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Article

Effect of Probiotic or Thymol Supplementation on Physiological and Biochemical Parameters in Growing Rats

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Abstract: The goal of the current study was to evaluate the effects of probiotic and thymol supplementation, both alone and in combination, for growth performance, hematological indices, serum biochemical parameters (urea, creatinine levels), lipid profile (TC), (TG), (LDL-C) and (HDL-C) in addition to antioxidant status (Catalase activity), inflammatory markers (interleukin-6). A total of thirty-two rats were assigned randomly in a block design experiment into four groups (n = 8): control, probiotic, thymol and probiotic + thymol for 56 days. Growth performance was assessed through body weight gain, feed intake, and (FCR). Hematological, biochemical, lipid, oxidative stress, antioxidant, and inflammatory parameters were subsequently determined. Final publication of probiotic and thymol supplementation will increase physiological/ biochemical variables to the control group. The combined treatment generated by highest final body weight and body weight gain and the lowest FCR. It also evidenced positive alterations in RBC, hemoglobin, PCV, liver- and kidney-associated biochemical markers, serum glucose, total protein, and albumin. Total cholesterol, triglycerides, and LDL were reduced, whereas HDL increased. We significantly improved oxidative status, showing a decline in (MDA) and rise in (SOD), (CAT), (GPx), and (TAC). Inflammatory markers, including TNF-α, IL-6, and IL-1β, also they lowered. In conclusion, the combination of probiotic and thymol had potent advantages as genomic approaches that have a synergistic action on growth, metabolic health, antioxidant defense capacity and inflammatory regulation in growing rats.

Keywords: Probiotic, Thymol, Oxidative Stress, Inflammatory Markers, Growing Rats

Introduction

In particular, nutrition is one of integral components to maintain normal growth, metabolic homeostasis and immune competence. In the active growth phase, the elevated need for nutrients and the requirement to regulate metabolism endow metabolic states with high sensitivity to both dietary composition and functional supplements. Consequently, during the last few years a many of attention is paid to natural nutritional strategies that can ameliorate growth performance while at the same time promoting metabolic, antioxidant and immune functions. Among these interventions, probiotics and plant-derived bioactivess compound has attracted considerable attention owing to their capacity to

modulate gut function, nutrients digestiones and absorptions , oxidative state as lipid metabolism and immune inflammatory processes without relying entirely on classical pharmaceuticals. The emergences with spreat of anti microbial resistance has led to a need for alternative and supplementary biological solutions that could potentially decrease the dependence on traditional antimicrobial therapies. Such approaches are especially pertinent for nutritional and biomedical studies that can be made on natural bioactive compounds as well as beneficial microorganisms, pharmacological potential properties including antibacterial, antifungal, anti-inflammatory bioactivities and other health-promoting benefits [1]. Microorganisms are a great source of biologically and nutritionally active compounds [1]. and have been exploited for many food and biotechnological applications for far better period of time. Especially fungi and yeasts can be sources of proteins, enzymes, vitamins, organic acids and various other important metabolites [2], thus indicating a wider category of microbial resources that could have functional nutrition and health-oriented appropriate amounts confer a healths benefiting to the hosts [3]. The biological effects that they exert are strain specific, which may involve interactions with resident gut microbes, epithelial cells, metabolites and molecules of host immune system. Probiotic organisms, especially species of Lactobacillus and Bifidobacterium have now become significant components of functional foods and nutriceuticals. According to a report, these benefits include the maintenance of balance of intestinal micro-flora, improvement of function of epithelial barriers, regulation of immune activities and generation or modulation with biologically active microbial metabolites [4].

The way from the gastrointestinal microbiota in nutrientes digestion, energy metabolisms , and immune homeostasis. Therefore, modulation of this microbial ecosystem by probiotics may thus impact on physiological processes that are not restricted to the gastrointestinal tract. The probiotics-associated metabolic activity can alter both the levels of little chain fat acids production and others microbials metabolites that will signal to intestinal epithelial and immune cells. Interventions of some bacteria might ameliorate gastrointestinal diseases and influence the functional regulation systemically [5]. The experimental evidence also shows that probiotic supplementation affects hematological and immune parameters. For example, Bibi et al. [6], which show similar changes in hematological indices, immunoglobulins and inflammatory markers after probiotic administration you also agree with broader relationship between probiotics intake and physiological and immune status of the host. An additional key domain in probiotic science pertains to oxidative stress. Oxidative stresses arises if the generations from reactives oxygen species (Nox) exceeds the availability of their endogenous antioxidants to neutralize them. Chronic oxidative imbalance may result in lipid peroxidation, cell injury, alterations in metabolism and the activation of inflammatory signaling pathways. Based on experimental studies, some probiotics increase the endogenous antioxidant defense. [7] revealed that, in a rat model of high-fat diet-induced obesity, oral administration of Lactobacillus fermentum DALI02 reduced malondialdehyde (MDA) and inflammatory mediators but increased (SOD), (CAT), (GPx), and total antioxidant capacity [7]. These findings demonstrated the strong interplay among modulation of gut microflora, oxidative homeostasis and systemic inflammation.

Probiotic supplementation has also attracted interest for its possible effects on lipid and hepatic metabolism. Al- Muzafar and Amin [8] demonstrated that a probiotic mixture improved lipid profiles and inflammatory biomarkers in rats subjected to a high-fat and high-sucrose diet. Similarly, [9] reported reductions in serum cholesterol, triglycerides, LDL, and hepatic enzyme activities following administration of selected probiotic bacteria in hypercholesterolemic rats. Although the magnitudes with directions of probiotic effects depend on bacterial strain, dose, diet, and physiological status, these observations support the concept that probiotic supplementation can influence systemic metabolic parameters in addition to its local effects within the intestinal environment.

There is also increasing evidence that oxidative stress and inflammation are functionally interrelated. In contrast, excess reactive oxygen species can activate inflammatory signalling pathways and sustained inflammation can further increase oxidative damage, leading to a self-reinforcing biological feedback loop. Probiotics have therefore been studied as nutritional alterations that can impact both processes simultaneously. Providing Clinical Evidence A systematics reviewing with meta- analysis by Naseri et al. [10] Probiotic or synbiotic supplementation was associated with reductions in several inflammatory and oxidative-stress biomarkers, and improved total antioxidant

capacity, Recent meta-analytic evidence also supports favourable effects of supplementation on an array of biomarkers such as TNF-α and MDA, although discrepant responses within different populations and experimental conditions have been noted regarding some specific biomarkers [11], [12]. Besides the probiotics, natural phytogenic compound has garnered more and more attention in nutrition and biomedical studies. Thymol [2-isopropyl-5-methylphenol] is a natural aromatic monoterpene phenol, and an importantes components of essential oils from Thymus species or other aromatic plants. Cause of its antimicrobial, antioxidant, anti-inflammatory and other biological activities, it has received considerable scientific attention. Gabbai-Armelin et al. [13] systematically reviewed the anti - inflammatories effects of thymol. The mechanisms discussed in the literature include modulation of mediators associated with NF-κB, MAPK, JAK/STAT, and arachidonic-acid signaling [14].

Thymol is the avid antioxidant that has a vital therapeutic relevance towards maintaining physiological and biochemical homeostasis in nature. Its phenolic structure is involved in its capacity for interaction with oxidative mechanisms, while experimental studies indicate that it may also act through regulation of endogenous antioxidant defenses and cellular signaling pathways. Only recently have some articles reported that thymol can affect pathways such as Nrf2, NF-κB and other oxidative stress and inflammatory molecular systems [15]. These effects lend phylogenic rationality to exploring thymol, not just as an antimicrobial phytochemical but also a dietary bioactive with systemic physiological consequences. Data from animal studies back these properties up even further. Thymol reduced hydrocortisone-induced hepatic oxidative damage in rats reported by Aboelwafa and Yousef [16], including better profiles of serum liver enzymes, oxidative markers, total antioxidant capacity, protein status, and TNF-α. Lahmi et al. [17] additionally found that thymol significantly decreased ALT, AST and TNF-α in diet-induced fatty liver disorders in rats. Our results suggesting that the biological effects of thymol may measure biochemical markers of hepatic and metabolic function. This research showed moderate evidence for antioxidant and anti-inflammatory activities of thymol (aged in the laboratory until October 2023). defense. Peirovy and Asle-Rousta [18] demonstrated that thymol reduced oxidative stress and pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, while influencing the Nrf2/HO-1 and NF-κB-associated pathways in stressed rats. Similarly, Abdelgawad et al. [19] reported that thymol attenuated hepatic oxidative stress, lipid peroxidation, inflammatory alterations, and dyslipidemia in rats exposed to imidacloprid. These experimental data taken together indicate that thymol has impact on multiple complementary physiological systems rather than through a specific biological mechanism.

There are substantial studies demonstrating effects of probiotics or thymol administered independently; however, fewer have investigated their combined administration under normal growing conditions. This is a key area of research because the two supplements may affect complementary biological targets. Probiotics have primary interactions between the gastrointestinal microbial ecosystem and host–microbe signaling while thymol has antioxidant, antimicrobial and anti-inflammatory effects respectively. Thus concurrent supplementation could invoke more systemic response than individual strategies. Still, such an effect alone should not be regarded as synergetic without appropriate experimental support and direct comparisons among the individual supplementation groups and the combined ones are needed. In addition, assessing the biological effects of nutritional interventions only from body weight or growth performance results in an incomplete picture. However, a more integrative assessment should include hematological, serum markers of liver and kidney functions, glucose and protein metabolism, lipid profile analysis, oxidative stress measurement as well as endogenous antioxidant capacity and inflammatory cytokines profiling. Acknowledging these results in unison may give an integrative view of the effect of dietary supplement on physiological homeostasis during growth. Therefore, the present study was planned to evaluate individual and combined impacts of supplemented probiotic together with thymol on some physiological and biochemical variables in growing rats. The study concentration include growth performance and feed utilization, hematological parameters, serum biochemical indices, lipid profile, oxidant-antioxidant balance as well as inflammatory cytokines including TNF-α, IL-6 and IL-1β. The study also determined if supplementation with both probiotic and thymol elicits a larger overall physiological response than either supplement alone.

Materials and Method

Experimental Design and Animals

In total, 32 healthy growing rats of approximately 135 g initial body weight was selected for the experiment. Animals were routinely maintained in standarding laboratory conditions and free reach to feed and drinking water during the entire duration of the experiment. Rats were adapted to the laboratory environment for a duration of time before to design(n=8). The groups included a control group with basal diet without supplementation, probiotic supplemented group (bacterium species and strain), thymol-supplemented group and combined probiotic plus thymol treatment. The experimental treatments continued for 56 consecutive days. All animals were feed the some basal diet and received each respective supplement according to their respective treatment. During the experimentals periods , data was collected regarding general health and feed consumption.

Growth Performance

The bodies weighting of individuals was measured in both the beginnings and end of a 56-day long experimental period. The gain in body weight was determined as the different of final and initial body weight. During the trial, feed intake was monitored by measuring the quantity of feed offered and residual feed. Feeding conversion ratio (FCR) was computed as the total amount of feed consumed per kilogram of body weight gain and was used as an efficiency measure for feed-utilization.

Blood Collection and Hematological Examination

Blood samples was taken from the appropriate animals under laboratory conditions at the end of experimental duration. A fraction of each blood samples was taken into tubeses with an anticoagulant and used for hematological measurements. The hematological parameters were (RBC), (Hb), (PCV), (WBC) and platelet count. The rest of the blood samples were allowed to clot and then centrifuged, in order to recover the serum. Serum separated was taked and stored at appropriate cooled/frozen temperatures until biochemical tests were conducted.

Serum Biochemical and Lipid Parameters

Method of Analyzing Biochemical Parameters Serum samples were analyzed for various possible metabolic correlates of hepatic and renal function. Includes alanines amino transferase , aspartate amino transferase , alkaline phos-phatase, urea, creatinine, glucose, total protein and albumin. Serum total cholesterol, triglycerides, HDL-C and LDL-C were determined. Biochemical and lipid parameter was measured using the standard recommended laboratory colorimetric and enzymatic methods for each analytic.

Inflammatory Markers

Serum concentrations of TNF-α, IL-6, and IL-1β were used to assess the systemic inflammatory response. Concentration of these inflammatory cytokines were measured by (ELISA) procedures designed for use with rat serum samples.

Statistical Analysis

The information were presenteding as mean ± standard deviation (SD). And (ANOVA) was used to evaluate differences among the four experimental groups. Using a multiple-comparison procedure means were significant for differences between groups. Statistically significants differences among the experimental groups are indicated by different superscript letteres within the same parameter.

Results

Growth performance

The impacts of probiotic and thymol supplementation on growthing performances of growing rats are presented in Table 1. The initial body weights were comparable among all experimental groups, with no significant differences noted at the beginning of the experiment ($P > 0.05$).After 56 days of supplementation, the supplemented groups displayed greater final body weight and body weight gain in comprised to the control group. The highest final body weight was recorded in rats receiving the combined probiotic and thymol treatment (274.6 ± 11.8 g), followed by the probiotic group (263.9 ± 11.3 g) and thymol group (258.7 ± 10.9 g), compared with 246.8 ± 10.7 g in the control group. Body weight gain showed a similar pattern, reaching 139.3 ± 9.0 g in the combined group compared with 112.2 ± 7.9

g in the control group ($P < 0.05$).Feed intake was slightly higher in the supplemented groups, whereas the feed conversion ratio was improved. The combined treatment produced the lowest FCR (2.82 ± 0.17), compared with 3.38 ± 0.21 in the control group ($P < 0.05$).

Table 1. Effect of probiotic and thymol supplementation on growth performance of growing rats.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
Initial body weight (g)	134.6 ± 5.2[a]	135.1 ± 5.0[a]	134.8 ± 5.4[a]	135.3 ± 5.1[a]
Final body weight (g)	246.8 ± 10.7[c]	263.9 ± 11.3[b]	258.7 ± 10.9[b]	274.6 ± 11.8[a]
Body weight gain (g)	112.2 ± 7.9[c]	128.8 ± 8.4[b]	123.9 ± 8.1[b]	139.3 ± 9.0[a]
Feed intake (g/day)	18.9 ± 0.8[a]	19.4 ± 0.7[a]	19.2 ± 0.8[a]	19.5 ± 0.7[a]
FCR	3.38 ± 0.21[a]	3.08 ± 0.18[b]	3.15 ± 0.19[b]	2.82 ± 0.17[c]

Values are presented as mean ± SD (n = 8). Different superscript letters within the same row indicate significant differences ($P < 0.05$).

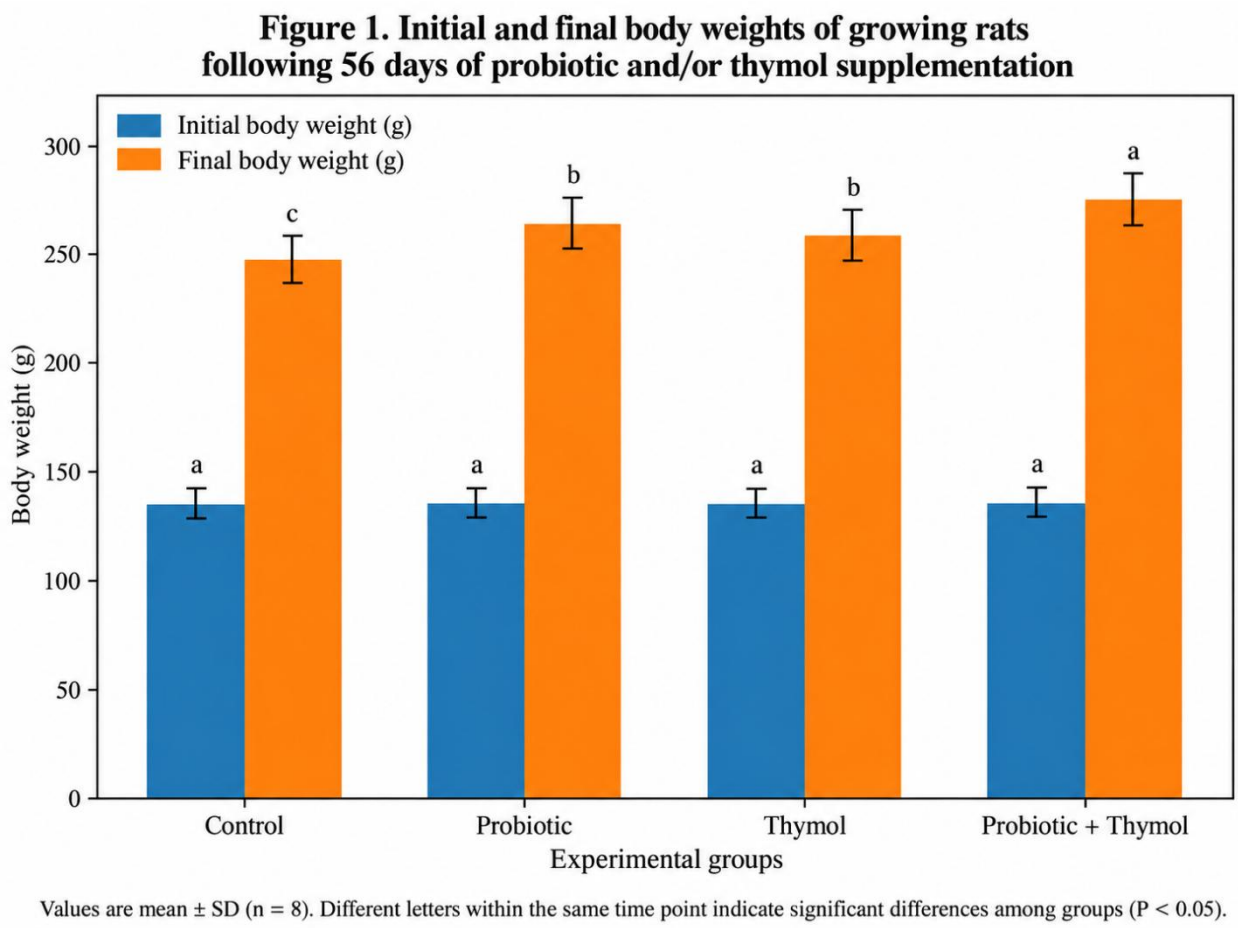

Figure 1. Initial and final body weights of growing rats following 56 days of probiotic and/or thymol supplementation. Values are expressed as mean ± SD (n = 8). Different superscript letters indicate significant differences among experimental groups at $P < 0.05$.

Hematological parameters

The hematological findings are summarized in Table 2. Probiotic and thymol supplementation resulted in moderate improvements in several hematological parameters. RBC count increasing from

7.12 ± 0.31 ×10⁶/μL in the control groups to 7.48 ± 0.34 and 7.39 ± 0.29 ×10⁶/μL in the probiotic and thymol groups, respectively. The highest RBC count were noted in the combined therapy group (7.71 ± 0.32 ×10⁶/μL).

Similarly, hemoglobin and PCV values were high ring in the supplementing groups comparative with control group . Hemoglobin reached 15.1 ± 0.5 g/dL in the combined therapy groups compared with 14.1 ± 0.6 g/dL in controls. WBC counts showed a slight increase following supplementation, while remaining within a physiologically acceptable range.

Table 2. Hematological parameters of growing rats following probiotic and thymol supplementation.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
RBC (×10⁶/μL)	7.12 ± 0.31^{c}	7.48 ± 0.34^{b}	7.39 ± 0.29^{b}	7.71 ± 0.32^{a}
Hb (g/dL)	14.1 ± 0.6^{c}	14.8 ± 0.5^{b}	14.6 ± 0.6^{b}	15.1 ± 0.5^{a}
PCV (%)	42.3 ± 1.8^{c}	44.5 ± 1.7^{b}	43.9 ± 1.9^{b}	45.6 ± 1.6^{a}
WBC (×10³/μL)	8.21 ± 0.55^{b}	8.76 ± 0.61^{a}	8.69 ± 0.58^{a}	9.02 ± 0.60^{a}
Platelets (×10³/μL)	742 ± 39^{a}	771 ± 42^{a}	764 ± 41^{a}	789 ± 40^{a}

Serum biochemical parameters

As seen in Table 3, probiotic and thymol supplementation significantly affected several serum biochemical parameters. Serum ALT and AST activity was lower in the supplemented groups than on the control groups . ALT decreased from 48.6 ± 3.9 U/L in controls to 38.9 ± 3.1 U/L in the combined treatment group.

A related trends were noted for AST, which decreased from 112.4 ± 7.8 U/L in the control group to 96.3 ± 6.5 U/L following combined supplementation. Urea and creatinine concentrations also showed moderate reductions, with the lowest values noticed in the combined treatment group.

Conversely , total protein and albumin concentrations increased following supplementation. Total protein reached 6.84 ± 0.27 g/dL in the combined treatment group compared with 6.31 ± 0.31 g/dL in controls.

Table 3. Serum biochemical parameters in growing rats.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
ALT (U/L)	48.6 ± 3.9^{a}	43.1 ± 3.5^{b}	41.8 ± 3.2^{b}	38.9 ± 3.1^{c}
AST (U/L)	112.4 ± 7.8^{a}	104.2 ± 7.1^{b}	101.6 ± 6.9^{b}	96.3 ± 6.5^{c}
ALP (U/L)	171.5 ± 11.4^{a}	163.2 ± 10.7^{b}	160.8 ± 10.2^{b}	153.6 ± 9.8^{c}
Urea (mg/dL)	39.4 ± 2.7^{a}	36.8 ± 2.5^{b}	35.9 ± 2.4^{b}	34.2 ± 2.3^{c}
Creatinine (mg/dL)	0.72 ± 0.06^{a}	0.68 ± 0.05^{ab}	0.67 ± 0.05^{b}	0.64 ± 0.05^{b}
Glucose (mg/dL)	108.6 ± 6.4^{a}	101.7 ± 5.9^{b}	98.9 ± 6.1^{b}	96.2 ± 5.7^{b}
Total protein (g/dL)	6.31 ± 0.31^{c}	6.67 ± 0.28^{b}	6.59 ± 0.30^{b}	6.84 ± 0.27^{a}
Albumin (g/dL)	3.72 ± 0.18^{b}	3.91 ± 0.17^{ab}	3.87 ± 0.16^{ab}	4.02 ± 0.18^{a}

Lipid profile

Probiotic and thymol supplementation improved the serum lipid profile (Table 4). Total cholesterol decreased from 92.7 ± 5.6 mg/dL in the control group to 84.9 ± 5.1 and 81.6 ± 4.9 mg/dL in the probiotic and thymol groups, respectively. The lowest cholesterol concentration was recorded in the combined treatment group (76.8 ± 4.7 mg/dL).

Triglyceride and LDL concentrations followed a similar pattern. In contrast, HDL concentration increased from 42.1 ± 2.8 mg/dL in the control group to 49.2 ± 2.6 mg/dL in the combined treatment group ($P < 0.05$).

Table 4. Serum lipid profile of growing rats.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
Total cholesterol (mg/dL)	92.7 ± 5.6[a]	84.9 ± 5.1[b]	81.6 ± 4.9[b]	76.8 ± 4.7[c]
TG (mg/dL)	78.4 ± 5.2[a]	70.6 ± 4.8[b]	67.9 ± 4.6[b]	63.1 ± 4.3[c]
HDL (mg/dL)	42.1 ± 2.8[c]	45.6 ± 2.7[b]	46.8 ± 2.9[b]	49.2 ± 2.6[a]
LDL (mg/dL)	34.9 ± 3.1[a]	29.8 ± 2.8[b]	27.5 ± 2.6[b]	24.7 ± 2.5[c]

Table 5. Oxidative stress and antioxidant parameters.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
MDA (nmol/mL)	4.82 ± 0.36[a]	3.91 ± 0.31[b]	3.47 ± 0.29[b]	2.91 ± 0.25[c]
SOD (U/mL)	7.84 ± 0.52[c]	9.12 ± 0.61[b]	9.48 ± 0.58[b]	10.37 ± 0.65[a]
CAT (U/mL)	28.6 ± 2.1[c]	33.4 ± 2.3[b]	35.1 ± 2.4[b]	38.7 ± 2.6[a]
GPx (U/mL)	6.42 ± 0.44[c]	7.38 ± 0.48[b]	7.71 ± 0.51[b]	8.43 ± 0.53[a]
TAC (mmol/L)	0.91 ± 0.07[c]	1.08 ± 0.08[b]	1.14 ± 0.09[b]	1.27 ± 0.09[a]

Figure 2a: MDA :

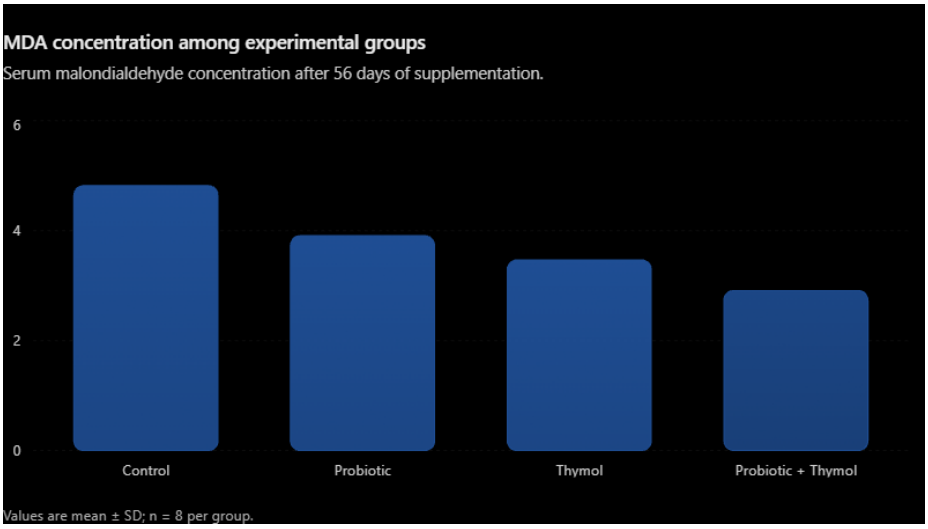

Figure 2b: Antioxidant enzymes: SOD, CAT, and GPx :

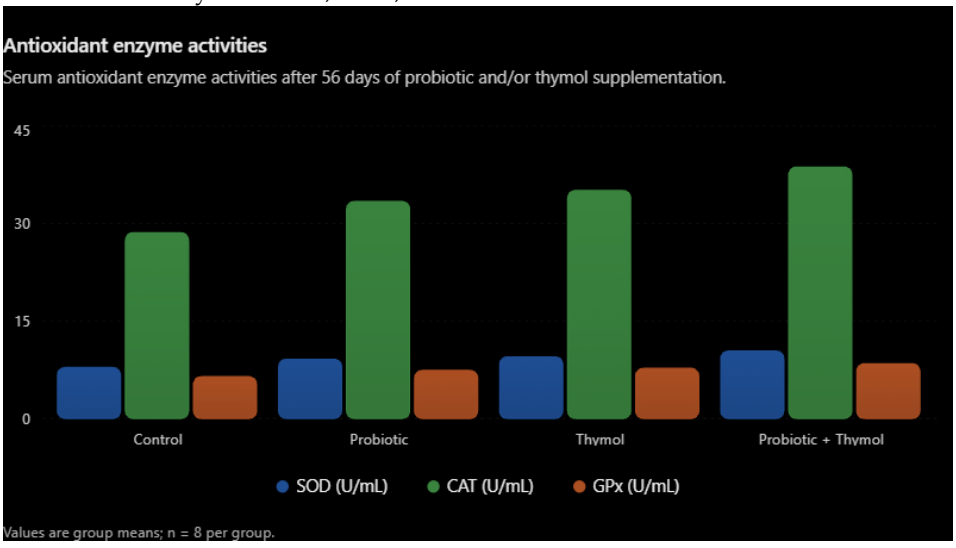

Figure 2. Effect of probiotic and thymol supplementation on oxidative stress and antioxidant status in growing rats. Values represent mean ± SD (n = 8). Different superscript letters indicate significant differences among groups at $P < 0.05$.

Inflammatory markers

The concentrations of inflammatory cytokines are presented in Table 6. In comparison to the control group, supplementation with probiotic or thymol resulted in lower serum concentrations of TNF-α, IL-6, and IL-1β. The combined treatment produced the greatest reduction in all measured inflammatory markers.

TNF-α decreased from 31.6 ± 2.7 pg/mL in the control group to 21.1 ± 1.9 pg/mL in the combined treatment group. Similarly, IL-6 decreased from 18.9 ± 1.8 to 12.1 ± 1.2 pg/mL, while IL-1β decreased from 12.7 ± 1.3 to 8.4 ± 0.9 pg/mL.

Table 6. Serum inflammatory markers.

Parameter	Control	Probiotic	Thymol	Probiotic + Thymol
TNF-α (pg/mL)	31.6 ± 2.7[a]	26.8 ± 2.3[b]	24.7 ± 2.2[b]	21.1 ± 1.9[c]
IL-6 (pg/mL)	18.9 ± 1.8[a]	15.7 ± 1.5[b]	14.2 ± 1.4[b]	12.1 ± 1.2[c]
IL-1β (pg/mL)	12.7 ± 1.3[a]	10.6 ± 1.1[b]	9.8 ± 1.0[b]	8.4 ± 0.9[c]

Figure 3. Inflammatory markers :

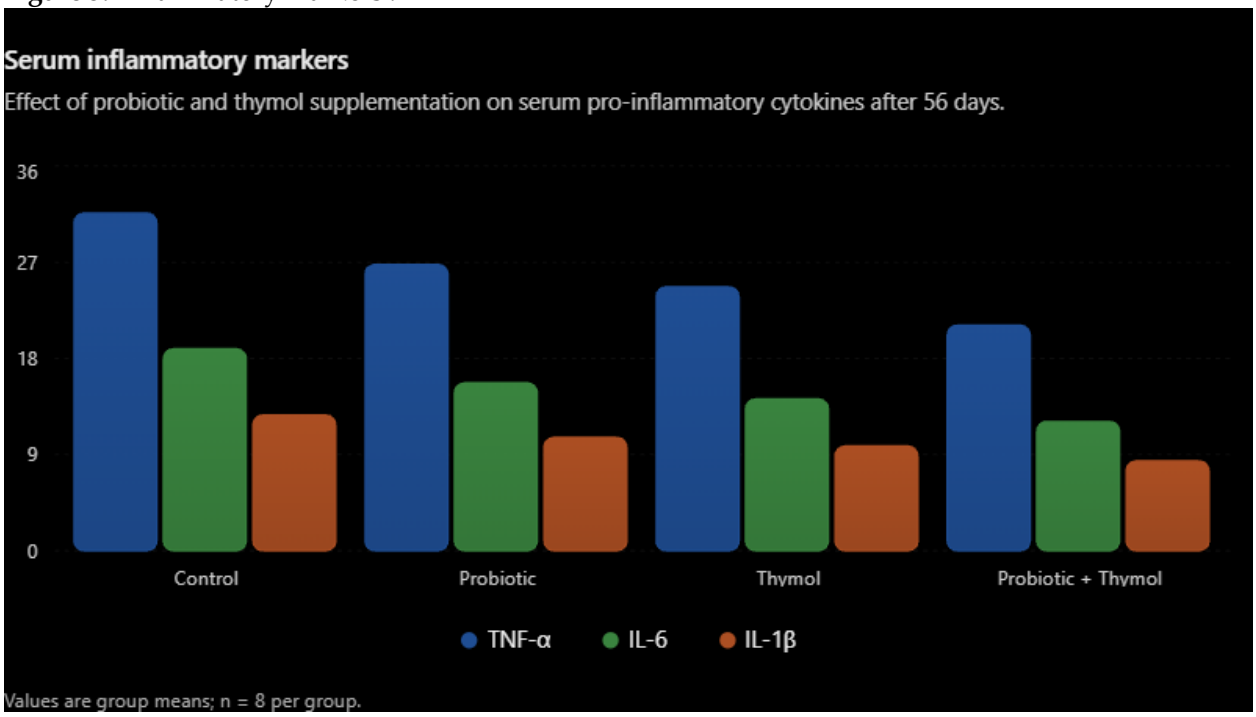

Figure 3. Effect of probiotic and thymol supplementation on serum inflammatory markers in growing rats. TNF-α, IL-6, and IL-1β concentrations are expressed as pg/mL. Values represent mean ± SD (n = 8), and different superscript letters indicate significant differences at $P < 0.05$.

Overall response to supplementation

Overall, we observed beneficial effects of both probiotic and thymol supplementation on growth performance, hematological parameters, serum enzymes profiles, lipids and antioxidant status, and inflammatory markers compared with the control group. The combined probiotic and thymol treatment generally produced the greatest numerical improvement across the measured parameters. The most clear response detected in the oxidative stress markers and combined supplementation significantly decreased MDA, meanwhile increased SOD, CAT, GPx and TAC. These results indicate that the treatment could offer an increased antioxidant protection in growing rats.

Discussion

The currents research was created to investigating the impacts of either single or dual administration of probiotic and thymol on growth rate, blood counts, blood chemistry, fat

absorption/utilization, body's defense systems against oxidation and inflammation in young rats. During the 56 day of the study, each treatment alone resulted in primarily beneficial physiological responses compared to the control groups . However, when used in combination, the two treatments showed the greatest number of positive numerical results for many of the variables tested. Therefore, it appears that combining a diet-based intervention that targets the gut microbiota with a naturally occurring bioactive substance from plants may provide advantages, although the mechanisms involved in producing such responses were not evaluated.

The animals receiving supplements showed better growth, especially those who received both the probiotic and thymol. Rats' final body weights increased from 246.8 ± 10.7 g (control) to 274.6 ± 11.8 g (probiotic+thymol), and their total body weight gain increased from 112.2 ± 7.9 to 139.3 ± 9.0 g. Also, there were a decreasing in the feed conversion ratio from 3.38 ± 0.21 to 2.82 ± 0.17 indicating improved feed efficiency. There are two potential ways that probiotics could improve nutrient utilization: by altering the rat's intestinal microbiota activity and/or by improving the rat's digestive processes. Thymol might also provide additional benefits to the rat's intestinal environment. Since feed digestibility, microbiota composition, and digestive enzyme activities were not assessed in this study, it is possible but unproven that these mechanisms exist. In contrast to this study which utilized normally growing rats, Al-Muzafar and Amin [8] utilized a probiotic mixture with rats fed a high-fat, high-sucrose diet. Their focus was primarily on metabolic and hepatic consequences of this type of diet. Therefore, due to the different types of diets used, it is not easy to compare the growth response of each study.

Hematology results indicated slight increases in total RBC count, Hb levels and PCV following supplementation, and overall the greatest increases occurred in the combined group. As an illustration of this point, RBC count increased from 7.12 ± 0.31 x 106/μL in the control group to 7.71 ± 0.32 x 106/μL after the combined supplementation, while Hb levels increased from 14.1 ± 0.6 to 15.1 ± 0.5 g/dL. Total WBC count was slightly higher; however, there were no significant differences in total platelet count among any of the groups. While these changes may represent differences in nutritional or physiological statuses, it is impossible to determine whether these changes reflect improved erythropoiesis or improved immune function without measuring both functions directly. Bibi et al. [6] studied hematologic and immune-related responses to probiotic treatment in Wistar rats, which provides a relevant animal model context for the potential that probiotic treatment may affect systemic indices; however, it must be recognized that there will be differences in probiotic preparation and study design.

Biochemical analysis of serum showed that supplemented rats had lower enzyme activity levels for ALT, AST, and ALP; lower levels of urea and creatinine; and higher total protein and albumin levels. For the combined-treatment group, there was a decrease in ALT enzyme activity level from 48.6 ± 3.9 units per liter (U/L) to 38.9 ± 3.1 U/L and a drop in AST enzyme activity level from 112.4 ± 7.8 U/L to 96.3 ± 6.5 U/L when compared to the control group. The results of the current study are line with the results of Aboelwafa and Yousef [16], which demonstrated that thymol decreased hydrocortisone-induced enzyme activities in the liver and increased oxidative stress in the liver while increasing indices related to protein synthesis in adult male rats. Similarly, Abdelgawad et al. [19] demonstrated that thymol decreased biochemical and oxidative abnormalities in the livers of rats exposed to imidacloprid. However, it should be noticed that both of these previous studies utilized models of experimentally produced damage to the liver whereas the present study investigated growing rats prior to receiving any damaging agents. Therefore, the differences observed in the current study should be seen as a providing evidence that the serum biochemical parameters were improved rather than providing direct evidence of protection against damage to the liver or kidneys. Histological examination and more precise assessments of function of each organ would be needed to provide more definitive evidence of protective effects.

As well as improving the blood lipid profile, supplementation had a positive effect on all components of the lipid profile. After supplementation, total cholesterol decreased from 92.7 ± 5.6 to 76.8 ± 4.7 mg/dL; triglycerides decreased from 78.4 ± 5.2 to 63.1 ± 4.3 mg/dL; and LDL cholesterol decreased from 34.9 ± 3.1 to 24.7 ± 2.5 mg/dL. On the other hand, HDL cholesterol rise from 42.1 ± 2.8 to 49.2 ± 2.6 mg/dL. These results are similar to those of Al-Muzafar and Amin (2017) who found that the administration of a probiotic mix improved lipid profiles and inflammatory markers in rats fed a

high-fat/high-sucrose diet. Additionally, Aminlari et al. [9] shown that the administration of specific probiotic bacteria resulted in improved lipid parameters in rats fed a cholesterol-rich diet. Abdelgawad et al. [19] have also shown that thymol administration can improve dyslipidemia caused by toxicant exposure. Although the studies mentioned above provide evidence for the biological feasibility of the lipid-modulating properties of various supplements, caution must be used when comparing the magnitude of these effects. The variations in dietary intake, methods of disease induction, supplement formulations and treatment durations among studies do not allow for direct comparison of the effect size.

Serum malondialdehyde levels decreased from 4.82 ± 0.36 nmol/mL in the control group to 2.91 ± 0.25 nmol/mL in the combined treatment group. In contrast, serum superoxide dismutase, catalase, glutathione peroxidase, and total antioxidant capacity increased. The results of this research show that one of the most consistent responses to the experimental treatments was a change in oxidative stress and antioxidant capacity. Similar to the current study, Huang et al. [7] demonstrated that Lactobacillus fermentum DALI02 reduced oxidative stress and inflammation when administered to rats fed a high-fat diet. Therefore, there appears to be a link between using probiotics and improving antioxidant-related parameters. Additionally, Aboelwafa and Yousef [16] reported that thymol administration improved oxidative-stress markers in rats treated with hydrocortisone. Furthermore, Abdelgawad et al. [19]demonstrated that thymol reduced hepatic lipid peroxidation and oxidative damage. Consequently, the finding of the present investigation are consistent with both types of preclinical data; however, measuring serum antioxidant levels alone cannot determine which particular cell pathway(s) are participate in producing these responses.

The finding of the analysis of inflammatory cytokine concentrations were related to those obtained for the analysis of MDA concentrations. In contrast to control groups, the combined administration of the supplements significantly reduced TNF-α concentration (from 31.6 ± 2.7 pg/mL to 21.1 ± 1.9 pg/mL), IL-6 concentration (from 18.9 ± 1.8 pg/mL to 12.1 ± 1.2 pg/mL), and IL-1β concentration (from 12.7 ± 1.3 pg/mL to 8.4 ± 0.9 pg/mL). Al-Muzafar & Amin (2017) found decreased concentrations of both TNF-α and IL-6 in rats receiving probiotics after exposure to a high-fat, high-sucrose diet. Aboelwafa & Yousef (2015) observed a drop in concentration of the inflammatory marker TNF-α after treating animals exposed to hydrocortisone with thymol. The consistency in direction of response to either treatment indicates that both types of treatments have potential to affect an animal's inflammatory state. Additionally, the fact that we were able to demonstrate decreases in MDA and inflammatory cytokine concentrations simultaneously is line with the generally accepted notion that oxidative stress/imbalance can contribute to inflammatory signaling. However, we cannot conclude that there are direct causal relationships among our measured variables based on this experiment alone.

The direct comparison of probiotic alone, thymol alone, and their combined use in the same growing rat model is an essential aspect of this research. While the combination group showed significantly better results than either of the single treatments in terms of many metabolic, antioxidant, and inflammatory measures, the fact that there was a greater response to the combination treatment does not provide evidence of synergy. In order to show evidence of synergy, it would be necessary to conduct an explicit interaction analysis (e.g., using a factorial model) and define an appropriate additive criterion. Additionally, interpretation of these findings is limited due to lack of direct assessment of the rats' intestinal microbiota, histopathological analysis of tissues, and evaluation of molecular signaling pathways. Future studies should examine dose-dependent effects; determine the specific probiotic strain(s) responsible for the observed effects; and perform direct testing of potential treatment interactions to assess if the combination provides additional benefit beyond what could be achieved through the use of each supplement separately.

Overall, the finding of this study are in line with other studies using rats that showed positive metabolic and inflammatory responses to using probiotics as a supplement [8], [9] and positive biochemical and antioxidant responses to thymol [16], [19]. This study has extended those findings to compare them in a nutritional context by studying both treatments alone and in combination while rats were growing. The combined treatment seemed to have beneficial effects on all of the physiological and biochemical variables we tested, however, we do not know what causes those benefits nor do we know if there is a true synergistic effect.

Conclusion

The use of probiotics and thymol had a positive effect on few physiological and biochemical variables in growing rats. Both treatments considerably improved growth performance, major hematological parameters, serum biochemical indices, lipid profiles, antioxidant defense mechanisms and inflammatory markers. In this regard, the results illustrate that, overall, the combined effects of probiotics + thymol resulted in more favorable changes than alone given either treatment on BWG and FCR (feed conversion ratio), lipid metabolism (total cholesterol and triglycerides), oxidative stress parameters, antioxidant enzyme activities) and important inflammation pathways by measuring systemic inflammatory cytokines levels. This study indicates that the joint supplementation of probiotics and thymol may have wider physiological benefits via their superior synergistic effects. However, additional histological, microbiological and molecular studies are necessary to understand the mechanisms underlying these beneficial effects.

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