aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2016.538.547

Research Article

Incubation Temperatures Affect Expression of Nutrient Transporter Genes in Japanese Quail

[1]Azhar Rashid, [1]Sanjeev Kumar Sharma, [1]Jagbir Singh Tyagi, [1]Jag Mohan, [2]Raj Narayan, [1]Gautham Kolluri and [3]Ram Pratap Singh

[1]Division of Physiology and Reproduction, ICAR-Central Avian Research Institute, 243122 Izatnagar, Uttar Pradesh, India
[2]Division of Avian Genetics and Breeding, ICAR-Central Avian Research Institute, 243122 Izatnagar, Uttar Pradesh, India
[3]Avian Physiology and Genetics Division, Salim Ali Centre for Ornithology and Natural History, Anaikatty, 641 108 Coimbatore, India

Abstract

Background and Objective: Elevated Incubation Temperature (EIT) plays an important role in the regulation of nutrient transporter gene (NTG) expression. Information on NTG expression in Japanese quail is scarce. Thus, an attempt was made to understand the effects of EIT on the mRNA expression of NTG in CARI-UTTAM and CARI-PEARL varieties of Japanese quail. **Materials and Methods:** Eggs of first treatment group (control) were subjected to 37.5°C throughout the entire incubation period (17 days). Eggs in 2nd and 3rd treatment group were incubated at 37.5°C for initial 10 days and there after at 38.5 and 39.5°C, respectively. Eight chicks (4 from each variety) from each treatment group were randomly sacrificed on Day Of Hatch (DOH), 3rd, 7th and 10th day of age to study mRNA expression of NTG. **Results:** Expression of SGLT1 gene was significantly up regulated on all the studied days except on DOH at 38.5°C and on 3rd day at 39.5°C in CARI-PEARL variety. Expression of GLUT5 gene was up regulated on all days except on DOH at 38.5°C in CARI-UTTAM variety. PepT1 gene expression was up regulated on 3rd day of age and over expression of EAAT3 gene was significant on 3rd and 10th day in both the varieties at elevated incubation temperatures. At incubation temperature 38.5°C UTTAM variety showed over expression for SGLT1 gene at DOH and 3rd day, for GLUT5 and PepT1 gene at 10th day and for EAAT3 gene at 7th and 10th day post-hatch in comparison to PEARL variety. At 39.5°C higher expression is observed on 7th post-hatch and DOH for SGLT1, 7th and 10th day post-hatch for GLUT5 and PepT1 and 3rd and 7th day post-hatch for EAAT3 gene. **Conclusion:** A significant up regulation in the mRNA expression of most of the studied nutrient transporter genes were reported at elevated incubation temperatures. In nut shell, it may be concluded that higher relative fold expression of most of the nutrient transporter genes in quail incubated at elevated temperatures may indicate higher susceptibility of jejunum to this temperature warranting higher fold expression as an adaptation mechanism.

Key words: Elevated incubation temperature, japanese quail, nutrient transporters

Received: December 08, 2015 Accepted: April 04, 2016 Published: August 15, 2016

Citation: Azhar Rashid, Sanjeev Kumar Sharma, Jagbir Singh Tyagi, Jag Mohan, Raj Narayan, Gautham Kolluri and Ram Pratap Singh, 2016. Incubation temperatures affect expression of nutrient transporter genes in japanese quail. Asian J. Anim. Vet. Adv., 11: 538-547.

Corresponding Author: Jagbir Singh Tyagi, Experimental Hatchery, Division of Physiology and Reproduction, ICAR-Central Avian Research Institute, 243122 Izatnagar, Uttar Pradesh, India Tel: + 91-0581-2303223

Copyright: © 2016 Azhar Rashid *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the tropical countries elevated ambient temperature is one of the major stressors in poultry production and it generates a wide range of behavioural and physiological responses[1]. An increase in body temperature above the regulated range, as a result of exposure to environmental extremes and/or excessive metabolic heat production may initiate a cascade of irreversible thermoregulatory events that could be lethal for the growing embryo[2]. In the first half of the incubation embryo absorbs heat from atmosphere due to temperature discrepancy and disperse heat to surrounding as metabolic rate and heat out-turn increases in second half[3]. Elevated incubation temperature (higher than 37.5°C) would impair chick quality and absorption of nutrients from the yolk sac and thus affect the performance of chickens[4] and other species.

The nutrient assimilation capacity depends on the mucosal surface area of the small intestine, digestive enzyme activity and on functional properties of specific nutrient transporters that are present in the basolateral and brush border membrane[5]. Nutrients are transported into enterocytes by transporters located in the brush border membrane. Glucose is transported from the lumen of the small intestine across the brush border membrane into the enterocyte primarily by the sodium-dependent glucose transporter (SGLT1)[6]. The facilitative fructose transporter (GLUT5) mediates the passive transport of fructose into enterocytes[7,8]. Amino acids are transported as free amino acids or small peptides by a variety of amino acid transporters or the peptide transporter (PepT1), respectively. Free glutamate and aspartate are transported across the brush border membrane of the enterocytes by the excitatory amino acid transporter (EAAT3)[9]. A temperature-by-age interaction was found for relative PepT1 gene expression at elevated incubation temperatures in Ross Broiler Chickens[10] however, the study could not found differences in the relative expression of SGLT1 or GLUT5 in broiler chicks incubated at temperature (39.6°C) from embryonic day 13-21. The elevated temperature causes metabolic and physiological changes in the intestinal transport of glucose through the increase of SGLT1, while the basolateral transporter GLUT2 is unaffected[11]. However, studies evaluating the effect of elevated temperature during incubation on the development of the gastrointestinal tract, more specifically nutrient transporter gene expression in Japanese quail are lacking. Thus, specific studies are warranted to understand the effect of elevated incubation temperatures on the pre and post-hatch performance coupled with expression of nutrient transporter genes in Japanese quail. Therefore, proposed study was planned to investigate the effect of various incubation temperatures on the expression of nutrient transporter genes in high and low body weight varieties of Japanese quail.

MATERIALS AND METHODS

The proposed research work was carried out as per the guidelines and approval of Institute Animal Ethical Committee and Committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Experimental design: A total of 720 hatching eggs (360 from each variety i.e., CARI-UTTAM-high body weight and CARI-PEARL-low body weight) were obtained from experimental quail farm, CARI, Izatnagar, India. The eggs were randomly allotted into three treatment groups (n = 240) (120 from each variety, which were further divided into 4 replicates). The first treatment group served as control, where in the eggs were set in an incubator (DAYAL, Electronic automatic incubator and New Delhi-46) at a temperature of 37.5±0.2°C and 55-57% RH up to 14 days, after that transfer to a hatcher at same temperature and 65-70% RH. In 2nd and 3rd treatment group, the eggs were incubated at 37.5±0.2°C for initial 10 days and thereafter at 38.5±0.2°C (Treatment 2) or 39.5±0.2°C (Treatment 3) in the different incubators and hatchers of same make at same RH. Care was taken to maintain same average initial egg weight (g) in all treatment groups of respective varieties. Eight birds from each treatment i.e., four from each variety were sacrificed on day of hatch, post-hatch day 3rd, 7th and 10th. Thus, total of 24 birds were sacrificed on each of these days. A small piece (1 cm) of anterior jejunum sample was collected aseptically, rinsed with nuclease free PBS, chopped and kept in TRIZOL solution (Invitrogen) at -20°C for further processing and estimation. The remaining Japanese quail chicks were reared under standard managemental conditions upto 5 weeks of age to study post-hatch performance.

RNA isolation and cDNA synthesis: Total RNA was isolated from jejunum sample kept in TRIZOL reagent following the manufacturer's instructions. The tissue samples were then homogenized using automated homogenizer (Polytron). The RNA samples were treated with DNase using RNase-free DNase (Biogene, CA, USA). The concentration and purity of RNA were determined spectrophotometrically at OD260 and OD280 using nanodrop (NanoDrop 1000, Thermo Scientific, Singapore). The integrity of the RNA samples was ascertained using agarose gel electrophoresis. Samples with 230/260

Table 1: Oligonucleotide primer sequences used in real-time PCR for the amplication of nutrient transporter gene in jejunum of Japanese quail

Gene	Primer sequence	Annealing temp (°C)	Product length (bp)	GenBan ID No.	Reaction efficiency (%)
SGLT1	F-TGTCTCTCTGGCAAGAAGTC	60	71	XM_415247	106.3
	R-TGTAAACCATGTAGTTCAGATCGA				
GLUT5	F-TTGCTGGCTTTGGGTTGTG	60	60	XM_417596	102.8
	R-GGAGGTTGAGGGCCAAAGTC				
PepT1	F-CCCCTGAGGAGGATCACTT	60	66	NM_204365	95.7
	R-CAAAAGAGCAGCAGCAACGA				
EAAT3	F-TGCTGCTTTGGATTCCAGT	60	79	XM_424930	97
	R-AGCAATGACTGTAGTGCAGAAGTAATATAG				
GAPDH	F-GCCGTCCTCTCTGGCAAAG	60	73	NM_204305	99
	R-TGTAAACCATGTAGTTCAGATCGA				
β-ACTIN	F- GGAAGTTACTCGCCTCTG	60	114	LO8165	101
	R-AAAGACACTTGTTGGGTTAC				

1.4-1.9 and 260/280 1.7-1.9 was used for cDNA preparation. The 1 µg of DNAse treated RNA sample was used for reverse transcription in a final volume of 20 µL using random hexamers according to the manufacturer's protocol (Revert Aid™ first strand cDNA synthesis kit, Fermentas). Synthesized single-stranded cDNA was stored at -20°C till further use.

Quantitative real-time PCR (RTqPCR) analysis: Expression of SGLT1, GLUT5, PepT1 and EAAT3 mRNAs on day of hatch, post-hatch 3rd, 7th and 10th day was quantified using real time PCR (IQ5 Multicolor real-time PCR detection system, Bio-Rad laboratories Inc. USA). Beta-actin (ACTB) and GAPDH was used as a reference gene. Gene specific primers reported by Mott *et al.*[12] were used in this study (Table 1). All PCR were performed in 20 µL volume in triplicates. The reaction mixture contained 1XSYBR Green I Master Mix (2×DyNAmoTM HS, Finnzymes, USA), 50 nM of each gene-specific primer (both forward and reverse) and 2 µL of cDNA template and the remaining volume was adjusted with nuclease free water. PCR cycling conditions included initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 sec annealing at 60°C for 30 sec and extension at 72°C for 45 sec. For each gene of interest, negative and positive controls were also included. A melting curve analysis was performed for each sample after completion of amplification and analyzed in comparison to negative and positive controls to determine the specificity of PCR reaction. Results were expressed in terms of the threshold cycle value (Ct). The efficiency of the primer pairs was calculated as follows: A serial dilution of the template was run with each primer pair and the log of the dilution was plotted against the cycle threshold (ct) value of each dilution. The slope of the resulting regression equation was used to calculate the efficiency of the PCR using E = 1+10^(1/slope) equation. The PCR efficiency for gene studied is given in Table 1.

Post-hatch performance parameters: Data on post-hatch performance like body weight, feed intake, body weight gain, Feed Conversion Ratio (FCR) at weekly intervals and daily mortality (%) was recorded up to 5 week of age.

Statistical analysis: Levels of expression of SGLT1, GLUT5, PepT1 and EAAT3 mRNAs were analysed using the relative expression software, REST-2009 (Corbet Research and M. Pfaffl) and expressed as fold change relative to control. The software analyses the Ct values of gene of interest and reference genes in control and treated samples. There are a set of control (CGOI) and sample (SGOI) Ct values for the gene of interest and similarly a set of controls (CREF) and sample (SREF) for the reference gene. An efficiency value (eGOI) for the gene of interest (GOI) and an efficiency value (eREF) for the reference gene are calculated. Data generated from post-hatch performance parameters were analyzed using analysis of variance and means were compared using Duncan's multiple range test/LSD.

RESULTS AND DISCUSSION

Post-hatch performance as influenced by elevated incubation temperature: Variety showed a significant effect on day old chick weight with the values being higher for UTTAM compared to PEARL. There was significantly ($p \leq 0.05$) decrease in day old chick weight at elevated incubation temperature in UTTAM variety. Weekly body weight, feed intake, body weight gain was significantly ($p \leq 0.05$) lower at 39.5°C in comparison to control (37.5°C) and 38.5°C (Table 2-4). Whereas, these were comparable in last two groups. Reduced ($p<0.01$) feed efficiency was found at elevated incubation temperature of 39.5°C during 2-4 weeks as compared to incubation temperature of 37.5 and 38.5°C (Table 5) and this might be due to poor maturation level of

Table 2: Effect of elevated incubation temperatures on the relative fold expression of SGLT1 gene in jejunal segments of Japanese quail as compared to normal incubation temperature (37.5°C)

			Fold expression of SGLT1 gene			
Temperature (°C)	Varieties	Variable	DOH	3rd day	7th day	10th day
38.5	PEARL	R. expression	1.807	3.743*	7.268*	6.454*
		sE[1]	0.932-4.519	1.587-7.601	4.224-12.754	4.847-8.770
		95% CI	0.806-4.972	1.143-10.612	3.441-18.293	4.197-11.921
		p-value	0.148	0.017	0.01	0.013
	UTTAM	R. expression	3.484*#	6.409*#	10.128*	5.997*#
		sE[1]	1.651-5.436	2.753-11.188	8.348-12.786	4.485-8.039
		95% CI	1.651-5.436	2.212-33.182	7.800-13.839	3.833-11.070
		p-value	0.003	0.004	0.006	0.006
39.5	PEARL	R. expression	2.329*	5.265	15.931*	2.591*
		sE[1]	1.238-5.562	2.445-12.144	6.470-34.538	1.483-5.056
		95% CI	0.990-6.902	1.378-17.316	4.142-51.476	1.370-5.625
		p-value	0.046	0.057	0.001	0.01
	UTTAM	R. expression	4.87*#	16.398*	22.265*	5.686*
		sE[1]	3.528-6.400	7.524-34.338	17.269-28.267	2.847-10.457
		95% CI	3.107-6.747	5.259-64.679	15.721-30.597	2.242-19.109
		p-value	0. 001	0.004	0.002	0.001

[1]Measurement of uncertainty in expression ratios is addressed by randomisation and bootstrapping to provide a range of possible estimates, *Indicates significant difference ($p \leq 0.05$) and #Indicates significant difference ($p \leq 0.05$) between varieties (PEARL vs UTTAM)

Table 3: Effect of incubation temperatures on the weekly feed intake (g)

		1st	2nd	3rd	4th	5th
Temperature	Variety	Weeks				
Interaction effect (°C)						
37.5	PEARL	23.29^{q}	47.00	65.00	95.33	107.33
	UTTAM	30.33^{r}	57.33	92.67	127.33	166.33
38.5	PEARL	23.22^{q}	47.00	65.00	95.33	104.00
	UTTAM	29.00^{r}	57.00	90.67	126.00	164.67
39.5	PEARL	15.33^{p}	38.33	56.67	85.67	87.33
	UTTAM	24.67^{q}	48.33	83.00	115.67	145.33
Pooled SEM		1.305	2.407	3.437	4.917	6.863
Temperature	37.5°C	26.81^{B}	52.17^{B}	78.83^{B}	111.33^{B}	136.83^{B}
	38.5°C	26.11^{B}	52.00^{B}	77.83^{B}	110.67^{B}	134.33^{B}
	39.5°C	20.00^{A}	43.33^{A}	69.83^{A}	100.67^{A}	116.33^{A}
Variety	PEARL	20.61^{a}	44.11^{a}	62.22^{a}	92.11^{a}	99.56^{a}
	UTTAM	28.00^{b}	54.22^{b}	88.78^{b}	123.00^{b}	158.78^{b}
Significance	Temperature	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
	Variety	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
	Interaction	p<0.05	NS	NS	NS	NS

^{p,q,r}Indicates significant difference (p<0.05), ^{a,b}Indicates significant difference (p<0.01) between varieties, ^{A,B,C}Indicates significant difference (p<0.01) between temperatures and NS: Non significant

enterocyte along the villus affecting carbohydrate nutrient uptake despite of feed availability in the gut. In general, significantly ($p \leq 0.05$) better FCR was observed in UTTAM variety of Japanese quail. Post-hatch mortality increased progressively at elevated incubation temperatures (Table 6), with comparable overall mortality in both varieties. Thus, hatching as well as post-hatch performance was affected negatively at elevated incubation temperatures.

Relative fold expression of SGLT1 gene: At incubation temperature 38.5°C, expression of SGLT1 gene (Fig. 1) was significantly ($p = 0.003$-0.006) upregulated at all the studied days (DOH, 3rd, 7th and 10th) in CARI-UTTAM variety in comparison to control (37.5°C). Whereas, the expression of SGLT1 gene was significantly ($p = 0.01$-0.017) upregulated in CARI-PEARL variety at 3rd, 7th and 10th day as compared to control. Except on day 10, the over expression was nearly two folds (1.40-1.95) higher in CARI-UTTAM as compared to CARI-PEARL variety. UTTAM variety showed relatively higher expression of SGLT1 gene at DOH on 3rd and 7th day but revealed lower than PEARL variety on 10th day.

Similarly, at incubation temperature 39.5°C expression of SGLTI gene was upregulated at all the studied days in CARI-PEARL ($p = 0.001$-0.057) as well as in CARI-UTTAM ($p = 0.001$-0.004) variety in comparison to their respective controls. The level of over expression was nearly two folds

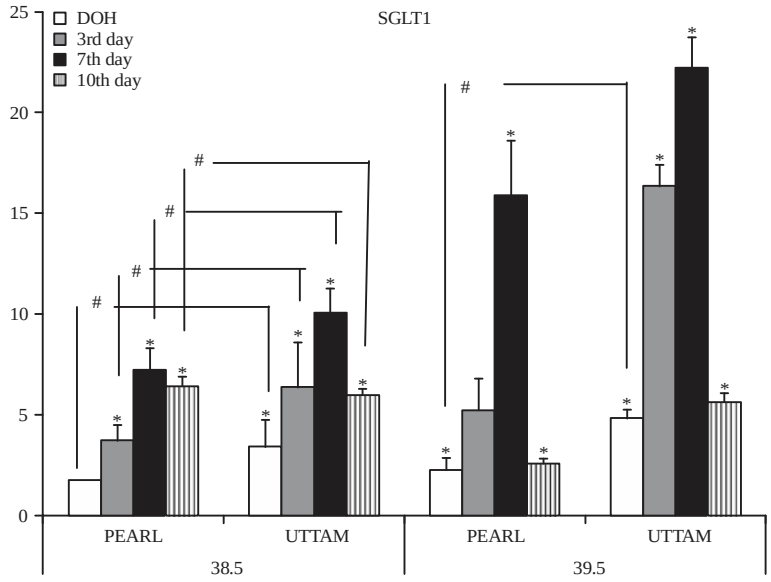

Fig. 1: Effect of elevated incubation temperatures on the relative fold expression of SGLT1 gene in jejunal segments of Japanese quail as compared to normal incubation temperature (37.5°C), *Significance within variety ($p \leq 0.05$) and #Significance between variety ($p \leq 0.05$)

Table 4: Effect of incubation temperatures on weekly body weight gain (g)

Temperature	Variety	1st	2nd	3rd	4th	5th
		----------	----------	Weeks	----------	----------
Interaction effect (°C)						
37.5	PEARL	12.12^{q}	20.62	25.99	35.85	38.65
	UTTAM	16.34^{s}	27.40	39.63	50.25	62.87
38.5	PEARL	11.94^{q}	20.44	26.37	36.41	37.00
	UTTAM	15.13^{r}	27.74	40.86	51.53	62.67
39.5	PEARL	7.07^{p}	14.97	21.03	31.74	30.00
	UTTAM	12.34^{q}	21.12	32.79	44.27	53.79
Pooled SEM		0.725	1.120	1.820	1.836	3.178
Temperature	37.5°C	14.23^{B}	24.01^{B}	32.81^{B}	43.05^{B}	50.76^{B}
	38.5°C	13.53^{B}	24.09^{B}	33.62^{B}	43.97^{B}	49.83^{B}
	39.5°C	9.705^{A}	18.05^{A}	26.91^{A}	38.01^{A}	41.90^{A}
Variety	PEARL	10.38^{a}	18.68^{a}	24.46^{a}	34.67^{a}	35.22^{a}
	UTTAM	14.6^{b}	25.42^{b}	37.76^{b}	48.68^{b}	59.78^{b}
Significance	Temperature	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
	Variety	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01
	Interaction	p<0.01	NS	NS	NS	NS

^{p,q,r,s}Indicate significant difference (p<0.01), ^{a,b}Indicates significant difference (p<0.01) between varieties, ^{A,B,C}Indicates significant difference (p<0.01) between temperatures and NS: Non significant

higher in CARI-UTTAM as compared to CARI-PEARL at DOH. Further results indicated higher expression pattern of SGLT1 was observed on 7th day in both varieties exposed to elevated incubation temperatures (38.5 and 39.5°C). Results in the present study indicate at elevated incubation temperatures (38.5 and 39.5°C) significant upregulation of SGLTI gene expression was observed in both the varieties in comparison to control (37.5°C) except in CARI-PEARL variety on DOH at incubation temperature 38.5°C and on 3rd day at incubation temperature 39.5°C. Relative fold expression increased progressively up to 7th day thereafter it decreased (Fig. 1). Overall the relative fold expression was comparably higher in CARI-UTTAM variety on DOH, 3rd and 10th day at incubation temperature 38.5°C, while at 39.5°C expression was higher on DOH. Results are well in agreement with the previous work of Garriga *et al.*[11] who observed that elevated temperature causes metabolic and physiological changes in the intestinal transport of glucose through the increase of SGLT1 gene.

Table 5: Effect of incubation temperatures on weekly feed conversion ratio

Temperature	Variety	1st	2nd	3rd	4th	5th
		----------	----------	Weeks	----------	----------
Interaction effect (°C)						
37.5	PEARL	1.92	2.28	2.50	2.66	2.78
	UTTAM	1.86	2.09	2.34	2.50	2.65
38.5	PEARL	1.95	2.30	2.47	2.62	2.79
	UTTAM	1.94	2.09	2.22	2.48	2.65
39.5	PEARL	2.18	2.56	2.70	2.71	2.92
	UTTAM	1.99	2.31	2.54	2.61	2.70
Pooled SEM		0.042	0.048	0.040	0.023	0.030
Temperature	37.5°C	1.89	2.19^{A}	2.42^{A}	2.58^{A}	2.71
	38.5°C	1.94	2.19^{A}	2.34^{A}	2.55^{A}	2.72
	39.5°C	2.08	2.43^{B}	2.62^{B}	2.66^{B}	2.81
Variety	PEARL	2.02	2.38^{b}	2.55^{b}	2.66^{b}	2.83^{b}
	UTTAM	1.93	2.16^{a}	2.37^{a}	2.53^{a}	2.67^{a}
Significance	Temperature	NS	p<0.01	p<0.01	p<0.01	NS
	Variety	NS	p<0.01	p<0.01	p<0.01	p<0.01
	Interaction	NS	NS	NS	NS	NS

^{a,b}Indicates significant difference (p<0.05) between varieties, ^{A,B,C}Indicates significant difference (p<0.05) between temperatures and NS: Non significant

Table 6: Effect of incubation temperatures on the total embryonic mortality (%) and post-hatch mortality (%)

Temperature (°C)	Variety	Embryonic mortality (%) Early	Mid	Late	Total	Post-hatch mortality (% overall)
37.5	PEARL	4.95	1.65	0	6.60	5.26
	UTTAM	5.64	1.13	0	6.67	4.81
38.5	PEARL	3.75	5.62	7.50	16.87	8.00
	UTTAM	6.74	8.42	3.36	18.52	9.80
39.5	PEARL	1.96	9.80	9.80	21.56	10.00
	UTTAM	5.02	11.73	6.70	23.45	12.00

Previous study reported that glucocorticoids released during stress, induce the expression of glucocorticoid-regulated kinase 1, which enhances glucose transportation by increasing SGLT1 abundance in the cell membrane Garriga *et al.*[11] and he also observed the capacity to take up hexose across the apical SGLT1 type transporter was increased by 50% in heat stressed chickens. Similarly, Mitchell and Carlisle[13] observed that chronic heat stress increases galactose absorption by 36%. Dale and Fuller[14] observed that the administration of corticosterone, hormone secreted during stress significantly increase calcium, phosphorus and glucose uptake in small intestines. Ferraris *et al.*[15] reported increase in active hexose by the intestine may be mediated by changes in number of actively absorbing cells, affinity of the transport systems and/or turnover. However, contrary to results Barri *et al.*[10] reported no significant difference due to elevated incubation temperatures in the relative fold expression of SGLTI gene in Ross Broiler Chickens. This difference may be due to different species used in the experiment.

Relative fold expression of GLUT5 gene: At incubation temperature 38.5°C, expression of GLUT5 gene (Fig. 2) was significantly (p = 0.02-0.046) upregulated at all the studied days in CARI-PEARL variety as compared to control. Whereas, expression of GLUT5 gene was significantly (p = 0.013-0.044) upregulated in CARI-UTTAM on 3rd, 7th and 10th day as compared to control. The level of over expression was comparably higher in CARI-UTTAM variety on 10th day as compared to CARI-PEARL variety. In general, an increased expression phenomenon of GLUT5 gene was observed in both varieties on 10th day compared to the respective controls. Likewise, at incubation temperature 39.5°C, expression of GLUT5 gene was significantly upregulated in CARI-PEARL (p = 0.012-0.04) as well as in CARI-UTTAM (p = 0.01-0.05) at all the studied days (DOH, 3rd, 7th and 10th) in comparison to control. The level of over expression was significantly higher in CARI-UTTAM as compared to CARI-PEARL except 7th and 10th day. Overall, an increased trend could be observed on 3rd day for GULT5 gene in both varieties.

In the present investigation significant upregulation in the relative fold expression of GLUT5 gene was observed (Fig. 2) except in CARI-UTTAM variety at incubation temperature 38.5°C on DOH as compared to control. At incubation temperature 38.5°C, there was progressive upregulation in

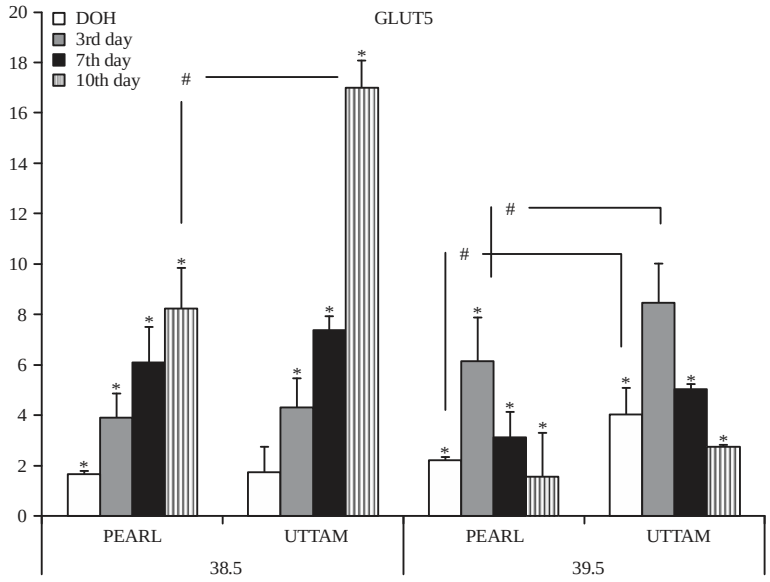

Fig. 2: Effect of elevated incubation temperatures on the relative fold expression of GLUT5 gene in jejunal segments of Japanese quail as compared to normal incubation temperature (37.5°C), *Significance within variety ($p \leq 0.05$) and #Significance between variety ($p \leq 0.05$)

relative fold expression. At incubation temperature 39.5°C higher gene expression was observed on 3rd day, thereafter it progressively decreased but remained significantly upregulated as compared to control. Between the two varieties comparably higher fold expression of GLUT5 gene was noticed in CARI-UTTAM variety at 10th day at 38.5°C and on DOH and 3rd day at 39.5°C. Barri *et al.*[10] reported no significant differences in the relative fold expression of GLUT5 gene at elevated temperature of incubation in broiler chicks. This discrepancy in findings may be due to different species used in the experiments. However, there is paucity of literature on this aspect in Japanese quail.

Relative fold expression of PepT1 gene: At incubation temperature 38.5°C, expression of PepT1 gene (Fig. 3) was significantly upregulated in CARI-PEARL ($p = 0.041$) as well as in CARI-UTTAM ($p = 0.04$) variety on 3rd day as compared to control. However, expression of PepT1 gene was higher in UTTAM variety on all studied days except 10th day as compared to CARI-PEARL. Whereas, at incubation temperature 39.5°C, significant ($p = 0.028$) upregulation of PepT1 gene was observed in CARI-UTTAM variety on day 3rd in comparison to control. In general, over expression of PepT1 gene was comparably higher in CARI-UTTAM as compared to CARI-PEARL variety at both the elevated incubation temperatures (38.5 and 39.5°C).

Results in the present study revealed significant upregulation in the relative fold expression of PepT1 gene on 3rd day post-hatch in CARI-UTTAM variety at both elevated incubation temperatures, whereas, significant upregulation was observed in CARI-PEARL variety at incubation temperature 38.5°C on 3rd day in comparison to control (Fig. 3). Further, CARI-UTTAM variety showed comparably higher fold expression on all studied day except 10th day at 38.5°C and on DOH and 3rd day at 39.5°C. Results are in accordance with Hu *et al.*[16] who reported increase in the expression of PepT1 gene during elevated temperatures. Barri *et al.*[10] reported no significant difference in relative gene expression of PepT1 on DOH and 2nd day post-hatch under elevated incubation temperature. However, they observed increase in the relative gene expression of PepT1 from 2nd-4th day in both control and treatment groups and by 6th day higher gene expression in control group. This discrepancy in results may be due to different sampling dates as well as due to varied species used in the investigations. Significant upregulation on 3rd day post-hatch in relative fold expression may indicate higher need of PepT1 transporter for uptake of dietary proteins for post-natal growth because by this time yolk sac had almost disappeared[17].

Relative fold expression of EAAT3 gene: At incubation temperature 38.5°C, expression of EAAT3 gene (Fig. 4) was significantly upregulated in CARI-PEARL ($p = 0.01$-0.028) as well as in CARI-UTTAM ($p = 0.04$-0.044) variety on 3rd and 10th day as compared to control (37.5°C). However, UTTAM variety showed higher expression at DOH and 7th day

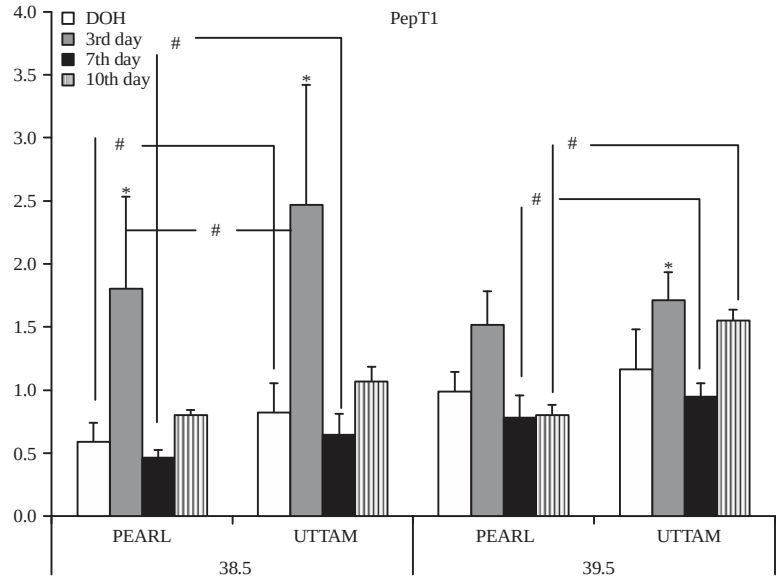

Fig. 3: Effect of elevated incubation temperatures on the relative fold expression of PepT1 gene in jejunal segments of Japanese quail as compared to normal incubation temperature (37.5°C), *Significance within variety ($p \leq 0.05$) and #Significance between variety ($p \leq 0.05$)

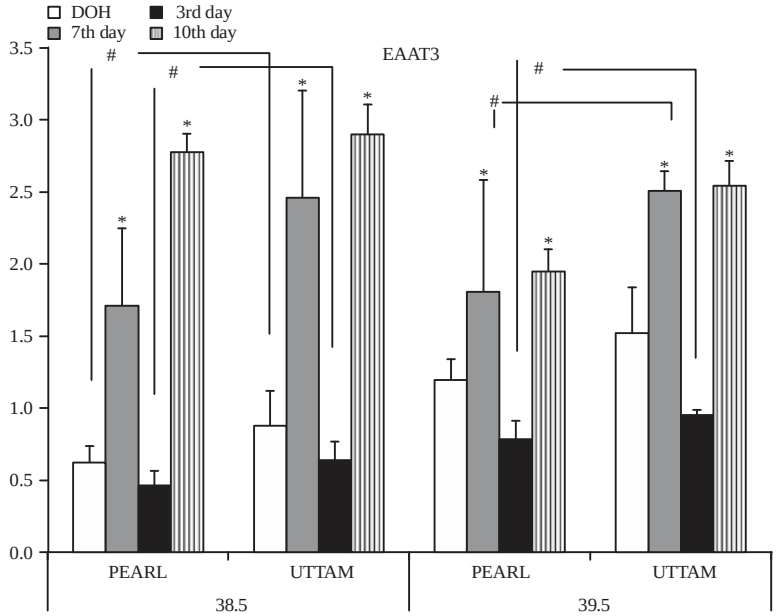

Fig. 4: Effect of elevated incubation temperatures on the relative fold expression of EAAT3 gene in jejunal segments of Japanese quail as compared to normal incubation temperature (37.5°C), *Significance within variety ($p \leq 0.05$) and #Significance between variety ($p \leq 0.05$)

post-hatch in comparison to PEARL variety. Similarly, at incubation temperature 39.5°C, expression of EAAT3 was significantly upregulated in CARI-PEARL ($p = 0.04$-0.046) as well as in CARI-UTTAM ($p = 0.022$) variety on 3rd and 10th day in comparison to control. The level of over expression of EAAT3 was comparably higher in CARI-UTTAM as compared to CARI-PEARL variety at 3rd and 7th day post-hatch.

Present investigation resulted in significant ($p = 0.01$-0.046) upregulation in the relative fold expression of EAAT3 gene on 3rd and 10th day post-hatch in both

varieties at elevated incubation temperatures (38.5 and 39.5°C) in comparison to control (37.5°C). Relative fold expression was comparably higher on 10th day post-hatch in both the varieties at both the elevated incubation temperatures. However, CARI-UTTAM variety showed comparably higher relative fold expression (Fig. 4). Hu *et al.*[16] reported that corticosterone administration increased the expression of intestinal nutrient transporter (EAAT3) mRNA of broiler chickens as a compensation for loss of absorptive area of the small intestines. Since, corticosterone is secreted during elevated temperatures, thus, results are in agreement with his study. However, Barri *et al.*[10] reported (in Ross Broiler Chickens) no significant changes in relative fold expression of EAAT3 at elevated incubation temperatures. This difference in results may be in part due to varied sampling dates and species used. Significant upregulation in the relative fold expression of EAAT3 gene on 3rd and 10th day post-hatch may indicate higher needs of excitatory amino acids aspartate and glutamate, the primary fuel for enterocytes[18].

This study indicates functional adaptation of quail embryos in response to elevated incubation temperatures. Intestinal mucosa is capable of rapid and extensive morphological and functional adaptations in response to evolutionary, genetic and ontogenetic demands[19], as well as to environmental and nutritional demands[20]. In general, all the transporter genes under study revealed a higher expression pattern at elevated incubation temperatures indicating a compensatory adaptation for prenatal growth depression as in chicken[21]. However, alternations in normal architecture of small intestine (weight and length[13]) might have posed long term effects on quail embryos with a transient decrease in post-hatch performance. In the present study, the birds exhibited lower weight gain as well as poor feed efficiency the possible reason may of reduced feed intake due elevated environmental temperature. Besides, thermal stress may reduce intestine (jejunum) weight and length[11] which may adversely affect performance. On the other hand, elevated incubation temperature caused increased expression of nutrient transporters, this may be due to adjustment in physiological needs as upregulation of intestinal transporters serves to provide a modest but adequate reserve of absorptive capacity in excess of current intake and downregulation serves to save biosynthetic energy and space by getting rid of transporters[22]. Furthermore, increased intestinal transport capacity is entirely dependent on adaptations of apical expression of transporters to heat stress and is not due to reduced food intake[11].

Further, among the two varieties studied CARI-UTTAM variety revealed higher relative fold expression of nutrient transporter genes. This may be due to differences in the sensitivities of the varieties to elevated incubation temperatures; it may also be due to the high body weight of CARI-UTTAM variety. Since, there is paucity of necessary information with regard to these varieties used; further detailed studies are needed on expression of nutrient transporter genes in Japanese quail.

In nut shell, it may be concluded that higher relative fold expression of most of the nutrient transporter genes in quail incubated at elevated temperatures may indicate higher susceptibility of jejunum to this temperature warranting higher fold expression as an adaptation mechanism. Further, it is also confirmed that heavier body weight CARI-UTTAM variety is comparably more sensitive to elevated incubation temperature as depicted by their overexpression of nutrient transporter genes studied.

REFERENCES

1. Minka, N.S. and J.O. Ayo, 2011. Behavioural responses, traumatic injuries and live weight losses in ostrich (*Struthio camelus*) chicks transported by road during hot-dry conditions. Arch. Tierz., 54: 636-648.
2. North, M.O. and D. Bell, 1990. Commercial Chicken Production Manual. 4th Edn., AVI Book, New York, USA., pp: 55-58.
3. French, N.A., 1997. Modeling incubation temperature: the effects of incubator design, embryonic development and egg size. Poult. Sci., 76: 124-133.
4. Geyra, A., Z. Uni and D. Sklan, 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci., 80: 776-782.
5. Amat, C., J.M. Planas and M. Moreto, 1996. Kinetics of hexose uptake by the small and large intestine of the chicken. Am. J. Physiol.-Regul. Integr. Comp. Physiol., 271: R1085-R1089.
6. Wright, E.M. and E. Turk, 2004. The sodium/glucose cotransport family SLC5. Pflugers Arch., 447: 510-518.
7. Kayano, T., C.F. Burant, H. Fukumoto, G.W. Gould and Y.S. Fam *et al.*, 1990. Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J. Biol. Chem., 265: 13276-13282.
8. Burant, C.F., J. Takeda, E. Brot-Laroche, G.I. Bell and N.O. Davidson, 1992. Fructose transporter in human spermatozoa and small intestine is GLUT5. J. Biol. Chem., 267: 14523-14526.
9. Kanai, Y. and M.A. Hediger, 1992. Primary structure and functional characterization of a high-affinity glutamate transporter. Nature, 360: 467-471.

10. Barri, A., C.F. Honaker, J.R. Sottosanti, R.M. Hulet and A.P. Mcelroy, 2011. Effect of incubation temperature on nutrient transporters and small intestine morphology of broiler chickens. Poult. Sci., 90: 118-125.
11. Garriga, C., R.R. Hunter, C. Amat, J.M. Planas, M.A. Mitchell and M. Moreto, 2006. Heat stress increases apical glucose transport in the chicken jejunum. Am. J. Physio. Regul. Integer. Comp. Physiol., 290: 195-201.
12. Mott, C.R., P.B. Siegel, K.E. Webb, Jr. and E.A. Wong, 2008. Gene expression of nutrient transporters in the small intestine of chickens from lines divergently selected for high or low juvenile body weight. Poult. Sci., 87: 2215-2224.
13. Mitchell, M.A. and A.J. Carlisle, 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (*Gallus domesticus*). Comp. Biochem. Physiol. A Comp. Physiol., 101: 137-142.
14. Dale, N.M. and H.L. Fuller, 1980. Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. Poult. Sci., 59: 1434-1441.
15. Ferraris, R.P., P.P. Lee and J.M. Diamond, 1989. Origin of regional and species differences in intestinal glucose uptake. Am. J. Physiol., 257: G689-G697.
16. Hu, X.F., Y.M. Guo, B.Y. Huang, L.B. Zhang and S. Bun *et al*., 2010. Effect of corticosterone administration on small intestinal weight and expression of small intestinal nutrient transporter mRNA of broiler chickens. Asian-Aust. J. Anim. Sci., 23: 175-181.
17. Romanoff, A.L., 1960. The Extraembryonic Membranes. In: The Avian Embryo: Structural and Functional Development, Romanoff, A.L. (Ed.)., Macmillan, New York, pp: 1042-1081.
18. Newsholme, P., M.M.R. Lima, J. Procopio, T.C. Pithon-Curi, S.Q. Doi, R.B. Bazotte and R. Curi, 2003. Glutamine and glutamate as vital metabolites. Braz. J. Med. Biol. Res., 36: 153-163.
19. Ferraris, R.P., 2001. Dietary and developmental regulation of intestinal sugar transport. Biochem. J., 360: 265-276.
20. Julian, R.J., 2005. Production and growth related disorders and other metabolic diseases of poultry-A review. Vet. J., 169: 350-369.
21. Tur, J.A., R.V. Rial, J. Tur and B. Planas, 1987. Effect of thyroidal state on the gastrointestinal transit and emptying of young broilers. Comp. Biochem. Physiol. A Comp. Physiol., 87: 665-670.
22. Diamond, J., 1991. Evolutionary design of intestinal nutrient absorption: Enough but not too much. Physiology, 6: 92-96.