)	NS (+2)	Cross et al. (2007)
165	28	Oregano	10	p<0.05 (-15)	NS (-10)	NS (+3)	Cross et al. (2007)
165	28	Rosemary	10	NS (+2)	NS (+1)	NS (-1)	Cross et al. (2007)
165	28	Thyme	10	NS (-2)	NS (-5)	NS (-4)	Cross et al. (2007)
120	42	Fennel	1	NS (-1)	p<0.05 (+6)	NS (-7)	Mohammed and Abbas (2009)
120	42	Fennel	2	NS (-8)	p<0.05 (+9)	p<0.01 (-16)	Mohammed and Abbas (2009)
120	42	Fennel	3	NS (-5)	p<0.05 (+11)	p<0.01 (-14)	Mohammed and Abbas (2009)

[1] Numbers in parentheses indicate percentage of change in comparison to control group.

[2] NS, not significant (*P>0.05*).

Table 4: Effects of different active components on feed intake, body weight (BW) gain and feed conversion ratio (FCR)

No. of Birds	Duration (days)	Supplementation	mg/kg	Feed intake[1]	BW gain[1]	FCR[1]	Reference
96	21	Thymol	100	NS[2] (-1)	NS (+1)	NS (-2)	Lee et al. (2003a)
72	28	Thymol	200	NS (+2)	NS (+2)	NS (0)	Lee et al. (2003b)
75	33	Thymol	100	NS (-2)	NS (+2)	NS (-5)	Lee et al. (2004b)
96	21	Cinnamaldehyde	100	NS (-3)	NS (-2)	NS (0)	Lee et al. (2003a)
75	33	Cinnamaldehyde	100	NS (+9)	NS (+6)	NS (-1)	Lee et al. (2004b)
72	28	Carvacrol	200	p<0.05 (-5)	NS (-3)	p<0.05 (-3)	Lee et al. (2003b)

[1] Numbers in parentheses indicate percentage of change in comparison to control group.

[2] NS, not significant (p>0.05).

Table 5: Effects of commercial essential oil (EO) blends on feed intake, body weight (BW) gain and feed conversion ratio (FCR)

No. of Birds	Duration (days)	Blend	mg/kg	Feed intake[1]	BW gain[1]	FCR[1]	Source
240	35	RepaXol®[2]	100	NS[3] (-4)	NS (0)	NS (-4)	Zhang et al. (2005)
240	35	RepaXol®	200	p<0.05 (-5)	NS (-3)	NS (+3)	Zhang et al. (2005)
240	35	RepaXol®	300	p<0.05 (-5)	NS (0)	p<0.1 (-4)	Zhang et al. (2005)
96	21	CRINA® Poultry[4]	100	NS (+3)	NS (+3)	NS (0)	Lee et al. (2003a)
120	35	CRINA® Poultry	25	NS (+4)	NS (+4)	NS (-1)	Jang et al. (2007)
	35	CRINA® Poultry	50	NS (+4.5)	NS (+3)	NS (+1)	Jang et al. (2007)
1656	35	CRINA® Poultry	100	p<0.05 (-4)	p<0.05 (-4)	NS (0)	Abildgaard et al. (2010)
1656	35	Crina® Poultry	200	NS (-2)	p<0.05 (-2)	NS (+1)	Abildgaard et al. (2010)
640	42	Enviva[5]	100	NS (+1)	NS (0)	NS (+1)	Cao et al. (2010)
336	41	XTRACT™ (maize)[6]	100	n.a.	NS (-1)	p<0.05 (-4)	Jamroz et al. (2005)
336	41	XTRACT™ (wheat and barley)	100	n.a.	NS (+3)	NS (-2)	
120	42	XTRACT™	200	NS (-2)	NS (0)	NS (-2)	Hernández et al. (2004)
312	49	XTRACT™	200	n.a.	NS (0)	p<0.05 (-17)	Garcia et al. (2007)
720	42	Rosemary-Oregano EO	75 + 75	NS (-7)	NS (-3)	NS (-4)	Basmacioglu et al. (2004)
720	42	Rosemary-Oregano EO	150 + 150	NS (-2)	NS (-1)	NS (-1)	Basmacioglu et al. (2004)
36000	42	Oregano-Thyme-Cinnamon- Eucalyptus-EO-mix	1000/750/500	NS (-2)	NS (+3)	p<0.05 (-5)	Ulfah (2006)
250	35	Oregano-Anise-Clove EO mix	100	NS (0)	NS (+3)	p<0.05 (-4)	Ertas et al. (2005)
250	35	Oregano-Anise-Clove EO mix	200	NS (+2)	p<0.05 (+16)	p<0.05 (-12)	Ertas et al. (2005)
250	35	Oregano-Anise-Clove EO mix	400	NS (+3)	NS (0)	NS (-3)	Ertas et al. (2005)
1320	46	Clove-Cinnamon EO mix	100	n.a.	NS (-1)	NS (0)	Isabel and Santos (2009)

[1] Numbers in parentheses indicate percentage of change in comparison to control group.

[2] Commercial blend of EO that contains oregano, cinnamon, thyme, capsicum and citrus EO (exact proportions of ingredients not stated).

[3] NS, not significant (p>0.05).

[4] Commercial blend of EO that contains 29% of active ingredients including thymol (exact composition not stated).

[5] Commercial blend of EO that contains two active ingredients including thymol and cinnamaldehyde (exact composition not stated).

[6] Commercial blend of EO that contains oregano, cinnamon, pepper (49.5 g/kg carvacrol, 29.7 g/kg cinnamaldehyde, 19.8 g/kg capsaicin).

7. Antimicrobial activities *in vivo*

On the basis of their *in vitro* antimicrobial activity, it is logical to consider EO application as feed additive alternative to antibiotic growth promoters in animal production. It would be expected that the intake of EO affects the gastrointestinal microflora composition and population. An "ideal flora" allows optimum growth performance while an alteration of the indigenous flora by diet or environment can be deleterious to the host (Schaedler, 1973).

In vivo studies on antimicrobial action of EO are rare and results are difficult to compare due to the use of different methods. Antimicrobial activity can be tested in all parts of the intestinal tract and methods to determine activity vary. Even though it has already been demonstrated that essential oils act as antimicrobial agents *in vitro*, these results sometimes do not appear in *in vivo* studies. This depends on several other factors like environment and basal diet. If the birds are housed under clean and healthy condition and if the diets are highly digestible it is possible that the antimicrobial effect of EO does not show. There are no improvements needed if the microflora is already in an equilibrium state.

Jang et al. (2007) used ten-fold dilution method to determine the number of colony forming units (CFU) for *Lactobacilli, E. coli* and *Salmonella* in digesta harvested from the ileo-cecum on broiler which were fed either 25 or 50 mg/kg CRINA® Poultry. The CFU of *Lactobacilli* were not influenced by dietary supplementation of feed additives. Slightly lower, but nonsignificant values were observed for *E. coli* CFU while no detectable forming unit of *Salmonella* were obtained. Jamroz et al. (2005) selected intestinal digesta from the final part of the small intestine and whole caeca to determine CFU for *E. coli, C. perfringens* and *Lactobacillus spp.* The diets were supplemented with 100 mg/kg XTRACT™, a commercial product that includes EO extracts from oregano, cinnamon, and pepper containing 49.5 g/kg carvacrol, 29.7 g/kg trans-cinnamaldehyde and 19.8 g/kg capsaicin. The supplementation reduced the CFU of *E. coli* to a limited extent, with a greater inhibition observed in older birds. There was a significant increase in *Lactobacillus spp.* while the number of *C. perfringens* was lightly reduced. Cross et al. (2007) also observed CFU of *E. coli, Lactobacillus* and *C. perfringens* by analysing caecal and faecal contents of chickens. Observations indicated no effects on either of the tested microorganisms when adding 10 mg/kg of selected EO including thyme, oregano, marjoram and rosemary. It was stated that the absence of effects may be due to an insufficient degree of replication as well as decreased exposure time to the air.

8. Conclusions and future research directions

There are only few *in vivo* studies that focus on EO in broiler diets. Conclusions of these studies tend to vary, although positive results dominate the observation. Increase of feed intake, BW gain, feed conversion as well as better efficiency to utilize nutrients, and inhibition of bacteria and fungi to stabilize the intestinal microflora were concluded in most studies. Nevertheless it has to be kept in mind that there is still insufficient significant evidence on EO as natural growth promoters. Mainly this is due to the lack of standardization in varying parts of experimental research. Differences between or within EO depend significantly on several variables, which makes it necessary to define the exact composition of the supplements which have been added to diets. Moreover, it is still unclear if certain single active components are responsible for actions or if the effects are due to a synergistic effect of multiple components. Unfortunately there are only a few studies that declare the exact compostion of the used supplement, which makes it difficult to compare results satisfactorily. This should be considered as an important focus in future research.

Another important aspect is, that the exact mechanism and the mode of action of EO are still not fully explained and understood. More *in vitro* and *in vivo* trials are needed to confirm theories and guarantee the safe use. Toxicology and over-dosage may result in negative, unwanted effects and should be excluded in advance.

Generally, it can be concluded that herbs, their EO and components have the potential to be considered as an alternative to antibiotic growth promoters. Nevertheless, there is still further research under standardized conditions needed to evaluate the exact mechanism of action and to determine the optimal dietary inclusion level in order to optimize growth performance and maintain healthy birds.

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4 Effects of thymol, carvacrol and their combination on feed intake and performance characteristics of broilers

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Abstract

This study was undertaken to evaluate the effects of different essential oil components on feed intake, growth characteristics and gastrointestinal microflora in growing broiler chicken. A total of 360 4-day-old Ross boiler chickens, 120 in each trial, were assigned to the basal control diet or the basal diet supplemented with either 100, 200, 300, 400 or 500 mg/kg thymol (trial 1), carvacrol (trial 2) or a thymol-carvacrol mixture (equal fractions of each compound; trial 3). Improvements for body weight gain, feed intake and feed conversion ratio ($P<0.05$) were observed in all three trials, however medium inclusions of 200 or 300 mg/kg carvacrol, thymol or their mixture in all three trials, resulted in overall best performance. Colony forming units (CFU) for *Lactobacillus spp*. were increased ($P<0.05$) for thymol and carvacrol supplemented groups (trial 1 and2), whereas the thymol-carvacrol mixture (trial 3) did not exhibit any changes. Likewise, CFU for *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* were either not detected or only observed in small numbers and no effect of supplementation with either essential oil component could be identified. In conclusion it can be stated that thymol, as well as carvacrol can be used as effective feed additives to improve performance and gut health of broiler chickens. However, future research is needed to determine the optimal dietary inclusion level and the exact mode of action of the examined active components.

1. Introduction

Since the EU banned antibiotic growth promoters in broiler nutrition (Anonymous, 2003) due to suspected cross and multiple resistance (Neu, 1992), much research has been conducted to explore the use of plants, plant extracts, essential oils and their active components as effective substitutes. Essential oils are secondary metabolites which are highly enriched in compounds based on isoprene structures that are called terpenes (Cowan, 1999). To date, there are more than 3,000 chemical compounds that have been isolated from essential oils. Monoterpenes, made by coupling of two isoprene units, constitute 900 g/kg of the essential oil molecules which allows for a wide variety of structures (Bakkali et al., 2008).

Thymol and its isomer carvacrol, components from thyme and oregano plants, are classified as monoterpene phenols and have already proven their antimicrobial effect in vitro (Ouwehand et al., 2010). This effect is mainly due to the lipophilic character of the active principles, which permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations of essential oils also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998).

Nevertheless, there is only limited data on studies reporting effects of thymol and carvacrol in vivo. For this reason, the aim of the present study implied an evaluation of the potential of different increasing levels of thymol and carvacrol as feed additives in broiler diets, in order to observe their influence on feed intake, performance and gastro-intestinal microflora.

2. Materials and Methods

2.1. Experimental animals and design, and feed preparation

A total of 360 1-day-old unsexed broiler chickens (Ross 308), 120 in each of three trials, were housed individually in metabolic wire cages in a temperature controlled room with a 23 h constant light schedule. The temperature of the animal facility was maintained at 32°C during the first week and than weekly reduced by 2°C to reach a final temperature of 24°C towards the end of the experiment.

During the first 3 days, chicks were fed a commercial starter diet (RWZ, Cologne, Germany), containing 200 g/kg crude protein (CP). From 4 to 42 days of age, the chicks were fed the experimental diet. The diets were formulated according to GfE (1999) to meet the nutrient

requirements (Table 6). The experiment has been subdivided into three trials. Each trial included 120 birds, which were divided into 6 groups (e.g. 20 birds per group). Each group was fed at different levels (0, 100, 200, 300, 400 or 500 mg/kg) of either thymol (Fluka No. 89330, Sigma-Aldrich, Steinheim, Germany) in the first trial, carvacrol (Aldrich No. W224502, Sigma-Aldrich, Steinheim, Germany) in the second trial, or a mixture of thymol and carvacrol (equal fractions of each compound) in the third trial.

All birds were offered the respective diets for ad libitum consumption and had free access to water for the entire period. Feed intake (FI) and body weight (BW) were recorded on a weekly basis, starting on day 4 until birds reached a final age of 42 days, to determine growth performance (i.e., BW gain) and feed conversion ratio (FCR). Mortality was recorded as it occurred during the entire experimental period.

Excreta were collected twice a day during the last week of each trial. At the end of the experimental period, total excreta from each bird were quantified. All excreta were kept in a freezer at a constant temperature of -20 °C until preparation for chemical analysis. Before chemical analysis the excreta were homogenised and DM was determined. Moreover, excreta were freeze dried (Christ, Osterode/Harz, Germany) and afterwards, ground finely using a 1 mm sieve with a centrifugal mill (KG type ZM1, Retsch, Haan, Germany).

Table 6: Ingredient composition of experimental diets fed to broiler chickens

Ingredients	g/kg
Maize grain	600
Soybean meal	305
Soybean oil	39
Maize gluten meal	20
Di-calcium phosphate	15
Limestone	14
Vitamin and mineral mix[1]	3
DL-Methionine	3
L-Lysine	1
Common salt	1

[1]Contained per kg: vitamin A, 10,000,000 IU; vitamin D, 2,000,000 IU; vitamin E, 10,000 mg; vitamin K3, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; biotin, 50 mg; BHT, 10,000 mg; pantothenic acid, 10,000 mg; folic acid, 1000 mg; nicotinic acid 30,000 mg; Mn, 60 g; Zn, 50 g; Fe, 30 g; Cu, 4 g; I, 3 g; Se, 0.1 g; Co, 0.1 g.

2.2. Microbial enumeration

Five birds from selected treatment groups were utilized to count the colony forming units (CFU) in the gastrointestinal tract (crop, small intestine and caecum). During the first trial, birds which received 0, 100, 300 and 500 mg/kg thymol were determined. Because no differences were detected, the second trial implied only birds receiving 0, 100, and 500 mg/kg carvacrol. No measurements of *Escherichia coli* and *Salmonella spp*. were performed for this group due to undetected bacteria during the first measurements. For the same reason, the third trial only implied birds that were fed the thymol-carvacrol mixture at levels of 0, 100 and 500 mg/kg. Measurements only included total viable count and *Lactobacillus* spp..

The digesta samples collected from crop, small intestine and caecum were decanted into separate sterile plastic containers and thoroughly mixed manually. Ten grams of homogenised sample along with ten-fold serial dilutions using physiological NaCl-Trypton was poured into a stomacher bag and shaken vigorously for 3 minutes. The plate media used were: Casein-peptone Dextrose Yeast Agar (Merck, Darmstadt, Germany) for total viable counts, MRS agar (Merck) for *Lactobacillus* spp., RAMBACH® (Merck) for the *Salmonella* sp., ChromoCult® (Merck) for *E. coli*, Baird Parker Agar (OXOID, Wesel, Germany) for *Staphylococcus aureus*, Brilliance TM (OXOID) for *Listeria monocytogenes*, PEMBA Selektivagar (OXOID) for *Bacillus cereus*.

2.3. Chemical analysis

Samples from the control and experimental diets, as well as the homogenized freeze dried excreta of each replicate for all experiments were milled to 1 mm using a centrifugal mill (ZM1, Retsch, Haan, Germany). Dry matter (DM) was determined by oven-drying at 100°C for 24 h. Total nitrogen (N) was estimated by combustion assay (LECO Instrumente, Mönchengladbach, Germany), CP was expressed as N x 6.25, ash (ID 942.05) and ether extract (ID 963.15) were analysed according to the standard methods of AOAC (1990). Starch and sugar contents of the diets were quantified using official European Union methods (Anonymous, 2009). The contents of neutral detergent fibre (assayed with a heat stable amylase, aNDF) and acid detergent fibre (ADF), both expressed inclusive residual ash, were determined sequentially without sodium sulphite by a modification of the method of Van Soest et al., 1991 P.J. Van Soest, J.B. Robertson and B.A. Lewis, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. (1991), pp. 3583–3597. View Record in Scopus |

Cited By in Scopus (4365)Van Soest et al. (1991) using semi-automated equipment (ANKOM, Macedon, NY, USA).

Calcium (Ca) was measured using atomic absorption spectrometry and phosphorus (P) was analysed colorimetrically (method 10.6.1; VDLUFA, 2007). Lysine and methionine (after oxidation) were analysed using an amino acid analyser after hydrolysis (6 M HCl) of the diets (method 4.11.1; VDLUFA, 2007). The GE contents of the diets and excreta were measured using an adiabatic bomb calorimeter (model C 4000; IKA, Heitersheim, Germany).

The N-corrected apparent metabolisable energy (AME_N) contents of the diets were calculated as follows (GfE, 1999):

AMEN (MJ/kg) = [energy intake (MJ) – energy of excreta (MJ) – F x N retention (kg)] / feed intake (kg)

F = Factor of correction (Titus et al., 1959): F = 36.5 kJ/g N retention.

2.4. Statistical analysis

The statistical analysis was performed separately for each trial using a completely randomized design and the general linear models (GLM) procedure of SAS 9.2 (SAS Institute, 2009). The model only included the level of supplementation and the bacteriological data required log transformation before statistical analysis. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of the increasing levels of supplementation in each experiment. Significance was declared at $P<0.05$, and a tendency toward significance was declared at $0.05<P<0.10$. *P*-values less than 0.001 are expressed as "<0.001" rather than the actual value.

3. Results

3.1. Diet composition

The proximate analysis carried out on treatment diets showed that there were no differences in the nutritional composition of the dietary treatments (Table 7). Diets for all three trials were similar with an approximate standard error of the means (SEM) between 0.01 and 3.29. Only ether extract expressed a relatively high SEM, which could be due to measurement faults. Nevertheless, this did not affect the results of the study.

Table 7: Proximate analysis of nutrients (g/kg dry matter (DM)), gross energy (GE) content, and standard error of the means (SEM) of diets fed to growing broiler chicken

Item	Trial			
	[1]	[2]	[3]	SEM
Dry matter (g/kg)	902.8	904.2	896.8	2.26
Organic matter	931.0	933.3	929.7	1.07
Ash	68.60	66.25	66.88	0.70
Crude protein	205.5	210.8	204.2	2.04
Ether extract	39.10	81.95	76.95	13.53
ADF	40.25	36.50	30.98	2.69
aNDF	90.28	78.92	84.07	3.29
Ca	17.80	17.53	16.18	0.50
P	7.30	6.66	7.38	0.23
Starch	458.0	466.2	458.3	2.67
Sugar	41.68	42.67	44.22	0.74
Lysine	1.06	1.10	1.10	0.01
Methionine	0.52	0.47	0.52	0.02
GE (MJ/kg DM)	20.88	19.37	19.20	0.54

ADF, acid detergent fibre expressed inclusive residual ash; aNDF, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive residual ash.

Trial [1]: Diet included 0, 100, 200, 300, 400 or 500 mg/kg thymol.

Trial [2]: Diet included 0, 100, 200, 300, 400 or 500 mg/kg carvacrol.

Trial [3]: Diet included 0, 100, 200, 300, 400 or 500 mg/kg thymol and carvacrol mixture.

3.2. Feed intake, performance and carcass traits

Table 8 presents growth performance, FI and FCR in broiler chickens fed the control diets as well as the diets that included different levels of thymol, carvacrol or the thymol-carvacrol mixture. In the Appendix (Table A1) the carcass characteristics and digestive measurements are shown. Improvements were observed in all three trials more precisely, quadratic improvements were observed for final BW, BW gain and FI. The FCR decreased linearly as the inclusion level of thymol increased. Similarly, linear improvements were observed for AME_N for all thymol and carvacrol supplementations, as well as a trend for the thymol-carvacrol mixture. Improvements in carcass weight were only detected for birds receiving carvacrol or the thymol-carvacrol mixture as a dietary supplementation. There were no mortalities in any phase of growth.

Table 8: Growth performance, feed intake and nitrogen-corrected apparent metabolizable energy (AME_N) from 42-day-old broiler chickens fed the basal diet and different additions of thymol, carvacrol or a mixture of thymol and carvacrol

Inclusion level (mg/kg)	Final Body weight[1] (g)	Body weight gain[1] (g)	Feed intake[1] (g)	FCR[1] (kg/kg)	AME_N[1] (MJ/kg)	Carcass weight[2] (g)
Thymol						
0	2029	1953	3286	1.699	15.2	1581
100	2070	1995	3285	1.653	15.0	1715
200	2109	2032	3342	1.647	14.9	1613
300	2218	2140	3484	1.631	14.5	1873
400	2207	2130	3423	1.612	14.8	1590
500	2024	1950	3194	1.623	14.9	1721
SEM	26.1	26.1	29.2	0.012	0.53	46.6
P- value lin.[3]	0.244	0.239	0.870	0.037	0.019	0.543
P- value quad.[4]	0.042	0.045	0.011	0.393	0.006	0.488
Carvacrol						
0	1682	1603	2681	1.676	15.2	1254
100	1773	1693	3032	1.793	15.3	1357
200	2040	1961	2997	1.535	15.7	1579
300	2029	1948	2927	1.508	15.5	1642
400	2028	1950	3092	1.592	15.7	1497
500	1874	1796	2851	1.590	15.8	1628
SEM	26.4	26.4	34.2	0.019	0.077	37.4
P- value lin.	<0.001	<0.001	0.13	<0.001	0.016	<0.001
P- value quad.	<0.001	<0.001	<0.001	0.039	0.960	0.051
Thymol and carvacrol mixture						
0	1695	1619	2681	1.666	13.9	1328
100	2055	1978	3032	1.533	13.4	1468
200	2001	1928	2997	1.554	13.6	1464
300	2084	2004	2927	1.461	13.0	1507
400	2022	1944	3092	1.591	13.4	1668
500	1965	1891	2851	1.508	13.3	1554
SEM	28.7	28.6	28.8	0.025	0.238	33.5
P- value lin.	<0.001	<0.001	0.805	<0.001	0.070	0.015
P- value quad.	<0.001	<0.001	0.057	<0.001	0.159	0.410

[1]Values in each row are means for 10 replicates of each treatment.

[2]Values in each row are means for 5 replicates of each treatment.

[3,4]Linear and quadratic responses, respectively, to the dietary inclusion levels.

SEM: Standard error of the means.

3.3. Microflora enumeration

Effects of thymol, carvacrol and their mixture on the gastro-intestinal microbial CFU are presented in Table 9. Birds supplemented with different levels of thymol expressed quadratic effects for total viable count in crop and small intestine, as well as quadratic and linear effects for *Lactobacillus spp*. in the crop and a trend for quadratic effects in the small intestine. Moreover, quadratic effects have been observed on CFU of *E. coli* in the small intestine and caecum. Birds in the second trial, which received different levels of carvacrol exhibited linear effects in the small intestine for total viable count, however more observations were absent. Birds receiving a mixture of thymol and carvarcrol (Trial 3) displayed neither linear nor quadratic findings for any CFU.

Table 9: Microbial enumeration (log CFU/g) in crop, small intestine and caecum digesta from 42–day-old broiler chickens

			Inclusion level (mg/kg)						
			0	100	300	500	SEM[4]	P- value lin.[5]	P- value quad.[6]
Total viable count	Crop	[1][1]	5.99	6.35	7.23	6.02	0.205	0.702	0.023
		[2][2]	9.82	9.77	n.a.[7]	9.82	0.169	0.220	0.820
		[3][3]	8.71	9.30	n.a.	8.85	0.162	0.870	0.140
	Small intestine	[1]	5.58	10.2	5.83	5.83	0.502	0.122	0.028
		[2]	7.85	8.49	n.a.	9.70	0.331	0.020	0.670
		[3]	8.62	9.35	n.a.	8.44	0.208	0.330	0.100
	Caecum	[1]	8.66	8.94	9.11	8.66	0.139	0.694	0.115
		[2]	10.4	10.2	n.a.	10.4	0.089	0.220	0.820
		[3]	9.44	9.82	n.a.	9.52	0.098	0.110	0.810
Lactobacillus spp.	Crop	[1]	4.91	9.30	8.84	8.97	0.024	0.042	0.065
		[2]	9.84	9.69	n.a.	9.84	0.140	0.420	0.540
		[3]	8.75	9.35	n.a.	8.82	0.190	0.750	0.190
	Small intestine	[1]	5.88	7.30	8.62	8.15	0.607	0.161	0.064
		[2]	7.87	8.72	n.a.	9.46	0.357	0.090	0.490
		[3]	8.48	9.31	n.a.	8.23	0.256	0.330	0.130
	Caecum	[1]	9.09	9.31	9.18	8.82	0.079	0.113	0.122
		[2]	9.40	9.87	n.a.	10.0	0.235	0.420	0.540
		[3]	8.94	9.42	n.a.	9.24	0.158	0.680	0.270
Staphylococcus aureus	Crop	[1]	1.23	1.24	2.14	1.24	0.396	0.825	0.469
		[2]	0.98	ND[8]	n.a.	ND	n.a.	n.a.	n.a.
	Small intestine	[1]	ND	ND	0.73	0.85	n.a.	n.a.	n.a.
		[2]	0.81	ND	n.a.	ND	n.a.	n.a.	n.a.
	Caecum	[1]	ND	0.79	ND	ND	n.a.	n.a.	n.a.
		[2]	ND	ND	n.a.	ND	n.a.	n.a.	n.a.
Bacillus cereus	Crop	[1]	1.31	ND	ND	ND	n.a.	n.a.	n.a.
		[2]	ND	0.70	n.a.	ND	n.a.	n.a.	n.a.
	Small intestine	[1]	1.19	ND	ND	ND	n.a.	n.a.	n.a.
		[2]	ND	ND	n.a.	0.64	n.a.	n.a.	n.a.
	Caecum	[1]	0.20	ND	ND	ND	n.a.	n.a.	n.a.
		[2]	ND	ND	n.a.	ND	n.a.	n.a.	n.a.
Listeria monocytogenes	Crop	[1]	2.02	1.43	1.90	0.82	0.734	0.418	0.713
		[2]	ND	ND	n.a.	ND	n.a.	n.a.	n.a.
	Small intestine	[1]	1.11	0.73	1.01	1.09	0.315	0.896	0.811
		[2]	ND	ND	n.a.	ND	n.a.	n.a.	n.a.
	Caecum	[1]	1.39	0.90	2.07	0.53	0.374	0.675	0.356
		[2]	ND	ND	n.a.	ND	n.a.	n.a.	n.a.
Escherichia coli	Crop	[1]	3.92	3.96	5.05	4.30	0.368	0.542	0.447
	Small intestine	[1]	4.77	5.93	5.49	4.63	0.354	0.738	0.085
	Caecum	[1]	6.48	8.54	9.16	8.50	0.393	0.056	0.038

Values in each row are means for 5 replicates of each treatment.
[1] Trial [1]: Diet included thymol.
[2] Trial [2]: Diet included carvacrol.
[3] Trial [3]: Diet included a thymol and carvacrol mixture.
[4]SEM = Standard error of the means.
[5,6]Linear and quadratic responses, respectively, to the dietary inclusion levels.
[7]n.a. = not analysed.
[8]ND = not detected.

4. Discussion

4.1. Growth Performance

Few studies have been performed investigating the effect of essential oils on broiler performance and even less studies concentrate on their active principles like thymol or carvacrol. For this reason the present experiment was conducted to investigate the effects of thymol, carvacrol or their mixture on growth performance and influence on the gastrointestinal system in broiler chicken. Improvements were observed in all three trials and all growth variables. The groups receiving thymol at levels of 300 and 400 mg/kg expressed up to 10% improvements in BW gain, 6% enhancements in FI and a 5% improvement in FCR when compared to the control group. This finding is not in agreement with observations made by Lee et al. (2003a, b; 2004). The supplementation of either 100 or 200 mg/kg thymol did not result in any improvement, although positive tendencies were noticed for BW gain and improved FCR. Feed intake only resulted in a positive drift when 200 mg/kg thymol was added (Lee et al. 2003b) while a dosage of 100 mg/kg expressed up to 2% less FI (Lee et al., 2003a; 2004). Lee et al. (2003a) concluded that the absence of the effects on growth performance may be due to a well balanced diet as well as a clean environment which could have masked the antimicrobial activity of thymol.

However, some positive results were observed when the essential oil of thyme was added to a broiler diet. Thyme essential oil usually contains between 300 and 550 g/kg of its major component thymol (European Pharmacopoeia, 2004). Bölükbasi et al. (2006) added 100 or 200 mg/kg thyme essential oil and observed a positive influence on FI, however, FCR was not improved, due to a stagnant BW gain. Cross et al. (2007) supplemented thyme essential oil at a level of 1000 mg/kg and found an improved BW gain, although FI decreased by almost 10%.

Within the groups supplemented with carvacrol, the birds receiving 200 or 300 mg/kg carvacrol showed up to 22% improvements in BW gain, a 15% higher FI and a 10% improved FCR. However, this finding is not in total agreement with previous studies. Lee et al. (2003b) performed a study on 72 broiler chicks which received a daily dosage of 200 mg/kg carvacrol added to a basal diet for a period of 28 days. They identified a lowered FI, yet the FCR was improved simultaneously. Possibly, carvacrol affected the FI by modulating appetite and in turn affected FCR by increased efficiency of energy and nutrient utilization. Also mentionable is that although several studies included oregano essential oil at different levels, which usually contains 400-700 g/kg carvacrol (European Pharmacopoeia, 2004), there is a lack of significant results. Moreover, the exact chemical composition of the used essential oils depends on several factors

such as plant species, physical and chemical soil conditions, harvest time, degree of plant maturity, technology of drying, duration of storage and extraction process (Burt, 2004; Bakkali et al., 2008). Data about the chemical composition of essential oils taken from different literature also suffers from this indisposition. This can also be due to lacks of standardisation implying differences in analysing methods (gas chromatography or mass spectrometry). Varying results lead to difficulties in using and discussing in vivo studies on essential oils, since optimal dosage and mixture are yet to be identified. Thus, it is meaningful to define active chemical components in essential oils, which are commonly used as feed additives in broiler diets (Hippenstiel et al., 2011).

Until now, there have been no studies investigating the effects of mixtures of thymol and carvacrol on feed intake and performance of broiler chickens. The mixture, fed at levels of 100 and 300 mg/kg persuaded the birds towards 24% improved BW gain, 13% enhanced FI and an improved FCR (12%). This could be due to the synergistic effect of thymol and carvacrol which has already been reported for these and other essential oil components in vitro (Ultee et al., 2002; Lambert et al., 2001). Several in vivo studies have been conducted to study and benefit from this effect with commercial and non-commercial blends of essential oils. A blend derived from oregano, clove and anise essential oil supplemented at a level of 200 mg/kg resulted in an increased BW gain by 16% as well as an improved FCR by 12%. It was concluded that these positive findings were due to the positive digestive stimulating effects of thymol and carvacrol (Ertas et al., 2005). However, Abildgaard et al. (2010) observed negative influences on broiler performance characteristics when 100 or 200 mg/kg of a commercial blend, which included 290 g/kg thymol, was added to the diet. Feed intake and BW gain decreased, maybe due to practical housing conditions, vaccination against coccidosis and a shift to a grower diet with high wheat content, which in turn led to certain degree of stress on the birds. Nevertheless, other studies with the same commercial products could not proof any beneficial effects on overall broiler performance (Lee et al., 2003a; Jang et al., 2007). Unfortunately, the exact amounts of comprised essential oils and their active components in the above mentioned commercial product were not stated. Even though most of the blends have had positive effects on performance, these are rather hard to compare to other studies. In order to achieve best results in BW gain when feeding essential oils, it is highly important to know the exact composition and formulation of the feed additive used (Hippenstiel et al., 2011).

4.2. Microbial enumeration

Several essential oil components exhibit antimicrobial action, some more strongly than others. Phenols, alcohols, ketones and aldehydes are mainly associated with the antibacterial actions, although the exact mechanism of actions has not been fully understood (Lambert et al., 2001). However, it is accepted that the antimicrobial activity depends on the lipophilic character of the components. The components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998).

On the basis of their reported in vitro antimicrobial activity (Helander et al., 1998; Cosentino et al., 1999; Lambert et al., 2001), it is logical to consider thymol and carvacrol application as feed additive alternative to antibiotic growth promoters in animal production. It would be expected that the intake affects the gastrointestinal microflora composition and population. An “ideal flora” ensures optimum growth performance while an alteration of the indigenous flora by diet or environment can be deleterious to the host (Schaedler, 1973).

The results of this study indicate that thymol and carvacrol increased *Lactobacillus* population in crop and small intestine digesta of broilers. Jin et al. (1998) showed that a diet which contained 500 or 1000 mg/kg *Lactobacillus* resulted in improved BW gain and FCR. *Lactobacillus* spp. is known as a probiotic, and may positively affect gut health, if bacteria strains are able to survive and colonize the gastrointestinal tract. Moreover, certain *Lactobacillus* strains are able to antagonize and competitively exclude some pathogenic bacteria (Jin et al., 1996). This effect may have strengthened the antimicrobial effect of thymol and carvacrol which could explain lack of detection or small numbers of harmful bacteria strains of *S. aureus*, *B. cereus*, *L. monocytogenes* and *E. coli.*

No effects could be noticed for the mixture of thymol and carvacrol. However, it has to be considered that for this trial only CFU for total viable count and Lactobacillus spp. were counted due to low numbers or lack of detection of other bacterial groups of interest in the two other trials. Similar observations were monitored by Jang et al. (2007) who used ten-fold dilution method to determine the number of CFU for *Lactobacilli, E. coli* and *Salmonella* in digesta harvested from the ileo-cecum. The birds were fed either 25 or 50 mg/kg of a commercial product containing 290 g/kg active ingredients, including thymol. The CFU of *Lactobacilli* were

not influenced by dietary supplementation of feed additives. Numerically lower numbers were observed for *E. coli* whereas *Salmonella* could not be detected. Jamroz et al. (2005) collected intestinal digesta from the most distal part of the small intestine and whole caeca to determine CFU for *E. coli, C. perfringens* and *Lactobacillus spp.*. The diets were supplemented with 100 mg/kg of a commercial product including 49.5 g/kg carvacrol, 29.7 g/kg trans-cinnamaldehyde and 19.8 g/kg capsaicin. The supplementation reduced the CFU of E. coli to a limited extent, with a greater inhibition observed in older birds. *Lactobacillus spp*. exhibited an ascent, while the number of *C. perfringens* was slightly reduced. Cross et al. (2007) observed CFU of *E. coli, Lactobacillus* and *C. perfringens* by analysing caecal and faecal contents of chickens. Observations indicated no effects on either of the tested microorganisms after adding 1000 mg/kg of thyme or oregano essential oil. The authors specified that the absence of effects could be due to an insufficient degree of replication as well as decreased exposure time to the air. Other reasons for missing effects in vivo may depend on several other factors like environment and basal diet. If the birds are housed under clean and healthy conditions and if the diets are highly digestible it is possible that the antimicrobial effect does not show. There are no improvements needed if the microflora is already in an equlibrium state.

5. Conclusions

In conclusion, this study shows that thymol, carvacrol and their combinations at different levels improved BW gain, FI and FCR without any side effect on carcass traits. Furthermore, increasing levels of thymol or carvacrol supplementation to broiler diets improved gut health and nutrient digestibility of the birds.

More detailed studies are still needed to determine the optimal dietary inclusion level and the mode of action of these herbal products to achieve the optimal growth performance and bacteria resistance in broiler production.

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5. Effects of thyme and oregano on feed intake and performance characteristics of broilers

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Abstract

This study was undertaken to evaluate the effects of different herbs on feed intake, growth characteristics and gastrointestinal microflora in growing broiler chicken. A total of 240 4-day-old Ross boiler chickens, 120 in each trial, were assigned to the basal control diet or the basal diet supplemented with either 10, 15, 20, 25 or 30 g/kg thyme (trial 1) or oregano (trial 2). Analysis of thyme and oregano chemical composition resulted in approximately 6.3 g/kg thyme essential oil, while oregano comprised 9.8 g/kg essential oil fraction. Moreover, essential oil of thyme yielded 500 g/kg thymol and 40 g/kg carvacrol, oregano essential oil consisted of 6 g/kg thymol and 500 g/kg carvacrol.

Improvements for body weight gain and feed conversion ratio ($P<0.05$) were observed in both trials, however feed intake was only affected by oregano addition. Colony forming units (CFU) of *Lactobacillus spp*. were increased ($P<0.05$) for thyme and oregano supplemented groups (trial 1 and 2), whereas no improvements could be observed for CFU of total viable count. In conclusion it can be stated that thyme, as well as oregano can be used as effective feed additives to improve performance and gut health of broiler chickens. However, future research is needed to determine the optimal dietary inclusion level and the exact mode of action including chemical composition and analysis of the essential oil fraction of the examined herbs.

1. Introduction

Natural feed additives of plant origin, also referred to as phytogenic substances are healthier, less regarded as chemical hazards and generally regarded as safe (GRAS; Burdock and Carabin, 2004). Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight (BW) gain, higher production rates and improved feed efficiency.

Since the EU banned antibiotic growth promoters in broiler nutrition (Anonymous, 2003) due to suspected cross and multiple resistance (Neu, 1992), much research has been conducted to explore the use of plants, plant extracts, essential oils and their active components as effective substitutes.

Thyme (*Thymus vulgaris*) and Oregano (*Origanum vulgare)* are popular medicinal plants, mostly grown in the Mediterranean region. Both plants are known for their antioxidant and antibacterial properties, mainly due to their active components thymol and carvacrol. Thymol and its isomer carvacrol are classified as monoterpene phenols and have already proven their antimicrobial effect *in vitro* (Ouwehand et al., 2010). This effect is mainly due to the lipophilic character of the active principles, which permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations of essential oils also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998).

Nevertheless, there is only limited data on studies reporting effects of thyme and oregano *in vivo*. For this reason, the aim of the present study implied an evaluation of the potential of different increasing levels of thyme and oregano as feed additives in broiler diets, in order to observe their influence on feed intake, performance and gastro-intestinal microflora.

2. Materials and Methods

2.1. Experimental animals and design, and feed preparation

A total of 240 1-day-old unsexed broiler chickens (Ross 308), 120 in each of two trials, were housed individually in metabolic wire cages in a temperature controlled room with a 23 h constant light schedule. The temperature of the animal facility was maintained at 32°C during the first week and than weekly reduced by 2°C to reach a final temperature of 24°C towards the end of the experiment.

During the first 3 days, chicks were fed a commercial starter diet (RWZ, Cologne, Germany), containing 200 g/kg crude protein (CP). From 4 to 42 days of age, the chicks were fed the experimental diet. The diets were formulated according to GfE (1999) to meet the nutrient requirements (Table 10). The experiment has been subdivided into two trials. Each trial included 120 birds, which were divided into 6 groups (e.g. 20 birds per group). Each group was fed at different levels (0, 10, 15, 20, 25 or 30 g/kg) of either thyme (product No. 128204, batch 13445, Alfred Galke, Gittelde/Harz, Germany) in the first trial or oregano (product No. 87804, batch 8242, Alfred Galke, Gittelde/Harz, Germany) in the second trial.

Table 10: Ingredient composition of experimental diets fed to broiler chickens

Ingredients	g/kg
Maize grain	600
Soybean meal	305
Soybean oil	39
Maize gluten meal	20
Di-calcium phosphate	15
Limestone	14
Vitamin and mineral mix[1]	3
DL-Methionine	3
L-Lysine	1
Common salt	1

[1]Contained per kg: vitamin A, 10,000,000 IU; vitamin D, 2,000,000 IU; vitamin E, 10,000 mg; vitamin K3, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; biotin, 50 mg; BHT, 10,000 mg; pantothenic acid, 10,000 mg; folic acid, 1000 mg; nicotinic acid 30,000 mg; Mn, 60 g; Zn, 50 g; Fe, 30 g; Cu, 4 g; I, 3 g; Se, 0.1 g; Co, 0.1 g.

All birds were offered the respective diets for *ad libitum* consumption and had free access to water for the entire period. Feed intake (FI) and body weight (BW) were recorded on a

weekly basis, starting on day 4 until birds reached a final age of 42 days, to determine growth performance (i.e., BW gain) and feed conversion ratio (FCR). Mortality was recorded as it occurred during the entire experimental period.

Excreta were collected twice a day during the last week of each trial. At the end of the experimental period, total excreta from each bird were quantified. All excreta were kept in a freezer at a constant temperature of -20 °C until preparation for chemical analysis. Before chemical analysis the excreta were homogenised and DM was determined. Moreover, excreta were freeze dried (Christ, Osterode/Harz, Germany) and afterwards, ground finely using a 1 mm sieve with a centrifugal mill (KG type ZM1, Retsch, Haan, Germany).

2.2. Microbial enumeration

Five birds from selected treatment groups were utilized to count the colony forming units (CFU) in the gastrointestinal tract (crop, small intestine and caecum). Measurements included total viable count and *Lactobacillus spp.* for birds which received 0, 10, and 30 g/kg thyme or oregano. The digesta samples collected from crop, small intestine and caecum were decanted into separate sterile plastic containers and manually, thoroughly mixed. Ten grams of homogenised sample along with ten-fold serial dilutions using physiological NaCl-Trypton was poured into a stomacher bag and shaken vigorously for 3 minutes. The plate media used were: Casein-peptone Dextrose Yeast Agar (Merck, Darmstadt, Germany) for total viable counts and MRS agar (Merck, Darmstadt, Germany) for *Lactobacillus spp.*.

2.3. Chemical analysis

Samples from the control and experimental diets, as well as the homogenized freeze dried excreta of each replicate for all experiments were milled to 1 mm using a centrifugal mill (KG type ZM1, Retsch, Haan, Germany). Dry matter (DM) was determined by oven-drying at 103°C for 24 h. Total nitrogen (N) was estimated by combustion assay (Type FP-238, LECO Instrumente, Mönchengladbach, Germany), CP was expressed as N x 6.25, ash (ID 942.05) and ether extract (ID 963.15) were analysed according to the standard methods of AOAC (1990). Starch (polarimetrical method) and sugar (Luff-Schoorl procedure) contents of the diets were quantified using official European Union methods (Anonymous, 2009). The contents of neutral detergent fibre (assayed with a heat stable amylase, aNDF) and acid detergent fibre (ADF), both expressed inclusive residual ash, were determined sequentially

without sodium sulphite by a modification of the method of Van Soest et al. (1991) using semi-automated equipment (ANKOM, Macedon, NY, USA).

Calcium (Ca) was measured using atomic absorption spectrometry and phosphorus (P) was analysed colorimetrically (method 10.6.1; VDLUFA, 2007). Lysine and methionine (after oxidation) were analysed using an amino acid analyser after hydrolysis (6 M HCl) of the diets (method 4.11.1; VDLUFA, 2007). The GE contents of the diets and excreta were measured using an adiabatic bomb calorimeter (model C 4000; IKA, Heitersheim, Germany). Thyme and oregano were analysed for essential oil content by volumetrical steam distillation according to European Pharmacopeia (2009). Thyme and oregano were extracted three times with dichloromethane (5 ml). Extracts were used to quantify thymol and carvacrol by gas chromatography with FID detection. Eicosane served as internal standard.

The N-corrected apparent metabolisable energy (AME_N) contents of the diets were calculated as follows (GfE, 1999):

AME_N (MJ/kg) = [energy intake (MJ) – energy of excreta (MJ) – F x N retention (kg)] / feed intake (kg)

F = Factor of correction (Titus et al., 1959): F = 36.5 kJ/g N retention.

2.4. Statistical analysis

The statistical analysis was performed separately for each trial using a completely randomized design and the general linear models (GLM) procedure of SAS 9.2 (SAS Institute, 2009). The model only included the level of supplementation and the bacteriological data required log transformation before statistical analysis. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of the increasing levels of supplementation in each experiment. Significance was declared at $P<0.05$, and a tendency toward significance was declared at $0.05<P<0.10$. *P*-values less than 0.001 are expressed as "<0.001" rather than the actual value.

3. Results

3.1. Diet composition

The proximate analysis carried out on treatment diets showed that there were no differences in the nutritional composition of the dietary treatments (Table 11). Diets for all two trials were similar with an approximate standard error of the means (SEM) between 0.01 and 5.75.

Analysis of thyme and oregano chemical composition resulted in approximately 6.3 g/kg thyme essential oil, while oregano comprised 9.8 g/kg essential oil fraction. Moreover, thyme essential oil yielded 500 g/kg thymol and 40 g/kg carvacrol, oregano essential oil consisted of 6 g/kg thymol and 500 g/kg carvacrol.

Table 11: Proximate analysis of nutrients (g/kg dry matter (DM)), gross energy (GE) content, and standard error of the means (SEM) of diets fed to growing broiler chicken

Item	Trial		
	[1]	[2]	SEM
Dry matter (g/kg)	923.8	912.3	5.75
Organic matter	929.5	929.2	0.17
Ash	70.50	70.83	0.17
Crude protein	209.0	210.3	0.67
Ether extract	65.98	61.32	2.33
ADF	36.10	40.48	2.19
NDF	80.77	86.00	2.62
Ca	16.23	16.67	0.22
P	7.40	7.35	0.03
Starch	449.8	456.2	3.17
Sugar	42.82	42.45	0.18
Lysine	1.05	1.03	0.01
Methionine	0.41	0.41	0.00
GE (MJ/kg DM)	19.57	19.60	0.02

ADF, acid detergent fibre expressed inclusive residual ash; aNDF, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive residual ash.

Trial [1]: Diet included 0, 10, 15, 20, 25 or 30 g/kg thyme.

Trial [2]: Diet included 0, 10, 15, 20, 25 or 30 g/kg oregano.

3.2. Feed intake, performance and carcass traits

Table 12 presents growth performance, FI and FCR in broiler chickens fed the control diets as well as the diets that included different levels of thyme or oregano. In the Appendix (Table A2) the carcass characteristics and digestive measurements are shown. Improvements were observed in both trials, more precisely, quadratic improvements were observed for final BW and BW gain within the thyme treatment groups, and for final BW, BW gain, FI and FCR

within the oregano treatment groups. When thyme was added to the diet, the FCR decreased linearly as the inclusion level increased. Similarly, linear improvements were observed for AME_N and carcass weight within the oregano supplementations, as well as a quadratic improvement for thyme addition groups. There were no mortalities in any phase of growth.

Table 12: Growth performance, feed intake and nitrogen-corrected apparent metabolizable energy (AME_N) from 42-day-old broiler chickens fed the basal diet and different additions of thyme or oregano

Inclusion level (g/kg)	Final Body weight[1] (g)	Body weight gain[1] (g)	Feed intake[1] (g)	FCR[1] (kg/kg)	AME_N[2] (MJ/kg)	Carcass weight[2] (g)
Thyme						
0	1555	1487	2465	1.663	13.9	1218
10	1680	1609	2558	1.590	14.8	1449
15	1724	1654	2578	1.559	15.4	1358
20	1715	1646	2515	1.529	14.8	1400
25	1676	1606	2458	1.529	13.8	1404
30	1636	1565	2410	1.545	13.7	1263
SEM	21.6	20.4	31.0	0.01	0.286	30.9
P- value lin.[3]	0.637	0.495	0.241	0.007	0.065	0.830
P- value quad.[4]	0.041	0.026	0.276	0.079	<0.001	0.034
Oregano						
0	1693	1631	2671	1.666	14.9	1469
10	1876	1813	2842	1.575	15.0	1555
15	1881	1817	2842	1.570	15.8	1662
20	1916	1853	2915	1.576	15.4	1446
25	1753	1691	2695	1.600	15.6	1438
30	1559	1497	2576	1.749	15.4	1278
SEM	69.6	69.2	69.5	0.04	0.22	75.3
P- value lin.	0.090	0.089	0.147	0.137	0.044	0.023
P- value quad.	<0.001	<0.001	<0.001	<0.001	0.057	0.022

[1]Values in each row are means for 10 replicates of each treatment.
[2]Values in each row are means for 5 replicates of each treatment.
[3,4]Linear and quadratic responses, respectively, to the dietary inclusion levels.
SEM: Standard error of the means.

3.3. Microflora enumeration

Effects of thyme and oregano on the gastro-intestinal microbial CFU are presented in Table 4. Birds supplemented with different levels of thyme expressed quadratic effects for *Lactobacillus spp*. in the crop. Moreover, oregano supplementation exhibited linear improvements for *Lactobacillus spp*. in the small intestine. However, there were no linear or quadratic effects on total viable count in crop, small intestine and caecum of broilers at any treatment groups, when digesta were sampled from 42 day old birds.

Table 13: Microbial enumeration (log CFU/g) in crop, small intestine and caecum digesta from 42–day-old broiler chickens

			Inclusion level (g/kg)			SEM[3]	P- value lin.[4]	P- value quad.[5]
			0	10	30			
Total viable count	Crop	[1][1]	9.43	9.10	9.52	0.10	0.332	0.122
		[2][2]	9.09	9.25	8.79	0.19	0.172	0.394
	Small intestine	[1]	9.08	8.66	9.39	0.18	0.246	0.238
		[2]	9.15	9.19	8.65	0.25	0.137	0.676
	Caecum	[1]	9.68	9.22	9.36	0.14	0.567	0.219
		[2]	9.63	9.34	9.18.	0.14	0.073	0.306
Lactobacillus spp.	Crop	[1]	9.36	8.85	9.41	0.11	0.292	0.028
		[2]	8.95	9.03	8.86	0.13	0.503	0.578
	Small intestine	[1]	8.49	8.86	9.12	0.16	0.264	0.264
		[2]	8.94	8.71	8.26	0.21	0.044	0.756
	Caecum	[1]	8.61	8.16	9.13	0.17	0.061	0.126
		[2]	9.34	8.99	8.89.	0.19	0.193	0.305

Values in each row are means for 5 replicates of each treatment.
[1] Trial [1]: Diet included thyme.
[2] Trial [2]: Diet included oregano.
[3]SEM = Standard error of the means.
[4,5]Linear and quadratic responses, respectively, to the dietary inclusion levels.

4. Discussion

4.1. Growth Performance

Throughout the last years several studies have been conducted to investigate the effect of herbs on broiler performance parameters. However, results of these studies vary to great extents. For this reason the present experiment was conducted to investigate the effects of thyme and oregano on growth performance and influence on the gastrointestinal system in broiler chicken. Body weight gain improvements were observed in both trials. Thyme supplementation of 15 g/kg resulted in 11% increase while the addition of 20 g/kg oregano

led to 13% improved BW gain. However, an inclusion of the maximal dose of 30 g/kg oregano induced a reduction of BW gain by 8%, when compared to the control group. In contrast to the present study, no improvements on BW gain were observed when oregano was supplemented at levels of 2.5, 5, 10 or 20 g/kg (Cross et al., 2007; Karimi et al., 2010). However, similar results were published by Toghyani et al. (2010) who observed a 6% increase in BW gain when 5 g/kg thyme was added to a broiler diet. As the dosage increased to 10 g/kg, improvements were no longer present. It was concluded that this effect might be due to an adverse effect on some beneficial microbial populations, which in turn prevented the herb from exhibiting its positive influence on performance. Unfortunately, Toghyani et al. (2010) did not include analysis of the chemical composition, nor were species or origin of the used thyme herb declared. The exact chemical composition of the used herb depends on several factors such as plant species, physical and chemical soil conditions, harvest time, degree of plant maturity, technology of drying and duration of storage (Burt, 2004; Bakkali et al., 2008). In order to achieve best results in BW gain, it is highly important to know the exact composition and formulation of the feed additive used (Hippenstiel et al., 2011).

Improvements of FI were observed only during the trial that included birds which were fed the oregano supplemented diet. An inclusion of 20 g/kg exhibited an increase of approximately 9%, while the addition of 30 g/kg suppressed FI by 4%. Possibly, carvacrol, a major principle of oregano essential oil, which is characterized by its dominant smell and taste, affected the FI by modulating appetite. Similar findings were observed by Cross et al. (2007), who observed a FI which was decreased by 15% when a diet included 10 g/kg oregano. It was suggested that there may have been bound tannins which could have had some negative influence. Similar to the present study, no influences on FI were sighted when different levels of thyme, ranging between 1 g/kg (Sarica et al., 2005) and 20 g/kg (Abd El-Hakim et al., 2009) have been added to diets. This leads to the suggestion that thyme herb does not affect appetite and FI, neither positive nor negative. Possibly, the essential fraction in the used herb was too little to express positive effects that have previously been observed with thyme essential oil or its main component thymol (Bölükbasi et al., 2006; Abdel-Wareth et al. 2011).

In the present study FCR was positively affected by 20 or 25 g/kg thyme and 15 or 20 g/kg oregano. However, when 30 g/kg oregano were added the FCR increased by approximately 5%. Almost all experiments that were conducted to evaluate the effects of herbs did not

observe any changes on FCR (Cross et al., 2007; Ocak et al., 2008; Karimi et al., 2010). The only exception is a study performed by Toghyani et al. (2010), who observed an increased FCR after the addition of 10 g/kg thyme.

However, some positive results were observed when the essential oil of thyme was added to a broiler diet. Thyme essential oil usually contains between 300 and 550 g/kg of its major component thymol (European Pharmacopoeia, 2004). Bölükbasi et al. (2006) added 100 or 200 mg/kg thyme essential oil and observed a positive influence on FI, however, FCR was not improved, due to a stagnant BW gain. Cross et al. (2007) supplemented thyme essential oil at a level of 1000 mg/kg and found an improved BW gain, although FI decreased by almost 10%. A blend derived from oregano, clove and anise essential oil supplemented at a level of 200 mg/kg resulted in an increased BW gain by 16% as well as an improved FCR by 12%. It was concluded that these positive findings were due to the positive digestive stimulating effects of thymol and carvacrol (Ertas et al., 2005). However, Abildgaard et al. (2010) observed negative influences on broiler performance characteristics when 100 or 200 mg/kg of a commercial blend, which included 290 g/kg thymol, was added to the diet. Feed intake and BW gain decreased, maybe due to practical housing conditions, vaccination against coccidosis and a shift to a grower diet with high wheat content, which in turn led to certain degree of stress on the birds. Nevertheless, other studies with the same commercial products could not proof any beneficial effects on overall broiler performance (Lee et al., 2003; Jang et al., 2007). Unfortunately, the exact amounts of comprised essential oils and their active components in the above mentioned commercial product were not stated. Even though most of the blends have had positive effects on performance, these are rather hard to compare to other studies.

4.2. Microbial enumeration

Unfortunately, only few studies have been performed to observe the effect of herbs on microbial enumeration in broiler chicken. This might be due to the minor part of essential oil fraction in herbs. Several essential oil components exhibit antimicrobial action, some more strongly than others. Phenols, alcohols, ketones and aldehydes are mainly associated with the antibacterial actions, although the exact mechanism of actions has not been fully understood (Lambert et al., 2001). However, it is accepted that the antimicrobial activity depends on the lipophilic character of the components. The components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron

flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al., 1998).

The results of the present study indicate that thyme and oregano increased *Lactobacillus* population in crop and small intestine digesta of broilers. Jin et al. (1998) showed that a diet which contained 500 or 1000 mg/kg *Lactobacillus* cultures resulted in improved BW gain and FCR. *Lactobacillus spp.* is known as a probiotic, and may positively affect gut health, if bacteria strains are able to survive and colonize the gastrointestinal tract. Moreover, certain *Lactobacillus* strains are able to antagonize and competitively exclude some pathogenic bacteria (Jin et al., 1996). Similar observations were monitored by Jang et al. (2007) who used ten-fold dilution method to determine the number of CFU for *Lactobacilli*, *E. coli* and *Salmonella* in digesta harvested from the ileo-caecum. The birds were fed either 25 or 50 mg/kg of a commercial product containing 290 g/kg active ingredients, including thymol. The CFU of *Lactobacilli* were not influenced by dietary supplementation of feed additives. Numerically lower numbers were observed for *E. coli* whereas *Salmonella* could not be detected. Jamroz et al. (2005) collected intestinal digesta from the most distal part of the small intestine and whole caeca to determine CFU for *E. coli, C. perfringens* and *Lactobacillus spp.*. The diets were supplemented with 100 mg/kg of a commercial product including 49.5 g/kg carvacrol, 29.7 g/kg *trans*-cinnamaldehyde and 19.8 g/kg capsaicin. The supplementation reduced the CFU of *E. coli* to a limited extent, with a greater inhibition observed in older birds. *Lactobacillus spp.* exhibited an ascent, while the number of *C. perfringens* was slightly reduced. Cross et al. (2007) observed CFU of *E. coli, Lactobacillus* and *C. perfringens* by analysing caecal and faecal contents of chickens. Observations indicated no effects on either of the tested microorganisms after adding 1000 mg/kg of thyme or oregano essential oil. The authors specified that the absence of effects could be due to an insufficient degree of replication as well as decreased exposure time to the air. Other reasons for missing effects *in vivo* may depend on several other factors like environment and basal diet. If the birds are housed under clean and healthy conditions and if the diets are highly digestible it is possible that the antimicrobial effect does not show. There are no improvements needed if the microflora is already in an equilibrium state.

5. Conclusions

In conclusion, this study shows that thyme at different levels improved BW gain and FCR, while oregano at different levels improved BW gain, FI and FCR. Furthermore, increasing levels of thyme and oregano supplementation to broiler diets improved gut health and nutrient digestibility of the birds.

More detailed studies are still needed to determine the optimal dietary inclusion level and the mode of action of these herbs, including their essential oils, to achieve the optimal growth performance and bacteria resistance in broiler production.

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General Conclusions

There are only few *in* vivo studies that focus on herbs and their active components in broiler diets. Conclusions of these studies tend to vary, although positive results dominate the observation. Increase of feed intake, BW gain, feed conversion as well as better efficiency to utilize nutrients, and inhibition of bacteria and fungi to stabilize the intestinal microflora were concluded in most studies. Thyme, oregano and their active components at different levels improved body weight gain, feed intake and feed conversion ratio without any side effect on carcass traits. Medium inclusions of 300 mg/kg carvacrol and thymol, or Medium inclusions of 20 g/kg thyme or oregano leave, resulted in overall best performance. Thyme and thymol increased *Lactobacillus* population in crop and small intestine digesta of broilers. Generally, it can be concluded that herbs, their EO and components have the potential to be considered as an alternative to antibiotic growth promoters. Nevertheless, there is still further research under standardized conditions needed to evaluate the exact mechanism of action and to determine the optimal dietary inclusion level in order to estimate the optimal level of active components and their effects on microbial count and disease resistance in broiler production.

6 APPENDIX

6 APPENDIX

Table A1: Carcass variables and digestive organs from 42-day-old broiler chickens fed the basal diet and different additions of thymol, carvacrol or a mixture of thymol and carvacrol

Inclusion level (mg/kg)	Live body weight (g)	Dressing %	Abdominal fat (%)	Giblets (%)	Small intestine weight (g)	Small intestine weight (cm)
Thymol						
0	2029	74.4	1.45	4.62	52.5	173.8
100	2303	74.3	1.43	4.78	52.4	178.5
200	2128	75.6	1.13	4.75	52.5	180.1
300	2522	74.3	1.28	4.48	60.6	186.3
400	2131	74.7	1.33	4.48	54.3	182.0
500	2290	75.0	1.25	4.70	59.0	190.2
SEM	*58.4*	*0.33*	*0.11*	*0.09*	*1.87*	*4.12*
P- value lin.[1]	*0.530*	*0.685*	*0.637*	*0.700*	*0.257*	*0284*
P- value quad.[2]	*0.444*	*0.835*	*0.674*	*0.852*	*0.977*	*0.951*
Carvacrol						
0	1681	74.5	0.94	4.94	38.5	169.4
100	1923	70.6	2.86	5.24	56.2	187.4
200	2122	74.3	1.44	4.66	49.2	173.6
300	2235	73.4	1.62	4.76	53.2	177.0
400	2044	73.2	1.46	4.70	49.5	175.4
500	2157	75.4	1.92	4.46	50.6	184.0
SEM	*45.6*	*0.04*	*0.01*	*0.01*	*1.86*	*2.49*
P- value lin.	*<0.001*	*0.207*	*0.60*	*0.08*	*0.21*	*0.42*
P- value quad.	0.008	*0.100*	*0.23*	*0.79*	*0.08*	*0.97*
Thymol and carvacrol mixture						
0	1842	72.1	1.33	4.67	44.8	178
100	2001	73.4	1.48	4.50	50.8	169
200	2002	73.2	1.35	4.72	50.3	176
300	2042	74.1	1.52	4.47	42.6	135
400	2196	75.9	1.85	4.41	50.8	176
500	2070	75.1	1.69	4.42	45.9	178
SEM	45.2	35.6	1.61	1.48	0.09	1.36
P- value lin.	0.066	0.010	0.086	0.398	0.932	0.869
P- value quad.	0.420	0.700	0.890	0.930	0.520	0.170

Values in each row are means for 5 replicates of each treatment.

[1,2]Linear and quadratic responses, respectively, to the dietary inclusion levels.

SEM: Standard error of the means.

Table A2: Carcass variables and digestive organs from 42-day-old broiler chickens fed the basal diet and different additions of thyme and Oregano.

Inclusion level (g/kg)	Live body weight (g)	Dressing %	Abdominal fat (%)	Giblets (%)	Small intestine weight (g)	Small intestine weight (cm)
Thyme						
0	1720	70.8	1.86	5.76	52.5	174
10	2054	70.6	1.85	5.65	63.5	183
15	1897	71.8	1.38	5.33	48.6	169
20	1908	73.5	1.72	5.61	54.1	160
25	1944	72.3	1.72	5.27	56.4	175
30	1771	71.5	1.31	4.84	49.4	169
SEM	44.7	0.31	0.11	0.12	1.85	3.28
P- value lin.[1]	0.944	0.113	0.212	0.520	0.386	0.403
P- value quad.[2]	0.085	0.033	0.449	0.030	0.598	0.619
Oregano						
0	2035	72.4	2.02	4.40	53.8	177
10	2149	72.4	2.01	4.35	50.7	177
15	2277	73.4	1.94	4.11	54.2	176
20	1980	0.415	1.27	4.32	50.8	169
25	2025	70.9	1.18	4.75	54.3	181
30	1705	74.9	1.26	4.76	42.4	175
SEM	99.2	1.13	0.16	0.20	3.60	5.93
P- value lin.	0.010	0.340	<0.001	0.068	0.115	0.968
P- value quad.	0.011	73.0	0.851	0.117	0.198	0.689

Values in each row are means for 5 replicates of each treatment.

[1,2]Linear and quadratic responses, respectively, to the dietary inclusion levels.

SEM: Standard error of the means.

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- **The 19th Conference on Society of Nutrition Physiology :** GfE (Gesellschaft für Ernährungsphysiologie)**:** 09.- 11 March 2010, Gottingen, Germany
- **The International Scientific Conference Biogas Science:** 2. - 4 December 2009, Erding, Germany
- **Media and communications in the SAARC country- conference**: AASF (Afrikanisch-Asiatischen studienförderung e.v.,): 9. – 11 October 2009, Gottingen, Germany.
- **17th European Symposium on Poultry Nutrition**: 23. - 27 August 2009, Edinburgh, Scotland, United Kingdom.
- **Biogas production in NRW (Nordrhein Westfalen) conference:** 24. -27 January 2009, University of Bonn, Germany.

6. TRAINING AND WORKSHOPS:

- **Animal Feed Science BFT (B**onner **F**örderkreis **T**ierernährung**-workshop compound feed optimization):** 08. November 2008, Animal Nutrition group, Institute of Animal Science, University of Bonn, Germany
- **Intercultural understanding workshop (Austrian cultural form):** 22. November 2007, South Valley University, Qena, Egypt.
- **Scientific research methodology workshop**: FLDP (Faculty and Leadership Development Projects), 2006, Qena, South valley university, Egypt.
- **Effective communication skills**. FLDP (Faculty and Leadership Development Projects), 2005, Qena, South valley university
- **Ethics and manners scientific research workshop**: FLDP (Faculty and Leadership Development Projects), 2005, Qena, South valley university

- **Preparation and writing of scientific research workshop:** FLDP (Faculty and Leadership Development Projects), 2004, Qena, South valley university.

7. PUBLICATION:

- Friederike Hippenstiel, A.A.A. Abdel-Wareth, Saskia Kehraus, K.-H. Südekum, 2011. Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review. Arch. Geflügelk. (in press).
- A.A.A. Abdel-Wareth, S. Kehraus, K.-H. Südekum, 2011. Effects of thyme and oregano on feed intake and performance characteristics of broilers. Under Journal review.
- A.A.A. Abdel-Wareth, S. Kehraus, F. Hippenstiel, K.-H. Südekum, 2011. Effects of thymol, carvacrol and their combination on feed intake and performance characteristics of broilers. Under Jouranl review
- A., Baiomy, A.A.A., Abdel-Wareth, O., Oduguwa, and J. A., Abiona, 2011. Effect of dietary zinc supplementation on semen characteristics of rabbit bucks in a tropical environment reproductive performance of rabbit on Zinc feeding. Under Journal review.
- A.A.A. Abdel-Wareth, S. Kehraus, F. Hippenstiel, K.-H. Südekum, 2010. Effects of thymol or carvacrol levels on feed intake and performance of broilers. Proc. Soc. Nutr. Physiol. 20: 107.
- A.A.A. Abdel-Wareth, S. Kehraus, K.-H. Südekum, 2010. Effects of thyme and oregano levels on feed intake and performance of broilers. Conference: Tagung Schweine-und Geflügelernährung, 11:151-153.
- Osman, A.M.A, Hassan, M.A., Hassanien, H.M., Abdel-Wareth, A.A.A., 2007. Evaluation of the growth performance of broiler chicks fed on plant diets supplemented with some feed additives. Journal of Agriculture Science Mansoura University, 32(1): 133-150.
- Abdel Wareth, A.A.A (2006). Evaluation of the growth performance of broiler chicks fed on plant diets supplemented with some feed additives. M. Sc. Mania University, Egypt.