EFFECT OF USING FERMENTED POMEGRANATE PEEL ON GROWING RABBITGROWTHPERFORMANCE, IMMUNITY, AND INTESTINAL HEALTH UNDER ENVIRONMENTAL HEAT STRESS

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(Received 6/8/2025, accepted 22/11/2025)

SUMMARY

This study aimed to evaluate the effect of dietary fermented pomegranate peel on growth performance, plasma metabolites, immune response, intestinal microbiota, and intestinal architecture in growing rabbits under environmental heat stress. Two hundred, 35-day-old New Zealand white rabbits were randomly assigned to five experimental groups with four replicates each. The experimental groups were as follows: CON, a basal diet serving as the control; supplemented with 1.5% pomegranate peel(PP1), 3% pomegranate peel(PP2), 1.5% fermented pomegranate peel(FPP1), and 3% fermented pomegranate peel(FPP2).The results showed significant improvements in body weight gain, feed conversion ratio, and carcass weight (P≤0.05)in rabbits fed PP2, FPP1, and FPP2 compared to the other groups; however, feed intake and other carcass characteristics were unaffected. Moreover, feeding FPP2 increased the digestibility of dry matter, crude fiber, and crude protein compared to the other groups. Furthermore, FPP2 supplementation significantly increased plasma total protein and high-density lipoprotein (HDL, (P≤0.05), while reducing triglycerides, total cholesterol, and aspartate aminotransferase (AST, (P≤0.05). FPP2 supplementation enhanced the immune response and oxidative stability by significantly increasing immunoglobulin A (IgA) and superoxide dismutase (SOD) levels, and reducing malondialdehyde (MDA, (P≤0.05). Adding FPP2 boosted gut health by significantly modifying the cecal microbiota, increasing the Lactobacillus count, and decreasing E. coli and Clostridium. Perfringens(P≤0.05) and increasing villi height and villi height: crypt depth ratio(P≤0.05). It can be concluded that supplementing with fermented pomegranate peel can enhance growth performance, lipid profile, immunity, oxidative stability, and intestinal integrity in heat-stressed rabbits. Adding fermented pomegranate peel may provide an effective way to mitigate the negative effects of heat stress on rabbits.

Keywords: *Pomegranate Peel; Rabbits; Performance; Heat Stress; Digestibility and Immunity.*

INTRODUCTION

Rabbit production may help meet the increasing demand for animal protein to alleviate meat shortages, especially since rabbit meat has a high nutritional value, due to its high protein content and low fat and low cholesterol content (Zeweil and Elgindy, 2016). However, rabbit farming suffers from high environmental temperatures during the summer, which leads to a decline in production performance through reduced daily weight gain, a decline in feed conversion ratio, and an increased mortality rate (Abdel-Moneim*et al.,*2021). In addition, it negatively impacts reproductive performance, carcass characteristics, and meat quality (Elbaz*et al.,*2025).Such approaches involve the substitution of

conventional feedstuffs or the inclusion of experimental additives designed to bolster immunity and antioxidant capacity. These additives often include natural antioxidants like essential oils, vitamin C, and pomegranate peel extract (Nassrallah *et al.,*2016; Mphahlele *et al.,*2016).

Recently, the global abundance of by-products generated by fruit and vegetable processing has garnered attention regarding their potential incorporation into animal diets (Hagag *et al.,*2023). Specifically, pomegranate by-products represent a promising reservoir of nutrients and antioxidants for rabbit formulations (Shadab *et al.,*2017). The functional phenolic properties of pomegranate and its derivatives are well-documented (Kamel *et al.,*2021; Elbaz, 2023). Given that peels account for roughly half of the fruit's total mass (Omer 2019), pomegranate peel (PP) is noted for offering significant health advantages driven by its dense concentration of bioactive compounds (Akuru *et al.,*2021). Extensive literature has verified the specific attributes of PP, citing its immunomodulatory, antibacterial, and antioxidant functions, in addition to its wound-healing and anti-atherosclerotic potential (Akuru *et al.,*2021; Haghighian *et al.,*2021; Hagag *et al.,*2023).

The application of fermentation to both conventional and alternative feed ingredients has yielded various positive outcomes. These benefits include the mitigation of anti-nutritional factors and the enrichment of feed with probiotics, prebiotics, enzymes, and antioxidants (Li *et al.,*2020; Elbaz *et al.,*2023). Within fermented substrates, probiotic bacteria and their metabolites generate lactic acid and other short-chain organic acids. These compounds promote intestinal health by reducing pH levels and suppressing pathogenic organisms (Leeuwendaal *et al.,*2022). Additionally, they bolster the digestive microbiota, enhance the uptake of minerals and amino acids, and modulate lipid profiles in the blood (Penha Filho *et al.,*2015; Katu*et al.,*2025).

These effects have been substantiated by trials involving monogastric species; for instance, incorporating fermented feeds in poultry diets has led to better animal health and production efficiency (Haghighian *et al.,*2021; Elbaz *et al.,*2023). Moreover, investigations by Czech *et al.,* (2024) indicate that including fermented rapeseed meal in rabbit diets aids immune system maturation and prevents intestinal dysbiosis. The modulation of gut microbiota further correlates with improved immune function, specifically the humoral response (including cytokine production, lysozyme activity, and immunoglobulin content), which alleviates oxidative stress and facilitates a robust defense against pathogens (Sayed *et al.,*2023; Al-Khalaifah *et al.,*2025).

In this experiment, PP was fermented utilizing a Bacillus subtilis strain. It was hypothesized that fermented pomegranate peel (FPP), via enhanced nutrient bioavailability, would lower anti-nutritional factors and potentiate immunomodulatory effects, thereby ameliorating rabbit health and feed efficiency under heat stress conditions. Consequently, the objective of this research was to assess the impact of dietary inclusion of varying levels of Bacillus subtilis-fermented FPP on the growth performance, nutrient digestibility, lipid profiles, and gut health of growing rabbits.

MATERIALS AND METHODS

The experiment was carried out at RasSadr station, South Sinai, Governorate, the Animal and Poultry Production Division, Desert Research Center, Ministry of Agriculture and Reclamation, Cairo, Egypt.

Preparation of fermented pomegranate peel (FPP):

Bacillus subtilis (KCTC 1103) was obtained from the Department of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. Pomegranate peel (PP, a by-product) was purchased from Juhayna Juice Manufacturing Company. The required amount of PP for the experiment was weighed and mixed with distilled water (to raise the humidity to 60%), then mixed with the microbe (at a rate of 1 gram per kilogram of PP), to start the fermentation process. The mixture was placed in sterile plastic bags for 48 hours after being tightly sealed, with stirring every two hours. The freshly dried fermented PP was ground and kept at room temperature for chemical analysis and preparing experimental diets.

Animals and diets:

Two hundred 35-day-old male New Zealand white rabbits, with an average weaning weight of 784 ± 8.3 g, were randomly assigned to five experimental groups with four replicates each, as follows: (CON), rabbits fed the basal diet serving as the control group; while the rabbits in the second and third groups were fed diets with 1.5 and 3% pomegranate peel added (PP1 and PP2), the rabbits in the fourth and fifth groups were fed diets with 1.5 and 3% fermented pomegranate peel added (FPP1 and FPP2),

respectively. The study lasted for 5 weeks, from 35 to 70 days of age. The basal diet was formulated to meet the nutritional requirements of growing rabbits according to the recommendations of NRC (1977), as shown in Table 1. The chemical composition of pomegranate peel was 92.3% dry matter, 16.4% crude protein, 6.1% crude fiber, and 2.9% crude fat; while the chemical composition of fermented pomegranate peel was 92.6% dry matter, 19.3% crude protein, 4.5% crude fiber, and 3.2% crude fat (AOAC, 2000). Pelleted diet and water were offered *ad libitum*. Temperature and humidity were measured during the experimental period inside the well-ventilated room where the rabbits were kept, twice daily (1 p.m. and 1 a.m.). The average daily humidity was 51.2 % and the daily temperature was 30.9°C during the experimental period.

The THI value was calculated as described by Marai *et al.,* (2001), yielding a value of 29. The equation was as follows: THI = db°C [(0.31-0.31 RH) (db°C - 14.4)], where db°C = dry bulb temperature in Celsius and RH= relative humidity percentage/100.The THI value was classified as follows: up to 27.8, absence of heat stress; from 27.8 to 28.9, moderate heat stress; from 28.9 to 30, severe heat stress; and above 30, very severe heat stress.

Performance and carcass traits:

At 70 days of age, the live body weight (LBW), and feed intake (FI) were recorded. Feed conversion ratio (FCR) and body weight gain (BWG) was calculated. At the end of the experiment, five rabbits were randomly taken from each experimental group, individually weighed, and slaughtered for carcass evaluation. Carcass, liver, kidney, heart, and lungs were weighed, and their relative weights to LBW were calculated.

Nutrient digestibility:

At the end of the experimental period, five rabbits/group were housed in metabolic cages and kept adapting for 12h before the collection period. Feces were collected for 5 days (3 times daily), dried (65 °C for 48 h), ground, and stored in polyethylene bags (− 20 °C) until the chemical analyses. Samples of feces and feed were analyzed for dry matter, crude fiber, crude protein, ether extract, and nitrogen-free extract following AOAC (2000) methods.

Table (1): Ingredients and chemical composition of experimental diets.

Item%	**Control**	**1.5 % pomegranate peel**	**3% pomegranate peel**	**1.5 % fermented pomegranate peel**	**3% fermented pomegranate peel**
Pomegranate peel	0	1.50	3.00	1.50	3.00
Yellow corn	16.9	16.9	16.9	16.9	16.9
Soybean meal (44%CP)	9.40	9.40	9.40	9.40	9.40
Alfalfa dehydrated	37.0	35.5	34.0	35.5	34.0
Wheat bran	14.0	14.0	14.0	14.0	14.0
Barley	10.0	10.0	10.0	10.0	10.0
Sunflower meal	6.00	6.00	6.00	6.00	6.00
Soybean Oil	2.00	2.00	2.00	2.00	2.00
Di-calcium phosphate	0.60	0.60	0.60	0.60	0.60
Limestone	1.20	1.20	1.20	1.20	1.20
DL-Methionine	0.15	0.15	0.15	0.15	0.15
NaCl	0.25	0.25	0.25	0.25	0.25
Premix *	0.50	0.50	0.50	0.50	0.50
Molasses	2.00	2.00	2.00	2.00	2.00
Chemical composition%					
Dry matter	90.6	90.3	89.4	90.1	90.4
Organic matter	82.6	82.4	81.7	82.1	82.3
Crude protein	18.14	18.09	18.04	18.13	18.12
Crude fiber	12.69	12.39	12.08	12.39	12.08
Ether extract	2.57	2.64	2.71	2.72	2.83

**Each kg of vitamin and mineral mixture (premix) contained: Vit A 2 000 000 IU; E: 10 mg; B1 400 mg; B2 1200 mg; B6 400 mg; B12 10 mg; D3 180000 IU; Choline chloride 240 mg; Pantothenic acid 400 mg; Niacin 1000 mg; Folic acid 1000 mg; Biotin 40 g; Manganese 1700 mg; Zinc 1400 mg; Iron 15 mg; Copper 600 mg; Selenium 20 mg; Iodine 40 mg and Magnesium 8000 mg.*

Blood biochemistry:

Upon slaughter, blood specimens were gathered into tubes containing anticoagulants and immediately centrifuged at 3000 rpm for 15 minutes to isolate plasma, which was then frozen at − 20 °C until analysis could be performed. The concentrations of plasma cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total protein, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assessed colorimetrically (Spectronic1201, Milton Roy, Ivyland, PA, USA) in accordance with the manufacturer's protocols (Spinreact Co., Girona, Spain). To quantify plasma immunoglobulin levels (IgA, IgM, and IgG), ELISA quantitation kits (Life Diagnostics Inc., PA, USA) were utilized. Additionally, plasma parameters for superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC) were analyzed using commercial kits (Spinreact Co. Girona, Spain), following the procedures outlined by Abdel-Moneim *et al.,* (2025).

Microbial count:

At slaughtering, digestasamples were collected from the cecum of five rabbits/group, placed in sterile bags, and kept at − 20 °C until microbial counts. Samples were diluted (10−1 to 10−7), immersed in an agar medium suitable for each microbe, and incubated under aerobic or anaerobic conditions and at the required temperature. *Clostridium perfringens* (*C. perfringens,*ontryptose sulfite cycloserine agar), *Escherichia coli* (*E. coli,*MacConkey agar), and *Lactobacillus* (MRS agar) were enumerated.

Histomorphometry:

To conduct the histological examination, the total length of the intestine was measured, and 3-cm tissue segments were excised from the ileum. These specimens were thoroughly rinsed with physiological saline (0.85% NaCl) and subsequently fixed in 10% formalin solution pending morphometric analysis. Tissue sections were cut to a thickness of 4 µm and stained using hematoxylin and eosin on prepared slides (Shehata *et al.,*2022). A light microscope fitted with a digital camera system (Labomed, LX 400; Labo America, Inc., USA) was employed to examine the slides and quantify villi height (VH) and crypt depth (CD), from which the villus height to crypt depth ratio (VH:CD) was calculated.

Statistical analysis:

Data were analyzed with the GLM procedure of SPSS using one-way analysis of variance (*P<0.05*). Tukey'spost-hoc test was used to estimate the statistically significant differences among the treatment groups.
The statistical model used for analyzing data was as following:

$$Y_{ij} = M + T_i + e_{ij}$$

Where:

Y_{ij} = observation of the parameter measured.
M = overall mean.
T_i = effect of treatment.
e_{ij}= random error.

RESULTS AND DISCUSSION

Growth performance:

Table 2 showed that the effect of adding fermented pomegranate peel on the growth performance of growing rabbits exposed to heat stress. Growth performance data indicated that adding pomegranate peel had a positive effect, particularly when fermented pomegranate peel was used. Feeding with eitherfermented or unfermented pomegranate peel supplementation increased BWG and improved FCR (P≤0.05) compared to the control group. However, FI was not affected (P≤0.05) by the addition of pomegranate peel. Consistent with our results, Akuru*et al.,* (2021) and Hagag*et al.,* (2023) found that adding pomegranate peel improved LBW and FCR. The growth-promoting effect of pomegranate peel supplementation may be attributed to its antioxidant and anti-inflammatory properties (Haghighian*et al.,*2021; Kamel*et al.,*2021), as well as its antimicrobial and immune-modulating effects. Nevertheless,

the best growth performance was observed in the rabbits that received fermented pomegranate peel. In line with these results, numerous reports indicate that the fermentation process of feed contributes to enhancing feeding efficiency and intestinal health by reducing pathogenic microbial and increasingvilli height(Katu*et al.,*2025), while also decreasing anti-nutritional compounds (Czech*et al.,*2024; Elbaz*et al.,*2023), which promotes nutrient utilization and consequently increases body weight.

Table (2): Effect of adding fermented or unfermented pomegranate peel on the growth performance of heat-stressedgrowing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
Initiallive body weight(g)	786	784	781	783	784	7.324	0.944
Final live body weight(g)	1641^{c}	1676^{bc}	1703^{b}	1695^{b}	1755^{a}	11.28	0.001
Body weight gain(g/day)	24.43^{d}	25.49^{c}	26.43^{b}	26.06^{b}	27.74^{a}	1.091	0.001
Feed intake (g/ day)	86.0	86.4	86.8	86.5	87.1	3.142	0.625
Feed conversion rate (gfeed:g gain)	3.52^{a}	3.39^{b}	3.28^{bc}	3.32^{b}	3.14^{c}	0.006	0.001

$^{a, b \text{ and } c}$*Means within the same row with different superscripts are significantly different*(P≤0.05)

CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.

Carcass traits:

Table 3 showed the effect of adding pomegranate peelon carcass characteristics in growing rabbits exposed to heat stress.The results showed that adding fermented pomegranate peel increased carcass weight (P≤0.05)compared to other groups, while other carcass characteristics, including heart, liver, kidneys, and lungs, were unaffected(P>0.05).Several studies have demonstrated that pomegranate peel supplementation can enhance carcass traits in poultry and rabbits, including increased (P≤0.05)carcass and muscle deposition (Hamady*et al.,*2015; Younis*et al.,*2025). Similarly, Elbaz*et al.,* (2023) observed improved carcass dressing percentages with fermented pomegranate peel supplementation, further supporting the potential of pomegranate peel to enhance carcass quality. The improvement in carcass weight observed in the present study may be attributed to several biological mechanisms associated with fermented pomegranate peel (El-Sissi*et al.,*2018; Kishawy *et al.,*2019). The bioactive compounds of fermented pomegranate peel are known to enhance intestinal integrity by improving antioxidant status and reducing inflammatory responses (Haghighian *et al.,*2021; Akur*et al.,*2021). In addition to its role in promoting a healthy gut environment by modifying the microbial content, lowering pH, and preserving intestinal membrane integrity, fermentation also plays a role in this process (Elbaz*et al.,*2023), thereby promoting a healthier digestive tract which further improves nutrient digestion and absorption (Al-Khalaifah*et al.,*2025). Collectively, these physiological improvements enhance feed utilization efficiency and support better growth performance, ultimately reflecting in improved carcass characteristics.

Table (3): Effect of adding fermented or unfermented pomegranate peel on carcass traits inheatstressed growing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
Live body weight (g)	1652^{d}	1688^{c}	1712^{b}	1706^{b}	1749^{a}	7.061	0.001
Carcass (%)	58.3^{bc}	58.7^{bc}	59.6^{b}	60.4^{ab}	61.1^{a}	3.004	0.020
Heart (%)	0.29	0.28	0.29	0.30	0.29	0.052	0.155
Liver (%)	3.15	3.08	3.11	3.13	3.09	0.881	0.307
Kidneys (%)	0.58	0.56	0.55	0.57	0.56	0.090	0.243
Lungs (%)	0.71	0.70	0.72	0.70	0.71	0.003	0.135

$^{a, b, c \text{ and } d}$*Means within the same row with different superscripts are significantly different* (P≤0.05).

CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.

Nutrient digestibility:

Table 4 shows that the addition of fermented pomegranate peel or pomegranate peel increased nutrient digestibility (P≤0.05) compared to the control group. Adding fermented pomegranate peel and pomegranate peel increased dry matter, crude protein, and crude fiber; however, it did not affect ether extract or nitrogen-free extract(P≤0.05). Similarly, several reports have indicated that the addition of fermented feeds enhanced nutrient digestibility in rabbits and chickens, increasing protein digestibility (Elbaz*et al.,*2023; Katu*et al.,*2025). Furthermore, the underlying mechanism contributing to these improvements is that pomegranate peel promotes the digestion and absorption of nutrients in the digestive tract by enhancing oxidative stability (Zeweiland Elgindy,2016; Shadab*et al.,*2017; Younis*et al.,*2025), which supports intestinal cell integrity. In addition, the marked improvement in nutrient digestibility in rabbits fed fermented pomegranate peel may be attributed to the fermentation process, which plays a crucial role in improving gut microbiota, epithelial cell integrity, and protein junctions (Li*et al.,*2020; Leeuwendaal*et al.,*2022), thus promoting gut health (Katu*et al.,*2025), and thereby enhancing nutrient digestion and absorption. Fermentation breaks down complex feed components (e.g., anti-nutritional factors, fiber, and large protein molecules) using microorganisms (Katu*et al.,*2025), which enhances protein and fiber digestibility.

Table (4): Effect of adding fermentedor unfermented pomegranate peel on nutrient digestion in heat-stressed growing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
Dry matter	59.1^{c}	59.8^{bc}	60.9^{ab}	61.4^{ab}	62.2^{a}	4.091	0.001
Crude protein	63.7^{c}	63.9^{c}	64.6^{b}	65.1^{b}	67.7^{a}	5.125	0.001
Crude fiber	41.8^{b}	41.6^{b}	42.2^{ab}	42.8^{ab}	43.5^{a}	2.331	0.031
Ether extract	76.2	75.9	76.5	77.0	77.2	4.252	0.150
NFE	51.3	51.5	52.0	51.8	52.3	1.032	0.208

$^{a, b \text{ and } C}$*Means within the same row with different superscripts are significantly different*(P≤0.05).

CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.NFE; Nitrogen-Free Extract.

Blood biochemistry:

The results of the current study showed that fermented pomegranate peel supplementation had a positive effect on lipid and protein metabolism and liver function, as demonstrated in blood biochemistry (Table 5). Adding fermented pomegranate peel to rabbit feed resulted in decreased triglyceride, cholesterol, and AST levels(P≤0.05), while increasing serum HDL and total protein levels(P≤0.05). However, ALT, albumin, and LDL levels remained unchanged(P>0.05). Consistent with our study, Kamel*et al.,* (2021) and Elbaz*et al.,* (2023) indicated that adding pomegranate peel or fermenting feed enhanced lipid metabolism and lowered blood lipid levels.

Table (5): Effect of adding fermentedor unfermented pomegranate peel on blood biochemistry in heat-stressedgrowing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
Triglycerides (mg/dL)	67.2^{a}	67.8^{a}	67.3^{a}	65.7^{ab}	64.5^{b}	2.008	0.017
Cholesterol (mg/dL)	90.3^{a}	89.7^{a}	90.1^{a}	88.4^{b}	87.3^{c}	1.955	0.001
HDL (mg/dL)	31.2^{c}	32.6^{b}	33.1^{b}	33.7^{ab}	34.6^{a}	0.547	0.001
LDL (mg/dL)	26.8	27.1	26.3	25.9	26.0	3.121	0.115
Total protein (mg/dL)	3.24^{b}	3.28^{b}	3.31^{b}	3.43^{a}	3.47^{a}	0.944	0.020
Albumin (mg/dL)	1.72	1.70	1.73	1.75	1.74	1.028	0.107
ALT (U/L)	16.4	16.1	16.2	15.8	16.0	0.847	0.082
AST (U/L)	57.4^{a}	56.1^{ab}	55.8^{b}	55.9^{b}	54.3^{c}	2.055	0.001

$^{a, b \text{ and } d}$*Means within the same row with different superscripts are significantly different*(P≤0.05).

CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase.AST: aspartate aminotransferase,

The lipid-lowering effects in rabbits fed fermented pomegranate peel can be attributed to the bioactive compounds in pomegranate peel, as well as the fermentation process itself, which improves lipid and protein digestion and metabolism (Elbaz*et al.,*2025).These effects can be attributed to various biochemical and metabolic changes, including alterations in adrenal hormone levels, reduced lipid oxidation, and decreased lipid transport and accumulation within tissues, all of which support lipid metabolism (Brownand Sharpe, 2016). Furthermore, the bioactive compounds in pomegranate peel stimulate bile acid production, leading to a decrease (P≤0.05) in blood cholesterol levels (Yilmaz andGül, 2024). Bile acids, steroid products resulting from cholesterol oxidation in the liver, play a key role in lipid digestion in the small intestine (Yilmaz *et al.,*2018).Fermented feed improves protein metabolism, which reduces the amount of undigested protein reaching the hindgut and lowers the liver's workload in processing excess nitrogen (Predescu*et al.,*2024).As a result, fermented feed helps maintain healthier liver function, which is reflected in lower AST levels. Because AST increases when liver cells are damaged during stress, the reduction of gut toxins, improved microbial balance, and enhanced antioxidant activity associated with fermented feed, and adding pomegranate peel collectively help protect liver cells (Zeweil and Elgindi.,2016; Li*et al.,*2020), thereby preventing elevations in AST.These findings demonstrate that fermented pomegranate peel effectively promotes lipid and protein metabolism by enhancing oxidative stability, thereby mitigating lipid oxidation problems in rabbits growing under heat stress.

Immuno-oxidant status:

The data in Table 6 showed that adding fermented or unfermented pomegranate peelenhances oxidative stability and modulates immunity in rabbits exposed to heat stress. IgA and SOD levels increased (P≤0.001 and P≤0.05)while MDA levels decreased in rabbits fed fermented or unfermented pomegranate peel (P≤0.05)compared to the control group; however, IgM, IgG, and TAC levels remained unchanged(P>0.05). In line with these findings, Shadab*et al.,* (2017),Haghighian*et al.,* (2021) and Hagag*et al.,* (2023) reported that adding pomegranate peel enhanced the oxidative state of rabbits by increasing oxidative enzymes.Similarly, several studies have reported a significant improvement (P≤0.05)in the oxidative state of chickens fed fermented feed (Li*et al.,*2020; Elbaz*et al.,*2023).Fermented feed improves chicken immunity by increasing beneficial gut microbes, enhancing gut barrier function, and stimulating immune cells, thereby strengthening both innate and adaptive immune responses (El-Sissi and Mohamed .,2017; Al-Khalaifah*et al.,*2025). At the same time, fermented feed contains bioactive metabolites and antioxidants that increase the activity of enzymes (Katu*et al.,*2025). Adding pomegranate peel to a rabbit's diet may improve immunity. It is rich in polyphenols, which boost antibody production and help reduce harmful gut microbes, thus supporting immune system development (Penha Filho*et al.,*2015; Kishawy*et al.,*2019). Its potent antioxidant compounds, such as ellagic acid and flavonoids, also enhance antioxidant enzymes (SOD, CAT and GPx) and reduce oxidation markers like MDA(Zeweil and Elgindy,2016; Shadab*et al.,*2017). As a result, fermented pomegranate peel enhances immune responses and protects chickens from oxidative stress.

Table (6): Effect of adding fermentedor unfermented pomegranate peel on immunity and oxidant status inheatstressed growing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
IgM (mg/mL)	184	180	189	191	193	1.091	0.064
IgA (mg/mL)	231^{c}	246^{b}	255^{ab}	257^{ab}	265^{a}	0.974	0.001
IgG (mg/mL)	306	301	309	311	309	2.108	0.137
SOD (U/ml)	23.6^{c}	24.2^{c}	25.1^{bc}	26.3^{b}	28.6^{a}	1.067	0.003
TAC (U/ml)	41.7	42.0	42.8	42.6	43.2	0.641	0.091
MDA (nmol/ml)	2.45^{a}	1.97^{b}	1.47^{c}	1.51^{c}	1.02^{d}	0.083	0.001

$^{a, b \text{ and } c}$*Means within the same row with different superscripts are significantly different*(P≤0.05)

*CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.*SOD: superoxide dismutase,TAC: total antioxidant capacity and MDA: malondialdehyde, .

Gut health:

The intestine functions as the principal organ responsible for digestion, absorption, nutrient utilization, and immunity. Furthermore, the gastrointestinal microbiota is fundamental to the health, immune competence, and productivity of rabbits. In this study, intestinal health was assessed through

histomorphometric analysis and evaluation of the microbial community. The results demonstrated an improved intestinal microbial profile in rabbits fed fermented pomegranate peel, characterized by a significant increase in Lactobacillus ($P \le 0.05$) and a reduction in pathogenic loads (E. coli and C. perfringens). These findings align with several previous reports (Predescu *et al.,*2024; Younis *et al.,*2025).The suppression of pathogenic bacteria observed in the groups treated with pomegranate peel is likely attributable to the antimicrobial properties of the peel's abundant bioactive compounds. It is well documented that constituents such as phenolics, flavonoids, and tannins within pomegranate peel can inhibit the proliferation of harmful microorganisms. Additionally, pomegranate peel serves as a significant source of organic acids—including fumaric, citric, malic, acetic, oxalic, tartaric, lactic, and ascorbic acids—as well as other essential nutrients (Younis *et al.,*2025). By acidifying the digestive tract and lowering the ambient pH, these organic acids may enhance resistance to infection and impede the colonization of specific intestinal pathogens, such as E. coli (Khan *et al.,*2022). Previous studies have similarly noted that E. coli counts were significantly lower in groups supplemented with pomegranate peel powder compared to controls (Ghasemi-Sadabadi *et al.,*2021; Mohammed *et al.,*2021).In the same context, the health benefits of fermented feeds for rabbits are derived from the establishment of a healthy gut ecosystem, marked by high Lactobacillus populations, reduced feed viscosity, lower pH, and increased concentrations of short-chain fatty acids (SCFAs), specifically lactic and acetic acids (Predescu *et al.,*2024). Accordingly, the fermentation of specific feed ingredients represents an effective strategy for mitigating enteric diseases through beneficial modulation of the gut microflora, which translates to enhanced gut health and improved performance (Elbaz *et al.,*2023). Furthermore, other research indicates that certain beneficial bacteria can synthesize essential vitamins and increase SCFA levels (Li *et al.,*2020; Katu *et al.,*2025), significantly contributing to the maintenance of beneficial microflora and supporting rabbit health and growth

The findings demonstrate that the consumption of fermented pomegranate peel exerted a significant regulatory influence on intestinal structure, with potential implications for its functionality. Specifically, rabbits in the fermented pomegranate peel group and the 3% pomegranate peel group displayed significantly longer villi relative to the control and 1.5% pomegranate peel groups, suggesting that elevated levels of pomegranate peel, particularly in its fermented form, may promote villus development. This observation aligns with the established physiological role of villi in nutrient uptake, where increased length offers an expanded absorptive surface area.The enhancement of villus length within the fermented group suggests that the combination of pomegranate peel supplementation and the fermentation process facilitates villus elongation, likely via intestinal cell proliferation. Furthermore, the fermented pomegranate peel group exhibited a significantly higher VH:CD ratio compared to both the control and unfermented pomegranate peel groups, indicating a stimulation of epithelial cell turnover. Conversely, no significant disparities were observed in crypt depth across the experimental groups. These data corroborate earlier studies investigating the positive impact of pomegranate peel on intestinal function through the improvement of histomorphometric metrics, including villus length (Younis *et al.,*2025).Likewise, numerous reports have substantiated that the fermentation of feed ingredients exerts beneficial effects on intestinal villus structure in both poultry and rabbits (Peng *et al.,*2022; Leeuwendaal*et al.,*2025). The fermentation process degrades anti-nutritional factors while generating advantageous metabolites—such as enzymes, organic acids, and probiotics—which enhance intestinal health. This results in augmented villus height and optimized goblet cell function (Peng *et al.,*2022; Elbaz *et al.,*2023; Katu *et al.,*2025). Consequently, this mechanism supports mucus secretion, reinforces the intestinal barrier, and expands the surface area available for nutrient absorption, thereby improving feed efficiency and growth performance.

Table(7): Effect of adding fermentedor unfermented pomegranate peel on microbial count (Log10 CFU g−1) and histomorphometry(µm) in heatstressed in growing rabbits.

Items	CON	PP1	PP2	FPP1	FPP2	SME	P-value
E. coli	3.84^{a}	3.51^{ab}	3.35^{b}	2.88^{c}	2.35^{d}	0.117	0.001
C. perfringens	4.37^{a}	4.09^{ab}	3.61^{b}	3.65^{b}	3.04^{bc}	0.099	0.001
Lactobacillus	5.19^{c}	5.82^{bc}	6.22^{b}	6.04^{b}	6.58^{a}	1.031	0.001
Villi height (VH)	621^{c}	638^{c}	674^{b}	681^{b}	752^{a}	22.18	0.012
Crypt depth (CD)	71.4	71.1	72.3	71.9	72.7	5.664	0.151
VH: CD ratio	8.70^{c}	8.97^{bc}	9.32^{b}	9.46^{b}	10.33^{a}	2.518	0.005

$^{a-d}$*Means within the same row with different superscripts are significantly different* ($P \le 0.05$).
CON: rabbits fed a basal diet without a feed additive, PP1: rabbits fed a basal diet with 1.5 % pomegranate peel, PP2: rabbits fed a basal diet with 3% pomegranate peel, FPP1: rabbits fed a basal diet with 1.5 % fermented pomegranate peel, FPP2: rabbits fed a basal diet with 3% fermented pomegranate peel.

CONCLUSION

It can be concluded that adding fermented pomegranate peel to the diets of heat-stressed growing rabbits has a positive effect on growth performance, nutrient digestion, lipid metabolism, and immune status, as well as enhancing oxidative stability and gut health, as it reduces the pathogenic microbial load and promotes intestinal histomorphometry.

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تأثير استخدام قشرالرمان المتخمر على الاداء الانتاجي والمناعي وصحة الأمعاء تحت تأثير الإجهاد الحراري البيئي في الارانب النامية.

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تهدف هذه الدراسة إلى تقييم تأثيرإضافة قشرالرمان المُتخمرعلى الاداء الإنتاجي ومكونات الدم والاستجابة المناعية وصحة الامعاء في الأرانب النامية تحت ظروف الإجهاد الحراري البيئي. تم توزيع 200 أرنب نيوزيلندي أبيض عمر 35 يومًا عشوائياً على خمسمجموعات تجريبية بأربع مكررات لكل منها. كانت المجموعات التجريبية على النحوالتالي:-

1- أرانب غذيت علي علائق بدون استخدام قشر الرمان (الكنترول).
2- أرانب غذيت علي علائق تحتوي علي 1.50% قشر رمان بدون تعامل PP1.
3-أرانب غذيت علي علائق تحتوي علي 3.00% قشر رمان بدون تعامل PP2.
4- أرانب غذيت علي علائق تحتوي علي 1.50% قشر رمان بدون تعامل FPP1.
5-أرانب غذيت علي علائق تحتوي علي 1.50% قشر رمان بدون تعامل FPP2.

أظهرت النتائج تحسنًا كبيرًا في وزن الجسم، ومعامل التحويل الغذائي، ووزن الذبيحة ($P < 0.05$) في الأرانب التي تغذت علىPP2 وFPP1وFPP2 مقارنة بالمجموعات الأخرى.في حين لم يتأثرالإستهلاك الغذائي وخصائص الذبيحة بالمعاملات التجريبية. علاوة على ذلك، أدى إضافة FPP2إلى زيادة هضم المادة الجافة والبروتين الخام والألياف الخام مقارنةً بالمجموعات الأخرى. كما أدى إضافة FPP2 إلى زيادة ملحوظة في البروتين الكلي في الدم والبروتين الدهني عالي الكثافة HDL ($P < 0.05$) ، مع خفض الدهون الثلاثية والكوليسترول الكلي وإنزيم AST ($P < 0.05$). بالإضافة الى ذلك عززت معاملة FPP2 الاستجابة المناعية والاستقرارالتأكسدي من خلال زيادة ملحوظة في مستويات IgA و SOD، وخفض MDA ($P < 0.05$). كماعززت معاملة FPP2 صحة الأمعاء من خلال تعديل ميكروبات الأعور بشكل ملحوظ، وزيادة عدد بكتيريا اللاكتوباسيلاس، وتقليل الإى كولاى والكوليستريديا ($P < 0.05$)، وزيادة ارتفاع خملات الأمعاء ونسبة VH:CD. يمكن الاستنتاج أن إضافة قشرالرمان المُتخمر إلى علائق الأرانب يمكن أن يعززأداءالنمو، ومستوى الدهون، والمناعة، والاستقرارالتأكسدي، وسلامة الأمعاء في الأرانب المُعرضةللإجهادالحراري.بالتالي يؤدي إضافة قشرالرمان المتخمرطريقة فعالة للتخفيف من الآثارالسلبية للإجهاد الحراري على الأرانب.

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