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Effect of *Ginkgo biloba* Leaves on Growth Performance and Thyroid Functions Markers in Normal and H₂O₂ Induced Oxidative Stress in Male Broiler Chicken

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ABSTRACT As antibiotic growth promoters are being phased out from poultry diets in different global regions, there is a significant focus on exploring substitute strategies. The complete randomized design applied to the study included 216 one-day-old male strain (Ross 307) broilers assigned into six equal number groups, three replicates, each consisting of 12 chicks. The control (group 1) was fed standard diet and group 2 was exposed to oxidative stress (OS) by 0.5% ml of hydrogen peroxide (50%) concentration per liter of water. Group 3 was exposed to OS and treated with 3.5 g/kg non-fermented Ginkgo biloba leaves (Gbl), group 4 was exposed to OS and treated with 3.5 g/kg fermented Gbl, group 5 comprised normal chicken treated with 3.5 g/kg non-fermented Gbl, group 6 normal chicken consists of 3.5 g/kg fermented Gbl. The result showed that the normal chicken in group 6 treated with F- Gbl caused a significant decline (P <0.01) in feed conversation ratio FCR (1.38) compared to all groups 2,3,4 and 5 (1.53,1.56,1.48 and 1.57) respectively, while non- significant change (P > 0.01) observed inverses to control group. the final body weight (FBW) and weight gain (WG) in group 6 significantly increased (P < 0.01) as compared to all other experimental groups, and the level of T_3 and T_4 in group 6 non-significant change (P > 0.01) observed compared to group1.In summary, incorporating F-Gbl within the recommended range into broiler diet improved growth performance, physiological condition and regulated thyroid hormone function thereby increasing FI, WG and FBW.

Keywords: *Ginkgo biloba* leaves, Broiler chicken, Growth performance, Thyroid hormone, Medicinal plant.

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INTRODUCTION

Ginkgo biloba leaves have been grown and valued for their medicinal attributes, although prior research predominantly focused on extracts from these leaves (Šamec et al., 2022). The preparation of such extracts involves a complex series of steps, driving up their cost. In contrast, the fermentation technique utilized in this current research avoids the need for chemical inputs and can be conveniently employed in on-farm settings or even at an industrial level (Cao et al., 2012). Chinese herbs, Ginkgo biloba leaves (family Ginkgoaceae) have been used worldwide for high flavonoid content (Liu et al., 2022). Diets supplemented with 0.5% starter and 1.0% grower phase Aspergillus niger-fermented Ginkgo biloba leaves have previously revealed beneficial impacts on intestinal health (Zhang, 2013). Aspergillus niger is a popular probiotic species in broilers because it is a fungus that may create enzymes, including *hemicellulases*, *hydrolases*, *pectinases*, *proteases*, amylase, lipases and tannases (Rangra et al., 2021). The two main probiotic strains used in broilers at the present are, Aspergillus niger and Candida utilis (Saleh et al., 2017). The beneficial effects of fermentation products on health were presumably mostly caused by dietary total flavonoids and polysaccharides (Zhao et al., 2020). After fermentation, flavonoid aglycones are absorbed in the intestines more rapidly and easily (Zhang et al., 2015). In mammals and birds, the thyroid gland and its hormones form an endocrine system that regulates vital bodily functions like growth, energy use, and physiology function. Numerous physiological processes in animals are influenced by triiodothyronine (T_3) , including growth, maintenance of body temperature, and lipid and carbohydrate metabolism (Mahmoud et al., 2014). An essential growth enhancer in chicken is T₃ (Saleh et al., 2014). This study aims to estimate the effect of Ginkgo biloba serum leaves (Gbl) on thyroid gland secretions, including triiodothyronine and thyroxine. Also, the study aims to investigate bl on growth performance concerning thyroids hormones in broiler chicken.

MATERIALS AND METHODS

Experimental Design

This study included 216 Ross-308 strain one-day-old male broiler chickens. The birds were purchased from a local hatchery in Erbil, placed in semi-enclosed cages ($2m \times 1.5m$ pens), litter thickness 5-6 cm, house sterilized with formalin and potassium permanganate at 0.5g/m3, and then closed for three days. All proportions were provided for heating, cooling, ventilation, and humidity. The temperature was set at 32 °C and then gradually decreased by 2 °C each week until it reached 22 °C at the end of the study. The 0.5 % ml of H2O2 50% concentration / one liter within drinking water started at the first week of life age; feed and water were given *adlibitum*. The chicken was put into six equal groups; every group consisted of three replicates. For each replicate, 12 chickens. Group 1 feeds the control standard diet and represents the control group. The second group was exposed to oxidative stress (OS) by 0.5 % ml (H₂O₂). The third group included induced OS chicken treated with 3.5 g/kg of fermented Gbl supplemented to the standard diet, the fifth group contain normal chicken treated with 3.5 g/kg non-fermented Gbl supplemented to the standard diet. The last group included normal chicken given 3.5 g/kg of fermented Gbl supplemented with a standard diet.

Serum (n=48) was collected at the end of the experimental study on day 42. Blood samples were taken in centrifuge tubes without anticoagulant from five randomly selected birds from each treatment. Serum was obtained from blood samples by centrifuging them at 3000 rpm for 10 minutes, and then they were frozen at -20°C for further examination. The research project applied Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI T₃ and T₄ to measure the levels of triiodothyronine (T₃), thyroxine (T₄), and thyroid stimulating hormone (TSH) in order to assess thyroid hormone levels.

Fungous Preparation

The microorganism employed in this investigation was Aspergillus niger, sourced from the research center at Koya University's Faculty of Science and Health. Aspergillus niger was

, using Sabouraud dextrose agar. The a

cultivated by Oxoid Ltd., based in Basingstoke, UK, using Sabouraud dextrose agar. The agar culture was then incubated at 24 °C for 7 days. To obtain spores of *Aspergillus niger*, the culture dish was inverted, and the top was repeatedly tapped. A total of 4.0×10^6 spores or 0.25 g of spores were collected using the Fuchs-Rosenthal technique (Cao *et al.*, 2012).

Ginkgo biloba Fermentation Process

The *Ginkgo biloba* leaves were divided into two halves for experimentation. One half remained untreated, while the other was subjected to fermentation using *Aspergillus niger*. The fermentation process involved utilizing a solid medium comprised of a blend of Radix astragali-ginkgo leaf, wheat bran, and corncob in a ratio of 8:1.5:0.5, with a total mass of 10g. Furthermore, this medium was supplemented with 16mL of a nutrient solution containing MgSO₄·7H₂O, KH₂PO₄, peptone, (NH₄)₂SO₄, urea, and glucose in proportions of 1:4:1:6:2:4 inoculation of the medium was done using 0.1% of *Aspergillus niger* seed, followed by incubation within a temperature range of 28–30°C for two days. The mixture was placed in a plastic container, lightly compressed, covered with adhesive film, and sealed. Subsequently, the mixture was spread onto a polythene sheet and air-dried at room temperature. After six days, approximately 900 g/kg of dried material was obtained, crushed, and passed through a 0.5 mm screen (Wang *et al.*, 2018).

Statistical Analysis

The Statistical (SPSS) program was applied to run one-way ANOVA test on the experimental data (SPSS 26,2019). The data analysis was supported by descriptive statistics. Means and standard error were estimated, according to Duncan (1955), the Duncan test was used to determine whether variations among the different parameters were significant at the 0.05 level.

RESULTS

Growth Performance

The determinations of this investigation indicated that chickens exposed to OS caused a significant decrease (P < 0.01) in FBW (2116g) in contrast to the control group (2730g). Also, groups 3 and 4 (2399-2327 g) were significantly reduced (P < 0.01) in (FBW) as compared to a control group (2730 g) (Table 1). Chickens given fermented Gbl (2700g) showed a significant increase (P < 0.01) in (FBW), as compared to all other treatments. Otherwise, non-significant differences (P > 0.01) were observed as compared to the control group (2730g), as shown in (Table 1).

The values obtained in the present study for FCR throughout the entirety of the experimental duration (0–6 weeks), the chickens in group 2 were shown a significant increase (P < 0.01) in FCR (1.53), as compared with the control group (1.31). Groups 3 and 4 were shown a significant increase (P < 0.01) in FCR (1.56-1.38), respectively, as compared with a control group (1.31). Normal chickens in group 6 caused non-significant change (P > 0.01) in FCR (1.58), as compared to CG (1.31). Chicken in group 5 caused a significant increase (P < 0.01) in FCR (1.57), as compared to the non-treated control group (1.31).

The data of this study revealed that broiler chicken-induced OS caused a significant decrease (P < 0.01) in weight gain WG (2078 g/birds) during 42 days of age, as compared with the non-treated control group (2692g). Also, groups 3 and 4 were significantly reduced (P < 0.01) in WG (2361-2288 g/birds), respectively, as compared to group 1 (2692 g/birds). Normal chicken in group 5 (2214 g/birds) was decreased significantly (P < 0.01) in WG, as compared to the control group. Normal chicken-fed fermented *Ginkgo biloba* leaves (group 6) showed a significant increase (P<0.01) in (WG) (2650 g/birds), as compared to other treatments, non-significant changes were observed as compared to the control group (2692 g/birds) (Table 1).

	Growth performance						
Treatments	Initial weight (g)	FBW (g)	WG (g/bird)	FI (g/bird)	FCR		
Control group 1	38.273 ± 0.530^{a}	2730.333± 14.678 ^a	2692.060± 14.735 ^a	3539.000± 27.790 ^a	$1.3145 \pm 0.006^{\circ}$		
(H ₂ O ₂) oxidative stress group 2	38.470 ± 0.485^{a}	2116.333± 77.076°	2078.310± 76.932°	3240.666± 53.405 ^b	1.534 ± 0.062^{a}		
Non-fermented Gbl stress group 3	38.023 ± 0.155^{a}	$\begin{array}{c} 2399.666 \pm \\ 56.854^{\rm b} \end{array}$	2361.200± 57.214 ^b	3616.000± 62.002 ^a	1.563 ± 0.054^{a}		
Fermented Gbl stress group 4	$\frac{38.716 \pm }{0.056^{a}}$	2327.333± 43.383 ^b	2288.616± 9.993 ^b	3164.333± 47.248 ^b	1.484 ± 0.050^{ab}		
Non- Fermented Gbl group 5	37.966 ± 0.536^{a}	2252.333± 34.743 ^{bc}	2214.366± 34.895 ^{bc}	3489.333± 25.115 ^a	1.576 ± 0.022^{a}		
Fermented Gbl group 6	38.466 ± 0.894^{a}	2700.000± 107.024 ^a	2650.530± 106.796 ^a	3560.000 ± 58.106^{a}	$1.382 \pm 0.015^{\rm bc}$		
P. Value	0.900	< 0.01	< 0.01	< 0.01	0.004		

Table 1: Impact of *Ginkgo biloba* leaves on growth performance in normal and (H₂O₂) oxidative stress broiler chicken

Thyroid Hormone

The current investigation found that induction of oxidative stress group by 0.5% mill (con 50%) (H₂O₂) /lit. Within drinking water, it caused a substantial decrease (P < 0.01) in serum triiodothyronine (T₃) (0.62 nmol/l), as compared to its concentration in the non-treated control group (2.46 nmol/l).

Chickens-supplemented non-fermented or fermented Gbl induced OS caused a significant decrease (P < 0.01) in serum T₃ concentration (1.52 and 2.05 nmol/l), respectively, as compared to its control group (2.46 nmol/l). While normal chickens were added, non-fermented Gb showed a significant decrease (P < 0.01) in Triiodothyronine hormone (1.12 nmol/l), as compared with its concentration in the control group (2.46 nmol/l).

Chickens in group 6 caused non-significant changes observed (2.39 nmol/l) in T3, as compared to its concentration of the control group (2.46 nmol/l), as shown in (Table 2).

The data shown in (Table 2), chickens induced OS increased significantly (P < 0.01) in T_4 level (24 nmol/l), as compared to the control group (13.5 nmol/l). The group that added non-fermented Gbl with OS significantly increased (P < 0.01) in T_4 level (17.7 nmol/l), as compared to the non-treated control group (13.5 nmol/l). Fermented Gbl with OS group increased significantly (P < 0.01) in the T_4 level (16.5 nmol/l). Fermented Gbl with OS group increased significantly chicken added non-fermented Gbl to the standard diet showed a significant increase (P < 0.01) in T_4 level (26.6 nmol/l), compared to the control group (13.5 nmol/l). At the same time, normal chickens added fermented Gbl (13.3 nmol/l) non-significant differences were observed (P < 0.01) in T_4 level inversus to the control group (13.5 nmol/l), as shown in (Table 2).

Parameters	Control (group) 1	H ₂ O ₂ oxidative stress (group) 2	Non-fermented Gbl with stress (group) 3	Fermented Gbl with stress (group) 4	Non- fermented Gbl (group) 5	Fermented Gbl (group) 6	P. value
T ₃ (nmol/l) Triiodothyro nine	2.462 ± 0.084^{a}	0.629± 0.155 ^e	1.526± 0.140°	2.058 ± 0.123^{b}	$1.126 \pm 0.054^{\rm d}$	2.398 ± 0.033^{a}	<0.01
T ₄ (nmol/l) Thyroxine	13.53± 0.544 ^d	24.52 ± 0.598^{b}	17.758± 0.421 ^c	16.504± 0.980°	26.652 ± 0.383^{a}	13.33± 0.495 ^d	<0.01

Table 2: Impact of *Ginkgo biloba* leaves on (T₃) and (T₄) in normal and (H₂O₂) oxidative stress broiler chicken

DISCUSSION

FBW and FCR of broiler chickens were decreased in the group that supplied 3.5 g/kg non-fermented Gbl during (42 days) of the experiment. This may be because *Ginkgo biloba* has a bitter taste and was not utilized much by chicken (Shareena and Kumar, 2022). The undisturbed glycosidic bonds within non-fermented leaves could potentially lead to diminished availability and absorption of certain compounds (Sknepnek and Miletić, 2022). Normal chickens fed fermented *Ginkgo biloba leaves* led to significantly increasing final body weight (2700g) compared to all other treatments (G2, G3, G4, and G5) (2116, 2399, 2327, and 2252g), respectively, while normal chickens added fermented Gbl, non-significant decrease (2700g) noted in FBW as, compared to the control group (2730g).

The fermentation processes decreased the bad taste of *Ginkgo biloba* and led to acceptance and increased consumption by chickens. Zhang et al. (2012) illustrated that fermentation leads to various biochemical transformations that enhance digestibility and bioactivity while decreasing the presence of antinutritive elements and inhibiting the pathogenic microorganism from leakage in the endothelial cells of the small intestine of the hosts. This study demonstrated that normal chickens non-fermented Gbl showed a significant increase in T₄ thyroxine level (26.6 nmol/l) given and a significant decrease in T_3 (1.12 nmol/l). This result showed that non-fermented Gbl negatively affected the T₃ hormone, decreasing FI (3489 g/bird). The current results, accepted with previous studies, reported that the hypothalamic-pituitary-adrenal axes (HPA) become stimulated due to this shift, which affects the neuroendocrine system in normal function (Gong et al., 2018), another case, non-fermented Gbl contains high amounts of Ginkgolic acid considered toxic compounds to humans and animals (Boateng, 2022). study investigated that biodetoxification of (ginkgolide acid) in *Ginkgo biloba* residue (GBLR) by solid-state fermentation (SSF) and inoculation with 1×10^7 fungi per 5 g residues, supplementation with 2 % maltose and peptone, the result showed that the Ginkgolic acid content in the GBLR was reduced from (14.8 to 1.5 mg/g) after SSF (Zhou et al., 2015). Short-chain fatty acids (SCFAs), which encompass acetic acid, propionic acid, and butyric acid, originate mainly from the microbial fermentation of dietary fiber. These substances are recognized for their ability to control gut microbial activity and contribute to the well-being of the host by engaging in tissue-specific processes connected to gut barrier integrity, glucose equilibrium, and immune response modulation (Markowiak-Kopeć and Śliżewska, 2020).

Also previously found, that is a positive linear association between plasma T_3 content with FI and WG in turkeys when the temperature is held constant (Morita *et al.*, 2016). Our result showed that decreasing in T_3 levels in groups (2, 3, 4, and 5) (0.62, 1.52, 2.05, and 1.12), respectively, can be attributed to the decrease in FI, along with the raised adrenocortical activity that was discussed by (Tabeekh *et al.*, 2016). Another study demonstrated that T_3 reduced growth and feed intake (FI) by 25% without effect on feed utilization efficiency.

Adding 3.5 g/kg non-fermented Gbl in normal chicken caused dramatically decrease in the T_3 (1.12) level, while T_4 (26.6) was significantly increased. Flavonoids found in *Ginkgo biloba* leaves may explain this discovery. Flavonoids are abundant in plant-based diets and are involved in a wide range of biological activities, one of which is the production of antithyroid effects in human and experimental animals. Flavonoids can be defined as a trio of carbon atoms that attach to two benzene rings (referred to as A and B rings) (Zhang *et al.*, 2023). The aglycone components in typical flavone glycosides of *Ginkgo biloba* consist of quercetin, kaempferol, and isorhamnetin (Gray *et al.*, 2007). Flavonoids inhibit thyroid peroxidase (TPO), the enzyme that catalyzes thyroid hormone biosynthesis (Hu *et al.*, 2020). The action of thyroperoxidase is inhibited by flavonoids, which leads to a reduction in thyroid hormone availability in tissues may result from flavonoids' ability to decrease *deiodinase* activity or displace T_4 from transthyretin (TTR). Studies have demonstrated that flavonoids can disrupt various stages of thyroid hormone biosynthesis (Ohlsson *et al.*, 2010). Fig. (1).

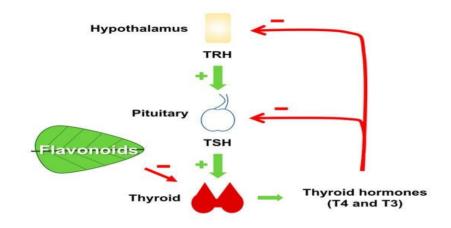


Fig. 1: Certain flavonoids possess the ability to influence the production of thyroid hormones. By inhibiting *thyroperoxidase* (TPO) (Ferreira *et al.*, 2006).

Our result revealed that normal chickens added fermented *Ginkgo biloba* leaves caused nonsignificant changes in (2.39 nmol/l) T_3 and T_4 (13.3 nmol/l) level, due to fermentation have been positive effect on thyroxine hormone and other physiological condition. During fermentation, microorganisms like bacteria and fungi can break down complex compounds present in *Ginkgo biloba* leaves, including flavonoid glycosides. β -glucosidase, an enzyme often produced by these microorganisms, plays a crucial role in the breakdown of glycosidic bonds, releasing aglycones and reducing sugar molecules, as shown in Fig. (2).

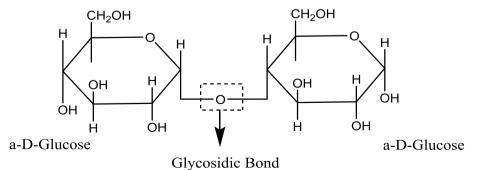


Fig. 2: Two α-D-Glucose molecules were linked by a glycosidic bond (Chen and Gibney, 2023)

In a study by Kooloth *et al.* (2019), an investigation was carried out on a β -glucosidase derived from *Aspergillus unguis* NII-08123, revealing that this particular enzyme exhibited strong resistance to elevated glucose levels. After being taken orally, flavonoids undergo specific absorption within the digestive system. Aglycones, due to their strong hydrophobic nature, are more effectively absorbed through the stomach compared to flavonoid glycosides, which do not exhibit the same efficiency in absorption (Chen *et al.*, 2022).

Fermentation is crucial within *Ginkgo biloba* leaves as it eliminates their bitter flavor and reduces the levels of flavonoids present (Wang *et al.*, 2018). In chickens, Thyroid hormones are influenced by environmental factors such as temperature, age, eating status, and pathophysiologic condition (Humphreys, 2020). In case it is generally recognized that T_3 is much more important than T_4 in the bio-oxidation processes that occur within cells (Sharma *et al.*, 2019). The ability of T_3 to bind with thyroid hormone receptors (TRs) is much higher than T_4 , which is characterized by prohormone (Lerro *et al.*, 2018).

Chicks fed 3.5 g/kg fermented Gbl-induced oxidative stress significantly decreased in triiodothyronine T_3 hormone. Meanwhile, decreasing in blood serum T_3 is in close agreement with the previous result found that diminishing blood T_3 content prevents the negative effects of catabolism in broilers exposed to heat stress. This result agrees with (Severo *et al.*, 2019), showing that heat stress causes an increase in the level of cortisol associated with to reduce the levels of T_3 and T_4 . However, in the current result, chickens fed fermented Gbl induced OS showed significant decrease in T_3 and significantly increase in T_4 . This may be due to the fact that the fermented Gbl with OS caused a negative effect and increase detrimental effects of stress content, high amounts of flavonoids and quercetin. It means that the number of hormones secreted by the thyroid gland is not represented by the actual concentration of T_3 and T_4 in blood plasma; instead, it relies on the intensity of peripheral deiodination of T_4 to the inverse form of T_3 (Abdullah *et al.*, 2022).

Initial investigations conducted on developing chickens indicated that moving the animals from their usual laboratory setting at a temperature of 23°C to a colder environment of 10°C leads to a swift decline in serum T_4 levels. This is then succeeded by a rise in serum T_3 levels. This sequence of events was hypothesized to occur due to an augmented conversion of T_4 to T_3 in the peripheral tissues (Kahl *et al.*, 2015).

CONCLUSION

Fermented *Ginkgo biloba* leaves had a positive effect on broiler thyroid gland, by regulating T_3 and T_4 level thereby control metabolic activity and increased feed intake, weight gain final body weight and overall growth performance in broiler chicks without effecting on feed utilization. The flavor of feed after fermentation was obviously enhanced by *B-glucosidase* because it reduced the number of flavonoid glycosides, broilers could improve their ability to produce chickens that healthy and affordable to consumers.

CONFLICT OF INTERESTS

No financial or personal links exist between the authors of this manuscript and any entities which could potentially influence or bias the content of this manuscript.

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تأثير اوراق نبات الجينكو على اداء النمو وعلامات وظائف الغدة الدرقية في الاجهاد التأكسدي الطبيعي والمستحث ببيروكسيد الهيدروجين في ذكور الدجاج اللاحم ريبين أزاد عمر أسماعيل صالح الكاكائي قسم علوم الحياة/ كلية العلوم والصحة/ جامعة كويه/ كردستان العراق الملخص

مع التخلص التدريجي من محفزات نمو المضادات الحيوية من وجبات الدواجن في مناطق عالمية مختلفة، هناك تركيز كبير على استكشاف استراتيجيات بديلة. شمل التصميم العشوائي الكامل المطبق على الدراسة 216 دجاجًا لاحمًا بعمر يوم واحد من السلالة (Ross 307) تم تقسيمها إلى ست مجموعات متساوية العدد، ثلاث مكررات، تتكون كل منها من 12 صوصاً. تم تغذية المجموعة الأولى على نظام غذائي قياسي (كونترول)، عُرضت المجموعة الثانية للإجهاد التأكسدي (OS) بنسبة 0.5٪ مل من بيروكسيد الهيدروجين (50٪) لكل لتر من الماء. تم تعريض المجموعة 3 بيروكسيد الهيدروجين ومعالجتها بـ 3.5 جم/ كجم من أوراق Ginkgo biloba غير المتخمرة (Gbl)، وعُرضت المجموعة 4 للإجهاد التأكسدي ومعالجتها بـ 3.5 جم/ كجم من أوراق Ginkgo biloba المتخمرة، وتتكون المجموعة 5 من دجاج عادي تم معالجته بـ 3.5 جم/ كجم. غير متخمر، المجموعة 6 دجاج عادي يتكون من 3.5 جرام/ كجم متخمر. أظهرت النتائج أن الدجاج الطبيعي في المجموعة السادسة المعاملة بتخمر Ginkgo biloba سبب انخفاضاً معنوياً (P <0.01) في نسبة كفاءة التحويل الغذائي FCR (1.38) مقارنة بجميع اوراق المجموعات 4,3,2 و5 (P > 0.01) على التوالي، في حين لم يظهر تغير ملحوظ (P > 0.01) مقابل مجموعة السيطرة. زاد وزن الجسم النهائي (FBW) وزيادة الوزن (WG) في المجموعة 6 بشكل ملحوظ (P <0.01) مقارنة بجميع المجموعات التجريبية الأخرى، ولم يتغير مستوى T₃ وT₄ في المجموعة 6 (P > 0.01). تمت ملاحظتها مقارنة بالمجموعة 1 كونترول. باختصار، أدى دمج تخمر اوراق Ginkgo biloba F-Gbl ضمن النطاق الموصى به في النظام الغذائي لدجاج التسمين إلى تحسين أداء النمو والحالة الفسيولوجية ووظيفة هرمون الغدة الدرقية المنظمة وبالتالي زيادة التغذية اليومية، زيادة الوزن، وزن الجسم النهائي على التوالي FI و WG و FBW.

الكلمات الدالة: أوراق نبات الجينكو، الدجاج اللاحم، أداء النمو، هرمونات الغدة الدرقية، النباتات الطبية.