

Concentration of Growth Factors in Lambs from Birth to 180 Days of Age

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Abstract: The SC of GH and IGF-1 in lambs from birth to 180 days of age and its relationship with age and body weight was evaluated. Blood samples were collected from five male lambs at each of the following ages: 1, 60, 120 and 180 days. The SC of GH and IGF-1 was analyzed by ELISA. Regression and correlation analyses between the concentration of growth factors and age and weight of animals were performed. The body weight of lambs at birth was not different but as expected it increased with age (P<0.05). The SC of GH and IGF-1 changed with the age of the lambs. The highest SC of GH was observed between d-60 and d-120 of age of the lambs, but it decreased at a level similar to that at birth. The highest concentration of IGF-1 was observed during the first 60 days of age followed by a gradual decrease as the lambs aged. The lowest serum IGF-1 was observed at 180 days of age. There was a negative relationship between IGF-1 and body weight at birth; the relationship between IGF-1 and age was also negative. These results show a close relationship between IGF-1 and the age and body weight of lambs.

Keywords: GH, IGF-1, Age, Serum concentration.

1. INTRODUCTION

Animal growth is determined by age in addition to nutritional [1], genetic, environmental and hormonal factors [2]. Hormonal regulation is mainly controlled by growth hormone (GH) and insulin-like growth factor type 1 (IGF-1), with its cellular transport and receptor proteins GHBP, GHR, IGFBP [3, 4], IGFR -1) [5, 6] Apparently, nutritional [7] and hormonal factors are the main determinants of animal growth [8]. Some studies in sheep [9, 10] report that the concentration of IGF-I in the blood and the relative expression in the liver gradually increases as the lamb grows up to three months. Likewise, the serum concentration (SC) of IGF-I varies with age and gender [11]. Control of the secretion and mechanism of action of GH and IGF-1 occurs through the signaling cascade that begins in the hypothalamus. The hypothalamus secretes GHreleasing hormone (GHRH) from the pituitary to release GH into the bloodstream. Once in the blood, GH travels to different tissues and promotes the synthesis and secretion of IGF-1. Both GH and IGF-1 bind to their receptors in their target tissues, which in this way are stimulated to continue or maintain their growth

[12]. The pulses generated on the release of GHRH is the cause of the pulsatile secretion of GH. This secretion pattern varies according to the genus and species. Regarding this, males have been reported to exhibit higher secretion pulses than females [13]. On the other hand, Ribeiroolivera and Barkan [14] mention that in rats and sheep, only the pulsatile administration of GHRH had an effect on growth in normal and GHRHdeficient individuals. While IGF-I has been recognized as the main regulator of postnatal growth and development, both IGF-I and IGF-II play an important role in the regulation of prenatal growth and development [15], in addition to IGF-I levels. they increase significantly after delivery [16]. Likewise, the serum concentration of IGF-I varies with age and gender [11]. Some studies in sheep [9, 10] have reported that the concentration of IGF-I in blood and the relative expression in the liver increases gradually as the lamb grows up to three months, likewise the expression of GHR increases, mainly responsible for the union of GH so that it can exert its effect. Likewise, they also reported that concentration and expression levels (IGF-I

and GHR) in lambs are affected by the level of feeding offered to mothers during pregnancy, that both overfeeding and restricted feeding decreased IGF expression. -I and GHR in the liver of the offspring, which negatively impacted the live weight of the offspring from birth to three months of age [17, 18, and 19]. However, until now the patterns of synthesis and secretion of growth factors in domestic animals have not been clearly determined. Their knowledge, specifically in lambs, will provide the basis for formulating strategies focused on increasing their growth rate and feed efficiency. The objective of the present study was to determine the SC of GH and IGF-I in lambs from birth to blood sampling weight and to analyze the impact of birth weight on growth of lambs.

2. MATERIALS AND METHODS

All lambs in this study were cared for in accordance with the guidelines established in the Official Mexican Regulation on Animal Care (NOM-062-Z00-1999). The study was carried out in the Posta Ovina and in the Nutrigenomics Laboratory of the Institute of Agricultural Sciences of the Autonomous University of Baja California (UABC), located in the ejido Nuevo León, Valle de Mexicali, B.C., in northwestern Mexico. The study was designed to compare serum GH and IGF-1 concentration in lambs from birth to 180 days of age and their relationship to age and body weight. Five male lambs (Dorper x Charolais), from birth to 180 days of age, were used in a pen equipped with (adlivitum) and feeders. troughs shade (galvanized sheet 2.5 m high) under the typical management of a commercial herd, dewormed vitamins, weaned at 60 d. The diets were formulated to meet the nutritional requirement indicated in the NRC (2007) to feed weaning lambs at 180 d of age. The diet was formulated with 30% chopped forage and 70% grain and a vitamin-mineral premix.

Blood samples were collected from the lambs at 1, 60, 120 and 180 days of age. To correct the variations in the GH concentration, due to its pulsatile secretion, each lamb was sampled every two hours for 12 continuous hours, starting at 0600 h. Each sample of one to three ml of blood was obtained from jugular vein using vacutainer tubes and 18G to 21G needles depending on the thickness of the blood vessel. Blood samples were kept on ice during their transfer to the laboratory, where they were centrifuged at 3,000 rpm for 10 min at 4°C, to separate the serum from the cell pack. Sub-samples of the same serum volume (300 µl, approx.) of all samples of the

same animal at the same age were mixed and stored at -80 °C until analysis. Serum concentrations of GH and IGF-1 were determined by Enzyme-linked immunoassays (ELISA). Commercial ELISA kits (Sheep-GH (Growth Hormone) ELISA Kit, E-EL-S1275 and Sheep Insulin-Like Growth Factor 1 ELISA Kit, CSB-E13753Sh for IGF-1) Wuhan, Hubei Province 430206, P.R China, was used, following the protocols described by the manufacturer. All samples were analyzed in duplicate. The SC of each growth factor was calculated on a Multiskan EX model ELISA plate reader (Thermo electron, USA).

The results of birth weight, weight at 60, 120 and 180 d, and the SC of GH and IGF-1 at the same age were subjected to analysis of variance. In addition, polynomial regression analyzes and Pearson correlation were performed between age, birth weight and weight at each sample blood date (1, 60, 120 and 180 d), and the SC of GH and IGF-1. The Statistix 10 program was used in the statistical analyzes of the data. Difference and significant trend were declared when P values were <0.05 and ≤ 0.10 , respectively.

3. RESULTS AND DISCUSSION

The weight at birth of the animals at the different ages was not different (P> 0.10), but as expected, the weight at blood sampling increased (P <0.05) with age. (Figure 1). The daily weight gain (Figure 2) from d-1 to d-60 of age was not different from that observed from d-60 to d-120 (P> 0.10). But the weight gain from d-120 to d-180 was markedly greater (almost twice) than that from d-1 to d-60 (P <0.05). The overall average weight gain in lambs was 284 g/d.

The SC of GH analyzed at different ages, from birth to 180 d, is presented in Figure 3. On d-60 and d-120 of age, the SC of GH increased (P <0.05) compared to the analyzed SC at birth but it was not different (P> 0.10) between d-60 and d-120. At 180 days of age, the serum GH decreased to a level similar to that observed at birth. In general, the SC of GH showed a quadratic response with respect to age (P < 0.001; $r^2 = 0.74$) and weight (P = 0.001, $r^2 = 0.73$), as shown in Figures 4 and 5, respectively. In contrast, the SC of IGF-1 (Figure 6) was not different between 1 and 60 days of age (P > 0.10) but gradually decreased as the age of the lambs increased (P < 0.05) on d 120 and 180. As shown in Figures 7 and 8, the IGF-1 SC decreased linearly with respect to age (P < 0.001, $r^2 = 0.81$) and weight (P <0.001, $r^2 = 0.80$) at blood sampling, respectively. On average, the SC

values of GH ranged from 4.9 to 9.1ng/ml while the IGF-1 values fluctuated between 58.5 and 106.2ng/ml. When data from d-1 to d-180 of age were included in the regression analysis, no relationship was found between the SC of GH and that of IGF-1. However, when the analysis included data from d-60 to d-180 of age (Figure 9), the SC of IGF-1 tended to increase with the increase of the SC of GH (P = 0.10, $r^2 = 0$, 31). The relationship between weight and SC of GH at birth was not significant. On the contrary, a negative linear relationship (P = 0.007, r2 = 0.34) was observed between weight and IGF-1 SC at birth (Figure 10). It is also important to mention that the weight gain of the lambs was linearly negative related to the SC of GH (Figure 11; P =0.006, r2 = 0.45) and that of IGF-1 (Figure 12; P = 0.002, r2 = 0.55).

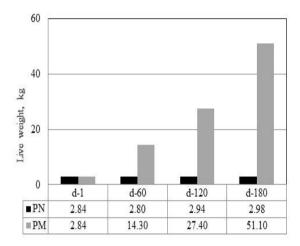


Figure1. Live weight at birth (WB) of the lambs (kg) and on days 56 (d-56), 126 (d-126) and 180 (d-180) of age, in which samples were collected from blood for GH and IGF-1 analysis. Different literal on each column means difference (P <0.05).

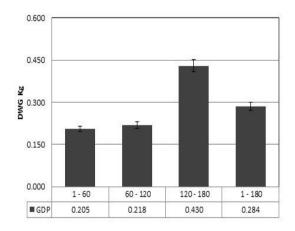


Figure2. Weight gain (g / d) of the lambs per period (1 to 60 d, 60 to 120 d, and 120 to 180 d of age) and overall (1 to 180 d of age). Different literal on each column means difference (P <0.05).



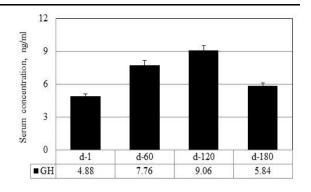


Figure3. Serum concentration (ng / ml) of GH in lambs on the first day of birth (d-1) and at 60 (d-60), 120 (d-120) and 180 (d-180) days of age. Different literal on each column means difference (P < 0.05).

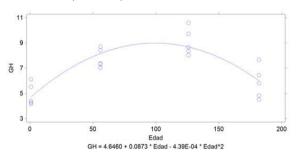


Figure4. Relationship (quadratic: P < 0.001, r2 = 0.74) between GH serum concentration and the age of the lambs.

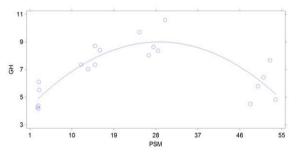


Figure5. Relationship between GH serum concentration and slaughter weight (SW) (quadratic: P = 0.001, r2 = 0.73)

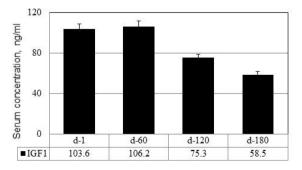


Figure6. Serum concentration (ng / ml) of IGF-1 in lambs on the first day of birth (d-1) and at 56 (d-56), 126 (d-126) and 180 (d-180) days of age. Different literal on each column means difference (P <0.05).

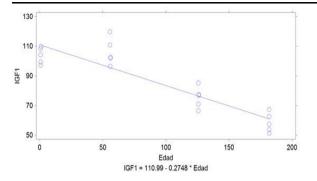


Figure7. Relationship between the serum concentration of IGF1 and the age of the lambs (linear: P < 0.001, r2 = 0.81)

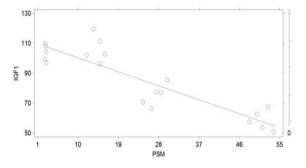


Figure8. Relationship between the IGF-1 serum concentration and the blood sampling weight of the lambs (linear: P = 0.001, r2 = 0.80)

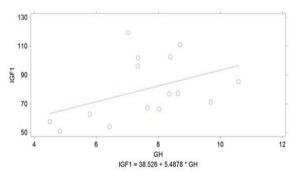


Figure9. Relationship between the serum concentration of GH and that of IGF-1, from d-60 (weaning) to d-180 of age (linear: P = 0.10, r2 = 0.31).

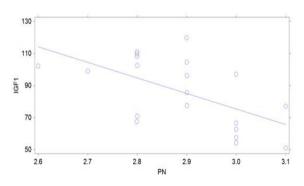


Figure10. Relationship between the serum concentration of IGF-1 and the birth weight of the lambs (linear: P = 0.007, r2 = 0.34).

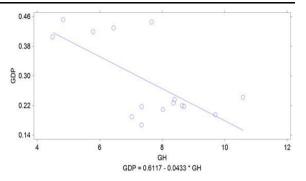


Figure11. Relationship between the serum concentration GH and the weight gain of the lambs (linear: P = 0.006, r2 = 0.45).

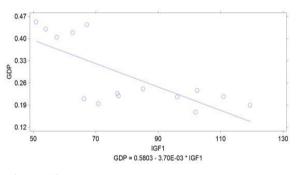


Figure12. Relationship between the serum concentration of IGF-1 and the weight gain of the lambs (linear: P = 0.002, r2 = 0.55).

The relationship between the variables studied was analyzed using Pearson's correlation tests (Table 1). The correlation between age and serum IGF-1 concentration was highly significant (P <0.001; r = -0.90), indicating that as the lamb grows, the IGF-1 concentration increases. Likewise, the serum concentration of IGF-1 has a high correlation with birth weight (P <0.005; r = -0.58) and highly significant with sample weight (P <0.001; r = -0.90). In addition, there is no response (P = 0.883; r =-0.04) when analyzing the concentration of IGF-1, that is, there was apparently no response in the secretion of IGF-1 directed by the concentration of circulating GH.

Table1. Correlation coefficients between age, birth weight, sampling weight, and serum concentrations of GH (GH-SC) and IGF-1 (IGF1-SC).

	Age	Body weight	Sampling weight	GH-SC
Sampling				
Weight	r=0.98	r= 0.52		
	P<0.001	P=0.020		
GH-SC	r= 0.26	r=-0.70	r= 0.11	
	P=0.276	P=0.778	P=0.643	
IGF1-C	r=-0.90	r=-0.58	r= -0.90	r=04
	P<0.001	P<0.005	P<0.001	P=0.883

Studies on SC of GH and IGF-1 in lambs are scarce, and in particular those related to birth weight, age and weight at blood sampling. The weight of the lambs used in this study, as expected, increased with age. Although the weight gain was not different between the 60 days before weaning and the first 60 days after weaning, this was approximately double during the last 60 days (d-120 to d-180 of age). These variations in weight gain allowed analyzing apparent associations with changes in the serum concentration of GH and IGF-1.

In the present study, the decrease in SC of GH from d-60 to d-180 is consistent with that found in sheep by Recabarren [11], who also showed that GH secretion in sheep decreases before puberty, although they mentioned that the physiological reasons for this decrease are not clear [4]. In agreement, Suttie [12] reported a decrease in the GH secretion, which was attributed to the increase in age or the decrease in GHRH secretion. Trenckle [14], suggests that the rate of growth the lambs decreases as age advances, it may be that with maturity the animal, the target tissues become less receptive to the low physiological effects values of GH, these results coincide with what was found in this study [3, 4]. In contrast, no differences in the CS of GH were observed in young cattle under tropical conditions during the first six months of life [13].

In the present work the concentration of IGF-1, decreased with the growth of the lambs. A significant decrease in the serum concentration of IGF-1 was observed in the post-weaning period (d-60), maintaining this decrease until 180 d, [5], which could indicate that weaning is a critical moment in the response to GH, in agreement with Lopez [10] they mention that the administration of rhGH at the time of weaning seems to interfere with the delicate mechanisms of adaptation to solid feeding characteristic of this period, and induce the cessation of growth. The decrease in circulating concentrations of IGF-I in plasma with the increase in the age of the lambs coincides with the results published by Mears [8].

The information published in relation to the SC of IGF-1 is variable depending on the animal species in which it is analyzed [5]. In horses [15] and rodents [16], the SC of IGF-1 gradually decreases with age; the last authors [15, 16] observed that the IGF-1 SC regulates longevity, the role of insulin/IGF-1 in longevity is probably related to stress resistance. [5]. In contrast, in works with pigs, Estany [17], observed that plasma IGF-1 increases steadily from 35 to 160 d, followed by a downward trend at 185 d. While

Muñoz [18], pointed out that there are other factors that affect the circulating concentrations of IGF-1; among the most important are race, sex, age and diet [19]. The lambs used in the present study were of the same race and sex, and consumed the same type of diet (formulated with 30% chopped forage and 70% grains and a premix of vitamins and minerals), they were weaned at 60 d age and were fed the aforementioned diet until 180 d of age.

The results obtained infer the fact that GH induces the synthesis of IGF-1 mainly in the liver and, to a lesser extent, in other tissues [20]. Likewise, Simmen [21] also mentions that the concentration of GH and Insulin regulate the molecular mechanisms of the gene for the synthesis of IGF-1. Similarly, Wester [22] found that administration of exogenous porcine GH to newborn pigs increased the CS of IGF-1 by 300% compared to the control group. In the present work, when GH and IGF-1 EC values were included in the regression analysis from birth to 180 days of age, no relationship was observed between GH and IGF-1 EC. However, when GH and IGF-1 values after weaning were considered in the analysis (after d-60), IGF-1 CS tended to increase linearly with increasing GH CS, which is consistent with [20, 21].

Moreover, it is important to mention that in the present study the feeding of the lambs before weaning was different from that received after weaning, especially with respect to the content of soluble carbohydrates and fats in the mothers' milk. This response indicates that the relationship between the SC of GH and that of IGF-1 is also dependent on the feed the animals receive, which coincides with that published by Treiber [23].

In this study the SC of GH and IGF-1 was not related to the weight of lambs at birth, indicating that the weight of animals at birth is independent of the SC of these hormones and that other maternal factors related to fetal development may have a greater influence on the weight of the newborn. On the other hand, the quadratic response observed in the SC of GH in relation to the weight and age of the lambs suggests a temporal association between GH and animal weight. Interesting was also the fact that the IGF-1 SC was negatively related to the age and weight of the lambs to blood sampling. Moreover, the weight gain of the lambs was negatively related to the SC of GH, coinciding with Müller [24] who published that the body mass index correlates negatively with the secretion of GH. On the other hand, Muñoz [18] mention in their research that IGF-1 concentrations experience an acute postnatal increase, possibly due to an increase in the number of GH receptors in various tissues, especially in the liver, they found an increase continuous IGF-1 from birth and decline with age. Likewise, Estany [17] also observed a positive association between live weight and IGF-1 concentration in pigs aged 35 d. The above indicates the existence of differences between species with respect to the SC of GH and IGF-1.

4. CONCLUSIONS

The results of this study show that the birth weight of lambs is independent of the SC of GH and IGF-1. Likewise, no consistency was found in the relationship between serum concentrations of GH and IGF-1 with respect to the age and weight of the lambs. Weight gain also does not seem to respond to the increase in the SC of GH and IGF-1. The SC of IGF-1 is related to that of GH but only in the post-weaning stage of the lambs. In general, the weight and weight gain of lambs are more related to the SC of GH than to IGF-1.

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