A METHOD FOR SPECIFIC DIFFERENTATION OF THE EGGS FROM OVINE GASTROENTERIC NEMATODES

(UN METODO PARA LA DIFERENCIACIÓN ESPECÍFICA DE HUEVOS DE NEMATODOS GASTROENTERICOS OVINOS)

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Key words: Trichostrongylidae, eggs, identification.
Palabras clave: Trichostrongylidae, huevos, identificación.

RESUMEN

Proponemos un método para la diferenciación específica de huevos de nematodos gastroentéricos ovinos, basado en una función discriminante que usa como parámetros la longitud y anchura de los huevos.

El estudio fue realizado en un rebaño de ovejas lachas en régimen extensivo en el Valle de Campoo (Cantabria). Los huevos se obtenían de heces en las que se conocía la presencia de una sola especie.

Nuestro método tiene una mejor capacidad discriminante que los métodos gráficos descritos y sin necesidad de infraestructura informática. Por otra parte, es menos laborioso que el método basado en la identificación de larvas de tercer estadio.

SUMMARY

We propose a method for specific differentation of the eggs from ovine gastroenteric nematodes, based on a discrimination function, which makes use of the parameters length and width of the eggs.

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The study was carried out on a flock of sheep of the lacha breed in the Campoo Valley, Cantabria (Spain), submitted to an extensive system of explotation. The eggs were obtained from faeces in which the presence of only one species was previously known. The species *Nematodirus filicollis, N. helvetianus, N.spathiger, Trichostrongylus axei, T. colubriformis, Haemonchus contortus* and *Ostertagia circumcincta* were studied.

Our method has a better discriminant capacity than the graphic methods described in the literature, and also without the necessity of an informatic infrastructure. Furthermore, it is less laborious than the coproculture method, based on the obtention of third-stage larvae, which makes its use in outdoor work adequate.

INTRODUCTION

The most important ovine gastroenteric helminthoses are caused by members of the superfamily Trichostrongyloidea. These etiologic agents have substantial dif rences in their pathogenicity and epizootiology, which makes an accurate knowledge of the genera and species in every situation desirable.

The nematodes of this superfamily laid eggs, segmenting but not in embryo, thin-shelled, ovoid or ellipsoid in shape, that go to the environment within the faeces of the host.

The genera and species identification is habitually carried out using faeces cultures and identifying the infective larvae (L3) using metrics and morphology. This method may be suitable in corologic investigations, but it has serious disadvantages in outdoor work because the professional does not generally have the adequate-infrastructure and enough time to make faeces cultures and the consequent larval identification. In this way, some authors have tried alternative methods in identifying nematode genera and species, using fresh egg metric and morphology.

The Shorbs's effort [9] appears to have been the first, but the identification key was very difficult and problematic.

Cunliffe y Crofton [5], being based on egg metric, length and width, and on their variability, establish the omnibus categories which comprise of those species with some degree of overlapping in their measurements. Obviously, this is only a part solution since this method can not separate the species within the same category. The poor development of the micrometric techniques over the fifty years obstructed these authors from going very far.

On the other hand, Christie y Jackson [3] proposed an identification method based on dimensional characteristics, length and width of the eggs, which complement embrionicevolution data, at low temeperature, with the idea of observing the development differences. This method and the faeces cultures method have the same or similar disadvantages.

Georgi y McCulloch [7] have proposed a method of specific differentation of eggs based on multidimensional analysis. The drawbacks of this method are the complexity of the metric data used: length, width, perimeter, area, polar areas, etc.., and the need for an informatic infrastructure.

In this contribution we suggest an alternative method, of an exclusively mathematical nature, to identify the gastrointestinal nematode eggs, which basically pursues its applicability under outdoor conditions.

MATERIAL AND METHODS

A 350 flock of sheep of the lacha breed in the Campoo Valley, Cantabria (Spain) between 1987 and 1988 was studied. The habitual pasture area were hillsides facing east, at an average higth of 900 meters, with argillaceous ground (Trias), grassland and oakwood *(Quercus pyrenaica* Willd.), and an abundance of little pools, with water most of the year. The average annual temperature and pluviosity were 9° C and 981 mm, respectively, without a dry season (Figure 1).

The faecal samples were directly collected from the rectum. Every mass of faeces was divided in two parts. One part (ca. 9/10) was designed for culture and the other stored at 1-2° C. If using the metric and morphology data of L3 obtained from the culture, we conclude that the larvae belong to only one species, then we take the eggs from the rest of the faeces, using the flotation technique with a saturate saline solution.

The length and width of the eggs were measured using a micrometric ocular. The number of eggs from every measured species was 121.

The nematode species *Nematodirus filicollis* (Rudolphi, 1802) (NF), *N.spathiger* (Railliet, 1896) (NS), *N.helvetianus* May, 1920 (NH), *Haemonchus contortus* (Rudolphi, 1803) (HC), *Trichostrongylus axei* (Cobbold, 1879) (TA), *T.colubriformis* (Giles, 1892) (TC) and *Ostertagia circumcincta* (Stadelmann, 1894) (OC) were obtained; according to the well-known morphology and metrics of the larvae [1, 6, 8]. They were gathered in two groups, *Nematodirus* species and the rest of the species, being based on the evident metric and morphologic differences. The average and the characteristic deviation were calculated.

It was necessary the use of 1728 coprocultures. So high number was due to the difficult to find pure coprocultures, here easier because the little number of eggs and larvae. An added difficult is the poor difference between some of the larvae; in these cases only when there was no doubt at all the coproculture was accepted.

The data obtained were graphically represented and mathematically processed. The graphic representation of these parameters in a two-axis system, y=width, x=length, allowed us to obtain dispersion ellipses, somewhat as large as the percentage of dispersion which we want to represent [3, 7, 10].

An overall mathematical analysis of the variables for each pair of species within the same group was carried out. In order to carry out this analysis, the discriminate function described by Chun Li [4] was resorted to which allow us, for each pair of species, to find out with a maximum degree of confidence, whether or not

significant, if an egg belong to one or to the other species. The function is a first degree equation of this type:

$$F = k_1 .l + k_2 w$$

k_1 and k_2 being constants calculated for each pair of species, l=length, w=width, and where F exceeds a determined value with one of the species and is lower than it, or than another inferior one, with the other species, for a determined degree of confidence.

RESULTS

The parameters of the distributions obtained, average and deviation, in the species studied allowed us to compile Table 1, where the average length and width, and their deviations, of the eggs of each species, and the upper and lower limits for an extensive series of percentages included in the dispersion, which range from 40% to 90% appear.

Table 1 data do it possible to make the graphic representation, getting the respective distribution ellipse for each species and dispersion; e.g. the *Nematodirus spathiger* distribution ellipse, for a 70% dispersion ($p \leq 0.3$), is made using length (199.1 μm) and width (107.7 μm) averages as the ellipse center, 185.3 and 212.9 μm as lower and upper extremes of the abscise axis, and 96.1 and 119.3 μm as lower and upper extremes of the ordinate axis.

Box

-First step: Calculation of the L, M and N intermediate values:

$n_a = n_b = 121$

$$L = \Sigma l_a w_a - \frac{(\Sigma l_a)(\Sigma w_a)}{n_a} + \Sigma l_b w_b - \frac{(\Sigma l_b)(\Sigma w_b)}{n_b} = -11.6$$

$$M = \Sigma l_a^2 - \frac{(\Sigma l_a)^2}{n_a} + \Sigma l_b^2 - \frac{(\Sigma l_b)^2}{n_b} = 23067.3$$

$$N = \Sigma w_a^2 - \frac{(\Sigma w_a)^2}{n_a} + \Sigma w_b^2 - \frac{(\Sigma w_b)^2}{n_b} = 9186.3$$

-Second step: System of equations to calculate k_1 and k_2:

$$M\ k_1 + L\ k_2 = (n_a + n_b - 2)(\bar{l}_a - \bar{l}_b)$$

$$L\ k_1 + N\ k_2 = (n_a + n_b - 2)(\bar{w}_a - \bar{w}_b)$$

$\bar{l}_a = 162.9$ $\bar{w}_a = 88.9$

$\bar{l}_b = 166.6$ $\bar{w}_b = 78.4$

$k_1 = -0.04$ $k_2 = 0.27$

-Third step: The assesment of the discriminate function for this pair of species:

$F = -0.04\ l + 0.27\ w$

Figures 2 and 3 show distribution ellipses of each considered species group, *Nematodirus* spp with a 70% dispersion ($p \leq 0.3$) in Figure 2, and the rest of the species with a 60% dispersion ($p \leq 0.4$) in Figure 3.

k_1 and k_2 constants of the discriminate function and the value reached for F with the highest possible signification degree for each pair of species within every group are shown in Table 2. The calculus of k_1 and k_2 constants was made following the one described by Chun Li [4]. Here we use, in the Box and as an example, the species *N.filicollis* (subindex a) and *N.helvetianus* (subindex b).

DISCUSSION AND CONCLUSIONS

The values obtained by us in the length and width are similar to those cited by others authors (Table 3), except in *H.contortus*, *Trichostrongylus* spp and *N.helvetianus*. The *H. contortus* and *Trichostrongylus* spp eggs have greater average widths, and *N.helvetianus* lesser than the range cited by some of these authors [2,11]. These differences migth be due to the geographic variations or to another reasons, and perhaps they would be inconvenient in the egg specific differentiation if we do not previously know the characteristic measurement of the local eggs.

The graphic method, used by us and based on dispersion ellipses [10], has overlappings which were muchlower than the stadistic signification. Christie y Jackson[3] had the same problem because there were overlappings in all the similar species at 50% dispersion. Furthermore, in order to establish the ellipse and to simplify the calculation, it is usually supposed that egg length and width have a normal distribution of values and are independent of each other. This is to say, their image in the plane is a cloud of points with regresion lines, or ellipse axis, parallel to the Cartesian axis and orthogonal between them. Our data support this asumption, except in *H. contortus* since in this species the shorter eggs were the wider ones.

Georgi y McCulloch [7] removed this possible relation in their complex graphic method and put in a lot of parameters which migth come from or be related to the egg length and width or even among them. This situation produces a redundancy in information and a loss of fiability.

The mathematical method proposed in this contribution does not come from any type of asumption, and makes an overall treatment of the egg length and width. Comparing the results obtained (Figures 2 and 3, Table 2), we observe certain discrepancies between the graphic and the mathematic methods. So, *N.filicollis* and *N.helvetianus*, using the geometric method (Figure 2), overlap at 70% significance; they do not do so using the mathematic method, since, at this significance level, *N.helvetianus* reaches a maximum value of 16.09 and *N. filicollis* a minimum value of 16.44. Another outstanding discrepancy is the one between *H.contortus* and *T.axei* which on using the geometric system overlap at 60% (Figure 3) and on using the mathematical system still do not do so at 70% (Table 2).

The capacity to discriminate of this mathematic method is higher than the graphic one due to the maximization, in the discriminate function, of the parameter which emphasizes the differences between the species of each pair. The fiability is good,

although Table 2 shows significances lower than acceptable statistical levels, but it is due to its reference to individual eggs and not to egg groups whose averages when closer to the populations averages, the bigger the egg group is.

Its applicability in outdoor work is easy, because of the sligth necessity for infrastructure, and it is only indispensable when establishing the discriminate functions between each nematode species pairs in every existing situations.

For this reasons and bearing in mind the general and checked use of this type of functions in Taxonomy, we consider our mathematic method as adequate to be used in the specific differentiation of nematode eggs, particularly in the everyday outdoor work.

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Table 1

Average in µm of the length and width of the eggs, measurements in relation to different levels of significance (L_u90 = upper limit at 90%,..., L_l90 = lower limit at 90%) and deviation.

	HC		TA		TC		OC		NS		NH		NF	
	l	w	l	w	l	w	l	w	l	w	l	w	l	w
L_u90	84.8	60.9	88.8	59.0	95.4	67.0	102.6	53.1	221.2	126.1	182.8	90.2	179.1	97.5
L_u80	83.4	59.3	87.4	57.2	93.5	64.8	100.7	51.3	216.2	122.0	179.2	87.6	175.5	95.6
L_u70	82.4	58.3	86.4	56.0	92.3	63.3	99.5	50.1	212.9	119.3	176.8	85.8	173.1	94.3
L_u60	81.6	57.4	85.6	55.1	91.3	62.1	98.5	49.2	210.3	117.1	174.9	84.4	171.2	93.2
L_u50	80.9	56.7	84.9	54.2	90.5	61.0	97.7	48.3	208.3	115.5	173.2	83.2	169.5	92.4
L_u40	80.3	56.1	84.3	53.5	89.7	60.1	96.9	47.6	206.1	113.5	171.8	82.1	168.1	91.6
Average	78.2	53.9	82.2	51.0	87.1	56.9	94.3	45.1	199.1	107.7	166.6	78.4	162.9	88.9
L_l40	76.1	51.7	80.1	48.5	84.5	53.7	91.7	43.6	192.1	101.9	161.5	74.7	157.8	86.2
L_l50	75.5	51.1	79.5	47.8	83.7	52.8	90.9	42.9	190.1	100.2	160.0	73.6	156.3	85.4
L_l60	74.8	50.4	78.8	46.9	82.9	51.7	90.1	42.0	187.9	98.3	158.3	72.4	154.6	84.6
L_l70	74.0	49.5	78.0	46.0	81.9	50.5	89.1	40.1	185.3	96.1	156.4	71.0	152.7	83.5
L_l80	73.0	48.5	77.0	44.8	80.7	49.0	87.9	38.9	182.0	93.4	154.0	69.2	150.3	82.2
L_l90	71.6	46.9	75.6	43.0	78.8	46.8	86.0	37.1	177.0	89.3	150.4	66.6	146.7	80.3
s	4.0	4.2	4.0	4.8	5.0	6.1	5.0	4.8	13.3	11.1	9.8	7.1	9.8	5.2

Table 2

Discriminate functions calculated for each pair of species and values obtained for these functions at the highest possible significant level.

Species involved 1	2	k_1	k_2	Max. value of F in species 1 with significance	Min. value of F in species 2 with significance	Significance
NH	NF	-0.04	+0.27	16.09	16.44	70%
NF	NS	+0.19	-0.20	-71.46	-70.02	80%
NH	NS	+0.19	+0.29	59.45	61.67	80%
TA	TC	+0.21	+0.17	26.80	26.87	40%
HC	TC	+0.44	+0.14	43.53	44.22	50%
HC	TA	+0.27	-0.11	15.84	16.00	70%
TA	ØC	+0.31	-0.09	22.64	23.11	70%
HC	ØC	+0.30	-0.10	20.17	21.24	80%
TC	OC	+0.02	-0.24	-10.27	-10.24	70%

TABLE 3

Measurements in µm given by various authors

		SOULSBY	BORCHERT	CHRISTIE & JACKSON	CUNLIFFE & CROFTON	OWN DATA
HC	l	70-85	68-80	78.0		78.2
	w	41-48	40-50	47.0		53.9
TC	l	79-101	73-96	87.0		87.1
	w	39-47	40-43	48.6		56.9
TA	l	79-92	70-112	82.8	86.5	82.2
	w	31-41	35-63	40.7	39.5	51.0
NF	l		130-200			162.9
	w		70-90			88.9
NS	l	175-260	175-260			199.1
	w	106-110	160-200			107.7
NH	l	160-230	160-230			166.6
	w	85-121	85-115			78.4
OC	l	80-100	92-110			94.3
	w	40-50	42-50			45.1

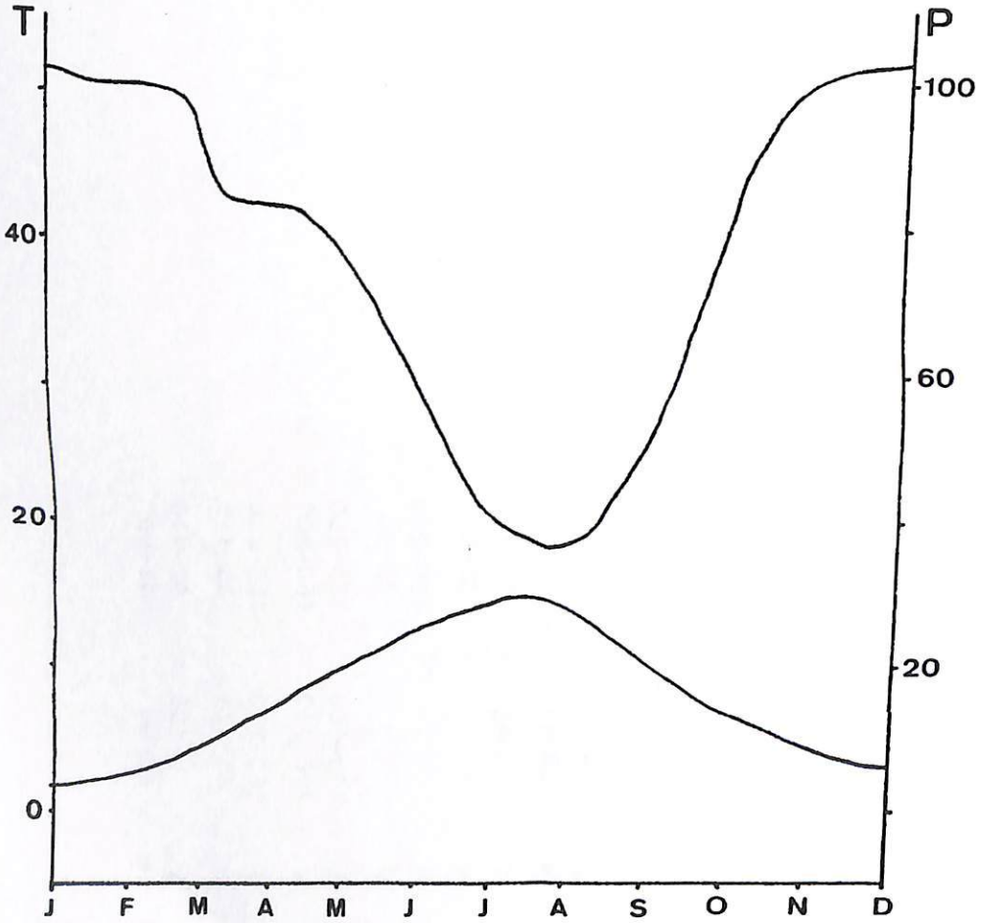

FIGURE 1
Ombrothermic diagram of the area of study

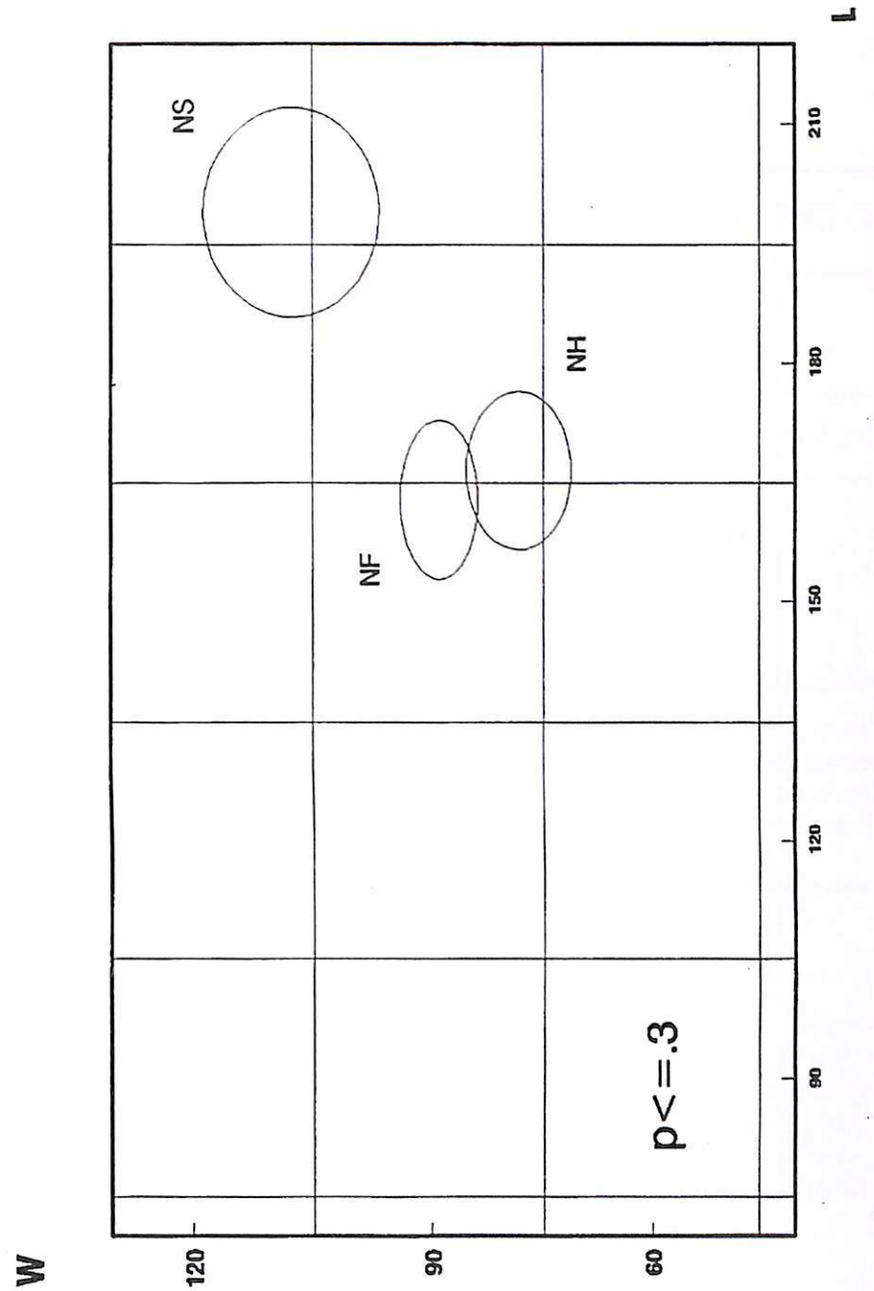

FIGURE 2
Distribution ellipses of the *Nematodirus* species, at a 70 % dispersion (L= length, W = width)

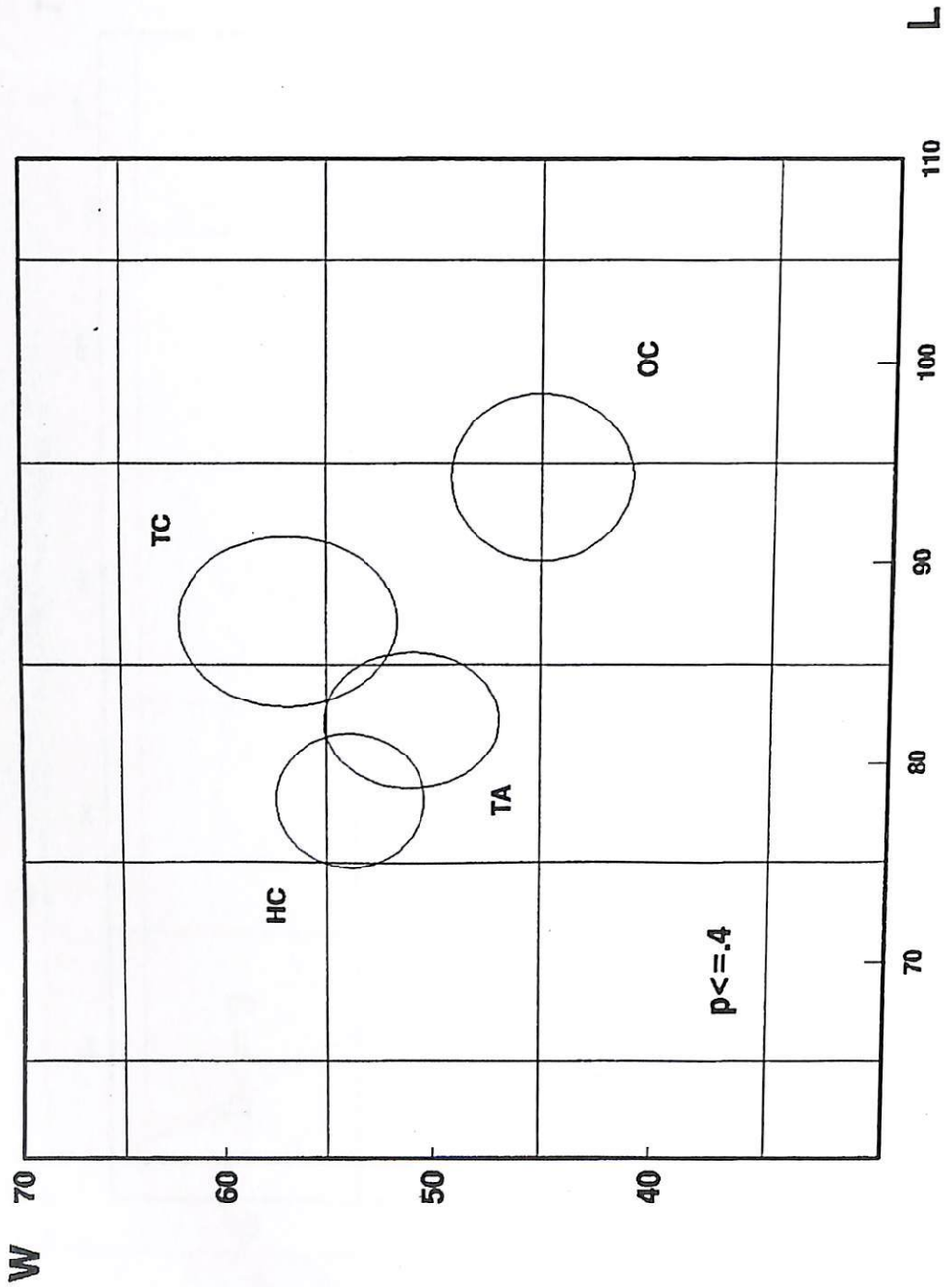

FIGURE 3
Distribution ellipses of the species with little eggs, at a 60%dispersion (L=length, W=width)

CAMBIOS EN LAS PROTEÍNAS DURANTE LA MADURACIÓN DEL CHORIZO.

(CHANGES IN PROTEINS DURING THE RIPENING OF CHORIZO).

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Palabras clave: Chorizo. Proteolisis. Nitrógeno no protéico.
Key words: "chorizo". Proteolysis. Non protein nitrogen.

SUMMARY

The extent of hydrolysis and loss extractability of proteins during ripening of "chorizo" -a dry fermented sausage- elaborated by traditional and industrial processess was studied. The amount of non-protein nitrogen (NNP), total α-amino nitrogen and ammonia increased during processing, while peptides decreased. The insolubilization is intense in both sarcoplasmic and myofibrillar proteins.

RESUMEN

En el presente trabajo se ha estudiado la intensidad de la proteolisis y la pérdida de extractibilidad de las proteínas durante la maduración del chorizo elaborado por procedimientos artesanales e industriales. A lo largo de la maduración se observa un aumento del nitrógeno no protéico (NNP), nitrógeno α-aminoacídico y nitrógeno básico volátil total (NBVT), mientras que el nitrógeno peptídico desciende. Tanto las proteínas miofibrilares como las sarcoplásmicas sufren una pérdida de solubilidad elevada.

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