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Parallelism of Nesfatin-1, Ghrelin and Leptin with Metabolic Parameters and Progesterone at Puberty in Murrah Buffalo Heifers

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ABSTRACT

Nesfatin-1, ghrelin and leptin, secreted mainly from the hypothalamus, gastrointestinal tract and adipose tissue, may be related with various metabolic parameters viz. age, body weight, productive performance, DMI, plasma metabolites. They may act on the central nervous system to regulate initiation of puberty. Present study was aimed at finding relationships between nesfatin-1, ghrelin, leptin, and age at onset of puberty, body weight (BW), dry matter intake (DMI) and plasma metabolites in Murrah buffalo heifers. For the present study, thirteen pubertal Murrah buffalo heifers, presenting ovary with an identifiable corpus luteum (CL), and plasma progesterone (P_4) concentration ≥1 ng/ml were selected. Blood samples were collected fortnightly until first signs of heat appeared. Nesfatin-1 was positively and significantly ($P<0.01$) correlated with BW, progesterone, DMI. Correlation was positive and significant ($P<0.05$) with average daily gain (ADG). However, correlation of nesfatin-1 was negative and significant ($P<0.01$) with glucose, non-esterified fatty acids (NEFA) and age at puberty. Ghrelin was significantly ($P<0.01$) and positively correlated with glucose and NEFA. However, it was significantly and negatively correlated with progesterone, BW and DMI. Leptin was significantly ($P<0.05$) and positively correlated with BW, progesterone, and DMI but negatively correlated with glucose and NEFA. The present study revealed that nesfatin-1, ghrelin and leptin may regulate the age at onset of puberty through regulating the body weight, DMI and plasma progesterone level.

Keywords: Nesfatin-1, ghrelin, leptin, metabolites, puberty, buffalo

Indian buffaloes usually suffer from delayed puberty, anestrus, sub–estrus, summer infertility, extended intercalving period and postpartum uterine disorders affecting their reproductive efficiency adversely and thus their production potential (Agarwal *et al.,* 2005). Buffalo heifers usually attain puberty when they achieve 55-60% of the adult body weight. Puberty contributes largely to lifetime reproductive efficiency. Regulation of age at the onset of puberty is known to be influenced by genetic factors, and factors such as body weight and growth (Rosales *et al.,* 2013; Zieba *et al.,* 2005; Wylie, 2011; Pittroff *et al.,* 2008). Early puberty is important to the overall animal's productive life. Delay in onset of puberty, and consequent delay in conception, is one of the causes of low reproductive efficiency in buffaloes, thus lengthening their non-productive life (Barile, 2005). During the last two decades, considerable focus has been on reproductive endocrinology and neuroendocrinology with aim of developing models to enhance reproductive efficiency. Studies have proved that puberty is affected by breed, season, climate, nutrition, management, growth rate and diseases (Borghese *et al.,* 1994; Polat *et al.,* 2009). It has been stamped that endocrine and neuroendocrine events play a major role in the commencement of puberty (Pineda *et al.,* 2010; Amstalden *et al.,* 2011; Hausman *et al.,* 2012 and Atkins *et al.,* 2013). Several reports suggest

that nesfatin-1, ghrelin and leptin alter the release of GnRH from the hypothalamus directly or indirectly and thus affect the onset of puberty (Garcia-Galiano *et al*., 2010; Könczöl *et al*., 2010; Fernandez-Fernandez *et al*., 2005a; Fernandez-Fernandez *et al*., 2005b; Lebrethon *et al*., 2007; Tena-Sempere 2008; Forbes *et al*., 2009; Zuure *et al*., 2013; Sheffer-Babila *et al*., 2013; Lents *et al*., 2013). These peptides also play an important role in neural pathways regulating feed intake and energy homeostasis.

The role of nesfatin-1, ghrelin and leptin in the metabolic control of onset of puberty, feed intake and energy homeostasis have been reported in cattle, human, rat, mice, pig, and fish (Anwar *et al.,* 2014; Könczöl *et al.,* 2010; Garcia-Galiano *et al.,* 2010; Fernandez-Fernandez *et al.,* 2005b; Zuure *et al.,* 2013; Carvalho *et al.,* 2013).

MATERIALS AND METHODS

Animals, housing and feeding

Thirteen Murrah buffalo heifers aged between 25 to 29 months (mean age 27 ± 0.83 months) with the mean body weight 298 ± 7.35 kg were selected from Livestock Research Centre (LRC), National Dairy Research Institute (NDRI), Karnal. The buffalo heifers were managed in a loose housing system having brick on the edge flooring during the study period. All the heifers were fed as per the standard feeding practices followed at LRC, NDRI farm that consisted of concentrate mixture, wheat straw and green fodder (Berseem, Oat, Maize and Jowar). Concentrate mixture had CP 19.81% and TDN 70% (Table 2). Quantity of concentrate mixture and roughages were calculated on dry matter basis at every 15-days interval as per the body weight of the animals. A weighed quantity of ration, which consisted of concentrate: wheat straw in the ratio of 40:60 along with 25-30 kg of green fodder/ animal/day was fed. The calculated amount of concentrate mixture and wheat straw was offered at 9.00 a.m. The green roughage was provided twice daily at 10.00 a.m. in the morning and 3.00 p.m. in the afternoon. Water was available throughout the day to all the heifers.

Body weight and feed parameters

All the animals were weighed consecutively for two days on a platform of electronic balance at 15 days intervals during morning time before offering any feed or water. Dry matter intake was recorded at fortnightly interval for three consecutive days during which weighed amount of concentrate mixture, green fodder, dry roughages were offered daily, and residues left were weighed. Dry matter content of fresh as well as leftover concentrate mixture, wheat straw and green fodder were estimated by drying the samples in electronic hot air oven at 100±1ºC for 24 hours.

Blood collection and biochemical estimation

Blood was collected from all the thirteen animals through jugular vein puncture method at fortnight intervals into 15 ml heparinized polypropylene tubes in the morning before offering the feed. Plasma was separated and frozen stored at -20°C to estimate biochemical attributes.

Plasma nesfatin levels were measured by EIA using the Ray-Biotech Inc. made (The Protein Array Pioneer Company) EIA kit. Plasma ghrelin levels were measured using the "Bovine Appetite-regulating Hormone (GHRL) ELISA Kit" (Catalog No. CSB-EL00941BO) from Cusabio Biotech Co. Ltd. Plasma leptin levels were measured using the "Bovine Leptin ELISA Quantization kit" (Catalog No. CSB-E06771b) from Cusabio Biotech Co. Ltd. The plasma progesterone was estimated using "Bovine Progesterone hormone (P_4) ELISA test kit" (Endocrine Technologies, INC., USA). Glucose was estimated in plasma samples by using God-Pap Trinder's method, purchased from Avecon Healthcare Pvt. Ltd. Modified copper soap solvent extraction method was adopted for the estimation of plasma NEFA (Shipe *et al.,* 1980)

Statistical analysis

Pearson's correlations coefficient were calculated among all variables using the Statistical Analysis System (SAS) 9.1 (SAS Institute Inc., USA, 2003) software package. Differences were considered significant at least at level $P<0.05$.

RESULTS AND DISCUSSION

Puberty is an event in animal's life, which is the most important deciding factor of animals overall productive

life span. It is affected by many factors, which includes neural, hormonal and metabolic parameters. The present study was conducted to find the relationship between plasma nesfatin-1, ghrelin, leptin, metabolic parameters and puberty.

Correlation between plasma nesfatin-1, progesterone, and metabolic parameters

Nesfatin-1 was positively and significantly (P<0.01) correlated with BW (r =0.867), progesterone (r =0.859), and DMI (r =0.876). The correlation was positive and significant (P<0.05) with ADG (r = 0.430) also. However, correlation between nesfatin-1 was negative and significant (P<0.01) with glucose (r = -0.616), NEFA (r = -0.735), and negative and significant (P<0.05) with age at puberty (r = -0.471) (Table 1). The results clearly suggest that nesfatin-1 plays important role in glucose and NEFA utilization. As a result, it favors DMI, ADG and increase in body weight. It also appears that it favors progesterone secretion by some complex neuroendocrine mechanisms. Due to these effects, it helps in early commencement of puberty. In the present study, nesfatin-1 showed strong positive correlations with progesterone and metabolic parameters. It appears that it favoured early commencement of puberty. It was in corroboration with reports of Lents *et al.* (2013), who stamped that nesfatin-1 in pigs had negative relationship with age at puberty and in turn was positively associated with body weight and body weight gain, which is an essential requirement for puberty to occur at younger age. Nesfatin-1 secretion increased during puberty in female rats (Garcia-Galiano *et al.,* 2010), and intracerebroventricular administration of nesfatin-1 significantly induced increase in gonadotropin secretion in pubertal rat (Garcia-Galiano *et al.,* 2012). Nesfatin significantly increased GnRH release from *in vitro* hypothalamic explants (Patterson *et al.,* 2011). These reports suggest that nesfatin-1 may favour secretion of progesterone, which is in agreement with our findings.

Correlation between plasma ghrelin, progesterone, and metabolic parameters

Ghrelin was significantly and positively correlated with NEFA (r = 0.665, P<0.01) and glucose (r = 0.554, P<0.05). Whereas it was significantly and negatively correlated with progesterone (r = -0.715, P<0.01), BW (r = -0.6, P<0.05) and DMI (r =-0.6, P<0.05) (Table 1). The results clearly suggest that ghrelin favors feed intake, thereby increasing blood glucose and NEFA level. As ghrelin suppresses luteinizing hormone (LH) pulse frequency, so it suppresses progesterone secretion. Ghrelin has negative effect on ADG, body weight gain, and DMI. In the present study, a negative correlation was observed between ghrelin and progesterone secretion. This is in agreement with previous reports which suggest that at pituitary level, ghrelin suppresses luteinizing hormone (LH) pulse frequency in sheep, monkey and human (Harrison *et al.,* 2008; Vulliemoz *et al.,* 2008 and Lanfranco *et al.,* 2008); and findings of Gupta *et al.* (2014) that ghrelin has inhibitory

Table 1: Correlation between different parameters

	Nes-1	Ghr	Lep	BW	ADG	Glu	NEFA	Prog	DMI	Puberty
Nes-1	1	-0.60**	0.87**	0.87**	0.43*	-0.62**	-0.74**	0.86**	0.88**	-0.47*
Ghr			-0.60**	-0.60*	-0.30	0.55*	0.67**	-0.72**	-0.60*	0.29
Lep				0.98**	0.05	-0.72**	-0.82**	0.74**	0.98**	-0.30
BW					0.17	-0.63*	-0.84**	0.76**	0.99**	-0.37
ADG						0.13	-0.14	0.48*	0.19	-0.81**
Glu							0.52*	-0.60**	-0.63**	0.16
NEFA								-0.71*	-0.84**	-0.05
Prog									0.78**	-0.45*
DMI										-0.39

Nes-1=Nesfatin-1, Ghr=Ghrelin, Lep=Leptin, BW=Body Weight, ADG=Average Daily Gain, Glu=Glucose, NEFA= Non-Esterified Fatty Acids, Prog= Progesterone, DMI= Dry Matter Intake, Puberty=Age at puberty. Correlation is significant at: *0.05% level of significance; **0.01% level of significance.

effect on production of progesterone from cultured luteal cells of buffalo. In the present study, ghrelin showed a positive correlation with glucose and NEFA, whereas it showed a negative correlation with DMI, BW and ADG. This was in agreement with reports of Fernandez-Fernandez *et al.* (2006) and Komarowska *et al.* (2012), who suggested that ghrelin plays an important role in the integrated control of energy balance and reproduction. Sim *et al.* (2014) reported that the activation of ghrelin receptors located in the spinal cord plays important role for the elevation of the blood glucose level. Broglio *et al.* (2003) reported that intravenous administration of ghrelin decreased insulin and increased blood glucose level. Huda *et al.* (2011) reported that ghrelin infusion increased NEFA in humans. Wertz-Lutz *et al.* (2008) reported that nutrient restriction in cattle resulted in elevated circulating ghrelin concentration. These reports were in agreement with the findings of the present study.

Table 2: Composition of concentrate mixture

Ingredient	Percentage (%)
Maize	33
Groundnut cake	21
Mustard cake	12
Wheat bran	20
Deoiled rice	11
Mineral mixture	2
Common salt	1

Correlation between plasma leptin, progesterone, and metabolic parameters

Leptin was significantly (P<0.01) and positively correlated with BW (r = 0.979), progesterone (r = 0.738) and DMI (r = 0.975). However, it was significantly (P<0.01) and negatively correlated with glucose (r = -0.716, P<0.01) and NEFA (r = -0.821) (Table 1). These findings suggest that leptin favors feed intake and body weight gain. It also stimulates progesterone secretion. It reduces blood glucose and NEFA levels, since it stimulates glucose utilization, and prevents adipose tissue mobilization. Present study found a positive correlation between leptin and BW, progesterone, and DMI. However, leptin showed negative correlation with glucose and NEFA. Present findings were in corroboration with previous reports in dairy cows, in which leptin was negatively related with age at puberty and positively related with progesterone secretion (Williams *et al.,* 2002). Kumar *et al.* (2012) reported a positive role of leptin in steroidogenesis; Caro *et al.* (1996) reported that leptin acts at hypothalamic level to cause LH release, favoring early commencement of puberty. These reports further support the findings of the present study. Effects of leptin on DMI was in validation with Blache *et al.* (2000), who reported that the concentration of leptin in plasma and in cerebrospinal fluid increased two folds after increasing the plane of nutrition.

Correlation between plasma nesfatin-1, leptin and ghrelin

The plasma hormones nesfatin-1, ghrelin and leptin were significantly (P<0.01) correlated with each other. Nesfatin-1 showed positive correlation with leptin and negative correlation with ghrelin. There was a negative correlation between ghrelin and leptin. This suggests that nesfatin-1 and leptin have synergistic action on blood glucose, NEFA, and metabolic parameters. They appear to be antagonistic in action with ghrelin. Therefore, present study elucidates that lower plasma concentration of ghrelin (having positive correlation with age at puberty), and higher plasma concentrations of nesfatin-1 and leptin (which were negatively correlated with age at puberty) may reduce the age at puberty in buffalo heifers.

CONCLUSION

This study indicates that nesfatin-1, ghrelin and leptin may regulate the age of puberty through an intricate orchestra of regulation of metabolism, feed intake, body weight gain and hormone regulation. These peptides can be used as markers for assessing the age at puberty in buffaloes.

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