**An-Najah National University**
**Faculty of Graduate Studies**

# Performance and Lipid Profile of Broilers Fed Two Medicinal Plants

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# Dedication

**To my mother, father, sisters and brothers.**

**To my wife and my family.**

**I dedicate this project.**

# Acknowledgements

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## الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

# Performance and Lipid Profile of Broilers Fed Two Medicinal Plants

# تأثير نوعين من النباتات الطبية على أداء ومستويات الدهن في دم دجاج اللحم

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء مـــا تمـــت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

### Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's name:** **اسم الطالب:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

# List of Abbreviations

| | |
|---|---|
| **HDL** | High density lipoprotein |
| **LDL** | Low density lipoprotein |
| **VLDL** | Very low density lipoprotein |
| **CHO** | Serum Cholesterol |
| **DM** | Dry matter |
| **CP** | Crude protein |
| **EE** | Ether extract |
| **GLC** | Gas liquid chromatography |
| **FCR** | Feed conversion ratio |
| **TCHO** | Total cholesterol |
| **TG** | Triglycerides |
| **ME** | Metabolizable Energy |
| **FI** | Feed Intake |
| **BW** | Body Weight |
| **WG** | Weight Gain |
| **G** | Garlic |
| **TH** | Thyme |
| **SI** | Small Intestine |
| **P** | Phosphorus |
| **CA** | Calcium |
| **D%** | Dressing Percentage |
| **LE** | Labiatae extract |
| **SAS** | Statistical Analysis System |
| **ANOVA** | Analysis of Variance |
| **A.O.A.C** | American Official Analytical Chemists |
| **CF** | Crude Fiber |
| **H M G** | 3-hydroxy-3-methyl glutaril |
| **CoA** | Coenzyme A |
| **DCP** | Di-calcium phosphate |
| **NRC** | National Research Council |

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**Performance and Lipid Profile of Broilers Fed Two Medicinal Plants**

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## Abstract

This experiment was conducted in faculty of agriculture at khadory-Tulkarem to investigate the effect of feeding garlic powder and dried thyme leaves on the performance, digestibility, dressing percentage, carcass and non carcass cuts and lipid profile of broilers. A total of 216 day-old Cobb-500 chicks were used in this experiment from 9/2/2011 to 22/3/2011.

Birds were divided into nine experimental treatments of 24 birds in each. Each treatment was composed of 4 replicates with 6 birds in each replicate. The control group was fed a commercial starter and finisher diet. The second and third groups were supplemented with garlic at the rate of 0.2 and 0.4%, respectively. Birds in forth and fifth groups were supplemented with thyme at the rate of 0.02 and 0.04%, respectively. The birds in the last four experimental groups were supplemented with a mixture of the supplements at the two levels (0.2%+0.02% for the sixth group, 0.4%+0.04% for the seventh group, 0.2% + 0.04% for the eighth group and 0.4% + 0.02% for the ninth group). In the last week of experiment, three birds from each experimental unit were used in metabolic trial. However, at the termination of the experiment, the same birds will be slaughtered for dressing percentage and giblets. Blood samples from all groups were collected on 3$^{rd}$, 4$^{th}$ and 5$^{th}$ weeks of age from wing vein for

lipid profile studies. Total cholesterol (TCHO), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) levels were determined. Results of this study showed that both garlic powder and the dried thyme leaves when fed separately had no significant effects on broilers weight gain, feed intake, feed conversion ratio, carcass cuts, visceral organs. However, both plants decreased ($P<0.05$) the levels of serum cholesterol (CHO), triglycerides (TG), low density lipoprotein (LDL) and increased the high density lipoprotein (HDL) compared to the control birds. The digestibility of dry matter (DM), crude protein (CP) and ether extract (EE) was improved by feeding garlic powder and dried thyme leaves individually. Diets supplemented with mixture of garlic powder and dried thyme leaves caused a significant improvement ($P<0.05$) in final body weight, feed intake, feed conversion ratio, dressing percentages, carcass weight, EE and the digestibility of DM, CP and EE compared to other treatments and the control chicks. These mixtures at different levels and separately caused a significant reduction ($P<0.05$) in the levels of CHO, TG, and LDL, and cause improvement in (HDL). The mixtures used had no effect on carcass cuts and visceral organ weight. It can be concluded that garlic powder and dried thyme leaves supplements can provide positive advantages in broilers performance.

# Chapter One

# Introduction

# Chapter One
# Introduction

Feed additives have been widely used in poultry industry since long time as tool to increase animals' performance in regard to growth and feed efficiency (Collington *et al*., 1990). Therefore, about 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Lee *et al*., 2001). With the status of banning of the use of antibiotics as feed additives has led to investigations of nonconventional feed additives in animal production (Cardozo *et al.,* 2004; Tipu *et al.,* 2006). Under the intensive management systems, herbal extracts are already being used as feed supplements to improve growth performance (William and Losa, 2001). These extracts when supplemented to animals diets can play a role in supporting both performance and health status of the animal (Janssen, 1989; Horton *et al*., 1991; Bakhiet and Adam, 1995; Skrabka Blotnicka *et al*., 1997; Gill, 2000; Manzanilla *et al*., 2001). Several studies indicated that these feed additives could be used in poultry diets as antifungal, antibacterial, antioxidant and or anti mutagenic compounds (Osawa *et al.,* 1995; Nielsen *et al*., 1999; Wuthi-udomler *et al*., 2000; Hernandez *et al*., 2004; Khosravi *et al.,* 2008).

Garlic (*Allium sativum*) is widely used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases (Javandel *et al*., 2008). Garlic has been found to lower serum and liver cholesterol (Qureshi *et al*., 1983a), inhibit bacterial growth (Cavallito and Bailey, 1994), inhibit

platelet growth and reduce oxidative stress (Horie *et al*., 1992). In broilers, it was reported that garlic as a natural feed additive, improved broiler growth and feed conversion ratio (FCR), and decreased mortality rate (Tollba and Hassan, 2003). Similarly, volatile oil from thyme (*Thymus vulgaris*) was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (Dorman and Deans, 2000). The pharmacological action of active plant substances or herbal extracts in humans is well known, but in animal nutrition the number of precise experiments is relatively low (Rahimi *et al*., 2011).

The objective of this study was to compare the effect of feeding different levels of two commercial herbs (garlic and thyme) on growth performance, digestibility, blood lipid profile, digestibility, visceral organs and dressing percentage in broiler chickens.

# Chapter Two

# Literature review

# Chapter Two
# Literature review

## The chemical composition of garlic and thyme and their components

It was estimated that there are 250000- 500000 species of plants on earth (Borris, 1996; Hashemi and Davoodi, 2010). Many scientists have searched for alternatives to antibiotics through utilization of the extracts of some of these plants (Longhout, 2000; Mellor, 2000; Wenk, 2000; Kamel, 2001; Alcicek *et al*., 2003).

The medicinal activities of some natural plants as garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) are well known and documented. Extracts from certain herbs have been proved to be good growth promoter (Hernandez *et al*., 2004; Stanley *et al*., 2004; Czech *et al.*, 2009)

Herbs or products containing plant extracts, essential oils or main components of the essential oil are among the alternative growth promoters that are already being used in practice (Williams and Losa, 2001; Lee *et al*.,2003; Acamovic and Broker , 2005; Bampidis *et al*., 2005; Griggs and Jacob, 2005).

Garlic is one of the oldest cultivated plants (Moyers, 1996; Ramaa *et al.,* 2006). Research results showed its effect on lowering blood cholesterol levels in human (Adler and Holub, 1997) and in animals (Aouadi *et al*., 2000). Garlic was considered as antibiotic growth promoters and have been used for about 50 years to enhance growth performance in poultry and swine (Dibner and Richards, 2005; Demir *et al.,* 2008). The search of

alternative feed supplements was necessary because of the ban on the use of antibiotics for broilers as growth promoters in European Union because of antibiotic resistant properties in human (Lee *et al*., 2003a ; Hernandez et al., 2004; Cabuk *et al.,* 2006; Demir *et al.,* 2008).

Prasad *et al.,* (2009) reported that garlic can prevent fat- induced hyperlipemia. Garlic has been cultivated in all over Middle East and used as flavoring agent and as a medicinal plant beside other several medicinal properties (Zargari, 1997). Different forms of garlic preparations are commercially available in the form of garlic oil, garlic powder, and pills. These are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (Elkayam *et al*., 2003).

It was suggested that garlic may decrease cholesterol (CHO) and triglyceride (TG) levels in patients with increased levels of these lipids (Zhang *et al*., 2001). Using garlic as natural feed supplement has several advantages as the inhibition of platelet aggregation (Apitz Castro *et al.,* 1983) reduction of arterial blood pressure (Mc Mohan and Vargas, 1993) prevention of fat infiltration of liver (Sand *et al.,* 1995).

Garlic consists of several organo sulfur constituents such as glutamyl cystein, s-allyl cysteine, alliin, allicin and ajoene (Agarwal *et al*., 1996). It is believed that allicin is the primary active compound responsible for inhibiting cholesterol biosynthesis (Gebhardt *et al.,* 1994)

Freshly crushed garlic (*Allium sativum*) contains allicin, alliin, ajoene, diallylsulfied, dithiin, s-allylcysteine (Onu, 2010).

Garlic contains vitamins and minerals (Gruenwald, 2004) and trace elements (selenium and germanium) (Skidmor-Roth 2003).

The effect of ground thyme on the performance of broilers was studied by Al-kassie (2009), who reported the positive effect of thyme on the live weight gain and the improvement of the health of broilers, in addition to other performance traits, feed conversion ratio and feed intake. The phenolic compounds carvacrol and thymol present in the essential oil from thyme exhibit considerable antimicrobial and antifungicidal activity (Basilico and Basilico, 1999; Hernandez *et al*., 2004).

Thymol is currently used to inhibit oral bacteria (Twetman and Peterson, 1997; Hernandez *et al*., 2004). Bostoglu *et al.* (2002) indicated that dietary oregano oil exerted no growth promoting effect on broilers when administrated at 50 or 100 mg/kg of feed. The growth promoting properties of mint, sage and thyme were studied by ( Deans and Ritchie, 1987; Hammer *et al*., 1999; Al-Ankari *et al*., 2004; Demir *et al.,* 2008; Ocak *et al.,* 2008).

## 2.1 Medicinal Plants in Poultry Feeds

Poultry feeds are regularly supplemented with pharmacological products, either for preventive purposes, as prevention of certain diseases or as growth stimulators, primarily for young chicks (Doyle, 2001). There are several non pharmacological products from the group prebiotics, probiotics, organic acids and other essential oils, medicinal plants or parts

of plants which are alternatives to antibiotics as growth stimulators (Simon, 2005).

Garlic *(Allium sativum L.)* is known to have antimicrobial, antioxidative and antihypertensive properties ( Prasad and Saharma 1981; Konjufca *et al*. 1997; Sivam, 2001).

Scientific research has shown that effects can be attributed to its bioactive components, the most important among which are sulphuric compounds, allin, diallylsulphide, allyldisulphide and allicin defined by Kumar and Berwal (1998).

**2.2 The Effect of (garlic and thyme) on chicken's performance:**

Garlic showed positive effects on the performance of different animals. Feeding garlic to pigs at 1% caused an increase in growth, feed conversion and meat quality(Cullen *et al.* 2005).Similar results were obtained when broilers were fed diets containing 1 or 2% garlic (Horton et al., 1991; Freitas et al., 2001**;** Bampidis et al., 2005).

Hernandez *et al*. (2004) showed that at the end of the second week, the highest body mass was achieved in the control group while in the experimental groups with garlic at 2% was slightly lower. That was probably due to reduced food consumption, resulting from the intense smell of garlic, which required a period of adaptation of chickens to this kind of feed. Horton *et al*. (1991) reported similar results. However, garlic manifested its stimulating effects and at the end of the fourth week, the

experimental groups had higher body mass than the control group. At the end of the experiment, garlic registered a statistically significant increase in body weight in comparison to the control group.

In regard to the weight of chicks, separated by gender, the same tendency is observed in both male and female birds at the end of that experiment.

Garlic powder at different levels (0.5 to 3%) had no significant effect on weight gain during the first 21 days of feeding trial compared to that of control birds (Raeesi *et al.,* 2010). However, for the period from 22 to 42 days, garlic level at 1% resulted in the highest gain weight. For the whole feeding period garlic levels of 1 and 3% significantly increased body weight gain as compared with 0.5% garlic supplemented groups but it was not significant in comparison with control group (Raeesi *et al*., 2010). The main active constituents of the essential oil are thymol (40-60%) and carvacrol (1 to 5%) (Radwan, 2003; Gibbons, 2005). Thyme is rich in essential oil as well as the monoterpene components; a- pinene and beta-pinene (Muhlbauer *et al*., 2003). Hassanein (1982) found that the oil contents of Egyptian thyme plant were 2.07% (on dry weight basis) and the essential oil extracted by gas liquid chromatography (GLC) contained 25.65% thymol (phenols), 2.6% limonene, 19.2% p-cymene and 7.2% cyclic terpenes. The essential oil derived from oregano is known to possess in vitro antimicrobial (Sivropoulou *et al*., 1996; Lambert *et al*., 2001), antifungal (Thompson, 1989), insecticidal (Karpouhtsis *et al.,* 1998) and

antioxidant (Botsoglou *et al*., 2002) activities . Oregano herb or essential oil or sole constituents of oregano essential oil have been tested as growth promoters in broiler chickens (Cross *et al*., 2002; Lee *et al*., 2003a, b) and pigs (Tsinas *et al*., 1998; Docic and Bilkei, 2003; Bampidis *et al.,* 2005). Thyme addition to minced broiler chicken meat similarly retarded the rate of lipid peroxidation (Onibi, 2003; Onibi *et al.,* 2009). Hernandez *et al.* (2004) observed that tow blends of plant extracts (oregani, cinnamon, and peeper at 200 mg.kg-1 and sage, thyme, and rosemary at 5000kg-1) affected digestibility and improved the performance slightly, but not significantly. Cross *et al*. (2007) reported that dietary thyme had a different effect when used as a herb or oil on weight gain and body mass. Thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*) have highly aromatic odor with a pungent and slightly bitter taste (El-Gendy *et al*., 2010). Thyme (*Thymus vulgaris*) and Sage (*Salvia officinalis*) cause a significant improvement in body weight, feed conversion, and mortality rate of broilers (Tollba, 2003 and Gibbons, 2005). Thyme is known for its ability to act as antimicrobial and growth promoter herb( Cross *et al*., 2002; Demir *et al*., 2003; Acamovic and Broker, 2005; Bampidis *et al*., 2005; Griggs and Jacob, 2005; Cross *et al*., 2007).

Mohan *et al*, (1996) studied the effect of thyme extracts on broilers. They reported that thyme extract decreased body weight gain as compared with control group. Sarica *et al*, (2005) found no significant effects of garlic and thyme powder on performance, when they added them to broilers diet. Amooz mehre *et al*., (2009) reported that garlic extract did not

influence broilers performance. Freitas *et al.* (2001) did not observe significant differences in the performance of 24-day-old broilers fed garlic or antibiotics, and attributed these results to the low health challenge to which birds were exposed. Studies on broilers, suggest that body weight will be higher in garlic supplemented groups than control group (Raeesi *et al*., 2010). Furthermore, garlic powder can facilitate activity of enzymes which are involved in the conversion of cholesterol to bilious acids and subsequently, there will be less cholesterol in the carcass (Bordia *et al.,* 1975; Raeesi *et al*., 2010). These results were in agreement with those of Schutte *et al*. (1993), Garcia *et al.* (1999) and Murphy *et al*. (2003).

Also, the inclusion of thyme oil did not affect body weight gain of broilers over a 42-d growth period (Cross *et al*., 2003). The feed intake of the birds were not ($P > 0.05$) affected by any of the supplemental treatments, in agreement with the findings of Schutte *et al*. (1993) and Jansman *et al*. (1999).There were no differences in feed conversion ratios between dietary treatments over the experimental period. These results are consistent with those of Engberg *et al.* (2000) and Van Campenhout *et al*. (2001).

Birds received garlic for the whole of the experiment, had higher feed intake (FI) (Raeesi *et al*., 2010). Chowdhuury *et al*, (2002) added different levels of garlic to layers diet. They reported no significant effects of this supplement on growth, feed intake and feed efficiency.

Supplementation of 1% garlic powder, decreased feed conversion rate (FCR) compared with 0.5% supplemented and control group (Raeesi *et al*. 2010). Birds received 3% garlic powder in their diets had better FCR than control group. Control groups significantly consumed more feed than the others, except those which were supplemented with 0.5% garlic powder. There were no significant differences between control and 0.5% supplemented group, although they had lower FCR. Groups which were supplemented with garlic powder in just the finisher diet had better FCR than those which were supplemented for the whole of the experiment. Control groups consumed more feed but they had no significant difference with which supplemented with garlic in starter diet (Raeesi *et al*., 2010). Jamroz *et al*. (2005) reported that capsaicin, cinamaldehyde and carvacrol decreased FCR significantly in broiler chickens, however they did not affect body weight gain (WG) at all. Demir *et al*. (2003) added thyme and garlic powder to broilers diet. They concluded that this supplementation did not affect growth, intake and feed conversion rate in whole of the experiment. Konjufca *et al*. (1997) reported that although performance was not affected when broiler diets were supplemented with 1.5, 3 and 4.5% garlic in powder form, their serum and liver cholesterol decreased significantly. They also indicated that this supplementation did not influence feed conversion rate. Lewis *et al*. (2003) reported that garlic extract increased body weight gain (WG) and also improved feed conversion rate (FCR) in broilers between 7-27d. Alcicek *et al*. (2003) indicated that broilers which received blend of essential oils, had higher

weight and feed intake (FI) and also lower feed conversion rate (FCR) than control group.

## 2.3 Effect of garlic and thyme additives on visceral organs and carcass cuts

Research studies have been focusing on improvement of chicken carcasses in order to meet the food industry standards. The best results are achieved through genetic selection, nutrition and breeding technology, which are reflected in a significant increase of overall carcass masses and the share of meat as well as a reduction of the abdominal fat content in 6-week-old chicks.

Carcass yield was higher in birds fed garlic. Diets supplemented with 1% garlic powder had higher carcass yield than those which received 0.5 and 3 %( Raeesi *et al*. 2010). Supplementation of garlic powder in finisher diet, resulted in higher carcass yield ($p<0.001$) than those which received garlic in starter diet or for whole of the experiment. Thigh yield was also higher in birds received garlic in their starter diet than finisher diet ($p<0.001$).

Breast yield was also higher in groups received garlic in their finisher diet than others ($p<0.001$). Relative weight of bursa was significantly higher in 3% supplemented groups. Relative weight of liver was higher in control and 3% supplemented groups (Raeesi *et al*. 2010). When birds fed garlic in their starter diets, they showed higher relative liver

weight ($p<0.001$) (Raeesi *et al*. 2010). Relative weight of gizzard was significantly higher in control groups (Raeesi *et al.* 2010). Relative weight of spleen also was higher, when starter diet was supplemented with garlic, while relative pancreas weight was higher when birds received garlic in starter and also for the whole of the experiment. Dieumou *et al*. (2009), studied the effects of ginger and garlic essential oils on growth performance reported that all organ weights and carcass characteristics were not affected by the treatments, except for a decrease ($P< 0.05$) in relative liver weight of birds fed garlic oil treatment compared with those given ginger oil and control.

There was no significant difference in relative weight of carcass, fat pad, or digestive organs among treatments except for the small intestine. These finding were in agreement with the results of Ocak *et al*. (2008) who found no differences in carcass and organs' weight of broilers fed a diet containing 2% thyme powder. Similar results were observed by Hernandez *et al* (2004) who found no difference in weight of organs of broiler chickens fed diets containing an extract from thyme and oregano.

Thyme has different effects on weight gain and carcass cuts (Cross *et al*., 2007). The oil of thyme and its different components are becoming increasingly popular as a naturally occurring antimicrobial and also as an antioxidant agent (Dursun *et al*., 2003; Thakar *et al*., 2004).

The effects of the supplements on relative weights of internal organs showed that the relative weights of the heart, liver, gizzard, spleen and

pancreas were not affected by dietary treatments, in agreement with the findings of Hashish *et al.* (1995). A similar observation was reported by Ceylan *et al.* (1998).

Hernandez *et al.* (2004) found no differences in gizzard, liver and pancreas weights of broiler chickens fed wheat-soybean meal based diets supplemented with an antibiotic and two plant extracts (an essential oil extract from oregano, cinnamon and pepper and a labiatae extract from sage, thyme and rosemary). The relative weights of the small intestines of broilers fed diets supplemented with an antibiotic, an antibiotic plus an enzyme, thyme plus the enzyme and garlic plus the enzyme were less than those of broilers fed the basal diet and the diets supplemented with thyme, garlic and the enzyme. The basal diet and garlic-supplemented diet increased the length of small intestine compared to that of the other treatments.

**2.4 Effects of garlic and thyme on blood lipids:**

Horton *et al.* (1991) reported that CHO concentrations were not significantly affected by the supplementation of dietary garlic powder at different levels (0 and 1 g/kg) over a 35-d growth period. Some studies suggested that commercial garlic oil, garlic powder and commercially available garlic extract may not be hypocholesterolemic (Berthold *et al.*, 1998; Isaacsohn *et al.,* 1998; McCrindle *et al.*, 1998; Chowdhury *et al.,* 2002). However, Chowdhury *et al*.(2002) reported in laying hens on wheat-maize based diet, a linear decrease in total serum cholesterol with

increasing levels (0, 2, 4, 6, 8 or 10%) of garlic. Qureshi *et al.* (1983a) found that serum cholesterol concentrations reduced by 18, 21, 24 and 25%, in broilers fed diets contained the equivalent of 1, 2, 4, 6 and 8% garlic paste, respectively, in male broiler chickens. Chowdhury *et al*. (2002) suggested that the relative stability of garlic and the duration of the study may affect responses, since Lawson *et al.* (1992) reported that alicin, the potentially active component in garlic, is unstable and poorly absorbed from the tract.

Garlic has hypocholesterolemic effects on chickens through inhibition of the most important enzymes that participate in the synthesis of cholesterol and lipids (trihydroxy- tri-methyl-glutaril coenzyme A reductase, cholesterol-7-.-hydroxylase and the synthesis of fatty acids). In addition, this additive has a relatively low price (Stanacev *et al*., 2011).

Garlic significantly reduced the serum levels of TC, LDL, and TG and significantly increased the level of HDL (Rahimi *et al*., 2011). There was no significant difference observed for TG levels among extracts in all of which a significant reduction from the control and antibiotic group was observed.

Cholesterol level was recorded to be the same in groups fed diet with thyme (*Thymus vulgaris*) and coneflower (*Echinacea purpurea)*. Thyme (*Thymus vulgaris*) improved hematocrit percentage and hemoglobin concentration, but not significantly (Rahimi *et al*., 2011).

## 2.5 Effects of garlic and thyme additives on digestibility:

Essential oils of garlic and thyme act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Lovkova *et al*., 2001; Williams and Losa, 2001; Cross *et al.,* 2007). Therefore, the main compound of peppermint may probably improve the digestibility of diet as a digestion stimulant, and hence increase the nutrient entry rate at an early stage of the bird's life without affecting feed conversion rate (FCR).

In a recent study, Cross *et al*. (2007) reported that the dietary inclusion (10 g per kg diet) of five culinary herbs including thyme had no effect on the intestinal microflora, metabolizable energy (ME) or the coefficients of digestibility.

# Chapter Three

# Material and Methods

# Chapter Three
# Material and Methods

## 3.1 Medicinal plants

Thyme leaves were bought from local market and then was sun dried for two days and grounded. Dry garlic powder was purchased from local market.

## 3.2 Birds and experimental design

The experiment was conducted at Khadory College from 9/2/2011 to 23/3/2011. A total 216 one day-old broiler chicks (Cobb 500) purchased from a local hatchery (Poultry Company of Palestine, Tulkarm, Palestine). Chicks were randomly assigned to their treatments and were divided into nine dietary treatment groups of 24 chicks each; each treatment was composed of four replicates with six birds in each in a complete randomized design. The birds were housed on floor and routinely managed as any other commercial broiler flock. Heating was provided by a single gas brooder, where the initial temperature was set at 32 $^{\circ}$C and decreased by 2$^{\circ}$C per week to final temperature of 20$^{\circ}$C at the end of experiment. Supplementary heating was provided as required by mobile butane gas heaters besides to electricity heater.

## 3.3 Experimental diets and management

The experimental diets in mash form and drinking water were provided *ad libitum*. Feed consumption and individual body weight were

measured on a weekly basis. All birds were vaccinated against Newcastle disease and Gumporo diseases. Mortality was recorded daily and it was very little to be mention.

All diets were formulated to meet nutrient requirements NRC (1994). The chicks were fed starter diet from day 1 to 21 and a finisher diet from day 22 to 42 (Tables 1 and 2). Tables (1 and 2) show the composition and the chemical composition of the starter and finisher rations respectively.

**3.4 The experimental diets were designed as**

Group 1: control ration.

Group 2: control ration + (0.2% garlic powder).

Group 3: control ration + (0.4% garlic powder).

Group 4: control ration + (0.02% thyme leaves).

Group 5: control ration + (0.04 thyme leaves).

Group 6: control ration + (0.2% garlic + 0.02% thyme leaves).

Group 7: control ration + (0.4% garlic + 0.04% thyme leaves).

Group 8: control ration + (0.2 garlic + 0.04% thyme leaves).

Group 9: control ration + (0.4% garlic + 0.02% thyme leaves).

**Table (1): Composition of the experimental starter diets fed to broilers and their chemical composition.**

| Treatments | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients% | Control | .2G | .4G | .02TH | .04TH | .2G+.02TH | .4G+.04TH | .2G+.04TH | .4G+.02TH |
| Corn | 55.8 | 55.8 | 55.8 | 55.8 | 55.8 | 55.58 | 55.36 | 55.56 | 55.38 |
| Soybean meal | 37.5 | 37.3 | 37.1 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 |
| Oil | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Limestone | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Salt | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Methionine and lysine | .2 | .2 | .2 | .2 | .2 | .2 | .2 | .2 | .2 |
| DCP | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Premix* | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Garlic powder | 0 | 0.2 | 0.4 | 0 | 0 | .2 | 0.4 | .2 | 0.4 |
| Thyme leaves | 0 | 0 | 0 | 0.02 | 0.04 | 0.02 | 0.04 | 0.04 | 0.02 |
| Chemical composition | 90.1 | 90 | 90 | 89.9 | 89.9 | 90.1 | 90.1 | 90.1 | 90.1 |
| Dry matter | 22 | 22.1 | 21.9 | 22 | 22.2 | 22 | 22 | 22 | 22 |
| Crude protein | 4.25 | 4.25 | 4.25 | 4.25 | 4.25 | 4.25 | 4.25 | 4.25 | 4.25 |
| Crude fiber | 5.46 | 5.46 | 5.46 | 5.46 | 5.46 | 5.46 | 5.46 | 5.46 | 5.46 |
| Crude fat | 5.04 | 5.04 | 5.04 | 5.04 | 5.04 | 5.04 | 5.04 | 5.04 | 5.04 |
| Ash | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Calcium | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Phosphorous | 2800 | 2850 | 2830 | 2810 | 2820 | 2800 | 2800 | 2800 | 2800 |
| ME, kcal/kg | | | | | | | | | |

Premix contents per 4.0 kg : Vitamin (A) 8.5 MIU. vitamin (D3) 2.5 MIU. vitamin (E) 50 KIU. vitamin (K3) 2.0 gr. vitamin (B1)0.80 gr vitamin (B2)6.00 gr.pantonec11.20 gr niacin 30.00gr. vitamin (B6)2.40gr vitamin (B12)8.00 mg. folic acid 0.80gr biotin 150.00 mg cholin 200.00gr anti oxidant 125.00gr manganese 80.00 gr. zinc 50 gr iron 20gr.copper 15 gr. iodine 1.2 gr. cobalt 0.2gr. Selenium 0.2 gr. bambermycin 10 gr. wheat enzyme 90 gr phytase 750 kfyt. Limestone 1818.67 gr. salinomycin 60 gr

**Table (2): Composition of the experimental finisher diets fed to broilers and their chemical composition.**

| Treatments | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
|---|---|---|---|---|---|---|---|---|---|
| **Ingredients%** | **Control** | **.2G** | **.4G** | **.02TH** | **.04TH** | **.2G+.02TH** | **.4G+.04TH** | **.2G+.04TH** | **.4G+.02TH** |
| **Corn** | 56.8 | 56.6 | 56.66 | 56.78 | 56.76 | 56.58 | 56.36 | 56.56 | 56.38 |
| **Soybean meal** | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| **Oil** | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 | 5.4 |
| **Limestone** | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 | 1.86 |
| **Salt** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Methionine and lysine** | .2 | .2 | .2 | .2 | .2 | .2 | .2 | .2 | .2 |
| **DCP** | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 |
| **Premix** | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 | 1.37 |
| **Garlic powder** | 0 | 0.2 | 0.4 | 0 | 0 | .2 | 0.4 | .2 | 0.4 |
| **Thyme leaves** | 0 | 0 | 0 | 0.02 | 0.04 | 0.02 | 0.04 | 0.04 | 0.02 |
| **Chemical composition** | | | | | | | | | |
| **Dry matter** | 90 | 90.1 | 89.9 | 90 | 90 | 90 | 90 | 90 | 90 |
| **Crude protein** | 19.48 | 19.48 | 19.48 | 19.48 | 19.48 | 19.48 | 19.48 | 19.48 | 19.48 |
| **Crude fiber** | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 |
| **Crude fat** | 7.93 | 7.93 | 7.93 | 7.93 | 7.93 | 7.93 | 7.93 | 7.93 | 7.93 |
| **Ash** | 4.87 | 4.87 | 4.87 | 4.87 | 4.87 | 4.87 | 4.87 | 4.87 | 4.87 |
| **Calcium** | 1.29 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 | 1.3 |
| **Phosphorous** | 0.5 | 0.48 | 0.49 | 0.49 | 0.49 | 0.5 | 0.5 | 0.5 | 0.5 |
| **ME, kcal/kg** | 3000 | 3050 | 3030 | 3010 | 3020 | 3020 | 3020 | 3020 | 3020 |

Premix contents per 4.0 kg: vitamin (A) 8.5 MIU. vitamin (D3) 2.5 MIU. vitamin (E)50 KIU. vitamin (K3) 2.0 gr. vitamin (B1)0.80 gr vitamin (B2)6.00 gr.pantonec11.20 gr niacin 30.00gr. vitamin (B6)2.40gr vitamin (B12)8.00 mg. folic acid 0.80gr biotin 150.00 mg cholin 200.00gr anti oxidant 125.00gr manganese 80.00 gr. zinc 50 gr iron 20gr.copper 15 gr. iodine 1.2 gr. cobalt 0.2gr. selenium 0.2 gr. bambermycin 10 gr. wheat enzyme 90 gr phytase 750 kfyt. limestone 1818.67 gr. salinomycin 60 gr

### 3.5 The performance trial

During the 42 days experimental period, growth performance was evaluated. Body weight and feed consumption were recorded weekly and body gain and feed conversion were then calculated. Mortality was recorded throughout the study.

#### 3.5.1 Feed consumption

Feed consumption is the amount of feed consumed every week; it was calculated for each treatment at weekly basis. At the end of the week, the residual amount of feed was weight and subtracted from the known weight of feed at the beginning of week. The product was divided by the total number of birds.

#### 3.5.2 Body weight and gain

Body weight was measured for all birds at the beginning of the experiment, and it was repeated weekly at the beginning of the week at the same time.

Live weight gain was calculated by subtraction the live weight at the beginning of the week from the live body weight of the next week.

### 3.5.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated every week at the amount of feed consumption per unit of body gain (average weekly feed consumption (g)/ average weekly gain (g))

## 3.6 Carcass cuts preparation and sampling

At the end of each experiment a sample of three randomly selected birds from each replicate within a treatment was slaughtered to estimate the dressing percentage. Before slaughtering, each bird was weighed and numbered and after that birds were slaughtered, dressing, carcass, giblets and fat pad percentages were then measured as follows:

1-Dressing % = Carcass weight/live weight * 100

2-Giblets % = (Liver+Gizzard+Heart) weight/Live weight *100

3- Fat pad % = (Fat pad weight/ Live weight)*100

## 3.7 Collection of blood samples

Blood samples were taken on 3rd, $4^{th}$, and $5^{th}$ week of age from the wing vein for all birds. Glass vials were used for blood collection. Plasma was separated and stored at -20 $^{\circ}$C for later analysis.

### 3.8 Digestibility trial

At day 35 of the trial 4 birds from each treatment were used in a digestion trial. Birds were housed individually in cages and fed the experimental rations. A three days collection of feed and feces period was performed after an adaptation period of 3 days. Digestibility of dry matter, crude protein and crude fat was determined.

### 3.9 Lipid profile measurements

Total plasma cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were determined in plasma using biochemical analysis. The very low density lipoprotein (VLDL) was calculated using the following formula where results were expressed in mg/dL.

VLDL Cholesterol = Plasma triglycerides/5

However, the low density lipoprotein (LDL) was estimated as the following:

LDL Chol = Total Chol – (VLDL Chol + HDL Chol) ((Adler and Holub 1997).

### 3.10 Chemical analysis

Feed and feces were analyzed for dry matter (DM), crude protein (CP), crude fat (CF), according to the A.O.A.C (1995) procedures.

### 3.11 Statistical analysis

All data were analyzed by ANOVA using the linear model procedure of SAS(2000) to determine the effect of medicinal plants supplementation on body weight gain, digestibility, mortality rate, dressing percentage, feed intake (FI), feed conversion (FCR), carcass cuts and lipid profile.

# Chapter Four

# Results

# Chapter Four
# Results

## 4.1 Effects of garlic and thyme on broilers performance

## 4.2 Feed intake

The feed intake by broilers under different treatments is shown in Table (3). Broilers fed both garlic powder and thyme separately or mixtures at different levels had more ($P<0.05$) intake compared to broilers of the control groups during the first 3 weeks of the trial. This trend was maintained until the termination of the trial at day 42 (Table 3).

**Table (3) The feed intake (gram) of broiler chicks fed different levels of garlic powder and dried thyme leaves.**

| Period | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Feed intake 0-21d | 693b | 752a | 798a | 842a | 823a | 876a | 867a | 865a | 857a | 0.05 |
| Feed intake 0-35d | 2787b | 2990a | 2953a | 2932a | 2971a | 2979a | 3023a | 2950a | 2951a | 0.05 |
| Feed intake 0-42d | 3690b | 3740a | 3736a | 3766a | 3853a | 3897a | 3823a | 3877a | 3853a | 0.05 |

### 4.3 Body weight

The study showed that birds fed with the mixture of garlic powder and thyme leaves had more ($P<0.05$) weight during the starter period compared to body weight in control birds and those fed with garlic powder or thyme leaves alone (Table 4). The body weight in the period from 21 to 35 days was similar to that in the first feeding period. This trend was similar in the period from 35 to 42 days. The average of body weight (gram/birds) for all diets at the different ages is shown in (Table 4).

**Table (4): Average weekly body weight (gram/bird) of broilers fed different levels of garlic powder and dried thyme leaves.**

| Period | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Initial wt/g | 47 | 47 | 47 | 47 | 48 | 48 | 50 | 48 | 48 | 0.05 |
| Wt 21d | 533b | 570b | 614b | 619b | 610b | 690a | 688a | 692a | 690a | 0.05 |
| Wt 28 d | 1156b | 1246b | 1255b | 1250b | 1244b | 1299a | 1301a | 1297a | 1310a | 0.05 |
| Wt 35d | 1689b | 1780b | 1768b | 1766b | 1790b | 1850a | 1866a | 1844a | 1856a | 0.05 |
| Wt 42 d | 2050b | 2079b | 2099b | 2128b | 2177b | 2320a | 2249a | 2267a | 2307a | 0.05 |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

**4.4 Feed conversion ratio:**

Feeding garlic powder and thyme leaves together had a significant influence on feed conversion (Table 5). The feed conversion ratio (FCR) for birds fed mixture of garlic powder and thyme leaves was improved ($P<0.05$) by 5% compared to that of birds fed the control or diets incorporated with powder garlic or thyme leaves individually.

**Table (5): The feed conversion ratios of broilers fed different levels of garlic powder and dried thyme leaves.**

| Period/d | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| FCR 0-21 | 1.30a | 1.32a | 1.30a | 1.36a | 1.35a | 1.27b | 1.26b | 1.25b | 1.24b | 0.05 |
| FCR 0-35 | 1.65a | 1.68a | 1.67a | 1.66a | 1.66a | 1.61b | 1.62b | 1.60b | 1.59b | 0.05 |
| FCR 0-42 | 1.8a | 1.78a | 1.78a | 1.77a | 1.77a | 1.68b | 1.70b | 1.71b | 1.67b | 0.05 |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

### 4.5 Carcass cuts

Both garlic powder and dried thyme leaves alone had no significant effect on broilers cuts, the cuts under investigation were neck, wing, back, thigh and breast (Table 6). However, the dressing percentages were higher ($P<0.05$) for broilers fed the mixture of garlic powder and the dried thyme leaves at both levels used. The effects of garlic powder and dried thyme leaves alone or in combination on average abdomen fat were not significant where the garlic groups, thyme groups and mixtures groups were lower in abdomen fat compared to fat in broilers fed the control diet (Table 6).

**Table (6): dressing percentages, Carcass cuts and abdominal fat of broilers fed different levels of garlic powder and dried thyme leaves.**

| | Control/g | .2G/g | .4G/g | .02TH/g | .04TH/g | .2G+.02TH/g | .4G+.04TH/g | .2G+.04TH/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| Live wt/g | 2050b | 2079b | 2099b | 2128b | 2177b | 2320a | 2249a | 2267a | 2307a | 0.05 |
| Carcass wt/g | 1466b | 1486b | 1488b | 1528b | 1552b | 1661a | 1597a | 1630a | 1679a | 0.05 |
| Dressing% | 70.5b | 70.5b | 70.9b | 71.0b | 71.3b | 72.3a | 72.7a | 72.9a | 72.8a | 0.05 |
| Abdominal fat% | 1.91a | 1.90a | 1.81a | 1.82a | 1.81a | 1.72b | 1.7b | 1.68b | 1.67b | 0.05 |
| Head% | 2.1 | 2 | 2 | 2 | 2 | 2 | 2 | 1.7 | 2 | 0.80 |
| Neck% | 5 | 4.7 | 5.3 | 3.7 | 4.7 | 5 | 5 | 4 | 3.7 | 0.20 |
| Wing% | 7.3 | 7.3 | 7.0 | 7.3 | 7.3 | 7.0 | 7.3 | 7.0 | 7.7 | 0.90 |
| Back % | 14.6 | 15.0 | 18.7 | 17.3 | 16.3 | 17.0 | 17.3 | 17.7 | 17.7 | 0.60 |
| Thigh % | 17.7 | 20.0 | 19.0 | 19.0 | 18.0 | 19.0 | 20.0 | 17.7 | 17.6 | 0.90 |
| Breast % | 20.0 | 21.6 | 21.3 | 20.1 | 19.3 | 19.3 | 21.0 | 21.7 | 19.3 | 0.90 |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

## 4.6 Visceral organs

### 4.6.1 Edible viscera (giblets)

The investigated tissues were the heart, gizzard and liver. Results of this study showed that addition of garlic powder and dried thyme leaves either separately or as a mixture had no significant effects on these tissues (Table 7).

**Table (7): Edible viscera weights in broilers fed different levels of garlic powder and dried thyme leaves.**

| | Control/g | .2G/g | .4G/g | .02TH/g | 0.04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.4$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Heart%** | **0.8** | **0.7** | **0.8** | **0.7** | **0.6** | **0.56** | **0.83** | **0.67** | **0.8** | **0.6** |
| **Liver%** | **2.7** | **3.0** | **2.3** | **2.7** | **2.7** | **2.7** | **3.0** | **2.3** | **2.3** | **0.7** |
| **Gizzard%** | **3.7** | **3.0** | **3.3** | **3.0** | **3.0** | **3.7** | **3.0** | **3.0** | **3.0** | **0.6** |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

### 4.6.2 The inedible viscera

The investigated organs are feet, small intestine and cecum. As observed inedible organs garlic powder and thyme leaves had no effect on the inedible organs. The mixture of thyme and garlic was significant higher ($P<0.001$) in small intestine and significant lower ($P<0.001$) in cecum length compared to other groups. However, these additives resulted in longer ($P<0.001$) small intestinal but shorter ($P<0.001$) cecum length (Table 8).

**Table (8): The inedible viscera weights of broilers fed different levels of garlic powder and the dried thyme leaves.**

| | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Feet %** | **4.0** | **4.3** | **3.7** | **3.7** | **3.3** | **3.3** | **4.3** | **4.0** | **4.3** | **0.8** |
| **Cecum %** | **1.0** | **0.9** | **1.3** | **1.3** | **0.9** | **0.9** | **0.9** | **0.9** | **0.9** | **0.9** |
| **SI wt%** | **4.3** | **4.7** | **5.3** | **5.0** | **5.0** | **5.3** | **4.7** | **5.3** | **5.0** | **0.6** |
| **SI length/cm** | **165b** | **160b** | **170b** | **157b** | **169b** | **195a** | **191a** | **193a** | **195a** | **0.001** |
| **Cecum length/cm** | **19a** | **20a** | **21a** | **21.3a** | **20.7a** | **17.7b** | **17.3b** | **17.9.0b** | **17.3b** | **0.001** |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

## 4.7 The blood lipids

Blood lipid parameters in broilers fed different diets are shown in (table 9)**.** The broilers blood levels of TG, CHO, (HDL) and LDL were investigated. Addition of garlic powder as well as dried thyme leaves separately or as a mixture at different levels caused a significant reduction ($P<0.05$) in the levels of blood TG, CHO and LDL (Table 9). At the same time, garlic powder and dried thyme leaves when fed alone or as a mixture increased the blood levels of HDL (Table 9).

**Table (9): Blood lipid parameter in broilers fed different levels of garlic powder and dried thyme leaves.**

| | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| TG* | 210.9a | 49.2b | 99.4b | 67.6b | 73.3b | 65.1b | 71.8b | 110.8b | 82.1b | 0.05 |
| CHO* | 149.1a | 98b | 90b | 102.6b | 102.3b | 112.6b | 99.6b | 97.1b | 97.9b | 0.05 |
| HDL* | 68.1b | 85.8a | 88.7a | 84.8a | 93.7a | 86.1a | 86.3a | 85.4a | 89.2a | 0.05 |
| LDL* | 187a | 110b | 112b | 109b | 115b | 115b | 119b | 118b | 114b | 0.05 |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

*** =units of TG, CHO, HDL, LDL (mg/dl)**

## 4.8 Effects on digestibility

Digestibility of broilers fed different levels of garlic powder and dried thyme leaves are shown in (table 10).

The digestibility of total tract DM, CP and CF was improved ($P<0.05$) by the addition of the garlic powder and the dried thyme leaves separately or as a mixture at different levels (Table 10) compared to that in the control diet.

**Table (10): Digestibility of dry matter, crude protein and ether extract of diets containing of garlic powder and dried thyme leaves.**

| | Control/g | .2G/g | .4G/g | .02TH/g | .04$^{TH}$/g | .2G+.02TH/g | .4G+.04$^{TH}$/g | .2G+.04$^{TH}$/g | .4G+.02TH/g | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| **DM%** | **71.0b** | **74.1a** | **74.4a** | **74.8a** | **74.7a** | **75.0a** | **74.7a** | **74.9a** | **74.7a** | **0.05** |
| **CP%** | **55.2b** | **60.0a** | **60.2a** | **60.4a** | **60.9a** | **60.8a** | **60.1a** | **60.4a** | **60.9a** | **0.05** |
| **EE%** | **86.0b** | **90.1a** | **90.4a** | **90.3a** | **90.2a** | **90.0a** | **89.9a** | **89.5a** | **89.8a** | **0.05** |

**Means within the same row with common superscripts do not differ significantly (P > 0.05)**

# Chapter Five

# Discussion

# Chapter Five
# Discussion

## 5.1 The broilers performance

Although it was expected that supplementing the dietary herbs (Cross *et al*., 2002, 2007; Bampidis *et al*., 2005) or plant extracts (Demir *et al.,* 2003; Lee *et al*., 2003) would stimulate the growth performance of broilers. Research on herbs, plant extracts, essential oil and/or the main components of the essential oil yielded contradicting results (Alcicek *et al*., 2003, Acamovic and Broker, 2005; Bampidis *et al*., 2005; Griggs and Jacob, 2005). The results of the present study contrasted some of the previous observations that indicated herbs, plant extracts, essential oil and/or the main components of the essential oil that did not affect body weight gain, feed intake or feed efficiency in broilers (Cross *et al*., 2002, 2007; Demir *et al*., 2003; Botsoglou *et al*., 2004; Hernandez *et al*., 2004; Bampidis *et al.,* 2005).

The improvement of performance observed in broilers fed the mixture of garlic powder and dried thyme leaves might be due to the improvement of nutrient digestibility observed in this study associated with the development of digestive tract and digestive organs (Lilja, 1983). The reduction in crypt depth (Lilja, 1983) in the ileum of broilers given dietary natural growth promoters such as garlic and thyme and the energy conserved by the reduced turnover rate of the epithelial cells might be utilized for lean tissue mass synthesis and may help to explain some

improvements seen in body weight gain and feed conversion ratio when mixtures of garlic powder and dried thyme leaves were fed to broilers.

Feed intake was not affected by herbal supplementation as single supplements. This finding is consistent with that of Chowdhuury *et al.,* (2002). However, our results were not consistent to with those reported by Raeesi *et al.* (2010) who reported no significant effect of garlic on growth, feed intake and feed efficiency. The absence of garlic or thyme effect on feed intake when fed separately to broilers was probably due to the intense smell of garlic or thyme, which required a period of adaptation of chickens to this kind of feed (Horton *et al*. 1991). The mixture of garlic powder and thyme leaves could improve the palatability of diets and had the positive effect on broilers feed intake and birds general performance observed in this experiment.

The results of this study showed that both garlic powder and dried thyme leaves had no significantly effect on feed conversion ratio when fed at different levels alone to broilers. However, the mixture of the two herbs at different levels had significantly improved the feed conversion ratio. These results are in disagreement with previous reports where the supplementation of 1% garlic powder, decreased feed conversion rate (FCR) compared with 0.5% supplemented and control group (Raeesi *et al*. 2010), and other reports showed that birds received 3% garlic powder in their diets had better FCR than control group(Raeesi *et al.* 2010). However, this result agrees with previous results Demir *et al*. (2008) who

concluded that garlic or thyme supplementation did not affect growth, intake and feed conversion rate in whole of the experiment (Raeesi *et al*. 2010). Konjufca *et al*. (1997) reported that although performance was not affected when broiler diets were supplemented with 1.5, 3 and 4.5% garlic in powder form, their serum and liver cholesterol decreased significantly (Raeesi *et al*. 2010). In the present study, the broiler chickens were kept in good hygienic conditions, which would probably result in a decreased efficacy of antibiotics or any dietary herbal additive. It has been proposed that immuno stimulation may have adverse effects on growth performance, because more nutrients will be repartitioned to synthesize antibodies and develop the immune organs, thereby decreasing the nutrients available for growth (Hevener *et al*., 1999; Takahashi *et al*., 2000)

### 5.2 Visceral organs and carcass cuts

Research studies have been focusing on improvement of chicken carcasses in order to meet the food industry standards. The best results are achieved through genetic selection, nutrition and breeding technology, which are reflected in a significant increase of overall carcass masses and the share of meat as well as a reduction of the abdominal fat content in 6-week-old chicks.

The lack of garlic powder and the dried thyme leaves effect on broilers visceral organs except in small intestine and cecum length observed in this experiment is similar to that reported by previous research. Raeesi *et al.* (2010) found no significant difference in relative weight of

carcass, fat pad, or digestive organs among thyme at different treatments except for the small intestine. Similar results were observed by Hernandez *et al*. (2004) who found no difference in weight of organs of broiler chickens fed diets containing an extract from thyme.

Results of this study showed that all carcass cuts (thigh, breast, back and neck) were the same in broilers fed control diet supplemented with garlic powder and dried thyme leaves when fed separately. This result is in disagreement with Raeesi *et al*. (2010*)* research where supplementation of 1% garlic powder caused higher thigh yield while the poorest thigh yield belonged to 3% supplemented group. Groups received 1% garlic powder significantly had higher breast yield than others (Raeesi *et al*., (2010).

When dietary treatments of garlic powder or thyme leaves or both at different levels, we find that relative weight of gizzard, heart and liver were not affected by these additives. Similar trend was observed in the inedible visceral organs (small intestine and cecum) relative weights. These findings are in agreement with the findings of Hashish *et al.* (1995) and Ceylan *et al.* (1998).

However, the mixture of garlic and thyme at different levels caused a significant increase in small intestine and decrease in cecum length.

Hernandez *et al.* (2004) found no differences in gizzard, liver and pancreas weights of broiler chickens fed wheat-soyabean meal based diets supplemented with an antibiotic and two plant extracts (an essential oil

extract from oregano, cinnamon and pepper and a labiatae extract from sage, thyme and rosemary). The relative weights of the small intestine of broilers fed diets supplemented with an antibiotic, an antibiotic plus an enzyme, thyme plus the enzyme and garlic plus the enzyme were less than those of broilers fed the basal diet and the diets supplemented with thyme, garlic and the enzyme. The basal diet and garlic-supplemented diet increased the length of small intestine compared to that of the other treatments.

However, the smaller length of the whole gut and lower weight of pancreas in the groups given peppermint or thyme compared to the control, although the reduction did not reach statistical significance, may support the idea that the active principles of herbs act as a digestibility enhancer, stimulating the secretion of endogenous digestive enzymes (Williams and Losa, 2001; Bampidis *et al.,* 2005). The second reason for the lack of effects of supplements may be related to the environmental conditions. The fact that none of the supplements caused a growth promoter effect at slaughter age indicates that the present trial was conducted in ideal conditions, which could affect the degree of growth promotion (Hernandez *et al.*, 2004).

### 5.3 Blood lipids

All of the blood lipid metabolites (CHO, TG, LDL and HDL) tested in broilers blood was significantly improved by all treatments. Addition of garlic powder as well as dried thyme leaves separately or as a mixture at

different levels caused a significant reduction ($P<0.05$) in the levels of blood (TG), (CHO) and (LDL). At the same time, garlic powder and dried thyme leaves when fed alone or as a mixture increased the blood levels of the (HDL) .These results agree with previous reports where dietary supplementation of garlic powder at both concentrations (i.e., 1.5 and 3.0%) in broiler chickens was found to cause a significant decrease in the mean values of total cholesterol as compared to control birds. However, higher levels of garlic (4.5%) showed no significant difference in mean values of plasma total cholesterol. This may probably be due to the possible mechanism of hypocholesterolaemic and hypolipidemic action of garlic products which depresses the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6-phosphatase dehydrogenase (Chi *et al*., 1982; Qureshi *et al*., 1983a) and 3-hydroxyl-3-methyl-glutaryl-CoA (HMG-CoA) reductase (Qureshi *et al*., 1983b, 1987). Afzal *et al*. (1985) reported that polyunsaturated fatty acids prevent atherosclerosis through the formation of cholesterol esters. They further reported the presence of higher polyunsaturated fatty acids like arachidonate and eicosapentenoate in garlic which could well be responsible for preventing atherosclerosis.

Furthermore, garlic powder can facilitate activity of enzymes which are involved in the conversion of cholesterol to bilious acids and subsequently, there will be less cholesterol in the carcass (Bordia *et al*., 1975; Raeesi *et al.*, 2010)

Garlic has hypocholesterolemic effects on chickens through inhibition of the most important enzymes that participate in the synthesis of cholesterol and lipids (trihydroxy- tri-methyl-glutaril coenzyme A reductase, cholesterol-7-.-hydroxylase and the synthesis of fatty acids (Stanacev *et al.,* 2011). In addition, this additive has a relatively low price.

**5.4 Effects on digestibility**

The digestibility of total tract dry matter, crude protein and crude fat digestibility was improved ($P<0.05$) by the addition of the garlic powder and the dried thyme leaves at different levels (Table 12) compared to that in the control diet. These finding are in agreement with previous research of Hernandez *et al*. (2004) who showed that plant extract supplementation improved apparent whole-tract and ileal digestibility of the nutrients. For starter feed, LE supplementation improved apparent fecal digestibility of DM, and all additives increased ether extract digestibility. However, no effect was detected for CP digestibility ($P > 0.1$). All additives improved apparent fecal digestibility of DM and CP of the finisher diet. In the present study, both plant extracts improved the digestibility of the feeds for broilers.

The improvement of total tract digestibility in broilers fed different levels of garlic powder and dried thyme leaves was probably due to herbal effects in increasing the microbial population especially the number of bacteria such as *E. coli*, *Clostridium* spp. and *Enterococci.* The active principles of essential oils act as a digestibility enhancer, balancing the gut

microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Lovkova *et al.,* 2001; Williams and Losa, 2001; Cross *et al*., 2007).

In a recent study, Cross *et al*. (2007) reported that the dietary inclusion (10 g per kg diet) of five culinary herbs including thyme had no effect on the intestinal microflora, metabolizable energy or the coefficients of digestibility.

The efficacy of any dietary feed additives observed under less hygienic housing conditions, especially under the separate floor pens equipped with wood shaving litter stimulates the activity of the feed additives. The isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract (Rahimi *et al*., 2011). The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism (Horton *et al*., 1991; Baratta *et al*., 1998; Jamroz *et al*., 2003; Rahimi *et al*., 2011).

# Chapter Six

# Conclusions and Recommendations

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## 6.1 Conclusions

From the present study the following conclusions can be addressed:

1. The garlic powder and dried thyme leaves had limited effect on general performance when fed separately to broilers. The only positive effect of these was on blood lipids and nutrient digestibility.

2. The broilers performance was improved when fed the mixture of the two additives at different levels in diets.

3. All carcass cuts and visceral organs were not affected by garlic and thyme when fed to broilers singly or as a mixture.

4. Final body weight, feed intake, feed conversion ratio were improved by feeding a garlic and thyme mixture at different levels.

## 6.2 Recommendations

1. It is recommended to use garlic powder and dried thyme leaves as feed supplements to broilers in their starter and finisher diets as a mixture according to the levels (0.2garlic+0.02 thyme)(0.4 garlic+0.04 thyme)(0.2 garlic+ 0.04 thyme)(0.4 garlic+ 0.02 thyme) used in this study. However, further research is required to assess present findings.

2. Sex, house conditions and management practices should be investigated in order to shed light on exact effect of herbal plants on the tested parameters.

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# Appendices

# Appendices1

## Procedure of blood lipid analysis

### HDL CHOLESTEROL

Precipitant and Standard, for Use with HUMAN CHOLESTEROL **liquicolor** Test Kit

**Package Size**

| | | | |
|---|---|---|---|
| REF | 10018 | 4 x 80 ml | Precipitant |
| | | 1 x 3 ml | Standard |

IVD

**Method**

The chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins) are precipitated by addition of phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL (high density lipoproteins) fraction, which is assayed for HDL Cholesterol with the HUMAN CHOLESTEROL **liquicolor** test kit.

**Contents, Reagent Composition**

| | | |
|---|---|---|
| PREC | **4 x 80 ml Precipitant** | |
| | Phosphotungstic acid | 0.55 mmol/l |
| | Magnesium chloride | 25.00 mmol/l |
| STD | **1 x 3 ml Standard** | |
| | Cholesterol | 50 mg/dl or 1.29 mmol/l |

**Reagent Preparation**

**Precipitant for Macro Assays** PRECa
Use undiluted PREC.

**Precipitant for Semi-micro Assays** PRECb
Dilute the contents of one bottle PREC with 20 ml distilled water, or dilute 4 parts of the bottle with 1 part distilled water (4+1).

STD
STD is ready for use and can directly be employed in the test. **No precipitation is required!** The factor in the calculation formula comprises the dilution ratio.

**Reagent Stability**

PREC is stable, even after opening, up to the stated expiry date when stored at 2...25°C. Contamination must be avoided.

**Specimen**

Serum, heparinised or EDTA-plasma.

**Assay**

See CHOLESTEROL **liquicolor**.

**1. Precipitation**

| Pipette into centrifuge tubes | Macro | Semi-micro |
|---|---|---|
| Sample | 500 µl | 200 µl |
| PRECa | 1000 µl | --- |
| PRECb | --- | 500 µl |

Mix well, incubate for 10 minutes at room temperature. Centrifuge for at least 2 minutes at 10000 g, alternatively for 10 minutes at 4000 g.

After centrifugation separate the clear supernatant from the precipitate within 1 hour and determine the cholesterol concentration using HUMAN CHOLESTEROL **liquicolor** reagent.

**2. Cholesterol Determination**

| Pipette into cuvettes | Reagent blank | STD | Sample |
|---|---|---|---|
| Dist. water | 100 µl | --- | --- |
| STD | --- | 100 µl | --- |
| HDL supernatant | --- | --- | 100 µl |
| Reagent | 1000 µl | 1000 µl | 1000 µl |

Mix, incubate for 5 minutes at 37°C or 10 minutes at 20...25°C. Measure the absorbance of the sample and the STD, respectively, against the reagent blank within 60 minutes (ΔA).

**Calculation of the HDL Cholesterol Concentration with Factor**

| Wavelength | | Macro | | Semi-micro | |
|---|---|---|---|---|---|
| | | C [mg/dl] = ΔA x | C [mmol/l] = ΔA x | C [mg/dl] = ΔA x | C [mmol/l] = ΔA x |
| Hg | 546 nm | 274 | 7.09 | 320 | 8.2 |
| | 500 nm | 180 | 4.65 | 210 | 5.43 |

**Calculation of the HDL Cholesterol Concentration with STD**

**1. Macro Method**

$$C = 150 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \text{ mg/dl}; \quad C = 3.87 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \text{ mmol/l}$$

**2. Semi-micro Method**

$$C = 175 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \text{ mg/dl}; \quad C = 4.52 \times \frac{\Delta A_{Sample}}{\Delta A_{STD}} \text{ mmol/l}$$

**Calculation of the LDL Cholesterol Concentration**[2,3]

The LDL Cholesterol concentration (LDL-C) is calculated from the total Cholesterol concentration (TC), the HDL Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald *et al.*[3]

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5} \text{ [mg/dl]}$$

or

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{2.2} \text{ [mmol/l]}$$

**Clinical Interpretation**[2]

**1. HDL Cholesterol**

| | Men | | Women | |
|---|---|---|---|---|
| | [mg/dl] | [mmol/l] | [mg/dl] | [mmol/l] |
| Prognostically favourable | > 55 | > 1.42 | > 65 | > 1.68 |
| Standard risk level | 35 - 55 | 0.9 - 1.42 | 45 - 65 | 1.16 - 1.68 |
| Risk indicator | < 35 | < 0.9 | < 45 | < 1.16 |

**2. LDL Cholesterol**

Suspicious: 150 mg/dl or 3.9 mmol/l
Elevated: 190 mg/dl or 4.9 mmol/l

**Performance Characteristics**

Typical performance date can be found in the Verification Report, accessible via
www.human.de/data/gb/vr/su-hdl.pdf or
www.human-de.com/data/gb/vr/su-hdl.pdf

**Quality Control**

All control sera with values for HDL-Cholesterol determined by this method can be employed.
We recommend the use of our animal based **HUMATROL** quality control serum or our human serum based **SERODOS**.

**Notes**

1. If the supernatant is not clear (high triglycerides level), dilute the sample before the precipitation 1:1 with 0.9% saline (multiply result by 2).
2. High concentrations of ascorbic acid (> 2.5 mg/dl) will give lower values.
3. Hemoglobin levels higher than 100 mg/dl and bilirubin levels higher than 10 mg/dl interfere with the test.

**References**


1. ISO 15223 Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied.
2. Gordon, T. *et al.*, Amer. J. Med. 62, 707 (1977)
3. Friedewald, W.T. *et al.*, Clin. Chem. 18, 499 (1972)



SU-HDL
INF 1001801 GB
09-2003-12


CE

Human

# TRIGLYCERIDES liquicolor mono

## GPO-PAP Method
## Enzymatic Colorimetric Test for Triglycerides with Lipid Clearing Factor (LCF)

**Package Size**

| | | | |
|---|---|---|---|
| REF | 10720P | 9 x 15 ml | Complete Test Kit |
| | 10724 | 4 x 100 ml | Complete Test Kit |
| | 10725 | 3 x 250 ml | Complete Test Kit |
| | 10163 | 9 x 3 ml | Standard |

IVD

**Method**

The triglycerides are determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

**Reaction Principle**

$$\text{Triglycerides} \xrightarrow{\text{lipases}} \text{glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate + } H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinoneimine + HCl + } H_2O + \text{4-chlorophenol}$$

**Contents**

RGT **15 ml; 100 ml or 250 ml Monoreagent**

| | |
|---|---|
| PIPES buffer | 50 mmol/l |
| 4-chlorophenol | 5 mmol/l |
| 4-aminophenazone | 0.25 mmol/l |
| Magnesium ions | 4.5 mmol/l |
| ATP | 2 mmol/l |
| Lipases | ≥ 1300 U/l |
| Peroxidase | ≥ 500 U/l |
| Glycerol kinase | ≥ 400 U/l |
| Glycerol-3-phosphate oxidase | ≥ 1500 U/l |

STD **3 ml Standard**

| | |
|---|---|
| Triglycerides | 200 mg/dl or 2.28 mmol/l |

**Reagent Preparation and Stability**

RGT and STD are ready for use.

The reagents are stable, even after opening, up to the stated expiry date when stored at 2...8°C. At 20...25°C the RGT is stable for 4 weeks. **Contamination must be avoided.**

Protect from light.

**Specimen**

Serum, heparinised plasma or EDTA plasma

Stability: 3 days at 2...8°C
4 months at -20°C

**Note:** Lipemic specimens usually generate turbidity of the sample reagent mixture which leads to falsely elevated results. The TRIGLYCERIDES liquicolor mono test avoids these falsely elevated results through its built-in Lipid-Clearing Factor (LCF). The LCF clears up totally a turbidity caused by lipemic specimens.

**Assay**

| | |
|---|---|
| Wavelength: | 500 nm, Hg 546 nm |
| Optical path: | 1 cm |
| Temperature: | 20...25°C or 37°C |
| Measurement: | against reagent blank (RB). Only one reagent blank per series is required. |

**Pipetting Scheme**

Please use only the HUMAN Triglycerides Standard provided with the test kits or separately available: REF 10163.

| Pipette into cuvettes | RB | Sample or STD |
|---|---|---|
| Sample / STD | --- | 10 µl |
| RGT | 1000 µl | 1000 µl |

Mix and incubate for 10 min. at 20...25°C or for 5 min. at 37°C. Measure the absorbance of the sample ($\Delta A_{sample}$) and the standard ($\Delta A_{STD}$) against the reagent blank within 60 min.

**Calculation of the Triglycerides Concentration**

$$C = 200 \times \frac{\Delta A_{sample}}{\Delta A_{STD}} \text{ [mg/dl] or } C = 2.28 \times \frac{\Delta A_{sample}}{\Delta A_{STD}} \text{ [mmol/l]}$$

**Performance Characteristics**

Linearity

The test is linear up to a triglycerides concentration of 1000 mg/dl or 11.4 mmol/l. Samples with a higher concentration have to be diluted 1 + 4 with physiological saline (0.9%) and retested. Multiply the result by 5.

Typical performance date can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/su-trimr.pdf or

www.human-de.com/data/gb/vr/su-trimr.pdf

**Clinical Interpretation for Atherosclerotic Risk**

Suspect: over 150 mg/dl or 1.71 mmol/l

Increased: over 200 mg/dl or 2.28 mmol/l

**Quality Control**

All control sera with triglycerides values determined by this method can be employed.

We recommend to use our HUMATROL control sera based on animal serum or our SERODOS based on human serum.

**Automation**

Proposals to apply the reagents on analyzers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**

1. To correct for free glycerol, subtract 10 mg/dl (0.11 mmol/l) from the triglycerides value calculated.
2. The test is not influenced by hemoglobin values up to 150 mg/dl or by bilirubin values up to 40 mg/dl. Ascorbate may give falsely low values at > 4 mg/dl.
3. The reagents contain sodium azide (0.05%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.

**References**


1. Schettler, G., Nüssel, E., Arb. Med. Soz. Med. Präv. Med. **10**, 25 (1975)
2. Jacobs, N. J., VanDemark, P. J., Arch. Biochem. Biophys. **88**, 250-255 (1960)
3. Koditschek, L. K., Umbreit, W. W., J. Bacteriol. **68**, 1063-1068 (1969)
4. Trinder, P., Ann. Clin. Biochem. **6**, 24-27 (1969)


SU-TRIMR INF 1072401 GB 02-2009-11

CE

Human

# CHOLESTEROL liquicolor

## CHOD-PAP-Method
## Enzymatic Colorimetric Test for Cholesterol with Lipid Clearing Factor (LCF)

**Package Sizes**

| | | | |
|---|---|---|---|
| REF | 10017 | 4 x 30 ml | Complete test kit |
| | 10019 | 3 x 250 ml | Complete test kit |
| | 10028 | 4 x 100 ml | Complete test kit |
| | 10015 | 9 x 3 ml | Standard |

IVD

**Method**

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.

**Reaction Principle**

$$\text{Cholesterolester} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{cholestene-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-amino-phenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4\,H_2O$$

**Contents**

RGT **4 x 30 ml, 3 x 250 ml or 4 x 100 ml Enzyme reagent**

| | |
|---|---|
| Phosphate buffer (pH 6.5) | 100 mmol/l |
| 4-Aminophenazone | 0.3 mmol/l |
| Phenol | 5 mmol/l |
| Peroxidase | > 5 KU/l |
| Cholesterolesterase | > 150 U/l |
| Cholesteroloxidase | > 100 U/l |
| Sodium azide | 0.05 % |

STD **3 ml Standard**

| | |
|---|---|
| Cholesterol | 200 mg/dl or 5.17 mmol/l |

**Reagent Preparation**

The RGT and the STD are ready for use.

**Reagent Stability**

The reagents are stable up to the given expiry date, even after opening, when stored at 2...8°C. The opened reagent is stable for 2 weeks at 15...25°C. Contamination must be avoided.

**Specimen**

Serum, heparinised or EDTA-plasma.

**Note:** Lipemic specimens usually generate turbidity of the sample/ reagent mixture which leads to falsely elevated results. The CHOLESTEROL liquicolor test avoids these falsely elevated results through its built-in Lipid Clearing Factor (LCF). The LCF clears up totally a turbidity caused by lipemic specimens.

**Assay**

| | |
|---|---|
| Wavelength: | 500 nm, Hg 546 nm |
| Optical path: | 1 cm |
| Temperature: | 20...25°C or 37°C |
| Measurement: | Against reagent blank. Only one reagent blank per series is required. |

**Pipetting Scheme**

| Pipette into cuvettes | Reagent blank | Sample or STD |
|---|---|---|
| Sample/ STD | --- | 10 µl |
| RGT | 1000 µl | 1000 µl |
| Mix, incubate 10 min. at 20...25°C or 5 min. at 37°C. Measure the absorbance of the sample/STD against the reagent blank (ΔA) within 60 min. | | |

**Calculation of the Cholesterol Concentration**

**1. With Factor**

| Wavelength | C [mg/dl] | C [mmol/l] |
|---|---|---|
| Hg 546 nm | 840 x ΔA | 21.7 x ΔA |
| 500 nm | 553 x ΔA | 14.3 x ΔA |

**2. With Standard**

Only the standard recommended by HUMAN (enclosed in kit or separately available, REF 10015) should be used.

$$C = 200 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}}\ [\text{mg/dl}]$$

or

$$C = 5.17 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}}\ [\text{mmol/l}]$$

**Performance Characteristics**

Linearity

The test is linear up to a cholesterol concentration of 750 mg/dl (19.3 mmol/l). Dilute samples with a higher cholesterol concentration 1 + 2 with physiological saline (0.9%) and repeat the determination. Multiply the result by 3.

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/su-chol.pdf

www.human-de.com/data/gb/vr/su-chol.pdf

**Clinical Interpretation**

| | | |
|---|---|---|
| Suspect over | 220 mg/dl or | 5.7 mmol/l |
| Elevated over | 260 mg/dl or | 6.7 mmol/l |

The European Atherosclerosis Society recommends to decrease the cholesterol level to approximately 180 mg/dl for adults up to 30 years and to approximately 200 mg/dl for adults over 30 years.

**Quality Control**

All control sera with values determined by this method may be employed. We recommend to use our quality control sera HUMATROL based on animal serum or our SERODOS based on human serum.

**Automation**

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

**Notes**

1. The test is not influenced by hemoglobin values up to 200 mg/dl or by bilirubin values up to 5 mg/dl.
2. The reagents contain sodium azide as preservative (0.05%). Do not swallow. Avoid contact with skin and mucous membranes.

**References**


1. Schettler, G. and Nüssel, E., Arb. Med. Soz. Med. Präv. Med. **10**, 25 (1975)
2. Richmond, W., Clin. Chem. **19**, 1350 (1973)
3. Röschlau, P. *et al.*, J. Clin. Chem. Clin. Biochem. **12**, 403 (1974)
4. Trinder, P., Ann. Clin. Biochem. **6**, 24 (1969)

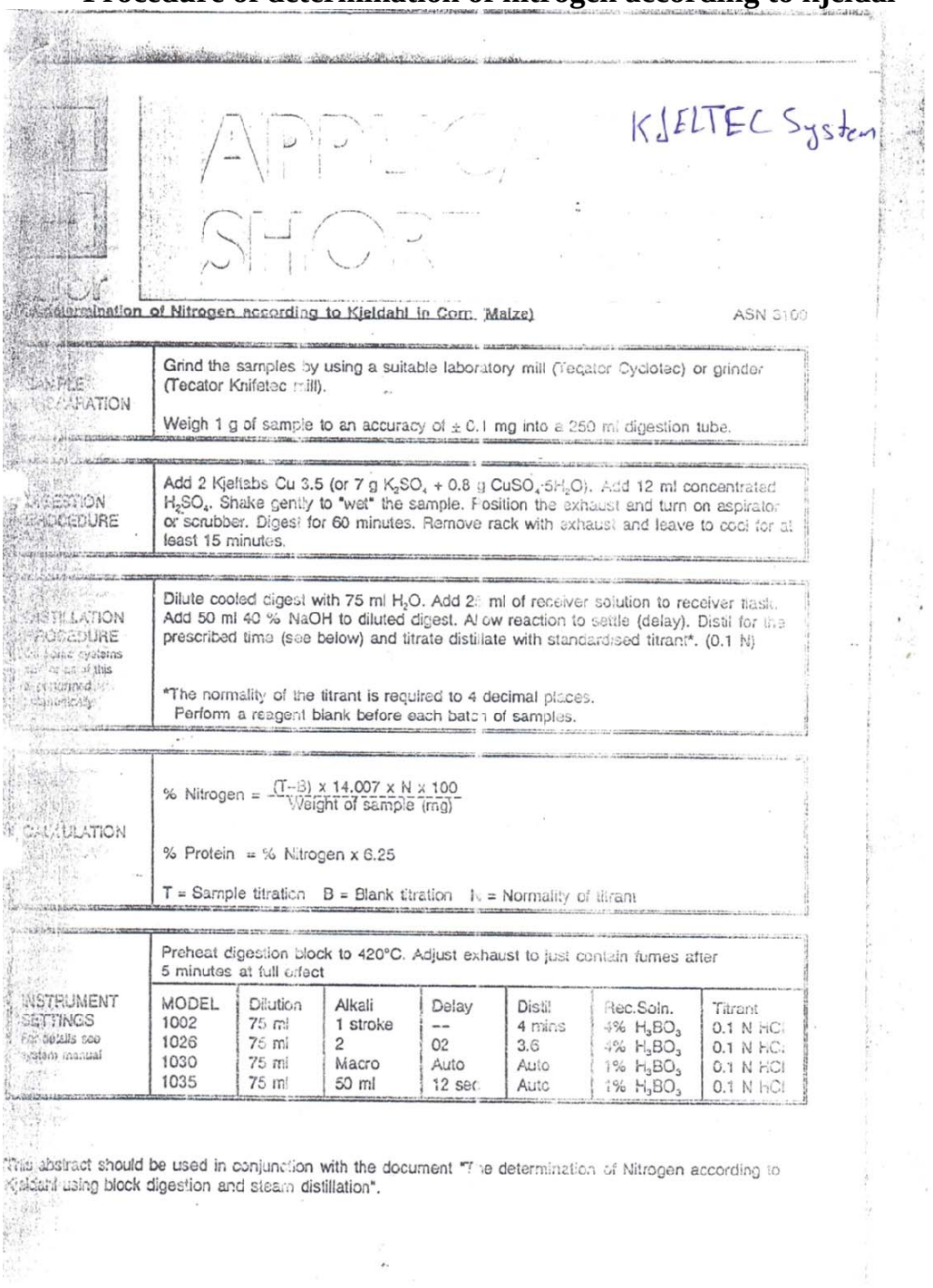

SU-CHOL INF 1001701 GB 07-2007-19

CE

Human

# Appendices2

## Procedure of determination of nitrogen according to kjeldal



APPLICATION SHORT

KJELTEC System

Determination of Nitrogen according to Kjeldahl in Corn, Maize)

ASN 3100

| | |
|---|---|
| SAMPLE PREPARATION | Grind the samples by using a suitable laboratory mill (Tecator Cyclotec) or grinder (Tecator Knifetec mill).<br><br>Weigh 1 g of sample to an accuracy of ± 0.1 mg into a 250 ml digestion tube. |
| DIGESTION PROCEDURE | Add 2 Kjeltabs Cu 3.5 (or 7 g $K_2SO_4$ + 0.8 g $CuSO_4 \cdot 5H_2O$). Add 12 ml concentrated $H_2SO_4$. Shake gently to "wet" the sample. Position the exhaust and turn on aspirator or scrubber. Digest for 60 minutes. Remove rack with exhaust and leave to cool for at least 15 minutes. |
| DISTILLATION PROCEDURE (For some systems refer to end of this application sheet) | Dilute cooled digest with 75 ml $H_2O$. Add 25 ml of receiver solution to receiver flask. Add 50 ml 40 % NaOH to diluted digest. Allow reaction to settle (delay). Distil for the prescribed time (see below) and titrate distillate with standardised titrant*. (0.1 N)<br><br>*The normality of the titrant is required to 4 decimal places. Perform a reagent blank before each batch of samples. |
| CALCULATION | $\% \text{ Nitrogen} = \frac{(T-B) \times 14.007 \times N \times 100}{\text{Weight of sample (mg)}}$<br><br>% Protein = % Nitrogen x 6.25<br><br>T = Sample titration B = Blank titration N = Normality of titrant |

Preheat digestion block to 420°C. Adjust exhaust to just contain fumes after 5 minutes at full effect

INSTRUMENT SETTINGS (For details see system manual)

| MODEL | Dilution | Alkali | Delay | Distil | Rec.Soln. | Titrant |
|---|---|---|---|---|---|---|
| 1002 | 75 ml | 1 stroke | -- | 4 mins | 4% $H_3BO_3$ | 0.1 N HCl |
| 1026 | 75 ml | 2 | 02 | 3.6 | 4% $H_3BO_3$ | 0.1 N HCl |
| 1030 | 75 ml | Macro | Auto | Auto | 1% $H_3BO_3$ | 0.1 N HCl |
| 1035 | 75 ml | 50 ml | 12 sec | Auto | 1% $H_3BO_3$ | 0.1 N HCl |

*This abstract should be used in conjunction with the document "The determination of Nitrogen according to Kjeldahl using block digestion and steam distillation".

جامعة النجاح الوطنية
كلية الدراسات العليا

# تأثير نوعين من النباتات الطبية على أداء ومستويات الدهن في دم دجاج اللحم

إعداد
كمال جمال جميل عيسى

إشراف
أ. د. جمال أبو عمر

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الإنتاج الحيواني في كلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2011م

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**أ. د. جمال أبو عمر**

## الملخص

أجريت هذه التجربة في كلية الزراعة / معهد خصوري في مدينة طولكرم لفحص تأثير تغذية دجاج اللحم على علف يحتوي على بودرة ألثومه وورق الزعتر الجاف المطحون على الأداء والهضم واللحم وغير اللحم وعلى أنواع الدهون لدجاج اللحم . استخدم 216 صوص عمر يوم واحد من سلالة كوب 500 تم استخدامها في التجربة التي مدتها 42 يوما حيث كان بداية التجربة من 2011/2/9 إلى 2011/3/22. تم تقسيم الصيصان إلى تسع مجموعات كل مجموعه تحتوي على 24 صوص كل مجموعه كانت مقسمه إلى أربع مكررات في كل مكرر 6 صيصان، مجموعة الشاهد تم تغذيتها على العليقة التجارية سواء في العليقة الابتدائية أو العليقه النهائية، ألمجموعه الثانية والثالثة تم إضافة بودرة ألثومه إلى العلف بمعدل 0.2% و0.4% بالترتيب ، والصيصان في ألمجموعه الرابعة والخامسة تم إضافة ورق الزعتر المطحون بمعدل 0.02% و 0.04% بالترتيب، أما بقية المجموعات الأخرى (السادسة، السابعة، الثامنة، والتاسعة تم إضافة خليط من المستويات (0.2%+0.02%) للمجموعة السادسة، و(0.4%+0.04%) للمجموعة السابعة، و( 0.2%+0.04%) للمجموعة الثامنة، و(0.02%+0.4) للمجموعة التاسعة. في الأسبوع الأخير من التجربة تم استخدام ثلاثة صيصان من كل مجموعه ليتم استخدامها في تجربة الهضم ، في نهاية التجربة تم ذبح نفس الصيصان لمعرفة صفات اللحم وغير اللحم.

تم سحب عينات الدم من كل المجموعات بواقع ثلاث عينات من كل مجموعه من المجموعات ابتداء من الأسبوع الثالث ثم على نهاية الأسبوع الرابع وفي نهاية الأسبوع الخامس

من الوريد تحت الجناح ليتم إجراء الدراسة عليها ومعرفة إشكال الدهون فيها، تم فحص مجموع الكلسترول و ترايجليسرايد والدهون ذات الكثافة العالية والدهون ذات الكثافة المنخفضة. تبين من نتائج هذه الفحوصات أن إضافة بودرة ألثومه وورق الزعتر المجفف المطحون بشكل منفصل في العلف فانه لا يؤثر على الزيادة الوزنية أو معدل استهلاك العلف أو على الكفاءة التحويلية وقطع اللحم والأعضاء الداخلية. .

إن كلا النباتين المستخدمين في التجربة أديا إلى خفض من مستوى مجموع الكولسترول في الدم والترايجليسرايد والدهون ذات الكثافة المنخفضة وزيادة في الدهون ذات الكثافة العالية بالمقارنة مع مجموعة الشاهد . وقد تحسنت عملية الهضم بالنسبة للوضع الجاف للعليقة والبروتين الخام والدهن المستخلص عندما تم إعطاء العلف المحتوي على بودرة الثومة وورق الزعتر المطحون كل على حده.

إن العليقة التي تحتوي على كل من ورق الزعتر المطحون وبودرة ألثومه كمزيج على عدة مستويات كان في هذه المجموعات زيادة واضحة في الوزن النهائي للقطيع ومعدل استهلاك العلف وعلى معدل التحويل الغذائي ونسبة التصافي ووزن الذبيحة والدهون المستخلص وهضم المادة الجافة والبروتين الخام مقارنة مع باقي المجموعات ومجموعة الشاهد. هذا الخليط عند مستويات مختلفة سبب نقص واضح في مستويات الكولسترول والترايجليسرايد والدهون ذات الكثافة المنخفضة وزيادة في الدهون ذات الكثافة العالية.

إن هذا الخليط المستخدم من بودرة ألثومه وورق الزعتر ليس له اثر واضح على صفات اللحم وقطعه وأيضا ليس له اثر على وزن الأعضاء الداخلية. فانه يمكننا القول أن بودرة ألثومه وورق الزعتر يستطيع أن يعطي مزايا ايجابية على أداء الصيصان من حيث تأثيره على مستويات الدهن في دم دجاج اللحم وهضم البروتين والدهن والمادة الجافة