SUPERIOR USAGE OF MARINE EPIBIOTA IN ZOOTECHNY

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ABSTRACT

In the Romanian Coastal area of the Black Sea, the marine epibiota that spontaneously develops has a high biodiversity and an explosive development, realizing average biomasses of 6 – 8 kg living material/m^2 of support in one growing cycle.
Marine organisms that form the epibiota (approx. 30 species) have a complex bio chemical composition and a superior content of essential bio active compounds such as amino acids, vitamins, enzymes and hormones, that can be superior used in zoo technology both in fresh and processed state.
Fresh marine epibiota obtained through marine aquaculture technologies can be processed into shell powder, additive that preserves totally the nutritive qualities and can be easily distributed in modern feeding equipment.
Distributed in concentrations of under 10% of the food ratio, the shell powder amplifies the growing rate: for chickens with 25.21% and for hens with 9%. The lipid concentration in yolk dropped with 31%, the cholesterol with 29%, the total fat acids with 5.6% and the triglycerides with 13.4%.
The producing of the marine epibiota offers the possibility of obtaining substantial profits by using the unexploited marine surfaces, and in the same time it is an ecological method of improving the quality of marine waters by the increase of the epibiotic bio filters.

KEY WORDS - epibiota powder, chickens, forage improvement

INTRODUCTION

On the littoral rocks, marine epibiota grows spontaneously, but it cannot be exploited because of its major role in the Black Sea ecosystem – natural biofilter.

Marine epibiota can though be obtained in big quantities by applying bivalve culturing technologies, and this is the way to obtain stabile productions and with known expenditures, through using an important marine resource – phytoplankton – and with the help of marine equipments adapted to the hydro meteorological conditions of the Black Sea.

MATERIAL AND METHOD

An efficient biotechnology for obtaining big quantities of marine epibiota needs the developing and locating between the 10 – 15 m isobaths of some floating equipments, capable to maintain in the water column the artificial supports destined for epibiont organisms fixation and development.

The fixing of the epibiont organisms on the artificial surfaces in the sea is naturally made by the larva from mussel populations reproductions. The high density of the organisms and their explosive growth ensures the obtaining of an average biomass of 3 tons/installation/culture cycle.

The culturing of the marine epibiota on floating surfaces is also an ecological method of improving the quality of the marine waters in the turistic areas by using the epibiotic biofilters.

The experiments were made at Lumina Farm, Constanta County, in a 30000 chickens capacity hall. At the end of this hall, 5 batches were prepared, in every one being distributed 10 chickens. The forage was mixed with epibiota powder in 0% (witness), 5%, 10%, 15% and 20% ratio. The birds were foraged twice a day with a dayly diet of 130 – 140 g.

For bio chemical analyses we used a Cellia spectrophotometer with kits by BALMED Bucharest.

RESULTS AND DISSCUSIONS

Technical means destined to produce marine epibiota

In the Romanian coastal zone of the Black Sea, the producing of the marine epibiota can be realized by arranging some floating equipments that will maintain the supports in the water column, at 1 – 5 m depth, where the phytoplanktonic food is more abundant (Fig. 1).

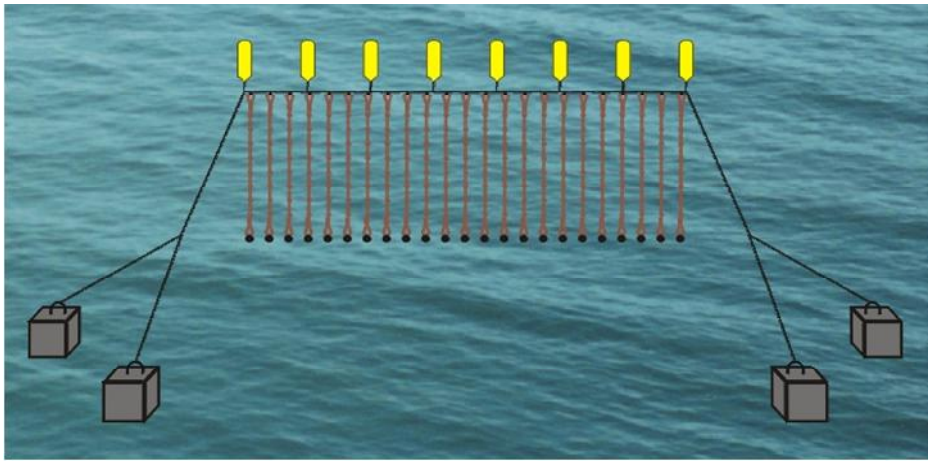

Fig. 1 - Floating equipment with artificial collectors

We projected a proper installation for the conditions at the Romanian littoral, which has two distinguished compounds:

- anchored floating superstructure;
- artificial supports suspended in the water column.

When projecting the equipments we wanted to cancel the destructive force of the hydro meteorological factors – wind, marine currents, waves, substrate – by using elements of marine engineering that conducted to the obtaining of an installation capable to resist in these conditions.

After five months from launching the culturing installations, organisms having average annual biomasses of 2 – 2.2 tons got fixed on the supports (average biomasses of 7.231 kg / l.m., approx. 68500 individuals/l.m.). The composition of the epibiota was 90% mussel juveniles, 8% cirripeds, 1.5% seasonal macro – algae, 0.5% other invertebrates (Fig. 2).

Considering the biofiltration capacity of about 73 m^3/24h and the total biomass on one installation's collectors (about 2.2 tons/year), we can say that the epibiotic biofilters may filtrate about 182500 m^3 sea water in 24 hours.

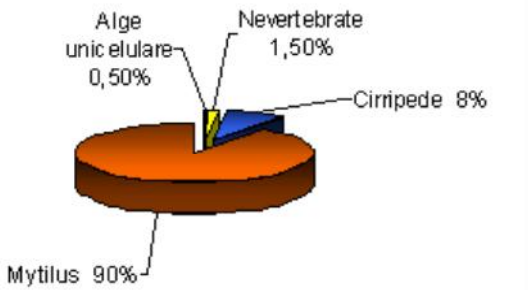

Fig. 2 - The epibiota structure after five months from launching

The technology of marine epibiota processing

The necessity of animal nutrition supplementing by adding natural products - algae, mollusks - that will ensure the mineral substances, proteins, glucides, and fats sustained the introduction of this matter in complex processing and bio technological analyses. The results are given as following:

Table 1 - Physic – chemical characteristics of marine epibiota

Look	Marine epibiota
Dry substance %	98.5 ± 0.3
Ash %	86.6 ± 0.4
Total organic substance %	11.5 ± 0.7
Total N %	1.3 ± 0.2
Water soluble substances mg/g	
proteins	8.5 ± 0.5
glucids	5.5 ± 0.5
Organic solvents soluble substances mg/g	
total fats	6.0 ± 0.5
pigments	4.2 ± 0.3
Mineral compounds % in ash	
CaO	50.5 ± 0.5
MgO	0.32 ± 0.5
MnO	0.12 ± 0.3
Oligoelements and heavy metals μg/g in ash	
Cu	4.70 ± 0.05
Cd	2.10 ± 0.05
V	0.15 ± 0.1
Fe	0.85 ± 0.05
Pb	2.50 ± 0.05

Table 2 - Bio-active characteristics of the thin extract

Amylase activity	130-150 mUml/min
Lipase activity	110 – 140 U/mg protein
Phosphatase activity	75 – 80 U/ mg protein

Table 3 - Microbiological characteristics of marine epibiota (germs no. /g)

NTG	5×10^4
Clostridia sulfur - reductants	20
Total Coli forms	60
Excrement *Staphylococcus*	4.4
E. coli	absent
Salmonella	absent
Staphylococcus coagulator positive	absent

For obtaining efficient results it is indicated the fresh epibiota to be dried at 65^0 C (in order not to depreciate the active natural compounds), crushed and preserved for as long as possible. We appreciate an outturn of 30% from the fresh product.

The processing of the epibiota constitutes of the following stages:

- drying in rotary ovens with hot air jet at maximum temperature of 65^0 C;
- crushing the product until obtaining homogenous powder with granulation between 0.20 - 0.80 mm or bigger, depending on consumers;
- packing in hygroscopic paper bags;
- storing in dry spaces.

Table 4 - Comparative characterization of marine epibiota and dry epibiota powder

No.	CHARACTERISTICS	EPIBIOTA	POWDER
I	**Organoleptic characteristics**		
1	look	mollusks, crustaceans, algae	powder under 1 mm (0.2 - 0.8)
2	color	green with red - brown aspects	gray - brown
3	smell	specific marine	specific marine
4	taste	-	specific marine
II	**Physical and chemical characteristics**		
1	water solubility at 20^0 C mg/ml	-	7.8 - 8.0
2	dry substance % of which	40.0 ± 2.0	98.2 ± 0.05
2.1	ash %	20.2 ± 2.0	50.47 ± 2.2
2.1.1	mineral compounds in ash mg/g		
	Na	-	-
	K	-	-

No.	CHARACTERISTICS	EPIBIOTA	POWDER
	CaO	50.5 ± 0.5	52.5 ± 0.5
	MgO	32.0 ± 0.5	34.0 ± 0.5
	MnO	1.25 ± 0.05	1.75 ± 0.05
	Fe	35.0 ± 0.5	38.5 ± 0.5
	Co	1.5 ± 0.1	1.7 ± 0.05
	Ni	1.2 ± 0.1	-
	Zn	2.8 ± 0.1	-
	Pb	0.2 ± 0.3	2.0 ± 0.5
	Cd	1.0 ± 1.0	1.2 ± 0.5
	Hg	-	0.2 ± 0.05
2.2	organic substance % of D.S. of which	20.0 ± 0.2	49.53 ± 2.2
2.2.1	total proteins	40.0 ± 5.0	18.5 ± 0.5
2.2.2	total glucides	30.0 ± 5.0	17.0 ± 0.5
2.2.3	total fats	8.0 ± 2.0	12.0 ± 0.5
2.2.4	bio-active compounds		
	vitamins μg/g SU		
	riboflavin	2.9 ± 4.6	0.5 ± 0.7
	niacin	10.2 - 10.8	-
	ascorbic acid	1.5 - 1.8	-
	carotenoids mg/g	30.0 - 39.0	10.0 - 12.0
2.2.4	enzymatic activity		
	amylase U/mg	39.0 ± 5	20 ± 5
	protease U Anson	44.0 ± 0.05	30.0 ± 9.0
	SOD / Umg prot.	0.420 ± 0.05	0.150 ± 0.005
	cathalase μ mol H_2O_2 min x mg prot	10.2 ± 0.05	7.20 ± 0.05
III	**Microbiological characteristics (no./g)**		
1.	NTG	10^5	5 X 10^3
2.	*Vibrio para haemoliticus*	absent	absent
3.	*Coli* forms	100	30
4.	*Streptococcus*	50	absent
5.	*E. coli*	absent	absent
6.	*Salmonella*	absent	absent
7.	*Staphylococcus* coaculozo positive	absent	absent
IV	**Toxicological characteristics**		
	Variation limits for heavy metals in ash		
1.	Cu	0.20 - 0.45	4.7 ± 0.05
2.	Cd	0.9 - 1.1	2.1 ± 0.05
3.	Pb	0.15 - 0.40	2.5 ± 0.05
4.	Hg	-	0.03 - 0.04

The modifications are obvious, as following:

- from the heterogeneous mixture of marine organisms, we obtained a homogenous powder, with granules between 0.20 – 0.80 mm;
- this powder maintained a specific marine smell;
- we noticed a concentration of bio active compounds in dry substance and in ash;
- the high concentrations of Ca, Mg, Mn, Fe, Co will contribute to mineralizing effect of adding powder into birds diet;
- the concentrations of heavy metals does not affect the sanitary attributes of the product; we also remind about the dropping of microorganisms concentrations;
- the organic compounds should be accumulated, though, we noticed a qualitative diminishing of proteins and glucids, probably due to some mineralization processes;
- some bio active compounds - vitamins, riboflavin, pigments - diminish both their concentrations and enzymatic activities, probably because of the thermic treatment that affected the substances.

The marine epibiota powder preserves some bio active characteristics that justify the necessity of adding it into birds' diets. These aspects are completed by an outturn of obtaining of about 32 – 35% and a digestion outturn of about 19 – 20% from the powder. Compared to other nutritive adjuvant, we noticed a significant activity of Superoxiddismutase and Cathalase, which may interfere in the regulation of animal metabolism.

Using the marine epibiota powder in birds diets

The results evaluation considered the gains during exploitation, the mortality during administration, the egg laying performances and the quality of the eggs. We also determined the average forage consumption and epibiota supplement.

The using of epibiota powder in meat chickens' diets evinced that a concentration between 5 - 20% dry powder resulted into a variable response of the morphological indices, depending on the concentration and chicken age.

Using a concentration of 10% powder in diet did not significantly influenced the growth, which was of 34, 46.5 and 50.8g at the age of 22-38, 38-46 and 47-54 days. The treated chickens had an excessive vitality, and red mucous membranes, probably because of the protein supplement that brought a higher level of blood at membranes.

Using a concentration of 20% epibiota powder in diet had negative effects and produced a mortality of 58%. The epibiota excess brings high levels of calcium, which produces cell death, because of the damages made to the control mechanisms.

The results are proven also by chemical and electronic microscopically exams of different tissues. These values are shown in the next table. It can be easily seen that the treatment of the chickens with 10% epibiota determines a significant growth of hepatic glycogen compared to the chickens that had only 5% epibiota in diet. The same growing of hepatic glycogen is found when comparing the chickens that had 10% epibiota with the ones with 15%. Between the individuals that had 5% and the ones with 15% there are no significant differences.

Table 5 - The values of glycogen, cathalase, superoxiddismutase (SOD) and reduced glutathione (GSH) activity in the hepatic tissue of chickens treated with 5%, 10% and 15% epibiota in diet

Experimental batch	**Hepatic Glycogen (mg/g tissue)**	**Chatalase activity (U/mg prot)**	**SOD activity (U/mg prot)**	**Reduced glutathione (GSH) (µg GSH/mg prot)**
5% X±ESn	10,02±0,406	1,61±0,206	0,32±0,016	37,14±0,846
X±ESnt10 % p± 5%	16,87±0,326 12,01 0,001 **+68,36%**	1,73±0,036 0,52 NS +7,45	0,32±0,016 0,30 NS 0%	39,62±1,326 1,43 NS +6,67
tp± 15%	11,45 0,001 **+45,05%**	5,77 0,001 **-37,14%**	0,55 NS -,03%	1,32 NS +6,11%
X±ESnt15 % p± 5%	11,63±0,256 0,34 NS +16,06%	2,40±0,096 3,18 0,02 **+49,06%**	0,33±0,026 0,38 NS +3,12%	37,33±1,996 0,079 NS +0,51%

Note: In the table are given average data ± standard errors (X±ES); n = samples number that constituted the average; p = statistic significance threshold; NS = statistically insignificant modification; ± 5% = the percentage difference between the considered batch and the one treated with 5% epibiota; ± 15% = the percentage difference between the batch treated with 10% epibiota and the one with 15%.

We can not explain the significant modification of the hepatic glycogen for the 15% epibiota batch and 5% one. We must advise that the

epibiota supplement brings a big Ca^{2+} quantity. It is possible that in the 15% batch, the calcium income was so big that it annihilated the positive effect of protein income. Actually, the weight of the individuals in the 15% batch was smaller than of the ones in 5% and 10% batches. The healthiest chickens were found in the 10% epibiota batch.

The chatalase activity is very high at the 15% epibiota batch. This proves that those chickens were the most stressed, probably because of the big calcium incomes. Both the SOD's and reduced glutathione's activities do not suffer significant variations at 10 and 15% batches compared to the 5% one.

The adding of a 10 – 20% dry epibiota powder in diets had a variable response of the productive indicators, depending on concentration and the age of the chickens.

- the 10% concentration significantly influences the growing rate: 34, 46.5 and 50.8g at the age of 22-38, 38-46 and 47-54 days; the untreated chickens of 22-54 days have an average natural growth of 40.61g, with 25.21% smaller than the treated ones;
- we noticed an obvious vitality of the chickens and red mucous membranes, probably because of the protein supplement that brought a higher level of blood at membranes.

The increasing of the epibiota ratio negatively influenced the growing speed in 29-36 days period (only 15g per day) and the mortality was of about 4%.

- the results suggest that a concentration above 10% may be given to chickens over 36 days, but before that they should be feed with 5 – 10% epibiota during 22-36 days period;
- the chickens over 38 days have an average growing of 44.4g, with 16% bigger than the untreated ones.

The 20% epibiota ratio in forage had negative effects on the chickens and produced a mortality of over 58%. The epibiota excess favours big calcium incomes that produce cellular death.

CONCLUSIONS

- after 7 months from launching the installations, the average biomass of epibiont organisms per collector is of 9.231kg/l.m. and the average bio productivity is around 2.5 – 3 tons, depending on seasonal conditions;
- the main organisms are the mussels, that form 90% of the biomass; the cirripeds have 8%, the algae have 1.5% and the rest of 0.5% represent small invertebrates;
- the introduction of a concentration of 3 – 20% dry epibiota in chickens diets conducted to a variable response, depending on concentration and chickens age;
- the introduction of a 10% concentration of epibiota powder in ratio, did not influenced significantly the growing of the chickens; the untreated chickens of same age (22-54 days) realized weights with 25.21% smaller than the ones supplementary foraged with epibiota powder;
- the studied bio chemical compounds had the following tendencies: the hepatic glycogen grew in normal limits at chickens fed with 5-10% epibiota; the cathalase and SOD had big values for excessive epibiota batches, proving the negative influence of these substances; the reduced glutathione had not big variations;
- at the 15% epibiota batch, we noticed corporal weights with 41% smaller than the normal batch; we also noticed the highest mortality rate at this 15% batch (27.77%);
- the research proves that the marine epibiota constitutes an alternative source of forages, which can be used in meat chickens diets, in ratios below 10% from normal diets.

REFERENCES

ADAM A.L., BOGATU D**.**, 1981 - *Pescuitul industrial*, Ed. Tehnica Bucureşti pg. 52 - 80;

ALBERTS B., 2004 - *Biologie molleculaire de la cellule*, Ed. Flammarion, Paris;

BLOKHUIS H.J., 1989 - The effect of a sudden change in floor type on pecking behaviour in chicks. *Appl.Anim.Behav.Sci*, 22, pg. 65-73;

BROOM D.M., 1969 - Effect of visual complexity during rearing on chiks reactions to environmental change, *Anim. Behav*. 17, pg. 773-780;

BURNE T.H.J., ROGERS L.J.., 1996 - Responses to odorants by the domestic chiks, *Physiol. Behav*. 60, pg. 1441-1447;

COLEN J., 1991 - *Production animals et produits animaux*. Institut National Agronomiques, Paris-Grignon, France;

EROSCHENKO V., 1993 - *Atlas of Histology*, Ed. Lea & Febiger, London, Philadelphia;

FORBES J.M**.,** 1995 - Application of diet selection by poultry with particular reference to whole cereals. *World's Poult. Sci. Journal* 51, pg. 149-165;

GANCHOROW J.R., STEINER J.E., 1990 - Behavioral reactions to gustatory stimuli in young chicks (*Gallus gallus domestecus*). *Dev. Psych*. 23, pg. 103-117;

GENTLE M.J., 1985 - Sensory involvement in the control of food intake in poultry. *Proc. Nut. Soc*. 44, pg. 313-321;

GENTLE M.J., 1986 - The bill tip organ of the chicken. *J.Anat*.145, pg. 79-85;

HALE C., 1988 - Effects of early ingestional experiences on the acquisition of appropriate food selection by young chiks. *Anim. Behav*. 36, pg. 211-224;

HAMILTON R.M.G., 1995 - Ingredient particle size and feed texture: effects on the performance of broiler chiskens. *Anim. Feed. Sci. Technol*. 51, pg. 203-210;

MEUNIER SALAUN M.C., PICARD M., 1996 - Les facteurs de choix alimentaires chez le porc et les volailles. *INRA Prod. Anim*., 9, pg. 339-348;

SARBULESCU V., STANESCU V., VACARU I., VINTILA C., 1983 - *Tehnologia si valorificarea produselor animale.* Ed. Didactică şi Pedagocică, Bucureşti;

URSACHE C., 1993 – Rezultate tehnice obţinute în testarea instalaţiilor flotante echipate cu colectori artificiali destinate creerii unui biofiltru natural. *Lucrările ştiinţifice a celei de a 3-a Conferinţe Naţionale de Protecţie a Mediului* - Braşov, pg. 37-40;

VACARU OPRIS I., 1993 - *Tehnologia creşterii păsărilor*. Centrul de multiplicare al Universităţii Agronomice, Iasi.