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ALP-AST/GOT-ALT/GPT-Bilirubin in Serum from Bos Taurus Cows in the Postpartum Period and Maintained by Grazing in the Humid Tropic Region

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Abstract: *The objective of this study was to determine the serum levels of liver enzymes ALP, AST/GOT, ALT/GPT and Bilirubin, thus establishing if there is affectation of these bovines in the humid tropical environment. 95 healthy crossbred Holstein cows in the postpartum period were used, where postpartum time of blood sampling (20, 75, 130 and 180 days), the height of the animal (high >1.3m and low <1.3m). Only between 17.5 to 23% of the population had normal values of the four liver elements; analyzing these values during the four postpartum periods, statistical difference was determined (P<0.05). The type of pasture had an effect significant (P<0.05) because the animals kept in Setaria grass showed higher values of the three liver enzymes than those kept on Axonopus grass. However for the group kept on pastures of Setaria there was a negative relationship between height and ALP (P<0.05) and height - GPT (P<0.10). In conclusion can be determined that this population has liver damage caused probably by the wrong dosage and frequency of use of all types of drugs, being the animals most affected those with little height and also those maintained on grassland of Setaria.*

Keywords: *Dairy cattle. Animal welfare. Xenobiotic. Liver damage. Longevity.*

Abbreviations

ALP: *alkaline Phosphatas*

AST/GOT*: Aspartate aminotransferase/Glutamic Oxaloacetic Transaminase*

ALT/GPT*: Alanine aminotransferase/Glutamic Pyruvic Transaminase*

1. INTRODUCTION

Currently the success in the systems of animal production are not based on the quantity, but if in the quality of the produced, just in this point where the animal welfare has a key role to obtain a safe product for human consumption., "Animal friendly" production system (Frewer *et al.*, 2005). The harmonious genotype-environment interaction in all animal production systems is the basis for the sustainability of animal production. The adaptation of a living organism to a given environment is characterized by a slow process, even more in severe environments like the tropics. An animal in welfare conditions will live and produces more and better, if all organs of the body are healthy, especially the liver, which plays a key role, as it is responsible for the processes of gluconeogenesis, biliary secretion, production of certain noble proteins and detoxification or metabolism of xenobiotics and all drugs that enter the body (Jeong *et al.*, 2013), malfunction of this organ will cause susceptibility, weakness and loss of productivity in the individual.

Liver enzymes as ALP (alkaline Phosphatase), AST/GOT (Aspartate aminotransferase/ Glutamic Oxaloacetic Transaminase), ALT/GPT (Alanine aminotransferase/Glutamic Pyruvic Transaminase) and Bilirubin are quantified in serum and routinely used as elements of reliable hepatic diagnostic (Koide *et al.*, 1959; Wang *et al.*, 2006; Tu *et al.*, 2007; Kang *et al.*, 2008; Quintela *et al.*, 2011; Jeong *et al.*, 2013) in all kinds of animals such as pigs (Wang *et al.*, 2006), fish (Kavitha *et al.*,

2011), sheep and goats (Tibbo *et al*., 2008); and cattle (Quintela *et al.*, 2011; Noro *et al*., 2013). It is known that the normal range of these liver enzymes is affected by internal and external factors such as the animals age and stage of lactation (Doornenbal *et al*., 1988; Otto *et al*., 2000), diet, season and management (Hawley and Peden., 1982: Quintela *et al*., 2011); and sex (Tibbo *et al*., 2008). The balance animal-environment allows serum levels of these enzymes to be normal and will determine an optimal liver diagnosis as, therefore, of him; but, what about those animals subjected to permanent environmental stress conditions, such as the *Bos Taurus,* managed to the level of the humid tropic that have to face the heat and humidity, submitted to the permanent presence of internal and external parasites (FAO 2004) and subject to a management that blocks their free movement on the grass in looks for refuge from environmental threats (Young *et al*., 1989).

The Amazon Region of Southern Ecuador (RAEsur), is an area dedicated to dairy cattle farming with a population composed primarily by Holstein cattle, carried to this environment from the high places in the Andes Mountains and have a slow process of adaptation, thereby having to endure the constant and excessive application of drugs to control health problems and parasites that affect them, so, the present study aimed to determine on cows in the postpartum period, the values of these liver enzymes and thereby establish whether the bovine *Bos Taurus* is affected by such tropical environment.

2. Material and Methods

This study was developed in the places of Zamora, Centinela del Condor, Paquisha, Yanzatza, El Pangui, Yacuambi and Chinchipe, belonging to the province of Zamora Chinchipe-Ecuador, South America, localized at 4° 04’09’’S and 78°57’24’’W, 900 m above the sea level, with an average temperature of 25°C and characterized by humid tropical equatorial climate, classified in the Köppen scale as Af. 95 healthy crossbred Holstein cows in the postpartum period, from 32 herds from Zamora Chinchipe (feed is basically grass), were used in the study; being the pastures established exclusively of gramineous, prevailing in a 87 percent the Setaria or merkeron (*Setaria sphacelata*) and a 13 percent the purple or white gramalote (*Axonopus scoparius)*. Animals were identified with a plastic tag and blood samples were taken (jugular vein) serially four times from each cow at intervals of 50 days from the postpartum up to 180 days. The blood samples were collected in an amount of 5 to 10 cc in Vacutainer tubes without anticoagulant, being maintained between 12 to 24 hours in a thermos with cooling gel (4 - 8°C) until they get to the Veterinary Diagnostic Laboratory of the National University of Loja for the corresponding analysis, conducted during the period of study 380 blood tests belonging to 95 postpartum cows that participated in the investigation, determining the values of liver enzymes ALP, AST, ALT and Bilirubin pigment liver. The Photometric System DiaSys® (Diasys Diagnostic Systems GmbH/Germany) was used for that. For this purpose the samples were centrifuged at 3000 r/15’ and the analyzer employed the Microlab® 300, the following methods were used:

- ALP in serum, the kinetic Photometric Test (DGKC).
- AST/GOT and ALT/GPT the UV Test, improved according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).
- Total Bilirubin in serum, the Photometric Test 2,4-dicloroanilina (DCA).

The variables considered in this study were: grass type (n=2) in which animals are kept (*Setaria sphacelata*, *Axonopus scoparius*), because, these grasses have different growth cycles, therefore the animal has also different frequency of graze and administration of drug to control the tick all year. The height (high >1.3 m, low <1.3 m), as higher animals are heavier and consequently receive more quantity of drugs. The other variable was the time (four) of collection of blood samples in the postpartum period. The results were analyzed using correlation-regression analysis and ANOVA, using the Statistical Analysis System program (SAS 9.3, 2010).

3. Results and Discussion

In Table 1, the biochemical elements investigated showed fluctuation in values that is different during the four postpartum times analyzed (P< 0,05), all of which are especially the ALP over the normal range for this species (Peter *et al*., 2002). Some studies, manifested that serum ALP levels fall during lactation (Doornenbal *et al*., 1988; Otto *et al*., 2000; Tibbo *et al*., 2008), but in our work they

remained high at this stage. The height had a significant influence on the values of ALP, small size animals had higher values (327.7±118.7U/l) than the ones of higher height (260.0±71.2U/l), the values of AST, ALT and Bilirubin were similar for this variable. As it concerns the effect of the ration, Hawley *et al.* (1982), Tibbo *et al*. (2008), Quintela *et al.* (2011), indicated that the type of food is one of the factors affecting the serum concentration of these elements and effectively affected (P< 0,05) our results because the animals kept in Setaria grass showed values of the three liver enzymes higher than those kept in Axonopus grass.

Table1. *Serum values (means±SD) of ALP, GOT/AST, GPT/ALT and Bilirubin, considering different sources of variation.*

Sources of variation	ALP (U/l)	GOT/AST(U/l)	GPT/ALT(U/l)	BILIRUBIN (mg/dl)
20 days postpartum	301.1±112.0^{a}	94.7±22.6^{b}	23.3±7.5^{a}	0.16±0.11^{b}
75 days postpartum	324.8±168.3^{b}	98.8±21.7^{b}	32.3±11^{b}	0.14±0.08^{b}
130 days postpartum	361.2±174.3^{c}	95.5±22.8^{b}	31.0±95^{b}	0.13±0.07^{b}
180 days postpartum	308.5±166.0^{a}	87.6±16.1^{a}	29.5±7.3^{b}	0.07±0.05^{a}
Low stature	327.7±118.7^{a}	95.8±16.9^{a}	27.7±7.8^{a}	0.14±0.05^{a}
High stature	260,0±71,2^{b}	92,2±18,0^{a}	25,7±5,5^{a}	0,15±0,07^{a}
Grass *Setaria sphacelata*	322.6±113.7^{a}	96.2±17.5^{a}	28.6±7.1^{a}	0.15±0.05^{a}
Grass *Axonopus scoparius*	256.6±102.8^{b}	86.3±10.9^{b}	19.9±5.2^{b}	0.14±0.08^{a}
Mean general	333.6±181.8	95.6±21,9	28.1±9,9	0.14±0,06

Same letter in each column and source of variation means not difference (P< 0.05).

The analysis by percentiles (Table 2) showed that only between 17.5% and 23% of the population presented values considered normal in the four biochemical elements analyzed while the rest of animals had high levels of ALP and Bilirubin negatively associated with the functional state of the liver parenchyma and degree of obstruction of the biliary tract (Gutman, 1959; Wang *et al*., 2006) and transaminases relating to liver damage (Tibbo *et al*., 2008; Kavitha *et al*., 2011; Noro *et al*., 2013), constituting all in biochemical markers for the diagnosis of liver diseases.

Table2. *Percentile of cows grouped into ranges for the serum value of ALP, AST, ALT and Bilirubin.*

	Ranges of serum value	% animals	Min and Max values found
ALP (U/l)	<200	17.5	Min. 74.0 Max. 660.0
	200-300	32	
	>300	51.5	
GOT/AST(U/l)	<80	20	Min. 62.1 Max. 169.6
	80-100	44	
	>100	36	
GPT/ALT(U/l)	<22	23	Min. 10.7 Max. 47.8
	22-30	49	
	>30	28	
BILIRUBIN (mg/dl)	<0.1	22	Min. 0.04 Max. 0.31
	0,1-0.15	42	
	>0.15	36	

When performing an analysis of the relationship between the variables considered in the investigation (Table 3), significant correlation was not found in the group of cows kept on pastures of Axonopus, between height and variables: ALP, GOT/AST, GPT/ALT, Bilirubin; but in the group of animals kept on pastures of Setaria a negative dependency ratio between height and ALP (P<0.05) and between height and GPT/ALT (P<0.10) was determined. These results are explained in a very common situation that occurs in that environment with animals *Bos Taurus* that graze *Setaria sphacelata*, because this grass has a fast growth cycle between 30 – 40 days (Ramírez *et al*., 1996), which facilitates the early return of the animals to its consumption, thereby not causing disruption of the biological cycle of ecto and endoparasites present in abundance in the tropics (Cardoso *et al*., 2013) and thereby causing a greater frequency of drugs administration for their control. While with the grass Axonopus this frequency decreases because the cycle of plant growth is slow and therefore the return of animals to its consumption is late between 150 and 180 days (personal communication with farmers).

Table3. *Grade of relationship between height and liver enzymes analyzed, considering the type of pasture where the animals stay.*

Pastures of *Setaria sphacelata:* **Correlation**				
Height	ALP	GOT/AST	GPT/ALT	BILIRRUBINA
	-0.286	-0.06	-0.187	0.008
Confidence interval	**0.0079***	0.57	**0.087****	0.94
Pastures of *Axonopus scoparius:* **Correlation**				
Height	ALP	GOT/AST	GPT/ALT	BILIRRUBINA
	-0.353	-0.432	0.26	-0.05
Confidence interval	0.26	0.16	0.41	0.87
***(P< 0,05) **(P< 0,10)**				
Regression: *Setaria sphacelata*				
Dependent variable	Independent	Intercept	Coef. Regressión	R^2
ALP	Height	800.17	-379.7	0.082
GPT/ALT	Height	47.74	-15.43	0.035

Another factor to consider in this negative dependency ratio between height and levels of ALP and GPT/ALT is the weighing of the animals for dosage of drugs, because in the absence of bascules or not using barimetrica tapes, the same is made by a "subjective" criteria of the farmer or technician, causing errors of notorious underestimation of body weight (Machilla *et al*., 2008) and thus causing under-dosing and resistance to anthelmintics (Soutello *et al*., 2007; Kaplan and Vidyashancar, 2012), especially macrocyclic lactones such as Ivermectin widely used for tick control (Castro-Janer *et al*., 2011), leading to use of Ivermectin of high concentration and intervals of diminishing time (Cotter *et al.*, 2014; Cruz *et al.*, 2014; Muñiz *et al.*, 2015). This has come to the fact that small animals receive higher amounts of drugs and bigger animals receive lower amounts just because of those errors of live weigh estimates.

Fig. 1 and 2 can see the scattering of data ALP and GPT/ALT, whose serum levels decrease as the height increases, it is showing that the person who is dosing the drug takes into consideration a criteria of overdosing for "the drug to act better", this causes levels of the cytosolic enzymes to be increased in blood by the high and continuous administration of corticosteroids (Gutman, 1959), therapeutic use of all kinds of chemical drugs (Koide and Oda, 1959; Lemaire *et al*., 1991; Manna *et al.*, 2006; Kavitha *et al*., 2011), causing liver cytolysis due to the release of oxidative radicals resulting from the splitting of these xenobiotics (Peter *et al.*, 2002; Ashworth *et al.*, 2006, Jeong *et al.*, 2013). Bilirubin also increases because of this liver damage and by the cholestasis because of the liver fluke, very common in this type of environment (Noro *et al*., 2013).

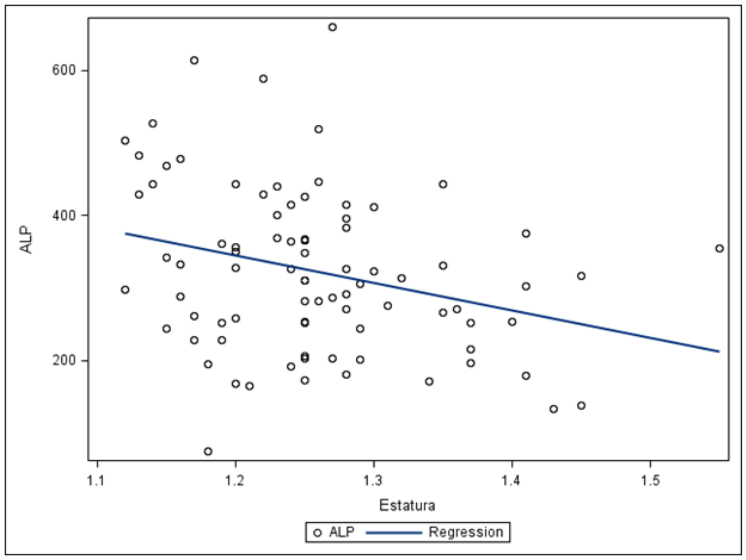

Figure1. *Negative linear regression between height and serum ALP of cows grazing in Setaria sphacelata*

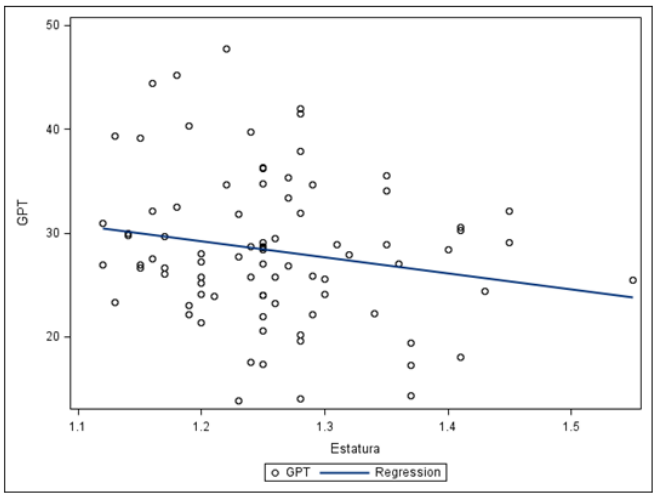

Figure2. *Negative linear regression between height and serum GPT/ALT of cows grazing in Setaria sphacelata*

4. CONCLUSION

Considering the susceptibility of *Bos Taurus* to health problems present in these humid tropic environments and the poor technical handling of chemical products to control these problems, it can be concluded by the high values of these biomarkers, especially ALP of a diagnosis of liver damage by probably continuous use of drugs that affect to it and causes malfunction for the process of glycogenesis, leading to malabsorption of nutrients, decreasing productive and reproductive performances, and longevity in the animals, being the most affected those with little height and also those maintained on grassland of *Setaria sphacelata*.

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