

## Determination of Coliform load and prevalence of *E. coli* in Broiler meat at Debre zeit Agricultural research center poultry farm

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**Abstract:** The current study was conducted from November 2007 to April 2008 with an attempt to determine the prevalence of *E. coli*, coliform load and the presence of *E. coli* 0157: H7 strains in broiler meat at DZARC Poultry farm. The Experimental broilers were fed ad libitum with commercial ration containing EM through all the study periods, which were available in form of solution and solid for commercial use and it was given at the dose rate of 1 ml/ ltr through drinking water to 1<sup>st</sup> groups, as biofeed at the dose rate of 30 gm/kg in feed to 2<sup>nd</sup> groups and both simultaneously to 3<sup>rd</sup> group continuously from 10 days of age to 56 days age. The forth groups served as control. Out of 100 broiler meat samples, 74 % of the isolates were *E. coli* positive. The overall isolation rate was 22(88%) in control groups, 15(60%) in groups fed with Bokash and normal water, 17 (68%) in groups fed with normal feed and EM with water, and 20 (80%) in groups fed with Bokash and EM with water. There is statistically significance difference ( $p < 0.05$ ) in isolation rates among the four treatment groups. The mean coliform count of positive samples was  $5.25 \times 10^3$  cfu/gm of chicken breast meat. The coliform load for each of the treatment groups was  $7.0 \times 10^3$  cfu/gm for control groups,  $4.0 \times 10^3$  cfu/gm for the groups fed on Bokash with normal water,  $4.5 \times 10^3$  cfu/gm for groups provided with normal feed and EM with water and  $5.5 \times 10^3$  cfu/gm for group fed with Bokash and EM with water. Result of the present study showed that there is higher *E. coli* contamination rate of broilers' meat.

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**Key words:** *Broiler, Coliform, Debre zeit, E. coli*

### 1. Introduction

Chicken meat is very popular all over the world, while it is cheap source of protein, nutritious, healthy and contains less calories. As a result of this, there is an increased demand for chicken meat. To satisfy the increased demand, chicken are produced under intensive husbandary system. Recently chicken are being fed with competitive or effective micro organisms to increase body weight gain in the shortest possible time. The major constituents of effective micro organisms are lactic acid bacteria, which multiplies and displace the normal micro flora of the gastro intestinal tract of chicken. Owing to the reduction of the PH of gastro intestinal tract due to lactic acid bacteria the normal micro flora will not have the chance to grow in the gut (Brashears *et al.*, 2011).

With this in mind research was conducted in collaboration with Agricultural research center, to find out whether exotic chicken kept under intensive husbandary system could increase their body weight by feeding effective micro organism. Besides it was assumed that the effective micro organisms administered orally will reduce the bacterial load of chicken carcasses so as to reduce bacterial spoilage. Chicken meat is prone to contamination with micro organisms. The rate of carcass contamination particularly increase during slaughtering operation.

Contamination of the carcass with gastro intestinal tract micro organisms takes place, during evisceration. Contamination of chicken carcass with GIT micro organisms results in meat borne infection intoxication and meat spoilage. *E. coli* is one of the major micro organisms which contaminates chicken carcasses (Quinn *et al.*, 2002).

The presence of *E. coli* in chicken carcass is an indication of faecal contamination and poor hygienic practice. *E. coli* has been chosen to be an indicator of contamination of food and water, because it has met a number of important criteria including (1) its presence in human and animal feces, (2) it is readily detectable by simple procedure that results in unambiguous identification of the *coli form* groups and (3) it is constantly present, where pathogens are present and (4) it show increased resistance to disinfectants applied to pathogens ( Redman *et al.*, 2003).

*E. coli* incurs severe economic losses in livestock and poultry industry and negatively affects the public health. *E. coli* is known to cause a number of poultry diseases including coli bacillosis, Hajarre's diseases, coli granuloma, peritonitis, salpingitis, synovitis and air sac diseases. The economic losses result from the high morbidity, mortality, reduced productivity and condemnation of chicken carcasses destined for human consumption ( Lignieres, 1994; Savou, 1973).

In addition to this *E. coli* O157: H7 causes food borne infection and intoxication in humans. Though chicken meat is not consumed raw, out breaks usually result from cross contamination of heat treated chicken meat and meat product. The disease in humans is characterized by diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (TTP). An estimated 10,000- 20, 000 causes of infection and 500 deaths due to *E. coli* O157: H7 have been reported in the USA annually (Read and Kaplan, 1996).

Recent report shows that the annual cost of illness due to *E. coli* O157:H7 is above \$ 405 million including \$370 million for medical case and \$5 million for lost productivity. The average cost per case, varied greatly, ranging from \$26 million for individual who did not obtain medical care to \$602 million for patient, who died from hemolytic uremic syndrome (HUS) (Frenzo *et al.*, 2005).

The severe economic losses in poultry industry and its negative impact on the public health qualifies *E. coli* amongst the serious food borne pathogen (Doyle *et al.*, 1997). Under Ethiopian conditions; however, no systematic work has been conducted on *E. coli* from different animal species. Then, there is a need to conduct in the survey and surveillance and monitor the occurrence of *E. coli* O157:H7 in food of animal origin including poultry and particularly chicken.

Therefore, the objectives of this study were to determine the prevalence of *E. coli*, asses the microbial load of *coliforms* and determine the presence of pathogenic *E. coli* O157:H7 in chicken carcasses.

## 2. Materials And Methods

### 2.1 Study area

The study was conducted from November 2007 to April 2008 at DZARC poultry farm. It is situated 45 km south east of Addis Ababa. The town is located in East Shoa zone of Oromia Regional State, central Ethiopia. Geographically it is situated at 9°N latitude and 40°E longitude. The altitude is about 1880 meter above sea level. The mean annual rainfall is 866 mm 84% of which falls during the long rainy season lasting from June to September. The mean annual maximum and minimum temperature is 26°C and 14°C, respectively; with mean relative humidity of 61.3% (NMSA, 2003). The major agriculture activity in the area is mixed farming where by crops are cultivated and different species of livestock are kept. Back yard chicken management system is practiced, where local breeds are allowed to scavenger, what nature provides them. Intensive chicken management is practiced with exotic breeds in Debre Zeit town. However, the

number is quite small when compared with indigenous breeds.

### 2.2 Study Animals

The study animals were broiler of exotic breeds kept by the Debre zeit Agriculture research center. Broilers are kept under intensive poultry management systems. Birds are provided with industrially produced poultry feed and water ad libitum.

### 2.3 Sampling method

Birds were sampled randomly using random table. 10 days old broiler chicken were divided in to as T1, T2 and T3. The control group were provided with normal feed and waters. Treatment group1 were given Bokash feed and water while in group 2 normal feed and effective micro organisms was administered with water. Birds in treatment groups 3 were provided with Bokash feed, effective micro organisms. The body weight gain was recorded at regular intervals and the birds were slaughtered at the end of 80 days using traditional slaughtering methods.

### 2.4 Laboratory Techniques

#### 2.4.1 Sample collection and transportation

After slaughtering and washing, broiler chicken breast muscles were aseptically removed from the broiler carcass, placed in sterile plastic bag and transported to the Micro biology Laboratory of the Faculty of Veterinary Medicine using cool box filled with ice. Up on arrival at the laboratory sample were put in a deep freezer at -20 °C until they are further processed. Before processing chicken breast muscles were tined at room temperature.

#### 1.4.2 Pre-enrichment

25 gram of breast meat samples were taken and chopped in to smaller pieces using sterile knife and cutter board. This was blended with a blender. The sample was then transferred to a sterile flask filled with 225 buffered peptone water and incubated at 35 °C for 18hrs (Quinn *et al.*, 2002).

#### 1.4.3 Viable coliform count

Serial 10 ml dilution rates were prepared first by taking 1 ml of the pre- enriched sample with a sterile pipette and transferring this to second test tube labeled 10<sup>2</sup>, which was previously filled with 9ml buffered peptone water. Further dilution rates were prepared with the same techniques until the dilution rate of 10<sup>5</sup> was reached. 0.1ml from each dilution rate 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> was taken and transferred to two pairs of petridishes containing Mac conkey agar. This was incubated at 37 °C for 18hrs. Following this colony forming unites in each pairs of petridishes were counted using colony counter. The final number of coliform colonies unites was determined using the formula indicated below.

$$C = \frac{\sum C}{n_1 + n_2} \times d$$

Where: C= is the mean coliform colony forming unit in 1 gm of broiler breast muscle

$\sum C$  = the total number of coliform colony forming counts in petridishes considered in the count

$n_1$  = number of petridish with the lowest dilution rate

$n_2$  = number of petridishes with the highest dilution rates

$d$  = the lowest dilution rates

#### 1.4.4 Biochemical identification of *E. coli*

Each presumptive *coli* forms positive samples were subjected to IMVIC test (indole, methyl red, Voges - proskauer reaction and citrate utilization test) to identify of they were *E. coli* as previously described (Quinn *et al.*, 2002).

##### Indole test

Indole test was conducted by inoculating colony of *coliform* onto medium and incubation of this at 37°C for 24 hrs. Following incubation kovac's reagent was added to the test tube. Red colouration suggested *E. coli* colonies (Quinn *et al.*, 2002).

##### Methyl red test

5 ml of methyl red voges proskauer bouillon was transferred to sterile test tube. The broth was inoculated with incubated at 37 °C for 24 hours. Following incubation 2 drops of 0.5 % methyl red solution was added to test tube. Red colouration was considered as indication for the presence of *E. coli* (Quinn *et al.*, 2002).

##### Voges- proskauer test

Methyl red - Voges proskauer bouillon was prepared as previously described 24 hrs culture of *coliform* colony was inoculated to the bouillon and incubated at 37 °C for 24 hrs. After incubation 5 ml of copper sulphate was added changing of the medium to red colour with 2-3 minutes was considered to be *E. coli* negative, where as no colour change suggested *E. coli* positive (Quinn *et al.*, 2002).

##### Citrate utilization test

Simmon's citrate agar was used in conducting the citrate utilization test. The slant agar was inoculated with the test organism and incubated at 37 °C for 24 hrs. Presence of blue colonies on the slant

agar, suggested *E. coli* negative where as absence of blue colour suggested *E. coli* positive sample (Quinn *et al.*, 2002).

#### 1.4.5 Confirmation of *E. coli* O157: H7

*E. coli* colonies proven biochemically were subjected to latex agglutination test (Oxoid, Basingstoke, Hampshire, England). One drop of the latex was placed near the edge of the circle on the slide and using a sterile wire loop, presumptive *E. coli* colony was picked up and emulsified in a drop of saline solution near the latex drop. After suspension the latex was mixed with the bacterial suspension. The card was rocked in circular motion for minute and examined for the presence of agglutination by naked eyes. Agglutination of the test latex with in 1 minute was considered as positive for *E. coli* 157: H7 and absence of agglutination was taken as *E. coli* O 157: H7 negative.

## 2.5 Data analysis

Prevalence of *E. coli* was defined as the proportion of meat sample positive for *E. coli* divided by the total number of meat examined in each treatment, multiplied by 100. The *E. coli* positive samples were statistically analyzed by chi square test. A confidence interval of 95% was used to interpret the statistically associations and significance was considered when p- value was less than 0.05 (Thrusfield, 2005).

## 2. Results

Out of the 100 poultry meat examined, 74% were found to be *E. coli* positive. The overall isolation rate was 22 (88%) in control groups, in groups fed with Bokash and Normal water, it was 15 (60%), in group fed with normal feed and EM with water, 17 (68%) in groups fed with Bokash and EM with water, 20 (80%) was found with significant difference ( $p < 0.05$ ) in each treatment group.

The mean *coli* form count of positive samples was  $5.25 \times 10^3$  cfu/gm of breast meat. The *coli* form count for each of the treatment groups was  $7.0 \times 10^3$  cfu/gm for the control group (T1),  $4.0 \times 10^3$  x cfu/gm for the group provided with Bokash and normal water (T2),  $4.5 \times 10^3$  provided with normal feed and EM with water (T4). Results of *coli* form counts on broiler meat is presented in table 3.

**Table1:** Isolation rate of *E. coli* in broiler breast meat

Treatment group	Examined N=25	Prevalence N=(%)	Feed group	X <sup>2</sup>	p-value
I control	25	22 (88%)	Normal feed + normal water	6.00	P< 0.05
Treatment (T2)	25	15 (60%)	Bokash feed + normal water		
Treatment (T3)	25	17 (68%)	Normal feed+EM with water		
Treatment (T4)	25	20 (80%)	Bokash feed + EM with water		

**Table 2:** Isolation rate and biochemical identification of *E. coli* isolates from broiler breast muscle

Treatment group	No of samples examined	Biochemical test	
		IMVIC	No. (%) positive
T1( control)	25	++--	22(88%)
T2	25	++--	15(60%)
T3	25	++--	17(68%)
T4	25	++--	20(80%)

**Key:-** IMVIC Test: I: indole, M: methyl red, V: voges- proskauer reaction, C: citrate utilization, +: *E. coli* positive, -: *E. coli* negative

**Table 3:** Viable *coli form* count/ gm of poultry breast meat

Treatment group	Mean viable <i>coli form</i> count on 2 plates	No of <i>coli form</i> count/ gm chicken breast meat	
		Dilution ratio	Viable count
T1 ( control)	70	10 <sup>-2</sup>	7.0 x 10 <sup>3</sup>
T2	40	10 <sup>-2</sup>	4.0 x 10 <sup>3</sup>
T3	45	10 <sup>-2</sup>	4.5 x 10 <sup>3</sup>
T4	55	10 <sup>-2</sup>	5.5 x 10 <sup>3</sup>
Mean	52.5	10 <sup>-2</sup>	5.25 x 10 <sup>3</sup>

The latex agglutination test was employed to detect the present of *O157: H7 E. coli* strains among the (n=74) *E. coli* isolates. All presumptive *E. coli* positive samples were found to be *O157:H7 E. coli* negative, suggesting that the presumptive *E. coli* positive samples were not pathogenic.

### 3. Discussion

A study on experimental broilers meat showed that 74% of the breast meat was *E. coli* positive. Chicken meat is prone to contamination with micro organisms. The rate of carcass contamination particularly increases during slaughtering operation. Contamination of the carcasses with gastro intestinal micro organisms takes place, especially during evisceration. *E. coli* is one of major micro organisms which contaminates chicken carcasses (Quinn *et al.*, 2002). The overall isolation rate was 22 (88%) in control groups, 15 (60%) in group fed with Bokash and normal water, 17 (68%) in groups fed with normal feed and EM with water and 20 ( 80%) in groups feed with Bokash and EM with water. This result is high when compared with the prevalence ranging from 36.8 % to 47.6% on cloacal swabs of broiler chicken and carcass swabs respectively from 100 chicken collected in abattoir in Chiang Mai and Thailand (Hanson *et al.*, 2002). A study on 212 retail chicken samples in greater Washington D.C, revealed, prevalence of *E. coli*, 82 (38.7%), while 11.9% of Turkey samples were *E. coli* positive (Bolton *et al.*, 1996).

There is statistically significance difference (p< 0.05) among the prevalence rates of *E. coli* positive in each treatment group (1 control = 88%, T2= 60%, T3=68% and T4 =80%). This indicates that, isolation rates was high in control when compared with chicken fed with EM supplement. And hence EM solution

contains lactic acid bacteria which suppress most pathogenic strains. The live body weight of broiler was significantly greater in all EM treated groups than control. These could be related to better digestibility of crude protein and crude fiber. EM appears to be a safe growth promoter without any associated risks. There are reports indicating that the use of EM in livestock and poultry, has an effect of EM on health and immune system of poultry under local conditions (Hussain *et al.*, 1996).

Study have shown that EM potentiated the immune response in experimental broilers. Lactic acid bacteria administered orally or peritoneally enhanced the activity the activity of mononuclear phagocytic system and increased the production of circulating antibodies for certain antigens in mouse. However, further investigation is required for the elucidation of the mechanism through which EM increases the immune response ( Kato *et al.*, 1983).

Another study has demonstrated that, the rate of microbial contamination of retail meats with *E. coli* ranged from 39% for chicken samples to 12% for turkey samples. The rate of *E. coli* contamination in different retail meats were not similar with the rates observed for campylobacter contamination. This may have been due to the frequency the presence of *E. coli* in the animal production and food processing environments. Infact, *E. coli* isolates identifies were part of the normal enteric flora that is present in animals and often identified in food production, processing and distribution environments (Bolton, *et al.*, 1996; Brooks *et al.*, 2001; Doyle *et al.*, 1987; Hevelink *et al.*, 1996; Samadpour *et al.*, 1994).

Our study has showed that all the four treatment groups were *E. coli O157: H7* negative. This results revealed that EM supplied contain lactic acid bacteria

which suppress pathogenic strains. Hussian *et al.*, (1996) report that EM treated broiler have better weight gain and immune response than the control. Doyle and Schoeni (1987) report that, on 263 chicken in USA, the prevalence of *E. coli* O157: H7 was 1.5%. In their base line studies of bacteria in or on beef and poultry carcasses and ground beef, the U.S Department of Agriculture ( USDA) findings as follows: *E. coli* was found in O157:H7 was found in 563 samples of ground beef; none in 1, 297 broiler carcasses, and none on the carcasses of 2,112 cows and bulls ( Jay, 2000).

The incidence and prevalence of enterohemorrhagic *E. coli* strains in meat, milk, poultry and sea food are highly variable. Considerably more positive cases were found when DNA probes are used to detect for EHEC strains than when *E. coli* O157: H7 is tested when used other test method are used alone although *E. coli* O157: H7 were not be isolated from sausage in the United kingdom, A DNA probe gave positive results on 25% of 1845 samples for other EHEC strains. 'None were found in 112 samples from 71 chicken. The following eight meat types poultry and sea food products gave the following positive results: 63 % of 8 veal, 48% of 21 lambs, 23 % of 60 beef, 18% of 51 pork, 12 % of 33 chicken, 10% of 62 fish, 7% of 15 turkey and 4.5 % of 44 Sheel fish ( Jay, 2000).

Little is known on the occurrence of VTEC O157 strains in chickens and poultry in Germany. The examination of rectal swabs taken from 144 chicken yielded no VTEC isolates (Beutin *et al.*, 1993). Absence of VTEC in rectal chicken was found in a British study ( Swith *et al.*, 1991 ), whereas 1-12 % of poultry meat investigated in the USA and Thailand were positive for VTEC or tested positive with VT-specific gene probes. Doyle and Schoei 1987; Suthinkul *et al.*, 1990; Samadpour *et al.*, 1994). However it is not clear whether the meat samples were contaminated with VTEC originating from chicken or from other sources. Further studies are necessary to explore poultry as possible reservoir of VTEC in nature.

The mean *coliform* count of positive samples was  $5.25 \times 10^3$  cfu/gm of chicks breast meat. This study revealed that the broiler meat was contaminated with *coliform* with significance difference ( $p < 0.05$ ) in each treatment groups. As compare to EM supplement, the control group was significantly loaded with *coliforms*. But a study on commercial broiler chicken by Cason and Hinton (2000) Shows the mean *coliform* concentrations in tanks 1, 2, and 3 were 4.6, 2.5 and 1.6 log<sub>10</sub>(CFU/ml) respectively. *E. coli* concentrations followed the same pattern with means of 4.4, 2.1 and 1.4 in tanks 1, 2 and 3 respectively with significance differences ( $p < 0.05$ ) in the concentration of both

*coliforms* and *E. coli* between tanks. The whole poultry meat tends to have a lower microbial count than cut up poultry meat most of the organism on such products are on the surface, so surface count/cm<sup>2</sup> are generally more valid than counts in deep tissue (Jay, 2000).

In the study of whole chickens from six commercial processing plants, the initial mean total surface count was log 3.30/cm<sup>2</sup>. After the chicken were cut up, the mean total count increased to log 3.81/ cm<sup>2</sup> and further increased to log 4.8 after packaging (Jay, 2000).

#### 4. Conclusion And Recommendations

Out of 100 broiler meat examined, 74% of the isolates were *E. coli* positive. The overall isolation rates was 22 (88%) in control groups, 15 (60%) in groups fed with Bokash and normal water, 17 (68%) was in groups fed with normal feed and EM with water, and 20 (80%) was isolated in groups fed with Bokash and EM with water. There is statistically significance difference ( $p < 0.05$ ) in four treatment groups of isolates. EM treated broiler have less *E. coli* isolates as compared to control. The mean *coliform* count of positive samples was  $5.25 \times 10^3$  cfu/gm of chicken breast meat. The *coliform* concentration of each treatment group was  $7.0 \times 10^3$  cfu /gm for control,  $4.0 \times 10^3$  cfu/gm in groups fed with normal water and Bokash,  $4.5 \times 10^3$  cfu/gm for the groups provided with normal feed and EM with water and  $5.5 \times 10^3$  cfu/gm for the group fed with Bokash and EM with water. No *E. coli* O157: H7 has been isolated from *E. coli* positive samples. In view of the present findings and available information, the following recommendations are forwarded:

- Implementation of the principle of hazard analysis critical control point (HACCP) under the umbrella of good manufacturing practice (GMP) and good hygienic practice (GHP) from farm to fork (from stable to table) should be adopted to ensure efficient food safety.
- Further study must be undertaken on the effect of EM in the broiler meat and the associated meat borne disease.
- Detailed microbiological studies on microbial load of *E. coli* on meat should be undertaken to know the limits of the bacteria on the broiler meat and to judge the fitness of meat for human consumption.
- The public should be made aware about effects on health associated with consumption of raw or under cooked broiler meat.
- Slaughter house operations should be managed properly to minimize contamination of meat by animal intestine contents.

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