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Thermal stress impaired expression of insulin-like growth factor binding proteins in pigs

S BORAH[1], S SOREN[1], R TALUKDAR[2], L KALITA[3✉], K PAME[1], A J NATH[1], B BORAH[1], P K PATHAK[4] and B N BHATTACHARRYYA[2]

Assam Agricultural University, North Lakhimpur, Assam 787 051 India

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ABSTRACT

This study aimed to investigate the correlation of IGFs, IGFBPs, and IGFBPRs of grower pigs with change in temperature humidity index (THI). THI was calculated to know the months of the year when pigs feel discomfort and samples of feed offered were analyzed to know the nutritional availability/deficit. The calculated average THI was above thermal comfort zone for seven months in a year. It was observed that crude protein (CP) and metabolizable energy (ME) availability to pigs were deficient. Gilts of two months age and similar body weight were randomly divided into two groups (n=6); viz. *Con-fed* animals (feed offered by the farmer) and *For-fed* animals (formulated feed supplement + Con-fed). The composition of formulated feed supplement was dried leaf of *Moringa oleifera*, soybean meal, rice mill waste, and mineral mixture. The plasma cortisol level increased in the experimental animals with accelerated THI as evidenced by the strong positive correlation of THI with HSP70 and HSP90. The level of leptin and ghrelin did not have significant relation with the expression level of HSPs in the present study, however, in the control animals, a low level of leptin was observed. The correlation between the growth factors and HSPs was not significant. During the season, where the THI was above the thermal comfort zone, the IGFBPR and IGFBP concentrations were non-significantly increased along with the increased THI.

Keywords: Binding proteins, Grower pigs, Insulin-like growth factors, Receptors,Temperature humidity index

An imbalance between metabolic heat production inside the animal body and its dissipation results in heat stress under high air temperatures and humid climates. Heat stress is considered one of the major concerns in pig farming as they do not possess functional sweat glands to dissipate body heat efficiently. Pig increases respiratory rates to enhance evaporative cooling during heat stress. An increase in environmental temperature has a direct negative effect on the appetite centre of the hypothalamus to decrease feed intake (Baile and Forbes 1974) thereby, reduces growth rate and (re)productive performance. Pre-pubertal gilts often undergo severe stress during critical weather conditions due to a marked negative influence on feed intake (Goodman *et al.* 2004). Furthermore, the association of higher relative humidity and high ambient temperature enhances the multiplication of pathogens leading to environmental pathogenic pressure. Increased pathogenic pressure results in the stimulation of the immune system to produce cytokines leading to alteration in the neuroendocrine system of the animals. Increased production cytokines and imbalanced neuroendocrine, negatively affect the growth of pigs (Johnson 2012). Poor feed conversion efficiency influences (re)productive performance of female pigs through delayed puberty, decrease ovulation rate, and embryonic survivability.

Kisspeptin binds with G-protein-coupled receptors in the hypothalamus resulting in the release of GnRH (Seminara and Crowley 2008). A disruption in kisspeptin signaling is associated with hypogonadotropic hypogonadism and can result in delayed onset of puberty (de Roux *et al*. 2003).

Heat stress also results in increased adipose tissue deposition (Kouba *et al*. 2001) and leptin secretion (Block *et al*. 2001), which influences appetite and energy expenditure (Friedman 2010). Moreover, ghrelin influence on appetite, fattening, and reproduction via input to the Hypothelamo-Pituitary-Gonadal axis (Tena-Sempere 2005). Because heat stress has a marked negative influence on feed intake and it may affect the secretion of ghrelin leading to decreased reproductive performances. Therefore the present experiment was conducted to study the correlation of productive and reproductive performances with temperature humidity index (THI) and heat shock proteins (HSPs). The study also investigated the ameliorative effect of nutrient supplementation on heat stress and productive performances in experimental animals.

Present address: [1]Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur, Assam. [2]College of Veterinary Science, Assam Agricultural University, Guwahati, Assam. [3]Kamrup Polytechnic, Baihata Chariali, Kamrup, Assam. [4]KVK, Assam Agricultural University, North Lakhimpur, Assam. ✉Corresponding author email: sbsborah06@gmail.com

MATERIALS AND METHODS

The present study was conducted in the North bank plain agro-climatic zone of Assam. To conduct the study, THI of previous five years (year 2014-2018) was calculated to know the months of the year when pigs feel discomfort. Data on the diet offered to the pigs were collected to know about the nutritional availability and deficit. On the basis of thermal stress data, the study was conducted from the month of March to October 2019, as it was observed that the average THI from March to October was >70 (Fig. 1). Serum biochemicals for growth [(Insulin-Like Growth Factor (IGF-1, IGF-2), its receptors (IGF-1R, IGF-2R) and binding proteins (IGFBP-1, IFGBP-2)]; nutritional stress (Leptin, Ghrelin); reproductive performance (Estradiol, Kisspeptin, FSH, and Progesterone) and thermal stress [Heat Shock Proteins (HSP70, HSP90), cortisol] were targeted for the present study.

Serum samples were collected and expression of different targeted biochemicals was estimated by using commercially available kits following standard procedures of ELISA. Twenty four hours of meteorological data was collected from Regional Agricultural Research Station, Assam Agricultural University, North Lakhimpur, Assam from the year 2014 to 2019, and THI was calculated (NRC (1971). The THI data of the year of experiment (year 2019) was considered to correlate with different targeted serum biochemicals.

A feed supplement was formulated based on nutritional availability/ deficiency (Table 1) to ameliorate thermal/ nutritional stress in growing pigs. For the experiment, gilts of two months of age with similar body weight were randomly divided into two groups (n=6); viz. *Con-fed* animals (feed offered by the farmer) and *For-fed* animals (formulated feed supplement + Con-fed). The composition of formulated feed supplement was dried leaf of *Moringa oleifera* (43.53%), soybean meal (43.53%), rice mill waste (broken rice-11.44%) and mineral mixture (1.5%). The leaves of *M. oleifera* were shade-dried for three days and subsequently pulverized using a grinder. The powdered samples were then sieved to remove the debris and were used for feed supplements. The aim of the study was to correlate THI, productive and reproductive performance with HSPs; to correlate growth factors (IGF-1, IGF-2), receptors (IGF-1R, IGF-2R), and binding proteins (IGFBP-1, IFGBP-2) with the THI. Estimation of serum growth factors, receptors, and binding proteins was done using commercially available kits following standard procedures of ELISA. Body weight of the animals was measured at 15 days intervals during the experimental period. Data generated were analyzed using the Pearson correlation coefficient and Student's t-test (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The calculated average THI revealed inclement weather for the productive and reproductive performances of pigs from April to October since 2014-2018 (Fig. 1) in the area of study. The THI ranged from 71.51 to 85.13 during the period and highest THI was observed during the month of July 2012 – 2019. During the month of July 2012 – 2019, the THI ranged from 79.58 to 83.75.

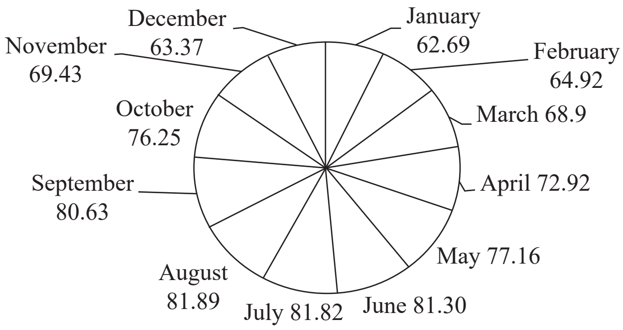

Fig. 1. THI in the area of study.

The THI can be considered as an indicator of thermal stress in animals as an estimation of values of meteorological variables associated with specific rectal temperatures of the animals (Dikmen and Hansen 2009). Livestock species are comfortable at THI values between 65 and 72. Mild, medium, and severe stress was classified (Habeeb *et al.* 2018) on the basis of THI range, i.e. 72-80 (mild), 80–90 (medium), and 90–98 (severe). In the present study, it was observed that the animals reared in the area of study are under thermal stress for seven months (April to October) in a year (Fig. 1). It is well described that when the THI exceeds from >72, the productive and reproductive performances can be affected in farm animals. Moreover,

Table 1. Average nutrient requirement and deficit per pig per day in various age groups of *Con-feed* animals (Calculated as per the DM requirement and CP and ME concentration)

Age group	Feed DM (Kg)		CP (kg)		ME (kcal)	
	Requirement	Deficit	Requirement	Deficit	Requirement	Deficit
3 months	1	0.157±0.04 (15.66%)	0.180	0.094±0.006 (52.57%)	3170	717±19 (22.6%)
4 months	1.25	0.231±0.05 (18.55%)	0.225	0.114±0.011 (50.78%)	3963	1005±40 (25.35%)
5 months	1.5	0.140±0.03 (9.35%)	0.270	0.120±0.013 (44.0%)	4755	763±22 (16.05%)
6 months	2	0.207±0.09 (10.35%)	0.360	0.150±0.015 (41.53%)	6340	1105±36 (17.44%)

pigs lack a well-developed cutaneous thermoregulatory mechanism that makes this species more uncomfortable with the changing environment.

The data on amount of feed offered to different age groups of pig (*Con-fed*) viz. 2-3 months, 3-4 months, 4-5 months and 5-6 months were collected. It was observed that nutrient availability to pigs (*Con-fed*) from the offered feed were deficient in CP and ME requirements as per NRC / BIS of pig feeding (Table 1). To calculate the nutrient requirement and deficit, it was assumed that the pigs acquired 25% of required nutrient through scavenging.

It revealed that the animals reared in the area of study were under thermal stress during seven months in a year (Fig. 1) and nutritional deficiency (Table 1) throughout the year.

Correlation coefficient of HSPs, THI and cortisol: The THI of the study area was observed higher than comfortable level (THI 72) during the month of May (THI 75), June (THI 80), July (THI 79), August (THI 82) and September (THI 78). Similarly, the expression level of HSPs (HSP70, HSP90) also increased ($p<0.05$) during these months. The expression of HSPs indicates the thermals stress (Cedraz *et al*. 2017) and most of the domestic animals suffer stressful conditions when the THI value increases beyond THI 72 (Mello *et al*. 2015). The plasma cortisol level of the samples from the growing pigs showed a positive correlation with the expression level of HSPs. Higher expression of HSP70 is well established in animals under thermal stress as HSP70 protect cellular damage through preventing aggregation of protein within the cell (Hassan *et al*. 2019). However, the expression of HSP90 is shown to increase in those animals that are under nutritional stress to protect the those proteasome of cells from oxidative inactivation (Castro *et al*. 2019). The positive correlation between HSPs and cortisol in the present study revealed the heat stress and nutritional stress of the growing pigs (Table 2) in the area of study. Further, the THI value also showed a strong positive correlation (r=0.881 & 0.919) with HSP70 and HSP90 (Table 2) respectively.

Table 2. Correlation coefficient of HSPs, THI and Cortisol

	HSP70	HSP90	THI	Cortisol
HSP70	1			
HSP90	0.965954	1		
THI	0.881895	0.919736	1	
Cortisol	0.596215	0.680336	0.708705	1

Correlation of THI, productivity and reproductive performance with HSP: Body weight gain was considered for the productive performance of the pigs in the present study. The body weight of the animals depends on different factors including the nutritional status of the animals. It was observed that the feed stuff offered (Table 1) to the pigs in our study was inadequate in protein and energy content as per requirement of the pigs. The body weight gain of the growing pigs was lower in *Con-fed* animals as compared to *For-fed* diet (Table 3). Lower body weight gain in *Con-fed* animals might be due to nutritional insufficiency and

Table 3. Body weight of experimental animals

Group	Period of treatment (days)															
	0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225
Con-fed	4.3± 0.31	5.55± 0.66	8.87± 0.77	10.67± 1.38	15.02± 1.02	15.87± 1.22	17.77± 0.97	20.25± 1.99	23± 1.59	24± 3.03	26.5± 2.58	30.25[a]± 1.35	34[a]± 0.93	38.73[a]± 1.98	42.23[a]± 0.53	44.98[a]± 2.00
For-fed	4.55± 0.16	5.72± 0.98	9.87± 0.96	11.05± 1.78	15.1± 2.05	16.1± 1.54	19.09± 0.43	21.10± 1.20	23.35± 1.91	26.45± 1.13	28.35± 2.03	31.70[b]± 2.50	36.50[b]± 5.32	39.42[b]± 0.24	44.52[b]± 3.26	48.52[b]± 3.23

Table 4. Correlation coefficient of THI, productivity and reproductive performance with HSP and hormones

	HSP70	HSP90	THI	Body weight	IGF-I	IGF-II	Leptin	Ghrelin	Estradiol	Kisspeptin	FSH	Progesterone
HSP70	1											
HSP90	0.965	1										
THI	0.881	0.919	1									
Body weight	0.250	0.377	0.448	1								
IGF-1	0.334	0.420	0.567	0.921	1							
IGF 2	0.124	0.264	0.328	0.984	0.878	1						
Leptin	0.412	0.536	0.584	0.880	0.926	0.861	1					
Ghrelin	0.178	0.315	0.352	0.980	0.866	0.995	0.875	1				
Estradiol	0.138	0.263	0.319	0.989	0.892	0.993	0.855	0.987	1			
Kisspeptin	0.215	0.275	0.353	0.875	0.718	0.856	0.586	0.852	0.863	1		
FSH	0.519	0.635	0.747	0.921	0.924	0.855	0.887	0.855	0.857	0.775	1	
Progesterone	0.195	0.172	0.432	0.546	0.589	0.458	0.321	0.4240	0.496	0.681	0.629	1

environmental stress. Because stress response comprises a series of reactions involving the hypothalamic–pituitary–adrenocortical and sympatho-adrenomedullary axes (Lopresti 2020) leading to improper growth. It is well established that the growth hormone influences the level of insulin like growth factors (IGFs) for the growth of the body mass.

The present study showed the positive correlation (0.921 & 0.984) between the IGF-1 & IGF-2 with the body weight (Table 4). However, the correlation between the growth factors and HSPs was not significant. In the present study, growth was observed in both the groups, however, the growth in *For-fed* animals was higher (P<0.01) from the day 165 of treatment. The higher growth in the *For-fed* animals might be due to incorporation of *M. oleifera* leaves that contain rich amount of vital phytonutrients and protein content, ranging from 22.99-29.36% (Sultana 2020). In *Con-fed* animals, the lower body weight gain might be due to deficiency of CP and ME requirements (Table 1).

The level of leptin and ghrelin hormone did not have significant relation with the expression level of HSPs in the present study (Table 4). This might be due to the synergistic effect to ameliorate thermal stress by the phytonutrients present in *M. oleifera* (Yasoob *et al*. 2021) and minerals (Weller *et al.* 2013) added in the feed supplement to ameliorate thermal stress in the experimental animals. In the present experiment, it was observed that the animals of *For-fed* group increased (P<0.01) body weight (Table 3) from the day 165 of experiment. The lower growth rate in *Con-fed* group clearly revealed that this group of animals was affected with nutritional and environmental stress in comparison to the *For-fed* animals. The higher body weight in *For-fed* animals might be due to higher (P<0.01) concentration of IGF-1 (Table 6) from day 150 of treatment.

The reproductive performance of animals can be analysed based on the hormonal status of the body. The levels of estrogen shown have a strong positive correlation (r = 0.989, 0.892, 0.995 and 0.987) (Table 4) with the body weight, IGF-1, IGF-2 and ghrelin. This indicated that the feed supplement offered to the *For-fed* animals could promote proper growth necessary for the reproductive performances. Further, the feed supplement used in the present study could establish a strong positive correlation of FSH, kisspeptin with the body weight, growth factors, estrogen in *For-fed* animals. It is necessary to mention that the plasma concentration of these hormones was elevated in *For-fed* animals compared to *Con-fed*. The age at puberty in *Con-fed* animals was delayed because the estimation of progesterone concentration indicated an almost steady level till the ninth month of age (day 210 of experiment) in *Con-fed* animals. In contrast, *For-fed* animals showed an increase (p<0.01) in progesterone concentration after eight months (day 210-225 of experiment) of age (Table 6) indicating ovulation (pubety). This revealed that the *For-fed* animals could acquire adequate protein, energy and minerals from the feed supplement to augment (re) productive performance. This finding also demonstrates that the stress (nutritional/thermal) decreases the secretion of hormones involved in the hypothalamic–pituitary–

Table 5. Correlation coefficient of growth factors, its receptors and binding proteins with THI

	THI	IGF-I	IGF-II	IGF BP-I	IGF BP-II	IGF-1R	IGF-2R
THI	1						
IGF-1	0.567	1					
IGF-2	0.328	0.878	1				
IGF BP-1	0.090	0.840	0.894	1			
IGF BP-2	0.768	0.795	0.621	0.434	1		
IGF-1R	0.515	0.959	0.957	0.836	0.755	1	
IGF-2R	0.5404	0.830	0.937	0.740	0.701	0.926	1

Table 6. Serum progesterone, IGF, IGFR and IGFBP concentration in different groups during different days of experiment

Parameter	Treatment	Period of treatment (days)															
		0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225
Progesterone (ng/ml)	*Con-fed*	0.64 ± 0.01	0.67± 0.02	0.68± 0.01	0.7± 0.007	0.6± 0.03	0.64± 0.02	0.74± 0.03	0.71± 0.01	0.72± 0.008	0.70± 0.007	0.62± 0.01	0.67± 0.02	0.65± 0.03	0.57± 0.01	0.71± 0.01	0.73± 0.02
	For-fed	0.67± 0.01	0.67± 0.03	0.69± 0.03	0.7± 0.06	0.68± 0.03	0.69± 0.008	0.74± 0.02	0.74± 0.02	0.74± 0.06	0.69± 0.04	0.65± 0.01	0.66± 0.04	0.66± 0.03	0.59± 0.06	0.87^{a}± 0.04	0.88^{a}± 0.04
IGF-1 (ng/ml)	*Con-fed*	125.31± 1.91	124.12± 1.96	125.31± 1.91	128.37± 1.19	131.98± 1.68	131.87± 1.69	126.6± 3.91	126.87± 2.41	123.3± 6.07	131.12± 4.81	131.9± 1.63	133.62± 5.23	134.31± 3.00	138.37± 2.34	146.6± 2.59	147.3± 1.90
	For-fed	126.98± 0.88	127.62± 0.96	120.31± 4.91	132.62± 4.39	136.98± 4.17	132.62± 8.78	140.3± 3.75	130.12± 3.22	136.9± 4.90	138.62± 2.80	146.98^{a}± 7.79	149.62^{a}± 3.83	162.65^{a}± 2.73	142.1^{a}± 2.53	154.3^{a}± 5.46	153.87^{a}± 4.24
IGF-1R (ng/ml)	*Con-fed*	0.96± 0.18	1.00± 0.17	1.04± 0.17	1.30± 0.08	1.44± 0.16	1.21± 0.06	1.91± 0.43	1.67± 0.20	1.59± 0.15	2.38± 0.39	2.09± 0.38	2.27± 0.41	2.14± 0.46	2.45± 0.22	2.69± 0.43	2.79± 0.41
	For-fed	0.97± 0.02	0.98± 0.01	1.34± 0.37	1.08± 0.05	1.18± 0.15	1.37± 0.12	1.34± 0.17	1.65± 0.04	1.76± 0.44	1.31± 0.16	2.26± 0.23	2.01± 0.07	1.69± 0.22	1.68± 0.23	2.31± 0.24	2.14± 0.06
IGF-BP-1 (ng/ml)	*Con-fed*	11.87± 0.88	13.12± 1.14	17.12± 1.29	18.13± 1.30	20.88± 1.25	21.13± 0.57	22.13± 1.27	22.63± 1.01	23.38± 1.05	24.63± 0.81	25.63± 0.37	26.13± 0.60	26.63± 1.04	29.38± 2.04	31.13± 1.98	31.88± 2.18
	For-fed	13.12± 0.52	14.62± 0.99	16.37± 0.98	16.13± 1.07	21.13± 1.81	23.13± 1.50	23.38± 1.30	23.63± 1.12	24.13± 1.08	25.38± 0.63	26.13± 0.51	27.13± 0.52	28.88± 0.74	30.13± 1.39	31.38± 1.91	32.63± 1.71

adrenocortical and sympathy-adrenomedullary axes (Lopresti 2020) leading to improper growth in pigs.

Correlation of growth factor (IGF-1, IGF-2), receptor (IGF-1R, IGF-2R) and binding protein (IGFBP-1, IFGBP-2) with THI: The plasma concentration of the growth factors (IGF-1 and IGF-2) increased along with the age of the pigs. The concentration of IGF-1 and IGF-II was higher ($P<0.01$) in *For-fed* animals compared to the *Con-fed* during the period of study. Growth factors play significant role in amelioration of heat stress (Mayorga *et al*. 2019) apart from controlling growth. Therefore, the higher level of growth factors can be considered as a good indicator to know the effectiveness of the experimental feed offered to *Con-fed* animals. However, significant correlation between the THI and the growth factors were not found in the present experiment. IGF-BP1 and IGF receptors did not have significant correlation with the THI except the IGF-BP2 (Table 5). The study was conducted on growing pigs (from the age of two months age) and the level of growth factors were increased with the growth of the experimental animals. However, the *Con-fed* animals showed lower growth along with lower concentration of growth factors as compared to the *For-fed* groups. During the summer season, when the THI (Fig. 1) was >75 (from the month of May to September), the expression of growth factor receptors increased non-significantly (Table 6) along with the increased THI. Similarly, the binding proteins for growth factors also increased (Table 6). The correlation study could not find any significant correlation between THI and the growth factors, receptors and the binding proteins (Table 5). These variations might be due to various other factors on the growing pigs reared under semi-intensive condition of rearing.

Thermal stress has a marked negative influence which may lead to decreased (re)productive performances in pigs as this species lacks well developed cutaneous thermoregulatory mechanism. HSPs protect cellular damage through preventing aggregation of protein within the cell when exposed to stress. Present study revealed a positive correlation of THI values with HSP70, HSP90 and IGF-BP2 that lead to impaired performances of pigs during thermal stress.

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