Journal of Thermal Analysis, Vol. 33 (1988) 1019–1026

DIRECT CALORIMETRY ON FREE SWIMMING GOLDFISH AT DIFFERENT OXYGEN LEVELS

J. van Waversveld, A. D. F. Addink, G. van den Thillart and H. Smit

DEPARTMENT OF ANIMAL PHYSIOLOGY, GORLAEUS LABORATORIES, STATE UNIVERSITY OF LEIDEN, P. O. BOX 9502, 2300 RA LEIDEN, THE NETHERLANDS

Heat production and oxygen consumption of groups of fasting, dark acclimated free swimming goldfish were measured at 20 °C during normoxia and anoxia. For this purpose a special 1 liter flow through microcalorimeter was constructed. It appeared that the normoxic values could be established at any time 2 days after introduction of the fish into the calorimeter, 700 j/h/MW and 1.6 mmoles/h/MW respectively. The normoxic oxycalorific value of 20 kJ/l O_2 indicates the use of a mixed substrate for oxidation. During anoxia, heat production was reduced to 30% of the normoxic level; 200 J/h/MW.

Goldfish (Carassius auratus, L.) at rest tolerate hypoxic and anoxic conditions, e.g. they can survive up till 16 hours of anoxia at 20°, using acetaldehyde instead of oxygen as electron acceptor, producing ethanol [1, 2]. The metabolic mechanism of this anaerobic ethanol production is still not completely clarified. Heat production during anoxia as an absolute measure of anaerobic metabolic rate will be used, together with biochemical data on metabolite concentrations and excretion products to solve the metabolic pathway of ethanol production. Direct calorimetry is the only available method to determine anaerobic heat production. Aerobic heat production, however, can be measured by both direct and indirect carolimetry.

Direct calorimetry on fish is sparse: Davies [3], Smith et. al. [4, 5] and Lowe quoted by Brafield [6]. Of these, only Davies measured heat production of goldfish. Davies used groups of 3 fed goldfish (mean weight 6.23 g) and measured heat production under preabsorptive conditions during daylight (0.71 cal/h/g), within 8 h after placing the fish into the calorimeter. As also concluded by Davies, these conditions do not result in measurement of basal metabolic rate. According to Van den Thillart [7] there is still an influence of handling of the fish on the metabolic rate. By simultaneous measurement of heat production and oxygen consumption, the oxycalorific value (kJ/l O_2) can be determined. This oxycalorific value can give an indication of the substrate used for oxydation. According to Elliot and Davison [8] the oxycalorific values for the 3 main substrates for oxydation are: carbohydrate 21.10, fat 19.60 and protein (ammonioteles) 19.13 kJ/l O_2.

John Wiley & Sons, Limited, Chichester
Akadémiai Kiadó, Budapest

So, for a mixed substrate for oxydation the oxycalorific value will be between 19.13 and 21.10 kJ/l O_2.

This paper deals with the results of heat production and oxygen consumption measurements of goldfish during normoxia and anoxia at 20°.

Methods

Fish

Ten healthy goldfish were kept in a flow through tank with air saturation at 20° and a 14 h light period. For each of the 7 experiments 4 fish were selected at random and afterwards they were replaced into the tank, after the selection of the next 4 fish.

The fish were kept on a low feeding level (trouvit pellets, Trouw, The Netherlands) to prevent rapid growth. During the first experiment (May, 1986), the mean weight of the group of 4 fish was 8.4 ± 0,9 g and during the last one (October, 1986) 9.8 ± 1.4 g. Before each experiment the 4 selected fish were kept in a vessel, identical to the calorimeter measurement vessel, to adapt the fish to fasting and the continously dark experimental conditions for approximately 4 days. One day before transferring the fish into the calorimeter, the fish were weighed. After removing the fish from the calorimeter, the were weighed again. The mean of both weights was used as experimental weight. Before the first experiment, all the animals were placed at least once into the calorimeter, to achieve an acquired behaviour during the experiments.

Heat production

Heat production measurements were performed stress free with a specially designed 1 liter flow through microcalorimeter (Sétaram GF108). As the calorimeter system will be described elsewhere [9], here only the main specifications will be summarized (Fig. 1). Air (or nitrogen for anoxia experiments) saturated water is pumped through a heat exchanger, for temperature stabilization, into the calorimeter. Final equilibration with the temperature of the calorimeter is established by means of three heat exchangers inside the calorimeter. The calorimeter is of the differential type to eliminate environmental influences. The differential signal of the heatflux detectors is

amplified and recorded on paper and stored into a computer for further calculations. The flow through the calorimeter was ca. 50 ml/min. The sensitivity of the calorimeter depends on the flow through the measuring vessel [9], and was established before and after each experiment with an exactly known electrical power of ca. 10 mW in the measurement vessel (ca. 87 μV/mW). The calibration was checked at the beginning and at the end of each experiment during the base line check (Fig. 2). The sensitivity was fed into the computer, after which the computer calculated the heat flow of base line, calibration check and fish in mW (Fig. 2). The heat production of the fish was calculated as the difference between the heat flow of the base line and the fish.

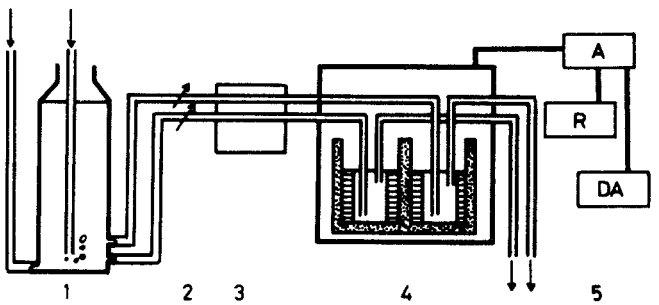

Fig. 1 Scheme of the calorimeter and its flow through system.
1. gas saturation (normoxia: air, anoxia: nitrogen); 2. pumps; 3. heat exchanger for temperature stabilization; 4. calorimeter (final temperature equilibration by means of 3 heat exchangers inside the calorimeter); 5. amplification, recording and data aquisition.

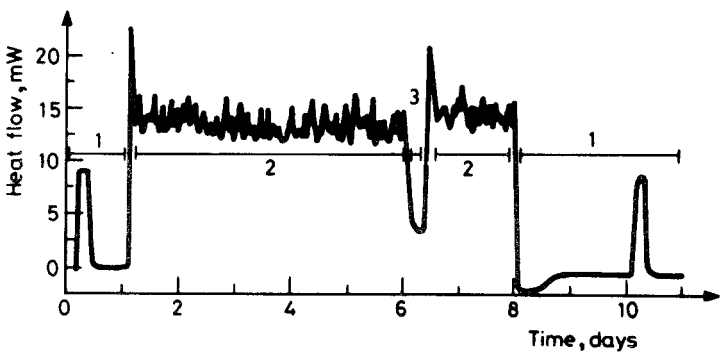

Fig. 2 Computer registration of an experiment.
1. base line and calibration check; 2. normoxia; 3. anoxia.

Oxygen consumption

For the oxygen consumption measurements, samples (ca. 2 ml/min) of the outflowing water (ca. 50 ml/min) of the measurement vessel were flown through a thermostated electrode cell (Radiometer, Denmark, D616), alternating with outflowing water of the reference vessel. The oxygen electrode (Radiometer, Denmark, E5046–0) was calibrated with air saturated (100% O_2) and nitrogen saturated (0% O_2) water of the reference vessel. The 100% O_2 level was determined with a Winkler oxygen titration: 100% O_2 = 8.86 ppm O_2 at 760 mm Hg and 20°. The oxygen electrode was connected to a Radiometer, Denmark, Digital Acid Base Analyser PHM 72 with a PO_2 module PHA 932. The oxygen concentration readings were recorded on paper. The concentration of the outflowing water of the reference vessel was taken as the concentration of the inflowing water of the measurement vessel. The oxygen consumption was calculated as the difference of the oxygen concentrations of the in- and outflowing water of the measurement vessel, multiplied by the flow through the measurement vessel.

The experiment

In Fig. 2 a computer registration of an experiment is shown. On day 1, the fish were introduced into the calorimeter. Nitrogen saturation on day 4 (5 experiments) or day 6 (2 experiments, as shown in fig. 2). Anoxia was reached after 3 h of nitrogen saturation. A stable anoxic heat production was reached after 1.5 h anoxia (this pattern is in accordance with the pattern found by Anderson, [10]) and lasted 2.5 h. Two days after anoxia, the fish were removed from the calorimeter. As also found by Van den Thillart [7], the influence of handling of the fish lasted approximately 2 days, while the stabilization of the calorimeter after introduction of the fish only took 12 h. Therefore, normoxic heat production calculations before anoxia were made after $2^3/_4$ days over a period of 6 h. The 2 experiments with 5 days before, anoxia served to check a further decline of heat production after 2 days. In that case heat production calculations were also made after $4^3/_4$ days. Anoxic heat production calculations were made over the last 2 h anoxia. After anoxia it took approximately 6 h before a stable normoxic level was reached again. Therefore, normoxic heat production after anoxia was calculated 7 h after over a period of 6 h. The normoxic heat production after anoxia was calculated also $1^1/_2$ days after anoxia.

Oxygen consumption measurements were performed over the same periods as decribed before for the calculations of the normoxic heat production; during anoxia, oxygen consumption was obviously zero.

All results were related to the metabolic weight (MW): $MW = \sum_{i=1}^{n} W_i^a$ (W in kg). According to Beamish and Mookherjii [11] and Ricker [12] for goldfish $a = 0.85$).

Statistical analysis was performed with the Wilcoxon signed rank test.

Results and discussion

In Table 1 the results of the heat production and oxygen consumption measurements are summarized. In every respect there is no significant ($\alpha > 10\%$) difference between the normoxic values of heat production ($\dot{H}$), oxygen consumption ($\dot{V}_{O_2}$) and oxycalorific value ($\dot{H}/\dot{V}_{O_2}$). This implies that normoxic heat production and oxygen consumption can be measured at any time 2 days after introduction of the fish into the calorimeter. The mean values of all normoxic values are also given in Table 1.

For 4 fish of 8.9 g the normoxic heat production value (706 J/h/MW) can be recalculated to 0.34 cal/h/g, which is approximately half the value given by Davies [3]; 0.71 cal/h/g. This lower heat production value is in accordance with the method of Davies: mesurement of heat production within 8 h after placing the fish into the calorimeter. Because of this stressing method Davies measured a raised heat production level.

Table 1. Heat production and oxygen consumption of goldfish at 20 °C. W (weight) = 8.9 ± 1.1 g; MW (metabolic weight) = $\sum_{i=1}^{n} W_i^{0.85}$ (W in kg)

Period	$\dot{H}$, J/h/MW	$\dot{V}_{O_2}$, mmoles/h/MW	$\dot{H}/\dot{V}_{O_2}$, kJ/lO$_2$
$2^3/_4$-3 days after introduction	683 ± 87	1.53 ± 0.23	20.1 ± 1.5
$4^3/_4$-5 days after introduction	646 ± 73	1.48 ± 0.01	19.5 ± 2.1
last 2 h anoxia	201 ± 15	0	X
7–13 h after anoxia	719 ± 61	1.63 ± 0.19	19.8 ± 1.8
$1^1/_2$–$1^3/_4$ days after anoxia	734 ± 80	1.61 ± 0.13	20.0 ± 1.2
Normoxia	706 ± 77	1.58 ± 0.18	19.9 ± 1.5
Anoxia	201 ± 15		

The normoxic oxygen consumption (1.58 mmoles/h/MW) can be recalculated for 4 fish of 8.9 g to 6.3 mg/h/100 g, according to the method of Van den Thillart [7], who corrected the oxygen consumption with a metabolic weight power $a = 0.8$ to that of a 100 g fish. Van den Thillart found a nightly oxygen consumption rate of 8 mg/h/100g, which is 27% more than the above calculated oxygen consumption rate of 6.3 mg/h/100 g. On the other hand, recalculation of the result of Van den Thillart with a metabolic weight power $a = 0.85$ gives 1.77 mmoles/h/MW, which is only 12% more than the presently found oxygen consumption rate of 1.58 mmoles/h/MW. This reduction of the difference between the result of Van den Thillart and the present result indicate that the metabolic weight power $a = 0.85$ gives a better relation between weight and metabolic rate. The higher oxygen consumption rate found by Van den Thillart can be explained in 3 ways. First, the metabolic weight power $a = 0.85$ gives a better relation between the result of Van den Thillart and the present result. However, based on a comparison of the result of Van den Thillart and the present result, a metabolic weight power $a = 0.9$ can be calculated, which is very well possible, according to Beamish and Mookherjii [11], who gave $a = 0.85$ as a mean value. Second, Van den Thillart measured the fish only once, which may have caused a minor raise in oxygen consumption, because of the fact that the fish were not used to the experimental conditions, while the present result was obtained with fish that were used to the experimental conditions before the first experiment. Unfortunately, no calculations were made during those tests. Third, the animals used by Van den Thillart were acclimated to a day and night cycle and it is possible that the oxygen consumption rate of these animals is different from that of animals which are acclimated to continously dark conditions.

The mean normoxic oxycalorific value of approximately 20 kJ/l O_2 indicates the use of a mixed substrate (carbohydrate, fat and protein) for oxydation.

During anoxia, heat production is reduced to approximately 30% of the normoxic value. Based on metabolite concentration measurements, Anderson [10] estimated a reduction of the heat production during anoxia to 20% at 18° and 30% at 5°. According to calculations by Van de Thillart [13], heat production during anoxia is about 30% at 20°, which is in good agreement with the present result. As discussed by Van den Thillart [14], the normoxic heat production levels of Anderson were probably above maintenance level (Anderson does not mention them), which could account for the lower relative anoxic heat production level.

Two additional experiments were performed, in which the 10 fish were divided into 2 groups of 5 fish at random. One group was taken out of the calorimeter during normoxia, without an anoxic period. The other group was taken out of the calorimeter at the end of an 8 h anoxic period. Both groups were analysed on metabolite concentrations of whole fish. After 8 h of anoxia the creatine-phosphate and glycogen concentrations, both part of the energy stock, had decreased. The concentrations of the end products of anaerobic metabolism, lactate and ethanol had increased. Most of the produced ethanol, however, is excreted into the water. The glucose concentration had increased as a result of the mobilization of glycogen.

It appeared that the stable anoxic heat production period, which was reached after ca. 1.5 h anoxia, proceeds not only for 2.5 h, but also for 8 h. Anderson [10] also found a stable heat production soon after the onset of anoxia, which lasted 9 h at 18° or 20 h at 5°.

* * *

The investigations were supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

1 E. A. Shoubridge and P. W. Hochachka, Science, 209 (1980) 308.
2 G. van den Thillart and A. van Waarde, Mol. Physiol., 8 (1985) 393.
3 P.M.C. Davies, Comp. Biochem. Physiol., 17 (1966) 983.
4 R. R. Smith, G. L. Rumsey and M. L. Scott, J. Nutr., 108 (1978) 1017.
5 R. R. Smith, G. L. Rumsey and M. L. Scott, J. Nutr., 108 (1978) 1025.
6 A. E. Brafield, in P. Tytler and P. Calow (ed.), Fish Energetics; New Perspectives, Croom Helm, London, 1985, p. 257.
7 G. van den Thillart, Ph.D. Thesis, University of Leiden, The Netherlands, 1977.
8 J. M. Elliot and W. Davison, Oecologia (Berl.), 19 (1975) 1985.
9 A. D. F. Addink, G. van den Thillart, H. Smit and J. van Waversveld, Nature, submitted (1987).
10 J. R. Anderson, Ph. D. Thesis, Australian National University, 1975.
11 F.W.H. Beamish and P. S. Mookherjii, Can. J. Zool., 42 (1964) 161.
12 W. E. Ricker, J. Fish. Res. Bd. Can., 30 (1973) 409.
13 G. van den Thillart, F. Kesbeke and A. van Waarde, Comp. Biochem. Physiol., 55A (1976) 329.
14 G. van den Thillart, Mol. Physiol., 2 (1982) 49.

Zusammenfassung – Die Wärmeerzeugung und der Sauerstoffverbrauch von Gruppen fastender, dunkelakklimatisierter freischwimmender Goldfische wurden bei 20 °C unter normalem Sauerstoffangebot (Normoxie) und in sauerstoff-freiem Wasser (Anoxie) gemessen. Hierfür wurde ein spezielles 1-Liter-Durchfluss-Mikrokalorimeter konstruiert. Anscheinend werden die Werte bei Normoxie (700 J h^{-1}/Stoffwechselgewicht bzw. 1.6 mmol h^{-1}/Stoffwechselgewicht) zu jeder Zeit später als 2 Tage nach dem Einsetzen der Fische in das Kalorimeter eingestellt. Der oxykalorische Wert von 20 kJ/l O_2 deutet auf den Einsatz eines Substratgemisches bei der Oxidation unter Normoxie. Bei Anoxie vermindert sich die Wärmeerzeugung auf 30% des Wertes bei Normoxie: 200 J h^{-1}/Stoffwechselgewicht.

РЕЗЮМЕ — Тепловыделение и потребление кислорода группой хорошо акклиматизированных своборно плавающих золотистых рыбок было измерено при 20° при нормальном уровне кислорода и при его недостатке. Для этой цели был осуществлен однолитровый поток через калориметр. Показано, что при нормальном уровне кислорода, величины теплот и потребления кислорода, установленные спустя два дня после впуска рыбок в калориметр, составляли, соответственно 700 дж·час$^{-1}$·МВ$^{-1}$ и 1,6 ммоль·час$^{-1}$·МВ$^{-1}$. Нормальное окситеплотворное значение, равное 20 кдж/1 O_2, указывает на использование смешанного субстрата в процессе окисления. При кислородном голодании тепловыделение понижалось до 30% от нормального уровня и составляло 200 дж·час$^{-1}$·МВ$^{-1}$.