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Impact of Dietary *Lactobacillus plantarum* Postbiotics on the Performance of Layer Hens under Heat Stress Conditions

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Abstract

This experiment was conducted to determine the performance of heat-stressed layers fed a diet containing the probiotic *Lactobacillus plantaru*m RS5 or its products of fermentation (postbiotics). Twenty-week-old Isa White layers, were subdivided into six treatments of 32 individually caged birds. Half of the birds were reared under regular temperature conditions, while the other half was subjected to cyclic daily heat stress. Layers were offered one of three diets: 1) Control; 2) Control + *Lactobacillus plantarum* RS5 probiotic; 3) Control + *Lactobacillus plantarum* RS5 postbiotics. Birds were tested for performance and visceral organ development for 5 months. Heat stress negatively affected the birds' feed intake, egg weight, shell weight percentage, Haugh unit, shell thickness, yolk color, body weight and spleen weight percentage. Postbiotics significantly increased egg production ($p < 0.05$) in comparison to the control and the probiotic fed group (94.8% vs 92.6% vs 93.1%, respectively). Birds under probiotic or postbiotic diet showed a significantly higher ($p < 0.05$) feed intake and egg weight, although the probiotic had a more pronounced and gradual effect. Specific gravity, yolk weight percentage and shell thickness didn't show differences among dietary groups. The Haugh Unit was significantly higher ($p < 0.05$) in probiotic group which also showed a significantly lower yolk color index ($p < 0.05$). The different feed treatments did not impact the bird's viscera weight percentage, except for the ileum that was significantly lower ($p < 0.05$) under postbiotic supplementation. Both probiotics and postbiotics could be used as a potential growth promoters and might alleviate heat stress impact in poultry industry.

Keywords

Lactobacillus plantarum, Layers, Heat Stress, Postbiotic, Probiotics,

Performance

1. Introduction

Heat stress is one of the most important environmental stressors challenging poultry production worldwide. Having adverse effects on animal health and productivity, heat stress can result in heavy economic losses due to increased mortality and reduced productivity [1]. Furthermore, birds' physiology and behavioral response to heat stress negatively affects productivity owing to lower feed intake and digestive capacity and alteration of the intestinal mucosa and microbiota ecology [2].

To combat some of the adverse effects of heat stress on poultry specifically on health and growth performance, the inclusion of feed additives such as antibiotics in the diet at sub-therapeutic levels is a common practice. The inclusion of antibiotics as growth promoters in layers' feed have been shown to alleviate the effect of heat stress and improve performance [3]. However, excessive and prolonged use of antibiotics in animal feeds has raised concerns regarding antibiotic residues in animal products and the development of antibiotic-resistant bacteria [4]. This has led to the banning of dietary growth promoter for animals in several countries [5].

To replace the use of antibiotics, probiotics have been used as feed additives in poultry to promote a healthy gut environment and improve growth performance [3]. It has been reported that probiotic strains can help maintaining the microbial balance in the gastrointestinal tract (GIT) as well as making changes in the composition of the intestinal microflora by increasing beneficial bacteria and decreasing harmful pathogens. This could be due to competitive exclusion by which beneficial bacteria compete with harmful ones for nutrients and attachment sites on the intestinal epithelial wall [6]; and/or produce of antimicrobial substances, such as organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins [7]. In addition, some probiotic cultures have been reported to be able to improve the morphology of chicken intestine toward increasing nutrient absorption and endogenous digestive enzymes secretion surface [6].

The use of probiotic supplementation containing beneficial bacteria, such as *Lactobacillus* spp., has a positive effect on the intestinal microbial population [3]. *Lactobacillus* strains have a high ability to attach to the intestinal epithelium and can establish in the chicken intestine within a day, so they are considered to be normal bacterial flora of the GIT of chickens [8]. *Lactobacillus* plantarum is classified as lactic acid bacteria categorized under probiotic microbial groups living in the digestive tract to improve its condition [6].

The possible mechanisms of probiotic action include, but are not limited to 1) Competitive exclusion of pathogenic micro-organisms; 2) Production of antimicrobial substances; 3) Competition for growth factors and nutrients; 4) En-

hancement of adhesion to intestinal mucosa to protect the gut lining from any damage; 5) Improvement of epithelial barrier function by increasing mucin expression and secretion, thereby limiting bacterial movement across the mucous layer; 6) Improvement of secretion of IgA the principal weapon protecting the body from pathogens and toxins that might otherwise penetrate mucosal surfaces [9].

In poultry, the administration of probiotics could improve the feed conversion ratio (FCR) and feed intake (FI), increase egg production, and stimulate growth rate [3]. However, it has been stated that probiotic bacteria may acquire and transfer antibiotic resistance genes between organisms [4].

Subsequently, postbiotics, which are metabolites of probiotics, have been used as feed additives in livestock as a potential replacement for antibiotics and probiotics [3]. Postbiotics have a similar mechanism of action and capacity as probiotics owing to the presence of secondary metabolites from probiotics but without a living cell [10]. The presence of antimicrobial metabolites, such as organic acids and bacteriocins, in postbiotics can reduce the gut pH and inhibit the proliferation of opportunistic pathogens in the feed and gut of animals [11]. It has been demonstrated that the application of postbiotics as a feed additive in livestock promotes the growth performance and health of broilers [11], layers [3] and pigs [12], as well as enhancing rumen fermentation and health in ruminants [13]. In addition, apart from their ability to promote a healthy gut environment, the potential antioxidant capacity of postbiotics obtained from *Lactobacillus* has been found to be particularly strong under heat-stress conditions [14].

Previous study showed that postbiotics obtained from *Lactobacillus plantarum* exhibit inhibitory action on various pathogenic bacteria, including Listeria monocytogenes, Salmonella typhimurium, Escherichia coli and vancomycin-resistant Enterococci [15]. In addition, postbiotics obtained from *L. plantarum* has been found to be particularly strong under heat-stress conditions [3]. In heat-stressed broilers, postbiotics from *L. plantarum* are expected to provide similar benefits to those from probiotic bacteria [3].

In layers, postbiotic dietary supplementation improves hen-day egg production, reduces the fecal pH and fecal Enterobacteriaceae population, increases the fecal lactic acid bacteria, reduces the plasma and yolk cholesterol, and increases the fecal volatile fatty acids content. Postbiotic metabolite combinations can be used as an alternative feed additive to achieve high productivity and better poultry health [3].

Since probiotic/postbiotic effect is strain dependent and may also depend on the host and its immunologic state, this study aims to evaluate the effect of dietary *L. plantarum* RS5 postbiotic preparations on performance and immunity parameters of layers under heat stress conditions. Performance parameters include live body weight; feed intake; egg production; egg quality namely specific gravity, yolk color shell thickness, HU score, percent white weight, percent yolk weigh and percent shell weight; and visceral organ indices namely liver, spleen,

gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat.

2. Materials and Methods

All experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee # 20-10-583.

Field Evaluation of the Preparation of Dietary Supplements

1) ***Lactobacillus Plantarum strain***

The *Lactobacillus plantarum* strain RS5 [NCIMB 701088] is obtained from NCIMB laboratory in the United Kingdom. This strain was isolated by A A Nichols from cheese.

The bacterial culture was resuspended in Man, Rogosa and Sharpe (MRS) broth and incubated at 37˚C for 48 hours. The solution was subjected to Gram staining for confirmation. Gram positive, non-spore-forming rods were inspected under microscope. The suspension was sub-cultured on MRS agar growth medium for 48 hours at 37˚C. A couple of white round colonies were randomly selected, part of which was sub-cultured on MRS agar (streaking for isolation), and the other part was inspected by Gram staining procedure for confirmation. Colonies were re-suspended in sterile 0.85% (w/v) saline solution. Transmittance of this bacterial suspension was adjusted to 3% at 450 nm wavelength. After a serial dilution, culture on MRS agar, and colonies count it has been shown that this mother solution contains 10^{15} CFU/ml. The initial bacterial cultures were preserved at −80˚C in MRS broth.

2) ***Preparation of Postbiotics from L. plantarum Strains***

Working cultures of *L. plantarum* were prepared by inoculating 10% (v/w) 10^9 CFU/mL active bacterial cells into MRS media and incubated at 30˚C for 10 h, followed by centrifugation [Eppendorf 5810 centrifuge, Eppendorf, Maryland, USA] at 10,000× g and 4˚C for 15 min. The cell-free supernatant (CFS) was then collected by filtration through a cellulose acetate membrane of 0.22 microns pore size [3]. The CFS was stored at −20˚C until the feeding trial was conducted. The liquid postbiotics were mixed with the feed using the three-way mixing technic in a horizontal feed mixer, at a concentration of 300 ml of solution (CFS in MRS broth) per 100 kg of feed.

3) ***Preparation of Probiotics from L. plantarum Strains***

The culture medium used for bacterial growth was MRS agar. The overnight culture of *Lactobacillu*s isolate was than inoculated for 24 to 48 h. The colonies were harvested and resuspended in phosphate buffered saline (PBS, pH 7.4) and count was adjusted to 3×10^9 CFU/mL using spectrophotometry. The suspension was mixed with the basal diet at a concentration of 200 ml of solution (RS5 in MRS broth) in every 100 kg of feed, using the three-way mixing technic in a horizontal mixer.

4) ***Birds Housing & Treatments***

This experiment was conducted at the research facilities (AREC) of the Amer-

ican University of Beirut in the Beqaa region in four identical environmentally controlled poultry houses. The trial was completed over a period of 6 months including one month of adaptation and 5 months of experimental phase. Birds egg production an initial body weight was recorded during the adaptation phase, based on these results birds were allocated into homogenous groups. A total of 192 twenty-week-old pullets of an Isa white strain, were equally subdivided into six groups of 32 birds individually caged, where each bird was considered a replicate. Birds in each group were subdivided into two houses, each pen holed 16 birds. Birds in the first two houses were reared under regular temperature, while those in the second two houses were reared under cyclic heat stress conditions, where the temperature gradually reached about 30˚C for 4 consecutive hours daily. Temperature was monitored daily at 10 am, 1 pm & 4 pm, and once a week at 4 am. Birds in each house were equally divided into 3 categories according to the offered diet: control, control+probiotic and control + postbiotc.

The birds were given water and feed ad libitum, provided as per the Manual recommendations (Institut de Sélection Animale BV, Villa "de Körver", Boxmeer, Netherland). At arrival, birds were granted a period of one month of adaptation. Afterwards, hens were allocated to different treatments according to live body weight and egg production to ensure homogeneous grouping at the beginning of the experiment. The study was granted the Approval of the Animal Care and Use Committee of the American University of Beirut (IACUC Approval# 20-10-583).

Birds were assigned to six different treatments as detailed in Table 1.

5) ***Evaluation of hens' production parameters***

The initial live body weight and the egg production were recorded for all birds at the end of the adaptation phase in order to allocate birds into different treatment homogeneously. Afterwards, egg production was recorded on a daily basis, and the live body weight of 4 birds per treatment was measured at sacrifice, *i.e.* at the middle and the end of the experimental phase.

The feed intake was measured once weekly. Twelve eggs per treatment were randomly collected to evaluate egg quality namely egg weight, Haugh unit, eggshell thickness, yolk color, density, white weight, yolk weight and shell weight. The egg quality was measured monthly for 3 consecutive days.

Table 1. Control and experimental groups.

Treatment	Temperature	Diet	Cages	Replication
1	Regular	Control	32	32 birds
2	Regular	Probiotic	32	32 birds
3	Regular	Postbiotic	32	32 birds
4	Cyclic heat stress	Control	32	32 birds
5	Cyclic heat stress	Probiotic	32	32 birds
6	Cyclic heat stress	Postbiotic	32	32 birds

6) *Evaluating hens' visceral organs weight index*

Four birds were sacrificed from each treatment in order to measure visceral organ indices namely: liver, spleen, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat. This process was carried out 2 times during the whole experimental phase at middle and again at the end of the trial.

7) *Statistical Design and Analyses*

The design of the trial is a factorial arrangement of treatments in a randomized block design, factorial 2 × 3 with 6 treatments and 32 birds/replicate per treatment. Univariate analyses were used to analyze the data and mean comparison at 95% confidence level. Analysis was performed using SPSS software (Statistical Package for the Social Sciences, V. 25).

3. Results and Discussion

3.1. Egg Production

Eggs from individual birds or cages were collected daily. The hen-day egg production was calculated as the percentage of production per treatment per month during the 5 months of the experiment. Results are presented in Table 2.

A numerical decrease in egg production was observed in heat stressed animals. Other reports show that heat stress significantly reduces egg production due to decrease in feed intake and the uptake of available nutrients and decreased digestibility of many components of the diet [16] [17]. Variable results

Table 2. Percentage egg production and percentage broken or shelless eggs of layer hens under different feed and temperature parameters during the 5 months of the experiment.

Treatment	Percentage egg production						Percentage Broken & Shelless eggs				
	Month 1	Month 2	Month 3	Month 4	Month 5	ALL	Month 2	Month 3	Month 4	Month 5	ALL
Feed											
Control	96.8	94.0[a]	92.4	93.1	90.6	92.6[a]	0.2	1.1	1.5	2.9	1.3
Probiotic	96.3	94.1[a]	93.1	91.7	91.1	93.1[ab]	0.3	1.6	1.6	2.3	1.2
Postbiotic	97.4	97.2[b]	94.8	92.6	93.8	94.8[b]	0.1	1.5	1.3	2.4	1.1
SEM	1.23	1.26	1.83	1.32	1.65	1.10	0.15	0.46	0.52	0.73	0.26
Temperature											
Control	97.7	95.4	93.9	92.7	91.8	93.9	0.3	1.6	1.8	3.0	1.4[a]
Heat Stress	95.9	94.9	93.0	92.3	91.9	93.2	0.2	1.3	1.2	2.1	0.9[b]
SEM	1.00	1.02	1.49	1.08	1.35	0.89	0.12	0.38	0.42	0.59	0.22
Variables											
Feed	0.649	0.017	0.387	0.578	0.109	0.105	0.266	0.497	0.802	0.636	0.782
Heat Stress	0.082	0.670	0.544	0.701	0.900	0.410	0.413	0.439	0.115	0.138	0.029
Feed * HS	0.291	0.643	0.252	0.674	0.614	0.189	0.225	0.267	0.748	0.409	0.337

[a,b]Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$). Total of 192 birds, 32 birds/treatment.

may be explained by the fact that our experiment adopted a different model in comparison to other studies. These differences include the use of birds of different age or genetic background, as well as variable intensity and duration of the heat stress treatments applied [18], knowing that temperature reached a maximum of 30˚C in this study. Another potential factor is that heat stress might also be accompanied by other stressors, such as limited housing space, insufficient ventilation, unbalanced feed ration and/or pathogens contamination [19] that were not observed in this experiment.

Postbiotic supplementation in feed showed a faster effect on percentage egg production than probiotic supplementation in this experiment. Overall, hens with supplemented postbiotic in their diet showed a significantly higher ($p < 0.05$) egg production than the control group and a numerically higher value ($p > 0.05$) than probiotic group. Especially for month 2 of the experiment, postbiotic diet had a significant impact ($p < 0.05$) in comparison to the other 2 diets (control and probiotic). This might be due to a slower effect of probiotics on egg production. Other research is in agreement with our findings knowing that probiotic supplementations increase laying, so it improves the egg production percentage [17] [20]. In other studies, postbiotics were shown to increase hen-day egg production [3] due to the increased feed conversion rate but also the improved immune response in chickens [11].

None of the interactions between the different feed and temperature parameters was significant for egg production or percent broken and shelless eggs for the entire experimental phase. A lower percentage of broken eggs was observed in heat stressed birds due to bad structure of few cages in the control group. However, probiotics were shown to ameliorate the quality of the eggs by increasing the eggshell strength and thickness leading to a decrease in the number of broken eggs [21].

3.2. Feed Intake

Feed intake was measured by subtracting the balance of feed from the quantity originally supplied to the laying hens. Results are presented in **Table 3**.

The heat stress negatively affected the birds during the first month (79.1 g vs 84.2 g for the control; $p < 0.05$) however they quickly adapted to the elevated temperature. In other studies, birds exposed to high ambient temperature show a significantly lower feed intake and a decreased digestibility of many components of the diet [16] [17] [22]. This might be due to the intensity of the heat in this experiment, reaching only 30˚C, and the adaptive capacity of the birds [18].

As per the changes in the diet, the individuals that were under probiotic or postbiotic diet showed a significantly higher feed intake (FI), especially during the first (82.9 g vs 82.2 g vs 79.7 for the control; $p < 0.05$) and third month (99.1 vs 99.8 vs 94.6 for the control; $p < 0.05$). Abundant research shows that probiotics prepared from *Lactobacillus* improved feed intake in chickens [23] [24] [25]. As for postbiotic, research reported a higher, though no significant, increase in

Table 3. Birds average feed intake under different temperature and feed parameters during the 5 months of the experiment.

Treatment	Average Daily Feed Intake (g)					
	Month 1	Month 2	Month 3	Month 4	Month 5	ALL
Feed						
Control	79.7[a]	94.8	94.6[a]	105.6[a]	112.1	93.8
Probiotic	82.9[b]	93.1	99.1[b]	101.4[b]	112.2	94.4
Postbiotic	82.2[b]	94.4	99.8[b]	106.5[a]	112.8	95.7
SEM	1.01	1.06	1.63	2.09	1.68	2.06
Temperature						
Control	84.2[a]	94.4	97.4	100.9[a]	111.4	94.8
Heat Stress	79.1[b]	93.8	98.5	108.1[b]	113.2	94.6
SEM	0.82	0.86	1.33	1.71	1.37	1.67
Variables						
Feed	0.004	0.268	0.004	0.031	0.888	0.648
Heat Stress	0.000	0.450	0289	0.000	0.192	0.901
Feed * HS	0.998	0.166	0.909	0.003	0.463	0.875

[a,b]Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$). Total of 192 birds, 32 birds/treatment.

feed intake [3] [11] which correlates with the overall result. However, the probiotic diet showed an unusual decrease in FI during the fourth month which might be consistent with the small decrease in egg production in probiotic groups during this month. Overall neither the temperature nor the feed had a significant effect on the birds feed intake.

3.3. Egg Quality

Twelves eggs were collected from each treatment monthly for 3 consecutive days. The eggs were tested on the same day. After weighing, the gravity was measured than the egg was broken and placed on to measuring plates. The yolk color, thickness of the shell, Haugh unit, yolk weight and the shell weight were measured, calculated, and recorded. Results are presented in **Table 4** & **Table 5**.

The heat stress showed a negative impact ($p < 0.05$) on the egg weight, percent shell weight, Haugh unit, shell thickness, and yolk color. Other studies agree with our findings, whereby decreased egg weight under high ambient temperatures has been reported extensively, and low egg weight is correlated with reduced feed intake. This might be an adaptive stress response to conserve metabolic energy [26] [27]. Additionally, it has been previously reported that exposure to high temperature negatively affects yolk weight, albumen weight, specific gravity, Haugh unit and yolk index [28] [29]. This might be due to the decline in feed digestibility such as proteins, fats, and starch [30]. Other reports show the negative effect of birds panting under heat stress on eggshell. Accelerated panting

Table 4. Percentage egg weight, shell weight, white weight and yolk weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

Treatment	Egg weight (g)					% Shell weight					% White weight					% Yolk weight				
	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All
Feed																				
Control	54.2^{a}	55.3	57.6^{a}	60.1^{a}	56.8^{a}	14.9^{ab}	14.9	15.4^{a}	15.1	15.1^{a}	60.4^{ab}	60.6^{a}	58.9^{a}	59.0	59.7^{a}	24.7	24.7^{a}	25.9	26.1	25.4
Probiotic	55.5^{b}	55.8	59.8^{b}	62.1^{b}	58.2^{b}	14.6^{a}	14.9	14.5^{b}	15.1	14.8^{b}	60.7^{a}	60.1^{ab}	60.2^{b}	59.3	60.1^{a}	24.7	25.1^{ab}	25.8	25.7	25.3
Postbiotic	54.4^{ab}	55.7	58.6^{ab}	61.4^{b}	57.5^{ab}	15.3^{b}	15.2	15.2^{a}	15.5	15.2^{a}	59.9^{b}	59.7^{b}	59.1^{a}	58.5	59.3^{b}	24.8	25.4^{b}	25.9	26.3	25.6
SEM	0.65	0.58	0.63	0.57	0.37	0.27	0.24	0.26	0.25	0.13	0.44	0.43	0.46	0.42	0.23	0.33	0.33	0.32	0.33	0.17
Temperature																				
Control	55.2^{a}	56.8^{a}	59.3^{a}	61.0	58.0^{a}	15.1	15.2	15.4	15.3	15.3^{a}	60.5	59.9	59.1	58.4^{a}	59.5^{a}	24.4^{a}	24.9	25.9	26.3^{a}	25.4
Heat stress	54.1^{b}	54.4^{b}	57.9^{b}	61.3	56.9^{b}	14.8	14.9	14.7	15.1	14.9^{b}	60.1	60.4	59.6	59.4^{b}	60.0^{b}	25.1^{b}	25.2	25.8	25.8^{b}	25.5
SEM	0.53	0.48	0.52	0.47	0.31	0.22	0.20	0.21	0.21	0.11	0.36	0.36	0.38	0.34	0.18	0.27	0.27	0.26	0.27	0.14
Variables																				
Feed	0.096	0.643	0.004	0.002	0.001	0.021	0.540	0.002	0.245	0.001	0.131	0.091	0.016	0.210	0.002	0.925	0.094	0.794	0.182	0.191
Heat stress	0.045	0.000	0.012	0.530	0.001	0.227	0.122	0.492	0.263	0.000	0.270	0.168	0.159	0.003	0.024	0.015	0.414	0.639	0.046	0.681
Feed * HS	0.269	0.212	0.093	0.000	0.012	0.827	0.525	0.147	0.527	0.524	0.714	0.581	0.128	0.165	0.079	0.320	0.706	0.016	0.105	0.020

^{a,b}Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$). Total of 192 birds, 32 birds/treatment.

Table 5. Specific gravity, Haugh Unit score, shell thickness and yolk color of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

Treatment	Gravity					Hu score					Shell thickness					Yolk color				
	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All	Jul	Aug	Sep	Oct	All
Feed																				
Control	1.095	1.093^{a}	1.091	1.091	1.091	96.0^{ab}	88.2^{a}	85.9	89.4	89.9^{a}	.36	.37	.38	.38	.37	7.7	7.7^{a}	6.6	6.9^{a}	7.2^{a}
Probiotic	1.095	1.091^{b}	1.093	1.090	1.090	98.2^{a}	91.3^{b}	87.0	90.5	91.7^{b}	.37	.36	.38	.38	.37	7.4	7.3^{b}	6.5	7.1^{b}	7.0^{b}
Postbiotic	1.091	1.091^{b}	1.092	1.090	1.090	94.6^{b}	89.6^{ab}	86.6	89.4	90.1^{a}	.37	.37	.37	.38	.37	7.5	7.3^{b}	6.7	7.0^{ab}	7.1^{ab}
SEM	0.001	0.001	0.001	0.001	0.001	10.24	10.37	10.31	10.28	0.72	0.004	0.004	0.004	0.006	0.002	0.17	0.12	0.01	0.09	0.07
Temperature																				
Control	1.096	1.093	1.092	1.091	1.091	96.4	91.0^{a}	87.2	90.8	91.3^{a}	.37	.37^{a}	.38	.38	.38^{a}	7.8^{a}	7.4	6.7	7.0	7.2^{a}
Heat stress	1.095	1.091	1.092	1.090	1.091	96.4	88.4^{b}	85.9	88.7	89.8^{b}	.36	.36^{b}	.37	.38	.37^{b}	7.3^{b}	7.5	6.5	6.9	7.1^{b}
SEM	0.001	0.001	0.001	0.001	0.000	10.01	10.12	10.07	10.05	0.59	0.004	0.003	0.003	0.005	0.002	0.14	0.09	0.08	0.08	0.06
Variables																				
Feed	0.557	0.010	0.287	0.452	0.689	0.017	0.094	0.702	0.590	0.020	0.317	0.753	0.222	0.979	0.881	0.279	0.000	0.168	0.030	0.114
Heat stress	0.344	0.059	0.644	0.611	0.106	0.747	0.021	0.547	0.052	0.008	0.156	0.028	0.077	0.999	0.020	0.000	0.849	0.253	0.492	0.003
Feed * HS	0.00.97	0.635	0.527	0.754	0.289	0.444	0.670	0.031	0.636	0.837	0.687	0.803	0.096	0.857	0.365	0.003	0.002	0.783	0.147	0.897

^{a,b}Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$). Total of 192 birds, 32 birds/treatment.

increases carbon dioxide levels and higher blood pH, alter acid-base balance (*i.e.*, alkalosis). This will hamper blood bicarbonate availability for eggshell mineralization, induces increased organic acid availability, and decreases free calcium and phosphorus concentration in the blood [27] [31].

The study showed that high ambient temperature did not have a significant effect on the specific gravity probably because the eggs were freshly tested. It did not have a significant impact on percentage yolk weight ($p > 0.05$) which could be due to the high impact heat stress had on the percentage shell weight.

Remarkably, a positive effect was demonstrated on egg white percentage. Although it contradicts other study findings [28] [29], the increment of total proteins and albumin concentrations in heat stressed birds was reported and can be considered as a sort of protection of muscle mass against injury induced by thermal challenge [32]. As reported this study, the percentage white weight increased however Haugh unit decreased which indicates a higher albumen content and a low albumen quality in the produced eggs.

As per the different diets, the supplementation of metabolites in the diets of laying hens may exert different effects compared to live probiotic cultures [3]. This study shows that both treatments, probiotics and postbiotics had a positive impact ($p < 0.05$) on egg weight, although probiotics had a more significant and gradual effect. This agrees with other studies that show an increase in egg weight in birds consuming probiotics. This increase could be due to the increase in albumen weight percentage [21] [24] [25]. However, for birds consuming postbiotics, other research revealed an increase in egg weight that wasn't significant [3]. The fluctuating results might be due the bacterial strain, concentration, and route of administration being used [33].

Probiotics showed a significantly ($p < 0.05$) lower percentage of shell weight; a different outcome in comparison to other studies [21] [24] [25]. That may be a consequence of the variation in mineral and protein absorption [34]. It can be also due to the harmful effect of heat stress on blood pH, the digestibility of many components of the diet, and the decreased plasma protein and calcium levels [16] [27].

The percentage egg white weight was significantly lower ($p < 0.05$) in postbiotic group. In Contrast, Mahdavi *et al.* [33] reported that the inclusion of lactic acid bacteria cultures did not affect any egg production parameters. There is a scarcity of reports documenting the effect of postbiotics on egg white weight. However, as previously stated, the variations in the results were most probably due to the difference in bacterial strains, concentration, and route of administration being used [33].

The Haugh Unit was significantly higher in probiotic group (91.7% vs 89.9% for the control; $p < 0.05$) due to the increase in albumen weight percentage [25]. The Haugh Unit can be associated with the increase in percentage white weight in the probiotic group.

Egg yolk color intensity has been correlated with cholesterol amount in the

yolk [35]. In this study, the yolk color was significantly lower in probiotic group in comparison to the control group (7.0% vs 7.2%, respectively; $p < 0.05$). As demonstrated in other studies, probiotic supplementation may play an important role in altering the lipid metabolism of chickens and subsequently reduce the cholesterol content of egg yolk [36]. Also, postbiotic group showed a lower yolk color that might be due to a reduced plasma and yolk cholesterol [3].

Specific gravity didn't show differences among groups, as mentioned before, probably because the eggs were freshly tested. Also, the percentage yolk weight did not show significant differences ($p > 0.05$) and neither the shell thickness. However, other studies reported an increase in eggshell thickness and strength under probiotic supplementation [37].

3.4. Visceral Organ Indices

Four birds per treatment were sacrificed at the middle and the end of the experiment in order to evaluate the visceral organ indices. Results are presented in Table 6 & Table 7.

Heat stress showed a significant effect ($p < 0.05$) on birds' weight and percentage spleen weight. This effect is initiated by the reduced plasma calcium and phosphorous concentrations under heat stress in laying hens which lowers relative weights of the thymus and the spleen [38]. In addition, growth rates decrease due to the decline of feed digestibility such as proteins, fats, starch [17] [30].

Table 6. Birds weight, percentage liver weight, spleen weight, gizzard weight and proventriculus weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

Treatment	Birds weight (g)			% Liver weight			% Spleen weight			% Gizzard weight			% Proventriculus weight		
	Mid	End	All	Mid	End	All	Mid	End	All	Mid	End	All	Mid	End	All
Feed															
Control	1598	1567	**1582**	3.01	3.30	**3.14**	0.102	0.095	**0.099**	1.35^{ab}	1.37	**1.36**	0.36^{a}	0.41	**0.39**
Probiotic	1535	1572	**1553**	2.99	3.47	**3.23**	0.099	0.093	**0.096**	1.44^{a}	1.33	**1.39**	0.41^{b}	0.41	**0.41**
Postbiotic	1511	1551	**1530**	2.99	3.29	**3.13**	0.091	0.093	**0.092**	1.29^{b}	1.39	**1.34**	0.39^{ab}	0.40	**0.39**
SEM	46.5	48.6	**35.7**	0.177	0.217	**0.149**	0.009	0.009	**0.006**	0.074	0.062	**0.050**	0.021	0.025	**0.017**
Temperature															
Control	1584	1579	**1581^{a}**	3.03	3.23	**3.12**	0.100	0.103^{a}	**0.101^{a}**	1.42	1.37	**1.39**	0.39	0.41	**0.40**
Heat stress	1512	1547	**1529^{b}**	2.97	3.47	**3.21**	0.095	0.084^{b}	**0.090^{b}**	4.29	1.36	**1.33**	0.38	0.40	**0.39**
SEM	37.9	39.7	**29.2**	0.145	0.179	**0.123**	0.007	0.007	**0.005**	0.060	0.051	**0.040**	0.017	0.021	**0.014**
Variables															
Feed	0.187	0.904	**0.367**	0.991	0.651	**0.752**	0.453	0.951	**0.554**	0.127	0.701	**0.594**	0.065	0.898	**0.423**
Heat stress	0.076	0.431	**0.084**	0.726	0.195	**0.482**	0.500	0.024	**0.028**	0.055	0.820	**0.114**	0.638	0.782	**0.613**
Feed * HS	0.016	0.516	**0.410**	0.745	0.742	**0.822**	0.889	0.269	**0.621**	0.579	0.422	**0.738**	0.490	0.344	**0.497**

[a,b]Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$).

Table 7. Birds weight, percentage liver weight, spleen weight, gizzard weight and proventriculus weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

Treatment	% duodenum weight			% jejunum weight			% ileum weight			% abdominal fat		
	Mid	End	All	Mid	End	All	Mid	End	All	Mid	End	All
Feed												
Control	0.56	0.53	**0.54**	1.6	1.7	**1.6**	1.42	1.42	**1.41[a]**	2.5	2.3	**2.4**
Probiotic	0.58	0.52	**0.55**	1.5	1.6	**1.6**	1.35	1.39	**1.37[ab]**	2.5	2.0	**2.2**
Postbiotic	0.51	0.54	**0.52**	1.4	1.5	**1.5**	1.26	1.26	**1.26[b]**	2.3	2.3	**2.3**
SEM	0.041	0.046	**0.030**	0.13	0.16	**0.09**	0.090	0.114	**0.071**	0.43	0.34	**0.27**
Temperature												
Control	0.55	0.53	**0.53**	1.5	1.5	**1.5**	1.36	1.37	**1.37**	2.4	2.1	**2.2**
Heat stress	0.55	0.54	**0.54**	1.6	1.6	**1.6**	1.32	1.35	**1.34**	2.5	2.3	**2.4**
SEM	0.034	0.038	**0.025**	0.11	0.13	**0.08**	0.075	0.093	**0.059**	0.35	0.28	**0.22**
Variables												
Feed	0.233	0.920	**0.656**	0.520	0.690	**0.438**	0.271	0.356	**0.114**	0.792	0.694	**0.848**
Heat stress	0.917	0.948	**0.912**	0.790	0.412	**0.504**	0.585	0.888	**0.606**	0.716	0.341	**0.380**
Feed * HS	0.841	0.886	**0.924**	334	0.693	**0.560**	0.079	0.748	**0.566**	0.504	0.433	**0.726**

[a,b]Means within a column in each comparison group with no common superscripts differ significantly ($p < 0.05$). Total of 192 birds, 32 birds/treatment.

However, heat stress didn't affect any of the other visceral organ; liver, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat. In contrast, Felver-Gant *et al.* [39] reported reduced liver weights in laying hens subjected to chronic heat stress conditions. The difference in the reported results may be explained, as mentioned before, by the fact that birds of different age or genetic background were used, as well as due to variable intensity and duration of the heat stress treatments applied [18]. Additional factors might have aggravated the situation such as limited housing space, insufficient ventilation, unbalanced feed ration and/or pathogens contamination [19].

The different feed treatments did not have any effect on the bird's visceral organ weight, except for the ileum that showed a significantly lower percentage weight ($p < 0.05$) under postbiotic supplementation and a slight decrease in ileum weight under probiotic supplementation to feed. In agreement with our findings, it's reported by Dizaji *et al.* [40] that weight of Proventriculus, Gizzard, Liver and Bursa did not show any significant difference by addition of probiotics. Moreover, probiotics have a positive effect on animals' physical properties of meat, namely poultry carcass quality by increasing overall carcass weight and reducing abdominal fat [41]. It is supposed that postbiotics mimic the impact of the microbial strain [42]. Furthermore, dietary probiotic did not affect the relative weight of the duodenum, jejunum, ileum, or small intestine in broilers, although ileum weight was numerically lower at day 40 [43]. In this experiment,

the reduced ileum size may reflect a more efficient absorption and utilization of nutrients [44].

4. Conclusions

The present study demonstrated that the heat stress negatively affected the birds feed intake especially during the first month resulting in a numerical decrease in egg production, then the birds quickly adapted. Although the individuals that were under probiotic or postbiotic diet showed a higher feed intake, postbiotic supplementation showed a faster positive effect on percentage egg production than probiotic supplementation. The latter had a positive effect on both percentage egg weight and Haugh unit under heat stress conditions; and postbiotic improved percentage egg white weight and percentage egg weight. Both postbiotic and probiotic groups resulted in a lower yolk color which might be due to reduced plasma and yolk cholesterol. The different feed treatments have an effect only on the bird's ileum weight percentage. The reduced ileum size may reflect a more efficient absorption and utilization of nutrients following the application of pro- or postbiotics.

Postbiotic metabolite can be an alternative feed additive to achieve high productivity while reducing the use of conventional chemotherapeutic agents such as in-feed antimicrobials under heat stress conditions. Further research is needed to study the changes induced by pro- or postbiotics at the molecular level. This will give a better insight into the role of such products in mitigating heat stress impact and explore in depth the interactions between these products with intestinal pathogens and epithelial cells. In addition, further study is needed to investigate the economic benefits of the use of postbiotics as a replacement feed additive in layer hens.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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