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EFFECT OF GREEN TEA ON SOME PHYSIOLOGICAL PARAMETERS AND LIVER FUNCTIONS IN MALE QUAIL EXPOSED TO DIFFERENT STOCKING DENSITY

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ABSTRACT

This study aims to investigate the effect of overcrowding alone and overcrowding combined with green tea (GT) extract on various physiological characteristics in Japanese quails. A total of 45 six-week-old male quails were utilized, and they were divided into three main groups. The first group consisted of 15 birds, served as the control group and was bred under normal conditions, with a stocking density of 15 birds per square meter. The second and third groups each included 15 birds divided into three replicates and were bred under overcrowded conditions, with 75 birds per square meter. The third group was given a water extract of GT in their drinking water. After 30 days, the birds' body weight, liver weight, serum and blood parameters, and liver function criteria were measured. The results showed that overcrowding had a negative impact on body weight, total protein, albumin, globulin, lymphocytes, and several haematological parameters (RBCs, WBCs, Hb, PCV, and MCHC). On the other hand, overcrowding led to an increase in parameters such as heterophil, basophil, liver weight, ALT, AST, MCV, and MCH. Quails treated with GT extract showed an increase in lymphocytes, basophils, neutrophils, and monocyte levels, as well as elevated body weight, total protein, globulin, and most haematological parameters (Hb, PCV, RBCs, WBCs, and MCHC). Additionally, levels of heterophil, ALT, MCV, and MCH decreased in the group treated with GT extract. In conclusion, overcrowding harms quail reproduction, behaviour, and nutrition. However, using the aqueous GT extract could mitigate these effects and improve the overall health of quail.

Keywords: Overcrowding, *Camellia sinensis*, Stocking Density.

INTRODUCTION

The oxidation of nutrients not only provides organisms with energy, but also helps in the defense action of cells by creating elements and compounds called free radicals. The production of free radicals at low to moderate levels is expected. However, these radicals can be harmful at higher amounts (Ponnampalam *et al*., 2022). If produced in excessive amounts beyond the body's ability to control them, free radicals will cause

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undesirable phenoma termed oxidative stress (Hutton & McGraw, 2016).

In efforts to improve poultry production, farmers try to use any available land, leading to increased stocking density in poultry housing. Stocking density is defined as the number of birds or the birds' live weight in a limited space. The over-crowding or overstocking in poultry farming usually leads to competition among them for space, feed, and water, which in turn results in bad feeding and less production, as well as oxidative stress (Mahrose *et al*., 2019; Nasr *et al*., 2021; Louis & Kloor, 2023). Due to the high sensitivity of poultry to oxidative processes, elevated ratios of unsaturated muscle lipids can result. The detrimental impact of oxidative stress extends to the protective barriers against pathogens and impairs immune system functions. In addition, oxidation has been suggested to be involved in a range of metabolic and physiological issues affecting birds' production, including congestive heart failure. Other features influencing poultry meat grade, include wooden breasts, pink colour defects, and white striping in broiler breast muscles (Jasim & Rasheed, 2012; Almoteoty *et al*., 2022).

Fortunately, cells and tissues are typically safeguarded against free radicals by an antioxidant defense system. This system contains various endogenous and exogenous compounds. Additionally, some animal and plant products can be categorized as exogenous antioxidants (Sies & Jones, 2020).

Green Tea, scientifically known as *Camellia sinensis,* is a widely enjoyed herbal plant that offers a wide range of health benefits. It is known to have anti-inflammatory and antioxidant properties. So, it has the potential to be an effective therapeutic agent for various diseases (Fang *et al*., 2019). It contains many effective materials like amino acids, purine alkaloids, polyphenols, and poly-saccharides. Such compounds affect various body organs and systems, including the immune system, by acting on different immune cells (Sun *et al*., 2022). Many studies have shown the beneficial effects of GT or its derivatives on liver enzyme levels due to their polyphenolic compounds (Mahmoodi *et al*., 2020).

Quail birds have significant potential to increase the supply of dietary protein for humans through their meat and eggs. Due to their fast growth, quails are preferred over other types of poultry. Quails also possess inherent immunity and great resistance against several poultry diseases, which reduces the need for in-feed antibiotics and growth promoters in their diets. Therefore, incorporating the GT or its by-products into the diet of quails could enhance their productivity, ultimately resulting in substantial economic benefits (Alagawany *et al*., 2020).

MATERIALS AND METHODS

Sample Size and Experimental Design

A total of 45 six-week-old male Japanese quail chicks were utilized in the current study. The birds were housed in a floor room that contained many ventilation fans. It was provided with artificial light for about 16 hours and kept in the dark for about 8 hours daily. Food and water were available *ad libitum*, and their containers were arranged in a designated space at home without impacting the stocking density. The bird's weights were about (150 ± 10 g). They were left for a week before applying for the experiments, which lasted for 30 days. The quails were randomly allotted to three main groups: The first control group comprised 15 birds fed and watered naturally in an *ad libitum* manner, with a stocking density of 15 birds / 1m^2. The second group consisted of 15 birds divided into three replicates (3*5). They were also fed and watered naturally, but they were bred in an overcrowded environment at a rate of 75 birds per square meter at a rate of 75 birds/1m^2. The last group also consisted of 15 birds divided into three replicates (3*5). they were housed under the same conditions

as the second group. in addition, they were watered with a GT water extract instead of normal drinking water.

GT extract preparation

The water extract of GT was prepared according to (Y Jasem *et al*., 2008) by soaking 10g of homogenized dry GT leaves (obtained from local markets in Mosul City) in 750 ml of (90°C after boiling) deionized water. The mixture was kept for 3 minutes before being filtered by a clean, dry gauze and cooled before being given to the quails.

Specimens & Lab tests

At the end of the experiments, the birds fasted overnight, their blood samples were collected according to (Rasheed & Al Nuaimmi, 2022), from the wing vein by using 3 ml syringes, each blood sample then divided into two parts, the first was saved in an EDTA tube and used to estimate blood picture parameters in plasma such as red blood cells (RBCs), white blood cells (WBCs), lymphocytes, monocytes, heterophils, basophils, haemoglobin (Hb), packed cell volume (PCV), and neutrophils, while the second was was centrifuged after coagulation, the resulting serum was used to test several biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransaminase (AST), total protein, albumin, and globulin. These tests were conducted using standard lab procedures and commercially available diagnostic kits (provided by Randox/UK). Additionally, the following parameters were also measured: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Statistical analysis

All data were analyzed using the linear procedure for one-way analysis of variance (ANOVA) and by using SPSS Version 16. The significance was determined by Duncan's multiple range test and considered at $P \leq 0.05$ (Heinisch, 1960).

RESULTS

Results of Table (1) show a significant decrease ($P<0.05$) in each of the total protein, albumin, and globulin levels as well as the body weight of the overcrowded quails, in contrast to the control. While a significant increase was shown in ALT and AST, as well as the liver weight of the overcrowded quails, compared to the control. The same table shows a significant increase ($P<0.05$) in body weight, total protein, and globulin levels in the GT-treated quails, compared to the overcrowded group and the control group. The significant increase was also noticed in albumin, but it did not rise over the control, while a significant decrease was noticed in each of the liver weight, ALT, and AST of the GT-treated quails, compared to the overcrowded group. It was also noticed that the liver weight of the GT-treated quails was comparable to that of the control.

The results of white blood cell differentiation in Table (2) showed a significant decrease ($P \leq 0.05$) in the percentages of lymphocytes, monocytes, and neutrophils in overcrowded quails, compared to the control group, while the percentages of heterophils and basophils were significantly increased. In the case of GT-treated quails, a significant increase occurred in lymphocytes compared to the over-crowded group, as well as the control group. A significant increase also occurred in neutrophils, which became like the control. While there were no significant changes in monocytes or basophils, the addition of GT extract caused a significant depression ($P \leq 0.05$) in heterophils, compared to the overcrowded and control groups. The ratio of stress heterophils/lymphocytes (H/L) showed a significant increase in the overcrowded group, compared to the control group, and a significant decrease in the GT-treated group, compared to the overcrowded group, as well as the control group.

The results in Table (3) show a significant decrease ($P<0.05$) in Hb, PCV, RBCs, WBCs, and MCHC of the overcrowded quails in contrast to the control group. The same parameters were increased in GT-treated quails; they became higher than those of the control group. While the values of MCV and MCH were significantly increased ($P<0.05$) in overcrowding and decreased in GT-treated quails.

Table 1: The effect of overcrowding and the addition of GT extract on levels of the total protein, albumin, globulin, ALT, and AST, as well as the body's weight and liver weight of male quails.

Groups	Body weight (grams)	Liver weight (grams)	ALT* IU / L	AST* IU / L	Total Protein (g / dL)	Albumin (g / dL)	Globulin (g / dL)
Control	208.4 ± 1.77b	1.52 ± 0.07b	34.89 ± 0.49b	62.14 ± 0.39c	6.04 ± 0.09b	o.93 ± 0.03a	5.1 ± 0.09b
Stress	143.93 ± 3.32c	2.55 ± 0.1a	78.4 ± 0.89a	76.57 ± 0.76a	3.62 ± 0.13c	o.51 ± 0.05c	3.1 ± 0.15c
Stress + GT extract	220.06 ± 3.01a	1.35 ± 0.05b	32.76 ± 0.73c	69.48 ± 0.67b	6.54 ± 0.12a	0.79 ± 0.04b	5.72 ± 0.1a

*ALT (alanine aminotransferase), AST (aspartate aminotransaminase)
*Values are expressed as mean ± SE,
*n = 5 animals
*Different letters in each column refer to the significance (P ≤ 0.05)

Table 2: The effect of overcrowding and the addition of GT extract on the white blood cell differentiation of the male quail.

Groups	Monocytes %	Lymphocytes %	heterophils %	Basophils %	Neutrophils %	Ratio of Stress H/L
Control	5.6 ± 0.13a	49.4 ± 0.21b	38.31 ± 0.25b	3.93 ± 0.21b	2.93 ± 0.11a	0.77 ± 0.007b
Stress	5.06 ± 0.21b	31.06 ± 0.22c	56.46 ± 0.53a	5.86 ± 0.29a	2.46 ± 0.13b	1.82 ± 0.02a
Stress + GT extract	5.2 ± 0.17ab	56.26 ± 0.37a	29.53 ± 0.37c	5.93 ± 0.18a	3.06 ± 0.18a	0.52 ± 0.009c

*Values are expressed as mean ± SE,
*n = 5 animals
* Different letters in each column refer to the significance (P ≤ 0.05)

Table 3: The effect of overcrowding and the addition of GT extract on the blood parameters of male quails.

Groups	Hb* g / dl	PCV* %	RBC* Cell* 10^6 /mm^3	WBC* Cell* 10^5/mm^3	MCV* fl	MCH* pg	MCHC* g / dl
Control	17.28 ± 0.22b	39.96 ± 0.37b	5.06 ± 0.22b	24.73 ± 0.35B	81.02 ± 3.55b	35.09 ± 1.69b	43.29 ± 0.70b
Stress	12.58 ± 0.22c	30.96 ± 0.20c	2.4 ± 0.13c	18.93 ± 0.53C	134.06 ± 6.70a	54.39 ± 2,76a	40.69 ± 0.84c
Stress + GT extract	20.34 ± 0.24a	41.06 ± 0.41a	7.26 ± 0.3a	29.8 ± 0.49A	57.94 ± 2.57c	28.88 ± 1.41c	49.83 ± 0.79a

*Values are expressed as mean ± SE,
*n = 5 animals
* Different letters in each column refer to the significance (P ≤ 0.05)

DISCUSSION

Our results unequivocally demonstrate that high stocking density has a detrimental impact on the body weight of quails, and we firmly assert that high stocking density significantly and adversely affects the body weight of quails in multiple ways. When birds are overcrowded in a limited space, they compete for water and food, affecting their physical access to the feeders and leading to malnutrition. Overcrowding leads to heat stress, which causes birds to eat less to regulate their body temperature (Ahmed *et al*., 2022). We also think that conducting our experiments during a hot season influenced the results we obtained, as the high environmental temperatures were enough to raise the birds' body temperatures, increasing heat stress. Together, malnutrition and heat stress could result in poor-quality housing, leading to severe oxidative stress in the quails (Goo *et al*., 2019; Simitzis *et al*., 2012). Oxidative stress itself can stimulate negative physiological changes and oxidative damage in proteins, lipids, and nucleic acids (Akbarian *et al*., 2016). Moreover, oxidative stress can also decrease the digestibility and absorption of nutrients (Aslam *et al*., 2021). All the reasons mentioned previously can account for the negative impact of overcrowding on quail body weight values. The results of the current study are consistent with the findings of Qaid *et al*. (2016), who discovered that birds stocked at lower densities consumed less food, gained more weight, and metabolized more efficiently. Similarly, Attia *et al*. (2012) suggested that an increase in stocking density from 12 to 24 birds per 2000 cm² resulted in reduced body weight. Askar *et al*. (2004) also confirmed the negative effects of high stocking density on quail growth and resting behaviour, which was attributed to disturbances caused by other birds. Furthermore, a study by Karthiayini *et al*. (2014) demonstrated a significant increase in live body weight and feed intake when stocking density decreased.

In response to overcrowding and poor food intake, quails may increase their fat synthesis and storage, which usually occurs in their livers. This may explain why the livers' weight increases during overstocking, and of course, this affects the livers' function (Son *et al*., 2022). AST and ALT were analyzed to indicate the liver damage, and we found that their concentrations were significantly increased. This is confirmed by the results of He *et al*. (2019), who demonstrated that stocking stress increased the serum levels of AST and ALT.

In the present study, we assessed all types of WBCs to understand the effect of GT supplementation. Additionally, they are used to determine the state of the immune system (Kang *et al*., 2016). The results indicated a fluctuation in WBC percentages due to overstocking, with some increasing and others decreasing. This variation may be correlated with the reduction in size of lymphatic organs caused by overcrowding (Rasheed *et al*., 2022). Overstocking led to a significant increase in heterophils and a decrease in lymphocytes, resulting in a higher H/L ratio compared to the control group. The H/L ratio is a useful measure of stress in poultry (Kang *et al*., 2016). This result agreed with El-Lethey *et al*. (2000), who confirmed the influence of housing conditions on the H / L ratio.

During the current study, the haematological parameters were assessed to evaluate the safety and effectiveness of GT in optimizing bird performance, without compromising their general health state (Mahlake *et al*., 2021). It was also used to indicate the patho-physiological status of the quails (Bostami *et al*., 2021). During the overcrowding, a significant deficiency occurred in nearly all

haematological parameters, except MCV and MCH. This may reflect a feed deficiency. Additionally, nutritional deficiency, especially during the stage of energy-consuming, may cause significant physiological stress, which manifests itself in decreased hematological indicators (Bostami *et al*., 2021). It was shown that overcrowded quails had significantly lower RBC values, and we suggest that this was due to the decreased lifespan of existing RBCs and the prevention of the evolution of new RBCs in the bone marrow (Rasheed *et al*., 2022). It is known that RBCs are very sensitive to oxidative damage, due to their high oxygen content. This sensitivity may cause adverse outcomes. RBCs are expected to be the first cells affected by oxidative stress, because haemoglobin is an effective stimulator of the oxidative stress process (Akinyemi & Adewole, 2021). Haemoglobin oxidation also leads to the production of Heinz bodies, which are removed by the reticuloendothelial macrophage system. However, this process results in more RBCs undergoing hemolysis (Rasheed *et al*., 2022). Our results were matched with Bratte (2011), who assumed that the feed-restricted broilers beyond 4 days had lowered RBCs, haemoglobin, and mean cell haemoglobin concentration compared to birds that were fed in an *ad libitum* pattern. Another study showed that elevated stocking density had stimulated hemodilution and PCV depression in broiler chickens (Abudabos *et al*., 2013).

The bioactive components of herbs, if used in correct amounts, will be beneficial for animal health. The digestive enzymes and immune system functions can be improved by powdered or extracted plant-based materials, and they have been utilized as a substitute for antibiotics (Aziz-Aliabadi *et al*., 2023). In the current study, there was a significant difference between over-stocking with GT consumption and overstocking without GT. These changes were represented in the body weight, immune cells, blood parameters and liver function. We will try to discuss these positive changes. GT contains a huge number of polyphenols, which have unique antioxidant, anticancer, antiinflammatory, and antibacterial properties (Cueva *et al*., 2020). Polyphenols may also improve the gut microbes by increasing beneficial bacteria in the digestive tract. These beneficial bacteria have an important role in the digestion of birds. They can produce many essential enzymes that facilitate their digestion (Reda *et al*., 2021). GT also contains vitamins, amino acids, and protein, which could improve the growth of quails (Kamil *et al*., 2021). Furthermore, the polyphenol content of GT can improve the liver antioxidant enzyme, including oxidized glutathione and glutathione peroxidase (Musial *et al*., 2020).

GT contains a powerful antioxidant phenolic compound, which protects the body in several ways: it prevents the oxidation of lipoproteins, damage to red blood cells, and platelet aggregation (Abdel-Moneim *et al*., 2022). Their chemical structures, especially in the number and locations of the hydroxyl groups, gave them antioxidant features (Mohamed *et al*., 2015). Phenolic compounds can neutralize free radicals by donating electrons or hydrogen atoms (El Gharras, 2009). They may also stop the generation of free radicals or deactivate the active species and precursors of free radicals (Tsao, 2010). Moreover, Phenolic compounds act as direct radical scavengers in the lipid peroxidation reactions, thus inhibiting this chain reaction. Polyphenols may also chelate metals, such as $Fe^{2}+$, which can lower the rate of Fenton reaction, thus preventing the oxidation of hydroxyl radicals (Tsao, 2010). It was observed that adding 300 mg/kg of tea polyphenols to broiler diets was enough to increase the total antioxidant capacity in the blood of broiler chickens (Li *et al*., 2012). Others investigated that adding GT powder to the diet can promote the growth of immune organs in white feather broilers

and improve the activity of the antioxidant enzymes and elevate the production of immunoglobulin (Yin, 2017). All the above explained how GT improved the general health of quails in the current study, and the action of GT was confirmed through a significant decrease of H/L index after GT treatment.

CONCLUSION

Based on the findings of the current study, it can be concluded that overcrowding has a significantly negative impact on the breeding, behaviour, and nutrition of quails. This negative influence may be due to elevated levels of oxidative stress, which in turn affects their weight, liver function, and various physiological parameters. However, the use of GT water extract has been shown to mitigate these negative effects and improve the overall health of the quails. Therefore, it is preferable to breed poultry in adequate space and provide them with optimal living conditions, as well as natural materials, to maximize their productivity.

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Conflict of Interest
There is no conflict of interest.

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تأثير الشاي الاخضر على بعض المعايير الفسلجية وضائف الكبد في ذكور طائر السمان المعرضة لظروف اكتظاظ مختلفة

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اجريت الدراسة الحالية بهدف إيجاد تأثير التكدس وتأثيراستخدام مستخلص الشاي الأخضر على المعايير الفسيولوجية المختلفة لطائر السمان. إذ تم استخدام 45 من ذكور طائر السمان بعمر ستة أسابيع، وتم تقسيمها إلى ثلاثة مجاميع رئيسية، ضمت المجموعة الاولى وهي المجموعة الضابطة ١٥ طائر تم تربيتها بظروف تغذية وسقي طبيعية وبمعدل اكتظاظ بلغ ١٥ طائر/١ م^{٢} ، بينما خضعت المجموعة الثانية التي بلغ تعدادها 15 طائر بواقع ثلاث مكررات (٣*٥) الى جهد اكتظاظ بلغ ٧٥ طائر/١ م^{٢} ، وكذلك المجموعة الثالثة ضمت 15 طائر بواقع ثلاث مكررات (٣*٥) وبمعدل اكتظاظ ٧٥ طائر/١ م^{٢} مع امداد الطيور في هذه المجموعة بالمستخلص المائي للشاي الأخضر في ماء الشرب. استمرت تربية المجاميع الثلاثة لمدة ٣٠ يوما قبل إجراء الفحوصات التي تضمنت وزن الجسم ووزن الكبد والعديد من الفحوصات المصلية ومقاييس الدم بالإضافة إلى الفحوصات المتعلقة بوظائف الكبد. أظهرت النتائج وجود تأثير سلبي للتكدس على كل من الخلايا الليمفاوية ووزن الجسم والبروتين الكلي والألبومين والجلوبيولين بالإضافة إلى التأثير السلبي على العديد من معايير الدم التي شملت (Hb، PCV، RBCs، WBCs، MCHC)، وسجلت الدراسة زيادة في مقاييس اخرى مثل (heterophil، basophil وزن الكبد، ALT، AST، MCV، MCH) في حين أن المعاملة بمستخلص الشاي الأخضر مع التكدس أدى إلى زيادة كل من الخلايا الليمفاوية والقاعدية والمتعادلة والوحيدات، وقد لوحظ أيضًا زيادة في وزن الجسم والبروتين الكلي والجلوبيولين وغالبية معايير الدم (Hb، PCV، RBCs، WBCs، MCHC) ، بينما انخفضت مقاييس أخرى مثل (heterophil، ALT، MCV، MCH) ، يمكن استنتاج أن الاكتظاظ له تأثير سلبي كبير على تكاثر السمان وسلوكه وتغذيته. ومع ذلك، فقد ثبت أن استخدام مستخلص GT المائي يخفف من هذه الآثار السلبية ويحسن الصحة العامة للسمان.

الكلمات الدالة: الإكتظاظ , طيور السمان , *Camellia sinensis*.