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Original article

Relationships between eye fluids and blood values after exercise in lidia cattle: mineral parameters

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Abstract

Eye fluids (aqueous humour and vitreous humour) may be helpful in estimating *ante-mortem* blood levels, since some parameters measured in these fluids have proved to be stable or to change in a predictable way after death. This would help in diagnosing the cause of death in some diseases or to evaluate *ante-mortem* blood levels in certain animals not easy to handle or with difficult access. In order to establish reference values of some parameters in blood and eye fluids (aqueous humour and vitreous humour), as well as the possible correlation among these three different fluids, various minerals and electrolytes (Ca, P, Mg, K, Na, Fe, Cr, Co, Ni, Cu, Zn, Se and Mo) were measured in 15 four to five year-old Lidia bulls, all dying after a period of significant stress and major exertion. Plasmatic values of Mg and P were much greater than reported in the literature. In general, mineral plasmatic values were greater than those found in ocular fluids (aqueous and vitreous), while Na, K and Cr were similar in the three fluids. We have verified the existence of correlations in P, Co and Mo among the three fluids measured, and between Se of plasma and vitreous humour. But the most marked correlations were observed in Mo (plasma -aqueous humour, r = 0.893, plasma-vitreous humour, r = 0.945, HA -HV, r = 0.849), in P (plasma-vitreous humour, r = 0.726) and in Co (plasma-vitreous humour, r = 879).

Key words: aqueous humour, vitreous humour, minerals parameters, intraocular fluids, blood, metabolism, Lidia cattle

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Introduction

Post-mortem diagnosis of abnormal metabolic states and cause of death in some pathology situations is often difficult due to the rapid deterioration of body fluids used for chemical analysis (McLaughlin and McLaughlin 1988). Ocular fluids (IOFs), aqueous humour (AH) and vitreous humour (VH), are easy to sample (Wittwer et al. 1992, McCoy 2004) and deserve special attention in the metabolites determination, particularly with regard to electrolytes and minerals, and could be of great use in veterinary medicine. The aqueous humour is a clear and watery consistency fluid, located in the anterior and posterior chambers of the eye (Wittwer et al. 1992, McCoy 2004). Vitreous humour is located between the lens and the retinae, filling the eye ball and has a gel consistency, formed by collagen fibers net joined by hydrophilic molecules of hyaluronic acid. Approximately 98% of this gel is water (McCoy 2004). The vitreous humour has a similar composition to that of aqueous humour and is considered to be more stable *post-mortem* than the aqueous, not easily contaminated and easy to sample, which made it suitable for *post-mortem* analysis (Mattioli et al. 2002, Garg et al. 2004, McCoy 2004).

According to Hanna et al. (1990) the aqueous fluid is as valid as vitreous humour to perform biochemical analysis, being as exact as the vitreous and easier to obtain and to analyse it does not always need previous centrifugation. However, the vitreous humour allows for a larger volume of sample, although it has to be centrifuged as it can contain impurities due to its viscous character (Wittwer et al. 1992). According to Gerometta (2005) the concentrations of various ions in the AH significantly differ from their plasma (PL) levels due to an asymmetry in the distribution of ion transporters in membranes of the epithelium, which is essential for the transport of solutes.

Ocular fluids have been used in human forensic medicine to estimate the approximate time of death, for previous diagnosis of diseases, for detection of levels of some drugs and toxins and for *ante-mortem* blood biochemical values determination. However, in veterinary medicine most research has been conducted in healthy animals under slaughterhouse conditions (Hanna et al. 1990), with serum values without or with moderate alterations (Madea and Musshoff 2007) although there are some publications on the death of animals by diseases such as uraemia, hypocalcaemia and hypomagnesaemia (Lincoln and Lane 1985a, Hanna et al. 1990, Wittwer et al. 1992, McCoy et al. 2001a,b, McCoy 2004, Gonzalez-Montaña et al. 2018). Also, there are some articles correlating *post-mortem* values in ocular fluids with blood values prior animal death (Hanna et al. 1990). In these fluids, the minerals and electrolytes *post-mortem* investigated were potassium, sodium, chloride, calcium, magnesium and phosphate. Some of these parameters, in particular sodium and chloride, proved to be quite stable in their *post-mortem* concentrations while other parameters showed significant changes in concentration (Thierauf 2009). A recent research in sheep has shown that serum concentrations of magnesium and creatinine were significantly higher than those determined in the aqueous humor, but that there is a high correlation between the magnesemia and the creatinine, with its values *post-morten* in aqueous humor (Athanasiou et al. 2018). For other minerals and especially for trace elements reference values have not been investigated yet, nor their relationship with blood parameters.

Lidia cattle (*Bos taurus brachiceros*) is an Iberian heterogeneous cattle population described in the Decree 60/2001 (BOE 2001). It is primarily bred free-range on extensive estates in southern Spain, France, Portugal and Latin American countries. This breed is characterized by their natural aggressiveness and resistance to traditional handling procedures, which does not allow for easy sampling (Escalera et al. 2013).

The aims of this study were to establish reference values of some parameters concentrations (macro and micro-minerals and electrolytes) in both blood and intraocular fluids (aqueous and vitreous humour) in the Lidia breed, specifically in stressed animals and after a major physical exertion. We also intended to verify the possible correlation between these biochemical parameters, and between the values found in blood and ocular fluids. Our final aim was evaluating the potential of *post-mortem* concentration of minerals investigated (macro and microminerals) in order to predict their corresponding values before death in the sampled bulls.

Materials and Methods

Animals and legal regulations

A total of 15 four to five-year-old Lidia bulls from different breeding farms were used in this study, all were fought in the Valladolid Arena. The Lidia cattle were kept under extensive conditions but the bulls were submitted to a fattening diet during the 9-10 months prior to the sampling collection that was performed immediately after death. Lidia bulls were fed on pasture and feed with an average of 10 kg of total mixed ration (TMR) made with 7 kg of concentrate and 3 kg of barley straw. The composition of the concentrate provided to bulls group is presented in Table 1.

The animals were slaughtered under the regulation of the local legislation law (BOCyL 2008). All experi-

Table 1. Chemical composition of concentrate rations.

Chemical composition (% kg)	
Crude protein	13.7
Crude fats and oils	5.3
Crude fiber	4.9
Crude ash	5.6
Calcium (carbonate)	0.8
Phosphorus	0.5
Sodium (chloride and bicarbonate)	0.5
Vitamins	
Vitamin A UI/kg	9.000
Vitamin D_3 UI/kg	1.800
Vitamin E mg/kg	15
Minerals (mg/kg)	
Cupper	7.4
Selenium	0.33*
Zinc	75
Cobalt	0.69
Iodine	0.81
Iron	50
Manganese	40

Minerals, Cu (as $CuSO_4$), Se (as Na_2SeO_3 and *0.12 mg/kg produced by *S. cerevisidae*), Zn (as ZnO), Co (as $CoSO_4$), Iodine (as KI), Iron (as $FeCO_3$), Mn (as MnO_2)

mental procedures were carried out in compliance with the provisions of the EU Directive of the European Parliament which regulates the use of animals for scientific purposes (European-Union 2010) and the Royal Decree which regulates in Spain experimentation and animal protection of animals used for scientific purposes (BOE 2013).

Informed consent

Informed consent has been obtained for client-owned animals included in this study.

Sampling and analytical procedures

As a result of the fight all the animals underwent significant physical exertion and stress situations for at least 15-20 minutes. Immediately after the end of the fight, blood, aqueous and vitreous humour samples were obtained. Blood was obtained directly from the bleeding in heparinized vacuum tubes (Vacutainer BD, Vacutainer® heparin lithium) (9 ml), while both the aqueous and vitreous humour were collected directly from the eye with 5 ml syringes. Aqueous humour was collected from the anterior chamber by gentle aspiration following corneal penetration with a 21-gauge needle. Vitreous humour was collected from the central portion of the vitreous body following insertion of a 18-gauge needle through the sclera and by aspiration (McCoy and Kennedy 1994). The blood samples were centrifuged at 4000 rpm (2200 x g) for 10 minutes (following the methodology described by Escalera et al. (2013), while eye fluids samples were centrifuged (Centrifuge Cencom II, JP Selecta, Barcelona, Spain) at approximately 1000 g for 10 min within 30 min after collection following the methodology described by Hanna et al. (1990). The free from impurities supernatant was placed in Eppendorf tubes and stored, first at 4ºC and then frozen to -20ºC for transportation in a cooler, until analyses in the Laboratory Instrumental Techniques (L.T.I.) in the University of León, Spain.

Calcium, magnesium and phosphorus were measured using an autoanalyser (Cobas Integra 400; Roche, Switzerland) and using Roche Diagnostics reagents (Roche, Switzerland), while for the determination of sodium and potassium an 1:100 dilution was necessary. The samples were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 2000DV, Perkin Elmer Instruments; Shelton, CT, USA) after addition of 5 ppm Sc, used as internal standard. The detection limit was approximately 0.1 ppm. The analysis of iron, chromium, cobalt, nickel, copper, zinc, selenium and molybdenum, in both plasma and ocular fluids was carried out by inductively coupled plasma mass spectrometry (ICP/MS, Varian 3900,

Table 2. Values of macrominerals and electrolytes in plasma and intraocular fluids (aqueous and vitreous humour) in the Lidia breed bulls.

Parameter	Fluids		Mean	SD	Minimum	Maximum
Calcium (mmol/l)	AH	a	1.30	0.22	0.62	1.50
	VH	a	1.55	0.35	1.09	2.37
	PL	b	2.99	0.30	2.59	3.49
Phophorus (mmol/l)	AH	a	1.34	0.08	0.71	1.81
	VH	b	0.46	0.01	0.31	0.68
	PL	c	3.93	0.23	3.07	4.70
Magnesium (mmol/l)	AH	a	0.74	0.13	0.41	0.97
	VH	b	0.93	0.16	0.76	1.34
	PL	c	1.36	0.15	1.13	1.71
Potassium (mmol/l)	AH	a	49.50	11.36	18.10	69.06
	VH	a	53.42	4.14	44.82	62.92
	PL	b	65.62	10.17	50.57	89.52
Sodium (mmol/l)	AH	a	1353.0	253.4	573.3	1549.2
	VH	b	1255.5	66.3	1117.2	1372.3
	PL	a	1376.1	81.9	1221.4	1556.7

Descriptive statistics indicating the mean value, the standard deviation (SD) and the minimum and maximum ranges. AH: aqueous humor; PL: plasma; VH: vitreous humor. Alls variables are measured in mmol/l. Different letters in each parameter group indicate significant statistical differences ($p<0.05$).

Varian Ibérica, Spain), capable of analysing elements in liquid matrix with lower sensibilities than 0.2 ppb. Samples were diluted 1:10 in a solution of 0.05% EDTA, 0.5% nitric acid to which was added 10 ppb of a solution mixture of elements used as internal standard (Sc, Y, Pt, Pd and Rh). The blanks and standards were prepared by the technique of the additions on a cow plasma also diluted 1:10 in EDTA-nitric solution previously mentioned. In order to check the accuracy of the analytical method, a multi-element standard solution (Multi-element standard solution IV, 23 elements, Merck, Darmstadt, Germany) with different concentrations (0, 10, 50 y 100 ppb) was used for the calibration. In all cases, the recovery rate was between 82.9% and 104.7%.

Statistical study

Concentrations of each parameter both in blood plasma and ocular fluids were calculated. The data were analysed using the SPSS 15.0 statistical program (SPSS Inc., Chicago, IL, USA). Descriptive statistics were carried out, indicating the mean value, the standard deviation (SD) and the minimum and maximum ranges. A statistical study was carried out by nonparametric tests, specifically by means of a Kruskal-Wallis test and when differences were found, the U-Mann Whitney test was performed to detect the differences between the groups of samples. The statistical differences are shown as different letters between the values of the fluids for each parameter. Correlation coefficients and principal components analysis method were used to measure the relationship between ocular fluids and between these and the plasma levels. A value $p<0.05$ was chosen for statistical significance.

Results

The results obtained in the present study are shown in Tables 2, 3 and 4. Except for chromium, plasma values were always greater than ocular fluids values (aqueous and vitreous).

The values found in sodium, potassium and chromium were similar in all 3 fluids. While plasma levels of calcium, magnesium and molybdenum were twice those found in the eye, plasma values of phosphorus, cobalt and nickel plasma values were slightly greater than those found in eye fluids. Thus, phosphorus plasma sample values were up to 3 times greater than in AH and up to 8 times greater than VH, while cobalt and nickel had values between 5 and 7 times greater in blood than and ocular fluids.

The value of plasma selenium, iron, zinc and copper was much greater than the one evaluated in ocular fluids. Zinc presented values 16 and 32 times greater

Table 3. Concentrations of trace minerals in plasma and intraocular fluids (aqueous and vitreous humour) in the Lidia breed bulls.

Parameter	Fluids		Mean	SD	Minimum	Maximum
Iron (mmol/l)	AH	a	1.007	0.675	0.2	2.4
	VH	a	0.636	0.582	0.1	2.1
	PL	b	36.28	18.94	11.2	75.9
Chromium (mmol/l)	AH	a	61.06	5.79	47.11	72.13
	VH	b	51.10	5.25	43.42	61.96
	PL	b	52.10	2.89	47.18	57.19
Cobalt (mmol/l)	AH	a	0.24	0.09	0.13	0.42
	VH	b	0.31	0.11	0.16	0.49
	PL	c	1.79	0.94	0.66	3.18
Nickel (mmol/l)	AH	a	0.45	0.32	0.04	1.00
	VH	a	0.39	0.21	0.10	0.81
	PL	b	2.36	0.55	1.78	3.68
Copper (mmol/l)	AH	a	26.27	29.5	7.87	114.92
	VH	b	6.298	2.041	4.241	12.441
	PL	c	1465.4	215.5	1120.3	1822.7
Zinc (mmol/l)	AH	a	90.72	40.57	43.92	194.28
	VH	b	53.2	51	26.3	231.1
	PL	c	1533.4	418.7	977.9	2514.6
Selenium (mmol/l)	AH	a	1.32	0.81	0.31	2.82
	VH	a	1.26	1.29	0.17	2.98
	PL	b	65.31	9.45	48.98	81.51
Molybdenum (mmol/l)	AH	a	2.48	1.34	0.76	4.36
	VH	a	4.08	2.84	0.90	10.71
	PL	b	8.11	4.06	2.60	14.86

Descriptive statistics indicating the mean value, the standard deviation (SD) and the minimum and maximum ranges. AH: aqueous humor; PL: plasma; VH: vitreous humor. Alls variables are measured in mmol/l. Different letters in each parameter group indicate significant statistical differences ($p<0.05$).

in blood than AH and VH, respectively. More marked differences were even registered in selenium and iron being between 40 and 60 times greater in blood than in eye fluids. Particularly noteworthy was the cupperhemic detected in these bulls, which was 57 times greater than that found in the aqueous humour and up to 240 times greater than that measured in the vitreous humour.

Most values obtained followed a normal distribution, except the copper into AH, which did not have a normal distribution ($p<0.05$) and showed that most of the values were concentrated below 20 ppb (µmol/l) although we found a value of 114.92 ppb in one of the bulls sampled. Studying the distribution of values in the vitreous humour we observed that these were especially concentrated in phosphorus, magnesium and potassium, while for the aqueous humour values were more clustered in calcium and sodium.

Significant correlations found in this study are shown in Table 4 and Fig. 1. There was a significant correlation of P, Co and Mo of the three sampled fluids. In the rest of macrominerals no significant correlations were found, although the Mg of the three fluids was projected in the same direction, the Na of both ocular fluids was projected in the same direction and in the opposite direction of the plasmatic Na, while the K of the different substrates were projected in different directions (Fig. 1). Only two trace elements (Co and Mo) showed significant correlation between all studied substrates. In addition, a correlation was found between

Table 4. Correlations between studied variables plasma and ocular fluids and between aqueous humour and vitreous humour concentrations.

Correlations			r	p
Phosphorus-PL	*vs*	Phosphorus-HA	0.519	0.047
Phosphorus-PL	*vs*	Phosphorus-HV	0.726	0.002
Phosphorus-HV	*vs*	Phosphorus-HA	0.498	0.059
Cobalt-PL	*vs*	Cobalt-HA	0.651	0.009
Cobalt-PL	*vs*	Cobalt-HV	0.879	0.000
Cobalt-HV	*vs*	Cobalt-HA	0.572	0.026
Selenium-PL	*vs*	Selenium-HV	0.524	0.045
Molybdenum-PL	*vs*	Molybdenum-HA	0.893	0.000
Molybdenum-PL	*vs*	Molybdenum-HV	0.945	0.000
Molybdenum-HV	*vs*	Molybdenum-HA	0.849	0.000

AH (aqueous humor), VH (vitreous humor), PL (plasma). Although all the correlations were made only those that had statistical significance are shown. The value $p < 0.05$ was chosen for statistical significance.

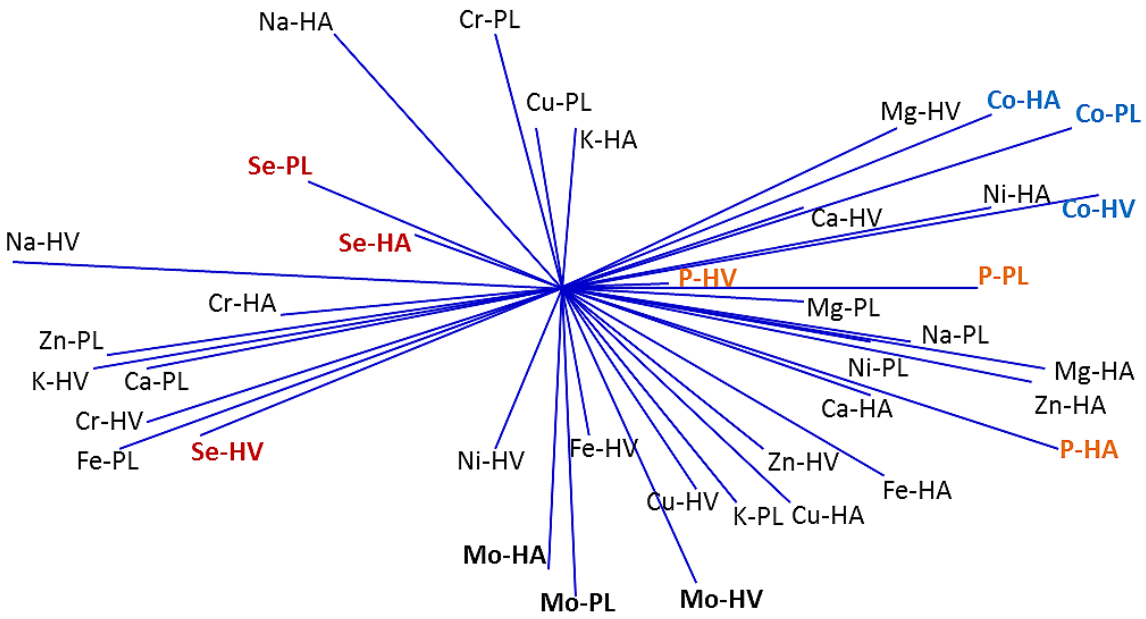

Fig. 1. Graphic image of correlations of all parameters through principal components analysis.

Se of aqueous humour and blood (Table 4). The most marked correlations were observed between plasmatic Mo with the values found in HA (r = 0.893) and in VH (r = 0.943) and between both ophthalmic fluids (r = 0.849). No correlations were found in any of the other variables studied, however the Cr, Fe, Cu, and Zn of both ocular fluids (HA, HV) were projected in the same direction, and in the last three their projection was contrary to the plasmatic values.

Discussion

It was hard to find research to compare our data, since most of the research was carried out in humans, and very few minerals present in eye fluids of animals have been measured. Furthermore, almost all measurements were taken from healthy animals sampled after being slaughtered. In addition, no publications exist which evaluate trace element concentrations in ocular fluids, neither in cattle nor in any other species of veterinary interest.

In our results, plasma levels were usually greater than those recorded from eye fluids, and not much

difference between the aqueous and vitreous humour was found, except for P, Cu, Zn and Mo, where the differences between the ocular fluids were very pronounced. Like Wittwer et al. (1992), we found that the concentrations of Na in both ocular fluids were similar to those measured in the blood of these animals, while the Ca and P concentrations were lower than those in blood. We have also observed that the aqueous humour showed lower values of Mg and K, as Witwer et al. (1992) did, while Ramirez et al. (1992) noted that the concentration of Mg in VH was greater than in plasma in 84% of cases (Ramirez et al. 1998). We also agree with Wittwer et al. (1992), comparing the concentrations found between aqueous humour and vitreous humour, and we observed that there were only similarities in the concentrations of Na, while the vitreous humour had greater concentrations of Ca, Mg and K.

In ewes the concentrations of serum Mg *ante morten* were significantly higher than those determined in the aqueous humor, but with a high correlation between the magnesemia and the *post-morten* values measured in aqueous humor, which allowed to estimate real serum values with a minimum error (-0.1%) (Athanasiou et al. 2018).

Concentration differences could be due to the existence of barriers. Firstly, there is a blood-aqueous barrier separating the aqueous humour and then there is a filtration mechanism between blood and vitreous humour (Bedford and Grierson 1986, Wittwer et al. 1992).

This explanation could also be used for most parameters evaluated in our study. In our case, the imbalance observed may be due to the fact that there was not enough time to balance these parameters which have changed considerably in the blood. Highly stressed and with a significant physical exertion, the sampled bulls showed significant modifications in their blood parameters, but as the fight lasts between 15 and 20 minutes and in these animals death is immediate, it is possible that not sufficient time has elapsed to balance blood and ocular fluids concentrations.

The plasma calcium values found in our research were similar to those reported in the literature (Radostits et al. 2006, Kaneko et al. 2008) and slightly greater than values given by McLaughlin and McLaughlin (1987) in cattle and in Lidia cattle (Carpintero et al. 1996, Sánchez et al. 1996, Chaves et al. 2001). However calcium values measured in IFOs is half of those found in blood, being slightly greater than bovine calcium vitreous values (McLaughlin and McLaughlin 1987), but lower than in equine (McLaughlin and McLaughlin 1988). The calcium values in IOFs of adult cattle could be used to estimate the *ante-mortem* serum calcemia values, but in the opinion of Hanna et al. (1990) it cannot be done with a high degree of precision. Vitreous calcium stability after death has been controversial since some researchers found that calcium remains fairly stable (Bito and Davson 1964, Blumenfeld et al. 1979, Dufour 1982, McLaughlin and McLaughlin 1988) and others had erratic results (McLaughlin and McLaughlin 1988). In our opinion an important fact to consider is the period necessary to balance the concentrations allowing calcium ions passage from blood into ocular fluids. In this way, controversial results have been published on the correlation between clinical hypocalcaemia and decreased vitreous calcium concentrations. In humans there is a poor correlation between serum and vitreous calcium levels in hypocalcemic patients. In cattle, however, a better correlation has been found (Wilkie and Bellamy 1982, McLaughlin and McLaughlin 1988, Hanna et al. 1990). This apparent disparity could be explained by considering the type of hypocalcaemia (acute or chronic) and the homeostatic mechanism by which the calcium concentration is maintained in the vitreous humour. In acute hypocalcaemia, the active transport system allows calcium pumping through the blood-retinal barrier (Bito and Davson 1964), but may not have enough time to replenish depleted vitreous calcium and *post-mortem* analysis may then reveal decreased vitreous levels of calcium. In chronic hypocalcaemia the transport system would have enough time to replenish the vitreous calcium, resulting in normal *post-mortem* concentrations (McLaughlin and McLaughlin 1988).

Both magnesemia and phosphatemia found in our bulls were much greater than those reported in the literature (Lincoln and Lane 1985a, McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988, Radostits et al. 2006, Kaneko et al. 2008), even in animals of the same breed (Sánchez et al. 1996, Chaves et al. 2001) but were similar to those published after a similar situation (Carpintero et al. 1996, Alonso et al. 1997), always being greater than those found in eye fluids. When considering phosphorus values they were slightly greater than those reported in cattle vitreous humour and considerably greater than those measured in horses, while the vitreous humour Mg concentration was similar to that reported in literature (Lincoln and Lane 1985a, McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988). We are of the opinion that the high average magnesemia value found could be due to the important physical exercise done, with marked muscular destruction, and also environmental stress. However, our results do not agree with consulted references which cited that stress causes a decrease in magnesium levels (Radostits et al. 2006), due to increased magnesium uptake by adipose tissue as a result of catecholamines release and possibly

an increased magnesium excretion. Magnesium deficiency also enhances catecholamines release and thus a vicious circle is created, increasing the shortage of magnesium (McCoy 2004).

The calemia and natremia are similar to those found in the literature (Lincoln and Lane 1985a, McLaughlin and McLaughlin 1987, Sánchez et al. 1996, Radostits et al. 2006, Kaneko et al. 2008). K plasmatic value is greater than that described in vitreous humour (McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988), while the Na value is slightly lower than that found in the vitreous humour (McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988). Ca, P, Mg and K values in blood are virtually identical to those reported in bulls of the same breed in a similar situation, after the fight (Carpintero et al. 1996, Alonso et al. 1997).

The ratios between ocular fluids and blood values were used to estimate serum *ante-mortem* values, as some parameters of the intraocular fluids remain constant or changed in a predictable way after death (Drolet et al. 1990, Athanasiou et al. 2018). Therefore, if the correlation and the regression equation are known, we could try to predict serum *ante-mortem* values from the ocular fluids value obtained after death (Drolet et al. 1990, Athanasiou et al. 2018). However, we keep in mind that almost all research have been conducted using healthy animals, and serum levels were always within normal limits. But it is possible that the correlations may be different when the measured parameters show significant alterations or in advanced stages of the disease (Drolet et al. 1990, McCoy 2004).

According to Drolet et al. (1990), only blood urea concentration could be estimated from the IFOs, while for Wittwer et al. (1992) aqueous humour samples could be used to estimate *ante-mortem* Na and urea blood concentrations up to 24 hours *post-mortem*, whereas vitreous humour could serve to estimate Mg, Na and uraemia *ante-mortem* values. The possibility to estimate the concentration of *ante-mortem* magnesium blood levels from samples of aqueous humour is feasible, but with a high margin of error, unlike urea which can be estimated fairly accurately (Wittwer et al. 1992).

A high correlation between the concentrations of Mg and urea in blood and both ocular fluids has been reported (Lincoln and Lane 1985b, Hanna et al. 1990, Wittwer et al. 1992, Ramirez et al. 1998, Mattioli et al. 2002, McCoy 2004), magnesium has been found to be very high in the vitreous humour (Wittwer et al. 1992) and in aqueous humour (Athanasiou et al. 2018).

However, for estimating *ante-mortem* serum values, while taking into account their concentration in IOFs, it is important to consider other factors such as the fluctuation in the concentration of some variables (Hanna et al. 1990, Gonzalez-Montaña et al 2018). This fluctuation may be due mainly to circumstances as time has passed since the death of the animal (Drolet et al. 1990, Hanna et al. 1990, Wittwer et al. 1992) and the ambient temperature (Lincoln and Lane 1985b, McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988, Hanna et al. 1990, Wittwer et al. 1992, McCoy and Kennedy 1994, McCoy et al. 2001b, Mattioli et al. 2002). In our study these circumstances, time between sampling and changes depending on the ambient temperature, are unimportant, since we have sampled eye fluids immediately after the death of the bull and the processing of the samples was carried out quickly.

Dufour et al. (1982) have shown a lack of correlation between the concentrations of serum and vitreous humour calcium concentrations, probably because of active transport mechanisms within the eye. In this way, the vitreous humour has a low utility when assessing *ante-mortem* calcemia (Farmer et al. 1985). The differences are due to the existence of a barrier between blood and ocular fluids (Wittwer et al. 1992), which would filter the components based on their molecular size. Thus, creatinine, with large molecular size, has it difficult to cross the blood-aqueous barrier, while conversely the sodium with a smaller molecular size, crosses the blood-aqueous barrier without being filtered, favouring concentrations similar to those found in blood, and thus shows a high correlation (Palmer et al. 1985, Gonzalez-Montaña et al 2018). The concentrations of Ca, P and Mg in aqueous humour should be lower than in blood (Wittwer et al. 1992, Athanasiou et al. 2018), as only the ionized fraction, corresponding to 46, 33 and 65%, respectively, could enter the aqueous humour (Wittwer et al. 1992).

It has been proposed the use of oculars fluids Mg concentration from the corpse as indicative of *ante-mortem* magnesemia. In order to evaluate this possibility, several authors measured the correlation between *post-mortem* HV Mg concentration and *ante-mortem* magnesemia with conflicting results. Thus, some researchers believe that VH could be useful (Lincoln and Lane 1985b, McLaughlin and McLaughlin 1987, Wittwer et al. 1992, Mattioli et al. 2002), while those who found lower correlations consider its use questionable (Hanna et al. 1990, McCoy and Kennedy 1994). In opinion of Athanasiou et al. (2018) the values of magnesium are aqueous humour are valid to estimate the magnesemia *ante morten* in animals such as sheep.

This could be due to the fact that Mg values in VH balance with Mg ion plasma concentration, which represents only 65% of the total Mg (Ramirez et al. 1998). In our research no correlation was found between the

levels of Mg measured in the eye fluids, or between them and the blood values. Perhaps because the blood levels were much greater than those reported in the literature (Lincoln and Lane 1985b, McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988, Alonso et al. 1997, Radostits et al. 2006, Kaneko et al. 2008), possibly as a result of the physical effort and the release of intracellular magnesium.

There is a possibility that the rapid water flow and diffusion of small molecules from the blood to the vitreous humour allow for quick changes in vitreous magnesium concentration (McCoy 2004). However, fluctuations in the concentrations of magnesium are relatively small and the vitreous magnesium can be considered quite stable (McLaughlin and McLaughlin 1988). Changes in Mg concentration in IOFs, depending on the ambient temperature must be taken into account in the diagnosis of hypomagnesemia. According to Mattioli et al. (2002) a 24-hour delay in sampling can increase intraocular magnesium concentration from 11 to 14 %, increasing significantly the possibility of error when the temperature exceeds 20°C (Mattioli et al. 2002). However, some authors have reported the decrease 48 hours *post-mortem* (McLaughlin and McLaughlin 1987) and 72 hours *post-mortem* (McCoy et al. 2001b), possibly due to bacterial growth (McCoy et al. 2001b).

Also, the increase in vitreous humour potassium concentration has been used in forensic studies as an indicator of the *post-mortem* time interval (McLaughlin and McLaughlin 1988, Drolet et al. 1990). However, due to the high margin of error its use has never been fully accepted (Coe 1993, McCoy 2004). The vitreous humour potassium concentration increases linearly with the time lapse after death and is independent of factors such as age, sex, temperature and environmental humidity (Garg et al. 2004). Moreover, the precision of the estimations decreases as the *post-mortem* interval increases, with 57 % precision between 5 and 36 hours *post-mortem*, but only 24% between 36-98 hours after death (Farmer et al. 1985).

Several animal studies have suggested that the biochemical and mineral analysis of eye fluids may be a useful tool in the *post-mortem* diagnosis of some metabolic diseases, including hypocalcaemia and grass tetany in ruminants (Wilkie and Bellamy 1982, Farmer et al. 1985, Lincoln and Lane 1985a,b, McLaughlin and McLaughlin 1987, McLaughlin and McLaughlin 1988, Hanna et al. 1990, McCoy 2004). For most researchers analysis of magnesium concentrations in the vitreous humour is a useful and practical marker for *post-mortem* diagnosis of grass tetany in ruminants and therefore should be recommended for clinical veterinarians as an important tool in the diagnosis of this disease (Wittwer et al. 1992, McCoy and Kennedy 1994, McCoy et al. 2001a, McCoy 2004).

With regard to other trace elements it is difficult to interpret our results, mainly because there are no other data to compare with. García-Belenguer et al. (1992) have noted an increase in cupremia in Lidia bulls in comparison to cattle, possibly as a consequence of stress and exercise causing transient cupremia, but of course values were not measured in eye fluids. Only McGahan and Bito (1983) have reported aqueous and vitreous humour copper levels in human eyes, indicating that the concentration of Cu in the IFOs and CSF is substantially lower than the plasma values, representing between 1 and 3%, while in our study they were between 0.5% and 2% in vitreous and aqueous humour, respectively. We also agree with these authors when stating that the valuation of the various trace elements in body fluids should always be carried out with regard to the blood concentrations because their physiology is intimately interrelated. According to McGahan and Bito (1983) it is important to note that both the IOFs as the LCR are separated from the blood by a barrier system, the blood-ocular barrier, with similar properties to the blood-brain barrier, which play an important role in the exchange between the blood and these fluids. Moreover these authors suggest that the Cu in these fluids is linked to proteins.

To sum up, we have found that mineral plasma values are generally always greater than those observed in ocular fluids (aqueous and vitreous) although sodium, potassium and chromium values are similar in all the three fluids. The selenium, iron, zinc and especially copper values in plasma are much greater than those measured in ocular fluids. Despite most parameters follow a normal distribution, we found a value of 114.92 ppb in blood copper in one of the bulls even though most values are concentrated below 20 ppb (mmol/l). The vitreous values of phosphorus, magnesium and potassium are particularly group together, while calcium and sodium values for the aqueous humour are less grouped. We have only verified the existence of correlations between phosphorus, cobalt and molybdenum of the three measured fluids, and between plasma and HV selenium.

The main interest of this research is to establish concentrations that could be used as reference values for minerals and electrolytes in different fluids in this breed. These could later been used in estimating the *ante-mortem* values. Especially in those animals which for their specific features are not easily sampled in life but could be accessible immediately after death.

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