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ASSESSMENT OF HISTO-PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN QUAILS (*COTURNIX COTURNIX*) SUPPLEMENTED BY ZINC AND PROTEXIN REARED UNDER HEAT STRESS

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Abstract

This study examined the effects of zinc and protexin supplementation on quails exposed to heat stress (HS). It was revealed that protexin and zinc positively influenced the metabolic parameters of heat-stressed quails, enhancing their overall health and immunity. A total number of one hundred thirty-five (n=135) quails were selected and equally divided into nine groups with each group having 3 replicates (n=5). These groups were designated as T_0, T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8 in which T_0 was set as control receiving only basal diet. T_1 and T_2 groups were supplemented with 100mg/kg of zinc+protexin and 150mg/kg of zinc+protexin respectively, without HS. The groups T_3, T_4, T_5 and T_6 were exposed to HS and supplemented 100mg/kg zinc, 150mg/kg zinc, 100mg/kg zinc+protexin and 150mg/kg zinc+protexin respectively. While T_7 was supplemented with protexin only and T_8 was kept only under HS without any supplementation. Results revealed that the birds of HS group had a reduced spleen weight 33.33%, bursa of fabricius 1.56%, liver weight 3.51%, villus width of the jejunum 0.11%, villus length of the jejunum 0.44%, villus length of the ileum 0.35%, Aspartate Aminotransferase (AST) 4.12%, glucose 3.26%, total protein 2.44% and albumin 0.30% concentrations compared to control. Whereas, total cholesterol 1.95%, triglyceride 2.67%, Alanine Aminotransferase (ALT) 10.45%, goblet cell of duodenum 6.75%, goblet cell of jejunum 4.47%, goblet cell of ileum 8.78%, villus width of the duodenum 2.24%, villus length of the duodenum 0.18%, villus width of the ileum 0.22%, intestinal length 10.29% were increased in heat stressed birds as compared to control. Zinc and protexin supplementation reduced the negative effects of heat stress (HS) by increasing spleen weight, glucose, albumin and total protein AST concentrations. The ALT, intestinal length, triglyceride and cholesterol level came back to the normal level with supplementation of zinc and protexin. It was observed that zinc either alone or in combination with protexin mitigated the effects of heat stress on the histo-physiological and biochemical parameters of quails.

Key words: Heat stress, Zinc, protexin, growth performance, biochemical analysis, histo-physiology.

Introduction

The Japanese quail (*Coturnix coturnix*) is reared for both meat and egg production due to its rapid growth rate and high reproductive potential (Ofori, 2020). Quail farming is considered promising for the growth of economy within country as it offers a viable source of income because it provides food security, income generation and alleviation of poverty (Kinyua, 2022). Heat affects general health of quails and has a detrimental effect on their welfare, productivity and reproductive function. It causes discomfort to bird and negatively influences production parameters (Ahmad et al., 2022). Quails exposed to heat stress have been reported to undergo damages in its liver, kidneys and testicles altering cellular functions which result in lung congestion and neuronal degeneration (Abdulkadir & Reddy, 2023). Heat can also produce detrimental effects on the quality of eggs, bones and quail productivity (Cruvinel et al., 2021). It has been observed that there was significant effect of heat on the welfare of quails by reducing reproductive performance and compromising product quality (Batool et al., 2023). Hatched quails from heat-stressed mothers when exposed to additional heat stress during rearing exhibited reduced weight gain, a greater feed conversion ratio and higher levels of protein oxidation (Santana et al., 2021). Heat stress can alter the intestinal microbiota of quails and reduces their growth (Qin et al., 2024). Quails that are subjected to prolonged heat stress experienced changes in blood parameters, including oxygen transport, electrolytes and acid-base balance. Initial physiological alterations have been reported in acute heat stress but no detrimental effects were noted (Truong et al., 2023). However, maintaining optimal production can be challenging, especially under environmental stressors like heat stress, which is increasing rapidly in most of the Asian countries because it disrupts physiological processes in birds leading to reduced feed intake, decrease in growth performance and less egg production (Nawaz et al., 2021). Zinc is an essential component of feed being crucial for appropriate growth and productivity in birds. It also improves immunity, appetite and feather creation (Alagawany et al., 2021). Zinc nanoparticles supplementation in quails develops faster and have better liver metabolism as well as can lower cholesterol levels. Moreover, this supplementation enhances immunity, supports useful gut flora and increases antioxidant activity (Reda et al., 2021). It has been reported that productivity and egg quality of Japanese quail greatly increased by supplementation of *moringa oleifera* seed powder (Abou-Elkhair et al., 2020). Protexin supplementation results in decreased lipid peroxidation and enhanced growth performance in Japanese quails as the number of useful bacteria in their stomach increased by this supplementation (Hazrati et al., 2020). As heat stress negatively impacts egg-laying quails leading to increased oxidative stress, gut health issues and gene expression changes so it has been noted that *spirulina platensis* (SP) can mitigate these effects in this regard by reducing E. coli bacteria, blood malondialdehyde levels and heterophil/lymphocyte ratio (Hajati et al., 2020). Previously significant effect of Zn as trace mineral has been found in improving the biochemical activities of poultry being fed under heat stressed environment and similarly protexin has been also found as potential probiotic (Alagawany et al., 2024). The purpose of this study was also to explore the potential of combat impacts of zinc and protexin for mitigating the heat under prevailing conditions of high temperature in Pakistan as well as behavior of quails towards these supplements.

MATERIALS AND METHODS

Experimental setup

The experiment was conducted in an environmentally controlled shed and examination of parameters was executed in the laboratory of Department of Environmental Science and Department of Bio-Sciences at Bahauddin Zakariya University (BZU) Multan. In this experiment, seven days old, one hundred thirty-five quails *(Coturnix coturnix)* were purchased from a local poultry breeder with an average body weight of 30.03 ± 0.10g. The experiment continued for 55 days. During the experiment, birds had free access to water. However, the feed intake was monitored on daily basis. Initially, a proper hygienical environment having a thermo-neutral temperature of 35℃ for twenty-one days was given while humidity level was adjusted to 65% to 70%.

Experimental design and treatment

On twenty second day of feeding, birds with equal body weights were randomly distributed into nine groups (T_0, T_1, T_2, T_3, T_4, T_5, T_6, T_7, and T_8) with each group having 15 quails. All groups were fed with basal diet on daily basis while control group (T_0) was kept in a thermo neutral environment (TNE at 35°C) and remaining groups were subjected to supplementation of zinc and protexin with heat stress for 12 hours each day at 37°C. The groups with treatments were divided as:

Group 1 = only basal diet (no supplement) as T_0
Group 2 = Basal diet with 100mg/kg of zinc + protexin as T_1
Group 3 = Basal diet +150mg/kg of zinc + protexin as T_2
Group 4 = Basal diet with 100mg/kg zinc heat of 37℃ as T_3
Group 5 = Basal diet + 150mg/kg zinc + heat of 37℃ as T_4
Group 6 = Basal diet + 100mg/kg zinc + protexin + heat of 37℃ as T_5
Group 7 = Basal diet +150mg/kg zinc + protexin + heat of 37℃ as T_6
Group 8 = Basal diet + protexin as T_7
Group 9 = Basal diet + 37℃ heat only as T_8

The experimental diets were formulated to contain all nutrients recommended by the National Research Council (NRC, 1994) for growing birds. The basal feed used in the experiment and its composition is shown in Table 1. The percentage composition of the ingredients for basal diet was formulated in line with those previously used by (Ojediran et al., 2022; Cakir et al., 2008).

Table 1. Percentage composition of ingredients for basal diet

Ingredients	Composition (g/kg)
Corn/ Wheat	54.0
Soybean meal	33.0
Vitamin and mineral premix	0.30
Dicalcium Phosphate	0.5
Vegetable Oil	4.0
Salt	0.4
Fish meal	4.0
Bone meal	2.50
Lysine %	0.10
Dl-methionine	0.20
Lime Stone	1.0

Analysis of selected parameters

Samples were analyzed by slaughtering the quails that were not fed by 12 hours and sampling was done randomly by taking three uniformed birds in each group. About 15ml of blood samples were taken in heparinized tubes from the jugular veins of the birds and serum was stored till further utilization (SALAKO, 2018). The growth and histo-physiological parameters such as liver weight (gm), spleen weight (gm), bursa of Fabricius weight (gm) were measured by using electric beam balance. About 3cm long small intestinal segments from midpoints of duodenum (segment encompassing the duodenal loop), jejunum (segment between duodenum and ileum) and ileum (distal segment before the ileocecal junction equaling the length of caecum) were taken and fixed in 10% neutral buffered formalin. Segments were then embedded in paraffin, stained by haematoxylin and eosin, and observed under microscope (Labomed, USA) at 4X. Measurements were made by using a commercial morphometric program (Prog Res 2.1.1). Villus length (VL) was measured from the tip of the villus to villus crypt junction. The villus width (VW) was measured from the base, middle, tip of villus and the average of the results was considered as VW (Saleem et al., 2018). The biochemical parameters such as Aspartate Aminotransferase (AST) (U/L), Alanine Aminotransferase (ALT), glucose(mg/dL), triglycerides (mg/dL), total cholesterol (mg/dL), triglyceride (mg/dL) and total protein (mg/dL) were measured by Diasis Diagnostica (Germany) (AOAC, 1925). The serum albumin and glucose were measured by using glucose oxidase/peroxidase method commonly known as GOD/POP method and albumin using the bromocresolgreen method (BCG) (Patra et al., 2019). The data was analyzed in MS Excel 2010 for calculating the mean and standard error while significant difference between the treatments was considered $P < 0.05$.

Results

Organ's Weight

Zinc and protexin produced significant impacts on the weight of spleen, liver and bursa of fabricius of quails. Weight of spleen in group T_1 and T_2 exhibited a significant decrease of 31.74% & 36.50% whereas, T_3 to T_8 displayed further reduction. The weights of liver and Bursa of fabricius were significantly changed by zinc and protexin under heat stress. The dietary supplementations used in the study exhibited great influence in overcoming the negative effects of stress. Liver weight of only heat stressed group showed slight decrease of 3.5% compared to rest of the groups. Moreover, it was observed that partial recovery of 6.68% & 13.59% was found in T_3 and T_4 respectively, after treating with Zn as compared to T_8. Whereas, T_5 and T_6 exhibited more pronounced recovery of 18.17% and 20.86% as compared to T_3, T_4, and T_8. Furthermore, only T_7 showed the increase of 24.85% in liver weight. Zinc and protexin together in T_1 and T_2 led to a slight increase in bursa weight of about 9.37% & 12.51% respectively. However, in stressed groups T_3, T_4, T_5, and T_6 showed reduction in bursa weight of quails. This decrease was most severe in T_7 near to 48.43% whereas, T_8 had a minimal impact of 1.56% compared to the rest of the groups at $P < 0.05$ as shown in Table.2.

Intestinal Goblet Cells Count

Shown in Table 2, in duodenum, only protexin supplementation caused a significant decrease in goblet cells by 32.77% while stressed group (T_8) had significant increase of about 6.75% as compared to control. All other groups from T_1 to T_6 showed decrease in goblet cells. T_1 and T_2 also showed increase in goblet cells by 4.58% and 6.87% respectively. The combination of Zn and protexin in T_5 and T_6 resulted in a decrease of 5.11% & 6.60% respectively compared to control group. Protoxins only applied in T_7 showed a minor negative effect while heat stress (T_8) led to a significant increase in goblet cells. Similar trends were observed in ileum. Zn and Protexin supplementation increased goblet cells, whereas heat stress caused reduction in cell amount. The combination of heat stress, protexin and Zn in T_5 and T_6 resulted in a decrease of about 2.48% to 4.80% compared to control and rest of the Zn-supplemented groups. Whereas, heat stress (T_8) caused increase of (8.78%) goblet cells within the ileum compared to the rest of the groups.

Intestinal Morphology

The results demonstrated that villus width of quail intestine changed by the application of stress, protexin and Zn as shown in Table.2. In jejunum, the normal villus width was found (99.43±0.30) in control group (T_0). Supplementation with protexin and Zn (T_1, T_2) showed slight increase of 0.38%

to 1.50% in villus width compared to control. While other groups from T_3 to T_8 demonstrated a significant decrease as compared to T_0, T_1 and T_2. Regarding duodenum and ileum a different pattern was observed as Zn supplementation under heat stress in T_1 and T_2 led to a non-significant increase in villus width (1.56% and 3.08% in duodenum, 0.71% and 1.33% in ileum) respectively. However, in all other groups (T_3-T_6, T_8) a significant increase in villus width of duodenum and ileum was observed compared to T_0, T_1. The most significant increase (14.23% and 7.29%) was observed in the villus width in the duodenum and ileum of T_7 group compared to rest of the groups. The villus length of duodenum, jejunum and ileum showed prominent fluctuations under supplementation and stress. The supplemented groups T_1 and T_2 led to slight decrease in villus length of T_1 and T_2 (35.75% to 36.73% in jejunum, 22.52% to 23.38% in ileum) compared to control. Addition of Zn and protexin under stress showed a greater decrease (48.14% & 50.04% in jejunum, 37.55% & 39.40% in ileum) in T_3 and T_4. Protexin supplementation (T_7) slightly decreased (34.18% in jejunum, 16.95% in ileum) compared to control. A minor decrease (0.44% in jejunum, 0.35% in ileum) was seen in stressed group (T_8) compared to control group. Slight fluctuations in villus length of duodenum in T_1 showed increases of about 0.02% while in T_2 slight decreases of about 0.07% was observed as compared to control. Conversely, Zn and protexin under heat stressed condition led to significant decrease (19.18% in T_3 & 24.52% in T_4) as compared to control. However, moderate decrease (19.30%) was seen in T_7 while heat stress in T_8 caused a significant increase of 0.18% as compared to control and rest of the groups at $P < 0.05$.

Serum's biochemistry

The effects of zinc and protexin supplementation on the biochemical parameters of quails under heat stress are shown in Table 3. Albumin, cholesterol, triglycerides and total protein levels were found to be in normal range however, Zn and protexin supplementation resulted in slight increases in albumin (1.84% & 0.30%), total protein (2.55% & 5.51%), triglycerides (9.62% & 10.59%) and cholesterol (3.23% to 4.39%) in T_1 and T_2 respectively. The groups (T_3 to T_8) demonstrated significant decrease in albumin (28.19% to 37.28%) and total protein (28.36% to 33.87%) at $P < 0.05$ whereas, triglycerides and cholesterol levels significantly increased. In heat stressed group triglycerides and cholesterol significantly increased compared to T_0. Supplementation of Zn and protexin to quails under heat stress showed significant effect on glucose and AST levels. In T_1 and T_2 glucose (1.91% to 2.69%) and AST (31.18% to 4.72%) increased as compared to control. However, all supplemented and heat stressed groups showed continuous decreases in glucose and AST levels. The normal range of ALT in quails was found 9.15±0.17 in control group while T_1 and T_2 demonstrated slight increase in AST levels whereas, T_3 and T_4 moderately increased these levels (11.90% & 14.42% respectively). However, T_7 and T_8 exhibited a significant increase of 15.25% & 10.45% respectively.

Table 2: Histo-physiological parameters of quails *(Coturnix coturnix)* fed with different levels Zn and protexins under heat stress (mean±SE, p≥0.001)

Parame	Treatment Groups								
	Control	100mg/kg Zn+Probiotics	150mg/kg Zn+Probiotics	100mg/kg Zn+heat 37ºC	150mg/kg Zn+heat 37ºC	100mg/kg Zn+probiotic+heat 37ºC	150mg/kg Zn+probiotic+heat 37ºC	Probiotic	Heat 37ºC
Liver Weight (gm)	2.84±0.16	3.13±0.16	3.26±0.12	3.03±0.30	3.23±0.22	3.36±0.21	3.44±0.16	3.55±0.19	2.74±0.15
Spleen Weight (gm)	0.21±0.05	0.14±0.04	0.13±0.04	0.07±0.03	0.09±0.03	0.11±0.04	0.11±0.03	0.06±0.02	0.14±0.04
Bursa of Fabricius Weight (gm)	0.21±0.07	0.23± 0.03	0.24±0.05	0.14±0.03	0.15±0.09	0.18±0.03	0.16±0.05	0.11±0.03	0.10±0.-2

Goblet Cells of Duodenum (μm)	3.95±0.08	3.84±0.12	3.94±0.12	3.76±0.17	3.35±0.18	3.55±0.04	3.94±0.14	2.65±0.21	4.21±0.31
Goblet Cell of Jejunum (per villus)	6.25±0.17	6.54±0.18	6.68±0.16	5.84±0.31	5.93±0.04	6.04±0.14	6.15±0.15	5.75±0.19	6.53±0.016
Goblet Cell of Ileum (per villus)	8.04±0.14	8.45±0.16	8.55±0.03	7.45±0.32	7.55±0.43	7.65±0.24	7.84±0.23	7.34±0.13	8.75±0.21
Villus Width of Duodenum (per villus)	132.65±0.31	134.73±0.30	136.75±0.32	147.04±0.03	138.03±0.12	136.3 ±0.32	134.7±0.13	151.54±0.24	135.63±0.23
Villus Width of Jejunum (μm)	99.43±0.30	99.81±0.29	100.93±0.15	99.17±0.22	99.23±0.45	95.04±0.20	95.94±0.32	96.04±0.24	99.32±0.28
Villus Width of Ileum (μm)	96.04±0.21	96.73±0.20	97.33±0.39	99.74±0.09	100.33±0.08	98.25±0.19	99.05±0.34	103.05±0.21	96.26±0.43
Villus Length of Duodenum (μm)	1152.64±0.19	1152.95±0.08	1151.73±0.17	872.75±0.31	869.97±0.39	929.14±0.16	931.45±0.32	930.15±0.23	1154.76±0.28
Villus Length of Jejunum (μm)	490.55±0.36	485.34±0.20	481.75±0.31	315.18±0.24	310.33±0.22	254.35±0.29	245.05±0.19	322.85±0.32	488.35±0.24
Villus Length of Ileum (μm)	395.14±0.21	391.87±0.15	387.36±0.17	306.14±0.22	302.74±0.31	246.76±0.19	239.45±0.25	328.14±0.21	393.74±0.21

Table 3: Biochemical parameters of quails *(Coturnix coturnix)* fed with different levels Zn and protexins under heat stress (mean±SE, p≤0.05)

Parameters	**Treatment Groups**								
	Control	**100mg/kg Zn+Probiotics**	**150mg/kg Zn+Probiotics**	**100mg/kg Zn+heat 37ºC**	**150mg/kg Zn+heat 37ºC**	**100mg/kg Zn+probiotic+ heat 37ºC**	**150mg/kg Zn+probiotic+ heat 37ºC**	**Probiotic**	**Heat 37ºC**
Albumin (g/dL)	2.16±0.13	2.20±0.10	2.21±0.16	1.46±0.12	1.55±0.12	1.76±0.11	1.86±0.16	1.36±0.06	1.34±0.25
Total Protein (g/dL)	3.27±0.05	3.35±0.14	3.45±0.24	2.26±0.13	2.34±0.20	2.75±0.14	2.95±0.03	2.16±0.21	2.98±0.17
AST (U/L)	169.35±0.18	174.75±0.12	177.36±0.37	170.05±0.32	166.85±0.28	163.15±0.24	162.05±0.28	176.75±0.29	162.36±0.31
ALT (U/L)	9.15±0.17	9.24±0.12	9.25±0.23	10.47±0.31	10.24±0.24	10.15±0.18	10.05±0.11	10.55±0.28	10.11±0.021
Glucose (mg/dL)	271.53±0.34	276.74±0.26	278.84±0.33	182.20±0.21	184.70±0.29	198.36±0.14	215.75±0.33	162.37±0.24	262.67±0.21

Triglyceride (mg/dL)	218.16±0.23	239.15±0.30	241.27±0.21	276.16±0.17	263.86±0.19	256.16±0.12	241.47±0.33	289.55±0.21	223.98±0.29
Cholesterol (mg/dL)	205.63±0.31	212.29±0.19	214.67±0.19	246.36±0.32	236.49±0.38	228.53±0.27	220.54±0.22	259.61±0.30	209.65±0.21

Discussion

In this study heat stress negatively affected the performance of quails while zinc and protexin have been seen as a beneficial agents for reducing the harmful effects of stress as zinc and protexin in combination increased goblet cell number in jejunum and ileum of quails approaching to the findings of Sandikci et al. (2004) in which similar effects were observed when *Saccharomyces cerevisiae* and Zn bacitracin were added to the basal diet of quails. Results have revealed that number of goblet cells within the villi had increased under stressed in duodenum, jejunum and ileum relating to study of (Leiper et al., 2001) in which mucin production and secretion are stimulated by bacterial peptides released by nonpathogenic bacteria in the colon mucosa. The decrease in goblet cell count of the quails under heat stressed conditions can be explained as a result of disruption in the microfloral balance caused by corticosteroids. Long-term stress induced in quails resulted in shorter intestinal segments which looks similar to Sandikci et al, (2004) who reported that villus width of jejunum significantly decreased due to high temperature. In previous study conducted by (Shamoto et al., 2000), feed intake decreased in heat stressed chickens validating results of present study in which heat stress caused the similar changes in quails. While the results of Bobek et al. (1980) also agrees with the findings of present study showing that heat stressed birds have higher body temperature than the normal birds which resulted reduction in the intake of daily feed. The current study also demonstrated the similar results as already conducted by by Ciftc et al, (2005) that the quails under stressed environment had considerably higher body temperature which caused the reduction in body weight. According to study, the failure of thermoregulation process in heat stressed group lowered the jejunal villus width which might have been caused by the exergonic reactions of free radicals generated due to heat stress as it was found that stress has raised total protein levels of the birds. Similarly results are in line with the findings of Yardibi et al, (2009), who also observed that heat stressed slightly decreased the blood total protein level in laying hens. It has been documented that vitamin E supplementation considerably raised the blood total protein level in broilers (Sahin and Kucuk, 2001), Japanese quails (Sahin as al., 2006) and laying hens (Ajakaiye et al., 2010) when exposed to heat stress. Vitamin E has been reported to play a significant role in decreasing adrenocorticotropic hormone in blood and increase protein levels in heat stressed birds (Sahin et al. 2003). In contrast to our findings El-Damrawy et al, (2013) found that chickens treated with powdered olive leaf significantly increased albumin level of birds which might be due to the fact that the birds of that study comparison to our study were not given heat stress. However, a previous study found that when three different organic extracts added to broilers grown under heat stress did not significantly change albumin levels (Akbarian et al., 2014). The current results demonstrate that heat stress reduced plasma glucose levels ($P \leq 0.05$) which is consistent with the findings of Sarica et al, (2017) who reported that dietary antioxidant supplementation and stress reduced glucose level. Sahin et al. (2003) found that vitamin C & E supplementation significantly decreased blood glucose levels in heat stressed birds which is comparable to the findings of the present. The results of this study about cholesterol and triglyceride levels are very similar with the findings of Hosseini-Mansoub et al, (2010) and Tawfeek et al, (2014). They observed that heat stress significantly increased serum cholesterol and triglyceride in broilers. The results exhibited an increase in blood lipids under heat stress which is comparable to the findings of Rashidi et al, (2010) in which they demonstrated that high temperature can breaks down body lipids, which raises plasma cholesterol and triglyceride levels but according to Ajakaiye et al. (2010) heat stress produced no significant effect on the blood cholesterol level of laying hens in the presence of prebiotic

Conclusion

As both zinc and protexin in single as well as in combination, not only produced significant effects in increasing the growth, biochemical and physiological functions of quails but also helped in sustaining against the negative impacts of heat stress. Hence, it can be concluded that although both have individual beneficial impacts, but if applied together, can be helpful in mitigating the effects of heat stress under the prevailing situation of high temperature.

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