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The Effect Of Garlic On Salmonella And Performance Of Broiler Meat

A Project Submitted to the College of Veterinary Medicine as Partial Fulfillments for the Requirement of The Bachelor Degree in Veterinary Medicine and Surgery

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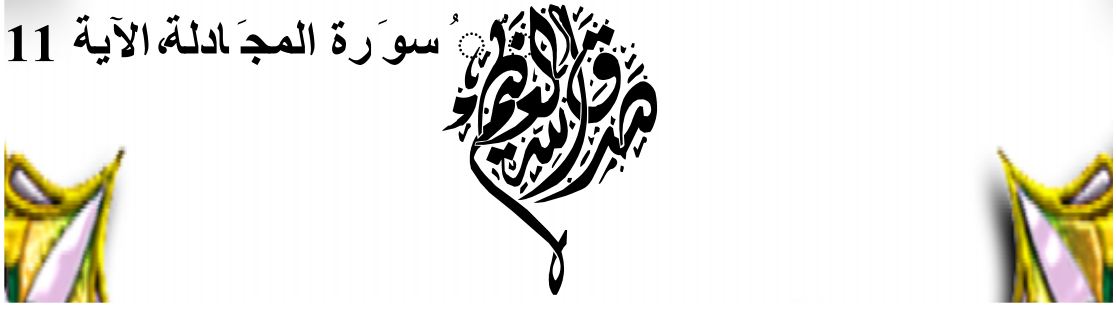
يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ
وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

سورة المجادلة الآية 11



Dedication

Dedicated with love to

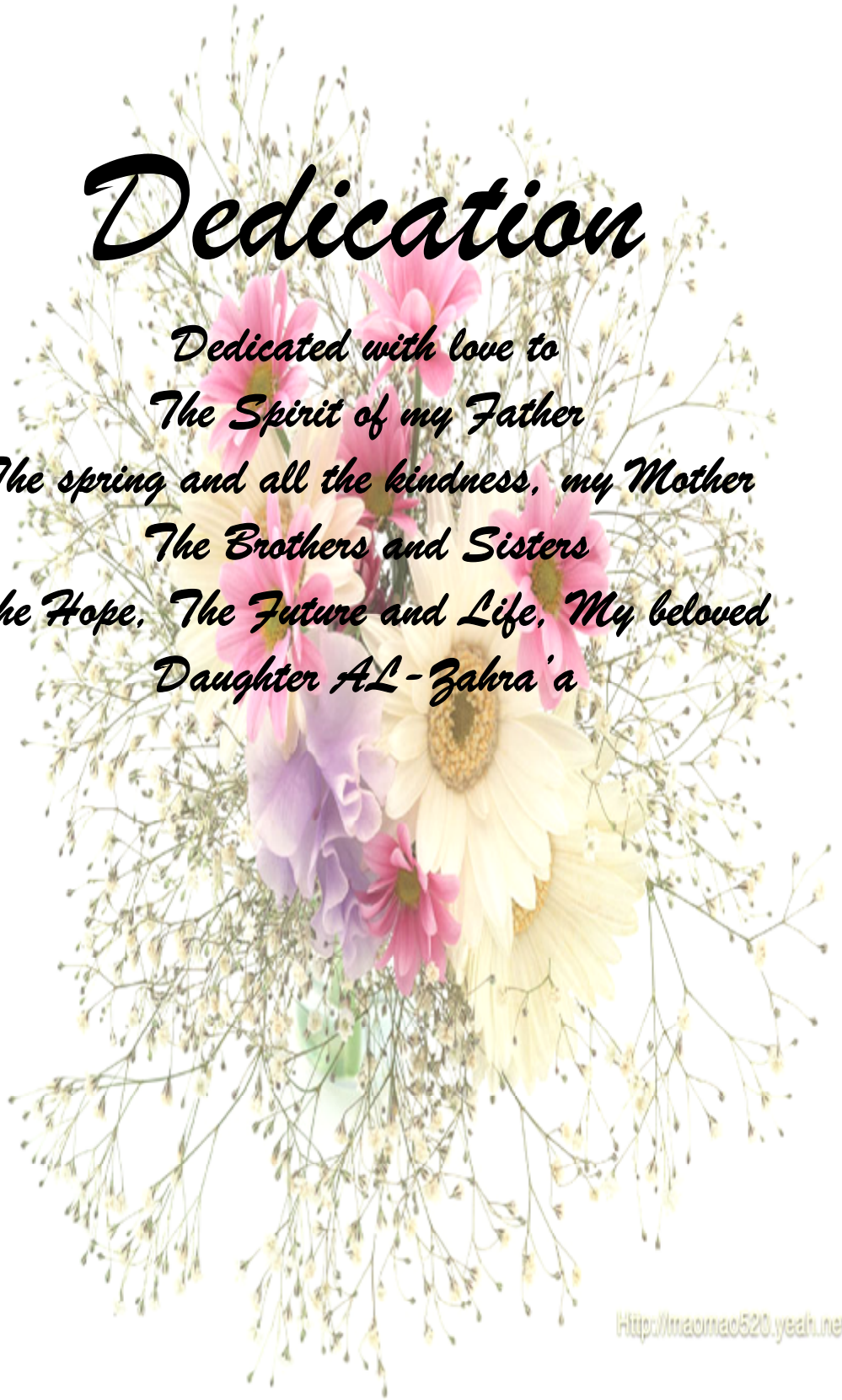
The Spirit of my Father

The spring and all the kindness, my Mother

The Brothers and Sisters

The Hope, The Future and Life, My beloved

Daughter AL-Zahra'a



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Summary

Garelic is one of the most common ingredients of several cuisines. No doubt, generation after generation has found garlic to be beneficial for several conditions, both as a preventive measure as well as a curative element.

This study has focused from review point of view on the effect of Garlic on Salmonella and performance of broiler meat. It has been found that Garlic had many benefits; one of them is antibacterial effect on Salmonella.

Salmonellosis is an infectious disease that can effect on broiler and their meat quality that can reflect a health problemes to human and economic loses. Feed additives are important materials that can improve the efficiency of feed utilization and animal performance.

However, the use of chemical products especially those of antibiotics and hormones may cause unfavorable effects. Many attempts in the field of animal nutrition are being done to achieve an increase in animal products and their products.

The possibility of using new natural alternative additives instead of antibiotics and hormones in animal diets is being recently used, some plants, containing various essential oils, have been used as alternative remedies by some workers. One of the most effective plant that can be used for such purposes is Garlic.

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Chapter One

Introduction:-

Broiler chicken meats, the most accepted white meat. The increased in domesticate populations used due to decreased of period which about 4-16 weak and lead to increase on the demand of chickens meat due to the ability of commercial form and hatchery to produce any numbers in any time of year.

The above-mentioned broilers and meat are the end products of the following genetic steps and is very important for human consumption. Broilers were reared for 35 days to reach a standard weight and quality of meat but infection with diseases like Salmonellosis will affect the weight and quality and cause heavy economic lose.

Salmonellosis is a major foodborne pathogenic bacterium and its important problem in poultry during the disease which causes and state poisoning which occur in human due to consumption the

meat and eggs contaminated by it. Its infected chickens and cause heavy mortality so that many antibiotics and plant additives or probiotics were used for prevention of the salmonellosis. In this study we focus on the use of feed additives Garlic. We investigate the direct effects of this plant on the salmonella and performance of broilers meats.

2.literature review:-

2.1. The History Of The Plant:-

Medicinal plants have been used for centuries and have become part of complementary medicine worldwide because of their potential health benefits (Gomez-Flores *et al*., 2008).

The medicinal value of these plants depends on bioactive phytochemical constituents that produce definite physiological action in the human and animal body, some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils and saponins (Krishnaiah*et al*., 2009).

Feed additives are important materials that can improve the efficiency of feed utilization and animal performance. However, the use of chemical products especially those of antibiotics and hormones may cause unfavourable effects. Many attempts in the field of animal nutrition are being done to achieve an increase in animal products and thereby profit (Abdou, 2001). The possibility of using new natural alternative additives instead of antibiotics and hormones in animal diets is being recently used, some plants, containing various essential oils, have been used as alternative remedies by some workers (Ceylan*et al*., 2003).

Recently, medicinal and aromatic plants have received much attention in several fields such as agroalimentary, perfumes, pharmaceutical industries and natural cosmetic products (Khorasaninejad*et al.,* 2010).

Several investigators reported that using medicinal and aromatic plants (MAP) in broiler diets improved body weight, body weight gain, feed conversion efficiency and reduce the cost of feed (Azouz, 2001;Tucker, 2002; Alcicek*et al*., 2004; Osman *et al*., 2004 and Abdel-Azeem, 2006).

Among Allium species Allium sativum L., Allium cepaL. and Allium porrum L. have beencultivated since ancient times as vegetables and characteristic pungent flavouring agents forfood but also for their pharmaceutical properties. The use of garlic, onion and leek is welldocumented by the Egyptians, Greeks and Romans. In the Middle ages ConstantinusAfricanus,Hildegard von Bingen, Albertus Magnus and many others mentioned the plants in theirmedicinal books [Sendl, 1995].

However, scientific investigations started only 200 years ago. One of the earliest studies wasperformed in 1844 by the German chemist Wertheim, who obtained some strongly

smellingsubstances named as allyl derivatives (from Allium) and sulphurlallyl derivatives [Wertheim,1844].

During the 19th and the 20th century scientific work on garlic extraction and identificationof many constituents has been performed. In recent years most investigations aimed atstandardisation of the active principles in pharmaceutical preparations. Further research work isstill needed to elucidate and quantify the active principles including pharmacokinetics and metabolism [Sendl, 1995].

Garlic used in Europe comes from Spain, Hungary, Czech Republic, Slovakia, Italy and France.Large amounts are cultivated in China, India, Egypt and Japan. In America the main producers ofgarlic are Mexico, Argentina and the United States, especially California.

It requires fertile, sandy, clay soil. The climate should be warm, sunny, not to windy or rainy. Garlic is vegetativelypropagated because of its sterility which precludes plant breeding in orderto obtain higher amounts of constituents [Sendl, 1995].

2.2. Description Of The Plants:-

Garlic is a bulbous perennial plant. A wild species of garlic is probably A. longicuspis REGEL,which grows wild in southwestern Asia. The bulb, which is commonly used for food flavouring,has 5-8 cm in diameter and is composed of several single bulblets (6-20) also known as cloves.The bulb varies slightly in shape, colour and flavour depending on variety and growingconditions. The foliage comprises a central stem up to 100 cm with erected flat or keeledleaves. The white, pink or purple flowers are arranged at the top of the stem.

2.3. Scientific Classification:-

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Amaryllidaceae
Subfami:	Allioideae
Genus:	*Allium*
Species:	*A. sativum*
Binomial name	
Allium sativum	

2.4. Chemical Structure Of The Garlic and Related Herbs:-

Garlic (Allium sativum L.) has been widely used as a foodstuffsince antiquity. It has acquired a reputation as a therapeutic agentand herbal remedy in many cultures to prevent and treat heart andmetabolic diseases, such as atherosclerosis, thrombosis, hypertension,dementia, cancer, and diabetes (Tyler 1993).Garlic and shallots (Allium ascalonicum) have antioxidant andfree radical-scavenging characteristics and identifiable odors atlow concentrations. They contain 2 main classes of antioxidantcompounds: flavonoids (flavones and quercetins)and sulfur-containing compounds (allyl-cysteine, diallyl sulfide,and allyltrisulfide).

The sulfur-containing amino acidderivative, alliin (S-allyl-L-cysteinsulfoxide), can be convertedinto allicin (diallyldisulfide-S-oxide), the compound commonlyassociated with garlic odor, by the enzyme alliinase. Thiosulfinates,such as allicin, give garlic its characteristic odor; however,they are not necessarily responsible for all of the various antioxidativeand health benefits attributed to it (Amagase 2006). Okadaand others (2005) have suggested that a combination of the allylgroup

(CH2CH=CH2) and the −S(O)S− group is necessaryfor the antioxidant action of thiosulfinates in garlic extracts. Sallylcysteine, S-allylmercaptocysteine, and nonsulfurcompounds, such as saponins, may contribute to the health benefits (hypolipidemic,antiplatelet, procirculatory, immune enhancement,anticancer, and chemopreventive activities) associated with garlic.

Gorinstein and others (2008) reported that trans-hydroxycinnamicacid (caffeic, p-coumaric, ferulic, and sinapic acids) concentrationsin garlic were twice that in onions.

The antioxidative effects of shallots are related primarily to theirphenol content (Leelarungrayub and others 2006). According toNuutila and others (2003), methanol extracts of onions have significantlyhigher radical-scavenging activities than garlic and redonion has higher activity than yellow onion. Quercetin content ishighest in red onions (Gorinstein and others 2008). The radicalscavengingactivities are positively correlated with the total phenolicsin these extracts.

2.5.Active ingredient of Garlic

Evidence of several investigation s suggests that the biological and medical function of Garlic mainly due to their high organusulphur compounds content (Amagas, 2006). The primary sulphur compounds constituents in this vegetable is the S-alk (en)yl-1-cysteinesulphoxides (ACSOS) such asalliin and y-glutamylcysteires which besides to serve as important storage peptides or biosynthetic intermediates for corresponding (ACSOS) from which and by different metabolic pathways in each

vegetable, volatile, such as allicin and lipid-soluble sulphar compound, such as diallulsulphide (DAS) diallyledisulphide (DADS) and other are originated (Lancaster and Shaw, 1989). These compounds provide to Garlic their characteristic odour and flavor as well as most of their biological properties (Lazotti, 2006). Non volatile water soluble sulphur compounds found in garlic as S-allylcusteine (SAC) (Amagas et al 2001) are also responsible for a great part of the health benefits of this vegetables. The biological effect of additional constituetes of intact garlic,such as lectins, prostaglandins, fructanpectinadenosine, Vit, B1, B2, B6 C and E, Biotin, nicotin C and D, fatty acid, glycolipids, phospholipids, and essential amino acids have been studied for over several decades (Lazotti, 2006). And important pharmacological activities and biological such as antifungal, antibacterial, antitumor, antiinfamatory, antithrombotic, and hypocholesterolemic properties of certain steroid, saponins and sapogenius such as B-chlorogenin (Lazotti, 2006)

2.6. Pharmaceutical Plants AndTheir Effects:-

Pharmaceutical plants have been commonly used byhumans long ago and the consumption rate has changedbased on the occasion and the requirement of the time.In recent years, Iranians and other people from aroundthe world have shown a tremendous interest in theseplants as a way to cure their illnesses. Today, there havebeen several attempts to develop these plants and thederivatives because the ever-increasing usage of thechemical drugs has led to serious problems and createdresistant types of insensitive micro-organisms, whileherbal drugs bring about fewer side effects because

thebiological balance. The herbal extracts are either usedalone as the drug itself or constitute a part of the drug.

Most essential oils consist of mixtures of compoundssuch as phenolics and polyphenols, terpenoides, saponins,quinines, esters, flavones, flavonoids, tannins,alkaloids and nonvolatile residues; and their chemical composition and concentration of compounds is variable.These components have many effects as antimicrobial,stimulating animal digestive systems, antioxidants, anticoccidial, increase production of digestive enzymesand improve utilization of digestive products by enhancingliver functions (Hernandez et al., 2009).

There have been some studies on the antifungalactivity of plant extracts (Wilson et al., 1997), inhibitoryeffects of aqueous extracts of garlic and onion (Shams etal., 2003), antimicrobial effects of garlic, ginger and lime(Onyeagba et al., 2004), antibacterial and antifungalactivity of Senecio (Loizze et al., 2004), antimicrobialactivity of garlic and onion extracts (Elnima et al., 1983)and effects of aromatic plants essential oils, lime and garlic skin on birds intestinal bacteria (Davis et al., 1994).Plant extracts represent a rich potential source ofalternative and environmentally acceptable control agentsfor infectious organisms due to their antimicrobial properties.Plants possess essential oils, which could be utilizedfor killing microorganisms.

Naturally occurring biologically active compounds fromplants are generally assumed to be more acceptable andless hazardous than synthetic compounds and representa rich source of potential disease-control agents.

Understandingof plant biochemistry, physiology and chemistryof natural products have shown that the secondarymetabolites may be used to control infectious organismsto overcome the earlier mentioned problems associated with synthetic chemicals (Delaquis and Mazza, 2008). Asa result, increased interest is being shown in developingalternative methods for microbial contamination control toreduce or eliminate reliance on synthetic pesticides. Oneof such method involves the use of plant-derived-productssuch as plant essential oils that has antimicrobialeffect.

Historically, plants have served as a useful resource for the development of novel drugs againsthuman and animal diseases. Plants produce a wide array of compounds, most of which as a defensemechanism against predation by pathogenic microorganisms and insects. Several plant compoundsform dietary constituents as well as active components in a number of herbal and traditionalmedicines. In recent years, the use of natural compounds has gained attention due to increasingconcerns over the safety of synthetic chemicals and emerging antibiotic resistance in
bacteria. The antimicrobial properties of several plant-derived essential oils have been previouslyreported, and a variety of active ingredients have been identified .

2.7.Salmonella SPP.:-

Salmonellosis is an infectious disease cauised by ant serotype of Salmonella and this divided into three groups:

A: host-adapted which includes:

1-Samonella pullorum causes white bacillary disease

2- Salmonella gallinarum causes fowl typhoid

B:in-vasive serotypes:

These infect intestine and migrates to visceral organs like liver, spleen, heart, kidneys or blood causing septicemia and this includes:

1-Salomonella typhimorium

2-Salmonella enteritidis

These serotypes cause acute infections with high mortality in birds and cause food poisoning to human through infected meat or eggs consumption and also during contacts with sick birds.

C: Non-invasive serotypes.

These serotypes infect intestines of birds and cause subacute infections bwith a different mortality. On the other hands, cause food poisoning to human via meat and eggs consumptions.

There are more than 200 serotypes in this group, most of them were isolated from poultry in Iraq like:

1-Salmonella hader

2-Salmonella Virchow

3-Salmonella Thompsopn

4-Salmonella Kedouqon

5-Salomonella London

6- Salmonella heidlberg

7-salmonella Newport

8-Slmonella Copenhagen

Etc…….until 200 or more serotypes.

Prevalence of Salmonella infection has increased markedly in both humans and domesticanimals. Probably as a consequence of the extensive use of antibiotics surveillance networkshave indicated that the incidence of human Salmonella food poisoning caused byantimicrobial resistant Salmonella is rising in many countries. In present, the anti-Salmonellaspp. properties of plant extract/essential oils from a variety of plant have been assessed.

It isclear from these studies that these secondary plant metabolites have potential as alternativeantibacterial in food conservation. The phenolic compounds are most active and appear toact principally as membrane permeabilisers. In addition, consumers are also demand forfood preservation from natural source. Therefore, the incorporating plant extracts in or ontofood packaging materials to against foodborne pathogen, especially Salmonella spp., is ofincreasing interest.

Salmonella spp., facultatively anaerobic gram-negative rod-shaped bacteria (Krieg & Holt,1984), is one of the most important food borne pathogens. If present in food, the bacteria donot affect the taste, smell or appearance of the food. Frequent hand washing, throwing outexpired food, avoid eating raw or undercooked eggs, meats, seafood or poultry are the key to preventing Salmonella food poisoning. Antibiotics (such as ampicillin,

chloramphenicol,streptomycin, sulfonamides and tetracycline) may be prescribed for moderate to severe casesof Salmonella food poisoning or when it occurs in a person who is at risk for complications.

At 2% level than those containing garlic and rosemary extracts ($P < 0.05$).Incorporation of garlic oil up to 0.4% v/v in alginate film, the clear zone of inhibition wasnot observed with S. typhimurium. However, incorporation of garlic oil at higher than 0.1%v/v revealed a weak inhibitory effect, indicated by minimal growth underneath film discs(Pranoto et al., 2005).

2.8.MechanismsofGarlic (Allium sativum) As Antibacterial Agent:-

Feed supplements with growth promoting activityincrease stability of feed and beneficially influence thegastrointestinal ecosystem mostly through growth inhibitionof pathogenic microorganism's growth. Due to improvedhealth status of digestive system, animals are lessexposed to the toxins of microbiological origin. Consequentlyherbs and spices help to increase the resistance ofthe animals exposed to different stress situations and increasethe absorption of essential nutrients, thus improvingthe growth of the animals (Windisch et al., 2008).

Numerous secondary metabolites formed by plantsserve as defence agents against physiological and environmentalstressors, predators and pathogenic microorganisms.Several in vitro studies showed strong antimicrobialactivity of certain plant extracts against Gram− andGram+ bacteria. Pasqa et al.

(2006) found a change inlong chain fatty acid profile in the membranes of E. coligrown in the presence of limonene or cinnamaldehyde.Similar observations were made with Salomonellaentericegrown in the presence of carvacrol or eugenol andwith Bronchotrixthermosphacta grown in the presence ofeither limonene, cinnamaldehyde, carvacrol or eugenol.In the case of Pseudomonas fluorescens in Staphylococcusaureus none of the tested phytochemicals changed thefatty acid profile. The changes in fatty acid compositioncan affect surviving ability of microorganisms.

Garlic (Allium sativum) has traditionaldietary and medicinal applications as ananti-infective agent (Ross et al., 2001).Distributed and used in all parts of theworld as a spice and herbal medicine forthe prevention and treatment of a variety ofdiseases, ranging from infections to heartdiseases (Rivlin, 2001).

Garlic is thoughtto have various pharmacologic propertiesand medical applications. It is mainlyconsumed as a condiment in variousprepared food (Amagase et al., 2001).Garlic is a strong antibacterial agentand acts as an inhibitor on both Grampositiveand Gram-negative bacteriaincluding such species as Escherichia,Salmonella, Streptococcus mutans,Porphyromonasgingivalis,Staphylococcus, Klebsiella, Proteus andHelicobacter pylori (Ankri and Mirelman,1999; Bakri and Douglas, 2005, Reuter etal., 1996).

The main antimicrobial constituent ofgarlic has been identified as the oxygenatedsulphur compound, thio-2-propene-1-sulfinic acid S-allyl ester, whichis usually referred to as allicin. Allicin isproduced catalytically when garlic clovesare crushed and the enzyme allinase (alliinlyase E.C. 4.4.1.4) of

the bundle sheathcells mixes with its substrate, alliin, whichis released from mesophyll cells (Miron etal., 2000; Curtis et al., 2004).

Allicin is one of the principals offreshly crushed garlic homogenates, is avolatile molecule that is poorly miscible inaqueous solutions liquid, responsible forthe pungent smell of garlic and ischemically an unstable and highly reactivemolecule. Allicin is a short lived molecules, this rather unstable compoundhas been suggested by Lawson and coworkersto transform rapidly intosecondary products (In vivo) such as allyl-mercaptan and others (Koch and Lawson1996; Lawson and wang, 1993).

The anti-microbial effects is due tothe chemical reaction of the allicine withthe thio groups of several enzymes such asARN Polymerase, by delaying andinhibiting DNA, RNA and proteinsynthesis (Ankri and Mirelman, 1999,Feldberg et al., 1988)

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The search for new antibacterialagents associated with specific plant families should be continued, and recentfocus has shifted to determining the antimicrobial activity of plant extracts usedin folk medicine (Rios and Recio, 2005).The screening of plant extracts and plantproducts for antimicrobial activity hasshown that higher plants are a potentialsource of novel antibiotic substitutes (Riosand Recio, 2005).

Although much has been reported onthe medicinal properties of garlic andallicin (Ali et al., 2000; Ankri andMirelman, 1999; Singh et al., 1998), notmuch is known about its proteinaceousconstituents (Terras et al., 1993; VanDamme et al., 1993). Lixin and Ng (2005)has isolated an antifungal

protein fromgarlic, designated alliumin, with amolecular mass of 13 kDa. Alliuminpresents antifungal activity againstMycospharellaarachidicola, inhibitoryactivity to the bacterium Pseudomonasfluorescences and exerted antiproliferativeactivity toward leukemia L1210 cells(Lixin and Ng, 2005).

2.9.Various Garlic Preparations:-

Various garlic preparations have beenshown to exhibit a wide spectrum ofantibacterial activity against Gramnegativeand Gram-positive bacteriaincluding such species as Esherichia,Salmonella, Streptococcus,Staphylococcus, Klebsiella, Proteus, andHelicobacter pylori (Ankri and Mirelman,1999; Small et al., 1947). Even acid-fastbacteria such us Mycobacteriumtuberculosis are sensitive to garlic (Uchidaet al., 1975).

Salmonella serovars was chosen asthe Gram-negative model organism, as it isone of the major human pathogens andfood poisoning cases (Reed, 1993). It'swell demonstrated the Salmonella candisseminate and survive in variousenvironmental niches for a long periods oftime. They are pervasive in nature andmany contaminate animals, vegetables,water and especially food during itsproduction and distribution (Davies andWray, 1996).

The growth of aerobic mesophilic bacteria on chicken carcasses cooled in cooling water containing selected concentrations of garlic extract . The initial assays for aerobic mesophiles provided nosignificant differences in microbial growth among the selected garlic extractconcentrations in the cooling water.

2.10.SalmonellaSP. In Chicken Feed AndOn Poultry Carcasses:-

Garlic extract did not effectively inhibit the growth ofSalmonellasp. Thelack of detectable numbers ofSalmonellasp. on chicken carcasses immersedin cooling water containing 15% garlic extract does not assure the absence ofcontaminatingSalmonellasp. The observed ineffectiveness of garlic extractto inhibit the growth ofSalmonellasp. in this experiment are contradictory tothe results reported by Kumar and Berwal (1998) that concentrations of 10%garlic extract in "in vitro" experiments were sufficient to inhibitSalmonellagrowth. The decrease of the inhibiting effect of garlic extract onSalmonellasp. growth may be related to adaptation of Salmonellasp. to the presence ofthe specific inhibiting activity of the garlic extract.Zaika and Kissinger (1981)reported that lactic cultures developed adaptation mechanisms against theinhibitory effects of selected spices.

In conclusion, the results presented suggest that there are disinfectantbenefits to adding garlic extract to the cooling tank water in poultry processingfacilities to reduce the bacterial load in the cooling water and inhibit crosscontaminationwithin the poultry processing facility, both promoting andextending a longer and safer refrigerated shelf life for chicken carcasses.

3-Recommendations

1- Preparation of suitable houses for rearing of chickens in the college.
2- Offering laboratory facilities for isolation and identification of Salmonella.
3- Offering machines and suitable clean room for mixing of Garlic in feed.
4- Offering the project loan and cost for experimrents for the researchers and student projects.

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الخـــــــلاصـــــــة

تبين من المعلومات المدونة عن الثوم بأنه يحتوي على مكونات مهمة لها دور كبير في التأثير على صحة الطيور وزيادة استهلاك العلف والتقليل من عدد البكتريا المرضية وخاصـــــة السالمونيللا وتبين ايضا ان السالمونيللا بانماطها المصلية التي تقارب200نمط مصـلي بالدواجن لها تأثير كـبير على صـحـة الطيور وارتفاع الهلاكات ومنها مايسبب التسمم الغذائي للانسان مثل s.enteritis.salmonella typhimurium وانماط اخرى وتبين ان فروج اللحم الذي يجهز الانسان باللحوم البيضاء والجيدة وسريعة النمو والتحويل الغذائي يحتاج الى اضافة اجراءات وقائية مختلفة ضد الامراض ومنها مرض السالمونيللا من خـلال التأثير المبـــــاشر على الاصابة بهذه البكتريا لذالك نوصي بوضع الثوم بالعلف بنســـب مقبولة لاتؤثر على نوعية اللحوم وتفي بالغرض.