APPENDIX

Appendix I – Table of correspondence of sample codes, broodstock tank, and date.

Code	Date	Tank	New Code
FE29.03.06	29-03-2006	A4	**1**
FE04.04.06	04-04-2006	A4	**2**
FE1	06-04-2006	A4	**3**
FE2	07-04-2006	A1	**4**
FE3	07-04-2006	A4	**5**
FE4	09-04-2006	A4	**6**
FE5	10-04-2006	A4	**7**
FE6	11-04-2006	A4	**8**
FE7	12-04-2006	A4	**9**
FE8	14-04-2006	A4	**10**
FE9	16-04-2006	A4	**11**
FE10	17-04-2006	A4	**12**
FE11	18-04-2006	A4	**13**
FE12	21-04-2006	A4	**14**
FE13	22-04-2006	A4	**15**
FE14	23-04-2006	A4	**16**
FE15	25-04-2006	A4	**17**
FE16	26-04-2006	A4	**18**
FE17	27-04-2006	A4	**19**
FE18	28-04-2006	A4	**20**
FE19	30-04-2006	A4	**21**
FE20	01-05-2006	A4	**22**
FE21	08-05-2006	A4	**23**
FE22	09-05-2006	A4	**24**
FE23	16-05-2006	A4	**25**
FE24	17-05-2006	A3	**26**
FE25	17-05-2006	A4	**27**
FE26	18-05-2006	A3	**28**
FE27	18-05-2006	A4	**29**
FE28	19-05-2006	A3	**30**
FE29	19-05-2006	A4	**31**
FE30	22-05-2006	A4	**32**
FE31	05-06-2006	A4	**33**
FE32	06-06-2006	A4	**34**
FE33	07-06-2006	A4	**35**
FE34	09-06-2006	A4	**36**
FE35	22-06-2006	A4	**37**
FE36	24-06-2006	A4	**38**
FE37	25-06-2006	A4	**39**

Appendix II – Other protocols used

Adenylate Kinase assay (Bergmeyer, 1985)

A triethanolamine buffer was prepared by diluting 5,6g of triethanolamine in 50 ml of distilled and de-ionized water (DDW). The pH was set to 7.6. After preparing the buffer, misture 1 was prepared by adding to 100ml of buffer, 480mg of magnesium sulphate ($MgSO_4$), 481mg of potassium chloride (KCl) and 23.1mg of dithiotheirtol (DTT).

Solutions of NADH (2.8mg/ 0.9ml of DDW), adenosine triphosphate (ATP; 21mg/ 1.8ml of DDW) and an adenosine monophosphate (AMP; 19.4mg/ 2ml of DDW) were prepared.

The working solution was prepared by mixing 20ml of mixture 1, 10µl of pyruvate kinase, 10µl of Lactic dehydrogenase, 4 mg of phosphor-enol-pyruvic acid, 0.9ml of the NADH solution, 1.8ml of ATP solution and 3.9ml of DDW.

In a cuvette, mix 640µl of the working solution and 20µl of sample. Incubate for 10 minutes and then add 50µl of AMP. After 15 seconds, absorvance 1 was measured (Abs1) at 340nm. Samples were left to incubate for 5min. Then, absorvance 2 (Abs2) was measured in the same conditions as above. The difference between Abs2 and Abs1 is adenylate kinase and can be quantified as described in MDH assay.

Transaldolase assay (Bergmeyer, 1985)

Triethanolamine buffer was prepared by dissolving 1.87g triethanolamine in 100ml DDW. The pH of this solution was adjusted to 7.6, by adding NaOH or HCl;. Solutions of erythrose-4-phosphate (E4P; 2mg/ 638µl of DDW; E0377: SIGMA), NADPH or NADH; 2mg/ 638µl DDW; N4505: SIGMA) and fructose-6-phosphate (F6P; 33mg/ 2ml DDW; F3627: SIGMA) were prepared.

This assay was performed by mixing in a cuvette 638µl buffer, 13µl F6P, 13µl NADH, 13µl E4P, 0.2µl triosephosphate isomerase (T2507: SIGMA), 0.2µl glycerophosphate

dehydrogenase (G6751: SIGMA) and 19µl DDW. Twenty-five microliters of sample was added and, after 15seconds, absorvance 1 (Abs1) was measured in the spectrophotometer at the wavelength of 340nm. After measuring, the samples were incubated for 10min at room temperature, and then absorvance 2 (Abs2) was measured in the same conditions as above. Temperature was set for 25ºC.

The activity of the enzyme was determined by the decrease in absorvance (*i.e.* Abs1-Abs2<0), and the use of the formula described in the MDH assay.

Pyruvate Kinase (Bergmeyer, 1985)

Trizma buffer was prepared by diluting 1.21g/ 10ml DDW. The pH of this solution was adjusted to 8.0 by adding 0.1M NaOH or0.1M HCl. Solutions of KCl; 7.456g/ 50ml DDW), NADH (2.8mg/ 2.4ml DDW; N4505: SIGMA), $MgCl_2$ (2.033g/ 100ml DDW), adenosine diphosphate (ADP; 17mg/ 1.2ml DDW; A2754: SIGMA) and phospho-(enol)-pyruvic acid (PEP; 14mg/ 1.4ml DDW: P0564: SIGMA) were prepared. Working solution was prepared by adding 2ml buffer, 2.4ml KCl solution, 2.4ml $MgCl_2$, solution, 1.2ml ADP solution, 2.4ml NADH, 10µl LDH and 10.2ml DDW.

The assay was performed by adding in a cuvette, 700µl of working solution and 20µl of sample and left to rest for 10min at room temperature. After this, 40µl PEP was added and 15 seconds later the first absorvance (Abs1) was measured in the spectrophotomer at 340nm. The samples were incubated for 5min, and after this period Abs2 was measured, in the same conditions as above. The activity of the enzyme was verified by the decrease in absorvance. The quantification of the enzyme activity was made by using the formula described in MDH assay.

Glucose-6-Phosphate & Fructose-6-Phosphate (Lahnsteiner & Patarnello, 2004a)

A buffer was prepared by dissolving 5.6g triethanolamine (T58300: SIGMA) in 50ml DDW. The pH of this solution was adjusted to 7.6. Solutions of $MgCl_2$ (1g/ 10ml DDW), NADP (10mg/ 500µl DDW; N5755: SIGMA), glucose-6-phosphate dehydrogenase (G6PDH, G7879: SIGMA; 400 units/ 400µl diluted buffer, 1:1 in DDW) and a

phosphoglucose isomerase (PGI, P2338: SIGMA; 10µl/ 400µl diluted buffer, 1:1 in DDW) were prepared.

Working solution was prepared by sample adding 340µl buffer, 7µl NADP solution, 7µl $MgCl_2$ and 300µl DDW. To perform the assay 20µl sample were added to 655µl working solution, in a cuvette and were left to incubate for 5 minutes. Absorvance 1 was then measured at 340nm. After the measurement of absorvance 1 was added to each cuvette 5µl G6PDH, the enzyme responsible for the degradation of glucose-6-phosphate. Some samples were checked in intervals of 5 minutes, until the end of the increase in absorvance, which took between 20-40 minutes. When the increase in absorvance ceased, Abs2 was measured at 340nm.

Having Abs2 been measured, 5µl of PGI were added. This enzyme acts in a reaction in which fructose-6-phosphate is the last metabolite). Again absorvance increased, and the samples underwent the same control of absorvance as described in the former paragraph. After this, Abs3 was measured in the same conditions of the prior measurements described for this assay. The presence of glucose-6-phosphate was determined by the difference between Abs2 and Abs1 (*i.e.* Abs2-Abs1>0) and the presence of fructose-6-phosphate by the difference between Abs3 and Abs2 (*i.e.* Abs3-Abs2>0). The concentration of these metabolites was calculated with the formula described below:

$$\mathbf{conc} = \frac{\mathbf{\Delta A \times V \times 1000}}{\mathbf{\varepsilon \times d \times v}}$$

In this expression, **ΔA** is the change in absorbance in the measured time interval, **V** is total assay volume (µl), **ε** is the absorption coefficient for NADH/NADPH, which is 6.3×10^3, **Δt** is the measured time interval in minutes, **v** is the sample volume (µl) and **d** is distance of the light path through the cuvette (=1 cm). The units of the concentration obtained are µmol/ l.