

Stress of alpacas caused by shearing in Hungary

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Abstract

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Alpacas are primarily kept for their wool in Hungary. Huacaya alpacas are sheared annually, usually in May, before the summer heat. Shearing is important to breeders because the wool is the primary product of the animal. However, it is important also for alpacas to avoid for example heat stress, similarly to sheep. The question has arisen whether shearing causes stress for alpacas. In 2014 we took saliva samples from 10 alpacas before, during and immediately after shearing and 30 min later. Then in 2015 I took samples from 12 animals on the same farm. The method was similar to the previous years, but I collected saliva samples from the alpacas on the day before the shearing as well. Both males and females were involved in the study. Alpacas responded to shearing differently. Compared to females, males were more nervous before they were sheared. This is probably due to their nature as there may be smaller dominance competitions on pasture (where space is wide). For most females it has been found that cortisol level in saliva has increased due to stress caused by shearing. The one named Contessa was the only female that had high cortisol level before shearing and the level decreased subsequently. Based on our preliminary experiment it can be concluded that shearing creates stress for the animals to a slight degree. By appropriate treatment strong stress can be avoided.

Keywords: alpaca; stress; cortisol; shearing; Hungary

Abbreviations: ACTH: adrenocorticotrophic hormone, FFA: free fatty acid, FPI: Fluorescence Polarization Immunoassay, RIA: radioimmunoassay

Introduction

Alpacas (*Lama pacos*) are classified into South American camelids and are domesticated like llamas (Wheeler et al., 1995; Wernery et al., 2002) for 6-7 thousand years. Products made from alpaca wool could have been worn only by the royal family. In South America animals are sheared before the rainy season (from November to December). Alpacas produce an average of 1300 grams wool (Westreicher et al., 2006). According to the statistics of the FAO, Suri alpacas give 2500 g, Huacaya alpacas give 2000-3500 g wool annually (Internet I.).

Various external factors affect farm animals, which may affect their health, development and production (Gere et al.,

2001). The body can respond to a strong stimulus in a variety of ways. This may be a specific response, for example, to an infection. However, if no specific defence is available, the body responds in an unspecific manner. This is called stress. Stress is caused by stressors which can be created by pain, weather, surgical intervention, pathogens, persistent hot or cold weather etc. This results in the release of ACTH (adrenocorticotrophic hormone) (Rudas et al., 1995). ACTH induces the release of corticosteroids in the adrenal cortex (Jensen, 2006). Cortisol belongs to the group of glucocorticoids, helps fat mobilization and raises FFA (free fatty acid) concentration in blood. Thus, cells do not supply glucose but fatty acids in stress or starvation (Bárdos et al., 2007). Cortisol also increases the rate of nutrient delivery, secretion in

stomach and intestinal tract. The permeability of the capillaries decreases; the tone is increased (Rudas et al., 1995).

The most common method for detecting stress hormones is blood sampling. However, this is a stressor for alpacas so it may be useful to choose another method. Stress hormones can be detected for example in milk, hair, saliva (Palme, 2012) and manure (Möstl et al., 2002; Arias et al., 2013). The unbound, biologically active hormone can be detected in saliva (Sheriff, 2011).

Fell et al. (1985) argue that after a little practice it is easier to take saliva than blood sample from sheep. They find this method useful also for other ruminants beyond sheep. In case of goats determination of the amount of cortisol from saliva was also applicable. There was no significant difference between the cortisol level of serum and of saliva (Greenwood et al., 1992). Furthermore, the amount of cortisol i.a. in saliva was also studied in Holstein-Friesian cows in 2013 (Kovács et al., 2016).

Majchrzak et al. (2015) examined dromedars' cortisol level in saliva before work (out of tourist season) and at work (in tourist season). During the work period samples were taken from the animals: when they were on pasture or when they were in the field - while resting, when there were less guests (50 people per day) and when there were more guests (150 people per day).

In case of alpacas, cortisol level was mainly monitored in blood. In 1978 Sumar et al. wrote that blood samples were taken also from foetuses and new-born alpacas. It has been proven that in both cases the main glucocorticoid was cortisol. Due to various stress conditions the level of cortisol metabolites increases in blood plasma, serum, saliva and manure for both alpacas and llamas (Smith et al., 1994; Grandon, 1997; Arias et al., 2015). 30 adult alpacas from the Andes plateau have been studied, of which cortisol levels have changed with daily biological activity: it was higher in the morning than in the afternoon (Raggi 1994, 2000). Anderson et al. (1999a) examined the effect of 30 min of transportation on alpacas. Blood samples were taken from animals before, immediately after and four hours after transportation. The level of cortisol was determined by Fluorescence Polarization Immunoassay (FPI) method. Bravo et al. (2001) took blood samples from alpacas in foaling and weaning period. Cortisol was measured with ELISA method. Andersen et al. (1999b) published also a cortisol level in saliva sample which was taken from the alpaca 30 min after transportation. Analysis was carried out with FPI / salivary ($1.25 \pm 0.35 \mu\text{g} / \text{dl}$) $34.48 + 9.65\text{nmol} / \text{L}$ /. Piccione et al. (2017) determined what is the least stressful method of shearing of alpacas by measuring the cortisol level i.a. in saliva. He found that shearing in standing position causes less stress for

animals because they are not bound or forced to the ground or to the table.

Apart from economic benefits shearing of alpacas is important also in order to prevent the increase of heat stress. In previous years in North America many alpacas have been affected by high temperature and humidity (Johnson, 1993). In 1995 approximately 1000 animals died due to heat stress (Fowler, 1993). Navarre et al. (2001) examined the effects of heat stress between sheared and non-sheared alpacas. Samples were taken every second week and cortisol level in blood were also checked. Un-sheared alpacas were less tolerant of heat than those that were sheared.

Alpacas are kept also in Hungary mainly for their wool. In order to shear the animals they are taken and put on table and their four legs are bound so the alpacas cannot move. I found it important to consider how much stress this method creates.

Material and Methods

In May 2014 and May 2015 I took saliva samples from alpacas using Salivette® tubes. The method can be carried out non-invasively, without penetration. I placed the tampon directly under the tongue. 30 seconds are sufficient to absorb saliva into the tampon in case of cattles (Kovacs et al., 2016). Alpacas produce less saliva so I kept the tampon in the mouth of the animals for 60 s (Picture 1). Similarly to the method of Wittek et al. (2017), I also took samples several times, first before shearing. This time the animals were in stable, where they are kept at night too. The second sample was taken during shearing when the alpaca was on the shearing table. The third one was taken right after shearing, when the animal was taken off the table. The last sample was taken 30 minutes after shearing.

The animals were treated gently all the time. The samples were delivered in cooling bag to the laboratory where the saliva samples were obtained from the tampons at 1000 x



Picture 1. Taking saliva sample in 2014

g centrifugation for 10 min at room temperature. The samples were frozen (-20°C) until analysis. The level of cortisol was measured by using the radioimmunoassay (RIA) method described by Csernus (1982) which was transformed into an analysis for samples from farm animals in order to determine the concentration of glucocorticoidate (Jurkovich et al., 2017). The cross-reactivity of the assay was as follows: cortisol: 100%, corticosterone: 19%, prednisolone: 9.5%, deoxycortisol: 6.4%, 17 α -OH progesterone: 5.7%, progesterone: 2.65%, and further 22 steroids from 0.54 up to 0.0001%. The standard assay (cortisol FW 362.5; Sigma Chemical Company, St. Louis, MO) was made in cortisol-free blood plasma (range per tube from 2000 to 31.25 fmol). The antibody binding and free fraction was separated by cold dextran-coated charcoal suspension after 18 to 24 hours incubation time. Radioactivity was measured with Tri-Carb liquid scintillation counter (Perkin Elmer Inc., Downers Grove, IL). The sensitivity of the assay was 11.37 fmol per tube. In the range of about 2.0 to 100.0 nmol/mL the coefficient of intra and interassay variation is 3-8% and 5-10%. Samples of cortisol concentrations greater than 100.0 nmol/L should be re-evaluated after dilution.

The same animal stock was examined in 2014 and 2015. The alpacas were closed to their place according to the usual daily routine. Samples were taken with Salivette® kits (Picture 2). However, compared to the previous year, saliva samples were taken from the animals on the day before the shearing, and then before, during, immediately after and half an hour after shearing.



Picture 2. Taking saliva sample in 2015

The transport of the samples and the measurement of the level of cortisol were similar to that of the previous year.

Results and Discussion

The cortisol level of samples that were taken 30 min before shearing was 100% of which values differed between

the animals examined and the changes were compared to those values.

2014

The males were nervous already before shearing (Figure 1). Generally males tend to get stressed by each other inside the stable once driven there from the spacious paddock. The cortisol level of Mega and Árpi decreased by shearing and after shearing even higher values were measured than initially. For Thorro the results showed stress all the time. Minor changes in cortisol level could be observed compared to the other two males.

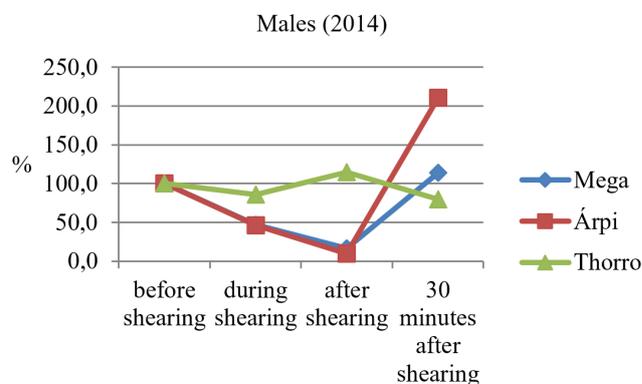


Fig. 1. Changes of cortisol level for males

The mares (Figure 2) were relatively relaxed before shearing. Magnolia and Balassagyarmat were the most nervous, their cortisol level in the saliva increased gradually during shearing. The cortisol values were the highest after shearing. The cortisol level of Melania also showed an upward trend but on a smaller scale than in case of the previous two animals. The cortisol level of Elegance, Szensácia and Chaquito slightly decreased compared to the pre-shearing

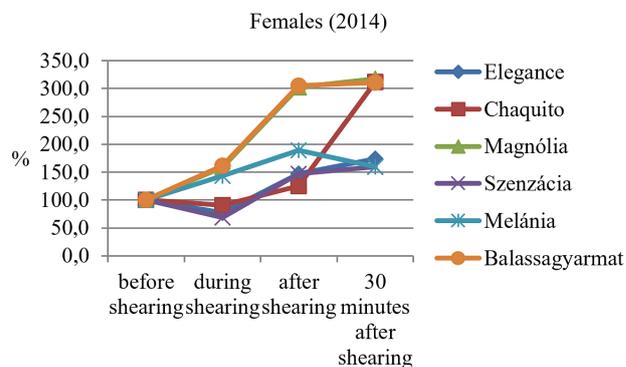


Fig. 2. Changes of cortisol level for females

values, and then gradually increased. For Chaquito, the level of cortisol was extremely high after shearing compared to other animals.

The reason why the evaluation of the cortisol level of Contessa (Figure 3) is separated is that her values showed difference from the other females' values. The cortisol level was higher in the pre-shearing period, and then it dropped off and stayed more or less on the same level. This animal was part of the group from the beginning, the other mares did not exclude her so this was not likely the reason for the initial high cortisol level.

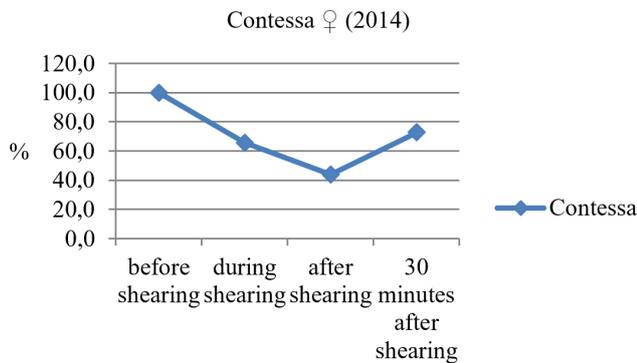


Fig. 3. Change of cortisol level of Contessa

2015

In 2015, the analysis of samples taken one day and 30 minutes before shearing (Table 1) showed that for the majority of alpacas the cortisol level the day before shearing was lower than shortly before shearing.

Table 1. Cortisol level (nmol/L) one day and 30 min before shearing (2015)

Name	One day before shearing	30 minutes before shearing
Melánia	1.89	6.2
Chaquito	2.99	2.93
Contessa	2.1	4.4
Harmony	3.09	2.56
Magnólia	1.8	6.32
Szenzácia	0.62	2.02
Elegance	1.46	0.88
Adonis	1.44	3.13
Árpi	0.96	2.94
Lubert	3.47	54.94
Thorro	0.6	1.32
Mega	3.81	5.5

For Chaquito, the values were nearly the same, while for Harmony and Elegance the cortisol level measured on the day before the shearing was higher.

The cortisol level of Thorro (Figure 4) has steadily increased from the pre-shearing value up to the value measured at the end of shearing, then the level decreased in the sample taken 30 min later. For Adonis, a higher stress hormone level was measured 30 min before shearing, which value has decreased during shearing and then it increased.

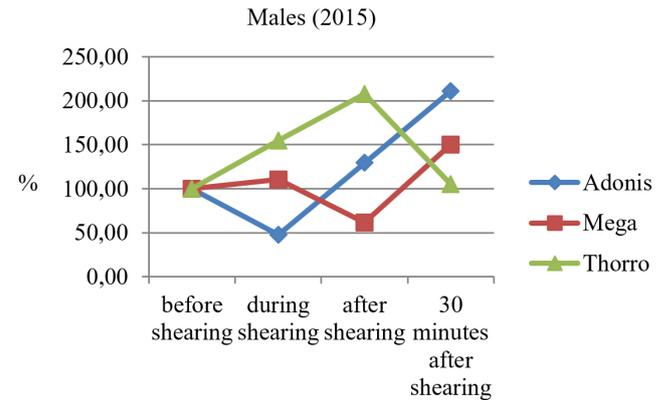


Fig. 4. Changes of cortisol level for males

For Mega, a slight increase of cortisol level could be observed, with its peak after shearing.

In case of Árpi (Figure 5) we measured extremely high values. The cortisol level was relatively low before and during shearing, however, it hit the multiple values after shearing that of the other alpacas. Lubert had high cortisol level all the time and it decreased by the last sampling.

The cortisol level of Melania and Magnolia (Figure 6) were about equal before and during shearing. Melania's value fell later on but the measured stress level increased for both animals after shearing again.

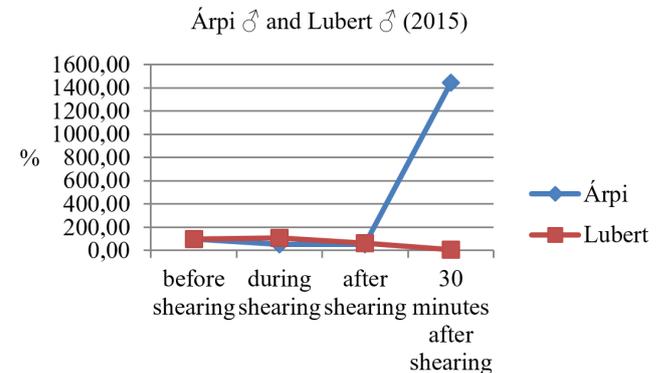


Fig. 5. Changes of cortisol level for Árpi and Lubert

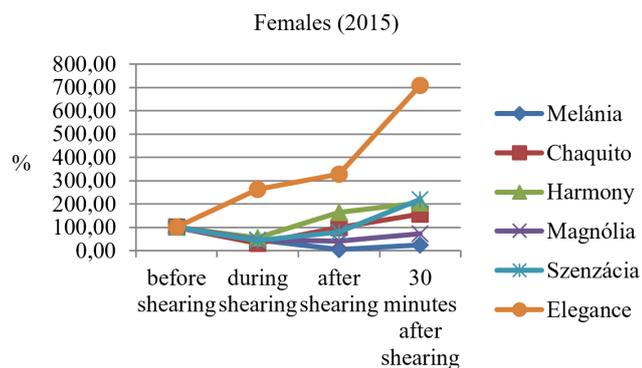


Fig. 6. Changes of cortisol level for females

In case of Elegance, Chaquito and Szenszácia the level of stress hormone has increased similarly, the highest value having been measured was of Elegance (Figure 7). The cortisol level of Harmony hardly changed since the value before shearing.

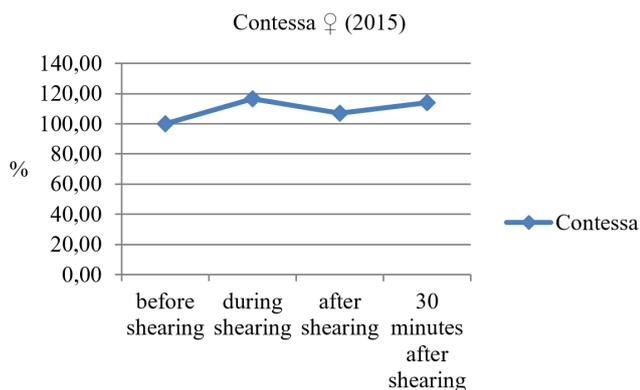


Fig. 7. Changes of cortisol level of Contessa

The cortisol level of Contessa increased from the level measured 30 min before shearing up to the level during shearing. No big change in the values was observed during and after shearing, unlike in the year 2014, when the high initial level of stress hormone decreased by the end of shearing.

Conclusions and Suggestion

From the preliminary experiment it can be stated that the annual shearing causes certain stress to alpacas. Analysing as per sex, it can be seen that males are more nervous and in several cases higher cortisol level was measured before than after shearing. The cortisol values of mares increased gradually during and after shearing. One of the females was nervous also before she was sheared. However, we could not find the reason for that, the reason what created stress

differed between animals (Figure 8 and Figure 9). Proper treatment can prevent unnecessary stress for alpacas. For example, it is advisable to avoid unnecessary movements when handling the animals. Moreover, teeth and nails should also be trimmed while the animals are on table. Thus, alpacas would be disrupted fewer times per year by their catching.

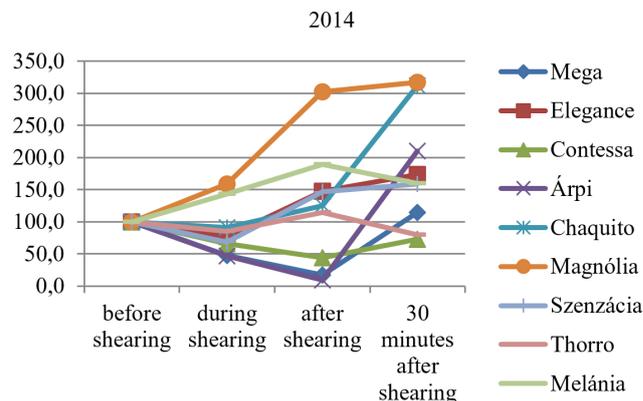


Fig. 8. Changes of cortisol level for alpacas in 2014

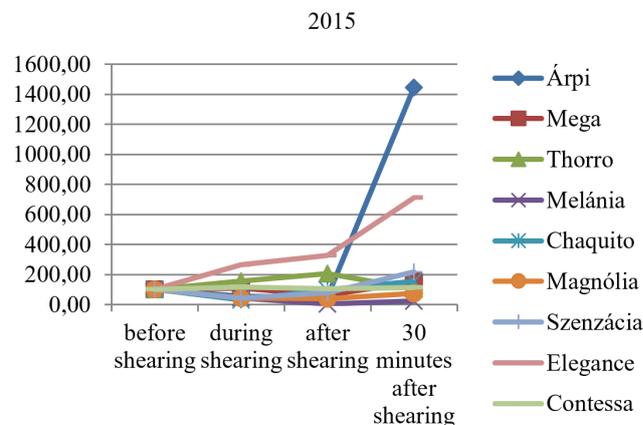


Fig. 9. Changes of cortisol level for alpacas in 2015

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