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Plasma Nesfatin-1 and Leptin in pubertal and non-pubertal Murrah buffalo heifers (*Bubalus bubalis*)

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A B S T R A C T

Buffaloes mostly suffer from delayed puberty, anestrus, sub–estrus, summer infertility, prolonged inter-calving interval and postpartum uterine disorders. Nesfatin-1 and Leptin are directly or indirectly related with body weight (BW), feed parameters and regulation of puberty. The objective of this study was to investigate the influence of Nesfatin-1 and Leptin in pubertal and non-pubertal Murrah buffalo heifers. The Murrah buffalo heifers (n=13) were randomly selected and divided into two groups; pubertal group (PG) and non-pubertal group (NG). Heifers with plasma progesterone (P_4) level of ≥1 ng/mL were classified as PG. Blood samples were collected at fortnight intervals for analysis of plasma Nesfatin-1, Leptin, P_4, glucose and non-esterified fatty acids. Body weight, dry matter intake and feed conversion efficiency were recorded at fortnight intervals. The mean (±SEM) plasma Nesfatin-1, Leptin, P_4, BW and feed conversion efficiency (%) were significantly (P<0.01) higher in PG as compared to NG. Dry matter intake by the heifers was also significantly (P<0.001) higher in PG than NG. Plasma metabolites (glucose and NEFA) did not differ significantly between the groups. The findings of this study suggest that Nesfatin-1 and Leptin have indispensable role in the onset of puberty in buffalo heifers by affecting BW and feed parameters.

Keywords

Buffalo, Leptin, Nesfatin-1, Puberty

ARTICLE HISTORY

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INTRODUCTION

Delayed puberty and consequently delayed conception is one of the reproductive problems leading to low reproductive efficiency in buffalo (*Bubalus bubalis*), thus lengthening the non-productive life. As good reproductive performance is essential for efficient livestock production, the female buffalo calves must grow rapidly to attain puberty followed by estrus cycle and fertile ovulation. Appropriate growth rate and onset of puberty are the two important factors, which directly affect the age at first calving. Onset of puberty is regulated by the energy reserves, and it is sensitive to different metabolic cues of the animal (Roa et al., 2010).

Sexual maturation involves activation of the hypothalamic-pituitary-gonadal axis, a process that results in fertile ovulation in females. Nesfatin-1 and Leptin affect the activation of the hypothalamic-pituitary-gonadal axis (Williams et al., 2002; Garcia-Galiano et al., 2010a; Kumar et al., 2012). Parameters like Nesfatin-1 and Leptin levels are directly or indirectly related with body weight (BW) and feed parameters. It was observed that when Nesfatin was administered directly into the brain of rats and pigs, it regulated or suppressed feed intake (Price et al., 2008; Shimizu et al., 2009; Lents et al., 2013).

It has been reported that alteration in feed intake affects circulatory concentration of Leptin in sheep (Nagatani et al., 2000), ewe (Thomas et al., 2001) and cattle (Amstalden et al., 2000). Moreover, it has been suggested that leptin is indispensable for attaining puberty, and has an important role in connecting metabolic activity and onset of puberty in humans and rats (Castellano et al., 2009; Roa et al., 2010). However, there is paucity of literature that reports the influence of plasma Nesfatin 1 and Leptin on attaining puberty of

buffalo heifer. Here, the role of plasma Nesfatin-1 and Leptin were evaluated for the onset of puberty in buffalo heifers.

MATERIALS AND METHODS

Animals, housing and feeding: Thirteen Murrah buffalo heifers within the age group from 25 to 29 months (mean±SD age 27±0.83 months) with the mean±SD BW 298±7.35 kg were randomly selected from the animal herd of National Dairy Research Institute (NDRI), Karnal, HARYANA. At the beginning of the experiment, none of the animals had attained puberty. At the end of experiment, the animals were divided into two groups, pubertal group (PG) and non-pubertal group (NG). Animals which exhibited signs of heat and corpus luteum could be palpated were considered as pubertal animals and plasma progesterone (P_4) level was ≥1 ng/mL in at least two samples. The buffalo heifers were housed in a loose housing system during the course of the experiment. All the heifers were fed as per the standard feeding practices of NDRI farm. Animals were fed on concentrate mixture (**Table 1**), wheat straw and green roughages (Berseem, Oat, Maize or Jowar fodder). The percentage of crude protein (CP) in concentrate mixture was 19.81% and total digestible nitrogen (TDN) was 70%. Concentrate mixture and roughage were calculated at every 15-day interval as per the requirement BW of the animals. The ration offered per animal/day consisted of concentrate: wheat straw in the ratio of 40:60 along with 25-30 kg of green fodder. The required amount of feed was offered at 9.00 am. The green roughage was provided twice daily, at 10.00 am in the morning and 3.00 pm in the afternoon. Fresh and clean tap water was available throughout the day.

Table 1. 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

BW and feed parameters: All the animals were weighed on electronic balance with platform facility, consecutively for two days at 15-days interval. BW of animals was recorded in the morning, before offering any feed or water. Dry matter intake (DMI) was estimated based on feed intake at fortnightly interval and weight of feed residue was recorded. Feed conversion efficiency (FCE) was calculated, taking the ratio of BW gain and DMI per day.

Blood collection and biochemical determinations: Blood was collected at fortnight intervals by jugular vein puncture into 15 mL heparinized polypropylene tubes in the morning before offering the feed. Plasma was separated and stored at -20°C to estimate biochemical parameters. Plasma Nesfatin level was estimated using the enzyme immunoassay kit (Ray-Biotech INC., Norcross, GA, USA). Plasma Leptin levels was measured using the "Bovine Leptin ELISA kit" (Cusabio Biotech Co., Ltd., Wuhan, China). The plasma P_4 was estimated using Bovine P_4 hormone (P_4) ELISA test kit" (Endocrine Technologies, INC., Newark, USA). Glucose was estimated in plasma samples by using GOD-PAP TRINDER'S method; using kit purchased from Avecon Healthcare Pvt. Ltd (Parwanoo, INDIA). The copper soap solvent extraction method modified by Shipe et al. (1980) was adopted for the estimation of plasma non-esterified fatty acids (NEFA).

Statistical analysis: Data were analyzed by using paired t-test with Statistical Analysis System (SAS) 9.1 (SAS Enterprise Guide) software package. The results are given as mean (±SEM). Differences were considered significant at least at $P<0.05$ level.

RESULTS AND DISCUSSION

At the end of experiment, after five fortnights, the Murrah buffalo heifers were divided into two groups as pubertal group (PG) and non-pubertal group (NG) as described in Materials and Methods section. Out of 13 animals, six of them attained puberty within 26-29 months of age and seven (NG) did not attain puberty. One animal of NG group was excluded because of its estimated values for the different parameters varied significantly from the rest of the animals of NG group. By 5th fortnight all heifers of PG group attained puberty.

Age and BW: Animals' mean (±SEM) age for PG group was 26.6±0.47 months with mean (±SEM) BW of 313.83±7.63 kg and mean (±SEM) age for NG group was 26.9±0.55 months with mean (±SEM) BW of 281.95±9.56 kg (**Table 2**). The mean (±SEM) BW was significantly ($P<0.01$) greater for PG group when compared with NG group. Body weights were 342.1±7.65 and 303.10±4.92 kg for PG and NG group, respectively (**Table 3**; **Figure 1**). In the present study, it was observed that animals in the PG group attained

Table 2. Initial age and body weight (Mean±SD) of pubertal group (PG) and non-pubertal group (NG) Murrah buffalo heifers.

Parameter	PG	NG	t
Age (months)	26.6±0.47	26.9±0.55	0.698
Body weight (kg)	313.83±7.63	281.95±9.56	2.495*

* $P<0.05$; the animals were classified after attaining puberty.

Table 3. Mean±SEM of different parameters of pubertal (PG) and non-pubertal (NG) groups after five fortnights.

Parameters	PG	NG	t
Progesterone (ng/mL)	0.85±0.09	0.53±0.01	3.30**
Nesfatin-1 (ng/mL)	1.96±0.05	1.75±0.01	4.24**
Leptin (ng/mL)	1.94±0.05	1.75±0.02	3.43**
Body weight (kg)	342.10±7.65	303.10±4.92	4.76**
Glucose (mg/dL)	66.80±0.96	67.12±0.70	0.27
Non Eesterified Fatty Acid (µmol/L)	197.58±3.67	206.93±4.04	1.71
Dry Matter Intake (kg/day)	8.89±0.20	7.74±0.12	5.01***
Feed Conversion Efficiency (%)	8.03±0.35	6.10±0.20	4.77**

*$P<0.05$, **$P<0.01$ and ***$P<0.001$. All the animals of NG group attained puberty by five fortnights.

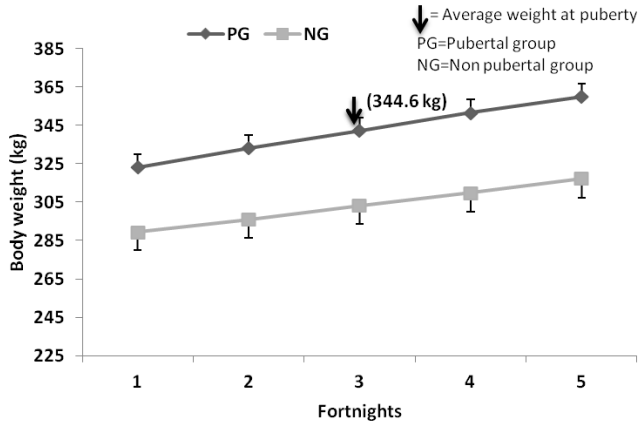

Figure 1. Mean±SEM of the body weight (kg) of pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

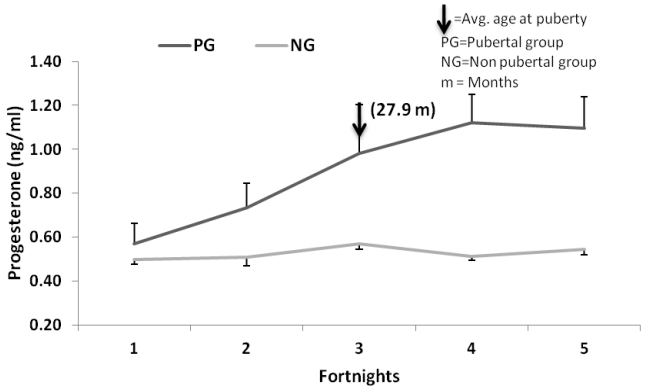

Figure 2. Mean±SEM of plasma P_4 concentration (ng/mL) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

puberty after 5 fortnights of study period when their BW was 57% of the adult BW. Available reports on buffalo species stated that they attain puberty when their body weight is 50-60% of the adult body weight (Anjum et al., 2012; Aydin, 2013).

P_4 and Puberty: It was observed in PG group, two animals attained puberty at 2nd, and rest four attained puberty at 3rd and 5th fortnight, respectively. The peak level of plasma P_4 of pubertal animals ranged from 1.12 to 1.46 ng/mL, during the mentioned fortnights (2nd, 3rd and 5th) the mean (±SEM) concentration of plasma P_4 was 0.73, 0.98 and 1.10 ng/mL, respectively in PG group (**Figure 2**). The mean (±SEM) concentration of plasma P_4 was 0.85±0.09 and 0.53±0.01 ng/mL for PG and NG groups, respectively (Table 3).

In the pre-pubertal period the mean P_4 concentration in both groups were at basal level, as reported by Haldar and Prakash (2005) and Terzano et al. (2007). When all the animals attained puberty in PG group, the P_4 concentration was significantly ($P<0.01$) greater than the NG group. The mean (±SEM) plasma P_4 level of the NG group after 5 fortnights was at basal level reflecting no cyclic activity. This is in agreement with reports of Júnior et al. (2010), Cooke and Arthington (2008), Haldar and Prakash (2005) and Terzano et al. (2007). This may be one of the reasons for the animals of PG group to come into puberty earlier.

Plasma Nesfatin-1: In the present study it was observed that the mean (±SEM) plasma Nesfatin-1 concentration for PG and NG group were significantly ($P<0.01$) different, the concentrations were higher for PG group. The mean (±SEM) plasma Nesfatin-1 concentration was 1.96±0.05 ng/mL and 1.75±0.01 ng/mL for PG and NG groups, respectively (**Table 3, Figure 3**). In the ruminants, plasma Nesfatin-1 has been estimated in lactating Holstein–Friesian cows in different physiological stages (Ingawale and Dhoble, 2004). The mean (±SEM) plasma Nesfatin-1 concentration increased from 1st fortnight to 5th

fortnight in PG group. The maximum mean (±SEM) observed was 2.10±0.07 ng/mL. The mean (±SEM) plasma Nesfatin-1 concentration was significantly greater for PG group when compared with NG group. This is in confirmation with the report available in humans (Anwar et al., 2014).

Lents et al. (2013) has reported that Nesfatin-1 has negative relationship with age at puberty in pigs, and in turn is positively associated with BW and BW gain, which is an essential requirement for early puberty. In the present study, it was also found that Nesfatin-1 concentration and BW were greater in animals of PG group than NG group.

No reports are available on plasma Nesfatin-1 and puberty in farm animals. Only one report is available (Anwar et al., 2014) in lactating Holstein–Friesian cows, which were at different physiological stage. In female rats hypothalamic NUCB2/Nesfatin-1 expression increased during puberty and a functional blockage of Nesfatin-1 in the hypothalamus delays an appearance of external sexual sign (Garcia-Galiano et al., 2010b).

Plasma Nesfatin-1 can cross the blood brain barrier without saturation (Pan et al., 2007). In rat, it has been shown that intracerebroventricular administration of Nesfatin-1 induced significantly increase in gonadotropin in pubertal rat and NUCB2 antisense-morpholino-oligonucleotides delayed puberty in female rats (Garcia-Galiano et al., 2012). Nesfatin significantly increased GnRH release from *in vitro* hypothalamic explants (Patterson et al., 2011). This study suggests that, greater plasma Nesfatin-1 concentration in PG group might have triggered puberty earlier through GnRH pathway. Further studies in detail have to be carried out to find out the mode of action of Nesfatin-1.

Plasma Leptin: In the present study, the mean (±SEM) plasma Leptin concentration was significantly (P<0.01) different between the groups. The mean (±SEM) values were 1.94±0.05 and 1.75±0.02 ng/mL for PG and NG groups, respectively (**Table 3**). It was observed that the Leptin concentration increased throughout the course of pubertal development in PG group (**Figure 4**).

The mean (±SEM) plasma Leptin concentration for PG group was significantly (P<0.01) higher when compared with NG group, reflecting that animals with higher circulatory Leptin concentration would attain puberty at younger age. This indicate that Leptin has positive effect on attainment of puberty in Murrah buffalo heifers; therefore, increase or decrease in plasma Leptin concentration may affect the age at puberty in murrah buffalo. This is in accordance with the findings of Garcia et al. (2002) and Williams et al. (2002), who reported that Leptin is negatively related with age at puberty and positively related with P_4 secretion in dairy cows.

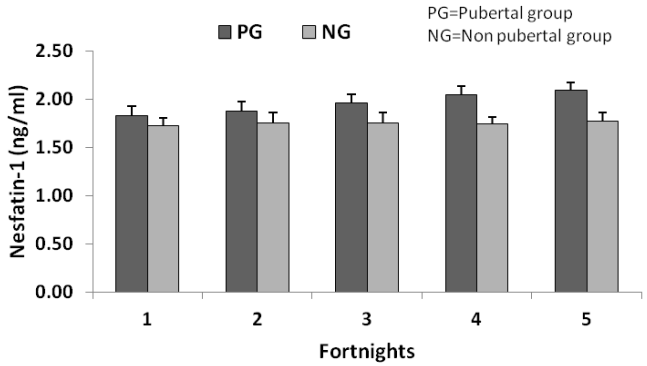

Figure 3. Mean±SEM of plasma Nesfatin-1 concentration (ng/mL) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

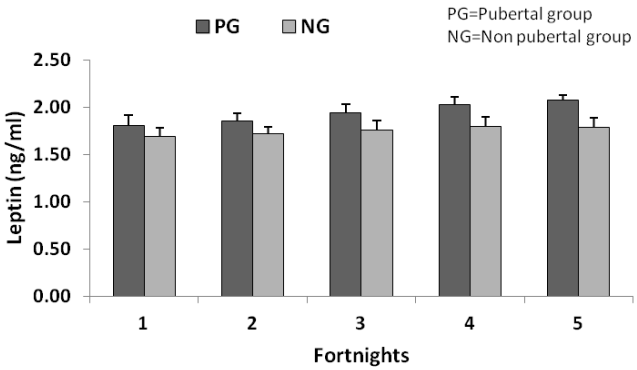

Figure 4. Mean±SEM of the plasma Leptin concentration (ng/mL) in pubertal (PG) and non-pubertal (NG) groupof Murrah buffalo heifers.

It is known that Leptin acts on hypothalamic level to cause LH release and for the induction of puberty (Caro et al., 1996). In PG group, the greater mean (±SEM) plasma Leptin concentration during five fortnights might have induced puberty in PG group. Sarkar et al. (2010) and Kumar et al. (2012) have reported that Leptin receptors are present on corpus luteum and can modulate secretion of P_4. Hence from the present study it can be suggested that due to increase in plasma Leptin concentration, had positive effect on corpus luteum function and P_4 synthesis. Hence Puberty could be attained at an earlier age in PG group.

Plasma Metabolites

Plasma Glucose and Plasma non-esterified fatty acids (NEFA): The mean (±SEM) plasma glucose concentration was not significantly different between the groups. The mean (±SEM) plasma glucose concentration was 66.80±0.96 and 67.12±0.70 mg/dL for PG and NG groups, respectively (**Table 3**).

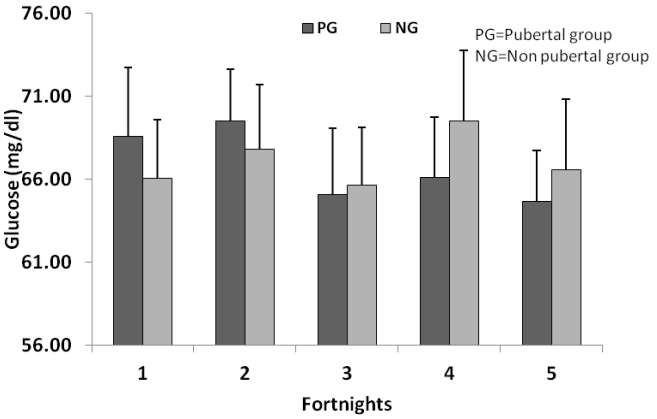

Figure 5. Mean±SEM of the plasma Glucose concentration (mg/dL) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

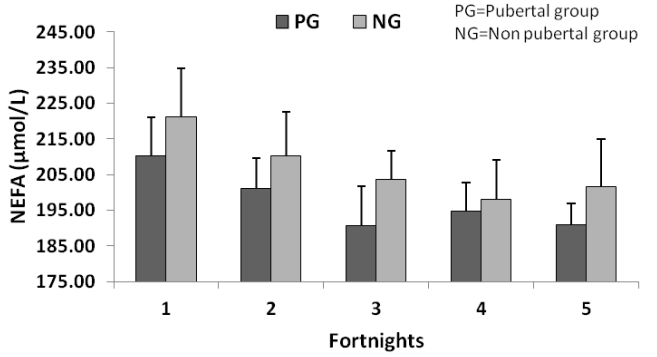

Figure 6. Mean±SEM of the plasma NEFA concentration (µmol/L) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

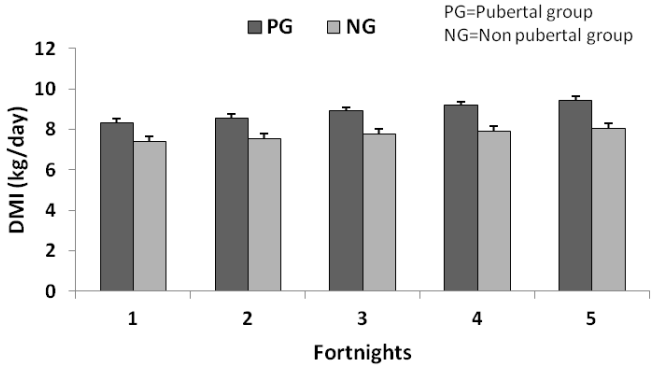

Figure 7. Mean±SEM of DMI (kg/day) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

The mean (±SEM) plasma NEFA concentration was also not significantly different between the groups. The mean (±SEM) plasma NEFA concentration was 197.6±3.7 and 206.93±4.04 µmol/L for PG and NG groups, respectively (**Table 3**).

The blood metabolites, mean (±SEM) glucose and NEFA were not significantly different between the groups. This indicates that although the BW (P<0.01), DMI (P<0.001) and nutrient efficiency (P<0.01) was significantly less in NG group when compared with PG group, it did not indicate negative energy balance in animals of NG group as indicated by the plasma level of NEFA and glucose. It can be suggested that without significant change in plasma metabolites glucose or NEFA level, the age at puberty may be delayed when compared with the animals attaining puberty at normal age.

Even though glucose and NEFA strongly affect the physiological state of animals where plasma NEFA level should not exceed the physiological level and plasma glucose concentration should not be below the physiological level. In the present study both were within the physiological range and there was no significant difference between the groups (**Figure 5 and 6**). Hence it can be said that buffalo heifers were not in negative energy balance.

Dry matter intake (DMI) and Feed conversion efficiency (FCE): At the end of experiment, the mean (±SEM) DMI difference was significantly (P<0.001) different between the groups, the value being higher for PG group. The mean (±SEM) values were 8.89±0.20 and 7.74±0.12 kg/day for PG and NG groups, respectively (**Table 3, Figure 7**).

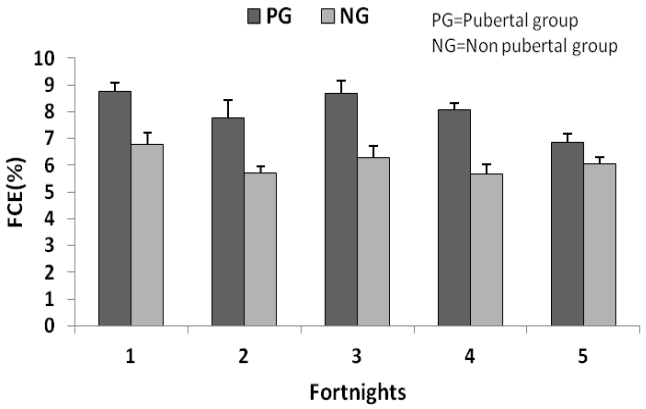

Figure 8. Mean±SEM of FCE (%) in pubertal (PG) and non-pubertal (NG) group of Murrah buffalo heifers.

Likewise, the mean (±SEM) FCE (%) was significantly higher (P<0.01, Table 3) in PG group when compared with NG group. The mean (±SEM) FCE (%) was 8.03±0.35 and 6.10±0.20 for PG and NG groups, respectively (**Table 3, Figure 8**).

No reports are available regarding Nesfatin-1 and feed parameters in ruminants. In humans and rodent models it reduces the feed intake and BW gain, suggesting a role as an anorexigenic factor. In the present study, when the mean (±SEM) DMI increased, the plasma Nesfatin-1 concentration also increased, suggesting a role for homeostasis. During pubertal time, both plasma Nesfatin-1 and DMI increased significantly which are important for attaining puberty. It is in agreement with the results of García-Galiano et al. (2010b) who reported similar trend in female rats. These results suggest that, during puberty, in buffalo heifers, uncoupling of inhibitory effect of Nesfatin-1 on DMI might have occurred removing inhibitory effect on DMI.

The effects of Leptin on feed intake are central and mediated via orexigenic and anorexigenic neuropeptides. It is well established that Leptin is an anorexic hormone. In humans it was shown that plasma Leptin concentration drops by 70% after 24 h fast (Boden et al., 1996).

The concentration of Leptin in plasma and in cerebrospinal fluid increased two fold after increasing the plane of nutrition (Blache et al., 2000). In contrast, complete feed deprivation causes a rapid fall in plasma Leptin in sheep (Marie et al., 2001).

In the present study the greater mean (±SEM) DMI and nutrient efficiencies PG group might have increased the plasma Leptin concentration. However, in NG group the lower mean (±SEM) DMI and nutrient efficiencies might have decreased the concentration of plasma Leptin. Since DMI is an essential parameter for increase in BW and onset of puberty (Archbold et al., 2012). In the present study also DMI and BW parameters increased significantly for the PG group of animals when compared with NG group resulting in earlier onset of puberty.

CONCLUSION

Plasma Nesfatin-1, Leptin, P_4, BW, DMI, FCE parameters were significantly higher in the pubertal group compared with non-pubertal group. This study suggests that Nesfatin-1 and Leptin may regulate the age of puberty by manipulating or increasing the BW gain, nutrient efficiency, and circulatory P_4 parameters.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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