

## EFFECT OF DIETARY SPINACH ON GENE EXPRESSION OF CGH, IGF-I GENES AND SOME BLOOD AND BIOCHEMICAL PARAMETERS IN BROILER CHICKS

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### ABSTRACT

This study was conducted at a commercial broiler farm located in AL-Diwaniyah city from 1/4/2018 to 1/6/2018 in order to estimate the effect of two-concentration dietary spinach (2% and 6%) on blood and some biochemical parameters of broiler chicks by using 180 Ross chicks at the age of one day, which was randomly divided into three groups by three replications per group (thirty chicks per replication). These broiler chicks are divided into following: C: control group representing the group which feeding basal diet without any addition. T<sub>1</sub>: representing the group which feeding basal diet plus 2% spinch powder. T<sub>2</sub>: representing the group which feeding basal diet plus 6% spinach powder. The results showed a significant increase in RBC count, WBC count, PCV, Hb, and total protein concentration in T<sub>1</sub> and T<sub>2</sub> at 6 and 8 weeks compared to the control group. Also, there are significant decrease in glucose concentration and H/L percentage when compared to the control group. The results of the current study showed that there was a significant increase in the level of gene expression (CGH, IGF-I) genes in the T<sub>2</sub> group over the T<sub>1</sub> compared with the control group for both genes and for both time periods in the current study. In conclusion, the results in a current study showed that there is good effect of dietary spinach on blood and biochemical parameters and enhancement of resistance in broiler chicks.

**Key words:** Spinach, broiler chicks, blood, biochemical parameters, CGH gene, IGF-I gene

### Introduction

Compared to the consumption of mutton and beef recently increase trending in the consumption of chicken meat worldwide mainly due to its affordable cost and high availability (Raza *et al.*, 2019; Mustafa and Baurhoo, 2017). Also, there are no strict affiliations that decide its utilization, specifically, on account of (hindu) hamburger and pork (for muslims) and has a by and large benefit food and taste profile. After effect of expanding accessibility and utilization of oven poultry, purchasers are currently beginning to show interest in its micronutrient profile, taste, juice and medical advantages (Hossain *et al.*, 2013). Business taking care of has upheld the fast development pace of broiler, however, purchasers frequently whine about helpless succulence and the flavor of chicken meat. In current years, a few endeavors have accepted that vegetables/Green verdant plants as hay, spinach and cauliflower that broiler feed on can further develop cell reinforcement status of meat and decrease destructive impacts of oxidative harm (Elagib *et al.*, 2013; Dorhoi *et al.*, 2006). Moreover, vegetables are rich supplement sources, possibly useful for providing fundamental amino acids, minerals to the birds, relatively modest, effectively accessible, simple to process and posture less danger of infection pollution (Ghazalah and Ali, 2008). Buyers lean toward the meat of grills only took care of with vegetable sources as they feel it is more delicious, succulent, and delicate. Spinach is tracked down filling ridiculously in tropical and subtropical nations, and is developed generally in China, Indonesia, Thailand, Vietnam, Myanmar, Philippines, Bangladesh, Arab nations and in India. It is a decent wellspring of protein on dry matter premise, and furthermore plentiful in minerals and nutrients, particularly vitamin A (carotene), B1, B2, C and iron (Ekenyem and Madubuike, 2006 and Gakuya *et al.*, 2014). This plant likewise has inflammatory prevention agent and hostile to ulcer action and moderate anticancer movement. Also, it medicinally

affects obstruction, liver illnesses and eye sicknesses. Changes in haematological boundaries are regularly used to assess pressure in broiler chicks due to, wholesome, ecological or potentially neurotic variables (Khan and Zafar, 2005). Moreover, haematological and serum organic chemistry boundaries are acceptable signs of the physiological status of broiler chicks and their progressions are significant in evaluating the reaction of such creatures to different physiological circumstances (Afolabi *et al.*, 2010 and Lee *et al.*, 2013). Hence, this experience came from planning it with numerous goals, specific to focus on the effect of more affordable vegetables dietary spinach on blood and some biochemical parameter in broiler chicks which in turn controls the health status of broiler chickens and thus, is reflected in the quality and taste of the meat product.

### Materials and Methods

#### Experimental site, diet, design and animals

This study was carried at a commercial broiler farm located in Diwaniyh city from 1/4/2018 to 1/6/2018 to estimate the effects of two-concentration dietary spinach (2% and 6%) on the blood and some biochemical parameters of broiler chicks. One hundred eighty unsexed chick type Ross was selected and randomized into three treatment groups (C, T1 and T2) (60 birds/ group) each treatment was divided into three replicates (20 birds/replicate). The birds reared on a deep litter system, feed and water were given *ad lib* during the experiment weeks. Two types of diets were given to birds, the first being feeding birds from 1 to 23 days of age and finisher that had been feeding birds from 24-48 day of age and chemical Table 1. The experimental groups handle as follows for eight weeks:

Control group (C): feeding basal diet without any addition.

Experimental group (T1): feeding basal diet plus 2% spinach powder.

Experimental group (T2): feeding basal diet plus 6%

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spinach powder.

**Preparation of spinach**

Fresh spinach had been purchased from local market and dried and grinded in the form of powder, also the chemical analysis conducted on the powder sample at nutrition laboratory in college of Veterinary Medicine/University of Al-Qadisiyah according to AOAC (1990).

**Lab detection of blood and biochemical parameters**

At the end of experiment, blood samples were collected randomly at 6 and 8 weeks (5 chicks/replicate) from bird's wing vein to RBC calculation according to Powers, in 1989 (Powers, 1989) method and PCV done according to Archer in 1972 (Archer, 1972) method and Hb according to Quinn *et al.* (1998), method of total WBC count prepared according to Sood (1989), method, L/H percentage performed according to Muir *et al.* (2000). Moreover, glucose concentration carried out

Table 1: Dietary material and chemical composition for starting and finishing basal diet

Ingredients	Starter (%)	Finisher (%)
maize	47	50
wheat	10.5	8.5
Soybean meal	27	28
Concentrated protein	10	10
Plants oil	4	2.5
Ground limston	1	0.5
salt	0.5	0.5
	100%	100%
Chemical investigation of sustenance		
Energy ME(Kcal/Kg)	3078	3105
Crude protein	22	19
Lysine%	1.2	1.1
Methoinin+cystein	0.8	0.7
Crude fiber	3.4	3.2
Ca%	1.3	1
Phosphorus%	0.4	0.4

Chemical investigation of sustenance calculated by the NRC (1994)

according to Trinder (1969) and total protein according to Wotton (1964).

**RNA extraction and primer**

RNA was isolated from rats (liver) tissues according to the protocol described by the TRIzol® reagent manufacturer by (Bioneer company, Korea). The primers are used in the quantification of gene expression levels by using qRT-PCR technique based SYBER Green DNA binding dye, which supported from (Bioneer company, Korea).

**Statistically investigation**

Static Package for Social Sciences form 20 (SSPS 20), programming along with Microsoft Excel 2010 were depended for measurable examination and just outcomes which had a likelihood esteem under 0.05 were considered statically Sciences (SPSS, 1990).

**Results and Discussions**

The proximate nutritional composition of the moisture,

CGH primer
F-CACCACAGCTAGAGACCCACATC
R-CCCACCCGGCTCAAACCTGC
IGF-I primer
F-GCGGTTAGCCATGGAAGGC
R-CGTGTGGCTATGGAATTGTTTCAGGT

protein, CHO, fat and sugar in spinach diet showed in Table 2. The moisture per cent is 91.5% spinach while protein, CHO, fat and sugar appeared in lower proportions (3.5%, 0.6%, 0.3% and 4.1%, respectively). The Table 3 revealed that T<sub>1</sub> and T<sub>2</sub> record significant elevation (p≤0.05) in RBC count as comparing to control, its levels (2.22, 2.38 and 2.07) million/mm<sup>2</sup> respectively, at 6 weeks and (2.33, 2.42 and 2.19) million/mm<sup>2</sup>, respectively, at 8 weeks. The Table 4 showed Hb levels which almost like those of RBC counts for three treatments. Besides, T<sub>1</sub> and T<sub>2</sub> record significant promotion (p≤0.05) as compared with control group, its levels (8.2, 8.5 and 7.8) gm/100 ml blood respectively at 6 week and (8.4, 8.6 and 8.0) gm/100 ml blood respectively at 8 weeks. Also the PCV values (Table 4) showed that T<sub>1</sub> and T<sub>2</sub> have significant values (p≤0.05) as compared with control group in both weeks, its levels (26.2, 30.8 and 23.0)%, respectively at 6 weeks and (27, 31 and 23.3)%, respectively at 8 week.

The Table 5 revealed significant difference in T<sub>1</sub> and T<sub>2</sub> in WBC count as compared with control group in both weeks. Its levels (27.9, 29.9 and 21.8) 1000 cell/ml<sup>3</sup>, respectively at 6 weeks while the values at 8 week is 28.2, 30.2 and 22.9 1000 cell/ml<sup>3</sup>, respectively. Likewise, there are critical diminishing (p<0.05) in rates of heterophil/lymph cells in both weeks, its levels is 0.25, 0.23 and 0.27% respectively at 6 week and 0.25, 0.23 and 0.28%, respectively at 8 weeks.

The Table 6 detected critical decreasing (p≤0.05) in T<sub>1</sub> and T<sub>2</sub> in glucose level as compared with control group in both weeks. its level is 239.8, 183.2 and 250.8 mg/dl, respectively at 6 week and 245.4, 185.9 and 253.2 mg/dl, respectively at 8 week whereas level of protein significantly increased by dietary spinach when the level of protein increased in T<sub>1</sub> and T<sub>2</sub> in compared with control group (3.8, 4.2 and 2.7, respectively) at 6 week and (3.9, 4.4 and 2.8, respectively) at 8 week.

In Figure (1, 2, 3, 4), the results of the current study showed that there was a significant increase in the level of gene expression (CGH, IGF-I) genes in the T<sub>2</sub> group over the T<sub>1</sub> compared with the control group for both genes and for both time periods in the current study. The significant increase of RBC count, Hb, PCV as result from dietary spinach (T<sub>1</sub> and T<sub>2</sub> groups) in both weeks (6 and 8) may be related to the good content of vitamins (ascorbic acid, riboflavin, niacin, and folic acid), minerals (iron and calcium) and dietary fibers in spinach

Table 2: Nutritional composition of Spinach

Nutritional compound	%
Moisture	91.5
Protein	3.5
CHO	0.6
Fat	0.3
Sugar	4.1

also spinach contains folic acid which is a maturation factor of RBC (Maung *et al.*, 2020; Khamparn and Preston, 2006). Consequences of PCV assessment for three gatherings had like that varieties in RBC count. PCV is worried about quantities of red cells with the goal that any addition in RBC includes lead to increment in PCV (Wotton, 1964). So the critical increase of Hb concentrations in both groups (T<sub>1</sub> and T<sub>2</sub>) as comparing to the control group could be directly associated with RBC count, this may be belonging to high percentage of iron in spinach (4

Table 3: The effect of dietary spinach in blood RBC (million/mm<sup>3</sup>) of broilers at 6 and 8 weeks

Time groups	6 week		8 week	
	Mean ± Standard deviation		Mean ± Standard deviation	
T <sub>1</sub>	2.22±0.005 <sup>a</sup>		2.33±0.015 <sup>a</sup>	
T <sub>2</sub>	2.38±0.003 <sup>b</sup>		2.42±0.014 <sup>b</sup>	
C	2.07±0.012 <sup>c</sup>		2.19±0.003 <sup>c</sup>	

Various letter indicates critical contrasts (P<0.05) between treatment

Table 4: The impact of dietary spinach in blood Hb and PCV of broilers at 6 and 8 weeks

Time Groups	Hb (gm/100ml)		PCV (%)	
	Mean ± Standard deviation		Mean ± Standard deviation	
	6 week	8 week	6 week	8 week
T <sub>1</sub>	8.2±0.042 <sup>a</sup>	8.4±0.006 <sup>a</sup>	26.2±0.008 <sup>a</sup>	27.2±0.02 <sup>a</sup>
T <sub>2</sub>	8.5±0.014 <sup>b</sup>	8.6±0.014 <sup>b</sup>	30.8±0.16 <sup>b</sup>	31.7±0.05 <sup>b</sup>
C	7.8±0.041 <sup>c</sup>	8.0±0.02 <sup>c</sup>	23±0.02 <sup>c</sup>	23.3±0.014 <sup>c</sup>

Various letter indicates critical contrasts (P<0.05) between treatment

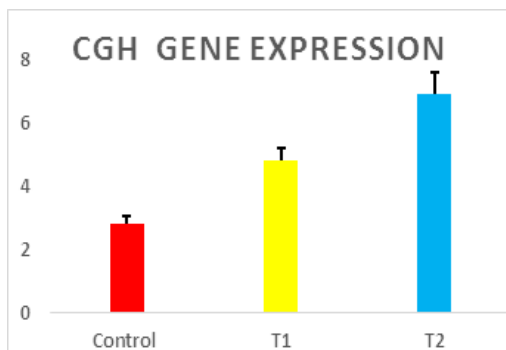


Fig 1: Effect of spinach on CGH gene expression for 6 weeks by qPCR

mg/100g) and folic acid which lead to elevation of Hb concentration (Men *et al.*, 2000). The improvement of these hematological boundaries for T<sub>1</sub> and T<sub>2</sub> bunches in contrast with control bunch was correlated to the action of spinach in general health of heart and liver. The number of erythrocytes in chicken influences the overall conditions of the bird (Ismail *et al.*, 2013).

Therefore, the numerical increases in PCV, haemoglobin and RBC counts of the birds fed ingredients are an indication that the oxygen-conveying limit of the blood was enhanced. Furthermore, it has been posited that high PCV reading (polycythemia) is an indication of either an arise in the counting of red blood cells or a decreasing in circulating plasma volume which might be due to a physiological adaptation to high altitudes or pathological response to chronic circulatory or respiratory disease (Hassan, 2006). It can also be as a result of iron storage disease, rickets, hypoxic increase in erythropoietin production or non-hypoxic autonomous increase in erythropoietin production. As a guide, a PCV value greater than 56% is an indication of dehydration in most birds. also the improvement of chicks resistance was uncovered by huge rise of WBC count in T<sub>1</sub> and T<sub>2</sub> groups as compared with control group which can be belong to contain the spinach of

Table 5: The impact of dietary spinach in blood WBC and H/L of broilers at 6 and 8 weeks

Time groups	WBC		H/L	
	6 week		8 week	
	Mean ± Standard deviation	Mean ± Standard deviation	Mean ± Standard deviation	Mean ± Standard deviation
T <sub>1</sub>	27.9±0.033 <sup>a</sup>	28.2±0.20 <sup>a</sup>	0.25±0.003 <sup>a</sup>	0.25 ±0.003 <sup>a</sup>
T <sub>2</sub>	29.9±0.057 <sup>b</sup>	30.2±0.10 <sup>b</sup>	0.23±0.003 <sup>b</sup>	0.23 ±0.003 <sup>b</sup>
C	21.8±0.91 <sup>c</sup>	22.9±1.18 <sup>c</sup>	0.27±0.003 <sup>c</sup>	0.28±0.003 <sup>c</sup>

Various letter indicates critical contrasts (P<0.05) between treatment

Table 6: The impact of dietary spinach in blood glucose and total protein of broilers at 6 and 8 weeks

Time; groups	Glucose mg/dl		Total protein	
	Mean ± Standard deviation		Mean ± Standard deviation	
	6 week	8 week	6 week	8 week
T <sub>1</sub>	239.8±0.44 <sup>a</sup>	245.4±0.30 <sup>a</sup>	3.8±0.016 <sup>a</sup>	3.9±0.008 <sup>a</sup>
T <sub>2</sub>	183.2±0.50 <sup>b</sup>	185.9±0.33 <sup>b</sup>	4.2±0.01 <sup>b</sup>	4.4±0.006 <sup>b</sup>
C	250.8±0.75 <sup>c</sup>	253.2±0.52 <sup>c</sup>	2.7±0.018 <sup>c</sup>	2.8±0.01 <sup>c</sup>

Various letter indicates critical contrasts (P<0.05) between treatment

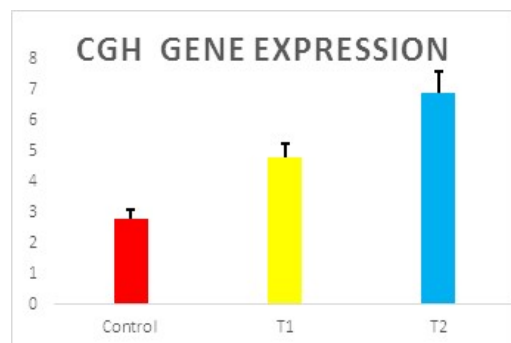


Fig 2: Effect of spinach on CGH gene expression for 8 weeks by qPCR

8100 UI from vitamin A this beta carotenes are consider as active factors (antioxidant) and contribute in improvement of physiological and immunity state of chicks (Hassan, 2006; Sevcikova *et al.*, 2008; Mohsen, 2007). The critical increment of WBC include rate in T<sub>1</sub> and T<sub>2</sub> bunches had given a view about the overall soundness of chicks (Al-Daragi, 1995). Expanding of H/L rate allude to openness of chicks to cut off pressure because of increment corticosteroid level in serum (Dosary, 2012). inhibition of this rate in T<sub>1</sub> and T<sub>2</sub> groups related to the role of spinach in improvement of general health of chicks. In general, an overwhelmingly high WBC count of generalized or localized infections, trauma, toxicities, neoplasms, and so on (Doneley and Doneley, 2010). No significant difference was observed in study of Aikpitanyi and Egweh (2020) when lymphocyte values obtained (75.50% - 83.33%) between T<sub>1</sub> (control), T<sub>2</sub> (diet with ginger) and T<sub>3</sub> (diet with pepper), but were higher than the referenced ranges of 45-70 per cent (Tvedten, 2010) and 54-73 per cent (Oleforuh-okoleh *et al.*, 2015). Also there are significant inhibition in glucose concentration in blood in the T<sub>1</sub> and T<sub>2</sub> groups as compared with control group, this results agree with researcher (Abdel, 2010) who recorded that the spinach is inhibit the glucose concentration in blood. Total protein concentration was increased significantly in T<sub>1</sub> and T<sub>2</sub> as compared with control group in both

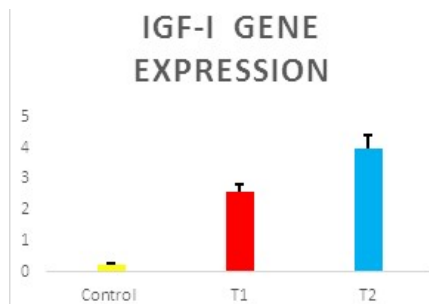


Fig 3: Effect of spinach on IGF-I gene expression for 6 weeks by qPCR

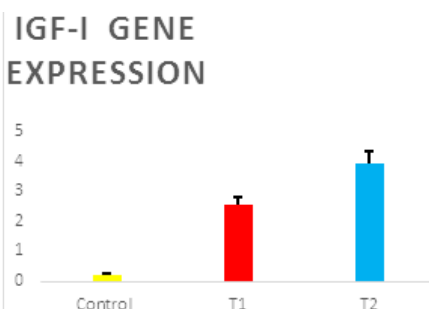


Fig 4: Effect of spinach on IGF-I gene expression for 8 weeks by qPCR

weeks; this result may be related to the role of dietary spinach in decreasing the bird's exposure to stress by stimulation thyroxin hormone secretion which increases the metabolism and biochemical reaction (Maung *et al.*, 2020).

Investigation of Nguyen and Ogle (2005) showed that basal ugar diet enhanced with water spinach to layer chickens had no critical impact on sugars level, proteins proportion, weight gain and feed transformation proportion when contrasted and the control diet (Nguyen and Ogle, 2005). Unigwe *et al.* (2016) likewise announced that grill chickens took care of diets containing various levels (0 %, 5 %, 10 % and 15 %) of yam (*Ipomoea batatas*) leaf feast showed no huge impact on normal body weight gain, feed admission, feed change proportion and protein productivity proportion (Unigwe *et al.*, 2016). The outcome from this review was likewise in concurrence with the perception by Paguaia *et al.* (2014) who set up that utilizing various measures of *Moringa oleifera* leaf feast (0%, 0.2%, 0.3%, 0.4%, 0.5%) in grill and layer proportion, didn't altogether impact the oven' feed admission, weight gain, last body weight and FCR, and furthermore had non-huge consequences for feed utilization, feed change proportion and egg creation in layer chickens.

It appeared to be that the consolidation levels of leaf dinners didn't influence the satisfactoriness of the weight control plans (Paguaia *et al.*, 2014; Odulate *et al.*, 2014). Nguyen and Ogle (2005) additionally proposed that the absence of impact of the green feeds on development rate, feed transformation and meat quality was at that point even in significant supplements with moderate impact on blood picture in bird (Nguyen and Ogle, 2005). On other hand, Yu *et al.* (2009) didn't observe any critical contrasts in the insusceptible reaction of ovens getting vegetables (incorporate spinach) with their eating routine, and reasoned that the expansion of one or the other 1 or 3% level in the eating regimen has no helpful impact on the immune response creation in broilers Yu *et al.* (2009). Besides, blood natural chemistry esteems got from

investigations of Yu *et al.* (2009) and Thrall (2007) were inside the typical reach for chickens *i.e.*, blood glucose levels in the reach 200 to 500 mg/dL, blood protein 2.5 to 4.5 g/dL, egg albumin and globulin in the scope of 0.5 to 1.8 g/dL (Yu *et al.*, 2009; Thrall, 2007).

Growth hormone (GH) is necessary for differentiation of muscle, adipocytes, and other cells to modulate development and growth (Kim, 2010). In the present study, increased expression of hepatic cGH gene was observed in T2 compared T1 and control group during 6 and 8 weeks period, indicating an effective way to increase the size of the chick (Ohta *et al.*, 1999). These results also correlated with the earlier studies where increase in body weight of broilers was reported when GH was administered in ovo at specific days of embryogenesis (Kocamis *et al.*, 1999). It is suggested that IGF-I synthesis is GH-independent during embryogenesis (Tanaka *et al.*, 1996). Generally, IGF-I and -II are responsible for proliferation of pre-adipocytes, chondrocytes, and fibroblasts through amino acid stimulation, glucose uptake, increased DNA synthesis, tissue growth stimulation, and overall embryogenesis regulation (Leach and Rosselot, 1992); Guernec *et al.*, 2003).

### Conclusion

Changes in blood markers and biochemical indicators are often used to evaluate stress in broiler chicks due to, nutritional, environmental and/or pathological factors. Product of the present study demonstrated that a clear and important effect of dietary spinach especially basal diet with 6% spinach on blood and biochemical parameters and enhancement of resistance in broiler chicks and this effect more evident with an increase in the period of administration as food to these birds at 8 weeks.

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