The effect of the *Capsicum annuum* in the diet of broilers on the isolation and shedding rate of *Salmonella paratyphoid*

Alaa Abdul Aziz

Al-Qadssiya University ,Vet. Med. Coll.

Abstract:

Capsicum annuum(Capsicum pepper) is widely consumed as a fresh vegetable(appetizer) by human community, although its extremely high capsaicin content has led to other uses such as medicinal agent for many case of humen , recently described antimicrobial peptides from *C. annuum* were very efficient in inhibiting growth in human and plant pathogenic bacteria and fungi.

At this research, in order to explore the inhibitory effect of the *C.annuum*, as a possible alternative to antibiotics against the challenge dose of *Salmmonella typhimurium* in broiler chickens were studied.

The results showed that the use of mixed diet with *C. annuum* at percent 1% and 2% ,were effective against *Salmonella typhimurium* infection through decreasing fecal shedding , isolation rate, bacterial count of *Salmonella typhimurium* and mortality rate also it cause elevation of serum total protein and decreasing total serum cholesterol level .

تأثير الفلفلِ annuum في حميةِ broilers على العزلةِ ويُريقُ نسبةَ بارا تيفوئيدِ السالمونيلا.

علاء عبد العزيز

كلية الطب البيطري- جامعة القادسية

الخلاصة:

يعتبر الفلفل الحار من النباتات التي تستهلك بصورة شائعة من قبل المجتمعات الإنسانية كأحد المقبلات الغذائية وكذلك كمادة علاجية للعديد من الحالات المرضية في الإنسان،حيث تم في السنوات الأخيرة اكتشاف ووصف العديد من المركبات و الببتايدات المعزولة من الفلفل التي لها تأثير تثبيطي ضد العديد من الجراثيم المرضية والفطريات في الإنسان وكذا النبات.

ولغرض معرفة الكفاءة التثبيطية أو القاتلة لاستخدام هذا النبات ضد الجراثيم المرضية كبديل محتمل عن استخدام المضادات الحياتية،تم في هذا البحث دراسة تأثير الفلفل الحار ضد جرعة التحدي لجراثيم السالمونيلا تايفيميوريم في أفراخ اللحم باستخدامه على شكل مسحوق جاف مخلوط مع العلف.

بينت النتائج إن استخدام مسحوق الفلفل الحار مع العليقة بالنسب 1% و2% أدى إلى تقليل نسب طرح السالمونيلا ونسب الإصابة وكذلك العد الجرثومي بالإضافة إلى انخفاض نسب الهلاكات، وبينت النتائج إن للفلفل الحار تأثير على مستوى البروتين الكلي في مصل الدم ومستوى الكولسترول حيث رفع الأول وخفض الثاني.

Introduction:

Avian salmonellosis , is responseble for heavy economic losses through morbidity, mortality, treatment, antibiotic resistance and contamination of meat و eggs and environment to the worldwide poultry industry (1, 2, 3, 4).

Salmonella infections in commercial poultry have long been an industry concern. the approach taken to *Salmonella* control depends on the type of infection (5), *Salmonella* needs special concern in the 3rd developing world because of the poor hygienic conditions that favours its spread and also need a good practical measures to control.

Antibiotic in agricultural animal production has been practiced for about 50 yr in the many developed country, evidence exists that antibiotic resistance genes can be and are transmitted from animal to human microbiota (6), monitoring and identifying resistance mechanisms and their dissemination into the food chain were recently reviewed by (7),pathogenic bacteria resistant to a number of antimicrobial agents emerged worldwide in the 1980s (8), as these were detected, several reports were published recommending a ban on antimicrobial use in food as precautionary measure.

The use of alternative medications to control salmonellosis is important in light of emergence of multiple drug-resistant strains of *Salmonellas* and the public's desire to have organically grown poultry ,and because of the recovery frequency of *Salmonella* from chickens fed antibiotics such as virginiamycin, bacitracin, nitrovin, tylosin, or flavomycin was similar or greater than that from chickens that did not receive antibiotics.(9 **).**

Plants have developed a number of defense mechanisms against pathogen attack (10), among these is the innate response, an ancient mechanism that provides relatively rapid host protection with low energy and biomass expenditure, this response is often afforded by small proteins with antimicrobial properties and broad bioactivity spectrums, (11, 12).

Capsicum pepper refers primarily to *C. annuum* and *C. frutescens*, plants used in the manufacture of selected commercial products known for their pungency and color, *C. annuum* is a herbaceous annual that reaches a height of one meter and has glabrous or pubescent lanceolate leaves, white flowers, and fruit that vary in length, color, and pungency (depending upon the cultivar), native to America, this plant is cultivated almost exclusively in Europe and the United States, *Capsicum* species are used as fresh or dried, whole or ground, the level of pungency of the *Capsicum* species depends upon the concentration of capsaicinoids, primarily of capsaicin, it's level in with 30 to 600 parts per million, or with 600 to 13,000 parts per million depending on the type of species (13).Capsicum peppers are important food ingredients, but also clearly have nutritional and medicinal properties, the ancient Maya recognized these properties and used them therapeutically, ethnobotanical

data suggest that the *Capsicum* species in particular harbor many potentially economically significant compounds yet to be discovered (14),it remains unclear exactly which properties led the ancient Maya to include *Capsicum* species in their pharmacopoeia and keeps them in use by traditional cultures (15), but it may have been in response to their therapeutic properties as antimicrobial and anti-hemolytic agents, the Capsicum continues to be a source of vitality and health in numerous countries including the Bahamas and Costa Rica, where it is used to overcome colic or indigestion, in Africa for vascular disorders and by North Americans who use it as a tonic and natural stimulant and Chinese physicians utilized it for physiologic conditions that needed stimulation (16) .

It's active compounds are alkaloids (capsaicin, 6,7-dihydrocapsaicin, homocapsaicin,homodihydrocapsain, and nordihydrocapsaicin), fatty acids volatile oil, rutin(flavonoids), high flavonoid content makes it a good antioxidant(17,18).),it is also high in carotenoids, (provitamin,A) mostly (capsanthin pigments also capsorubin, carotene, and lutein) , which significantly contributes for vital normal vision, cellular activity, growth, the red color of *Capsicum* is due in part to its very high content of vitamin A , and rich in Vitamin C which it has a tonic effect with Vitamin A on the immune system, making the body less vulnerable to microorganism invaders, also contain B-complex vitamins,B1, B2, B3, B5, B6, B9, folic acid and Zinc, , iron, calcium, potassium, magnesium, cobalt, phosphorus, sulphur, , sodium, selenium, fats(9-17%) and proteins(12-15%) (16,19, 20), Capsicum also contain phenyl-propanoids (L-phenylalanine, t-cinnamic acid, o-coumaric acid, mcoumaric acid, ferulic acid, and caffeic acid) which are intermediates of the capsaicinoids pathway (21).

The presence of the capsaicin in these species has long been associated with strong analgesic properties (19), alterations in the pH of gastrointestinal tract epithelial cells, prevention of microbial infection (22), and possible anti-carcinogenic effects in human (23),recent study, however, has shown that pepper species also contain peptides with strong antimicrobial activity and that these peptides are encoded in the pepper genome (24, 25).

It has been shown to significantly lower both blood cholesterol and triglycerides, but even more important, lower the LDL-HDL ratio, the mechanism for this is not understood blood serum cholesterol levels was significantly inhibited., (26, 27).

The aim of study is to explore the role of *Capsicum annuum* as antimicrobial agent which can be contribute with other control methods for *Salmonella* infection or contamination.

Materials and Methods:

Feeding trial

Broilers (Ross) (n=375), were obtained from local market, on the day-of hatch were randomly

assigned to tow treatment diets groups, and control group of basal diet of 125 birds each group, vaccinated as ordinary schedule, with ND,IB and IBD vaccines, birds were given diet to end of experiment, the administration of treatments begin at age 1day old, 23 hours light and 1 hr. darkness, each group was fed *ad libitum* its own diet for a period of experiment ,the composition of the basal diet is presented in Table 1.meeting NRC requirements (28) *C.annuum* (fresh pepper) were purchased from local market , left to dry and then were grinded.

Group A - No added Basal diet (Control).

Group B - Basal diet plus (1%) *C.annuum* dry powder

Group C –Basal diet plus (2%)of *C.annuum* dry powder

Biochemistry:

At the experimental period some birds from each treatment group were selected to determine some blood chemistry parameters, assay of serum biochemistry randomly analytical procedures, blood samples were centrifuged at 2500/5 min and serum samples were stored in a deep freezer for further analysis serum samples were analyzed for serum total protein, total cholesterol, glucose by spectrophotometer (PD-303, APEL , Japan) with enzymatic kits for each parameter which include the following kits :

Total protein kit, (Cambridge), with 546 nm wave length.

Cholesterol SL kit ,(Giesse Diagnostic Snc, Italy), with 510nm wave length.

Cholesterol SL kit , (Giesse Diagnostic Snc, Italy), with 510nm wave length.

Glucose SL kit, Giesse Diagnostic Snc, Italy,with 500nm wave length. .

Bacteriology:

The orally challenge dose at age of 3 days old were 4 $\times 10^4$cfu/ml,the procedure was according to (29)

The shedding rate which represents by positive samples of *Salmonella* were achieved by cotton swab inserted to the cloaca of birds then transferred to the selenite broth then incubated for 18-24 hr.40C^O then cultured on selective media BG agar and BSagar ,24hr.at 37C^o.

The isolation rate and *Salmonella* counts (most probable number, MPN-Log$_{10}$) has been done by collecting ceacal contents from randomly selected birds weekly, were achieved after birds slaughtering, ceacal contents samples were taken immediately and serially diluted with 1:10 deionize water followed by culture analyses. during counting procedure, selenite broth and tow agars were used, BG agar and BS agar,TSI agar and LI agar for more confirmation and other ordinary lab. biochemicals.

Statistical Analysis

The data obtained in the experiment were analyzed by ANOVA test, t-test for bacterial count and chai square for comparing the shedding and isolation rate and treatment means were separated

using Duncan s New Multiple Range Test.(30)

TABLE-1 Percentage of the different ingredients in the diets

Ingredients	Diet 1starter	Diet 2.grower
Corn	359	400
Soybean Meal	250	210
Protein	100	100
Wheat	270	270
Vitamin Premix	4	4
Lysine	1	0.75
Methionine	0.5	0.40
Dicalcium Phosphate	0.5	5
Limestone	9.5	8
Salt	2	2

Met.Energy	2910 kcal/kg*	2986 kcal/kg**
Crude protein	% 21.6	%20.3
Fat	% 2.49	%2.63
Fiber	% 3.13	%3
Ca	% 1.38	%1.31
P	%0.66	%0. 66
Methionine	% 0.70	%0.71
Lysine	% 1.4	%1.23
Na	% 0.27	%0.27
Choline	1400mg/kg	1400mg/kg
Potassium	% 0.93	%0.85
Met. energy/ Protein	135	147

Chemical analysis

*Diet 1.starter

**Diet 2.grower

Table.2 . Represents the *Salmonella* fecal shedding rate

Group	1st week	2nd week	3rd week	4th week	5th week	Total& %
A	18/20	14/20	8/20	6/20	3/10	49/90 [a] 54.5%
B	16/20	14/20	5/20	4/20	2/10	41/90 [a] 45.5%
C	12/20	5/20	6/20	1/20	3/10	27/90 [b] 30%

A. (control group), B. (1% treated group), C. (2% treated group) , results with in one column indicated by different superscripts differ (P>"0.05).

Table.3.Represents the *Salmonella* isolation rate of cecal content and *Salmonella* count

Group	1st week	2nd week	3rd week	4th week	Total & %	5th week
						MPNlog_{10}/gm of cecal content
A	5/5	7/10	3/5	1/5	16/25 [a] 64%	2.5 [a]*
B	5/5	4/10	2/5	1/5	12/25 [a] 48%	1.9 [b]**
C	3/5	3/10	2/5	0/5	8/25 [b] 32%	1.5 [b]***

A. (control group), B. (1% treated group), C. (1% treated group) , results with in one column indicated by different superscripts*=(0.35×10^3cfu) **=(0.85×10^2cfu) *** =(0.35×10^2cfu)

Table.4. Represents the chickens mortality rate

Group	1st week	2nd week	3rd week	4th week	5th week	Total &%
A	17/125	5/108	3/103	2/100	1/98 (97*)	28/125 [a] 22.4%
B	15/125	4/110	2/106	1/104	0/103 (103*)	22/125 [a] 17.6%
C	9/125	2/116	0/114	2/114	1/112 (111*)	14/125 [b] 11.2%

A.(control group), B. (1% treated group), C. (2% treated group) , results with in one column indicated by different superscripts.*: remaining live birds

Table.5. Represents the serum biochemistry mean values at 38days old

Group	Total protein g/dl	Total cholesterol Mg/dl	Glucose Mg/dl
A	12.82 [a] ±0.38	250.97 [a] ±9.99	93.79 [a] ±7.4
B	14.55 [b] ±1.12	190.93[b]±4.71	89.78 [a] ±4.6
C	14.96 [b]±1.30	189.87 [b] ±8.10	88.67 [a] ±4.4

A.(control group), B. (1% treated group), C. (2% treated group) , results with in one column indicated by different superscripts differ (P>"0.05).

Results and Discussion:

The antimicrobial effect of the many medicinal plants is well documented (31),the results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (32).

The results showed in table 2 and 3, that the *Salmonella typhimurium* shedding rate was numerically differed at group B ,and decreased significantly for group C comparing to control, isolation rate numerically differed with in group B and significantly with in group C

comparing to control and there is a decreased in bacterial counting of *Salmonella* of both treated groups with significant difference comparing to control , these results agreed with the results obtained by McElroy and co-workers(1994) (34),who used *Salmonella enteritidis*, suggest that capsaicin administration increases resistance to *S. enteritidis* colonization and organ invasion without detrimental effects on growth in broiler chickens, and this results confirmed the observation of earlier studies in herbal medicine (14,33),it had been suggested that *Capsicum* species are used as antimicrobials because of the capsaicin and dihydrocapsaicin contents (14),and that some specific *Capsicum* phenylpropanoids had a bacteriostatic activity, such as the t-cinnamic, o-coumaric, m-coumaric, ferulic acids, or bactericidal activity such as caffeic acid (21), our results also agreed with the results obtained by Acero-Ortega and co-workers (2005) (21),they confirmed that *L. monocytogenes* is inhibited by pepper extracts,also Capsicum extract inhibited *Salmonella typhimurium* and *Pseudomonas aeruginosa* growth of contaminated meat and at high dose the extract showed a bactericidal effect on *Pseudomonas aeruginosa* (33),for this we can presume that the Capsicum mixed with feed may have the ability to reduce *Salmonella* and other bacteria contaminated feed, also the extracts of Capsicum pepper were found to exhibit varying degrees of inhibition against *Bacillus cereus, Bacillus subtilis, Clostridium sporogenes, Clostridium tetani, and Streptococcus pyogenes*.(14).

So from the results that we obtained and other researchers results showed that the Capsicum pepper have a broad spectrum effect on pathogenic bacteria.

The table 4. showed that the chickens mortality rate was numerically differed at group B and significantly differed with group C, comparing to control this may refers to a good health status of the body (no adverse effect on body weight or feed efficiency were observed after dietary administration of 1% or 2% of *C. annuum* in this study) by decreasing the pathogenic bacterial load or may be due to the augment of the immunity ,and the effect of antioxidant action of *Capsicum* (due to high flavonoid contents) that may interfere with oxygen radical transfer mechanism common to lipoxygenase and cyclooxygenase pathways ,this in turn may have beneficial effect on the general health status and decreasing mortality rate.

The positive results in our study seems to be correlated with high dose and long duration of pre-challenge treatment and post challenge treatment with *C. annuum*, and the challenge dose in relation to reduction of fecal shedding ,isolation rate and in mortality caused by *S. typhimurium.*

As shown in table 5. the capsicum increase the level of total protein in blood serum with significance difference between the treated groups and control this effect may be due to the enhancement of digestion of food by changing the PH and may

be increasing acids production , serum total proteins are a significant indicator of the health condition and production features of the body because of their numerous roles in the physiology , the level of total cholesterol is lowered ,there is a significant difference between treated groups and control, so we can say that capsicum is blood cholesterol reducer, but there is no significant difference in the level of blood glucose between treated groups and control.

In conclusion, our study showed a good antibacterial activity of *C. annuum* against *S. typhimurium*, the results both confirm and highlight the antimicrobial effects of *C. annuum* which may have a future pharmaceutical and agricultural applications ,we can suggest that the using of *C. annuum* which considers a method to contributes with other control methods or even as an alternative medicine to control avian *Salmonellas*, the *C. annuum* may conceders as environmentally friendly alternative medicine, and may have potential economic advantages over commercial synthetic agrochemicals and medicines since its preparing as solution extract or dry powder is far less expensive than the chemical synthesis usually required to manufacture artificial compounds and,.as well as the consumer can get a good healthy chickens with less contaminated chickens and relatively low level of cholesterol .

References:

1-Lee,Y.J.; K.S Kim,.; Y.K. Know,. and R.B. Tak,.2003.Biochemical characteristic and antimicrobial susceptibility of *Salmonella gallinarum* isolated in Korea. Korea J.Vet. Sci.4:161-166.

2-Glosnicka, R. & D.Kunikowska, 1994. The epidemiological situation of *Salmonella enteritidis* in Poland. Int. J. Food Microbiol., 21:21-30.

3-Coburn, B.G.; A. Grassl, & B.B. Finlay,. 2007. *Salmonella*, the host & disease: Abrief review. Immunol. Cell Biol., 85:112-118.

4-CDC, 2006. *Salmonella* annual summary, 2005. Centers for disease control and prevention. www.cdc.gov

5-Breytenbach J. H.,2004. *Salmonella* Control in Poultry Intervet International b.v. Wim de K.rverstraat 35, 5830AA, Boxmeer, The Netherlands.

6-Greko, C. 2001. Safety aspects on non-use of antimicrobials as growth promoters. Pages 219–230 in Gut Environment of Pigs. A. Piva, K. E. Bach Knudsen and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.

7- Roe, M. T., and S. D. Pillai. 2003. Monitoring and identify- ing antibiotic resistance mechan- isms in bacteria. Poult. Sci.82:622–626.

8-Aarestrup, F. M. 2003. Effects of termination of AGP use on anti-

microbial resistance in food animals. Pages 6–11 inWorking papers for the WHO international review panels evaluation, Document WHO/CDS/ CPE/ZFK/2003.1a. World Health Organization, Geneva, Switzerland.

9-Anonymous ,2000. Effect of the use of antimicrobials in food-producing animals on pathogen load: Systematic review of the published literature Prepared for Center for Veterinary Medicine US Food and Drug Administration Rockville, MD

10-Lay, F. T. and M. A. Anderson, 2005.Defensins components of the innate system in plants.Curr. Protien Peptide Sci.,6:85-101.

11-Hancock, R.E.W. and R.I.Lehrer ,1998. Cationic peptide: A new source of antibiotics. Trends Biotechnol.,16: 82-88.

12-Zasloff,M.,2002.Antimicrobial peptides of multi-cellular organisms .Nature,415:389-395 .

13-Simon, J.E., A.F. Chadwick and L.E. Craker. 1984. Herbs: An Indexed Bibliography. 1971-1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books, 770 pp., Hamden, CT.

14-Cichewicz R.H.; P.A. Thorpe .,1996.The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. J Ethnopharmacol. 1996 Jun;52(2):61-70

15-Alcron,J.B.1984.Huastic Mayan Ethnobotany.1st ed., Austin, University of Texas Press, Austin ,ISBN: 0292715439,pp:982.

16-Anonymous[a] ,1996.*Capsicum* Therapeutic Powerhouse and Herbal Catalyst, a book of Woodland Publishing, Inc. P.O. Box 160 Pleasant Grove, UT 84062

17- Lee,Y.; L.R. Howard, and B. Villalon, 1995.Flavonoids and antioxidant activity of fresh pepper (Capsicum annuum) cultivars. Journal of Food Science. May: 60 (3): 473-476.

18- Howard,L.R.; R. T. Smith; A. B. Wagner; B.Villalon; E. E.Burns,. 1994. "Provitamin A and ascorbic acid content of fresh peppercultivars (Capsicum annuum) and processed jalapeños." Journal of Food Science., 59: (2): 362-365.

19- Cordell, G.A. and O.E. Araujo, 1993. Capsaicin: Identification, nomenclature, and pharmaco-therapy." Ann. Pharmacother. 27: 330-336.

20-Anonymus[b], 2005. Capsicum information, drug interaction, uses and benefits. http:/www.Ayurvedic-Medicines.org.

21- Acero-Ortega**,** C. L. Dorantes-Alvarez**,** H. Hernández-Sánchez, G. Gutiérrez-López**,** G. Aparicio**,** M. E. Jaramillo-Flores, 2005. Evaluation of Phenylpropanoids in Ten *Capsicum annuum* L. Varieties and Their Inhibitory Effects on Listeria

monocytogenes Murray, Webb and Swann Scott A. Food Science and Technology International, Vol. 11, No. 1, 5-10

22-Tellez,G.I.Jaeger,C.E. Dean, D. E. Corrier and J. R. Deloach,1993 . Effect of prolonged administration of dietary capsacin on *Salmonella* enteritis infection in leghorn chicks. Avian Dis.,37:143-148.

23-Surh, Y.J. S.S.Lee,1995. Capsaicin, a double-edged sword: Toxicity, metabolism and chemo-preventive potential . Life Sci.,56: 1845-1855.

24-Texeira, F.R.,M.C.O.P.Lima, H.O.Almeida, R.S.Romeiro and D.J.H. Silva,2006 Bioprospection of cationic antimicrobial peptides from bell pepper leaves for inhibition of Ralstonia solanacearum and Clavibacter michiganesis ssp. Michiganesis growth. J. Phyto-pathol.,154:418-421.

25-Diz M.S.S.,A.O.Ccarvalho , R.Rodriguez , A.G.C.Neves-Ferreira M.Da Cunha,2006. Antimicrobial peptides from chili pepper seeds causes yeast plasma membrane permeabilization and inhibits the acidification of the medium by yeast cells. Biochem. Biophys. Acta, 1760:1323-1332.

26- Sambaiah ,K. and N. Satyanarayana. 1980.Hypocholester-olemic effect of red pepper and capsaicin." Indian Journal of Experimental Biology. 18: 898-899

27- Visudhiphan,S., S, Poolsuppasit, O. Pibolnukarintr, 1982. The relationship between high fibrinolytic activity and daily capsicum ingestion in Thais. *Am. J. Clin. Nutr*.35: 1452-1458.

28- -National Research Council, 1994. Nutrient Requirements of Poultry. (9th rev. ed.). National Research Council. National Academy Press. Washington, D.C., USA.

29- Pivnick, H.; B. Blanchfield,; J. Y. D'aoust,. 1981.Prevention of *Salmonella* infection in chicks by treatment with fecal culture from mature chickens (Nurmi culture) . J. Food Prot. 44: 909 – 916.

30- Duncan, D B.; Multiple range and multiple. Biometrics, 1955.

31-Valero, M., and M. C. Salmeron. 2003. Antibacterial activity of 11 essential oils against*Bacillus cereus* in tyndallized carrot broth. Int. J. Food Microbiol. 85:73-81

32- Kone, W. M., K. K. Atindehou, C. Terreaux, K. Hostettmann, D. Traore, and M. Dosso., 2004. Traditional medicine in North Cote-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol. 93:43-49.

33-Careaga, Mónica M; Fernández, Elizabeth E; Dorantes, Lidia L; Mota, Lydia L; Jaramillo, Maria Eugenia ME; Hernandez-Sanchez, Humberto H., 2003. Antibacterial activity of Capsicum extract against *Salmonella typhimurium* and

Pseudomonas aeruginosa inoculated in raw beef meat. Int J Food Microbiol; vol 83 (issue 3) : pp 331-335.

34-McElroy, A. P., J. G. Manning, L. A. Jaeger, M. Taub, J. D. Williams, B. M. Hargis. 1994.Effect of prolonged administration of dietary capsaicin on broiler growth and *Salmonella enteritidis* susceptibility. Avian Dis. 38:329-333.