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Effect of protein synthesis modulator and acute heat stress on jejunal digestive enzymes in broiler chicken

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Abstract
A total one ninety two day old chick of CARIBRO Vishal broiler chicken were housed in multi-tier brooder cages up to five weeks of age and reared under uniform husbandry conditions. On 36th day divided into 6 groups with three treatments (32 birds in each group's) including control, enhancer and inhibitor, receiving normal saline, glutamine @ 0.75 mg kg^{-1} BW and quercetin @5 mg kg^{-1} BW respectively. After, 24 hours of administration each of the six groups were again divided into two with three groups in each, one being exposed to acute heat stress (40±1 °C; 55% RH) for different duration of 0, 2, 5 and 10 hours in psychometric chamber and another one were kept as unexposed to heat stress. The result indicated that concentrations of lipase (91.88 ± 2.25), amylase (111.49 ± 3.06), pepsin (71.66 ± 1.33) and trypsin (85.10 ± 2.84) were significantly (*$p<0.05$) higher in enhancer group. Heat stress has significantly (*$p<0.05$) increased the activity of trypsin (71.96 ± 2.98) and pepsin (61.01 ± 1.88) in jejunum and simultaneously decreased the amylase activity; however the lipase activity did not show any change in response to acute heat stress exposure. A significant difference observed (*$p<0.05$) on enzymatic activity of pepsin and trypsin and the activity of amylase and lipase was not significant under different duration of heat stress exposure. It can be concluded that protein synthesis modulator; glutamine significantly increases digestive enzymes activity and improve performance of broiler chicken under acute heat stress condition.

Keywords: Protein synthesis modulator, digestive enzymes, heat stress

Introduction
Heat stress is one of the most challenging environmental conditions affecting commercial poultry compare to other species of live stock (Mahmoud *et al*., 2003) [9] and considered to be the major cause of loss of production and reduced profit in the poultry production worldwide (Sejian *et al*., 2012) [17]. Broiler chicken is more sensitive to heat stress because of higher, growth rate, body temperature and absence of sweat gland (Geraert *et al*., 1996) [4]. At high temperatures, birds tend to lose appetite and feed intake (Dale & Fuller, 1980) [2]. Feed intake and utilization are closely related to digestion and absorption, which are in turn affected by environmental temperature changes (Temim *et al*., 2000, Itoh *et al*., 2001) [18, 7]. According to when environmental temperature increased from 22 ºC to 32 ºC there was a decrease in feed efficiency from 0.485 to 0.340 in poultry, indicating that the functional digestive tract adaptation could be related to compensatory adjustment on production rate and enzymatic contents of digestive secretions (Osman & Tanios, 1983) [11].
Protein synthesis modulator like glutamine is non-essential amino acids that play an important role in alleviating the heat stress. Dietary supplementation of glutamine mainly targets small intestine and improves digestive function in rodents (Larson *et al*., 2007) [8] and pigs (Wu *et al*., 1996) [19]. Digestive enzymes activity increased by Protein modulator glutamine in chicken (Hao *et al*. 2012 and Devi-Priya *et al*. 2010) [6, 3] by over-expressing heat shock protein during heat stress conditions**.** Another protein synthesis modulator is quercetin that is bioflavonoid act as a negative control in present study.
Changes in digestive enzyme activity may be one reason for the change in digestive function under heat stress. However, studies focused on the effects of protein modulator and heat stress on the poultry digestive enzyme activity are few at present. Thus the present study was designed to effect of this protein modulator and acute heat stress on digestive enzymes in broiler chicken.

Materials and Methods

Experimental birds

Total one ninety two day old chicks of CARIBRO Vishal broiler chicken were obtained from Central Avian Research Institute, Izatnagar, India and housed in multi-tier brooder cages up to five weeks of age and reared under uniform husbandry conditions. The birds died during the study period were subjected to necropsy examination.

Experimental design

A total of one hundred ninety two broiler chickens with similar body weight were housed in cages and reared straight run chicks up to five weeks of age under standard managemental conditions. On 36th day divided into 6 groups with three treatments (32 birds in each group's) including control, enhancer and inhibitor, receiving normal saline, glutamine @ 0.75 mg kg^{-1} BW and quercetin @5 mg kg^{-1} BW respectively. After, 24 hours of administration each of the six groups were again divided into two with three groups in each, one being exposed to acute heat stress (40±1 °C; 55% RH) for different duration of 0, 2, 5 and 10 hours in psychometric chamber and another one were kept as unexposed to heat stress and during the 0, 2, 5, and 10 hours in the heat-stress and non heat stress condition chickens that weighed similarly in each replicate per treatment (n-4 for each exposure duration) average were selected and immediately killed by cervical dislocation. Longitudinal sections of jejunum tissue were obtained and flushed with 5 ml of saline. A homogeneous mucosal homogenate sample was farmed method given by Majumdar *et al*., (1992) [10] after that stored immediately at −70 °C until analysis.

Estimation of total protein in intestinal mucosal homogenate

The total protein in intestinal mucosal homogenate was estimated by modified Lowry's method as described by Rosenberg (1996) [14].

Estimation of digestive enzymes in intestinal mucosal homogenate

The digestive enzymes namely amylase, lipase, pepsin and trypsin in saline based intestinal mucosal homogenate were estimated by method of Gomori (1957) [5] Schmid (1974) [16], Rick and Fritsch (1974) [13] and Rick (1974) [12] respectively.

Statistical analysis

The data obtained from experiment were analysed by 2x3x4 factorial method using SPSS V.20 for both interaction and main effect. The means were compared using Tukey test.

Results and Discussion

The interaction and main effect of protein synthesis modulator (Mean values ± SE) on jejunal enzymatic activity are represented in table.1 and table.2 respectively. Results indicated significant (*p<0.05) effect of protein synthesis modulator on activity of various enzymes i.e. amylase, lipase, pepsin and trypsin was observed in broilers exposed to varied periods of heat stress.The main effect of protein synthesis modulator significantly modulates activity of various enzymes in jejunum. Concentrations of lipase (91.88 ± 2.25), amylase (111.49 ± 3.06), pepsin (71.66 ± 1.33) and trypsin (85.10 ± 2.84) were significantly (P<0.05) higher in enhancer group when compared to that of control and inhibitor group.

Results of the present study are in agreement with the finding of Hao *et al.* (2012) [6] and Devi-Priya *et al.* (2010) [3] who reported that glutamine significantly increases the digestive enzymes activities under heat stress. Hence, there is a possibility that the glutamine over-express heat shock protein that improved intestinal digestion and absorption function under acute heat stress by deploying positive effects on jejunum enzymatic activity of amylase, lipase, trypsin and pepsin with increased concentrations.

Results pertaining to the effect of acute heat stress exposure and duration on enzymatic activity are presented in table.3 and 4 respectively. Heat stress has significantly (P<0.05) increased the activity of trypsin (71.96 ± 2.98) and pepsin (61.01 ± 1.88) in jejunum and simultaneously decreased the amylase activity (62.59 ± 3.39). However, lipase activity did not show any change in response to acute heat stress exposure. A significant difference observed (P<0.05) on enzymatic activity of pepsin and trypsin and the activity of amylase and lipase was not significant under different duration of heat stress exposure.

Reduction in feed intake and compromised digestive function in broiler chicken during heat stress is generally noticed. one possible reason of change in digestive function may be changes in digestive enzyme activity that why changes in activities of digestive enzymes such as pepsin, trypsin, amylase, lipase etc. of poultry needs to explore with respect to exposure of heat stress however; studies focused on the effects of heat stress on the poultry digestive enzyme activity are few at present.

In present experiment exposure of heat stress reduce amylase activity and unaffected lipase activity in broiler chicken which reaffirm the previous findings of Routman *et al*, (2003) [15], however, Osman and Tanios, (1983) [11] found significant increase level of intestinal and pancreatic amylase in broiler chicken during exposure of heat stress. In our study, we have also evaluated the activity of pepsin and trypsin during exposure of heat stress and found significant increment of both enzyme activity in broiler chicken with respect to unexposed group while Routman and co-worker's found trypsin activity of heat stressed exposed broiler chicken remain unaffected which might be due to different exposed experimental conditions such as heat stress mode, the duration of heat stress, and the feed and water system however, we have observed a significant change in the enzyme concentrations of pepsin, and trypsin with the different duration of heat stress exposure.

Table 1: Effect of protein synthesis modulator at different periods of heat stress exposure on jejunal enzymatic activity (Units mg^{-1} of protein)

Heat stress	Protein modulator	Hours	Pepsin	Trypsin	Amylase	Lipase
Unexposed	Control	0	49.37^{ab}±2.19	46.95^{abcd}±4.28	59.94^{abc}± 9.94	49.69^{abcd} ±6.94
		2	49.90±3.06	47.37^{abcd}±3.76	57.58^{abc}± 8.87	52.39^{abcde}±4.72
		5	49.58^{ab}±3.00	46.73^{abcd}±5.28	61.45^{abc}± 11.28	48.52^{abc}±2.40
		10	49.27^{ab}±1.49	49.57^{abcd}±6.50	62.63^{abc}± 9.91	52.14^{abcde}±6.79
	Enhancer	0	65.20^{cd}±3.70	76.88^{cde}±10.63	118.65^{d}± 11.58	96.00^{fg}±9.72
		2	65.35^{cd}±5.09	78.93^{cd}±11.19	117.09^{d}± 15.54	98.10±7.56
		5	65.03^{cd}±2.91	77.91^{cd}±9.35	112.07^{d}± 13.10	92.93^{defg}±17.76
		10	64.93^{cd}±2.51	75.90^{bcde}±7.71	119.58^{d}± 16.64	94.90^{efg}±5.34
	Inhibitor	0	36.52^{a}±1.94	23.87^{a}±4.20	34.08^{a}± 3.43	42.14^{a}±3.32
		2	36.80^{a}±4.02	21.76^{a}±2.46	35.46^{a}± 5.01	44.25^{ab}±2.77
		5	36.69^{a}±3.02	24.95^{ab}±3.74	36.22^{a}± 5.55	43.25^{a}±5.09
		10	36.45^{a}±2.02	25.74^{abc}±4.40	38.74^{a} ±7.34	47.82^{abc}±5.97
Exposed	Control	0	49.66^{ab}±1.16	47.73^{abcd}±3.56	54.49^{abc}± 2.43	53.16^{abcdef}±5.07
		2	70.13^{cde}±2.28	82.03^{cd}±12.47	44.50^{ab}± 4.61	45.56^{ab}±9.15
		5	75.71^{cdef}±2.12	91.88^{cd}±17.65	55.03^{abc}± 5.81	43.41^{ab}±6.97
		10	61.08^{bc}±4.21	58.03^{abcde}±4.48	50.64^{abc} ±3.03	50.45^{abcd}±8.62
	Enhancer	0	65.08^{cd}±1.65	78.02^{cd}±9.46	121.27^{d}± 9.43	90.25^{cdefg}±12.18
		2	85.77^{f}±2.56	95.46^{cd}±14.00	90.28^{cd}± 3.63	79.06^{abcdef}±10.97
		5	78.86^{def}±2.91	94.65^{cd}±10.42	113.70^{d}±11.20	97.03^{g}±6.69
		10	84.74^{ef}±1.53	103.13^{e}±12.76	88.23^{bcd}± 4.68	86.80^{bcdefg}±11.83
	Inhibitor	0	36.58^{a}±2.12	26.16^{abc}±2.76	35.69^{a}± 2.94	45.02^{ab}±7.67
		2	48.38^{ab}±1.32	58.43^{abcde}±18.27	29.02^{a}±2.44	39.81^{a}±4.94
		5	39.18^{a}±2.72	57.11^{abcde}±5.83	34.74^{a}± 4.18	51.77^{abcde}±9.30
		10	37.15^{a}±2.53	54.03^{abcde}±15.08	30.95^{a}± 4.69	46.80^{ab}±5.29
P-value			0.000	0.000	0.000	0.000

[a-g] Mean values bearing different superscripts within columns differ significantly (*$p<0.05$*)

Table 2: Effect of protein synthesis modulator on jejunal enzymatic activity (Units mg^{-1} of protein)

Protein synthesis modulator	Pepsin	Trypsin	Amylase	Lipase
Control	57.04^{b} ± 1.46	58.78^{b} ± 2.89	54.37^{b} ± 1.82	49.41^{a} ± 1.53
Enhancer	71.66^{c} ± 1.33	85.10^{c} ± 2.84	111.49^{c} ± 3.06	91.88^{b} ± 2.25
Inhibitor	38.46^{a} ± 0.75	36.50^{a} ± 2.88	34.27^{a} ± 1.37	45.10^{a} ± 1.30
P-Value	0.000	0.000	0.000	0.000

[abc]Mean values bearing different superscripts within columns differ significantly *(*$p<0.05$*)*

Table 3: Effect of heat stress exposure on jejunal enzymatic activity (Units mg^{-1} of protein)

Heat stress	Pepsin	Trypsin	Amylase	Lipase
Unexposed	50.43^{a} ± 1.32	49.73^{a} ± 2.62	70.83^{b} ± 4.14	62.22 ± 2.70
Exposed	61.01^{b} ± 1.88	71.96^{b} ± 2.98	62.59^{a} ± 3.39	61.99 ± 2.57
P-value	0.000	0.000	0.000	0.409

[ab]Mean values bearing different superscripts within columns differ significantly *(*$p<0.05$*)*

Table 4: Effect of duration of heat stress exposure on jejunal enzymatic activity (Units mg^{-1} of protein)

Duration heat stress exposure	Pepsin	Trypsin	Amylase	Lipase
0 hour	50.49^{a} ± 1.80	49.93^{a} ± 3.40	71.05 ± 5.76	62.71 ± 3.70
2 hours	59.18^{b} ± 2.50	63.99^{ab} ± 4.59	62.41 ± 5.12	59.86 ± 3.61
5 hours	57.57^{b} ± 2.59	66.15^{b}± 4.57	68.93 ± 5.44	62.81 ± 4.10
10 hours	55.63^{b} ± 2.56	61.06^{ab} ± 4.07	64.45 ± 5.20	63.15 ± 3.43
P- value	0.000	0.027	0.325	0.888

[ab]Mean values bearing different superscripts within columns differ significantly (*$p<0.05$*)

Conclusion

Based on the result it is concluded that glutamine significantly increases digestive enzymes activity under acute heat stress condition and may be beneficial incorporating in feed of poultry under heat stress conditions However, further more studies are required for establishing the relationship the digestive enzymes activities with respect to acute heat stress.

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