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In vivo study of the use of *Thymus vulgaris* essential oil in avian therapy

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Abstract

An *in-vivo* therapeutic trial based on the essential oil, in order to solve the antibiotic resistance problem in *ISA15* strain chickens. *Escherichia coli* choose for this study has a resistance to several antibiotics of veterinary interest. The study of antimicrobial activity by aromatogram of *Thymus vulgaris* oil, gave diameters of inhibitions between 13.33 and 35.33 with a large inhibitory effect. The LD50 calculate of EO is 4500mg/kg. The macrodilution method in a liquid medium made it possible to obtain a MIC of 5µl/mL with a bactericidal effect. Four groups were prepared (control); G II (oral receipt of 450mg/kg/day essential oil for five days); G III (intramuscularly received 0.1ml of an inoculum of 88.10^7CFU/ml of *E. coli*) G IV (received intramuscularly 0.1ml of an inoculum of 88.10^7CFU/ml of *E. coli* then treated with a dose of 450mg/kg/day orally for five days). The limits of the usual values were calculated for the main biochemical and hematological parameters. The differences statistically assessed versus placebo, group GIII recorded a significant disturbance ($P < 0.05$) for blood glucose, CRP, GB, ASAT and ALAT. While the GIV group has recorded a significant disruption for blood glucose, CRP, ASAT and creatinine. Group III recorded lesions of different organs (liver, air sacs, pericarditis) of an average (m ± esm) of 10.60 ± 0.50 larger than that of Group IV 3.60 ± 1.28, which statistically shows a significant difference between two samples $t = 5.052$ ($p < 0.05$). Group III recorded 80% of deaths, double the IV group. No mortalities and lesions were reported to groups I and II. In this work, we have demonstrated the importance of the essential oil of *T. vulgaris* as an antimicrobial agent and as an alternative to improve some hematological and biochemical parameters in broiler chickens.

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Introduction

In industrial breeding, the possibilities of treatment of avian infections has become more and more limited in the face of therapeutic problems.

The concept of antimicrobial resistance is given as the loss of bacteriostatic or bactericidal activity of an antimicrobial to eliminate targeted bacterial agents (Jorgensen, 2004) Avian colibacillosis is one of the dominant pathological entities reported in the health surveillance of antimicrobials. poultry farms. Antibiotic resistance monitoring of specific germs such as *E. coli* requires particular importance in the human and veterinary health policy of countries (Rahmatallah, Nassik *et al.*, 2016). The control of avian colibacillosis is mainly ensured by antibiotic treatments, used either in prevention during viral attacks or in curative treatment (Rahmatallah, Nassik *et al.*, 2016). *Escherichia coli* that possess a fairly pathogenic character are divided into two distinct groups according to the system. They invade: either intra-intestinal or extra-intestinal (Tenaillon, Skurnik *et al.*, 2010). This phenomenon of resistance of *E. coli* to Antibiotic is more and more marked especially resistance to the family betalactamines. In general, colibacilli are classified as enterobacteria naturally sensitive to several antibiotics (Kadir and Touati, 2017)

Merazi *et al* (2016) report by study of the Ethno-Veterinary Approach of Medicinal Plants Used in the Region of Sidi Bel Abbes-Algeria, that the most used plants are *Thymus capitatus* 20% followed by 15% *Thymus vulgaris*, use is dominated by decoction 31.67%.

Recent studies have shown that essential oils and their constituents have significant potential as antimicrobial agents (Domans and Deans, 2000). *Thymus vulgaris* essential oil has a broad spectrum of action against Gram-negative bacteria, with very large inhibitory diameters (Berrada, Bennani *et al.*, 2016). To combat the problem of failure antibiotic treatment, and replace it with a biological treatment. Aromatherapy allows to consider a cure in the experimentally contamination of expensive chicken by *Escherichia coli*.

In our previous study who wears a title of In vitro study of the activity of essential oils of *Thymus capitatus* and *Thymus vulgaris* against enterobacteria. We examined an alternative to antibiotics such as essential oil of thyme in vitro. *Thymus vulgaris* oil showed bactericidal antibacterial activity against all microorganisms studied, including *Enterobacteriaceae* (Merazi and Hammadi, 2018)

The present study begins with direct activity tests (Aromatogram) of *Thymus vulgaris* oil on the antibiotic-resistant agent (*E. coli*), this in vitro test is completed by an in vivo test which consists of the effect of the essential oil as an antimicrobial agent by replenishing the antibiotic.

Induction of disease in chicken *E. coli* and treatment with a therapeutic dose of the essential oil after a toxicity test. Macroscopic and microscopic observations of surviving and dead chicken organs were evaluated to determine the effectiveness of the oil on the health and status of hens.

Materials and methods

Breeding

To carry out our study, we carried out a breeding of 120 ISA15 strain chicks, the chicks were raised on the ground on litter under conditions of humidity, ventilation and temperature. The means of sanitary prophylaxis such as hygiene, crawlspace, and prevent the appearance of possible pathologies. The animal feed is provided by a balanced diet with a composition adapted to the period of washing. Food and water are distributed abundantly.

Plant material

The choice of our plants is based on a survey conducted previously in the axis of the Ethno-Veterinary Approach of Medicinal Plants Used in the Region of Sidi Bel Abbes-Algeria (Merazi, Hammadi *et al.*, 2016).

Essential oils

The essential oils are obtained by hydro distillation with a Clevenger type apparatus (Clevenger, 1928).

The distillation was carried out by boiling for three hours 250g of fresh plant material (aerial part) with 750ml of water.

Aromatogram

The aromatogram technique (Vincent's method) is based on the principle of antibiogram(NCCLS, 1997). Sterile disks of Wattman paper 6 mm in diameter, containing essential oil to be tested, are deposited on the surface of an agar medium suitable for the studied strain, previously inoculated.

The MIC and the MBC

For each extract, a series of sterile concentration, ranging from 80 to 1.25mg/ml with distilled water. An inoculum whose turbidity is adjusted to 0.5 McFarland which corresponds a 10^8CFU/ml in a MH broth. A mixture for the same volume (1ml) of each concentration is the bacterial inoculum. Bacterial growth is examined in each tube, after incubation, the first clear tube gives us the MIC (Bolou, Attioua *et al.*, 2011).

A loop of broth was collected from the tubes which showed no visible signs of growth and were inoculated on streaked MH agar, then incubated at 37 degrees Celsius for 18h. A series from which no growth is visible is considered MBC.

The antibacterial effect has been judged bactericidal or bacteriostatic by the following ratio: MBC/MIC. The effect is bactericidal if the ratio lower to 2, and bacteriostatic if the ratio higher to 4 (Berche, Gaillard *et al.*, 1991).

Determination of LD50

For the essential oil of *Thymus vulgaris*, the LD50 is determined by the method described in the European guideline OECD code 423.

On a farm, the 7-day-old chicks were divided into 6 batches (each set contains 3 chicks randomly placed in cages of ½m2). Chicks, weighing about 156.14 ± 6.57g.

One of the lots is used as a control (receiving 10ml/kg of 1% Tween 80) while the other lots were treated, each with a single dose of *Thymus vulgaris* oil (solubilized in Tween 80 at 1% and adjusted to 10ml/kg per dose). Administration was by oral gavage at doses ranging from 0 to 6000mg/kg body weight).

We observed the chicks daily to record all physiological and behavioral changes compared to the placebo group.

The LD50, expressed inmg/kg of body weight, is determined by the method of calculating Dragstedt and Lang, (1957).

The LD50 is calculated by the following formula.

$$LD50 = \frac{50(X2-X1)+X1.Y2-X2.Y1}{Y2-Y1}$$

X2: Upper dose surrounding LD50; X1: Lower dose surrounding LD50; Y2: Percentage of mortality corresponding to X2; Y1: Percentage of mortality corresponding to X1.

Escherichia coli strain

Avian *Escherichia coli* strain, is a strain isolated from a spontaneous case of death of broiler, has a resistance of 83.33%. The sensitivity profile of *Escherichia coli* resistant to antibiotics is shown in (Tab. 1)

Table 1. The sensitivity profile of *Escherichia coli* resistant to antibiotics.

	R	I	S
AM 10µg	0 mm		
TIO 30 µg	0 mm		
N 30 µg	0 mm		
UB 30 µg	0 mm		
CS 10 µg		9 mm	
GM 10 µg			22 mm

The choice of the dose of bacteria

The chosen inoculated dose is 0.125v/v or 88.10^7 bacteria/ml per chicken, this dose is intended to cause 33.33% mortality in 5 days (A mortality approach in a chicken production unit).

Preparation the groups of experience

4 batches of chicken aged 17 days, chickens were chosen with a weight of 450.4 ± 26.76

• Group I received distilled water orally.

• Group II received oral oil of 450mg/kg/day for five days.
• Group III intramuscularly received 0.1ml of an inoculum of 88.10^{7}CFU/ml of *E. coli*.
• Group IV received intramuscularly 0.1ml of an inoculum of 88.10^{7}CFU/ml of *E. coli* and then treated with a dose of 450mg/kg/day orally for five days.

Induction of an inflammatory syndrome

The induction of avian colibacillosis is achieved by inoculation of a pathogenic strain of experimental *Escherichia coli*. The inoculum of 88.10^7 CFU/ml is prepared the day of its use. Chickens are fasted for 12 hours. Inoculation occurred intramuscularly in the keel bone muscles at a volume of 0.1ml.

Blood analysis

Blood sample

The blood samples, about 3ml, are collected in 2 different tubes ; one EDTA tube used for the examination of hematological parameters and the second heparin container is used the examination of biochemical parameters.

Histology study

The digestive organs and accessory glands removed were subjected to a macroscopic and microscopic observation.

Macroscopic examination is based on a recording of lesions according to a scoring grid allowing to calculate a lesion score. The determination of the lesional score according to the method described by (Johnson and Reid, 1970).

It is first necessary to subject the tissues that are taken, fixation with formalin buffered to 10%. Pieces of cut organs become solid in contact with paraffin. The blocks were cut with a microtome at 5 µm thickness and stained with hematoxylin-eosine-safran (HES) for microscopic examination.

Statistical analysis

According to the test of variance (ANOVA) the mean differences were determined with significance at p≤0.05 with a 95% confidence interval. Using SPSS v 19.0 for windows. The difference was considered statistically significant when the value of p is <00.5.

Result

Essential oils

The yield of essential oil of *Thymus vulgaris* is 2.75% compared to the dry matter.

Aromatogram

Effect of *T. vulgaris* oil on *Escherichia coli* at a concentration of 5µl, 10µl and 15µl gave inhibition diameters respectively (expressed as means ± standard deviations) 13.33 ± 1.53, 35.33 ± 1.53, 29.00 ± 1.00 with a large inhibitory effect (Tab. 2).

Table 2. Antibacterial activity of different concentrations of essential oils.

	Tymus vulgaris		
	5 µl	10 µl	15 µl
Escherichia coli	+	+++	+++

According to a symbolic rating scale from - to +++, the reading of which (Jirovetz, Buchbauer *et al.*, 2000) is as follows: Ø <10mm: essential oil without inhibitory action (-); 16> Ø ≥ 10mm: HE at an intermediate inhibitory action (+); 25> Ø ≥16mm: HE with significant inhibitory action (++); Ø≥25 mm: HE with a very effective inhibitory action (+++)

MIC and MBC

The macrodilution method in a liquid medium made it possible to obtain a MIC at a value of 5µL/mL with a bactericidal effect (Tab. 3).

Table 3. MIC and MBC.

(µL.mL^{-1})	*Tymus vulgaris*		
Escherichia coli	MIC	MBC	MBC/MIC
	5	5	1

Determination of LD50

A dose lower than 4000 mg/kg causes no mortality, but some temporary symptoms appeared such as confusion, tired, diarrhea, vomiting. A dose above 4000 mg/kg causes mortality that increases with the dose.

The control batch retained 0mg/kg of the essential oil remained without any physiological and behavioral changes. The calculated LD50 is equal to 4500mg/kg

The lesion rate of dead and alive chickens

The appearance of the liver and heart during avian colibacillosis represented in (Fig. 1)

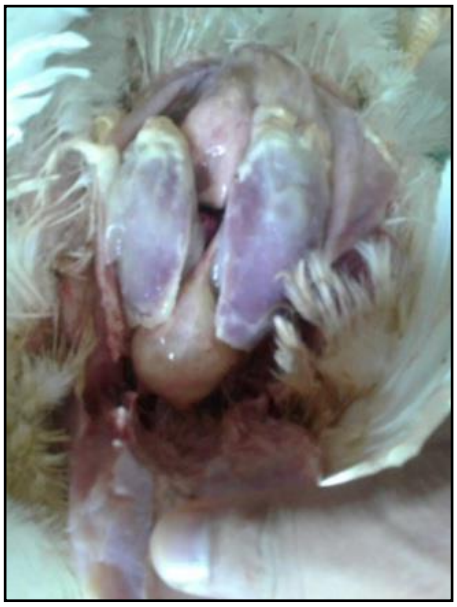

Fig. 1. Fibrin covering the entire organ of the liver and heart.

The statistical analysis carried out on the different lots is a Student's t-test for the case of independent samples. Group III infected to recorded lesions of different organs (liver, air sac, pericarditis) of an average of 10.60 ± 0.5099 larger than that of Group IV infected and then treated with the essential oil 3.60 ± 1.28841.

The statistical study carried out according to T test shows that there is a difference significant between the two samples t = 5.052 (p <0.05).

Death rate

Inoculation occurred intramuscularly in the keel bone muscles volume of 1/10ml. Infected Group III recorded four deaths out of five chickens 0.8 ± 0.44 (a percentage of 80%). Group IV infected and then treated with the essential oil recorded two deaths in five chickens 0.4 ± 0.54 (a percentage of 40%) (Fig. 2). For group I and II its survival.

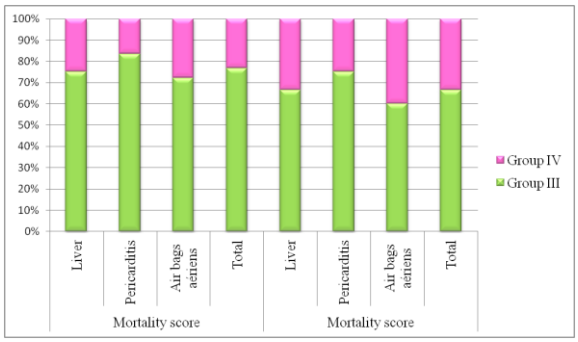

Fig. 2. Lesion score of dead and sacrificed chickens.

For group III 1/5 of death at D5 ; 2/5 of death to D6 with a cumulative of 3/5 ; 1/5 of death on day 7 with a cumulative 4/5. For group IV 1/5 of death at D6 ; 1/5 of death at D8 with a cumulative of 2/5.

Biochemical and hematological parameters

The results are expressed on mean ± SEM; * (Significant, p <0.05); ns (not significant); G I (control); G II (uninfected treated); G III (untreated infected); G IV (infected + treated). The results indicate a significant increase in CRP, GB, ALAT, ASAT with a significant decrease in glycemia in the untreated (GIII) infected group compared with control (GI). However, there has been reported a decrease in blood glucose, ASAT and a significant increase in creatinine, CRP in the GIV group compared to GI control. The parameters studied showed no significant changes in the oil-treated and uninfected group (GII) compared with control (Tab. 4)

Table 4. Variations in Blood and Biochemical Parameters.

Tests	G I	G II	G III	G IV
Glycemia g/L	2.21± 0.14	2.502± 0.16^{ns}	0.94± 0.09*	1.23± 0.17*
Cholestérol g/L	1.298± 0.13	1.29± 0.16 ^{ns}	0.808± 0.18 ^{ns}	1.126± 0.17 ^{ns}
ALAT UI/L	10.2± 0.58	9± 0.70 ^{ns}	23± 1.22 *	12.2± 1.90 ^{ns}
ASAT UI/L	276± 7.88	236.6± 7.18 ^{ns}	285.8± 5.32 *	193.6± 25.87*
Creatininemg/L	9.8± 0.86	11.2± 0.37 ^{ns}	10.4± 0.74 ^{ns}	16.2± 0.96*
CRPmg/L	≤6	≤6 ^{ns}	30± 3.70*	15.6± 1.77*
Hématocrit %	45.8± 2.15	52.4± 2.24 ^{ns}	48.6± 4.48 ^{ns}	51.6± 1.96 ^{ns}
GB *10^{3}/mm^{3}	15.2± 2.76	12.8± 3.41 ^{ns}	43.2± 4.48*	26.6± 1.56 ^{ns}

ns : not significant difference, * : significant difference.

Histology study

Microscopic lesions observed in the intestinal mucosa of older animals of 22 days are characterized by lamina propria congestion, the presence of necrotic cell debris in the lumen, intestinal villi lined by a deformed epithelium, some mucosa also showed dilation of some crypts, total absence of crypts at the level of the zone of necrosis (Fig. 3).

The intestine present mucosa describes villi and crypts. The villi take an enlarged form in the small intestine, and the crypts are deeper (Fig. 4). The thick mucosa with a velvety appearance that is due to many long villi. Between the villi we find crypts which occupy the lower portion of the mucosa. These are the Lieberkükn glands (Fig. 5).

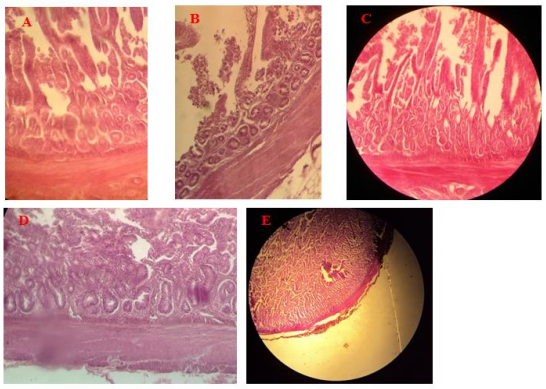

Fig. 3. The microscopic lesions of G III.

A. Congestion of Propria lamina (G × 40); B. the presence of necrotic cell debris at the level of light (G × 40); C. Intestinal motility covered by a deformed epithelium; D. Dilation of crypts and flattening of epithelial cells of the epithelium lining these crypts (G × 40); E. Total absence of crypts at the area of necrosis (G × 16).

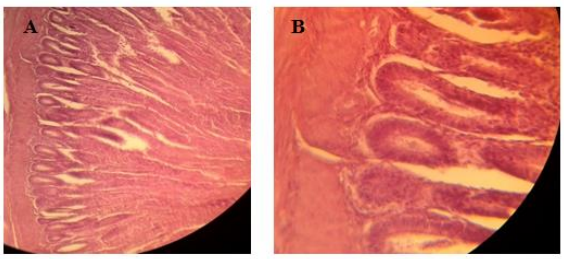

Fig. 4. The microscopic lesions of G II.

A. intestinal motility (G × 40); B. The Lieberkühn crypts (G × 100)

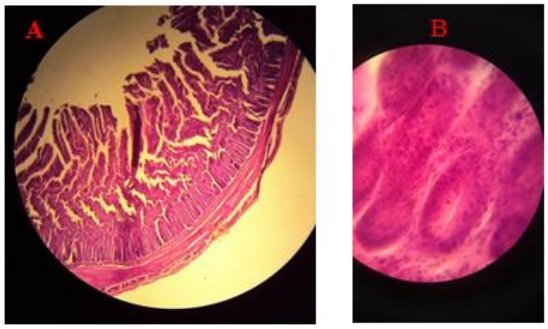

Fig. 5. The microscopic lesions of G IV.

A: Improved intestinal villi (G × 40) B: Normal appearance of crypts (G × 100)

Discussion

Our yield in essential oil of *Thymus vulgaris* is 2.75% of dry matter was obtained from the aerial part. The yield of the essential oil from several studies was variable for various circumstances: stage of development (Sangwan, Farooqi *et al.*, 2001), stage of growth, pedoclimatic conditions, extraction technique. (Satrani, Ghanmi *et al.*, 2007) and harvest period (Aafi, Ghanmi *et al.*, 2011).

Our result resulted in a sensitivity of *Escherichia coli* in contact with *Thymus vulgaris* oil for concentrations of oil of 10µl and 15µl. This activity could mainly be due to the majority compounds according to (Dorman and Deans, 2000, El Ouali Lalami, Fouad *et al.*, 2013).

This oil has potent antimicrobial activity against a range of microorganisms, including *E. coli* (Van Vuuren, Suliman *et al.*, 2009). It has shown bactericidal effects at concentrations <1.5µL/ml for the bacterial strains tested, in particular *Escherichia coli* (Mith, Dure *et al.*, 2014). Soković *et al* (2010) ishowed the best antibacterial activity were those of *Thymus vulgaris* (16-30mm) against various bacteria and particularly against *Escherichia coli* which generates an inhibition zone (22mm). Our results confirmed that the essential oil is active by their strong inhibition on *E. coli* tested with a MIC is equal to 5µl/ml which gives them a bactericidal effect. The results of this work corroborate those of other studies, which demonstrates the bactericidal effect of *Thymus vulgaris* face to *E. coli* (Soković, Glamočlija *et al.*,

2010), and also incompatible with those Hammer *et al.* (1999). *Thymus vulgaris* had the lowest MIC against *E. coli* (Hammer, Carson *et al.*, 1999).

For the LD50, the adverse effect is related to the dose, the route of absorption, the type, the severity of the lesions and the time required for the appearance of a lesion. Toxic effects result in mortality; changes in behavior and physiological state; digestive problems, changes in hematological and biochemical parameters (Gomes, Lourenço *et al.*, 2012). Based on this LD50 oral, it will be possible to classify the molecules by level of toxicity according to the scale of (Viau and Tardif, 2003, OECD, 2001). When: 500mg/kg <LD50 <5000mg/kg: the substance is slightly toxic. In the present study the LD50 of the essential oil of *Thymus vulgaris* calculate is equal to 4500mg/kg, she belongs to category 5.

The amount of inoculated bacteria is involved in the disease (Nakamura, Imada *et al.*, 1987). Chickens aged between 7 and 21 days are likely to colibacillosis. In the field, however, colibacillosis is usually observed in chickens over four weeks old (Goren, 1978). Intramuscular inoculation is by deep injection of *E. coli* 88.10^7CFU/ml in a volume of 0.1ml in the pectoral muscles at the tip of the keel bone, according to the inoculation protocol performed by (Mogenet, Bezille *et al.*, 1997) cited by (Lezzar and Benmakhlouf, 2006). We observed a mortality of 33.33% of chickens inoculated with 88.10^7CFU/ml of *E. Coli*, but this rate rises to 67.66% when the dose increases to 164.10^7CFU/ml. The high mortality among untreated inoculated chickens may be due to a high dose at 164.10^7CFU/ml.

This experimental method, which was used to produce colibacillosis, was the same when Lezzar and Benmakhlouf (2006) induced colibacillosis by intramuscular injection of 10^9CFU/ml *E. coli* serotype O78: K80. All avian species are susceptible, especially young birds (Martin, 2010). The lesion is characterized by a green liver and congested pectoral muscles. While a less virulent colisepticemia results in pericarditis and peritonitis.

The lesions observed correspond to intestinal inflammation, large thickened and edematous plaques containing blood and mucus (Jeffrey, Nolan *et al.*, 2002). Air sacs, liver, heart and abdominal cavity are the most affected organs. Organ damage is characterized by congestion, tissue thickening and fibrin deposition. This deposit is sometimes so important that the surface of the organ takes on the appearance of a pancake (Stordeur and Mainil, 2002). According to a study carried out in English slaughterhouses, 43% of carcasses seized with colibacillosis showed lesions of pericarditis, perihepathitis (Yogaratnam, 1995).

Birds have significant metabolic activity (Koochaksaraie, Irani et al., 2010).

Glucose is used by birds as a source of energy, glycogen synthesis, fatty acid synthesis, as well as non-essential amino acid synthesis, vitamin C synthesis (Braun and Lefebvre, 2008). Changes in blood glucose rate demonstrated a significant ($p < 0.05$) decrease in the G III (untreated infected) group, and a less significant ($p < 0.05$) decrease in the GIV group (infected and then treated with oil). Compared to control group 2.21 ± 0.14g/L. The level of plasma glucose is influenced by carbohydrate consumption and endogenous carbohydrate synthesis, and release by the liver on the one hand, and storage, and its elimination on the other (Nwaoguikpe, 2010).

Cholesterol is a major lipid, it is a steroid essential for the renewal and synthesis of membranes of all cells of the body in birds, unlike many mammalian species, lipid synthesis is essentially hepatic (Griffin, Guo *et al.*, 1992), adipose tissue being mainly storage tissues. Our diagnostic values for this parameter indicate cholesterol stability in four groups of poultry. ALAT (alanine aminotransferase) and ASAT (aspartate aminotransferase) enzymes of interconversion. In poultry, liver and muscle contain large amounts of ASAT.

In our experience, an influence marked by a significant decrease ($P < 0.05$) in the ASAT levels is found in the GIV group (infected and then treated with oil) compared to control group 276 ± 7.88UI/L.

The activity of this enzyme is considered today as very sensitive but non-specific liver lesions in poultry. ASAT) has a much wider distribution, in the liver (in the 90% mitochondria), but also in the heart, skeletal muscles, kidneys, brain (Imbert, Colombat *et al.*, 2003).

While ALAT values did not show significant differences, values ranged from 9 to 12UI/L in all four groups of animals. Significant increases in activities of both transaminases were demonstrated in birds at 4 days post-infection (Koynarski, Mircheva *et al.*, 2010). This increase may be related to lesions of intestinal cells whose enzymes are released into the circulation (Pascalon-Pekelniczky, Michoudet *et al.*, 1996).

Variations in creatinine demonstrated a significant ($p < 0.05$) increase in the G IV group (infected and then oil treated) compared to the control group (GI) 9.8 ± 0.86mg/L. creatinine is an important indicator of protein metabolism, resulting from a breakdown of muscle creatine (Wyss and Kaddurah-Daouk, 2000). Among the factors that influence on creatinine : muscle, mass and age and physical activity (Rajman, Juráni *et al.*, 2006).

The creatinine is eliminated by the kidneys, it appears that the renal function requires a certain time to be fully functional (Mahangaiko, 2016). The results indicate a significant increase in CRP in two groups G III (untreated infected), and GIV (infected and then treated with oil) compared to control group.

The CRP is a determinant of inflammation (Faugere, 2015, Tremblay, 2015). When the CRP is greater than 10mg/l, acute infection should be suspected (Yeh and Willerson, 2003). Determination of hematological parameters of animals is important to diagnose many diseases (Diaby, Yapo *et al.*, 2016). No changes in hematocrit were recorded in all other groups compared to control group. The Hematocrit level of the placebo group is consistent, as a whole, with data reported in different research.

Group G III indicates a significant elevation of GB compared to control (15.2 ± 2.76) $10^3/mm^3$. The alteration of white blood cells and red blood cells is an indicator of early exposure to toxins that affect tissues (Vinodini, Chatterjee *et al.*, 2015). There is an awareness of lymphocyte populations involved in the production and regulation of immunoglobulins (antibodies) and they illustrate their participation in immune reactions (Allardyce and Bienenstock, 1984).

Histology study with broilers demonstrated the *in vivo* antimicrobial efficacy of the essential oil against *E. coli* (Jamroz, Wertelecki *et al.*, 2006). The first microscopic signs consist of the appearance of edema followed by infiltration of heterophiles. Then, in a second time appear the phagocytes which quickly become majority. The lesions are then characterized by the presence of these, giant cells and caseous necrotic debris (Stordeur and Mainil, 2002). (Koynarski, Mircheva *et al.*, 2010) reported that the group infected only by *E. coli* exhibits fibrinous air-sacculitis, perihepatitis or pericarditis, and inflammation of the gallbladder.

This mild treatment can significantly reduce infections of different organs (air sac, liver, heart), and improve the appearance of the bowel compared to group G III (untreated infected) which highlighting the Aromatherapy interest by oil of *Thymus vulgaris*.

Conclusion

In vitro the essential oil of *Thymus vulgaris* shows a bactericidal effect. This strain has an antimicrobial resistance, and capable of inducing pathologies characteristic of colibacillosis at a dose of 88.10^7 CFU/ml.

An influence of oral treatment with the essential oil of *Thymus vulgaris* at 450mg/kg orally for five days on the effects of colibacillosis has been noted. *In vivo E. coli* causes disruption of hematological and biochemical parameters: blood sugar; CRP; GB; ASAT; ALAT. While the essential oil treatment stabilizes some hematological and biochemical parameters: GB; ALAT; Hematocrit; Cholesterol. The treatment with essential oil decreases macroscopic lesions of different organs (air sac, liver, heart) observed in chickens infected with *E. coli*. With the treatment dose 450mg/ml ensures a 40 p100 mortality reduction.

The essential oil of *Thymus vulgaris* tested has shown efficacy as an anti-infective agent in the treatment of colibacillosis. This work confirms the importance of the essential oil of *Thymus vulgaris* as an antimicrobial agent and amelioration certain hematological and biochemical parameters in broilers. The development of an aromatherapy based on the essential oil of *Thymus vulgaris* makes it possible to fix several hematological and biochemical parameters, to reduce kept chicken alive and to reduce tissue damage.

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