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THE EFFECTS OF TEMPERATURE ACCLIMATION ON GROWTH PERFORMANCE AND SOME HAEMATOLOGICAL ASPECTS OF CARP CYPRINUS CARPIO

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ABSTRACT

*Growth performance and some haematological aspects of carp, **Cyprinus carpio** were evaluated under laboratory conditions for fish acclimated at three different temperatures (20, 25 and 30°C). Fish were maintained for 42 days at ration of 1% wet body weight per day (limited energy). The mean survival rate of fish reared at different temperatures were 100%. There was a statistically significant effect of temperature on growth performance. The optimum temperature for best growth performance was achieved at 20°C. On the other hand, blood constituents were measured in an attempt to identify the effects of exposure to different temperatures. Plasma total protein, total lipid, triglyceride, cholesterol and sodium ions all decreased with increasing temperature while red blood cell counts, haemoglobin content and plasma glucose levels were increased. It is concludea that, an increase of acclimation temperature can place a stress of considerable magnitude on the homeostatic mechanisms of fish.*

INTRODUCTION

Temperature has been shown to be one of the most important environme factors affecting the growth of fishes. Temperature affect several feed related factors on fish including digestion rate (Jobling ***et al.***, 1977); satia time (Grove ***et al.***, 1978); appetite (Waiwood, 1978); feed frequenc maximum meal size (Gwyther and Grove, 1981) and finally metaboli

(Rice, 1990). An increase in temperature should lead to increased maintenance requirements and food intake. Therefore, the rate of growth will vary with the ability of the fish to digest more food than is required for maintenance. The expected relationship between temperature and growth is that there will be little or no growth below a certain temperature, above this, the growth rate should increase with temperature to a maximum and then decrease, perhaps-becoming negative at temperature, approaching the lethal limit. This relationship was found by Al-Jerian and Younis (1998) for ***Oreochromis mossambicus*** with an optimum temperature for rapid growth at 25°. For *Salmo trutta*, however, the relationship is different. Brown (1957) found two optimum temperature ranges for two-year-old trout, 7-9°C and 16-19°C. Below, between and above these temperatures, the growth rate were lower. Relatively little quantitative information is available concerning the thermal ecology of carp particulary with respect to growth performance and haematological aspects. These parameters are currently recognized as a sensitive indication of physiological condition and water quality (Murray, 1984; Rice, 1990).

The prime purpose of this study is to determine how temperature affects growth performance and some haematological aspects of carp under limited source of energy to a better understanding of the temperature requirements of this species.

MATERIALS AND METHODS

Specimens of carp, ***Cyprinus carpio***, weighing 101.4 + 4.9g, were obtained from Dolna Odra, Szczecin, Poland and acclimated under appropriate experimental conditions for at least two weeks at three different temperatures (20, 25 and 30°). Only those individuals that appeared healthy at the end of acclimation were used in the tests. Ten fish were randomly distributed in three glass aquaria containing 200L of aerated dechlorinated tap-water. The physico-chemical characteristics of water in each aquarium (according to the Standard Methods for the Examination of Water and Wastewater, 1975) are shown in Table (1). The fish were fed on a diet containing 35% crude protein and 421.5 Kcal/100g. The ingredients and composition of the pellet feed are shown in Table (2). Fish were fed two times daily (9.00 an and 14.00pm) for seven days a week at a rate of 1% of their wet biomass per day (limited energy) and readjusted bi-weekly after the biomass of fish in each aquarium was

Table (1): The physico-chemical characteristics of water in each different temperature acclimation

Parameter	Temperature		
	20°C	25°C	30°C
pH	7.89	8.12	8.33
Conductivity μohm/cm^2	457.0	462.0	444.0
T. Hardness (mg/l)	4.5	4.2	4.3
T. Solid (mg/l)	678.0	655.0	639.0
S. Solid (mg/l)	12.0	10.0	10.0
D. Solid (mg/l)	666.0	645.0	629.0
Biochemical oxygen demand (mg/l)	6.20	7.80	8.20
Chemical oxygen demand (mg/l)	24.0	32.0	40.0
Ammonia (mg/l)	0.982	1.056	1.543
Nitrite (mg/l)	0.012	0.014	0.015
Nitrate (mg/l)	0.786	0.798	0.812
Sulphate (mg/l)	32.6	28.4	33.7
Phosphate (mg/l)	0.023	0.031	0.042
Chloride (mg/l)	71.0	71.0	71.0

Table (2): Chemical analysis (%) in dry matter of the experimental diet

Item	%
A) Chemical analysis:	
Crude Protein	35.0
Lipid	9.0
Nitrogen free extract	33.0
Fiber	4.0
Ash	10.0
Total phosphorus	1.3
Calculated gross energy (Kcal/100g)	421.5
B) Additives:	
Vitamie A (IE/Kg)	2500
D (IE/Kg)	500
E (mg/kg)	100
Ethoxyquin (mg/kg)	100

determined. Accumulated wastes were removed and fixed amount of water was exchanged daily over the experimental period (42 days).

By the end of the experiment, fish in each aquarium were netted, counted and weighed. Body composition analysis were performed using standard AOAC (1980). Gross energy of the diets as well as carcass energy count was estimated according to NRC (1993).

The following formulae were used in these experiments :

Relative growth rate = Weight changes for specific time period / initial weight X 100
Average daily gain = Average body gain / experimental period
Specific growth rate = Ln final weight – Ln initial weight / time in days
Condition factors = Weight X100 / Length ^{3}
Feed conversion ratio = g. dry feed / g . body gain
Feed efficiency ratio = g . body gain / g . dry feed
Protein efficiency ratio = g . body gain / g. protein intake
Protein productive value = g . protein gain / g . protein intake X100
Energy utilization = Energy gain / energy intake X100

To study the effects of different temperatures on some haematological aspects of carp, blood was collected directly from the caudal artery into heparinized capillary tubes, then pooled in eppendorf tubes. Red blood cell counts (RBC's) were counted according to the method of Kokot (1969). Haemoglobin content (Hb) was measured according to Wintrobe (1981). Plasma glucose, total lipid, triglyceride, cholesterol, total protein and sodium ions were measured using Roche Diagnostic Systems (COBAS INTEGRA).

Statistical analysis of data was computed by the analysis of variance and the least significant differences (LSD) between means according to Snedecor and Cochran (1974).

RESULTS

The mean survival rate of carp reared at different temperatures (20, 25 and 30°) was 100% which means that thermal stress is not obvious i.e. the fish was feeding properly and no external signs of abnormal behaviour.

As shown in Table (3), the differences in growth performance of carp reared at 20, 25 and 30°C were statistically significant ($P< 0.01$). Fish reared at 20° grew better than those reared at 25°C and 30° e.g. relative growth rate for fish reared at 20°C was 26.8% while those reared at 25°C and 30°C were 22.5% and 20.7%, respectively. Average daily gain (ADG), specific growth rate (SGR %) and the fish condition (K_f) showed the same trend . Feed and nutrient utilization showed also significant variations ($P < 0.01$) between treatments The best feed conversion ratio (FCR), feed efficiency ratio (FER), efficiency of protein utilization in term of protein productive value (PPV %) and protein efficiency ratio (PER) as well as the utilization of energy (EU %) all were achieved at 20°C. Chemical composition of carps showed also significant differences ($P < 0.01$) when reared at different temperatures. Fish reared at 20°C had the highest value of crude protein (21.3 %), lipid (1.4 %), dry matter (23.2 %) and gross energy (573.4 Kcal/100 g).

As shown in Table (4), red blood cell counts (RBC's) and haemoglobin content (Hb) of fish reared at 20°C were 1.870 million/ml and 7.81g/100 ml. A rise in water temperature to 25°C and 30°C exhibited a significant increases in RBC's counts to 2.040, 2.133 million/ml and Hb content to 8.48, 8.75 g/100 ml respectively. Fish acclimated at 20°C had mean plasma glucose levels of 102.8 mg/100 ml. An increase of temperature to 25°C resulted in an increase of plasma glucose levels to 119.4 mg/100 ml. This change was statistically insignificant ($P > 0.05$). On the other side, a rise of temperature to 30°C exhibited a significant ($P < 0.01$) disturbance in fish carbohydrate metabolism as shown by the developed hyperglycemia (134.3 mg/100 ml). Plasma total lipid, triglyceride and cholesterol as an indicator of lipid metabolism decreased significantly ($P < 0.01$) with increasing temperature from 746.4, 291.9, 168.0 mg/100 ml at 20°C to 629.8, 214.8, 154.6 mg/100 at 25°C and to 597.9, 191.2, 148.1 mg/100 ml at 30°C, respectively. Plasma total protein showed also the same trend where it decreased significantly ($P < 0.01$) from 3.0 g/100 ml at 20°C to 2.5 and 2.3 g/100 ml at 25°C and 30°C. Finally, significant ($P < 0.01$) reduction in plasma sodium ions (127.6 mmol/L) showed in fish adapted to 30°C compared to those adapted at 20°C and 25°C (137.9 and 131.6 mmol/L).

Table (3): Effects of different water temperature acclimation on growth, feed utilization and body composition of carp, *Cyprinus carpio*

Item	Water temperature acclimation			LSD	
	20°C	25°C	30°C	P< 0.05*	P< 0.01**
A) Growth:					
Relative growth rate (%)	26.8 ± 0.28	22.5 ± 0.27**	20.7 ± 0.33**	0.275	0.371
Average daily gain (ADG)	0.65 ± 0.04	0.65 ± 0.06**	0.52 ± 0.06**	0.047	0.064
Specific growth rate (SGR%)	0.57 ± 0.04	0.48 ± 0.03**	0.45 ± 0.05**	0.035	0.047
Fish condition factor (k_f)	1.904 ± 0.02	1.786 ± 0.05**	1.771 ± 0.03**	0.034	0.047
B) Feed utilization:					
Feed conversion ratio	2.58 ± 0.20	3.30 ± 0.20**	3.53 ± 0.52**	0.319	0.429
Feed efficiency ratio	0.58 ± 0.03	0.50 ± 0.03**	0.46 ± 0.04**	0.031	0.042
Protein efficiency ratio	1.64 ± 0.03	1.41 ± 0.02**	1.31 ± 0.02**	0.07	0.094
Protein productive value	63.0 ± 0.62	51.2 ± 0.46**	44.7 ± 0.46**	1.515	2.041
Energy utilization	31.4 ± 0.62	24.4 ± 0.44**	19.4 ± 0.48**	1.527	2.059
C) Body composition (%):					
Crude protein	21.3 ± 0.53	20.3 ± 0.38**	19.7 ± 0.38**	0.401	0.540
Crude lipid	1.4 ± 0.15	1.2 ± 0.15**	0.9 ± 0.10**	0.125	0.169
Water content	76.8 ± 0.49	77.7 ± 0.37**	78.3 ± 0.45**	0.403	0.543
Dray matter	23.2 ± 0.49	22.2 ± 0.38**	21.7 ± 0.49**	0.425	0.573
Ash	7.2 ± 0.39	7.4 ± 0.55	8.15 ± 0.43**	0.368	0.496
Gross energy (Kcal/100g)	573.4 ± 3.3	566.2 ± 2.8**	550.2 ± 2.6**	2.664	0.590

Average of 10 fish ± standard error

**** Significant differences (P < 0.01) in comparison to 20°C.**

Table (4): Effects of different water acclimation on some haematological aspects of carp, *Cyprinus carplo*

Item	Water temperature acclimation			LSD	
	20°C	25°C	30°C	P< 0.05*	P< 0.01**
RBC'S (million/ml)	1.870 ± 0.04	2.040 ± 0.04*	2.133 ± 0.06**	0.127	0.171
Haemoglobin (g/100ml)	7.81 ± 0.21	8.48 ± 0.19*	8.75 ± 0.22**	0.596	0.803
Total protein (g/100ml)	3.0 ± 0.11	2.5 ± 0.14**	2.3 ± 0.07**	0.329	0.447
Glucose (mg/100ml)	102.8 ± 7.8	119.4 ± 7.1	134.3 ± 7.6**	22.13	30.111
Total lipid (mg/100ml)	746.4 ± 38.4	629.8 ± 38.9*	597.9 ± 14.2	95.888	130.463
Triglyceride (mg/100ml)	291.9 ± 15.0	214.8 ± 23.2**	191.1 ± 10.7**	50.336	68.486
Cholesterol (mg/100ml)	168.0 ± 3.7	154.6 ± 4.8	148.1 ± 4.5**	12.898	17.549
Sodium (m mol/l)	137.9 ± 0.9	131.6 ± 3.3	127.6 ± 2.1**	6.851	9.327

Average of 10 fish ± standard error

*** Significant differences (P < 0.05) in comparison to 20°C.**

**** Significant differences (P < 0.01) in comparison to 20°C.**

DISCUSSION

The median tolerance limit of carp reared at 20°C, 25°C and 30°C were 100% which indicate that, this range of temperature meets the tolerance zone of fish and not included the upper and lower critical ranges. For our experimental animal, the common carp, the preferendum was reported at 22-29°C and the upper and lower avoidance should be at 31-35 and 24°C (Coutant, 1977). These data are not really convincing because it was seen by Gluth and Hanke (1984) that carp can really be kept for a longer time at 12°C and are relatively sensitive to infection when kept higher than 22°C. Other references to thermal requirements of carp are given by Alabaster and Lloyd (1980); Elliott (1981). They referred to a normal feeding range from 15-32°C with optimum growth between 24-28°C. Stress should be caused below 15°C and higher than 30°C. Temperatures higher than 33°C could be lethal.

The present study revealed a significant effect of temperatures adaptation on fish growth performance, feed utilization and body composition of carp fish. The optimum temperature for best growth was achieved at 20°C when the ration was 1% of the total body weight (limited energy). The obtained results support the hypothesis that the optimum temperature for growth would drop as the ration decreased, accompanied by a reduction in conversion efficiency. This was based on the supposition that the decrease in maintenance metabolism that accompanies reduced temperature would permit comparatively better growth at lower temperatures when the source of energy was restricted. Brett ***et al.***, (1969) found a change in the optimum growth from 15 to 5°C occurring when the ration was reduced from 6 to 1.5% day. Moreover, the higher metabolic costs caused by elevated water temperature can have large effects on weight loss and lower body condition. Rice (1990) reported that, a 10% increase in metabolic rate accompanied by elevation of temperature reduced net growth in the largemouth bass simulation by 22% while 20% increase caused a reduction of 42% in net growth. The obtained results are in agreement with Letcher and Bengtson (1993) who found that growth rate of ***Menidia beryllina*** decreased with increasing in temperature for fish eating specific weight of food. It is worthy to note that, the optimum temperature range for the carp in a particular habitat may differ from that found in the present study because of the effects of temperature on food requirements and efficiency of utilization of the food ingested for growth. Therefore, it becomes clear that, the requirements for food intake are increased as temperature increases (Winberg, 1956) and the

efficiency of food conversion to growth is reduced (Mc Cromick, 1960). In consequence, as food become limiting, the temperature for optimum growth is progressively lowered (Brett ***et al.***, 1969).

In this study, temperature acclimation modified haematological aspects of carp. Red blood cell counts and haemoglobin content increased significantly with increasing temperature. This response may be attributed to increased demand of fish for more oxygen at higher temperature or a disturbance in water balance (hemoconcentration) might have occurred. The obtained results are in agreement with Lie ***et al.***, (1989) and Frey ***et al.***, (1998).

Plasma total protein in this study was found to decrease significantly with increased temperature. The reduced of plasma protein concentration could be attributed to several processes including plasma dissolution, decrease liver protein synthesis or alteration in hepatic blood flow. Moreover, Houston (1973) suggested that plasma protein in fish are important in cardiovascular adjustment to temperature changes and in immune response mechanisms. The obtained results are in accordance with Castritsi-Catharios and Kavadias (1993). On the other hand, plasma glucose levels of carp increased with increasing temperature. A rise in plasma glucose levels was probably a consequence of the increased epinephrine secretion which occurs with stress and the conversion of lactate to glucose via the Cori cycle (Robertshaw, 1977). The obtained results are in agreement with Mourad (1995) who found that blood glucose level of the fish was temperature dependent However, significant changes in plasma glucose levels were not included in fish when temperature rose from 20°C to 25°C or from 25°C to 30°C. On the other hand, a rise of 10°C in temperature was enough to alter the glycemic level of fishes. The effect of temperature on plasma glucose levels was found to be inconsistent e.g. Dean and Goodnight (1964) reported that at low temperature, ***Ictalurus melas*** and ***Pomoxis annularis*** become hyperglycemic, ***Lepomis macrochirus*** become hypoglycemic and ***Micropterus salmoides*** remains unchanged. In general, other environmental factors may be contributed to the reported variations.

Lipid metabolism as indicated by plasma total lipid, triglyceride and cholesterol were affected by the acclimated temperature. These levels were decreased significantly with increased temperature. One explanation for the decreased plasma lipid could be increased hepatic metabolism together with

biliary secretion (temperature stimulated). Moreover, reduced feeding rate (1% of total wet biomass) was also probably initiating factor. The report of Irvine ***et al.***, (1957) that the resistance of goldfish to elevated holding temperature is enhanced by increasing dietary lipid levels may be pertinent here. The obtained results are in accordance with Wallaert and Babin (1994) who found that, in rainbow trout, temperature variations modified plasma concentrations of lipid and the peak of dietary lipid absorption occurred at low compared with high temperature.

Finally, acclimation to temperature within the normal environmental range generally resulted in small changes in plasma sodium ions content. Carp adapted to 30°C showed a slight, but significant reduction in plasma sodium concentration compared to those adapted to 20°C and 25°C. The obtained results are in accordance with Houston ***et al.***, (1968) who found that the rainbow trout does not exhibited a very marked temperature dependant variation in the ionic composition of its blood plasma.

From the above it becomes clear that temperature can act as a loading stress on fish by affecting functions such as growth and metabolism especially when food intake in reduced. The present study has led to a better understanding of the temperature requirements of this species.

REFERENCES

Alabaster, J. and Lloyd, R., 1980. Water Quality Criteria for Freshwater Fish. pp. 297. Butterworths, London.

Al-Jerian, A and Younis, E., 1998. The effect of temperature on the growth of ***Orechromis mossambicus*** reared in tanks. Pakistan J. Zool., 30 (1): 74-76.

AOAC (Association of Official Analytical Chemists), 1980. Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, VA, 1141 pp.

Brett, J., Shelbourn, J. and Shoop, C., 1969. Growth rate and body composition of fingerling sockeye salmon, ***Oncorhynchus nerka***, in relation to temperature and ration size, J. Fish. Res. Bd. Can. 26: 2363-2394.

Brown, M., 1957. Experimental studies on growth. In: The Physiology of Fishes (Brown, M. E. ed). Vol I, pp. 361-398, Academic Press Inc. New York.

Castritsi – Catharios, J. and Kavadias, S., 1993. An experimental study of some haematological parameters of cage aquacultured sea bass ***Dicentrarchus labrax*** L. under extreme environmental conditions. Preliminary results. Mar. Life, 3 (1-2): 95-98.

Coutant, C., 1977. Compilation of temperature preference data. J. Fish. Res. Bd. Can., 34: 739-745.

Dean, J. and Goodnight, C., 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. Physiol. Zool., 37: 280-299.

Elliott, J., 1981. Some aspects of thermal stress on freshwater teleosts. In: Stress and Fish (Pickering, A.D. ed.) pp: 209-245. Academic Press, London.

Frey, J.; Roy, E.; Willie, J. and Fago, A., 1998. The hemoglobin system of the mudfish, ***Labeo capensits***: Adaptation to temperature and hypoxia. Comp. Biochem. Physiol. B. 120 (4): 735-742.

Gluth, G. and Hanke, W., 1984. A comparison of physiological changes in carp, ***Cyprinus carpio*** induced by several pollutants at sublethal concentration. II. The dependancy on the temperature. Comp. Biochem. Physiol. 79 C (1): 39-45.

Grove, D.; Lozides, L. and Nott, J., 1978. Satiation amount, frequency of feeding and gastric emptying rate in ***Salmo gairdnerii***. J. Fish Biol. 12: 507-516.

Gwyther, D. and Grove, D., 1981. Gastric emptying in ***Limanda limanda*** (L.) and the retrurn of appetite. J. Fish Biol. 18: 245-259.

Houston, A. 1973. Environmental temperature and the body fluid system of the teleost. In: Response of fish to environmental changes (Chavin, w. ed.). pp. 87 – 162. Thomas, Spring field.

Houston, A.; Reaves, R.; Madden, J. and DeWild, M. 1968. Environmental temperature and the body fluid system of the freshwater teleost. I. Ionic regulation in thermally acclimated rainbow trout, ***Salmo gairdneri***. Comp. Biochem. Physiol. 25: 563 – 581.

Irvine, D.; Newman, K. and Hoar, W. 1957. Effects of dietary phospholipid and Cholesterol on the temperature resistance of goldfish. Can. J. Zool. 35: 691-709.

Jobling, M.; Gwyther, D. and Grove, D. 1977. Some effects of temerature, meal size and body weight on gastric evacuation time in the dab ***Limanda limanda*** (L.). J. Fish Biol. 10: 291-298.

Kokot, F. 1969. Method Badan Labortoryjnch stosowanych w klinice. PZWI, Warszawa, Polska pp. 568.

Letcher, B. and Bengtson D. 1993. Effects of food density and temperature on feeding growth of young inland silversides (***Menidia beryllina***). J. Fish Biol. (43): 671-686.

Lie, O.; Lied, E. and Lambertsen G. 1989. Haematological values and fatty acid composition of erythrocyte phospholipids in cod (***Gadus morhua***) fed at low water temperatures. Aquaculture, 79 (1-4): 137-144.

Mc Cromick, J. 1960. Efficiency of food utilization by three species of forage fish. MS thesis. Colorado State Univ., Fort Collins, Colo. pp. 213.

Mourad, M. 1995. The influence of some factors on blood glucose levels of ***Tilapia zillii*** Gerv. Bull. High Inst. Public Health, 25 (4): 953-960.

Murray, S. 1984. Hematological studies of the bluegill, ***Lepomis macrochirus*** Raf. Comp. Biochem. Physiol. 78 A (4): 787-791.

NRC (National Research Council), 1993. Nutrient Requirements of Warmwater Fishes and Shell Fishes. National Academy of Sciences, Washington DC. pp. 102.

Rice, J. 1990. Bioenergetics modelling approaches to evaluation of stress in fish. American Fisheries Society Symposium 8, 80-92.

Robertshaw, D. 1977. Role of adrenal medulla in thermoregulation. In: International Review of Physiology, Environmental Physiology II (Robertshaw, D. ed.), vol. 15 pp. 198-215, University Park Press, Baltimore, Maryland.

Snedecor, G. and Cochran, W. 1974. Statistical Methods. Iowa State Univ. Press, Aress, Ames. Iowa, USA, pp. 341.

Standard Methods for the Examination of Water and Wastewater 1975. 14th ed., Am. Pub. Health Asso., Washington D.C.

Waiwood, K. 1978. Growth of Cod (***Gadus morhua***). A laboratory study. CAFSAC Res. Doc. 78/16.

Wallaert, C. and Babin, P.. 1994. Effects of temperature variations on dietary lipid absorption and plasma lipoprotein concentrations in trout (***Oncorhynchus mykiss***). Comp. Biochem. Physiol. 109B (2-3): 473-487.

Winberg, G. 1956. Rate of metabolism and food requirements of fishes. Fish. Res. Bd. Can. Transl. Ser. No. 194, 1960.

Wintrobe, M.; Lee, G.; Boggs, D.; Bithell, T.; Foester, J.; Athens, J. and Lukens, J. 1981. Clinical Hematology. 8th ed. Lee and Febiger, Philadephia.