Original Research (2026) 3(1); 37 - 45
https://doi.org/10.22103/VCBR.2025.25915.1096

Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

ORIGINAL ARTICLE

Efficacy of Probiotic (BioSAF47) and Prebiotic (BioMos) on Physiological Performance of Broiler Chickens under Heat Stress Conditions

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Online ISSN: 3060-7663 **https://doi.org/10.22103/VCBR.2025.25915.1096**

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Article History

Received: *15 September 2025*
Revised: *2 November 2025*
Accepted: *13 March 2026*
Published: *19 March 2026*

Keywords

Heat Stress
Bio-SAF47
Bio-MOS
Immune System
Chicken

Running Title

The Effect of Probiotics on Heat Stress in Broiler Chicken

Abstract

Prebiotic and probiotic products seem to have beneficial effects on the physiological performance of broiler chickens. This study aims to evaluate the effects of dietary supplementation of mannan-oligosaccharide prebiotic (Bio-MOS) and Saccharomyces cerevisiae probiotic (Bio-SAF47), either alone or in combination, on some of the biological markers of heat stress in broiler chickens. 300 one-day-old chicks (Cobb 500) were randomly divided into five groups (n = 12) with 5 repetitions. All groups had access to a basal diet, and experimental groups each additionally received 0.05, 0.1, and 0.2 percent of their respective supplementation regimen in starter, grower, and finisher diets, respectively. Dietary supplementation of Bio-SAF47 and Bio-MOS reduced ($p < 0.05$) the elevated serum corticosterone concentration during 1-21 and 22- 42 days of age, respectively. Combination of Bio-SAF47 and Bio-MOS reduced ($p < 0.05$) the elevated serum corticosterone concentration and H/L ratio; while increasing ($p < 0.05$) the reduced IgG titer, bursa index, BWG, heterophil and lymphocyte count during 35-42 (finisher) days. Our results suggest that prebiotics and probiotics reduce the negative effects of heat stress in broilers and the combination of Bio-SAF47 and Bio-MOS diet (synbiotic effect) was advantageous compared to the control diet with respect to feed efficiency. Moreover, separate supplementation of Bio-SAF47 and Bio-MOS had a positive effect on serum corticosterone levels. These results emphasize the role of prebiotic and probiotic supplementation in improving physiological performance and open a new window to future studies.

How to cite this article: *Seyed Saeid Taheri, Javad Cheraghi, Hamidreza Mohajerani. Efficacy of Probiotic (BioSAF47) and Prebiotic (BioMos) on Physiological Performance of Broiler Chickens under Heat Stress Conditions. Veterinary and Comparative Biomedical Research, 2026, 3(1): 37 – 45. https://doi.org/10.22103/VCBR.2025.25915.1096*

Introduction

Elevated ambient temperature, especially in tropical areas, is one of the most important stressors affecting the health and overall physiology of animals, which may eventually lead to alterations in body composition (1). Reports show that heat stress can adversely change the metabolic status and physiological equilibrium (2), and may manifest as aggressive behavior, leading to health problems and a high mortality rate (3). Moreover, heat stress reportedly increases serum concentrations of corticosterone (4), glucocorticoids (5); glucose, triglycerides, total cholesterol and low-density lipoprotein (LDL)-cholesterol (6). Decreased antibody production as a result of heat stress may limit immunocompetence, as studies suggest (6, 7). Heat stress (HS) is also associated with high mortality, reduced food intake and body weight gain, higher feed conversion ratio (8, 9), and increased thirst (10), increased H/L ratio, impaired intestinal morphology and barrier integrity of chickens (11, 12), leading to impaired digestive and absorptive capacity and increased permeability to luminal antigens and toxins. At the molecular level, stress may change the expression of a number of genes, e.g., heat-shock protein (HSP) HSP40 and HSP90 that are involved in self-regulation and compensation to maintain homeostasis (13). Recently, indication of drugs and natural herbal compounds to strengthen the immune system has increased, due to fewer side effects (14-16). The main natural growth promoters that have just entered the market are prebiotics, probiotics, acidifiers and synbiotics. They are currently used as feed additives in broilers to enhance nutrient utilization by means of diverse mechanisms (15, 17-19). Studies exploring the effects of antibiotic growth promoters (AGPs) have demonstrated immunity improvement (20, 21), reduced feed conversion ratio, increased growth and daily live weight gain (22), improvement of production factors (16, 23, 24), and beneficial effects counteracting the adverse influence of HS in broiler chicken (3).

Other commercial direct-fed microbial additives previously used have been shown to improve villus length, width, and total intestine weight (25, 26). Prebiotics are defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity of the gastrointestinal microbiota that confer benefits upon host well-being and health” (27, 28). Probiotics have been defined as “living microorganisms that, on ingestion in sufficient numbers, exert health benefits beyond basic nutrition” (29). Using these substances in poultry diet provides healthy meat without drug residues and improved nutrient digestibility (30), and also alleviates heat stress (4). Inclusion of Manan-oligosaccharide (MOS) in chicken diets may also enhance immune function and improve growth of the intestinal mucosal layer, and diversity of intestinal microbiota in hens (31). It has been shown that probiotic supplementation is more beneficial during stressful conditions (32, 33). This study intends to compare the effects of probiotic (Bio-SAF47) and prebiotic (Bio-MOS) and their synergistic effects in stimulating the immune system and body weight gain (BWG) in heat-stressed broilers.

Materials and Methods

Experimental Design

300 one-day-old broiler chickens (Cobb 500) were reared on wood shavings litter in an environmentally controlled house equipped with hot air heaters and cooling pads in standard brooding conditions. Chickens were randomly divided into five groups of 12 broilers and studied for 42 days. Group 1 was kept as a control with a standard diet, without induction of HS, while in the rest of the experimental groups, for investigating the effects of prebiotics, probiotics, and their synergistic effects, HS was induced.

Group II was housed at cyclic heat conditioning (34°C in 4 hours per day) and only had access to the basal diet. Groups III to V, in addition to a basal diet, each received Bio-MOS prebiotic (Mannan-oligosaccharide, Alltech, Nicholasville, KY), Bio-SAF probiotic (Saccharomyces cerevisiae, Alltech, Nicholasville, KY), supplemented diet, and both supplementation regimens, respectively (34).

Dietary treatment included the basal diet as control, plus 0.2, 0.1 and 0.05 percent of Bio-MOS or Bio-SAF-supplemented diets in starter, grower and finisher diets, respectively. Table 1 was balanced to meet or exceed minimum nutrient recommendations (35). Birds had free access to clean water and a standard diet and were vaccinated against bronchitis and IBD diseases. The complete vaccination protocol for all chickens was executed as follows: One-day-old chicks initially received the bronchitis vaccine (S120 strain) via spray application. Subsequently, the IBD vaccine (IBD 78 strain) was delivered in the drinking water on days 7 and 14." They were kept under a standard 23 h light/1h darkness program and standard ambient temperature. Chickens were reared on a concrete floor covered with ~5 cm of new wood shaving as litter material. All experimental procedures were approved by the Animal Ethics Committee of Ilam University.

Table 1. Diet composition and calculated analysis of the basal diet

Ingredient (g/kg diet)	Starter feed	Grower Feed	Finisher Feed
Maize	596	596	660
Wheat	56	50	50
Soybean meal (44% crude protein)	266.5	160.5	101.2
Corn gluten meal 60% crude protein	100	114.8	115
Soybean oil	35	33.4	31
Limestone	14.5	12.3	10
Dicalcium phosphate[A]	19.5	18	18.3
Sodium chloride	3.6	3.6	3.6
Vitamin premix[B]	2.5	2.5	2.5
Mineral premix[C]	2.5	2.5	2.5
DL-Methionine	5.2	5.8	5.7
L-Lysine, HCL	2.5	0.6	0.4
Calculated analysis			
ME, MJ/kg	12.60	13.19	13.40
Crude protein (g/kg)	230	200	180
Ca (g/kg)	10	9	9
Available phosphorus (g/kg)	5.5	4.5	4.5
Lysine (g/kg)	14.1	11.6	10.5
Methionine+cysteine (g/kg)	10.9	8.1	7.8

Measurements

Serum Corticosterone and Immunoglobulins

On days 21, 35 and 42, blood samples from chickens were collected in sterile blood collection tubes. The tubes were centrifuged at 1500 rpm for 10 min. The samples were stored in -20 °C until analysis. Serum concentrations of corticosterone, IgG and IgM were analyzed using ELISA kits (Dia Plus, Inc, USA).

Immune organ and body weight gain (BWG)

On day 42, the birds were euthanized by cervical dislocation. Bursa and spleen were collected and the relative weight as an index of immunosuppression was calculated using the following formula: immune organ weight (g) × 1000 / body weight (g) (36). Also, BWG was measured on days 21, 35 and 42 of age as an indicator of heat stress influence. During the experiment, the mortality rate in each group was also measured.

Leukocyte counts

White blood cell (WBC) counts of samples including heterophils, lymphocytes, basophils, eosinophils, and monocytes, were performed using methods from previous studies (37, 38).

Statistical Analysis

Statistical analysis of data was carried out using SPSS software (version 19.0 for Windows, Inc., Chicago, IL). Data were presented as Mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Results

Corticosterone

The serum concentrations of Corticosterone are shown in Table 2. As the table shows HS increases serum cortisol significantly in comparison to control ($p < 0.05$). Bio-SAF

and Bio-MOS reduced the elevated serum cortisol concentration during 1-21, and 22- 42 days of age, respectively ($p < 0.05$) (Table 2).

Lymphoid Organ Index

The lymphoid organs index shown in Table 3 indicates that HS significantly decreased the bursa and spleen weight index during days 1-42 in comparison to the control group ($p < 0.05$). Co-administration of supplementary regimens significantly increased the bursa weight index ($p < 0.05$), while spleen weight index was not significantly influenced in comparison to control ($p > 0.05$).

Immunoglobulins (Antibody) titers

Heat stress showed a decrease in serum IgG level on days 35 and 42, and reduced IgM titers on day 42 of age ($p < 0.05$). While neither probiotic nor prebiotic could increase serum levels of IgG and IgM significantly ($p > 0.05$), co-administration significantly increased serum IgG & IgM levels ($p < 0.05$).

Leukocyte Counts

Results showed that HS increased blood heterophil and basophil count, and H/L ratio, while it decreased lymphocyte count significantly ($p < 0.05$) (Table 5). Bio-MOS synbiotic reduced the elevated serum heterophil/lymphocyte (H/L) ratio, heterophil and lymphocyte count during 35-42 (finisher) days ($p<0.05$) (Table 5).

BWG

While HS decreased BWG significantly ($p < 0.05$), Bio-SAF and Bio-MOS co-administration increased BWG on days 21, 35, and 42 significantly ($p < 0.05$) (Table 6).

Table 2. Effects of heat stress and dietary additives on serum cortisol hormone concentration in broilers up to the age of 42 days.

Day	TN	HS	HS+Bio-SAF	HS+Bio-MOS	HS+COM
21	0.78 ± 0.03^{c}	2.74 ± 0.60^{a}	1.73 ± 0.08^{c}	2.06 ± 0.73^{b}	2.01 ± 0.46^{b}
35	1.58 ± 0.21^{b}	2.67 ± 0.72^{a}	2.03 ± 0.66^{a}	1.74 ± 0.53^{b}	2.28 ± 0.71^{a}
42	1.38 ± 0.23^{b}	2.09 ± 0.39^{a}	1.61 ± 0.42^{b}	0.73 ± 0.08^{c}	0.54 ± 0.05^{c}

TN: Thermal; HS: Heat stress; Bio-SAF: Probiotic; Bio-MOS: Prebiotic; COM: Combination of probiotic and prebiotic. a,b,c Means in row with no common superscript differ significantly ($p < 0.05$).

Table 3. Effects of heat stress and dietary additives on lymphoid organ index in broilers at 42 days of age.

Lymphoid Organ Index	TN	HS	HS+Bio-SAF	HS+Bio-MOS	HS+COM
Bursa Index	0.78 ± 0.08^{a}	0.59 ± 0.05^{b}	0.61 ± 0.03^{b}	0.63 ± 0.02^{b}	0.68 ± 0.11^{a}
Spleen Index	1.26 ± 0.08^{a}	1.09 ± 0.07^{b}	1.12 ± 0.04^{b}	1.10 ± 0.05^{b}	1.13 ± 0.08^{b}

TN: Thermal; HS: Heat stress; Bio-SAF: Probiotic; Bio-MOS: Prebiotic; COM: Combination of probiotic and prebiotic. a,b Means in row with no common superscript differ significantly ($p < 0.05$).

Table 4. Effects of heat stress and dietary additives on immunoglobulins in broiler serum up to the age of 42 days.

	Day	TN	HS	HS+Bio-SAF	HS+Bio-MOS	HS+COM
IgG	**21**	1.07±0.05	0.96±0.04	0.99±0.05	1.04±0.03	1.01±0.05
	35	1.22 ± 0.09^{a}	0.88 ± 0.04^{b}	0.76 ± 0.06^{b}	0.83 ± 0.04^{b}	0.91 ± 0.08^{b}
	42	1.43 ± 0.07^{a}	1.14 ± 0.14^{b}	1.06 ± 0.08^{b}	1.16 ± 0.05^{b}	1.48 ± 0.25^{a}
IgM	**21**	1.03±0.07	0.98±0.06	1.06±0.05	1.05±0.02	1.08±0.07
	35	2.08±0.09	1.93±0.08	1.99±0.08	1.88±0.07	1.09±0.06
	42	2.03 ± 0.21^{a}	1.63 ± 0.13^{b}	1.66 ± 0.05^{b}	1.52 ± 0.17^{b}	1.69 ± 0.23^{b}

TN: Thermal; HS: Heat stress; Bio-SAF: Probiotic; Bio-MOS: Prebiotic; COM: Combination of probiotic and prebiotic. a,b Means in row with no common superscript differ significantly ($p < 0.05$).

Table 5. Effects of heat stress and dietary additives on the leukocyte count of broilers up to the age of 42 days.

	Day	TN	HS	HS+Bio-SAF	HS+Bio-MOS	HS+COM
Heterophil	**21**	15.6± 2	16.1± 3	16.3± 8	17.7± 5	16.5± 2
	35	15.2± 4	19.4± 9	20.8± 5	19.4± 7	18.7± 3
	42	16.5± 4^{c}	27.5± 8^{a}	27.6± 6^{a}	25.1± 7^{a}	21.8± 2^{b}
Eosinophil	**21**	2.2±0.1	2.3±0.4	2.6±0.7	2.9±0.4	2.5±0.6
	35	3.1±0.6	2.9±0.2	2.8±0.3	2.9±0.8	3.3±0.7
	42	3.7±0.2^{a}	3.6±0.1^{a}	3.7±0.3^{a}	3.4±0.5^{a}	2.9±0.7^{a}
Basophil	**21**	2.3±0.9	3.2±0.5	3.4±0.8	3.5±0.2	3.9±0.1
	35	1.9±0.2	2.9±0.6	3.5±0.7	2.9±0.1	3.7±0.3
	42	2.3±0.5^{b}	4.3±0.1^{a}	4.4±0.5^{a}	4.4±0.3	4.6±0.6^{a}
Monocyte	**21**	1.9±0.3	2.2±0.4	2.5±0.1	2.7±0.4	2.8±0.3
	35	2.2±0.6	2.7±0.2	2.8±0.5	2.6±0.1	2.4±0.9
	42	2.5±0.4^{a}	2.1±0.3^{a}	2.9±0.3^{a}	2.6±0.6^{a}	2.4±0.5^{a}
Lymphocyte	**21**	66.5±11	64.3± 7	68.9± 9	66.8± 7	70.8± 12
	35	71.2± 9^{a}	62.8± 8^{b}	66.5± 9^{b}	65.1± 7^{b}	69.8± 9^{a}
	42	74.7± 12^{a}	51.6± 11^{b}	60.6± 5^{b}	63.3± 11^{b}	69.7± 5^{a}
H/L ratio	**21**	0.23±0.09	0.25±0.04	0.24±0.01	0.267±0.02	0.23±0.01
	35	0.21±0.05	0.31±0.01	0.31±0.02	0.29±0.01	0.27±0.06
	42	0.22±0.40^{c}	0.53±0.6^{a}	0.45±0.3^{a}	0.43±0.1^{a}	0.31±0.5^{b}

TN: Thermal; HS: Heat stress; Bio-SAF: Probiotic; Bio-MOS: Prebiotic; COM: Combination of probiotic and prebiotic. a,b,c Means in row with no common superscript differ significantly ($p < 0.05$).

Table 6. Effects of heat stress and dietary additives on body weight gain (BWG) from 21 to 42 days of age.

Day	TN	HS	HS+Bio-SAF	HS+Bio-MOS	HS+COM
21	631.8±13^{b}	574.8±9^{c}	609.5±11^{b}	628.5±8^{b}	687.3±10^{a}
35	927.2±11^{b}	618.8±12^{c}	904.7±13^{b}	939.4±11^{b}	1042.5±9^{a}
42	483.1±14^{c}	422.7±9^{d}	429.8±7^{d}	536.2±8^{b}	587.5±12^{a}
T-B 1-42	2042.1±12^{c}	1616.3±11^{d}	1944.0±9^{c}	2104.1±9^{b}	2317.3±10^{a}

TN: Thermal; HS: Heat stress; Bio-SAF: Probiotic; Bio-MOS: Prebiotic; COM: Combination of probiotic and prebiotic. a,b,c,d Means in row with no common superscript differ significantly ($p < 0.05$).

Discussion

Heat exposure induces behavioral, physiological, and immunological responses in broilers (39). Furthermore, the results of this study indicated that heat stress induces increased corticosterone, decreasing leucocyte count and antibody titer, leading to a decline in immune response, and finally decreasing weight gain and body weight. Co-administration of prebiotic and probiotic supplements works to counteract the adverse effects of HS, while neither supplement had such effects when administered alone. Generally, supplementation with probiotics for 21 days, prebiotics for 35-42 days synbiotic. In the final days of the raising period, it could prevent and improve the undesirable effects of heat stress on the corticosterone level. The results of this study are in accord with previous results reported by (9, 34). It was reported that increased levels of corticosterone due to stress caused by newborn separation from parents in mice were attenuated by probiotics supplements such as *Bifidobacterium* and *Lactobacillus* (40, 41). Increasing the activity of the hypothalamus-pituitary-adrenal axis in thermal stress causes increased secretion of hypothalamic releasing hormones and adrenocorticotropic hormones of the pituitary and adrenal corticosterone, leading to a decline in immune response (40). In this study, decreased IgM and IgG levels caused by heat stress were attenuated through co-administration of prebiotic and probiotic supplementation. Thermal stress in birds can increase corticosterone levels and lead to suppression of the immune system (16, 41-44). Usually, heat stress causes a decline in antibody production and increases the production of inflammatory cytokines, which enhances the secretion of the corticotropin-releasing hormone from the hypothalamus. Corticosterone, in turn, inhibits antibody production and weakens the immune system. Synbiotic consumption has been demonstrated to potently modulate the humoral immune system. This adaptive mechanism, driven by B-lymphocytes, is essential for generating protective antibodies. Studies frequently report that synbiotics lead to enhanced serum concentrations of specific immunoglobulins, including IgG, IgA, and IgM. Thermal stress causes changes in leukocyte count. It has been reported that the H/L ratio increases by approximately 43%. During this experiment, lymphocyte counts were reduced regularly in parallel with the increase in heterophile counts. These results are in agreement with (7, 33). Generally, an increased H/L ratio is a mild response to the increase of ambient temperature, but an increased H/L ratio is considered as the sensitive index of stress, and heterophiles are considered the receptor of thermal stress in chicks (45). Thermal stress decreases the production of lymphocyte proliferation factors, such as Interleukin-I (45, 46). In this study, body weight gaining was decreased by heat but prebiotic and probiotic supplements and especially co-administration during 23-25 days of age improved body weight gain. The results of this experiment were consistent with the results of, who stated that probiotics *Lactobacillus* supplementations increase body weight gain. However, this study is not consistent with the results of (15,47). Probiotics supplementation can probably stimulate secretion of cytokines indirectly through neuroendocrine pathways leading to reduction of the blood level of ACTH and corticotropin. The effects of probiotic, prebiotic and co-administration on weight gain may be due to enhanced nutrient absorption ability of intestinal epithelium and also influencing the balance of intestinal microflora so that competition with microbial pathogens inhibits pathogen growth, which indirectly improves nutrient absorption.

Conclusion

Finally, as a conclusion, it is estimated that perhaps a combination of probiotics and pre-biotics in diets of broilers can have beneficial effects on physiological and immunological functions.

Acknowledgements

Not applicable

Authors' Contributions

Seyed Saeid Taheri: Project Execution, **Javad Cheraghi:** Project Outline- Writing. Editing, **Hamidreza Mohajerani:** Editing

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

All animal experiments were conducted in accordance with the ethical guidelines for the use of animals in clinical practice.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable

Funding

The authors received no financial support for the current research.

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