

Comparison of Certain Haematological and Biochemical Parameters Regarding Pre-slaughter Stress in Saanen, Maltese, Gokceada and Hair Goat Kids

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ABSTRACT

In farm animals, pre-slaughter period is the most stressful stage of their lifetime. The current study was conducted to investigate comparatively the stress responses of Saanen, Maltese, Gokceada and Hair Goat kids to the stressors related with pre-slaughter management procedures. A total of 40 goat kids from 4 different breeds (11 Saanen, 11 Maltese, 9 Gokceada and 9 Hair Goat) were used in the study. Two blood samples (EDTA and heparinised) were collected from each animal at two different times (at home pen and at exsanguination). Packed cell volume (PCV) was measured in EDTA blood samples by the standard capillary microhematocrite method. Heparinised samples were used for determination of plasma glucose, lactate dehydrogenase (LDH), creatine kinase (CK), cortisol and total protein concentrations. For each breed, plasma glucose and cortisol concentrations measured at exsanguination were higher than those of determined at home pen. Breed had no significant effect on plasma glucose and cortisol concentrations at both sampling times ($P>0.05$). However, Maltese kids had higher concentration of plasma LDH at exsanguination than Saanen kids ($P<0.05$), and they had higher plasma CK level than Gokceada, Saanen and Hair Goat kids ($P<0.05$). The effect of breed on PCV and total protein level was significant at both sampling times. Plasma total protein concentration measured at home pen and at exsanguination in Hair Goat kids was lower than those measured in other breeds ($P<0.01$). It is concluded that, pre-slaughter procedures resulted in a stress response in Saanen, Maltese, Gokceada and Hair Goat kids. However, breed had no significant effect on cortisol and glucose responses of Goat kids to the stressors related with pre-slaughter management procedures. Moreover, significant differences were observed among breeds in terms of CK and LDH levels measured at exsanguination, which is probably resulted from the difference in temperament.

Key Words: Goat kid, breed effect, stress parameters at exsanguination

ÖZET

SAANEN, MALTA, GÖKÇEADA VE KIL KEÇİSİ İRKi OĞLAKLARDA KESİM STRESİNE İLİŞKİN BAZI HEMATOLOJİK VE BİYOKİMYASAL PARAMETRELERİN KARŞILAŞTIRILMASI

Çiftlik hayvanlarında kesim öncesi dönem hayatlarının en stresli dönemidir. Bu araştırmada Saanen, Malta, Gökçeada ve Kıl Keçisi ırkı oğlakların kesim öncesi uygulanan prosedürlere karşı verdikleri stres yanıtının karşılaştırmalı olarak incelenmesi amaçlanmıştır. Araştırmada dört farklı ırktan toplam 40 adet erkek oğlak

(11 Saanen, 11 Malta, 9 Gökçeada, 9 Kıl Keçisi) kullanılmıştır. Her bir hayvandan, hem oğlaklar besi bölümünde iken hem de kesim sırasında ikişer adet kan örneği (EDTA'lı ve heparinli) alınmıştır. Hematokrit değeri (PCV) EDTA'lı kan örneklerinde standart kapillar mikrohematokrit yöntemi ile belirlenmiştir. Plazma kortizol, glukoz, kreatinin kinaz (CK), laktat dehidrojenaz (LDH) ve total protein, düzeyleri ise heparinli kan örneklerinde belirlenmiştir. Araştırma kapsamında incelenen her bir keçi ırkı için kesim sırasında belirlenen plazma kortizol ve glukoz düzeyleri hayvanlar besi bölümünde iken yapılan ölçümlerden daha yüksek bulunmuştur. Her iki kan alma döneminde de ırkın plazma kortizol ve glukoz düzeyleri üzerine etkileri önemsiz bulunmuştur ($P>0,05$). Bununla birlikte, kesim sırasında alınan kan örneklerinde Malta ırkından oğlakların plazma LDH düzeyi Saanen ırkı oğlaklarından ($P<0,05$), plazma CK düzeyi ise Saanen, Gökçeada ve Kıl Keçisi ırkı oğlaklarından ($P<0,05$) önemli düzeyde yüksek bulunmuştur. ırkın PCV ve total protein düzeyi üzerine etkisi her iki kan alma dönemi için de önemli bulunmuştur. Plazma total protein konsantrasyonu hem besi bölümünde hem de kesim sırasında yapılan ölçümlerde diğer ırklara oranla Kıl Keçisi ırkı oğlaklarda önemli düzeyde düşük bulunmuştur ($P<0,01$). Araştırma sonucunda kesim öncesi hayvanlara uygulanan prosedürlerin Saanen, Malta, Gökçeada ve Kıl Keçisi oğlaklarında bir stres yanıtına neden olduğu, fakat kesim öncesi yapılan uygulamalara ilişkin stres uyaranlarına verilen kortizol ve glukoz yanıtı üzerine ırkın önemli bir etkisinin olmadığı belirlenmiştir. Diğer yandan, kesim sırasında belirlenen CK ve LDH düzeyleri üzerine ırkın etkisinin önemli olduğu ve bunun da muhtemelen ırklar arasında ki mizaç farklılığından kaynaklandığı sonucuna varılmıştır.

Anahtar Kelimeler: Oğlak, ırk etkisi, kesim sırası stres parametreleri

Introduction

Small ruminant breeding is important for economic assessment of natural pastures that cannot be used for other purposes in the country, such as the public nutrition, and provision of raw material requirements for food, textile, carpet and leather industries (Yalçın, 1985). Goat breeding, which takes place in small ruminant breeding, plays an important role in livestock breeding of Turkey in respect of both economy and provision of nutritive requirements of the public (Koşum et al., 2003).

In animal production systems, besides profitability, animal welfare should also be taken into consideration during the determination of optimal production systems (Mejdell, 2006). Especially in developed western countries, people pay more attention to the issues such as the conditions in which animals are managed and reared in intensive livestock farms, how they are transported, what kind of procedures are applied to the animals and requirement of these applications. Therefore, the importance of "animal welfare" concept increases day by day (McGlone, 2001). Scientific researches regarding farm animal welfare are generally focused on (a) the effects of intensive breeding on animal welfare, and establishment of more comfortable shelters, (b) transportation stress, and improvement of transportation conditions, (c) lairage procedure before slaughter, and

lessening of pain before and during slaughter. In farm animals, pre-slaughter period is the most stressful stage of their lifetime. Animals can express a stress response with increased plasma cortisol and glucose concentrations due to the procedures applied during transportation and pre-slaughter period. Furthermore, removal of animals from their shelters, loading and unloading onto vehicles, and injuries during the management of animals in the slaughterhouse cause an increase in plasma LDH and CK concentrations (Adenkola and Ayo, 2010; Ferguson and Warner, 2008). On the other hand, excessive stress during these procedures causes depletion of glycogen reserves in muscles, which in turn may cause darker meat color and tougher meat (Ekiz et al., 2012a; Kadim et al., 2006; Kadim et al., 2010; Sañudo et al., 1998).

Stress responses of animals can differ due to various environmental or individual factors. One of the factors of different stress responses observed among animals is the genetic structure of animal such as breed or genotype (Broom, 2005). Kadim et al. (2006) found significant differences between Batina and Jabal Akdhar goat breeds in terms of concentration of dopamine, cortisol, adrenalin and noradrenalin in blood samples taken immediately prior to slaughter. Hall et al. (1998) found that breeds reared in highlands had higher plasma cortisol concentration measured after transport than those

of breeds reared in lowlands. About 99% of goat population in Turkey compose of native breeds such as Hair Goat, Gokceada Goat and Angora, and number of goats from improved breeds is quite low. However, an obvious increase in demand to the dairy breeds, such as Maltese and Turkish Saanen, by the breeders who intend to produce goat milk through intensive production system, has been observed in recent years.

In the current study it was aimed to investigate comparatively the packed cell volume (PCV), and plasma concentrations of glucose, cortisol, lactate dehydrogenase (LDH), creatine kinase (CK), and total protein in blood samples taken at home pen and at exsanguination from Saanen, Maltese, Gokceada and Hair Goat kids slaughtered at 5.5-6 months of age, and therefore, to assess breed differences in responses of animals to the stressors that they experienced during the pre-slaughter period.

Materials and Methods

All experimental protocols of the study were approved by the Ethic Committee of Istanbul University (No: 53, 28.05.2009).

Animals, housing and fattening

Animal material of the study was 40 male kids from Saanen (n=11), Maltese (n=11), Gokceada (n=9) and Hair Goat (n=9) breeds. The kids suckled their mothers until they reached to 3-3.5 months of age. Following weaning, kids were purchased from a private farm, and they were transported to Istanbul University Veterinary Faculty. Kids were placed into four pens (each pen was 13 m²) prepared for each breed. Finishing period started after two weeks of adaptation period and continued for 56 days. Kids had ad libitum access to the grower concentrate feed (16.9% CP and 2820 kcal/kg ME) and good quality alfalfa hay (7.34% CP and 2050 kcal/kg ME), and continuous access to clean/fresh water. Following 56 days of finishing period, kids were slaughtered in experimental abattoir in Faculty of Veterinary Medicine immediately after electrical stunning.

Blood sampling and analyses

In order to assess the stress response of goat kids during the pre-slaughter period blood samplings were performed at two different times: 1) one week prior to slaughter, when the animals were at their home pen, 2) at exsanguination. Two blood samples (EDTA and heparinised) from each animal were collected at each blood sampling time. Packed cell volume was determined in EDTA blood samples by the standard capillary microhematocrite method. Plasma samples obtained by centrifugation (3500 rpm for 15 min) of heparinised blood samples within 1 h of blood collection were kept at -85°C until analysed. Plasma cortisol concentration was measured by using commercial diagnostic ELISA kit (DiaMetra, Foligno, Italy). Assay sensitivity, and intra- and inter-assay variations were 5 ng/ml, and 7 and 9.32% respectively. In order to determine plasma CK, total protein, glucose and LDH concentrations, a computer process-controlled auto-analyser (TMS 1024, Tokyo-Boeki Medical System, Tokyo, Japan) and appropriate commercial kits (Spinreact, Girona, Spain) were used.

Statistical analyses

One-way ANOVA was performed using SPSS 10.0 programme (SPSS, 1999) in order to determine the effect of Goat breed on cortisol, glucose, LDH, CK and total protein concentrations, and on PCV, measured in blood samples collected at home pen and at exsanguination. If the influence of Goat breed on these parameters were significant, "Duncan's multiple range test" was applied in order to identify the significance of the differences between pairs of breeds. Paired samples t-test was applied to detect the significance of the differences between blood sampling times for the same breed.

Results

The influence of goat breed on plasma cortisol, glucose, LDH, CK, total protein concentrations and on PCV measured in blood samples collected at home pen and at exsanguination were given in Table 1. The

effects of breed on plasma cortisol and glucose concentrations at both sampling times were not significant ($P>0.05$). On the other hand, the effect of breed on plasma CK and LDH concentrations in blood samples collected at home pen were not significant ($P>0.05$), while differences between breeds in terms of these parameters were significant at exsanguination ($P<0.05$). In the blood samples taken at exsanguination, Maltese kids had higher plasma LDH concentration than Saanen kids ($P<0.05$), and had higher plasma CK level than Saanen, Gokceada and Hair Goat kids ($P<0.05$). In both sampling times, breed had significant effect on PCV, and Saanen kids had higher PCV than the other breeds ($P<0.001$). Furthermore, mean PCV value determined for Maltese kids at exsanguination was higher than those of Gokceada and Hair Goat kids. Plasma total protein concentration of Hair Goat kids was lower than those of other breeds ($P<0.01$) at each blood sampling time.

For each breed, mean values for plasma glucose and cortisol concentrations obtained at exsanguination were higher than those means obtained at home pen. Plasma CK and LDH concentrations in Saanen and Gokceada kids were not influenced from sampling time. However, significant rises in LDH and CK concentrations for Maltese kids and in CK for Hair Goat kids were observed at exsanguination. Blood sampling time had no significant influence on plasma total protein concentration for all investigated breeds. Moreover, while any significant change in PCV was not observed in Saanen and Maltese kids, PCV of indigenous breeds (Gokceada and Hair Goat) decreased significantly in blood samples taken at exsanguination.

Discussion

Slaughtering is the final stage of production systems aimed to produce meat in farm animals (Grigor et al., 2004). Meat producing animals are subjected to various stressors such as gathering, loading, transport, unloading, and associated handling procedures, social disruption, physical discomfort, fatigue and noise during the pre-slaughter period (De la

Fuente et al., 2010; Ferguson and Warner, 2008; Terlouw et al., 2008). These stressors lead to an imbalance of homeostasis, and then the animal endeavour to restore the balance of its body. Due to pre-slaughter stress, an animal may encounter increased physical activity, fear, fatigue and physical injury, which in turn cause evident physical and emotional stress in the animal (Ferguson and Warner, 2008). Since certain blood parameters such as PCV, and plasma concentrations of CK, LDH, glucose and cortisol may change in animals exposed to various stressors, these parameters had been used as stress indicators in most of the previous researches (Bórnez et al., 2009; Ekiz et al., 2012a; Ekiz et al., 2012b; Tadich et al., 2009).

When an animal is exposed to various stressors, blood cortisol concentration increases as a consequence of the stimulation of hypothalamo-pituitary-adrenal axis. The elevated cortisol concentration may cause an increase in blood glucose level. Moreover, increased sympatho-adrenal activity stimulated by physical and psychological stress also leads hyperglycemia by increasing breakdown of glycogen in the liver (Adenkola and Ayo, 2010; Ali et al., 2006). In the current study, Saanen, Maltese, Gokceada and Hair Goat kids had similar plasma concentrations of glucose and cortisol in blood samples collected at home pen and at exsanguination. These results indicate that cortisol and glucose responses of Saanen, Maltese, Gokceada and Hair Goat kids to the stressors related with pre-slaughter management treatments were similar. Supporting the current results, Ekiz et al. (2012b) found no significant differences among Sakız, Karakul, Red Karaman and Imroz rams in terms of cortisol and glucose concentrations in blood samples taken immediately after 2 h lairage period. However, Hall et al. (1998) found significant differences in plasma cortisol concentration of different sheep genotypes, and the authors noted that cortisol responses to transportation stress was higher in sheep originated from highlands than lowlands.

Table 1. Certain haematological and biochemical parameters in blood samples collected at home pen and at exsanguination from kids of Saanen, Maltese, Gokceada and Hair Goat breeds.

Parameters	Sampling Time	Saanen (n=11)		Maltese (n=11)		Gokceada (n=9)		Hair Goat (n=9)		Significance
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Cortisol, ng/ml	At home pen	14.85	4.88	14.93	4.03	17.57	3.43	14.45	5.67	NS
	Exsanguination	33.54	7.40	62.53	22.53	32.44	7.24	42.87	11.61	NS
	Significance ^d	**		*		*		*		*
Glucose, mg/dl	At home pen	60.18	4.51	56.64	4.21	55.22	1.51	59.67	2.43	NS
	Exsanguination	78.36	2.31	70.18	3.39	74.67	3.00	70.56	3.79	NS
	Significance ^d	**		*		***		*		*
CK, U/l	At home pen	285.36	30.74	541.45	132.56	312.56	27.86	301.33	46.45	NS
	Exsanguination	481.91 ^b	120.23	1440.55 ^a	409.67	581.67 ^b	159.67	589.00 ^b	137.44	*
	Significance ^d	NS		*		NS		*		*
LDH, U/l	At home pen	970.64	48.10	1106.73	79.37	1030.33	76.98	1067.56	31.39	NS
	Exsanguination	1096.00 ^b	45.14	1532.64 ^a	154.88	1229.78 ^{ab}	95.14	1213.44 ^{ab}	105.46	*
	Significance ^d	NS		**		NS		NS		NS
Total Protein, g/dl	At home pen	6.88 ^a	0.12	6.40 ^a	0.22	6.53 ^a	0.18	5.80 ^b	0.19	**
	Exsanguination	6.99 ^a	0.15	6.69 ^a	0.27	6.79 ^a	0.21	5.74 ^b	0.23	**
	Significance ^d	NS		NS		NS		NS		NS
PCV, %	At home pen	33.55 ^a	1.07	25.27 ^b	1.57	22.78 ^b	1.42	22.33 ^b	1.07	***
	Exsanguination	33.82 ^a	1.09	24.45 ^b	1.57	19.11 ^c	1.50	18.89 ^c	1.10	***
	Significance ^d	NS		NS		*		***		***

^{a, b, c}: Differences between the mean values carrying different letters in the same row are significant.

NS : Not significant (P>0.05); * : P<0.05; ** : P<0.01; ***: P<0.001.

^d : Significance level of differences between blood sampling times for the same column.

Significant breed effect on plasma cortisol level was also found by Kadim et al. (2006), who investigated stress responses of Jabal Akdhar, Dhofari and Batina goats regarding to transportation and pre-slaughter treatments. Plasma cortisol and glucose levels obtained in the present study were in agreement with the levels found for Alpine goats (Kannan et al., 2003) and Spanish Goats (Kannan et al., 2002). On the other hand, Zimmerman et al. (2011) reported greater cortisol values for crossbred male kids subjected to different pre-slaughter stressors.

The level of plasma CK increases as a consequence of muscle damage or high levels of physical activity. LDH is also released into the bloodstream in an increased amount following tissue damage (Bórnez et al., 2009; Kannan et al., 2003). Therefore, these two enzymes are considered as indicators of trauma, vigorous exercise or tissue damage resulted from pre-slaughter management and transport (Bórnez et al., 2009; De la Fuente et al., 2010; Tadich et al., 2009). In the current study, environmental conditions and facilities of fattening pens, handling and feeding practice during fattening programme, and handling and management applications during pre-slaughter period were similar for Saanen, Maltese, Gokceada and Hair Goat kids. Moreover, differences among breeds in terms of CK and LDH measured at home pen were not significant. But, mean plasma LDH concentration in blood samples taken at exsanguination from Maltese kids was higher than that of Saanen kids. Moreover, at exsanguination, Maltese kids had higher CK concentration than Gokceada, Saanen and Hair Goat kids. Shaw and Tume (1992) noted that blood samples taken at exsanguination can supply valuable information regarding the effects of pre-slaughter management. Results for CK and LDH in the current study may indicate greater amount of tissue damage due to traumatic events and/or physical stress resulted from catching of animal in the lairage pen, driving of the animal to the stunning area, restraining of the animal for stunning and the process of stunning itself in Maltese kids than

kids of other investigated breeds during the pre-slaughter period. Kannan et al. (2007) noted that the breed differences in CK activity may be explained by difference in the temperament of breeds. The authors also noted that, agonistic encounters among animals occurred during the lairage and pre-slaughter period may also cause an increase in the concentration of blood CK. Furthermore, Van de Water et al. (2003) reported that elevated CK level determined at bleeding might be result from mounting and butting behaviours expressed during the lairage period.

Packed cell volume may increase as a result of splenic contraction induced by sympathetic nerve activity or catecholamines released into the blood stream due to various stressors. PCV can also increase as a result of dehydration (Adenkola and Ayo, 2010). In the current study, indigenous goat breeds (Gokceada and Hair Goat) had significantly lower PCV values at exsanguination than Maltese and Saanen breeds. However, this result cannot be attributed to the higher stress responses of Maltese and Saanen breeds compared with those of indigenous breeds to the pre-slaughter management procedures, since the four goat breeds investigated in the present study had similar glucose and cortisol levels in blood samples collected at exsanguination. Furthermore, PCV values of Maltese and Saanen breeds are within the normal reference ranges reported for healthy goats (Jones and Allison, 2007). On the other hand, PCV values of indigenous breeds (Gokceada and Hair Goat) determined at exsanguination may indicate anemia, which presumably, was resulted from a factor related with the fattening period, since Gokceada and Hair Goat kids had also lower PCV values measured at home pen compared with Saanen kids.

An increase in total protein concentration due to several stressors has been reported by various authors (Bornez et al., 2009; Ekiz et al. 2012a; Kadim et al. 2006). In the current study, Hair Goat kids had lower total protein concentration than the other 3 breeds both at home pen and at exsanguination. However, this result did not indicate a higher stress response

in Maltese, Saanen and Gokceada kids, since total protein concentrations of these breeds are within normal physiological ranges of 6-7,5 g/dl reported for healthy goats (Jones and Allison, 2007).

According to the results of the current study, it is concluded that pre-slaughter procedures resulted in a stress response in Saanen, Maltese, Gokceada and Hair Goat kids which coincides with increased cortisol and glucose levels at exsanguination compared with home pen values. However, differences among breeds were not significant in terms of cortisol and glucose responses to the stressors related with pre-slaughter management procedures. However, significant differences among breeds, in terms of CK and LDH levels, were determined at exsanguination, which indicate physical trauma and/or tissue damage occurred during the pre-slaughter period, and probably resulted from the differences in temperament.

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