First haematic results for the sea bass (*Dicentrarchus labrax*) metabolic profile assessment

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ABSTRACT

The assessment of blood reference ranges of farmed fish can be extremely useful in improving production and product quality. A first attempt to establish the normality ranges for the most important haematochemical parameters of farmed sea bass was carried out by analysing the trend of Haematocrit, Glucose, Total Protein, Albumin, Globulin, Total Cholesterol and some electrolytes in 353 sea bass farmed with two different farming systems within 1 year. A strong seasonal effect was found with regard to each parameter; the role of some environmental conditions was evaluated; and some reference ranges were proposed for the culturing methods considered.

Key Words: Sea bass, Metabolic profile, Blood parameters, *Dicentrarchus labrax*.

RIASSUNTO

PRIMI RILIEVI EMATICI TENDENTI ALLA DEFINIZIONE DEL PROFILO METABOLICO DI *DICENTRARCHUS LABRAX*

*La definizione dei limiti di normalità dei valori ematici dei pesci allevati può essere un valido strumento per controllare la produzione e la qualità del prodotto. Sono stati compiuti prelievi di sangue mensili su spigole (*Dicentrarchus labrax*) allevate con due diverse tipologie di allevamento per un totale di 353 campioni tendenti ad una prima definizione del profilo metabolico della specie.*

E' stata rilevata un'ampia variabilità dovuta alla stagione del prelievo per tutti i parametri considerati. L'effetto di alcuni parametri ambientali è stato discusso all'interno di ogni parametro ematico considerato e sono stati proposti dei range di normalità per i sistemi di allevamento presi in esame.

Parole chiave: *Spigola, Profilo metabolico, Parametri ematici,* Dicentrarchus labrax.

Introduction

The determination of some haematochemical parameters is considered by many authors (Payne, 1972; Caldwell and Hinshaw, 1994; Ravarotto *et al.*, 1996) an extremely useful instrument for assessing the health status of farmed animals. Intensive aquaculture conditions have placed increasing demands on the fish, which must be able to cope with many stress factors that may affect their basic physiological functions, thus also affecting production and product quality. Periodic blood analyses are an inexpensive and easy method to point out metabolic disorders, deficiencies and chronic stress status before they appear in a clinical setting.

It is necessary to establish the reference ranges of species-specific haematic parameters for blood analysis to be affirmed as a standard method for the evaluation of the health status of cultured fish and for the correct interpretation of the results of haematochemical analyses.

Data reported in the literature refer mainly to acute stress status (Arends *et al.*, 1999; Erikson *et al.*, 1999; Kubokawa *et al.*, 1999; Skjervold *et al.*, 2001; Person Le-Ruyet *et al.*, 2003) in different farmed fish species, while few works refer to the normality of the haematic parameters under rearing conditions (Kavadias *et al.*, 2003; Roncarati *et al.*, 2003).

The purpose of the present study was, therefore, to contribute to the collection of data for the exact determination of haematic reference ranges in two different rearing conditions for sea bass (*D. labrax*), one of the most commonly farmed seawater fish in the Mediterranean sea.

Material and methods

Blood samples of 353 reared sea bass were collected in two different farms in the province of Grosseto (Italy): the semi-intensive plant "Il Padule" in Castiglione della Pescaia and the intensive one "Il Vigneto" in Ansedonia. Sea bass of the semi-intensive farm were reared in rectangular ponds and fed commercial diets (Marine Basic, Trouvit, Hendrix) (Table 2) supplemented by natural food; water was the Diaccia Botrona brackish marshes. Sea bass of the intensive farm were reared in concrete tanks with ground water and fed with formulated diets only (Marine MRF, Trouvit, Hendrix) (Table 2). Blood samples were drawn in each farm once a month, from September 2002 to September 2003, from 15 fish that fasted for 12 hours. In the semi-intensive farming system samples were not collected in December, January and February because of the "wintering" season and the impossibility of fish handling in this period. Rearing conditions were those shown in Table 1.

Experimental subjects all belonged to the same batch, and were different for each rearing method. Sea bass were randomly caught in the morning and anaesthetised (Ethylene glycol monophenyl ether, 0.4 cc/l); all fish were weighed and measured; blood was taken from each subject by puncturing the dorsal aorta with a 2.5 ml sterile plastic syringe and was divided into BD Vacutainer serum and EDTA (K3) tubes. The Haematocrit (Hct %) was measured (Redacrit centrifuge, 3600 rpm, 5 min) with micro-haematocrit heparinized capillary tubes, then sample tubes were refrigerated and carried to the laboratory of the Dipartimento di Scienze Zootecniche (Università di Firenze, Italy) where plasma and serum were obtained by centrifugation with Refrigerated Centrifuge ALC 4227R (3000 rpm, 30 min). Samples were frozen at -20°C and the following analyses were performed with UV/VIS Spectrometer Lambda EZ 150 (PerkinElmer) using Sclavo Diagnostics Inc. kits:

- Plasma Glucose (GLU): colorimetric determination with oxidase-peroxidase;
- Plasma Total Protein (TP): colorimetric determination with biuret-tartrate;
- Plasma Albumin (ALB): colorimetric determination with BCG;
- Plasma Globulin (GL): calculated as [Total Protein – Albumin];
- Plasma Total Cholesterol (TCho): enzymatic colorimetric determination;
- Serum Calcium (Ca): colorimetric determination with o-Cresolphthalein;
- Serum Inorganic Phosphorus (IP): colorimetric determination with phosphomolibdate;
- Serum Magnesium (Mg): colorimetric determination with Xylidine-Blue;
- Serum Chloride (Cl): colorimetric determination with Mercury Thiocyanate;

Table 1. Culturing conditions.

	Initial Weight (g)	Final Weight (g)	Stocking Density (Kg/m^3)	T range (°C)	Dissolved Oxygen (ppm)	Salinity (‰)
Semi-Intensive	563.17 ± 98.43	679.33 ± 45.66	7.5	3.5-30	5-8	12-35
Intensive	281.66 ± 52.12	438.33 ± 92.98	27-35	19-24	3.5-6	12-15

Table 2. Chemical composition (% on wet basis) of the feed.

	Moisture	Protein	Total lipid	Crude fiber	N-free extract	Ash	Ca	P
Semi-Intensive	7.70	44.00	15.55	1.45	23.40	7.90	1.50	0.39
Intensive	6.20	47.15	16.45	1.80	18.25	10.15	1.78	1.24

Monthly values of dissolved oxygen (DO), water temperature, oxygen percentage saturation and salinity were registered for the semi-intensive farm only, while for the intensive farm annual ranges were collected through farmer information.

Seasonality and farming system were analysed by 1-Way ANOVA ($P<0.05$) considering month or farm as fixed effect, respectively. Means separation was computed by Fisher's test. Sampling dates were divided into classes according to the Fisher's test results: a number was assigned to any combination of significant letters — the smaller the number, the higher the monthly mean. Relationships between environmental and blood parameters were investigated by regressions and Pearson correlation.

Results and discussion

From results obtained by comparing the 2 farming conditions, the Haematocrit showed values in accordance with the literature (Roche and Bogé, 1996; Pavlidis *et al.*, 1997; Papoutsoglou *et al.*, 1998) and no difference was found between the semi-intensive and the intensive farmed stocks (34.4 ± 6.3, 35.1 ± 5.4, respectively).

A wide variability within each parameter occurred, especially for the Glucose content, which showed the highest Coefficient of Variability: 70.86 and 55.53 % for the semi-intensive and intensive farm, respectively. The comparison between the two farming conditions highlighted a difference in the haematic Glucose content with a higher value for the semi-intensive system (Table 4). This difference is probably not due to the farming conditions, but can be attributed to the difficulty in catching the fish due to the pond characteristics: catching fish in the Padule farm was more time-consuming and more difficult than in the Vigneto one, resulting in a stressed fish stock. In fact, many authors reported Glucose as an index of stress, capture stress included (Benfey and Biron, 2000; Sadler *et al.*, 2000).

The ANOVA carried out on monthly means within the same parameter indicated a strong effect of the sampling date on all the blood parameters in both the farming systems with the exception of the Glucose content in the intensive farm (Table 3). Many authors agree in the assertion that haematic parameters are affected by the sampling season (Kavadias *et al.*, 2003) because the food intake increases as temperature rises and so does

Table 3. Seasonality of blood parameters.

	Semi-Intensive System				
	GLU (mmol/l)	TP (g/dl)	Alb (g/dl)	GL (g/dl)	TCho (mmol/l)
September 2002	3.46 ± 0.54[7]	3.86 ± 0.36[5]	1.23 ± 0.18[6]	2.64 ± 0.34[6]	2.95 ± 0.54[6]
October 2002	4.82 ± 0.82[5]	5.31 ± 1.28[2]	1.27 ± 0.17[5]	3.81 ± 0.85[2]	3.86 ± 1.02[4]
November 2002	4.11 ± 0.88[6]	4.08 ± 0.62[5]	1.22 ± 0.29[6]	2.86 ± 0.45[6]	2.85 ± 0.56[7]
December 2002					
January 2003					
February 2003					
March 2003	5.21 ± 1.93[4]	5.06 ± 0.52[3]	1.53 ± 0.10[3]	3.53 ± 0.49[3]	4.84 ± 1.24[2]
April 2003	4.55 ± 1.09[5]	3.90 ± 0.84[5]	1.08 ± 0.19[7]	2.70 ± 0.54[6]	3.64 ± 1.19[5]
May 2003	5.73 ± 1.26[3]	4.98 ± 0.55[3]	1.44 ± 0.13[4]	3.55 ± 0.49[3]	4.26 ± 0.78[3]
June 2003	6.74 ± 2.29[2]	5.13 ± 0.74[3]	1.94 ± 0.25[1]	3.19 ± 0.83[4]	5.55 ± 0.85[1]
July 2003	8.15 ± 3.43[1]	4.53 ± 0.66[4]	1.60 ± 0.27[2]	2.92 ± 0.69[5]	4.15 ± 0.88[4]
August 2003	5.86 ± 2.72[3]	5.02 ± 0.76[3]	1.44 ± 0.17[4]	3.58 ± 0.67[2]	4.18 ± 0.84[4]
September 2003	6.88 ± 3.15[2]	6.53 ± 2.09[1]	1.26 ± 0.33[5]	5.27 ± 2.06[1]	4.13 ± 0.74[4]

	Ca (mmol/l)	IP (mmol/l)	Mg (mmol/l)	Cl (mmol/l)
September 2002	2.65 ± 0.31[5]	2.92 ± 0.43[5]	0.90 ± 0.06[4]	119.52 ± 6.09[2]
October 2002	3.69 ± 0.51[1]	3.53 ± 0.42[2]	1.22 ± 0.15[1]	116.29 ± 5.33[3]
November 2002	2.68 ± 0.21[6]	2.75 ± 0.25[5]	5.86 ± 0.05[4]	103.82 ± 8.49[5]
December 2002				
January 2003				
February 2003				
March 2003	2.07 ± 0.48[3]	3.16 ± 0.54[3]	1.15 ± 0.12[2]	114.36 ± 9.74[3]
April 2003	2.33 ± 0.47[8]	2.64 ± 0.61[4]	1.03 ± 0.08[3]	100.25 ± 11.79[5]
May 2003	2.64 ± 0.48[7]	2.72 ± 0.45[4]	1.03 ± 0.07[3]	102.36 ± 3.56[5]
June 2003	3.27 ± 0.56[5]	2.87 ± 0.48[1]	1.33 ± 0.07[1]	135.04 ± 10.12[1]
July 2003	2.03 ± 1.13[8]	2.62 ± 0.38[6]	0.85 ± 0.06[5]	119.49 ± 8.79[2]
August 2003	2.57 ± 0.58[2]	3.21 ± 1.05[4]	1.04 ± 0.09[3]	111.54 ± 5.96[4]
September 2003	2.65 ± 0.69[4]	3.06 ± 0.62[4]	1.03 ± 0.05[3]	120.23 ± 7.20[2]

	Intensive System				
	GLU (mmol/l)	TP (g/dl)	Alb (g/dl)	GL (g/dl)	TCho (mmol/l)
September 2002	5.70 ± 3.18	4.87 ± 2.41[4]	1.03 ± 0.23[8]	3.83 ± 2.24[1]	4.32 ± 1.19[3]
October 2002	4.43 ± 2.01	4.16 ± 0.89[8]	1.37 ± 0.25[4]	2.80 ± 0.72[8]	4.16 ± 1.13[4]
November 2002	4.83 ± 2.49	3.90 ± 0.73[9]	1.30 ± 0.27[5]	2.60 ± 0.56[9]	3.39 ± 0.85[6]
December 2002	4.28 ± 1.07	4.60 ± 0.59[5]	1.35 ± 0.19[5]	3.24 ± 0.54[4]	3.91 ± 0.98[5]
January 2003	3.83 ± 1.12	5.05 ± 0.80[3]	1.42 ± 0.16[4]	3.63 ± 0.74[3]	3.86 ± 0.79[5]
February 2003	4.40 ± 1.42	4.35 ± 0.86[7]	1.23 ± 0.35[6]	3.12 ± 0.70[7]	3.65 ± 1.04[5]
March 2003	4.74 ± 2.86	4.34 ± 0.60[7]	1.13 ± 0.21[7]	3.20 ± 0.68[5]	3.69 ± 0.90[5]
April 2003	4.81 ± 2.49	5.15 ± 0.69[2]	1.85 ± 0.30[1]	3.30 ± 0.46[4]	3.90 ± 0.73[5]
May 2003	5.01 ± 2.22	5.47 ± 0.56[1]	1.74 ± 0.71[2]	3.73 ± 0.37[2]	3.96 ± 0.70[5]
June 2003	4.93 ± 3.10	5.25 ± 0.63[2]	1.75 ± 0.40[2]	3.49 ± 0.48[3]	4.33 ± 0.84[3]
July 2003	3.91 ± 1.59	4.98 ± 1.15[4]	1.60 ± 0.46[3]	3.48 ± 0.77[3]	5.00 ± 0.98[2]
August 2003	4.56 ± 1.09	4.72 ± 0.79[5]	1.44 ± 0.20[4]	3.27 ± 0.70[4]	5.02 ± 1.15[1]
September 2003	5.28 ± 1.01	4.54 ± 0.67[6]	1.39 ± 0.20[4]	3.15 ± 0.55[6]	5.05 ± 1.08[1]

	Ca (mmol/l)	IP (mmol/l)	Mg (mmol/l)	Cl (mmol/l)
September 2002	2.59 ± 0.26[7]	2.81 ± 0.45[6]	1.09 ± 0.09[9]	123.44 ± 13.88[8]
October 2002	2.74 ± 0.63[5]	2.96 ± 0.40[6]	1.25 ± 0.12[8]	131.93 ± 13.12[7]
November 2002	2.79 ± 0.40[4]	3.04 ± 0.55[6]	1.26 ± 0.11[7]	137.57 ± 15.84[4]
December 2002	2.66 ± 0.49[6]	2.82 ± 0.48[6]	1.40 ± 0.17[6]	129.27 ± 9.50[7]
January 2003	2.97 ± 0.34[4]	3.27 ± 0.44[5]	1.57 ± 0.27[3]	136.58 ± 11.83[5]
February 2003	2.84 ± 0.48[4]	3.56 ± 0.86[4]	0.80 ± 0.11[11]	146.72 ± 14.75[2]
March 2003	3.01 ± 0.45[3]	3.85 ± 1.16[3]	0.93 ± 0.15[10]	130.54 ± 14.28[7]
April 2003	3.62 ± 0.50[1]	3.96 ± 1.04[3]	1.73 ± 0.11[1]	138.14 ± 1.72[4]
May 2003	3.10 ± 0.53[2]	4.20 ± 1.08[2]	1.38 ± 0.19[6]	152.16 ± 7.61[1]
June 2003	4.23 ± 0.57[1]	5.31 ± 0.91[1]	1.66 ± 0.18[2]	141.83 ± 10.03[3]
July 2003	3.36 ± 0.46[1]	4.11 ± 0.68[2]	1.46 ± 0.14[4]	109.70 ± 16.04[10]
August 2003	3.46 ± 0.49[1]	4.22 ± 0.57[2]	1.38 ± 0.11[6]	115.55 ± 18.63[9]
September 2003	3.62 ± 0.49[1]	3.06 ± 0.44[6]	1.42 ± 0.14[5]	134.31 ± 5.34[6]

Different numbers indicate a difference ($P < 0.05$) in the blood content due to the sampling period

Hct (%) = Haematocrit; GLU (mmol/l) = Glucose; TP (g/dl) = Total protein; Alb (g/dl) = Albumin; GL (g/dl) = Globulin; Tcho (mmol/l) = Total Cholesterol; Ca (mmol/l) = Calcium; IP (mmol/l) =Inorganic Phosphorus; Mg (mmol/l) = Magnesium; Cl (mmol/l) = Chloride

the general metabolism. Due to the special condition of the intensive farm - supplied by ground water with constant temperature all the year the feeding is constant in time and quantity and it reflects on the glycaemic blood content (Table 3). The semi-intensive farm, on the contrary, suspends the fish feeding in winter (December, January and February in this study) and gradually starts again as soon as the water temperature allows the fish to experience normal metabolic activity. This hypothesis is even confirmed by the growth: in the intensive farm fish gained 155% of their body weight in 13 months, while in the semi-intensive farm they gained only 120%.

From Table 3 it is clear that in the semi-intensive system Glucose, Total Protein and Globulin have a strong trend to higher values during the summer season (from May to September). The same trend was also found in the other parameters, although it was less marked. In the intensive system the period showing higher haematic values is longer, starting already in April for all parameters, with the exception of the Total Cholesterol, which was higher only in June.

A difference between the two farming methods was also found in the blood electrolytes that showed wider ranges (Figure 1) and higher values (Table 4) in the intensive farm.

The differences in the haematic content of Calcium, Inorganic Phosphorus, Magnesium and Chloride can be attributed to the water quality and the feed. In fact, ground water undergoes remarkable fluctuations during wet and dry seasons and this explains the wider ranges in the blood electrolytes of the intensive farming; moreover, the feed used in the intensive farm had a higher Inorganic Phosphorus and Calcium content (Table 2).

No effect of the fish weight on the haematic parameters was found.

Correlations between blood parameters and environmental parameters, carried out for the semi-intensive system only, showed a strong positive correlation between the water temperature and the Haematocrit, Glucose, Albumin, Chloride and Total Cholesterol (r=0.295; 0.362; 0.402; 0.423; 0.207, respectively). The same blood parameters are negatively correlated to the DO (r = -0.231; -0.323; -0.510; -0.398; -0.335, respectively). Moreover it seems that Haematocrit, Glucose, Calcium and Chloride tend to rise as salinity rises (r = 0.360; 0.201; 0.181; 0.279, respectively), while Magnesium decreases (r=-0.182). Finally, the oxygen saturation was negatively correlated to the Haematocrit, Glucose, Albumin and Chloride (r=-0.344, r = -0.310, r = -0.252, r = -0.389).

Table 4. One-way ANOVA between farming systems within each parameter.

	Hct (%)		GLU (mmol/l)		TP (g/dl)		Alb (g/dl)		GL (g/dl)	
Semi-Intensive	34.39 ± 6.30	ns	5.60 ± 2.42	a	4.86 ± 1.21	ns	1.41 ± 0.32	ns	3.41 ± 1.13	ns
Intensive	35.13 ± 5.40	ns	4.66 ± 2.12	b	4.73 ± 1.04	ns	1.44 ± 0.40	ns	3.30 ± 0.88	ns
	TCho (mmol/l)		Ca (mmol/l)		IP (mmol/l)		Mg (mmol/l)		Cl (mmol/l)	
Semi-Intensive	4.07 ± 1.15	b	2.67 ± 0.75	b	2.95 ± 0.62	b	1.06 ± 0.10	b	114.32 ± 12.77	b
Intensive	4.18 ± 1.07	a	3.11 ± 0.59	a	3.61 ± 0.95	a	1.34 ± 0.18	a	132.85 ± 16.94	a

a, b = P < 0.05.

Hct (%) = Haematocrit; GLU (mmol/l) = Glucose; TP (g/dl) = Total protein; Alb (g/dl) = Albumin; GL (g/dl) = Globulin; Tcho (mmol/l) = Total Cholesterol; Ca (mmol/l) = Calcium; IP (mmol/l) =Inorganic Phosphorus; Mg (mmol/l) = Magnesium; Cl (mmol/l) = Chloride

Figure 1. Box plots showing blood ranges in the semi-intensive and in the intensive farming system.

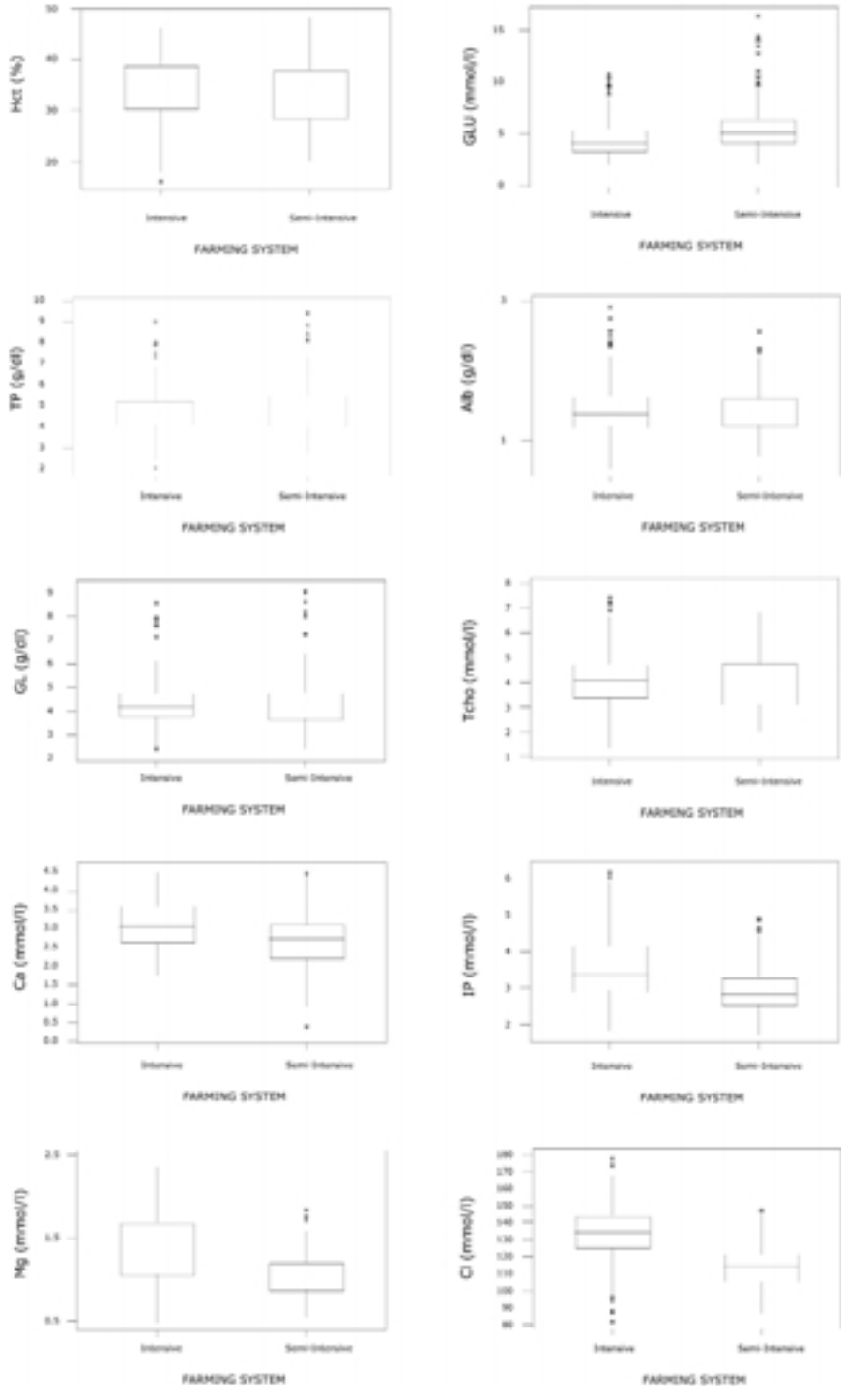

Table 5. Variances resulting from the regression of each environmental parameter over time for the semi-intensive system.

	Sum of squares									
	Hct	GLU	TP	Alb	GL	TCho	Ca	IP	Mg	Cl
Dissolved oxygen (DO)	304.21	89.07	0.02	3.85	3.68	20.56	0.03	0.16	0.00	3712.10
Temperature	199.23	23.97	11.17	0.03	14.19	3.57	2.52	0.07	0.13	634.20
Salinity	273.36	7.20	0.00	1.85	0.55	17.18	1.08	3.07	0.23	15.00
Oxygen saturation	56.54	18.68	0.11	0.20	0.13	0.26	11.99	0.01	0.19	12.30

Hct (%) = Haematocrit; GLU (mmol/l) = Glucose; TP (g/dl) = Total protein; Alb (g/dl) = Albumin; GL (g/dl) = Globulin; Tcho (mmol/l) = Total Cholesterol; Ca (mmol/l) = Calcium; IP (mmol/l) =Inorganic Phosphorus; Mg (mmol/l) = Magnesium; Cl (mmol/l) = Chloride

Table 6. Reference ranges proposed for haematic parameters of farmed sea bass in two different farming systems.

		Semi-Intensive		Intensive	
		12° percentile	87° percentile	12° percentile	87° percentile
Hct	%	26.3	41.6	28.7	40.9
GLU	mmol/l	2.70	8.50	2.30	6.70
TP	g/dl	3.10	6.40	3.20	6.00
Alb	"	0.90	1.70	0.80	1.80
GL	"	2.00	4.90	2.10	4.50
TCho	mmol/l	2.63	5.43	2.80	5.41
Ca	"	1.77	3.52	2.35	3.80
IP	"	2.07	3.71	2.36	4.75
Mg	"	0.29	0.57	0.33	0.74
Cl	"	96.4	129.50	111.60	151.50

Hct (%) = Haematocrit; GLU (mmol/l) = Glucose; TP (g/dl) = Total protein; Alb (g/dl) = Albumin; GL (g/dl) = Globulin; Tcho (mmol/l) = Total Cholesterol; Ca (mmol/l) = Calcium; IP (mmol/l) =Inorganic Phosphorus; Mg (mmol/l) = Magnesium; Cl (mmol/l) = Chloride

The comparison of variances that resulted from the regressions (Table 5) (carried out for the semi-intensive farming system only) indicated that the DO explains most of the variance in most of the blood parameters considered. As shown by the correlations, in fact, the dissolved oxygen plays an important role on Haematocrit, Glucose, Albumin, Chloride and Total Cholesterol blood content, while the oxygen saturation affects the values of Calcium and Magnesium only. Temperature and salinity seem to have a secondary role with respect to DO. The former affects the haematic values of Total Protein, Globulin and Magnesium while the latter affects Total Cholesterol, Inorganic Phosphorus and Magnesium.

As already described by Caldwell and Hinshaw (1994), the decreasing trend of Haematocrit as DO increases suggests, that exposure to hyperoxic conditions results in moderate anaemia (Edsall and Smith, 1990), but also confirms a capacity of the spleen of sea bass to adapt its blood cell producing activity to changes in environmental conditions, as found in trout by Wells and Weber (1990) and Pearson and Stevens (1991).

Conclusions

The effects of season on all the measured parameters are confirmed by the present study, but it seems that the age/weight of the fish does not affect the haematic content. This study has highlighted the importance of the rearing method and the evaluation of blood composition and has demonstrated the variability in the blood parameters even when using the same fish stocks. As confirmed by this study, the DO affects the values of most of the blood parameters, while Oxygen saturation, temperature and salinity seem to have a secondary role. Thus, the blood analyses should always consider the farming system and conditions.

The ranges proposed in Table 6 refer to the semi-intensive and the intensive systems separately and consider the means of the 75% of the sampled population for each parameter during the year. Values out of the given range cannot be considered pathological, but should prompt the repetition of the analyses, perhaps increasing the number of samples and the control of the common farming practices. It is should be noted that a slight increase in summer or decrease in winter of some parameters is normal. Despite the great variability observed, the proposed ranges must be taken as a first assessment of normal values, characteristics of sea bass and farming method.

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