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Alterations in prolactin and cortisol levels in heat stressed pigs from arid tracts in India

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Abstract. A study was carried out in order to evaluate modulations in prolactin levels caused by heat stress in indigenous pigs from arid tracts in Rajasthan, India. Porcine serum prolactin and cortisol levels were determined by radioimmunoassay, from samples collected during two seasons when maximum environmental temperatures ranged between 27°C and 29°C (moderate period) and between 45°C and 46°C (hot period). The samples collected during the moderate period served as controls and those collected during the hot period reflected the heat stress. Heat stress caused significantly ($p \leq 0.05$) higher levels of serum prolactin and cortisol; the mean rise in prolactin and cortisol levels was 4.68 fold and 4.60 fold, respectively. Higher serum prolactin levels reveal thermal load associated modulations in prolactin hormone. Also, a significant ($p \leq 0.05$) correlation ($r=0.971$) between the prolactin and cortisol levels was observed. Increased levels of stress hormone (cortisol) recorded in the present study lead to the premise that prolactin can also be used as an indicator of stress.

Key Words: prolactin, cortisol, pigs, heat stress, thermal load.

Introduction. Limited capacity to lose heat by water evaporation from the skin by pigs is well known (Ingram 1965). At higher ambient temperatures pigs may show increased respiration rate, rectal temperature and decreased voluntary feed intake (Quiniou et al 2000). At increased thermal load, respiratory evaporation becomes inadequate for sufficient heat loss to keep the rectal temperature constant. Under such conditions, the animals supposedly are above the thermo neutral zone. As a consequence, rectal temperature increases and then there is an adaptive depression of the heat production (Quiniou et al 2000a). Increased thermal load switches on many adaptive mechanisms. It is also believed to be one of the important factors to cause stress which can disturb the physiological functioning of an animal. There are many measurable parameters of stress and cortisol hormone is considered as a key indicator. This hormone is responsible for many stress related physiological modulations in animals. If increased cortisol levels persist for a longer period, modulations mechanisms may acquire a negative turn, culminating in chronic stress.

Prolactin (PRL), secreted by the pituitary gland, is a multi-functional hormone, supposed to be involved in several biological processes. Relationship between PRL and ambient temperature has been investigated by many researchers and may indicate the involvement of PRL in acclimatization responses to higher ambient temperature (Kataria & Kataria 2010; Alamer 2011). PRL has also been shown to influence cortisol secretion (Kataria & Kataria 2010). The role of PRL in the correction of stress, with the immune system and in thermal regulation is documented (Ahmadzadeh et al 2006). PRL is now being used as a stress marker in animals (Kataria & Kataria 2010, 2011). PRL is reported to be involved in the regulation of reproduction in male animals as it is a mediator of photoperiodic changes in a number of species. PRL secretion increases with increasing day length, suggesting that photoperiodism may be an external regulator and the

presence of high concentrations of PRL before peak testosterone concentrations suggests its role in regulating seasonal changes in the testes (Tsubota et al 1995). Regardless of advances in temperature management systems, the hot period of the year is still a financial burden for pig breeders. In the pork industry, reasons for economical losses derived from heat stress are: reduced growth, poor sow performance and decrease in carcass quality, in addition to increased veterinary costs (Sanz Fernandez et al 2012).

The emerging concepts of management for these animals include early detection of stress and its timely alleviation. If levels of stress are continuously elevated for prolonged periods, physiological modulations may turn into pathological consequences. The ability of pigs to neutralize the reactive oxygen species plays a key role in their welfare and scientists have recommended the application of such methods for the assessment of farmed animals' welfare (Brambilla et al 2001). The scientific community has started looking towards stress as a budding problem due to its implications in health and production of animals.

Generation of proper data of stress markers in native breeds is considered to be an important mandate of diagnostic laboratories for timely detection of stress. Pig farming is an important trade of marginal animal owners in arid tracts. Paucity of research to relate thermal load with development of stress in the pigs emphasised the need of a study to find out the relation of stress indicators with higher environmental temperatures.

Materials and methods. Serum PRL and cortisol levels were determined in indigenous pigs found in the arid tracts of Rajasthan state, India. The animals were maintained under similar management and feeding regimens by private breeders. Blood samples were collected in sterile tubes without anticoagulants, for harvesting sera, from a private slaughter house, during periods of the year when maximum environmental temperature ranged between 27°C and 29°C (moderate period) and between 45°C and 46°C (hot period). These animals were free from endo- and ecto-parasites, as assessed by routine faecal and skin examination, respectively. The samples were collected from adult male animals (seven each).

The serum PRL was determined by immunoradiometric assay, using a RIA kit (IRMA CT, RADIM, Italy) as per the manufacturer's protocol. The method employed the use of two anti-PRL monoclonal antibodies which recognised two different epitopes of the molecule. One antibody was adsorbed in solid phase in the coated tube (mouse monoclonal anti-PRL antibody) and the other as radioactive conjugate labelled with iodine-125 (¹²⁵I anti-PRL mouse monoclonal antibody in serum matrix). The serum samples and labelled antibodies were incubated simultaneously in the coated tubes. The amount of bound conjugate was directly proportional to the hormone concentration in the sample and standard. At the end of the incubation period, the unbound material was removed by an aspiration and washing cycle (Tris-HCl and Tween 20). The radioactivity in the tubes was measured in a ¹²⁵I Gamma counter (ECIL, India).

Serum cortisol was determined using the Gamma coat (¹²⁵I) cortisol radioimmunoassay kit procedure based on the competitive binding principles of radioimmunoassay (DiaSorin, USA). Serum samples and standards were incubated with cortisol tracer in antibody-coated tubes (Rabbit anti-cortisol serum coated) where the antibody was immobilised onto the lower inner wall of the Gamma Coat Tube. After incubation the contents of the tubes were decanted and the tubes were placed in a ¹²⁵I Gamma counter (ECIL, India).

Statistical significance between moderate and hot periods, for each parameter, was analysed using Student's *t*-test (Kaps & Lamberson 2004).

Results and discussions. The mean ± SEM values of serum PRL and cortisol in the pigs are presented in Table 1. There is paucity of literature regarding information on serum PRL levels in the indigenous pigs. The mean value of serum PRL in pigs was compared with the values available for other animals and found lower (Kataria & Kataria 2010, 2010a, 2010b, 2011). Earlier researchers have validated a radioimmunoassay to measure PRL in bear serum and described seasonal changes in serum PRL concentrations

with a rise during May (Tsubota et al 1995). Later many researchers have reported the use of commercially available human radioimmunoassay (RIA) kits (Kataria & Kataria 2010, 2010a, 2011) for PRL determination, as carried out in the present study.

Table 1

Serum levels of prolactin and cortisol in indigenous pigs during moderate and hot periods of the year

Serum biomarkers	N	Period of the year		Differences (\pm d)
		Moderate	Hot	
Prolactin (pmol/L)	7	252.44 \pm 1.81	1181.42 \pm 81.43	928.98*
Cortisol (nmol/L)	7	22.57 \pm 1.00	104.00 \pm 1.92	81.43*

Significantly ($p \leq 0.05$) higher levels of serum PRL and cortisol were observed during the hot period of the year as compared to that in the moderate period. The mean rise in serum PRL and cortisol levels during the hot period was 4.68 fold and 4.60 fold, respectively. A significant ($p \leq 0.05$) correlation ($r = 0.971$) between the PRL and cortisol was observed during the hot period which showed similar pattern of changes in markers of stress. Heat stress associated variation in serum PRL was accompanied by a rise in serum cortisol levels, a well documented marker of stress in animals (Kataria et al 2000). Kataria and Kataria (2010) also reported hot ambience related increase in serum cortisol levels in donkeys accompanied by PRL and advocated the use of PRL as a stress marker.

Sanz Fernandez et al (2012) also reported increase in blood PRL levels in growing pigs due to heat stress. Variations in PRL levels point towards its significant role in physiological adjustments of stress in pigs. Stress associated variations in serum PRL levels have also been reported by previous studies (Kataria & Kataria 2010a; Kataria & Kataria 2010b; Kataria & Kataria 2011). Heat stress can develop a cascade of changes in the physiological reactions of the animals to withstand the stress. Higher cortisol concentration substantiated the significance in meeting the energy crisis during physical stress, since cortisol increases glucose supply, by the glucogenolytic and gluconeogenic properties (Kataria et al 2000). In animals, elevated blood concentrations of cortisol, have been accepted as an indicator of acute stress. Stress is an important stimulus for the release of CRH and hence the release of ACTH. Heat stress through photoperiodic response is generally coupled with low quality nutrition and is found to be associated with increased PRL secretion (Brown & Forbes 1980). Stress-induced PRL release is a rapid and strong response of the organism, reflecting defensive behaviour and immunomodulatory influence (Kataria & Kataria 2010). PRL hyper-secretion during heat exposure may be involved in the enhancement of some thermoregulatory mechanisms, possibly by supporting the defence against heat or reducing heat increment. Heat stress induces marked alterations in water turnover rate and metabolism which boost the requirements for water during heat stress. Evidence suggests that PRL may affect body fluid regulation by maintaining extracellular fluid volume during heat exposure and hence supporting heat dissipation (Alamer 2011). Probably the PRL response to heat exposure might be involved in meeting the expanding water demands of heat stressed pigs. Association between hot period and PRL levels in serum also indicates towards modulations in water turnover. Combined effects of environmental temperature and level of energy intake on plasma concentrations of PRL have been investigated in pigs by earlier researchers (Dauncey & Buttle, 1990) who concluded that circulating levels of plasma PRL have a role in maintaining the differences in growth and morphology of young pigs kept in widely different environmental conditions.

Available literature suggests that PRL release rises in response to various forms of stressors (Kataria & Kataria 2010a, 2010b, 2011). Serum PRL increase in heat-stressed animals was reported (Kataria & Kataria 2010) but such reports in pigs are scarce and the scientific community is eager to find out the reasons for increase in PRL levels in heat stressed animals. Researchers have observed significant correlation between rectal temperature and peripheral PRL concentration during heat stress in several species (Hill & Alliston 1981; Chemineau & Ravault 1984; Low et al 2005). The results of the present

study showed that the secretion of PRL represented a modulation of the physiological response to high ambient temperature. Increase in serum PRL and cortisol levels in pigs was a way to attenuate the homeostatic disruption due to thermal load. An increased PRL level may modulate some thermoregulatory processes during Heat stress, probably associated with the mechanisms of heat production and heat loss, in order to support heat balance in the body. The rising levels of the hormones were suggestive of stress adaptation to unfavourable ambience.

Conclusions. Higher serum PRL levels indicated heat stress-associated modulations in PRL hormone. Higher levels of stress hormone cortisol in the present study helped to put forward the hypothesis that PRL can also be used as an indicator of stress. It is concluded that heat load during the hot period of year affected pigs which experienced potent stress. The observations of the present study advocated the importance of timely detection of stress in pigs for improved management, by protecting them from thermal stress. Data generated can help in diagnosis of stress related problems in pigs.

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