Italian Journal of Animal Science

ISSN: 1828-051X (Online) Journal homepage: www.tandfonline.com/journals/tjas20

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To cite this article: Moataz Fathi, Magdy Abdelsalam, Mohamed Abd El-Razik, Salah El-Safty, Gamal Rayan, Osama Abou-Emera & Raed Alayouni (2025) Impact of strain and probiotic supplementation on carcase characteristics, antioxidant status and meat quality of growing rabbits under hot climate, Italian Journal of Animal Science, 24:1, 2095-2108, DOI: 10.1080/1828051X.2025.2559192

To link to this article: https://doi.org/10.1080/1828051X.2025.2559192

Published online: 29 Sep 2025.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2025, VOL. 24, NO. 1, 2095–2108
https://doi.org/10.1080/1828051X.2025.2559192

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RESEARCH ARTICLE

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Impact of strain and probiotic supplementation on carcase characteristics, antioxidant status and meat quality of growing rabbits under hot climate

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ABSTRACT

This study investigated the interactive effects of probiotic supplementation and strain on growth performance, antioxidant status and meat quality in growing rabbits under hot climate conditions. Ninety weaned male rabbits (45 V-line and 45 Alex strain), aged four weeks, were assigned to a 6-week trial in a 2 × 3 factorial design involving three levels of probiotics (control, low and high). Each subgroup (15 rabbits) was divided into five replicates of three rabbits. Probiotic supplementation, particularly at high and low levels, significantly improved final body weight and weight gain compared to the control. V-line rabbits consumed more feed, while Alex rabbits showed better feed conversion ratio (FCR). Both probiotic level and strain significantly influenced muscle pH values. High-level probiotic supplementation markedly reduced total bacterial count (TBC), spore-forming colonies, *E. coli* and *Salmonella sp*. Alex rabbits had lower overall bacterial loads, though V-line rabbits recorded lower spore-forming colonies and *E. coli* counts. Probiotic addition significantly enhanced total antioxidant capacity (TAC) in plasma, with a greater effect observed in V-line rabbits. The interaction between strain and probiotic level was significant for most parameters, excluding *Salmonella sp*. Meat quality traits (appearance, colour, taste, aroma, tenderness, juiciness and overall acceptability) were significantly improved by probiotics, while strain had no effect on these traits. In conclusion, both probiotic supplementation and strain influenced growth and health parameters, with probiotics notably enhancing performance, antioxidant capacity, microbial status and meat quality in rabbits reared in hot climates.

HIGHLIGHTS

- In this experiment, a 2 × 3 factorial study examined the interaction between rabbit strain (V-line *vs.* Alex) and probiotic supplementation level under hot climate conditions.
- Significant strain × probiotic interaction was found in most traits, except *Salmonella sp*.
- Probiotics improved meat quality attributes (appearance, flavour, tenderness, juiciness and acceptability), while strain had no effect on these traits.

ARTICLE HISTORY
Received 13 June 2025
Revised 4 September 2025
Accepted 4 September 2025

KEYWORDS
Genotype; probiotics; carcase; meat quality; hot climate

Introduction

The increasing demand for animal products due to the growing global population has posed an ongoing challenge for the animal production sector worldwide (Wicks et al. 2019). The production and development of rabbit meat are concentrated in Mediterranean countries, such as Spain, France, Italy, Algeria and Egypt, as well as in Asian countries like Korea and China (Kumar et al. 2025). Unlike other farm animals, rabbits have a high capacity to produce significant amounts of meat in a short production cycle. Additionally, rabbit meat is a protein-rich food source, containing high levels of vitamins and minerals while being low in cholesterol (Ologbose et al. 2018). Rabbit farming is considered a promising solution for producing white meat at the household level, especially for small-scale farmers in developing countries.

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Following the European Union's ban on the use of antibiotics in livestock feed in 2006, natural feed additives have emerged as a promising alternative for improving animal productivity and health. As consumer demand shifts towards antibiotic-free animal products, probiotics have gained attention as a natural, safe and sustainable alternative to synthetic growth promoters in rabbit farming systems. The supplementation of rabbit diets with probiotics has been widely explored as a strategy to enhance growth performance, carcase yield and meat quality (Fathi et al. 2017; Mancini and Paci 2021; Adli et al. 2023; Osti et al. 2025).

Heat stress has a detrimental effect on rabbits' digestive efficiency, immune function and overall growth performance, ultimately leading to reduced productivity (Liang et al. 2022; Abdelsalam and Fathi 2023; El-Raghi et al. 2023). Probiotics have been shown to alleviate these negative effects, particularly under heat stress conditions, by improving nutrient absorption, strengthening the immune system and balancing the gut microbiota. Supplementing rabbit diets with probiotics enhances gut health by promoting a balanced microbiota, which is essential for efficient nutrient digestion and absorption, factors that contribute to improved feed conversion and weight gain (Fathi et al. 2017; Chandrasekaran et al. 2024). In addition, probiotics modulate immune function by stimulating gut-associated lymphoid tissue, thereby enhancing immune responses and increasing resistance to environmental stressors (Adli et al. 2023). This study aimed to evaluate the interaction between probiotic supplementation and strain on growth performance, antioxidant status and meat quality in growing rabbits raised under hot climatic conditions.

Materials and methods

Study site and ethical statement

The current experiment was conducted during the hot summer season (July–August) of 2024 at the Poultry Research Centre, Alexandria University, Alexandria, Egypt. All procedures involving live experimental rabbits were performed and approved by the Committee of Experimental Animal Care and Ethics at Alexandria University (Protocol No. Alex.Agri.092401101). Efforts were made to minimise animal suffering throughout the study.

Animals and management

Two different strains of rabbit were used in the present experiment. V-line is an exotic Spanish rabbit line imported from Valencia Polytechnic University, Spain, that was selected for giving more weaned kittens per litter. The Alex rabbit strain is a developed line produced from crossing between the V-line and Egyptian native breed to be more acclimatised for hot environmental conditions. A total of 90 weaned rabbits representing two strains (45 V-line and 45 Alex strain) aged four weeks of age were utilised in a 6–week experiment. The rabbits of each strain were randomly distributed into 3 levels of probiotic supplementation in a 2×3 factorial arrangement experimental design comprising 6 sub-groups (15 rabbits each). The initial body weight was not significantly different (590.3 ± 16 g) for all sub-groups. The rabbits of each sub-treatment were collectively divided into 5 wire-fenced cages (5 replicates and 3 rabbits each). The dimensions of the cage are 50 cm $\times$ 40 cm $\times$ 38 cm. The cages were installed in one-level cage construction located in an open-sided building. Each cage was supplied with a hopper feeder and two drinking nipples. The experiment started after a one-week adaptation period, during which the rabbits were acclimatised to the new environment and to the presence of the staff. The animals were raised under similar environmental and managerial conditions. This experiment was conducted during the summer season of 2024. The rabbits were not exposed to heat stress, as the temperature-humidity index (THI) throughout the entire experimental period was 84.9, based on the minimum and maximum daily temperatures. The lighting schedule applied during the whole experiment was 16 L:8D. All animals were given *ad libitum* access to a commercial pelleted ration containing 18% crude protein, 12% crude fibre and 2,650 kcal/kg metabolisable energy. The feed and water were offered to rabbits *ad libitum* throughout the whole experimental period. For probiotic supplementation, a substance containing 6.67*109 CFU/g of *Bacillus licheniformis* (GalliPro-Tect-WS, Milwaukee, WI) was used in two levels. For low and high concentration levels of probiotics, 1 g and 2 g were dissolved in each litre of drinking water, respectively. The actual *Bacillus licheniformis* concentration was 6.67*109 CFU/L and 13.35*109 CFU/L for low and high levels, respectively. The rabbit group that drank fresh water without supplementation represents a non-supplemented group. To ensure the effective dissolving of the probiotics in drinking water and delivery in a fresh manner to the rabbits, the mixture was prepared and placed daily in a water tank for each group.

Body weight and feed intake

The growth performance, including initial body weight, final body weight and overall feed intake were

determined. The total body weight gain from the initial weight over six weeks was calculated. Feed conversion ratio (FCR) was calculated based on overall body weight gain and feed intake for each experimental unit (3 rabbits).

Slaughter and carcase evaluation

At the end of the experiment, 10 rabbits were randomly assigned from each group (60 rabbits in total) for carcase characteristics. They were fasted for 12 h with free access to clean drinking water. Then, the rabbits were weighed and slaughtered by cutting carotid and jugular veins. Upon bleeding, the rabbits were dissected. After skinning, the carcase was eviscerated, and all organs and offal were removed. Hot carcase, skin, head, liver, heart, kidney and lungs were excised and weighed. Caecum with content was weighed, and the length was measured using a measuring tape. Also, lymphoid organs (spleen and thymus) were removed and trimmed. The carcase was divided into three cuts: fore part, mid part and hind part. All carcase traits were expressed as a percentage of the live body weight.

Blood collection and determination of antioxidant status

Blood samples were collected post-mortem from each rabbit into heparinised tubes for determination of biochemical analysis. The blood samples were centrifuged (3000 rpm for 15 min at 4 °C), and the harvested plasma was stored at −20 °C until further analysis. Total antioxidant capacity (TAC), Malondialdehyde (MDA) and Superoxide dismutase (SOD) were determined in the plasma using commercial kits (Biomerieux, Craponne, France). The globulin was calculated as the difference between the total protein and albumen.

Bacterial count

Upon slaughter, the meat samples were collected from the hind legs and carefully hand-stripped into sterile containers. Total bacterial count (TBC) and spores-forming colony (SFC) were determined. Total aerobic bacteria, *Salmonella sp*., and *E. coli* were analysed in the caecal digesta according to the procedures described by van Horn et al. (1996). Briefly, 1/10 serial dilutions of digesta were prepared in buffered peptone water (1 g/L peptone, 8 g/L NaCl and 0.5 g/L L-cysteine hydrochloride) for the enumeration of total aerobic bacteria, *E. coli* and *Salmonella sp*. Each dilution was cultured on selective media for each bacterial strain to be counted or detected. Nutrient agar was used for the total aerobic bacteria count, and MacConkey agar was used for the *E. coli* count. Detection of *Salmonella sp*. was accomplished by multiplying the dilutions in tetrathionate broth for 24 h and culturing onto modified brilliant green agar supplemented with 20 mg/mL novobiocin. The culture plates for total aerobic bacteria, *E. coli* and *Salmonella sp*. were incubated at 37 °C in an aerobic environment. The colony-forming units (cfus) in log10 per gram for total aerobic bacteria and *E. coli* within digesta were counted based on the colony morphology and characteristics. *Salmonella sp*. was expressed as a percentage of detection.

Meat quality analysis

pH

After slaughter, the carcases were chilled at 4 °C for 24 h to minimise rigour mortis and allow for proper storage of meat samples. The pH of meat samples was measured at 24 h post-mortem using a digital pH metre (model XYZ) equipped with a penetrating electrode. The probe was inserted into the centre of the meat samples to obtain the readings. The pH was measured in triplicate per sample.

Water holding capacity and plasticity

The water holding capacity (WHC) of the meat was determined by Grau and Hamm's filter-paper press method described by Janocha et al. (2021) based on the surface of meat juice on the filter-paper. Approximately, 0.3 g of minced meat was placed between two pieces of filter paper and subjected to a standardised pressure with 1 kg weigh for 10 min, then the weight was removed and the area of water spot (outer spot) and compressed meat sample (inner spot) on the filter paper were defined by a graphite pencil. Using the Digiplan Digital Planimeter 300 planimeter (Gebrüder Haff GmbH, Pfronten, Germany), the spot areas (outer and inner) were determined. WHC was determined as the difference between the inner and outer spot cm^2/0.3 g, where plasticity was determined as the area of compressed meat sample (inner spot) as cm^2/0.3 g.

The colour of the meat samples was measured using a Minolta CR-400 chromameter (Konica Minolta, Osaka, Japan) as described by Combes et al. (2008). Colour values were recorded in the CIE Lab* colour space, where L* relative to lightness, a* relative to redness and b* relative to yellowness. Three readings

were taken at different locations on the surface of each meat sample, and the average values were calculated to use in the statistical analysis.

To determine the precise meat colour, the chroma (the colour intensity or saturation) and hue (the dominant colour on the colour wheel) were calculated using the following formulas:

$$\text{Chroma}(C^*) = \sqrt{(a*)^2 + (b*)^2}$$

$$\text{Hueangle}(\text{hab}) = \boldsymbol{tan}^{-1} * \left(\frac{a*}{b*}\right)$$

Sensory evaluation

Sensory analysis was performed 72 h after slaughter according to the method of Pelicano et al. (2003). Briefly, the muscle samples were treated with 1% (w/w) salt, then meat samples were cooked in a pre-warmed oven (170 °C) until the internal temperature reached 75 °C. Sensory evaluation of meat samples were determined based on a nine-point hedonic scale on which scale 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) for appearance, colour, taste, aroma, tenderness, juiciness and overall acceptability.

Shear force measurement (tenderness)

Meat tenderness was assessed using a texture analyser (model XYZ, TA Instruments, New Castle, DE) with a Warner–Bratzler shear blade. The meat samples (approximately 2.5 cm thick) were sheared across the fibres of the muscles. The maximum shear force (measured in Newtons) required to cut through the meat was recorded as a measure of tenderness. Three replicates per sample were analysed.

Statistical analysis

Data were subjected to a two-way ANOVA using JMP software version 13.0, NC, USA with strain and probiotic level as fixed effects (SAS Institute 2013). The statistical model is described as follows:

$$Y_{ijk} = \mu + P_i + S_j + (PS)_{ij} + e_{ijk}$$

where:

Y_{ijk} = the observation taken on the kth individual,

μ = overall mean,

P_i = the fixed effect of the jth probiotic supplementation level,

S_j = the fixed effect of the ith strain,

$(PS)_{ij}$ = interaction between strain and probiotic supplementation level,

e_{ijk} = random error assumed to be independent normally distributed with mean = 0 and variance = σ^2.

All results were presented as mean, and the variability in the data was expressed as pooled standard error of the mean. The significance of difference among the groups was assessed using Tukey's test. Differences between means were considered significant at $p \leq 0.05$. Orthogonal polynomial contrast test for linear and quadratic effects was applied to describe the shape of the response to increasing concentrations of probiotic supplementation and to determine the best model fit. The responses in optimal parameters to the probiotic supplementation level can be modelled using the following quadratic equation:

$$Y = a + b_1X_1 + b_2X_2 + e$$

where Y = optimal response, a = intercept, bs = coefficients of the quadratic equation, Xs = probiotic levels and e = error.

Results

Productive performance

Table 1 describes the effects of probiotic levels (P) and rabbit strain (S) and their interaction (P*S), including linear and quadratic trends on productive performance in growing rabbits. All rabbits started the experiment at a similar body weight (no significant differences), ensuring a fair comparison of treatment effects. A linear significant ($p < 0.01$) difference among probiotic levels was found in final body weight. Animals receiving higher levels of probiotics had significantly higher body weights (4536.3 g) compared to the control treatment (4373.4 g). The low level was intermediate (4462.5 g). Rabbits receiving a higher level of probiotics gained more weight ($p < 0.05$). On the other hand, probiotic treatment did not affect either FI or FCR. Neither body weight nor weight gain was influenced by the strain of the rabbit. However, V-Line rabbits consumed (9525.9 g) significantly ($p < 0.01$) more feed than Alex ones (8718.9 g). Alex rabbits had significantly ($p = 0.05$) better FCR (3.26) compared to V-Line (3.56). No significant interactions between probiotic level and rabbit strain for all growth traits were observed.

Table 1. Productive performance of growing rabbits as affected by rabbits' strain and probiotics levels.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Parameter	Control	Low	High	Alex	V-Line	SEM	Linear	Quad	S	P*S
Initial body weight, g	1763.7	1765.8	1783.2	1737.9	1803.9	47.1	0.87	0.94	0.50	0.98
Final body weight, g	4373.4^{b}	4462.5^{ab}	4536.3^{a}	4418.1	4496.7	55.8	<0.01	0.96	0.09	0.07
Δ body weight, g	2587.2^{b}	2694.9^{ab}	2754.3^{a}	2676.9	2675.4	51.3	0.05	0.26	0.97	0.64
FI, g	9037.2	9076.5	9253.5	8718.9^{b}	9525.9^{a}	158.4	0.90	0.50	<0.01	0.14
FCR	3.49	3.37	3.36	3.26^{b}	3.56^{a}	0.08	0.80	0.89	0.05	0.18

Values in this table depended on experimental unit (3 rabbits/cage).
SEM: standard error of the mean
^{a,b}Means in the same rows followed by different superscripts significantly differ.

Table 2. Physico-chemical attributes of rabbits' meat given different levels of probiotics.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Trait	Control	Low	High	Alex	V-Line	SEM	Linear	Quad.	S	P*S
pH	6.67^{a}	6.53^{b}	6.39^{c}	6.51^{b}	6.54^{a}	0.02	<0.01	0.96	0.05	0.07
WHC	13.09^{a}	12.04^{b}	13.49^{a}	13.38^{a}	12.37^{b}	0.19	0.27	<0.01	<0.01	0.05
Plasticity	5.64^{b}	6.03^{ab}	6.24^{a}	6.28^{a}	5.66^{b}	0.11	<0.01	0.61	<0.01	0.03
Colour attributes										
Redness (a*)	6.05	5.81	7.06	4.51^{b}	8.10^{a}	0.54	0.29	0.37	<0.01	<0.01
Yellowness (b*)	7.08^{a}	5.74^{b}	4.29^{c}	6.80^{a}	4.61^{b}	0.42	<0.01	0.90	<0.01	<0.01
Lightness (L*)	48.52^{b}	53.25^{a}	49.98^{b}	52.14^{a}	49.04^{b}	0.66	0.26	<0.01	<0.01	0.19
b/a	1.71	1.98	1.39	2.43^{a}	0.95^{b}	0.25	0.56	0.37	<0.01	0.75
Chroma	10.24	9.79	9.26	8.50^{b}	11.04^{a}	0.55	0.25	0.95	<0.01	<0.01
Hue angle	51.20	44.73	41.18	55.86^{a}	35.55^{b}	3.62	0.19	0.82	<0.01	0.04

Alex: Alexandria strain; SEM: standard error of the mean; WHC: water holding capacity
^{a,b,c}Means in the same rows followed by different superscripts significantly differ.

Physico-chemical meat quality

Physico-chemical meat quality attributes of rabbit strains given different levels of probiotics are presented in Table 2. There was a high significant impact of probiotic treatment on pH measure. However, the lowest pH values in carcase meat were recorded by a high level of probiotic group followed by low level and control groups, respectively ($p < 0.01$). Moreover, the rabbit strain had a significant impact on the pH of meat. However, the pH values of Alex meat were lower than those of V-Line ones ($p < 0.05$). Regarding the WHC of meat, the low level of probiotics resulted in a significant decrease in WHC compared to other groups ($p < 0.01$). Additionally, WHC in V-Line meat significantly decreased compared to Alex counterparts. The interaction between the rabbit strain and the probiotic level was not significant ($p < 0.055$). With respect to the plasticity of meat, adding probiotics *via* water significantly improved ($p < 0.01$) this trait, especially at a high level compared to the control group. Plasticity value of Alex meat was significantly ($p < 0.01$) higher (6.28) than those of V-Line counterparts (5.66). Moreover, the interaction between rabbit strain and probiotic level was significant ($p < 0.03$).

Regarding the colour of meat, V-Line meat exhibited significantly ($p < 0.01$) more redness (8.1) compared to Alex meat (4.5). Although probiotics did not affect the redness of meat, the interaction between the two main factors was highly significant ($p < 0.01$). There was a highly significant difference (linear manner) among probiotic levels in meat yellowness. Where the meat of the control group was more yellow-coloured (7.1), followed by the low (5.7) and high (4.3) probiotic levels, respectively. Moreover, the rabbit strain significantly ($p < 0.01$) affected this trait, where the Alex meat recorded dark yellowness (6.8) compared to the V-Line one (4.6). Moreover, the interaction between rabbit strain and probiotics was highly significant ($p < 0.01$). The lightness of meat was also significantly affected by both probiotic treatment and rabbit strain. The meat of low probiotic level recorded a lighter colour (53.3) compared to control (48.5) and high level (50.0). Likewise, Alex meat was significantly ($p < 0.01$) lighter in colour compared to V-Line meat. The percentage between yellow and red meat was higher and significant ($p < 0.01$) in the Alex strain compared to the V-Line. Concerning chroma (the colour intensity or saturation index), the effect of probiotic supplementation was not significant. Whereas V-Line chroma was significantly higher ($p < 0.01$) compared to the Alex strain. Furthermore, the interaction between the rabbit strain and probiotic treatment was highly significant ($p < 0.01$). The values of the hue

angle (the dominant colour on the colour wheel) were not influenced by probiotic supplementation. On the other hand, the Hue angle recorded a significantly higher value (55.7) in the Alex strain compared to the V-Line counterparts (35.6). In addition, the interaction between the two factors was significant ($p < 0.04$). As shown in Figure 1, the effect of probiotic supplementation on certain physico-chemical attributes is not consistent across the two rabbit strains. The high level of probiotics in the Alex strain resulted in the highest WHC, while the low level in V-Line rabbits showed the lowest mean. Interestingly, supplementation with a high level of probiotics produced opposite effects on plasticity and redness between the two strains. Conversely, both strains responded differently in terms of chroma and hue angle when given a high level of probiotics (Figure 2).

Meat microbial profile

Table 3 summarises the effects of different probiotic levels and rabbit strain on several microbial traits in rabbits. Supplementation of probiotics *via* drinking water, especially at high levels, significantly reduced the TBC (linear trend) compared to the control group ($p < 0.01$). Likewise, Alex rabbits recorded a significantly ($p < 0.01$) low level of bacterial count (5.01) compared to V-Line ones (5.45). Furthermore, the interaction

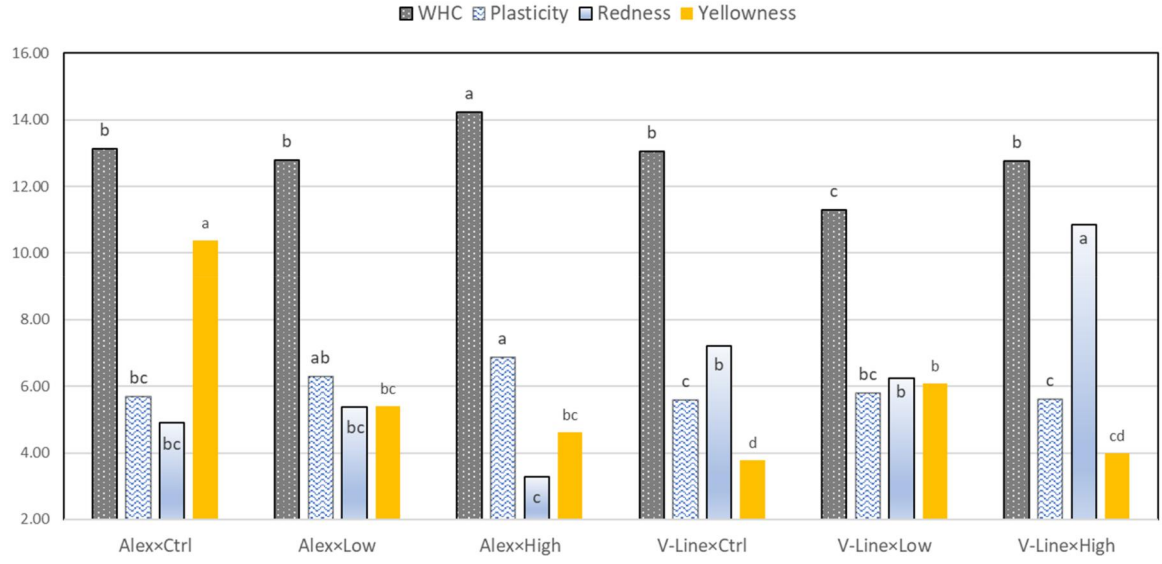

Figure 1. Effect of interaction between probiotic supplementation level (ctrl, low and high) and rabbit strain (Alex and V-Line) on physico-chemical attributes of rabbit meat; WHC: water holding capacity; [a–d]significance letters.

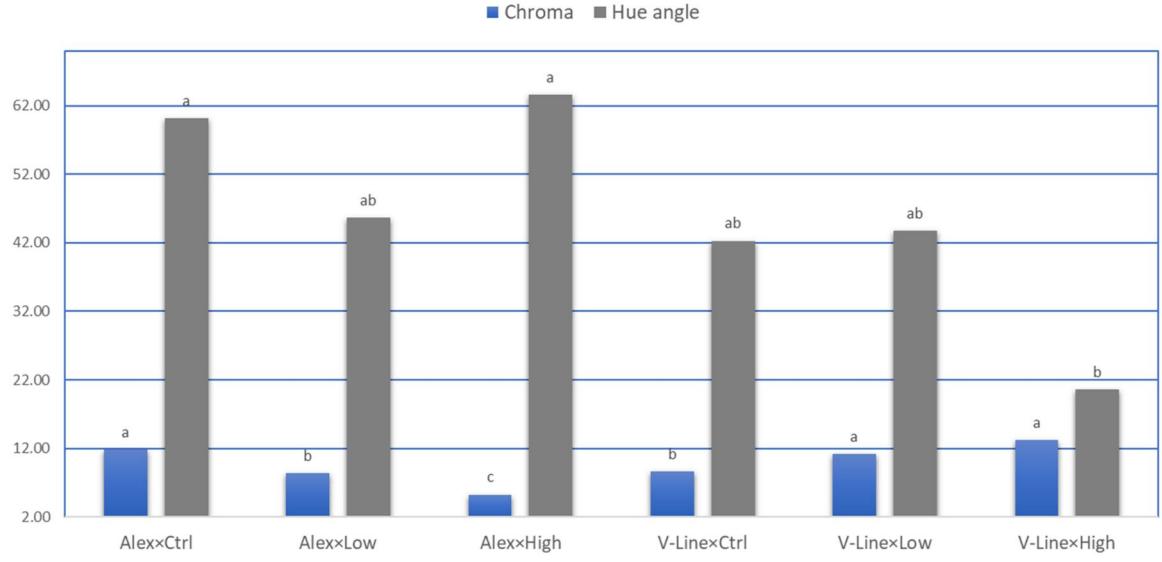

Figure 2. Effect of interaction between probiotic supplementation level (ctrl, low and high) and rabbit strain (Alex and V-Line) on chroma and hue angle attributes; [a–c]significance letters.

Table 3. Impact of rabbit strain and probiotic level on meat microbial profile of growing rabbits.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Trait	Control	Low	High	Alex	V-Line	SEM	Linear	Quad.	S	P*S
TBC	5.50^{a}	5.20^{ab}	4.99^{b}	5.01^{b}	5.45^{a}	0.09	<0.01	0.74	<0.01	<0.01
SFC	4.40^{a}	3.45^{b}	3.38^{b}	3.89^{a}	3.60^{b}	0.10	<0.01	<0.01	<0.01	<0.01
E. coli	5.53^{a}	5.22^{b}	4.87^{c}	5.54^{a}	4.87^{b}	0.10	<0.01	0.89	<0.01	<0.01
Salmonella sp.	100.00^{a}	100.00^{a}	20.00^{b}	73.33	73.33	6.37	<0.01	<0.01	1.0	1.0

Alex: Alexandria strain; SEM: standard error of the mean; TBC: total bacterial count; SFC: spores forming colony; *E. coli: Escherichia coli*
^{a,b}Means with no common superscripts within each factor are significantly different.

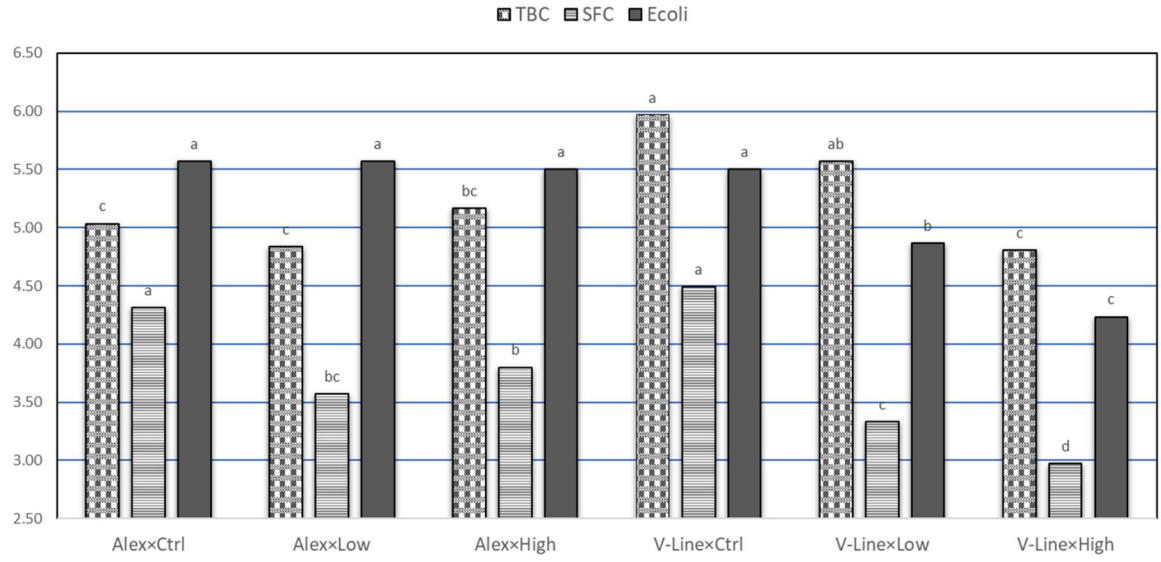

Figure 3. Effect of interaction between probiotic supplementation level (ctrl, low and high) and rabbit strain (Alex and V-Line) on meat microbial profile; TBC: total bacterial count; SFC: spores forming colony; *E. coli: Escherichia coli*; $^{a-d}$significance letters.

between rabbit strain and probiotic level was highly significant ($p < 0.01$). Similarly, the positive effect of probiotic supplementation (in both levels) was observed for SFC compared to the control group. Furthermore, V-line rabbits recorded a significantly lower figure ($p < 0.01$) of 3.60 compared to Alex rabbits (3.89). Additionally, a significant interaction between rabbit strain and probiotic level was recorded. A significant linear reduction ($p < 0.01$) in *E. coli* count was realised with probiotic supplementation, especially with the high level. V-line rabbits recorded the lowest value (4.87) of *E. coli* count compared to Alex rabbits (5.54). Furthermore, significant interaction between the two factors was observed (Figure 3). The untreated V-Line rabbits exhibited the highest TBC compared to the Alex rabbits. Probiotic supplementation with a high level significantly reduced *E. coli* count in the V-Line strain only, with no significant effect observed in the Alex rabbits. Regarding *Salmonella sp.* isolation, a high level of probiotics dramatically reduced ($p < 0.01$) *Salmonella sp.* compared to the other groups (control and low probiotic level).

Carcase yield and internal organs

The impact of rabbit strain and supplemented probiotic level on carcase yield and internal organs of growing rabbits is shown in Table 4. No significant effect of probiotic level was observed for dressed carcase. The dressed carcase percentage was significantly affected by the strain ($p < 0.05$), with V-Line rabbits exhibiting a higher yield (51.7%) compared to Alex rabbits (50.0%). Regarding carcase organs, the head of rabbits as a percentage of live body weight with a low level of probiotics recorded a significantly higher ($p < 0.02$) percentage (6.6%) compared to both control and high-level groups (6.3%). Heart weight (relative to body weight) was significantly influenced by rabbit strain ($p < 0.01$). V-Line rabbits had a larger relative heart weight (0.5%) compared to Alex rabbits (0.3%). Significant differences were observed among probiotic groups and rabbit strains in kidney percent. The Alex rabbits recorded higher kidneys (0.6%) than those of the V-Line rabbits (0.5%). There were no effects due to probiotic treatment or rabbit strain on liver, lungs, thymus and caecum percents. A significant increase

Table 4. Impact of supplemented probiotic level and rabbit strain on carcase yield and internal organs (expressed as a relative figure of body weight) of growing rabbits.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Trait	Ctrl	Low	High	Alex	V-Line	SEM	Linear	Quad.	S	P*S
Dressed carcase	50.3	52.0	50.4	50.0^{b}	51.7^{a}	0.45	0.82	0.09	0.05	0.87
Head	6.3^{b}	6.6^{a}	6.3^{ab}	6.4	6.4	0.06	0.74	0.02	0.64	0.08
Heart	0.3	0.4	0.4	0.3^{b}	0.5^{a}	0.03	0.14	0.18	<0.01	0.15
Kidneys	0.6^{a}	0.6^{ab}	0.5^{b}	0.6^{a}	0.5^{b}	0.02	0.02	0.55	<0.01	0.58
Liver	2.6	2.6	2.5	2.6	2.6	0.05	0.45	0.96	0.49	0.28
Lungs	0.5	0.5	0.6	0.5	0.5	0.01	0.21	0.92	0.71	0.89
Spleen	0.075	0.075	0.070	0.062^{b}	0.084^{a}	0.003	0.61	0.81	<0.01	0.27
Thymus	0.23	0.25	0.28	0.25	0.25	0.01	0.07	0.79	0.94	0.85
Caecum	7.6	7.0	6.4	6.7	2.6	0.04	0.07	0.91	0.33	0.19
Front	18.2^{b}	19.2^{a}	18.6^{ab}	18.3	19.0	0.21	0.38	0.05	0.11	0.43
Mid	10.9	11.0	10.5	10.5	11.1	0.17	0.44	0.47	0.12	0.82
Hind	21.2	21.8	21.3	21.2	21.6	0.20	0.79	0.19	0.24	0.86

Alex: Alexandria strain; SEM: standard error of the mean
[a,b]Means with no common superscripts within each factor are significantly different.

Table 5. Impact of rabbit strain and supplemented probiotic level on antioxidant indices in blood plasma.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Trait	Ctrl	Low	High	Alex	V-Line	SEM	Linear	Quad.	S	P*S
TAC, mmol/L	0.42^{b}	0.65^{a}	0.67^{a}	0.44^{b}	0.69^{a}	0.03	<0.01	0.02	<0.01	0.02
MDA, nmol/mL	0.38	0.40	0.34	0.38	0.37	0.02	0.41	0.32	0.93	0.08
SOD, mmol/L	251.3	246.1	240.8	249.1	243.2	8.16	0.57	0.92	0.68	0.42

Alex: Alexandria strain: SEM: standard error of the mean; TAC: total antioxidant capacity; MDA: malondialdehyde; SOD: superoxide dismutase
[a,b]Means with no common superscripts within each factor are significantly different.

($p < 0.01$) of spleen percent was recorded in V-Line rabbits (0.084%) compared to Alex strain (0.062%). Furthermore, the rabbits received low-level probiotics, which recorded a front limb percentage significantly ($p < 0.01$) higher (19.2%) than that of the control and high-level groups (18.2% and 18.6%, respectively). Additionally, neither probiotic supplementation nor rabbit strain influenced the mid and hind limbs of the carcase. Concerning the interaction effect between the two factors, no significant differences were observed for all studied traits.

Antioxidant status

Table 5 presents the influence of different probiotic supplementation levels and rabbit strains on key antioxidant biomarkers in growing rabbits. It could be observed that there was a significant positive impact (linear and quadratic $p < 0.01$; $p < 0.02$) of probiotic addition (low or high) on TAC in blood plasma compared to the control group. In addition, V-Line rabbits had superior ($p < 0.01$) TAC compared to Alex ones. Moreover, the interaction between the two main factors was significant ($p < 0.02$). Regarding the other measures of oxidative status (MDA and SOD), no significant differences were observed among either probiotic treatment or rabbit strain.

Meat quality

The results of sensory meat quality traits in growing rabbits as affected by probiotic supplementation level and strain are illustrated in Table 6. Probiotic supplementation had a significant positive effect on all sensory traits, either in a linear or in a quadratic manner. The highest improvement was found in rabbits that received a high level of probiotics, followed by the lowest probiotic level. On the other hand, no significant differences due to the rabbit strain or the interaction between the two affecting factors were detected.

Discussion

Probiotic supplementation has been used as a growth promoter to increase body weight and improve feed efficiency in rabbits (Phuoc and Jamikorn 2017; Abdelsalam and Fathi 2023; Ashour et al. 2024). In this study, probiotic supplementation positively influenced growth, with a clear dose-response trend (although not strictly linear). Body weight gain followed a linear

Table 6. Impact of rabbit strain and supplemented probiotic level on sensory attributes of meat of growing rabbits.

	Probiotic level (P)			Strain (S)			*p* Value			
							P			
Trait	Control	Low	High	Alex	V-Line	SEM	Linear	Quad.	S	P*S
Sensory attributes										
Appearance	6.95^{c}	7.80^{b}	8.40^{a}	7.58	7.85	0.11	<0.0001	<0.0001	0.10	0.84
Colour	6.95^{c}	7.80^{b}	8.35^{a}	7.55	7.85	0.11	<0.0001	<0.0001	0.07	0.69
Taste	7.17^{c}	7.80^{b}	8.35^{a}	7.68	7.87	0.10	0.003	<0.0001	0.27	0.29
Aroma/flavour	7.17^{c}	7.80^{b}	8.35^{a}	7.68	7.87	0.10	0.003	<0.0001	0.27	0.29
Tenderness	7.10^{c}	7.80^{b}	8.40^{a}	7.68	7.85	0.11	0.001	<0.0001	0.33	0.49
Juiciness	7.10^{c}	7.80^{b}	8.40^{a}	7.68	7.85	0.11	0.001	<0.0001	0.33	0.49
Acceptability	7.22^{c}	7.82^{b}	8.50^{a}	7.85	7.85	0.10	0.003	<0.0001	1.00	0.28

Alex: Alexandria strain; SEM: standard error of the mean
^{a,b,c}Means with no common superscripts within each factor are significantly different.

pattern, with significantly higher values observed in rabbits receiving probiotics compared to the control. In agreement with our findings, Phuoc and Jamikorn (2017) reported an approximately 5% increase in the body weight of New Zealand rabbits fed a diet supplemented with lactic acid bacteria as a probiotic feed additive compared to the control.

The current results revealed that feed intake was numerically higher in rabbits supplemented with a high level of probiotics, which agrees with the findings of Shehu et al. (2014) and Ezema and Eze (2014). Contrarily, Oso et al. (2013) reported an improvement in FCR, but no effect on apparent nutrient digestibility in rabbits fed a diet supplemented with *Prediococcus acidilacts* and *Bacillus cereus*. Similarly, Bhatt et al. (2017) stated that probiotic administration with *Lactobacillus acidophilus* and *Lactococcus lactis* in growing Chinchilla rabbits greatly improved feed conversion, average daily gain and final body weight.

However, the lack of a significant effect of probiotic inclusion on feed intake and FCR may be attributed to multiple factors, such as rabbit strain, ambient temperature, the type and concentration of probiotics, as well as the method of administration. Rabbit strain did not significantly influence final body weight or weight gain. Feed consumption was not affected by probiotic supplementation but was influenced by rabbit strain, with V-Line rabbits consuming more. This may explain the higher body weight observed in V-Line rabbits. Although probiotic supplementation did not significantly affect FCR, Alex rabbits converted feed into body weight more efficiently than V-Line rabbits, indicating better feed utilisation. No significant interactions between probiotic level and rabbit strain were observed, suggesting that the effects of probiotics were consistent across both strains.

Regarding physico-chemical meat quality, a strong correlation between muscle pH and meat colour, particularly lightness, has been widely reported. Muscles with a high pH tend to be darker in colour than those with a low pH (Fletcher et al. 2000). Our findings revealed that the administration of probiotics *via* drinking water to rabbits significantly decreased muscle pH in a linear pattern. As is well known, meat colour is the primary visual attribute that attracts consumers at the time of purchase.

The probiotics increased the yellowing and luminosity of meat compared to the control group (Nóia et al. 2020). Additionally, probiotic-supplemented broilers' leg and breast muscle exhibited higher colour lightness and greater WHC, along with lower pH values (Mohammed et al. 2024). Furthermore, Alex rabbits recorded the lowest pH values compared to V-line ones. Regarding WHC, the current findings revealed a significant decrease in this parameter in rabbits receiving a low level of probiotics, compared to other groups. These results align with the findings of Elkhateeb et al. (2018), who reported that WHC decreased in low-level probiotic groups. Probiotic administration (*Saccharomyces boulardii*) has been shown to enhance the physical properties of rabbit meat, such as cooking yield and WHC (Jiya et al. 2018). In contrast, Nóia et al. (2020) reported no effect of probiotic inclusion on WHC. Similarly, Jiya et al. (2018) found no significant differences in WHC, cooking yield or cooking loss among various inclusion rates of *Saccharomyces boulardii*. Moreover, Pogány Simonová et al. (2020) reported no significant differences in meat pH values, colour, proximate composition or WHC between the probiotic-supplemented group and the control group when using *Enterococcus faecium* in rabbit meat. Rotolo et al. (2014) also indicated that yeast used as a probiotic did not affect the performance or meat quality of rabbits reared in controlled environments. In this context, the administration of *Enterococcus faecium* to rabbit diets did not influence meat pH values, colour or WHC (Mancini and Paci 2021). The researchers attributed these findings

to the specific probiotic strain, which may not markedly influence meat quality attributes. Concerning rabbit strain effect, results showed that rabbit strain had a significant impact on pH and WHC. Additionally, meat colour values exhibited significant differences between the two strains across all attributes. However, Paci et al. (2012) found that pH and WHC were not significantly influenced by the genetic origin of rabbits, whereas meat colour attributes exhibited significant differences.

Concerning carcase yield and internal organs, the results indicated that probiotic supplementation, particularly at low level, affected some traits (e.g. head, kidney and front limb), but its overall effect on carcase yield was modest. The results revealed that receiving probiotics at low level may enhance meat deposition in the forequarters. Rabbits in all treatment groups had almost similar dressed carcases, which aligns well with several earlier reports (Marounek et al. 2007; Rotolo et al. 2014). In addition, our findings are consistent with those reported by Bhatt et al. (2017), who did not observe significant differences in carcase characteristics among the treatment groups. The lack of response to probiotic supplementation in carcase yield suggests that the probiotic strains or doses used may not sufficiently influence nutrient absorption or muscle deposition to affect overall yield (Mancini and Paci 2021). In contrast to these findings, Ashour et al. (2024) concluded that using *Bifidobacterium bifidum* in rabbit feed could enhance growth performance and carcase characteristics, especially under hot weather conditions. Rabbits receiving higher levels of *Bacillus subtilis* (400 g/ton) showed improved carcase traits, particularly the mid and hind parts under high temperatures (Fathi et al. 2017). Rabbit strain had notable effects on carcase yield and certain organ traits, with V-Line rabbits generally superior in meat yield and organ development. The significantly higher carcase yield in V-Line rabbits aligns with the breed's genetic selection for meat production traits. V-Line, developed for fast growth and efficient feed conversion, tends to have superior carcase characteristics (Al-Saef et al. 2008). On the other hand, Belabbas et al. (2019) found no significant differences in carcase traits between the local population and the developed rabbit line. The increased heart size observed in V-Line rabbits likely reflects higher metabolic demands and greater cardiovascular development due to their higher growth potential. This suggests a genetic predisposition in V-Line rabbits for enhanced heart development, possibly related to metabolic or cardiovascular efficiency. The significant reduction in kidney relative weight of rabbits received higher probiotic level agrees with the findings of Wang et al. (2021), who stated that the probiotic supplementation did not significantly increase kidney weight in rats. The spleen is an immune-related organ, and the observed difference suggests greater immune organ development in V-Line rabbits, possibly contributing to strong immune system and better health (Smith and Hunt 2004).

Probiotic supplementation markedly enhanced antioxidant capacity in both low and high levels showing similar improvements. V-Line rabbits exhibited superior TAC compared to Alex, indicating better innate or responsive antioxidant defence. The significant interaction between the two factors suggests that the probiotic effect is dependent on rabbit strain, with V-Line likely gaining benefit more from supplementation. This suggests that probiotics exert a positive effect on systemic antioxidant defence, likely by modulating gut microbiota, enhancing nutrient absorption (particularly of antioxidant vitamins and minerals) and stimulating endogenous antioxidant systems. In agreement with the findings of Abd El-Aziz et al. (2022), prebiotic supplementation can improve antioxidant status, and caecal microbiota. Using probiotics as lactic acid bacteria can produce antioxidant enzymes such as SOD and catalase, which help scavenge free radicals (Feng and Wang 2020). The rabbit strain also had a significant effect, with V-Line rabbits showing higher than Alex rabbits. This may reflect a superior antioxidant defence system in V-Line rabbits. This may be due to the differences in metabolism, growth rate and immune function associated with selective breeding for performance traits (Fathi et al. 2017). Moreover, the significant interaction between probiotic level and rabbit strain suggests that the magnitude of the probiotic effect differs between genetic lines. This implies a strain–nutrient interaction, where genetic potential influences the animal's responsiveness to dietary interventions (Abd El-Hamid et al. 2022). Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative cell damage, was not significantly affected by probiotic supplementation or rabbit strain. The high level of probiotic administration had a slightly lower MDA level, indicating reduced oxidative damage to lipids. Regarding SOD activity, no significant differences were observed among probiotic treatments or rabbit strains. This insignificant effect suggests that probiotics may not have directly altered SOD enzymatic activity under experimental conditions. These findings are consistent with those of Abdel-Wareth et al. (2021), who reported that while probiotics enhanced TAC and reduced oxidative markers, they had variable or negligible effects

on individual antioxidant enzymes like SOD under heat stress.

Meat microbial quality is a key indicator of food safety and shelf life, particularly in rabbit meat, which is prone to microbial contamination due to its relatively high pH and moisture content. In this study, supplemented probiotics and rabbit strain significantly influenced the microbial profile of the meat. Probiotics may reduce TBC through competitive exclusion of pathogens, production of bacteriocins (natural antimicrobial peptides) and modulation of host gut microbiota. These lead to improved gut health and reduced translocation of pathogens to the meat (Pogány Simonová et al. 2015; Lyasota et al. 2020). Furthermore, the reduction in microbial contamination may stem from improved intestinal barrier function and lowered gut bacterial shedding during slaughter (Gaggìa et al. 2010). Increasing probiotic supplementation appeared to reduce total bacterial load, especially in Alex rabbits. The observed linear and quadratic significance suggests both gradual and possible threshold effects. Probiotic supplementation also suppressed spore-forming bacteria, although the response may differ between strains. The Alex rabbits were more responsive. The significant interaction observed indicates that the impact of probiotics depends on rabbit strain. This may reflect breed-specific differences in gut microbiota composition and immune function (Fathi et al. 2017). These findings highlight the importance of considering genetic background when implementing dietary interventions to improve meat hygiene. Spore-forming count (SFC), often dominated by *Clostridium* and *Bacillus spp.*, was also markedly reduced by probiotic supplementation. This reinforces the antimicrobial role of probiotics, particularly against spore-formers, which are often heat-resistant and pose a risk during storage. In this context, the use of spore-forming probiotics such as *Bacillus subtilis* has been shown to not only survive processing but also inhibit harmful spore-formers in meat animals (Payne et al. 2024). Probiotics may also stimulate the host's immune response and mucosal immunity, resulting of reduced microbial colonisation in the gut and, consequently, in meat post-slaughter. Rabbit genetic strain also greatly influenced SFC, with Alex rabbits showing higher values than V-Line rabbits. This may reflect differences in gut physiology or hygiene management between rabbit genetic lines. In addition, a strong linear reduction in *Escherichia coli* counts was found by probiotic supplementation. The results of Al-Shawi et al. (2020) and Sachdeva et al. (2025) support our findings. They stated that the probiotics reduced *E. coli* colonisation by creating an unfavourable environment (lower pH, competition for adhesion sites and nutrient competition). Consistently, feeding probiotics such as *Lactobacillus* spp. and *Bacillus* spp. can inhibit the growth of these pathogens, either through competition for nutrients or by producing antimicrobial substances. The absence of a significant strain effect suggests that the action of probiotics on *E. coli* is robust across different genetic backgrounds. Regarding *Salmonella sp.* prevalence in meat, the reduction of *Salmonella prevalence* from 100% in both control and low probiotic groups to just 20% in the high probiotic level indicates a powerful antimicrobial effect at higher probiotic doses. Rabbit strain did not influence this outcome. The action potential for probiotics as a natural control strategy against *Salmonella* contamination is well documented. Several studies have reported that high doses of probiotic inclusion inhibit *Salmonella* by enhancing gut integrity, promoting beneficial flora (e.g. *Lactobacillus*), and stimulating local immune responses (Mancini and Paci 2021; Abd El-Hamid et al. 2022).

Concerning meat quality attributes, the results indicate that the probiotic administration (at both levels) significantly enhanced sensory attributes compared to control rabbits. The positive impact of probiotic supplementation on meat quality has been well documented (Mancini and Paci 2021; Saha et al. 2023). Supplementing rabbit diets with *Bacillus subtilis* at levels of 200 and 400 g/t markedly improved the proximate meat composition, including higher percentages of dry matter, organic matter, protein and fat (Fathi et al. 2017). The effect of probiotics on gut microbial load may also influence meat quality. Some studies have reported that reducing pathogenic microbial populations in animals given probiotics can promote beneficial bacteria and reduce the risk of meat contamination, thereby enhancing food safety. Probiotics may also improve meat tenderness and reduce spoilage due to their influence on gut and immune system functions (Al-Shawi et al. 2020). Additionally, probiotic supplementation has been shown to improve various aspects of meat quality, including enhanced WHC, meat texture and oxidative stability (Shah et al. 2020). Based on rabbit strain, numerically higher sensory scores were recorded for the V-Line rabbits compared to the Alex ones. This suggests a genetic predisposition to superior meat quality. The quality of meat can be influenced by animal breed and environmental factors (Ha et al. 2022). Similarly, Tůmová et al. (2014) and Wang et al. (2016) reported that different rabbit

breeds exhibited variations in meat quality traits, including tenderness and juiciness.

Conclusions

We concluded that probiotic supplementation significantly improves growth performance, antioxidant status and meat quality in growing rabbits under hot climate conditions, with the effects varying by strain. Both high and low probiotic doses enhanced final body weight and weight gain compared to the control group. V-line rabbits exhibited higher feed intake, while Alex rabbits showed better feed conversion. Probiotic supplementation, especially at higher levels, significantly improved muscle pH and reduced microbial loads (TBC, SFC, *E. coli* and *Salmonella sp.*), with strain influencing bacterial counts differently. Notably, rabbits that received probiotics showed superior antioxidant capacity in blood plasma, with an advantage observed in V-line rabbits compared to Alex ones. Meat quality traits were positively affected by probiotic supplementation across all measured characteristics, although rabbit strain had no significant effect on sensory meat quality. Importantly, the interaction between strain and probiotic level was significant for most traits, emphasising the need to consider both factors for optimal rabbit production under heat stress conditions.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU253158].

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Data availability statement

The data that support this study will be shared upon reasonable request to the corresponding author.

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