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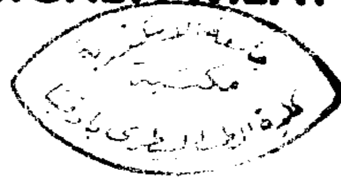
# EFFECT OF NIGELLA SATIVA (BLACK SEED) ON QUALITY OF BROILER CHICKEN MEAT



*A Thesis Presented*

*By*

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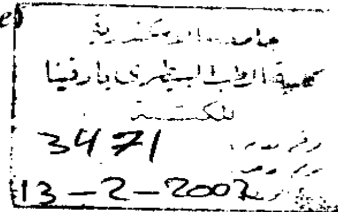
**(B.V.Sc., Fac. Vet. Med., Alexandria Univ., 1998)**

**For**

**The Degree of M.V.Sc.**

*(Meat Hygiene)*



**To**

Department of Food Hygiene

Faculty of Veterinary Medicine

Alexandria University

**EGYPT**

**2001**

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## UNDER THE SUPERVISION OF


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## قرار لجنة الحكم والمناقشة

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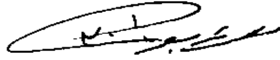

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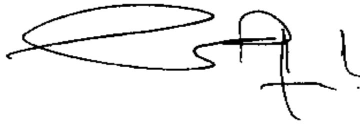

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To

My Family and

My Fiancée

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# ACKNOWLEDGMENT

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**ARABIC SUMMARY**

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# Introduction

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# 1. INTRODUCTION

From the earliest times, herbs have been used for their pain-relieving and healing abilities, and today we still rely on the curative properties of plants in about 75% of our medicines. Over the centuries, societies around the world have developed their own traditions for medicinal plants. Some of these traditions and practices may seem strange and magical, others rational and sensible, but all of them are attempt to overcome illness and suffering, and to enhance the quality of life where the life of the consumer is our life's work. Many of the thousands of plant species growing throughout the world have medicinal uses, containing active constituents that have a direct action on the body. They are used both in herbal and conventional medicine and offer benefits that pharmaceutical drugs often lack, helping to combat illness and support the body efforts to regain good health ***(Chevallier, 1996)***.

Spices and herbs therefore play an important role in human life. They are used not only for flavoring foods, but also for their antioxidant, antibacterial and preservative effects as well as for their medical properties ***(Farag et al., 1985)***. The word herb comes from the Latin herba, which simply means grass or green plant ***(Jay, 1999)***.

Artificial and synthetic feed additives may have a great public health hazards, owing to their carcinogenic, immunosuppressive and hypersensitivity effects which may resulting in death. So, the most actual solution to avoid the residues of these additives in food is back to nature by using available and worldwide herbs and most beneficial one, black seed (*Nigella sativa*).

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Also, there is little scientific evidence to support the public's perception of risk. Due to good surveillance and detection methods, the residues of agrochemicals and veterinary drugs found in meat very seldom exceed regulatory maximum residue levels (MRLs) ***(O'Keeffe, 1998)***. Continued vigilance is required, however; to prevent abuse and to assure consumers; instances of misuse of chemicals do occur and these have a negative impact on consumer's confidence.

In the case of antibiotics; an additional problem of bacterial resistance and to what extent the use of antibiotics in intensive animal production systems contributes to this problem. This is of course a microbiological hazard rather than strictly a chemical residue one, and also may be more dangerous than the disease itself. The magnitude of the problem is not known, but there is enough evidence to cause concern. Because the same antibiotics are largely used in human medicine as they are used in treatment of animals, it is not easy to establish the contribution from each source to the development of resistant bacteria. Antibiotics are used in poultry as they growth promoters (at sub-therapeutic doses), prophylactically for disease prevention or therapeutically for the treatment of infection. Microbiological and clinical evidence is growing that resistant bacteria might be passed from animals to humans, resulting in infections that are more difficult to treat. Recent examples of food poisoning from meat products infected by resistant pathogens included emerging strains of *Salmonella typhimurium* DT104 and *Campylobacter jejuni*.

The genus Nigella belongs to the family Ranunculaceae and includes about twenty species ***(Schmauder and Doebel, 1989)***. *Nigella sativa L.* is

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an annual herbaceous plant growing in countries bordering Middle Europe, Mediterranean sea and Western Asia. It is cultivated in different areas of Egypt for the purpose of its seeds ***(Toppozada et al., 1965)***. The seeds are tiny, angular, deep black in colour and have a nutty, peppery flavor ***(Mohamed and Katsumi, 1997)***. The seeds vary from 2.5 to 3.5 mm in length, and from 1.5 to 2.0 mm in width. The weight of 100 seeds varies from 0.26 to 0.33 gram ***(Ihab, 1999)***.

The seeds of *Nigella sativa L.* are known in Arab countries under the following names; Habbah Sowda, Black seed, Habbet El-Baraka, Kamun Aswad (Kalounji). In English, the *Nigella sativa* plant is commonly referred to as "Black cumin" ***(Karawya et al., 1994)***. It is also known as the "Blessed seeds" or "Black caraway".

Prophet **MOHAMED** (P.B. U.H) said "In the black seed, there is healing for every illness except death." The Arabian authors particularly Ibn El-Bitar and Ibn Sina reported that the seeds are useful in the treatment of headache, respiratory depression, asthma and expelling urinary calculus ***(Soliman, 1978)***.

In Egyptian folk medicine, the seeds are used as diuretics, carminative and as a flavoring agent to bread, while its expressed oil is used for treatment of respiratory depression and cough ***(Abou-Donia and Abdel-Kader, 1979)***. Moreover, the whole seed alone or in combination with honey or garlic are promoted for treatment of hypertension. Many reports and articles have been included, indicating the significant role of *Nigella sativa L.*

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oil in enhancing immunity and maintaining good health ***(El-Kadi and Kandil, 1987)***.

It was only in 1959, when the active principle (nigellone) was isolated from the oil ***(Mahfouz and El-Dakhakhny, 1960)***, they concluded that the essential oil has bronchodilating effect which is useful in the treatment of asthma. Another active principles were reported latter by ***El-Dakhakhny (1994)*** (thymoquinone) and by ***El-Alfy et al. (1975)*** (thymohydroquinone), which were used in treatment of asthma and diabetes.

Proximate analysis of *Nigella sativa L.* seeds showed a composition of 21% protein, 3.5% fat, 5.5% moisture, 3.7% ash and the rest being total carbohydrate ***(Babayan et al., 1978)***.

Amazingly, black seed's chemical composition is very rich and diverse. A side from its active ingredient, black seed contains 15 amino acids, proteins, carbohydrates, both fixed oils (84% fatty acids, including linolenic, and oleic), and volatile oils, alkaloids, saponin, and crude fiber as well as minerals such as calcium, iron, sodium and potassium. There are still many components in black seed that have not been identified.

Therefore, this study was planned to investigate and clarify the effect of *Nigella sativa L.* (black seed) on quality of broiler chicken meat.

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# 2. REVIEW OF LITERATURE

## 2.1. *Nigella sativa Linn.* (Black seed, Black cumin)

***Chopra et al. (1956)*** mentioned that seeds of *Nigella sativa* were used in the indigenous system of medicine and possess carminative, digestive, diuretic and antiseptic properties. Moreover, he reported that *Nigella sativa* is commonly as Kalajira or Kalonji.

***Toppozada et al. (1965)*** revealed that *Nigella sativa* seeds were used for delayed menses, lactation, flatulence, respiratory depression, abtundent in toothaches and as a diuretic.

***Nagib (1978)*** mentioned that *Nigella sativa* was known to be indigenous and it grows well in different localities in Egypt. *Nigella sativa L.* is an annual herbaceous erect branched plant widely distributed in the countries bordering the Mediterranean sea, Middle Europe and Western Asia. It is cultivated in different districts in Egypt for the purpose of the seeds.

***Babayan et al. (1978)*** mentioned that *Nigella sativa* is a herbaceous plant which is a member of the Ranunculaceae family. It is known as Nigella or black cumin. Its seeds composed of 35.49% fat, 33.96% carbohydrate, 21.26% crude protein, 5.52% moisture, 5.50% crude fiber, 3.77% ash, 1.06% calcium, 0.582% potassium, 0.098% sodium and 0.014% iron while *Nigella sativa* seed oil had 0.16% myristic, 12.08% Palmitic, 3.11% Stearic, total saturated fatty acids, 24.46% oleic, 56.12% linoleic, 0.70% linoleic and 2.53% eicosadienoic acids.

***Salomi et al. (1992)*** investigated that the main constituent of the volatile oil of *Nigella sativa* seeds is thymoquinone (TQ) which has been subjected to a

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range of pharmacological investigations in recent years due to its various effects.

***Abd El-Aal and Attia (1993)*** revealed that *Nigella sativa* seeds and oil maintaining good health. This may be attributed to the increment of serum protein and its fractions which may be due to the stimulating effect of *Nigella sativa* to the liver exhibiting an anabolic action favouring protein synthesis or due to its preserving effect to the body protein from degeneration.

***Mandour and Rady (1997)*** found that the administration of *Nigella sativa* revealed a significant decrease in the concentration of all amino acids in sera except histidine which showed a significant increase in ducklings.

***Mandour et al. (1998)*** concluded that feeding *Nigella sativa* seeds at low level for a short duration has a non-toxic effect on liver and kidney and decreased serum uric acid. Moreover, they recorded that feeding low doses of *Nigella sativa* decreases serum calcium, zinc, magnesium, and increases serum copper and iron level in broiler chicken for sixty days.

**2.2. Effect of *Nigella sativa L.* on body weight**

***Brander et al. (1982)*** considered that *Nigella sativa L.* seeds have a growth promoting effect because the seeds have antimicrobial activity.

***Rathee et al. (1982)*** recorded that the carcass traits of experimental rabbits indicated significant increase in meat and fat yields which appeared as a result of improvement of feed conversion and the efficient utilization of ration.

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***Damyanova et al. (1983)*** and ***Grobner et al. (1985)*** recorded that the rabbits fed 1% *Nigella sativa L.* oil and 3% *Nigella sativa L.* seeds exhibited a higher dressing percentage than the control group.

***Nair et al. (1991)*** found that *Nigella sativa L.* seeds prevented decrease in body weight caused by cisplatin, a cytotoxic substance.

***El-Mahdy (1997)*** found that birds infected with *Salmonella typhimurium* treated with ethereal extract of *Nigella sativa L.* revealed a significant increase in live body weight after the 3rd week from treatment compared with chickens experimentally infected with *Salmonella typhimurium* while there was no significant difference in live body weight between infected treated, non-infected treated ethereal extract of *Nigella sativa L.* and control birds after the 3rd week.

***El-Tabakh (1999)*** mentioned that the crushed *Nigella sativa L.* seeds at 2% concentration for 8 weeks produced the highest weight gain. Moreover, the total weight gain was significantly higher in the same group than other groups.

***Awadalla and Kamal (2000)*** reported that the growth stimulatory effect may be attributed to the antimicrobial properties of the black seeds which were of values as a growth promoting agent by treating the subclinical cases and so improving the health of the animal which indicated by a significant increase in body weight and a better feed consumption.

**2.3. Flavouring effect of *Nigella sativa L.***

***Chopra et al. (1956)*** concluded that black seeds posses carminative properties.

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***Furia (1968)*** recorded that *Nigella sativa L.* is one of the spices which have preservative qualities, besides their obvious function as flavour components.

***Hedrick (1972)*** mentioned that *Nigella sativa L.* seeds were used by oriental people as a carminative. Also, he recorded that the seeds, on account of their aromatic nature, were used as a spice in cooking, particularly in Italy and Southern France.

***Taha et al. (1975)*** reviewed that the volatile oil of *Nigella sativa L.* seeds was a pale yellow liquid with characteristic aromatic odour and aromatic taste.

***Babyan et al. (1978)*** discussed that *Nigella sativa L.* seeds, as carminative and flavouring agent were also used in western Cuisine. Some possible applications for the seeds have been attempted in making bakery-type products. Pizza, a favourite American dish, may be made with the seeds sprinkled on top with other pizza flavours.

***Abou-Donia and Abdel-Kader (1979)*** reported that the black seeds were used by the Syrians for cheese flavouring and by Americans for flavouring cheese and bakery products.

***Baytop (1984)*** reported that *Nigella sativa L.* was cultivated in areas of Turkey and the seeds were sold in the markets to be used as condiment and native medicine.

Freshness is the most important single criterion of quality for meat and it is essential that anyone concerned with the quality of meat must be able to

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estimate freshness with an appropriate degree of accuracy. However, the circumstances under which freshness has to be measured will vary in the chain from port to the retailer and different checks and tests will be applied accordingly. It must be borne in mind too, that freshness is not an easy property to define or to measure. Loss of freshness followed by spoilage is a complex combination of physical, chemical and microbiological processes and there is no single component to be measured which will give a freshness rating. As meat spoils it goes through a sequence of changes, which are readily detected by the human senses of sight, touch and smell. The spoilage patterns are constant for different types of meat or closely related groups of meat types ***(Ranken, 1986)***.

***Akhtar and Riffat (1991)*** examined that the sensory evaluation of organoleptic properties achieved the best scores at concentration of 0.2%. Therefore, addition of *Nigella sativa L.* oil to the processed cheese as a natural and effective preservative agent was recommended.

***Hanna (1992)*** recommended that measurement of sensory quality had influenced by variables such as the sample under investigation, the method of assessment, and the judges.

***Allen et al. (1997)*** reported that sensory evaluation represented mainly by odour evaluation. A three-member panel characterized subjective odour evaluation. The odour scores were determined by opening the sample bag, sniffing the carcass and recording a score. Scores of 1 to 5 were used according to the following descriptors: odour; 1 = fresh chicken odour; 2 = no change odour; 3 = slight odour development but still acceptable odour; 4 = definite off odour indicative of spoiled chicken odour; 5 = very strong off odour

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associated with spoiled chicken meat. Scores 1 to 3 were considered indicative of acceptable chicken meat; whereas, scores of 4 or 5 represented unacceptable and spoiled chicken meat. The scores from the three panel members were averaged and the mean was used as the score for chicken meat.

**2.4. Antimicrobial effect of *Nigella sativa Linn.***

**2.4.1. Antibacterial effect:**

***Mahfouz and El-Dakhakhny (1960)*** reported that the phenolic compound obtained from the volatile oil of *Nigella sativa L.* seeds has antimicrobial activity.

***Toppozada et al. (1965)*** concluded that the antibacterial activity of crude *Nigella sativa L.* oil was tested by different methods and it was found that the oil inhibited the growth of Gram positive and Gram negative bacteria (7 strains of *Staphylococcus aureus*, 5 strains of *Streptococcus pyogenes, 3 strains of Streptococcus faecalis,* 3 strains of pseudo-anthrax bacilli, 2 strains of *Streptococcus pneumoniae,* 3 strains of *Staphylococcus albus,* 2 strains of diphtheroid bacilli and *Bacterium coli*). Moreover, it was found that, the phenolic fraction of *Nigella sativa L.* is more potent than that of systemic antibiotic which was demonstrated by the radiological examination. Some strains of *Pseudomonas pyocyanea* were resistant to the crude *Nigella sativa L.* oil.

***Guendi et al. (1968)*** recorded that the active principle of *Nigella sativa L.* has been used successfully for treatment of rhinoscleroma (Gram –ve Von Frisch bacillus). No side actions were recorded during treatment.

***El-Alfy et al. (1975)*** reported that on refrigeration of the volatile oil of *Nigella sativa L.* (obtained by steam distillation of the oily product prepared by

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expressing the seeds), a white crystalline substance was obtained. This substance was proved to be thymohydroquinone which was tested to show its high antimicrobial activity against Gram +ve microorganisms and with low sporicidal but high sporostatic activity.

***Juknevicíene et al. (1977)*** concluded that on comparison with ampicillin, volatile oil of *Nigella sativa L.* showed high antimicrobial activity particularly against Gram positive bacteria.

***Abou Donia and Abdel-Kader (1979)*** recorded that *Nigella sativa L.* is one of the spices which are used in Syrian cheese, and it is known that these spices have preservation qualities. They found that the averages of aerobic plate counts were decreased.

***Clealand and Grunberg (1979)*** mentioned that the ether extract of *Nigella sativa L.* successfully eradicated localized infection with *Staph. aureus* in the mice when injected at the site of infection. Thus, *Nigella sativa L.* seeds can possibly provide the basis for a successful antibacterial preparation for the chemotherapy of localized infections.

***Krogstad and Moellering (1979)*** reviewed that the ether extract of *Nigella sativa L.* showed antibacterial synergism with streptomycin and gentamicin. These findings suggest that preparations from the plant, if given with these antibacterial drugs, would enhance their efficacy.

***Agrawal et al. (1979)*** reported the presence of pronounced antibacterial activity even in high dilutions with organic solvent against Gram positive and Gram negative bacteria. Moreover, they recorded that the essential oil of *Nigella*

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*sativa L.* was more effective against Gram positive bacteria e.g. *Bacillus cereus, B. anthracis, B. pupillus, Staphylococcus lutea, Staph. aureus* and *Staph. albus* than that of Gram negative ones e.g. *Escherichia coli, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella niger* and *Shigella sonnei*. Also, it has a wide activity against *Bacillus pumilus, Staphylococcus lutea* and *Vibrio cholera*.

***Brander et al. (1982)*** reviewed that black seed as any antimicrobial agent possesses a growth promoting activity because of preventing subclinical infections.

***Rathee et al. (1982)*** reported that the essential oil of *Nigella sativa L.* possesses carvone which has a very strong antimicrobial properties compared with sodium penicillin G and streptomycin sulphate in cases of *Salmonella typhi, Pseudomonas aeruginosa* and *Shigella shigae*. Nevertheless, both the fixed oil and the unsaponifiable matter do not possess any appreciable antimicrobial properties.

***Namba et al. (1985)*** examined that the addition of *Nigella sativa L.* seed is greatly useful in the preservation of food and prevention of food poisoning since some of the bacterial species inhibited by *Nigella sativa L.* extract are known to be involved in food poisoning. The odour and taste of the ether extract of *Nigella sativa L.* are weak and not disagreeable, if not pleasant, which would favour its use in food technology. Moreover, they found that methanolic extract of *Nigella sativa L.* seeds prevented adhesion of cells of *Streptococcus mutans* to smooth surfaces, which indicated that this plant could be of value in preventing dental plaques and dental caries.

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***Knobloch et al. (1986)*** found that the phenolic components of essential oils of *Nigella sativa L.* had the strongest antimicrobial activity followed by aldehydes, ketones and alcohols.

***Saxena and Vays (1986)*** reported that the petroleum ether extract of *Nigella sativa L.* seeds showed antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Streptococcus faecalis*. Also, it was shown that aqueous, ethanolic and hexane extracts of seeds exhibited strong antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus viridans* while none of these extracts has any effects on *Diplococcus pneumoniae* and *Corynebacterium diphtheriae*.

***Atilla (1989)*** reported that the most sensitive bacteria to the essential oil of *Nigella sativa L.* were *Enterobacter aerogenes, Bacillus cereus, B. subtilis* and the most resistant were *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium*.

***Kgul (1989)*** revealed that black seed has a potent antibacterial effect and an immune enhancing effect.

***Akhtar and Riffat (1991)*** showed that the addition of *Nigella sativa L.* oil to the processed cheese at concentration of 0.2 – 0.3% led to elimination of some hazardous e.g. Coliforms and Staphylococci, in addition to reducing other spoilage bacteria by more than 80% and inducing minimal growth of anaerobic spore formers. Also, the sensory evaluation of organoleptic properties achieved the best scores at concentration of 0.2%. Therefore, addition of *Nigella sativa L.* oil to the processed cheese as a natural and effective preservative agent was recommended.

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***Hanafy and Hatem (1991)*** reported that the diethyl ether extract of *Nigella sativa L.* seeds (24 – 400 µg of extract of *Nigella sativa L.*/filter paper disc) caused concentration dependant inhibition of Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). It showed antibacterial synergism and additive activity when combined with various antibiotics. It successfully eradicated a non-fatal subcutaneous *Staphylococcus aureus* infection in the mice when injected at the site of infection.

***Islam et al. (1992)*** mentioned that the antibacterial activity of the volatile oil of *Nigella sativa L.* seeds was studied against 37 isolates of *Shigella sonnei, Shigella dysenteriae, Shigella flexneri* and *Shigella baydii* and 10 strains of *Vibrio cholera* and *Escherichia coli.* Most strains were clinically resistant to ampicillin, cotrimoxazole and tetracycline. All strains tested showed promising sensitivity to the volatile oil. The minimum inhibitory concentration of the volatile oil for Shigella, *Vibrio cholera* and Escherichia strains tested was between 50 – 400 µg/ml.

***Roy et al. (1992)*** investigate the possible effects of *Nigella sativa L.* and fresh juice of *Allium sativa* L. (commonly known as garlic) and their combination against *E. coli,* Klebsiella, *Staph. aureus* isolated from urinary tract infection. A synergistic effect was obtained by using the combination of garlic juice and *Nigella sativa L.* extract. *E. coli* was found to be sensitive to the herbal combination in 90% of the cases. Similar pattern of sensitivity was also observed with Klebsiella and *Staph. aureus.*

***Mahdy (1993)*** described inhibitory action of *Nigella sativa L.* seeds against *Staph. aureus* in vitro and in vivo.

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***Mahmoud (1993)*** found that 0.5% (W/W) and 1.5% (V/V) from seeds and their oil, respectively were enough to prevent the growth of all tested strains of *Listeria monocytogenes*.

***Karawya et al. (1994)*** reported that the expressed oil has a more pronounced antimicrobial activity compared with the extracted oil especially against Gram positive microorganisms e.g. *Staph. aureus,* Micrococcus spp. and Streptococcus spp. and Gram negative microorganisms; *E. coli, Pseudomonas aeruginosa* and *Proteus vulgaris*.

***El-Kayata et al. (1995)*** reviewed that the volatile oil of *Nigella sativa L.* seeds had a high antimicrobial activity against Gram positive as well as the Gram negative ones. The minimum inhibitory concentration of the oil was between 50 and 400 mg/ml for various strains.

***Hussain and Tobji (1997)*** reported that the ethanol extract of the *Nigella sativa L.* had potent antimicrobial activity against *Escherichia coli, Staph. aureus, Staph. epidermidis* and *Bacillus cereus*.

***Mahmud and Shaila (1999)*** concluded that the *Nigella sativa L.* showed moderate antibacterial action against *E. coli.* The ethanolic and diethyl ether extracts exhibited 64.83 and 65.04% bacterial inhibition using the agar diffusion method, respectively.

***Minakshi and Banerjee (1999)*** reviewed that *Nigella sativa L.* (black cumin) had potent antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*.

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***Sokmen et al. (1999)*** mentioned that the crude extract prepared from *Nigella sativa L.* seeds showed antibacterial activity in vitro against mainly 3 bacteria and a yeast (*Candida albicans*). No activity against herpes complex viruses.

***Awadalla and Kamal (2000)*** reported that the growth stimulating effect in rabbit fed on black seeds and its oil may be attributed to the antimicrobial properties which are of value as a growth promoting agent by treating the subclinical cases and so improving the health of the animal.

***Cutter (2000)*** mentioned that the extracts of plants, especially *Nigella sativa L.* extract has potent effect against *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes* when the extract dispersed in sodium citrate (protetca one) or sodium chloride (protecta two) and the number of these organisms were reduced when the extract was applied in vitro.

***Morsi (2000)*** mentioned that different crude extracts of *Nigella sativa L.* were tested for antimicrobial effectiveness against different bacterial isolates. These isolates comprised 16 Gram negative and 6 Gram positive strains. They showed multiple resistance against antibiotics, specially the Gram negative ones. Crude extracts of *Nigella sativa L.* showed a promising effect against some of the test organisms. The most effective extracts were the crude alkaloid and water extracts. Gram negative isolates were more sensitive than Gram positive ones.

**2.4.2. Antifungal effects**:

***Taha et al. (1975)*** reported that *Nigella sativa L.* has potent activity against *Candida albicans*.

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***Agrawal et al. (1979)*** proved that the oil of *Nigella sativa L.* had an excellent antifungal activity. The growth of fungi was completely checked even with 1:100 dilution compared with hamycin and resorcinol, particularly against Aspergillus species (*A. niger* and *A. flavus*), *M. gypseum, Trichoderma viride, Candida albicans, Curvularia lunata* and *Curvularia indica*.

***Rathee et al. (1982)*** mentioned that the essential oil of black seed has a potent antifungal effect as well as a wide effect against Gram positive and Gram negative bacteria.

***El-Shayeb and Mabrouk (1984)*** revealed that *Nigella sativa L.* seeds caused reduction in mycelial growth and aflatoxin formation that ranged from 15 to 33% and 38 to 88% of the control according to *Nigella sativa L.* concentration in the medium. The study provided evidence of the activity of the seeds as anti-aflatoxigenic than anti-fungistatic.

***Saxena and Vyas (1986)*** showed that the petroleum ether and ethanol extracts of *Nigella sativa L.* have a strong activity in vitro against human pathogenic fungi (*Aspergillus fumigatus, Trichophyton mentagrophytes* and *Candida albicans*).

***Hanafy and Hatem (1991)*** discussed that discs containing the diethyl ether extract of *Nigella sativa L.* caused inhibition zones in plates inoculated with a pathogenic yeast (*Candida albicans*).

***Kandil et al. (1994)*** concluded that the different fractions of *Nigella sativa L.* seeds were found to possess antifungal activity against *Candida*

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*albicans, Aspergillus niger, Aspergillus flavus,* Penicillium species, *Microsporum gypseum* and *Microsporum canis.*

***Abdel Kader et al. (1995)*** reported that the addition of *Nigella sativa L.* by 2.5, 5 and 10% to the growth media inhibited the growth of dermatophytes isolated from clinical cases of sheep by 35.13 to 100%.

***El-Kayati et al. (1995)*** concluded that the *Nigella sativa L.* oil had antimicrobial activity against yeast and mould.

***Mandour et al. (1995)*** reviewed that the supplementation of *Nigella sativa L.* seeds significantly increased the gamma-globulin level. Furthermore, its addition to the diet containing 500 ppb aflatoxin protected the depression of gamma-globulin that may minimize or avoid the harmful effects of aflatoxins.

***Siddiqui and Sharma (1996)*** reviewed that the antifungal activity of *Nigella sativa L.* was attributed to the essential oil of the seed.

***Minakshi and Banerjee (1999)*** concluded that black cumin (*Nigella sativa*) had potent antimicrobial properties against *Saccharomyces cerevisiae.*

***Sokmen et al. (1999)*** showed that *Nigella sativa L.* exhibited antimicrobial activities in vitro against a yeast (*Candida albicans*).

**2.4.3. Antiparasitic activity of *Nigella sativa L.*:**

***Dhar et al. (1968)*** showed that the ethanolic extract (50%) of *Nigella sativa L.* has antiprotozoal activity against *Entamoeba histolytica* strain STA.

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***Agrawal et al. (1979)*** concluded that the essential oil of *Nigella sativa L.* was found to have a good activity against earth and tape worms. The activity against hook and nodular worms was somewhat higher in comparison with that of hexylresorcinol. Also, they discussed that the essential oil from seeds showed in vitro activity against earth and tape worms, the action being comparable with that of piperazine phosphate.

***Akhtar and Javed (1991)*** reviewed that the *Nigella sativa L.* seeds and niclosamide were equally effective in treating the infested sheep with *Moniezia expansa*.

***Akhtar and Riffat (1991)*** reported that single oral administration of 40 mg powdered *Nigella sativa L.* seeds per kg body weight and the equivalent amount of its ethanolic extract produced reduction of faecal egg count of *Taenia saginata* and *Hymenolepis nana* in children not significantly different from 50 mg niclosamide per kg body weight. The crude did not produce any side effects in the treated doses.

***Korshom et al. (1998)*** studied the trematocide activity of *Nigella sativa L.* seeds compared with Hapadex against rumen fluke (Paramphistomum) in sheep. They reported that the use of *Nigella sativa L.* seeds reflexed a very good efficacy against Paramphistomum in sheep, in addition, it is economically cheap.

**2.5. Biological effects of *Nigella sativa* seeds**:

***Edward (1976)*** reported that polyunsaturated oil may stimulate cholesterol excretion into the intestine and its oxidation to bile acids.

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***Al-Awadi et al. (1991)*** concluded that *Nigella sativa L.* extract caused a decrease of blood glucose level through inhibition of hepatic gluconeogenesis.

***Nair et al. (1991)*** found that an extract of *Nigella sativa* seed tend to protect mice from cisplatin (a cytotoxic drug) induced fall in haemoglobin levels and leucocytic counts.

***El-Dakhakhny et al. (1994)*** found that the administration of *Nigella sativa L.* oil in hypertensive rats for 6 weeks resulted in significant decrease in serum total cholesterol and high density lipoprotein.

***Abd El-Aziz et al. (1995)*** emphasized ~~that the~~ feeding of black seed increases the number of B cells of islets of Langerhan's of pancreas which resulted in hypoglycemic effect.

***Houghton et al. (1995)*** reported that the intraperitoneal administration of the volatile oil of *Nigella sativa L.* seeds (50 mg/kg b.wt.) to fasting normal and diabetic rabbits produced significant hypoglycemic effect. These effects were consistent and time dependent. In normal animals, 15 and 23% decreases in fasting plasma glucose were detected 4 and 6 h after treatment, respectively. While, the same treatment produced 12 and 21% decreases in the fasting glucose levels in diabetic rabbits at the 4 h and the 6 h time intervals, respectively. The mode of action of the hypoglycemic effects exhibited by the volatile oil from *Nigella sativa L.* seeds remain to be elucidated.

***Abdel-Khalek et al. (1996)*** mentioned that the addition of *Nigella sativa L.* seeds to hyperlipidemic diet led to significant decrease in total cholesterol

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and triglyceride content, but no significant effect on serum glucose was observed.

***Abd El-Maksoud et al. (1996)*** investigated that the causative potency of *Nigella sativa L.* was attributed to serum total cholesterol, LDL cholesterol and triglycerides were lowered, while HDL cholesterol was elevated.

***El-Badrawy (1996)*** concluded that the hydroalcoholic extract of *Nigella sativa L.* seeds had no hypoglycemic effects in normal and alloxan induced diabetic rabbits.

***Hassanin and Hassan (1996)*** recorded that the administration of *Nigella sativa L.* produced significant increase in serum glucose by about 25% over the control level. When it was supplemented at 1, 2 and 10% of the diets, there was significant increase in all serum parameters (glucose, total lipids, total cholesterol, triglycerides, total protein, uric acid and urea). It was concluded that *Nigella sativa L.* seeds had no hypoglycemic effect.

***Hedaya (1996)*** found that the oral administration of low dose of *Nigella sativa L.* oil to the rats caused a significant increase in haemoglobin concentration, PCV, RBCs count and lymphocyte %, while the WBCs count, neutrophil, acidophil, monocytes percentages were not significantly changed.

***Khodary et al. (1996)*** reported that low dose of crushed *Nigella sativa L.* seeds (1 and 2% of the diet) in chickens caused a significant increase of haemoglobin %, PCV%, RBCs, WBCs and thrombocytes counts whereas, the high dose (3%) did not elicit the same effect.

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***Mandour and Rady (1997)*** showed that the concentrations of amino acids were significantly decreased except for histidine which was greatly increased in the sera of ducklings fed 2.5% black seeds for 12 days.

***Al-Gaby (1998)*** discussed that the supplementation of diet with *Nigella sativa L.* seeds caused significant increases in serum total protein and its two fractions albumin and globulin and did not have any adverse nutritional effects in the levels of lipid fractions in the serum.

***Awadalla and Kamal (2000)*** observed that the hypolipemic and hypocholesterolemic effect was more pronounced in animals fed with 1% *Nigella sativa L.* oil and 3% *Nigella sativa L.* seeds which may be attributed to its higher content of unsaturated fatty acids.

**2.6. Other effects of *Nigella sativa L.*:**

**2.6.1. Effect of *Nigella sativa L.* on the immune system:**

***El-Kadi et al. (1987)*** tested the effect of *Nigella sativa L.* on T-cell subset distribution in volunteers with a low helper T-cell ($T_H$) to suppressor T-cells ($T_S$) ratio. They suggested that the intake of *Nigella sativa L.* may enhance T-cell mediated immunity through improvement of $T_H$:$T_S$ ratio. This effect may exert an important role in cases involving cellular immunodeficiency particularly cancer and AIDS.

***Abdel Aal and Attia (1993)*** reported that *Nigella sativa L.* seed oil played a significant role in increasing immunity and maintaining good health.

***Basil and Erwa (1993)*** studied the effect of *Nigella sativa L.* on ingestion ability of mice peritoneal macrophages. They found that there was a significant

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enhancement of ingestion against dead *Candida albicans*. Electron microscopical studies also showed no differences between macrophages of the two groups (*Nigella sativa L*. fed group and control group) except the degree of ingestion.

***Mahdy (1993)*** investigated the effect of *Nigella sativa L*. seeds (1 g seeds to be chewed twice daily for 2 months) on the level of IgG and IgM in 20 cirrhotic patients. He found that, there was no significant changes in either IgG or IgM levels.

***Abdel-Azim (1996)*** concluded that the oil of *Nigella sativa L*. can be considered as an immunostimulant by increasing the lymphocyte count.

***Nassar (1997)*** found that short term use of *Nigella sativa L*. seeds and oil led to beneficial effects in the form of improvement of the body immunity represented by an increase in serum globulins. But the long term intake of *Nigella sativa L*. did not exert the same effect.

***Abo Khalil (1999)*** found a significant increase in total serum protein in chickens receiving higher level of *Nigella sativa L*. seeds (4 – 8%) for 36 days. He suggested that the hyperproteinaemia in chickens may be due to immunostimulant effect of seeds on liver.

***Awadalla and Kamal (2000)*** reported that the *Nigella sativa L*. has important positive immunostimulant effect and antimicrobial effect which prevented the subclinical infections.

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***Swamy and Tan (2000)*** recorded that the ethanolic extract of *Nigella sativa L.* seeds has potent immunopotentiating effect on the cellular immune response.

**2.6.2. Anticancer activity of *Nigella sativa L.*:**

***Atta-Ur-Rahman et al. (1985)*** could isolate the novel alkaloid (nigellicine) from the *Nigella sativa L.* seeds. Nigellicine was claimed to be analogous to some anticancer agents.

***Hassan (1985)*** reported that no dysplastic alterations or carcinoma were observed in the cheek pouches of the groups of hamsters treated with *Nigella sativa L.* She concluded that the oil imparted a protective effect against chemical carcinogenesis and/or retarded the carcinogenic process.

***Siegal et al. (1987)*** mentioned that the unsaturated fatty acids of *Nigella sativa L.* represented by oleic, linoleic, linolenic and arachidonic acids were significantly effective on the tumour cells in vitro.

***Salomi et al. (1989)*** concluded that the aqueous extract of *Nigella sativa L.* seeds along with *Smilax china* and *Hemidesmus indicus* (1:3:2) was reported to be beneficial in curing the oral cancer diagnosed by modern method.

***Salomi et al. (1991)*** recorded that topical application of *Nigella sativa L.* and *Crocus sativus* extract (common food spices) inhibited skin carcinogenesis in mice. Moreover, intraperitoneal administration of *Nigella sativa L.* (100 mg/kg body weight) restricted tumour incidence to 33.3% compared with 100% in treated controls.

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***Salomi et al. (1992)*** reported that the active principle of *Nigella sativa L.* seeds containing fatty acids was completely inhibited the Erlich ascites carcinoma (EAC).

***Badary et al. (1998)*** recorded that the administration of thymoquinone (active ingredient of *Nigella sativa*) in drinking water inhibited both the incidence and multiplicity of forestomach papillomas induced by benzopyrene in female Swiss albino mice.

***Korshom (1998)*** concluded that subcutaneous administration of methanolic extract of *Nigella sativa L.* seeds in rats induced the hepatic glutathione S transferases which may explain the antitumour activity of *Nigella sativa L.* seeds.

***Badary et al. (1999)*** discussed that the thymoquinone was the main constituent of the volatile oil of *Nigella sativa L.* seed, has a powerful anticarcinogenic potential.

**2.6.3. Analgesic, anti-inflammatory and antioxidant activity of *Nigella sativa L.* seeds**:

***Ibn El-Bitar, Ibn Sina (428 higriah)*** and ***Dawood El-Antakia (1094 higriah)*** mentioned that the seeds were useful in treatment of headache.

***Vohora and Dandllya (1992)*** reported that *Nigella sativa* showing promising narcotic analgesic activity.

***Khanna et al. (1993)*** reported that the seeds of *Nigella sativa L.* have shown analgesic activity in mice.

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***Houghton et al. (1995)*** reported that the unsaturated fatty acids of *Nigella sativa L.* have antioxidant activity.

***Saad et al. (1998)*** recorded that the *Nigella sativa* seeds showed a significant decrement in lipid peroxidation which attributed to the increased activities of glutathione reductase and glutathione transferase.

***Awadalla and Kamal (2000)*** reviewed that the *Nigella sativa* exhibited an anabolic action due to its preserving effect to the body protein from degeneration which express its effect on growth performance.

**2.6.4. Antihistaminic effect of *Nigella sativa L.*:**

***Gomaa et al. (1982)*** reported that nigellone (the active principle of *Nigella sativa*) inhibited the histamine release from the sensitized rat mast cells due to the antigen action.

***Chakyavarty (1993)*** reported that nigellone in relatively low concentration is very active in inhibiting histamine release from rat peritoneal mast cells induced by several secretogauges, antigen in sensitized cells, compound 48/80 and the calcium – ionophore A23187. He suggested that this action of nigellone seems to be due to decreasing intracellular calcium or by inhibition of protein kinase C. He added that there was also an interaction for a mild inhibition of oxidative energy metabolism.

***Mandour and Rady (1997)*** reported that feeding *Nigella sativa L.* seeds (2.5%) in birds led to significant increase in the serum concentration of the amino acid histidine. This observation was attributed to the inhibitory effect of the active principle (thymoquinone) of *Nigella sativa* seeds on the synthesis of histamine.

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# 3. MATERIAL AND METHODS

## 3.1. Material

### 3.1.1. Birds

A total of 120 broiler chicks (Arber acres) of 20 days old and weighing from 550 – 650 grams was obtained from Cairo Company for poultry production. The chicks were housed in a separate place of poultry farm at El-Montazah area and kept with free access to water and feed.

### 3.1.2. Feeding

The birds were fed on a balanced ration obtained from Cairo Company for poultry production as labeled by the producer contained not less than 21% raw protein, 2.9% crude fat and not more than 4% fibers which were achieved using the following constituents: 600 kg maize, 250 kg soya bean, 100 kg concentrates, 15 kg bone meal, 25 kg oil, 1 kg methionine, 1 kg zinc bacitracin, 1 kg calcium chloride, 1 kg dicalcium phosphate, 3 kg premixes, 1 kg antifungal (Sorbatox), 1 kg anticoccidial and 1 kg antimycotoxins per ton ration.

### 3.1.3. *Nigella sativa* (black seed)

The whole seeds were obtained from the market (Barghash market for black seed and its oil) in tightly closed plastic bags.

### 3.1.4. Media, chemicals, glasswares and apparatus and instruments

#### 3.1.4.1. Media

- Standard plate count agar (Oxoid)
- Violent red bile glucose (VRBG) agar (Biolife)

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- Salmonella-Shigella (SS) agar (Difco)
- Mannitol salt agar (Oxoid)
- Sabouraud's dextrose agar (Oxoid)
- Baird-Parker agar (Oxoid)
- Brain heart infusion broth (Oxoid)
- Simmon's citrate agar (Oxoid)
- Triple sugar iron agar (Difco)
- Urea agar
- Rice agar.

**3.1.4.2. Chemicals**

- Concentrated sulphuric acid ($H_2SO_4$)
- 0.1 N sulphuric acid
- Sodium hydroxide (40%) solution
- 0.1 N sodium hydroxide
- Screened methyl red indicator
- Catalyst mixture
- Distilled water
- Petroleum ether
- Copper sulphate solution 5%
- Eber's reagent
- Indole reagent
- 0.2% bromocresol purple indicator
- Hydrogen peroxide ($H_2O_2$) 3%
- Paraffin wax
- Methyl red reagent
- Chloroform
- Ethyl alcohol
- Xylol
- Formalin

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- Kits for determinations of serum cholesterol, triglycerides, total protein and albumin (Bimeda Co.).

**3.1.4.3. Glasswares**

- Test tubes
- Wassermann tubes
- Petri dishes (10 – 15 cm)
- Pipettes (1, 2, 5 and 10 ml)
- Four glass plates (1 kg weight)
- Conical flasks (100, 200, 250, 500 and 1000 ml)
- Homogenizer flask
- Kjeldahl flasks 500 ml
- Bent glass rod
- Glass rods
- Disposal sterile plastic bags
- Dissecators
- Glass slides
- Cover slides
- Durham's tubes

**3.1.4.4. Instrument and apparatus**

- Forceps
- Scissors
- Scalpel
- Spatula
- Homogenizer, operating at 1400 rpm
- Benzene flame
- Hot air oven (panacea)
- Balance (Sartorius)
- Autoclave (Anchor-China)

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- Incubator (MLW Electro)
- Centrifuge, operating at 3000 rpm
- Filter paper (Whatmann No. 1)
- Water bath (MLW Electro)
- pH meter (Hanna)
- Soxhlet apparatus
- Venipuncture
- Eppendorff.

## 3.2. Methods

### 3.2.1. Preparation of *Nigella sativa* for feeding

The seeds were finely crushed, the crushing was done just before adding the seeds to the ration at the time of offering ration to the birds.

### 3.2.2. Experiment

The chicks were classified into six groups (20 chicks each). The groups were fed on ration containing *Nigella sativa* at different concentrations as shown in the following Table:

| Groups | No. of birds | Concentration (%) of *Nigella sativa* in ration | Period of Feeding (days) |
|---|---|---|---|
| First | 20 | 0.5 | 20 |
| Second | 20 | 1.0 | 20 |
| Third | 20 | 1.5 | 20 |
| Fourth | 20 | 2.0 | 20 |
| Fifth | 20 | 2.5 | 20 |
| Sixth (control) | 20 | 0.0 | 20 |

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### 3.2.3. Sampling

#### 3.2.3.1. Collection and preparation of blood samples

Blood samples were collected from the wing vein by venipuncture in a clean dry sterilized Wassermann tubes after 20 days of feeding the ration from treated groups as well as from the control group. The sample volume was about 5 ml. After clotting, serum was separated from the whole blood, kept in Eppendorff vials, identified and stored at – 20 °C till assayed for the biochemical parameters.

#### 3.2.3.2. Collection of muscle samples

Following feeding broiler chicks for twenty days on rations supplemented with *Nigella sativa*, 12 broiler birds were slaughtered from each group. Each bird was opened and samples from each of breast and thigh were divided into 4 portions for organoleptic, chemical, microbiological and histopathological examinations. All samples were kept in a clean sterile plastic bag and transferred with a minimum of delay to the laboratory.

### 3.2.4. Organoleptic evaluation

#### 3.2.4.1. Sensory examination

Samples were evaluated for their colour, odour and consistency according to the method reported by ***Morr-Marry (1970)***. This method was carried out as follows: five panelists examined the samples for different physical characters (colour, odour and texture), everyone of them gave a score from 5 (maximum intensity) to 1 (nil). The scores were recorded as the following Table:

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| Factor | Scores 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Colour | Very dark | Dark red | Red patches | Dull colour | Bright colour |
| Odour | Offensive | Very bad | Bad | Fair | Normal odour |
| Texture | Hard | Tough | Doughy | Soft | Firm |

### 3.2.4.2. Physical examination

#### 3.2.4.2.1. Thermal shrinkage

Thermal shrinkage was carried out according to ***Walczak (1959)*** as follows: 20 g muscle samples were weighed and chopped (W1) in a sterile pored aluminum foil. The surface of the foil was sprayed with distilled water then kept in an oven at 85 °C for 10 minutes. The foil was then opened and the meat was transferred to another aluminum foil and left for 4 hours in contact with the atmosphere of the laboratory then weighed (W2).

$$\text{Thermal shrinkage} = \frac{W1 - W2}{W1} \times 100$$

#### 3.2.4.2.2. Water holding capacity (WHC)

WHC was measured according to ***Grau and Hamm (1957)*** as modified by ***Volovinskaia and Merkolova (1958)***, which was done as follows: 0.3 g of muscle sample was weighed and placed on filter paper (Whatmann No. 1) and pressed for 10 minutes using 1 kg weight glass plates (25 x 25 cm in diameter). Two zones were formed on the filter paper (the first zone was the pressed meat and the second zone was the water imbibed). The diameters of the first and second zones were measured and the area of each zone was calculated and by using the following formula.

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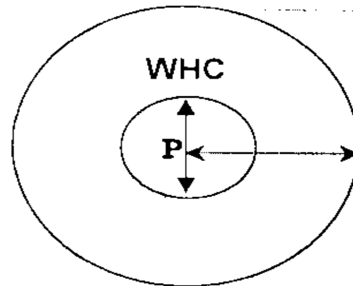
The area of the outer zone represents the water holding capacity in $Cm^2$. Each $Cm^2$ of the outer zone is equivalent to 8.4 mg free water. Water holding capacity was calculated as follows:

WHC =

$$\frac{\text{Moisture content} - \left[\dfrac{8.4 \times cm^2 \text{ outer zone area} \times 100}{0.3 \times 1000}\right]}{\text{Moisture content}} \times 100$$




WHC = Water holding capacity of the muscle.

P = Plasticity of the muscle.

### 3.2.4.2.3. Plasticity

According to ***Grau and Hamm (1957)***, the internal zone formed due to the pressed meat sample indicates only the plasticity in $cm^2$. Also, plasticity was calculated as $cm^2$ of the internal zone per 1 g T.N. as follows:

$$\text{Plasticity } (Cm^2/g \text{ T.N.}) = \frac{(Cm^2 \text{ internal zone} \div 0.03)}{\% \text{ T.N. on wet weight basis}} \times 100$$

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### 3.2.5. Chemical evaluation

#### 3.2.5.1. Preparation of samples for chemical examination *(AOAC, 1990)*:

50 g from each muscle sample were weighed and homogenized in meat homogenizer, then they were kept chilled in glass container with air and water tight cover to prevent loss of water and samples decomposition.

#### 3.2.5.2. Determination of keeping quality

#### 3.2.5.2.1. Measurement of pH *(Reagon et al., 1981)*:

The pH value was determined by using digital pH meter, the electrode was immersed in prepared meat extract (10 g of meat and 10 ml of distilled water). The results were recorded.

#### 3.2.5.2.2. Copper sulphate test *(Mousa, 1991)*

Five ml of meat extract were placed in a test tube, then 3 drops of 5% copper sulphate were added to the tube and left for 5 minutes. Positive results were indicated by formation of precipitate or gel sediment. The results were recorded.

#### 3.2.5.2.3. Eber's test *(Mousa, 1991)*

Two ml of Eber's reagent were placed in a test tube, to a thin glass rod, a stopper was attached at one end and a part of the tested meat was fixed at the other end. The rod was placed in the test tube just above the surface of the reagent (1 part HCl, 1 part ether and 3 parts ethyl alcohol 96%) about 1 cm. The stopper was fitted on the mouth of the test tube. Positive reaction was indicated by the appearance of grayish-white cloudiness after few seconds later. The results were recorded.

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**3.2.5.3. Determination of moisture percent *(Pearson, 1972)***

Accurately 5 g of each sample were weighed into a flat bottomed porcelain dish and placed in a thermostatically controlled oven at 100 °C for 20 minutes the dish was removed and placed in a dissecator to cool then weighed, the process of heating and dissecation was repeated till two successive constant weights were obtained. The moisture content was calculated and recorded as follows:

$$\text{Moisture \%} = \frac{\text{Weight loss (g)}}{\text{Weight of the sample (g)}} \times 100$$

**3.2.5.4. Determination of total protein content *(AOAC, 1985)***

**3.2.5.4.1. Digestion**

Two grams of the prepared sample were placed in a dry 500 ml Kjeldahl digestion flask (which contains a clean and dry glass beads) and 8 grams of catalytic mixture (10 grams of potassium sulphate and 0.7 gram copper sulphate) were added, then 24 ml concentrated nitrogen free sulphuric acid were added carefully down the side of the flask. The flask was then kept in decline position with gentle heating till frothing subsides, then followed by vigorous heating till the liquid became clear (complete digestion two hours after the liquid became clear). The flask was cooled and the mixture was diluted with 200 ml distilled water then transferred to one liter distillation flask.

**3.2.5.4.2. Distillation**

The flask was rinsed several times and the rinse was added to the distillation flask until the total volume was about 400 ml, then 75 ml of sodium hydroxide 40% (40 grams sodium hydroxide/100 ml distilled water)

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were added and the flask was fixed in its place in distillation unit with extended of distillator outlet to the dip into conical flask containing 100 ml of 0.1 N sulphuric acid (28 ml sulphuric acid/1 liter distilled water) with few drops of methyl red indicator (1 part 0.2% methyl red + 2 parts 0.2% bromocresol green). The distillation was continued until 300 ml of the distillate were received.

**3.2.5.4.3. Titration**

The distillate was titrated against 0.1 N sodium hydroxide solution (4 grams sodium hydroxide/100 ml distilled water) until the appearance of yellow colour (end point).

**3.2.2.6.4. Calculation:**

$$\text{Total nitrogen \%} = \frac{(Y - R) \times 0.0014 \times 100}{\text{Weight of sample taken}}$$

Protein % = total nitrogen % x 6.25.

Y = ml of 0.1 N sulphuric acid.

R = ml of 0.1 N sodium hydroxide.

1 ml ammonium sulphate = 0.0014 gram nitrogen.

1 g nitrogen = 0.0014 (Y - R).

1 g protein = Y - R x 0.0014 x 6.25.

**3.2.5.5. Determination of total fat *(Pearson, 1972)***

Using Soxhlet extraction method for crude fat determination or total fat.

**3.2.5.5.1. Drying**

Ground samples of breast and thigh (50 g) in meat homogenizer were placed into a weighed Petri dish then placed in an oven controlled at 80 °C for 24 hours till complete drying.

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#### 3.2.5.5.2. Extraction

Two grams of moisture free samples were weighed and placed in the extraction tube of the Soxhlet apparatus. 150 ml of petroleum ether were introduced into a weighed Soxhlet flask (W1) and was connected to the extraction tube and the latter was connected to the condenser. Heating of the flask was controlled in such a way that the ether remained boiled. The extraction was continued for 18 – 24 hours, cooled, evaporated the ether in weighed Soxhlet flask and dried to constant weight (W2) in an oven at 150 °C.

#### 3.2.5.5.3. Calculation

Weight of fat is the gain weight of Soxhlet flask = W2 – W1.

$$\text{Percentage of fat in sample} = \frac{W2 - W1}{\text{Weight of the sample (g)}} \times 100$$

### 3.2.6. Biochemical evaluation

The serum samples were thawed at room temperature then the different biochemical parameters were measured the following:

#### 3.2.6.1. Determination of serum total cholesterol:

Quantitative enzymatic determination of total cholesterol in serum was used according to ***Allain et al. (1974)*** using kits from Stanbio (Stanbio Laboratory Inc., 2930 East Houston Street, San Antonio, Texas 78202).

**Principle:**

Cholesterol esterase (CE) hydrolyses esters to free cholesterol and fatty acids. The free cholesterol so produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase ($CO_X$) to cholest-4-en-3-one and hydrogen peroxide. A quinoneimine chromogen is produced

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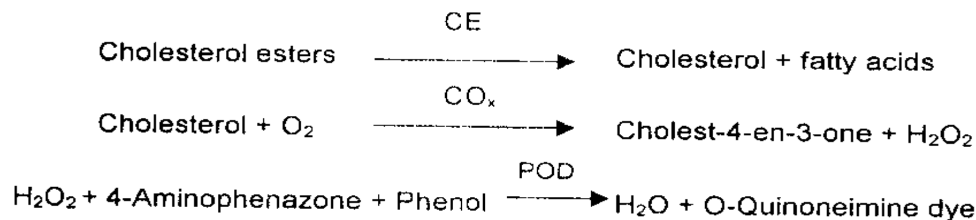
when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase (POD), with hydrogen peroxide. The intensity of the final red color is proportional to the total cholesterol concentration. Measurements were performed at 500 nm.

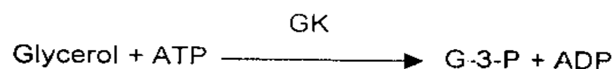



**3.2.6.2. Determination of serum triglycerides:**

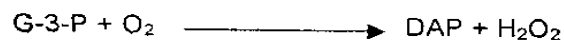
Measurement of triglyceride levels in the serum samples followed the method of ***Fossati and Preneipe (1982)*** described in the kits of Stanbio (Stanbio Laboratory Inc., 2930 East Houston Street, San Antonio, Texas 78202). (Wavelength = 500 nm).

**Principle:**

1. Glycerol and fatty acids are first formed by lipase action on the triglycerides.
2. Glycerol is then phosphorylated by adenosine-5 -triphosphate (ATP) to produce glycerol-3-phosphate (G-3-P) and adenosine-5 -diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK):




3. The G-3-P is oxidized by glycerylphosphate oxidase (GPO) producing dihydroxyacetone phosphate (DAP) and hydrogen peroxide:

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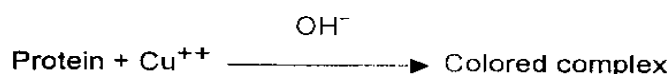
4. Peroxidase reacts with a 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase (POD) to form quinoneimine.

**3.2.6.3. Determination of serum total protein:**

Serum total protein was determined according to ***Doumas et al. (1981)*** using a commercial kit from Chemroy (Biochemical Trade Inc., Miami, Florida 33166, USA).

**Principle:**

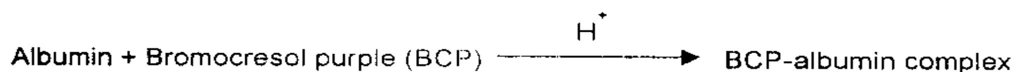
In this method, the Biuret reaction is used to quantitate serum total protein. The method depends on the determination of colored complex (purple) between peptide chains and cupric ions in strongly alkaline solutions. The amount of complex formed, determined spectrophotometrically at 540 nm, is proportional to the protein concentration.




**3.2.6.4. Determination of serum albumin:**

Serum albumin was determined according to ***Reinhold (1953)*** using kits of Chemroy (Biochemical Trade Inc., Miami, Florida 33166, USA).

**Principle:**




The amount of the complex formed which can be measured photometrically at 605 nm, is proportional to the albumin concentration.

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### 3.2.6.5. Determination of serum globulin:

Globulin was estimated as total protein minus albumin ***(Coles, 1974).***

### 3.2.7. Microbiological evaluation

### 3.2.7.1. Preparation of the samples for microbiological examination

The samples were taken from the thigh and breast of each group. Samples of 10 g were homogenized in 90 ml of 0.1% sterile peptone water in stomacher lab blender for 30 seconds to give a dilution of 1/10, then the decimal dilutions up to $10^{-5}$ were prepared.

### 3.2.7.2. Total mesophilic bacterial count ***(Nickerson and Sinskey, 1972)***

One ml from prepared serial dilutions were transferred with sterile pipette to each of two separate sterile Petri dishes from both thigh and breast. Then about 10 ml of the sterile standard plate count agar melted and cooled at 45 °C were poured to each Petri dish. Inoculated plates after being mixed and solidified were incubated at 37 °C for 48 hours. Colonies were counted and recorded as the total colony count per gram of the sample.

### 3.2.7.3. Determination of total Coliforms

The technique adapted was that recommended by ***ICMSF (1978)***. On each duplicate pre-dried surface of violet red bile (VRB) agar plates, 0.1 ml from each of serial dilutions prepared was plated and spread evenly on the surface of the agar using sterile bent glass rod then applying a thin layer of melted VRB agar at 45 °C on the inoculating surface. After solidification, inoculated plates were then incubated at 37 °C for 24 hours. Dark red colonies of a diameter 1 – 2 mm, mostly surrounded by a reddish halo area were counted and recorded as a Coliform count per gram.

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### 3.2.7.4. Enterobacteriaceae count *(ICMSF, 1978)*

From each of the previously prepared serial dilutions 0.1 ml aliquots were delivered into a duplicate set of Petri dishes, previously inoculated 10 – 15 of a sterile violet red bile glucose agar (VRBG) after sufficient spreading a cover layer (tempered promptly to about 45 °C) of VRBG agar was poured over all the plates. The plates were incubated at an inverted position at 37 °C for 24 hours. Suspected colonies (clearly visible purple colonies surrounded by a purple haloes) were counted. The results were calculated and recorded as Enterobacteriaceae count per gram.

### 3.2.7.5. Detection of *Staphylococcus aureus*

#### 3.2.7.5.1. *Staphylococcus aureus* count

The use of Baird-Parker agar is highly recommended for enumeration of coagulase positive *S. aureus (ICMSF, 1978)*. From each of prepared serial dilutions 0.1 ml was inoculated onto the surface of separate plates and spread with a sterile bent glass rod with the surface of the medium appears dry. Duplicate plates were prepared for each dilution. The plates were incubated at an inverted position at 35 - 37 °C for 24 – 48 hours. Black shiny colonies surrounded by clear haloes were counted as a *Staphylococcus aureus*. The number per gram was calculated and recorded. Suspected colonies were stabbed in semi-solid agar tubes for further biochemical identification.

#### 3.2.7.5.2. Identification of *Staphylococcus aureus*

A loopful from purified isolates was inoculated into tubes containing 5 ml brain heart infusion (BHI) broth. Inoculated tubes were incubated at 37 °C for 18 hours. The growth cultures from BHI were identified as follows.

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**3.2.7.5.2.1. Morphological examination**

**3.2.7.5.2.1.1. Staining *(Cruickshank et al., 1975)***

Films were prepared from the pure culture of isolated organisms stained with Gram's stain and examined microscopically for a Gram positive clusters.

**3.2.7.5.2.1.2. Motility test *(ICMSF, 1978)***

The medium was inoculated by stabbing to a depth of 1 mm and incubated at 37 °C for 24 hours. A circular growth from the line of stabbing was considered a positive result.

**3.2.7.5.2.2. Biochemical identification according to *ICMSF (1982)* and *(APHA, 1984)*:**

**3.2.7.5.2.2.1. Catalase test**

One drop of hydrogen peroxide (3%) was added to a loopful from purified colonies on a clear dry glass slide, then the cover slide was applied. Appearance of gas bubbles indicates presence of catalase enzyme.

**3.2.7.5.2.2.2. Mannitol test**

Suspected colonies were subcultured on mannitol salt agar and incubated at 37 °C for 24 hours and examined for presence of yellow colonies surrounded by a yellow halo.

**3.2.7.5.2.2.3. Coagulase test (tube method)**

0.1 ml from overnight brain heart infusion broth culture was added to 0.3 ml of sterile rabbit plasma in a Wasserman tube and then incubated at 37 °C. After 24 hours, all incubated tubes were observed for visible fibrin clot

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formation. Tubes showed no clot were incubated and examined every 2 hours up to 24 hours. Tubes showed clot formation were recorded as positive.

#### 3.2.7.5.2.2.4. Thermostable nuclease production (TNase test)

In toludine blue-O-DNase agar, 2 mm diameter wells were cut using a sterile capillary tube and agar plugs were removed by aspiration of 10 µl of heated broth culture (15 minutes in boiling water bath) were added to each well. The plates were incubated at 37 °C for 4 hours. Appearance of a bright pink halo was recorded as positive thermostable deoxyribonuclease which is a characteristic reaction of *Staphylococcus aureus*.

### 3.2.7.6. Detection of Salmonellae *(ICMSF, 1982)*

#### 3.2.7.6.1. Isolation of Salmonellae

From the previously prepared homogenate, 1 ml was aseptically inoculated into 10 ml of tetrathionate brilliant green broth tubes and thoroughly mixed before being incubated at 43 °C for 18 hours. 0.1 ml from enriched culture was streaked onto duplicate plates of Salmonella Shigella (SS) agar, the inoculated plates were incubated at 37 °C for 24 hours. Large creamy with blackish center colonies were counted as Salmonella species per gram. The suspected colonies were picked up and stabbed into semi-solid agar tubes for further identification.

#### 3.2.7.6.2. Identification of Salmonellae

#### 3.2.7.6.2.1. Morphological examination

#### 3.2.7.6.2.1.1. Staining *(Cruickshank et al., 1975)*

Films were prepared from pure suspected cultures, stained with Gram's stain and examined microscopically for Gram negative bacilli.

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**3.2.7.6.2.1.2. Motility test *(ICMSF, 1978)***

Suspected organisms were inoculated into semi-solid agar and incubated at 37 °C for 18 hours. Motility was assessed by circular growth in the medium.

**3.2.7.6.2.2. Biochemical identification according to *ICMSF (1982)* and *APHA (1984)*:**

Suspected isolates were biochemically identified using the following biochemical reactions.

**3.2.7.6.2.2.1. Indole production test**

Tubes of 1% peptone water were inoculated with pure suspected cultured and incubated at 37 °C for 48 hours. About 0.5 ml of indole reagent was trickled down the side of each tube. The development of rosy red ring (on the surface) after one minute indicates positive reaction.

**3.2.7.6.2.2.2. Methyl red test**

Tubes containing buffered glucose broth inoculated with pure suspected cultured and incubated for 48 hours at 37 °C. To each tube, few drops of methyl red reagent were added. Positive reaction was indicated by appearance of a red colour.

**3.2.7.6.2.2.3. Voges-Proskauer test**

The suspected cultures were inoculated into tubes of buffered glucose broth and incubated at 37 °C for 48 hours. From each culture, 1 ml was taken in a sterile test tube then 0.6 ml of alpha-naphthol solution and 0.4 ml KOH 40% solution were added. Developing of pink colour within 15 – 20 minutes in the mixture was recorded as a positive result.

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**3.2.7.6.2.2.4. Citrate utilization test**

A loopful from pure suspected cultures was inoculated into butt and streaked onto the slant of Simmons citrate agar tubes, then incubated at 37 °C for 48 hours. The development of blue colouration indicates utilization of citrate.

**3.2.7.6.2.2.5. Sugar fermentation**

A loopful from suspected culture was inoculated into a tube containing 1% peptone water and 0.2% bromocresol purple indicator to which 1% of the following sugars were added; glucose, mannitol, sorbitol and inositol. Durham's tubes were inverted into the tubes for collection of gas. After incubation at 37 °C, the reaction of inoculated tubes was noticed daily for 7 successive days.

**3.2.7.6.2.2.6. Hydrolysis of urea**

Suspected isolates were stabbed into the butt as well as streaked onto the slant of urea agar tubes. The inoculated tubes were incubated at 37 °C and the preliminary reading was made at the end of 2 – 4 hours and daily for 5 days. Development of red colour due to splitting of ammonia which acts on phenol red denotes hydrolysis of urea.

**3.2.7.6.2.2.7. Hydrogen sulphide ($H_2S$) production test**

To triple sugar iron agar (TSI) tubes, the isolated organism was stabbed into butt, then streaked onto the slant. The tubes were incubated at 37 °C for 24 – 48 hours. The tubes having alkaline slant (red), acid butt (yellow) with or without $H_2S$ production (black colour) were noticed and recorded.

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### 3.2.7.7. Total mould and yeast count

The total mould and yeast count was done by using Sabouraud's dextrose agar medium ***(Cruickshank et al., 1975)*** supplemented with chlortetracycline (100 mg) as described by ***Koburger (1970)***. One ml from the previously prepared dilutions was aseptically transferred into sterile Petri dishes. Then about 15 ml of the sterile Sabouraud's dextrose agar (previously melted and cooled at 45 °C) were added and mixed thoroughly in a horizontal position. After solidification, inoculated as well as uninoculated control agar plates were incubated at an inverted position at 25 °C for 3 – 5 days. The first examination of plates was done after 3 days incubation to determine the degree of yeast growth, and if large numbers are visible, a count was made and reported on the fifth day. The yeast and mould counts per gram of the sample was then calculated and recorded.

#### 3.2.7.7.1. Identification of moulds

Isolated moulds were cultured onto malt extract plates for 3 – 5 days at 25 °C then identified macroscopically according to ***Raper and Fennel (1965)*** and ***Samson et al. (1995)*** for genus Aspergillus, ***Samson et al. (1995)*** for genus Penicillium, while other genera were identified according to ***Zycha et al. (1969); Barnnett and Hunter (1972)*** and ***Samson et al. (1995)***.

##### 3.2.7.7.1.1. Macroscopical examination

The macroscopical examination of mould colonies includes the rate and pattern of growth as colour, texture, basal and surface mycelia were examined by help of stereomicroscope.

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#### 3.2.7.7.1.2. Microscopical examination

A triangular piece was transferred from the periphery of 4 to 5 days old mould colonies to a clean glass slide with two mycological needles. The piece of the colony was distributed on the slide, then one to two drops of lactophenol stain was added and then covered by a clean slide (24 x 24 mm) followed by gentle pressure to remove the excess fluid. The prepared slides were then examined under low power and oil emersion lens to characterize the measurements and morphological structure of the mould growth, concerning the conidial stage, head, vesicle, sterigmata, conidiophore and conidia.

#### 3.2.7.7.2. Identification of isolated yeasts

The identification of isolated yeasts was carried out according to ***Lodder and Kreger (1967)*** and ***Kreger (1984)*** using the following tests:

#### 3.2.7.7.2.1. Ascospore formation

The ability of the isolated yeast to form ascospore was studied on Gorodkowa agar and rice agar medium. These media were inoculated with the isolates and incubated at 25 °C. Stained films were prepared after 3, 10, 20 and 30 days for detection of ascospore.

#### 3.2.7.7.2.2. Sugar fermentation

Test tubes, supplemented with Durham's tubes containing 10 ml of 1% peptone water, 2% sugar (glucose, maltose, galactose, lactose and sucrose) and bromocresol purple as an indicator, were inoculated by the isolated yeasts. Inoculated tubes were incubated at 25 °C for 3 days, the positive results were recorded as collection of gas in Durham's tubes.

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### 3.2.7.7.2.3. Sugar assimilation

A nitrogen base medium devoid from source of carbon was melted, cooled to 45 °C and poured into a Petri dish containing 2 ml of a heavy yeast suspension then thoroughly mixed. After solidification, paper discs soaked in 2% solutions of sugar (glucose, maltose, galactose and sucrose) were put on the surface of the medium. The plates were incubated at 25 °C for 1-3 days then examined for the ability to assimilate sugars and growth around the discs.

### 3.2.7.7.2.4. Vegetative reproduction

Rice agar in a thin layer of 2 mm thickness in Petri dishes were inoculated with the suspected yeast by a fine needle and covered by a sterile cover slide. The Petri dishes were incubated at 25 °C for 2 – 3 days, then examined directly under microscope for pseudo and true mycelia with blastospore.

### 3.2.8. Histopathological evaluation

Following complete necropsy, fresh specimens from the muscles of the thigh and breast were removed and rapidly fixed in 10% neutral buffered formalin. Therefore, these specimens were processed through the conventional paraffin embedding technique (dehydration in ascending grades of ethanol, clearing in chloroform and embedding in paraffin wax). Paraffin blocks were cut into 5 – 7 microns thick sections which were stained by Hematoxylin and Eosin (H,E) according to the method described by ***Harris (1960)***.

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# 4. RESULTS

**Table (1): Organoleptic examination of meat (thigh and breast) from broilers fed on different concentrations of *Nigella sativa L* seeds.**

| Factor | Control group | *Nigella sativa* supplemented groups | | | | |
|---|---|---|---|---|---|---|
| | | 0.5% | 1% | 1.5% | 2.0% | 2.5% |
| **Appearance (colour)** | Pale white with red patches | Red white with red patches | Pale white | Light red | Bright and light red | Homogenous bright red |
| Scores | 2 | 2 | 3 | 3 | 4 | 5 |
| **Flavour (odour)** | Normal little odour | Normal little odour | Normal little odour | Normal little odour | Spicy odour | Nearly similar to spiced and mutton meat |
| Scores | 3 | 3 | 3 | 3 | 4 | 4 |
| **Taste** | Acceptable | Fair taste | Good taste | Good taste | Very good taste | Excellent taste |
| Scores | 2 | 2 | 4 | 4 | 5 | 5 |
| **Texture** | Soft on touch | Soft on touch | Soft on touch | Soft on touch | Firm on touch | Firm on touch |
| **Scores** | 3 | 3 | 3 | 3 | 4 | 4 |

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**Table (2):** **Statistical analytical results of thermal shrinkage (%) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 60.12 | 63.85 | 62.19 ± 0.58 cb | 70.73 | 73.00 | 71.87 ± 0.40 bc | ** |
| *Nigella sativa* 1.0% | 62.00 | 64.99 | 63.12 ± 0.47 cb | 70.48 | 74.82 | 72.86 ± 0.67 ab | ** |
| *Nigella sativa* 1.5% | 59.98 | 65.42 | 63.94 ± 0.82 b | 71.21 | 75.00 | 73.25 ± 0.61 ab | ** |
| *Nigella sativa* 2.0% | 63.98 | 67.97 | 65.92 ± 0.69 a | 73.00 | 75.48 | 74.28 ± 0.39 a | ** |
| *Nigella sativa* 2.5% | 66.90 | 68.80 | 67.38 ± 0.30 a | 72.70 | 76.22 | 74.59 ± 0.57 a | ** |
| Control | 58.45 | 65.00 | 61.18 ± 0.99 c | 67.85 | 72.94 | 71.11 ± 0.73 c | ** |

SE = Standard error.

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (3): Statistical analytical results of water holding capacity (%) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 60.59 | 65.32 | 62.16 ± 0.75 d | 60.00 | 61.84 | 61.01 ± 0.30 d | NS |
| *Nigella sativa* 1.0% | 63.90 | 67.41 | 66.08 ± 0.56 c | 61.16 | 64.24 | 63.14 ± 0.49 c | * |
| *Nigella sativa* 1.5% | 66.13 | 68.59 | 67.62 ± 0.42 b | 63.12 | 66.69 | 66.69 ± 0.54 b | ** |
| *Nigella sativa* 2.0% | 72.91 | 75.00 | 73.89 ± 0.28 a | 67.93 | 69.41 | 68.43 ± 0.25 a | ** |
| *Nigella sativa* 2.5% | 72.99 | 74.59 | 73.67 ± 0.29 a | 66.89 | 69.92 | 68.37 ± 0.42 a | ** |
| Control | 57.94 | 61.13 | 59.58 ± 0.50 e | 56.13 | 61.03 | 58.72 ± 0.74 e | NS |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

NS = No difference ($P>0.05$) between thigh and breast muscles.

* Significant ($P<0.05$) difference between thigh and breast muscles.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (4): Statistical analytical results of plasticity (%) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 0.31 | 0.34 | 0.325 ± 0.005 c | 0.33 | 0.36 | 0.343 ± 0.005 c | * |
| *Nigella sativa* 1.0% | 0.33 | 0.35 | 0.337 ± 0.003 bc | 0.32 | 0.37 | 0.352 ± 0.007 bc | NS |
| *Nigella sativa* 1.5% | 0.33 | 0.37 | 0.348 ± 0.005 ab | 0.36 | 0.39 | 0.370 ± 0.004 ab | ** |
| *Nigella sativa* 2.0% | 0.34 | 0.36 | 0.353 ± 0.004 a | 0.35 | 0.39 | 0.368 ± 0.006 ab | NS |
| *Nigella sativa* 2.5% | 0.34 | 0.37 | 0.358 ± 0.005 a | 0.36 | 0.40 | 0.382 ± 0.007 a | * |
| Control | 0.30 | 0.34 | 0.322 ± 0.007 c | 0.28 | 0.35 | 0.322 ± 0.012 c | NS |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

NS = No difference ($P>0.05$) between thigh and breast muscles.

* Significant ($P<0.05$) difference between thigh and breast muscles.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (5):** **Statistical analytical results of pH values of meat from broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 5.00 | 6.12 | 5.42 ± 0.185 |
| *Nigella sativa* 1.0% | 4.62 | 5.20 | 5.02 ± 0.085 |
| *Nigella sativa* 1.5% | 4.30 | 5.90 | 5.16 ± 0.264 |
| *Nigella sativa* 2.0% | 4.10 | 6.11 | 5.57 ± 0.304 |
| *Nigella sativa* 2.5% | 4.40 | 6.00 | 5.50 ± 0.204 |
| Control | 5.20 | 6.04 | 5.94 ± 0.192 |

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**Table (6):** **Incidence of positive results in copper sulphate and Eber's test within 10 minutes post-slaughtering.**

| Treatment | No. of examined samples | Copper sulphate test | | Eber's test | |
|---|---|---|---|---|---|
| | | Positive samples | % | Positive samples | % |
| *Nigella sativa* 0.5% | 6 | 4 | 66.67 | 0 | 0 |
| *Nigella sativa* 1.0% | 6 | 0 | 0 | 0 | 0 |
| *Nigella sativa* 1.5% | 6 | 0 | 0 | 0 | 0 |
| *Nigella sativa* 2.0% | 6 | 0 | 0 | 0 | 0 |
| *Nigella sativa* 2.5% | 6 | 0 | 0 | 0 | 0 |
| Control | 6 | 5 | 83.33 | 0 | 0 |

Clear and transparent aspect = negative copper sulphate test.
Gel sediment and turbid aspect = positive copper sulphate test.
Grayish white cloudiness = positive Eber's test.

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**Table (7): Statistical analytical results of moisture percent of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 61.13 | 63.01 | 62.10 ± 0.17 d | 60.31 | 71.13 | 68.53 ± 0.96 a | ** |
| *Nigella sativa* 1.0% | 61.00 | 63.00 | 61.76 ± 0.19 d | 69.93 | 71.92 | 70.81 ± 0.20 a | ** |
| *Nigella sativa* 1.5% | 61.96 | 64.91 | 63.07 ± 0.28 c | 53.03 | 72.56 | 66.83 ± 2.58 a | NS |
| *Nigella sativa* 2.0% | 62.31 | 67.72 | 65.66 ± 0.48 b | 54.50 | 74.31 | 69.05 ± 2.21 a | NS |
| *Nigella sativa* 2.5% | 65.93 | 68.31 | 67.11 ± 0.22 a | 55.31 | 74.95 | 70.36 ± 2.00 a | NS |
| Control | 60.51 | 62.00 | 61.52 ± 0.17 d | 65.31 | 76.51 | 68.35 ± 0.99 a | ** |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

NS = No difference ($P>0.05$) between thigh and breast muscles.

* Significant ($P<0.05$) difference between thigh and breast muscles.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (8):** **Statistical analytical results of protein percent of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 16.93 | 22.16 | 19.64 ± 0.51 bc | 14.30 | 22.30 | 19.28 ± 0.93 a | NS |
| *Nigella sativa* 1.0% | 19.13 | 23.61 | 20.63 ± 0.39 ab | 13.60 | 21.45 | 18.11 ± 0.98 a | * |
| *Nigella sativa* 1.5% | 20.00 | 24.69 | 21.04 ± 0.52 ab | 13.99 | 24.01 | 19.76 ± 0.87 a | NS |
| *Nigella sativa* 2.0% | 16.31 | 23.17 | 20.69 ± 0.61 ab | 14.60 | 21.69 | 19.45 ± 0.78 a | NS |
| *Nigella sativa* 2.5% | 20.00 | 26.06 | 22.51 ± 0.58 a | 18.24 | 23.39 | 20.25 ± 0.52 a | ** |
| Control | 13.37 | 24.50 | 18.49 ± 1.02 c | 13.98 | 21.31 | 18.99 ± 0.76 a | NS |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

NS = No difference ($P>0.05$) between thigh and breast muscles.

* Significant ($P<0.05$) difference between thigh and breast muscles.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (9):** **Statistical analytical results of fat percent of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | BREAST | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean ± SE | Minimum | Maximum | Mean ± SE | |
| *Nigella sativa* 0.5% | 2.39 | 2.63 | 2.51 ± 0.02 b | 1.59 | 1.80 | 1.67 ± 0.02 ab | ** |
| *Nigella sativa* 1.0% | 2.34 | 2.52 | 2.45 ± 0.02 c | 1.50 | 1.68 | 1.59 ± 0.02 bc | ** |
| *Nigella sativa* 1.5% | 2.30 | 2.48 | 2.41 ± 0.02 c | 1.49 | 1.61 | 1.55 ± 0.01 dc | ** |
| *Nigella sativa* 2.0% | 2.29 | 2.41 | 2.35 ± 0.01 d | 1.31 | 1.61 | 1.49 ± 0.03 d | ** |
| *Nigella sativa* 2.5% | 2.29 | 2.40 | 2.34 ± 0.01 d | 1.10 | 1.50 | 1.38 ± 0.05 e | ** |
| Control | 2.59 | 2.70 | 2.63 ± 0.01 a | 1.65 | 1.89 | 1.72 ± 0.02 a | ** |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

** Highly significant ($P<0.01$) difference between thigh and breast muscles.

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**Table (10): Statistical analytical results of serum cholesterol (mg/dl) of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 91 | 137 | 114.00 ± 11.68 a |
| *Nigella sativa* 1.0% | 102 | 125 | 112.25 ± 6.01 a |
| *Nigella sativa* 1.5% | 102 | 120 | 109.50 ± 4.50 a |
| *Nigella sativa* 2.0% | 102 | 120 | 109.50 ± 4.50 a |
| *Nigella sativa* 2.5% | 97 | 108 | 102.25 ± 2.25 a |
| Control | 102 | 125 | 115.25 ± 4.96 a |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

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**Table (11): Statistical analytical results of serum triglycerides (mg/dl) of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 70 | 140 | 102.50 ± 14.93 a |
| *Nigella sativa* 1.0% | 80 | 150 | 100.00 ± 16.83 a |
| *Nigella sativa* 1.5% | 90 | 100 | 97.50 ± 2.50 a |
| *Nigella sativa* 2.0% | 70 | 110 | 87.50 ± 8.54 a |
| *Nigella sativa* 2.5% | 70 | 100 | 85.00 ± 6.45 a |
| Control | 90 | 120 | 103.75 ± 6.25 a |

Means in the same column followed by the same letter do not differ significantly at $P = 0.05$.

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**Table (12): Statistical analytical results of serum total protein (g/dl) of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 3.00 | 4.30 | 3.65 ± 0.32 b |
| *Nigella sativa* 1.0% | 3.60 | 3.90 | 3.75 ± 0.09 ab |
| *Nigella sativa* 1.5% | 3.40 | 4.30 | 3.90 ± 0.23 ab |
| *Nigella sativa* 2.0% | 3.90 | 4.50 | 4.15 ± 0.13 ab |
| *Nigella sativa* 2.5% | 4.20 | 4.30 | 4.25 ± 0.03 a |
| Control | 3.40 | 3.90 | 3.58 ± 0.12 b |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

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**Table (13): Statistical analytical results of serum albumin (g/dl) of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 1.20 | 2.20 | 1.65 ± 0.21 ab |
| *Nigella sativa* 1.0% | 1.60 | 2.20 | 1.90 ± 0.17 ab |
| *Nigella sativa* 1.5% | 1.60 | 2.20 | 2.05 ± 0.15 ab |
| *Nigella sativa* 2.0% | 1.20 | 1.60 | 1.40 ± 0.12 b |
| *Nigella sativa* 2.5% | 1.60 | 2.80 | 2.20 ± 0.35 a |
| Control | 1.20 | 1.60 | 1.50 ± 0.12 b |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

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**Table (14): Statistical analytical results of serum globulin (g/dl) of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 1.40 | 2.70 | 2.00 ± 0.27 b |
| *Nigella sativa* 1.0% | 1.40 | 2.30 | 1.85 ± 0.19 b |
| *Nigella sativa* 1.5% | 1.20 | 2.10 | 1.85 ± 0.22 b |
| *Nigella sativa* 2.0% | 2.50 | 2.90 | 2.75 ± 0 10 a |
| *Nigella sativa* 2.5% | 1.50 | 2.60 | 2.05 ± 0.32 b |
| Control | 1.80 | 2.40 | 2.08 ± 0.16 b |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

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**Table (15): Statistical analytical results of serum albumin to globulin ratio of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | Minimum | Maximum | Mean ± SE |
|---|---|---|---|
| *Nigella sativa* 0.5% | 0.59 | 1.16 | 0.87 ± 0.16 ab |
| *Nigella sativa* 1.0% | 0.70 | 1.57 | 1.09 ± 0.21 ab |
| *Nigella sativa* 1.5% | 0.80 | 1.83 | 1.18 ± 0.22 ab |
| *Nigella sativa* 2.0% | 0.41 | 0.64 | 0.51 ± 0.05 b |
| *Nigella sativa* 2.5% | 0.62 | 1.87 | 1.25 ± 0.36 a |
| Control | 0.50 | 0.89 | 0.75 ± 0.09 ab |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

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**Table (16):** **Statistical analytical results of total mesophilic bacterial count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 6 | 100 | $2.12 \times 10^6$ | $4.00 \times 10^6$ | $2.94 \times 10^6 \pm 2.67 \times 10^5$ ab | 6 | 100 | $1.50 \times 10^6$ | $3.55 \times 10^6$ | $2.33 \times 10^6 \pm 3.15 \times 10^5$ ab | * |
| *Nigella sativa* 1.0% | 6 | 100 | $1.85 \times 10^6$ | $4.45 \times 10^6$ | $2.86 \times 10^6 \pm 3.89 \times 10^5$ ab | 6 | 100 | $1.05 \times 10^6$ | $2.55 \times 10^6$ | $1.88 \times 10^6 \pm 2.14 \times 10^5$ b | NS |
| *Nigella sativa* 1.5% | 6 | 100 | $2.30 \times 10^6$ | $5.30 \times 10^6$ | $3.48 \times 10^6 \pm 5.51 \times 10^5$ ab | 6 | 100 | $7.00 \times 10^5$ | $3.40 \times 10^6$ | $1.96 \times 10^6 \pm 3.68 \times 10^5$ b | * |
| *Nigella sativa* 2.0% | 6 | 100 | $1.30 \times 10^6$ | $4.80 \times 10^6$ | $2.74 \times 10^6 \pm 6.57 \times 10^5$ ab | 6 | 100 | $1.24 \times 10^6$ | $2.10 \times 10^6$ | $1.77 \times 10^6 \pm 1.55 \times 10^5$ b | NS |
| *Nigella sativa* 2.5% | 6 | 100 | $1.15 \times 10^6$ | $3.95 \times 10^6$ | $2.10 \times 10^6 \pm 4.03 \times 10^5$ b | 6 | 100 | $9.50 \times 10^5$ | $2.35 \times 10^6$ | $1.56 \times 10^6 \pm 2.23 \times 10^5$ b | NS |
| Control | 6 | 100 | $2.30 \times 10^6$ | $5.61 \times 10^6$ | $3.74 \times 10^6 \pm 5.41 X 10^5$ a | 6 | 100 | $2.55 \times 10^6$ | $4.50 \times 10^6$ | $3.29 \times 10^6 \pm 2.95 \times 10^5$ a | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

* Significant (P<0.05) difference between thigh and breast muscles.

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**Table (17): Statistical analytical results of total Coliform count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 6 | 100 | $1.00 \times 10^5$ | $3.40 \times 10^5$ | $2.20 \times 10^5 \pm 3.61 \times 10^4$ a | 6 | 100 | $1.80 \times 10^5$ | $2.60 \times 10^5$ | $2.05 \times 10^5 \pm 1.13 \times 10^4$ a | NS |
| *Nigella sativa* 1.0% | 6 | 100 | $1.20 \times 10^5$ | $2.20 \times 10^5$ | $1.65 \times 10^5 \pm 1.61 \times 10^4$ a | 6 | 100 | $4.50 \times 10^4$ | $2.00 \times 10^5$ | $1.38 \times 10^5 \pm 2.19 \times 10^4$ a | NS |
| *Nigella sativa* 1.5% | 6 | 100 | $9.00 \times 10^4$ | $3.50 \times 10^5$ | $1.98 \times 10^5 \pm 3.50 \times 10^4$ a | 4 | 65.67 | $7.00 \times 10^4$ | $1.50 \times 10^5$ | $1.18 \times 10^5 \pm 1.97 \times 10^4$ cb | NS |
| *Nigella sativa* 2.0% | 6 | 100 | $4.50 \times 10^4$ | $1.85 \times 10^5$ | $1.08 \times 10^5 \pm 2.22 \times 10^4$ b | 5 | 83.33 | $7.00 \times 10^4$ | $1.85 \times 10^5$ | $1.11 \times 10^5 \pm 1.97 \times 10^4$ cb | NS |
| *Nigella sativa* 2.5% | 6 | 100 | $5.50 \times 10^4$ | $1.10 \times 10^5$ | $8.58 \times 10^4 \pm 9.70 \times 10^3$ b | 5 | 83.33 | $3.80 \times 10^4$ | $1.10 \times 10^5$ | $7.96 \times 10^4 \pm 1.26 \times 10^4$ c | NS |
| Control | 6 | 100 | $1.50 \times 10^5$ | $3.05 \times 10^5$ | $2.38 \times 10^5 \pm 2.77 \times 10^4$ a | 6 | 100 | $1.95 \times 10^5$ | $3.40 \times 10^5$ | $2.28 \times 10^5 \pm 2.28 \times 10^4$ a | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

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**Table (18): Statistical analytical results of total Enterobacteriaceae count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 6 | 100 | $1.50 \times 10^5$ | $3.00 \times 10^5$ | $2.32 \times 10^5 \pm 2.30 \times 10^4$ a | 6 | 100 | 9.00 x 104 | 2.30 x 105 | $1.37 \times 10^5 \pm 2.06 \times 10^4$ ab | * |
| *Nigella sativa* 1.0% | 6 | 100 | $1.30 \times 10^5$ | $2.60 \times 10^5$ | $1.87 \times 10^5 \pm 2.38 \times 10^4$ abc | 6 | 100 | 7.00 x 104 | 1.70 x 105 | $1.33 \times 10^5 \pm 1.48 \times 10^4$ ab | NS |
| *Nigella sativa* 1.5% | 6 | 100 | $1.50 \times 10^5$ | $2.25 \times 10^5$ | $1.94 \times 10^5 \pm 1.19 \times 10^4$ ab | 6 | 100 | 1.00 x 105 | 2.01 x 105 | $1.40 \times 10^5 \pm 1.68 \times 10^4$ ab | * |
| *Nigella sativa* 2.0% | 6 | 100 | $1.15 \times 10^5$ | $2.10 \times 10^5$ | $1.58 \times 10^5 \pm 1.67 \times 10^4$ bc | 5 | 83.33 | 1.10 x 105 | 1.65 x 105 | $1.28 \times 10^5 \pm 1.01 \times 10^4$ ab | NS |
| *Nigella sativa* 2.5% | 6 | 100 | $1.00 \times 10^5$ | $1.70 \times 10^5$ | $1.35 \times 10^5 \pm 1.15 \times 10^4$ c | 6 | 100 | 6.00 x 104 | 1.30 x 105 | $9.67 \times 10^4 \pm 9.32 \times 10^3$ b | NS |
| Control | 6 | 100 | $1.35 \times 10^5$ | $3.40 \times 10^5$ | $2.40 \times 10^5 \pm 2.95 \times 10^4$ a | 6 | 100 | 9.50 x 104 | 2.50 x 105 | $1.80 \times 10^5 \pm 2.50 \times 10^4$ a | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

* Significant (P<0.05) difference between thigh and breast muscles.

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**Table (19): Statistical analytical results of total *Staphylococcus aureus* count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa* (n = 6).**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 5 | 83.33 | $1.50 \times 10^4$ | $3.50 \times 10^4$ | $2.40 \times 10^4 \pm 3.67 \times 10^3$ a | 5 | 83.33 | $5.00 \times 10^3$ | $1.50 \times 10^4$ | $8.00 \times 10^3 \pm 2.00 \times 10^3$ a | NS |
| *Nigella sativa* 1.0% | 5 | 83.33 | $1.00 \times 10^4$ | $1.50 \times 10^4$ | $1.34 \times 10^4 \pm 1.03 \times 10^3$ ab | 4 | 66.67 | $5.00 \times 10^3$ | $1.50 \times 10^4$ | $1.00 \times 10^4 \pm 2.04 \times 10^3$ a | NS |
| *Nigella sativa* 1.5% | 3 | 50.00 | $5.00 \times 10^3$ | $1.00 \times 10^4$ | $6.67 \times 10^3 \pm 1.67 \times 10^3$ bc | 5 | 83.33 | $1.00 \times 10^3$ | $1.20 \times 10^4$ | $6.60 \times 10^3 \pm 1.96 \times 10^3$ ab | NS |
| *Nigella sativa* 2.0% | 4 | 66.67 | $1.00 \times 10^3$ | $1.50 \times 10^4$ | $7.75 \times 10^3 \pm 3.04 \times 10^3$ bc | 3 | 50.00 | $5.00 \times 10^3$ | $1.00 \times 10^4$ | $8.33 \times 10^3 \pm 1.67 \times 10^3$ a | NS |
| *Nigella sativa* 2.5% | 3 | 50.00 | $1.00 \times 10^3$ | $1.20 \times 10^4$ | $6.00 \times 10^3 \pm 3.21 \times 10^3$ c | 3 | 50.00 | $1.00 \times 10^3$ | $4.00 \times 10^3$ | $3.00 \times 10^3 \pm 1.00 \times 10^3$ b | NS |
| Control | 5 | 83.33 | $1.00 \times 10^4$ | $3.80 \times 10^4$ | $2.44 \times 10^4 \pm 4.57 \times 10^3$ a | 5 | 83.33 | $5.00 \times 10^3$ | $2.00 \times 10^4$ | $1.30 \times 10^4 \pm 2.50 \times 10^3$ a | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

n = Number of examined samples.

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**Table (20):** **Incidence of Salmonella species isolated from thigh and breast muscles of broilers fed on different concentrations of *Nigella sativa*.**

| Treatment | No. of samples | THIGH | | BREAST | |
|---|---|---|---|---|---|
| | | No. of isolates | % | No. of isolates | % |
| *Nigella sativa* 0.5% | 6 | 4 | 66.67 | 2 | 33.33 |
| *Nigella sativa* 1.0% | 6 | 2 | 33.33 | 0 | 0 |
| *Nigella sativa* 1.5% | 6 | 0 | 0 | 0 | 0 |
| *Nigella sativa* 2.0% | 6 | 1 | 16.67 | 0 | 0 |
| *Nigella sativa* 2.5% | 6 | 0 | 0 | 0 | 0 |
| Control | 6 | 4 | 66.67 | 2 | 33.33 |

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Table (21): **Statistical analytical results of total mould count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 6 | 100.0 | $1.00 \times 10^3$ | $5.00 \times 10^3$ | $3.17 \times 10^3 \pm 6.01 \times 10^2$ ab | 6 | 100.0 | $2.00 \times 10^3$ | $4.00 \times 10^3$ | $3.00 \times 10^3 \pm 2.58 \times 10^2$ ab | NS |
| *Nigella sativa* 1.0% | 3 | 50.00 | $1.00 \times 10^3$ | $6.00 \times 10^3$ | $3.00 \times 10^3 \pm 1.53 \times 10^3$ ab | 6 | 100.0 | $2.00 \times 10^3$ | $4.00 \times 10^3$ | $3.17 \times 10^3 \pm 3.07 \times 10^2$ a | NS |
| *Nigella sativa* 1.5% | 5 | 83.33 | $2.00 \times 10^3$ | $5.00 \times 10^3$ | $3.00 \times 10^3 \pm 6.32 \times 10^2$ ab | 4 | 66.67 | $1.00 \times 10^3$ | $4.00 \times 10^3$ | $2.00 \times 10^3 \pm 7.07 \times 10^2$ ab | NS |
| *Nigella sativa* 2.0% | 6 | 100.0 | $1.00 \times 10^3$ | $3.00 \times 10^3$ | $2.00 \times 10^3 \pm 2.58 \times 10^2$ b | 4 | 66.67 | $1.00 \times 10^3$ | $4.00 \times 10^3$ | $2.00 \times 10^3 \pm 7.07 \times 10^2$ ab | NS |
| *Nigella sativa* 2.5% | 3 | 50.00 | $1.00 \times 10^3$ | $2.00 \times 10^3$ | $1.67 \times 10^3 \pm 3.33 \times 10^2$ b | 3 | 50.00 | $1.00 \times 10^3$ | $3.00 \times 10^3$ | $1.67 \times 10^3 \pm 6.67 \times 10^2$ b | NS |
| Control | 5 | 83.33 | $3.00 \times 10^3$ | $6.00 \times 10^3$ | $4.40 \times 10^3 \pm 6.00 \times 10^2$ a | 4 | 66.67 | $2.00 \times 10^3$ | $4.00 \times 10^3$ | $2.75 \times 10^3 \pm 4.79 \times 10^2$ ab | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

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**Table (22): Statistical analytical results of total yeast count (cfu/g) of thigh and breast muscles of broilers fed different concentrations of *Nigella sativa*.**

| Treatment | THIGH | | | | | BREAST | | | | | Difference between thigh and breast |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive samples | | Minimum | Maximum | Mean ± SE | Positive samples | | Minimum | Maximum | Mean ± SE | |
| | No. | % | | | | No. | % | | | | |
| *Nigella sativa* 0.5% | 4 | 66.67 | $1.00 \times 10^3$ | $3.00 \times 10^3$ | $2.00 \times 10^3 \pm 5.77 \times 10^2$ a | 5 | 83.33 | $1.00 \times 10^3$ | $2.00 \times 10^3$ | $1.40 \times 10^3 \pm 2.45 \times 10^2$ a | NS |
| *Nigella sativa* 1.0% | 4 | 66.67 | $2.00 \times 10^3$ | $6.00 \times 10^3$ | $4.00 \times 10^3 \pm 9.13 \times 10^2$ a | 3 | 50.00 | $1.00 \times 10^3$ | $3.00 \times 10^3$ | $1.67 \times 10^3 \pm 6.67 \times 10^2$ a | NS |
| *Nigella sativa* 1.5% | 5 | 83.33 | $1.00 \times 10^3$ | $7.00 \times 10^3$ | $3.40 \times 10^3 \pm 1.17 \times 10^3$ a | 4 | 66.67 | $1.00 \times 10^3$ | $5.00 \times 10^3$ | $2.25 \times 10^3 \pm 9.46 \times 10^2$ a | NS |
| *Nigella sativa* 2.0% | 3 | 50.00 | $1.00 \times 10^3$ | $3.00 \times 10^3$ | $1.67 \times 10^3 \pm 6.67 \times 10^2$ a | 3 | 50.00 | $2.00 \times 10^3$ | $3.00 \times 10^3$ | $2.67 \times 10^3 \pm 3.33 \times 10^2$ a | NS |
| *Nigella sativa* 2.5% | 1 | 16.67 | $1.00 \times 10^3$ | $1.00 \times 10^3$ | $1.00 \times 10^3$ a | 2 | 33.33 | $2.00 \times 10^3$ | $3.00 \times 10^3$ | $2.50 \times 10^3 \pm 5.00 \times 10^2$ a | NS |
| Control | 6 | 100.0 | $2.00 \times 10^3$ | $7.00 \times 10^3$ | $3.67 \times 10^3 \pm 9.18 \times 10^2$ a | 6 | 100.0 | $1.00 \times 10^3$ | $7.00 \times 10^3$ | $3.83 \times 10^3 \pm 8.33 \times 10^2$ a | NS |

Means in the same column followed by the same letter do not differ significantly at P = 0.05.

NS = No difference (P>0.05) between thigh and breast muscles.

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Table (23): **Incidence of mould isolated from thigh and breast muscles of broilers fed on different concentrations of *Nigella sativa*.**

| Treatment | No. of samples | THIGH | | | | | | BREAST | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aspergillus niger* | | Mucor spp. | | Cladosporium spp. | | *Aspergillus niger* | | Mucor spp. | | Cladosporium spp. | |
| | | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % | +ve | % |
| *Nigella sativa* 0.5% | 6 | 2 | 33.33 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 2 | 33.33 | 2 | 33.33 |
| *Nigella sativa* 1.0% | 6 | 2 | 33.33 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 2 | 33.33 | 2 | 33.33 |
| *Nigella sativa* 1.5% | 6 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 2 | 33.33 |
| *Nigella sativa* 2.0% | 6 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 1 | 16.67 | 0 | 0 |
| *Nigella sativa* 2.5% | 6 | 1 | 16.67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Control | 6 | 2 | 33.33 | 4 | 66.67 | 3 | 50.00 | 1 | 16.67 | 3 | 50.00 | 2 | 33.33 |

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**Table (24): Incidence of yeast isolated from thigh and breast muscles of broilers fed on different concentrations of *Nigella sativa*.**

| Treatment | No. of samples | THIGH | | | | BREAST | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Candida spp. | | Torulopsis | | Candida spp. | | Torulopsis | |
| | | +ve | % | +ve | % | +ve | % | +ve | % |
| *Nigella sativa* 0.5% | 6 | 3 | 50.00 | 3 | 50.00 | 3 | 50.00 | 2 | 33.33 |
| *Nigella sativa* 1.0% | 6 | 3 | 50.00 | 2 | 33.33 | 3 | 50.00 | 1 | 16.67 |
| *Nigella sativa* 1.5% | 6 | 2 | 33.33 | 2 | 33.33 | 2 | 33.33 | 1 | 16.67 |
| *Nigella sativa* 2.0% | 6 | 1 | 16.67 | 2 | 33.33 | 1 | 16.67 | 1 | 16.67 |
| *Nigella sativa* 2.5% | 6 | 1 | 16.67 | 1 | 16.67 | 0 | 0 | 0 | 0 |
| Control | 6 | 4 | 66.67 | 3 | 50.00 | 3 | 50.00 | 3 | 50.00 |

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**Histopathological results:**

It was observed that by addition of *Nigella sativa L.* seeds at concentrations of 2 and 2.5%, the muscle fibers were increased and the connective tissue was decreased, particularly, in thigh muscles which showed by using light microscope a high increase in muscle fibers on account of connective tissue content.

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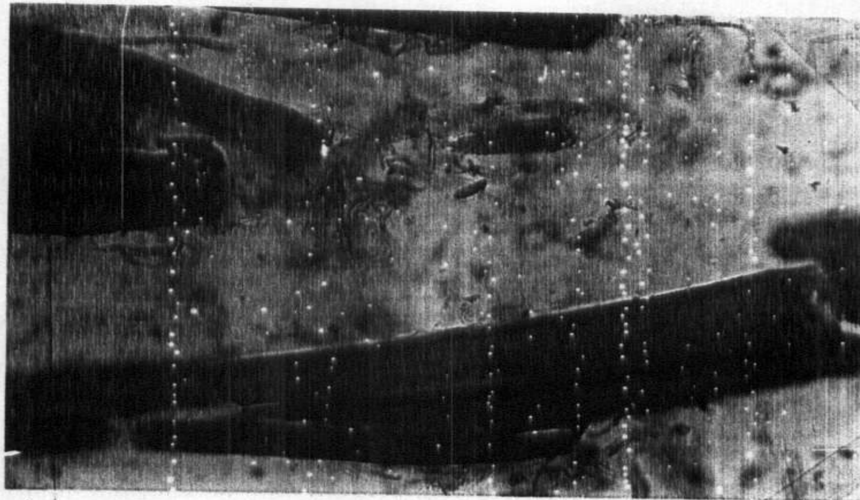

**Fig. (1):** Thigh muscle of group 6 (control). Note the amount of connective tissue. H&E X40.

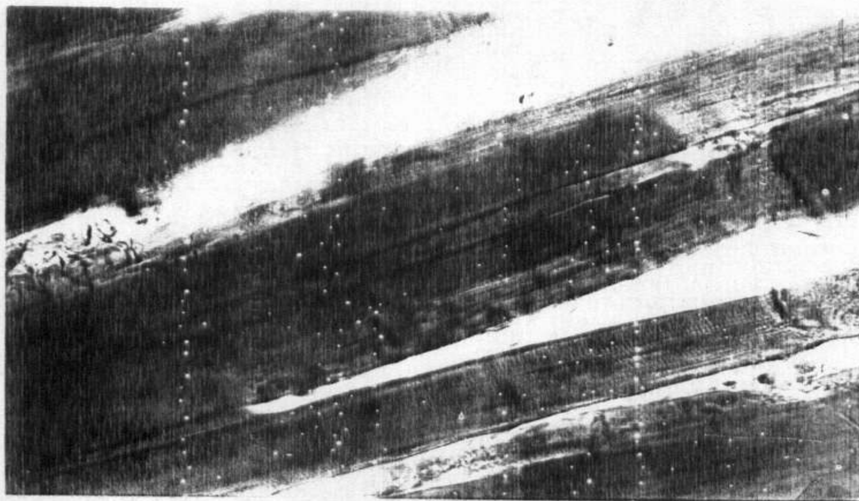

**Fig. (2):** Breast muscle of group 6 (control). Note the amount of connective tissue. H&E X40.

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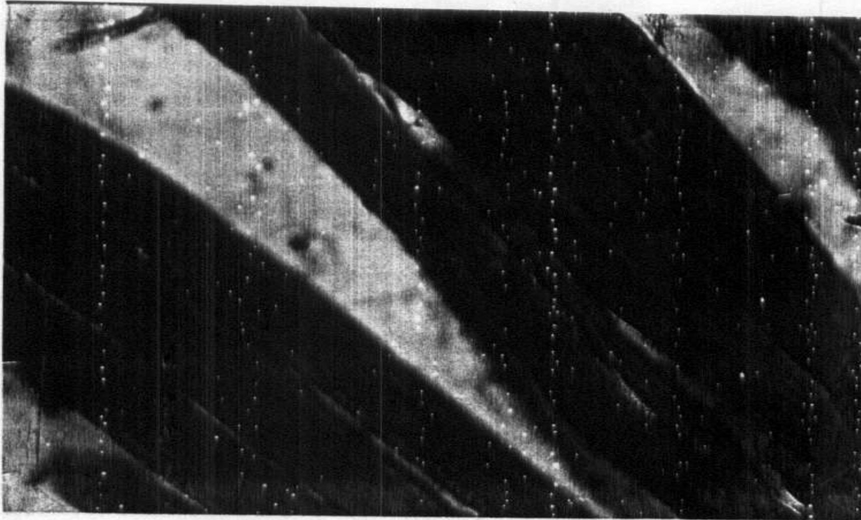

**Fig. (3):** Thigh muscle of group 5 (*Nigella sativa* 2.5%). Note the amount of connective tissue: moderately decreased compared to the control. H&E X40.

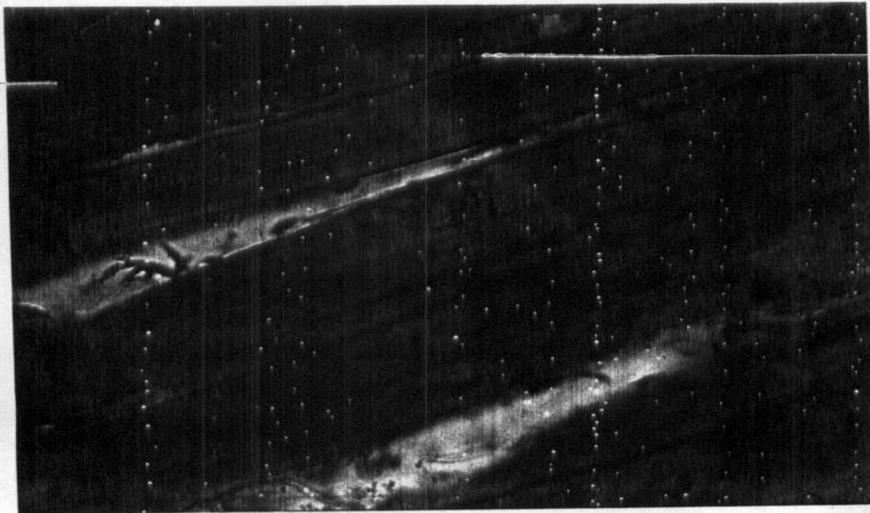

**Fig. (4):** Breast muscle of group 5 (*Nigella sativa* 2.5%). Note the amount of myofilaments: moderately increased compared to the control. H&E X40.

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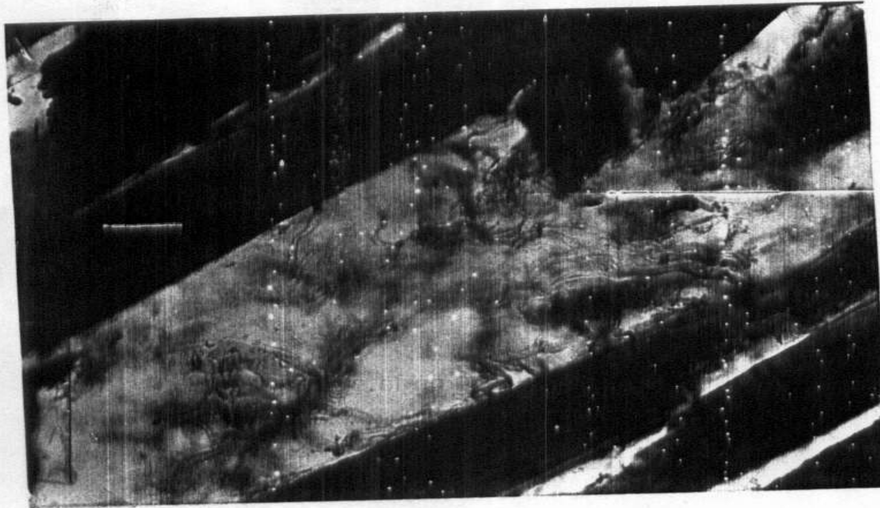

**Fig. (5):** Thigh muscle of group 4 (*Nigella sativa* 2%). Note the amount of connective tissue: slightly decreased compared to the control. H&E X40.

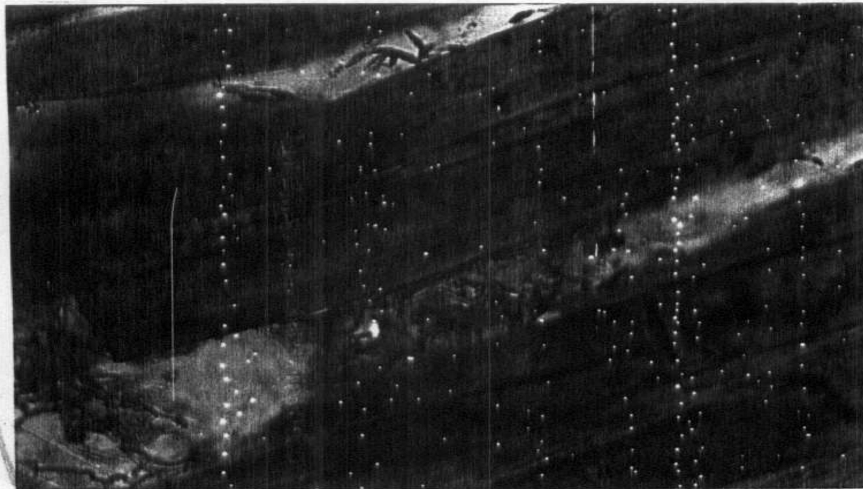

**Fig. (6):** Breast muscle of group 4 (*Nigella sativa* 2%). Note the amount of myofilaments: slightly increased compared to the control. H&E X40.

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# 5. DISCUSSION

## 5.1. Physical examination:

Physical and sensory analyses use scientific principle drawn from food science, physiology, psychology and statistics. Its purpose is to elicit objective responses to properties of food as perceived by senses as smell, touch, vision, ..... etc. Sensory techniques must meet the requirements of all measurement method which must be accurate and valid ***(Piggott, 1995)***.

### 5.1.1. Sensory evaluation:

#### 5.1.1.1. Colour:

Colour is an important quality aspect of poultry meat. Colour is a quality attribute which together with flavour and texture play an important role in food acceptability. The colour of meat is mainly due to haeme proteins which represent the chief colour constituents of meat, the predominant components being myoglobin and haemoglobin ***(June, 1976)***.

As shown in Table (1) the colour of poultry meat fed on ration contained 2.5 and 2% *Nigella sativa L.* seeds was homogenous bright red colour. While, the colour of meat from poultry fed on ration contained 1.5, 1 and 0.5% *Nigella sativa L.* seeds and from control group were light red, pale white and pale white with red patches, respectively. This may be attributed to the increased serum globulin and porphyrin which produced under the stimulating effect of *Nigella sativa L.* seeds on the liver. These results agree with ***Mandour and Rady (1997)***.

#### 5.1.1.2. Flavour:

Flavour of meat has been defined as the sensation produced by a material taken by the mouth, perceived principally by the senses of taste and

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smell. Flavour is mainly composed of taste and odour. Uncooked poultry meat has little odour and only a blood-like taste, and cooking is necessary to develop the flavour ***(Hall, 1968)***.

Table (1) illustrates that the flavour obtained from poultry meat fed on ration containing 2.5% *Nigella sativa L.* was very slight spicy taste and nearly similar to the odour of the mutton meat, while in 2% concentration gave spicy flavour. In case of meat from groups fed on ration containing 1.5, 1 and 0.5% as well as from the control group, there was no great difference between them which was normal little odour.

The spicy taste obtained due to the flavour compounds of *Nigella sativa L.* seeds such as carvone, vanillin and ethyl vanilline which have flavouring effect at high concentrations and also due to the presence of the volatile oils found in the seeds. The above results agree with those of ***Jay and Rivers (1984)***.

**5.1.1.3. Texture**:

Texture was defined as the way in which the various constituents and structural elements are arranged and combined into a micro- and macro-structure and the external manifestations of this structure in the term of deformation and releasing of juice. The nature of these structures is of particular significance to meat quality ***(Sherman, 1969)***.

As shown in Table (1) the meat of poultry fed on rations containing 2.5 and 2% was firm on touch while meat from other groups (1.5, 1 and 0.5%) and the control one was soft on touch. This is attributed to the antioxidant

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effect of *Nigella sativa L.* which may protect against oxidative damage of cell membrane. These results agree with those of ***Mandour and Rady (1997)***.

**5.1.2. Physical evaluation:**

**5.1.2.1. Thermal shrinkage**:

The considerable decrease of water holding capacity (WHC) during the heating of poultry meat, which result in the release of juice is due to tightening of the myofibrillar network by heat denaturation of the proteins which results in what is called "thermal shrinkage" ***(Bouton and Harris, 1972)***.

The data presented in Table (2) shows that the differences between the mean values of thermal shrinkage percentage in thigh muscle of control (untreated) group and each of the groups fed on rations containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds were significant (P<0.05). On the other hand, the mean values of thermal shrinkage percentage of thigh muscle in control group and each of the groups fed on ration containing 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). Table (2) also showed that the difference between the mean values of thermal shrinkage percentage in breast muscles of the control group and each of the groups fed on rations containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds were significant (P<0.05), while the same comparison was not significant (P>0.05) between the control group and the group fed on a ration containing 0.5% *Nigella sativa L.* seeds. The same Table (2) also shows that the difference between the mean values of thermal shrinkage in both thigh and breast muscles in all groups were highly significant (P<0.01). From the above results, it was observed that the mean values of thermal shrinkage

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percentages in both thigh and breast muscles were slightly increased by increasing the percentages of *Nigella sativa L.* seeds in the ration.

**5.1.2.2. Water holding capacity (WHC):**

The cross-striated muscles of poultry meat contain about 70% water. The power with which this water is bound by the muscle proteins is of great importance for the quality of meat, this power is known as water-holding capacity (WHC). Thus, investigation of the WHC of poultry meat is of considerable economic interest where the great economic problem of weight losses during storage, cooking or freezing and thawing of poultry meat is related to the binding of water within the muscle. The changes in the WHC are very sensitive indicator of changes in pH and structure of muscle proteins, since the myofibrillar proteins are primarily responsible for the binding of water in muscles ***(Hamm, 1972)***.

The differences between the mean values of water holding capacity percentages in thigh muscles in control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* were significant ($P<0.05$). On the other hand, the differences between the mean values of water holding capacity percentages in breast muscles of control group and each of groups fed rations containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* were significant (Table 3).

The differences between the mean values of water holding capacity percentage in breast and thigh muscles in groups fed ration containing 2.5, 2 and 1.5% *Nigella sativa L.* were highly significant ($P<0.01$). Also, the difference between the mean values of water holding capacity percentage in

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thigh and breast muscle in both control group and the group fed on 0.5% *Nigella sativa L.* was significant (P>0.05). However, this difference was significant in group fed 1% *Nigella sativa L.* seeds (Table 3).

From the above results it was observed that, the water holding capacity percentage was increased by increasing the percentage of *Nigella sativa L.* seeds in the feed of poultry. These results may be attributed to the increased myofibrillar proteins to which a relatively small part of tissue water (4 – 5% of the total water) was tightly bound on the surface of such protein molecules as hydration water. The above results agree with ***Harmon and Muller (1971)***.

**5.1.2.3. Plasticity**:

Plasticity is a term of the method of measurement of meat consistency depending on pressing of the meat sample between two plates (e.g. the "filter paper press method") which resulting in release of hydration water which vary between 15 and 36 g water/100 g protein ***(Betton et al., 1972)***.

The differences between the mean values of plasticity percentages in thigh muscles in control group and each of the groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* were significant (P<0.05). While, the same difference were non-significant between the control group and each of the groups fed on ration containing 1 and 0.5% *Nigella sativa L.* seeds. In breast muscles, a significant difference was also found between the control group and each of the groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds. On the other hand, the difference between the mean values of plasticity percentage in breast and thigh muscles in groups fed on ration containing 2 and 1% *Nigella sativa L.* and control group were

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not significant ($P<0.05$). Also, the differences between the mean values of plasticity percentage in breast and thigh muscles in groups fed on ration containing 2.5 and 0.5% *Nigella sativa L.* seeds were significant ($P<0.05$) while the differences between the mean values of plasticity percentage in breast and thigh muscles in groups fed ration containing 1.5% *Nigella sativa L.* seeds were highly significant ($P<0.01$). These results are attributed to the WHC where by increasing the WHC the plasticity was increased as the shortening of the muscle fibers was decreased. These results are nearly similar to the results obtained by ***Bouton et al. (1973)***.

**5.2. Chemical examination:**

**5.2.1. Keeping quality tests:**

**5.2.1.1. pH value:**

Hydrogen ion concentration is the most important intrinsic factor with regard to the shelf life of poultry meat ***(Jan, 1995)***. However, increasing pH can increase susceptibility to microbial growth and decreasing shelf life ***(Banks et al., 1998)***.

As shown in Table (5), there was no significant difference between the mean values of pH in control group and each of the treated groups. Moreover, all tested samples were fresh. These results may be attributed to that the pH examination was done immediately after slaughtering of the birds.

**5.2.1.2. Copper sulphate test:**

Copper sulphate test is a qualitative test based on the precipitation of albuminous material in decomposed meat. The test was carried out within 10 minutes after slaughter.

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As shown in Table (6), it was found that 83.33% of the control group were positive for copper sulphate test (gel sediment) while about 66.67% of the group fed on ration containing 0.5% *Nigella sativa L.* seeds were also positive for the same test. On the other hand, the groups fed on rations containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds proved negative for copper sulphate test.

From the above results it was observed that in spite of carrying the test after 10 minutes of slaughter, the control group and the group fed on ration containing 0.5% *Nigella sativa L.* seeds seem to be not fresh but this is actually not true and these positive results may be attributed to the deposition of urates in muscles of control group and group fed on ration containing 0.5% *Nigella sativa L.* seeds, which prevented at high concentrations of the seeds in ration (2.5, 2, 1.5 and 1%) where the seeds have a diuretic and uricosuric effects. The results are nearly similar to the results obtained by ***Mandour et al. (1998)***.

In conclusion, feeding chickens on rations containing *Nigella sativa L.* seeds will reduce uric acid content in the muscles and so copper sulphate test cannot be considered as a reliable method for spoilage assessment of poultry meat.

**5.2.1.3. Eber's test:**

The results achieved in Table (6) reveals that all tested samples were fresh and this is attributed to that, the test was done immediately after slaughter of the chickens.

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**5.2.1.4. Moisture**:

The differences between the mean values of moisture percentage in thigh muscles in control group and each of the groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds were significant (P<0.05). On the other hand, the differences between the mean values of moisture percentage in thigh muscles in control group and each of the groups fed on ration containing 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05) (Table 7).

Also, Table (7) shows that the differences between the mean values of moisture percentages in breast muscles in control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). The same Table (7) shows that the differences between the mean values of moisture percentage in both thigh and breast muscles in control groups fed on ration containing 1 and 0.5% *Nigella sativa L.* seeds were highly significant (P<0.01). While, the differences between the mean values of moisture percentage of both thigh and breast muscles in groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds were not significant (P>0.05).

These results may be attributed to the increased protein content in muscles due to feeding *Nigella sativa L.* seeds at high concentrations in ration which resulted in increased moisture content in muscles but within the standard levels. These results agree with ***(El-Magoli et al., 1995)***.

**5.2.1.5. Protein**:

The data presented in Table (8) show that the differences between the mean values of protein percentage in thigh muscles in control and each of

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the groups fed on ration containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds were significant (P<0.01). On the other hand, the differences between the mean values of protein percentage in thigh muscles in control group and groups fed on ration containing 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). Also, there was no significant difference (P>0.05) between the mean values of protein percentage in thigh muscles in groups fed on ration containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds.

On the other hand, the difference between the mean values of protein percentage in breast muscles of control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05).

Table (8) also shows that the differences between the mean values of protein percentage in both thigh and breast muscles of control group and groups fed on ration containing 2, 1.5 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), while the difference was highly significant (P<0.01) between thigh and breast muscles in the group fed a ration containing 2.5% *Nigella sativa L.* seeds but the difference between the mean values of protein percentage in both thigh and breast muscles in groups fed on ration containing 1% *Nigella sativa L.* seeds was significant (P>0.05).

The above results may be attributed to the increased serum amino acids content due to feeding *Nigella sativa L.* seeds at high concentrations in the ration. These results agree with ***Mandour and Rady (1997)***.

**5.2.1.6. Fat:**

Flavour intensity, juiciness and tenderness of poultry meat are directly related to fat content ***(Taki, 1991)***. The presence of high fat lowers the

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quality of poultry meat, especially if the poultry meat is to be kept for long periods under improper storage conditions before consumption, because of rancidity ***(Berry, 1993)***.

The results achieved in Table (9) reveal that the differences between the mean values of fat percentage in thigh muscles of control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were significant (P<0.05).

The differences between the mean values of fat percentage in breast muscles of control group and each of the groups fed on ration containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds were significant (P<0.05). While, the difference between the mean values of fat percentage in breast muscle of control group and the group fed on ration containing 0.5% *Nigella sativa L.* seeds was not significant (P>0.05). Also, the difference between the mean values of fat percentage in breast muscles of groups fed on ration containing 1.5 and 1% *Nigella sativa L.* seeds and groups fed on ration containing 2 and 1.5% *Nigella sativa L.* seeds were not significant (P>0.05). The difference between the mean values of fat percentage in both thigh and breast muscles of control groups and each of the groups fed on rations containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were highly significant (P<0.01). These results may be due to the hypolipidemic effect of *Nigella sativa L.* seeds which reduces the level of cholesterol and triglycerides as recorded by ***Abd El-Aal and Attia (1993), Hedaya (1996)*** and ***Awadalla and Kamel (2000)***.

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### 5.3. Serum biochemical analysis:

#### 5.3.1. Serum cholesterol:

The difference between the mean values of serum cholesterol from control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). From these results it is proved that feeding *Nigella sativa L.* seeds has no effect on serum cholesterol level (Table 10).

#### 5.3.2. Serum triglycerides:

The differences between the mean values of serum triglycerides from control group and each of the groups fed on rations containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05) (Table 11).

These results do not agree with those reported by ***Weigand et al. (1973), Abd El-Aal and Attia (1993)*** and ***Mandour et al. (1998)*** who reported that the oil of *Nigella sativa L.* seeds contain high amounts of unsaturated fatty acids reaching 72% of the total fatty acids which may be considered the main cause of its hypolipidemic effect which caused a significant decrease in serum cholesterol and triglycerides which preventing hepatic fatty acid synthesis. These disagreement between the results of this study and those of the investigators mentioned above may be due to the differences in the feeding period with *Nigella sativa* seeds and species variation.

#### 5.3.3. Serum total protein:

The differences between the mean values of serum total protein from the control group and each of groups fed on ration containing 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05) (Table 12). Also,

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the same Table showed that the difference between the mean values of serum total protein from the control group and group fed on a ration containing 2.5% *Nigella sativa L.* seeds was significant (P<0.05). These results agree with ***Khodary et al. (1996)***.

**5.3.4. Albumin:**

It is evident from Tables (13 and 15) that The differences between the mean values of serum albumin from the untreated control group and each of the groups fed on ration containing 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), but the difference between the mean values of serum albumin from control group and that from the group fed on a ration containing 2.5% *Nigella sativa L.* seeds was significant (P<0.05). These results agree with those of ***Hedaya (1996)*** who reported that serum albumin was increased at feeding ration containing *Nigella sativa L.* seeds at high concentrations.

**5.3.5. Globulin:**

The differences between the mean values of serum globulin from the untreated control group and each of the groups fed on ration containing 2.5, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05) (Tables 14 and 15). On the other hand, the differences between the mean values of serum globulin from the control group and the group fed on ration containing 2% *Nigella sativa L.* seeds was significant (P>0.05). The same result was recorded by ***Nassar (1997)*** who found that the total serum protein and globulins were significantly increased in chickens received a ration containing 2% *Nigella sativa L.* seeds.

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**5.4. Microbiological examination**:

The microbiological examination of poultry meat to determine conformance to the poultry meat specification (i.e. microbiological criteria) is often used, testing for conformance to such criteria provides only limited protection to consumer against food poisoning and/or food borne disease, this often in fact the reason for carrying out the tests to provide assurance of poultry meat ***(Wilkie, 1998)***.

**5.4.1. Total mesophilic bacterial count**:

Realize that demonstration of bacterial density of poultry meat can give some indications about hygienic quality of poultry meat investigated. Moreover, the high mesophilic bacterial count often indicates contamination of poultry carcasses or unsatisfactory handling ***(ICMSF, 1978).***

The results given in Table (16) show that the difference between the mean values of total mesophilic bacterial count in thigh muscles of control group and each of the groups fed on ration containing 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). But, the difference between the mean values of total mesophilic bacterial count in thigh muscles of control group and the group fed on a ration containing 2.5% *Nigella sativa L.* seeds was significant (P<0.05).

The same Table showed that the difference between the mean values of total mesophilic bacterial count in breast muscles of control group and each of the groups fed on ration containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds were significant (P<0.05). While, the difference between the mean values of total mesophilic bacterial count in breast muscle of the control

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group and that from the group fed on a ration containing 0.5% *Nigella sativa L.* seeds was not significant (P>0.05).

The differences between the mean values of total mesophilic bacterial count in both thigh and breast muscles in control group and each of the groups fed on ration containing 2.5, 2 and 1% *Nigella sativa L.* seeds were not significant (P>0.05). On the other hand, there was a significant difference (P<0.05) between the mean values of total mesophilic bacterial counts of breast and thigh muscles in groups fed on ration containing 1.5% or 0.5% *Nigella sativa L.* seeds.

Also, Table (16) shows that the minimal count in thigh muscles (1.15 x $10^6$) was observed in group fed on ration containing 2.5% *Nigella sativa L.* seeds and this count seems to be high in comparison with the statement of ***Shiffman (1961)*** who said that the fresh meat and meat products of a bacterial content up to $10^5$/g have not been implicated in food poisoning. Therefore, the microbiological standard for meat and meat products should be established at $10^5$/g. These high counts may be attributed to post slaughtering contamination which reduced by feeding ration containing *Nigella sativa L.* for a long period (over 30 days) beside its effects on the immunity status of the live birds due to the presence of thymohydroquinone and also carvone which have a strong antibacterial activity ***(Jay and Rivers, 1984)***.

**5.4.2. Total Coliform count**:

Although the total mesophilic bacterial count was used in microbiological examination to reflect the hygienic quality of poultry meat, however, it is evident that the examination of Coliform is considered of much greater value in assessing its quality ***(Cruickshank et al., 1975)***.

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The data presented in Table (17) show that the differences between the mean values of total Coliform count in thigh muscles of control groups and each of the groups fed on ration containing 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), however, the differences between the mean values of total Coliform count in thigh muscles of control group and each of the groups fed on ration containing 2.5 and 2% *Nigella sativa L.* seeds were significant (P<0.05).

The same Table also showed that the differences between the mean values of total Coliform count in breast muscles of control group and each of the groups fed on rations containing 1 and 0.5% *Nigella sativa L.* seeds were not significant (P<0.05). On the other hand, the differences between the control group and each of the groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds were significant (P<0.05).

The differences between the mean values of total Coliform count in both thigh and breast muscles in control and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% were not significant (P>0.05).

Also, from the results mentioned in Table (17), high counts of Coliforms were observed in both thigh and breast muscles and this high count may be attributed entirely to post slaughtering contamination.

**5.4.3. Total Enterobacteriaceae count:**

The differences between the mean values of total Enterobacteriaceae count in thigh muscles of control group and each of the groups fed on ration containing 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant

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(P>0.05), while the differences between the mean values of total Enterobacteriaceae count in thigh muscles of control and each of the groups fed on ration containing 2.5 and 2% *Nigella sativa L.* seeds were significant (P<0.05) (Table 18).

Also, the same Table shows that the differences between the mean values of total Enterobacteriaceae count in breast muscles of control group and each of the groups fed on ration containing 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), while the difference between the mean values of total Enterobacteriaceae count in breast muscles in control group and the group fed on ration containing 2.5% *Nigella sativa L.* seeds was significant (P<0.05).

On the other hand, the differences between the mean values of total Enterobacteriaceae count in both thigh and breast muscles of control group and groups fed on ration containing 2.5, 2 and 1% *Nigella sativa L.* seeds were not significant (P>0.05).

These results agree with ***Jay and Rivers (1984)*** who reported that Enterobacteriaceae count was decreased due to the presence of thymohydroquinone compound which is formed in poultry muscles due to feeding *Nigella sativa L.* seeds at high concentrations.

**5.4.4. Total *Staphylococcus aureus* count:**

*Staphylococcus aureus* in poultry meat may originate from Staphylococcal infection in poultry or through contamination from food handlers ***(Frazier and Westhoff, 1983)***.

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The differences between the mean values of total *Staphylococcus aureus* count in thigh muscles of control group and each of groups fed on ration containing 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), while the differences between the mean values of total *Staphylococcus aureus* count in thigh muscles in control group and each of the groups fed on ration containing 2.5, 2 and 1.5% *Nigella sativa L.* seeds were significant (P<0.05) (Table (19).

The same Table also shows that the difference between the mean values of total *Staphylococcus aureus* count in breast muscles in control group and each of the groups fed on ration containing 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05), while the difference between the mean values of total *Staphylococcus aureus* count in breast muscles of control group and the group fed on ration containing 2.5% *Nigella sativa L.* seeds was significant (P<0.05).

The same Table showed that the differences between the mean values of total *Staphylococcus aureus* count in both thigh and breast muscles of control and in all treated groups were not significant (P>0.05). These results were in agreement with ***Branen et al. (1980)*** and ***Davidson et al. (1981)***.

**5.4.5. Detection of Salmonellae**:

The results obtained in Table (20) reveal that the incidence of Salmonellae in thigh muscles was higher in control group and the group fed on ration containing 0.5% *Nigella sativa L.* seeds and lower in groups fed on ration containing 2 and 1% *Nigella sativa L.* seeds and no isolates were

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found in thigh muscles in both groups fed on ration containing 2.5 and 1.5% *Nigella sativa L.* seeds. Also, the same Table showed that the same incidence (33.33%) of isolated Salmonella species was found in breast muscles of both control group and group fed ration containing 0.5% *Nigella sativa L.* seeds, while no strains were isolated from breast muscles in groups fed on ration containing 2.5, 2, 1.5 and 1% *Nigella sativa L.* seeds. These results may be owing to the fact that the *Nigella sativa L.* seeds at high concentrations can reduce the number of Salmonellae. This is in agreement with results obtained by ***(Cutter, 2000)***.

**5.4.6. Total mould count**:

The moulds are used as an index of the proper sanitation. Mould can assist in the putrefactive processes and in other cases they may impart a mouldy taste and odour to poultry meat. Moulds are likely to render the meat slightly unacceptable rather than render it unfit for human consumption ***(Frazier, 1967)***.

Table (21) shows that the differences between the mean values of total mould count in thigh muscles of control group and each of the groups fed on ration containing 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). Also, the differences between the mean values of total mould count in thigh muscles of control and the groups fed on ration containing 2.5 and 2% *Nigella sativa L.* seeds were significant (P<0.05) (Table, 21).

On the other hand, the differences between the mean values of total mould count in breast muscles of control and each of the groups fed on

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ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05) and also, there was no significant difference in between all treated groups in total mould count in breast muscles.

The differences between the mean values of total mould count in both thigh and breast muscles were not significant (P>0.05) in control group and each of the treated groups.

These results may be attributed to the antifungal compounds present in the *Nigella sativa L.* seeds such as thymoquinone, carvone, vanillin and ethyl vanillin. These results agree with ***(Lin, 1983)*** and also agree with ***Hanafy and Hatem (1991)*** who reported that *Nigella sativa L.* seed extract has antifungal activity against *Candida albicans* and Aspergillus.

**5.4.7. Total yeast count:**

Yeasts normally play a small role in spoilage because they constitute only a small portion of the initial population, because they grow slowly in comparison with most bacteria and because their growth may be limited by metabolic substances produced by bacteria. Spoilage yeasts are those which find their way into poultry meat because of their wide distribution in nature resulting in undesirable changes in physical appearance of the meat ***(Walker, 1976)***.

As shown in Table (22) the differences between the mean values of total yeast count in thigh muscles of control group and each of the groups fed on ration containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds were not significant (P>0.05). Also, the difference between the mean values of

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total yeast count in breast muscles of control group and each of the groups of all treated groups were not significant (P>0.05). On the other hand, there was no significant difference (P>0.05) between the mean values of total yeast count in both thigh and breast muscles in control and each of all treated groups.

Also, Table (23) shows that the isolated mould strains were *Aspergillus niger,* Mucor species and Cladosporium species. The incidence of *A. niger*, Mucor species and Cladosporium species isolated from thigh muscles were decreased by increasing the concentration of *Nigella sativa L.* seeds in ration. Moreover, both Mucor species and Cladosporium species were absent in thigh muscles obtained from chickens fed on ration containing 2.5% *Nigella sativa L.* seeds.

The same Table (23) also shows that the incidence of these moulds could be isolated from breast muscles were also decreased by increasing the concentration of the *Nigella sativa L.* seeds in the ration. On the other hand, these mould strains in breast muscles were completely absent at 2.5% concentration. The above results agree with ***Agrawal et al. (1979)*** who proved that the oil of *Nigella sativa L.* seeds had an excellent antifungal activity, particularly against Aspergillus species.

The data presented in Table (24) show that the incidence of Candida species and Torulopsis in both thigh and breast muscles was decreased by increasing the concentration of *Nigella sativa L.* seeds in the ration. On the other hand, the growth of these yeasts was completely prevented in breast

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muscles at 2.5% concentration of *Nigella sativa L.* seeds in the ration. These results agree with ***Hanafy and Hatem (1991)*** and ***Sokmen et al. (1999)*** who reported that the growth of *Candida albicans* was inhibited by using *Nigella sativa* extract.

**5.5. Histopathological studies**:

The histopathological results were attributed to the increased level of myofibrillar proteins which resulted from the significant increase of some amino acids by feeding ration containing 2.5% *Nigella sativa L.* seeds. These results agree with ***Mandour and Rady (1997)***.

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# 6. CONCLUSION AND RECOMMENDATIONS

Information given by the obtained results allow to conclude that the appearance, taste and odour of the broiler meat were improved by feeding rations containing 2.5% *Nigella sativa L.* seeds and also, the physical examination revealed that the water holding capacity and plasticity of the broiler meat were improved by feeding ration contained 2.5, 2 or 1.5% *Nigella sativa L.* seeds.

Also, the chemical examination showed that the beneficial increase in protein and moisture contents and the required decrease in fat content were achieved at concentrations of 2.5, 2 and 1.5 *Nigella sativa L.* seeds in the ration of broiler chicks.

On the other hand, the significant increase in biochemical parameters such as albumin and globulin was obtained at concentrations of 2.5 and 2% *Nigella sativa L.* seeds in the ration, respectively.

On the other hand, the microbiological examination revealed that the microbial count was the lowest in thigh and breast muscles in group fed on ration contained 2.5% *Nigella sativa L.* seeds in comparison with other examined muscle samples from other treated and control groups.

Also, the histopathological studies revealed that the lowest connective tissue percentage in the broiler was obtained from group fed on a ration containing 2.5% *Nigella sativa L.* seeds.

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Therefore, the following precautions and recommendations should be carried out:

- The use of natural feed additives such as herbaceous plants, particularly *Nigella sativa L.* seeds is more beneficial and safe than artificial additives.
- The *Nigella sativa L.* seeds must be obtained from official or well known sources to avoid its adulteration by other seeds or herbs.
- The seeds must be free from any foreign materials and other unknown herbs.
- Avoid storage of crushed seeds for long time to avoid the release of the volatile substances or rancidity of its oil. So, if we have to store black seed, it must be stored without crushing in tightly closed plastic bags.
- The ration must be moistened by oil or water before adding the crushed seeds.
- The seeds should be added in crushed form and the process of crushing should be performed just before offering the ration to the broiler chicks to avoid the unacceptability and losses of the seeds.
- Proper mixing of the crushed seeds with the ration is necessary.
- Addition of finely crushed seeds is advisable than coarsely crushed ones and also, better than the seeds extract and its oil.
- Feeding of *Nigella sativa L.* seed in the ration should be done at age above 20 days old to avoid the over load and to be economic.
- The most beneficial and safe concentrations of the black seeds were 2 and 2.5%.

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# 7. SUMMARY

A total of 120 broiler (20 days old) chicks was classified into 6 groups (20 broiler chicks each). The groups were fed on rations containing *Nigella sativa L.* seeds at different concentrations as shown in the following Table:

| Groups | No. of birds | Concentration (%) of *Nigella sativa* in ration | Period of Feeding (days) |
|---|---|---|---|
| First | 20 | 0.5 | 20 |
| Second | 20 | 1.0 | 20 |
| Third | 20 | 1.5 | 20 |
| Fourth | 20 | 2.0 | 20 |
| Fifth | 20 | 2.5 | 20 |
| Control | 20 | Untreated | 20 |

Following feeding broiler chicks for 20 days, 12 broiler chicks were slaughtered from each group. The blood samples were collected for biochemical analysis then breast and thigh muscles of each group were taken immediately to the laboratory and examined physically, chemically, microbiologically and histopathologically. The obtained results revealed the following:

1. **ORGANOLEPTIC EXAMINATION** of both thigh and breast muscles revealed that the **colour** of meat was red and homogenous at 2.5% concentration, while at 2% and 1.5% concentrations it was bright and light red. On the other hand, it was patchy (pale white and red patches) in control and each of the groups fed on rations containing 1 or 0.5 *Nigella sativa L.* seeds. The **flavour** was spicy and nearly similar to

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spiced mutton meat in muscles obtained from birds fed on ration containing 2 or 2.5% *Nigella sativa L.* seeds. However, other treated and control groups had normal little odour. The taste of the meat was excellent, very good, good, fair and acceptable for groups fed on rations containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds and control group, respectively. The **texture** of the meat was firm on touch in both groups fed on rations containing either 2.5 or 2% *Nigella sativa L.* seeds and soft on touch in other treated and control groups.

Physical examination also revealed that the mean values of **thermal shrinkage** in thigh muscles from groups fed on rations containing 2.5, 2, 1.5, 1 or 0.5% *Nigella sativa L.* seeds and control group were 67.38±0.30, 65.92±0.69, 63.94±0.82, 63.12±0.47, 62.19±0.58 and 61.18±0.99, respectively. Meanwhile, in breast muscles the mean values of thermal shrinkage percentage were 74.59±0.57, 74.28±0.39, 73.25±0.61, 72.86±0.67, 71.87±0.40 and 71.11±0.73, respectively. Also, the mean values of **water holding capacity** percentage in thigh muscles were 73.67±0.29, 73.89±0.28, 67.62±0.42, 66.08±0.56, 62.16±0.75 and 59.58±0.50, while in breast muscles were 68.37±0.42, 68.43±0.25, 66.69±0.54, 63.14±0.49, 61.01±0.30 and 58.72±0.74, respectively. The mean values of **plasticity** percentages in thigh muscles were 0.358±0.005, 0.353±0.004, 0.348±0.005, 0.337±0.003, 0.325±0.005 and 0.322±0.007, while in breast muscles the mean values were 0.382±0.007, 0.368±0.006, 0.370±0.004, 0.352±0.007, 0.343±0.005 and 0.322±0.012, respectively.

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2. **CHEMICAL EXAMINATION** of poultry meat samples revealed that the mean values of **pH** of meat from groups fed on rations containing 2.5, 2, 1.5, 1 and 0.5% *Nigella sativa L.* seeds and control groups were 5.50 ± 0.204, 5.57 ± 0.304, 5.16 ± 0.264, 5.02 ± 0.085, 5.42 ± 0.185 and 5.94 ± 0.192, respectively. Also, the incidence of positive copper sulphate test was 0, 0, 0, 0, 66.67 and 83.33%, respectively. Meanwhile, the incidence of Eber's test was 0% for all examined samples from treated and control groups.

On investigating the **moisture percentages** in thigh muscles, the highest mean values (67.11 ± 0.22) was observed in birds fed 2.5% while the lowest mean value (61.52 ± 0.17) was recorded in the control untreated birds. Also, the mean values of moisture percentage in breast muscles in birds fed on a ration containing 2.5% *Nigella sativa L.* seeds and control birds were 70.36 ± 2.00 and 68.35 ± 0.99, respectively.

The mean value of **protein percentage** was the highest in thigh muscles (22.51 ± 0.58) and the lowest (18.49 ± 1.02), while in breast muscles the highest mean value (20.25 ± 0.52) and the lowest (18.99 ± 0.76) were observed in group fed a ration containing 2.5% *Nigella sativa L.* seeds and the control group, respectively.

The mean values of **fat percentage** in both thigh and breast muscles decreased at high concentrations of *Nigella sativa L.* seeds where the lowest mean value (2.34 ± 0.01) and the highest (2.63 ± 0.01) in thigh muscle, while in breast muscles the lowest (1.38 ± 0.05) and the highest (1.72 ± 0.02) were observed in groups fed on 2.5% *Nigella sativa L.* seeds and control group, respectively.

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3. **BIOCHEMICAL EXAMINATION** revealed that there was no significant difference between the mean values of both **cholesterol** (mg/dl) and **triglycerides** (mg/dl) in the control and all treated groups where the mean values of cholesterol were 102.55 ± 2.25 and 115.25± 4.96, while the mean values of triglycerides were 85.00 ± 6.45 and 103.75 ± 6.25 the groups fed on 2.5% *Nigella sativa L.* seed and control group, respectively.

The mean values of **serum total protein** (g/dl) were 4.25 ± 0.03 the highest and 3.58 ± 0.12 the lowest while the mean values of **serum albumin** (g/dl) were 2.20 ± 0.35 the highest and 1.50 ± 0.12 the lowest in group fed on 2.5% *Nigella sativa L.* seeds and control group, respectively. On the other hand, the mean values of **serum globulin** (g/dl) were the highest (2.75 ± 0.10) and lowest (1.85 ± 0.19) in groups fed on ration containing 2 and 1% *Nigella sativa L.* seeds but in control group the mean value of serum globulin was 2.08 ± 0.16.

4. **MICROBIOLOGICAL EXAMINATION** revealed that the mean values of **total mesophilic bacterial count** (cfu/g) in thigh muscles were the highest ($3.74 \times 10^6 \pm 5.41 \times 10^5$) and the lowest ($2.10 \times 10^6 \pm 4.03 \pm 10^5$) in control group and group fed on ration containing 2.5% *Nigella sativa L.* seeds, respectively. While, in breast muscles, the mean values were highest ($3.29 \times 10^6 \pm 2.95 \times 10^5$) and lowest ($1.56 \times 10^6 \pm 2.23 \times 10^5$) in control and group fed on a ration containing 2.5% *Nigella sativa L.* seeds, respectively.

The mean value of **total Coliform count** (cfu/g) in thigh muscles were highest ($2.38 \times 10^5 \pm 2.77 \times 10^4$), lowest ($8.58 \times 10^4 \pm 9.70 \times 10^3$)

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while, in breast muscles were highest ($2.28 \times 10^5 \pm 2.28 \times 10^4$), lowest ($7.96 \times 10^4 \pm 1.26 \times 10^4$) in control group and group fed on ration containing 2.5% *Nigella sativa L.* seeds, respectively.

The mean values of **total Enterobacteriaceae count** (cfu/g) in thigh muscles were highest ($2.4 \times 10^5 \pm 2.95 \times 10^4$), lowest ($1.35 \times 10^5 \pm 1.15 \times 10^4$) and in breast muscles were highest ($1.8 \times 10^5 \pm 2.5 \times 10^4$), lowest ($9.67 \times 10^4 \pm 9.32 \times 10^3$) in control group and group fed on ration containing 2.5% *Nigella sativa L.* seeds, respectively.

The mean values of **total *Staphylococcus aureus* count** (cfu/g) in thigh muscles were highest ($2.44 \times 10^4 \pm 4.57 \times 10^3$), lowest ($6 \times 10^3 \pm 3.21 \times 10^3$) and in breast muscles were highest ($1.3 \times 10^4 \pm 2.5 \times 10^3$), lowest ($3 \times 10^3 \pm 1 \times 10^3$) in control group and group fed on ration containing 2.5 *Nigella sativa L.* seeds, respectively.

Isolated **Salmonella strains** from positive samples of thigh muscles were at an incidence of 66.67, 33.33, 0, 16.67, 0 and 66.67%, while of breast muscles were at an incidence 33.33, 0, 0, 0, 0 and 33.33% from groups fed on ration containing 0.5, 1, 1.5, 2, 2.5% *Nigella sativa L.* seeds and control group, respectively.

The mean values of **total mould count** (cfu/g) of thigh muscles were highest ($4.40 \times 10^3 \pm 6 \times 10^2$) and lowest ($1.67 \times 10^3 \pm 3.33 \times 10^2$) in control group and group fed on ration containing 2.5% *Nigella sativa L.* seeds, respectively. While, the mean values in breast muscles were highest ($3.17 \times 10^3 \pm 3.07 \times 10^2$) and lowest ($1.67 \times 10^3 \pm 6.67 \times 10^2$)

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and $2.75 \times 10^3 \pm 4.79 \times 10^2$ in groups fed on rations containing 1, 2.5% *Nigella sativa L.* seeds and control group, respectively.

The mean values of **total yeast count** (cfu/g) in thigh muscles were $4 \times 10^3 \pm 9.13 \times 10^2$, $1 \times 10^3$ and $3.67 \times 10^3 \pm 9.18 \times 10^2$ in groups fed on ration containing 1, 2.5% *Nigella sativa L.* seeds and control group, respectively. While in breast muscles, the mean values were $3.83 \times 10^3 \pm 8.33 \times 10^2$ and $1.40 \times 10^3 \pm 2.45 \times 10^2$ in control group and group fed on ration containing 0.5% *Nigella sativa L.* seeds, respectively.

Isolated **mould strains** from positive samples in thigh and breast muscles were *Aspergillus niger* at incidence 33.33, 33.33, 16.67, 16.67, 16.67 and 33.33 in thigh muscles and at incidence of 16.67, 16.67, 16.67, 16.67, 0 and 16.67 in breast muscles; Mucor species at an incidence of 16.67, 16.67, 16.67, 16.67, 0 and 66.67% in thigh muscles and at an incidence of 33.33, 33.33, 16.67, 16.67, 0 and 50% in breast muscles; Cladosporium species at an incidence of 16.67, 16.67, 16.67, 16.67, 0 and 50% in thigh muscles and at an incidence of 33.33, 33.33, 33.33, 0, 0, and 33.33% in breast muscles from groups fed on rations containing 0.5, 1, 1.5, 2, 2.5 *Nigella sativa L.* seeds and control group, respectively.

Isolated **yeast strains** from positive samples were Candida species at incidence of 50, 50, 33.33, 16.67, 16.67 and 66.67% in thigh muscles and at an incidence of 50, 50, 33.33, 16.67 and 50% in breast muscles and Torulopsis species at an incidence of 50, 33.33, 33.33,

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33.33, 16.67 and 50% in thigh muscles and 33.33, 16.67, 16.67, 16.67, 0 and 50% in breast muscles from groups fed on ration containing 0.5, 1, 1.5, 2, 2.5% *Nigella sativa L.* seeds and control group, respectively.

5. **HISTOPATHOLOGICAL STUDIES** showed that the muscle fibers were increased on account of the connective tissue content at concentrations of 2 and 2.5% *Nigella sativa L.* seeds in the ration.

The results of organoleptic, chemical, biochemical, microbiological and histopathological examinations as well as the suggestive measures to improve the quality of broiler meat using black seed were discussed.

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# Arabic Summary

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# الملخص العربي

## تأثير النيجيلا ساتيفا (الحبة السوداء) على نوعية لحوم دجاج المائدة

بذور حبة البركة (الحبة السوداء) نبات عشبي من عائلة رانكلاسيا التي تنمو في بلاد وسط أوروبا و دول البحر الأبيض المتوسط و غرب أسيا و هي منتشرة في إنحاء جمهورية مصر العربية. و لما ذاع صيت حبة البركة في الآونة الأخيرة بصفتها فارس الأعشاب الطبيعية و بما تنفرد به عن مثيلاتها في خدمة الكائن البشري، حاولنا الإستفادة من الطبيعة بإضافتها إلى علائق الدواجن و دراسة تأثيراتها عند تركيزاتها المختلفة و أجريت الدراسة على ١٢٠ كتكوت تسمين من نوع أربر أكرز عمر ٢٠ يوم و تم تقسيم الكتاكيت إلى ستة مجموعات كل مجموعة تتكون من ٢٠ كتكوت و تم معاملة المجموعات لمدة ٢٠ يوم كالتالي:

المجموعة الأولى: تم تغذيتها على عليقة أساسية مضاف إليها مطحون حبة البركة بنسبة ٠,٥٪ (٥٠٠ جرام لكل ١٠٠ كيلو جرام من العليقة).

المجموعة الثانية: عليقة أساسية مضاف إليها مطحون حبة البركة بنسبة ١٪ (١ كيلو جرام لكل ١٠٠ كيلو جرام من العليقة).

المجموعة الثالثة: عليقة أساسية مضاف إليها مطحون حبة البركة بنسبة ١,٥٪ (١,٥ كيلو جرام لكل ١٠٠ كيلو جرام من العليقة).

المجموعة الرابعة: عليقة أساسية مضاف إليها مطحون حبة البركة بنسبة ٢٪ (٢ كيلو جرام لكل ١٠٠ كيلو جرام من العليقة).

المجموعة الخامسة: عليقة أساسية مضاف إليها مطحون حبة البركة بنسبة ٢,٥٪ (٢,٥ كيلو جرام لكل ١٠٠ كيلو جرام من العليقة).

المجموعة السادسة: تم تغذيتها على عليقة أساسية دون إضافة حبة البركة.

و قد أخذت عينات الدم بعد ٢٠ يوم من التغذية على الحبة السوداء في العليقة لإجراء بعض الإختبارات البيوكيميائية و بعد الذبح تم تقسيم عينات اللحم إلى صدور

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و أوراك ثم أجريت على هذه العينات إختبارات فيزيائية (ظاهرية و حسية) و كيميائية و ميكروبيولوجية و هستوباثولوجية. و كانت أهم النتائج هي:

١- **بفحص العينات فيزيائياً (ظاهرياً و حسياً)** وجد أن **لون لحم الدجاج** الذى تم تغذيته على عليقة تحتوى على ٢,٥٪ و ٢٪ حبة البركة كان أحمر متجانس بينما في المجموعات الأخرى كان لون لحومها أحمر باهت (١,٥ و ١٪) أو به بقع حمراء و بيضاء (٠,٥٪ و المجموعة الضابطة).

و كانت **رائحة اللحم** في كلتا المجموعتين اللاتى تناولت عليقة بها ٢,٥ و ٢٪ حبة البركة مثل رائحة لحم الضأن المتبل و اللحم المتبل فقط على التوالي بينما كانت الرائحة في باقي المجموعات هي رائحة لحم الدجاج الطبيعي، كذلك لوحظ تحسن في **المذاق** بزيادة نسبة حبة البركة في العليقة و أيضاً تم ملاحظة **ملمس اللحم** و الذى أيضاً كان متماسك في كلتا المجموعتين اللاتي تناولت عليقة بها ٢,٥ و ٢٪ حبة البركة عن باقي المجموعات. و لوحظ أيضاً أن متوسط **معدل الإنكماش الحراري** للعضلات يزداد بزيادة نسبة حبة البركة في العليقة حيث كان أعلى متوسط إنكماش حراري (٦٧,٣٨ ± ٠,٣٠) في الأوراك و أعلى متوسط (٧٤,٥٩ ± ٠,٥٧) في الدجاج الذي تم تغذيته على عليقة بها ٢,٥٪ حبة البركة و كذلك تم قياس نسبة **الماء المتحد (ماء العددنه)** و الذي لوحظ أنه يزداد بزيادة تركيز حبة البركة في العليقة و كانت المتوسطات عند تركيز ٢,٥٪ هي ٧٣,٦٧ ± ٠,٢٩ و ٦٨,٣٧ ± ٠,٤٢ في كلاً من الأوراك و الصدور على التوالي.

٢- **بفحص العينات كيميائياً: أولاً لتقدير طزاجة اللحم** باستخدام كلاً من الأس الهيدروجيني وجد أن المتوسطات كانت ٥,٥٠ ± ٠,٢٠٤، ٥,٥٧ ± ٠,٣٠٤، ٥,١٦ ± ٠,٢٦٤، ٥,٠٢ ± ٠,٠٨٥، ٥,٤٢ ± ٠,١٨٥ و ٥,٩٤ ± ٠,١٩٢ في عينات اللحم من الكتاكيت التي تناولت عليقة بها حبة البركة بنسبة ٢,٥، ٢، ١,٥، ١، ٠,٥٪ و العليقة الضابطة على التوالي. و تم استخدام **اختبار كبريتات النحاس** و كانت النتائج الإيجابية بنسبة ٧١,٤٣ و ٨٥,٧١٪ في العينات التي أخذت من المجموعات التي تناولت حبة البركة بتركيز ٠,٥٪ و المجموعة الضابطة على التوالي. كما أجرى **اختبار إيبر** و كانت نتائج كل العينات سلبية.

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**ثانياً: تقدير نسبة الرطوبة، البروتين و الدهن:** أوضحت النتائج أن أعلى متوسطات **للرطوبة و البروتين** كانت عند تركيز ٢,٥٪ في الأوراق (٦٧,١١ ± ٠,٢٢) و (٢٢,٥١ ± ٠,٥٨) و في الصدور و كانت (٧٠,٣٦ ± ٢) و (٢٠,٢٥ ± ٠,٥٢) على التوالي و كانت أقل متوسطات في المجموعة التي تناولت عليقة دون إضافات و كانت في الأوراك (٦١,٥٢ ± ٠,١٧) و (١٨,٤٩ ± ١,٠٢) و في الصدور كانت (٦٨,٣٥ ± ٠,٩٩) و (١٨,٩٩ ± ٠,٧٦) على التوالي. على العكس كانت أقل المتوسطات **للدهن** عند تركيز ٢,٥٪ حبة البركة حيث كانت في الأوراك (٢,٣٤ ± ٠,٠١) و في الصدور (١,٣٨ ± ٠,٠٥) و أعلى متوسطات للدهون في المجموعة الضابطة و كانت في الأوراك (٢,٦٣ ± ٠,٠١) و في الصدور (١,٧٢ ± ٠,٠٢) على التوالي.

٣- **الفحص البيوكيميائي**: أوضحت النتائج أنه لا يوجد فرق معنوى بين المجموعات في مستوى كلاً من **الكوليسترول و الجليسريدات الثلاثية فى المصل** حيث كان أعلى متوسط (١١٥,٢٥ ± ٤,٩٦) و (١٠٣,٧٥ ± ٦,٢٥) على التوالي في المجموعة الضابطة و كانت أقل المتوسطات هي (١٠٢,٢٥ ± ٢,٢٥) و (٨٥,٠٠ ± ٦,٤٥) على التوالي في المجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة و بمقارنة متوسطات **البروتين الكلى في المصل** كان أعلى مستوى (٤,٢٥ ± ٠,٠٣) في المجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة و أقل مستوى (٣,٥٨ ± ٠,١٢) في المجموعة التي تناولت عليقة اساسية فقط و كان أعلى متوسط **للألبيومين في المصل** (٢,٢ ± ٠,٣٥) و أقل متوسط (١,٥ ± ٠,١٢) أيضاً في هاتين المجموعتين على التوالي بينما لوحظ أن أعلى متوسط **للجلوبيولين في مصل الدم** في المجموعة التي تناولت عليقة بها ٢٪ حبة البركة و كان ٢,٧٥ ± ٠,١ و أقل متوسط كان ١,٨٥ ± ٠,١٤ في المجموعة التي تناولت عليقة بها ١٪ حبة البركة.

٤- **بالفحص الميكروبيولوجي**: وجد أن متوسط **العد الكلى للبكتريا أليفة الإعتدال** في كلاً من الأوراك و الصدور (٣,٧٤ × ١٠^٦ ± ٥,٤١ × ١٠^٥) و (٣,٢٩ × ١٠^٦ ± ٢,٩٥ × ١٠^٥) على التوالي في المجموعة الضابطة و كان (٢,١٠ × ١٠^٦ ± ٤,٠٣ × ١٠^٥)

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و (١,٥٦ × ١٠$^{٦}$ ± ٢,٢٣ × ١٠$^{٥}$) على التوالي في المجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة.

وجد أن متوسط **العد الكلي للبكتريا القولونية** في الأوراك (٢,٣٨ × ١٠$^{٥}$ ± ٢,٧٧ × ١٠$^{٤}$) و (٨,٥٨ × ١٠$^{٤}$ ± ٩,٧ × ١٠$^{٣}$) وفي الصدور (٢,٢٨ × ١٠$^{٥}$ ± ٢,٢٨ × ١٠$^{٤}$) و (٧,٩٦ × ١٠$^{٤}$ ± ١,٢٦ × ١٠$^{٤}$) في المجموعة الضابطة والمجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة على التوالي.

وجد أن متوسط **العد الكلي للبكتريا المعوية** في الأوراك (٢,٤ × ١٠$^{٥}$ ± ٢,٩٥ × ١٠$^{٤}$) و (١,٣ × ١٠$^{٥}$ ± ١,١٥ × ١٠$^{٤}$) وفي الصدور (١,٨ × ١٠$^{٥}$ ± ٢,٥ × ١٠$^{٤}$) (٩,٦٧ × ١٠$^{٤}$ ± ٩,٣٢ × ١٠$^{٣}$) في المجموعة الضابطة والمجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة على التوالي.

وجد أن **العد الكلي للميكروب العنقودي الذهبي** في الأوراك (٢,٤٤ × ١٠$^{٤}$ ± ٤,٥٧ × ١٠$^{٣}$) و (٦ × ١٠$^{٣}$ ± ٣,٢١ × ١٠$^{٣}$) وفي الصدور (١,٣ × ١٠$^{٤}$ ± ٢,٥ × ١٠$^{٣}$) و (٣ × ١٠$^{٣}$ ± ١ × ١٠$^{٣}$) في المجموعة الضابطة والمجموعة التي تناولت عليقة بها ٢,٥٪ حبة البركة على التوالي.

تم عزل ميكروب **السالمونيلا** من عينات الأوراك بنسبة ٦٦,٦٧، ٣٣,٣٣، صفر، ١٦,٦٧، صفر و ٦٦,٦٧٪ ومن الصدور بنسبة ٣٣,٣٣، صفر، صفر، صفر، صفر و ٣٣,٣٣٪ من المجموعات التي تناولت علائق بها حبة البركة بتركيز ٠,٥، ١، ١,٥، ٢ و ٢,٥٪ والمجموعة الضابطة على التوالي.

وجد أن متوسط **العد الكلي للفطريات** في الأوراك هو (٤ × ١٠$^{٣}$ ± ٦ × ١٠$^{٣}$) و (١,٦٧ × ١٠$^{٣}$ ± ٣,٣٣ × ١٠$^{٣}$) في المجموعة الضابطة والمجموعة التي تناولت ٢,٥٪ حبة البركة في العليقة على التوالي وفي الصدور (٣,١٧ × ١٠$^{٣}$ ± ٣,٠٧ × ١٠$^{٣}$) و (٢,٧٥ × ١٠$^{٣}$ ± ٤,٧٩ × ١٠$^{٣}$) في المجموعة التي تناولت ٢,٥٪ و ١٪ حبة البركة على التوالي.

وجد أن متوسط **العد الكلي للخمائر** في الأوراك (٤ × ١٠$^{٣}$ ± ٩,١٣ × ١٠$^{٣}$) و (١ × ١٠$^{٣}$) و (٣,٦٧ × ١٠$^{٣}$ ± ٩,١٨ × ١٠$^{٣}$) في المجموعات التي تناولت عليقة بها ١، ٢,٥٪ حبة البركة والمجموعة الضابطة على التوالي. وفي الصدور كان متوسط

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العد الكلى للخمائر هو (٣,٨٣ × ١٠^٣ ± ٨,٣٣ × ١٠^٢) و (١,٤ × ١٠^٣ ± ٢,٤٥ × ١٠^٢) فى المجموعة الضابطة و المجموعة التى تناولت عليقة بها ٠,٥٪ حبة البركة.

**تم تصنيف بعض الفطريات و الخمائر** التى تم عزلهامن الأوراك و الصدور كالأسبرجلس نيجر و الميكور و الكلادوسبوريم و الكانديدا و التوريولوبسيس و كانت بأعلى نسب من الأوراك ٣٣,٣٣، ٦٦,٦٧، ٥٠، ٦٦,٦٧و ٥٠٪ على التوالى من المجموعة الضابطة و من الصدور كانت أعلى النسب هى ٦٦,٦٧، ٥٠، ٣٣,٣٣، ٥٠ و ٥٠٪ على التوالى من المجموعة الضابطة.

٥- **بالفحص الهستوباثولوجى**: وجد أن نسبة الألياف العضلية (myofilaments) تزداد على حساب الأنسجة الضامة (connective tissue) عند تركيز ٢٪ و ٢,٥٪.

هذا و قد تم مناقشة أهمية إضافة الحبة السوداء (حبة البركة) لعلائق الدواجن و إقتراح الطرق المثلى للإستفادة منها لتحسين جودة لحوم دجاج التسمين.

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## تحت إشـــراف

الأستاذ الدكتور/ محمد محمد موسى

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**رئيس قسم الرقابة الصحية على الأغذية**

**كليـة الطب البيطـرى**

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# تأثير النيجيلا ساتيفا (الحبة السوداء) على نوعية لحوم دجاج المائدة

جامعة الإسكندرية مكتبة كلية الطب البيطري

رسالة مقدمة من

ط.ب./ حسام عبد الجليل على إبراهيم

بكالوريوس العلوم الطبية البيطرية – كلية الطب البيطرى – جامعة الأسكندرية – ١٩٩٨

للحصول على

درجة الماجستير فى العلوم الطبية البيطرية

{الرقابة الصحية على اللحوم و منتجاتها}

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