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# The incidence of adding Gingko biloba and Curcumin extracts in drinking water on Body growth, hematological and immune statues of New Zealand White rabbit

## ABSTRACT

This study was performed to investigate the effect of adding ginkgo biloba (GB) and curcumin extracts in rabbit drinking water, as growth promoter, serum biochemistry, antioxidant enzyme activities and immunoglobin statues. A total of 40 New Zealand White rabbit (NZW) at 5 weeks of age assigned to 4 treatments with four replicates. Gingko biloba (T2) and Curcumin extracts (T3) and their mix (T4) had no significantly differences among all treatments in initial weight, feed and water intake, also in body temperature. While, body weight and body weight gain had significantly ($P \leq 0.05$) increased in the all groups of water additive compared with control (T1). As well as FCR (feed conversion ratio) had significantly ($P \leq 0.05$) improved in the treatments T2, T3 and T4, compared with the control group T1. Otherwise, the heart rate (beat) was significantly ($P \leq 0.01$) decreased in the treatments respectively compared with the control T1. Quite good results were achieved that approve the curcumin and ginkgo biloba extract has a direct influence on the production and blood parameter, improve immunoglobins and antioxidant enzymes concentrations in blood serum.



## INTRODUCTION

Rabbits are members of the Leporidae family, raised in small-holder and large-scale, commercial production systems, is marketed as whole or half carcasses, although interest in cuts and ground meat is increasing. Rabbit meat has high nutritional value but in developed countries continues to be considered for rural usage or limited to ethnic groups despite its outstanding dietetic properties (Zotte, 2014).

Ginkgo biloba are from an ancient Chinese tree that has been cultivated and held sacred for its health-promoting properties. There is substantial experimental evidence to support the view that Ginkgo biloba extract (EGB), which is the leaf extract of Ginkgo biloba, has many pharmacological effects (Zhang *et al*., 2013). Most of the studies indicated that supplementation of

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Ginkgo biloba products had favorable influences on feed efficiency, growth performance, intestinal morphology, absorption functions, and immune responses, antioxidation (Zhao *et al.*, 2011), anti-inflammation (Zhou et al., 2006). Moreover, EGB, which contains 24% flavonoid glycosides, 6% terpene lactones, and less than 5 ppm ginkgolic acid (Mohammad and Anvari, 2015).

Curcumin is a phenolic compound collectively extracted from turmeric is a dietary food and is claimed to have a number of medicinal properties such as anti-inflammatory, anti-oxidant and anti-cancer activities (Cooper *et al*., 1994). Curcumin is proving to be a potential drug molecule, and freedom from toxicity (Allen *et al.*, 1998).

This study aims to evaluate the incidence of Gingko biloba and curcumin extracts additive in drinking water on growth and some physiological parameters of New Zealand white rabbit.

## MATERIAL AND METHODS

This study was carried out at the rabbit research house in the department of animal production, Collage of agriculture/ Kirkuk University. A total of forty New Zealand white growing rabbits at age 5 weeks, with initial body weight about (640±4) g which brought from local markets. Rabbits were randomly allocated to 4 treatment groups, each of which included four replicat. The experiment lasted for 8 wk, dietary treatments were as follows: T1 (control - drinking water (DW) without adding), T2 (adding 0.25 g ginkgo biloba extract to 100 ml DW), T3 (adding 0.25 ml curcumin to 100 ml DW), T4 (adding extract mixture of 0.125 ml ginkgo biloba + 0.125 ml curcumin to 100 ml DW). Animal housed in individual cages at distance (40 cm high × 30 cm wide × 40 cm long). All animals in the four treatments were given 100 gm diet at age (5-8) weeks and 120 gm diet at age (9-13) weeks, and water offered *ad libitum*. Animals were provided photoperiod16 L: 8 D with 80 lux intensity. House temperature kept under 20±2 during winter. Rabbits were fed to fill their requirements according to NRC (1994). The ginkgo biloba and curcumin extracts were obtained from local market. The consumed feed contains 20.5% crude protein, 3100 kcal/ kg feed metabolic energy, 3.62Ca 0.9 %, % crude fiber, 0.51% P, 0.16% Na.

At the end of the experimental period, Live body weight (LBW) and body weight gain (BWG) were recorded, feed intake (FI), and feed conversion ratio (FCR) were calculated.

Blood was collected from the heart of all animals in heparinized and non-heparinized tubes. Blood samples in non-heparinized tubes centrifuged at 4000 rpm for 15 min, the serum was separated and stored at -20 °C until used in the biochemical analysis. The complete blood count (Cbc) by Swelab device made in Sweden: Red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), and a granulocyte count, the serum biochemical parameters: total protein, albumin, globulin, total cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), concentrations were estimated in serum using commercial Bioscience kits made in China. Immunoglobulins (IgG, IgA and IgM) as reported by kits from Wondfo Biotech Co. Ltd, and serum antioxidant enzyme activity of malondialdehyde (MDA), super oxide dismutase (SOD), total antioxidant capacity (TAC), glutathione Peroxidase (GSH-Px), catalase (CAT) were determined with assay kit (Sigma- Aldrich) by ELISA technique.

Statistical data were analyzed using CRD (Completely Randomized Design) by the SAS institute program (SAS, 2005). Duncan's multiple range tests were used to compare differences among the treatments at levels 0.01 and 0.05.

## RESULTS AND DISCUSSION

### RESULTS

The table 1 shows that adding GB (T2) and Curcumin extracts (T3) and their mix (T4) in drinking water of rabbits had no significantly differences among all treatments in initial weight, feed intake, water consumption and body temperature among all treatments of the study. While, body weight and body weight gain had significantly ($P \leq 0.05$) increased in the all groups of water additive, also FCR had significantly ($P \leq 0.05$) improved in T2, T3 and T4 compared with control (T1). On the other hand, heart rate is significantly ($P \leq 0.01$) lower in the treatments T2, T3 and T4 respectively.

**Table (1): The incidence of adding Gingko biloba and Curcumin extracts in New Zealand White rabbits drinking water on body weight, gain, feed intake, FCR and water consumption**

| Traits | Treatments | | | | S.E | S.L |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | | |
| IBW (g) | 268 [a] | 260 [a] | 268 [a] | 265 [a] | 6.33 | N.S |
| BW (g) | 1079 [b] | 1429 [a] | 1453 [a] | 1482 [a] | 13.37 | * |
| BWG (g) | 811 [b] | 1169 [a] | 1185 [a] | 1217 [a] | 11.67 | * |
| BWG (g/d) | 14.49 [c] | 20.78 [b] | 21.15 [ab] | 21.74 [a] | 1.59 | ** |
| FI (g/d) | 110 [a] | 110 [a] | 110 [a] | 110 [a] | 0.00 | N.S |
| FCR (g FI/ g BWG) | 7.60 [b] | 5.27 [a] | 5.20 [a] | 5.06 [a] | 0.213 | * |
| Body temperature °C | 37.8 [a] | 37.9 [a] | 37.9 [a] | 38.0 [a] | 1.48 | N.S |
| Heart rate (beats/minute) | 251 [a] | 193 [c] | 205 [b] | 200 [b] | 9.95 | ** |

IBW: Initial body weight, BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, WI: water intake, NS: means insignificant rows with the same superscripts.

The results in Table 2. shows significantly (P≤0.05) increased in total RBCs count, PCV, total WBC and lymphocytes, also Hb and Platelets count were significantly (P≤0.01) higher in the treatments of additive in drinking water compared with the control (T1). On the other hand, neutrophils had significantly (P≤0.05) lower in the treatments T2, T3 and T4 compared with control T1.

**Table (2): The incidence of adding Gingko biloba and Curcumin extracts their mix in in drinking water of New Zealand White rabbits on red blood cells, white blood cells, and platelets profiles**

| Traits | | T1 | T2 | T3 | T4 | SE | S.L |
|---|---|---|---|---|---|---|---|
| RBC profile | TRBC ($10^{12}$/l) | 4.60 [b] | 5.22 [a] | 5.68 [a] | 5.71[a] | 0.36 | * |
| | Hb (g/l) | 105 [c] | 111 [b] | 118 [ab] | 123 [a] | 6.72 | ** |
| | PCV % | 30.5 [b] | 36.5 [a] | 35.3 [a] | 36.7 [a] | 2.05 | * |
| WBC profile | TWBC ($10^{9}$/l) | 11.13 [b] | 11.97 [a] | 12.25 [a] | 12.94 [a] | 0.42 | * |
| | Lymphocyte | 44.8 [b] | 58.3 [a] | 56.03 [a] | 57.63 [a] | 2.80 | * |
| | Neutrophils | 52.10 [a] | 40.00 [b] | 42.47 [b] | 41.17 [b] | 1.79 | * |
| Platelet ($10^{9}$/l) | | 380.3 [c] | 455.3 [a] | 422.3 [b] | 463.0 [a] | 25.3 | ** |

T1= Control (Drinking water without adding), T2=0.25 ml Ginkgo biloba extract/ 1 L drinking water, T3= 0.25 ml curcumin extract/ 1 L drinking water, T4= 0.125 ml Ginkgo biloba extract + 0.125 ml curcumin extract / 1 L drinking water. [a, b, c] Means within rows with different superscripts differ significantly at (P≤ 0.05) & (P≤ 0.01).

The results in table 3. shows the impact of adding ginkgo biloba, curcumin extracts and their mix in drinking water were significantly (P≤0.01) decreased the concentrations of Triglycerides (TG), Total cholesterol (TCH), LDL, VLDL, RBS and c- reactive protein (CRP), and creatinine significantly (P≤0.05) decreased in blood serum compared with the control T1. Otherwise, HDL and protein concentration were significantly (P≤0.01) increased in the additive groups compared with the control.

**Table (3): The incidence of adding Gingko biloba and Curcumin extracts their mix in in drinking water of New Zealand White rabbits on serum lipid profile, RBS, creatinine, total protein and c-reactive protein**

| Traits | | T1 | T2 | T3 | T4 | S.E | S.L |
|---|---|---|---|---|---|---|---|
| Lipid profile (mg\dl) | TG | 169.7 [a] | 113.5 [c] | 126.7 [b] | 122.3 [bc] | 4.38 | ** |
| | TCH | 129.7 [a] | 84.3 [b] | 86.5 [b] | 79.8 [c] | 3.26 | ** |
| | HDL | 20.3 [c] | 33.7 [ab] | 32.3 [b] | 35.0 [a] | 1.67 | ** |
| | LDL | 81.2 [a] | 37.3 [bc] | 40.4 [b] | 35.5 [c] | 1.35 | ** |
| | VLDL | 28.2 [a] | 13.3 [b] | 13.8 [b] | 9.3 [c] | 1.06 | ** |
| RBS (mg\dl) | | 149.3 [a] | 102.3 [b] | 106.0 [b] | 101.3 [b] | 5.14 | * |
| creatinine (mg\dl) | | 0.635 [a] | 0.427 [b] | 0.471 [b] | 0.407 [b] | 0.038 | * |
| Total protein (g/l) | | 3.65 [c] | 4.75 [b] | 5.22 [ab] | 5.47 [a] | 0.29 | ** |
| CRP (mg/l) | | 5.33 [a] | 2.75 [bc] | 3.33 [b] | 1.62 [c] | 0.25 | ** |

TG: Triglycerides, TCH: Total cholesterol, RBS: random blood sugar- glucose, CRP: c-reactive protein.

Table 4 referred to the effect of adding ginkgo biloba, curcumin extracts and their mix in drinking water shows significantly (P≤0.05) higher concentration of immunoglobins IgA and IgM in rabbits blood serum in the treatments T4, T3 and T2 respectively compared with the control T1. Also, IgG and total immune globulins had significantly (P≤0.01) higher in the all treatments of water additive during compared with the control T1.

**Table (4): The incidence of adding Gingko biloba and Curcumin extracts their mix in in drinking water of New Zealand White rabbits on the immunoglobins profile**

| Traits | Immunoglobins profile (mg/dL) | | | | S.E | L.S |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | | |
| IgA | 0.487 $^{b}$ | 0.542 $^{a}$ | 0.560 $^{a}$ | 0.638 $^{a}$ | 0.042 | * |
| IgG | 8.79 $^{c}$ | 12.00 $^{ab}$ | 11.45 $^{b}$ | 12.75 $^{a}$ | 0.527 | ** |
| IgM | 0.293 $^{b}$ | 0.488 $^{a}$ | 0.510 $^{a}$ | 0.532 $^{a}$ | 0.048 | * |
| Total Ig | 9.57 $^{c}$ | 13.03 $^{ab}$ | 12.52 $^{b}$ | 13.92 $^{a}$ | 0.204 | ** |

T1= Control (Drinking water without adding), T2=0.25 ml Ginkgo biloba extract/ 1 L drinking water, T3= 0.25 ml curcumin extract/ 1 L drinking water, T4= 0.125 ml Ginkgo biloba extract + 0.125 ml curcumin extract / 1 L drinking water. $^{a, b, c}$ Means within rows with different superscripts differ significantly at (P≤ 0.05) & (P≤ 0.01).

Table 5 explain the impact of adding ginkgo biloba, curcumin and their mix in drinking water on rabbit oxidative enzymes in blood serum of rabbits. The results show significantly (P≤0.05) decreases in the concentration of MDA the treatments of water additives. At the same table TAC, GSH-Px and CAT were significantly (P≤0.01) higher and SOD (P≤0.05) in the treatments of the additives in drinking water compared to the control T1.

**Table (5): The incidence of adding Gingko biloba and Curcumin extracts their mix in in drinking water of New Zealand White rabbits on some oxidative indicators**

| Traits | Anti-oxidant enzymes profile | | | | S.E | L.S |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | | |
| MDA (µmol/ml) | 18.33 $^{a}$ | 12.88 $^{b}$ | 11.63 $^{b}$ | 12.09 $^{b}$ | 0.41 | * |
| TAC (U/mL) | 1.48 $^{c}$ | 2.45 $^{ab}$ | 2.19 $^{b}$ | 2.73 $^{a}$ | 0.035 | ** |
| SOD (U/mL) | 18.4± $^{b}$ | 23.39 $^{a}$ | 21.55 $^{a}$ | 23.00 $^{a}$ | 1.73 | * |
| GSH-Px (U/mL) | 31.67 $^{c}$ | 44.10 $^{a}$ | 40.91 $^{b}$ | 42.53 $^{a}$ | 2.13 | ** |
| CAT (U/mL) | 3.94 $^{c}$ | 7.50 $^{a}$ | 6.33 $^{b}$ | 7.25 $^{a}$ | 0.39 | ** |

T1= Control (Drinking water without adding), T2=0.25 ml Ginkgo biloba extract/ 1 L drinking water, T3= 0.25 ml curcumin extract/ 1 L drinking water, T4= 0.125 ml Ginkgo biloba extract + 0.125 ml curcumin extract / 1 L drinking water. MDA: Malondialdehyde (µmol/ml), TAC: total antioxidant capacity (U/mL), SOD: Super oxide dismutase (U/mL), GSH-Px: Glutathione Peroxidase (U/mL), CAT: Catalase (U/mL).

$^{a, b, c}$ Means within rows with different superscripts differ significantly at (P≤ 0.05) & (P≤ 0.01).

**DISCUSSION**

As it is briefly explained ginkgo biloba and curcumin and their mix play an important role in body weight, gain, feed intake, FCR and water consumption this result agrees with (Sadowska-Krępa, 2017), curcumin may help fight infections and some cancers, reduce inflammation, and treat digestive problems (Zorofchian *et al.*, 2014), Curcumin and Ginkgo biloba leaves extract contains many flavonoids: quercetin, kaempferol, biflavone, ginkgetin, sciadopitysin, bilobetin, roanthocyanidines, sterols and terpene lactones (Drieu and Jaggy, 2000), flavonoids and terpenes inclusion in Ginkgo biloba and curcumin extract can stimulate the release of endothelium-derived

relaxing factor (EDRF), which may increase muscle tissue blood flow through improved microcirculation and can thus improve aerobic endurance by enhancing muscular energy production, so disagree with (Teich, 2017). The additive of curcumin or ginkgo biloba are essential for health and well-being led to increase of WBCs of blood., may indicate that the immune system is working to destroy infection, an increase in white blood cells is known as leukocytosis, and it's an infection immunosuppression (Calder, 2020). Its briefly that curcumin plays an important role on increased white blood cell, curcumin showed protective effects against aflatoxine-$B_1$ induced toxicity by modulating red blood cell count, white blood cell count and Hb percentage to some extent (Sharma, 2011). Curcumin can affect different immune cells, such as various T lymphocyte subsets, macrophages, dendritic cells, B lymphocytes and natural killer cells, which results in decreasing severity of various diseases with immunological etiology (Abdollahi, 2018). Ginkgo biloba has strong anti-inflammatory and antioxidant capacities and an ability to improve circulation and have the potential to affect numerous body systems and diseases, although the science behind it still has some catching up to do, (Al-Achi, 2008). Ravi (2018) shown that ginkgo improves blood circulation as vasodilation during lower the blood viscosity and increase blood flow, it has improved the cerebral blood supply and increase tolerance to hypoxia and increase brain levels of ATP and glucose (Bridi *et al.*, 2001). Ginkgo biloba reduce cholesterol and triglycerides levels in blood, minimizes lipid peroxidation, and decreases the level of lipoprotein (Diamond, 2000 and Yao 2007). Also, curcumin can lower total cholesterol, triglycerides and LDL (Lee, 2016), and raising HDL-C, which is associated with lower risk of heart disease. (Qin, 2017 and Al-Zakaria *et al.*, 2018).

All feed additive has components that provide unique health benefits by minimizing free radical and raising the antioxidant capacity of blood and improves the body health of animal that may be enhanced immune statues (Kuan, 2012). Curcumin can help in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia. It may also help in the management of exercise-induced inflammation and muscle soreness, (Balaramnavar, 2021).

The extract of Ginkgo biloba played its neuroprotective roles by increasing the expression of SOD, CAT, and GSH-Px or by decreasing both ROS and MDA to exert a direct free radical scavenging effect (Tönnies and Trushina, 2017 and Chen et al., 2019). Many studies indicate curcumin has ability to reduce serum MDA levels as well as increase SOD and GPx activity (Wang, 2017; Yonar, 2017; Xie, 2017), However, often these studies include curcuminoids with piperine or extracts that are a mixture of different compounds (Pakfetra, 2015; Panahi, 2016), the effect of reducing the level of oxidative stress markers and increasing antioxidant capacity seems to be closely related to the mechanism of direct action of curcumin and ginkgo biloba by cleansing the body of free radicals, as well as increasing the activity of antioxidant enzymes (Nasseri *et al.*, 2017, Alizadeh, 2018). The reduction in oxidative stress depended on the duration of treatment and the curcumin dose administered, as well as the presence of piperine (Alizadeh, 2019). Under the conditions of the present study, supplementation of Ginkgo biloba and curcumin extracts in drinking water of rabbit acts as a natural antioxidant which enhanced body weight and gain, feed conversion ratio, heart rate, body temperature, whole blood and serum parameters through enhancing immune globulins and antioxidant statues.

## CONCLUSIONS

In view of the above findings and discussion, we concluded that ginkgo biloba and curcumin supplementation in drinking water, increased growth performance and body weight, and also improved the immunity responses; in addition, rabbits water supplemented with medicinal plant ginkgo biloba and curcumin improved lipid profile, immune and antioxidant indicators statues.

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## تأثير إضافة مستخلصات الجنكو بيلوبا والكركمين في مياه الشرب على نمو الجسم والحالة الدموية والمناعية للأرنب النيوزيلندي الأبيض

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**الكلمات المفتاحية:**
الأرانب ، الكركمين ، الجنكو بيلوبا ، الأداء الآنتاجي ، معاييرالدم ، الإنزيمات المضادة للأكسدة.

**الخلاصة**

أجريت هذه الدراسة لمعرفة تأثير إضافة مستخلصات الجنكو بيلوبا والكركمين في مياه شرب الأرانب ، كعوامل محفز للنمو على الأداء الانتاجي والكيموالحيوية للدم ، ونشاط الإنزيمات المضادة للأكسدة والغلوبينات المناعية. استخدمت 40 أرنب بيضاء نيوزيلندية (NZW) بعمر 5 أسابيع وزعت على أربع معاملات و بأربعة مكررات. :T2 مستخلص جينكو بيلوبا وT3: مستخلص الكركمين و T4مزيجهما في مياه الشرب للأرانب. لوحظت من نتائج هذه الدراسة عدم وجود فروق معنوية بين جميع المعاملات في الوزن الأولي، العلف وكمية الماء المستهلك، وكذلك في درجة حرارة الجسم. بينما ارتفع وزن الجسم والزيادة الوزنية للجسم معنويا ($P\leq0.05$) في جميع معاملات الأضافة للماء مقارنة مع مجموعة السيطرة T1 .وكذلك تحسن معامل التحويل الغذائي (FCR) معنويا ($P\leq0.05$) في المعاملات T2 و T3 وT4، مقارنة بمجموعة التحكم T1. وبخلاف ذلك ، انخفض معدل ضربات القلب معنويا ($P\leq0.01$) في معاملات الإضافة مقارنة بمعاملة السيطرة T1. تم تحقيق نتائج جيدة جدًا باضافة خلاصة الكركمين والجنكو بيلوبا مما كان لهما تأثير مباشر على الإنتاج ومعايير الدم وتحسين تركيز الغلوبيولينات المناعية و الأنزيمات المضادة للأكسدة في مصل دم الأرانب.