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COMPARISON OF HUSBANDRY AND EGG QUALITY OF CONVENTIONAL AND FREE RANGE COMMMERCIAL LAYERS

by

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AN ABSTRACT OF THE THESIS OF

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The thesis is divided into two parts namely, studies A and B. The objective of study A was to present the degree of compliance of Lebanese intensive system (IS) and free range chicken layers (FRCL) farms to the EU standards, targeting the welfare of chicken and the improvement in its egg quality. A questionnaire was implemented to uncover the adopted husbandry system on IS and FRCL. The improvement in egg quality included also an experiment that was conducted to asses quality parameters, and heavy metal content of eggs produced by IS versus FRCL. Five IS and five FRCL farms were selected and 5 eggs were randomly collected from each farm. The percentage of compliance of farms in both systems with EU regulations ranged between 0 and 66.7 % namely, for the parameters of housing management, feeding, watering, vaccines, medication, packaging and egg quality. The egg quality parameters were not significantly different between the two systems, in relation to egg weight, porosity, shell thickness, density, yolk %, albumen%, shell% and percentage of eggs with AA quality. However, the IS eggs had significantly higher Haugh unit scores and yolk color index compared to the FRCL eggs. Copper was the only metal that was significantly higher in yolk of the FRCL as compared to that of the IS eggs.

The objective of study B was to evaluate the growth promotion in broilers by two natural essential oil preparations (Mentofin and modified Mentofin) versus synthetic Maxiban in the period from d1 till d21(growth promotion period), under controlled temperature versus open system. Another objective was to study the protection and performance of broilers, 7 days following an eight *Eimeria spp*. cocktail challenge at 21 days of age. Eighty birds were equally distributed into 8 groups of 10 each. Birds of group 1-5 were reared under a controlled environment and were challenged with the *Eimeria* cocktail. Birds of groups 6-8 were reared in an open system and left without *Eimeria* challenge. Feed conversion during growth promotion in the period between d1-d21 was the best when treating non challenged broilers by Modified Mentofin under controlled environment. The Maxiban treatment had the lowest feed conversion during the first life cycle of *Eimeria spp*. The oocyst output reduction were comparable between Maxiban (63.4%), Mentofin (54.9%) and Modified Mentofin (46.3%), confirming the coccidiostat effect of both essential oil preparations.

Key words: layers; intensive system; free range; compliance; EU Standards; broilers; coccidiostats; synthetic; essential oil; growth promotion; coccidiosis

CONTENTS

ABBREVIATIONS

Trt	Treatment
CFU	Colony Forming Unit
NOP	National Organic Program
IS	Intensive System
FRCL	Free Range Chicken Layers
NRC	National Research Council
WHO	World Health Organization
EU	European Union
EEC	European Economic Community
KNC	Korean Native Chicken
HU	Haugh Unit
DSR	Driving Force State Response
OECD	Organization For Economic Cooperation And Development
NGO	Non-Governmental Organization
SCARM	Standing Committee On Agriculture And Resource Management
NOP	National Organic Program
USDA	United State, Department Of Agriculture
PVM	Parasitophorous Vacuole
IMC	Inner Membrane Complex
ITS	Internal Transcribed Spacer
SCAR	Sequenced Characterized Amplified Region
DAPS	Diaminopyrimidine
DHFR	Dihydro Folate Reductase
MIC	Minimum Inhibitory Concentration
EO	Essential Oil

MBC	Minimum Bacterial Concentration
EDS	Egg Drop Syndrome
IB	Infectious Bronchitis
NCD	New Castle Disease

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CHAPTER I

INTRODUCTION

Free range poultry farming is described as an agriculture approach in which the goal is to produce integrated, humane, economically and environmentally sustainable production system. Extreme reliance is implemented in such a system on farm-derived or locally renewable resources. Recent studies demonstrated the consumers' increasing awareness of pollution issues and food safety of IS products, forming an augmentation of determinants for the transformation towards the purchase of free range farming and organic foods. The price of free range products and lack of availability appear to be key limitations to the purchase of these products (Blair, 2008). In 1981, the first movement against battery cages, and the first large scale free-range units were established. "It was as if they are teaching them how to walk again" (Katie Thear, 1997). Organic and free range animal production has increased rapidly in many countries over the past few years. This development was a response to an expanded interest for nourishment that is seen to be healthy, tasty, fresh and free from any additives such as antibiotics, chemicals, hormones, and produced in a manner that is free from gene-modified crops and environmentally sustainable.

Free range products are usually more expensive than those used in IS, such as feed, this results in meat and eggs being more expensive. Recent data proved that there is a constant growing market for meat and free range eggs, if they reach the consumer in a reasonable price. This will be tougher for northern regions which are challenged with harsh climates. Since there is a lower supply of organic feedstuffs than southern, warm weather,

close to some grain sources, where labor is abundant, and it is much cheaper to cool with fans than to heat with gas (Blair, 2008).

Disease occurrence in free range poultry farm versus IS farming is still debatable, according to Permin and Pedersen, (2002). Many studies revealed that laying hens kept in free-range systems and litter-based housing systems have a significantly higher occurrence of parasitic diseases and bacterial infections as compared to layers kept in cages. In parallel viral disease occurrence was significantly lower in cages as compared to indoor litter-based housing system (Fossum et al., 2009).

However, other studies revealed that *Salmonella*, the leading cause of food-related human death and hospitalization, and Coccidiosis, one of the most economically damaging and prevalent poultry diseases, are much more prevalent in big cage egg operations (Bell and Weaver, 2002). Kaufmann-Bart and Hoop, in 2009 found a consistent decrease in parasitism (mostly helminths and coccidiosis) and viral diseases (mostly Marek's disease) during the twelve-year period after the ban of battery cages in Switzerland. The difficulty found in free range system, in which drugs are not commonly used as in IS , is that birds are exposed to pathogens reservoirs and carriers such as wild birds, insects, and rodents. However, better management practices and enhancing biosecurity led to a decrease in cannibalism, feather pecking, parasitism and viral disease in cage-free systems (Kaufmann-Bart & Hoop, 2009).

The major problem in intensive farming is wastes; one million hens, produces 125 tons of wet manure a day; intensive egg farming is a huge waste of agricultural resources,

as only 23 % of protein feed is converted to animal protein in eggs. Local water ways are also polluted by ammonia run-off and waste spills by factory egg farming (Gerber and Steinfeld, 2007).

Free range poultry system in Lebanon constitutes an important source of protein and ready income in rural areas and smallholder farmers since they can be integrated in other farming activities in a sustainable way. Because of the ease with which poultry products can be supplied and sold to different areas with their relatively low economic values, the main objectives behind rural poultry are home consumption and gifts to visitors and relatives. Money from the sale of the birds is used to buy immediate household requirements such as food, dairy feeds and to pay school fees. By eating leftovers from the kitchen and insects such as cockroaches, birds perform a valuable sanitary function in villages. Poultry manure can be used as soil conditioner or as feed supplement for ruminants

A change from conventional cages system to either a non-cage or enriched cage system may affect both the safety and quality of the eggs laid by hens raised in this new system. The eggs safety may be affected either through pathogens or microbiologically for example through contamination of the inner content of the egg with *Salmonella* enteritidis, and chemically by the contamination of the internal quality with pesticides, heavy metals or dioxins. Egg quality parameters may be affected by the changes in the integrity of the shell, yolk, albumen along with changes in composition, nutrition, or function. Flock age, season, flock disease-vaccination status, and hen breed must be taken into account since they also interact, affecting the egg quality and safety. Before any large-scale change to an

alternative housing system is undertaken an understanding of the different factors stated above is prudent **(**Holt et al., 2011)

The most recent EU legislative additions underline the importance of housing systems concerning commercial egg production. The European Council Directive 1999/74/CE (EU, 1999a) set the minimum standards for the welfare protection of laying hens in cages, free range housing systems and barn. Regulation 2295/2003 (EU, 2003) mandated that the housing system must be labeled on the egg shell and on the box and Regulation 1804/1999/CE (EU, 1999b) outlined organic production methods for animal origin products. In Lebanon, Free range system farming doesn't follow any rules or regulations, due to the lack of regulations and absence of governmental surveillance and control. It is worth noting that very few private beneficiaries are being monitored by the USAID in the south region of Lebanon (Hidalgo et al., 2008).

Eimeria spp. infection in poultry is an economically important disease, in an industry raising 40 billion chickens, in which 2.4 billion $ are annual losses due to mortality, poor performance and loss in productivity (Quiroz and Dantán, 2015). The continual use of coccidiostats in the feed is causing the emergence of serious resistance of coccidia to the drugs used during production (Chapman, 1997; Stephen *et al*., 1997). In addition, most broiler operations in developing countries are not following the proper withdrawal periods of coccidiostats from the feed before slaughter, resulting in significant residues in chicken carcasses (Mortier *et al*., 2005).

The most common coccidial sp. involved in broilers infection (Lee *et al*., 2011) are: *Eimeria tenella* (Railliet and Lucet, 1891), *Eimeria brunette* (Hein, 1974), *Eimeria acervulina* (Assis *et al*., 2010), *Eimeria mivati (*Vrba *et al*., 2011), *Eimeria hagani* (Joyner and Long, 1974), *Eimeria necatrix* (Conway and Mckenzie, 2007), *Eimeria praecox* (Reperant *et al*., 2012) and *Eimeria maxima* (Schnitzler and Shirley, 1999).

Modeling of the intestinal pathogenesis by a controlled challenge with the eight species of *Eimeria* is of primary importance, before the evaluation of any anti-coccidial drug (Elmusharaf *et al*., 2010). In addition, the multiplication and pathogenesis of *Eimeria* spp. is related to innate immunity of different chicken breeds (Lillehoj, 1994); thus, the inclusion of a certain breed in the *Eimeria* spp. challenge requires a detailed optimization to reproduce disease signs and lesions of *Eimeria* in its birds. The establishment of a chicken model for achieving Koch's postulate, using mixed infection by the eight species of *Eimeria*, was recently documented by Barbour *et al*., (2013 a,b).

The anti-parasitic activity of essential oils is sporadically reported in the literature (Fatani and Hilali, 1994; Kamsuk *et al*., 2007; Grabensteiner *et al*., 2008; Khater, 2014). The literature reporting the coccidiostat activity of terpenes present in essential oils against *Eimeria* spp. in poultry is scarce (Giannenas *et al*., 2003; Oviedo-Rondon *et al*., 2006). A blend of eucalyptus and peppermint has been assessed with regard to its protection abilities against different pathogens in poultry including infectious bronchitis virus , avian influenza virus and *Mycoplasma gallisepticum* (Barbour *et al*., 2006, 2011). This blend showed acceptable degrees of protection and production improvement. Other essential oils were reported to cause an improvement in broiler performance when administered as a dietary supplement (Suk *et al*., 2003;

Hernández *et al*. 2004; Cross *et al*., 2007). Timbermont *et al*. (2010) showed that broiler diets supplemented with essential oil of eucalyptus, prevented necrotic enteritis induced by interaction between *Clostridium perfringens* and Paracox-5™ anticoccidial vaccine. The active ingredients of eucalyptus oil enabled the stimulation of the immune system response by triggering the phagocytic activity of monocytes (Serafino *et al*., 2008). In addition, the cineole active ingredient in the eucalyptus essential oil has been proved to control mucosal secretions in the epithelial layer of the respiratory system (Juergens *et al*., 2004). The *in vivo* and *in vitro* activity of terpenes in eucalyptus and peppermint essential oils against poultry viruses was previously documented (Schuhmacher *et al*., 2003; Barbour *et al*., 2006, 2010; Siddiqui *et al*., 1996; Sivropoulou *et al*., 1997; Ocak *et al*., 2008).

The objective of study A is to present the degree of compliance of Lebanese intensive system (IS) and free range chicken layers (FRCL) farms to the EU standards, following the welfare of chicken and the improvement in their egg quality parameters and a reduction in their heavy metals content. To our knowledge no one has done this study before in Lebanon. Study B investigates the coccidiostat activity of essential oils Mentofin and Modified Mentofin in an attempt to replace a commonly used synthetic coccidiostat namely, the Maxiban, against eight *Eimeria* species which are frequently involved in coccidiosis of chicken worldwide. Performance parameters of the experimental chicken and their quantified oocyst shedding will be assessed.

CHAPTER II

LITERATURE REVIEW

A. Problems in quality of poultry products

1. Heavy metals

Metals usually accumulate in the eggshell and/or egg contents in the course of metal sequestration by the female. The higher the concentration of the metals pollutant in the soil, the more metals will be sequestered in the egg and the more metals will reach the avian tissue. Quantitation of contaminants, mainly heavy metals, in eggs is now gaining considerable interest by scientists as a biological tool to assess and monitor spatiotemporal pollutant trends in the environment. (Hashmi et al., 2015)

The essential trace metals with known biochemical functions in chicken eggs are chromium, cobalt, copper, iron, zinc and manganese. On the other hand the non-essential metals with known toxic effect are cadmium, lead and mercury (Kirkpatrick et al., 1975). There are some essential heavy metals like Cu and Fe that maintain the appropriate metabolic activity in living organisms (Abdulkhaliq et al., 2012).

In a study conducted by Giannenas et al., (2009), it was reported that the husbandry system significantly affect specific metal content in the egg shell, egg yolk and/or the egg albumen (Table 1). Zn and chromium contents of eggs in backyard poultry were

significantly higher than that found in conventional or organic system, which was similarly reported in the works of Waheed et al., (1985) (Table 2). Se concentration in yolk of eggs collected from backyard poultry was significantly lower, in comparison to that collected from conventional and organic system while its concentration in the albumin was significantly lower. Most of the other trace elements were in comparable concentrations among different measuring systems.

Table 1.Trace element concentration in yolk and albumen of eggs from three different husbandry systems.

Trace elements	Egg yolk (ng/g)[1]			Egg albumen (ng/g)[1]		
	Conventional	Organic	Courtyard	Conventional	Organic	Courtyard
Se	313 ± 16^{b}	410 ± 26^{a}	217 ± 14^{c}	62 ± 4.4	54.5 ± 4.6	66 ± 6.1
Zn	20676 ± 923^{b}	18225 ± 857^{c}	21196 ± 908^{a}	1003 ± 54^{b}	1029 ± 96^{b}	1385 ± 141^{a}
Mn	836 ± 79^{a}	797 ± 44^{a}	705 ± 41^{b}	33 ± 3.5	35 ± 4.7	35 ± 4.1
Co	4.6 ± 0.5	4.6 ± 0.4	4.9 ± 0.3	1.36 ± 0.2	1.14 ± 0.2	1.15 ± 0.3
Cu	1357 ± 111	1233 ± 104	1282 ± 108	212 ± 24	189 ± 28	254 ± 34
Mo	260 ± 14	246 ± 16	236 ± 21	26 ± 1.3	19.5 ± 3.8	23.3 ± 2.3
V	12.5 ± 0.4	13.2 ± 0.8	12.6 ± 0.8	13.2 ± 0.2	13.6 ± 0.3	13.9 ± 0.2
Cr	66.2 ± 8^{b}	$82.9 \pm 11^{a,b}$	90.5 ± 12^{a}	48.2 ± 5.2^{b}	48.2 ± 3.4^{b}	68 ± 4.8^{a}
Ni	63.3 ± 5.6	58.4 ± 3.6	59.2 ± 4.7	64.2 ± 4.2	56.3 ± 4.3	74 ± 7.2
Tl	1.4 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	0.72 ± 0.2	0.51 ± 0.1	0.78 ± 0.2
As	13.9 ± 1.8	12.5 ± 2.6	15.4 ± 2.9	5.4 ± 0.5	4.4 ± 0.2	5.8 ± 0.6
Cd	1.4 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.3 ± 0.2

[1]Values are means ± S.E.M.

[a-c]Numbers with different superscript are significantly ($p < 0.05$) different with respect to row for yolk or albumen.

Source: (Giannenas et al., 2009)

Table 2. Trace element normal concentration of Zn in egg-white and yolk produced by IS and FRCL on dry weight basis

Element	Egg –yolk (IS poultry)	Egg-white (IS poultry)	Egg-yolk (FRCL)	Egg- yolk (FRCL)
Zn (ppm) Arithmetic mean	57±21	2±0.7	82±20	3±1.2

Source: (Waheed et al., 1985)

To ensure consumer health while consuming poultry products, it is essential that heavy metals must be restricted in the diet to acceptable levels. Research has been carried out at National Research Council (NRC) in USA indicating both, maximum tolerable levels concentrations of these metals in dietary poultry eggs as well as dietary maximum tolerable levels of these elements in poultry feed. (Islam et al,. 2014)

Table3. Permissible limits of heavy metals in table hen eggs

Metal	Permissible Limit (mg kg-1)
Copper	10
Zinc	20

Source: (Zmudzki and Szkoda 1996)

According to world health organization (WHO), copper is a vital element, with daily copper consumption of 1 to 5 mg is needed for adults which Corresponds to a Tolerable daily intake of 140 µg Cu/kg bw/day. Copper has been reported to cause acute poisoning with estimated doses of 6 to 647 mg of copper per kg body weight (Baars et al., 2001).

On the other hand Zinc toxicity in humans is not reported. It's rather an essential element, most prominent Zinc deficiencies in humans are skin lesions. The daily requirement of zinc is up to 22 mg per day, which is equivalent to 0.3 mg/kg bw/day according to the WHO (Baars et al., 2001).

B. Factors affecting the physical structure of the egg

1. Weight

The weight of the egg also determines the nutrient content and the weight of the day old chicks. Many factors affect the laying hen's egg such as strain, age, heat stress, water quality, disease, heredity, body size, feed and water consumption (Roberts, 2004). Egg weight is positively correlated to age, body size and feed and water consumption while factors such as disease, low water quality, and heat stress negatively affect the egg weight. Chicken breeds such Leghorn, Minorca, New Hampshire Red are known to lay eggs of higher weight as compared to Black Australorp , Buckeye, Hamburg and Dorking (Nelson et al., 2010).

According to the Canadian egg size standardization, egg weights are characterized by egg size benchmarks: small (42.0–48.9 g), medium (49.0–55.9 g), large (56.0–63.9 g), extra-large (64.0–69.9 g) and jumbo (70.0 g or higher). These egg size evaluations are fundamentally the same as those used in the USA. Classification of egg size is according to weight: jumbo (≥70 g), extra-large (65–70 g), large (56–65 g) and medium (49–56 g). The

most commonly available classifications are Medium, large and extra-large (Şekeroğlu, and Altuntaş, 2009).

According to EU standards, the egg weight classifications are: small (under 53g), medium (from 53 g up to 63 g), large (63 g up to 73 g) and extra-large- very large (73 g and more) (Commission regulation, European Economic Community (EEC) No 1274/91, 15 May 1991).

Egg weight is an imperative financial attribute. For instance, in egg-type hens, every addition of 1 g in mean egg weight may enhance its grading and income by around 4%.

Impacts of egg weight on egg quality attributes were experimented on brown layers strain of Lohmann hens raised in a confined system. Jumbo eggs had the darkest shell color. Medium eggs had a significantly lower redness of the egg shell color as compared to the other egg sizes ($P < 0.05$). Egg shell thickness ($P < 0.05$) was the lowest in extra-large eggs (0.382mm) and the highest in medium egg (0.400 mm). There was an increase in albumen height and yolk height with egg weight. A darker yolk color on the scale was seen with an increase in egg weight. Medium-weight egg had the highest breaking strength. (Shalev and Pasternak et al., 1993).

In the study by Van Den Brand et al. (2004), caged layers had a higher egg weight at an early age as compared to outdoor layers. But with increase in age the outdoor layers egg weight surpassed them. Eggshell quality remained constant or even increased in outdoor layers with age while in caged layers egg quality decreased.

2. Egg shell quality

Egg shell quality could be affected by many factors including the age and strain of hen, water quality, disease, induced molt, nutritional factors such as phosphorus, calcium, vitamins, non-starch polysaccharides, enzymes, contamination of feed, general stress, heat stress and production system (Roberts, 2004).

C. Factors affecting egg shell weight % and shell quality

1. Bird Strain and age

Different strains of laying hen vary significantly in egg shell quality, production and egg size as a result of genetic selection which lead to a clear difference between traditional breeds and commercial birds of laying fowl. Selection for unique characteristics such as production can have an effect on other characteristics such as egg shell quality (Roberts, 2004).

A number of studies have revealed that as birds grow, egg shell quality decreases while at the same time egg size increases. Usually the increase in egg weight is not supplemented by a proportional increase in shell weight so that the ratio of egg weight to shell weight (often referred to as percentage shell) decreases. Studies have shown that the hen doesn't have the ability to produce an increased amount of egg shell. This is related to an enzyme involved in calcium homeostasis know as hydroxycholecalciferol hydroxylase. Egg shell quality in older hens can be adjusted by dietary manipulations that decrease egg size (Roberts, 2004).

The shell in Isa Brown was significantly ($P < 0.05$) thicker at 45 week of age than that at 55, 60, 70, or 75 week of ages. The trend for shell thickness in Isa brown are the same with the studies made by Curtis et al. (1986), who stated that with increasing hen age the percentage of shell thickness dropped in all of the three white and three brown commercial layer strains. The shell weight is also related to breed as we can see in the following study. The average shell weight of ISA brown is 0.747 g while the average shell weight of the Korean Native Chicken is 4.8 ± 0.57 with a significance of ($p< 0.01$) (Suk and Park, 2001).

2. Water quality

Water containing excess amount of electrolytes (saline drinking water) may cause long-term negative impacts on egg shell quality. The water supplied to chicken should likewise be hygienic to guarantee that the disease wouldn't be transmitted by this course. The temperature of the water given to laying hens is likewise critical, particularly amid hot climate. It creates the impression that hens diminish water consumption or may even stop drinking, if the water gets excessively hot (Roberts, 2004).

3. Heat Stress

Heat stress decreases feed consumption and restricts the availability of blood Ca for egg shell formation. It might likewise lessen the movement of carbonic anhydrase, an

enzyme that results in the production of bicarbonate that contributes the carbonate to the egg shell. This ultimately results in reduced shell quality and smaller eggs.

4. Housing system

Hidalgo et al (2008) study on the quality characteristics of eggs from different housing systems showed that the shell (%) in cage is 11.0a ± 0.19 which is significantly higher than free range 10.2b ± 0.20. The largest eggs in the cage system had the lowest shell percentage (Casiraghi et al., 2005).

In another example, the shell thickness (mm) is significantly greater in free range 0.50a ± 0.01 then in caged chicken 0.41c ± 0.01. The conflicting results found in literature prevent to conclude on the effect of housing system on shell thickness (Hidalgo et al., 2008).

5. Disease

Egg shell quality has been reported to be influenced by a number of diseases. Defective eggs and egg shells can appear if the health of the bird is compromised by any disease. Problems with egg shell formation can be caused by any pathogen that develops in the tissues of the conceptive tract. For instance Infectious bronchitis has been accounted for causing egg shells with paler shading and in some cases wrinkled in appearance. (Roberts, 2004).

6. Density

Specific gravity of egg (density) is measured by immersing eggs in salt solutions at different concentrations. Any score above 1.075 of specific gravity indicated good shell quality. This measure is only accurate if eggs have a very small air cells, that is, only fresh eggs (Holder and Bradford, 1979).

Density is a decent pointer of egg freshness. The maturing procedure of eggs relies upon the capacity conditions, for example, stockpiling temperature and dampness. During storage the most noticeable physical change in the egg is the increase in air cell volume, predominantly because of the loss of H_2O and CO_2 through the egg shell, hence prompting lower densities (Aboonajmi et al., 2014). Eggs density is positively correlated with shell quality and shell weight % (Holder and Bradford, 1979).

The value of egg specific gravity was demonstrated to diminish alongside the reduction in the shell thickness .It goes in parallel with the bird's age (Amem and Al-Daraji, 2011) for instance when the mature hens were at the age of 54, 58 and 62 weeks, the estimated values of their egg densities where 1.081, 1.077, and 1.060 g/cm^3, respectively (Kontecka et al., 2012).

7. Yolk weight percentage

Theories suggest that the highest amounts of cholesterol are found in eggs with the heaviest yolks and the largest yolk to albumen ratios. The average yolk weight for ISA

Brown is 15.5 ± 1.38 while the average weight for Korean Native Chicken 16.3 ± 1.38. (Suk & Park, 2001).

Yolk dry matter content increases with layer age, whereas dry albumen matter and albumen height content get reduced with age. Albumen weight decreased with layers age, whereas yolk weight increases regardless of the housing system, resulting in an increase in yolk: albumen ratio with advancing age (Van Den Brand et al. 2004). The outcome of the present study demonstrated that the different impacts on the Yolk to Albumen proportions were because of breed as opposed to age (Suk and Park, 2001).

However in an experiment evaluating the impact of the housing system on egg quality parameters, it was found that the yolk weight percentage was not significantly different in back yard poultry versus cage hens (24.5 versus 25.2 %) respectively (Suk and Park, 2001).

8. Albumen

During storage, thick albumen liquefies with time and becomes more slender, this is why old eggs tend to spread out while fresh ones broken into a plate sit high and firm. The main reason behind this phenomenon is the pH increment increase during the storage period which causes a change in the lysozyme–ovomucine complex (Aboonajmi et al. 2014).

In another study, the dry matter content and albumen height decrease with age as reported by Van Den Brand et al., (2004).

In literature, various factors were reported to cause significant changes in albumen height and weight %, for instance Benton et al., (2001) revealed that fertilized eggs have a lower albumen height and higher albumen pH than unfertilized eggs. Since cockerels are housed together with layers in free range system, while conventional layers were housed individually, higher albumen pH and lower albumen height in the free range systems could be predicted.

Commercial egg-type chicken ISA Brown had an albumen weight average of 40.6 ± 4.16 while the dual-purpose pure breed of the Korean Native Chicken (KNC) had an average albumen weight of 31.5 ± 3.48 which indicates a difference of albumen weight caused by breed.(Suk and Park, 2001).

9. Yolk color

Yolk color is an indicator for acceptability and preference for the consumer. Yolk color characteristics are highly influenced by hen diet. There's a tendency for lower values of yolk color in organic eggs as compared to cage eggs. The addition of synthetic xanthophyll in organic feeds was banned (EU, 1999) which explains the low yolk pigmentation in organic eggs (Hidalgo et al., 2008). However it was reported by literature that yolk color is significantly affected by the housing system. The free range system is expected to have a darker yolk, because layers can consume xanthophyll rich feedstuffs, such as herbs or grass (Van Den Brand et al., 2004).

Chang-Ho et al., (2014) reported a positive correlation between the hen age and yolk color, while the intensity of the eggshell color was relatively constant.

10. Haugh unit and percentage of eggs with AA quality

Haugh unit (HU) is an indicator of egg freshness. HU consistently decreases as the hen ages (Chang-Ho et al., 2014). HU calculations depend on the height of the thick albumen and the egg weight, as shown in the below formula (Roberts, 2004):

$$HU = 100 * log(h - 1.7w^{0.37} + 7.6)$$

Where:

- HU = Haugh unit
- *h* = observed height of the albumen in millimeters
- *w* = weight of egg in grams

Higher storage temperature leads to significant increase in egg weight loss and albumen weight, but lower HU unit. It is well reported that longer storage duration or higher temperature increased egg weight loss, albumen pH and decreased HU unit. Khan et al., (2013) stated that those deteriorations in albumen quality are the consequence of the evaporation of moisture and carbon dioxide, thus led to pH increase and concomitant

structural change in albumen protein. Egg quality is more affected by temperature or storage duration, but not by hen age as reported by Chung and Lee, (2014).

Quality grading of eggs depends on HU in ascending order of quality; grades are chosen as B, A, and AA (Vaclavik and Christian, 2014).

In the USA a, B grade egg has, lower than 60 HU, while the A grade, has 60 – 72 HU, and the AA grade eggs score 72 HU or higher. Eggs with 60 HU and lower are considered to be un-fresh (Lin et al., 2015).

D. Consumer pressure on poultry industry in relation to intensive management

The Driving Force State Response (DSR) model Organization for Economic Cooperation and Development (OECD, 1996) essentially recognizes and comprehend the procedures involved in domesticated animals environment interaction. It concentrates first on the human exercises that make pressures, which are plants and animal agriculture with all their related processes. Positive or negative forces are created by these activities (for instance methane discharge, improvement of soil structure the recycling of nutrients, soil compaction), which affect the quantity and quality of all natural resources from water , flora and fauna, soil, and non- renewable resources. Data on the condition of that asset are either fortified or weakened by the valuation of the general public of ecological qualities, prompts a societal reaction. The reaction of the society to this information could be through sectoral policies, general economic and environmental change. Changes in these

approaches will, for the most part, be through motivating forces to utilize certain advancements, for instance, contamination relief, diminishment of methane emission or positive cooperation between wild-life and livestock. The model is particularly appropriate to direct the identification of most important parameters to be used in an environmental impact assessment of proposed policies and livestock projects.

For bio-economic assessments of free range versus conventional laying hens, in Lebanon involves the following parameters:

1. Driving force

a. Indirect demand

- Environmental resources: Commonly provided range area (Specifically in rural areas)

-Climate: Mediterranean climate characterized by long, hot, dry summers and short, cool, rainy winters suitable for free range production.

- Economics and social markets: this demographic increment, along with the steady increase of Lebanon population has increased the demand for food, especially the low cost source of protein (eggs).

-Population: following the Syrian emigration to Lebanon, the population rose from 4.5 million to an approximate of 6.5 million, in the last three years (Balsari et al., 2015).

-Government policies: the rules on free range and conventional egg production in Lebanon, is minimal due to the total control of the private sector over this production, and the

absence of governmental surveillance, which facilitates with ease for almost anyone to start their own production system.

-Institutions: Private institutions and non-governmental organization (NGO) are readily available for all services, facilitating the establishment of small scale poultry farms.

-Endowment: usually comes from NGOS, like for example the USAID in Jezzine that provided financial support for the disabled and war casualties to start their own free range production system.

b. Direct demand

- Farm inputs: The backyard poultry products represent 15% of the total market poultry products. Consequently, the number of animals should be increased in order to meet the market demand for the backyard poultry eggs (Markou and Stavri, 2005).

-Energy: the most widely used form of direct energy in the production process is the fossil energy (diesel, electricity etc.), thus the production cost will be, continuously affected by the undulating prices of oil.

- Feed: feed constitute almost 70 % of the poultry production cost.

- A good system for animal welfare.

-Environmental sustainability.

2. Conditions on the farm

-Ecosystem: The aim is to create integrated, economically and environmentally sustainable agricultural production systems. Thus reliance is placed on farm or locally-derived resources which are renewable.

-Biodiversity: through free-range production in which even low-producing, disease resistant, local breeds like "baladi" are used.

-Natural habitats: free-range maintains healthy natural habitat, if the rules of production set by the European Union are followed.

3. Responses

-Consumer reactions and change in diet and agro feed chain responses: This growing interest in backyard and organic poultry production is a direct response to satisfy the demand for food that's supposed to be fresh, healthy, tasty and free from any additives such as chemicals, hormones, antibiotics, produced in a way which doesn't contain gene-modified crops and that is environmentally sustainable (Havinga and van Waarden, 2015).

- Farmer behavior: shifting systems increases the production to satisfy the demand for the niche market with proper management.

- Input use: vaccines, production of feed, infrastructure, feeders and drinkers.

- Cooperation between stakeholders: for a unified strong cooperative unit, and if possible one logo and one seller (Kline, 2015).

- Government policy: government should check the sector out, and put policies and follow up on implementation. The government should provide incentives, such as subsidies to farmers (Wei and Hennessy, 2015).

4. A case study from EU

European Union and its member states have permitted the use of antibiotics as growth promoters in animal feed for the past 50 years. Nevertheless, transmission of antibiotic resistance genes from animal to human microbiota and concerns regarding the development of antimicrobial resistance, led to the approval of withdrawing antibiotics as growth promoters in the EU starting from January 1, 2006.

Regulation 1831/ 2003 that was set by the European Parliament, concerning additives for use in animal nutrition indicated that antibiotics, other than histomonostats and coccidiostats, might be labeled and given as feed additives up until December 31, 2005. Anti-coccidial medications, such as ionophores, will also be banned as feed additives before 2013. After this date, medication in animal feed is only allowed with a veterinary prescription and very limited therapeutic use. Finally, the international trade of poultry products is affected by this ban since the EU will only allow feed that weren't supplemented with antibiotics to be imported to Europe. However, because drug-resistant pathogens concerns are rising especially that it could be transmitted to humans through the food chain (World Health Organization,l 2003, 2004), it is anticipated that the utilization of

antimicrobials in animal production will diminish overtime, especially in farms with better management and hygiene conditions (Castanon, 2007).

Backyard rearing could be the answer to diminish, or even halt, the use of antibiotics. Disease outbreaks are less likely to occur when the chicken are raised outdoors, as a result of minimizing the infective dose of a circulating pathogen (Grace, 2015). The European Union is the biggest supporter of animal welfare and legislative attention as compared to the rest of the world. Countries exporting to the EU have lower animal welfare standards particularly in the developing countries. That's why European Commission objectives are to further expand the body of regulatory standards in a recent action plan. For example broiler production around the world commonly takes place on litter so the EU established a new directive to set new criteria for the maximum bird density. However, this change will not greatly influence the global trade. In Brazil and Thailand, the animal condition differ greatly from EU for instance bird density is very limited as compared to EU also the majority of commercial layers are kept in cages during egg production. There is wide variation in space allowance per bird for instance the current minimum of 550 cm^2 per hen in the EU while Brazil, Ukraine and India space allowance is 300 to 400 cm^2. Since 2013, hens in the EU will have a space allowance of 750 cm^2 per hen and are kept in enriched cages. This is expected to have an impact on the world trade in egg products particularly egg powder. Local regions will continue to control the trade in local eggs. Labeling is being considered by the EU to provide the consumer with all the information concerning all production standards (Van Horne and Achterbosch, 2008).

It’s impossible for hens to express their natural behavior in conventional cages since the space for hens is very limited to flap their wings and to sand bath. That’s why EU developed an alternative housing system to accommodate the societal concerns and improve the animal’s welfare.

There are three main housing systems for egg producers to pick from nowadays:

- Enriched houses are large and are equipped with nest boxes litter and perches; Conventional houses have smaller enclosures with a slopping floor constructed from welded wire mesh.

– Barn systems. The barn is constructed to be one large enclosure and the birds are raised on the floor and have freedom of movement within the poultry house.

– Free range systems: Is the same as the barn systems, but with access to an outdoor space. (Van Horne and Achterbosch, 2008).

Considering all the choices, we have the most economical way to produce eggs is through laying cages and it’s also the best system for prevention of diseases (Hulzebosch, 2006)

The EU has the widest variation of housing system. The only countries outside EU that have some commercial non-cage systems are New Zealand and Australia (Van Horne and Achterbosch, 2008).

The EU directive (1999/74/EC) which manages the welfare regulations for layers has established standards for enhancing the welfare of commercially raised hens. It was planned that enriched cages or alternative housing systems should replace all conventional cages by the end of 2012. The specifications of the enriched cage system are that each hen should have at least 750 cm^2, a nest box, litter and a perch at least. In the present circumstance, layers have access to at least 550 cm^2 per hen in conventional cages within the EU (Van Horne and Achterbosch, 2008).

5. A case study from Canada

The poultry production in Canada consists of more than 2600 regulated chicken producers who have a legitimized access to various antibiotics, accepted as feed additives for poultry. Feed formulations and blends vary tremendously from a farm to the other, and geographically also which makes linking between production yield and the use of specific antibiotic feed additives or selection of the exact antibiotic resistant bacteria very difficult to establish. Many experimental tests were done on farms and the results concluded that there's a prevalent presence of antibiotic resistant bacteria in broiler farms. These antibiotic resistant bacteria were linked to the use of feed supplemented with antibiotics; on top of that, recent studies couldn't pin out the benefits of antimicrobial growth promoters on production yields and performance. With the development of better hygienic practices and biosecurity measures there will be less concern about the cessation of utilization of

antimicrobial growth promoters or antibiotics in feed. Pressure coming from the public, concerning environmental and food safety (antibiotic-resistant pathogens, antibiotic residues) stimulated researchers to come up with replacements for antibiotics. Some of these substitutes include essential oils, organic acids and pre- and probiotics. (Diarra and Malouin, 2014).

Authorities are being pressured to eliminate the use of antibiotics as growth promoters and any medication with medical importance in animal production in order to regain the effective use of medical antibiotics as well as safe food. The Canadian Veterinary Medical Association is creating a strict antimicrobial guideline that should be followed by veterinarians working with dairy herds, swine, beef and poultry flocks. The Veterinary Drugs Directorate of Canada, which has the responsibility of registration and approval of all antimicrobials for use in agriculture, is assessing a risk management strategy to decrease the human health impact of antimicrobial resistance due to the use of antimicrobials in animals (Diarra and Malouin, 2014).

6. A case study in Australia

In Australia and most developed countries free range eggs grew in popularity because of animal welfare activists. Movements were made to ban battery cages; food lovers, consumers and alternative farmers wanted to reduce the distance between people producing their food and themselves. Activists supported small-scale producers, pastured, organic, and environmentally sustainable farming practices (Loughnan, 2012; Miele et al.,

2010). Royal Society for the Prevention of Cruelty to Animals and Animals Australia are both animal welfare advocates that have campaigned to ban cage egg production completely in Australia, and at the meantime, all consumers should "boycott" caged eggs.

The welfare problems of raising chicken in caged egg are well recognized, especially hens raised on a barren wire cage, known as battery cages. It is frequently noted that layers egg production is the most intensively farmed animal that endures the cruelest conditions in commercial agriculture (Loughnan, 2012). The regulation in Australia provides that the minimum floor space provided for each hen is almost the size of an A4 sheet of paper (627cm^2) (Primary Industries Standing Committee, 2002). It also says that chickens must have access to water at all times and must be able to stand naturally in the cage, but no requirements were found regarding perches, access to a feed trough, or nests. The following is an example of the extreme cruelty the layers have to go through. Houses have artificially set climate and lighting with a controlled atmosphere. There are two conveyer belts one for delivering grain mix to the bird while the other belt collects the eggs (Loughnan, 2012). The hens are finally considered spent after 18 month of production with an estimate of 300 eggs per hen, and they are ground up to make stock and pet food (Loughnan, 2012). The provided space is inadequate to allow hens to turn around or spread their wings (Weis, 2007), or to perform regular practices of perching, foraging, preening, nesting and dust bathing. Hens show a variety of problematic practices and wellbeing issues. They get their feet trapped or stuck in the wire floor; develop broken legs and weakened bones (due to absence of cramping, exercise, and sunlight); they eventually become de-feathered (since they rub their bodies against the wire of the cage). Hens display

an array of wellbeing issues and problematic behaviors such as pecking to death, trampling, and cannibalism (due to lack of ability to exercise cognitive and social behaviors followed by cramped conditions). To hinder pecking, which is mainly caused by bad management, chicks are de-beaked with a heated blade often causing trauma to the bird and chronic pain if done incorrectly (Sharman, 2009). Manipulation of lighting and feed has routine cycles in production to force industrial hens to continue and maximize the production cycle while reducing the hens' rest periods. This reduces the hens' health, immunity to disease, and bone strength (Loughnan, 2012.). Finally the problem of the voluminous amounts of fecal matter the pen was sprayed with chemical disinfectants and antibiotics that is often added to the feed creates another issue (Weis, 2007).

Alternative free-range farm practices are on a rise after all the promotion and campaigns to ban battery cages and better the animal welfare. The most impressive modifications are taking place where foodies and farmers are concerned with the agro-ecological issues and intensification of animal production (Wiley et al., 2004).

In mixed integrated farms the manure from small groups of animals acts as plant fertilizer for legumes, grains, and pasture for example, thus maintaining rich, productive, and healthy soils. While in industrial-scale egg farms, chicken wastes must be gathered, sold, and shipped to be used as fertilizers in other farms; otherwise it is dumped in surrounding areas around the farms contaminating the water and air (Weis, 2007). A small amount of farmers in the United States, Australia, and elsewhere developed accreditation bodies to recognize alternative farming methods to lessen the environmental impacts of industrialized egg production. For example members of the Free Range Farmers'

Association in Australia, started independent auditing of all farms, no beak-trimming of hens, prohibition of mixed systems in the same farm (i.e., farming both barn-laid eggs and free-range). Moreover, we should be able to trace the eggs back to every individual farm (Parker, 2013).

a. Supply chains constraints to consumer choices

The ministers of agriculture in Australia found that egg production in both non-caged and caged could not be compared and ranked decisively and raised diverse animal welfare problems. However, most consumers' first choice was non-caged eggs, which lead for the report to be "industry-led," a "consumer choice" approach in which three sorts of eggs would be unmistakably separated and named:

1- "Cage," where birds "are continuously housed in cages within a shed (generally battery cages)";

2-"barn," where birds "are free to roam inside the shed which may have multiple levels";

3- "Free range," where birds "have access to an outdoor range and housed in sheds" (SCARM Working Group, 2000).

The feedback depicted a "vision" of the "quality-guaranteed well-being and welfare of chicken in an economically feasible industry, environmentally friendly and competitive egg industry generating a reliable, regular and reasonably priced supply of eggs. Allowing

the consumer to have the option of an informed choice, of the contemporary standards for occupational health and food safety" (Standing Committee on Agriculture and Resource Management (SCARM) Working Group, 2000). Hence, animal welfare was classified as a private good for which any consumer have the option to pay an ethical premium for it, not a public good that necessitates more than marginal obligatory regulation (Parker, 2013).

This was also confirmed by the Independent Panel for the Review by Food Labelling Law and Policy in 2011, in Australia and New Zealand Food Regulation Ministerial Council, which concluded that the choice of caged versus non-caged should not be the subject of mandatory regulation, not even legislatively authorized classifications of what counts as "free range" (Parker, 2013).

E. The slow transition towards free range and organic poultry farming

1. Constraints of this transition

a. Health

A change from the usual conventional cages to non-cage system or enriched cage may cause the eggs raised in this environment to have an effect on the quality or safety, and in some cases both. The eggs safety may be compromised either chemically through

contamination of the albumen and yolk with pesticides, dioxins, or heavy metals, or contamination might occur microbiologically, through contamination of internal contents with *Salmonella* enteritidis, or other type of pathogens. The quality of the egg can be affected by many changes like the integrity of the shell, albumen, or yolk accompanied by changes in composition, nutrition, or function. The egg safety is also affected by the interaction of flock age, season, hen breed, and vaccination. Before any large-scale move to an alternative housing system is undertaken it's critical to understand these different effects (Holt, et al, 2011).

The contamination levels of free-range floor eggs and free-range nest box eggs were always larger than the conventional cage eggs, which is consistent throughout the whole study (0.42–0.02 log cfu/mL) conducted by Jones et al. (2011). Mold levels and shell yeast were significantly lower in conventional cage eggs and free-range nest box eggs as compared to free-range floor eggs throughout the entire study. It was observed that the season had a role to play as well; the lowest level of populations monitored for all treatments was seen during winter time, excluding aerobic free-range floor egg shell emulsions, which increased (Jones et al, 2011).

b. Cost

Generally, the cost of production/kg of eggs is by far lower in the intensive system as compared to that in the backyard system (SCARM Working Group, 2000).

In Australia the minister of agriculture reported in the year 2000 that a great proportion of the public opposes the conventional laying system on animal welfare grounds (SCARM Working Group, 2000), since it was not fitting to burden industry with the expense of mandating enriched cages (SCARM Working Group, 2000). The most important factor behind this decision is that the most effective and fastest aspect of the egg industry is to sell these eggs to be used in processing of food. This part of industry consists of 32 percent of the market for eggs in 2000 and 42.1 percent in 2012, and all of this could be lost to producers from Asia of liquid or dried products, if the ban on battery cages occurred (SCARM Working Group, 2000). The animal welfare was overruled by the promotion of Australian production (Parker, 2013)

c. Management

Legislation and regulations governing the management of layers differ between both conventional and backyard systems according to countries; the EU being the most demanding for backyard poultry production in term of housing, feed (use of meat-and-bone meal) and general practice (Van Horne and Bondt, 2005)

d. Assurance system

To help the industry in complying with all legal necessities for egg production, a national quality assurance system is needed for the production of eggs above all bio-risk security, food safety and animal health was most urgently needed. In order to be on the list of accredited producers and use the "Egg Corp (Egg Corporation Limited) Assured" logo

the egg producers should implement a proper management system that would be later audited by Egg Corp. In parallel with the general policy approach, and an assurance that the eggs are properly labeled as barn-raised, free range or caged (Parker, 2013).

2. Consumer acceptability of free range versus organic products

a. Economically and socially

The findings revealed that for proximity of production (local, national over imported and regional) and improved practice of production (that of barn, organic and/or free-range instead of cage produced eggs), buyers are willing to pay a premium price (Gracia et al., 2014).

The following study compares acceptability of organic versus free range eggs. The results revealed that the number one determinant of consumer preference is cost, then comes rearing conditions and hens feed. The preference, percentage wise, was as follows: rearing conditions (19.68%), price (30.14%), feed (19.20%), and choosing different egg sizes (14.41%). This study revealed that not all consumers are willing to pay a premium price for alternative methods of production (Mesías et al., 2011)

3. The need for conformity in regulations covering the free range and organic products

England, Sweden and Norway are the leaders in taking strict regulations in the husbandry of all animal farms in the EU; on the other hand, countries like Italy are at the minimum level of EU standards. Furthermore, the food industry in cooperation with non-governmental animal protection organizations are setting down, developing and promoting additional welfare schemes. Some of the schemes set down focus on numerous aspects of animal welfare issues (e.g. Label Rouge in France; Shechita in the UK; organic labelling), others focus only on animal welfare such as (Freedom Foods). France is the leader in egg production with (14%) of the total production; Italy is just behind France with a production percentage of 11%, and the same for Germany and Spain. There is 45 million hens reared for eggshell production, 86 % of them are reared in cage system and only 3 % live in enriched cages. The remaining are divided between free range system (2%), organic system (3 %) and 9% are reared in barn systems (Vecchio and Annunziata, 2012).

Table 4. Comparison between EU and French regulations for organic poultry production

	Council Regulation (EEC) 1804/99	French specifications
Stock	No requirement	Agreement from a certification body needed
Maximum farm buildings area	1600 m^2	800 m^2
Minimum slaughter age	81 days	91 days
Fish meal allowed?	Yes	No
Products from conversion	30% maximum	20% maximum
Products from conventional farming	20% maximum	10% maximum

	Council Regulation (EEC) 1804/99	French specifications
Cereals	65% minimum	70% minimum
Allopathic or antibiotics	One treatment allowed for curative purposes	No treatment allowed
Link to the farm?	Yes	Yes

(Von Borell and Sørensen, 2004).

Between the UK, Scandinavian countries and Italy, Italy sets a far lesser score behind in compliance to animal welfare. This could be a result of Italy's resistance to recent changes in the EU's animal welfare legislation as well as conformity to pre-existing, outdated standards (Vecchio and Annunziata, 2012).

F. The EU versus US regulations covering the free range and organic poultry production.

1. Organic products

The Council Regulation in EU (EC) No, 1804/1999 (1999) has formalized the basic rules for organic animal production. The rules include animal welfare, disease prevention and veterinary treatments, husbandry practices, management of manure and foodstuffs. Organic production excludes any modified organisms or products derived from genetically modified organisms. The genetic diversity should be maintained around the farm and in the agriculture system. Animals should be provided by a housing system that allows them to perform all aspects of their innate behavior.

The following principles are set to prevent diseases in production of organic livestock:

–Breeds selected should be resistant to diseases, have the ability to cope with the environmental conditions found, and should not suffer from the same prevailed health problems and diseases found in the conventional system.

–livestock should be raised in a way which satisfies all the species requirement's and supports a good resistance against infections and diseases.

–Application of outdoor areas for grazing and good quality feed is necessary to make the natural immune system of the animal resilient to infection.

–There should be a suitable space for the animal to prevent overcrowding which is associated with many health problems.

Immediate care and treatment should take place for sick animals. Allopathic medicine should be chosen if efficient instead of non-allopathic medicine. It's also not allowed to be used as preventative medication. A veterinarian is required in order to diagnose and instruct if medical treatment is needed. A record should be kept by the organic farmer of all the disease control agents and treatments used by the veterinarian.

Only one allopathic medicine treatment is allowed for animals with a life time less than a year, such as pigs or fattening bulls. For example if such an animal receives allopathic medicine (such as antibiotics) more than two times a year, it's not considered organic anymore. Double the time is needed for the withdrawal period in organic food, than what the veterinary authorities require.

The only artificial reproduction method allowed is artificial insemination; others, such as embryo transfers, are forbidden. And in order for the product to be sold as organically grown, the farmer should use the certified organic practices for 2 years.

The provided indoor area should include a maximum of 10 broilers per m^2 and six layers per m^2 with a maximum of 21 kg live weight/m^2 .The outdoor space should include 4 m^2 per layer and broiler (Von Borell and Sørensen, 2004).

Table 5. Comparison of highlights of poultry requirements of selected organic programs

	USDA National Organic Program (NOP)	**European Union**
Living Conditions		
Flooring	No rules	At least 1/3 of house must be solid with litter
Perches	No rules	18 cm/layer
Nests	No rules	8 layers/nest
Maximum indoor density	No rules	6 layer/m^2 10 meat poultry/ m^2 (21 kg/ m^2 max)
Outdoor area	Outdoor access required	At least 1/3 of birds' lives; mainly covered by vegetation; shelter required on pasture; access to pond for waterfowl
Popholes or "bird doorways"	No rules	4 m of Pop hole per 100 m^2 house
Maximum outdoor	No rules	4 m^2 per chicken; 4 m^2 per layer; 10 m^2 per turkey; 4-5 m^2 per duck

density		
Maximum flock/farm size	No rules	4,800 meat chickens; 3,000 layers; 2,500 turkeys; maximum total house area (entire farm) is 1,600m^2
Health		Downtime between flocks required
Antibiotics	Not permitted	withdrawal is double
Beak trimming	Permitted	Permitted
Artificial insemination	Permitted	Permitted
Caponization	No rules	Permitted for traditional product
Stock		
Origin	Under organic management after 2 d	Organic must be used if available; or under organic management after 3 d
Minimum age at slaughter	No rules	81 d for chicken, 140 d for Turkey and 49 d for Duck (Peking)
Genetics	No rules	using slow-growing strains
Feed		
% organic Feed	100% feed required	100% organic feed is required
Nutrient level	No rules	At least 65% of finishing feed must be cereal
Roughage	No rules	required
Synthetic amino acids	Prohibited, temporary exception for methionine	Prohibited
Transport/processing		Should be low stress

(Fanatico 2008).

2. Free range

For laying hens in non-cage systems the main issues found in several private standards beyond or additional to EU legislation were: maximum flock size, maximum stocking densities (lower than EU legislation), requirements for perches and nests, dust/sand bath and regular visits. In systems with free range area the main differences were a higher number of pop-holes, additional requirements for outdoor run, stocking density, duration of outdoor access and pasture management (Schmid and Kilchsperger, 2010).

In order to group the animal welfare status of EU third countries with regard to the main animal categories as well as to transport and slaughter four main categories were made:

- Group A: beyond EU legislation. More than 4 main aspects clearly beyond EU rules ex. Switzerland
- Group B: comparable to EU legislation in main points (deviations on minor points) ex. Argentina and New Zealand
- Group C: slightly below EU legislation (in more than 4 main aspects deviations) ex. Australia, Canada and Brazil
- Group D: clearly below EU rules (many main aspects not regulated by national legislation) ex. China and USA. (Schmid and Kilchsperger, 2010).

Free range hens in EU must have free access to outdoor runs, as well as indoor housing for the night-time. The EU Hens Directive specifies that indoor housing for free

range hens and barns must (1) provide a litter area that occupies at least 1/3 of the ground surface of the house, which include 250 cm^2 (38.75 square inches) of littered area per hen at least; and (2) the maximum stocking density should be 9 hens per square meter (m^2) of usable area. By 1 January 2007 these requirements will come into force.

By the year 2004 mandatory labeling for eggs and egg packs of the code that allows the farming method to be identified should be applied in EU. One of the following terms: "organic eggs", "eggs from caged hens" or "barn eggs" and "free-range eggs" is required to be used according to the legislations. In addition the egg packs must have a visible and clearly legible type of farming on the outer surface. This is of great importance since it will be the first time the industrial type of the product will be clearly labeled "battery cages". The legislations also lay down the welfare condition that must be achieved for the use of these labels. For example, egg packs coming from free range hens labeled free-range must by law have free access to continuous daytime to open air runs covered by vegetation. Furthermore, the maximum outdoor stocking density must be one hen per $4m^2$ at all times.

Regarding debeaking, the 1999 Hens Directive forbids all mutilations, but then continues to provide that, for the farmers to prevent cannibalism and feather pecking, beak trimming could be carried out for chicks that are less than 10 days old by qualified staff.

Concerning forced molting, the Council Directive 98/58/EC prohibits it when it involves depriving hens of feed for long periods of time. As for the protection of animals kept for farming purposes, "Animals must be nourished an adequate amount of feed to sustain them in good health and satisfy their nutritional needs as well as a wholesome diet

which is proper to their age and species. Following that, hens from which feed is being withdrawn for numerous days are not being given the appropriate intervals of feed in order to satisfy their physiological needs "All animals must have access to feed at intervals appropriate to their physiological needs" (Stevenson, 2012).

In USA, the coverage of governmental non-organic animal welfare legislation is voluntary, while in Europe it is a mandatory Code of Practice.

The United States, Department of Agriculture (USDA), indicated that the animals are allowed access to open air runs. These guidelines do not specify the duration and time an animal must have access to the outside, nor the size or quality of the outside range.

The labeling of products as free range in USA isn't properly regulated; there has been a proposition by USDA to set specific rules for this issue in hand. But until now these rules haven't been applied (Shears, 2010).

G. The baseline data about the average heavy metals levels in poultry eggs of free range vs organic systems

In a study conducted by Giannenas et al., (2009) the concentrations of heavy metals in yolks were found to be much higher than those found in egg albumen. The results showed significant differences in some trace elements among the tested eggs, and which belong to different husbandry systems. It has been noted that major differences were observed in the yolk rather than albumen, in comparing the different husbandry systems.

In egg yolks, organic eggs showed the highest values for Se (410 ng/g) where as in free range it was much lower (217 ng/g). Values of Zn were significantly higher in free range (21196) ng/g as compared to organic chicken (18225 ng/g). Mn values were higher in organic eggs (797 ng/g) as compared to free range (705 ng/g). Cr values were higher in free range (90.5 ng/g) as compared to organic eggs (82.9 ng/g). As for Mo, As, Cu, Co, V, Ni, Tl and Cd, these heavy metals did not have any significant differences among the eggs from the different housing systems, i.e., free range versus organic.

In egg albumen, the highest values for Zn 1385 ng/g and Cr 68 ng/g were obtained in free range eggs as compared to organic eggs which are 1029 ng/g and 48.2 ng/g for Zn and Cr, respectively. As for Se, Mn, Co 1.14, Cu, Mo, V, Ni, Tl, As and Cd in albumen, these heavy metals did not differ amongst the eggs from the different housing systems (Giannenas et al., 2009)

A study conducted by Zhu et al., (2015) found that the Ca, Cu, Zn and Se contents of the conventional eggs were significantly higher than those of free-range eggs and the Mn and Pb contents of the conventional eggs were significantly lower. The P and Cd values were not significantly different between the two rearing systems (Zhu et al., 2015) (Table 6).

Table6. Trace element concentration in yolks of eggs from the two different husbandry systems (fresh samples) (Zhu et al., 2015)

Element	Conventional	Free-range
Ca (mg/kg)	1058±122a	946±129b
P (mg/kg)	4152±133	4058±121
Se (µg/kg)	311.4±15.6a	111.9±9.9b
Zn (µg /kg)	26360±185a	24906±468b

Cu (µg /kg)	1117±123a	946±73b
Mn (µg /kg)	861±74a	1379±135b
Cd (µg /kg)	1.547±0.210	1.365±0.209
Pb (µg /kg)	19.96±3.24a	45.50±6.42b

Values are means±S.E.M; [a,b]: Numbers with different superscripts in the same row are significantly different ($p<0.05$)

H. The *Codex Alimentarius* regulations related to free range vs organic chicken layer system.

The *Codex Alimentarius* has no policies discriminating between free range and organic chicken systems; however, there are general rules and regulating different aspects of safe egg production namely.

1. Environmental hygiene

The egg laying institution should be suitable such that sources of potentially damaging substances are diminished and none existent at unacceptable levels on or in eggs.

2. Flock Management and Animal Health

Eggs must come from flocks of either breeders or laying hens that are in good health in order not to adversely affect the suitability and safety of the eggs.

3. Areas and Establishments for Egg Laying Systems

Egg laying open spaces and establishments should, to the extent feasible, be constructed and designed, sustained and used in a manner that minimizes contact with domesticated birds or their eggs to pests and hazards.

4. Watering

Water used in production operations should be suitable for its intended purpose Water should not be a contributor in the transmission of chemical hazards or microbiological hazards into or on the egg; it should be managed in a way to lessen the direct or indirect potential of transmission of hazards.

5. Feeding

While feeding breeders and/or laying flocks unwanted chemical or microbiological contaminants should not be introduced into the eggs especially if it affects the suitability of eggs products and eggs and present unacceptable health risk to the consumer.

6. Pest control

Pests are known as vectors for pathogenic organisms and should be controlled using a suitably designed pest control program. Pest control measures shouldn't cause undesirable levels of residues in or on eggs.

7. Agricultural and Veterinary Chemicals

Procurement, transport, storage and use of agricultural and veterinary chemicals should be undertaken in such a way that they do not pose a risk of contaminating the eggs, flock or the egg laying establishment.

8. Collection, handling, storage and transport of eggs

Minimal damage and/or contamination should take place during collection, handling, storage and transport of eggs or egg shells. Appropriate attention is required for temperature fluctuations and time temperature considerations. Proper measures should be applied during disposal of unsuitable and unsafe eggs in order to protect other eggs from contamination.

9. Egg collection equipment

Collection equipment should be made from materials that are non-toxic, and constructed, installed; maintained, and designed in a manner to facilitate descent hygiene practices.

10.Packaging and storage

Packaging equipment and egg packaging should be designed in a manner that avoids the damage and introduction of contaminants in or on eggs. And no matter where eggs are stored introduction of contaminants, or growth of existing microorganisms should be avoided whether it in or in the egg while taking into consideration the temperature and time conditions.

11.Transport, Delivery Procedures and Equipment

Minimal damage and contaminants should be introduced to the eggs while transportation is taking place.

12. Cleaning and maintenance of egg laying establishments

Health, safety and suitability of the eggs should be maintained and ensured during cleaning of egg laying establishment.

13.Personal hygiene and health status

Health and hygiene of the persons that come in direct contact with the flock or the eggs should be ensured, in order not to transmit illness or disease between the birds or cause any contamination that might affect the safety and suitability of the eggs.

14.Personal cleanliness

In order not to introduce contamination into the laying areas the persons working indirect contact with the animals should maintain a high degree of cleanliness, and wear suitable and appropriate clothing and footwear.

15.Sanitary facilities

Facilities should be available to ensure that an appropriate degree of personal hygiene can be maintained.

16.Documentation and record keeping

Records should be kept, as necessary and where practicable, to enhance the ability to verify the effectiveness of the control systems. Documentation of procedures can enhance the credibility and effectiveness of the food safety control system. (CODEX ,1976).

I. Coccidiosis disease

1. Nature of the disease

The most important protozoan disease affecting the industry of poultry worldwide is Coccidiosis with an estimated loss of more than 4 billion US $ per year (Williams, 1999a; Shirley et al., 2004). Eight % losses are caused by temporary loss of egg production in layers, reduced weight, and inefficient feed conversion as well as mortality (Dalloul & Lillehoj, 2005). The etiologic agents of coccidiosis are from the various *Eimeria spp*. found in nature, which occupy the lining of the intestine through ingesting sporulated oocysts found in the environment which are then transmitted from bird to bird. The life cycle of Eimeria is complex, having both asexual and sexual stages. *Eimeria* infections are site specific by which each *Eimeria spp* infects specific part of the intestine, and host specific, with varying pathogenicity in different poultry breeds (Jeffers et al., 1970; Levine, 1985; Lillehoj, 1988; Lillehoj et al., 1989). Seven species of Eimeria are considered crucial in

pathogenesis namely, *E. maxima, E. necatrix, Eimeria acervulina, E. tenella, E. brunetti, E. praecox and E. mitis*; (Long, 1973; Shirley et al., 1983; Barta et al., 1997; Tsuji et al., 1997; Vrba et al., 2011).

Infection with *Eimeria* induces protective immunity that last lifelong and is species-specific to that particular Eimeria species (Akhtar et al., 2005). Large numbers of oocysts are required to generate a protective immune response against Eimeria, but not in case of E. maxima that is highly immunogenic, in which lower numbers of oocysts are needed to give almost full immunity. Moreover, the early endogenous stages of the Eimeria life cycle are more immunogenic than the later stages (Rose et al., 1984). However, gamete antigens of *E. maxima* were confirmed to be immunogenic (Akhtar et al., 2005).

2. Etiology, pathogenesis and diagnosis of Eimeria spp.

a. Etiology

The coccidia is a spore-forming intracellular parasite belonging to the subkingdom Protozoa of the phylum Apicomplexa, and genus Eimeria. Apicomplexans express characteristic organelles at the anterior end of the parasite, the apical complex after which the phylum was named. The apical complex consists of secretory organelles namely, the rhoptries and micronemes, as well as the polar rings surrounding the conoid. The apical organelles play a key role in attachment and invasion to host cells (Fig. 1).

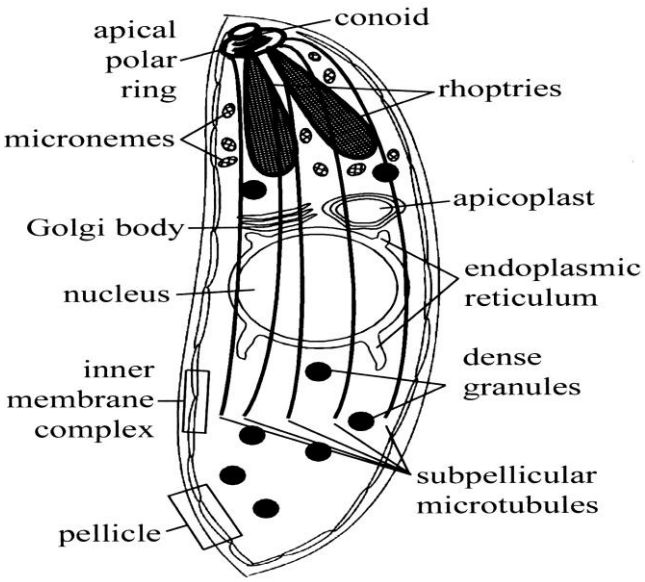


Fig.1. The morphology of Apicomplexans.

Apicomplexans contains a group of organelles that are distinctive to the phylum. Source: Morrissette and Sibley (2002).

Invasion of the parasite to host cells is an important stage of its life cycle. Extracellular parasites do not multiply outside the cells of the host, thus they need a parasitophorous vacuole (PVM) inside host cells to grow and replicate, making them obligate intracellular parasites (Morrissette & Sibley, 2002). The PVM occurs by invagination of the host cell membrane after contact of the parasite with the host cell (Shirley, 1992). During contact there are some secretions from the micronemes, rhoptries and dense granules. Micronemes secrete their contents first and are involved in host attachment, motility and recognition followed by rhoptry protein secretions which aid in the movement of the parasite into the host and formation of PVM. Finally, the dense granules secrete their proteins inside PVM which aid in the remodeling of the PVM (Shirley, 1992; Bromley et al., 2003).

The inner membrane complex (IMC) (Figure 1) lies directly under the parasite plasma membrane and is closely associated with it, producing a three-layered pellicle typical of the Apicomplexa. Most Apicomplexia are motile by actin- and myosin-based machine situated at the pellicle (Frénal et al., 2010), and their motility is coupled with their invasion. Motility and invasion is accompanied by the discharge of soluble proteins minimum inhibitory concentration (MICs) from the micronemes (Soldati et al., 2001). MICs encode adhesive motifs (Tomley & Soldati, 2001), and are important for motility and attachment; compounds that interfere with their secretions blocks the parasite motility, invasion, and attachment (Wiersma et al., 2004). TRAP (thrombospondin-related anonymous protein) possesses multi- adhesive domains, stored in the micronemes and is exposed at the sporozoite anterior tip when parasite comes in contact with host cells (Akhouri et al., 2008). The TRAP family is a trans-membrane protein of the microneme involved in attachment to the host and gliding locomotion and is conserved among Apicomplexian (Kappe et al., 1999; Tomley & Soldati, 2001). Another type of parasite proteins involved in invasion are GPI-linked surface antigens (glycosylphosphatidylinositolanchored variant surface proteins) (SAGs) found on the surface of sporozoites and merozoites (Tabares et al., 2004; Jahn et al., 2009). Obviously, the apical complex and invasion protein gives valuable source of antigens to regard as components of a recombinant vaccine to coccidiosis.

Coccidiosis is referred to adequate number of coccidia to produce clinical signs while Coccidiasis is referred to mild infections without any clinical signs. Coccidiasis is more common than coccidiosis. All species of Eimeria invade the lining of the intestine. Seven

species of Eimeria are considered valid these are *Eimeria acervulina, E. maxima, E. tenella, E. necatrix, E. brunetti, E. mitis and E. praecox*; however, *Eimeria hagani* and *E. mivati* validity is under review (Long, 1973b; Shirley et al., 1983; Barta et al., 1997; Tsuji et al., 1997; Vrba et al., 2011). Simultaneous infection with two or more species of Eimeria is common, and each specie causes a separate and recognizable disease independent of the other species. Coccidia is transmitted to the host through fecal-oral route. Development of the parasite Eimeria includes both exogenous stages where oocysts sporulation takes place in the environment to become infective and endogenous phase of asexual and sexual stages of development and lysis of the host intestinal tissue. A generalized life cycle sketch of *Eimeria spp*. in chickens can be found in a previously published document (Conway and Mckenzie 2007)

b. Pathogenicity and Gross lesions of Eimeria species

i- *Eimeria acervulina* (Tyzzer, 1929)

Eimeria acervulina infects the duodenal loop of the intestine; heavy infections may expand down the intestine. *E. acervulina* causes shortening of villi and reduction in the absorptive area of the intestine (Assis et al., 2010), resulting in reduced broiler growth. Heavy infection (106oocysts) of *E. acervulina* causes marked reduction in growth rate. Suppression of weight again may appear 3-4 weeks after infection but is most evident at one week after infection (Reid & Johnson, 1970). According to Reid and Johnson in 1970,

gross lesions scoring scale from 0 to 4 was used to determine the pathogenicity of Eimeria species. Score +1 and Score +2 (Figs. 2 & 3) show mild infection of *E. acervulina*. The mucosa is covered with white plaques resembling a leader form. Scraping of these white lesions when viewed under the microscope reveals unsporulated oocysts and gametocytes. These mild lesions might cause a little loss of skin pigmentation and very little or no effect on weight gain and feed conversion. Score +3 and Score +4 (Figs. 4 & 5) show the intestine that looks pale and containing watery fluid attributable to mucous secretions causing diarrhea. The lesions show more coalescing causing thickening of the intestinal wall. Definitely weight gain and feed conversion efficiency are depressed, besides pigment loss in skin (Conway and Mckenzie, 2007).

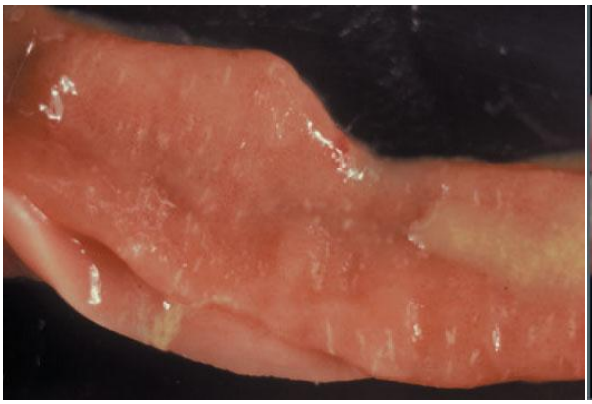


Figure.2. E. acervulina Score +1

Conway & McKenzie (2007)

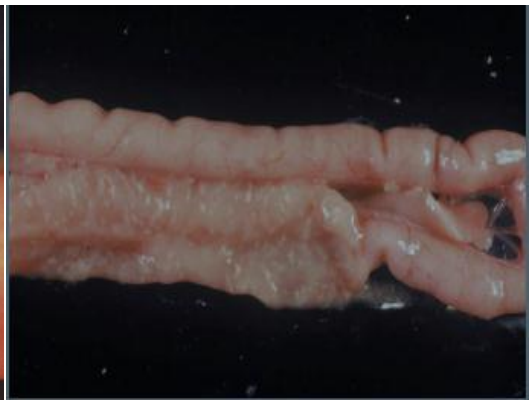


Figure.3. E. acervulina Score +2

Conway & McKenzie (2007)

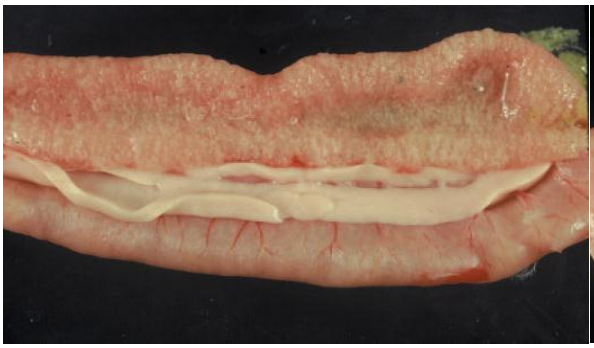


Figure.4. E.acervulina Score +3

Conway & McKenzie (2007)

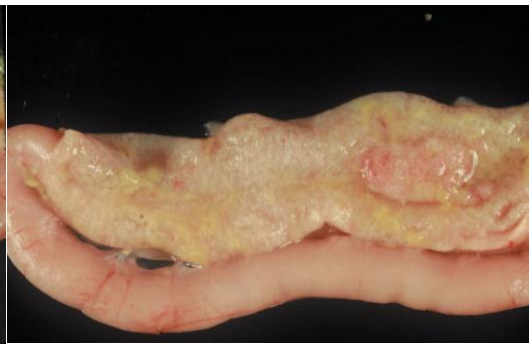


Figure.5. E. acervulina Score +4

Conway & McKenzie (2007)

ii- *Eimeria mivati* (Edgar and Siebold, 1964)

This specie was first recognized as a strain of *E. acervulina* or mixture of *E. acervulina* and *E. mitis* and later named as separate specie (Long1973b; Shirley et al., 1983; Barta et al., 1997; Vrba et al., 2011). *E. mivati* moves down the intestine more than *E. acervulina* as the infection progress. *E. mivati* endogenous stages are most numerous in the lower small intestine and proximal ceca (Norton & Joyner, 1980). In light infections lesions looks like *E. acervulina* but are more circular in shape. These lesions are colonies of gametocytes and oocysts, and may be seen from the serosal surface of the intestine. Score +1 and Score +2 (Figs. 6 & 7) may cause no or mild weight loss, and skin pigment loss. Score +3 and Score +4 (Fig 8 & 9) reveal more coalescing of lesions and the intestinal wall is thickened. Weight loss occurs at these lesion scores.

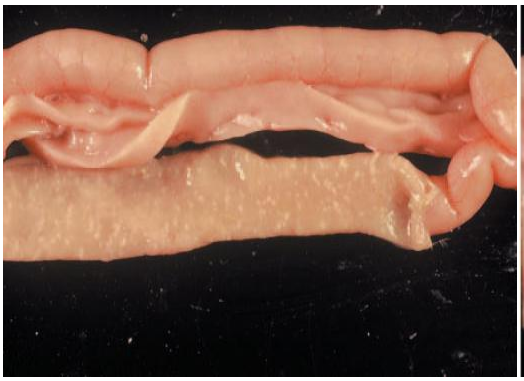


*Figure.6. E. mivati*Score +1

Conway and McKenzie(2007)

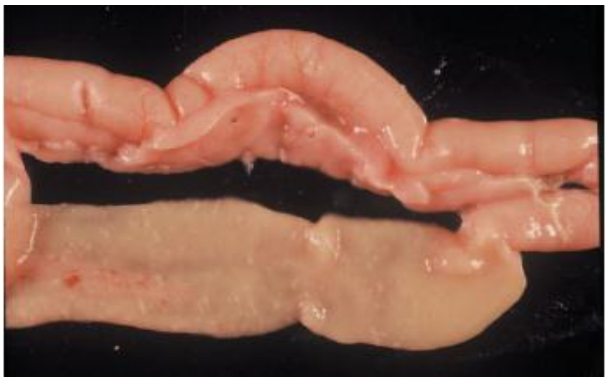


*Figure.7. E. mivati*Score +2

Conway and McKenzie (2007)

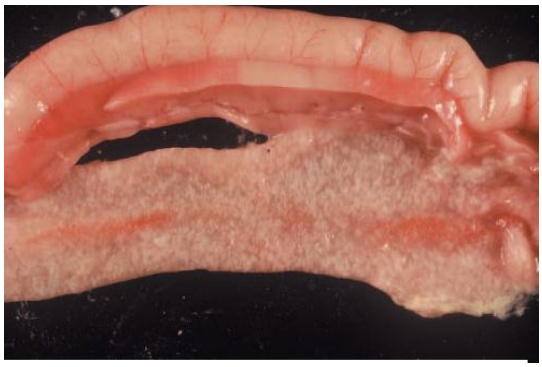


*Figure.8. E. mivati*Score +3

Conway and McKenzie(2007)

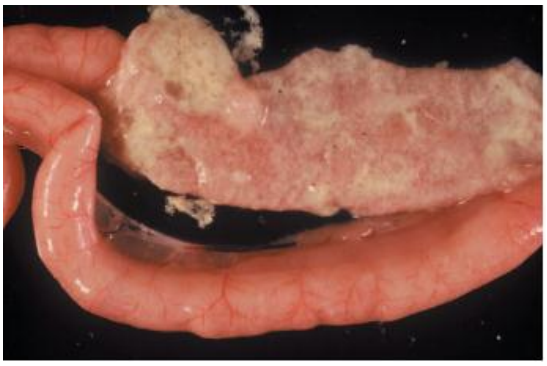


Figure.9. E. mivati Score+4

Conway and McKenzie(2007)

iii- *Eimeria praecox* (Johnson, 1930)

E. praecox often infect the duodenum (Fig. 10) and is overlooked sometimes in the field because no typical gross lesions exist. The basis of low pathogenicity may be due to short prepatent period (83 hours), but there may be watery or mucoid droppings, loss of

pigmentation, loss of weight, and depressed feed conversion (Williams et al., 2009). Pathogenicity of *E. praecox* and its effect on performance is debatable. Lowest administered dose ($5x10^3$oocysts per bird) had a significant impact on weight gain (Répérant et al., 2012). Jenkins et al. 2008 did not notice any significant effect of *E. praecox* on weight gain with an infective dose of 10^4 oocysts, although Répérant et al. (2012) observed a significant effect with a dose of 5×10^3. This conflict can be explained by variations in pathogenicity of the different strains of this specie (Répérant et al., 2012).

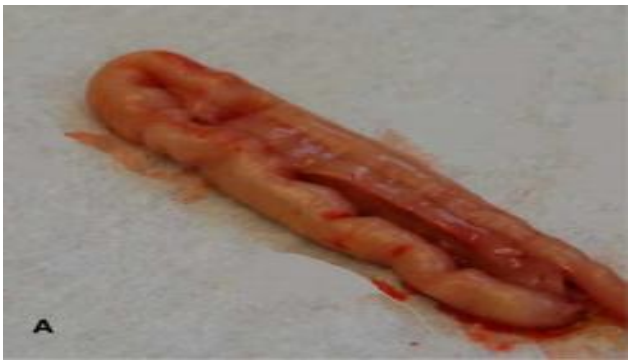


Figure.10. Birds infected with 10^6 sporulated oocyts of *E. praecox* showing wrinkled duodenum (Williams *et al..*, 2009).

iv- *Eimeria hagani* (Levine, 1938)

This specie is of low pathogenicity and infects the anterior part of the small intestine; it may produce mucosal inflammation and watery contents in the intestine.

v- *Eimeria necatrix* (Johnson, 1930)

E. necatrix is very well known specie by poultry producers because of the well-known lesions in the intestine. The lesions are found in mid intestinal area as that caused by *E. maxima*; however developing oocysts are found in the cecum. This feature is valuable in the diagnosis of this specie. The oocysts that are located in the cecum are near in size to oocysts of *E. tenella*. This highly pathogenic specie typical signs include high morbidity, mortality, loss of skin pigmentation, and reduced growth that are associated with hemorrhagic enteritis. Layer pullets (7-20 weeks) infected with *E. necatrix* may suffer decreased flock uniformity, and decreased egg-laying capacity. *E. tenella and E. necatrix* maximum damage occur during the asexual phase when large schizonts rupture. The range of $2x10^4$ -$8x10^4$ oocysts of *E. necatrix* were enough to cause severe weight loss, morbidity, and mortality (Hein, 1971). Chickens that were inoculated orally with C. perfringens after *E. necatrix* inoculation, had significantly increased numbers of C. perfringens especially in the jejunum and ileum where the endogenous stages of *E. necatrix* take place (Baba et al. 1997). Score +1 and Score +2 (Figs. 11 & 12) show petechiae and white plaques on the serosal surface - salt and pepper appearance - associated with ballooning and increased mucus secretion. Score +3 and Score +4 (Figs. 13 & 14) show more packed petechiae and white plaques on the serosal surface, intestinal mucosa is thickened and contents are tinged with blood and mucus while contents with E. maxima may be orange. Definitely weight loss and poor feed conversion occur, and birds do not eat or drink. Microscopic examination on day 4-5 post inoculation may show many aggregates of large schizonts (66µm) (Figs. 15 & 16), with hundreds of merozoites. These aggregates are found deep in

the mucosa and submucosa causing damage to the layers of smooth muscle and blood vessels.

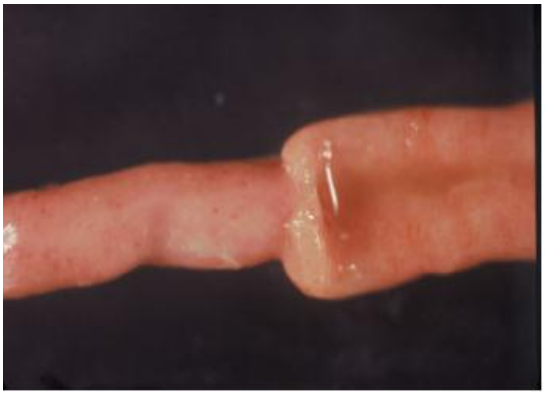


Figure.11. *E. necatrix* Score +1
Conway and McKenzie (2007)

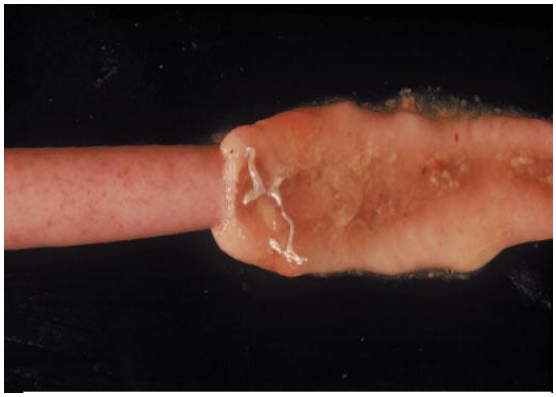


Figure.12.*E. necatrix* Score+2
Conway and McKenzie (2007)

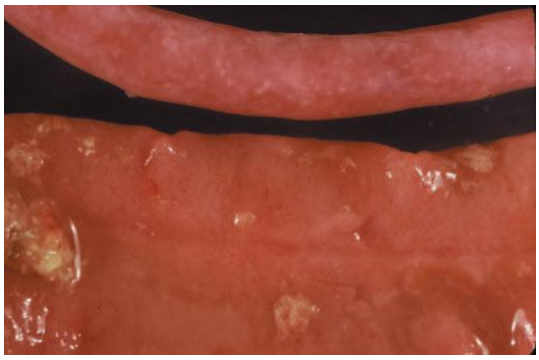


Figure.13.*E. necatrix* Score +3
Conway and McKenzie (2007)

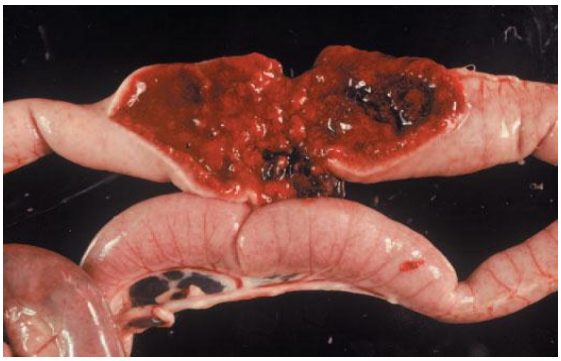


Figure.14. *E. necatrix* Score +4
Conway and McKenzie (2007)

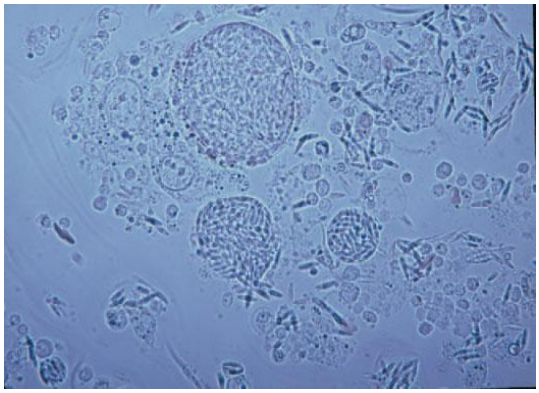


Figure.15. E. necatrix schizonts (low power). Conway and McKenzie (2007)

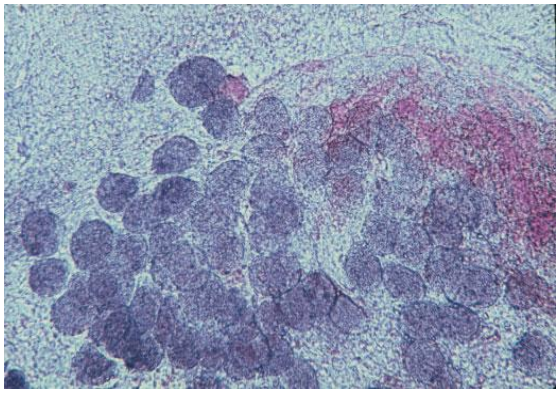


Figure.16. E. necatrix schizonts (high power). Conway and McKenzie (2007)

vi- *Eimeria maxima* (Tyzzer, 1929)

E. maxima as *Eimeria necatrix* is often found in the mid intestinal area. In heavy infections, the lesions may extend throughout the small intestine. *E. maxima* is easy to recognize because of the characteristic large oocysts (21-42 x 16-30µm), and was named for its large oocysts. This specie can be differentiated from *E. necatrix* by the absence of large schizonts. *Eimeria maxima* is a moderately pathogenic specie. Infections with $5x10^4$ - $20x10^5$oocysts cause morbidity, mortality, diarrhea, loss of skin pigment, anorexia and weight loss (Schnitzler & Shirley, 1999). Poultry producers concerned about good skin color are also concerned about subclinical infections of this specie that causes significant effect on skin color due to decreased absorption of xanthophyll and carotenoid pigments in the midgut. The majority of tissue damage occurs with sexual stages (oocysts) of *E. maxima.* Score +1 and Score +2 (Figs. 17 & 18) rated as mild infections, showing few petechiae on the serosal surface. Intestinal contents are slightly orange. These lesion scores are accompanied by some weight and pigment loss from the blood and skin. Score +3 and

Score +4 (Figs. 19 & 20) show thickening of the intestinal wall and ballooning that may occur with moderate and severe infections.

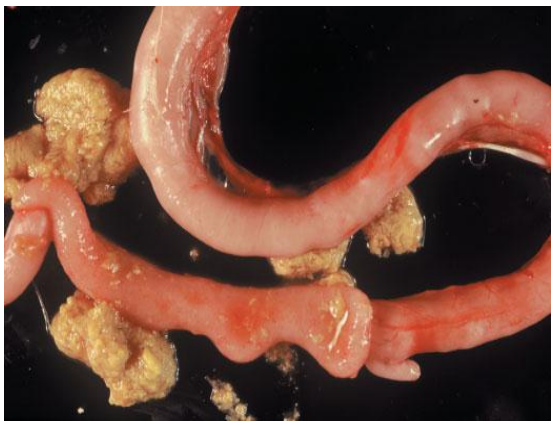


Figure.17. *E. maxima Score* +1

Conway and McKenzie (2007)

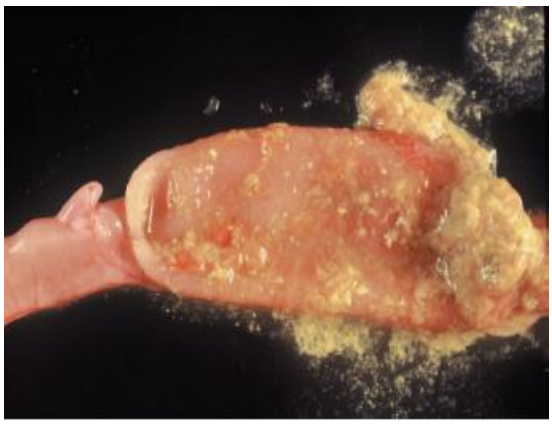


Figure.18. *E. maxima Score* +2

Conway and McKenzie (2007)

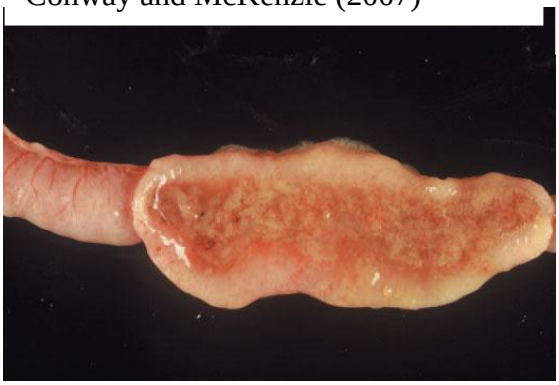


Figure.19. *E. maxima* Score +3

Conway and McKenzie (2007)

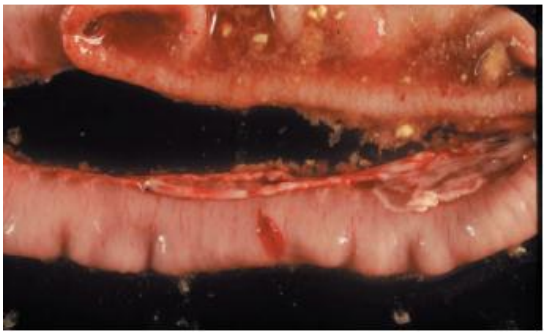


Figure.20. *E. maxima* Score +4

Conway and McKenzie (2007)

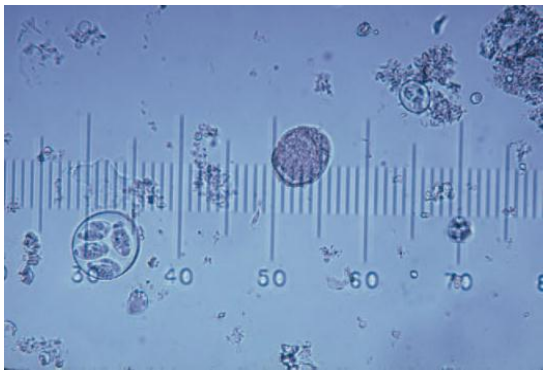


Figure 21. The center of the figure shows large oocyst of *E.maxima* and its characteristic brownish red color. Sporulated oocyst of *E. maxima* to the left and greatly smaller sporulated oocyst characteristic of *E. mivati* or *E. mitis* are found to the right. Source: Conway and McKenzie (2007)

vii- *Eimeria brunetti* (Levine, 1942)

This specie is found in the lower small intestine, and more to the large intestine and ceca in severe cases. Field infections are hard to recognize because of no typical lesion. *E. brunetti* is less pathogenic than *E. tenella or E. necatrix*. Two weeks old chicks inoculated with $8x10^5$oocysts of E. brunetti can show morbidity, loss of weight and mortality of about 30% (Hein, 1974). Score +1 and Score +2 (Figs. 22 & 23) show few petechiae on the serosal surface and roughened mucosal surface in the lower small intestine that might be detected by feeling it more than by sight. Score +3 (Fig. 24) shows hemorrhagic bands and coagulated materials that were sloughed off the mucosa and mixed with intestinal contents. Drying up of the cecal contents may occur on days 6 and 7 of *E. brunetti* infection. Weight loss and feed conversion efficiency is reduced at this stage. Score +4 (Fig. 25) shows the mucosa that is badly damaged, and the whole mucosal membrane is eroded due to heavy infection. A core (cottage cheese like) may form from this coagulated material, and may

obstruct the intestine resulting in death. Score +4, is rare in the field. Mild and moderate infections are more common and are mostly overlooked.

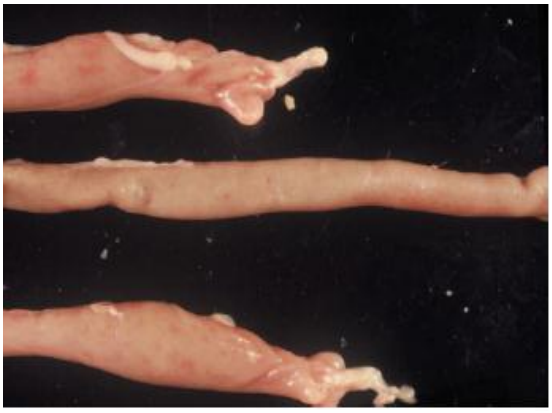


Figure.22. *E.brunetti* Score +1

Conway and McKenzie (2007)

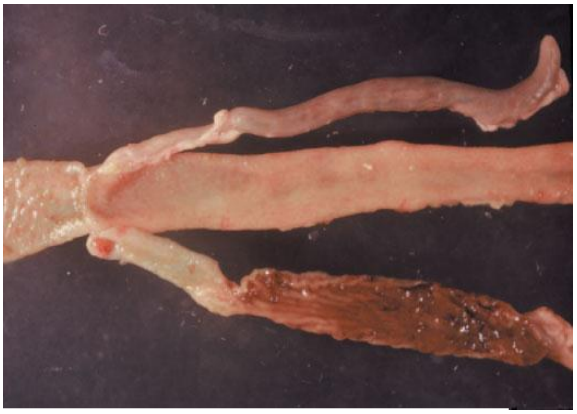


Figure.23. *E. brunetti* Score +2

Conway and McKenzie (2007)

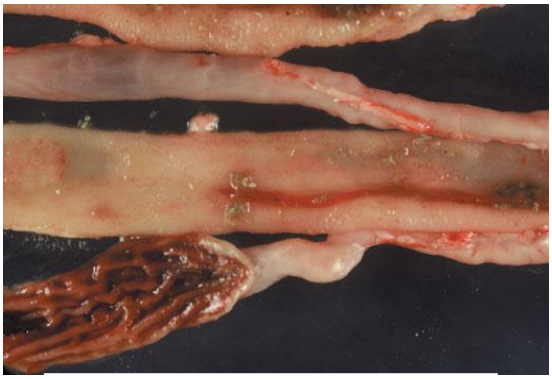


Figure.24. *E. brunetti Score* +3

Conway and McKenzie (2007)

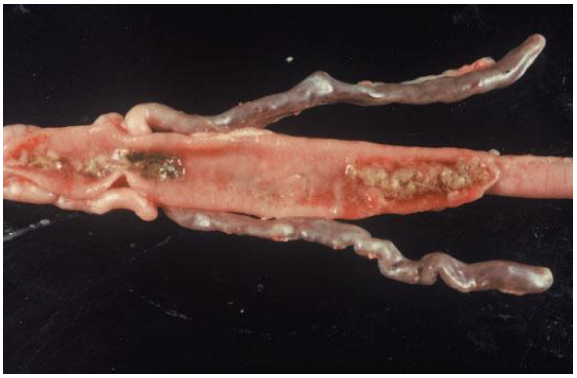


Figure.25. *E. brunetti* Score +4

Conway and McKenzie (2007)

viii- *Eimeria tenella* (Railliet & Lucet, 1891)

E.tenella is one of the famous species among poultry producers due to the high mortality and characteristic cecal lesions. *E. tenella* invades the epithelium of the ceca, and causes mortality, morbidity, loss of weight, loss of skin pigment, and bleeding. The cecal cores show clear blood that often forms firm bloody cores. Large schizonts and oocysts are often found in the cecal lesions. Inoculation with 104oocysts is enough to cause mortality, morbidity, and severe weight loss making it one of the most pathogenic species. *E. tenella* as *E. necatrix* produces large second generation schizonts at day 4 post infection that are the most pathogenic stage. Hematocrite value and erythrocytes count are reduced by 50 % due to blood loss. Score +1 (Fig. 26) shows few scattered petechiae that are seen on the opened and unopened cecum. Cecal contents (not shown) and cecal wall thickening are normal. Score +2 (Fig. 27) shows more petechiae on the the ceca and more hemorrhage on the mucosal surface. Thickening of the mucosal surface is slight, and clinical signs at this stage are evident in infected chicks. Score +3 and Score +4 (Figs. 28 & 29) show more bleeding and clotting, this clot will harden joining the sloughed mucosa. Clinical signs include bloody droppings.

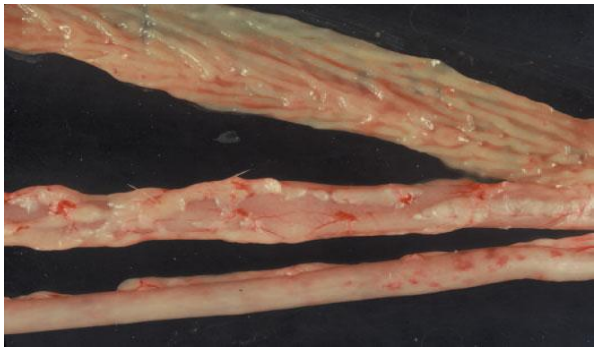


Figure.26. E.tenella Score +1

Conway and McKenzie, (2007)

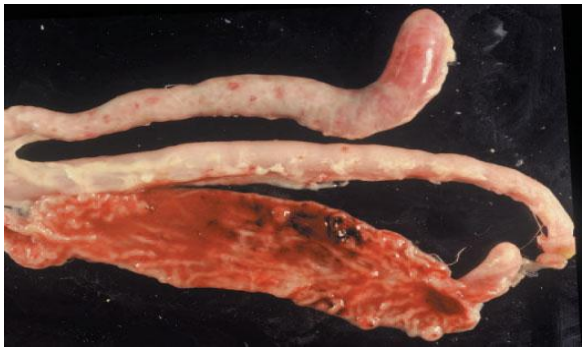


Figure.27. *E. tenella Score* +2

Conway and McKenzie, (2007)

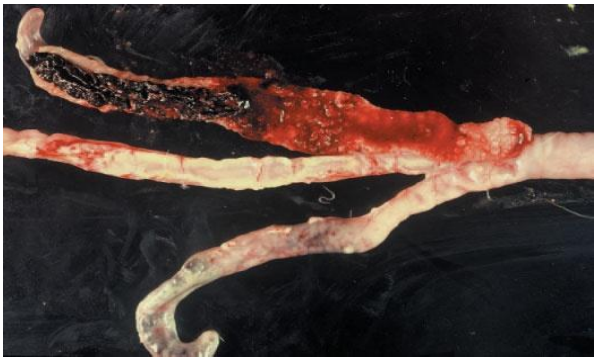


Figure.28. E. tenella Score +3

Conway and McKenzie, (2007)

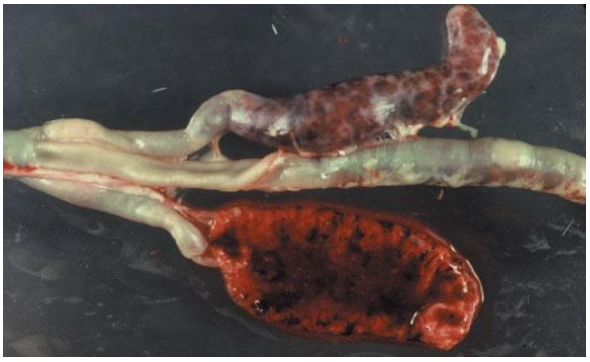


Figure.29. E.tenella Score +4,

Gangrene is seen in purple area. Conway and McKenzie, (2007)

ix- *Eimeria mitis* (Tyzzer, 1929)

Eimeria mitis is found in the lower small intestine, from the yolk sac diverticulum to the ileocecal junction. No typical lesions are found, but E. mitis has a negative impact on weight gain, feed conversion, morbidity, and pigment absorption was recently indicated (Fitz-Coy & Edgar, 1992). Average of $5x10^5$ -$1.5x10^6$ oocysts of *E. mitis* will cause weight

loss, morbidity and loss of skin pigment. This specie is overlooked sometimes especially in mild infections due to indistinctive lesions. Table 7 summarizes *Eimeria species*, pathogenicity, oocysts morphology and size besides location in the intestine.

Table 7. Oocyst, habitat, morphology and pathogenicity of *Eimeria* spp. (Levine 1985)

Species	Host /Habitat	Oocysts size (µm)		Shape	pathogenicity	Reference
		length	width			
E. acervulina	Small intestine	12-23	19-17	Ovoid	Low	Tyzzer (1929)
E. brunetti	Small intestine, rectum, ceca, cloaca	14-34	12-26	Ovoid	Moderate	Levine (1942)
E. hagani	Small intestine	16-21	14-19	Ovoid	Low	Levine (1938)
E. maxima	Small intestine	21-42	16-30	Ovoid	Low to moderate	Tyzzer (1929)
E. mitis	Small intestine	10-21	9-18	Subspheric	Low	Tyzzer (1929)
E. mivati	Small intestine, large intestine	11-20	12-17	Ellipsoid or ovoid	Low to moderate	Edgar & Siebold (1964)
E. necatrix	Small intestine, ceca	12-29	11-24	Ovoid	High	Johnson (1930)
E. praecox	Small intestine	20-25	16-20	Ovoid	No	Johnson (1930)

E. tenella	Ceca	14-31	9-25	Ovoid	High	Raillet & Lucet (1891)

c. Identification and diagnosis of Eimeria species

i- Identification

Identification of different *Eimeria spp*. is dependent on traditional (Conway and Mckenzie, 2007) and molecular methods as:

- Zone parasitized in intestine
- Gross lesions
- Oocyst morphology
- Minimum prepatent period determined experimentally
- Schizont size and location
- Minimum sporulation time
- Parasite location in the host intestinal epithelium
- Immunogenicity tests (cross-immunization tests)
- Molecular techniques.

Due to overlapping of morphological characteristics of *Eimeria spp*, identification was recently improved by classification through computer examination using microscope digital images. Different species of Eimeria oosyts vary in size, contour, thickness and color of the oocyst wall (Castañón César et al., 2007 & Fig. 30).

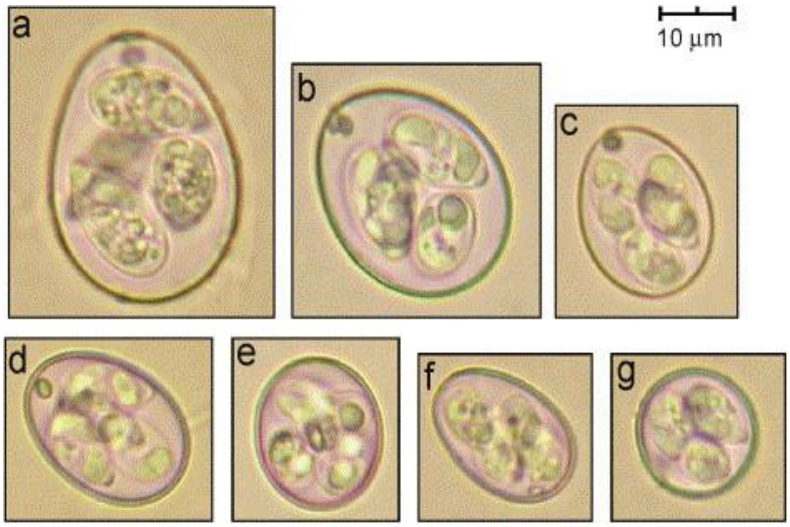


Figure.30. Photomicrographs of oocysts of seven Eimeria species of chicken. *E. maxima*, (b) *E. brunetti*, (c) *E. tenella*,(d)*E. necatrix*,(e) E. praecox,(f*) E. acervulina*, and (g) E. mitis. Source: Castañón, César et al., (2007).

ii- Diagnosis

Oocyst counts technique

Oocyst counting is one of the most extensively used parameters of Eimerian infection in chickens (Holdsworth et al., 2004). An oocyst count is done using McMaster chamber method, this method is used in intestinal, litter and fecal oocyst counts. The % sporulation and oocysts dimensions are not essential for this method. Variations of this method have been described by (Long & Rowell 1958); (Hodgson 1970); Williams (1973, 1995); Long et al. (1976); Ministry of Agriculture, Fisheries and Food (1986); Eckert et al. (1995); Peek & Landman (2003); Haug et al. (2006) and Conway & Mckenzie (2007). Williams et al. (2001) developed a new method for counting eimerian oocysts at very low

concentrations in aqueous suspensions. Methods used for counting eimerian oocysts either by isolation from intestine, litter or fecal samples (salt-flotation, McMaster chamber method) include a dilution step due to high number of oocysts needed to be counted. Multiplication factor to determine number of oocysts /ml may be no less than one thousand depending on the degree of dilution. This is undoubtedly unsatisfactory level of sensitivity if the sample contains as low as a few tens of oocysts/ ml.

Molecular tools for identification of *Eimeria spp*. is of importance. PCR procedure can provide a sensitive means for diagnosis based upon the internal transcribed spacer-1 (ITS-1) regions of ribosomal DNA (rDNA) that were sequenced and regions of distinctive sequences identified (Schnitzler et al., 1998; 1999). This test was used as a base for molecular diagnosis of *Eimeria spp*., and to study intrastrain variation of chicken Eimeria (Lew et al., 2003; Su et al., 2003). However, due to the polymorphic nature of ITS-1 of E. maxima strains and occurrence of variability in amplification, species RAPD-derived markers, the SCAR (sequenced -characterized amplified region) which can stand for species-specific or strain-specific genetic DNA markers was found (Paran & Michelmore, 1993).

3. Control by synthetic coccidiostat

a. Control of coccidiosis: Prophylactic coccidiostatic drugs and their mode of action

Coccidiosis has been a major cause of poor performance and loss of productivity in poultry industry. Early recommendations to control coccidiosis were established through

improvements in management and hygiene. This management practice is still found till today but the intensive nature of the poultry industry ensures the continuous presence of coccidia. Attempts to eliminate the disease were ineffective.

The report about sulfonamide effect to control Eimeria infections led to research about properties of these compounds (Levine, 1939). At that point in time it was thought that sulfonamides were just used to treat sick birds. Later, a continuous in feed low concentration of sulfaquinoxaline to control Eimeria was reported by Grumbles et al. (1948). These workers showed for the first time that it was achievable to control coccidiosis by the continuous inclusion of a low level of a drug in the feed of chickens (prophylaxis or prevention). In the same year sulfaquinoxaline was introduced as a commercial product and the age of chemotherapy started. Horton Smith (1951) wrote that "we believe that continuous feeding of any drug, even at a low level, as a means of preventing disease is unwise because of our ignorance of the possible long-term effects on the bird itself". He pointed out that resistance to Eimeria may develop due to inclusion of low concentrations of the drug.

The use of suboptimal levels of the anticoccidial drugs may increase the probability of selecting drug-resistant strains (Chapman, 1984). Shortly after introduction of Sulphaquinoxaline and Nitrofurazone resistance had been reported (Cuckler & Malanga, 1955; Waletzky et al., 1954). Since that time, many reports concerning anticoccidial drugs resistances were reported. It is therefore possible that in medicated birds adequate parasite multiplication may occur and permit immunity development, a fact that permitted producers to increase the duration of the withdrawal period (less cost or less food residues),

with a risk of susceptibility to coccidiosis and outbreaks (McDougald and Reid, 1971). From a practical point of view, it is important not to withdraw anticoccidial drugs ahead of time, since birds may not have developed solid immunity. Solid immunity in medicated birds did not develop until birds were 6 to 7 weeks of age (Chapman, 1999a,b).

b. Classification

Anticoccidial drugs are classified as either chemical, ionophores and mixed as mentioned below according to Chapman (1999 a, b); Allen & Fetterer (2002); Conway & Mckenzie (2007); Peek & Landman (2011).

i- Synthetic drugs (chemicals)

These are created by chemical synthesis, and have definite modes of action in face of parasite metabolism.

ii- Polyether antibiotic (ionophores)

These are created by fermentation of *Streptomyces* species or Actinomadura species. Ionophores work throughout common mechanisms of shifting ion transport and disturbing osmotic balance. These drugs are today the basis of coccidiosis control (Jeffers, 1997). Ionophores are classified into the following groups:

iii- Monovalent ionophores

monensin, narasin and salinomycin

iv- Monovalent glycosidic ionophores

maduramicin and semduramycin

v- Divalent ionophores

Lasalocid

vi- Mixed products

Maxiban® (nicarbazine/narasin)

Lerbek® (Meticlorpindol/methylbenzoquate)

c. Mode of action

The investigation for new drugs would be easier if the approach is based upon thorough knowledge of the biochemical pathways found in the parasite and how they vary from those of the host. This may lead to the finding of new enzymes that could be a goal for drug inhibition (Table 8). Important examples of pathways in Eimeria, and not in the host are folic acid and purine salvage pathways (Eimeria cannot make purines by itself, it has to have the ability to take exogenous purines with the aid of purine salvaging enzymes) (Wang, 1978; Wang & Simashkevich, 1981). Recently companies did not introduce new

anticoccidial drugs to the market. However, two drugs might have this capacity, and those target enzymes of mannitol cycle (Allocco et al., 1999, Smith et al., 1998) and trophozoite histone deacetylase (Smith et al., 1998). Table 8 gives a brief view of the metabolic processes that are affected by the anticoccidial drug, their mode of action and speed of resistance.

Table 8. Metabolic process affected by anticoccidial drugs, mode of action and speed of resistance. Source: Chapman (1997)

Metabolic process	Drug	Mode of action	Resistance[a]
Membrane function	lonophores	Cation transport	Slow
Cofactor synthesis	Amprolium	Thiamine uptake	Slow
	Sulphonamides + DHFR	Folate synthesis	Slow
	inhibitors		
Mitochondrial function	Quinolones	Electron transport	Rapid
	Clopidol		Moderate
	Nicarbazin	?	Slow
	Robenidine	?	Moderate
Unknown	Halofuginone	?	Moderate
	Diclazuril	?	Moderate

[a]Resistance has eventually developed to all drugs that have been introduced.

?Mechanism unknown

d. Products affecting cofactor synthesis

i- Amprolium

Amprolium was used extensively as a prophylactic drug, but with the introduction of ionophores its use has declined. Like the sulphonamides, amprolium is used as water treatment for clinical coccidiosis. Amprolium is a thiamine antagonist. A thiamine antagonist may be defined as a compound that can compete with thiamine or thiamine derivatives in enzymatic reactions. Amprolium blocks thiamine absorption in coccidia at relatively low levels and have an antagonistic effect on Vitamin B1. Thiamine is converted to thiamine pyrophosphate in the cell an important coenzyme in carbohydrate metabolism. However, Amprolium due to the lack of hydroxyl group that is found in thiamine it cannot be pyrophosphorylated, thus these reactions does not take place. Thiamine transport in the parasite is more sensitive to Amprolium than that of the host, making Amprolium efficacious against the parasite (Rogers, 1962). Amprolium competitively inhibits the uptake of thiamine by second generation schizonts of *E. tenella* (James, 1980). It is effective against cecal species as *E. tenella and E. necatrix* and to a lesser degree against *E. acervulina, E. maxima and E.brunetti* (McLoughlin and Gardiner, 1962). Amprolium does not affect immunity development (Karlsson and Reid, 1978). Since amprolium has poor spectrum of activity, it is used in mixtures with the folic acid antagonists as ethopabate and sulfaquinoxaline.

ii- Folate antagonists and inhibitors

According to Conway & Mckenzie (2007), this class includes:

-Ethopabate

-Sulfonamides

Sulfadimethoxine, Sulfaguanidine, Sulfamethazine, Sulfanilamide, and Sulfaquinoxaline

-2, 4 Diaminopyrimidine (DAPs)

-Diaveridine, pyrimethamine, and ormetoprim that are used in combination with sulfonamides for improved efficacy several products with anticoccidial activity act by blocking a biochemical Pathway in the parasite through affecting an important cofactor in the pathway (Greif et al 2001). Folic acid, or tetrahydrofolic acid, plays an important role in the synthesis of purines and thymidylate, and therefore is a vital cofactor for nucleic acid synthesis and for cellular replication (Bertino and Johns, 1967). Thus, interference with folate metabolism can affect cell growth.

- Ethopabate is a folate antagonist that inhibits folic acid pathway and blocks a step in the synthesis of *para*-amino benzoic acid (PABA) (Rogers et al., 1964). Ethopabate affects 2nd generation schizonts and mostly active against E. maxima and E. brunetti (Reid, 1975). Ethopabate is used in conjunction with Amprolium to improve its efficacy and spectrum of activity.

-Sulphonamides also inhibit the folic acid pathway by inhibiting the enzyme dihydropteroate synthase (not present in the host) that is important in the synthesis of dihydrofolate, blocking the conjugation of pteridine and PABA. Sulphonamides affect 2nd

generation schizonts (Reid, 1975). These products are effective against intestinal coccidiosis as *E. brunetti, E. maxima, and E. acervulina* and to lesser degree against cecal coccidiosis as *E. tenella and E. necatrix* (Ryley & Betts, 1973). An important concern about the use of these sulfonamide drugs is their high ability for toxic effects in chickens at doses close to or within the range of their recommended levels, especially at recommended dose for therapeutic treatment (Peckham, 1978; Julian, 1991). Care in estimating the dose for treatment is very important when given in feed or water. Signs of toxicity may include decreased egg production, loss of eggshell pigment, hemorrhagic syndrome, bone marrow depression, thrombocytopenia and depression of immune system.

- 2, 4 Diaminopyrimidine (DAPs): Another step in the folate pathway is the reduction of dihydrofolate to tetrahydrofolate (Active form of folic acid and important in the synthesis or purines and pyrimidines in coccidial parasite) by the enzyme dihydrofolate reductase (DHFR) and this enzyme is inhibited by DHFR inhibitors aspyrimethamine (DAPs). Pyrimethamine alone has little anticoccidial activity and is able to potentiate the action of sulphonamides and is used in mixture with sulphonamide shaving a clear synergistic effect (Kendall & Joyner, 1956). Similar to sulphonamides, DAPs affect 2nd generation of schizonts (Reid, 1973).

e. Products affecting mitochondrial function

i- 4-hydroxyquinolone group

Three products under 4-hydroxyquinolone group affect the mitochondrial function namely:

- Buquinolate
- Decoquinate
- Nequinate (methyl benzoquate).

These products show an anticoccidial activity at very low doses, and inhibit respiration of the coccidial parasite (E. tenella) by blocking the electron transport system in the mitochondria of this parasite and are shown to inhibit respiration of sporocysts and sporozoites of E. tenella, with 100 folds less activity against chicken liver mitochondria (Wang, 1975; Wang, 1976); however, quinolones rapidly induce resistance (McManus et al., 1968) that have limited their use in the field. Quinolones arrest sporozoite stage, thus affecting adequate immunity development (Reid, 1973;Yvoré, 1968). These Quinolone drugs are effective against *E. acervulina*, *E. brunetti*, *E.maxima, and E. mivati and to lesser extent E. tenella and E. necatrix* (Ryley and Betts, 1973).

ii- Pyridone group

- Clopidol
- Meticlorpindol

The most important member of the group is Meticlorpindol that acts as quinolones by inhibiting electron transport system in the mitochondria, but at another level due to the fact that cross- resistance with quinolones does not exist. Synergism between Meticlorpindol and 4-hydroxyquinolone drugs was found (Challey and Jeffers, 1973). The known widely used product Lerbek ® (Meticlorpindol/methylbenzoquate) is an example of this Synergism. Similar to Quinolone this pyridone group affects early stages of the life cycle of

all Eimeria (Reid, 1973; Ryley and Wilson, 1975), thus inhibiting immunity development (Bennejean et al., 1970).

iii- Nicarbazine (4, 4'-dinitrocarbanilide)

Studies by Ott et al. (1956) and Sherwood et al. (1956) indicated that Nicarbazine adversely affect eggshell pigmentation, egg production, and egg hatchability depending on the in-feed dose of nicarbazin. Low level of 50 ppm of nicarbazine given in layers feed resulted in extensive mottling of egg yolks (Polin et al.,1957). Studies by Beers et al. (1989) indicated that Nicarbazin (125ppm in broiler feed) increases body temperature in heat-stressed birds, which resulted in adverse effect on blood acid-base balance, blood lactate, and heart rate than in control-fed birds. The exact mode of action of Nacarbazine in not known , and is thought to be through inhibition of succinate-linked nicotinamide adenine dinucleotide (NAD) reduction in mitochondria of beef hearts and the energy-dependent transhydrogenase and calcium accumulation in the rat liver mitochondria (Dougherty, 1974). Nicarbazine affects 2ndgeneration schizonts but earlier stages are also affected (McLoughlin & Wehr, 1960). Nicarbazine is mostly effective against *E. tenella, E. necatrix* and *E. acervulina* and to a *minor extent E. maxima* and *E. brunetti* (Morrison et al., 1961). Ott et al.(1956), and McLoughlin et al. (1957) indicated that Nicarbazin allowed immunity development, although Berg et al. (1956) indicated that there was no immunity development against *E. necatrix* with Nicarbazin.

iv-Robenidine hydrochloride

The exact mode of action is not known; however in mammals studies showed that Robenidine at high concentrations inhibit oxidative phosphorylation of mitochondria, assuming that it has the same function in the parasite (Wong et al., 1972). Robenidine acts on 1st generation schizonts, allowing development of immunity (Ryleyand Wilson, 1971; Karlsson and Reid, 1978). Robenidine is active against all species of Eimeria (Kantor et al., 1970). Robenidine sensitivity is best managed when used in rotation with other anticoccidial drugs (Chapman, 1989).

v- Toltrazuril

Toiltrazuril is possibly affecting mitochondrial function and belongs to the triazine class of compounds that have a high degree of activity against Eimeria species when given in feed or water at relatively low doses of treatment. The anticoccidial action of this class is cidal, not static (Chappel et al., 1974; Ryley et al., 1974; Haberkorn& Stoltefuss, 1987). Harder, & Haberkorn (1989) indicated that the activities of some enzymes of the respiratory chain, such as NADH oxidase, succinate-cytochrome C reductase, and succinate oxidase from mouse liver, decreased in the presence of toltrazuril. Toltrazuril also exhibited an inhibitory effect on the dihydroorotate-cytochrome C reductase from mouse liver. Concluding that toltrazuril has an effect on respiratory chain. Recently, Hackstein et al. (1995) indicated that Toltrazuril targets plastid-like organelles in the parasite. Toltrazuril

affects all intracellular stages and is active against *Eimeria tenella, E. acervulina* and *E. maxima* (Mehlhorn et al., 1984, 1988; V´azquez, and V´azquez, 1990; Mathis et al., 2003). Toltrazuril does not affect immunity development (Greif & Haberkorn, 1997; Greif, 2000).

f. Products with effect on the cell membrane

Polyether ionophores: These drugs are not effective for therapeutic treatment in poultry and are able to form lipid-soluble complexes with mono- or divalent cations as Na+, K+, Ca++, and Mg++, and transporting them across cell membranes causing osmotic damage (Pressman, 1976; Smith and Strout, 1979). These later writers found that both narasin and lasalocid caused accumulation of considerable quantities of ionophores in extracellular sporozoite (prior to invasion) of E. tenella with no consequences on the host cell. Studies on monensin, salinomycin, and lasalocid showed that these ionophores altered Na-K pump in the cytoplasmic membrane (Smith and Rozengurt, 1978; Austic and Smith, 1980; Smith and Galloway, 1983). The entry of sodium into the parasite exceeded its ability to remove it, and resulted in death. These ionophores allow some degree of infection and immunity development depending upon the *Eimeria spp*, dosage of ionophore used, and the intensity of infection (Jeffers, 1989; Chapman & Hacker, 1993; Chapman, 1999 a, b).

g. Products with unknown mode of action

i- Diclazuril

Diclazuril belongs to the nucleoside analogue group and it is effective at 1ppm in diet against *E. tenella, E. acervulina, E. necatrix, E. brunetti, E. maxima,* and *E.mitis* (Vanparijs et al., 1989), however its exact mode of action in not known. Diclazuril affected both the asexual and sexual development of *Eimeria tenella*, (Verheyen et al., 1988) resulting in complete degeneration of schizonts and gamonts. The establishment of normal pattern of oocyst wall was completely disturbed, resulting in thickened and incomplete oocyst wall (Verheyen et al., 1989).

ii- Halofuginone

Halofuginone is of unknown mode of action. Christensen et al., (1994) indicated that halofuginone hinders collagen synthesis, and reduced skin strength causing increased incidence of skin tears at slaughter time. Halofuginone was greatly effective against E. *tenella, E. maxima, E. acevulina, E. necatrix, E. brunetti*, or *E.mivati* at 3 ppm in broiler feed, and not effective against *E. acervulina* as other species. The drug was found to be cidal rather than static (Edgar and Flanagan, 1979).

4. Essential oils as anti-protozoal alternatives

The use of natural therapeutic antimicrobial plant products, such as eucalyptus and peppermint oils hosts many useful bioactive substances for the treatment of various infections. For example, the use of essential oils against zoonotic *Salmonella* bacteria and many other antibiotic resistant pathogens. The antibacterial activity of plant essential oils

was reported for the first time in 1881 (Boyle, 1955). According to Burt (2004), Eucalyptus globulus Labill is the main source of eucalyptus oil in the world. It's an antiseptic used for relieving symptoms of cold, sore throat, cough, and other infections. While on the other hand peppermint is known for its antibacterial, antifungal, anti-inflammatory, and decongestant properties. Eucalyptus and peppermint are responsible for enhancing the efficacy of the immune system.

The mechanism of action of these essential oils can't be confirmed since different components display antimicrobial mode of action not only at a particular location but also at different cell sites (Carson, Mee, Riley, 2002). For this reason, the phenolic, volatiles and terpene compounds found in plant essential oils where the main attraction (Sikkema et al., 1995).

Terpenes have the ability to disrupt and penetrate the lipid structure of the cell membrane of bacteria resulting in the changes of cell function, leading to denaturing of proteins, and release of ionic molecules, causing starving conditions. Destruction of cell membrane leads to cytoplasmic leakage, cell lysis and eventually cell death (Fisher & Phillips, 2008; Oussalah, Caillet Lacroix, 2006).

Published studies have showed less sensitivity of negatively charged bacteria in comparison with positively charged ones. This is attributed to the additional polysaccharide layer as compared to positively charged bacteria lacking this outer cell wall coverage (Weidenmaien 2008).

- Peppermint and eucalyptus

Essential oil (EO) of peppermint (*Mentha piperita*) was tested against biofilm formation of S. enterica serotype Enteritidis S64 on stainless steel surface. The minimum inhibitory concentration (MIC) for peppermint oil was 7.80 μL/mL; at the MIC, the antimicrobial activity of peppermint EO was 13.000 ± 4.68 and 16.900 ± 1.30 AU/MI. Sanitizing solution formulated with sodium hydroxide NaOH 1% added to Tween 80 0.5% and essential oil at 7.8 μL/mL1 showed powerful anti-biofilm effect after treatment. (Valerianoa et al, 2012).

In a study conducted by Bianchini et al. (2014), 25 essential oils where tested against *Salmonella typhimurium*. They tested it by disc diffusion and quantified by agar dilution. The best inhibitors for Salmonella were cinnamon essential oil (EO) at 0.05% and thyme EO at 0.1%. Lime, thyme and cinnamon had the highest antimicrobial activity against salmonellae (gram negative) while eucalyptus citridora, thyme, peppermint, cinnamon and spearmint had the highest effect against P. roqueforti (gram positive). Eucalyptus citridora and peppermint had no effect against salmonellae while Eucalyptus globulus had a slight effect (8 mm<diam<11 mm) (Bianchini et al 2014).

Using an agar dilution method, eucalyptus and peppermint were examined for activity against, Salmonella *enterica* subsp. *enterica* serotype *typhimurium*, and other gram negative bacteria namely, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii, Aeromonas veronii* biogroup *sobria, Serratia marcescens* Peppermint inhibited all organisms except Ps. aeruginosa at (MIC) ≤2•0% (v/v). On the other hand eucalyptus had an inhibition effect on all organisms between

(MIC) 0.5 and ≤2.0% (v/v). Eucalyptus had ≤2.0% (v/v) effect on salmonella while peppermint had a 1.0% (v/v) (Hammer, 1999).

In another study, peppermint was tested to be used for the preservations of freshly cut vegetables based on its anti-microbial properties and appealing aroma. The major volatile constituents of peppermint (M. piperita) were menthol, menthone, menthyl acetate, menthofuran. They had antimicrobial effect against B. brevis, V. Choleraei, E. coli, K. pneumoniae, P. aeruginosa, A. flavus, A. niger, P. corylophilum (Ayala Zavala et al 2009).

Four different forms of peppermint (Mentha piperita) aqueous infusion, decoction, juice and essential oil, where tested against antimicrobial activity of 11 gram negative bacteria. Both infusion and decoction peppermint had no effect on all gram negative bacteria; on the other hand essential oil peppermints exhibited the highest antimicrobial activity in the standard disc diffusion method. Both essential oils and juice peppermints had an inhibition effect on all Salmonella and other gram negative bacteria which are the following S. typhi ,S. paratyphi , S. paratyphi , S. dysentriae , P. mirabilis , P. vulgaris , E. coli , K. pneumoniae , P. aeruginosa , Y. enterocolitica and E. aerogenes (Saeed et al 2006).

Eucalyptus globulus Labill , was analyzed by gas chromatography-mass spectrometry. It's leaf essential oil is composed of mainly of 12 compounds with, 1,8-cineole (85.8%) being the most abundant. Monoterpene hydrocarbons (12.45%) and oxygenated monoterpenes (87.32%) is where most of the antimicrobial activity takes place. Eucalyptus oil showed zero effect against salmonella at 5µl but a significant activity at the

concentration of 30 µl, with the diameter of the growth inhibition zone of 20 mm (with the use of different antibiotics, the diameters of the growth inhibition zones vary from 15 mm to 39 mm). while the antimicrobial activity of Eucalyptus globulus Labill against salmonella infantis expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was 3.13 mg/ml which is considered one of the lowest activity compared to the other 11 Gram-negative bacteria E. coli, Escherichia coli, Klebsiella pneumoniae , Acinetobacter baumannii, Morganella morganii, Proteus mirabilis, Providencia stuartii, Enterobacter cloacae, Citrobacter freundii, P. aeruginosa ATCC and Pseudomonas aeruginosa. (Damjanović-Vratnica et al 2011).

Main volatile components of eucalyptus oil where tested against salmonellae. The (MIC) Minimum inhibitory concentrations (% vol/vol) of the heavy end fraction of eucalyptus oil was 30 %(vol/vol), Piperitone had a 33%(vol/vol) the second most abundant component, terpinen-4-ol (87.9%) had a MIC of 0.17%(vol/vol) which is particularly effective against gram negative bacteria (Delaquis, 2002).

In the study made by Barbour et al 2015, a chemically characterized essential oil of eucalyptus and peppermint was tested on the most predominant Eimeria spp. involved in the economic disease of coccidiosis. The experimental groups were assigned to different treatments as shown in Table.9.

The assessed parameters included performance and pathological observations such as:

i- The mean live body weight and percent of weight increase in each broiler group,

ii- The mean feed conversion ratio,

iii-The mean percent mortality,

iv- The mean sore of lesions (0–3 scores) in each of the four intestinal organs (duodenum, jejunum, ileum and caecum)

v- Eimeria oocysts count per gram of each of the four intestinal organs, observed at the end of the 6-day period following each of the four challenges.

Table 9. Description of the different treatments

Groups and subgroups	Number of birds	Treatment with essential oil	Treatment at age (days)	*Eimeria* spp. challenge	Age at challenge (days)	Age at sacrifice (days)
G_1	40	No	–	No	–	
G_{1a}	10	No	–	No	–	20
G_{1b}	10	No	–	No	–	27
G_{1c}	10	No	–	No	–	34
G_{1d}	10	No	–	No	–	41
G_2	40	Yes		No	–	
G_{2a}	10	Yes	4–7, 16–18	No	–	20
G_{2b}	10	Yes	4–7, 16–18, 25–27	No	–	27
G_{2c}	10	Yes	4–7, 16–18, 25–34	No	–	34
G_{2d}	10	Yes	4–7, 16–18, 25–39	No	–	41
G_3	40	No	–			
G_{3a}	10	No	–	Yes	14	20
G_{3b}	10	No	–	Yes	21	27
G_{3c}	10	No	–	Yes	28	34
G_{3d}	10	No	–	Yes	35	41
G4						
G_{4a}	10	Yes	4–7, 16–18	Yes	14	20
G_{4b}	10	Yes	4–7, 16–18, 25–27	Yes	21	27
G_{4c}	10	Yes	4–7, 16–18, 25–34	Yes	28	34
G_{4d}	10	Yes	4–7, 16–18, 25–39	Yes	35	41

(Barbour et al 2014)

The authors reported that the Essential oil enhanced the performance and reduced the pathological effect of coccidial challenge.

In the absence of coccidial challenge, the mean per cent weight increase was greater in essential oil-treated group (G_2) as compared to that observed in the control group

(G_1). In addition, the essential oil improved the percent weight increase in challenged birds (54.6%) compared to the challenged-untreated birds (18.6%) ($P < 0.05$).

The mean feed conversion, mortality, intestinal lesion scores and oocyst counts were significantly reduced in the challenged-treated birds compared to the challenged-untreated birds ($P < 0.05$). (Barbour et al 2015)

Another study conducted by Giannenas et al (2003) aimed to investigate the possible use of oregano essential oil at the concentration rate of 300 mg/kg in feed against *Eimeria tenella* in broilers, which is known to be an extremely pathogenic *Eimeria species* that causes coccidiosis in the caeca. The efficiency of the oregano essential oil was compared with 75 mg/kg of anticoccidial lasalocid. Oregano oil consists of 30 ingredients; a large portion of it is constituted from phenolic compounds with varying antioxidative, antifungal or antimicrobial activity. Major constituents are thymol and carvacrol that constitute about 78 – 82% of the total essential oil. The supplementation with dietary oregano oil after two weeks from the infection with *E. tenella*, resulted in feed conversion ratios and body weight gains not significant from the non- infected group, but worse than those of the lasalocid group and better than those of the infected control group. These parameters are in correspondence with the survival rate, lesion score, oocyst numbers and extent of bloody diarrhoea. In conclusion, the study suggests that oregano essential oil proved to have an effect on *E. tenella*, but less than the effect exhibited by lasalocid. The oregano oil under the trade name Ecodiar is approved by the EU legislation as a feed additives and an appetite enhancer (Giannenas et al 2003).

An additional study was conducted by Oviedo et al (2006) in order to evaluate the effects of TWO essential oil blends Crina® ALTERNATE (CA) and Crina® POULTRY (CP) at 100 ppm). In broilers infected with mixed viable Eimeria spp. 8 treatments were evaluated which consisted of 3 treatments vaccinated at day of hatch with Advent® coccidia vaccine, 2 unvaccinated treatments and 3 controls. The results showed that the two specific EO blends had similar efficacy to maintain growth, reduce intestinal lesions and oocyst shedding after an induced mixed coccidia infection in non-vaccinated chicken. However, cocci-vaccinated broilers fed diets without essential oils had the best live performance, lowest oocyst shedding and anticoccidial index responses 7 days after a challenge with mixed *Eimeria spp* which was at 19 d old. The supplementation of these 2 specific essential oil blends to coccidia vaccinated broilers did not show any benefits under the present experimental conditions.

CHAPTER III

MATERIAL AND METHODS

A. Degree of compliance to Lebanese intensive system (IS) and free range chicken layers farms (FRCL) with the EU standards.

Experimental Design

A Completely Randomized Design was followed in this research, based on two different egg production systems, namely IS and FRCL.

1. Questionnaire

A questionnaire was prepared including data related to the compliance of practices, IS and FRCL with the EU standards, (Official Journal of the European Communities). (Appendices A and B).

2. Farms

Five farms were selected from each system, and 5 eggs were sampled per farm as per the below (Table 10).

Table 10. Selected farms in Study A

Farmer	Location	Number of collected eggs	System (IS / FRCL).
Mriti khawan	Wadi Jezzine	5	Free range
Lour Haddad	kfarhouna	5	Free range
Alfred Abed Al Nour	kfarhouna	5	Free range
Tony Abed Al Nour	kfarhouma	5	Free range
Pier Abou Rashid	Jezzine	5	Free range
Rashad Haj Hasan	Zahle	5	Conventional
Mohamad Sahili	Dalhama	5	Conventional
Aabes Masri	Baalbek	5	Conventional
Robert Sarkis	Aayen Kfar Zabad	5	Conventional
Nasri Khoury	Hezzine	5	Conventional

FRCL farms were located at Jezzine area, while the IS farms, from which the eggs were collected, were located in Bekaa region.

NB: The eggs where all collected in the same month. Hyline was the only breed used in both systems.

3. Determination of egg quality parameters

The determination of egg quality parameters included the measurement of the following parameters:

a-Weight of the eggs: measured on a Mettler Toledo balance

b- Porosity of the eggs: measured using a Schlueter candling machine (The Schlueter company, Janesville,Wisconsin USA) and were assessed according to subjective scale (either 1=porous, 2=semi porous, 3=firm)

c- Density (D) of the eggs was measured using 8 sets of NaCl solutions (D=1.065, 1.07, 1.075, 1.08, 1.085, 1.09, 1.095, 1.1).

d- Yolk color was determined using the yolk color fan (Royal DSM, Aargua, Switzerland).

e- Yolk, albumin and shell weight were separated using an egg separator and weighed separately.

f- HU was measured using Baxlo micrometer (Instumentos de medida y precision, s.l. Barcelona, Spain) for egg quality measuring in MM.

g- Egg shell thickness was measured by following the removal of the inner shell membrane using BAXLO thickness gauge (Instumentos de medida y precision, s.l. Barcelona, Spain).

4. Determination of Copper (Cu) and zinc (Zn) metal content in egg yolk and albumen of the collected eggs

a. Microwave digestion

Yolk and albumen were digested using a Milestone ETHOS PLUS with HPR-1000/10S high pressure rotor (Mileston, Sorisole, Italy), according to the below protocol:

i. Place a Teflon (TFM) vessel on the balance plate, tare it, and weigh 1.5 g of egg yolk
ii. Place a TFM vessel on the balance plate, tare it ,and weigh 1.5 ml of egg albumen
iii. Introduce the TFM vessel into the plastic (HTC) safety shield
iv. Add the acids, 8 ml of nitric acid 65% and 2ml of H_2O_2. Swirl the solutions to homogenize it.
v. Close the vessel and introduce it into the rotor segment , then tighten using the torque wrench
vi. Insert the segment into the microwave cavity and connect the temperature sensor
vii. Run the microwave program to completion (Table 11).
viii. Cool the rotor by air or by water until the solution reaches room temperature
ix. Open the vessel and transfer the solution to a marked flask

Table11. Microwave[1] program for digestion of Cu and Zn.

Step	Time	Temperature
1	2 min	85^0C
2	3.5 min	135^0C
3	4.5 min	230^0C
4	15 min	230^0C

[1]Microwave power up to 1000 Watt

b. Atomic absorption photometry

Digesta were transferred into 50 ml capacity conical tubes. Each sample was diluted up to 20 ml volume with Deionized water. The flame atomic absorption spectrophotometry

method was used to determine Zn and Cu contents using SOLAAR atomic absorption spectrophotometer (Thermo Lab Systems, USA) with ASX-510 autosampler. The wavelength of the spectrometer was set at 324.8 nm for Cu and 213.9 nm for Zn. A blank test was run as a control. A standard curve was built for Zn and Cu determine their percentage in the sampled egg portions. The Zn concentrations used to build the standard curve were:

- for yolk: 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 ppm
- for albumen: 0.01, 0.02, 0.04, 0.08, and 0.1 ppm

As for the Cu, the following standard concentrations were used:

- for yolk: 0.0125, 0.025, 0.05, 0.1, and 0.2 ppm

5. Statistics

One way ANOVA and Tukey's test) were used to compare means of egg quality parameters using SPSS version 22 (SPSS Inc., USA). The confidence interval was 95%.

Chi-square test was used to compare the % of compliance of the farms with EU standard practices.

B. Evaluation of essential oils replacing synthetic drugs for control of coccidiosis

The purpose of this study is to evaluate the growth promotion of broilers by each of two essential oil preparations namely Mentofin and modified Mentofin versus Maxiban in the period that precede the challenges with coccidial sporulated oocysts namely, at d1-d21. We also assessed the protection and immunity against the challenge with 8 Eimeria spp. cocktail at day 21.

Experimental Design

A Completely Randomized Design was followed in this research, with eight included treatments, as seen in Table 12.

1. Experiment

A total of 80 day-old birds were divided into 8 groups, with 10 birds/group. Birds of each group were divided into isolation units at AUB facility.

Table12. Treatments of the experiment of Study B

Group#	Treatments	Challenge with *Eimeria* spp	System
1	Mentofin	+	Controlled environment
2	Modified Mentofin	+	Controlled

			environment
3	Maxiban	+	Controlled environment
4	Maxiban	+	Controlled environment
5	None	+	Controlled environment
6	Maxiban	-	Open system
7	Mentofin	-	Open system
8	Modified Mentofin	-	Open system

-Challenged with Eimeria at d21 adjusted at $1x10^6$ sporulated oocyst of *Emiria* spp. Per bird.

- The controlled environment had an average daily temperature of 27 C versus 24 C at the open system. Chicks where reared at 30 C for the first 10 days in both systems.

- Birds were weighed individually at the age of 1day and distributed into 8 pens of 10 birds each.

2. Treatment

The constituents of the Mentofin and the modified mentofin are the proprietory information of the producer. The treatments with Mentofin and Modified Mentofin in drinking water were according to the concentrations recommended by EWABO Co., namely 0.02% in drinking water for the whole experimental period (27 days). The Maxiban treatment in feed was according to Eli Lilli instructions (625 g/t of feed), the producer of this coccidiostat.

3. Challenge: Eimeria inoculum

The challenge was with 8 Eimeria spp. at 21 days of age. Vials of non-attenuated strains of Coccivac-D, designed to vaccinate 1000 birds (Intervet Inc., Summit, NJ 07901, USA), and containing eight *Eimeria* spp. (*E. necatrix, E. acervulina,E. mivati, E. tenella, E. brunetti,E. maxima, E. hagani* and *E. praecox,*) were used in the challenge. Each vial was diluted with saline water 9/1000 NaCl to a volume of 10ml. Each bird at challenge time was inoculated intra-esophageally with 1 ml of the prepared inoculum which is equivalent to 100 times the vaccine dose/bird, and adjusted to $1x10^6$sporulated oocysts/*Eimeria* spp./bird (Hong *et al.,* 2006; Danforth *etal.,* 1997; Danforth, 1998). It is worth noting that the study focused on post challenge period of one cycle (6 days).

4. Oocysts counting technique

a. An amount of approximately 4g of feces were collected from three different floor spots of each pen and pooled in a sterile conical tube.
b. 20ml of 30% NaCl were added in the tube containing the fecal pool.
c. The pooled samples were homogenized using a vortex for a period of 15 seconds.
d. Oocysts were allowed to float for a period of 10 minutes.
e. Mc Master Chamber was filled with 20 microliters of the prepared samples using Pasteur pipette, and oocysts were counted using *leica* DFC300 FX microscope at a magnification of 400x.

f. Number of oocyts/g of feces was obtained using the following formula

Number of oocyts/g of feces= N/0.15 x volume of sample x 1000/wt of sample

N = the number of counted oocysts in one square of the Mc Master Chamber.

0.15 ml = the volume of Mc Master counting chambers.

Volume of sample = 20 ml of 35% saline added over the 3 pooled samples.

Wt of sample = total weight of the 3 pooled samples range between 3 to 5 grams.(Step number 3).

5. Diet formulation

Basal diet was formulated according to NRC, 1992. The respective protein and energy in the diet were 23 % and 3200 KCal/Kg. The basal diet was deprived of any coccidostat and fed *ad libitum* all through the rearing period.

6. Broiler chicks

Day-old Ross 308 broiler chicks were obtained from Tanmia (Zahle) and were apparently healthy and not vaccinated.

a. The % weight gain and feed conversion ratio were calculated at intervals of d1-d21 and d21-d27.
b. The Oocyst output in feces of the birds was calculated at d14, 21, and 27.
c. The mortality was recorded at intervals of d1-d21 and d21-d27.

d. Samples of fecal material were collected starting at d14, and 21, from all 8 groups, to confirm absence of oocysts in the control and treated groups before the challenge is administered at day 21.

7. Statistics

The Completely Randomized design allowed for analysis by ANOVA, followed by Tukeys Test for mean separation (P = 0.05). Frequencies are analyzed by Chi-Square.

CHAPTER IV

RESULTS AND DISCUSSION

STUDY A

A- Degree of compliance to Lebanese intensive system (IS) and free range chicken layers farms (FRCL) with the EU standards.

Table 13 represents the egg quality measurement between IS versus FRCL

Table13. Egg quality parameters of eggs from produced by IS versus FRCL

Egg quality parameter	**Rearing system[1]**		**Standard Error of the Mean (SEM)**
	IS	**FRCL**	
Weight (g)	62.0	64.8	0.73
Porosity[2]	2.24	2.00	0.12
Density	1.0874	1.0896	0.00091
Yolk percentage	26.5	25.2	0.36
Albumen percentage	60.3	61.8	0.43
Shell percentage	13.2	13.0	0.21
Shell thickness (mm)	0.3676	0.3794	0.0037
Haugh unit	94.76[a]	85.62[b]	1.10
Yolk color index	8.24[a]	5.56[b]	0.23
Percentage of eggs with AA quality[3]	100.0	88.0	

[1] Eggs were collected from 5 farms/system, with 5 eggs/farm,

[2] Porosity was given a score from 1 to 3: 1 = porous shell, 2 = semi-porous shell, 3 = firm shell

[3] AA quality eggs are those having a Haugh unit greater than 78

[a,b] Means in a row with different alphabetical superscript are significantly different ($P < 0.05$)

No significant difference was observed for most of the egg quality parameters namely weight, porosity, density, yolk percentage, albumen percentage, shell thickness and percent of eggs with AA quality. Similar results were obtained in previous literature (Sekeroglu et al, 2010; Hidalgo et al, 2008).

The free range system is expected to have a darker yolk, because layers can consume xanthophyll-rich feedstuffs, such as herbs or grass according to Van Den Brand et al., (2004). In this study, the yolk color index of conventional eggs was significantly higher than that of free range (8.24 versus 5.56 respectively). This could be due to the low presence of herbs found in the free range area, and could also be due to feed synthetic pigmentation added to the feed in conventional system (Hidalgo et al., 2008).

The same pattern was observed for HU unit. Measurement of conventional eggs showed an average Haugh units of 94.76 , significantly higher than that of free range eggs (85.62 HU). This difference could be related to various parameters, specifically the age of the hen, where the HU of eggs decreases by around 1.5 to 2 units for each month in lay (Chang-Ho et al., 2014; Gerber, 2006). Chickens in free range system could be exposed to more diseases such as certain strains of Egg Drop Syndrome (EDS), Infectious Bronchitis (IB), New Castle Disease (NCD) and Infectious Laryngotracheitis (ILT) can all cause a decrease in albumin consistency (Coutts and Wilson, 1991).

It is worth noting that all of the eggs showed an AA quality reflecting high HU unit for conventional and free range eggs which indicated that the eggs were all fresh.

Table14 represents the concentration of heavy metals in the egg yolk of IS and FRCL.

Table14. Percentage of metals in yolk of eggs collected from IS versus FRCL

Metals	**Rearing system[1]**		**SEM**
	IS	**FRCL**	
Cu (mg/100g yolk)	0.158257[a]	0.173926[b]	0.0038
Zn (mg/100g yolk)	2.071265	2.134122	0.0272

[a,b] Means in a row with different alphabetical superscript are significantly different ($P < 0.05$)

[1] Eggs were collected from 5 farms/system, with 5 eggs/farm

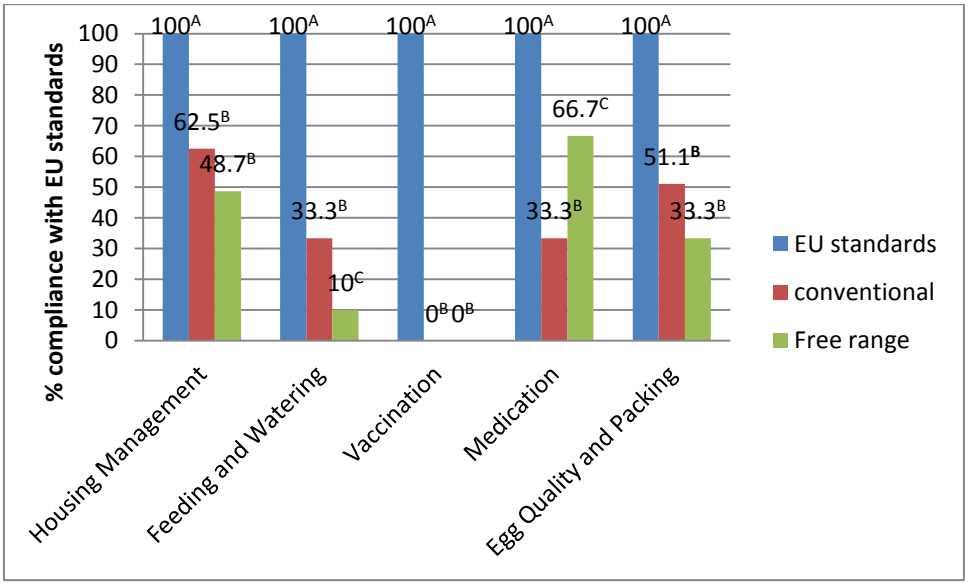
The percentage of Zn in the yolk was not significant in both the conventional and free range system, as cited in literature (Kiliç et al., 2002; Giannenas et al., 2009).

On the other hand, Cu was significantly higher in the yolk of eggs from free range system as compared to conventional system. This could be due to the copper contamination of the pasture with chemicals such as pesticides, fertilizers, etc. These results do not agree to those reported by Zhu et al., (2015) and Giannenas et al., (2009), who mentioned that Cu levels in yolk were significantly lower in free range as compared to those of the conventional system.

Fig. 31 represents the mean % compliance of layers husbandry in free range and conventional system, as compared to European Union standards for free range and

conventional system farming regulations. Stevenson, (2012). Directive, C. (1991). Directive, C. (1998). Directive, E. U. (1999). (Fanatico 2008).

Fig31.Percentage compliance of layers husbandry in IS versus FRCL to EU standards



[a,c] Means over a histogram with different superscripts are significantly different (P <0.05)

Both conventional and free range didn't comply with the EU standards regarding housing management, feeding and watering, vaccination, medication and egg quality and packing. This is due to the absence of governmental supervision, surveillance, and chaotic husbandry and farm practices.

Conventional Farms were more complying with EU regulations as compared to free range farms regarding housing management, feeding, watering and egg quality and packaging. In this context, the intensive farmers are following the instructions followed by poultry breeders and companies to a certain extent, while neglecting the welfare and environmental aspect of the poultry production. However free range farmers were more

complying with the EU regulations that are concerned with medication; since the inclusion of medication in feed is not an acceptable practice in free range layers system.

STUDY B

A. Coccidiosis

Mortality and performance parameters

The mortality % and feed conversion of birds of different groups are presented in Tables 15 and 16 respectively.

Table15. Mean frequency of mortality in eight different treatments of broilers at two age intervals namely, growth promotion period (d1-d21), and Eimeria cycle period (d21-d27).

Group	Treatments	Challenge[a]	% Mortality (d1-21)	% Mortality (d21-27)	% Cumulative Mortality (d1-d27)
1	Mentofin	Cocci	0	0	0
2	Modified Mentofin	Cocci	0	0	0
3	Maxiban	Cocci	0	0	0
4	Maxiban	Cocci	0	0	0
5	None	Cocci	0	0	0
6	Maxiban	None	0	0	0
7	Mentofin	None	0	0	0

8	Modified Mentofin	None	0	0	0

[a]Challenge with 8 Eimeria spp. at 21 days of age according to previously documented research (Barbour et al., 2015).

There was no mortality in any treatment throughout the whole experiment (Table 1); this could be due to using a low oocysts count in the challenge. Comparable results were obtained by Barbour et al (2014), reporting low mortality (0-5%) of birds with similar challenge.

Table16. Mean feed Conversion in different treatments of broilers at two age intervals namely, growth promotion period (d1-d21) and first Eimeria cycle period (d21-d27).

Group	Treatment	Challenge[a]	Feed Conversion (d 1-21)	Feed Conversion (d 21-27)
1	Mentofin	Cocci	1.57	3.11
2	Modified Mentofin	Cocci	1.44	3.18
3	Maxiban	Cocci	1.48	1.97
4	Maxiban	Cocci	1.61	2.32
5	None	Cocci	1.61	4.97
6	Maxiban	None	1.75	1.68
7	Mentofin	None	1.85	Missing value
8	Modified Mentofin	None	1.82	1.68

[a]Challenge with 8 Eimeria spp. at 21 days of age according to previously documented research (Barbour et al., 2015).

It is worth noting that during the growth promotion period (d1-d21), in absence of challenges in Trt 1 to Trt 5, and under controlled environment, the Modified Mentofin (Trt 2) had the lowest feed conversion of 1.44. However, in open system rearing of Treatments 6, 7, and 8, and in absence of challenge too, and in the same growth period of d1-d21, the

Maxiban treatment (Trt 6) was superior to the other two treatments by essential oil. This shows clearly the effect of controlled environment versus open system that has fluctuation in the temperature during the rearing period.

During the first Eimeria spp. cycle (d21-d27), the two Maxiban-treated groups (TrT 3 and TrT 4 did the best in feed conversion., in comparison to control-positive TrT 5 that was none treated with essential oils or Maxiban resulting in birds with higher feed conversion of 4.97. The Mentofin treatment in TRT 1 birds reduced the feed conversion by 37.4%, the Modified Mentofin in TrT 2 reduced the feed conversion by 36.0%, and the Maxiban TrT 3 reduced the feed conversion by 57.7%. This proves that all treatments (Mentofin, Modified Mentofin or Maxiban) have coccidiostat effects, but to different degrees (Barbour et al., 2015; EFSA, 2010). This fact was further confirmed in the Oocyst output data presented in Table 18, which we will interpret in later text of this thesis..

Table 17 below shows the mean % weight increase in the differently treated birds at different time intervals.

Table17. Mean % weight increase in different treatments of broilers at two age intervals namely, growth promotion period (d1-d21), and first Eimeria cycle period (d21-d27)

Group	Treatment	Challenge[a]	% weight gain (d1-21)	% weight gain (d21-27)
1	Mentofin	Cocci	1600.7	32.23
2	Modified Mentofin	Cocci	1759.6	31.69
3	Maxiban	Cocci	1683.1	58.61
4	Maxiban	Cocci	1474.5	57.69

5	None	Cocci	1406.5	29.63
6	Maxiban	None	1457.8	64.73
7	Mentofin	None	1303.8	92.45
8	Modified Mentofin	None	1400.7	65.91

[a]Challenge with 8 Eimeria spp. at 21 days of age according to previously documented research (Barbour et al., 2015).

In comparing % weight gain in controlled environment from d1 to d21, Trt 2 showed the highest value with 1759.6 % as compared to 1474.5 % in Trt 4 and 1600.7 in Trt 1. In the open system, Trt 6 (Maxiban treated group) showed the highest weight gain % value as compared to those of groups 7 and 8 (Mentofin and Modified Mentofin treatments, respectively). This shows that modified Mentofin works better in controlled system, while Maxiban was more efficient in the open system. During the first cycle of Eimeria challenge (d21-d27), Maxiban treated group showed the highest % weight gain in the controlled system with a 58% weight gain. However, in the outdoor system Mentofin had the upper hand (92.45% weight gain) while Maxiban and Modified Mentofin treatments had almost the same effect (64.73 and 65.91% respectively).

Table18 shows the Mean Oocyst count and % Oocyst reduction due to essential oils and Maxiban in relation to untreated and cocci challenged-group in different treatments of broilers at three ages namely, during growth promotion period (d 14), at Eimeria spp. challenge time (d21), and at the end of the first life cycle of Eimeria spp. (d 27).

Table18. The % reduction in oocyst output among different treated broilers.

Group	**Treatments**	**Challenge[a]**	**Oocyst count (per gram feces)-D14**	**Oocyst count (per gram feces)-D21**	**Oocyst count (per gram feces) at D27**	**% reduction in comparison to Group 5**
1	Mentofin	Cocci	0	0	164940[b]	54.9 %
2	Modified Mentofin	Cocci	0	0	196721[b]	46.3%
3	Maxiban	Cocci	0	0	133822[b]	63.4%
4	Maxiban	Cocci	0	0	111531[b]	69.5%
5	None	Cocci	0	0	366006[c]	Not applicable
6	Maxiban	None	0	0	0[a]	Not applicable
7	Mentofin	None	0	0	0[a]	Not applicable
8	Modified Mentofin	None	0	0	0[a]	Not Applicable
SEM					17220	

[a]Challenge with 8 Eimeria spp. at 21 days of age according to previously documented research (Barbour et al., 2015).

The % reduction in Oocyst output at the end of the first Eimeria cycle (d 27) in challenged treatments, in reference to the non-treated-challenged birds of Trt 5 was in the following decreasing order (Table 4): (Trt 4)-Maxiban treated, and challenged (69.5%), (Trt 3)-Maxiban treated, and challenged (63.4%), (Trt 1)-Mentofin treated, and challenged

(54.9%), and (Trt 2)-Modified Mentofin, and Challenged (46.3%). This confirms the coccidiostat effects of Mentofin and Modified Mentofin (Barbour et al., 2015).

It is worth noting that there is a small difference of 8% reduction of Oocysts output at the end of the Eimeria cycle (d27), caused by Mentofin Treatment TrT 1 versus the Maxiban TrT 3 in comparison to the output by TrT 5 (Non treated with Essential oils or Maxiban). Thus, there is a place of improvement of the essential oil preparations to get even with Maxiban coccidiostat effect, or even a better effect. It is worth noting that birds that were non-challenged kept an oocyst output of zero count, a reflection of the proper management in the isolation and open system units.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

1- Feed conversion during growth promotion through d1-d21 is best obtained when treating non challenged broilers by Modified Mentofin under controlled environment; however, parallel groups raised under open system had the Maxiban treatment resulting in best feed conversion during the d1-d21 period, an indication of the negative effect of essential oils under open system.

2- The Maxiban treatment had the lowest feed conversion during the first life cycle of Eimeria spp. (d21-27), while both the Modified Mentofin and Maxiban had the same feed conversion during the same period in parallel non challenged birds raised under open system.

3- The Oocysts output reductions at the end of the first life cycle, in comparison to Control positive group by Mentofin and Modified Mentofin were 54.9% and 46.3%, respectively, values that are not far away from the reduction obtained by Maxiban (63.4%). These values confirm again the coccidiostat effect of both essential oil preparations.

4- Regarding the comparison of the IS to FRCS, there was no significant difference in most of the egg quality parameters except for HU and yolk color index that were significantly higher in conventional eggs. This reveals the misconception of higher quality of the free range eggs.

5- Free range eggs have higher concentrations of Zn and Cu in their yolk, most likely due to ingestion of plants and soil contaminated by heavy metals.
6- There was no compliance between IS and FRCS with EU regulations, due mostly to the absence of regulations and governmental surveillance.

Modified Mentofin is more effective than Mentofin in feed conversion under open system. During complex challenges, under environmentally controlled pens, both the Mentofin and Modified Mentofin result in similar feed conversion, while the Modified Mentofin results in better % weight gain compared to that of Mentofin. The Mentofin still gives a better reduction in Oocyst output compared to Modified Mentofin, however, both are having coccidiostat effect, when compared to control positives.

The Modified Mentofin can be recommended as a growth promoter in future marketing strategy, while keeping the Mentofin as a preparation that is useful against coccidiosis. Further improvement of the essential oil constituents is needed to close the gap between these preparations and the Maxiban, a gap that is not very wide, as shown in this fulfilled task.

It’s recommended to include further parameters in comparing free range and conventional egg quality. Namely Egg shell, internal egg , yolk , albumen and the overall quality and some important heavy metals like iodine, selenium, iron, manganese, calcium, magnesium, potassium, lead, cadmium and arsenic.

Regulation must be set to control egg production in a way to respect animal welfare and the ecosystem. And regulations need continuous governmental supervision and surveillance in order for it to be applied.

APPENDIX

Questionnaire for FRCL. STUDY A

Farm:

Local region:

Housing and management:

1-What is the number of chickens in the house?

2- Chicken breed?

3-Type of nests:

-individual

-group

4- If individual: -1 nest per 7 hens

-more

- Less

-If group nests are used: -dimensions of the nest

-number of hens

5- Do you use perches? - Yes

- No

6- If yes total length?

7-What's the horizontal difference between them?

-bigger or equal to 30 cm

-less

8-And what's the horizontal difference between perches and the wall?

–bigger or equal to 20 cm

-less

9-Do you mount the perches above the litter?

-yes

-no

10- What's the littered area?

11- What percent of the littered area is occupied with litter?

-bigger or equal to one third of the area

-less

12- Floor type indoors:

-concrete

-wood

-wire

-soil

-others

13- Presence of rearing shelves?

-yes

-no

14-If yes how many

15-Distance between them?

16- What is the surface area indoors ?

17-Do you perform beak trimming?

-yes

-no

18-If yes at what age?

-older than 10 days

-below ten days

19-If not do you observe cannibalism and or feather pecking?

-yes

-no

20- Litter state?

-dry friable

-moist

21- Do you provide refuge area?

-yes

-no

22- Do you have partitions if house is large? (Barriers to form smaller groups)

-yes

-no

Feeding and watering

1-Is there equal access for all hens when feeding and drinking is provided?

-yes

-no

2-Shape of feeders:

-circular

-linear

-else

-perimeters or length of circular or linear feeders respectively

3-If linear feeding how much distance provided per bird? -greater or equal to 10cm/hen

-other

-If circular distance provided per bird? -greater or equal to 4cm /hen

-other

4- Drinking system:

-continues

-nipples

5-If continuous drinking troughs what's the shape of the drinkers?

-linear

-circular

-else

6-If circular drinking troughs what is the distance provided per hen?

-greater or equal to 1 cm

-less

7-If linear drinking troughs what is the distance provided per hen? (Measure the length)

-greater or equal to 2.5 cm

-less

8-If nipple drinkers are used how many nipples are there?

-greater or equal then 1 for each hen

-less than one nipple for each hen

Vaccination

1-Do you vaccinate?

-yes

-no

2-If yes, do u vaccinate?

-Preventative vaccination -yes

-no

-Emergency vaccination -yes

-no

3- If yes, vaccinate for which disease?

Medication

1- Do you use medication?

-yes

-no

2- Is use of medicine based on medical prescription?

-yes

-no

3- Do you comply with the medical prescription?

-yes

-no

4- Is antimicrobial medicinal products used to?

-prevent disease

-enhance the bird’s performance

-else

5- If yes, what’s the duration of use? -one month

-two weeks

-other

6- Do u respect the withdrawal period?

7-Do you have records for the medication used?

-yes

-no

8-If yes for how many years?

-greater or equal to 5 years

-else

9-Use of medicine in?

-feed

-water

10- If medicine mixed in feed, do you have a homogeneous incorporation of medicine in the feed?

-yes

-no

11- Do you have a partition/hospital for injured hens?

-yes

-no

Egg quality and packaging

1-Do you have a candling equipment? -yes

-no

2- A machine for grading the eggs by weight? -yes

-no

3-One or more adjusted balances for weighing eggs?

-yes

-no

4- Equipment for stamping eggs?

-yes

-no

5- Are equipment clean and free of extraneous odors?

-yes

-no

7- Are the packs labeled?

-free-range eggs

-barn eggs

-eggs from caged hens

-free range eggs

- Else

- no

8-Do you wash or clean the eggs?

-yes

-no

9-At what temperature do u store the eggs?

-less or equal to 5 Co

-between 5 and 15 Co

- Above 15 Co

10- How many days do you store the eggs before marketing?

11-Do you remove cracked egg shells?

-yes

-no

Questionnaire for IS STUDY B

Farm:

Local region :

Housing and management

1-What is the number of chickens in the house?

2-Chciken breed?

3-Type of nests:

-individual

-group

4- If individual: -1 nest per 7 hens

-more

- Less

-If group nests are used: -dimensions of the nest

-number of hens

5- Do you use perches?

-yes

- No

6-If yes total length?

7-What’s the horizontal difference between them?

-bigger or equal to 30 cm

-less

8-And what’s the horizontal difference between perches and the wall?

-bigger or equal to 20 cm

-less

9-Do you mount the perches above the litter?

-yes

-no

10- What’s the littered area?

11- What percent of the littered area is occupied with litter?

-bigger or equal to one third of the area

-less

12- Floor type indoors:

-concrete

-wood

-wire

-soil

-others

13- Free movement and access inside /outside:

-yes

-no

14- Presence of rearing shelves?

-yes

-no

15-If yes how many

16-Distance between them?

17- Number of pop holes?

18-What's the dimensions of each pop hole?

-35x40 cm ^{2}

-less

-more

19- Outdoors surface area?

20- Do you have a shelter in the outdoor area?

-yes

-no

21-If yes

- Shaded area?

-providing partial protection against predators?

22- What is the surface indoors?

23 – Do you perform beak trimming?

-yes

-no

24- If yes at what age?

-older than 10 days

-below ten days

25-If not do you observe cannibalism and or feather pecking?

-yes

-no

26- Do you lock them at specific times during the day?

27- Litter state?

-dry friable

-moist

28- Do you provide refuge area?

-yes

-no

29- Do you have partitions if house is large? (Barriers to form smaller groups)

-yes

-no

Feeding and watering

1-Is there equal access for all hens when feeding and drinking is provided?

-yes

-no

2-Shape of feeders:

-circular

-linear

-else

-perimeters or length of circular or linear feeders respectively

3-If linear feeding how much distance provided per bird?

-greater or equal to 10cm/hen

-other

-If circular distance provided per bird?

-greater or equal to 4cm /hen

-other

4- Drinking system:

-continues

-nipples

5-If continuous drinking troughs what's the shape of the drinkers?

-linear

-circular

-else

6-If circular drinking troughs what is the distance provided per hen? (Get the radius)

-greater or equal to 1 cm

-less

7-If linear drinking troughs what is the distance provided per hen? (Measure the length)

-greater or equal to 2.5 cm

-less

8-If nipple drinkers are used how many nipples are there?

-greater or equal then 1 for each hen

-less than one nipple for each hen

9- Is there drinking troughs outdoors?

-yes

-no

10- Is the outdoor area covered with vegetation when the hens are outside?

-yes

-no

11-Do you spread the feed?

-yes

-no

Vaccination

1-Do you vaccinate?

-yes

-no

2-If yes, do u vaccinate?

-preventative vaccination

-yes

-no

-emergency vaccination

-yes

-no

3- If yes, vaccinate for which disease?

Medication

1- Do you use medication?

-yes

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-enhance the bird’s performance

-else

6- If yes, what’s the duration of use?

-one month

-two weeks

-other

7- Do u respect the withdrawal period?

8-Do you have records for the medication used?

-yes

-no

9- If yes for how many years?

-greater or equal to 5 years

-else

10- Use of medicine in?

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-water

11- If medicine mixed in feed, do you have a homogeneous incorporation of medicine in the feed?

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-no

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-no

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-yes

-no

4- Equipment for stamping eggs?

-yes

-no

5- Are equipment clean and free of extraneous odors?

-yes

-no

7- Are the packs labeled?

-free-range eggs

-barn eggs

-eggs from caged hens

-free range eggs

- Else

-no

8-Do you wash or clean the eggs?

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-no

9-At what temperature do u store the eggs?

-less or equal to 5 C^o

-between 5 and 15 C^o

- Above 15 C^o

10- How many days do you store the eggs before marketing?

11-Do you remove cracked egg shells

-yes

-no

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