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Artigo

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Comparative Trace Elemental Analysis in Cancerous and Noncancerous Animal Tissues Using Logistic Regression

Abstract: Essential metals are important in the normal functioning of the body. However, tissues or organs can be affected when there are inappropriate concentrations of these elements. In the literature, there are works indicating that essential elements may be related to the emergence of neoplasms. Therefore, it is important to determine the metal species in biological samples in order to relate the concentration of these species to the emergence of neoplasms. This study reports the application of the Flame Atomic Absorption Spectroscopy (FAAS) for the quantification of Cu, Fe, Mn and Zn in 31 samples of canine tissue (11 of mammary glands, 4 of pancreas, 3 of skin and 13 of testicles) with and without neoplasms, using alkaline solubilization as sample preparation method. The concentration of these metals was used as co-variables for the construction of models of logistic regression. The concentration of Zn in breast and Fe in pancreas was significant ($p < 0.05$) in the logistic models. The findings of this study describe that the elements Fe and Zn play a major role in the carcinomas development, since high levels of these metals were observed in neoplastic tissues.

Keywords: Metals; canine tissue; neoplasms; logistic regression.

Resumo

Os metais essenciais são importantes no funcionamento normal do organismo, porém tecidos ou órgãos podem ser afetados, quando existem concentrações inadequadas desses elementos. Na literatura existem trabalhos indicando que elementos essenciais podem estar relacionados ao surgimento de neoplasias. Portanto, destaca-se a importância da determinação de espécies metálicas em amostras biológicas, a fim de relacionar a concentração dessas espécies ao surgimento de neoplasias. Este estudo relata a aplicação da Espectrometria de Absorção Atômica em Chama (FAAS) para a quantificação de Cu, Fe, Mn e Zn em 31 amostras de tecido canino (11 de glândulas mamárias, 4 de pâncreas, 3 de pele e 13 de testículos) com e sem neoplasias, utilizando a solubilização alcalina como método de preparo de amostra. A concentração desses metais foi utilizada como co-variáveis para construção de modelos de regressão logística. A concentração de Zn em mama e de Fe em pâncreas mostrou-se significativa ($p < 0.05$) nos modelos logísticos. Os achados deste estudo descrevem que os elementos Fe e Zn têm um papel importante no desenvolvimento de carcinomas, pois foram observados altos níveis destes metais em tecidos neoplásicos.

Palavras-chave: Metais; tecido canino; neoplasias; regressão logística.

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Análise Comparativa de Elementos Traços em Tecidos Animais Cancerosos e Não Cancerosos Usando Regressão Logística

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1. Introduction

Essential elements, such as Fe, Zn, Cu and Mn, are chemical species used to keep body homeostasis. The importance of these metals is unquestionable due to their physiological functions when present in specific concentrations, while, in turn, in inadequate concentrations to the body, they may cause unbalance, triggering illnesses.[1,2]

When there is deficiency or excess of the elements, the body cannot complete its normal cycle or have a healthy development, breaking the homeostasis and causing illnesses. Some specific biological functions need the presence of these elements in the syntheses of proteins to exercise their functions, like Zn and Cu, which take part as co-factors of enzymes or directly take part in several reactions in the body, and are considered essential.[2-7]

Fe is an important element which helps in the transport of oxygen in the blood, but high concentrations in the body produce free radicals which can be connected to illnesses such as malignant neoplastic processes, cancer, early aging, damage in the liver and pancreas. Elements such as Ni, Cu and Fe were also observed in high concentrations in gastric cancer in human beings in relation to the normal tissue adjacent to the lesion.[8]

The etiology and the development of the neoplastic processes are multi-factorial and they can be idiopathic forms, that is, when their nature is unknown, or hereditary, by physical agents, biological agents, chemical agents, nutritional, therapeutic and environmental ones. These factors may act in any stage of cell development, this is, in the stages of initiation, promotion and progression.[9-11]

Therefore, it is relevant to carried out studies relating the concentration of essential metals to the emergence of neoplasms, since it is unknown whether higher or lower contents of Cu, Fe, Mn and Zn are caused by the presence of neoplasms, or if the alterations in the concentrations of these metals cause neoplasms.[11-13] This way, a better understanding of the role of different elements regarding the emergence of neoplasms in animal organs is being sought.

The aim of this work was quantify Cu, Fe, Mn and Zn in biological samples of canine tissues (mammary glands, pancreas, skin and testicle) with and without neoplasms, using alkaline solubilization and the Flame Atomic Absorption Spectroscopy (F AAS). The correlation of the concentrations of such metals with the presence of neoplasms using logistic regression was also performed.

2. Methodology

2.1. Canine Tissue Samples

The samples chosen for the study were all from animals of the canine species. The parts collected were from the mammary glands, testicle, pancreas and skin, because they are samples which present higher frequency of neoplasms as identified by Veterinary Pathological Anatomy Laboratory of the UNICENTRO in previous studies.[14-20] Table 1 shows informations about the identification and of the characteristics of the animals of which the canine tissues were sampled. The samples of the tissues of mammary glands (M), pancreas (P), skin (S) and testicles (T) were codified as M1 to M11, PA1 to PA4, S1 to S3 and T1 to T13, respectively.

The samples collected were kept in plastic bags of polyethylene and stored at a temperature of – 4°C. Mammary glands and skin tissues were obtained in the Veterinary Medicine Clinic School of the UNICENTRO, using the mastectomy surgery technique of (full removal of the breast) and by skin biopsy. Pancreas and testicles samples were obtained and labeled through necropsies; these neoplasms were not the main illness of causa mortis of the animal, since they were not clinically determined as primary factors.

2.2. Sample Preparation for F AAS Analysis

After defrosted, the biological samples described in Table 1 were cut in fragments of ~2.0 mm, using a glass support and a stainless steel knife to avoid contaminations. The sample preparation procedure using alkaline solubilization with tetramethylammonium hydroxide (TMAH) (Sigma-Aldrich, 25% m v^{-1}) was carried out in triplicate out according to methodology described by BUTIK *et al.*[21]. In 0.3 g of tissue canine, 1.0 mL of TMAH and 0.5 mL of water were added; the tissue was then heated in a block digester at 60°C for 1 h.

2.3. Metal Analysis by F AAS

The determinations of the elements Cu, Fe, Mn and Zn in the biological samples described in item 2.1 were carried out by Flame Atomic Absorption Spectrometry (F AAS) in the instrument of Varian, Spectra AA-220, equipped with hollow cathode lamps (Varian and Agilent). Table 2 shows the instrumental parameters used in the measurements of the metals contents by F AAS.

In this work, standard solutions of Cu, Fe, Mn and Zn (1000 µg mL^{-1} P.A. -Biotec) were used. All the solutions were prepared using deionized water obtained by the Gehaka OS 10LX system. All the flasks and glassware employed were properly decontaminated in a nitric acid (HNO_3) 5% (v v^{-1}), for 24 hours, and they were subsequently washed with deionized water for three times.

2.4. Logistic Regression Analysis

A completely randomized design (CRD) was used in this research. The set of data of the metal contents Cu, Fe, Mn and Zn determined by FAAS in the organs of mammary glands, pancreas, skin and testicles were adjusted at the logistic regression[22] using the SPSS Statistics software, version 20. The logistic models were adjusted using the Forward method. The answers in the logistic models were codified as 0 for absence and 1 for the positive diagnosis of cancer (without and with neoplasm, respectively). The significance of the coefficients in the logistic model was analyzed using the *p*-value, and values of *p* lower than 0.05 were considered significant and were kept in the models.

3. Results and Discussion

The metal Cu, Fe, Mn and Zn were quantified in different biological tissues (mammary glands,

Table 1. Informations about the animals of which the canine tissues (mammary glands, pancreas, skin and testicles) were sampled.

Sample	Breed	Genre	Age (Yearsold)	Neoplasm	Nutrition
M1	Pincher	F	4.5	Inflammatory papillary adenocarcinoma	Dog food and homemade food
M2	Poodle	F	2.0	Papillary adenocarcinoma	Dog food
M3	NDB	F	4.5	Mixedcystic adenocarcinoma	Dog food and homemade food
M4	NDB	F	2.5	Mixedcystic adenocarcinoma	Dog food
M5	Poodle	F	5.5	Difuse nodular adenocarcinoma	Dog food and homemade food
M6	Poodle	F	5.0	Inflammatory adenocarcinoma	Dog food
M7	Pit Bull	F	4.0	Mixed adenocarcinoma	Dog food
M8	Poodle	F	5.0	Mixed malignant papillary adenocarcinoma	Dog food
M9	NDB	F	6.0	Papillary adenocarcinoma	Homemade food
M10	NDB	F	5.5	Mixedmalignant nodular adenocarcinoma	Homemade food
M11	NDB	F	4.0	Inflammatory adenocarcinoma	Dog food and homemade food
PA1	NDB	M	6.5	Pancreatic adenocarcinoma	Dog food
PA2	York Shire	M	4.5	Pancreatic adenoarcinoma	Dog food
PA3	NDB	F	Adult	Pancreatic hemangioma	Homemade food
PA4	NDB	F	Adult	Pancreatic adenocarcinoma	Homemade food
S1	NDB	M	4.5	Fibroma	Homemade food
S2	NDB	M	3.0	Epidermoidcyst	Dog food
S3	Boxer	M	6.0	Mastocytoma	Dog food and homemade food
T1	NDB	M	4.0	Seminoma	Dog food and homemade food
T2	GERMAN SHEPHERD	M	4.0	Sertolioma	Dog food and homemade food
T3	POODLE	M	3.0	Seminoma and Sertolioma	Dog food and homemade food
T4	LABRADOR	M	6.0	Seminoma and Sertolioma	Dog food
T5	NDB	M	3.0	Sertolioma	Dog food and homemade food
T6	POODLE	M	2.5	Sertolioma	Dog food
T7	ROTWEILER	M	3.5	Seminoma and Sertolioma	Dog food and homemade food
T8	NDB	M	4.0	Seminoma and Sertolioma	Dog food and homemade food
T9	NDB	M	5.0	Seminoma and Sertolioma	Dog food and homemade food
T10	NDB	M	5.0	Sertolioma	Dog food
T11	POODLE	M	4.5	Sertolioma	Dog food
T12	NDB	M	5.0	Seminoma and Sertolioma	Homemade food
T13	POODLE	M	5.0	Seminoma and Sertolioma	Dog food

NDB – Non-defined Breed; F – Female; M – Male; Adult – older than 7 years old.; M: mammary gland; PA: pancreas; S: Skin; T: testicle

pancreas, skin and testicle) with and without neoplasms. The metal concentrations found for each canine tissue are shown in Table 3.

Several samples evaluated in this work presented significant differences in metal concentrations when were compared the healthy tissues with the neoplastic tissues. Figure 1 presents graphs of distribution and frequency of the levels of metals in the organs analyzed, in order to present the results in a concise way that allows extracting information on the behavior of the data.

It was observed that the concentrations of Cu (Fig. 1A) and Zn (Fig. 1B) were higher for the neoplastic tissues than for the healthy tissues. As malignant neoplastic processes need higher vascularization for their development, Cu helps in the process of neovascularization, and Zn in the protein synthesis. In the neoplastic cells, this synthesis is taking place in an exacerbated way by the atypical mitosis,

Table 2: Instrumental parameters of the metals determination by F AAS

Metal	λ (nm)	Current Lamp (mA)	Slit width (nm)	Flame
Cu	324.7	4	0.5	air/C_2H_2
Fe	248.3	5	0.2	air/C_2H_2
Mn	279.5	5	0.2	air/C_2H_2
Zn	213.9	5	1.0	air/C_2H_2

Table 3. Concentration (mg kg^{-1}) of Cu, Fe, Mn and Zn in samples of canine tissues of mammary glands, pancreas, skin and testicles with and without neoplasms (n=3).

Sample	Cu		Fe		Mn		Zn	
	With neoplasm	Without Neoplasm	With Neoplasm	Without neoplasm	With neoplasm	Without neoplasm	With neoplasm	Without neoplasm
M1	0.6 ± 0.1	0.6 ± 0.0	57.4 ± 4.0	129.0 ± 5.0	0.5 ± 0.0	<LD	33.0 ± 2.0	2.5 ± 0.2
M2	0.6 ± 0.1	1.0 ± 0.0	185.0 ± 12.0	186.1 ± 7.5	<LD	<LD	10.4 ± 0.6	6.6 ± 0.4
M3	6.7 ± 0.3	4.7 ± 0.3	121.5 ± 0.3	171.0 ± 8.0	<LD	<LD	9.6 ± 0.5	6.6 ± 0.2
M4	0.9 ± 0.1	0.9 ± 0.1	88.3 ± 4.0	57.0 ± 1.5	<LD	<LD	0.7 ± 0.0	0.8 ± 0.1
M5	0.2 ± 0.0	0.1 ± 0.0	111.1 ± 4.0	36.2 ± 0.3	<LD	<LD	6.3 ± 0.3	0.6 ± 0.0
M6	8.8 ± 0.1	2.3 ± 0.1	32.5 ± 0.2	137.0 ± 8.5	<LD	0.2 ± 0.0	9.0 ± 0.4	9.0 ± 0.7
M7	1.6 ± 0.1	1.3 ± 0.1	197.0 ± 13.0	266.0 ± 16.0	<LD	<LD	5.0 ± 0.2	5.8 ± 0.5
M8	2.8 ± 0.0	5.9 ± 0.2	12.4 ± 1.0	117.0 ± 11.0	<LD	0.4 ± 0.0	1.2 ± 0.1	8.1 ± 0.5
M9	3.5 ± 0.3	4.4 ± 0.2	139.1 ± 5.0	72.5 ± 6.0	<LD	<LD	7.0 ± 0.5	7.8 ± 0.5
M10	1.2 ± 0.0	0.4 ± 0.0	91.5 ± 7.5	79.0 ± 6.0	0.2 ± 0.0	<LD	9.5 ± 0.4	6.8 ± 0.5
M11	0.6 ± 0.0	1.8 ± 0.0	103.0 ± 3.0	138.0 ± 2.0	0.2 ± 0.0	0.3 ± 0.0	6.8 ± 0.3	9.0 ± 0.0
PA1	1.7 ± 0.0	0.3 ± 0.0	79.1 ± 1.0	48.5 ± 0.7	<LD	< LD	8.8 ± 0.3	5.1 ± 0.2
PA2	4.3 ± 0.2	12.0 ± 0.5	142.6 ± 3.5	111.5 ± 2.4	0.2 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	19.5 ± 0.9
PA3	1.6 ± 0.1	1.1 ± 0.1	111.0 ± 4.5	49.9 ± 2.5	1.2 ± 0.1	0.3 ± 0.0	20.5 ± 0.3	33.4 ± 3.0
PA4	1.7 ± 0.1	1.5 ± 0.1	74.1 ± 3.0	81.4 ± 2.0	1.3 ± 0.1	1.2 ± 0.1	7.3 ± 0.6	7.3 ± 0.5
S1	0.7 ± 0.1	0.4 ± 0.0	10.8 ± 0.6	98.5 ± 2.5	< LD	<LD	12.0 ± 0.6	5.2 ± 0.1
S2	0.9 ± 0.1	1.1 ± 0.1	36.1 ± 3.0	50.0 ± 4.0	<LD	<LD	3.2 ± 0.2	3.1 ± 0.2
S3	0.3 ± 0.0	2.2 ± 0.1	42.1 ± 1.0	45.0 ± 4.0	<LD	<LD	4.0 ± 0.3	6.6 ± 0.5
T1	0.6 ± 0.1	0.8 ± 0.1	35.0 ± 1.0	36.0 ± 1.7	<LD	<LD	17.3 ± 1.6	22.5 ± 0.3
T2	0.8 ± 0.0	0.7 ± 0.0	33.6 ± 2.7	26.5 ± 2.1	<LD	<LD	17.3 ± 0.4	13.5 ± 0.1
T3	2.2 ± 0.2	1.1 ± 0.1	56.0 ± 4.0	87.0 ± 1.4	<LD	<LD	9.0 ± 0.8	2.8 ± 0.2
T4	0.8 ± 0.0	1.1 ± 0.0	24.6 ± 1.0	24.9 ±1.1	<LD	<LD	28.6 ± 0.6	25.2 ± 0.6
T5	1.0 ± 0.0	0.7 ± 0.0	30.5 ± 0.2	24.1 ± 0.8	0.2 ± 0.0	<LD	13.5 ± 0.1	11.4 ± 0.2
T6	0.7 ± 0.0	0.7 ± 0.0	29.1 ± 0.2	26.8 ± 0.7	0.2 ± 0.0	<LD	11.4 ± 0.3	11.3 ± 0.1
T7	1.1 ± 0.0	1.0 ± 0.0	27.9 ± 1.4	33.3 ± 0.1	<LD	0.4 ± 0.0	16.0 ± 0.8	16.4 ± 1.5
T8	0.9 ± 0.0	0.9 ± 0.0	21.4 ± 0.7	25.4 ± 0.4	0.3 ± 0.0	0.2 ± 0.0	11.6 ± 0.2	10.7 ± 0.3
T9	0.8 ± 0.0	1.1 ± 0.1	35.6 ± 3.0	43.5 ± 0.6	0.4 ± 0.0	0.4 ± 0.0	12.9 ± 0.4	18.3 ± 1.0
T10	0.8 ± 0.0	0.9 ± 0.0	45.6 ± 2.7	23.4 ± 0.8	0.3 ± 0.0	<LD	14.7 ± 0.4	15.5 ± 0.2
T11	0.8 ± 0.0	0.6 ± 0.1	29.0 ± 1.4	22.9 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	12.0 ± 0.5	12.6 ± 0.7
T12	1.0 ± 0.0	1.0 ± 0.0	23.7 ± 1.0	23.0 ± 1.5	<LD	<LD	10.8 ± 0.1	9.0 ± 0.1
T13	1.2 ± 0.1	1.2 ± 0.0	22.5 ± 0.5	25.1 ± 0.4	0.2 ± 0.0	<LD	12.1 ± 0.3	11.6 ± 0.4

LD: Limit of detection (0.16 mg kg^{-1}); M: mammary glands; P: pancreas; S: skin; T: testicles.

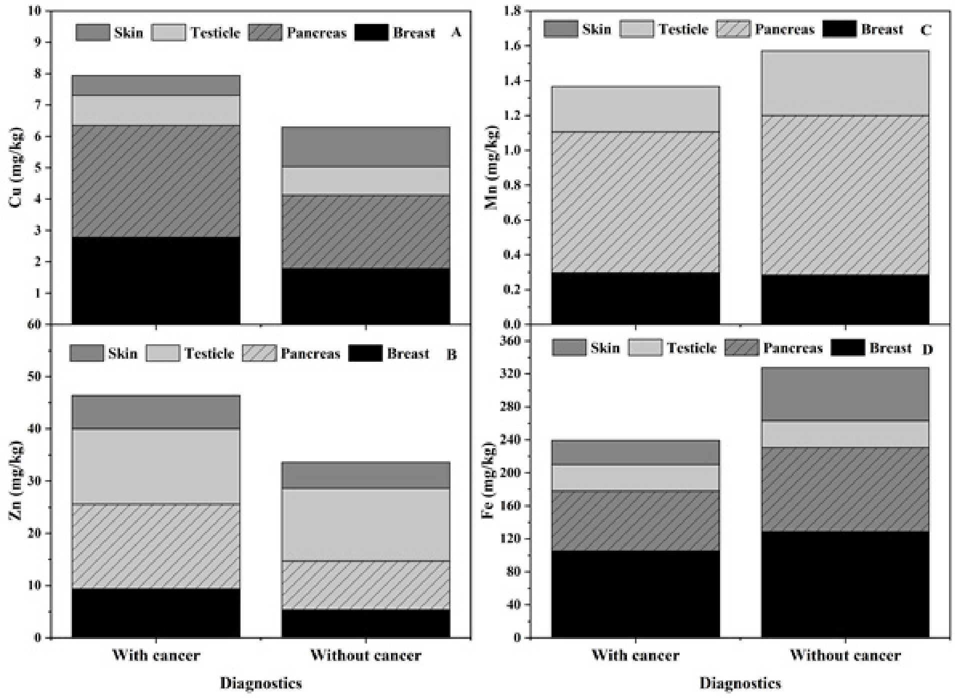

Figure 1. Distribution and frequency of the metal concentrations for Cu, Fe, Mn and Zn in different canine tissues with and without neoplasms

beyond the fact that neoplasms inflame and the Zn helps in the inflammatory process considering the activation of lymphocytes. The fact that these elements are present in neoplastic tissues suggests that they may not even act as primary factors for the development of neoplasm, as an initiation factor, but they act as promoters and/or mainly as factors of progression.[23]

Regarding Fe and Mn (Fig. 1C and D), the opposite was observed; the healthy tissues presented slightly higher contents of these metals. The Fe element is in a smaller amount, since the neovascularization formed is disorganized and has undermined vascular structural integrity. Being so, the Fe that is connected to the red blood cells is degraded, since they break, forming local bleeding. Mn has its use in great concentration for the formation of conjunctive tissue, forming the stroma of the neoplasms, which functions as the sustenance for the growth of the neoplasm, and, this way, its concentration will be decreased.[24,25]

It can be still observed that in healthy tissues of breasts and skin, the level of Zn proportionally increases when higher levels of Fe are detected. In normal tissues, the distribution of Fe takes place in a regular way on the surface of red blood cells, which does not happen in malignant neoplastic processes, since the neovascularization formed is not integrated, occurring, thus, the lysis of the red blood cells. In normal tissues, mainly in breast and skin, it becomes necessary the formation of sustenance tissue, conjunctive tissue, which is dependent on Zn and needs vascularization for its formation, mainly because these two organs are directly connected to hormonal factors and have a lot of structural variants due to their physiological specificities.[26, 27]

However, the opposite took place in breast and skin tissues with neoplasm, since when the Zn contents are increased, the Fe contents are decreased. The Fe contents are decreased because the neovascularization formed is not integrated and favors the lysis of red blood cells and, as a result, the Fe contents decrease making Zn contents increase, since the latter is associated to the cell protein synthesis, necessary to atypical mitoses, as well as to help in the activation of inflammatory reaction and malignant neoplastic process.[26-28]

In tissues of canine testicles and pancreas, the levels of Zn detected were always inversely proportional to the levels of Fe regardless the kind of tissue, with or without neoplasm. This variation takes place in these organs in both normal and neoplastic tissues, since the proportion of these elements vary according to the organ metabolism. These organs have different functions, being dependent on the concentration of hormones originated from other organs, and also produce their own hormones; this way, they act with greater cell activity. High levels of Zn are required when the organ synthesizes hormones, and so they need higher vascularization, making it available more circulating integrated blood cells and, as a result, higher Fe concentration.[26-29]

The levels of Cu and Zn are directly correlated in the healthy tissues of breast, testicle, pancreas and skin, as well as in skin tissues with neoplasm. On the other hand, the levels of Cu and Zn are always inversely correlated in neoplastic tissues of breast, testicles and pancreas. Zn is necessary for cell protein synthesis, atypical mitosis. However, there comes a point in which the protein synthesis is no longer necessary. Thus, the cell in the stage of cell progression, third stage of the neoplastic cell development, is no longer dependent on protein synthesis.[28,29]

The standard of metals in healthy and neoplastic tissues of breast, testicles, pancreas and skins always presented the lowest contents of Mn with comparison the concentrations of other metals (Cu, Fe and Zn). As element Mn is used for the formation of sustenance of tissue, stroma, it will be increased until the tissue is formed; once the tissue is formed, the neoplastic process already in development does not need sustenance tissue anymore, since it already exists, and so does the sustenance tissue itself on the action of the lysis by the inflammatory reaction installed; this way, its concentration tends to decrease.[11,12]

In order to help the interpretation of the results and verify possible correlations among the elements and their respective neoplasms, the data were modeled using logistic regression. Table 4 shows the coefficients and the statistics associated with the models of logistic regression by using the metals as co-variables for mammary gland canine tissues.

The logistic regression with all the predictors revealed that the levels of Cu (p-value = 0.518), Fe (p-value = 0.100) and Mn (p-value = 0.969) did not present a statistically significant effect on the probability of occurrence of neoplasm in mammary gland tissues. On the other hand, the variable Zn was statistically significant (p-value < 0.05) on the occurrence of neoplasm in the mammary tissues in this model of logistic regression. The final logistic model in this case can be represented by equation 1:

$$P = \frac{1}{1 + e^{(0.220 \cdot [Zn])}} \quad \text{(equation1)}$$

Where P is the probability of occurrence of breast cancer and [Zn] is the concentration of zinc in the tissue in (mg kg^{-1}).

The profile of behavior of the occurrence of neoplasm according to the Zn contents, generated from equation 1, with the real values analyzed and simulated, can be seen in Figure 2.

In low Zn contents, for instance, of 0.100 mg kg^{-1}, the probability of occurrence of neoplasm will be of 50.55%. However, in Zn contents in the order of 10.00 mg kg^{-1}, this probability will be of 90% (Figure 2).

In Table 5 is shown the classification matrix of the cases of mammary tissue diagnosed with and without neoplasms. Approximately 82% of the cases with a positive diagnosis of cancer can be predicted by the logistic model. In turn, a low percentage (48.5%) of the cases can be correctly predicted for normal cells, non-carriers of cancer. The global percentage of hits of the model of logistic regression for this organ (mammary gland) was 65.2%.

Considering that the Zn is involved in enzymatic reactions and in reactions with molecules which

Table 4. Coefficients of logistic regression model for mammary glands according Cu, Fe, Mn and Zn contents.

		B	S.E.	Wald	DF	Sig.
Step 1	Cu	0.092	0.143	0.417	1	0.518
	Fe	-0.008	0.005	2.709	1	0.100
	Mn	0.070	1.833	0.001	1	0.969
	Zn	0.220	0.106	4.335	1	0.037
	Constant	-0.676	0.738	0.837	1	0.360

B: coefficients of the logistic model; S.E.: Standard error; Wald: wald chi-square test; DF: degrees of freedom; Sig.: p-value.

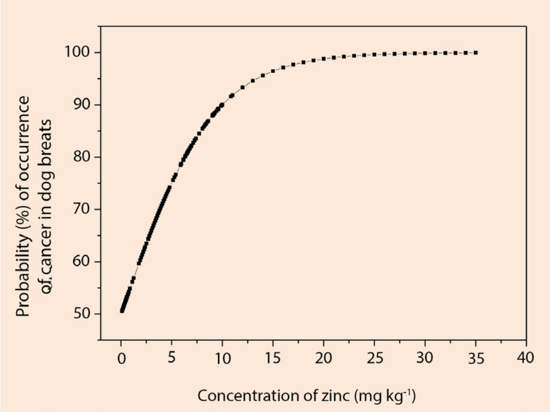

Figure 2. Occurrence probability of neoplasm in mammary glands predicted by logistic regression model according to the Zn contents

control the cell activity, this element, when in excess, can contribute to the formation and progression of neoplasm and to the metastasis.[30-32] Lappano *et al*.[32] also verified higher concentrations of Zn in tissues of breast whit neoplasm in human beings than in healthy tissues.

Table 6 shows the coefficients and the statistics associated with the models of logistic regression by using the metals as co-variables for pancreas tissues. The levels of Cu, Fe, Mn and Zn present in the pancreas were also analyzed using logistic regression. In this case, the levels of Cu (p-value = 0.077), Mn (p-value = 0.511) and Zn (p-value = 0.682) did not presented statistically significant effects for the occurrence of neoplasm in pancreas canine tissue.

Only the level of Fe (p-value = 0.031) in this organ showed to be significant for the positive diagnosis of the occurrence of neoplasm. The final logistic regression model in this case can be represented by equation 2:

$$P = \frac{1}{1 + e^{-(0.130[Fe])}} \qquad \text{(equation2)}$$

Where P is the probability of occurrence of neoplasm in pancreas tissue and [Fe] is the Fe concentration (mg kg^{-1}) dosed in this organ.

The profile of the behavior of the occurrence of neoplasm according to Fe contents generated from equation 2 with the real values analyzed and simulated can be seen in Figure 3.

In general, higher Fe contents, when compared to the levels of Zn were found in pancreas tissues. For example, for the Fe concentration of 1 mg kg^{-1}, there is the probability of 53.2% of occurrence of neoplasm in this organ. Higher Fe concentrations, such as 17 mg kg^{-1}, increase the probability of occurrence of this event to 90.0% (Figure 3).

Table 7 shows the classification matrix of the cases of pancreas tissues diagnosed with and without neoplasm.

This model of logistic regression presented an overall percentage of hits of 79.2%. Cases with pancreas neoplasm were classified with 75% of hits, whereas 83.3% of cases diagnosed without neoplasm were correctly classified.

Fe can have a direct involvement in the progression and development of tumors, since the highest Fe levels are associated with the higher risks of occurrence of neoplasm, providing metastasis in animal tissues and activating neurovascularization, such as the process which is necessary for the development of malignant neoplasm, the so-called cancer.[30,33-34] The storage

Table 5. Classification matrix of the logistic regression model generated for mammary gland tissue samples

Observed	Predicted			Correct Percentage
	Pathology			
	0	1		
Without câncer	0	16	17	48.5
With câncer	1	6	27	81.8
Overall Percentage				65.2

Table 6. Coefficients logistic regression model pancreas canine tissues according to Cu, Fe, Mn and Zn

		B	S.E.	Wald	DF	Sig.
Step 1	Cu	-0.737	0.417	3.12	1	0.077
	Fe	0.130	0.06	4.669	1	0.031
	Mn	-0.758	1.154	0.432	1	0.511
	Zn	-0.057	0.14	0.168	1	0.682
	Constant	-7.459	3.84	3.773	1	0.052

B: coefficients of the logistic model; S.E.: Standard error; Wald: wald chi-square test; DF: degrees of freedom; Sig.: p-value.

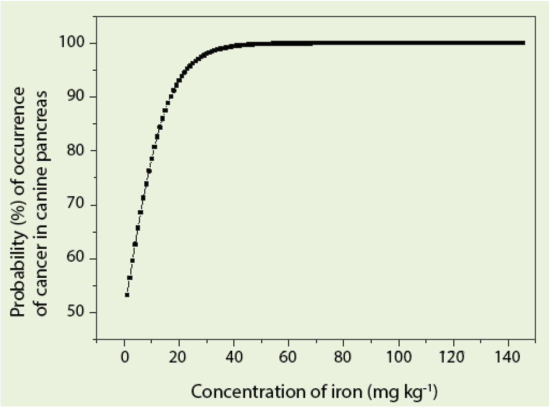

Figure 3. Occurrence probability of neoplasm in pancreas predicted by logistic regression model according to the Fe contents

Table 7. Classification matrix of the logistic regression model generated for pancreas tissue samples

Observed	Predicted			Correct Percentage
	Pathology			
	0		1	
Without cancer	0	9	3	75.0
With câncer	1	2	10	83.3
Overall Percentage				79.2

and transport of Fe can be present at different levels depending on the kind of tissue where it is found; this differentiation of concentration takes place due to the kind of tissue where the neoplasm is being developed, and the concentration tends to decrease because of the lysis of the red blood cells by the compromised vascular integrity. Lappano *et al.*[32] and Rahman[35] for example, found in human, higher Fe levels cancerous mammary tissue than in healthy tissues. Carvalho *et al.*[36] also found higher concentrations of Fe, Cu and Zn in lung tissues with cancer, and Mn, Cu and Zn in tissues with prostate tumors, both of human tissue. Fe is associated to the progression step because of the neovascularization, and in the progression stage the neoplasm already exists; what is happening is the appearance of metastasis.[37]

The contents of the metals Cu, Fe, Mn and Zn dosed in skin and testicles tissues were also adjusted to the models using logistic regression, but the models can't apllied for this tissues using these metals because none of the metals were statistically significant (data not shown).

4. Conclusions

It is suggested that Zn is potentially relevant in the occurrence of neoplasm in mammary gland, since the mammary tissue has great epithelial, mesenchymal and histological distribution of tissues; the accelerated physiological function needs accelerated cell protein metabolism, which, in malignant neoplastic processes, needs

Zn for its occurrence. Because it is an organ with exocrine and endocrine functions, the pancreas already has specialized vascularization and with the neovascularization increase, the Fe levels are raising too. However, when the neoplasm is in the stage of progression due to the cell lysis, there will be decrease in the Fe concentration. Logistic regression models can be used in the detection of positive cases of neoplasm in cells of canine mammary gland and pancreas tissues, when there is the presence of high concentrations of Fe and Zn.

Studies regarding the concentrations of trace metals are important, since the adoption of more specific diets for each neoplastic process tends to help in its therapeutic practice, as well as in the decrease of stages of promotion and progression of neoplasm.

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