

RELATIONSHIPS BETWEEN CONCENTRATIONS OF BIOLOGICAL VARIABLES IN EYE FLUIDS AND BLOOD AFTER EXERCISE IN LIDIA CATTLE

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Lidia cattle are a heterogeneous Iberian cattle population known for its natural aggressiveness and resistance to traditional handling procedures making in vivo blood sampling and biological fluid collections extremely difficult. Blood variables are influenced by physical exertion and stressful situations; consequently, post-mortem blood analysis does not reflect basal concentrations for this species. Nevertheless, ocular fluids (aqueous and vitreous humour) maintain their stable composition after death and maybe could be used to estimate ante-mortem blood concentrations. So, 15 bulls which had fought (for 15-20 minutes) and, subsequently after death, blood, aqueous and vitreous humour were sampled. Total protein, albumin, triglycerides, uric acid, urea, AST, ALT, GGT, AP, CK, LDH, cholesterol, creatinine, glucose and lactate were measured. Statistical analysis and correlation coefficients between the three fluids were carried out. All variables showed high plasma concentrations of glucose, uric acid, LDH and CK, compared with normal bovine concentrations. Apart from urea, all plasma concentrations were greater than those found in ocular fluids. The measured enzymes activities were higher in the vitreous than in the aqueous humour, but only marked differences in uric acid, lactate, AP and AST were found. There was a significant correlation between creatinine in the plasma and aqueous humour, and between albumin and GGT in the plasma and vitreous humour. Glucose, creatinine and urea exhibit a high correlation between ocular fluids. All plasma concentrations were clearly modified, however ocular fluids do not seem affected, thus establishing important correlations between the blood and intraocular fluids.

Key words: aqueous humor, vitreous humor, biochemical variables, intraocular fluids, blood, metabolism, Lidia cattle.

INTRODUCTION

The evaluation of certain biochemical variables ante-mortem is helpful in diagnosing many diseases or at least studying the metabolic state of animals [1]. It is sometimes

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impossible to obtain blood samples from live animals, either due to the management system or simply the animal may have died. This happens with wild animals, difficulty with handling domestic animals or when the owners prevent access to the animals [1].

After death a variety of physical and biochemical changes occur in the biological body fluids. Thus, blood, urine and cerebrospinal fluids are not always suitable for evaluating post-mortem biochemical analytes [2,3] due to contamination by bacteria, release of intracellular compounds and post-mortem metabolism of certain serum substances [3]. Instead, ocular fluids [aqueous humour (AH) and vitreous humour (VH)] seem to deteriorate more slowly after death and are an easily accessible source to sample [2]. The analysis of ocular fluids may be helpful in estimating ante-mortem blood values [1,4,5] due to the fact that the eyeball behaves like a watertight compartment, maintaining both the aqueous and the vitreous humour isolated from the rest of the body and so the post-mortem composition of these fluids can remain more stable [2,6,7].

The aqueous humour is a clear and watery fluid produced by the ciliary body, located in the anterior and posterior chambers of the eye. A semi-transparent and semi-viscous liquid forms the vitreous humour or vitreous body, which is located between the lens and the retina [7,8]. They have been described as solutions composed of all the plasma (PL) components in equal or lower concentrations, with some substances that are secreted actively [7]. Both fluids, and especially the vitreous humour, have been widely used for post-mortem biochemical analysis because they are easier to collect than the cerebrospinal fluid and because their composition changes more slowly after death than it does in the blood [9,10].

In human medicine, especially in forensic medicine, ocular fluids are used to estimate the approximate time of death [7,10,11], ante-mortem blood biochemical values [10-12], levels of some toxins [7], drugs detection [9], as well as in sudden infant death studies [1,10].

In veterinary medicine there are several publications attempting to correlate post-mortem fluid levels of some parameters with blood levels before the death of animals [1], although most research has been conducted under slaughterhouse conditions or on healthy animals [1]. Analysis of ocular fluids has been used for post-mortem biochemical determinations, in order to diagnose pre-existing diseases that could have caused the death [9-11], to know the relationships between eye fluids with blood values [1,11,14], to understand the changes that occur from the interval death to the sample collection [2,6,7] and also to estimate the time interval between death and the discovery of the corpse [11]. Limited research has been done in hypocalcemic, uraemic or hypomagnesemic animals [1,7,8,13]. Thus, aqueous humour samples collected during necropsy provide adequate data for the extrapolation of the ante-mortem 3-OH butyrate serum values in ewes dead due to pregnancy toxemia [14] and the magnesium concentration in the vitreous humour has been used to confirm the diagnosis of hypomagnesemic tetany, both in sheep and cows found dead [8,18-20].

The Lidia cattle breed (*Bos taurus brachiceros*) is an Iberian heterogeneous cattle population described in the Decree 60/2001 [21]. It is mainly an open-range breed reared on extensive estates in central and 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 [22].

Blood sampling prior to the fight, even under natural farm conditions, is difficult to obtain. There are several reasons for this, one being the aggressive behavior of the animal, which increases the risk of injury for both humans and animals, and mainly to keep bulls with minimum human interaction so as to prevent abnormal behaviors during the fight. For these reasons it is practically possible only to carry out sampling on bulls once they have been killed [22].

The aim of this paper was to establish reference values for some biochemical variables in both blood and intraocular fluids (aqueous and vitreous humour) in the Lidia breed, specifically in stressed animals and after major physical exertion. We also tried to verify the possible correlations among these biochemical variables and also between the values found in the blood and ocular fluids. These data could be used to estimate baseline ante-mortem blood values in bulls, which are almost impossible to sample in bulls in vivo. Also the results may provide information about the relationship between the variables studied and the plasma concentration before death, and this information could be used in other cattle breeds, as well as in other species which are difficult to sample, such as wild animals.

MATERIAL AND METHODS

Animals and legal regulations

A total of 15 four to five-years-old Lidia bulls from different breeding farms were used in this study and they all fought in the Valladolid Arena. The animals were slaughtered under the regulation of the local legislation law (Decree 57/2008) [23]. All experimental procedures were performed in compliance with the provisions of the EU Directive of the European Parliament (Directive 2010/63/EU) that regulates the use of animals for scientific purposes [24] and the Royal Decree (Real Decreto 53/2013) that regulates experimentation and animal protection in Spain [25].

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 animals underwent significant physical exertion and stress situations for at least 15-20 minutes. Immediately after the end of fighting, blood,

aqueous and vitreous humour samples were obtained. Blood was obtained directly from the bleeding, while both the aqueous and vitreous humour were collected directly from the eye with 5 ml syringes. The aqueous humour was collected from the anterior chamber by gentle aspiration following corneal penetration with a 21-gauge needle. The vitreous humour was collected from the central portion of the vitreous body following insertion of a 18-gauge needle through the sclera by aspiration [18]. Immediately after collection, the blood samples were centrifuged at 4000 rpm (2200 x g) for 10 minutes (following the methodology described by Escalera *et al.* [22]), while fluid eyes samples were centrifuged at approximately 1000 x g for 10 min (Centrifuge Cencom II, JP Selecta, Barcelona, Spain) within 30 min after collection following the methodology described by Hanna *et al.* [1].

The supernatant free of impurities was placed in Eppendorf tubes and stored firstly refrigerated at 4 °C and then frozen at -20 °C, for transportation in a cooler. The samples were stored, until analyzed at the Laboratory Instrumental Techniques (LTI) at the University of León to determine biochemical variables using a Cobas Integra 400 multi-analyte analyzer (Roche, Mannheim, Germany), by means of Roche Diagnostic reagents (Roche Diagnostics GmbH, Germany). The measured biochemical variables were: total proteins, albumin, triglycerides, uric acid, urea, cholesterol, creatinine, glucose, lactate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (AP), creatine kinase (CK), and lactate dehydrogenase (LDH).

Statistical study

Concentrations of each parameter both in blood plasma and ocular fluids were measured. The data were analyzed using the SPSS 15.0 statistical program. Descriptive statistics was 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 values. The $p < 0.05$ value was chosen for statistical significance.

RESULTS

The results obtained in the present study are shown in Tables 1 and 2. It was not possible to evaluate triglycerides and cholesterol in both ocular fluids, and ALT, GGT and CK in the aqueous humour.

Table 1. Values of biochemical variables in plasma and intraocular fluids (aqueous and vitreous humor) in the Lidia breed

Parameter	Fluids		Mean	SD	Minimum	Maximum
Total protein (g/L)	AH	a	1.02	1.05	0.10	3.30
	VH	a	0.51	0.33	0.20	1.30
	PL	b	84.59	78.00	2.97	89.00
Albumin (g/L)	AH	a	0.69	0.55	0.20	2.30
	VH	a	0.56	0.25	0.10	0.90
	PL	b	40.03	35.20	2,97	44.70
Triglycerides (mmol/l)	AH	–	n.d.	n.d.	n.d.	n.d.
	VH	–	n.d.	n.d.	n.d.	n.d.
	PL	–	0.40	0.20	0.20	0.70
Glucose (mmol/l)	HA	a	2.33	1.06	0.93	4.26
	HV	a	2.84	0.70	1.85	4.35
	PL	b	24.36	0.98	12.77	51.11
Cholesterol (mmol/l)	AH	–	n.d.	n.d.	n.d.	n.d.
	VH	–	n.d.	n.d.	n.d.	n.d.
	PL	–	2.30	0.40	1.80	3.10
Uric acid (mmol/l)	AH	a	75.0	24,5	40.0	121.0
	VH	b	5,4	2.8	2.0	14.0
	PL	c	354.4	78.5	235.0	513.0
Creatinine (μmol/l)	AH	a	49.9	17.4	21.2	83.9
	VH	b	73.9	23.8	55.7	149.4
	PL	c	255.7	24.8	221,0	302,3
Urea (mmol/l)	AH	a	4.61	0.92	3,26	6.39
	VH	b	5.83	1.87	3,46	9.54
	PL	b	6.12	1.08	4.31	9.26
Lactate (mmol/l)	AH	a	17.7	3.8	9.4	24.1
	VH	b	9.7	2.3	6.1	15.1
	PL	c	43.5	5.6	34.0	53.4

Descriptive statistics indicating the mean value, the standard deviation (SD) and the minimum and maximum ranges. AH: aqueous humor; n.d.: Not detected; PL: plasma; VH: vitreous humor; albumin and total proteins (in g/L); creatinine (in μmol/l), rest of parameters (in mmol/l). Different letters in each parameter group indicate significant statistical differences ($p < 0.05$).

Mean plasma values of all analyzed variables were higher than those found in ocular fluids (AH and VH). In some variables such as total protein, albumin, glucose, uric acid and some enzymes (AP, AST and LDH) they were remarkably higher. Thus plasma values of total proteins were up to 95 and 180 times higher than the values found in ocular fluids, and 160 and 85 times higher LDH enzyme activity, and 100 and 20 times higher AP activity and 60 and 75 times higher albumin concentration (considering always the first value for AH and the second for the VH). For CK enzyme, plasma value was 160 times the value found in the vitreous humour. Plasma concentrations of uric acid and AST were between 5 and 72, and 55 and 3 times higher than those found in AH and VH, respectively. Glucosemia value and GGT plasma values were

approximately 10 times higher than in the ocular fluids (we should bear in mind that GGT was only measured in AH). The creatinine and lactate plasma concentrations were between 2 and 5 times higher than in ocular fluids, and only urea values were comparable among the three fluids measured.

Table 2. Biochemical enzyme values (in IU/l) in plasma and intraocular fluids (aqueous and vitreous humor) in the Lidia breed

Parameter	Fluids		Mean	SD	Minimum	Maximum
AP	HA	a	1.1	0.7	0.1	4.6
	HV	b	3.6	2.0	1.4	8.1
	PL	c	84.9	32.3	43.8	170.3
ALT	HA		n.d.	n.d.	n.d.	n.d.
	HV	a	1.5	1.2	0.1	4.6
	PL	b	62.6	24.9	33.9	115.9
AST	HA	a	9.2	7.1	2.5	27.4
	HV	b	155.3	132.5	47.7	555.8
	PL	c	506.2	346.8	220.1	1408.9
CK	HA		n.d.	n.d.	n.d.	n.d.
	HV	a	28.2	30.7	3.0	103.0
	PL	b	4599.7	4979.8	862.0	19776.0
GGT	HA		n.d.	n.d.	n.d.	n.d.
	HV	a	3.7	3.2	0.2	13.3
	PL	b	32.4	8.1	21.5	52.1
LDH	AH	a	16.5	15.5	4.2	50.1
	VH	a	38.1	27.3	3.3	178.0
	PL	b	2695.6	1149.7	1479.0	5502.0

Descriptive statistics indicating the mean value, the standard deviation (SD) and the minimum and maximum ranges. AH: aqueous humor; VH: vitreous humor; PL: plasma; ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; CK: creatine kinase; GGT: gamma-glutamyl transpeptidase; LDH: lactate dehydrogenase; n.d.: not detected. Different letters in each parameter group indicate significant statistical differences ($p < 0.05$).

In some studied variables (uric acid, lactate, AP and AST) we found marked differences in ocular humours concentrations. Uric acid and lactate values were higher in AH while all the enzymes values were higher in the VH than in AH. Most variables followed a normal distribution but LDH enzyme values showed a great dispersion. In general values found in the aqueous humour were more grouped than those found in the vitreous humour and in plasma, particularly in total protein, albumin, AP and LDH.

The significant correlations found in this study are shown in Table 3 and Figure 1. The biological variables that showed a higher correlation between both ocular fluids are glucose ($r = 0.74$), creatinine ($r = 0.64$) and urea ($r = 0.75$), albumin and GGT between plasma and vitreous humour ($r = 0.56$ and $r = 0.59$, respectively) and creatinine between plasma and aqueous humour ($r = 0.72$) being the only variable with a correlation between plasma and AH. Proteins, enzymes (apart from GGT)

and bilirubin showed a weak trend of correlation between plasma and ocular fluids, and also between both eye fluids and no correlations were found in any of the other variables studied.

Table 3. Correlations between studied variables plasma and ocular fluids, and between aqueous humor and vitreous humor concentrations

Correlations			r	P
Albumine-PL	vs	Albumine-VH	0.559	0.030
Glucose-AH	vs	Glucose-VH	0.738	0.002
Creatinine-VH	vs	Creatinine-AH	0.644	0.010
Creatinine-PL	vs	Creatinine-AH	0.724	0.002
Urea-AH	vs	Urea-VH	0.754	0.001
GGT-PL	vs	GGT-VH	0.591	0.020

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

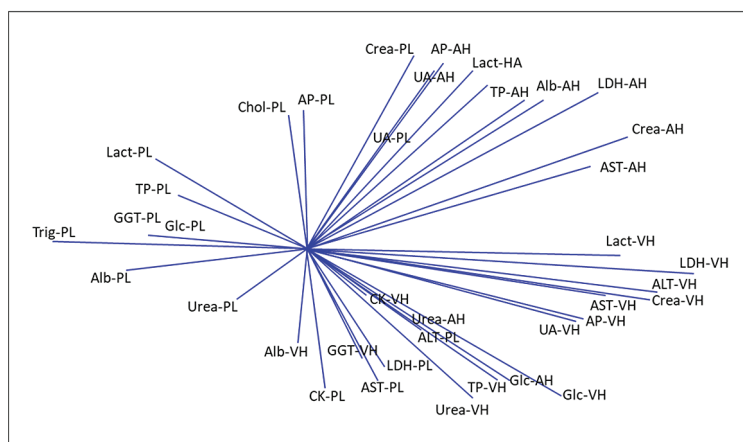


Figure 1. Graphic image of correlations of all parameters through principal components analysis

DISCUSSION

Considering the important diagnostic value of eye fluids [4,6], one of the aims of this study was to evaluate the possibility of using the eye fluid concentrations values as estimators of ante-mortem plasma values of some biochemical variables in bulls, prior to death. It is important to note that, before the present research, only normal or moderately modified values, either elevated or decreased, had been investigated [10] but never with important modifications or after situations of great exercise and stress. In addition, we have only found a few reference values to compare the levels found in bovine blood after these situations, and even less in ocular fluids.

We decided to carry on this research in both ocular fluids as, according to Hanna *et al.* [1], the AH is very useful for performing these analyses, because it is at least as accurate as VH, easier to collect and analyze and does not always need to be centrifuged [7]. However, the VH allows to obtain a higher sample volume than AH, although it should be centrifuged, since it carries natural viscous impurities.

All studied plasma variables concentrations were greater than those referred for cattle [26-28] and are comparable to those published in similar situations in the Lidia breed [26,29-32]. It could be explained by the special characteristics of the Lidia cattle breed. It must be considered to be a particular bovine breed, intended for combat and that has been sampled after extreme circumstances. The fighting not only represents a period of maximum exertion against the matador, but also includes stressful situations that accompany the animal from the field to the bullring. These include transport, penning and exposure to people, since this breed is reared under extensive conditions with little human contact [22]. The increased values could also be due to muscle cells destruction together with fluid loss, and consequent hemoconcentration [22] as well as a metabolic acidosis situation [22,29,30].

The blood glucose values found in our research were 8 to 10 times higher than reported cattle basal values [26-28]. The extraordinarily high glycaemia is an attempt to compensate the increase in energy demand due to the fight. The major muscle exercise, which lasts no more than 15-20 minutes, was obviously not enough to consume all the mobilized glucose that remains in the blood.

Glucose originated in gluconeogenesis process has not been metabolized, either aerobically or anaerobically, as it is used in situations of great physical effort. There could even be problems in their consumption by the muscle fibre, that is not able to use glucose adequately [31]. On the other hand, the values that we found in ocular fluids were not as high, and were even slightly lower than those cited by Hanna *et al.* [1]. Considering all the above mentioned factors and according to the high correlations between blood glucose concentrations and ocular fluids reported by Hanna *et al.* [1], the possibility of using the glucose concentration in AH and VH as an indicator of plasmatic values in animals difficult to sample ante-mortem could be validated.

Both total protein and albumin values were much lower in the ocular fluids than in the plasma (Table 1). This is in accordance with the literature reviewed, with AH values of 0.5% of plasmatic proteins [5] and between 1 and 2 % in VH [33], and furthermore, these proportions remained constant with age [33,34]. It is important to note that only a small percentage of large molecules (cholesterol, bilirubin, proteins, etc.) are present in eye fluids due to the difficulty they encounter in order to pass through the blood-ocular fluid barrier [1,7]. This could explain why the concentration of the majority of the studied variables were not elevated in ocular fluids, even when the plasma values raised considerably, and perhaps because there was not enough time for the exchange, especially for those molecules of higher molecular size, such as proteins and albumin [4].

A similar explanation allows us to understand why despite the marked elevation of plasma concentrations of the analyzed enzymes, they were not reflected in the values of eye fluids, particularly those related to muscle disorders and tissue damage (CK, AST and LDH), due to the important exercise performed by the bull. According to Lane and Lincoln [4], permeability not only depends on the molecular size but also on lipid solubility. In the case of cholesterol, even with high amounts in the plasma, it could not be determined in ocular fluids, perhaps because the amounts were negligible, around 1 µg/ml, at 30 minutes post-mortem in cows in a slaughterhouse [35].

The average uremia value found was comparable to that mentioned as baseline in Lidia cattle [26, 36], and similar to values found in both ocular fluids (Table 1). However, the urea value found in the vitreous humour in our study is more than twice the value reported by Lane and Lincoln [4].

Also, plasma creatinine levels are twice those previously reported [4,15,26], whereas in ocular fluids (Table 2) are slightly higher than those published [4,15]. We disagree with Lane and Lincoln [4], who reported vitreous values significantly lower than blood values for BUN and creatinine (15.7 and 0.7 mg/dl in VH and 20.0 and 1.5 mg/dl in the blood, respectively). The urea values determined in ocular fluids could be used to determine the ante-mortem blood concentration, because as Hanna et al. [1] and Wittwer et al. [7] indicated, a long time is required to balance the changes concentrations of urea through the blood-eye barrier and, after death, changes in the vitreous occurs more slowly than in the blood [9].

The average blood lactate value is similar to that reported in similar situations Escalera et al. [22], but lower than those presented by Aceña et al. [29] and Picard et al. [32]. The levels in eye fluids are even higher than blood concentrations in anesthetized animals [36], which could reflect the ability of lactate to cross the blood- eye barrier.

While we might think that there is an important correlation between plasma levels and those found in ocular fluids, different papers reported that, under physiological conditions, this does not occur. Most variables showed a poor correlation between ocular fluids and blood concentrations [2]. According to Hanna et al. [1], the correlation of proteins, enzymes, bilirubin and cholesterol is very weak, both between serum and eye fluids, as well as between both ocular fluids. In our research, we only found a correlation between plasma and VH values of albumin and GGT, as well as between plasma levels of creatinine and AH (Table 3).

However, correlations have been reported in certain parameters such as urea [2,4,5,7], glucose [1,4] and magnesium [13,18,20]. Therefore, these concentrations in ocular fluids could be extrapolated to ante-mortem blood levels, with an important diagnostic value [4,6], since the vitreous behaves like a stable compartment after death, and values while being low vary in a consistent and predictable way [4].

This correlation could be significantly influenced by the size of the molecule due to the difficulty to cross the blood-eye barrier. Therefore, only a small percentage of large molecules could be present in the ocular fluids [1,7].

The significant correlation found, in the creatinine, between plasma values and in aqueous humour (Table 3) agrees with those reported by different authors [1,4,5], shown both at the time of death [5] and in the following 24 hours [1,5]. On the contrary, we were not able to find the high correlation cited by some researchers between urea concentrations [1,4,5]. It is possible that the predictive utility of ante-mortem blood values from post-mortem vitreous values decreases in cattle with renal failure or with high concentrations of blood urea nitrogen (BUN) [4], which may be similar to the situation observed in our study, with high values of plasmatic creatinine and urea.

On the other hand, in our opinion the fact that we have not found correlations could be due to unusually high blood levels measured in certain variables, such as glycaemia that cannot be “balanced” with eye fluids, since changes in the vitreous humour occurs much more slowly after death than in the blood [9].

This work should be considered a preliminary study of the use of ocular fluids to establish basal levels in bulls before the fight, as well as in wild animals, without having to manipulate or stress them, avoiding the risk for both people and animals due to the difficulties in sampling [26].

CONCLUSIONS

All plasma variables measured showed greater concentrations, especially glucose, uric acid, LDH and CK, compared with baseline bovine values. The greater concentrations detected in the Lidia breed can be justified by the important physical overexertion, stress situation, muscle cells destruction and fluid loss experienced by those animals as a consequence of intense fighting. However, the values found in ocular fluids do not seem to have been affected. Perhaps, because there was not enough time to balance concentrations and cross the blood-ocular fluids barriers, but there is also a possibility for some variables to be considered as “baseline” values.

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Authors' contributions:

GMJR, GVR and AME designed the experiments and interpreted the results. EF and LJM harvested and prepared samples. All authors helped in the overall execution of

the experiment. GMJR and EF wrote the paper. AME and GVR revised it critically. All authors have approved the final version of the manuscript.

Declaration of conflicting interests:

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REFERENCES

1. Hanna PE, Bellamy JE, Donald A: Postmortem eye fluid analysis in dogs, cats and cattle as an estimate of antemortem serum chemistry profiles. *Can J Vet Res* 1990, 54: 487-494.
2. Drolet R, D'Allaire S, Chagnon M: The evaluation of postmortem ocular fluid analysis as a diagnostic aid in sows. *J Vet Diagn Invest* 1990, 2: 9-13.
3. McLaughlin BG, McLaughlin PS: Equine vitreous humor chemical concentrations: correlation with serum concentrations, and postmortem changes with time and temperature. *Can J Vet Res* 1988, 52: 476-480.
4. Lane VM, Lincoln SD: Changes in urea nitrogen and creatinine concentrations in the vitreous humor of cattle after death. *Am J Vet Res* 1985, 46: 1550-1552.
5. Gallardo C, Paredes E, Perez J: Estudio histopatológico de hígado y riñón de caninos y su relación con las concentraciones de urea, creatinina, proteínas, enzimas (ALT y SAP) en sangre premortem y en humor acuoso a las 0 y 24 horas postmortem. *Arch Med Vet* 2003, 35: 61-74
6. Lincoln SD, Lane VM: Postmortem chemical analysis of vitreous humor as a diagnostic aid in cattle. *Mod Vet Pract* 1985, 65: 883-886.
7. Wittwer F, Urcullu F, Contreras PA, Böhmwald H: Concentraciones postmortem de minerales, urea y creatinina en humor acuoso y vítreo en vacas como indicadores de sus concentraciones sanguíneas premortem. *Arch Med Vet* 1992, 24: 61-68.
8. McCoy MA: Hypomagnesaemia and new data on vitreous humour magnesium concentration as a post-mortem marker in ruminants. *Magn Res* 2004, 17: 137-145.
9. McNeil AR, Gardner A, Stables S: Simple method for improvising the precision of electrolyte measurements in vitreous humor. *Clin Chem* 1999, 45: 135-136.
10. Madea B, Musshoff F: Postmortem biochemistry. *Forensic Sci Int* 2007, 165: 165-171.
11. Thierauf A, Musshoff F, Madea B: Post-mortem biochemical investigations of vitreous humor. *Forensic Sci Int* 2009, 192: 78-82.
12. Coe JI: Use of chemical determinations on vitreous humor in forensic pathology. *J Forensic Sci* 1972, 17: 541-546.
13. Lincoln SD, Lane VM: Postmortem magnesium concentration in bovine vitreous humor: comparison with antemortem serum magnesium concentration. *Am J Vet Res* 1985, 46: 160-162.
14. Scott PR, Sargison ND, Penny CD, Strachan WD: Aqueous humour and cerebrospinal fluid collected at necropsy as indicators of ante mortem serum 3-OH butyrate concentration in pregnant sheep. *Br Vet J* 1995, 151: 459-461.

15. McLaughlin PS, McLaughlin BG: Chemical analysis of bovine and porcine vitreous humors: correlation of normal values with serum chemical values and changes with time and temperature. *Am J Vet Res* 1987, 48: 467-473.
16. Iqbal Z, Muhammad Z, Shah MT, Bashir S, Khan T, Khan MD: Relationship between the concentration of copper and iron in the aqueous humour and intraocular pressure in rabbits treated with topical steroids. *Clin Exp Ophthalmol* 2002, 30: 28-35
17. Sauer MJ, Pickett RJH, Mac Kenzie AL: Determination of clenbuterol residues in bovine liver, urine, and eye by enzyme immunoassay. *Anal Chim Acta* 1993, 275: 195-203.
18. McCoy MA, Kennedy DG: Evaluation of post mortem magnesium concentration in bovine eye fluids as a diagnostic aid for hypomagnesaemic tetany. *Vet Rec* 1994, 135: 188-189.
19. McCoy MA, Hutchinson T, Davison G, Kennedy DG: Postmortem biochemical markers of experimentally induced hypomagnesaemic tetany in cattle. *Vet Rec* 2001, 148: 233-237.
20. Mattioli GA, Tittarelli C, Giuliadori MJ, Costa EF: Valor diagnóstico del magnesio en humor vítreo en cinco establecimientos con brotes de tetania hipomagnésica. *Analecta Veterinaria* 2000, 20, 39-41.
21. BOE: Real Decreto 60/2001, de 26 de enero, sobre prototipo racial de la raza bovina de lidia. *Boletín Oficial del Estado*, 2001, 5255-5261.
22. Escalera-Valente F, Gonzalez-Montaña JR, de la Varga MEA, Lomillos-Perez JM, Gaudioso-Lacasa VR: Influence of intense exercise on acid-base, blood gas and electrolyte status in bulls. *Res Vet Sci* 2013, 95: 623-628.
23. BOCyL: Decreto 57/2008, de 21 de agosto, por el que se aprueba el Reglamento General Taurino de la Comunidad de Castilla y León. (Decree 57/2008, of 21 August, General Taurino Regulation of Castilla and Leon Community). *Boletín Oficial de Castilla y León*, 2008, 17317-17333.
24. European-Union: Directive 63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* 2010, 33-79.
25. BOE: Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. *Boletín oficial del Estado*, 2013, 11370- 11420.
26. Sánchez JM, Castro MJ, Alonso ME, Gaudioso VR: Adaptive metabolic responses in females of the fighting breed submitted to different sequences of stress stimuli. *Physiol Behav* 1996, 60: 1047-1052.
27. Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2006): *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*, 10 th. Philadelphia, USA: WB Saunders Ltd.: 2006, 2065 pp.
28. Kaneko JJ: Carbohydrate metabolism and its diseases. In: Bruss JJ, Kaneko JW , Harvey ML (eds). San Diego, USA: Academic Press; 2008, 45-80.
29. Aceña MC, García-Belenguer S, Gascón M, Purroy A: Modifications hématologiques et musculaires pendant la corrida chez le taureau de combat. *Rev Med Vet (Toulouse)* 1995, 146: 277-282.
30. Alonso ME, Sánchez JM, Robles R, Zarza AM, Gaudioso VR: Relation entre la fréquence des chutes et différents paramètres hématologiques chez le taureau de combat. *Rev Med Vet (Toulouse)* 1997, 148: 999-1004.

31. Agüera Buendía E, Rubio MD, Vivo R, Escribano BM, Muñoz A, Villafuerte JL, Castejón F: Adaptaciones fisiológicas a la lidia en el toro bravo. Parámetros plasmáticos y musculares. *Vet México* 1998, 29: 399-403.
32. Picard B, Santelhoutellier V, Ameslant C, Micol D, Boissy A, Hocquette JF, Compan H, Durand D (): Caractéristiques physiologiques de taureaux de la race Brave à l'issue de la corrida. *Rev Med Vet (Toulouse)* 2006,157: 293-362.
33. Chen CH, Chen SC (1981): Studies on soluble proteins of vitreous in experimental animals. *Exp Eye Res* 32, 381-388.
34. Hockwin O, Kietzmann MT: Enzyme activities of bovine aqueous humor in dependence on age. *Graefes Arch Clin Exp Ophthalmol* 1978, 206: 179-181.
35. Cenedella RJ: Lipoproteins and lipids in cow and human aqueous humor. *Biochim. Biophys. Acta, Lipids Lipid Metab* 1984,793: 448-454.
36. Requena F, Escribano BM, Rubio MD, Tovar P, Santiesteban R, De Miguel RJ, Agüera E: Estimación de los cambios fisiológicos producidos por la anestesia en el toro de lidia. [Estimation of physiological changes caused by anesthesia in the bull]. *Revista Universitaria de Sanidad* 2006, 2: 159-159.

POREĐENJE KONCENTRACIJE BIOLOŠKIH PROMENLJIVIH VREDNOSTI U FLUIDIMA OKA I U KRVI POSLE IZLAGANJA NAPORU GOVEDA RASE „LIDIA“

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Lidia rasa je populacija heterogenih iberijskih goveda koja su poznata po prirodnoj agresivnosti i pružanju otpora na tradicionalne metode fiksiranja što čini da su procedure tokom in vivo uzimanja uzoraka krvi i bioloških fluida ekstremno teške i zahtevne. Promenljive vrednosti krvnih parametara su pod uticajem fizičkih napora i stresnih situacija što čini da analiza krvi posle klanja ne predstavlja pravi odraz koncentracija osnovnih sastojaka krvi. Ipak, okularne tečnosti (očna vodica i staklasto telo) održavaju stabilni sastav posle uginjavanja i mogu da se koriste prilikom procene koncentracija krvnih parametara pre žrtvovanja životinje. U ogledu je bilo 15 bikova koji su prethodno bili u borbi (15 do 20 minuta) i kod kojih je posle žrtvovanja obavljeno uzorkovanje fluida: krvi, oka i staklastog tela. U uzorcima je obavljeno ispitivanje koncentracija ukupnih proteina, albumina, triglicerida, mokraćne kiseline, uree, AST, ALT, GGT, AP, CK, LDH, holesterola, kreatinina, glukoze i laktata. Obavljena je statistička analiza kao i koeficijent korelacije između ove tri tečnosti. Sve su promenljive vrednosti pokazale visoke koncentracije glukoze, mokraćne kiseline, LDH i CK u plazmi, u poređenju sa normalnim vrednostima kod bovida. Sa izuzetkom ureje, sve koncentracije u plazmi su bile veće nego koncentracije u fluidima oka. Izmerene aktivnosti enzima su bile veće u staklastom telu u poređenju sa očnom tečnošću, ali su značajne razlike uočene samo kod mokraćne kiseline, laktata, AP i AST. Postojala je značajna

korelacija između kreatinina u plazmi i koncentracije u očnoj tečnosti kao i između albumina i GGT u plazmi i staklastom telu. Koncentracije glukoze, kreatinina i ureje su pokazivale visok stepen korelacije između fluida oka. Sve koncentracije u plazmi su bile promenjene, međutim po svemu sudeći to nije imalo posledica po fluide oka. Može da se zaključi da se na osnovi prikazanih rezultata može uspostaviti značajna korelacija među parametrima krvi i intraokularnih fluida.