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Full Length Article

Combination of Probiotics and Phytase Supplementation Positively Affects Health of Japanese Quails

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Abstract

Phytase is an enzyme that breaks down phytate into inositol and inorganic phosphate. This study aimed to investigate the effects of *Bacillus cereus* and microbial phytase on numerous health measurements in Japanese quails individually and in combination. For 28 days, 200-day-old Japanese quails were divided into four groups and fed a commercial corn-based basal diet (BD) enhanced with probiotics and phytase. The results showed that the combination of probiotics and phytase supplementation positively impacted the health of quails. The birds in the supplemented groups had higher total protein and albumin levels and lower aspartate transaminase (AST) levels than the untreated group. However, the supplementation had no influence on the levels of urea, uric acid, creatinine, and alanine transaminase (ALT) in the serum of the quails. Furthermore, the serum triiodothyronine level decreased in the birds that received probiotics and the combination of probiotics and phytase, while serum glucose level increased in all supplemented groups associated with the control group. In conclusion, combining probiotics and phytase supplementation improved liver health and thyroid hormone levels in Japanese quails, increasing serum proteins.

Keywords: *Bacillus cereus*; *Coturnix japonica*; Microorganisms; Phytate; Physiological processes

Introduction

The increasing demand for protein has led to the need to enhance the production of protein sources. Japanese quails (*Coturnix japonica*) are being raised as a potential alternative broiler protein source. Quails are disease-resistant and offer a distinct meat taste (Priti and Satish 2014; Mnisi *et al.* 2021). Animal feed supplements such as enzymatic products (phytase) and living microorganisms (probiotics) enhance animal performance. Phytase is naturally found in seeds of legumes and their by-products and other feedstuffs that serve as microbial sources (Rabie and Abo El-Maaty 2015). The stomach plays a crucial role in utilizing supplemental phytase as an enzyme due to its acidic environment and high resistance to pepsin. The phytase enzyme hydrolyzes phytate into inositol and inorganic phosphate (Alam *et al.* 2020). Microbial phytase enhances the digestibility of dietary phytate phosphorus and positively influences the digestion of other nutrients in *Coturnix japonica*. However, there is a lack of evidence on the impact of phytase on bone growth and development in quails (Mansoori *et al.* 2012; Ahiwe *et al.* 2021).

Phytase supplementation in poultry diets has become increasingly common due to public concerns regarding phosphorus pollution and its ability to increase non-phytate phosphorus consumption. However, it also decreases trypsin, pepsin, and α-amylase activity. The addition of dietary phytase leads to increased bioavailability and digestibility of phytate-bound minerals such as zinc, copper, calcium and phosphorus. Furthermore, microbial phytase improves the

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digestibility of crude protein and amino acids while reducing nitrogen and phosphorus excretion (Alagawany *et al.* 2020).

Probiotics, living microorganisms, are critical in enhancing the bioavailability and digestibility of nutrient content in the gastrointestinal tract (GIT) (Gul and Alsayeqh 2023). These microorganisms are derived from various sources such as yeast, fungi and bacteria, including *Lactobacillus, Bifido bacterium, Cerus bacillus* and *Saccharomyces* (Hossain and Momu 2022; Ramaiyulis *et al.* 2023). Probiotics enhance the absorptive area of gastrointestinal tract, weight gain, reduce infection rate and stimulate immune system. Additionally, these microorganisms play a crucial role in lowering serum cholesterol levels. This decrease in cholesterol levels could be due to the assimilation or uptake of cholesterol by live microbial cells such as *Lactobacillus* or through the co-precipitation of cholesterol with de-conjugated bile salts (Abd El-Moneim *et al.* 2019; Ramaiyulis *et al.* 2023).

Several studies have demonstrated that probiotics and phytase alone positively affect the health of poultry animals (Dim *et al.* 2022). While a large amount of data is available on the separate use of these supplements, there is limited literature on their combined use, particularly in Japanese quails. This research aimed to investigate the impact of probiotics and microbial phytase, individually and in combination, on the biochemical profiles and physiological processes in *C. japonica*.

Materials and Methods

The Avian Research and Training Center at the University of Veterinary and Animal Sciences in Lahore provided 200 day-old quails (*Coturnix japonica*) for an experiment. The quails were kept in experimental sheds for 8-10 days and separated into four groups of five replicates and ten birds per replicate. For 28 days, the quails were fed a commercial corn-based basal diet (BD) supplemented with probiotics and phytase and given free access to water. Group A acted as a control and received a regular diet, whereas groups B, C and D received BD + 0.01% Microbial phytase, BD + 0.1% *Bacillus cereus* and BD + 0.01% phytase + 0.1% probiotics, respectively. The temperature and humidity were kept at appropriate levels during the experimental course. At the end of the trial, blood specimens of two birds from every replicate were collected for serum analysis and liver and kidney samples were taken and preserved at -40ºC. In addition to serum total protein, albumin, globulin, urea, creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triiodothyronine (T3), thyroxin (T4), glucose, oxidant and antioxidant levels and lipid profile (TC, TG, HDL, and LDL), oxidant and antioxidant levels were also measured in homogenized muscles and tissues (liver and kidney).

The recorded data were analyzed with Statistical Package for Social Sciences (SPSS) in one-way analysis of variance (ANOVA). The findings were reported as means, standard error of the means (SEM). The means were compared using Duncan's multiple range tests, with the significance level set at $P<0.05$.

Results

The results revealed that serum proteins, group D showed a critical rise ($P<0.05$) in total protein, while groups C and D exhibited a rise ($P<0.05$) in albumin concentration when linked to other groups (Table 1). Renal function tests (RFT) (urea, uric acid and creatinine) were non-significant ($P<0.05$) results in all treated groups (Table 1). When compared to the control group, AST levels were significantly reduced ($P<0.05$) in groups C and D, but serum ALT levels were non-significant ($P<0.05$) in all supplemented groups (Table 1). Thyroid hormones showed that T3 levels were significantly reduced in groups C and D, while T4 was non-significant in all groups (Table 1). When contrasted with the untreated group, all supplemented groups demonstrated a significant increase ($P<0.05$) in serum glucose (Table 1).

The lipid profile revealed that total cholesterol concentrations in treatment groups were not significantly different ($P>0.05$) from the control group (Fig. 1A). However, when contrasted with the untreated group, triglyceride levels were significantly lower ($P<0.05$) in all treatment groups. In contrast, serum high-density lipoprotein (HDL) levels were significantly higher ($P<0.05$) in supplemented groups C and D (Fig. 1B).

Oxidative status results showed that the activity of serum and muscle Malondialdehyde (MDA) was considerably lower ($P<0.05$) in all treatment groups when contrasted to the control group (Fig. 1C, E). Group D was found to decrease liver MDA levels when contrasted to groups B and C (Fig. 1D). The serum catalase activity was found to be considerably higher ($P<0.05$) in all treatment groups (Fig. 1F), while all treatments had a non-significant effect ($P<0.05$) on muscle catalase activity (Fig. 1H). The concentration of catalase in the liver was estimated to be significantly higher ($P<0.05$) in group D when contrasted with control and other supplemented groups (Fig. 1G).

These findings are essential in understanding the effect of probiotics and phytase supplementation on quail health and may have implications for developing dietary strategies to enhance quail health and productivity.

Discussion

The present study evaluated the potential positive effects of probiotics (*B. cereus*) and Phytase Supplementation on the health of Japanese Quails. The probiotic *B. cereus* is a vital food pathogen most commonly used in livestock and humans. Probiotics have been shown to improve the antioxidant status in birds by reducing the production of free radicals. They enhance the absorptive area of the gut, improve feed conversion ratio, support weight gain, reduce mortality and disease infection, and stimulate the immune

Table 1: Mean and SEM of different parameters in quails supplemented with basal diet, phytase, and combination

Parameters	Experimental Groups				
	Control	Microbial phytase (0.1 g/kg)	*Bacillus cereus* (1 g/kg)	Microbial phytase + *B. cereus*	P - value
Serum proteins	3.86±0.49^{c}	4.12±0.58^{c}	8.58±1.17^{b}	14.00±1.04^{a}	0.000
Serum albumins	2.28±0.16^{b}	2.78±0.45^{b}	3.60±0.19^{a}	3.13±0.17^{a}	0.006
Serum urea	30.24±0.68	32.63±0.94	34.64±2.52	30.50±2.52	0.302
Serum uric acid	12.78±0.22	13.64±0.49	13.53±0.41	13.22±0.34	0.406
Serum creatinine	6.49±1.42	5.09±1.27	5.01±1.01	8.83±1.01	0.074
Serum AST	37.12±3.21^{a}	26.20±3.01^{a}	3.27±0.63^{b}	8.73±6.55^{b}	0.000
Serum ALT	2.62±0.43	4.80±1.27	3.27±1.09	2.91±0.46	0.294
Triiodothyronine (T3)	329±16.81^{a}	330±10.09^{a}	289±10.25^{b}	269±12.54^{b}	0.003
Thyroxine (T4)	2.36±0.18	2.17±0.35	1.83±0.10	1.90±0.16	0.299
Serum glucose	293.5±25.5^{c}	397.8±71.7^{ab}	336.3±56.8^{bc}	393.4±48.3^{a}	0.001

Values represent the Mean ± SEM of experimental groups of quails
Different superscripts $^{a\text{-}c}$ indicates an essential difference between groups (P < 0.05)

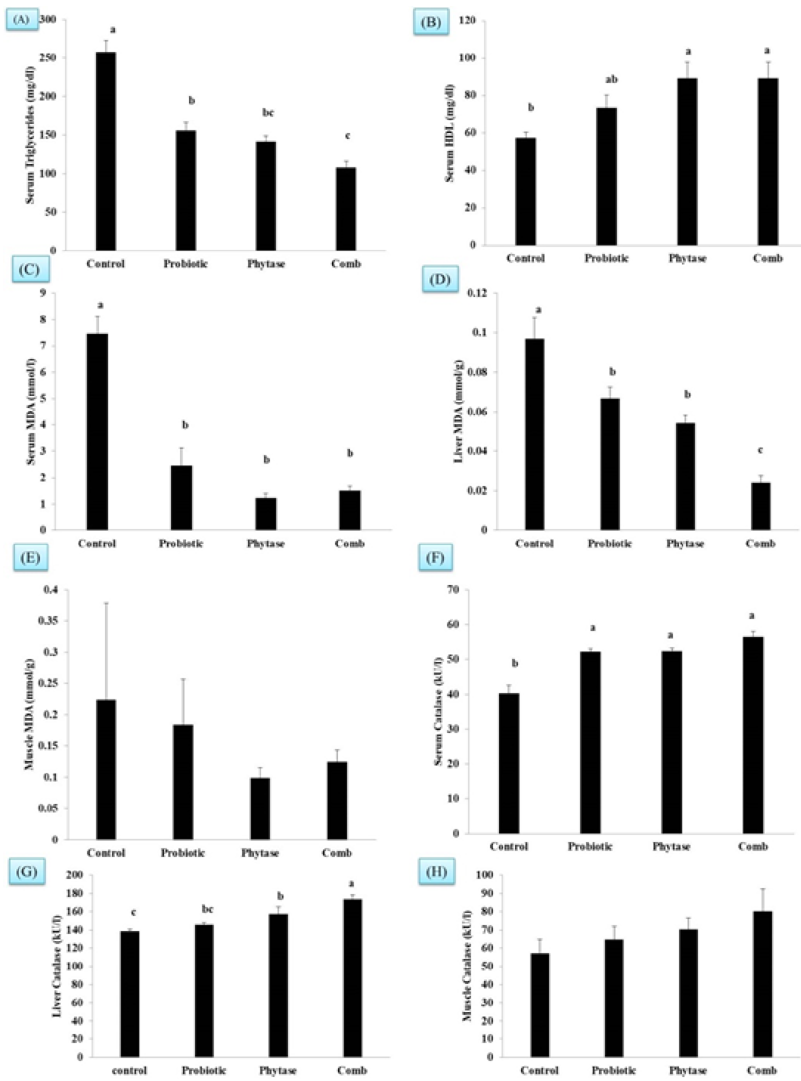

Fig. 1: Effects of phytase and probiotics supplementation on quail serum, liver, and muscle oxidative stress biomarkers and serum catalase activity. Quails in Group A served as the control (Control), Group B received BD + 0.01% Microbial phytase (Phytase), Group C received BD +0.1% Bacillus cereus probiotic), and Group D received BD + 0.01% phytase + 0.1% probiotics (comb). (A) Serum triglycerides concentration, (B) Serum HDL concentration, (C) Serum MDA concentration, (D) Liver MDA concentration, (E) Muscle MDA concentration, (F) Serum catalase concentration, (G) Liver catalase concentration, and (H) Muscle catalase concentration are presented as Mean ± SEM. Different superscripts a-c represent significant differences (P<0.05) among the groups

system (Ramlucken *et al.* 2020). This study found that Group D had significantly higher serum total proteins and albumin levels. In addition, the probiotic-supplemented group (Group C) showed a significant increase in albumin levels because the probiotic-supplemented group was given *B. cereus* (Fig. 1C–D). These findings are consistent with previous research where concentrations of albumin and serum proteins were similar to other bird species, such as ostriches, peregrine falcons, amazons and pigeons (Yalçin *et al.* 2008; Scholtz *et al.* 2009). Our research findings indicated no significant changes in RFT, including urea, uric acid and creatinine, in the serum of supplemented quails. These results are partially consistent with previous studies where globulin, total serum proteins, albumin, urea and creatinine, in addition to ALT, AST activities had no significant (p<0.05) differences among control and feed additive supplemented groups (Algedawy *et al.* 2011; Owosibo *et al.* 2013).

Results showed that serum AST levels were significantly lower in groups C and D of quails, whereas all groups had no significant effect on serum ALT levels. These results are consistent with the findings of (Sarangi *et al.* 2016). The probiotic supplementation appears to have a nurturing effect on the liver by lowering the activity of liver enzymes and mitigating the impact of stress due to decreased concentration of AST. Our results demonstrated a significant decrease in serum T3 levels in birds group C and group D. Non-significant results were found for T4 levels in all supplemented groups (Fig. 1C–D). These findings have been consistent with (Zhan *et al.* 2007). The blood glucose level was high in all supplemented groups, and the results agreed with (Shata *et al.* 2017). However, phytase responses against blood glucose are still unknown because 80% of dietary glucose is absorbed through sodium active transport systems; due to this, phytase indirectly increases blood glucose levels through effects on sodium (Cowieson *et al.* 2004).

The current study revealed that the total cholesterol concentration in serum was not considerably (P<0.05) exaggerated by treatments. These results are supported by the study conducted by (Rajput *et al.* 2013). This decrease in cholesterol levels could be due to the assimilation or uptake of cholesterol by live microbial cells such as *Lactobacillus* or through the co-precipitation of cholesterol with de-conjugated bile salts. The serum triglyceride (TG) was significantly decreased in group C with probiotics alone, supported by a study conducted by (Rajput *et al.* 2013; Behrouz *et al.* 2020).

The consequences of our study designated that the addition of phytase led to a significant increase (P<0.05) in HDL cholesterol compared to probiotic supplementation alone. However, the combination of probiotic and phytase appeared to have a synergistic effect on serum HDL cholesterol, leading to a significant increase (P < 0.05). The results of (Priyodip *et al.* 2017) research showed similar results. Our study demonstrated that the novel combination of phytase and probiotic as a feed supplement resulted in a significant (P<0.05) increase in HDL and a decrease in TG (P<0.05) but had no critical effect on TC. These findings align with our expectations, as both phytase and probiotics are known to reduce bad cholesterol, and the combination of the two was more effective in achieving this.

The present study on quails showed an effective decrease in the activity of liver MDA in all supplemented groups, which corroborated the research results of (Arendt and Allard 2011; Chalasani *et al.* 2012; Rozman 2014). The observed decrease in liver MDA's activity is due to decreased oxidative stress, and decreased liver MDA's activity is beneficial because MDA is an indicator of lipid peroxidation. In the present study, serum catalase activity was higher in all treated groups in broiler chicks (Aluwong *et al.* 2012). Our study found that the liver level of catalase was considerably higher (P<0.05) in group D contrasted with the untreated group and other supplemented groups. As a novel feed supplement, the combination of phytase and probiotics significantly (P<0.05) boosted the catalase activity and reduced (P<0.05) MDA in serum and liver tissue in all the supplemented groups. These findings are according to our expectations, as both phytase and probiotics are responsible for decreasing oxidative stress.

Conclusion

Probiotics and phytase, alone and in combination, have positive effects on the biochemical profiles and physiological processes of Japanese quails. The supplementation of probiotics and phytase improved liver health and thyroid hormones, preventing energy loss by storing glucose in glycogen. The combination of probiotics and phytase was more effective than either supplementation alone. However, additional study is required to determine the mechanisms underlying these impacts and evaluate the long-term impact of supplementation on other physiological and immunological parameters.

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Author Contributions

CN, MB and MA performed material preparation, data collection, and analysis. The original draft of the manuscript was written by CN & MA. RS, AH, UM, and HM completed the revision and editing of the manuscript. MA reviewed, edited and approves the final version of manuscript. SAF provided valuable supervision.

Conflict of Interest

The authors declare no conflict of interest among them.

Data Availability

The datasets generated and analyzed during this study are included in this published article.

Ethics Approval

All the procedures adopted to perform this experiment were adopted by the ethical review committee of the University of Veterinary and Animal Sciences, Pakistan

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