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EFFECT OF FORTIFICATION OF EGGS WITH FOLIC ACID DURING INCUBATION AND POST HATCH ASCORBIC ACID SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE, BLOOD CONSTITUENTS AND IMMUNE STATUS OF BROILER CHICKS

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SUMMARY

The present study aimed to evaluate the effect of in ovo injection with Folic acid and adding ascorbic acid (VC) into the drinking water on growth performance, carcass yield, some blood biochemical changes and immune response of broiler chicks. A total of 150 eggs were randomly divided into three groups. The 1st one has no treatment (control) , while the 2nd was in ovo injected with 0.1 ml distilled water/egg (Sh - group) . The third one was given injected by folic acid 10% (0.1 ml/egg). Feeding and flock management were done in accordance with commercial practices. The results indicated that chicks hatched from eggs treated with folic acid or folic acid and then given VC in the drinking water had higher body weight, carcass (%), breast muscle yield and lymphoid organs weight than other treatments. The visceral organs of the birds likewise increased. Plasma concentration of total lipid, cholesterol, triglycerides and LDL were significantly decreased by both folic acid injection and VC addition to drinking water . Addition of VC was effective on increasing intestinal total count of bacteria while, decreasing intestinal count of *Escherichia coli*. It could be successfully used as alternative to antibiotic growth promoters as a tool of controlling intestinal pathogenic bacteria.

Keywords: *Broiler chicks, Embryo, Folic acid, Vitamin C, Muscle, Blood Parameters, pathogenic bacteria.*

INTRODUCTION

Nowadays, there is a linear enhancement in the growth performance and meat yield of commercial poultry with the best input efficiency all over the year. Development of the embryo, promotes early gut development and improve immune status by introducing nutrients to the incubating embryo through *in ovo* injection **(**Havenstein *et al*., 2003; Tako *et al.*, 2005 and Foye *et al*., 2007). *In ovo* injection is excessively used for plenty of purposes, such as injecting eggs with immunological materials (Jochemsen and Jeurissen, 2002), raising the post-hatching body weights of birds (Ohta *et al*., 1999), enhanced immune response; enhanced early growth by refine intestinal function and development (Tako *et al*., 2004**);** increased skeletal growth (Hargis *et al*., 1989), breast muscle yield (Hajihosaini and Mottaghitalab, 2004), and body market weight (Selim *et al*., 2012). Folic acid is major for cell division and body functions , therefore it is essential for embryo development. A possible approach to counteracting the negative effects of heat stress among chickens was the supplementation of birds with Vitamin C; which plays a major role in the biosynthesis of corticosterone (Bain, 1996). Several researchers have reported beneficial effects of Vitamin C supplements (given either in diets and or in drinking water) in enhanced performance of broiler chickens with experimentally induced hypothyroidism (Takahashi et al., 1991), and improved disease resistance of the birds (Amakye-Anim *et al*., 2000). Skinner *et al*. (1991) reported that vitamins supplementation via the drinking water can be an effective alternative method to provide the birds with extra nutrients to maintain good performance especially during unusual conditions

Therefore, the present study was conducted to elucidate the influence of *in ovo* injection of folic acid (FA) during incubation and post hatching vitamin C addition in drinking water on productive performance, some blood parameters and immune responses of broiler chicks.

MATERIALS AND METHODS

Experimental Procedures:

A total number of 150 eggs with an average weight of 67g were obtained from a commercial broiler breeder flock (Ross). Before incubation, eggs were randomly divided into three groups of 50 eggs each . The first group, was intact non-injected eggs, considered as control (C), while the second and third groups were injected into air cell with 0.1ml distilled water/egg as solvent and 0.1ml FA (10%) respectively . All eggs were normally incubated in a forced draught Laboratory Incubator at the recommended incubation temperature (37.6-37.7ºC) and relative humidity (RH) between 55 to 60% in an automatic incubator. Egg injection procedure was carried out at day 7of embryonic development. Thus, the large end of each egg (location of air cell) was disinfected by ethyl alcohol. The point site of injection was punctured by hard and thin stylus and the tested material was injected into the air cell of each egg by using graded insulin syringe (1 ml) and the punctured site was sealed with non-toxic glue sticks. The injected dose was 0.1 ml/egg. At the 18^{th} day of incubation, all eggs were transferred to the Hatcher and kept till hatching at 37.5°C and 70% RH. The hatched chicks from each group were sub-divided into two vitamin C (VC) – treatment groups. VC was supplied in drinking water of newly hatched chicks at 0 (control) or 40 mg/L of drinking water. Since 6 expermintal treatments were existed (2 Vit. C levels X 3 in ovo folic acid treatments). All chicks were fed *ad-libitum* on commercial starter (1 to 16 d), grower (16-30 d) and finisher (30-35d) diets. The birds were given the same management and vaccination program as those done in commercial farms.

Slaughter test:

At the end of the experimental period (5 weeks of age) , three chicks from each group were randomly taken and slaughtered by severing the carotid arteries and jugular veins. After complete bleeding, chicks were scalded in hot water bath then eviscerated and weighed to determine the hot carcass weight as well as giblets (liver and heart) and lymphoid organs (Spleen, bursa of Fabricius and all thymic lobes) weight. All weights were expressed as percentages of live body weight.

Blood biochemical Analysis:

Blood samples were collected from three chicks per treatment, during their exsanguinations, in heparinized tubes, centrifuged at 4000 rpm for 15 min, plasma samples were decanted and then stored at -20^{o}C until analysis. Plasma samples were assigned for the determination of total protein, albumin, total lipids, triglycerides, cholesterol and HDL-cholesterol using available commercial kits, while, LDL-cholesterol was calculated by the following equation:

LDL Cholesterol (mg/dl) = Total cholesterol- (Triglycerides/5) – HDL Cholesterol.

Globulin was calculated by subtraction of plasma albumin from total protein.

Microbiological examination :

Samples from intestinal contents were randomly collected, and transferred to the laboratory without delay to be examined.

Cultivation media:

1- (NA) Nutrient Agar (Merck 1994, Cat. No.10233)
2- MacConkey agar (Merck 1994, Cat. No. 5465)

Preparation of examined broiler samples:

Samples were collected at slaughter, after scalding and de-feathering , but before washing and cooling of carcasses, then placed individually in plastic jars without transport medium. One gram of the tested broiler sample from the intestinal content was placed in a sterile plastic bag. An appropriate amount of sterile phosphate buffer of pH 7.0 was added, massaged for 2 minutes. Before the examination, the sample was then removed into a sterile empty Petri dish to be cut to small pieces using an alcohol sterilized cutter under aseptic conditions.

Microbial cultivation media:

Digestive tract *E. coli* colonies counts were determined as colony forming units (cfu) per gram of sample.

Statistical analysis:

Data were subjected to the analysis of variance by using the General Linear Models Procedure of the Statistical Analysis System (SAS, 2003). Differences among treatment means were detected using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The effects of in ovo injection and adding vitamin C into the drinking water on the production parameters of broiler chicks during the whole experimental period (1-35 days of age) are shown in Table (1).

It is clearly noted from the present results that both of *in ovo* injection with FA treatments during incubation and VC (40 mg /L) into the drinking water of post hatched chicks significantly improved body weight, carcass parts yield and giblets of broiler chicks as compared to other groups. This may be due to the anabolic effect of FA treatment on muscle building. This study affirmed the findings of Robel (1993), who used folic acid treatments in turkey, and Gursu *et al*. (2004) who studied effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress. The improvement in body weight due to vitamins administration in the present study was in agreement with several previous investigations which used same vitamins in poultry feed (El Barkouky *et al*., 2010; Khan *et al*., 2010). Vitamin C (ascorbic acid) is a well-known low molecular weight antioxidant that protects the cellular compartment from water-soluble oxygen and nitrogen radicals (Jurczuk *et al*., 2007).

Table (1). Effect of *in ovo* injection by folic acid and post hatch addition of Vitamin C on carcass yield and giblets weight of broiler chicks.

Treatment	Live body weight (g)	Carcass weight (%)	Breast weight (%)	Thigh weight (%)	Liver weight (%)	Heart weight (%)	Fat weight (%)
Injection -effect (T)							
T1	2150.0±168.9^{b}	72.9±1.8^{b}	25.7±1.5^{b}	23.3±0.9	2.6±0.2^{b}	0.64±0.08	2.80±0.61^{a}
T2	2271.7±174.9^{b}	72.4±3.1^{b}	26.9±1.2^{ab}	23.3±1.1	2.7±0.2^{ab}	0.63±0.05	2.14±0.20^{ab}
T3	2837.8±62.2^{a}	81.8±0.5^{a}	31.5±0.6^{a}	26.1±0.6	3.2±0.1^{a}	0.67±0.03	1.44±0.14^{b}
Sig.	**	***	*	NS	*	NS	*
Vitamin C – effect							
C1	2132.8±154.5^{b}	72.1±2.7^{b}	26.0±1.4^{b}	23.09±0.7^{b}	2.56±0.17^{b}	0.57±0.04^{b}	2.50±0.35^{a}
C2	2706.9±65.3^{a}	79.3±0.8^{a}	30.04±0.7^{a}	25.4±0.56^{a}	3.09±0.04^{a}	0.72±0.04^{a}	1.70±0.12^{b}
Sig.	***	***	***	*	***	**	**
Interaction T X C							
T1*C1	1776.7±20.3	69.1±0.5	22.7±1.1	21.8±0.1	2.2±0.2	0.47±0.02	3.54±0.45
T1*C2	2523.3±53.7	76.6±0.9	28.7±1.3	24.8±1.3	3.0±0.1	0.80±0.07	2.00±0.14
T2* C1	1881.7±31.1	65.6±0.6	24.1±0.3	21.9±0.4	2.3±0.1	0.53±0.04	2.59±0.06
T2* C2	2661.7±6.1	79.3±0.4	29.6±0.2	24.7±0.7	3.2±0.1	0.61±0.03	1.70±0.05
T3*C1	2740.0±75.1	81.7±1.7	31.1±0.5	25.5±1.1	3.2±0.1	0.71±0.04	1.38±0.29
T3* C2	2935.7±64.3	82.0±0.4	31.9±1.2	26.7±0.4	3.1±0.1	0.74±0.04	1.49±0.11
Sig.	***	***	**	NS	***	**	**

T1: (control); T2:in ovo injection with distilled water/egg; T3: in ovo injection with folic acid; C1 and C2 : vitamin (C) into the drinking water as follow (0 and 40 mg/l water, respectively).
** P 0.05, ** P 0.01, *** P 0.001, NS= non-significant.*
A and b Means within columns with no common superscripts differ significantly

Vitamin C efficiently inhibits in vitro lipid peroxidation due to a combination of direct radical interception and interaction with α-tocopherol as a co-antioxidant (Verma *et al*., 2007). Kultu, (2001) reported that VC supplementation increased body weight gain, carcass weight and concluded that ascorbic acid supplementation improved the productive performance of broiler chicks with experimentally induced hypothyroidism. In addition, VC is required for the hydroxylation of essential amino acids and of several oxidase enzymes (Dawson *et al*., 1990). Vitamin C could still play a role in

these findings as it has been shown that it takes part in the synthesis of leukocytes especially phagocytes and neutrophiles which play a part in the defense system of the chickens. In respect to VC, McKee and Harrison (1995) found that vitamin C increased the growth rate and improved the tolerability to stress and reduced the mortality. Also, Mona *et al*. (2004) reported that VC improved average body weight and this effect may be related to collagen synthesis, calcium and vitamin D3 metabolism as well as carnitine synthesis required for oxidation of fatty acid to obtain energy.

The results shown in Table (1) indicated that in ovo injection with FA during incubation and VC addition into drinking water caused a significant increase in the breast and thigh muscles weight of chicks at 5 weeks of age. This effect may be due to that the positive effects of FA and VC on breast muscles yield reflect their beneficial use in improving muscles yield of broilers. The tibia bone is the fastest growing bone in the body and is considered as the most sensitive during embryogenesis. Pirsljin *et al*. (2008) reported that one of the methods to assure an adequate nutrient content in the egg is in ovo administration of nutrients, which increases hatching weight and the size of the breast muscle. The improved performance could possibly be effected by vitamin C in neutralizing the negative impact of heat stress. VC is capable of minimizing oxidation within the tissues (Phoprasit *et al*., 2014).

The results in Table (1) indicated that in ovo injection by FA and VC administration in water drinking was significantly reduced the total body fat followed by folic acid compared with the control groups.

The birds whose drinking water was supplemented with vC showed increased body weight and carcass parts weight. However, these treatments did demonstrate high influence on percent of abdominal fats. These results are in agreement with those reported by **(**Phoprasit *et al*., 2014)**.** Kultu (2001) stated that Vit. C supplementation reduced carcass fat content.

Table. (2) shows the effect of in ovo injection with FA and VC supplementation in water on the relative weights of lymphoid organs . There were significant increases in lymphoid organs weight (%) in chicks that hatched from FA- in ovo injected eggs compared with the other groups. Also, VC addition significantly improved lymphoid organs weight, regardless in ovo FA injection. Moreover, the interaction between both in ovo FA injection and VC was significant in this respect.

Table (2). Effect of *in ovo* injection by folic acid during and post hatch addition of Vitamin C on lymphoid organs weight and some plasma constituents.

Treat.	Bursa %	Thymus%	Spleen%	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (g/dl)
Injection Treatment (T)							
T1	0.20±0.01^{b}	0.61±0.01^{b}	0.14±0.02^{b}	6.16±0.26^{b}	3.35±0.10^{b}	2.81±0.23^{b}	197.17±12.78
T2	0.24±0.02^{b}	0.63±0.07^{b}	0.15±0.02^{b}	6.08±0.34^{b}	3.30±0.14^{b}	2.78±0.21^{b}	206.83±7.83
T3	0.33±0.01^{a}	0.78±0.02^{a}	0.19±0.01^{a}	7.23±0.23^{a}	3.96±0.15^{a}	3.27±0.13^{a}	178.83±5.38
Sig.	**	*	*	**	*	*	NS
Vitamin C – effect							
C1	0.24±0.03^{b}	0.57±0.05^{b}	0.13±0.02^{b}	6.40±0.36	3.49±0.19	2.91±0.21	210.11±6.72^{a}
C2	0.28±0.01^{a}	0.78±0.02^{a}	0.19±0.01^{a}	6.58±0.33	3.59±0.16	3.00±0.18	178.44±5.62^{b}
Sig.	**	*	**	NS	NS	NS	**
Interaction T X C							
T1*C1	0.19±0.01	0.43±0.09	0.10±0.01	5.79±0.43	3.16±0.12	2.63±0.45	222.00±2.89
T1*C2	0.22±0.01	0.80±0.05	0.18±0.01	6.52±0.13	3.53±0.05	2.99±0.15	172.33±13.86
T2* C1	0.19±0.01	0.50±0.01	0.10±0.02	5.93±0.53	3.28±0.19	2.65±0.33	222.33±4.84
T2* C2	0.29±0.01	0.75±0.03	0.20±0.01	6.23±0.52	3.32±0.24	2.91±0.31	191.33±6.57
T3*C1	0.33±0.01	0.76±0.04	0.20±0.01	7.17±0.78	3.98±0.42	3.19±0.37	186.00±8.62
T3* C2	0.33±0.01	0.80±0.04	0.20±0.03	7.29±0.74	3.94±0.38	3.35±0.35	171.67±4.33
Sig.	*	*	*	NS	NS	NS	NS

T1: (control); T2:in ovo injection with distilled water/egg; T3: in ovo injection with folic acid; C1 and C2 : vitamin (C) into the drinking water as follow (0 and 40 mg/l water, respectively).
** P 0.05, ** P 0.01, *** P 0.001, NS= non-significant.*
A and b Means within columns with no common superscripts differ significantly

This improvement could possibly be ascribed to the anabolic effect of various treatments of injection folic acid and vitamin C on muscle building where heart consists mainly of muscles. VC plays a part in the defense system of the chickens. The antioxidant function of these vitamins could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells and hence decrease animal morbidity and mortality (Chew, 1995). The protective effects of VC on health may partially be a result of reducing circulating levels of glucocorticoids (Nockels, 1990). Since during stress, glucocorticoids, which suppress the immune response, are elevated.

***Blood Constituents**:*

Results in Table (2) showed that in ovo injection with folic acid significantly increased plasma total proteins, albumin and globulin of chicks at 5 weeks compared with chicks of other groups. However, VC had no effect. This may reflect the effect of folic acid on protein metabolism during embryogenesis. Moreover, folic acid results are in close agreement with the findings by Hebert *et al*. (2005) who worked on folic acid. The interaction effect of FA and VC on plasma protein fraction was not significant.

Vitamin C may increase oxygen metabolism of tissues and activity in broiler, due to increased nutrients utilization and use of blood glucose, as observed in Table-2 where VC administration significantly decreased blood glucose, indicative of higher metabolic rate.

The results concerning the effects of in ovo injection of folic acid on plasma total lipids and triglycerides are presented in Table (3).

Results showed that folic acid and vitamin C treatments significantly decreased plasma level of total lipids, cholesterol and triglycerides.

Table (3). Effect of *in ovo* injection by folic acid during embryogenesis and adding Vitamin C into the drinking water on some plasma constituents(mg/dl):

Treatment	Cholesterol	Total lipids	Triglycerides	HDL	LDL	L/ H ratio
Injection Treatment (T)						
T1	170.4±12.7 [a]	698.4±23.7 [a]	80.2±3.5 [a]	58.6±0.2[b]	95.7±12.3 [a]	1.6±0.2
T2	145.7±3.7 [b]	682.9±39.6 [a]	80.1±3.8 [a]	58.9±0.9[b]	70.8±4.0 [b]	1.21±0.1
T3	141.3±7.7 [b]	623.3±10.6 [b]	69.2±1.6 [b]	61.3±0.3[a]	66.2±7.5 [b]	1.08±0.1
Sig.	*	*	*	**	*	NS
Vitamin C –effect						
C1	164.3±9.7[a]	717.4±22.1[a]	81.8±3.0[a]	58.9±0.6[b]	89.0±9.6[a]	1.5±0.1[a]
C2	140.6±3.1[b]	619.1±10.2[b]	71.2±1.7[b]	60.2±0.4[a]	66.2±3.1[b]	1.1±0.05[b]
Sig.	***	***	**	***	***	***
Interaction T X C						
T1*C1	87.22±1.89	198.55±2.55	749.76±11.38	58.30±0.12	122.81±3.01	2.11±0.06
T1*C2	73.18±2.69	142.15±2.92	646.95±5.07	58.90±0.12	68.62±3.47	1.17±0.06
T2* C1	87.75±1.88	151.88±5.07	763.34±28.54	57.40±0.55	76.93±5.99	1.35±0.12
T2* C2	72.47±3.09	139.57±2.13	602.38±23.10	60.41±0.50	64.67±2.68	1.07±0.05
T3*C1	70.37±1.30	142.53±13.80	638.96±15.08	61.18±0.06	67.27±3.80	1.10±0.09
T3* C2	68.04±3.02	140.11±9.93	607.68±9.51	61.32±0.62	65.18±5.64	1.06±0.15
Sig.	**	*	NS	**	**	**

T1: (control); T2:in ovo injection with distilled water/egg; T3: in ovo injection with folic acid; C1 and C2 : vitamin (C) into the drinking water as follow (0 and 40 mg/l water, respectively).
** P 0.05, ** P 0.01, *** P 0.001, NS= non-significant.*
A and b Means within columns with no common superscripts differ significantly.

The pronounced reduction in plasma total lipids, cholesterol and triglycerides may be associated with increasing triglycerides metabolism as a source of energy in absence of other metabolites required for energy (VFA and glucose levels). Folic acid results are in close agreement with the recent findings by Abd El-Azeem, *et al*. (2014) who used in ovo injection of folic acid. It is of interest to note that the present differences in both triglycerides and total lipids concentrations may be attributed to change in the concentration of total cholesterol, where the change in total lipids concentration was mainly attributed to change in triglycerides and cholesterol concentration.

In ovo injection with FA during embryogenesis or supplementing drinking water with VC affected the level of HDL while folic acid insignificantly decreased cholesterol at 5 weeks comparable to the other groups. The beneficial effect of drinking water with VC administration reducing the plasma concentration of triglycerides, cholesterol and LDL. Folic acid and VC reduced the concentration of cholesterol, triglycerides, free fatty acids, phospholipids and very low density lipoproteins (VLDL). Therefore, it may be concluded that vitamin C may be used with water to get best result in terms of body weight gain, physical appearance and blood profiles without any detrimental effects on broilers.

Microbiological examination :

Total microbial counting of intestinal contents :

The total microbial count (cfu x 10^3/g sample) of intestine contents of the tested broilers was measured and the obtained results are listed in Table (4).

Table (4). Effect of *in ovo* injection by folic acid and adding Vitamin C into the drinking water on total bacterial count of intestinal contents (cfu×10^3/g) .

Treatment	Total Count of bacteria	Count of *E. Coli*
Injection Treatment (T)		
T1	849±47.9^b	110.3±21.8^a
T2	837.0±46.4^b	111.6±27.7^a
T3	1161.2±52.4^a	64.3±18.2^b
Sig.	***	**
Vitamin C into the drinking water treatment		
C1	847.9±56.5^b	136.6±12.2^a
C2	1050.2±53.6^a	54.2±12.8^b
Sig.	***	**
Interaction T X C		
T1*C1	745.0±14.4	140.7±11.1
T1*C2	953.0±20.8	80.0±36.5
T2* C1	736.0±10.4	171.5±15.6
T2* C2	938.0±20.8	51.7±3.8
T3*C1	1062.7±58.3	97.7±5.5
T3* C2	1259.7±24.6	31.0±2.7
Sig.	*	*

T1: (control); T2:in ovo injection with distilled water/egg; T3: in ovo injection with folic acid; C1 and C2 : vitamin (C) into the drinking water as follow (0 and 40 mg/l water, respectively).
** P 0.05, ** P 0.01, *** P 0.001, NS= non-significant.*
A and b Means within columns with no common superscripts differ significantly

The results showed that the addition of vitamin C significantly decreased the count of Escherichia coli compared to the control treatments, which had more effective than the control group on decreasing intestinal count of *Escherichia coli.* However, VC and folic acid treatments increased the total viable bacterial count. It appears that VC with its acidic properties can affect the bacterial counts in the digestive tract of chicks . In this concern , Lampromsuk *et al.* (2012) reported that organic acids increase the population of lactic acid bacteria in the intestinal tract which produce lactic and acetic acids . This in turn could be used by chicks as an energy source for intestinal epithelial cell growth that improves nutrient absorption . In the present study ,both folic acid and vitamin C significantly increased the total viable bacterial count which in close agreement with the previous results .

CONCLUSIONS

It is concluded that in ovo injection of FA at the 7th day of embryogenesis followed by Vit. C supplementation to drinking water of hatched chicks, could be applied to improve live body weight, carcass characteristics, lymphoid organs weight, reduce plasma lipids and pathogenic bacterial count in the cecal contents.

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تأثير حقن بيض التفريخ بحمض الفوليك ثم إضافة فيتامين (ج) لمياه الشرب بعد الفقس على الأداء الإنتاجى والمناعى وبعض مكونات الدم لكتاكيت اللحم

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أجريت هذه الدراسة لتقييم تأثير حقن بيض التفريخ بحمض الفوليك واضافة فيتامين (ج) لمياه الشرب بعد الفقس على الأداء الإنتاجى وبعض قياسات الدم والحالة المناعية لكتاكيت اللحم. حيث تم تقسيم عدد 150 بيضة غلي ثلاث مجموعات : المجموعة الأولى هى مجموعة الكنترول بدون معاملة والمجموعة الثانية هى مجموعة الكنترول الموجبة التى تم حقنها بالمحلول الفسيولوجى (المذيب) في اليوم السابع من فترة التفريخ بينما المجموعة الثالثة فتم حقنها في نفس العمر بواسطة حمض الفوليك . وبعد الفقس تم تقسيم كتاكيت كل مجموعة غلي مجموعتان الأولي للمقارنة والثانية تم إضافة فيتامين (ج) لماء الشرب بمعدل 40 ملليجرام / لتر طوال فترة التجربة . وكانت أهم النتائج المتحصل عليها من هذه الدراسة أن المعاملة بواسطة حامض الفوليك وفيتامين (ج) أدت لزيادة الأداء الإنتاجى ورفع المناعة والنسبة المئوية لمحصول اللحم كما كان هناك تأثير معنوى لحمض الفوليك وفيتامين (ج) على خفض مستوى بلازما الدم من الدهون الكلية والكوليسترول والكوليسترول ذو الكثافة العالية والكوليسترول ذو الكثافة المنخفضة وزيادة معنوية فى تركيز البروتينات الكلية والألبيومين والجلوبيولين والجليسريدات الثلاثية . بالإضافة للتأثير المعنوى لفيتامين (ج) على زيادة العدد الكلى للبكتريا بالأمعاء مع انخفاض معنوي في محتوى الأمعاء من البكتريا الممرضة. وقد خلصت الدراسة إلي أن تدعيم محتوي البيضة بحمض الفوليك عن طريق الحقن أثناء فترة التفريخ ثم إضافة فيتامين " ج " إلي ماء الشرب للكتاكيت بعد الفقس له تأثير جيد وعنوي علي الأداء الإنتاجي والإستجابة المناعية لكتاكيت اللحم .

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