Contribution to the definition of the metabolic profile of farmed rainbow trout *(Oncorhynchus mykiss)*

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ABSTRACT

The haematic outline of 339 rainbow trout *(Oncorhynchus mykiss)* from two different farms in the Lucca province was studied for a preliminary assessment of the metabolic profile of this species and for the investigation of the influence of some endogenous and exogenous factors on the variability of the studied parameters. The sampling time, as well as the weight, appears to have caused significant variations on most of the parameters analysed. The present study gives the annual means and the seasonal trends for each farm where the study was carried out.

Key Words: Metabolic profile, Blood parameters, Rainbow trout, *Oncorhynchus mykiss*.

RIASSUNTO

CONTRIBUTO ALLA DEFINIZIONE DEL PROFILO METABOLICO IN TROTE IRIDEE *(ONCORHYNCHUS MYKISS)* ALLEVATE

È stato studiato il quadro ematico di trote iridee (Oncorhynchus mykiss) *allevate in due aziende della provincia di Lucca per un totale di 339 campioni, tendente alla definizione del profilo metabolico della specie e per individuare l'influenza di fattori endogeni ed esogeni sulla variazione dei parametri studiati. Il periodo di prelievo, insieme al peso, sembra abbiano determinato modificazioni significative su quasi tutti i parametri analizzati. Vengono quindi fornite le medie annuali e gli andamenti mensili di entrambe le aziende in cui è stato condotto lo studio.*

Parole chiave: *Profilo metabolico, Parametri ematici, Trota iridea,* Oncorhynchus mykiss.

Introduction

In the '70s in Great Britain, Payne and colleagues (1970) introduced for the first time the idea of a "metabolic profile," (MP) considered as a set of haematic analyses meant to provide a better understanding of the species-specific physiological characteristics, especially with respect to nutritional and farming aspects. Through the MP analysis it is possible to identify functional disorders and to point out nutritional alterations that might not otherwise be noticed using traditional methods. The MP can be considered a valuable method in preventing deficiencies that affect the productive and reproductive performances of animals, at least in homeothermic animals (Cappa, 1988).

The MP determination makes it possible to validate the efficiency of a particular method of food rationing that has been adopted and, thus, to intervene on possible nutritional disorders (Cappa, 1988).

The practical utility of this diagnostic technique is thus clear, as it permits the verification of possible errors in the farming practice so that they

can be dealt with before they show up clinically (Melotti *et al.*, 2004).

The most difficult thing when establishing the MP of a species is the extreme variability of the blood parameters due to endogenous and exogenous factors: sex and age (Pickering, 1986; Bau *et al.*, 1994), weight (Sano, 1960; Martinez *et al.*, 1994), food (Jeney *et al.*, 1997) season and environmental conditions (Luskova, 1998; Kavadias *et al.*, 2003), photoperiod (Boujard *et al.*, 1993; Pavlidis *et al.*, 1997), physiological status (Railo *et al.*, 1985; Roche and Bogé, 1996), density (Melotti *et al.*, 2004) and genetics (Benfey and Biron, 2000) are all factors that affect the haematic outline of the fish and make the definition of normal values extremely difficult.

The literature on aquatic species is poor and often dated and the values reported from many authors disagree.

The rainbow trout haematic outline still has not been studied sufficiently and the aim of the present study is to contribute to a greater knowledge of the MP of this species.

Material and methods

Blood samples of 339 immature farmed rainbow trout (2N) were collected in two intensive farms in the province of Lucca (Italy); they are indicated as Farm 1 and Farm 2. The food used was the Select ME (Trouvit, Hendrix, 1:100) manually distributed twice a day (8:30 and 16:30) in both facilities. Farm 1 used additional oxygen during the hottest months. Farming conditions are reported in Table 1; both the farms are approved as free of Viral Haemorrhagic Septicaemia (VHS) and Infectious Haematopoietic Necrosis (IHN). Blood samples were drawn in the morning on each farm every 28-32 days, from November 2003 to October 2004. These samples were drawn from about 15 fish that had fasted for 12 hours.

The experimental subjects all belonged to the same batch and were different for each farm. On Farm 2 the drawings were interrupted in August because of the opening of a dam by the electric company. This dam caused much damage to plant life and resulted in the death of most of the animals used for the present study. Trout were randomly caught in the morning and anaesthetized (Ethylene glycol monophenyl ether, 0.4 cc/l); all fish were weighed (g) and measured for total length (cm). 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 (+4°C) and carried to the laboratory of the Dipartimento di Scienze Zootecniche (Università di Firenze, Italy) where plasma and serum were obtained by centrifugation with refrig-

Table 1. Rearing conditions on the two farms.

		Farm 1	Farm 2
Initial weight	g	52.50 ± 9.66	171.47 ± 33.67
Final weight	"	769.30 ± 238.50	377.30 ± 47.1
Initial length	cm	17.32 ± 1.18	25.91 ± 1.55
Final length	"	40.33 ± 3.99	32.77 ± 1.75
Cage size	m^3	430	430
Density	Kg/m^3	5-25	5-18
T	°C	4 to 18	2 to 16
pH		7.3-8.3	7.5-8.2
DO	mg/l	8.0-12.3	8.0-11.2

DO: Diluted Oxygen

Table 2. Tukey's test results: monthly and annual trends of the haematic outline on Farm 1.

	GLU (mmol/l)	TP (g/dl)	ALB (g/dl)	GL (g/dl)	TCho (mmol/l)
Nov 2003	bc	f	e	e	f
Dec 2003	abc	de	d	cd	e
Jan 2004	bc	ab	a	bc	de
Feb 2004	bc	cde	bc	de	cd
Mar 2004	bc	e	cd	de	e
Apr 2004	c	de	a	e	bc
May 2004	abc	cde	a	de	bc
Jun 2004	ab	ab	b	ab	abc
Jul 2004	bc	abc	bcd	abc	abc
Aug 2004	ab	bcd	bcd	abc	cd
Sept 2004	a	a	bc	ab	ab
Oct 2004	ab	a	bc	a	a
Annual mean	4.86 (CV=32.51%)	3.64 (CV=23.35%)	1.83 (CV=23.50%)	1.81 (CV=38.67%)	3.88 (CV=33.76%)

	Ca (mmol/l)	IP (mmol/l)	Mg (mmol/l)	Cl^- (mmol/l)
Nov 2003	d	g	e	b
Dec 2003	b	bc	abcd	a
Jan 2004	a	d	de	a
Feb 2004	a	def	abcde	a
Mar 2004	a	b	abcd	a
Apr 2004	b	cd	f	a
May 2004	a	a	e	a
Jun 2004	cd	efg	cde	a
Jul 2004	cd	fg	a	a
Aug 2004	cd	g	abc	a
Sept 2004	c	efg	ab	a
Oct 2004	cd	de	bcde	a
Annual mean	3.16 (CV=31.33%)	4.83 (CV=25.05%)	0.85 (CV=29.41%)	118.48 (CV=9.83%)

a,b,c: P< 0.05.
GLU = Glucose; TP = Total Protein; ALB = Albumin; GL = Globulin; TCho = Total Cholesterol; Ca = Calcium; IP = Inorganic Phosphorus; Mg = Magnesium; Cl^- = Chloride.

erated Centrifuge ALC 4227R (3000 rpm, 30 min, 0 ± 2°C). Samples were frozen at -20°C and the following analyses were performed with the spectrophotometer Mitsubishi Super Z-818 biochemical analyser using Sclavo Diagnostics International S.r.l. reagents:

- Serum Glucose (GLU): colorimetric determination with oxidase-peroxidase;
- Serum Total Protein (TP): colorimetric determination with biuret-tartrate;
- Serum Albumin (ALB): colorimetric determination with BCG;
- Serum Globulin (GL): calculated as [Total Protein - Albumin];
- Serum Total Cholesterol (TCho): enzymatic colorimetric determination;

Table 3. Tukey's test results: monthly and annual trends of the haematic outline on Farm 2.

	GLU (mmol/l)	TP (g/dl)	ALB (g/dl)	GL (g/dl)	TCho (mmol/l)
Nov 2003	d	d	d	e	c
Dec 2003	bcd	c	cd	d	ab
Jan 2004	de	b	c	bc	ab
Feb 2004	cd	b	b	cd	ab
Mar 2004	ab	cd	b	e	bc
Apr 2004	abc	c	cd	de	c
May 2004	e	b	a	cd	c
Jun 2004	a	a	ab	a	a
Jul 2004	a	ab	c	ab	bc
Annual mean	4.10 (CV=36.10%)	3.80 (CV=24.74%)	1.94 (CV=18.04%)	1.86 (CV=42.27%)	4.44 (CV=26.35%)
	Ca (mmol/l)	IP (mmol/l)	Mg (mmol/l)	Cl^- (mmol/l)	
Nov 2003	de	cd	bc	bcd	
Dec 2003	ab	b	c	bcd	
Jan 2004	e	b	b	bc	
Feb 2004	cd	d	b	cd	
Mar 2004	cd	bcd	bc	d	
Apr 2004	bc	cd	d	b	
May 2004	bc	a	d	a	
Jun 2004	e	bc	a	a	
Jul 2004	a	b	a	bcd	
Annual mean	4.04 (CV=28.71%)	5.17 (CV=17.60%)	0.97 (CV=36.08%)	119.08 (CV=4.95%)	

a,b,c: P< 0.05.
GLU = Glucose; TP = Total Protein; ALB = Albumin; GL = Globulin; TCho = Total Cholesterol; Ca = Calcium; IP = Inorganic Phosphorus; Mg = Magnesium; Cl^- = Chloride.

- 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.

Seasonality was analysed by the GLM considering the month as fixed effect. Means separation was computed by Tukey's test results. Simple regressions were also carried out to investigate the influence of the fish weight on variability of blood parameters for each farm.

Results and discussion

The GLM between months on Farm 2 showed a strong influence ($P < 0.05$) of the month factor on all the analysed parameters (Table 3): the Tukey's test outlined a seasonal trend with highest values concentrated between May and July (Figure 1,

Figure 1. Annual trend of the haematic parameters on Farm 1 and 2.

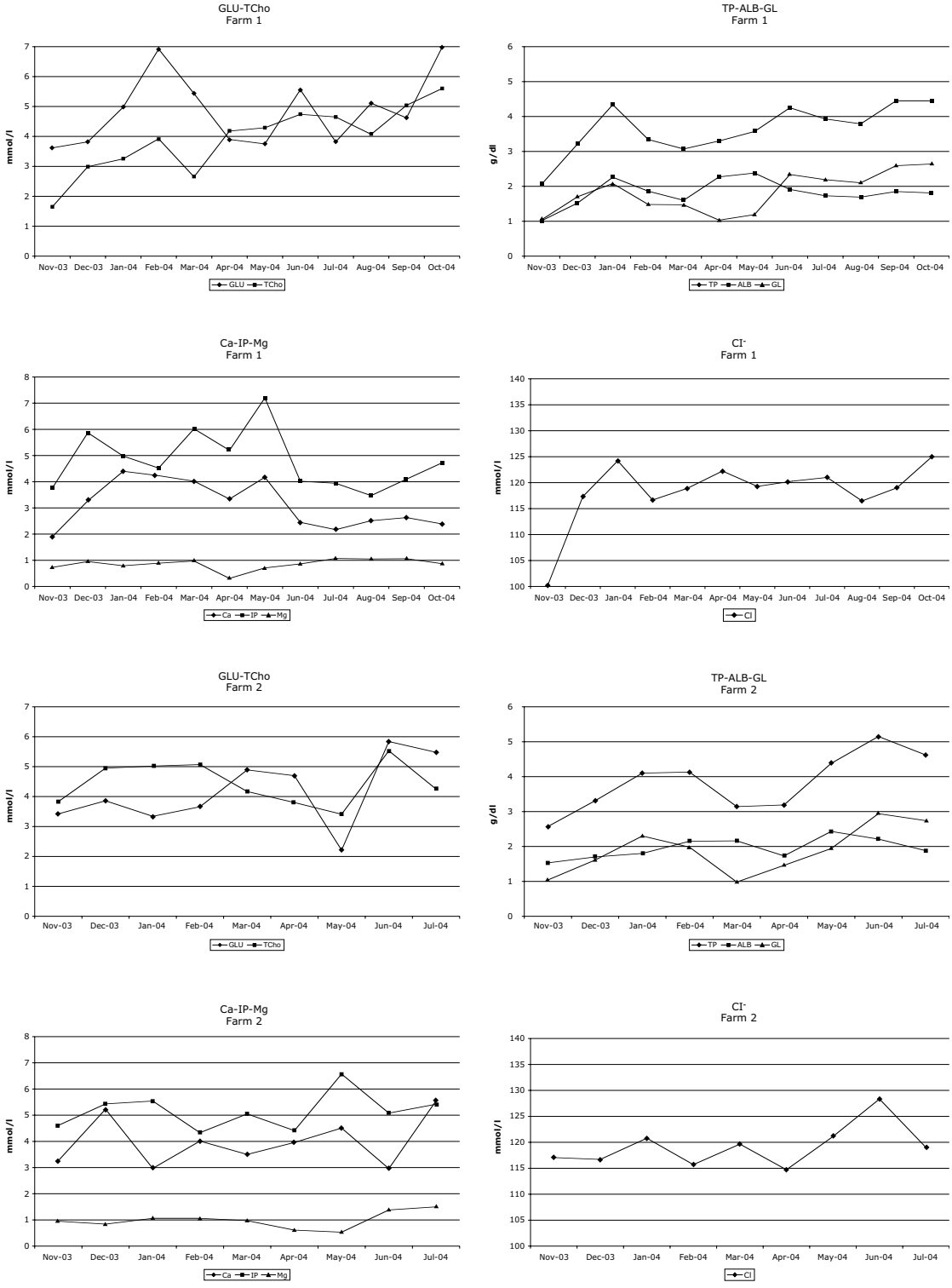

Table 4. Bibliographic values for the parameters analysed compared to values found in the present study.

	GLU (mmol/l)	TP (g/dl)	ALB (g/dl)	GL (g/dl)	TCho (mmol/l)
Farm 1 & Farm 2 (respectively)	4.86-4.10	3.64-3.80	1.83-1.94	1.81-1.86	3.88-4.44
Melotti *et al.*, 2004	2.55-4.15	2.52-3.16			2.43-5.83
Velíšek and Svobodova, 2004	6.85	2.92	0.40	2.52	1.97
Ceschia *et al.*, 1978	2.89-4.33	3.7-4.7			6.24-10.0
Giorgetti and Ceschia, 1977	3.05	3.4			5.26
McCarthy *et al.*, 1973/75	6.5-7.11	4.9-5.0			
Perrier *et al.*, 1978a	3.72	3.7			7.15
Jeney *et al.*, 1997	< 2.00				
Railo *et al.*, 1985	6.72-7.66				

Table 5. Bibliographic values for the parameters analysed compared to values found in the present study.

	Ca (mmol/l)	IP (mmol/l)	Mg (mmol/l)	Cl^- (mmol/l)
Farm 1 & Farm 2 (respectively)	3.16-4.04	4.83-5.17	0.85-0.97	118.48-119.08
Amend and Smith, 1974/75	5.0-5.4	4.30-4.97		115-120
Barnett *et al.*, 1979	8.8-10.1	2.16-5.94	2.3-2.8	
Ceschia *et al.*, 1978	2.8-3.4			120.00-129.0
Giorgetti *et al.*, 1977	5.00			122.00
Grant and Mehrle, 1973	6.5	1.49	2.7	122.00
Houston and Smeda, 1979	4.7		1.4	127
Madden and Houston, 1976	4.1		2.0	107
Perrier *et al.*, 1978b	2.6	2.87		132
Schreck et al., 1976	5.0		1.9	
Wedemeyer and Nelson, 1975	3.4-5.3	2.71	1.2-3.3	
Railo *et al.*, 1985				124.3-131.5
Zeitoun *et al.*, 1977	12.8	5.23	3.4	

Table 3). The same seasonality was not found on Farm 1, where the peaks in the haematic outline were spread over the year (Figure 1, Table 2), but a strong influence of the sampling period was found in any case ($P < 0.05$).

As shown in Table 2 and 3, the variability of the parameters is great, sometimes exceeding 30%, except for the Cl^-. The highest values in the annual means were found on Farm 2.

The absence of seasonality found on Farm 1 can be ascribed to the different management of the farm, even though the culture system was the same for both facilities. Farm 1, in fact, mitigated the effect of climate by adding oxygen to the culture water during the hot season. The lower annual means may demonstrate the effort of the fish in adapting their metabolism to the changing environmental conditions.

The fish weight, as resulted from the regressions carried out for each farm, can only partially explain the variability found in the haematic values: in both farms most of the parameters measured were influenced by the weight factor ($P < 0.01$), but some with very low r^2 values. On Farm 2 GLU, Ca, Mg and TCho resulted as not affected by the weight factor and within the parameters affected only TP and ALB demonstrated r^2 higher than 30% ($r^2 = 43.2\%$ and 31.9%, respectively). On Farm 1 GLU and Cl^- resulted not affected by the weight and the most affected parameters were TP ($r^2 = 32.8\%$), GL ($r^2 = 32.6\%$) and TCho ($r^2 = 48.8\%$). TCho and TP normally tend to increase with age (Sano, 1960) and the result for TCho on Farm 2 can be explained by the higher initial weight of the animals and the shorter period of sampling which reduced the differences between the samplings.

The comparison of the annual means found in the present study with the bibliographic values (Table 4 and 5) outlines an uncertain situation: the GLU, extremely variable even in the literature, fluctuates between <2 to 8 mmol/l. The TP showed steady values around 3 g/dl both in the present study and in the literature. The shortage of bibliographic data does not make it possible to infer a trend for ALB and GL. The values found in the present study disagree with Velíšek and Svobodova (2004), however we must take into consideration that the initial weight of the fish used by the authors was the half that of the fish used in the present study and that Sano (1960) reported an increase in the haematic TP related to the age.

The microelements are affected by many factors and their variability can be ascribed to the tendency of the organism to maintain an internal balance and to the opposite influence of the external factors in increasing or decreasing the amount of these parameters in the blood.

From the values reported in Table 2 and 3 and the monthly trends in Figure 1 the more constant values all throughout the year seem to be the Cl- within the microelements and the proteins (TP, ALB and GL) within the macroelements.

Conclusions

The influence of the sampling time on the haematic outline of rainbow trout is clear, independent of the farm management. The farms, which exert a certain control on the environment, seem to be able to mitigate their effect, resulting in more constant values throughout the year and in low or negligible seasonal effects. Thus, if the farming system is an important factor for the determination of the blood parameters, the farm management is also important, modifying the blood outline and its annual trends.

The parameters which suggest a certain stability, independent of the farm management, are the TP, the ALB and the GL which imply a possible use in the assessment of the health status of farmed rainbow trout.

A great variability of the blood parameters was also found in the annual means, which require further study to enlarge the dataset on the haematic outline and so to permit the identification of a normal range. The reported values require further, more in-depth studies, yet they can be considered representative enough for an initial haematic characterisation of the farmed rainbow trout and can contribute to the MP assessment of the entire species.

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