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Comparative Effect of Zinc Oxide and Silymarin on Growth, Nutrient Utilization and Hematological Parameters of Heat Distressed Broiler

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ABSTRACT

The purpose of the study was to investigate the comparative beneficial effects of dietary supplementation of silymarin (*Silybum marianum*) and zinc oxide (ZnO) in broilers during the finishing stage exposed to heat stress (HS). Two hundred and seventy broiler chicks were randomly assigned to 6 dietary groups: three dietary groups in thermoneutral temperature (TN) and three dietary groups under high temperature. One group of birds received a basal diet only (control group), second group of birds received a diet supplemented with silymarin at the level of 10g/kg and third group of birds received a diet supplemented with ZnO at the level of 60 mg/kg in both TN and HS birds. It was found that supplementation of ZnO significantly ($P<0.05$) improved feed intake, dressing percentage, body weight, feed conversion ratio, blood parameters and nutrient digestibility at both levels of thermal zones. The results of silymarin on growth, nutrient digestibility and hematological parameters were comparatively lower ($P<0.05$) in broilers during TN and HS zones. The current results indicate that the supplementation of feed with silymarin and ZnO improved the growth performance, blood parameters and nutrient digestibility in broiler during heat stress, however, the positive effect of ZnO was better than silymarin.

INTRODUCTION

In subtropical countries, high ambient temperature and humidity have harmful effects on production (Chand *et al*., 2016) and reproduction (Ihsanullah *et al*., 2017) leading to huge economic losses (Khan *et al*., 2011a; Zia ur Rehman *et al*., 2018). As a general rule, animals have known zones of survival and adaptability. For birds, the thermoneutral (TN) zone ranges between 18 to 22°C. Birds are considered heat stressed, when the temperature of their bodies exceeds the upper limit of the TN zone (Khan *et al*., 2012a; Zia ur Rehman *et al*., 2017a). Heat stress (HS) adversely affects the growth performance and nutrient digestibility in broiler (Zia ur Rehman *et al*., 2017b). A number of remedies have been suggested to combat HS, however, the most suitable among them is the dietary supplementation (Khan *et al*., 2014; Chand *et al*., 2014; Shakirullah *et al*., 2017).

The use of antibiotic as feed additive has recently been banned in many countries of the world due to the potential threat of bacterial resistance and antibiotic residues in meat of broiler (Khan *et al*., 2012b, c; Abudabos *et al*., 2016; Zia ur Rahman *et al*., 2017; Alhidary *et al*., 2017). Therefore, the poultry researchers and nutritionists are looking for alternative to antibiotics, which maintain the growth the birds and do not compromise the health of the consumers (Abudabos *et al*., 2017a, b; Khan *et al*., 2012d). Milk thistle (*Silybum marianum*) is a natural herb and contains various flavonolignans such as silybin, isosilybin, silychristin and silydianin (Surai, 2015). Silymarin has renal, hepatic, anti-carcinogenic and anti-inflammatory effects (Manna *et al*., 1999). In addition, silymarin and silybin are the two most important flavonoids in milk thistle, which have been reported to stimulate the immune system and scavenge free radical (Thyagarajan *et al*., 2002; Wilasrusmee *et al*., 2002). Studies are scarce on the effect of milk thistle in broiler under heat stressed condition. To the best of our knowledge, Silymarin has not been used in the heat stressed broiler. Milk thistle contains some very important

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compounds such as silybin and flavonolignans, isosilybin, silydianin, dehydrosilybin and silychristin (Alhidary *et al*., 2017). Silymarin stabilizes and protects the cell membrane against the injury, prevents the deposition of collagen fibre and disrupts free radicals (Surai, 2015).

Zinc (Zn) is a vital element and involved in many biochemical reactions of animal body (Khan *et al*., 2011b; Rahman *et al*., 2014). The zinc ion is a cofactor of many enzymes and an element of matallo enzymes that contributes in metabolic processes (Zowczak Drabarczyk *et al*., 2004; Naz *et al*., 2016). High ambient temperature can reduce the retention of Zn in poultry birds with increased Zn elimination and alleviates the effects of high temperature in broilers (Sahin *et al*., 2005). A number of studies have reported the effect of ZnO and silymarin in broilers; however, to the best of our knowledge, their comparative effects have not been reported.

The objective of the present study was to investigate the comparative effect of silymarin and zinc oxide (ZnO) on broiler growth performance, nutrient digestibility and hematology under TN and HS conditions during the finishing phase.

MATERIALS AND METHODS

Experimental design and bird husbandry

A total of 270 experimental broiler chicks were divided into two groups and sub-divided into six sub-groups with 3 replicates each. Birds in TN zone were maintained at 35°C and then the temperature was gradually brought and maintained at 23°C. The average temperature and humidity in heat stressed (HS) zone was 38°C and 61%, respectively. A standard diet was prepared as per National Research Council (1994) requirements of the bird (Table I). Zinc oxide and milk thistle powder were added at 60 mg/kg and 10 g/kg of diet, respectively. The experiment lasted for three weeks.

Performance measurement

Offer amount of feed and measure refused feed every day for the determination of feed intake. On weekly basis, the data were analyzed. Bird's weight was measured on day first of the week and then at the end of the week. On weekly basis body weight gain was determined. The FCR given was recorded on a weekly basis. From each replicate two birds were randomly selected, weighed and slaughtered. The edible and non-edible parts were removed after scalding to find the carcass weight.

Hematology

For hematological study, at the end of the experiment, three ml blood samples per treatment were collected from wing vein in separate ethylene diamine tetra acetate (EDTA) tubes. Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV) and hemoglobin (Hb) contents were determined with the aid of Cell-DYN 3500 Hematology Analyzer.

Nutrient digestibility and chemical analysis

On day 30, 4 birds per replicate were shifted to metabolic cages for digestibility study. Fecal material was collected for four days and feed intake was determined. Fresh feces were stored in labeled plastic bags and stored at -20°C. Excreta samples were analyzed for nitrogen (Kjeldahl method; AOAC, 1990), CP (N × 6.25), dry matter (drying in oven at 103°C for 8 h), crude fibre (AOAC, 1990), ether extract (AOAC, 2000) and ash (Vogtmann et al., 1975). Metabolizable energy (ME) was determined with the help of bomb calorimeter as described by Sultan *et al*. (2015).

Statistical analysis

The data were statistically analyzed with the standard procedure (ANOVA) using two factorial designs (2 x 3) in SAS (2004). LSD test was applied according to measure means for significant difference (Steel and Torrie, 1981).

Table I.- Composition of basal diets.

Ingredients	**(%)**
Maize	60.7
Soybean meal	35.6
Vegetable oil	0.5
Limestone	1.4
Dicalcium phosphate	1.2
DL Methionine	0.15
Salt	0.4
Vitamin + trace mineral premix	0.3
Total	100
Calculated nutrient composition	
Protein, %	22
Poultry ME, kcal/kg	2960

Each kg of premix consisted: pyridoxine, 1mg; folic acid, 0.4mg; molybdenum, 0.32mg; ethoxyquin, 25mg; choline chloride, 60mg; dl-α-tocopherol acetate; 4mg; iodine, 0.2mg; thiamine, 0.3mg; Ca pantothenate, 3mg; cyanocobalamin, 3µg; biotin, 0.02mg; Mn, 15mg; Zn, 10mg; iron, 4mg; Cu, 1mg; Co, 0.06mg; Se, 0.02mg; cholecalciferol, 0.018mg; *trans*-retinol, 0.66mg; menadione, 0.4mg; riboflavin, 1.6mg; niacin, 6mg.

RESULTS

The effect of supplementation of ZnO and milk thistle on the weekly feed intake and weight gain in broiler is given in Table II. Feed intake and weight gain was significantly ($P<0.05$) high in birds supplemented with ZnO in TN zone. Birds in HS condition showed significantly higher ($P<0.05$) feed intake and weight gain in response to the supplementation of ZnO.

Table II.- Effect of supplementation of zinc oxide and silymarin on feed intake (g), body weight gain (g), feed conversion ratio (FI/BW) and carcass yield (%) of heat stressed broilers.

Temperature zone	Treatments	Feed intake (g)			Body weight gain (g)			FCR (FI/BW)			Carcass yield (%)
		Day 28	Day 35	Day 42	Day 28	Day 35	Day 42	Day 28	Day 35	Day 42	
TN	Control	1523.33^{c}	2121.67^{c}	2830.00^{c}	1010.00^{c}	1318.33^{d}	1656.67^{c}	1.50^{b}	1.60^{a}	1.70^{b}	60.35^{c}
	Silymarin	1588.33^{b}	2248.33^{b}	2896.67^{b}	1086.67^{b}	1446.67^{b}	1776.67^{b}	1.45^{c}	1.55^{b}	1.63^{d}	61.15^{b}
	Zinc oxide	1620.00^{a}	2326.67^{a}	3023.33^{a}	1150.00^{a}	1533.33^{a}	1873.33^{a}	1.40^{d}	1.51^{c}	1.61^{e}	62.34^{a}
HS	Control	1436.67^{d}	1970.00^{d}	2720.00^{d}	920.00^{e}	1183.33^{e}	1550.00^{d}	1.55^{a}	1.66^{a}	1.75^{a}	59.76^{d}
	Silymarin	1485.00^{c}	2148.33^{c}	2813.33^{c}	995.00^{d}	1351.67^{c}	1670.00^{c}	1.49^{c}	1.58^{c}	1.68^{c}	60.15^{c}
	Zinc oxide	1518.33^{b}	2226.67^{b}	2923.33^{b}	1036.67^{c}	1426.67^{b}	1780.00^{b}	1.46^{d}	1.56^{d}	1.64^{d}	61.64^{b}
Pooled SEM		12.01	13.23	14.09	11.09	12.03	13.07	0.07	0.07	0.09	0.95
Temperature	P value	0.04	0.05	0.04	0.02	0.04	0.04	0.01	0.04	0.03	0.03
Treatment	P value	0.03	0.03	0.02	0.01	0.02	0.03	0.02	0.02	0.02	0.02
Temp × Treatment	P value	0.05	0.06	0.05	0.04	0.05	0.05	0.04	0.05	0.05	0.04

Means within the same column with different superscript are significantly different (P<0.05). TN, thermoneutral; HS, heat stressed.

Table III.- Effect of supplementation of zinc oxide and silymarin on blood parameters of heat stressed broilers.

Temperature zone	Treatments	WBC (× 10^{3}/µl)	RBC (× 10^{6}/µl)	Hb (%)
TN	Control	7.33^{c}	2.53^{c}	9.38^{d}
	Silymarin	8.53^{b}	3.54^{b}	12.37^{b}
	Zinc oxide	9.50^{a}	4.45^{a}	13.50^{a}
HS	Control	6.33^{d}	2.13^{e}	8.12^{e}
	Silymarin	7.46^{c}	2.89^{d}	11.18^{c}
	Zinc oxide	8.43^{b}	3.21^{b}	12.17^{b}
Pooled SEM		0.13	0.19	0.25
Temperature	P value	0.02	0.05	0.04
Treatment	P value	0.01	0.03	0.02
Temp × Treatment	P value	0.04	0.04	0.05

Means within the same column with different superscript are significantly different (P<0.05). TN, thermoneutral; HS, heat stressed.

The effect of supplementation of ZnO and milk thistle on the FCR in broiler is given in Table II. The FCR was significantly (P<0.05) high in birds supplemented with ZnO during HS condition compared to the control. Improved FCR was also found in TN condition in ZnO supplemented birds at the end of the experiment.

The carcass yield during TN and HS condition in broiler fed with ZnO and milk thistle is given in Table II. The carcass yield was significantly (P<0.05) high in ZnO supplemented group compared to the control in TN zone. Similarly, ZnO supplemented birds also showed significantly (P<0.05) higher carcass yield during HS condition.

The effect of supplementation of ZnO and milk thistle on the hematological parameters in broiler is given in Table III. The WBC, RBC and Hb were significantly (P<0.05) high in ZnO supplemented birds in TN zone. Similarly, in HS condition, ZnO supplemented birds showed better hematological values compared to the control.

The effect of supplementation of ZnO and milk thistle on the nutrient digestibility in broiler is given in Table IV. ZnO supplementation significantly (P<0.05) increased DM, ash, CP, EE, CF and AME were significantly (P<0.05) high in ZnO supplemented group in TN condition. Similarly, birds during HS, showed better nutrient digestibility in response to ZnO supplementation compared to the control.

Table IV.- Effect of supplementation of zinc oxide and silymarin on nutrient digestibility (%) of heat stressed broilers.

Temp. zone	Treatment	DM (%)	Ash (%)	CP (%)	EE (%)	AME (Kcal/kg)
TN	Control	70.18^{e}	30.22^{e}	63.18^{e}	53.25^{e}	2922.74^{c}
	Silymarin	73.16^{c}	31.35^{b}	64.15^{c}	54.44^{c}	3011.89^{b}
	Zinc oxide	74.19^{a}	32.15^{a}	65.08^{a}	55.45^{a}	3113.00^{a}
HS	Control	69.55^{e}	29.22^{e}	62.15^{e}	52.35^{e}	2984.30^{e}
	Silymarin	72.22^{d}	30.26^{d}	63.22^{d}	53.49^{d}	3082.67^{c}
	Zinc oxide	73.25^{b}	31.18^{c}	64.25^{b}	54.50^{b}	3186.11^{a}
Pooled SEM		1.9	0.91	1.8	2.7	13.01
Temp	P value	0.04	0.02.	0.04	0.05	0.01
Treatment	P value	0.03	0.01	0.02	0.03	0.03
Temp × Treatment	P value	0.05	0.04	0.05	0.04	0.04

Means within the same column with different superscript are significantly different (P<0.05). TN, thermoneutral; HS, heat stressed; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude protein; AME, apparent metabolizable energy.

DISCUSSION

The feed intake of broilers was significantly high during TN as compared to HS zone. Above 32°C is considered a high ambient temperature for birds and the high ambient temperature suppress the feed intake. Zinc oxide and silymarin combat heat stress via its anti-oxidative and free radical scavenging properties. Silymarin supplemented group had higher feed intake as compared to control and ZnO (supplemented) groups in both the zones. Chand *et al*. (2011) reported that feed intake of broiler chicken fed on Silymarin supplemented diets significantly improved. It may be due to its positive effects on metabolism and digestion. ZnO scavenges free radicals and alleviates stress (Naz *et al*., 2016).

The feed efficiency and body weight gain per birds were significantly affected by high environmental temperature and treatments assigned. High ambient temperature exerts some hazardous effects on bird's performance, creating some physiological changes and stresses in body (Chand *et al*., 2017). Supplementation of silymarin and ZnO attenuate the deleterious heat-induced stress. Supplementation of Zn in diet is necessary for optimal growth, because Zn ion is a cofactor of many enzymes and contributing in metabolism (Zowczak-Drabarczyk *et al*., 2004). Silymarin promotes digestibility and absorption of protein in animal's body. All the above reasons might explain, why high weight gain and better FCR were observed in treatment groups compared to the control group. Chand *et al*. (2011) recoded better performance of birds fed on toxin contaminated feed with silymarin as compared to other birds. Similarly, Tedesco *et al*. (2004) reported better FCR in birds supplemented with silymarin. Sahin *et al*. (2005) and Ezzati *et al*. (2013) reported improved performance of birds in heat stressed condition, when supplement with dietary Zn. The increment in dressed weight is strongly correlated with the improved performance of bird fed on silymarin and ZnO. Increase dressed weight of treated groups is the positive outcome of silymarin and ZnO supplementation in ration. Higher dressing percentage was reported by Chand *et al*. (2011) and Zahid and Durrani (2007) by feeding milk thistle at different levels in broiler chicken.

In the current study, there is a significant relationship between heat stress and decrease in hematological parameters. Heat stress inhibits production and decreases the life span of RBCs and reduces the Hb level. The WBC showed a significant reduction at high temperature. This might be linked to the atrophy of lymphoid tissue. Also stressed hormones (corticosteroids) have suppressive effects on lymphoid tissue and total leucocytes count. It is pertinent to note that silymarin and ZnO were not much effective in HS zone as compared to the TN condition. At TN zone, the stress was lowered, so beneficial effects of silymarin and ZnO were more pronounced than HS zone. Our results are in agreement with Osman (1996) who reported that the blood parameters revealed a reduction with heat stress.

The digestibility of DM, ASH, CP, EE, CF and Apparent Metabolisable Energy had significant differences. Heat stress reduces glucose and minerals metabolism and produces lesions in gastro intestinal tract (GIT), resulting in low digestibility (Khan *et al*., 2011). This will lead to an alteration in starch, amino acids, fat, and mineral metabolism, that results in the deposition of abdominal fat and reduction of structural protein. Virden *et al*. (2007) reported that stress results in significant changes in digestibility of carbohydrates, protein, lipid, and mineral metabolism. It is speculated that supplementation of silymarin and ZnO overcome the heat stress problems in birds, probably due to the reduction in oxidative stress (Chand *et al*., 2011; Naz *et al*., 2016).

CONCLUSION

Heat stress adversely affected the bird performance, nutrient digestibility and hematology. The supplementation of silymarin and ZnO decreased the negative effects of high stress and improved bird performance. The positive effect of ZnO was better than silymarin in birds both during TN and HS conditions.

Statement of conflict of interest

Authors have declared no conflict of interest.

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