

RESEARCH

Open Access



# Physiological responses and reproductive performance of naturally heat-stressed rabbit does treated with postbiotic of *Bacillus subtilis* and *Saccharomyces cerevisiae* in free and nano-encapsulated forms

N. S. Hosny<sup>1</sup>, A. S. Morsy<sup>1</sup>, Z. R. Abo-elezz<sup>2</sup> and N. M. Hashem<sup>2\*</sup>

## Abstract

**Background** Heat stress negatively affects the physiology and reproductive functions of rabbits. In order to mitigate these effects, palm date seeds were fermented with *Bacillus (B.) subtilis* and/or *Saccharomyces (S.) cerevisiae* to generate microbial-derived active metabolites, postbiotics, which were subsequently characterized using GC-MS. Notably, the postbiotic generated by the combined microbial fermentation (BYP) exhibited the highest concentration of secondary active metabolites. This postbiotic was then incorporated into rabbit diets in either free (BYP) or nano-encapsulated (NBYP) formulations.

**Methods** Forty-five nulliparous rabbit does were randomly assigned to one of three groups and received dietary supplementation with either 0.9 g/kg BYP, 0.9 g/kg NBYP, or no supplementation (C) over 30 days spanning mating and the first and second semesters of pregnancy.

**Results** Supplementation with BYP and NBYP significantly increased feed intake compared to the C group, while concurrently reducing rectal temperatures and respiratory rates. Both treatments markedly enhanced hematological, immunological, and redox parameters, as well as progesterone levels during pregnancy. The NBYP demonstrated superior effects for most variables, particularly during pregnancy. Additionally, the kindling rate and litter size and litter weight at birth were significantly higher in the BYN and NBPY groups compared to the C group.

**Conclusions** The incorporation of NBYP is recommended as an innovative natural microbial-derived supplement to enhance the health status, heat tolerance, and reproductive efficiency of rabbit does maintained under natural thermal stress conditions.

**Keywords** Nano-encapsulation, Palm date by-product, Postbiotic, Fertility, Antioxidant, Heat stress

\*Correspondence:

N. M. Hashem  
nesreen.hashem@alexu.edu.eg

<sup>1</sup> Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application (SRTA-CITY), New Borg El Arab, Alexandria, Egypt

<sup>2</sup> Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University, Alexandria 21545, Egypt

## Background

The raising of global warming poses a significant threat to livestock production, particularly in rabbit farming, due to the high susceptibility of rabbits to heat stress [1]. The optimal ambient temperature range for rabbits is 15–25 °C, whereas temperatures above 30 °C cause heat stress, resulting in significant changes in physiology, behavior,



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

and metabolism. In adult rabbit does, heat stress correlates with reduced fertility and estrogen and progesterone secretion, impaired ovum integrity, and lower fertilization and conception rates, ultimately affecting litter size and litter weight [2–4].

Dietary manipulation is an effective strategy for alleviating the effects of heat stress in rabbit farming. Targeted supplementation may improve redox status, regulate inflammatory and immune responses, stabilize hormonal balance, and enhance overall reproductive performance. Microbial-derived supplements, including probiotics and postbiotics have been extensively studied for their health-promoting properties. Probiotics are acknowledged for their advantages. However, concerns persist regarding their viability, the possible presence of antibiotic-resistant genes, and their sensitivity to gastrointestinal and storage conditions, which may compromise their efficacy [5]. Furthermore, the sensitivity of probiotics to gastrointestinal conditions, industrial processing, and storage can limit their biological activity and restrict their utility as feed supplements [6, 7]. The concept of a “postbiotic” has emerged as an alternative in order to extend the scope of the probiotics concept beyond its inherent viability. The term “postbiotic” denotes secondary metabolites obtained from probiotic strains, such as *Lactobacillus* (*L.*), *Bifidobacterium*, *Streptococcus*, and *S. cerevisiae* yeast [6, 7]. In 2019, the International Scientific Association of Probiotics and Prebiotics (ISAPP) broadened the conceptual framework of the term ‘postbiotic’ to encompass “the production of microorganisms that are non-living and/or their components that confer health benefits to the host organism.” Postbiotics provide an advantage over probiotics as their benefits do not rely on cell viability, which can vary, and the presence of dead cells may even exceed that of viable cells. The health benefits of postbiotics stem from multiple mechanisms, including antimicrobial, antioxidant, and immunomodulatory activities, along with improving gut function and preserving eubiosis. The observed effects are primarily due to postbiotic compounds such as bacteriocins, organic acids, enzymes, and vitamins [8, 9].

Although few studies have evaluated the health benefits of postbiotics in farm animals, current research suggests potential advantages. For instance, in heat-stressed broiler chickens, the addition of postbiotics derived from *L. plantarum* improved growth performance, intestinal morphology, immune response, the mRNA expression levels of hepatic insulin-like growth factor-1, plasma immunoglobulins, and lactic acid bacteria count while reducing the *Enterobacteriaceae* count [10]. Another study demonstrated that the administration of postbiotic-stabilized non-viable *Lactobacilli* fermentation products in both dry and aqueous formulations

ameliorated colisepticemia [11]. Postbiotics produced by fermentation of *S. cerevisiae* in transitional dairy cows improved milk yield, antioxidant capacity, and immunity [12]. Additionally, lactic acid bacteria-based postbiotics enhanced sperm quality and overall health in male rabbits [13].

Postbiotic quality and its enrichment with secondary metabolites mainly depend on the microbial strains used and the composition of the fermentation media, which influence the metabolic output of these strains. For instance, the addition of inulin to the fermentation medium enhanced the inhibitory activity of *L. plantarum* postbiotics against various pathogenic bacteria, including *S. enterica*, *E. coli*, and *L. monocytogenes* [14]. Consequently, the product enhanced the broiler chickens’ fecal microbiota, meat quality, growth performance, and growth-linked gene expressions [15]. Chang et al. [16] found that the media type affected the composition and functional characteristics of postbiotics generated by *L. plantarum*, and specific metabolites, particularly pyrrole compounds, can be generated by regulating fermentation media composition.

Based on previous findings, we aimed to develop a postbiotic enriched with diverse secondary metabolites to mitigate the adverse effects of heat stress on rabbit does during mating and pregnancy while ensuring product safety by eliminating synthetic media in the production of microbial secondary metabolites. Therefore, we utilized two well-established safe and effective probiotics, *B. subtilis* and *S. cerevisiae* [17, 18]. Moreover, we used palm date (*Phoenix dactylifera* *L. Arecaceae*) seeds by-product as a fermentation medium due to their cost-effectiveness, particularly in arid and semi-arid regions, as well as their high nutritional value and enrichment with nutrients and vitamins that facilitate microbial growth [19]. Additionally, we evaluated the efficacy of free versus nano-encapsulated postbiotics to determine whether nano-encapsulation enhances the biological activity of the secondary metabolites, as recommended by previous studies [20, 21].

## Methods

### Study location

The fieldwork was conducted at the Rabbit Physiology Research Laboratory, Agricultural Experimental Station, Faculty of Agriculture, Alexandria University, Egypt. The postbiotics preparation and laboratory analyses were performed at the Nanoencapsulation and Biotechnology Laboratories (NBL, Animal and Fish Production Department, Faculty of Agriculture, Alexandria University) and at the Laboratory of Livestock Research (Arid Land Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria). All procedures

were approved by the Committee for Institutional Animal Care and Use at Alexandria University (Approval No. 082211212118).

#### Postbiotic preparation

Dates (*Phoenix dactylifera*) were obtained from a palm farm in El Kharga Oasis, The New Valley, South Egypt (25°26'18"N, 30°33'30"E). The date seeds were manually separated from the flesh, thoroughly washed, and stripped of excess husk. The seeds were then dehydrated at 50 °C for 2 days and finely milled to a particle size of 1.0 mm using a heavy industrial disc mill (Buhler-Miag laboratory disc mill, model DLFU, Shandong Province, China).

Probiotic strains *B. subtilis* (EMCC 1009, isolated from natto food, Japan) and *S. cerevisiae* (ATCC MYA-795, isolated from cream) were obtained from Cairo MIRCEN (Faculty of Agriculture, Ain Shams University, Cairo, Egypt).

*B. subtilis* was activated on DSM agar plates (composition: peptone, 5 g; meat extract, 5 g; agar, 15 g per liter; pH 7.0) to prepare activated colonies for subsequent inoculation. Nutrient broth (composition: D(+)-glucose, 1 g; peptone, 15 g; sodium chloride, 6 g; and yeast extract, 3 g per liter; pH 7.5) was used for *S. cerevisiae* activation. Both media were prepared in 500 mL flasks, sterilized by autoclaving at 121 °C for 15 minutes, and cooled before inoculation [1]. Each microbial strain was cultured on agar plates at 30 °C for 48 hours.

To produce postbiotics, date seeds powder (1 kg) was soaked in distilled water (1 L) and inoculated with either *B. subtilis* ( $8 \times 10^{11}$  CFU), *S. cerevisiae* ( $8 \times 10^{11}$  CFU), or both strains combined ( $8 \times 10^{11}$  CFU of each). The mixtures were incubated at room temperature for three days [22]. Following fermentation, the contents were centrifuged (Benchtop Microfuge 20 R, Beckman Coulter, Germany) at 10,000 g for 15 minutes at 4 °C. The resulting cell-free supernatants (postbiotics) were collected, lyophilized, and stored at -80 °C for further analysis.

#### Identification of the active components of postbiotics

The chemical constituents of non-fermented date seeds extract and *B. subtilis*, *S. cerevisiae*, and *B. subtilis* and *S. cerevisiae* postbiotics were characterized using gas chromatography coupled with mass spectrometry (GC-MS; Thermo Scientific TRACE-1300 series GC; Thermo Fisher Scientific Inc., Austin, TX, USA). The analysis was performed on a fused silica DB-5 capillary column (30 m length, 0.32 mm inner diameter, 0.25 µm film thickness; Thermo Fisher Scientific Inc., TSQ 8000 Evo). The temperature profile for the column oven was initially held at 50 °C for 2 minutes, subsequently increased at a rate of 5 °C/min to reach 250 °C for an additional 2 minutes,

and finally elevated to 300 °C at a rate of 30 °C/min for 2 minutes. Helium was employed as the carrier gas, maintaining a continuous flow rate of 1 mL/min. The injector and detector temperatures were set at 250 °C and 290 °C, respectively. Mass spectra were recorded at 5 scans per second over a mass-to-charge ratio of 40 to 700 amu. The NIST 14 mass spectral database, incorporating retention index data, was used for the identification of the chemical constituents [23]. Based on the GC-MS findings, postbiotic date seeds fermented with both *B. subtilis* and *S. cerevisiae* (BYP) was selected for further investigation.

#### Creation and characterization of the nano-encapsulated postbiotic

As per the ionic gelation procedure, sodium alginate and calcium chloride (CaCl<sub>2</sub>) were utilized to synthesize NBYP [23]. First, a 1% (w/v) sodium alginate solution was mixed with 0.9 g of BYP under continuous gentle magnetic stirring. Subsequently, two portions of the mixture were injected dropwise using a syringe pump (Model: SK 500I, Shenzhen, China) into one portion of CaCl<sub>2</sub> solution (2.2 mol/L). The resultant nanoparticles were collected by centrifugation at 8,000 rpm for 20 minutes and stored at -80 °C. A scientific nanoparticle analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) was used at 25 °C to assess the physicochemical properties, including particle size, polydispersity index (PDI), and zeta potential of both the sodium alginate-CaCl<sub>2</sub> complex and the NBYP.

#### Animals and experimental design

A total of 45 nulliparous rabbit does, aged 5 months and weighing  $2.920 \pm 0.10$  kg, were used. The does were individually housed in standard wire cages under same management conditions [24]. Rabbit does were fed a pelleted diet containing 28% alfalfa hay, 18% barley, 25% wheat bran, 6% yellow corn, 18% soybean, 2% molasses, 1% di-calcium phosphate, and 1% NaCl, and 1% premix, covering daily maintenance as recommended by the National Research [25]. The chemical analysis of the diet (g/100 g DM) was: 17.50 crude protein, 2.05 ether extract, 2.53 crude fiber, 9.43 ash, and 59.45 nitrogen-free extract. Rabbit does were divided into three homogenous experimental groups and received 900 mg/kg of diet free postbiotic (BYP), 900 mg/kg of diet nano-encapsulated postbiotic (NBYP), or no supplementation (control, C). Treatments were administered for 30 days, including 10 days pre-mating and 20 days post-mating (first and second semesters of pregnancy). Each rabbit doe received 25 IU of equine chorionic gonadotropin (Gonaser®, Hipra, Spain) intramuscularly to achieve estrous synchronization. Forty-eight hours later, 0.8 µg of gonadotropin-releasing hormone (0.8 µg buserelin; Receptal, Boxmeer,

Holland) was administered intramuscularly to induce ovulation. This was followed by artificial insemination of 0.2 mL of freshly diluted (1:5) pooled semen containing  $15 \times 10^6$  sperm/insemination [21].

#### **Metrological variables and heat-tolerance indices**

An electronic digital thermo-hygrometer was used to record the rabbitry's ambient temperature ( $^{\circ}\text{C}$ ) and relative humidity during the experimental period. The formula  $\text{db } ^{\circ}\text{C} - [(0.31 - 0.31 \text{ RH}\%) (\text{db } ^{\circ}\text{C} - 14.4)]$  was used to calculate the temperature-humidity index (THI), where RH% represents the percentage of relative humidity, while  $\text{db } ^{\circ}\text{C}$  denotes the dry bulb temperature measured in degrees Celsius. The THI values were classified as follows: the absence of heat stress (27.8), moderate heat stress (27.8–28.8), severe heat stress (28.9–29.9), and extremely severe heat stress ( $> 30.0$ ) for rabbits [26]. The mean values for ambient temperature, relative humidity, THI, and day length were  $30.30 \pm 0.12$   $^{\circ}\text{C}$ ,  $72.40 \pm 0.52\%$ ,  $28.91 \pm 0.113$ , and  $15.08 \pm 0.12$  h, respectively. Rectal temperature, respiration rate, and feed intake of rabbit does were monitored and recorded to assess their heat-tolerance capacity [23].

#### **Hematobiochemical attributes**

Blood samples were collected from the marginal ear veins on the day of insemination (day 0) and on days 10 and 20 post-mating (first and second semesters of pregnancy) using heparinized vacuum tubes (blood collection vacuum tubes, REF: G40111, NEW VAC, China). Each sample was divided into two subplots to assess immunological variables and biochemical characteristics: whole blood and separated blood plasma. Blood plasma samples were obtained by centrifugation at 3,000 g for 20 min at  $4^{\circ}\text{C}$  [27]. Red blood cells (RBCs), white blood cells (WBCs) and their types, and hemoglobin levels were assessed according to El-Desoky et al. [23]. Blood plasma samples were analyzed for total protein, albumin, and glucose concentrations using a BioSystem SA kits (Barcelona, Spain), with method linearity ranges of  $\leq 10.0$  g/dL,  $\leq 7.0$  g/dL, and  $\leq 500$  mg/dL, respectively. Globulin concentrations were calculated by subtracting albumin values from total protein values. Furthermore, the antioxidant capacity of blood plasma was evaluated using colorimetric commercial kits (Biodiagnostic, Giza, Egypt) by measuring the activity of reduced glutathione enzyme (GSH-Px), malondialdehyde (MDA, linearity of the methods was up to 100 nmol/mL), and total antioxidant capacity (TAC, linearity of the methods was up to 2 mM/L). The plasma lysozyme activity (LA) analysis was conducted following the methodology outlined by Hashem et al. [28]. The interleukin-1 $\beta$  ELISA (IL-1 $\beta$ ) kit (Cat. No. MBS262525, My BioSource, San Diego,

CA 92195–3308, USA) was utilized to measure IL-1 $\beta$  levels in blood plasma, with sensitivity limit of 5 pg/mL and intra- and inter-assay precisions of  $\geq 8\%$  and  $\geq 12\%$ , respectively [24]. Insulin-like growth factor I (IGF-I) concentrations were assessed using an immunoassay kit (Quantikine IGF-I Immunoassay, R&D Systems, Minneapolis, MN, USA). An enzyme-linked immunosorbent assay (IBL America Immuno-Biological Laboratories, Inc., Minneapolis, MN, USA) was used to measure the amounts of immunoglobulin G (IgG), -A (IgA), and -M (IgM). The assay demonstrated a sensitivity of 90.0% (95% confidence interval: 68.3% - 98.7%) for IgM and IgG and 100% (95.0% confidence interval: 95.2% - 100%) for IgA.

Progesterone concentrations in blood plasma samples collected during pregnancy (days 10 and 20) were analyzed using commercial solid-phase enzyme immunoassay ELISA kits purchased from Pointe Scientific Inc, MI, USA. The analysis demonstrated a sensitivity of 0.0625 ng/mL. The corresponding intra-assay and inter-assay coefficients of variation were 2.4% and 2.6%, respectively.

#### **Reproductive performance**

Reproductive and pregnancy variables, including litter size at birth (total of kits, both viable and non-viable rabbits), weight of the litter at birth, and kindling rate ([number of delivered females/number of inseminated females  $\times 100$ ]) were measured [23].

#### **Statistical analyses**

All statistical analyses were conducted using the Statistical Analysis Software program (SAS, Version 8, Cary, NC, USA: SAS Institute; 2001). The fixed effects of treatment (C, BYP, and NBYP), status (physiological status at the time of sampling and/or data collection), and the treatment by status interaction were assessed for physiological and hematochemical variables, redox status, and hormonal profiles using the MIXED procedure for repeated measurement. The effects of treatments on litter size, litter viability, and litter weight were evaluated using one-way ANOVA, while the effect of treatments on the kindling rate was evaluated using the Chi-square test. Differences between treatment means were evaluated using Duncan's multiple range.

## **Results**

#### **GC-MS and physicochemical analyses**

The analysis of non-fermented date seeds extract revealed eight secondary metabolites, with oleic acid (39.10%) and 9,19-cyclolanost-23-ene 3 $\beta$ ,25-diol (22.11%) identified as the predominant compounds (Table 1). Post-biotic derived from *B. subtilis*-fermented date seeds contained ten compounds, with glycerin (63.12%) and

**Table 1** GC-MS analysis of active metabolites of date seeds extract and postbiotics of *Bacillus subtilis*, *S. cerevisiae*, and both microorganisms cultured in date seeds

Compounds	Area, %
<b>Date seeds extract</b>	
Oleic acid	39.10
9,19-cyclolanost- 23-ene 3 β,25 diol	22.11
1,3,5-triazine- 2,4,6 -triamine	11.17
Methyl palmitate	10.56
Stigmast- 5-en- 3-yl 9-octadecenoate	8.52
9,19-cyclolanost- 24-en- 3-ol,(3,β)	7.00
Docosane	0.84
2-methyl- 6-propyl dodecane	0.70
<b>Bacillus subtilis postbiotic</b>	
Glycerin	63.12
psi.,psi.-Carotene,1,1',2,2'-tetrahydro- 1,1'-dimethoxy	9.58
l-Gala-l-ido-octonic	4.60
d-Gala-l-ido-octonic amide	3.50
2,4-Difluorobenzene, 1-benzyloxy-	3.27
Benzaldehyde,3-benzyloxy – 2-fluoro- 4-methoxy	3.27
10-Phenyldecanoic acid	3.27
5-Benzyloxy- 3,8,9-trioxa-tricyclo[4.2.1.0(2,4)]nonane	3.27
á-D-Glucopyranose,4-O-á-D-galactopyranosyl	3.09
Lactone	3.01
<b>Saccharomyces cerevisiae postbiotic</b>	
Glycerin	36.06
d-Lyx-d-manno-nononic- 1,4-lactone	20.30
d-Glycero-l-gluco-heptose 1-	12.30
Heptatriacotanol	11.68
d-Gala-l-ido-octonic amide	10.15
Trilinolein	4.65
Linolenic acid, 2-hydroxy- 1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	3.69
2,4-Difluorobenzene, 1-benzyloxy-	1.17
<b>Bacillus subtilis and Saccharomyces cerevisiae Postbiotic</b>	
(Z)– 18-Octadec- 9-enolide	19.70
Trilinolein	18.52
Desulphosinigrin	17.76
Oleic Acid	10.64
1,1,3,3,5,5-Hexamethyl- 1,5-bis(2-methylpropoxy)trisiloxane	9.76
[1,1'-Bicyclopropyl]– 2-octanoic acid, 2'-hexyl-, methyl ester	8.05
d-Mannose	5.39
Estra- 1,3,5(10)-trien- 17á-ol	4.81
Strychane, 1-acetyl- 20à-hydroxy- 16-methylene-	2.74
Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	2.63

psi,psi-carotene- 1,1',2,2'-tetrahydro- 1,1'-dimethoxy (9.58%) being the most abundant. The postbiotic derived from *S. cerevisiae*-fermented date seeds produced eight compounds, including glycerin (36.06%),

**Table 2** Physicochemical characteristics of the alginate–calcium chloride nanoparticles (A) and prepared nano-encapsulated postbiotic (B)

Item	A	B
Size, nm	195.10	325.3
Zeta potential, mV	– 3.41	8.22
Poly dispersity index	0.457	0.551

d-lyxo-d-manno-nononic- 1,4-lactone (20.30%), and d-glycero-l-gluco-heptose (12.30%). Co-fermentation involving both microbial strains yielded ten bioactive compounds postbiotic, with (Z)– 18-octadec- 9-enolide (19.70%), torilinolein (18.52%), desulphosinigrin (17.76%), and oleic acid (10.64%) identified as the predominant components (Table 1).

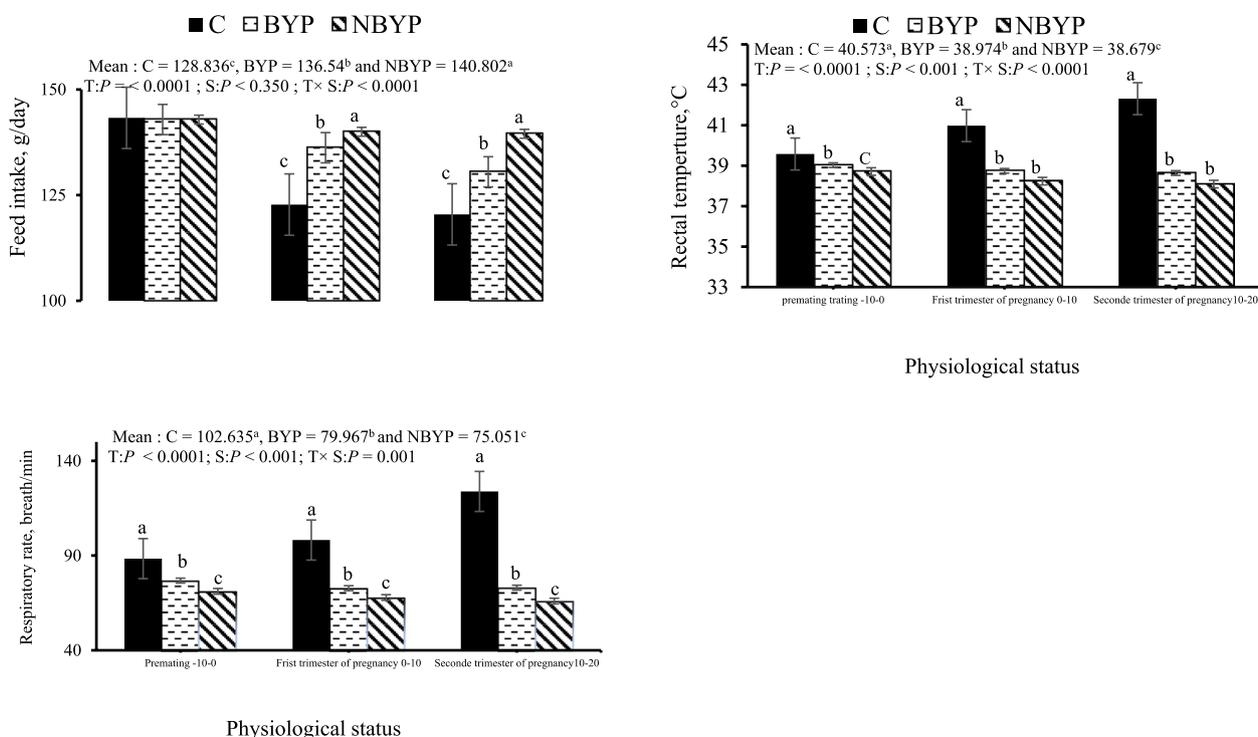
The physicochemical assessment of alginate-CaCl<sub>2</sub> nanoparticles and alginate-CaCl<sub>2</sub> nano-encapsulated BYP revealed average particle sizes of 195.10 nm and 325.3 nm, zeta potential measurements of – 3.41 mV and 8.88 mV, and polydispersity index (PDI) values of 0.457 and 0.551, respectively (Table 2).

**Heat-tolerance indices**

The effects of BYP (free postbiotic) and NBYP (nano-encapsulated postbiotic), at a dosage of 900 mg/kg diet on heat-tolerance indices, including feed intake, rectal temperature, and respiratory rate of rabbit does in various physiological conditions are shown in Fig. 1. Treatment with BYP or NBYP significantly increased the overall mean of feed intake. However, the interaction between treatment and physiological status indicated that this enhancement occurred during the first and second semesters of pregnancy (day 10–20 post-mating) but not during the pre-mating period. BYP and NBYP treatments significantly reduced rectal temperatures and respiratory rates in females across various physiological states (pre-mating, first and second trimesters of pregnancy) compared to the C treatment, with the lowest values recorded for NBYP treatment.

**Hematobiochemical attributes**

The effects of BYP (free postbiotic) and NBYP (nano-encapsulated postbiotic), at the level of 900 mg/kg diet, on hematobiochemical attributes (RBCs, PCV, hemoglobin, total protein content, albumin content, glucose content, IGF-I, GSH-Px activity, TAC and MDA content) compared to control are shown in (Fig. 2). Both PBYP-based treatments significantly increased overall count of RBCs ( $p < 0.0001$ ), hemoglobin levels ( $p = 0.012$ ), and PCV ( $p = 0.009$ ) compared to the C treatment. These increases were observed during days 10 – 20



**Fig. 1** Changes (means ± SEM) in the feed intake, respiratory rate and rectal temperature of rabbit does during the experimental period. C = 0 mg/kg diet, BYP= 900 mg/kg diet, and NBYP = 900 mg/kg diet. Means within the same physiological status having different superscripts (a, b, c) differ significantly at  $p < 0.05$

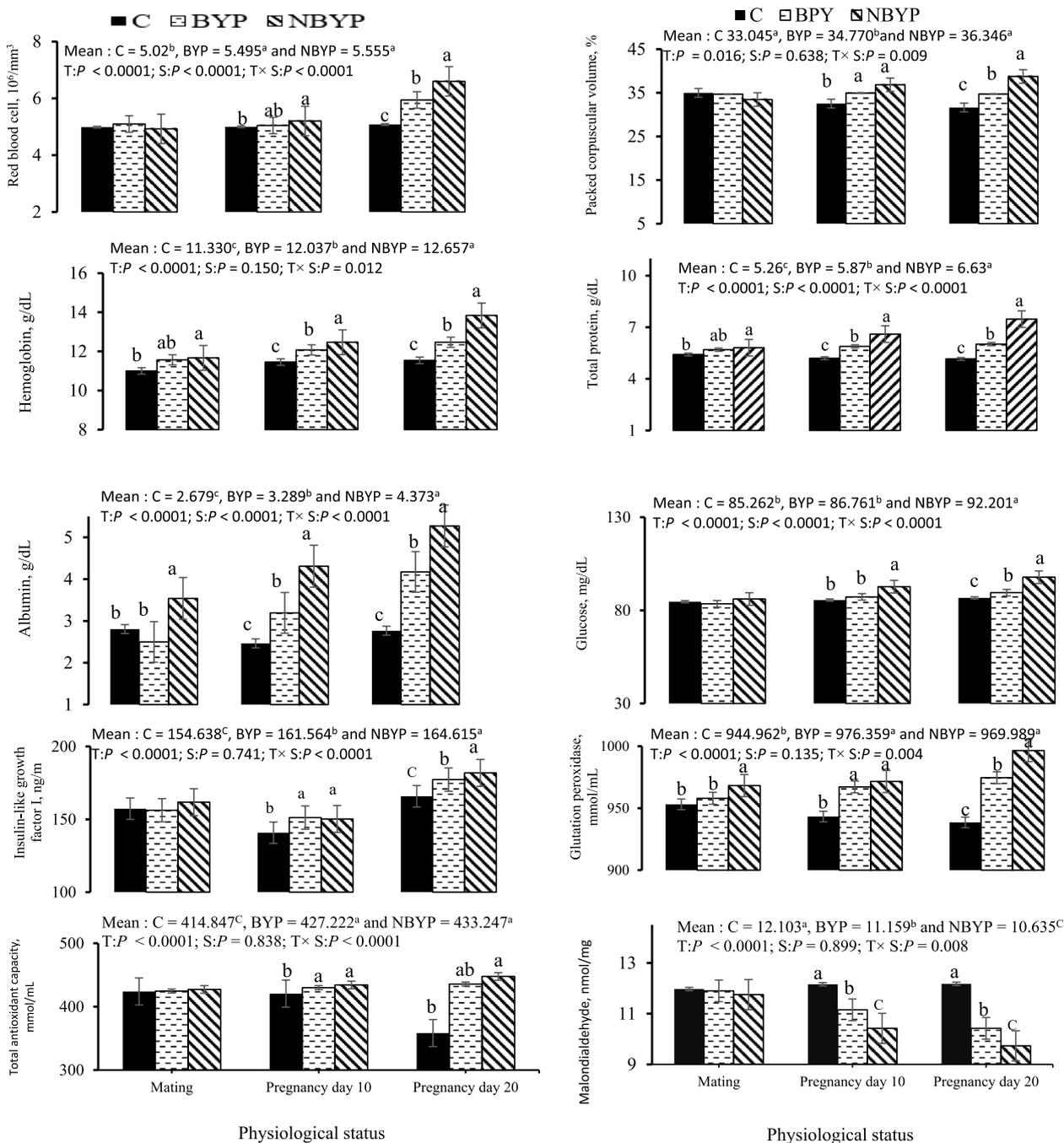
of pregnancy. The highest values were observed in the NBYP treatment on day 20 of pregnancy. Both BYP and NBYP treatments significantly increased overall concentrations of blood plasma metabolites (total protein, albumin, glucose, and insulin-like growth factor,  $p < 0.0001$ ). These increases were noted between days 10 and 20 of pregnancy. The NBYP treatment exhibited the highest values on day 20 of pregnancy. Both BYP and NBYP treatments significantly increased the overall concentrations of blood plasma metabolites, including total protein, albumin, glucose, and IGF-I ( $p < 0.0001$ ). These increases were noted in total protein and albumin across various physiological statuses, with the highest values recorded in the NPBY treatment on day 20 of pregnancy. During day 20 of pregnancy, blood plasma glucose concentrations and IGF levels increased. Both BYP and NBYP treatments significantly elevated overall concentrations of TAC ( $p < 0.0001$ ) and GSH-Px ( $p = 0.004$ ) while reducing MDA ( $p = 0.008$ ) compared to the C treatment. These increases in TAC and GSH-Px and decreases in MDA levels were noted between days 10 and 20 of pregnancy.

Both BYP and NBYP treatments significantly increased overall concentrations of WBCs count, lymphocytes, and monocytes ( $p = 0.05$ ) compared to the C treatment (Fig. 3). Such increases were observed between days 10

and 20 of pregnancy for WBCs count and lymphocytes, while monocyte concentrations increased only on day 20 of pregnancy. Both BYP and NBYP treatments significantly elevated overall concentrations of blood plasma immunoglobulin G, M, and A ( $p < 0.0001$ ) and lysozyme activity ( $p = 0.142$ ), while reduced interleukin-1 $\beta$  levels ( $p = 0.026$ ) compared to the C treatment (Fig. 3). These increases were noted across various physiological statuses for IgA. During days 10 to 20 of pregnancy, an increase in IgG, IgM, and lysozyme activity was observed, with the highest values recorded in the NPBY treatment on day 20 of pregnancy. The concentrations of blood plasma interleukin-1 $\beta$  significantly decreased between days 10 and 20 of pregnancy (Fig. 3).

**Reproductive performance**

The effects of BYP (free postbiotic) and NBYP (nano-encapsulated postbiotic), at the level of 900 mg/kg diet, on reproductive performance indicators, including progesterone levels, kidding rate, litter size at birth, number of live and dead litter sizes, and litter weight at birth is presented in Fig. 4 and Table 3. Both BYP and NBYP treatments significantly increased overall concentrations of progesterone ( $p < 0.0001$ ), with the highest concentration recorded in the NBYP treatment on day 20

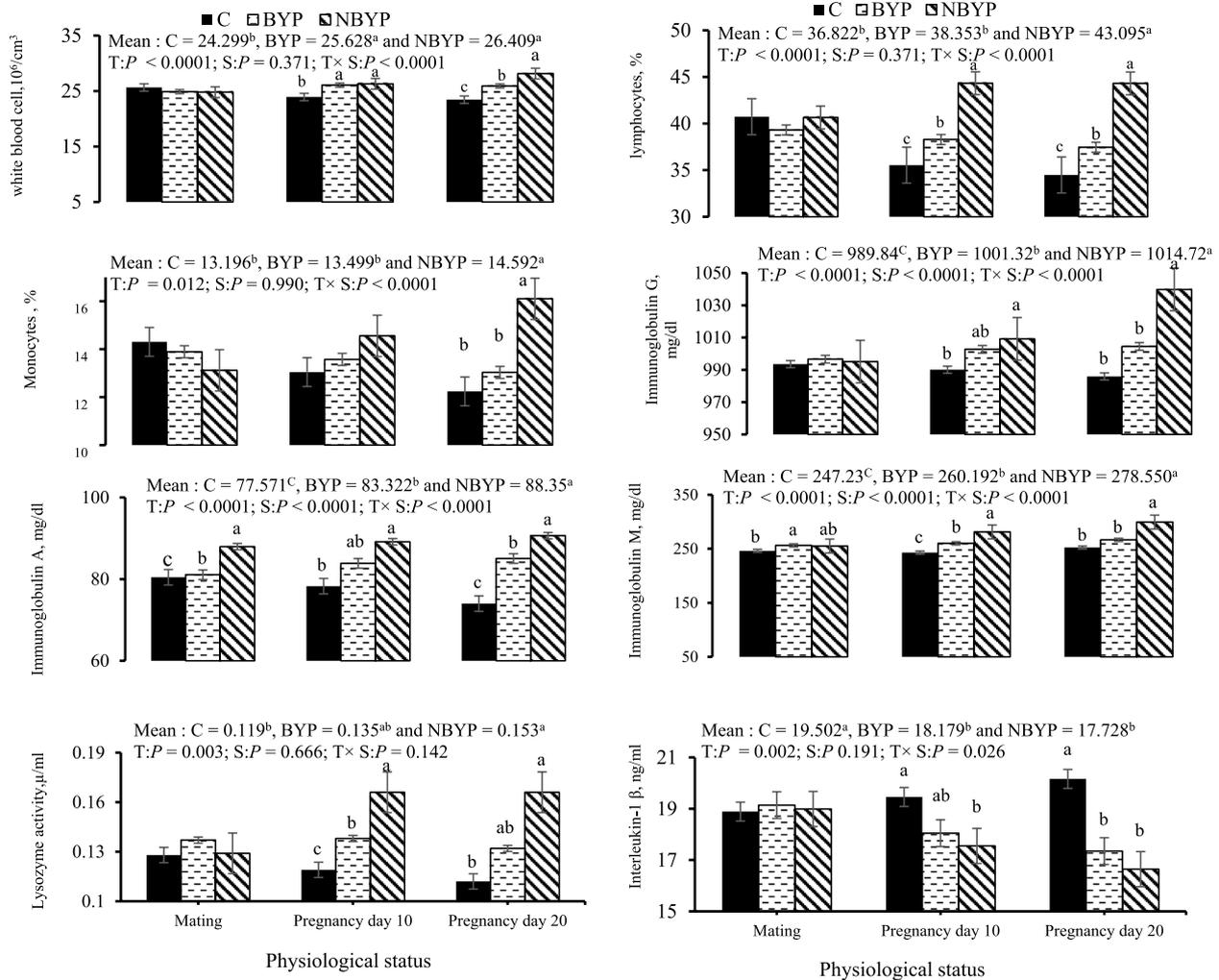


**Fig. 2** Changes (means  $\pm$  SEM) in the blood plasma metabolites of rabbit does during the experimental period. C = 0 mg/kg diet, BYP= 900 mg/kg diet, and NBYP = 900 mg/kg diet. Means within the same physiological status having different superscripts (a, b, c) differ significantly at  $p < 0.05$

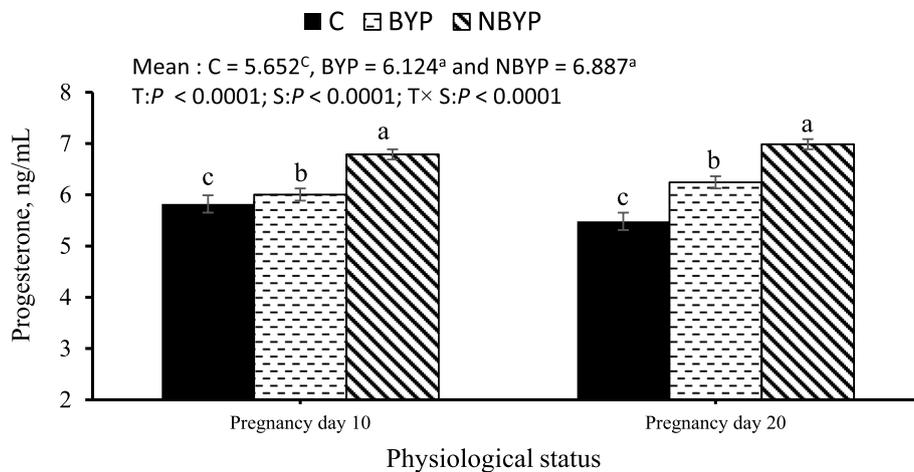
of pregnancy (Fig. 4). Both BYP and NBYP treatments significantly enhanced kidding rates and live litter sizes, while reduced the number of dead litters compared to the C treatment (Table 3). NBYP demonstrated superior efficacy, yielding the highest values for all reproductive traits.

### Discussion

This study investigated the potential of a postbiotic formulation to ameliorate heat stress negative impacts on thermotolerance and reproductive performance in rabbits. To safely promote animal health, date palm (*Phoenix dactylifera* L.) seed by-products were employed as a



**Fig. 3** Changes (means ± SEM) in immunity variables of rabbit does during the experimental period. C = 0 mg/kg diet, BYP= 900 mg/kg diet, and NBYP = 900 mg/kg diet. Means within the same physiological status having different superscripts (a, b, c) differ significantly at  $p < 0.05$



**Fig. 4** Changes (means ± SEM) in progesterone hormone of rabbit does during the experimental period. C = 0 mg/kg diet, BYP= 900 mg/kg diet, and NBYP = 900 mg/kg diet. Means within the same physiological status having different superscripts (a, b, c) differ significantly at  $p < 0.05$

**Table 3** Effect of treatments on the reproductive performance of rabbit does

Variable	Treatment <sup>1</sup>			SEM	P-Value
	C	BYP	NBYP		
Kindling rate, %	53 <sup>c</sup> (8/15)	66 <sup>b</sup> (10/15)	80 <sup>a</sup> (12/15)	-	0.096
Litter size at birth	5.37	5.38	7.25	1.31	0.001
No. live litter sizes	3.87 <sup>b</sup>	4.25 <sup>ab</sup>	6.59 <sup>a</sup>	1.48	0.03
No. dead litter sizes	1.51 <sup>b</sup>	1.13 <sup>ab</sup>	0.66 <sup>a</sup>	0.92	0.04
Litter weight at birth, g	333.87 <sup>b</sup>	401.63 <sup>b</sup>	693.51 <sup>a</sup>	74.6	0.001

<sup>1</sup> C = 0 mg/kg BW, BYP = 900 mg/kg BW and nano encapsulated NBYP = 900 mg/kg diet

Means within the row having different superscripts (a, b, c) differ significantly at  $p < 0.05$

fermentation substrate for the probiotic strains *B. subtilis* and/or *S. cerevisiae*. Gas chromatography-mass spectrometry (GC-MS) profiling revealed that fatty acids, such as oleic acid, are the predominant secondary metabolites in the non-fermented date seeds extract. In contrast, postbiotics obtained from the fermentation of *B. subtilis* or *S. cerevisiae* postbiotics exhibited a higher concentration of carbohydrates, especially glycerol. Co-fermentation with both microbial strains produced novel secondary metabolites, such as trilinolein and desulfosinigrin, which were not present in single-strain postbiotics or non-fermented extracts. These findings highlight a synergistic metabolic interaction between *B. subtilis* and *S. cerevisiae*, which enhances the diversity of bioactive compounds and their functional effectiveness. Cross-strain interactions in mixed microbial cultures enable the integration of complementary biosynthetic pathways, optimizing the production of biologically active metabolites with improved host-targeted activity [29]. For example, Jia et al. [30] found that fattening lambs supplemented with *B. licheniformis* and *S. cerevisiae* combination had better growth performance, antioxidant capacity, immune function, ruminal fermentation, and microbial diversity than those supplemented with a single-strain.

Rabbit does subjected to severe heat stress (Temperature-Humidity Index =  $28.91 \pm 0.113$ ) and supplemented with either BYP or NBYP demonstrated improved thermotolerance, as evidenced by increased feed intake, decreased rectal temperature, and respiratory rate, along with enhanced hematological and immunological functions, optimized blood metabolites, and better antioxidant status relative to the non-supplemented rabbit does (C). These findings are consistent with research indicating that *Aspergillus oryzae* postbiotics enhanced heat stress resilience in cattle and Holstein calves [31].

In poultry, dietary supplementation with *L. plantarum* strains (RS5, RI11, UL4) attenuated heat stress-induced lipid peroxidation, restored antioxidant enzyme activity, and enhanced meat quality [32].

The hematobiochemical improvements observed in BYP/NBYP-supplemented rabbits, including elevated packed cell volume and hemoglobin levels are critical for sustaining thermoregulatory capacity under heat stress [33]. Both packed cell volume and hemoglobin levels improve oxygen transport and tissue delivery, facilitating cellular respiration and metabolic requirements during hyperthermia [23]. Albumin plays a crucial role in maintaining plasma colloid osmotic pressure, thereby preserving protein integrity and regulating fluid balance. Glucose serves as the main energy source in monogastric species, offering easily metabolizable energy and decreasing dependence on energy-demanding catabolic processes, particularly under high thermal conditions [23].

Heat stress adversely affects immune function, increasing infection susceptibility [3]. This study demonstrated that BYP and NBYP treatments significantly improved cellular immunity, as evidenced by increased lysozyme activity and decreased proinflammatory cytokines, as well as enhanced humoral immunity, indicated by elevated levels of immunoglobulins (IgG, IgA, IgM) relative to C treatment. These findings are consistent with the immunomodulatory effects of postbiotics documented in other studies. *Bifidobacterium coagulans* postbiotics induce T helper 2 cytokines (IL-4, IL-6, IL-10), suppress IL-2, and promote B-lymphocyte proliferation, exerting anti-inflammatory effects [34]. In another study, lactic acid bacteria postbiotic reduced tumor necrosis factor- $\alpha$  levels and increased interferon- $\gamma$  levels in vaccinated rabbits, thereby alleviating the severity of myxomatosis lesions in wild rabbits [35].

Interestingly, none of the previously published studies on postbiotics in farm animals have identified secondary metabolites of used postbiotics or their potential biological activities. In contrast to our study, the positive biological effects can be attributed to the distinct array of secondary metabolites found in BYP. For instance, trilinolein and desulfosinigrin exhibit antioxidant activity. Moreover, trilinolein is classified as a proinflammatory inhibitor of mediators for nitric oxide synthase, cyclooxygenase-2, nuclear factor tumor necrosis factor- $1\beta$ , IL-1, IL-6, and mitogen-activated protein kinases [36]. Furthermore, oleic acid has antimicrobial activity. D-mannose plays a role in inhibiting bacterial adhesion [37] and is involved in the synthesis of glycoproteins, which are essential for immune system regulation and exhibit anti-inflammatory and antibacterial properties [38]. Additionally, [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-,

methyl has anti-inflammatory, antitumor, antiviral, antibacterial, and antifungal properties [39].

The exposure of pregnant rabbit does to heat stress can evoke several reproductive hazards, including oocyte incompetence and decreased fertility, conception, and kindling rates, and increased embryonic loss and abortion rate [40, 41]. In this study, rabbit does supplemented with either BYP or NBYP expressed higher kindling rate, number of live litter size at birth, and litter weight at birth than the C rabbit does. This is primarily due to the decrease in proinflammatory cytokine levels, leading to increased uterine health and functioning [42]. Moreover, improved progesterone concentrations can contribute to these positive effects on conception rate and pregnancy outcomes [42]. Progesterone is responsible for the preparation of the myometrium for implantation by reducing muscle sensitivity to nervous or hormonal stimulation (oxytocin) and inhibiting lymphocyte proliferation and its activity [43]. Furthermore, several metabolites and hormones, notably glucose and insulin-like growth factor-1, which showed significant improvement in the treated groups, can positively influence pregnancy development [43]. Additionally, the improvements in reproductive performance seen in this study may be directly attributable to the biological role of certain postbiotic metabolites. For example, oleic acids can enhance the development of one-cell rabbit embryos into morulae by serving as a reservoir for metabolic precursors found in uterine and oviductal fluids and embryos [44].

Finally, a key finding of this study is that NBYP exhibited greater biological activity than BYP, as indicated by the values for each variable, which were significantly higher or lower than those of BYP. These findings align with previous studies regarding the capacity of nanoparticles to traverse various biological barriers and sustain release over extended periods, thereby enhancing their cellular uptake and availability compared to larger-sized particles [20, 23].

## Conclusion

The secondary metabolites of postbiotics are affected by the type of the microorganism. This study demonstrates that the richest postbiotic, containing various secondary metabolites, was produced through the fermentation of date seeds using a combination of the microorganism strains *B. subtilis* and *S. cerevisiae*, rather than a single strain, thereby emphasizing the synergistic effects of both microbes. This postbiotic enhanced the health status and reproductive performance of rabbit does raised in natural heat conditions. Furthermore, the nano-encapsulation of the postbiotic improved its biological function, resulting in improved reproductive performance. Therefore, postbiotics represent a novel category of feed supplements

that may effectively alleviate the effects of heat stress in rabbit does. Applying nano-encapsulation technology can yield further benefits. Future research should investigate alternative postbiotic formulations utilizing various fermenting materials and microorganisms for diverse applications in livestock production.

## Abbreviations

BYP	Free postbiotic
C	Control
CaCl <sub>2</sub>	Calcium chloride
GC-MS	Gas chromatography/mass spectrometry
GSH-Px	Reduced glutathione enzyme
IgA	Immunoglobulin-A
IgG	Immunoglobulin-G
IGF-I	Insulin-like growth factor- I
IgM	Immunoglobulin-M
IL- 4	Interleukin- 4
IL- 6	Interleukin- 6
IL- 10	Interleukin- 10
LA	The plasma lysozyme activity
MDA	Malondialdehyde
NBYP	Nano-encapsulated postbiotic
P4	Progesterone
RBCs	Red blood cells
WBCs	White blood cells

## Acknowledgments

The authors would like to acknowledge Dr Nagwa El-Desoky, the Laboratory of Rabbit Physiology Research, supervised by NMH, Faculty of Agriculture, Alexandria University for help in sample collection.

## Authors' contributions

N.M.H., A.S.M., Z.B.E., and N.S.H. conceptualization and validation and N.S.H. and N.M.H. methodology, validation, writing and editing and N.S.H. statistical analysis and N.M.H. project management and facilities and N.M.H. and N.S.H. laboratory analysis. N.M.H., A.S.M., and Z.B.E. supervision. All authors contributed to drafting the final manuscript. The authors read and approved the final manuscript.

## Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). NMH and NSH are grateful for the project entitled "Designing an industrial prototype for innovating microbial-based feed additives using nano-encapsulation technology for improving performance and immunity of farm animals" funded by Academy of Scientific Research and Technology (ASRT), Science and Technology Center (STC), Egypt for partial funding of the current article.

## Data availability

Data is provided within the manuscript and any further information can be achieved by contacting the corresponding author.

## Declarations

### Ethics approval and consent to participate

The experimental protocol was conducted according to the guidelines for the care and use of animals in research at Alexandria University (Protocol Approval no. 082211212118).

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

Received: 2 December 2024 Accepted: 1 April 2025  
Published online: 25 April 2025

## References

- Hashem NM, Hosny NS, El-Desoky N, Soltan YA, Elolimy AA, Sallam SMA, Abu-Tor ESM. Alginate nanoencapsulated synbiotic composite of pomegranate peel phytochemicals and multi-probiotic species as a potential feed additive: physicochemical, antioxidant, and antimicrobial activities. *Animals*. 2023;13(15):2432–16.
- García ML, Argente MJ. Exposure to high ambient temperatures alters embryology in rabbits. *Int J Biometeorol*. 2017;61:1555–15.
- Liang ZL, Chen F, Park S, Balasubramanian B, Liu WC. Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity and production performance, and the potential mitigation strategies of nutritional intervention. *Front Vet Sci*. 2022;9:906084–15. <https://doi.org/10.3389/fvets.2022.906084>.
- Ebeid TA, Aljabeili HS, Al-Homidan IH, Volek Z, Barakat H. Ramifications of heat stress on rabbit production and role of nutraceuticals in alleviating its negative impacts: an updated review. *Antioxidants*. 2023;12(7):1407–29.
- Ji J, Jin W, Liu SJ, Jiao Z, Li X. Probiotics, prebiotics, and postbiotics in health and disease. *MedComm*. 2023;4(6):1–23. <https://doi.org/10.1002/mco2.420>.
- Chaney E, Miller EA, Firman J, Binnebose A, Kuttappan V, Johnson TJ. Effects of a postbiotic, with and without a saponin-based product, on turkey performance. *Poult Sci*. 2023;102(5):102607–5.
- Oliveira JM, Marchi DF, Geronimo BC, Oba A, Soares AL. Effect of diets containing *S. cerevisiae* fermentation products on broiler performance and meat quality. *J Agric Sci Res*. 2022;2:2–8.
- Sadeghi A, Ebrahimi M, Kharazmi MS, Jafari SM. Effects of microbial-derived biotics (meta/pharma/post-biotics) on the modulation of gut microbiome and metabolome; general aspects and emerging trends. *Food Chem*. 2023;411:135478.
- Ringø E, Van Doan H, Lee SH, Soltani M, Hoseinifar SH, Ramasamy H. Probiotics, lactic acid bacteria and Bacilli: interesting supplementation for aquaculture. 2020;129:116–20. <https://doi.org/10.1111/jam.14628>.
- Djaoudene O, Bey MB, Louaileche H. Physicochemical characteristics and nutritional compositions of some date (*Phoenix dactylifera* L.) fruit cultivars. *Acta Univ Cibiniensis Ser E Food Technol*. 2019;23(2):129–9.
- Abd El-Ghany WA, Fouad H, Quesnell R, Sakai L. The effect of a postbiotic produced by stabilized non-viable *Lactobacilli* on the health, growth performance, immunity, and gut status of colisepticaemic broiler chickens. *Trop Anim Health Prod*. 2022;54(5):286–11.
- Dai D, Kong F, Han H, Shi W, Song H, Yoon I, Wang Sh, Liu X, Lu NA, Wang W, Li SH. Effects of postbiotic products from *S. cerevisiae* fermentation on lactation performance, antioxidant capacities, and blood immunity in transition dairy cows. *J Dairy Sci*. 2024;107:105840–14.
- Diaz Cano JV, Argente MJ, García ML. Effect of postbiotic based on lactic acid bacteria on semen quality and health of male rabbits. *Animals*. 2021;11(4):1007.
- Kareem KY, Hooi Ling F, Teck Chwen L, May Foong O, Anjas Asmara S. Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. *Gut Pathog*. 2014;6:1–7.
- Van Thu T, Foo HL, Loh TC, Bejo MH. Inhibitory activity and organic acid concentrations of metabolite combinations produced by various strains of *Lactobacillus plantarum*. *Afr J Biotechnol*. 2011;10(8):1359–4.
- Chang HM, Foo HL, Loh TC, Lim ETC, Abdul Mutalib NE. Comparative studies of inhibitory and antioxidant activities, and organic acids compositions of postbiotics produced by probiotic *Lactiplantibacillus plantarum* strains isolated from Malaysian foods. *Front Vet Sci*. 2021;7:602280.
- Cai L, Indrakumar S, Kiarie E, Kim IH. Effects of a multi-strain *Bacillus* species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn–soybean meal-based diets. *J Anim Sci*. 2015;93(9):4336–6.
- Sun X, Wang Y, Wang E, Zhang S, Wang Q, Zhang Y, Wang Y, Cao Z, Yang H, Wang W, Li S. Effects of *S. cerevisiae* culture on ruminal fermentation, blood metabolism, and performance of high-yield dairy cows. *Animals*. 2021;11(8):2401.
- Al-Thubiani AS, Ahmad Khan MS. The prebiotic properties of date palm (*Phoenix dactylifera* L.) seeds in stimulating probiotic *Lactobacillus*. *J Pure Appl Microbiol*. 2017;11(4).
- El-Raghi AA, Hassan MAE, Hashem NM, Abdelnour SA. Struggling thermal stress impacts on growth performance and health status of newly weaned rabbits using nanoemulsion of *Origanum majorana* considering the economic efficiency of supplementation. *Animals*. 2023;13(11):1772.
- Hashem NM, Hosny NS, El-Desoky NI, Shehata MG. Effect of nanoencapsulated alginate-synbiotic on gut microflora balance, immunity, and growth performance of growing rabbits. *Polymers (Basel)*. 2021;13(23):4191.
- Kouhsar M, Kashaninia E, Mardani B, Rabiee HR. CircWalk: a novel approach to predict CircRNA-disease association based on heterogeneous network representation learning. *BMC Bioinformatics*. 2022;23(1):1–16. <https://doi.org/10.1186/s12859-022-04883-9>.
- El-Desoky NI, Hashem NM, Gonzales-Bulnes A, Elkomy AG, Abo-Elezz ZR. Effects of a nanoencapsulated moringa leaf ethanolic extract on the physiology, metabolism and reproductive performance of rabbit does during summer. *Antioxidants*. 2021; 10(8). <https://doi.org/10.3390/antiox10081326>.
- El-Desoky NI, Hashem NM, Elkomy AG, Abo-Elezz ZR. Improving rabbit doe metabolism and whole reproductive cycle outcomes via fatty acid-rich moringa oleifera leaf extract supplementation in free and nano-encapsulated forms. *Animals*. 2022; 12(6). <https://doi.org/10.3390/ani12060764>.
- Council NR. Nutrient requirements of rabbits second revised edition, 1977. Washington: The National Academies Press; 1977. 10.17226/35.
- Marai IFM, Habeeb AAM, Gad AE. Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livest Prod Sci*. 2002;78(2):71–90.
- Hosny NS, Hashem NM, Morsy AS, Abo-Elezz ZR. Effects of organic selenium on the physiological response, blood metabolites, redox status, semen quality, and fertility of rabbit bucks kept under natural heat stress conditions. *Front Vet Sci*. 2020;7:290.
- Hashem NM, Morsy AS, Soltan YA, Sallam SM. Potential benefits of boswellia sacra resin on immunity, metabolic status, udder and uterus health, and milk production in transitioning goats. *Agric*. 2021;11(9). <https://doi.org/10.3390/agriculture11090900>.
- Chen HJ, Yang WY, Wang CY. The review on the function of intestinal flora and the regulatory effects of probiotics on the intestinal health of rabbits. In 2017 2nd International Conference on Biological Sciences and Technology (BST 2017). Atlantis Press. 2018;6:49–6.
- Jia P, Cui K, Ma T, Wan F, Wang W, Yang D, Wang Y, Guo B, Zhao L, Diao Q. Influence of dietary supplementation with *Bacillus licheniformis* and *S. cerevisiae* as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. *Sci Rep*. 2018;8(1):16712.
- Rius AG, Kaufman JD, Li MM, Hanigan MD, Ipharraguerre IR. Physiological responses of Holstein calves to heat stress and dietary supplementation with a postbiotic from *Aspergillus oryzae*. *Sci Rep*. 2022;12(1):1587.
- Humam AM, Loh TC, Foo HL, Izuddin WI, Awad EA, Idrus Z, Samsudin AA, Mustapha NM. Dietary supplementation of postbiotics mitigates adverse effects of heat stress on antioxidant enzyme activity, total antioxidant, lipid peroxidation, physiological stress indicators, lipid profile and meat quality in broilers. *Animals*. 2020;10(6):1–21. <https://doi.org/10.3390/ani10060982>.
- Li H, Gu Y, Jin R, He Q, Zhou Y. Effects of dietary rutin supplementation on the intestinal morphology, antioxidant capacity, immunity, and microbiota of aged laying hens. *Antioxidants*. 2022;11(9):1843.
- Cao J, Yu Z, Liu W, Zhao J, Zhang H, Zhai Q, Chen W. Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases. *J Funct Foods*. 2020;64:103643.
- García-Vicente EJ, Rey-Casero I, Martín M, Pérez A, Benito-Murcia M, Risco D. Oral supplementation with postbiotics modulates the immune response produced by myxomatosis vaccination in wild rabbits. *Vaccine*. 2024;42(13):125978.
- Huang SS, Deng JS, Lin JG, Lee CY, Huang GJ. Anti-inflammatory effects of trilinolein from *Panax notoginseng* through the suppression of NF- $\kappa$ B and MAPK expression and proinflammatory cytokine expression. *Am J Chin Med*. 2014;42(6):1485–506.

37. Nunzio CDE, Bartoletti R, Tubaro A, Simonato A, Ficarra V. Role of D-mannose in the prevention of recurrent uncomplicated cystitis: state of the art and future perspectives. *Antibiotics*. 2021;10(4):373.
38. Dhanalakshmi M, Sruthi D, Jinuraj KR, Das K, Dave S, Andal NM, Das J. Mannose: a potential saccharide candidate in disease management. *Med Chem Res*. 2023;32(3):391–17.
39. Abbas FM. Evaluation of antibacterial and antifungal activity of bioactive chemical compounds isolated from *Candida albicans*. *J Curr Med Res Opin*. 2023;6(08):1677–84.
40. Rosell JM, de la Fuente LF, Carbajo MT, Fernández XM. Reproductive diseases in farmed rabbit does. *Animals*. 2020;10(10):1873.
41. Hashem NM, Abdelnaby EA, Madkour M, El-Sherbiny HR. Melatonin administration during the first half of pregnancy improves physiological response and reproductive performance of rabbits under heat stress conditions. *Reprod Fertil Dev*. 2024;36(9):RD23139.
42. Hashem NM, Essawi WM, El-Raghi AA. Ovarian activity, hormone profile, pro-inflammatory cytokines and reproductive performance of buffalo cows fed diets with different estrogenicity. *J Anim Physiol Anim Nutr (Berl)*. 2024;108(1):1–12.
43. Chmurska-Gasowska M, Sowińska N. Hormonal regulation of pregnancy and related behavioral changes in rabbits. 2020.
44. Hadjadj I, Hankele A-K, Armero E, Argente M-J, de la Luz García M. Fatty acid profile of blood plasma at mating and early gestation in rabbit. *Animals*. 2021;11(11):3200.

### **Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.