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Serine protease-loaded inulin microsphere with *Bacillus subtilis* qb1-based synbiotic for improved growth indices in Ross 308 broilers

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Abstract: The use of antibiotics in poultry has raised concerns about antibiotic resistance, increasing interest in alternative approaches to enhance broiler performance. The current study evaluated the effects of combining serine protease (SP) loaded inulin microspheres with *Bacillus subtilis* (QB1) on the growth indices, health indicators and meat quality of Ross 308 broilers. A total of 105 chicks were equally divided into six treatment groups and one control group: control (S1), 0.5 g/kg Bacitracin (S2), QB1 at 10^6 CFU/g (S3), 1g/kg inulin (S4), 10 mg/kg SP (S5), synbiotics (1 g/kg inulin + QB1 at 10^6 CFU/g) (S6) and SP loaded inulin microspheres (10 mg/kg) + QB1 at 10^6 CFU/g (S7). S7 significantly outperformed others, with body weight gain of 1907 g, feed intake of 3182 g, feed conversion ratio of 1.554, energy efficiency ratio of 23.23 and protein efficiency ratio of 3.417 ($P<0.05$). S7 improved breast (6.42) and thigh (6.50) pH, water holding capacity (80.27% breast and 76.17% thigh), protein (26.60% breast and 24.71% thigh), extract release volume (33.36 thigh and 32.63 breast) ($P<0.05$). These results suggest that SP loaded inulin microspheres combined with QB1 offer a promising antibiotic alternative, enhancing growth indices, meat quality, and health in broilers.

Keywords: *Bacillus subtilis*; inulin; serine protease; microsphere; antioxidant

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INTRODUCTION

The use of antibiotics in poultry has generally improved the health and productivity of broiler chicks (Dev *et al*., 2020). However, the widespread use of antibiotic growth promoters (AGPs) aggravated concern about the contagion of potentially fatal antibiotic-resistant diseases that may pose potential threats to both human and animal health (Polidoro *et al*., 2024). This pressing issue has led to advocacy worldwide for reducing AGPs. Animal scientists need to find alternative solutions to maintain poultry production while minimizing animal suffering and safeguarding food quality (Hafsan *et al*., 2021). Synbiotics which combine probiotics and prebiotics have gained so much attention as potential alternatives to antibiotics due to the possibility of improving the growth and health of chickens both economically and physiologically. Inulin and other non-digestible carbohydrates termed 'prebiotics' stimulate the beneficial intestinal bacteria to bulk up the immune system and improve nutrient assimilation (Du *et al*., 2023, Shang, 2024). Literature reports that a combination of prebiotics and probiotics is much more effective for promoting intestinal health, quality of meat, and antioxidant capacity in broiler chickens (Qiu *et al*., 2023, Ogwiji *et al*., 2024).

Exogenous enzymes like serine proteases have emerged as potential solutions to the ongoing challenges faced by poultry particularly feed conversion efficiency and nutrient bioavailability (Gazani *et al*., 2024). The inclusion of serine protease in poultry diets has been shown to enhance the digestibility of proteins which in turn influence growth rate and feed conversion ratio, and reduce nitrogen emission (Wealleans *et al*., 2024). However, administrating serine protease alone can result in its degradation in the gastrointestinal tract, limiting its bioavailability and effectiveness for improved growth and digestion. To overcome this limitation micro spheres in drug delivery systems have improved the potential of bioactive compounds (Khandale *et al*., 2024). These bioactive compounds are encapsulated within micro spheres to protect them from the harsh GIT environment while simultaneously controlling the release of probiotics and enzymes (Liu *et al*., 2022). In broilers, this encapsulation method improves physical characteristics and the bioavailability of serine proteases improves digestion, growth and feed conversion (Ozaltin *et al*., 2019). Studies have shown that HPMC-AA microsphere formulations can improve the efficacy of dietary supplements by protecting the compounds from gastrointestinal degradation and enhancing their functional responsiveness in the colon (Wang *et al*., 2024).

However, the cumulative effects of *Bacillus subtilis* and serine protease-encapsulated inulin beads on growth performance, meat quality, and antioxidant capacity in broiler chicks have not been investigated thus far. Therefore, this study was designed to assess the effects of *Bacillus subtilis* and serine protease encapsulated inulin beads on the growth rate, antioxidant status and the meat

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quality of broiler. In this study, we aimed to provide a comprehensive understanding of how serine protease-loaded inulin microspheres and *Bacillus subtilis* (QB1) supplementation could improve broiler productivity, meat quality, and health, thereby contributing to the development of sustainable poultry production alternatives to AGPs.

MATERIAL AND METHOD

Experimental design and dietary treatments

Study groups (treatment and control groups) were designed by selecting chicks based on their initial weight to maintain uniformity while using health as their main selection factor. The experimental subject pool excluded chicks displaying illness signs, abnormal growth patterns, or failing weight specifications. A total of 105 Ross 308 broiler chicks (15chicks/group) were assigned to six treatment groups and one control group was provided with a basal diet (corn, soybean meal and corn gluten meal). Treatments: S1 (Control), S2 (0.5g/kg Bacitracin), S3 (*Bacillus subtilis* 10^6 CFU/g), S4 (1g/kg Inulin), S5 (10 mg/kg Serine Protease), S6 (Synbiotics: 1 g/kg Inulin + *Bacillus subtilis* 10^6 CFU/g), and S7 (Serine Protease-loaded Microsphere: 10mg/kg + *Bacillus subtilis* 10^6 CFU/g). Chicks were fed ad libitum with starter, grower, and finisher diets for 45d, with free access to water. The Institutional Review Board approved the study (GCUF/ERC/319, dated October 5, 2023) following the ethical guidelines.

Bacillus subtilis (Accession No. OQ932777) and freshly prepared micro spheres were used in this trial. Serine protease-loaded inulin micro spheres were prepared through an emulsion-assisted technique. Micro spheres were collected after coating with inulin, gelling with $CaCl_2$, centrifugation (3,000 rpm, 5 min), washing, freezing, and storage at 20°C (Liu *et al*., 2022). The micro sphere size distribution was around 45 to 70 µm with minimal dispersion variation. Analysis of micro sphere structure demonstrated porous structure patterns that facilitated optimum conditions for enzyme encapsulation. The micro spheres released enzymes in a controlled manner through a gradually increasing pH environment that replicated gastrointestinal conditions from pH 1.2 for 0 to 2 hours to pH 4.5 from 2 to 5 hours and following pH 6.8 from 5 to 7 hours then reaching pH 7.2 between 7 to 10 hours.

Growth performance

Body weight gain (BWG in g), feed intake (FI in g), feed conversion ratio (FCR), energy efficiency ratio (EER), protein efficiency ratio (PER) and performance efficiency factor (PEF) were determined under the respective dietary treatment (over 45 days) using the formulas (eq. (i), eq. (ii) and eq. (iii) (Dev *et al*., 2020).

$$PEF = \frac{Final\ body\ weight\ (kg) \times Livability\ \%}{\text{Age in days} \times \text{FCR}} \times 100 \ldots\ldots\ldots\ldots eq.(i)$$

$$PER = \frac{\text{Weight gain}}{\text{Protein Intake}} \ldots\ldots\ldots\ldots\ eq.\ (ii)$$

$$EER = \frac{\text{Weight gain (g)}}{\text{Total energy intake (ME kcal)}} \times 100 \ldots\ldots\ldots eq.(iii)$$

Sample collection and biochemical profiles

Blood samples from individual birds at the time of slaughter were collected in non-heparinized tubes, centrifuged at 5000rpm for 15 minutes to harvest serum, and stored at -80°C for further analysis. To evaluate, physicochemical parameters, the antioxidant profile and lipid peroxidation, thigh and breast meat samples were collected from individual birds. Liver samples were also collected to study antioxidant enzymes. ALP and ACP activities were determined to evaluate the health status of bone tissues and livers. Total proteins, globulins, albumin, and A:G (albumin globulin ratio), Triglyceride (TG) total cholesterol (TC) and high-density lipoprotein (HDL) were estimated to determine the health status.

Antioxidant and Physicochemical properties of meat

The antioxidant status of broiler chicken meat was estimated using the 2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging and lipid oxidation was evaluated using the Thiobarbituric Acid Reactive Substances assay (Dev *et al*., 2020). The pH of breast and thigh meat was estimated by homogenizing 5 g of meat in 25mL distilled water, water holding capacity (WHC) by homogenizing 10 g in 0.6 mol/L NaCl and extract release volume (ERV) by homogenizing 15 g in 60mL of 0.05 mol/L phosphate buffer, and protein percentage was calculated using the AOAC method (1995) (Zhao *et al*., 2024).

STATISTICAL ANALYSIS

The data was analyzed using GraphPad Prism version 7.04 and Microsoft Excel 2013. The data are expressed in mean ± SD and were tested by one-way Analysis of Variance and Tukey's Multiple Comparisons tests. Statistical significance was considered as ****P< 0.0001, ***P< 0.001, **P< 0.01, and *P <0.05.

RESULTS

Prior to treatment no significant differences were observed between the groups in BWG, FI, or efficiency indices. After treatment, the chicks in treatment group S7 showed significantly superior outcomes compared to control group (S1). Specifically, S7 exhibited significantly higher body weight gain (1907g), energy efficiency ratio (23.23), protein efficiency ratio (3.417), and productive efficiency factor (279.8) (fig. 1a, 1c, 1d, 1e, 1f). Additionally, the feed intake in S7 (3182 g) was significantly higher than S1 (***P < 0.001) (fig. 1b).

Table 1: Comparative effect of serine protease-loaded synbiotics and conventional supplementation on serum biochemistry indices in Ross 308.

Item	Treatments								
	S1	S2	S3	S4	S5	S6	S7	SEM	P-value
Glucose (mg/dL)	194.7 [a]	174.8 [b]	175.1 [c]	184 [d]	174.3 [e]	165.6 [f]	153.9 [g]	1.412	<0.0001
SGOT (U/L)	184.1 [a]	187.3 [a]	197.4 [a]	188.2 [a]	191.7 [a]	215.5 [b]	219.9 [c]	4.023	<0.0001
SGPT (U/L)	149.3 [a]	147.7 [a]	156.2 [b]	148.1 [a]	154 [a]	165.6 [c]	191 [d]	3.154	<0.05
ALP (U/L)	26.19 [a]	28.56 [b]	31.41 [c]	26.14 [d]	31.55 [e]	32.78 [f]	32.54 [g]	0.597	<0.001
ACP (U/L)	27.11 [a]	30.36 [b]	32.02 [c]	28.63 [d]	30.25 [e]	25.42 [f]	28.32 [a]	0.5059	<0.05
TG, mg/dL	127[a]	120.6b	110.2[c]	115.7[d]	108.5[e]	105.1[f]	101.4[g]	0.05091	<0.0001
TC , mg/dL	97.25[a]	93.01[b]	85.59[c]	88.33[d]	84.51[e]	82.25[f]	78.44[g]	0.05466	<0.0001
HDL, mg/dL	50.86[a]	51.97[b]	55.35[c]	53.95[d]	56.7[e]	58.45[f]	60.22[g]	0.05244	<0.0001

SGOT=serum glutamic oxaloacetate; SGPT=serum glutamic pyruvic transaminase; ALP=Alkaline phosphatase; ACP=acid phosphatase; TG=Triglycerides; TC=Total Cholesterol; HDL=High density lipoproteins. Mean values within a row with superscripts [a to g] are significantly different (P < 0.05).

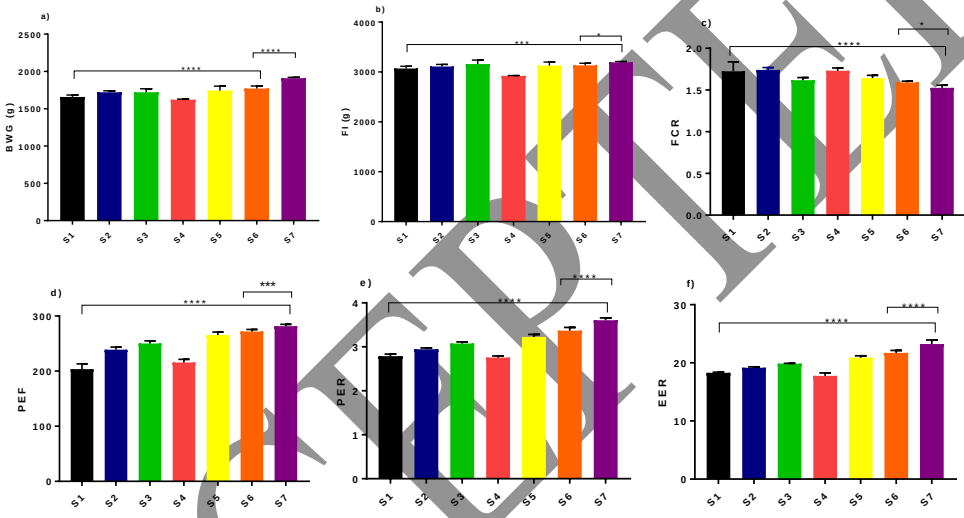

Fig. 1: Comparative Effect of Serine Protease-Loaded Synbiotics and Conventional Supplementation on Growth, Feed Efficiency and Productivity in Ross 308. Data are expressed as mean ± standard error. (a)Body weight gain. (b) Feed Intake (c) Feed conversion ratio. (d) Production efficiency factor. (e) Protein efficiency ratio. (f) Energy efficiency ratio. Control (S1), 0.5 g/kg Bacitracin (S2), QB1 at 10^6 CFU/g (S3), 1g/kg inulin (S4), 10mg/kg serine protease (S5), synbiotics (1 g/kg inulin + QB1 at 10^6 CFU/g) (S6), and serine protease-loaded inulin microspheres (10 mg/kg) + QB1 at 10^6 CFU/g (S7). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test: ****P<0.0001, ***P<0.001, *P<0.05.

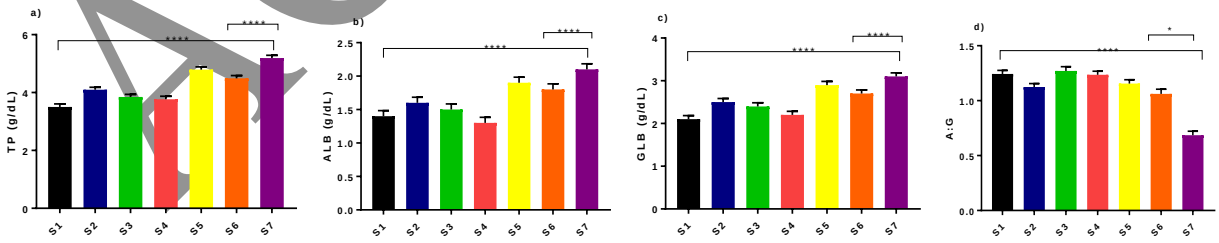

Fig. 2: Comparative Effect of Serine Protease-Loaded Synbiotics and Conventional Supplementation on Protein Content. Data are expressed as mean ± standard error. (a)Total protein. (b)Albumin (c) Globulin. (d)Albumin to globulin ratio. Control (S1), 0.5 g/kg Bacitracin (S2), QB1 at 10^6 CFU/g (S3), 1g/kg inulin (S4), 10 mg/kg serine protease (S5), synbiotics (1 g/kg inulin + QB1 at 10^6 CFU/g) (S6), and serine protease-loaded inulin microspheres (10 mg/kg) + QB1 at 10^6 CFU/g (S7). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test: ****P<0.0001, *P<0.05.

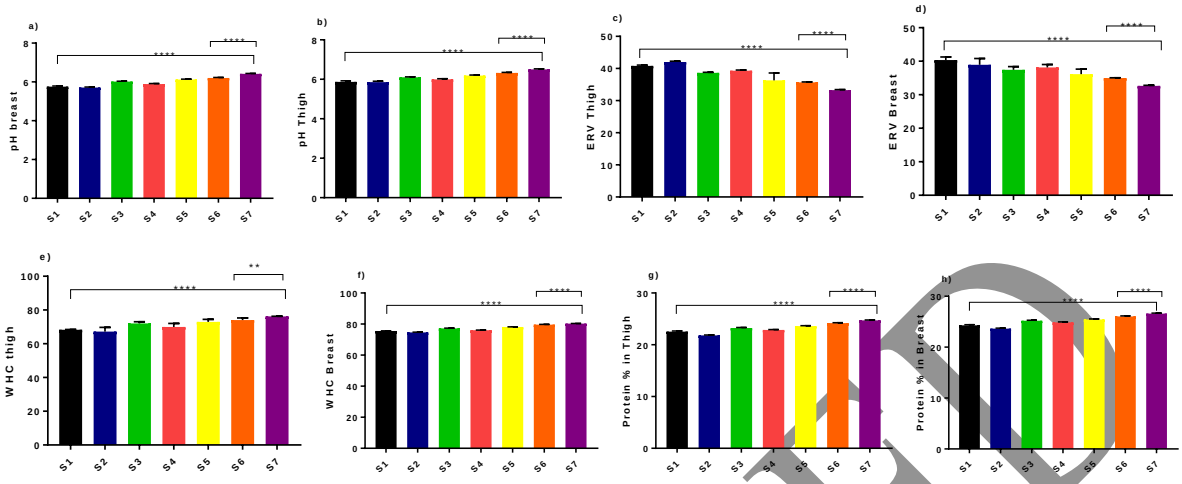

Fig. 3: Comparative Effect of Serine Protease-Loaded Synbiotics and Conventional Supplementation on Physicochemical Parameters of Ross 308 Meat. (a) pH of breast meat. (b) pH of thigh meat. (c) Extract release volume of thigh. (d) Extract release volume of breast meat. (e) Water holding capacity of thigh meat. (f) Water holding capacity of breast meat. (g) Protein percentage in thigh meat. (h) Protein percentage in breast meat. Data are expressed as mean± standard error. Control (S1), 0.5 g/kg Bacitracin (S2), QB1 at 10^6 CFU/g (S3), 1g/kg inulin (S4), 10 mg/kg serine protease (S5), synbiotics (1 g/kg inulin + QB1 at 10^6 CFU/g) (S6), and serine protease-loaded inulin microspheres (10 mg/kg) + QB1 at 10^6 CFU/g (S7). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test: ****P < 0.000, **P < 0.01.

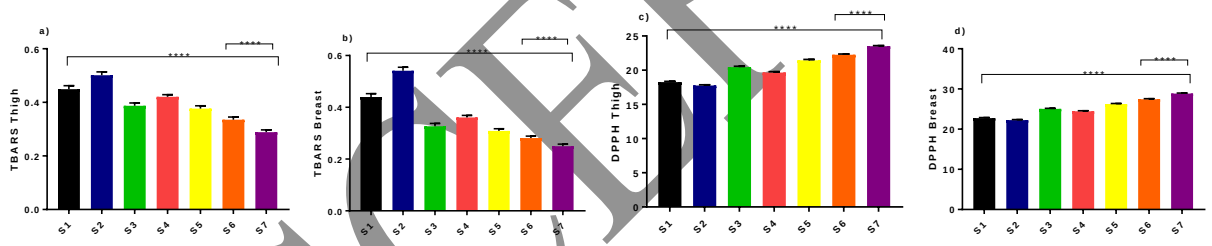

Fig. 4: Lipid peroxidation and antioxidant activity in thigh and breast meat of broilers under different treatments. (a, b) TBARS values (mg MDA/kg) indicate lipid peroxidation. (c, d) DPPH radical scavenging activity (%) reflecting antioxidant potential. Data are expressed as mean ± standard error. Control (S1), 0.5 g/kg Bacitracin (S2), QB1 at 10^6 CFU/g (S3), 1g/kg inulin (S4), 10 mg/kg serine protease (S5), synbiotics (1 g/kg inulin + QB1 at 10^6 CFU/g) (S6), and serine protease-loaded inulin microspheres (10 mg/kg) + QB1 at 10^6 CFU/g (S7). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test: ****P < 0.0001.

Similarly, treatment group S7 demonstrated significantly improved serum biochemistry parameters compared to control group S1 (*P< 0.05) (table 2). Specifically, S7 had the lowest glucose (153.9 mg/dL) and the highest SGOT (219.9 U/L), SGPT (191 U/L), albumin (1.991 g/dL), globulin (3.098 g/dL), and A:G ratio (0.6891). Furthermore, S7 exhibited the lowest triglycerides (101.4 mg/dL) and total cholesterol (78.44 mg/dL) levels, while HDL levels were significantly elevated (60.22 mg/dL), indicating enhanced lipid metabolism. The greatest protein metabolism agonistic action was observed in treatment group S7 compared to control S1 (fig. 2). Among the evaluated groups S7 demonstrated the maximum protein content and combined protein fractions of total protein (5.19), albumin (2.1) and globulin (3.1) (fig. 2a, 2b, 2c). These differences were statistically significant (****P < 0.0001) compared to other treatment groups. Additionally, treatment group S7 resulted in

reduced albumin-to-globulin (0.686) ratio compared to S1 (****P < 0.0001), reflecting changes in protein distribution pattern (fig. 2d). These findings indicate that treatment group S7 was the most effective treatment in improving protein content through the combined actions of serine protease-loaded inulin microspheres and *Bacillus subtilis* QB1.

Similarly, treatment group **S7** significantly (****P< 0.0001) improved pH, extract release volume (ERV), water holding capacity (WHC) and protein content in both breast and thigh meat compared to S1 (fig. 3). S7 had the highest pH in both the breast (6.417) and thigh (6.503) tissues (fig. 3a, 3b)**,** ERV thigh (33.26), and ERV breast

(32.62) followed by S1 (fig. 3c, 3d)**.** Additionally, treatment group S7 exhibited the highest WHC (****P< 0.0001) in both the thigh (76.17%) and breast (80.27%) meat (fig. 3e, 3f), as well as the highest protein content in both the thigh (24.71%) and breast (26.60%) (fig. 3g, 3h) compared to S1. The consistent out performance of the S7 highlights its superior effect on meat quality.

Moreover, treatment group S7 significantly (****P< 0.0001) reduced lipid peroxidation and enhanced antioxidant activity in both thigh and breast meat compared with S1 (fig. 4). S7 exhibited the lowest TBARS values in thigh meat (0.2887 mg MDA/kg) (fig. 4a) and breast meat (0.25 mg MDA/kg) (fig. 4b), indicating minimal lipid peroxidation. Regarding antioxidant activity, S7 achieved the highest DPPH radical scavenging percentage, with 23.51% inhibition in thigh meat (fig. 4c) and 28.85% in breast meat (fig. 4d), demonstrating superior antioxidant effects compared to S1. S7 consistently yielded the most pronounced improvements, emphasizing its effectiveness in enhancing the overall meat quality.

DISCUSSION

Antibiotic inclusion in poultry diets is an issue of food safety and resistance among poultry chicks. Therefore, several strategies are being practiced for better growth and health of the broilers. The influence of a newly developed synbiotic approach, consisting of *Bacillus subtilis* QB1 and serine protease-loaded inulin microspheres in Ross 308 broiler chickens, was investigated in the present study. This study aimed to assess this combination's impact on meat quality, growth rate, protein metabolism, biochemical profile and antioxidant activity. Literature reported that synbiotic supplementation is superior to individual probiotic or prebiotic applications as in combination prebiotics necessarily provide a food source to probiotics which facilitates the improved growth of Ross broiler chicken thereby increasing resistance to temperature, low pH, and oxygen (Dev *et al*., 2020). In line with these findings, S7 significantly improved growth parameters, with body weight gain (BWG) of 1907 g, feed intake (FI) of 3182 g, feed conversion ratio (FCR) of 1.554, energy efficiency ratio (EER) of 23.23, and protein efficiency ratio (PER) of 3.417 (P<0.05). Meat quality parameters in S7 were also improved, including breast and thigh pH (6.42 and 6.50, respectively), water holding capacity (WHC; 80.27% breast and 76.17% thigh), protein content (26.60% breast and 24.71% thigh), and extract release volume (ERV; 33.36 thigh and 32.63 breast). Inulin as a prebiotic, stimulates healthy gut microbiota while boosting nutrient absorption and digestion efficiency. The use of synbiotic supplementation is reported to be superior to the individual use of probiotics or prebiotics because prebiotics act a necessary food source for probiotics and also increase their resistance to temperature, oxygen, and low pH (Sekhon and Jairath, 2010) which results in better growth performance in broiler chicken (Dev *et al*., 2020).

(Khandale *et al*., 2024) confirmed that delivery mechanisms including microspheres improve the functional capacity of synbiotics. Microspheres help to deliver the desired bioactive across the GIT in a site-specific manner while at the same time protecting probiotics and enzymes not only from extremes of pH but also digestion in the gut (Liu *et al*., 2022). The solubility and stability from the discovered bioactive compounds like serine proteases used for augmenting the protein digestibility, growth rate, and feed conversion ratio in the broilers are protected under this process of encapsulation (Ozaltin *et al*., 2019). According to (Wang *et al*., 2024), microsphere formulations can enhance the efficacy of supplements by minimizing their degradation in the gastrointestinal tract and enhancing their functional utility in the intestinal regions. Apart from improving the beneficial effects mentioned in the present study, the advancement in drug delivery promotes the rationale of synbiotics formulation with serine proteases as a robust substitute to AGP in poultry production.

Beneficial bacteria such as *Bacillus subtilis* enhance intestinal morphology through the directed growth of villi and improve the overall gut barrier function enhancing growth rates and more efficient nutrient use (Qiu *et al*., 2023). Similarly, Song *et al*. (Song *et al*., 2023) showed that exogenous enzymes improve the rate of crude protein and amino acid degradation. Based on the observations of this study, the supplementation of probiotics, prebiotics, and enzymes yielded the highest post-weaning growth rates and feed conversion efficiencies. (Angel *et al*., 2011) provided additional information on synergism by demonstrating that protease improves feed conversion, growth, and performance when protein supplementations are scarce.

From the present study, the synbiotic group supplemented with enzymes displayed the least value of TBARS in the breast and thigh meats and the highest value of DPPH scavenging activity. These outcomes are per those reported by (Ogwiji *et al*., 2024), based on evidence the fact that probiotics and prebiotics enhance systemic antioxidant protection, and as a result cultivate the decreased oxidative strain and lipid peroxidation. *Bacillus* and *Lactobacillus* strains have also been shown to increase antioxidant enzyme activity; the subjects in the study (Ragab *et al*., 2024, Du *et al*., 2023) experienced a similar reduction in oxidative damage. Contrary to our studies, (Erdoğan *et al*., 2010) reported that prebiotics or probiotics don't positively affect broiler chicken growth. This variation can be explained based on the delivery of serine protease, amounts and inclusion levels of synbiotics, etc. (Erdoğan *et al*., 2010, Baurhoo *et al*., 2009, Manafi, 2015). The variations in the results about

the growth performance of broiler chicken in response to synbiotic supplementation can partly be explained by the difference in genetics of the birds used for the experiment, the strain and amount of probiotic, the source and inclusion level of prebiotic, etc. From the enhanced quality of meat by the enzyme-loaded synbiotic group, it was clear that the breast and thigh meat had better water-holding capacity, protein content and low pH values. Synbiotic supplements in the present study have augmented the muscle protein content and enhanced the protein profile (Popova, 2017). The pH stability is likely due to the decrease in postmortem glycolysis by which improved meat texture is also achieved with a minimal loss in drip. (Devnath, 2021) and (Purslow *et al*., 2021), also reported that synbiotics increase WHC and meat tenderness. According to recent studies by (Giannenas *et al*., 2017) and (Saleh *et al*., 2020), the addition of Ronozyme® ProAct (serine protease) has been pointed to contribute to the improved growth performance and physical characteristics of the meat in broiler production in Greece.

Chicks in the synbiotic groups that received enzymes had fared better in glucose, albumin, and total protein levels indicating better liver function and metabolic health. The intensity of the triglycerides-totaled cholesterol, and high-density lipoprotein concentrations, differed significantly between the groups. Thus, the enzyme-loaded synbiotic group was reported to have improved lipid status regarding TG, TC, and HDL levels indicating beneficial effects on lipid metabolism. In line with our findings, (Saleh *et al*., 2020) investigated that synbiotics improve people's metabolic health by optimizing nutrient intake and reducing energy utilization in the digestive process. In the present study, lower serum TBARS levels; uncover synbiotics' systemic antioxidant potential and ability to counter free radical attacks.

The findings presented in this paper may prove that synbiotics containing specific serine proteases are an effective option for oral antibiotics in chickens. Apart from enhancing the quality of meat and growth performance, they lower inflammation and provide customers with antibiotic-free chicks. It similarly aids sustainability and getting the best out of any input feed because it has improved feed conversion efficiency. Rather, focused on the long-term effects of synbiotics, disease prevention by synbiotics, and the application of metagenomics and proteomics methods, future studies should explore the economic efficiency of synbiotics in commercial poultry.

CONCLUSION

It can be concluded from the present study that supplementation of Ross 308 broiler chicks with serine protease-loaded inulin microspheres (10 mg/kg) along with *Bacillus subtilis* (QB1) at 10^6 CFU/g can be used as an effective feed additive for excellent growth performance, meat quality and improved body antioxidant system with significant inhibition of lipid peroxidation. Similarly, better physiochemical properties of meat are observed in chicks fed with the same novel synbiotics compared to the conventional diet. This supplementation results in enhanced intestinal integrity and improved meat quality with better health indices in Ross 308.

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REFERENCE

Angel C, Saylor W, Vieira S and Ward N (2011). Effects of a monocomponent protease on performance and protein utilization *in*: 7-to 22-day-old broiler chickens. *Poult. Sci*. **90(10)**: 2281-2286.

Baurhoo B, Ferket P and Zhao X (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations and carcass parameters of broilers. *Poult. Sci.,* **88(11)**: 2262-2272.

Dev K, Mir NA, Biswas A, Kannoujia J, Begum J, Kant R and Mandal A (2020). Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. *Anim. Nutr.*, **6(3)**: 325-332.

Devnath R (2021). The productivity and meat quality of broiler chicken fed mushroom supplemented diet.*PhD Diss.*, chattogram veterinary and animal sciences university chattogram-4225, bangladesh, pp.51-52.

Du M, Cheng Y, Chen Y, Wang S, Zhao H, Wen C and Zhou Y (2023). Dietary supplementation with synbiotics improves growth performance, antioxidant status, immune function, and intestinal barrier function in broilers subjected to cyclic heat stress. *Environ. Sci. Pollut. Res*., **30(7)**: 18026-18038.

Erdogan Z, Erdogan S, Aslantas O & Celik S (2010). Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. *J. Anim. Physiol. Anim. Nutr*.,**94(5)**: E40-E48.

Gazani S, Rezaei M and Kazemifard M (2024). Use of protease enzyme in diets with different levels of protein and amino acids on performance, carcass characteristics, nutrient digestibility and some blood parameters concentrations in broiler chicks. *Iran. J. Anim. Sci*.,, **55(1)**: 163-180.

Giannenas I, Bonos E, Anestis V, Filioussis G, Papanastasiou DK, Bartzanas T, Papaioannou N, Tzora

A and Skoufos I (2017). Effects of protease addition and replacement of soybean meal by corn gluten meal on the growth of broilers and on the environmental performances of a broiler production system in greece. *PLOS ONE*., **12(1)**: E0169511.

Hafsan H, Kiramang K, Thaha AH and Rasyid MR (2021). Broiler farms practice without agp as an islamic conceptionin the strategy to achieve "asuh" food. *J. Islam. Sci*., **8(1)**: 29-37.

Khandale NB, Alam MS, Birla DS, Vishwas S, Gulati M, Babu MR and Singh SK (2024). Synbiotics and drug delivery: An introduction. *synbiotics in human health: biology to drug delivery.* **1st ed.**, Springer, Int publishing, Switzerland, pp. 311–318**.**

Liu S, Fang Z and Ng K (2022). Incorporating inulin and chitosan in alginate-based microspheres for targeted delivery and release of quercetin to colon. *Food Res. Int.*, **160(2022)**: 111749.

Manafi M (2015). Comparison study of a natural non-antibiotic growth promoter and a commercial probiotic on growth performance, immune response and biochemical parameters of broiler chicks. *J. Poult.Sci*., Sci., **52(4)**: 274-281.

Ogwiji M, Jatau I, Natala A, Mohany M, Al-Rejaie S and Zhu M (2024). Effect of prebiotic, probiotic, and synbiotic products on oxidative status, performance, and parasitological parameters of broiler chickens induced with cecal coccidiosis. *J. Appl. Poult.* Res., **33(4)**: 100472.

Ozaltin K, Postnikov PS, Trusova ME, Sedlarik V and Di Martino A (2019). Polysaccharides based microspheres for multiple encapsulations and simultaneous release of proteases. *Int. J. Biol. Macromol.*, **132(2019)**: 24-31.

Polidoro B, De Oliveira M, Braga F and Polycarpo G (2024). Mannan oligosaccharide as an alternative to infeed antibiotics to improve growth performance of broilers: a meta-analysis. *Br. Poult. Sci.*, 66(1):98-106.

Popova T (2017). Effect of probiotics in poultry for improving meat quality. *Curr. Opin. Food Sci.*, **14(2017)**: 72-77.

Purslow PP, Gagaoua M and Warner RDJMS (2021). Insights on meat quality from combining traditional studies and proteomics. *Meat Sci*., **174(2021)**: 108423.

Qiu H, Gao S, Hou L, Li A, Zhu LQ, Dong J and Chen F (2023). Selenium-enriched bacillus subtilis improves growth performance, antioxidant capacity, immune status and gut health of broiler chickens. *Biol. Trace Elem. Res.*, **201(12)**: 5756-5763.

Ragab R, Hassan AH, Emeash HH and Khalil F (2024). Influence of moringa oleifera leaf extract and synbiotic supplementation on mitigating stress of broilers subjected to high stocking density. *J. Adv. Vet. Res.*, **14(4)**: 696-703.

Saleh AA, Dawood MM, Badawi NA, Ebeid TA, Amber KA and Azzam MM (2020). Effect of supplemental serine-protease from bacillus licheniformis on growth performance and physiological change of broiler chickens. *J. Appl. Anim. Res.*, **48(1)**: 86-92.

Sekhon BS and Jairath S (2010). Prebiotics, probiotics and synbiotics: an overview. *J. Pharm. Educ. Res.*, 2(2010):13-36.

Shang Q (2024). Inulin alleviates inflammatory response and gut barrier dysfunction via modulating microbiota in lipopolysaccharide-challenged broilers. *Int. J. Biol. Macromol*., 282(2024):137208..

Song X, Anas MA, Kurniawati A, Hanim C, Aprianto M A, Madani AMA, Wang Q and Chen H (2023). Effects of reduced-protein diets with protease supplementation on growth, carcass yield, intestinal morphology, organ development, nutrient digestibility and blood biochemical of broiler chickens. *Transl. Anim. Sci*.,**7(1)**: Txad098.

Wang M, Wang S, Zhang C, Ma M, Yan B, Hu X, Shao T, Piao Y, Jin L and Gao J (2024). Microstructure formation and characterization of long-acting injectable microspheres: the gateway to fully controlled drug release pattern. *Int. J. Nanomed*.,19(2024): 1571-1595.

Wealleans AL, Ashour RA, Ishmais MAA, Al-Amaireh S and Gonzalez-Sanchez D (2024). Comparative effects of proteases on performance, carcass traits and gut structure of broilers fed diets reduced in protein and amino acids. *J. Anim. Sci. Technol*., **66(3)**: 457-470.

Zhao Z, Liswaniso S, Qin N, Cao S, Wu X, Ma C, Yan C, Xu R and Sun X (2024). Effects of a novel synbiotics-enzyme complex as a replacement for antibiotics on growth performance, slaughter and meat characteristics, immune organ index and intestinal morphology of broilers. *Front. Vet. Sci*., **11(2024)**: 1468847.