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Original article

Levels of hormones and cytokines associated with growth in Honamlı and native hair goats

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Abstract

This study was designed to assess alterations of hormone and cytokine levels associated with growth period during puberty in Honamlı goats which were identified as a new goat breed and had one of the highest meat production potential among the other goat breeds in Turkey. Honamlı goats are originated from native hair goats, so parallel studies of sampling and analyzing were conducted also in native hair goats which have moderate meat production. Blood serum samples of Honamlı (n=90) and native hair goats (n=90) were obtained from the pure herds in Korkuteli and Ka districts of Anatolia. Concentrations of growth hormone (GH), myostatin (MSTN), insulin-like growth factor (IGF), growth hormone releasing hormone (GHRH), growth hormone releasing peptide (GHRP), leptin, transforming growth factor-betal (TGF-β1) and vascular endothelial cell growth factor (VEGF) levels were measured by ELISA in each breed in the age groups of 4, 8 and 12 months. The present results indicate interesting correlations among the age groups and all the examined hormone and cytokine parameters exhibited significant ($P<0.05$ and $P<0.001$) differences. The parameters investigated were usually begun to increase after 4 months of age in the both breeds and sexes. Therefore, this paper supported the view that the beginning of hormonal alterations of goats could occur at 4th month of age. The results reported here emphasize the primary role played by GH, MSTN, IGF-1, leptin, GHRH, GHRP, TGF-βi and VEGF in the first year growth period of goats.

Key words: growth endocrinology, honamli goat, native hair goat, pubertal development

Introduction

Honamlı goats were previously considered within native hair goat breed, and later on they were defined as a new breed through their distinctive morphology and yield properties (Elmaz et al. 2012a). They are reared by the Yörük nomads who spend the winter on the Mediterranean coasts and migrate to the plateaus at the beginning of the summer. Thus the literature is very limited on Honamlı goats due to the nomadic life style of Yörüks. In the previous study performed on Honamlı goats, it was reported that in terms of meat production, Honamlı goats had one of the highest potential among the other goat breeds reared in Turkey (Elmaz et al. 2012b).

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Table 1. The characteristics of the each study group (n=15).

Group ID	Sex	Breed	Age (months)
1	Female	Native Hair Goat	4
2	Female	Honamlı Goat	4
3	Male	Native Hair Goat	4
4	Male	Honamlı Goat	4
5	Female	Native Hair Goat	8
6	Female	Honamlı Goat	8
7	Male	Native Hair Goat	8
8	Male	Honamlı Goat	8
9	Female	Native Hair Goat	12
10	Female	Honamlı Goat	12
11	Male	Native Hair Goat	12
12	Male	Honamlı Goat	12

Skeletal muscle development is a prominent and complex biochemical process in meat animals, and it directly affects the meat production (Swatland 1994). In this regard, growth hormone (GH), myostatin (MSTN) and insulin-like growth factor (IGF) play a direct role in the growth and development in muscle, and they are decisive on the meat production traits (Biga et al. 2004).

Growth hormone releasing hormone (GHRH) increases lipolysis by stimulating the release of GH from the anterior pituitary gland, and promotes protein synthesis and the growth of long bones through IGF (Groenewegen et al. 1990; Binelli et al. 1995). It is also known that both growth hormone releasing peptide (GHRP), which is a synthetic oligopeptide and leptin, an adipocyte-derived hormone, exert stimulatory effects on GH secretion (Hashizume et al. 1997, Watanobe et al. 2002). Additionally transforming growth factor-betal (TGF-β1), which is closely related to MSTN, has been shown to inhibit proliferation and differentiation of myogenic cells in cell culture. It was reported that TGF-β1, independently or together with MSTN plays an important role in the muscle growth (Dayton et al. 2008).

Vascular endothelial cell growth factor (VEGF) has been reported to be a key regulator on physiological angiogenesis, skeletal development and reproductive functions (Ferrara et al. 2003). Besides induction of new blood vessels, VEGF is effective in vessel viability. In addition, there are studies that reported autocrine effects of VEGF on non-vascular cells such as muscle (Germani et al. 2003) and bone (Byun et al. 2007).

To the best of our knowledge, the present study is the first work performed in order to identify endocrinological characteristics of goat growth during puberty. The aim of the present study was to detect alterations of hormone and cytokine levels associated with the growth period of Honamlı and native hair goats widely grown in the same region.

Materials and Methods

Experimental procedures were approved by Mehmet Akif Ersoy University, Ethics Committee (21.11.2012, meeting/decision: 3/09). Blood samples of Honamlı (n = 90) and native hair (n = 90) goats were obtained from the pure herds in Korkuteli and Kaş districts of Turkey. The kids were identified using ear tags. Twelve groups were formed and each group included 15 goats. Study group details are given in the Table 1.

The kids were weighed by an electronic balance which was sensitive up to ±10 gr and recorded. No experimental applications or dietary changes were made to the animals. The inception and the end of the puberty period (Khanum et al. 2000, Ozder 2006) were considered in the creation of the age groups. Healthy animals of 4, 8 and 12 months of age were included in the study. The blood samples (5-7 mL) were taken to the vacuum blood collection tubes (yellow cap, 8 mL) from jugular veins. The sera were obtained with the centrifugation of the blood samples at 2000 × g for 10 minutes. The samples were stored at -20°C until the ELISA analyses were performed.

ELISA tests were performed using blood serum samples. GH, MSTN, IGF, GHRH, GHRP, leptin, TGF-βi and VEGF assays were performed at least two times spectrophotometrically (Multiskan GO, Thermo) using commercial kits (EASTBIOPHARM) according to manufacturer's instructions.

Results of the ELISA were evaluated separately for statistical analyses. MINITAB statistical software package version 16.1 was used in the evaluation of the data. One-way analysis of variance (One-Way ANOVA) was performed for the study parameters. Significance of statistical difference between the groups was determined by the Tukey test.

Table 2. Mean live weight in the different periods of female Honamlı and native hair Goats (kg) ($\bar{x} \pm s_{\bar{x}}$).

Breed	n	Birth weight	4 months	8 months	12 months
Honamlı	15	4.21 ± 0.10[a]	29.8 ± 0.73[a]	35.7 ± 0.48[a]	33.8 ± 0.57[a]
Native Hair	15	3.84 ± 0.11[b]	26.7 ± 0.50[b]	28.9 ± 0.66[b]	25.8 ± 0.68[b]

[a, b] Differences between values within the same column signed as different letter is significant (p<0.05).

Table 3. Mean live weight in the different periods of male Honamlı and native hair Goats (kg) ($\bar{x} \pm s_{\bar{x}}$).

Breed	n	Birth weight	4 months	8 months	12 months
Honamlı	15	3.52 ± 0.21[b]	32.3 ± 1.64[a]	35.8 ± 1.88[b]	45.6 ± 1.52[a]
Native Hair	15	3.92 ± 0.15[a]	32.7 ± 0.40[a]	39.8 ± 0.50[a]	36.8 ± 0.51[b]

[a, b] Differences between values within the same column signed as different letter is significant (p<0.05).

Table 4. Group (n = 15) means (mean ± standard error) of the females according to the age groups. Different letters in the same rows refer to statistical difference (p<0.05), NS: Not significant.

	Native Hair				Honamlı			
	4 months	8 months	12 months	*P*	4 months	8 months	12 months	*P*
GH (ng/mL)	1.95±0.07[b]	3.73±0.15[a]	3.79±0.16[a]	<0.001	1.93±0.10[b]	3.72±0.18[a]	3.85±0.14[a]	<0.001
MSTN (ng/mL)	12.14±0.84[b]	13.67±166[b]	18.60±1.41[a]	<0.05	10.84±0.70[b]	11.93±1.58[a,b]	15.10±1.14[a]	<0.05
IGF-1 (ng/mL)	225.1±20.2[b]	323.8±23.9[a]	389.1±15.4[a]	<0.001	189.9±9.33[b]	278.9±28.4[a]	282.5±17.3[a]	<0.05
Leptin (ng/mL)	24.89±4.61[b]	36.20±2.46[a]	41.62±2.20[a]	<0.05	19.65±1.01[b]	31.56±3.78[a]	36.41±1.52[a]	<0.001
GHRH (ng/L)	221.7±14.4[b]	329.6±23.9[a]	335.7±17.7[a]	<0.001	214.1±14.5[b]	301.5±27.0[a]	342.8±18.3[a]	<0.001
GHRP (ng/mL)	2.19±0.13[b]	3.04±0.17[a]	3.18±0.20[a]	<0.05	1.80±0.08[b]	2.52±0.24[a]	2.57±0.20[a]	<0.05
TGF-β1 (ng/L)	225.1±30.4[b]	760.1±31.2[a]	709.5±25.9[a]	<0.001	181.3±13.8[b]	669.1±63.0[a]	619.1±33.4[a]	<0.001
VEGF (ng/L)	312.5±24.5[b]	473.1±29.2[a]	549.4±35.3[a]	<0.001	285.9±19.7	364.4±60.8	429.0±36.0	NS

Table 5. Group (n = 15) means (mean ± standard error) of the males according to the age groups. Different letters in the same rows refer to statistical difference (p<0.05), NS: Not significant

	Native Hair				Honamlı			
	4 months	8 months	12 months	*P*	4 months	8 months	12 months	*P*
GH (ng/mL)	1.90±0.09[b]	3.51±0.19[a]	3.87±0.34[a]	<0.001	1.90±0.11[b]	3.53±0.30[a]	3.94±0.37[a]	<0.001
MSTN (ng/mL)	10.94±0.54	12.83±1.84	15.72±1.96	NS	11.61±0.95	11.87±2.37	18.91±3.61	NS
NGF-1 (ng/mL)	201.36±6.84[b]	321.1±28.1[a]	350.5±24.8[a]	<0.001	187.3±8.00[b]	274.9±22.3[b]	380.3±43.9[a]	<0.001
Leptin (ng/mL)	2L25±0.72[b]	35.38±3.44[a]	36.27±3.02[a]	<0.05	20.01±1.60[b]	30.89±3.74[a,b]	41.57±5.84[a]	<0.05
GHRH (ng/L)	216.2±9.83[b]	327.1±41.5[a]	360.8±30.0[a]	<0.05	212.3±17.2[b]	311.5±29.1[a,b]	371.9±42.2[a]	<0.05
GHRP (ng/mL)	2.01±0.06	2.88±0.33	2.69±0.28	NS	1.96±0.15[b]	2.44±0.25[a,b]	2.93±0.32[a]	<0.05
TGF-β1 (ng/L)	182.1±9.13[b]	698.1±21.7[a]	680.9±35.8[a]	<0.001	175.5±10.9[c]	566.2±27.5[b]	667.6±42.4[a]	<0.001
VEGF (ng/L)	275.4±14.0[b]	466.1±40.6[a]	453.1±51.1[a]	<0.05	277.5±21.2	302.8±46.2	418.0±53.9	NS

Results

The results from the Table 2 and 3 indicate the alterations in the average live weights of the females and males, respectively. The average weight of Honamlı females was higher than that of native hair females in all the age groups. As for the males, average live weight of native hair goats was significantly (p<0.05) higher than that of Honamlı kids for birth and 8 months period. On the contrary, the average live weight of the Honamlı males was increased significantly (p<0.05) and exceeded that of native hair kids on 12 months period.

In the groups of all breeds and sexes there were significant ($p<0.001$) GH increases (nearly 2 folds) after 4 months of age (Tables 4, 5). As for MSTN, there were no significant differences in the groups of males (Table 5). However the female groups exhibited significant ($p<0.05$) MSTN increases after 8 months of age in native hair goats and between age groups of 4 and 12 months in Honamlı goats (Table 4). In the groups of females, IGF-1 concentrations increased significantly ($p<0.001$) after 4 months of age (Table 4). In the males, IGF-1 levels increased significantly ($p<0.001$) after 4 months in native hair goats, and after 8 months in Honamlı goats (Table 5). Leptin values increased nearly a half fold in the females of native hair ($p<0.05$) and Honamlı ($p<0.001$) goats (Table 4). As for the males, leptin concentrations increased significantly ($p<0.05$) in native hair goats after 4 months of age and in Honamlı goats on 12 months of age (Table 5).

GHRH levels were increased after 4 months of age in both females ($p<0.001$) and males ($p<0.05$). GHRP values were increased significantly ($p<0.05$) after 4 months of age in the group of females. As for group of males, there was a significant ($p<0.05$) increase in 12 months compared to 4 months period only in Honamlı goats. TGF-β1 concentrations were increased significantly ($p<0.001$) in both breed groups of females and also in the male native hair goats after 4 months of age. As to the males of Honamlı, TGF-β1 levels were increased ($p<0.001$) gradually with age periods. In both sexes of Honamlı goats, there were no significant changes in VEGF values among the age groups. Conversely, in both female ($p<0.001$) and males ($p<0.05$) of native hair goats, the levels of VEGF were increased after 4 months of age.

Discussion

It was reported that hormonal alterations in goats could begin to occur at the 4th month after the birth (Khanum et al. 2000). In the present study, sampling studies were carried out on three different age groups in the periods of 4, 8 and 12 months, considering the beginning and the end of the puberty period (Ozder 2006).

The present results showed that in the females, Honamlı kids had higher live weights than the native hair goat kids in all the age groups. As for the males, Honamlı kids slammed nearly 10 kg live weight as compared to the native hair goats on the 12th month. So, it can be concluded that at the end of the 12-month period, Honamlı males generate live weight gain more slowly but higher than native hair males.

In the literature, there is a small number of studies reporting serum GH levels in goats and they usually deal with the period of pregnancy. In a study (Juárez-Reyes et al. 2004) that examined the effects of semi-arid grazing conditions on metabolic changes in pregnant goats, a negative correlation was determined between serum insulin and GH levels during advanced pregnancy (December) period and the highest serum GH levels were reported as 20.2 ng/ml. In another study examining milk production and breast development, the serum GH levels were reported in the pregnant goats as 3.22±0.05, 2.84±0.1 and 2.75±0.22 ng/ml on prenatal 1, 7 and 14th days, respectively (Zhang et al. 2014). Considering these reports, it can be concluded that GH levels vary on a wide scale in the period of pregnancy. On the other hand, mean GH values found in the present study were determined between 1.93±0.10 and 3.85±0.14 ng/ml in the female groups up to the 12 months of age. In this respect, it can be concluded that in the period of the first one year of intensive growth, the GH values obtained in the present study would contribute to overcome the lack of the literature.

In the present study, GH increases were observed after 4 months of age in both Honamlı and native i hair goats having high and moderate meat productions, respectively. Considering this situation, it can be evaluated that the increases of GH after the 4 month age period would probably be the characteristics of the species of goat. Although we could not find any studies reporting goat serum GH values of the similar age periods' the increase observed after 4 months of age is considered to play an important role in the intensive growth period during pubertal development.

In the female native hair goats, there was a MSTN increase in 12-months age group, compared to 4 and 8 months of ages. As for the females of Honamlı, MSTN values were increased in 12 months of age, compared to that determined at 4 months. That probably means MSTN rise starts after 4 to 8 months of ages in females. In the both breeds, MSTN rise becomes significant at the age of 12 months. However, there was no significant difference between the age groups of the males. This situation may be peculiar to male goats and should be investigated by new studies to be carried out in goats over 12 months of age. Although there were some MSTN gene polymorphism and characterization studies (Singh et al. 2014a, b), we could not find any report about the blood serum levels of MSTN in goats.

In the male and female groups of both breeds, IGF-1 levels were increased after 4 months and reached the highest level in 12 months of age. Considering these findings, it could be seen that IGF-1 increases occurred in male Honamlı groups formed

later. Serum IGF-1 levels were stated as 312.79 ng/mL in a study (Park et al. 2012) that was carried out on the 7 month old male goats in various nutrition alternatives. This value is in the interval of the IGF-1 values (Table 5) of our 8 month groups.

Significant leptin increases were determined between 4 and 8 months of age in the female groups. As for the males, leptin levels were increased in native hair and Honamlı goats, after 4 and in 12 months of ages, respectively. Considering all these findings together, it can be concluded that there are increases in leptin levels after 4 months of age. There are reported leptin levels (Whitley et al. 2005, Celi et al. 2008, Walker et al. 2011) below 10 ng/mL. However, these differences could be arising from the impact of nutritional conditions and design of the age groups.

Significant increases were observed in GHRH values of the female goats after 4 months of age. In the males, GHRH values increased in native hair and Honamlı goats after the 4th and 8th month, respectively. Considering the data of GH and GHRH together, it could be concluded that these parameters revealed parallel courses and they appear to correspond with those observed in previous studies (Mogi et al. 2004, Yonezawa et al. 2011). There were significant GHRP increases in Honamlı bucks and in the females of the both breeds after 4 months period. These findings suggest that, as similar to the course of GH and GHRH levels, GHRP starts to increase after a period of 4 months of age and exhibits significant rises, especially in Honamlı goats.

Increasing VEGF values were observed after 4 months of age in both sex groups of native hair goats, and Honamlı goats revealed non-significantly rise in VEGF values parallel to age. Probably, statistically significant VEGF increases would be obtained with the greater number of Honamlı goats.

TGF-β1 increases occurred in females after 4 months of age. As for the males, TGF-β1 increased after 4 months in native hair goats and periodically in all age groups of Honamlı goats. Considering these findings, it can be concluded that in both sexes, an increase in TGF-β1 levels begins from 4 months of age but it occurs later in the females in the first year period. In a study of Jing et al. (2012), serum TGF-β1 levels were reported as 40-60 pg/ml in adult (3-4 years old) healthy goats in the second and third lactation period. Although these values are lower than those found in the present study dealing with TGF-β1 levels, it is concluded that investigated age period in the present study is the main period of the growth and could present higher values.

Consequently, most of parameters determined in the present study started to alter after 4 months of age. In this regard, it can be suggested to consider the 4 months of age as a critical period for hormonal alterations in goats. The data reported in the present study emphasize the primary role, played by GH, MSTN, IGF-1, leptin, GHRH, GHRP, TGF-β1 and VEGF during the puberty period of goats. Also, the levels of hormones and cytokines associated with the growth period of goats were reported for the first time in the present study. It is concluded that the present study would contribute to the lack of data about growth endocrinology in goats and expected to constitute a reference for designing the new studies in the future.

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