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Growth, gonadal histology and liver lipid composition in *Pagellus acarne*

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SUMMARY - Growth, survival and food conversion rates, as well as sexual development and fat metabolism, were investigated in the Sparid fish Pagellus acarne reared at different densities, in order to assess the most convenient conditions for farming of this species. Three different population densities were tested. The group reared at lowest density showed an increase in weight of 75 g over 220 days, with FCR increasing from 4.7 to 8.5. Since this species is hermaphroditic, special attention was paid to maturity stages of the bisexual gonads. A close relationship exists between such aspect and the results of the biochemical analysis of liver fatty acid fraction. From these data, together with the other results, conclusions can be drawn which allow to penetrate deeper inside the development processes of a fish population intensively reared.

Key words: *Pagellus acarne*, growth, gonads, fatty acids, digestive enzymes.

RESUME - La croissance, survivance et les index du conversion alimentaire, ainsi que le développement sexuel et le metabolisme des lipides ont été étudiés chez le poisson Sparidé Pagellus acarne élevé à des différentes densités, visant à établir les meilleurs conditions pour l'élevage de cette espèce. Trois différentes densités de population ont été testées. Les poissons maintenus à la densité la plus bas ont montré une augmentation du poids de 75 g au cours de 220 jours, avec un FCR qi l'est passé de 4.7 à 8.5. Vue l'hermaphrodisme de cette espèce, le développement sexuel des gonades a été aussi étudié dans les différentes conditions d'élevage et mis en relation avec les résultats des analyses biochimique engagées sur la fraction hepatique des acides gras. Des résultats obtenus, on a tiré des conclusions sur plusieurs aspects de l'élevage intensif de cette espèce.

Mots-clés: *Pagellus acarne*, croissance, gonades, acides gras, enzimes digestifs.

INTRODUCTION

Pagellus acarne is one of the three species of breams described by Risso (1826). The characteristics of this species were later studied by Dordelain (1889) on a 250 mm long specimen caught in Sicily, now held in the British Zoological Museum. De Gaetani (1935) reported floating eggs gathered in the Straits of Messina "with spherical capsule, without any special sculpture, with a single envelope and with a single oil droplet".The eggs examined by De Gaetani were all embryonated and after about 48 hours after the collection, kept in culture, hatched larvae of about 2.40-2.50mm . In more recent times a detailed study was carried out by Morales and Fauquet (1984) on the scales of the lateral line of the three pagellus species caught along the Spanish littoral. The investigation by means of electron microscopy allowed the authors to conclude that the species may be determined from the scales , in that, other than a number of common characteristics such as the shape of the denticles and the number of the basi ctenidiali , a series of characteristics are sufficiently differentiated to be used as taxonomic characteristics.

The species is present in the Mediterranean and on the eastern Atlantic coasts, from England to Senegal. Detailed research has been done in Spain (Oliver and Bruno, 1981) and in Morocco (Domanevskaya, 1982) on the percentages of catch and vertical distribution. It is very common along all the Sicilian coasts with sandy or muddy bottoms. It is one of the most caught fish in small scale fishing in the Straits of Messina, with a percentage incidence of 21% (Andaloro, 1983). It is caught by trammel net, circular net and trowl net. Investigations carried out on the stomach content of specimens caught inthe Straits of Messina both on the Tyrrhenian and the Jonian side revealed the presence of 6 Phyla in the diet, variable between stations but constant in time, with a prevalence of fish and crustaceans.

The present research was carried out on *P. acarne* as a part of a wider programme on the family Sparidae, with the aim of evaluating the suitability of this species for intensive rearing. Furthermore, the choice of P. acarne, unusual on the Italian continent, was made for the good commercial value that it has in Sicily, where it is known and appreciated for its palatability, comparable to those of saragi.
In particular, the growing-on at different densities and with different diet regimes has been studied at the experimental mariculture plant of the Istituto Sperimentale Talassografico in Messina (Sicily). Moreover, in the awareness of the importance that basic research has for the best exploitment of any cultured fish, the seasonal changes of gonads, the lipid content of the liver and the digestive enzymes occuring in the gastro-intestinal tract have been investigated.

MATERIALS AND METHODS

The bronze breams used in the present research were collected in the Strait of Messina.A preliminary experience was performed on these specimens to evaluate the growth parameters with two different diet regimes, a dry pelletted one given in daily rations amounting to 5% of body weight, and a fresh one made of fish offal, given in daily rations amounting to 25% of body weight. The two experimental groups (P and F), which were quite homogenous (mean body weight= 45.3⇧ 2.5 g), were reared at a density of 8 fish/m^2. The trial lasted 120 days.
Further detailed investigations were carried out at different densities, taken into consideration that this environmental parameter can be easily manipulated to improve the

profitability of fish culture plants. For this purpose, three homogenous groups (mean body weight= 11.05 ⇧ 1.5 g, mean size = 6.3 ⇧ 0.7 mm) were made of 10 (group A), 20 (group B) and 40 (group C) fish/m^2 respectively, all fed dry pellets. The rearing tanks used in this experiment were weekly controlled for temperature, salinity, dissolved oxygen and micronutrients. The trial lasted 220 days.

Gonads were excised from 10% random samples from each group at the end of growing trial (January), after which the study of gonadal histology proceeded in groups B and C only, with seasonal samplings until September. All gonads were weighed, fixed in Bouin's solution, dehydrated through graded alcohols, cleared in xylene and finally embedded in plastified paraffin. Transverse sections were taken at 5 ↘m and stained with haematoxylin-eosin-orange G and Rasmussen-Ignesti trichrome for observation under the light microscope.

The gonosomatic index was calculated as gonadal weight x 100/ total body weight.

Fatty acid composition of the total lipids of the liver were compared at the different densities. The liver of each fish was excised and frozen immediately at -20 °C under oxygen -free nitrogen atmosphere, in order to avoid oxidation phenomena.The total lipids were extracted from each sample (Folch *et al.,* 1957) and from this the fatty acids. The methyl esters of fatty acids were analysed by using a silica layer in 10% AgNO3 catalytic hydrogenation and HRGC. A Fractovap 2150 gas chromatograph ,equipped with a FID detector and fused silica capillary column was used. Experimental conditions were: column temperature (isotherm) 210°C; injector and detector temperatures 275°C ; hydrogen carrier gas 0.6 bar. The relative percentage of each detected component as well as the total content in saturated, mono-unsaturated and poly-unsaturated fatty acids were determined in each sample.

The effect of two diets (fresh offals and pellets) was evalueted on the digestive enzymes of specimens. The digestive tract of 5 samples was divided into stomach, pyloric caeca and intestine.They were separately weighed and homogenized in Tris buffer 50 mM ,pH 8.4 (in a volume 15 times the weight), and then centrifugated at 6000 rpm at 40°C for 10 minutes to obtain the enzymatic extract, used to measure the acid and alkaline proteolytic (Maskel and Di Capua, 1988), lipolytic (Tietz and Fiereck, 1966), amylolytic (Bernfield, 1955), elastase (Mandl, 1955) and leucine aminopeptidase (Berger and Broida, 1977) activities.

RESULTS AND DISCUSSION

Growth

In Tables 1 and 2 the growth data obtained during the two experiments is reported.

Examining in detail growth with respect to diet and observing the distribution in frequency classes (Fig.1) of lot F one can see that 38% of the animals did not reach 100 g, while 62% went beyond this. Of this latter about 68% went over 125 g.From the same analysis, with reference to lot P, about 62% did not reach 100 g and among those that exceeded this ,only a negligible number went beyond 125 g.

Data relative to growth with respect to density show a significant difference between the three experimental lots, with an increase in the lot of lowest density (lot A) which passes from a mean weight of 11.05±0.89 g. to a final weight of 86.24±1.93 g.. The other two lots, B and C, show comparable growth rates up to 150 days of the experiment and then consolidate at 65.62 ±2.38 and 54.38 ±2.35 g , respectively.

With regard to percentual distribution of weight classes a substantial homogeneity can be observed in the development of lot A, with individuals not excessively dispersed with respect

to the mean value in which nearly 50 % of the entire lot falls within. In the various stages of periodical sampling, in lot B at double density, a few weight classes below the mean value were represented until the end of the test and the population gathered at 220 days within a weight range comprised between 70 and 90 g. In the weight increase of lot C this phenomenon of dispersion was accentuated, with individuals blocked at very low weights to the advantage of another fraction of the population which, on the contrary, reached individual weights of 120 and 130 g, well beyond the confidence interval at the end of the test.
The trend of the main physical-chemical parameters in the growing tanks does not show an excessive trophic load in any of the experimental sectors, with the only foreseeable exception of $N\text{-}NH_3$ (from a minimum of 0.24 µg-a/l in the incoming water to a maximum of 11.91 µa/l inside the tank).

	DAYS		
	0	60	120
Lot F	43.6 ±2.6	81.6±2.9	113.2±4.0
Lot P	45.3±2.2	68.1±2.5	92.9±2.7

Table 1 : Mein weights of P. acarne fed different dieys over 120 days

	DAYS	
	0	220
Lot A	11.05±0.89	86.24±1.93
Lot B	11.05±0.89	65.62±2.83
Lot C	11.05±0.89	54.38±2.35

Table 2 : Mean initial and final weights of P. acarne reared at different densities over 220 days.

Fat metabolism

In Table 3 the mean composition in franctions of the hepatic fatty acids of the lots of *P.acarne* reared at different densities is reported.

TABLE 3. Mean composition in classes of liver fatty acid in three group of P. acarne reared at different population density .

Fatty acid	Lot A	Lot B	Lot C
Tot.sat.	36.55±3.2	35.10±2.1	41.87±2.5
Tot. mono.	51.12±4.2	53.03±2.5	50.02±4.2
Tot. poly.	12.38±3.5	11.82±4.5	8.11±2.3
Poly (n-3)	4.78±0.2	5.84±0.3	2.69±0.6
Poly (n-6)	7.60±0.5	6.04±0.2	5.42±0.5

The analysis of the mean values obtained for the various classes of fatty acids with respect to the three lots showed no significative differences between Lot A and Lot B both regarding the saturated and mono-unsaturated fractions as well as for the poly-unsaturated one. In lot C, on the other hand, a fair increase in the saturated fraction was noted while a decrease in the poly-unsaturated, particularly those of ω-3 series were found.

Digestive enzymes

The study of the digestive capacity of *P.acarne* showed (Fig. 2a-2b) that the protease and elastase content of fish fed on fresh offals are higher than those of fish fed on pellets; with regard to enzyme distribution, the intestine has the highest concentration of lipase only. As observed in other carnivorous fish (Alliot *et al.*, 1974; Ash, 1988) the pyloric caeca represent a "reservoir" of digestive enzymes. The proteolytic activity exhibits an antero-posterior gradient: it is higher in the stomach and it decreases along the gastrointestinal tract. The digestive capability of fish fed on fresh offals to degrade proteic molecules is always higher than those fed the other diet, because of the exogenous origin of proteases. Exogenous enzymes which are present in food, in fact, may be involved in the digestive processes of fish, in particular for larval nutrition and the evaluation of their contribution is very difficult.

Histology of gonads

Gamete maturation

Four stages of sperm development were distinguished, namely spermatogonia, spermatocytes, spermatids and spermatozoa. Spermatogonia were present in all specimens at all samplings, whereas the other three spermatogenic stages occurred only in functional males.

Seven oocyte developmental stages were identified. The first four, namely oogonia, chromatin-nucleolus, early perinucleolus and late perinucleolus stages, were present in all specimens, including functional males, at all samplings. Yolk vesicle stage oocytes were occasionally found in the predominantly female gonads in January and March and normally found at the following samplings. Primary and secondary yolk granule stage oocytes were

found in September only in the predominantly female gonads of group C. Occasional atresia of yolk vesicle stage oocytes occurred as from May. On the other hand, massive atresia of yolk granule stage oocytes was evident in the maturing females of group C found in September.

Seasonal changes of the gonads

All fish had bisexual gonads (ovo-testes) in which the ventro-lateral male and dorso-medial female territories were separated by a thin layer of connective cells and fibres. The relative proportion of male and female areas in transverse sections varied. The larger between the two territories was also found to contain the most advanced gametogenic stages. Thus, the relative size of each germinal tissue within the ovotestis, together with the maturity stage of germ cells, was considered as indicative of the functional sex of the individual. The seasonal changes in GSI and gonadal histology in the fish of the three groups are summarized in Table 4, 5 and 6, respectively. Data for group A (lowest density) were obtained from the single sampling carried out at the end of growing trial (January).

There was no difference between the three experimental groups in the maturity stage reached by the fish at the end of the growing trial at different densities. On the other hand, the higher was the rearing density, the lower was the percentage of predominantly female gonads. These gonads contained oogonia, primary growth phase oocytes and, occasionally, some yolk vesicle stage oocyte; the male territory appeared extremely small and was populated by spermatogonia. At the same sampling the predominantly male gonads were in active spermatogenesis, as demonstrated by the presence of spermatocytes and spermatids. The ovarian portion in these gonads appeared as a tiny stripe of oogonia and chromatin-nucleolus stage oocytes. In all groups the gonosomatic index (GSI) of functional males was lower than that of the fish with predominantly female gonads.

At the following samplings the number of predominantly male gonads was always higher than that of the predominantly female ones in both groups B and C. As indicated by GSI and histological findings, spermatogenesis proceeded in males, so that spermatozoa were formed by March and emitted by some individuals in May. Spawning proceeded in all the males of group B in June and July, while it occurred only in some ones of group C in the same months. The other ones had pre-spawning gonads. In September the males of group B were at the end of spawning, as shown by the decreased GSI and presence of spermatogonia and spermatozoa only, with no intermediate spermatogenic stages. On the other hand, the males of group C kept on showing an asynchronous maturation, that is some of them were spawning and some other were still pre-spawning. Such a difference in the maturity stage reached by the males of group C from June through September is reflected by the high standard deviations of GSI recorded in that period (Table 6).

The predominantly female gonads underwent considerable growth from January onwards, as shown by increasing GSIs, and this was accompanied by advancement of oogenesis, so that yolk vesicle stage oocytes were found in great amounts by May in both groups B and C and vitellogenic oocytes up to the secondary yolk granule stage occurred in September in group B. The testicular tissue in the maturing females remained very small and contained few spermatogonia among which clusters of yellowish pigment, suggesting a degenerative process, were apparent.

On the basis of histological observations it is suggested that a rearing density of 20 fish/m^2 is advisable to yield the best exploitation of *Pagellus acarne* as a breeder. In fact, spawning of all functional males and vitellogenesis in the predominantly female gonads occurred at this density.

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Table 4: Seasonal changes in gonadal histology of *Pagellus acarne* reared at a density of 40 fish/m^2.

Month	n	GSI*	Histological features		Functional stage
			Testicular portion	Ovarian portion	
January	19	0.38± 0.10	Spermatogonia	Oogonia, PGP oocytes, occasional yolk vesicle stage oocytes	Resting female
	29	0.08± 0.02	Spermatogonia,spermatocytes,spermatids	Oogonia, PGP oocytes	Maturing male
March	2	0.70± 0.13	Spermatogonia,yellowish pigment	Oogonia, PGP oocytes, occasional yolk vesicle stage oocytes	Resting female
	5	0.18± 0.06	All spermatogenic stages	Oogonia, PGP oocytes	Maturing male
May	3	0.66± 0.20	Spermatogonia, yellowish pigment	Oogonia, PGP oocytes, yolk vesicle stage oocytes; occasional atresia	Maturing female
	4	0.41± 0.22	All spermatogenic stages	Oogonia, PGP oocytes	Pre-spawning/spawning male
June	-	-	-	-	-
	7	0.68± 0.40	All spermatogenic stages	Oogonia, PGP oocytes	Pre-spawning/spawning male
July	-	-	-	-	-
	7	1.88± 1.18	All spermatogenic stages	Oogonia, PGP oocytes	Pre-spawning/spawning male
September	1	1.26	Spermatogonia, yellowish pigment	Oogonia, PGP oocytes, yolk vesicle stage oocytes; occasional atresia	Maturing female
	6	2.31± 1.49	All spermatogenic stages	Oogonia, PGP oocytes	Pre-spawning/spawning male

* Values expressed as mean ± S.E.

Table 5: Seasonal changes in gonadal histology of *Pagellus acarne* reared at a density of 20 fish/m^2

Month	n	GSI*	Histological features		Functional stage
			Testicular portion	Ovarian portion	
January	13	0.29± 0.08	Spermatogonia	Oogonia, PGP oocytes, occasional yolk vesicle stage oocytes	Resting female
	11	0.07 ±0.03	Spermatogonia,spermatocytes,spermatids	Oogonia, PGP oocytes	Maturing male
March	1	0.58	Spermatogonia	Oogonia, PGP oocytes, occasional yolk vesicle stage oocytes	Resting female
	6	0.14± 0.05	All spermatogenic stages	Oogonia, PGP oocytes	Maturing male
May	2	0.87± 0.15	Spermatogonia	Oogonia, PGP oocytes, yolk vesicle stage oocytes; occasional atresia	Maturing female
	5	0.77± 0.20	All spermatogenic stages	Oogonia,PGP oocytes	Pre-spawning/spawning male
June	1	1.22	Spermatogonia, yellowish pigment	Oogonia,PGP oocytes, yolk vesicle stage oocytes; occasional atresia	Maturing female
	6	1.78± 0.50	All spermatogenic stages	Oogonia, PGP oocytes	Spawning male
July	2	1.13± 0.19	Spermatogonia,yellowish pigment	Oogonia, PGP oocytes, yolk vesicle stage oocytes; occasional atresia	Maturing female
	5	1.71± 0.34	All spermatogenic stages	Oogonia, PGP oocytes	Spawning male
September	2	1.72± 0.83	Spermatogonia, yellowish pigment	Oogonia, PGP oocytes and SGP ooc.up to II yolk granule st.; atresia	Maturing female
	5	1.37± 0.35	Spermatozoa, spermatogonia	Oogonia,PGP oocytes	Male at end of spawning

* Values expressed as mean ± S.E.

Table 6 : Gonadal histology of *Pagellus acarne* reared at a density of 10 fish/m^2

Month	n	GSI*	Histological features		Functional stage
			Testicular portion	Ovarian portion	
January	7	0.45 ±0.09	Spermatogonia	Oogonia, PGP oocytes, occasional yolk vesicle stage oocytes	Resting female
	4	0.11± 0.03	Spermatogonia, spermatocytes, spermatids	Oogonia, PGP oocytes	Maturing male

* Values expressed as mean ± S.E.

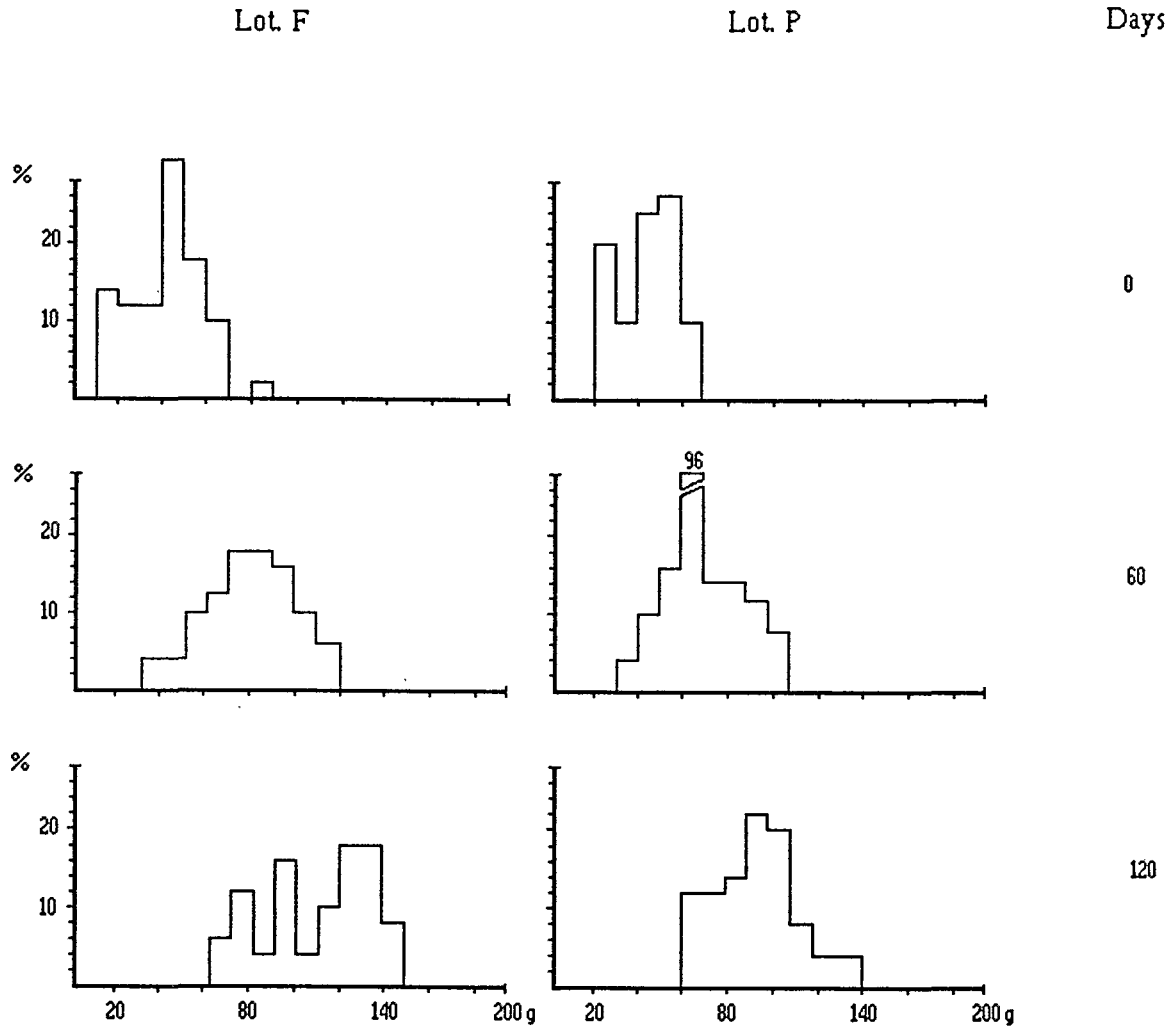

Fig. 1. Frequency histogram of body weight distribution in the two groups of *P. acarne.*

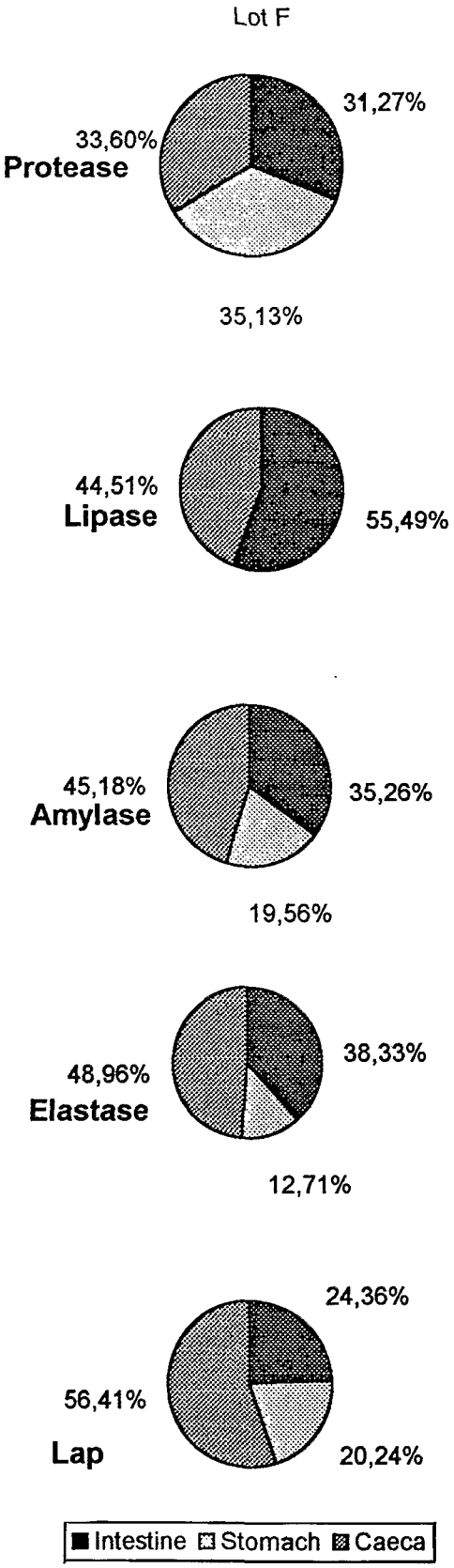

Fig. 2a. Distribution of the enzymatic activities in relation to diets.

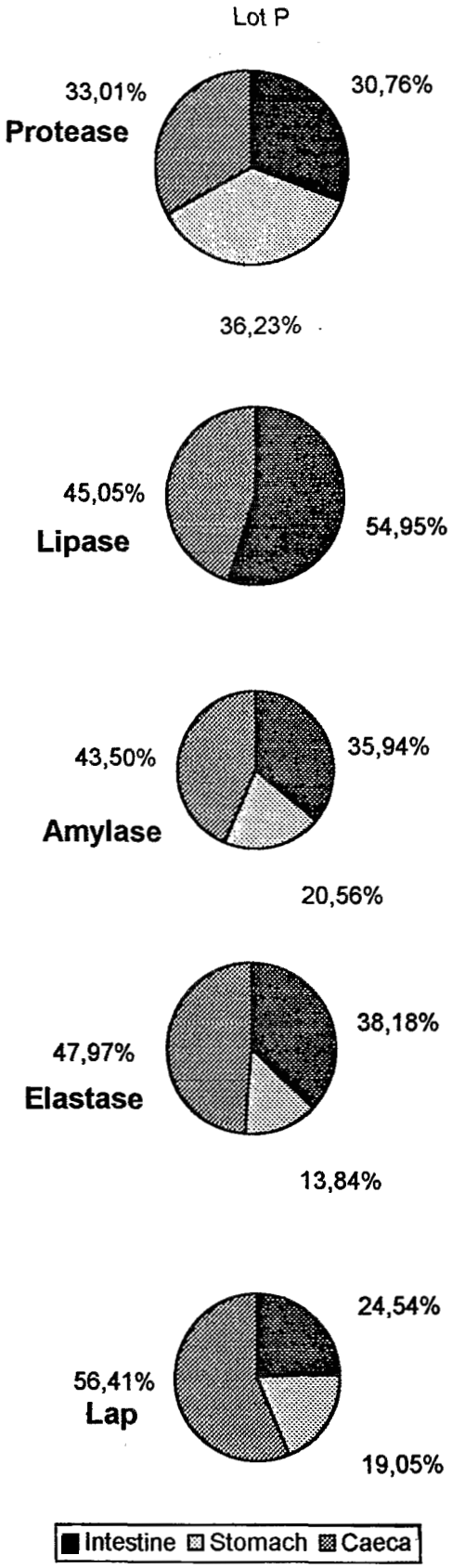

Fig. 2b. Distribution of the enzymatic activities in relation to diets.