



EFFECT OF PROBIOTICS ON PRODUCTIVE, PHYSIOLOGICAL AND MICROBIOLOGICAL PARAMETERS OF NEW ZEALAND WHITE RABBITS REARED UNDER HOT SUMMER CONDITIONS

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ABSTRACT: The effect of two kinds of probiotics different in their mode of actions was tested on rabbits' performance grew under hot summer conditions. Fifty-six New Zealand White rabbits (5-weeks-old) were fed on growing diet and orally supplemented with *Saccharomyces cerevisiae* (*S. cerevisiae*) 0.5, 1 and 1.5 g., *Lactobacillus acidophilus* (*L. acidophilus*) 1, 2 and 3×10^9 CFU/kg body weight or distilled water as a control. Calculated temperature humidity index was classified as severe heat stress. Significant improvements in productive parameters, (average daily gain and feed conversion ratio) were detected in the high-dose group (3×10^9 CFU/kg) of *L. acidophilus*. Similarly, the haemoglobin concentration, red blood cell count and fT3 serum levels were considerably higher in the *L. acidophilus* groups, with significantly lower cortisol levels. The mean duration of anxiety-related behavioural responses in the open field test showed significant improvements in treated groups. Also, microbiological investigation showed an absence of some pathogenic bacterial species (*Salmonella spp*, *Clostridium spp* and *Enterobacteria spp*) in treated groups and the presence of beneficial yeast species (*Yarrowia lipolytica*) in the *L. acidophilus*-supplemented groups. Conclusively, administration of *S. cerevisiae* and *L. acidophilus* for consecutive 8 weeks may counteract the consequences of chronic heat stress in growing rabbits.

Keywords: *Oryctolagus cuniculus*-Heat stress-Probiotics-Physiology-Behaviour-Intestinal Microbiota

INTRODUCTION

Superiority of rabbit's prolificacy and productivity to other livestock are severely limited under hot summer conditions (Sharma and Choudhary, 2017). The adverse effect on heat stressed rabbits may initiate certain physiological and behavioural mechanisms to dissipate surplus body heat (Donnelly, 2004). If such mechanisms fail to mitigate hyperthermia, dysregulation of thermal balance can take place and physiological parameters directly decline, which ultimately compromises performance and affects productivity (Zeferino et al., 2013). These impairments in productive traits have been clearly demonstrated in many comparable studies (Fathi et al., 2017). Hyperthermia also provokes oxidative processes, which might cause cellular damage, reduce antioxidant activities, decrease immune responses and consequently increase mass mortalities (Phuoc and Jamikorn, 2017).

Several studies have highlighted the beneficial effects of synthetic drugs to address heat stress in rabbits during summer months (Fann and O'Rourke, 2001). Furthermore substantial research has been devoted to identifying alternative substances as growth promoters in parallel with global reservations to limit antibiotics and pharmaceutical agents in the animal production industry (Mingmongkolchai and Panbangred, 2018). Probiotics may produce a favourable effect on microbiological responses by aiding in the establishment of an intestinal population of bacteria and by preventing enteric diseases of rabbits. Yeast and most of the administered lactic acid bacteria can resist stomach acid and arrive active at their sites of action and can also act on the digestive caecal ecosystem (Kimsé et al., 2012). Addition of yeast to rabbit feed has a direct action on the surroundings of the microflora by increasing the redox

without altering the acidity of the gastrointestinal tract. Gut colonization is largely affected by probiotics, which have competitive biomass growth against harmful microorganisms, reducing the intestinal pH and enhancing digestion efficiencies. This high efficiency in digestibility might strengthen the immune defence against pathogenic microorganisms (Fortun-Lamothe and Drouet-Viard, 2002). It is hypothesized that oral administration of probiotics to weaning rabbits could possibly improve the gut microbial population and digestion, resulting in enhanced productivity. Furthermore, the addition of yeast, *S. cerevisiae*, increased the proportion of *Ruminococcus albus* (Belhassen et al., 2016) but did not modify the structure or diversity of the bacterial community. Probiotics have a positive effect on animals as bioregulators of the intestinal microflora and fortify the host's natural immunity (Kechagia et al., 2013).

In addition to the function of probiotics as a growth enhancer and regulator, probiotics have been investigated as an effectual approach to alleviate the unfavourable effects of hyperthermia on rabbits (Fathi et al., 2017). There are limited studies of probiotic supplementation effects on microbial populations in rabbits (Phuoc and Jamikorn, 2017). Therefore, the present work aimed to assess the effects of oral supplementation of both *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Lactobacillus acidophilus* (*L. acidophilus*), on the growth performance, carcass traits, blood parameters, behavioural traits and intestinal microbiological characteristics of rabbits reared under hot summer conditions.

MATERIALS AND METHODS

Animals and management

This study was carried out at the rabbit farm of Animal Production Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, during the summer season. Fifty-six New Zealand White rabbits (NZW) at five-weeks-old and average weight 0.97 ± 0.07 kg were used. Animals were randomly assigned to seven experimental groups (eight rabbits each, 4 rabbits per cage). Rabbits were housed in galvanized wire cages equipped with feeding troughs and drinking nipples and reared under a semi-closed management system. Feed and water were freely provided and the light cycle was constant at 12L: 12D. All groups were fed isonitrogenous and isocaloric commercial diets that contained 17% crude protein, 2.8% fat, 10% crude fibre and 10.89 MJ/kg diet.

Ambient temperature and relative humidity (RH) were recorded daily inside the rabbitry using a digital thermo-hygrometer apparatus. Temperature-humidity index (THI) was calculated according to following equation: $THI = db^{\circ}C - \{(0.31 - 0.31RH)(db^{\circ}C - 14.4)\}$, which was formulated by LPHSI (1990) and adapted by Marai et al. (2001). The obtained THI value (28.94) was classified as severe heat stress.

Administration of probiotics

Two kinds of commercial probiotics [*Lactobacillus acidophilus* (LacteolFort[®], Rameda Pharma, Egypt) and *S. cerevisiae* (Dietary Supplement[®], Mepaco Pharma, Egypt)] were used. Before starting the experiment, *L. acidophilus*, supplied as a powder, was cultured on MRS agar media and *S. cerevisiae*, supplied as tablets was cultured on Sabouraud dextrose agar (SDA) to evaluate their viability. The studied probiotics were dissolved in 5 ml distilled water and orally administered twice weekly for 8 consecutive weeks. Probiotics were administered using plastic

droppers and the animals were assigned to the following treatments: (C) control group (received distilled water), (S1) *S. cerevisiae* low dose (0.5 g/kg body weight (BW)), (S2) medium dose (1 g/kg BW), (S3) high dose (1.5 g/kg BW), (L1) *L. acidophilus* low dose (1×10^9 colony-forming units (CFU)/kg BW), (L2) medium dose (2×10^9 CFU/kg BW) and (L3) high dose (3×10^9 CFU/kg BW).

Growth performance and carcass traits

Live body weight and feed intake were recorded weekly to the nearest 0.1 (g). The productive parameters, including average daily gain (ADG), relative growth rate (RGR), feed intake (FI) and the feed conversion ratio (FCR), were calculated for all groups. At the end of the experiment period (animals aged 14 weeks), all animals were fasted for overnight, weighed and then slaughtered. The internal organs were removed from the body cavity such as heart, liver, kidney, spleen and abdominal fat and weighed to the nearest 0.01g. Giblets (heart, liver, kidney and spleen) were calculated as the percentage of the carcass weight. Edible meat (empty carcass + giblets) and abdominal fat were expressed as a percentage of the live body weight. For each animal, the pH of the fresh duodenum content was determined using an ADWA pH meter (AD1040, Romania). The length of the caecal appendix was measured to the nearest 0.01 (cm) using a calliper.

Physiological parameters

Two blood samples were collected during the sacrificing procedure. One in a heparinized tube for determination of certain haematological parameters, such as the haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs) and platelet (PLT) count. A complete blood cells count and white blood cell differential count were assessed shortly after collection by a conventional method using a

haematology analyser (SFRI H18 light blood cell counter, France). Hence, the relative proportion of neutrophils to lymphocytes (N/L ratio) was calculated. The 2nd blood sample was collected in a non-heparinized tube for serum biochemical analysis. Blood serum was separated by centrifugation at 1800×g for 20 min and was stored at -20°C until analysis. *In vitro* quantitative determinations of free triiodothyronine (fT3), free thyroxine (fT4) and cortisol hormone were performed using Automated Cobas e411/601 Immunoassay Analysers (Cobas 6000, Germany) based on electrochemiluminescence immunoassay (ECLIA) technology. The sensitivity of the assay was 0.2 ng/ml, 0.4 ng/dl and 18.0 µg/dl for fT3, fT4 and cortisol, respectively.

Behavioural measurements

One day before been slaughtered, the animals were individually tested in an open field apparatus, which offered the opportunity to analyse anxiety-related behaviours in response to heat stress. The apparatus was described by EL-Azzazi et al. (2017). Each testing session was recorded with a video network camera (Panasonic® WV-NS202AE) mounted above the testing apparatus and linked to a computer. Each rabbit was placed in the central square and allowed to freely explore the arena for a 10-min testing session. After the 10-minute test, rabbits were returned to their home cages and the open field arena was cleaned between each test using 30% ethanol. The following behavioural activities were measured: locomotion, grooming, freezing, rearing and resting. Focal sampling and continuous recording techniques were used during the 10-minute recording session for all individuals. Each testing session was analysed using Behavioural Observation Research Interactive Software (BORIS, v. 2.95, University of Torino, Torino, Italy).

Microbiological investigation

After the slaughtering procedure, microbial activities were assessed on four rabbits from each group. At least 2 g of duodenal and caecal contents were aseptically collected from each animal and then preserved in sterile glass tubes. Representative samples of digested contents from the caecum and duodenum, extracted inside an anaerobic chamber, were resuspended in 5 ml of PBS (pH 5.5) and stored at -20°C until analysis. Bacterial isolation was performed using nutrient agar and selective media that allow the growth of specific genera. Finally, identification was confirmed by staining, motility testing and biochemical tests as described by (Forbes et al., 2007). For the identification of yeast biota, routine laboratory methods were used, including isolation on Sabouraud dextrose agar (Merck, Germany) supplemented with 0.1 mg/ml chloramphenicol and then incubated aerobically at 37°C for 48 h. After incubation, the isolates were identified by standard-taxonomic procedures, including the production of germ-tubes, typical microscopic appearances on cornmeal agar supplemented with Tween-80, chlamyospore production, colony morphology and pigment production on chromogenic medium and identification was confirmed using an API 32°C AUX (BioMerieux, Marcy-l'Etoile, France) according to Neppelenbroek et al. (2014).

Statistical analysis

SPSS 22 software (version 22.0; IBM Corp., Armonk, NY, USA) was used for all analyses. One-way analysis of variance (one-way ANOVA) was used with treatment groups as fixed variables, while growth traits, physiological parameters, behavioural responses and total bacterial and yeast counts were dependent variables. Significant differences amongst the groups were detected using Duncan's post hoc test at $\alpha = 0.05$.

RESULTS**Productive parameters**

Overall mean values of the initial and final average body weights of all animals were 970.3 ± 72.82 g and 2134.8 ± 68.27 g, respectively (Table 1). The highest ADG was obtained in L3 (22.66 ± 0.87), followed by S2 (21.52 ± 0.70), while the lowest value was observed in L1 (19.62 ± 0.41). Differences between all treated groups and the control were not significant, except for L3 ($P \leq 0.05$). RGR did not differ significantly among all studied groups, but L3 was the highest without significant difference compared to control (Table 1). The highest value of FCR was recorded in the control (4.31 ± 0.21), while the best value was measured in the L3 group (3.73 ± 0.14). A significant difference ($P \leq 0.05$) in FCR values was observed between the S3 and L3 groups compared with the control (Table 1).

Table (2) shows the effects of the probiotic supplements on certain carcass traits. The percentage of edible meat did not differ significantly among all treatment groups and the overall mean of all values was $57.75 \pm 0.33\%$. Giblets ratio of S1 (5.98%) and S2 (5.89%) groups were higher ($P \leq 0.05$) than those of control (5.71%) group while the giblet percentages in the L1 (5.05%), L2 (5.15%) and L3 (5.40%) groups did not significantly differ from the control group. Similarly, there were no significant differences in abdominal fat yield among all treatments. Although the S2, S3 and L3 groups represented the longest caecal appendices (10.75, 10.91 and 10.75 cm, respectively), the differences were not statistically significant in compare to control. Finally, the probiotic supplementation groups had lower duodenal pH values than the control group (Table 2), as the lowest pH values were recorded in the S2 group, but not significant different.

Physiological parameters

As presented in Table (3), Hb recorded the highest value in L3 (11.77 ± 0.44) and L2 (11.54 ± 0.63) groups with significant ($P \leq 0.05$) difference in compared to the control (9.82 ± 0.76 g/dl). No other treatment groups differed significantly from the control. Additionally, RBCs count were significantly higher in the L3 (5.41 ± 0.13) and L2 (5.36 ± 0.25) groups compared with the control (4.59 ± 0.40) group, while no other experimental groups differed significantly from the control. WBC counts were significantly ($P \leq 0.05$) lower in the L3 group compared with S1-treated group, while both highest and lowest values were not differed significantly from the control. However, a significant increase in PCV value was found in the group that received the highest level (L3) of *L. acidophilus* and lowest level (S1) of *S. cerevisiae* compared with L1-treated group but they did not differ significantly when compared with the control. PLT values exhibited a similar trend to that observed for blood RBCs and Hb. A significant difference ($P \leq 0.05$) in PLT values was observed between the L1 compared with the S2, L2 and control group. In contrast, the control group had a higher N/L ratio than all treated groups, but a significant difference was only observed in the L2 and S3 groups in comparison with the control. On the other hand, cortisol concentration increased significantly ($P \leq 0.05$) in the control, while it significantly ($P \leq 0.05$) declined in the *L. acidophilus* treated groups and the lowest level was observed in the L2 group (Table 3). In addition, the level of fT3 was highest ($P \leq 0.05$) in the L3 group (100.47 ng/dl) compared with that observed in L1, but the differences were not significant in comparison with the control (95.70 ng/dl). Although there were some variations in serum fT3

concentrations among treatments, FT4 did not display a similar trend.

Behavioural measurements

There were no significant differences in locomotion or grooming between groups (Table 4). However, the treatment groups showed significant differences ($P \leq 0.05$) in freezing, that the highest duration of freezing was found in the control and S1 groups. Similarly, rearing and resting activities differed significantly ($P \leq 0.05$) between groups and the highest duration of rearing was found at the highest concentrations of the *L. acidophilus* (L3) while the highest duration of resting was found in S2-treated group.

Microbiological parameters

The data in Table (5) show differences in bacterial and yeast taxa isolated from the control and treatment groups. The bacterial flora isolated from control animals were *Actinobacter spp.*, *Actinobacillus spp.*, *Salmonella spp.*, *E coli*, *Serratia spp.*, *Clostridium spp.*, *Streptococci spp.*, *Enterobacteria spp.* and *Bacteroides*, while no yeast species were isolated. In the *S. cerevisiae*-supplemented groups, the isolated bacterial flora was *Actinobacter spp.*, *Actinobacillus spp.*, *E coli*, *Serratia spp.* and *Bacteroides*, while the yeast species were *S. cerevisiae* and *Rhodotorula mucilaginosa*. However, in the *L. acidophilus*-supplemented groups, the isolated bacterial flora was *Actinobacillus spp.*, *Pseudomonas spp.*, *Serratia spp.*, *L. acidophilus* and *bacteroids* and the yeast species were *Yarrowia lipolytica* and *Rhodotorula mucilaginosa*.

As presented in Table (6), the total bacterial count from the duodenum of the *S. cerevisiae*-supplemented groups revealed that the S1 group was not significantly different from the control, unlike S2 and S3. The total caecal

bacterial count showed that S1, S2 and S3 differed significantly ($P \leq 0.001$) from the control. Additionally, there was a significant ($P \leq 0.001$) difference between the control and *L. acidophilus*-treated groups with respect to the total bacterial count of the duodenum and caecum. Yeast fungi were isolated only from the caecum of both treated groups ($P \leq 0.01$); the duodenum of the control and treated groups revealed no yeast isolates.

DISCUSSION

Productive traits

Our results showed that administration of high dose (L3) of *L. acidophilus* showed the best effective level of probiotics on productive performance of rabbits grown during summer condition. Whereas the highest dose of *L. acidophilus* significantly increased average daily gain of growing rabbits and also improved feed conversion ratio when compared to other experimental groups. Ondruska et al. (2011) concluded that there was a negative relationship between hyperthermia and the daily feed intake, body weight and feed conversion ratio of NZW rabbits. The beneficial use of probiotics in the present results was confirmed by microbiological examination of the caecum and duodenum of experimental rabbits, which showed the absence of pathogenic bacteria from the *S. cerevisiae*-treated group. The improvement in growth and feed efficiency could be explained by the retention of beneficial microbiota in the gut and an increase in both feed digestion and absorption. The results of our study agreed with those of Phuoc and Jamikorn (2017), who concluded that supplementation of *L. acidophilus* alone or combined with *B. subtilis* at a partial dose may enhance the number of gut-beneficial bacterial inhabitants, nutrient digestibility, caecal fermentation, feed

efficacy and growth performance, but rabbits fed only *B. subtilis* alone were not different from the control. By contrast, Belhassen et al. (2016) reported that neither weight gain nor feed intake was affected by yeast supplementation during the whole period of the experiment except in the first week after weaning. Another explanation could be that the probiotics can act on non-digestible carbohydrates and give rise to short-chain fatty acids, which result in better absorption of minerals and nutrients (Simonová et al., 2015) and consequently, increased weight gain.

It is worth noting that the addition of probiotics influenced the dressing percentage, where the treated rabbits showed a higher cut parts percentage and earlier organ development (Fathi et al., 2017). In our study, there were no significant variations in carcass traits, caecal appendix and duodenal pH, in contrast to giblet %, between the treated groups (Table 2). However, a slight increase in edible meat parts was observed for the rabbits that received probiotics. The opposite trend was observed for pH values, which indicates that the lower pH associated with probiotic treatments may be considered a good indicator of increased nutrient digestibility and, in turn, enhanced feed efficiency utilization. Phuoc and Jamikorn (2017) reported that the higher organic acid concentration in the intestine of treated rabbits should be expected to decrease the pH, which probably has a favourable effect on nutrient digestibility. In addition, the caecal appendix has thick walls that hold lymphoid tissue and produce bicarbonate ions in the caecal lumen to neutralize volatile fatty acid production (Davies and Davies, 2003). This could also be evident by the increased need for

lymphoid tissue due to altered microbial populations in such rabbits.

Physiological parameters

Our results has clearly demonstrated two aspects: First, the deleterious consequences of chronic heat stress on physiological parameters, Second, the improvement in the studied parameters upon administration of different doses of *S. cerevisiae* and *L. acidophilus* to heat-stressed rabbits. Heat is a potent driver of multiple malfunctions in stressed organisms, including compromised blood constituents (Akbarian et al., 2016), endocrine function (Mete et al., 2012) and immune response (Lara and Rostagno, 2013), which consequently may lead to numerous abnormalities and failure of the individual to cope with the stress level. Some measured haematological parameters, including the Hb concentration and RBCs count, were significantly higher in the L3 group than in the control. This significant decrease in Hb concentration and RBC count (Table 3) in the control group is simply explained by the fact that during heat stress, the rate of nutrient intake and utilization would be differently affected compared with normal circumstances (Recep and Halit, 2016). Similarly, impairment of iron intake and output may take place during chronic stress, which further affects iron metabolism or iron distribution and eventually inhibits the synthesis of Hb and erythropoiesis (Wei et al., 2008). In this study, all probiotic-treated rabbits had a lower N/L value than control animals. The same trend of lymphocyte populations and total WBCs count may be indicative of higher activity of immune responses in treated rabbits and, on the other hand, suppressed immune responses under chronic heat stress in the control group (Davis et al., 2008). A similar finding was found by

Fathi et al. (2017), who reported that the inclusion of a probiotic (*Bacillus subtilis*) in the feed of rabbits reared under hot environmental conditions greatly enhanced humoral and cell-mediated immune responses. The latter results indicated that the treated rabbits were less stressed than the control under hyperthermia conditions.

The changes in cortisol and fT3 levels may best describe the mechanism deployed to increase resistance and adaptability to the thermal load by altering the levels of certain hormones, such as increasing cortisol levels (Nakyinsige et al., 2013). Additionally, fT3 elevation in the L3 group might indicate better adaptation to chronic heat stress, reducing the rate of glucose oxidation and increasing the amount of metabolic heat production (Kumari and Nath, 2017). The increase in cortisol might be helpful to the control group in coping with the disadvantages of long-term high temperature (Khalil et al., 2015), while the low level of cortisol in the treated group signified that the strength of thermal resistance which might be due to the administration of a high dose of *L. acidophilus*.

Behavioural analysis

Rabbits from the control group that were exposed to the open field paradigm exhibited increased anxiety-related responses compared to other treatment groups (Table 4). This is clearly illustrated by the increased freezing and decreased rearing and resting durations in the control group. Freezing is a typical defensive response that is displayed in response to a variety of environmental stresses, including novelty, and has been among the most powerful predictors of anxiety and stress responses to a novel context (Gould et al., 2009). In our experiment,

the control group displayed the highest durations of freezing, which is an indicator of higher levels of anxiety due to thermal stress. Similar results were also recorded by Sinha and Kumar (2004), who found that heat-stressed rats showed higher durations of freezing than control. Similarly, rearing is a specific exploratory behaviour that is used to gather information from the surrounding environment. Upon facing an unusual context, animals engage in systematic exploratory activities that are considered a priority to uncover the novel environment (Russell et al., 2010); however, when animals are stressed, they exhibit lower levels of exploration (Shors and Dryver, 1992) and rearing (Sinha and Kumar, 2004). This confirms our assumption that animals in the control group that displayed the lowest rearing durations were more stressed and anxious due to heat stress. Conversely, the longer resting time that was observed mirrors the lowered levels of stress and anxiety in the yeast-treated groups.

Finally, two behavioural variables were also measured in all groups: locomotion and grooming, which showed non-significant differences among the treated groups. We assume that the short 10-min testing time in the open field test promoted similar locomotor activities in all of the treated groups. Although grooming has been extensively studied as an indicator of low-stress situations, our experiment failed to unveil any difference in grooming time between treated groups. To explain our results, grooming can typically be higher in two opposing situations, under high and low stress, and higher stress levels can effectively provoke bouts of purposeless stereotypical grooming (Xu et al., 2012).

Microbiological investigation

Heat stress lead to symbiosis of beneficial gut bacteria, which might increase disease susceptibility (Zhang et al., 2017). In the present study, the caecum and duodenum bacterial flora of both the control and treated rabbits with probiotics were similar in diversity. Additionally, no yeast species were isolated from the duodenum of the control or treated rabbits and yeast species were only isolated from the caecum of treated animals (Table 6). This finding is consistent with the results of Kimsé et al. (2012), who reported that the caecal digestive ecosystem of the control rabbits which did not receive dietary probiotics appears to lack fungi. The significant increase in the bacterial count in the supplemented groups compared with the control (Table 6) may refer to the existence of certain factors that might cause changes in the gastrointestinal ecosystem. These factors including physical stress, antibiotics, prebiotics, probiotics, radiation, altered intestinal peristalsis and nutritional dietary changes, can ultimately change the microbial population (Prakash et al., 2011).

In our study *Yarrowia lipolytica* was isolated from the *L. acidophilus* groups and may have beneficial effects. *Yarrowia lipolytica* and *S. cerevisiae* have a similar structure, but *Yarrowia lipolytica* stimulates the immune defence mechanisms, by increasing the percentage of phagocytic cells and plasma lysozyme levels (Merska et al., 2015). Also, *Lactobacilli spp.* was isolated only from *L. acidophilus*-supplemented groups, which increase the feed efficiency. Bernardeau et al. (2006), who stated that rabbits fed diets supplemented with *L. acidophilus* had the highest number of intestinal lactobacilli, could enhance intestinal hydrolytic enzyme activity, increase nutrient digestibility and

feed efficiency. In addition, this may lead to an increase in caecal acetic acid (Oglesbee and Jenkins, 2012) and decrease intestinal coliform population, which consequently improves gastrointestinal performance. Also, Högberg and Lindberg (2006) stated that gut function might have been improved by feeding a diet supplemented with *L. acidophilus* due to the increase in lactase and sucrase activities in the small intestinal mucosa. Certain pathogenic bacterial species, for example, *Salmonella spp.*, *Clostridium spp.* and *Enterobacteria spp.* were isolated from control but not isolated from either of the probiotic-treated groups. This was consistent with the previous work of Price et al. (2010) who explained that the mode of action of *S. cerevisiae* as a probiotic in animals initiates beneficial effects in terms of productive performance. These positive effects generally due to stimulation of nonspecific resistance, inhibition of toxin actions and their antagonistic effect against pathogenic microorganisms, which finally neutralize the impact of heat stress.

CONCLUSION

In conclusion, it was elucidated that supplementation of probiotics such as *S. cerevisiae* and *L. acidophilus* may offer a managerial strategy for rabbit farms to overcome the deleterious effects of thermal stress, especially during the hot summer months. It seems that further studies on the mode of action of probiotics are needed to expand knowledge on the use of such additives in industrial practice.

Conflicts of Interest:

The authors declare that they have no competing interests.

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Table (1): Effect of probiotics supplementation on body weight, feed intake, average daily gain and feed conversion of rabbits reared under hot summer conditions.

Parameters	Control	<i>Saccharomyces cerevisiae</i>			<i>Lactobacillus acidophilus</i>			SEM	P-value
		S1	S2	S3	L1	L2	L3		
Initial body weight (g)	939.3	968.3	952.0	981.8	989.5	985.4	975.6	72.89	0.904
Final body weight (g)	2086.1	2129.1	2157.1	2123.8	2088.1	2115.0	2244.6	68.27	0.745
Average daily gain (g/head/day)	20.48 ^b	20.73 ^{ab}	21.52 ^{ab}	20.39 ^b	19.62 ^b	20.17 ^b	22.66 ^a	0.61	0.045
Relative growth rate (%)	2.18	2.14	2.26	2.08	1.98	2.05	2.32	0.20	0.775
Feed intake (g/head/day)	86.95	84.48	83.48	79.21	80.36	82.23	83.71	--	--
Feed conversion ratio (FCR)	4.31 ^b	4.08 ^{ab}	3.91 ^{ab}	3.89 ^a	4.11 ^{ab}	4.11 ^{ab}	3.73 ^a	0.12	0.050

Means with different superscripts within the same row are significantly different at the $P \leq 0.05$ level.

Table (2): Effect of probiotics supplementation on carcass traits, caecal appendix and duodenal pH of rabbits reared under hot summer conditions.

Parameters	Control	<i>Saccharomyces cerevisiae</i>			<i>Lactobacillus acidophilus</i>			SEM	P-value
		S1	S2	S3	L1	L2	L3		
Edible meat (%)	57.98	58.62	58.10	58.92	58.52	58.01	58.21	0.46	0.167
Giblets (%)	5.71 ^{ab}	5.98 ^a	5.89 ^a	5.45 ^{ab}	5.05 ^b	5.18 ^{ab}	5.40 ^{ab}	0.25	0.050
Abdominal fat (%)	0.96	1.19	1.12	1.09	0.92	0.87	1.25	0.15	0.501
Caecal appendix (cm)	10.50	10.19	10.75	10.91	9.90	9.63	10.75	0.51	0.599
Duodenal pH	7.06	6.77	6.58	6.97	6.64	6.81	6.95	0.15	0.285

Means with different superscripts within the same row are significantly different at the $P \leq 0.05$ level.

Table (3): Effect of probiotics supplementation on blood parameters and hormonal levels of rabbits reared under hot summer conditions.

Physiological Parameters	Control	<i>Saccharomyces cerevisiae</i>			<i>Lactobacillus acidophilus</i>			SEM	P-value
		S1	S2	S3	L1	L2	L3		
Haematology									
Hb (g/dl)	9.82 ^c	10.07 ^{bc}	9.99 ^{bc}	10.46 ^{bc}	10.20 ^{bc}	11.54 ^{ab}	11.77 ^a	0.43	0.028
RBC (x10 ⁶ /mm ³)	4.59 ^c	4.76 ^{bc}	4.49 ^c	4.72 ^{bc}	4.72 ^{bc}	5.36 ^{ab}	5.41 ^a	0.19	0.015
WBC (x10 ³ /mm ³)	6.15 ^{ab}	7.20 ^a	5.40 ^{ab}	5.47 ^{ab}	4.75 ^{ab}	5.05 ^{ab}	3.70 ^b	0.72	0.050
PCV (%)	42.88 ^{ab}	45.44 ^a	42.45 ^{ab}	40.90 ^{ab}	38.72 ^b	42.27 ^{ab}	45.37 ^a	1.51	0.040
PLT (x10 ³ /mm ³)	198.25 ^b	236.33 ^{ab}	198.66 ^b	267.66 ^{ab}	308.20 ^a	199.25 ^b	288.75 ^{ab}	27.42	0.050
N/L ratio	1.74 ^a	1.21 ^{abc}	1.47 ^{ab}	0.82 ^{bc}	1.14 ^{abc}	0.61 ^c	1.13 ^{abc}	0.22	0.021
Hormones									
Cortisol (µg/dl)	1.92 ^a	1.10 ^{ab}	1.19 ^{ab}	1.23 ^{ab}	0.94 ^b	0.61 ^b	0.73 ^b	0.27	0.050
fT3 (ng/dl)	95.70 ^{ab}	93.75 ^{ab}	78.30 ^{ab}	76.75 ^{ab}	67.00 ^b	79.45 ^{ab}	100.47 ^a	7.49	0.046
fT4 (µg/dl)	2.78	3.10	2.48	2.93	2.80	2.58	3.30	0.38	0.425

Means with different superscripts within the same row are significantly different at the $P \leq 0.05$ level.

N/L means neutrophils to lymphocytes ratio; fT3 and fT4 refers to free triiodothyronine and free thyroxine, respectively.

Table (4): Effect of probiotics supplementation on behavioural activities (sec) in an open field test of rabbits reared under hot summer conditions.

Behavioural Parameters	Control	<i>Saccharomyces cerevisiae</i>			<i>Lactobacillus acidophilus</i>			SEM	P-value
		S1	S2	S3	L1	L2	L3		
Locomotion	235.90	235.75	176.32	193.78	224.48	252.71	239.62	22.06	0.220
Grooming	3.73	4.98	3.45	5.91	3.68	3.38	3.49	1.62	0.920
Freezing	24.59 ^a	21.78 ^a	10.26 ^b	4.57 ^b	5.52 ^b	6.69 ^b	3.68 ^b	3.18	0.001
Rearing	6.41 ^b	17.14 ^b	15.80 ^b	12.17 ^b	9.92 ^b	11.92 ^b	33.03 ^a	4.21	0.009
Resting	0.46 ^b	0.50 ^b	48.50 ^a	36.28 ^{ab}	0.24 ^b	0.41 ^b	15.40 ^{ab}	9.32	0.049

Means with different superscripts within the same row are significantly different at the $P \leq 0.05$ level.

Table (5): Effect of probiotics supplementation on bacteria and yeast fungi isolated from the caecum and duodenum of the rabbit groups.

Groups	Control	<i>Saccharomyces cerevisiae</i>	<i>Lactobacillus acidophilus</i>
Isolated bacterial species	<i>Actinobacter spp.</i> <i>Actinobacillus spp.</i> <i>Salmonella spp.</i> <i>E. coli</i> <i>Serratia spp.</i> <i>Clostridium spp.</i> <i>Streptococci spp.</i> <i>Enterobacteria spp.</i> <i>Bacteroides</i>	<i>Actinobacter spp.</i> <i>Actinobacillus spp.</i> <i>E. coli</i> <i>Serratia spp.</i> <i>Bacteroides</i>	<i>Actinobacillus spp.</i> <i>Pseudomonas spp.</i> <i>Serratia spp.</i> <i>Lactobacillus acidophilus</i> <i>Bacteroides</i>
Isolated yeast species	-ve Yeast species	<i>Saccharomyces cerevisiae</i> <i>Rhodotorula mucilaginosa</i>	<i>Rhodotorula mucilaginosa</i> <i>Yarrowia lipolytica</i>

Table (6): Total bacterial (CFU/g) and yeast/fungal (cells/g) counts in dilutions of 10⁻¹² isolated from the duodenum and caecum of rabbits supplemented with probiotics under hot summer conditions.

Bacteria/yeast fungi count	Source	Control	<i>Saccharomyces cerevisiae</i>			<i>Lactobacillus acidophilus</i>			SEM	P-value
			S1	S2	S3	L1	L2	L3		
Bacterial count (CFU/g)	Duodenum	12.50 ^e	15.00 ^e	48.42 ^d	47.17 ^d	55.38 ^c	61.50 ^b	68.17 ^a	0.82	0.001
	Caecum	18.25 ^g	22.58 ^f	52.50 ^e	65.73 ^d	76.00 ^c	83.83 ^b	91.00 ^a	1.31	0.001
Yeast Species Count (Cells/g)	Duodenum	00.00	00.00	00.00	00.00	00.00	00.00	00.00	0.00	NS
	Caecum	00.00 ^d	12.67 ^b	13.08 ^b	17.33 ^a	8.25 ^c	8.75 ^c	13.75 ^b	0.33	0.010

Means with different superscripts within the same row are significantly different at the $P \leq 0.05$ level. NS: Non-significant. CFU = colony forming units.

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الملخص العربي

تأثير البروبيوتيك على الصفات الانتاجية والفسيوولوجية والميكروبيولوجية للأرانب النيوزيلندي الأبيض المرباة تحت ظروف الصيف الحار

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تهدف هذه الدراسة إلى بحث تأثير نوعين من البروبيوتيك مختلفان في الأثر الفعال لهما على الأرانب المرباة في ظروف الصيف الحار. استخدم في هذه الدراسة عدد 56 من الأرانب النيوزيلندي الأبيض النامية عمر 5 أسابيع وتم تغذيتها على عليقة نامية مع امدادها عن طريق الفم بتركيزات مختلفة من *S. cerevisiae* (S1): 0.5 و S2: 1 و S3: 1.5 جم) ومن *L. acidophilus* (L1: 1 و L2: 2 و L3: 3 بليون وحدة) لكل كجم من وزن الجسم ومجموعة مقارنة أعطيت ماء مقطر. وبحساب دليل الحرارة والرطوبة كان 28.94 مما يدل على أن الحيوانات تتعرض لاجهاد حراري. وقد لوحظ تحسن في الصفات الانتاجية (معدل النمو اليومي والتحويل الغذائي) في المجموعة المعطاة تركيز عالي من L3 مقارنة بالمجموعات الأخرى. أيضاً تركيز الهيموجلوبين وعدد كرات الدم الحمراء ومستوى الثيرونين ثلاثي اليود (ft3) بالسيرم كانت أعلى في مجموعات اللاكتوباسيلاس مقارنة بمجموعة الكنترول مع انخفاض مستوى الكورتيزول معنوياً. وكان متوسط الاستجابات السلوكية بها تحسن ملحوظ في مجموعات المعاملة. أيضاً من الناحية الميكروبيولوجية غابت أنواع البكتريا الممرضة (أنواع السالمونيلا والكوليستريديوم والانثيروباكتيريوم) في المجموعات المعاملة، مع تواجد الخمائر النافعة (*Yarrowia lipolytica*) في المجموعات المعاملة باللاكتوباسيلاس. ويمكن نستخلص مما سبق أن المعاملة بخمائر *S. cervisiae* وبكتريا اللاكتوباسيلاس *L. acidophilus* لثمانية أسابيع متتالية يمكن أن يحسن الأداء الانتاجي والفسيوولوجي للأرانب النامية تحت ظروف الصيف الحار.