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Adaptation to Heat Stress in Broilers Using Dried Tomato Pomace and Zinc: Effects on Growth Performance, Oxidative Stress, Intestinal Features and Humoral Immunity

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Key words
Antioxidant, Broiler, Dried tomato pomace, Heat stress, Zinc

ABSTRACT

Dried tomato pomace (DTP) is a low-cost and nutritionally beneficial byproduct of tomato processing that is obtained during the production of tomato paste. This research aimed to assess the impact of Zinc (Zn) and DTP supplementation on the growth, carcass quality, blood metabolites, intestinal histology and antibody levels in broiler chickens exposed to heat stress. A total of 625 Hubbard broilers were distributed among 25 floor pens, with 25 birds per pen. The birds subjected to heat stress (HS) were provided with a basal diet (control), Zn at a concentration of 50 mg/kg (Zn-50), and two levels of DTP at rates of 10g/kg (DTP-10) and 20g/kg (DTP-20) of feed over a span of 35 days. The results demonstrated significantly higher (P<0.01) total feed consumption, weight gain, and feed conversion ratio for both the DTP-10 and DTP-20 groups as compared to the control group. Additionally, the dressing percentage was also notably higher (P<0.01) in these supplemented groups. Notably, the supplementation of DTP-10 and DTP-20 led to a reduction (P<0.01) in the levels of MDA (malondialdehyde) in the broilers experiencing heat stress. In conclusion, the findings of this study suggest that supplementation with DTP-10 and DTP-20 significantly improved growth performance, lowered MDA, and strengthened humoral immunity in thermal stressed broilers.

INTRODUCTION

Heat stress is regarded as a severe problem with significant physiological implications for animal performance (Khan *et al*., 2012; Bai *et al*., 2023). Lack of sweat glands birds are compelled to rely on open mouth respiration, which can result in terrible physiological disequilibrium (Khan *et al*., 2011, 2023; Chand *et al*., 2018; Ahmad *et al*., 2020; Hafeez *et al*., 2021). To minimize chicken heat stress, a multidisciplinary approach is used to adjust the environment, ventilation system, and nutritional management (Khan *et al*., 2021). Vitamins, minerals, and phytochemicals help in order to mitigate the adverse impacts of heat stress (Zia *et al*., 2017; Sultan *et al*., 2018; Shah *et al*., 2023).

Several studies have recognized medicinal herbs as agents that promote growth in broiler chickens (Khan *et al*., 2012; Ahmad *et al*., 2020). Medicinal herbs can

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be used safely in animal and poultry feeds because they are natural products (Khan *et al*., 2012). Botanicals and their components can assist broiler chicks in growing and remaining healthy without changing their blood profile or generating any visible pathological concerns. This suggests that botanicals are efficient growth promoters that can be substituted for antibiotics (Haq *et al*., 2020).

Zinc (Zn) is a mineral that is used to combat the effects of environmental stress (Shah *et al*., 2019). Because Zn is a cofactor for over 200 enzymes, it plays a variety of critical activities. Its presence in the antioxidant defense system is one of their main roles. Free radicals cause oxidative damage to the cell membrane during zinc deficiency (Naz *et al*., 2016), affecting antioxidant enzymes and substances. It is thought that the antioxidant role of Zn is linked with higher production of metallothionein that act as antioxidant agent (Chand *et al*., 2020).

The seeds make up 44% of dry tomato pomace (DTP), while the pulp and flesh make up 56%. Seed includes 35.1 % total dietary fibre (TDF), 22.2-33.9 % crude protein (CP), 20.5-29.5 % fat, and 3.9-9.6 % ash, making it an important component of tomato pomace (Silva *et al*., 2019; Szabo *et al*., 2019; Concha-Meyer *et al*., 2020). According to an estimate, one g includes 4.5 % fat, 11 % CP, 0.13 mg of carotene, 0.8 mg of lycopene, and 1.73 mg of vitamin C (Sahin *et al*., 2008). Lycopene, folate, tocopherols, vitamin C, flavonoids, and phenolics are all antioxidants found in tomato pomace (Sahin *et al*., 2008; Selim *et al*., 2013; Khan *et al*., 2022). Supplementation at a dosage rate of 1% has been found to improve antioxidant potential and lower malondialdehyde (MDA) concentration in broilers grown at high temperatures (Selim *et al*., 2013). Supplementation of DTP was found to boost growth, immunity, and antioxidant status in many experiments in broilers (Sahin *et al*., 2008; Abdel-Baset *et al*., 2009; Palozza *et al*., 2011; Yitbarek, 2013; Hosseini-Vashan *et al*., 2016). The impact of DTP on broilers under heat stress has only been studied in a few research reports. Consequently, the goal of this study was to compare DTP and Zn under heat stress to see how it affected growth, redox balance, intestinal histology and immunological response in broilers.

MATERIALS AND METHODS

Preparation of dried tomato pomace powder (DTPP)

Fresh cleaned tomatoes were chopped into small pieces and dried under the shed for two weeks at room temperature and then milled into fine powder.

Animals husbandry and experimental protocol

A total of 625 male Hubbard broiler chicks, each only one day old, was methodically divided into 25 separate floor pens, accommodating 25 birds within each pen. Wood shavings were employed as the bedding material for these pens. To ensure proper sustenance, every pen was equipped with plastic feeders and water dispensers. Throughout the course of the trial, the broiler chickens were granted unrestricted access to both feed and water. Routine vaccinations were administered to safeguard against various diseases, including the New Castle disease virus. The broiler chickens were subjected to continuous illumination lasting 23 h each day. Furthermore, the thermal stress regimen, delineated in Table I, was imposed on the broiler chickens. The fundamental composition of the feed is outlined in Table II.

Table I. Thermal stress conditions during the experimental period.

H	Relative humidity (%)	Temperature (°C)
08:00	82.32	34.72
12:00	94.16	35.21
16:00	70.85	38.57
20:00	69.58	35.63
24:00	75.27	33.71
04:00	77.32	31.54

Table II. Composition of starter and finisher diets and their chemical composition as fed basis.

Ingredients (%)	Starter phase (1-21 days)	Finisher phase (22-35 days)	Dried tomato pomace
Corn	56.2	55.00	
Soybean meal (44%)	29.5	26.5	
Canola meal	5.95	5.41	
Sunflower meal (28%)	3.5	3.9	
Vegetable oil	2.15	2.2	
Molasses	1.5	1.00	
Dicalcium phosphate	1.8	1.95	
Limestone	1.10	1.10	
NaCl	0.01	0.01	
$NaHCO_3$	0.02	0.01	
DL-Methionine	0.15	0.12	
Lysine-HCl	0.21	0.33	
Vitamins minerals premix[1]	0.3	0.27	
Chemical composition			
ME (kcal/kg)	2995	3000	
Crude protein (%)	22.5	22.30	
Crude protein (%)			22.67
Crude fibre (%)			8.75
Crude fats (%)			8.34

Table III. Feed intake, weight gain and feed conversion ratio of broilers under thermal stress and supplemented with dried tomato pomace.

Treat-ments	Feed intake (kg)			Weight gain (kg)			Feed conversion ratio (kg/kg)		
	Starter phase	Finisher phase	Overall	Starter phase	Finisher phase	Overall	Starter phase	Finisher phase	Overall
Control	1.29±0.01c	1.75±0.01c	2.84±0.01c	0.65±0.01d	1.07±0.01c	1.72±0.01d	1.83±0.01a	1.98±0.01a	1.65±0.01a
Zn-50	1.26±0.01b	1.82±0.01b	2.95±0.01b	0.69±0.01c	1.11±0.01b	1.82±0.01c	1.82±0.01a	1.63±0.01b	1.62±0.01b
DTP-10	1.35±0.01a	1.96±0.01a	3.15±0.01a	0.72±0.01b	1.19±0.01a	1.97±0.01b	1.80±0.01b	1.64±0.0b	1.59±0.01b
DTP-20	1.36±0.01a	1.99±0.01a	3.09±0.01a	0.85±0.01a	1.20±0.01a	2.02±0.01a	1.60±0.01c	1.65±0.01b	1.52±0.01c
P value	0.0001	0.0012	0.0001	0.0001	0.001	0.001	0.001	0.0001	0.0001

Mean values in the same column with dissimilar superscript are statistically significant (P<0.05). Zn-50, Zn at the rate of 50 mg/kg; DTP-10 and DTP-20 indicate the supplementation of dried tomato pomace at the rate of 10 and 20 g/kg, respectively.

Experimental protocols and samplings

Four diets consisting of a control, and two levels of dried tomato pomace (DTP) at the rate of 10 (DTP-10) and 20 (DTP-20) and zinc (Zn-50) at the dose of 50 mg/kg of feed were included and allocated in a completely randomized design for 35 days, except the first week. The supplements were well mixed in the experimental diets. Feed intake was assessed on daily basis while body weight gain and feed conversion ratio (FCR) were assessed on a weekly basis. Three birds per pen were taken and killed on day 35. Dressing percentage was determined for individual birds and pooled on pen basis. Weight of spleen, bursa, and thymus were taken with help of sensitive scale. Blood samples of 3 ml was aseptically taken from the slaughtered birds, centrifuged (3000 rpm for 10 minutes) resulting in the separation of serum, which was stored at -80°C until it analysis.

Serum MD and antibody titre against New Castle disease (NDV)

The thiobarbituric acid (TBA) reaction was used to determine MDA in serum samples as published by Ohkawa *et al*. (1979). The colour generated was spectrophotometrically quantified at 532nm (IRMECO Model U2020). A haemagglutination inhibition (HI) test was conducted to find the humoral response against the ND virus.

Intestinal histology

Two birds were chosen from each pen, subsequently slaughtered, and their ilium were handled under aseptic conditions for the purpose of conducting histological examinations. To accomplish this, sections measuring 1 cm^2 were meticulously excised from the ilium and subsequently placed in a solution of 10% buffered formalin. Following this, the tissue samples underwent dehydration using a freshly prepared alcoholic solution. Utilizing a microtome, the tissue blocks were carefully sliced and then subjected to H & E staining. Ultimately, the stained sections were thoroughly examined under a microscope at a magnification of 40x.

Statistical analysis

Data were analysed using statistical analysis software (Statistix 8.1) in a Completely Randomized Design. Means were compared using Tukey test at the level of 5% probability.

RESULTS

Table III presents the impacts of DTP and Zn additives on parameters such as feed intake, body weight gain, and feed conversion ratio (FCR) in broilers experiencing thermal stress. The broiler chicks in the DTP-10 and DTP-20 groups exhibited the most substantial (P<0.01) average feed intake values throughout the starter, finisher, and entire growth phases. Conversely, the control group displayed the lowest feed intake levels. Notably, the total feed consumption was significantly higher (P<0.01) in the DTP-10 and DTP-20 groups over the entire duration of the study, followed by the Zn-50 group. Conversely, overall feed intake was the lowest in the the control group.

In the initial phase, the DTP-20 group exhibited a higher weight gain (P<0.05), while the control group displayed a notably smaller weight gain (P<0.01). In the final phase, both the DTP-10 and DTP-20 groups demonstrated significantly larger weight gains (P<0.01) when compared to the control group, with the Zn-50 group following suit. When considering the overall weight gain, both the DTP-10 and DTP-20 groups exhibited higher values (P<0.01), followed by the Zn-50 group, in contrast to the control group.

During the initial phase, the DTP-20 group exhibited a significantly lower (P<0.01) and comparable feed conversion ratio (FCR), whereas both the control group and Zn-50 group displayed poorer FCR. Upon comparing all treatment groups to the control group in the concluding phase, there was an improvement in FCR (P<0.05). In general, higher mean FCR values were observed in the DTP-20 group, followed by the DTP-10 and Zn-50 groups. Conversely, the control group exhibited a lower FCR value.

Table IV depicts the impact of DTP and Zn supplementation on variables including dressing percentage, as well as the weights of the immune organs. Remarkably, the DTP-10 group exhibited significantly higher dressing percentages (P<0.01) compared to the DTP-20 group, followed by the Zn-50 group. Conversely, the control group presented the lowest dressing percentage values (P<0.01). It's noteworthy to mention that no significant changes were observed in the weights of the spleen, bursa, and thymus across the treatment groups.

Table IV illustrates the serum concentrations of MDA, and HI titres against New Castle disease (ND) in broilers facing thermal stress. Notably, the inclusion of DTP-10 and DTP-20 supplements led to a significant reduction in MDA levels among heat-stressed broilers (P<0.01). Among the treatment groups, the control group showed the lowest mean MDA value. Regarding antibody titers, the DTP-10 group displayed the highest levels, followed by the DTP-20 and Zn-50 groups, respectively.

Table V show the effect of supplements on the intestinal histology of broilers under heat stress. No significant change was observed in intestinal dimensions of broilers in the control and treatment groups.

DISCUSSION

This study reveals that ambient temperature exerts a detrimental impact on broiler development, antioxidant status, and immunological response. However, the introduction of DTP supplementation has shown to mitigate these adverse effects. Specifically, the DTP-10 and DTP-20 groups exhibited notably higher total mean feed consumption in comparison to the control group. This corroborates with previous findings from studies such as Abdel-Baset *et al.* (2009) and Yitbarek (2013). Moreover, similar positive outcomes were observed in terms of weight gain, where the DTP-10 and DTP-20 groups displayed significant improvements in broilers subjected to heat stress when contrasted with the control group.

The disposal of DTP has emerged as an environmental concern in multiple countries. Elevated ambient temperatures have been found to detrimentally affect broiler development, antioxidant status, and immunological responses, as evidenced by this study. However, the supplementation of DTP has shown promise in alleviating these adverse effects.

Table IV. Dressing percentage and weight of spleen, bursa and thymus, MDA, and ND titre of broilers supplemented with Zn and dried tomato pomace (DTP) under thermal stress. The values are Mean±SEM.

Groups	Dressing percentage	Spleen weight (g)	Bursa weight (g)	Thymus weight (g)	MDA (nmole/ml)	ND Titer (Log_{10})
Control	59.77±0.64b	0.11±0.01	0.10±0.01	0.22±0.01	1.97±0.26a	4.33±0.33c
Zn-50	60.43±0.81b	0.12±0.01	0.11±0.01	0.22±0.01	1.37±0.2b	4.66b±0.33c
DTP-10	64.75±0.5a	0.13±0.01	0.12±0.01	0.22±0.01	1.20±0.31c	6.66±0.33a
DTP-20	64.39±0.580a	0.12±0.01	0.11±0.01	0.21±0.01	1.10±0.27c	5.66±0.33b
P value	0.00002	0.0030	0.0007	0.0000	0.0000	0.0044

Within the same column mean values having different superscripts are significantly different (P<0.05). Zn-50, Zn at the rate of 50 mg/kg; DTP-10 and DTP-20 indicate the supplementation of dried tomato pomace at the rate of 10 and 20 g/kg, respectively.

Table V. Villus dimensions of broilers supplemented with Zn and dried tomato pomace (DTP) under thermal stress. The values are Mean±SEM.

Groups	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height to crypt depth ratio
Control	851.67±4.87	179.12±3.98	200.43±4.87	4.23±2.12a
Zn-50	823.36±2.34	1180.17±1.34	210.23±4.43	4.55±2.11e
DTP-10	856.67±0.88	182.37±2.43	204.45±4.26	4.65±2.11b
DTP-20	845.00±3.48	181.12±3.67	211.76±4.35	4.60±02.22d
P value	0.324	0.650	0.08	0.07

In the context of this study, both the DTP-10 and DTP-20 groups exhibited significantly higher total mean feed consumption compared to the control group. Similar findings have been documented previously, echoing the works of Abdel-Baset *et al*. (2009) and Yitbarek (2013). Moreover, DTP-10 and DTP-20 supplementation led to substantial increases in weight gain among broilers exposed to heat stress, in contrast to the control group. However, in the realm of feeding rabbits, tomato pomace led to a remarkable 20% improvement in feed conversion ratio, as observed by Abdel-Azeem *et al*. (2009). The outcomes of this current study lend support to such assertions.

The mean dressing percentage exhibited an increase in both the DTP-10 and DTP-20 groups, while it decreased in the control group. The principal factors influencing higher FCR in the respective groups can be attributed to weight gain and FCR. The observed augmented feed intake and efficiency could be attributed to the presence of lysine in tomato pomace, a crucial amino acid that stimulates the synthesis of body muscle protein and consequently enhances weight growth. One hypothesis posits that the elevated feed intake might be linked to the elevated fiber content in the diets, which could potentially cause an expansion of the digestive tissue due to prolonged meal retention time for digestion, an idea in line with the work of Colombino *et al*. (2020).

In this particular study, the HI (hemagglutination inhibition) titers against New Castle disease virus (NDV) were notably higher in the DTP-10 group compared to the control group. This phenomenon could potentially be attributed to the presence of lutein in tomatoes. Lutein, functioning as a carotenoid, is known to modulate immune responses by enhancing immunological activity and promoting the proliferation of lymphocytes (Ribaya-Mercado *et al*., 2004).

The impact of DTP on antibody titers during instances of heat stress has been explored in only a limited number of cases. For instance, Selim *et al*. (2013) reported that tomato puree augmented humoral immunity against NDV in broilers subjected to thermal stress. Similarly, Hosseini-Vashan *et al*. (2016) found that administering DTP to broilers led to an increase in the production of antibodies against NDV during heat stress.

The observed elevation in antibody titers among groups supplemented with tomato pomace may be attributed to the presence of lutein in tomatoes. Lutein, a carotenoid, exerts an influence on the immune system by enhancing immune functionality and promoting the proliferation of lymphocytes, thereby elevating antibody titers against NDV (Ribaya-Mercado *et al*., 2004). Additionally, the presence of genistein, another substance found in tomato pomace, has been noted to increase the relative weight of lymphoid organs, consequently intensifying their responsiveness to antibodies against viral antigens. Genistein has been found to enhance immunological capacity and elevate blood lymphocyte counts in broilers (Rasouli and Janania, 2015).

Our research aligns with their discoveries. Our study indicates significantly reduced MDA levels in both the DTP-10 and DTP-20 groups. Tomato-based products have been shown to enhance the body's antioxidant capacity and influence the status of these essential constituents within the body, as noted by Sahin *et al*. (2008). Lycopene, a primary carotenoid present in tomatoes, has the capacity to scavenge harmful free radicals and curtail the generation of reactive oxygen species, as highlighted by Palozza *et al*. (2011). This investigation also resonates with Sahin *et al*. (2011), who found that supplementing quails exposed to thermal stress with tomato pomace led to a reduction in liver MDA concentration and an increase in liver lycopene levels, along with heightened concentrations of antioxidant enzymes.

Lycopene found in tomato pomace has demonstrated the capability to upregulate key components of the antioxidant response. It triggers the synthesis of antioxidant enzymes, which collectively play a protective role against cellular damage from free radicals (Sahin *et al*., 2014). Saed *et al*. (2018) reached similar conclusions, observing that the inclusion of one percent tomato puree in the diets of broiler chicks exposed to high-temperature conditions resulted in improved antioxidant capacity and lowered MDA values. These outcomes also correspond with the findings of Hosseini-Vashan *et al*. (2016), who documented that supplementing broiler with 5% DTP led to decreased levels of plasma MDA. Sahin *et al*. (2011) further reinforced these findings by indicating that tomato powder supplementation enhanced the activity of antioxidant enzymes in the livers of thermally stressed quails.

CONCLUSION

From the results of the present study, we concluded that DTP-10 and DTP-20 enhanced the growth performance and MDA status and humoral immunity in broilers exposed to heat stress.

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Ethical statement and IRB approval

This study has been approved by the departmental committee on ethics and animal welfare the University of Agriculture, Peshawar (12/PS/2020).

Competing interest

There is no potential competing interest with this study.

Consent to participate and consent to publish

All the authors have equally participated in this study and agreed to publish this work in this journal.

Data availability

Data is available in the thesis.

Statement of conflict of interest

The authors have declared no conflict of interest.

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