

## Expression profile of growth hormone receptor (GHR), insulin like growth factor receptor (IGFR) and leptin genes, with associated blood bio-chemicals and endocrines profiles in black Bengal kids (*Capra hircus*) during different stages of pre-pubertal growth

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### ABSTRACT

The aim of this study was to evaluate the expression profile of growth hormone receptor (GHR), insulin like growth factor receptor (IGFR) and leptin genes, together with some blood biochemical (glucose, aspartate aminotransferase, AST; alanine aminotransferase, ALT) and endocrine (growth hormone and cortisol) parameters. For this purpose, blood samples were collected from 25 male and 25 female kids of the Black Bengal goat (*Capra hircus*), at days 15, 30, 60, 90, 120 and 150. Male kids gained more body weight compared to females. The glucose and cortisol levels significantly ( $P \leq 0.01$ ) decreased with the advancement of age. AST, ALT, and growth hormone increased significantly ( $P \leq 0.01$ ). In both sexes, plasma glucose level was negatively ( $P \leq 0.01$ ) correlated with AST. In males both cortisol and GH levels were positively ( $P \leq 0.05$ ) correlated with plasma glucose levels. In female kids only GH showed a positive ( $P \leq 0.05$ ) correlation with glucose levels. Female kids had greater expression of the GH receptor, IGFR mRNA and Leptin mRNA on day 30 ( $P \leq 0.05$ ) and day 150 ( $P \leq 0.01$ ). GHR was only positively ( $P \leq 0.05$ ) correlated with IGFR and LEP in female kids. On the basis of the results obtained from our research, we are able to conclude that the lower growth rate in female kids expressed more leptin, which stimulated the somatotrophic axis for the expression of GHR and IGFR during the prepubertal growth stage.

**Key words:** black Bengal goats; growth hormone receptor; IGF receptor; leptin genes; prepubertal growth

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### Introduction

India ranks second in terms of its goat population (133.0 million) after China (188.0 million) in the world (FAOSTAT, 2014) along with being largest exporter of sheep & goat meat to the world

(APEDA, 2018). Black Bengal goats (colloquially Bengal goats) are one of the best goat breeds of India and known globally for their high prolificacy and good quality of meat and hide (DIXIT and

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SHUKLA, 1995) despite its slower growth rate, low milk production and higher kid mortality (HUSSAIN et al., 1997; AMIN et al., 2001). As Black Bengal goats are reared primarily for meat production, their prolificacy and growth are the two important economic factors. The prolificacy of black Bengal goats has been well validated with the Polymorphism of fecundity genes (POLLEY et al., 2009) but the studies on growth have mainly focused on the polymorphisms of growth hormone, growth hormone receptor genes and insulin like growth factor genes, and their effects on growth traits in goats (AN et al., 2011; RASOULI et al., 2017) as a whole and black Bengal goats in particular (GUPTA et al., 2007). However, the literature is scant on the alterations in these gene expression patterns throughout the pre-pubertal growth stage, particularly in black Bengal goats. The somatotrophic axis, consisting of the growth hormone (GH) and insulin-like growth factors (IGF), governs growth homeostasis in conjugation with other endocrine factors (leptin, glucocorticoids) (PADGETT and GLASER., 2003; KEOGH et al., 2015; AFSAL and SEJIAN, 2018). Leptin is a suitable biomarker from the somatotrophic axis for nutritional stress tolerance in goats (HYDER et al., 2013), and is required for maintaining physiological normalcy (ZIEBA et al., 2005), and the interrelationship between GH, IGF and leptin during the pre-pubertal phase of growth may reveal the growth homeostasis in a comprehensive manner in black Bengal goats. Therefore, the present investigation was designed to explore the expression profile of GHR, IGFR, leptin receptor throughout the pre-pubertal growth phase in male and female black Bengal kids, in relation to some blood biochemical parameters (glucose, plasma AST, ALT) as markers for hepatic functions and endocrine parameters related to growth (GH and cortisol).

### Materials and methods

*Study area and period.* The study was carried out at Eastern Regional Station-Indian Veterinary Research Institute, Kalyani Goat Farm of Nadia district, West Bengal, India. The farm is located at an altitude of 11 meters above the mean sea level, at a latitude of 22°98'N and at a longitude of 88°44'E.

It is in the lower Gangetic Plain Region of India. The soil is loamy pattern clay with pH varying from 6.5 to 8.75. The environmental temperature was  $38.79 \pm 3.44$  °C in summer and  $14.98 \pm 4.64$  °C in winter. Relative humidity was 64.36% and 57.86% in summer and winter respectively.

*Selection of experimental animals and sampling.* Thirty clinically healthy pregnant does of the black Bengal goat breed were randomly selected with parity of 4 - 6 ( $4.38 \pm 0.12$ ). The age of the does was recorded as  $38.67 \pm 6.48$  Mo with body weight of  $21.13 \pm 2.89$  Kg. The experimental animals were kept in dry, clean and well-ventilated hygienic sheds with a concrete floor. The litter size of the does was recorded as  $2.14 \pm 0.04$ . In this study, 25 male and 25 female kids were selected randomly from the chosen does. The mean birth weights of the experimental kids were  $1.23 \pm 0.11$  Kg (males) and  $0.92 \pm 0.05$  (females) respectively. All kids were fed with mother's milk up to weaning and were maintained under a free range rearing system. The vaccination and deworming schedules followed in the experimental animals were as per the standard schedule. Each animal was turned out for natural grazing at 8.00 am to 12.30 pm, and again at 2.30 pm to 5.30 pm. No extra concentrated feed was provided at the shelter. Animals were provided with adequate quantities of clean and fresh drinking water.

Blood samples (5 mL) were collected from the jugular vein in heparinised vacutainers at 7.00 am before any feed had been offered to the experimental animals on days 15, 30 (1 Mo), 60 (2 Mo), 90 (3 Mo), 120 (4 Mo), and 150 (5 Mo). Some of the blood samples were kept for molecular study, and another portion was subjected to centrifugation (3000 rpm for 30 min) for separation of plasma.

*Measurement of body weight gain.* The weights of the new born kids were measured as soon as the kids were cleaned and dried immediately after birth. Subsequently, the body weight of each animal was recorded as per schedule in the morning before feeding and watering. Body weight was recorded by making the animals stand individually on the weighing machine (Balance Avery, Bombay, India). Body weight and weight gain were expressed respectively in Kg and gram.

*Measurement of blood biochemical parameters (Blood glucose, AST and ALT).* Blood glucose, AST and ALT were measured using commercially available kits specific for goats (Ecoline-Merck diagnostic kit, Merck Specialities Pvt. Ltd., Mumbai, India).

*Measurement of endocrine parameters (GH and cortisol).* GH and cortisol were measured by enzyme immune assay, using the commercially available ELISA kit specific for goats (MyBioSource, Inc., San Diego, CA, USA).

*Expression profile of GHR, IGFR and leptin.* Total RNA was extracted from blood using the Trizol method as reported by (CHOMCZYNSKI and SACCHI, 1987). The quality of RNA was checked electrophoretically using agarose gel (1.5%). The RNA samples with two distinct intact bands representing 18 S and 28 S subunits were purified by DNAase treatment (DNA-free™ Kit, Ambion), and stored in RNA storage solution (Ambion). RNA was quantified by spectrophotometry. The samples with acceptable purity (*i.e.* ratio 1.65-2.0) were used for reverse transcription.

First strand cDNA was prepared from 1µg of RNA using the commercially available cDNA synthesis Kit (Novagen first strand cDNA synthesis kit, La Jolla, CA, USA).

Optimum annealing temperatures for all the primers of the target (GHR, IGFR and Leptin) and housekeeping ( $\beta$ -actin) genes were standardized by gradient PCR (MyCycler, Bio-Rad Lab. Inc., USA) of 47-59 °C temperature range. The single bands of the expected sizes for all the genes at 55 °C signify that the primers were highly specific to their target sequence.

Roche's Lightcycler 480 instrument was used for qPCR as per the method of PFAFFL (2001). Primers for the caprine specific target (GHR, IGFR and leptin) and housekeeping ( $\beta$ -actin) genes (Table 1) were designed and synthesized (Sigma chemicals Co., St. Louis, Missouri, USA) so that they have an equal melting temperature range. First, strand cDNA was diluted to 1:1 ratio before the qPCR study. The master mix for qPCR for all the samples was prepared as follows: 1 µL template + 5 µL (2X) SYBR green mix + 0.5 µL each of reverse and

forward primer and 3 µL nuclease free PCR grade water.

Table. 1. Primers used for gene expression studies

Sl. No.	Gene	F/R	Sequence (5 →3)
1	GHR	F	CAGTGACATGTGCCGATGGTA
		R	ATGTGTGATTACGCCCTCGAC
2	LEP	F	TGGAAGAGAGGAGTTCCGAG
		R	AGGGGCAAAGGTCATTTATTG
3	IGFR	F	GAGAAAGGGGAATTTGGTCCC
		R	GGTGATATCCGCAGGTTGT
4	b-ACTIN	F	CCTCAAGTACCCCATGAGC
		R	TGATGTCACGGACGATTCC

The Light Cycler was programmed at Initial denaturation at 95 °C for 5 minutes, then denaturation at 95 °C for 60 seconds, followed by annealing at 55 °C for 40 seconds, and elongation at 72 °C for 60 seconds. Cycles were repeated 35 times. The final extension was at 72 °C for 7 minutes, and kept at the holding temperature of 4 °C.

Calculation of the CPs (crossing points) was automatically performed by the instrument's Second Derivative Max method of absolute quantification control at each time point. The relative expression ratio was calculated as per the formula given below:

$$\text{Ratio} = (E_{\text{target}})^{\Delta C_p_{\text{target}} (\text{control} - \text{sample})} / (E_{\text{ref}})^{\Delta C_p_{\text{ref}} (\text{control} - \text{sample})}$$

Where,  $E_{\text{target}}$  = Real Time PCR efficiency of the target gene transcript,  $E_{\text{ref}}$  = Real Time PCR efficiency of the reference gene transcript,  $\Delta C_p_{\text{target}}$  = CP deviation of control sample and experimental sample with target gene,  $\Delta C_p_{\text{ref}}$  = CP deviation of control sample and experimental sample with reference gene.

*Statistical analysis.* The experimental data were subjected to appropriate statistical analysis using the method described by SNEDECOR and COCHRAN (1967) by the IBM-SPSS software (Ver. 20.0). The General Linear Model technique repeated over age-groups and sex was considered for all variables under study. F values, if found significant, the main effect means of age group, n and sex were further compared by the LSD test at 5% level of significance. Pearson's correlation

coefficients between all possible pairs of variables were calculated using sex-wise and overall sets of data.

### Results

**Average weight gain (gm/day).** Average weight gain (gm/day) found in male and female black Bengal kids on different days of pre-pubertal growth are presented in Table 2. The average weight gain altered significantly ( $P \leq 0.01$ ) from day 15 to 180 days. Male kids ( $41.38 \pm 2.26$  g/day) gained more body weight (23%) compared to females ( $33.56 \pm 2.23$  g/day).

Table 2. Average daily weight gain (gm/day) in male and female black Bengal kids on different days of pre-pubertal growth

Day	Average daily weight gain (gm/day)	
	Male	Female
15	$50.17 \pm 6.23$	$40.61 \pm 3.93$
30	$34.06 \pm 4.07$	$13.81 \pm 2.81$
60	$34.06 \pm 4.38$	$34.06 \pm 4.13$
90	$58.17 \pm 5.57$	$53.31 \pm 7.97$
120	$60.44 \pm 4.95$	$36.47 \pm 4.89$
150	$26.06 \pm 2.68$	$32.47 \pm 6.58$

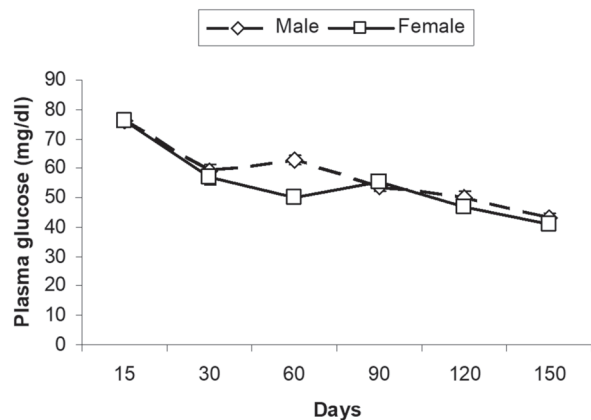


Fig. 1. Alterations in the plasma glucose levels in male and female black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE.

**Blood glucose level.** Alterations in the plasma glucose levels in male and female black Bengal kids on different days of pre-pubertal growth are presented in Fig. 1. The glucose level significantly ( $P \leq 0.01$ ) decreased with the advancement of age, and the maximum level occurred on day 15 whereas

the lowest level was at 150 days, with an overall of  $54.40 \pm 1.00$  mg/dL in both males and females. There was no significant difference between plasma glucose levels in male and female kids.

**Plasma AST and ALT.** Fig. 2 shows alterations in the AST and ALT levels in male and female black Bengal kids on different days of pre-pubertal growth. The overall AST and ALT levels, irrespective of sex and growth phase, were  $82.73 \pm 1.28$  and  $23.43 \pm 0.55$  IU/L respectively. Both AST and ALT levels increased significantly ( $P \leq 0.01$ ) from day 15 ( $67.01 \pm 1.97$  IU/L) and reached the maximum level of AST at day 90 and ALT at day 150. Our results indicated that sex has no influence on AST and ALT levels.

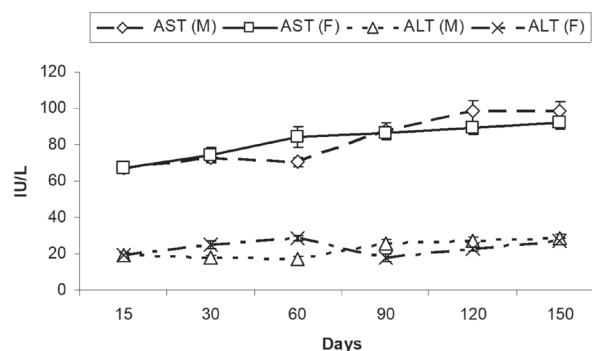


Fig. 2. Alterations in the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in male (M) and female (F) black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE.

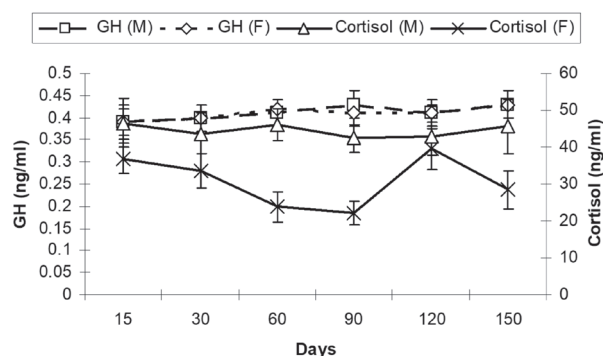


Fig. 3. Alterations in the growth hormone (GH) and cortisol level in male (M) and female (F) black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE

**GH and cortisol.** Alterations in the growth hormone and cortisol levels in male and female black Bengal kids on different days of pre-pubertal



growth are presented in Fig. 3. The growth hormone increased significantly ( $P \leq 0.01$ ) from day 15 to 180 days, and cortisol level declined with the advancement of age, and was lowest at 90 days in both sexes. The overall levels of GH and cortisol were  $0.41 \pm 0.01$  and  $38.12 \pm 1.45$  respectively. Sex had no significant influence on GH and cortisol levels.

Table 3. Correlation coefficients between blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), growth hormone (GH) and cortisol in male black Bengal kids during the pre-pubertal growth stage

	Blood glucose	AST	ALT	Growth hormone
AST	- 0.314**			
ALT	- 0.126	0.792**		
Growth hormone	0.274*	0.468**	0.687**	
Cortisol	0.248*	0.291**	0.430**	0.529**

\*\*  $P \leq 0.01$  \*  $P \leq 0.05$

Table 4. Correlation coefficients between blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), growth hormone (GH) and cortisol in female black Bengal kids during the pre-pubertal growth stage

	Blood glucose	AST	ALT	Growth hormone
AST	- 0.265*			
ALT	0.016	0.625**		
Growth hormone	0.244*	0.537**	0.715**	
Cortisol	0.213	0.354**	0.503**	0.607**

\*\*  $P \leq 0.01$  \*  $P \leq 0.05$

*Correlation coefficients between blood glucose, AST, ALT, GH and cortisol.* Correlation coefficients between blood glucose, AST, ALT, GH and cortisol in male and female black Bengal kids during the pre-pubertal growth stage are presented in Tables 3 and 4 respectively. In both sexes, plasma glucose level negatively ( $P \leq 0.01$ ) correlated with AST. In males, both cortisol and GH levels were positively ( $P \leq 0.05$ ) correlated with plasma glucose levels, but in female kids only GH showed a positive ( $P \leq 0.05$ ) correlation with glucose levels. AST and ALT were positively ( $P \leq 0.01$ ) correlated with growth hormone and cortisol in both male and female kids.

There was a significant positive correlation between growth hormone and cortisol in both sexes.

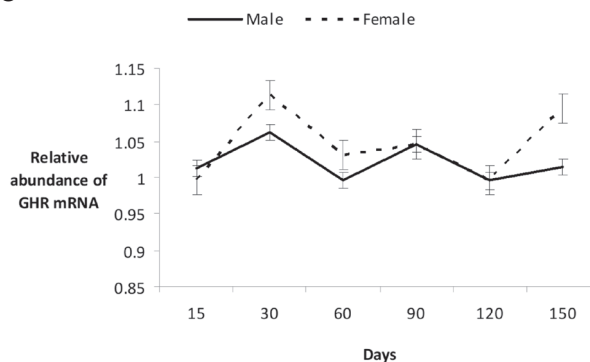


Fig. 4. Alterations in the relative abundance of growth hormone receptor (GHR) mRNA in male and female black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE.

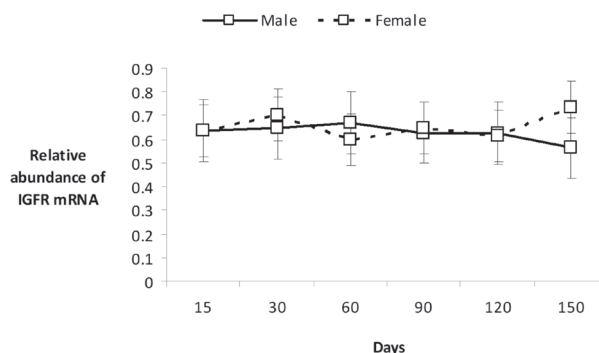


Fig. 5. Alterations in the relative abundance of insulin like growth factor receptor (IGFR) mRNA in male and female black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE.

*Relative abundance of GHR, IGFR and leptin mRNA.* The relative abundance of growth hormone receptor, insulin like growth factor receptor and leptin mRNA in male and female black Bengal kids on different days of prepubertal growth phase is presented in Fig. 4, 5 and 6 respectively. The expression of all these genes varied significantly ( $P \leq 0.05$ ) between different days of the prepubertal growth phase in both male and female kids, with the highest at day 30. Female kids had greater expression of the GH receptor on day 30 ( $P \leq 0.05$ ), day 60 ( $P \leq 0.05$ ) and day 150 ( $P \leq 0.01$ ). IGFR mRNA expression was greater in female kids on day 30 ( $P \leq 0.05$ ) and day 150 ( $P \leq 0.01$ ), and in males on day 60 ( $P \leq 0.01$ ). Leptin mRNA expression was observed more in the female kids on day 30 ( $P \leq 0.01$ ) and day 150 ( $P \leq 0.01$ ).

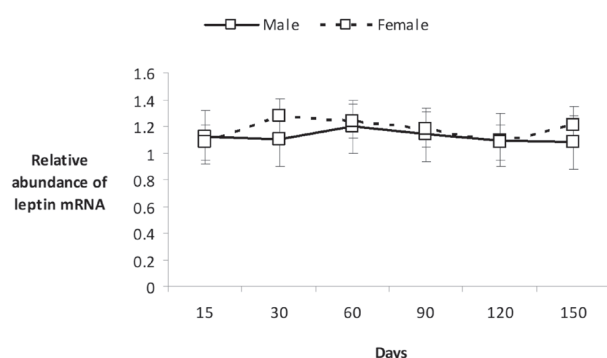


Fig. 6. Alterations in the relative abundance of leptin mRNA in male and female black Bengal kids on different days of pre-pubertal growth. Values are expressed as mean  $\pm$  SE.

*Correlation coefficients between blood glucose and expression of GHR, IGFR and Leptin.* Correlation coefficients between blood glucose and expression of GHR, IGFR and Leptin in male and female black Bengal kids during the pre-pubertal growth stage are presented in Tables 5 and 6 respectively. GHR was positively ( $P \leq 0.05$ ) correlated with IGFR and LEP in only female kids.

Table 5. Correlation coefficients between blood glucose and expression of growth hormone receptor (GHR) mRNA, insulin like growth factor receptor (IGFR) and Leptin (Lep) in male black Bengal kids during the pre-pubertal growth stage

	GHR	IGFR	LEP
IGFR	0.053		
LEP	- 0.235	0.703	
Blood glucose	- 0.007	0.675	0.420

Table 6. Correlation coefficients between blood glucose and expression of growth hormone receptor (GHR) mRNA, insulin like growth factor receptor (IGFR) and Leptin (Lep) in female black Bengal kids during the pre-pubertal growth stage

	GHR	IGFR	LEP
IGFR	0.834*		
LEP	0.847*	0.446	
Blood glucose	- 0.374	- 0.248	- 0.376

\*  $P \leq 0.05$

## Discussion

Blood glucose level have been reported to provide an index of nutritional status in goats and are influenced by the growth rate in animals (MORANT-FEHR et al., 1977). The blood glucose levels in black Bengal kids obtained in this investigation were in accordance with the earlier reports by PRADHAN (2016) and SHAIKAT et al. (2013). Declining levels of blood glucose with the advancement of age were in accordance with earlier reports in goats (UPADHAYAY and RAO, 1985; PRADHAN, 2016). In this investigation, sex had no influence on blood glucose levels, which was corroborated by earlier findings in different breeds of goats, including black Bengal (BEHERA et al., 1993; PARMAR et al., 2017) and sheep (KIRAN et al., 2012).

The values of AST and ALT were with the range reported earlier in black Bengal goats (GODARA et al., 2015) and West African Dwarf goats (ONI et al., 2012), but slightly lower than the reports by DEVRIM et al. (2015a) in Honamli and Native Hair goats. Both AST and ALT increased significantly with age in this study. Similar trends were reported earlier in goats (ELITOK, 2012; DEVRIM et al., 2015; NJIDDA et al., 2013), sheep (ANTUNOVIC et al., 2012) and cattle (MOHRI et al., 2007). Our study showed that AST and ALT did not differ significantly between male and female kids, which is in accordance with the earlier reports by GWAZE et al. (2012), but disagrees with NJIDDA et al. (2013) and TIBBO et al. (2008) who reported increased ALT in males and AST in female goats.

The values of GH in the present study were lower than the values reported in Shiba goats (HASHIZUME et al., 1999), Honamli and native hair goats (DEVRIM et al., 2015b), Tellicherry goats (SUGANYA and GOMATHY, 2009) and cross bred goats (KHAN and LUDRI, 2012). The lower GH levels in black Bengal Kids may be one of the causes of stunted growth in this breed of goats. The growth hormone increased significantly ( $P \leq 0.01$ ) from day 15 to 180 days, which was in accordance with the earlier reports by DEVRIM et al. (2015b). In contrast, HASHIZUME et al. (1999) reported a declining trend of GH in Shiba goats. He postulated that higher levels of GH in the maternal blood is

carried to the foetus, which increased the level of GH at birth in kids and it gradually decreased after activation of the hypothalamo-hypophyseal axis. Higher GH in neonates may be derived from milk since milk contains higher GH during the postpartum period (RONGE and BLUM, 1988). In our investigation, sex had no significant influence on GH level. A variable response of GH between sexes was reported earlier in goats (HODATE et al., 1985) and cattle (JOHKE et al., 1984; KELLER et al., 1979). It has also been reported that the plasma GH concentration in male lambs was significantly higher than that in castrated male lambs. In bulls, the circulating GH level was higher than in heifers (JOHKE et al., 1984). It has also been reported that ovariectomy induces weight gain in goats (ZHANG et al., 2014). This higher basal level of GH in males compared to females may be due to the higher sensitivity of growth hormone releasing factors in males (HODATE et al., 1985) or due to the effect of oestrogen, through modulation of GH and IGF-I (FISHER et al., 1998).

The value of cortisol in goat kids was in accordance with earlier reports by CAMARGO et al. (2010) in kids. The cortisol levels were higher at day 15, and gradually decreased from day 60 to day 90, then remained stationary. Higher levels of cortisol at birth were reported by CAMARGO et al. (2010). On the contrary, WALTMAN et al. (1991) reported that cortisol secretion levels remain stable during ageing. There is also some evidence suggesting that ageing is associated with significant activation of the hypothalamic pituitary adrenal (HPA) axis (HALBREICH et al., 1984), resulting in increased production of cortisol. In our investigation, sex had no influence over cortisol level. In contrast, DOERR and PIRKE (1976) reported that cortisol had a direct effect on gonads, and could decrease testosterone and oestrogen secretions. The different cortisol response between the sexes could be due to the binding of cortisol with transcortin and this affinity decreases with higher androgen levels (SLAUNWHITE, 1988). In contrast to these findings, HOWLAND et al. (1985) reported that increased aggressive behaviour and reduced voluntary food intake in male goats is due to an acute rise in cortisol and testosterone.

AST and ALT are the important markers for liver function in many animals, including goats (MEYER and HARVEY, 1998; MOHAMED, 2014) and are reported to be influenced by age and metabolic activity (WEIGERT et al., 1980). In this study, plasma glucose level was negatively ( $P \leq 0.01$ ) correlated with AST. Similar findings were reported in sheep (ANOUSHEPOUR et al., 2014) and cattle (KALAITZAKIS et al., 2010). Increased AST values with decreased blood glucose may be explained by protein mobilization from muscles for gluconeogenesis (CARDOSO et al., 2008).

In this study, GH was positively correlated with blood glucose levels in both male and female kids. The hyperglycaemic effects of GH have been well documented in laboratory animals. GH increases blood glucose levels by increasing gluconeogenesis in the liver and kidneys (SCHWARZ et al., 2002; KIM et al., 2012) decreasing glycogenolysis (HOYBYE et al., 2008; CHO et al., 2006) and suppressing glucose uptake in the adipose tissue (KILGOUR et al., 1995)

The positive correlation between plasma glucose levels and cortisol in male kids was in accordance with the earlier reports by KANNAN et al. (2000) and AJION et al. (2001) in goats. Cortisol increases blood glucose levels via glycogenolysis and gluconeogenesis (BOND et al., 2004). No significant correlation was found between plasma glucose level and cortisol in females. This may be due to the fact that female animals are less excitable and stress sensitive than male animals, as indicated by lower concentrations of cortisol under the same conditions (OKEUDO and MOSS, 2005).

The positive correlation between GH, cortisol, AST and ALT obtained in this study can be explained by higher hepatic activity since both of these hormones induce gluconeogenesis in the liver (SCHWARZ et al., 2002; KIM et al., 2012; BOND et al., 2004)

The somatotrophic hormone, or growth hormone, and its receptor, along with the insulin-like growth factor I axis are considered to be one of the most important regulatory systems, because of their broad range of effects and central role in growth (KATOH et al., 2008). Leptin acts as a unique nutritional signal to the growth axis, as

high leptin levels have been observed to inhibit feed intake through binding to a specific receptor in the hypothalamus (AGARWAL et al., 2009). Growth hormone (GH) exerts its action after binding with transmembrane receptor and leads to the activation of an intra-cellular and inter-cellular signal transduction pathway, inducing transcription of many genes, including IGF1 (ROTWEIN et al., 1994; ARGETSINGER and CARTER-SU, 1996) and leading to growth.

Novel polymorphisms of the growth hormone, growth hormone receptor genes and insulin like growth factor genes and their effects on growth traits have been reported earlier in goats (AN et al., 2011; RASOULI et al., 2017), cattle (MAJ et al., 2005) and sheep (VALEH et al., 2009). In our investigation, female kids had greater expression of GHR, IGFR and leptin compared to males, and a significant, positive correlation exists between these genes in female kids. The probable explanation for this is the higher bodyweight gain of male kids compared to females, as BERRYMAN et al. (2010) reported that growth hormone receptor gene-disrupted (GHR<sup>-/-</sup>) mice exhibited a greater percentage of body mass throughout life. This may be further validated by the study by DEVRIM et al. (2015) who reported higher levels of GH, IGF and leptin in the plasma of female goats compared to males.

Leptin acts as a unique nutritional signal to the somatotrophic axis, especially in nutritionally stressed animals (ZIEBA et al., 2005) as high leptin levels have been observed to inhibit feed intake through binding to a specific receptor in the hypothalamus (AGARWAL et al., 2009). Thus, leptin gene expression is considered to be an indirect indicator of the nutritional status of the animal, and may be used as a suitable biomarker from the somatotrophic axis of nutritional stress tolerance in goats (HYDER et al., 2013). Animals have a characteristic physiological ability to survive nutritional stress by altering the leptin levels required for maintaining the physiological normalcy (ZIEBA et al., 2005). Accordingly, leptin gene expression levels could act as markers for growth and feed conversion efficiency in kids, which has potential welfare applications (AGARWAL et al., 2009). BAGATH et al. (2016) also stated that GHR, LEP

and IGFR mRNA transcript expression was higher in nutritionally stressed Osmanabadi goats, but in our investigation, there was no significant difference in blood glucose levels in male and female kids. So, the higher expression of leptin may be explained by body weight gain, which is significantly lower in females compared to male kids. Male goats grew more quickly, retained more nitrogen and used feed with greater efficiency than females (ASH and NORTON, 1987). The expression and secretion of leptin are associated with body fat mass (ZIEBA et al., 2005; ROCHA et al., 2001) and attributed to the levels of feed intake, and the physiological and growth stages of these animals (AGARWAL et al., 2009).

### Conclusion

The present study permitted us to conclude that the lower growth rate in female kids expressed more leptin which stimulated the somatotrophic axis for expression of GHR and IGFR during the prepubertal growth stage.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

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**PERVEEN, S., P. K. DAS, P. R. GHOSH, D. BANERJEE, J. MUKHERJEE, M. MONDAL: Profil ekspresije receptora hormona rasta (GHR), receptora čimbenika rasta sličnog inzulinu (IGFR) i gena leptina s biokemijskim i endokrinim profilom u jaradi crne bengalske koze (*Capra hircus*) tijekom različitih razdoblja prepubertetskog rasta. *Vet. arhiv* 90, 243-254, 2020.**

#### SAŽETAK

Cilj istraživanja bio je procijeniti profil ekspresije receptora hormona rasta (GHR), receptora čimbenika rasta sličnog inzulinu (IGFR) i gena leptina zajedno s krvnim biokemijskim pokazateljima (glukoza, aspartat-aminotransferaza AST, alanin-aminotransferaza ALT) i endokrinim pokazateljima (hormon rasta i kortizol). U tu svrhu 15., 30., 60., 90., 120. i 150. dan života uzeti su uzorci krvi od 25 muške i 25 ženske jaradi crne bengalske koze (*Capra hircus*). Muška je jarad imala veći prirast tjelesne mase u usporedbi sa ženskom. Razine glukoze i kortizola s porastom dobi znakovito su se smanjivale ( $P \leq 0,01$ ). AST, ALT i hormon rasta znakovito su porasli ( $P \leq 0,01$ ). Kod oba je spola razina glukoze u plazmi bila u negativnoj korelaciji ( $P \leq 0,01$ ) s AST-om. Kod muške su jaradi kortizol i hormon rasta bili u pozitivnoj korelaciji ( $P \leq 0,05$ ) s razinama glukoze u plazmi. Samo je u ženske jaradi hormon rasta pokazao pozitivnu korelaciju ( $P \leq 0,05$ ) s razinama glukoze. Ženska je jarad imala veću ekspresiju receptora hormona rasta, mRNA IGFR i mRNA leptina 30. dan ( $P \leq 0,05$ ) i 150. dan ( $P \leq 0,01$ ). GHR je bio u pozitivnoj korelaciji ( $P \leq 0,05$ ) samo s IGFR-om i leptinom u ženske jaradi. Na temelju rezultata ovoga istraživanja možemo zaključiti da niža stopa rasta u ženske jaradi dovela do veće ekspresije leptina, što je stimuliralo somatotropnu os za ekspresiju hormona rasta i IGFR-a u prepubertetskom razdoblju.

**Ključne riječi:** crna bengalska koza; receptor hormona rasta; receptor IGF; geni leptina; prepubertetski rast

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