Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 91, Number 6, November 1982, pp. 523–532.

Toxic and sublethal effects of endosulfan on *Barbus stigma* (Pisces : Cyprinidae)*

T MANOHARAN and G N SUBBIAH
Zoological Research Laboratory, Thiagarajar College, Madurai 625 009, India

MS received 4 December 1981 ; revised 12 July 1982

Abstract. Toxicity and the effect of sub-lethal concentrations of endosulfan on a fresh water fish *Barbus stigma* had been studied. Endosulfan proved to be lethal to *B. stigma* at a concentration of 0·01 ppm and above. The LC_{50} was 0·0043 ppm and the sub-lethal concentration was 0·003 ppm and below. At sub-lethal concentrations the fish exhibited erratic swimming activity and at lethal concentrations it lost the sense of balance. The rate of feeding was reduced by 5·94% to 9·02% and assimilation by 6·44 to 9·60% in different sub-lethal concentrations. Growth (weight) retarded from 11·6 mg/day in the control fish to 7·3, 6·0 and 5·1 mg/day in the endosulfan treated fishes. Respiratory rate of the pesticide treated fish also dropped by 10 to 16·6%. Due to the over all effect of the toxicity, the fish. *B. stigma* comparatively showed a poor nutritive value by displaying a drop of nearly 35% in the organic constituents.

Keywords. Endosulfan ; pesticide ; lethal concentration ; sublethal concentrations

1. Introduction

Although pesticides produce good many results in the control of pests, their harmful effects on the non-target animals are also not ruled out. Pesticides leave residues in water and mud even several days after their spray in the adjacent crop fields. This poses a constant threat to the non target organisms, especially to the fishes, because pesticides are known to alter their behaviour pattern (Anderson 1971), growth and nutritional value (Korschgen and Murphy 1967; Arunachalam *et al* 1980 ; Yaganobano *et al* 1981), reproductive potential (Johnson 1967), cellular morphology (Mukhopadhyay and Dehadrai 1980) and Physiology (Baskaran 1980 ; Natarajan 1981). Though a good number of literatures are available on the toxicity of pesticides in fishes, studies on the sublethal concentrations of toxicants are meagre. The objective of the present study is to find out the effect of sublethal concentrations of endosulfan (the most effective and widely used pesticide in the field) on survival, behaviour, energy budget, respiratory pattern and the organic constituents of *B. stigma.*

* A part of the M.Phil. dissertation submitted by T. Manoharan to the Madurai Kamaraj University.

2. Materials and methods

The fish *B. stigma,* used in the present experiment, is edible, commercially valuable, and distributed all over India. The fishes were obtained from the Public Works Department and stocked in glass aquaria, after dipping in a 3·5% salt solution to prevent any parasitic attack. They were acclimatized to the laboratory condition for ten days and were fed on Oligochaete worm *Tubifex tubifex.* Preliminary tests were conducted in five aquaria containing five individuals each, to find out the toxicity range of the toxicant. The mortality range was assessed by using five arbitrarily chosen concentrations of endosulfan. For pesticide dilutions the static bioassay method (APHA 1971) was employed. After determining the mortality range (100% mortality) of the pesticide, desired concentrations down to the sublethal dose were prepared by diluting 35% EC endosulfan.

To find out the LC_{50} of 1·0 g unit weight of *B. stigma,* mortality rate was observed for 96 hrs at different arbitrarily chosen concentrations of endosulfan. At a concentration of 0·0043 ppm 50% of the fishes died at the end of 96 hrs. At 0·003 ppm and below, all fishes survived over a period of 30 days. Thus, 0·0043 ppm and 0·003 ppm of endosulfan were taken as LC_{50} and sublethal concentrations respectively. Following the method of Sprague (1973), the LC_{50} curve was drawn and mortality rate and concentration were expressed in probit and log values.

2.1. *Experiments in sublethal concentrations*

Experiments were conducted in three different concentrations (0·003, 0·002 and 0·001 ppm) of endosulfan. In each concentration three replicates and one control (without insecticide) were used simultaneously and the experiment was carried out for 20 days at 28 ± 1° C. The fishes were fed on freshworms of *T. tubifex ad libitum* for 3 hrs/day. The unfed food was collected carefully by a pipette and the faeces by filtering the water daily. Water was changed once in a day. Both the left over food and the excreta were dried to constant weight at 90°C. Water content of the fish and the worms were determined by using the sacrifice method (Maynard and Loosli 1962). The scheme of energy balance was expressed by IBP formula (Petrusewicz and MacFadyen 1970), *i.e.,*

$$C = P + R + F + U$$

Where C = Food consumed ; P = Production (*i.e.* difference between the initial dry weight and the final dry weight) ; R = Respiration ; F = Faeces and U = Nitrogenous excretory products.

Assimilation was estimated by substracting "F" from "C". Assimilation efficiency was calculated as the percentage of food assimilated in relation to food consumed, gross (K_1) and net (K_2) conversion efficiencies were represented as percentage of food converted in relation to food consumed and assimilated respectively. Rates of feeding, assimilation and production were calculated to the respective quantities of food consumed, assimilated and converted relating to per unit live weight (g) of the fish per unit time (day).

2.1a. *Statistical analysis* : Different sublethal concentrations of endosulfan were correlated with rates of feeding, assimilation and conversion.

2.1b. *Specific growth rate*: Specific growth rate (mg/day) was calculated using the method adopted by Kosi Onodera (1962).

2.2. *Respiratory studies*

Control and experimental fishes were introduced into separate troughs containing two litres of water. A thin layer of Kerosene was layered carefully on the surface of the water to avoid the diffusion of atmospheric oxygen. After 30 min, 200 ml of water was siphoned out from each of the troughs and the oxygen content was estimated (Winkler 1948). The oxygen consumed by the experimental and control fishes were calculated by subtracting the value from the initial oxygen content of water.

2.3. *Bio-chemical analysis*

At the end of experiment (after 20 days), 5 mg of dried powder of total homogenate of control and experimental fishes were used and the total protein (Lowry *et al* 1951), the total lipid (Bragdon 1951) and the total sugar (Seifter *et al* 1950) were colorimetrically estimated.

3. Results

Endosulfan caused 100% mortality within 24 hrs of exposure at a concentration of 0·01 ppm (lethal). The LC_{50} (figure 1) was 0·0043 ppm during the 96 hrs of exposure. At the concentration of 0·003 ppm and below no mortality was observed (sublethal).

3.1. *Behaviour*

There was a marked increase in the swimming activity of the fishes immediately after they were transferred to lethal and sublethal concentrations.

3.2. *Feeding rate* (*table* 1)

Sublethal concentrations of endosulfan affected almost equally all the intermediary processes connected to food utilization (figure 2). The average feeding rate of test fish reared in fresh water (control) was 18·84 mg dry food/g live fish/day. This value decreased to 17·72 (5·9%), 17·52 (7%) and 17·14 (9·02%) mg dry food/g live fish/day, when they were reared in 0·001, 0·002 and 0·003 ppm concentration of endosulfan respectively.

3.3. *Assimilation rate* (*table* 1)

Assimilation rate also decreased from 17·07 mg dry food/g live fish/day (control) to 15·97 (6·44%), 15·89 (6·91%) and 15·43 (9·60%) in the experimental fishes (figure 3).

3.4. *Production rate* (*table* 1)

Fish growth was found to have retarded with increased concentrations of endosulfan in the medium (figure 4). The average production rate of *B. stigma* was

Table 1. Effects of different sublethal concentrations of Endosulfan on food utilization and efficiencies in *Barbus stigma*.

Parameters	Concentration			
	0·000	0·001 ppm	0·002 ppm	0·003 ppm
Feeding rate (Cr)	18·84±0·98	17·72±1·10	17·52±0·33	17·14±0·85
Assimilation rate (Ar)	17·07±0·65	15·97±0·98	15·89±0·99	15·43±0·54
Production rate (Pr)	2·47±0·17	1·41±0·40	1·23±0·33	1·14±0·50
Assimilation efficiency	90·61±0·74	90·07±0·39	90·65±0·51	89·76±0·31
Gross conversion efficiency	13·09±0·96	7·97±0·35	7·07±0·89	5·88±0·85
Net conversion efficiency	14·54±0·95	8·81±0·33	7·80±0·82	7·37±0·49

Each value represents the average performance of three (mean ± SD) individuals observed for 20 days at 28° C ± 1° C. Rates are expressed in mg dry weight/g live fish/day and the efficiencies are expressed in percentage.

2·47 mg dry substance/g live fish/day. The growth rate dropped to 1·41 (42·90%) in 0·001 ppm, 1·23 (50·20%) in 0·002 ppm and 1·14 (53·84%) in 0·003 ppm concentration of endosulfan.

Correlation coefficient values obtained between different sublethal concentrations of endosulfan and feeding ($r = -0·95$), assimilation ($r = -0·93$) and conversion ($r = -0·88$) rates were negatively correlated and the values were significant at 0·1% level.

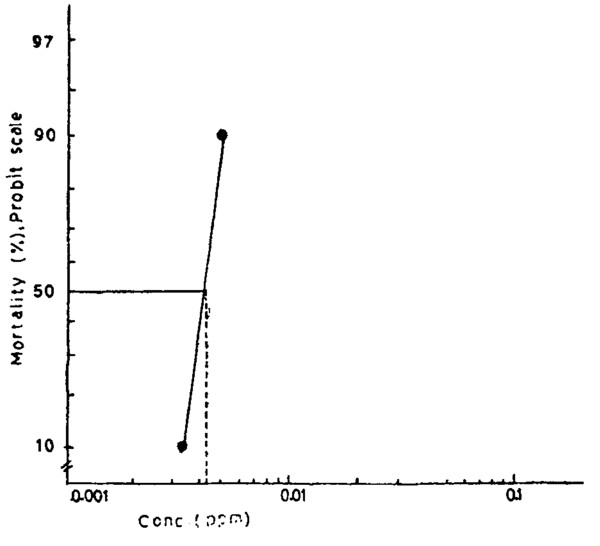

Figure 1. Dotted line indicates the LC_{50} value at 96 hr exposure.

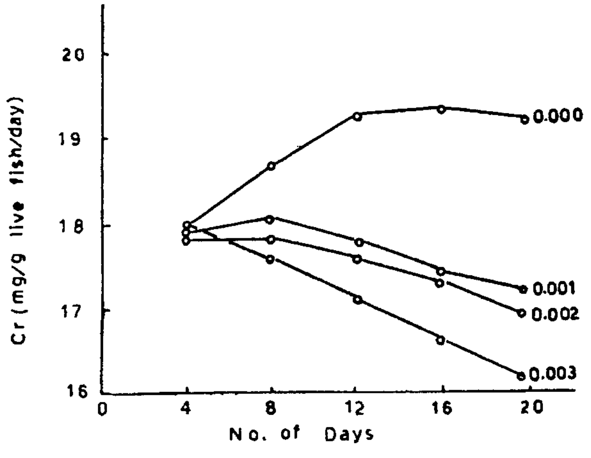

Figure 2. Effects of different sublethal concentrations of Endosulfan on the feeding rate of *B. stigma*.

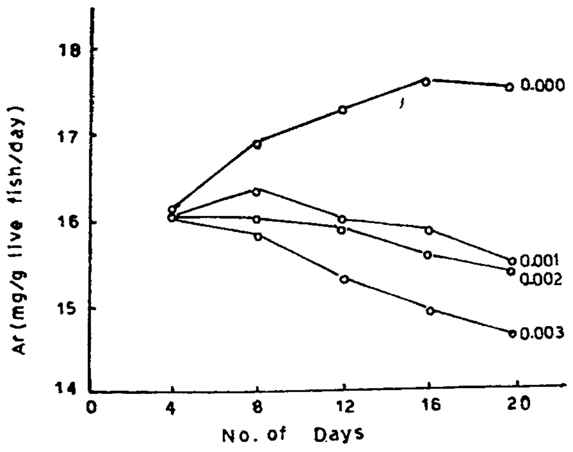

Figure 3. Effects of different sublethal concentrations of Endosulfan on the assimilation rate of *B. stigma*.

3.5. *Efficiencies of Assimilation, gross* (K_1) *and net* (K_2) *conversion* (*table* 1)

Endosulfan did not affect assimilation efficiency which averaged as 90%. The gross conversion efficiency (K_1) of the fish reared in fresh water was 13·09%, while the value was decreased in different sublethal concentrations (7·97%, 7·07% and 5·88% in 0·001, 0·002 and 0·003 ppm respectively). Net conversion efficiency (K_2) of the control fish was 14·54%, while the value was reduced to 8·81% in 0·001 ppm, 7·89% in 0·002 ppm and 7·39% in 0·003 ppm (figure 5).

3.5a. *Specific growth rate*: Specific growth rate of the control fish was 11·60 mg/day. This value decreased to 7·3, 6·0 and 5·1mg/day in 0·001, 0·002 and 0·003 ppm of endosulfan media respectively (figure 6).

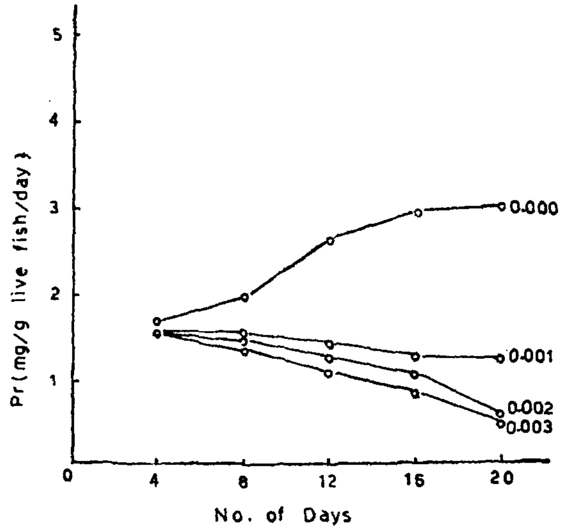

Figure 4. Effects of different sublethal concentrations of Endosulfan on the production rate of *B. stigma*.

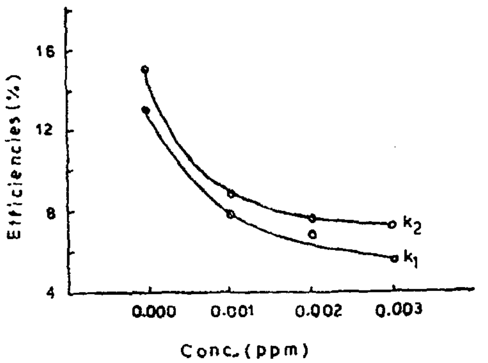

Figure 5. Effects of different sublethal concentrations of Endosulfan on the gross (K_1) and net (K_2) conversion efficiencies of *B. stigma*.

3.6. *Respiration*

The respiratory rate of the fish reared in pesticide-free water (control) was 1·08 mlo_2/g live fish/hr. In 0·001 ppm of endosulfan, the fish maintained more or less identical value. However, when the concentration was increased to 0·002 and 0·003 ppm the respiratory rate declined by 10·8% and 16·6%.

3.7. *Organic constituents*

The average values of protein, lipid and sugar content in the control fish were 280, 118·30 and 50·50 mg/g dry weight of the fish. The experimental fishes

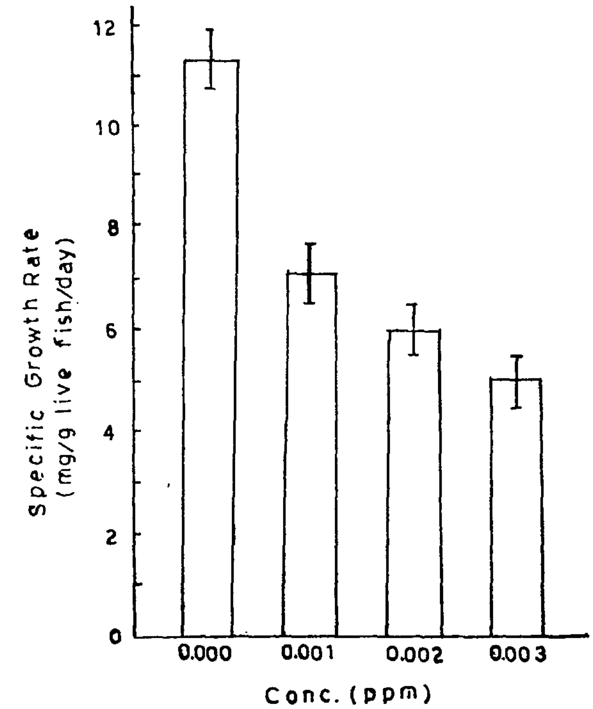

Figure 6. Effects of different sublethal concentrations of Endosulfan on the specific growth rate of *B. stigma*.

exhibited a significant drop in their organic constituents. Protein content was found to have dropped to 233, 221 and 180 mg/g in 0·001, 0·002 and 0·003 ppm endosulfan treated fishes. The value of lipid also declined from 118·30 to 97·56, 91·40 and 77·70 mg/g. Similarly sugar content of the experimental fishes also reduced from 50·50 to 45·00 in 0·001 ppm, 43·50 in 0·002 ppm and 40·00 in 0·003 ppm concentration.

4. Discussion

4.1 LC_{50} *and sublethal concentration*

The pesticide endosulfan has produced lethal effect at 0·01 ppm in *B. stigma*. The LC_{50} is 0·0043 ppm, at which 50% of fishes died within 96 hrs of exposure. Fishes survived at 0·003 ppm and below, indicating that 0·003 ppm is the sublethal level. In their toxicity studies, using endosulfan, Amminikutty and Rege (1971) observed that 0·0016 ppm is the LC_{50} for the fish *Gymnocorymbus ternetzi*. The data obtained in the present work indicates that different fishes have different tolerance range against the toxic effects of the same pesticide. In this way *B. stigma* seems to be more tolerant (about three times) to endosulfan than *G. ternetzi*, 4.1a. *Behaviour* : At sublethal concentrations the fish becomes restless, exhibiting erratic swimming activity and at lethal doses it loses its balance. A similar observation has been made in *Ictalurus punctatus* and *Channa punctatus* by Carter (1971) and Munawar Ahmed Anees (1975) respectively. Desi *et al* (1974) and Kingsley (1973), from their neurotoxicological studies, concluded that cholinesterase activity of various parts of nervous system is affected by the pesticide leading to the imbalance of the animal. In the present case also the disturbed swimming activity of the fish may be explained in the same line.

4.2. *Rates of feeding, assimilation and production*

Rate of feeding, assimilation and growth declined in *B. stigma* with increased concentrations of endosulfan in the medium. This observation falls in line with the findings made by Arunachalam *et al* (1980) and Baskaran (1980), in *Mystus vittatus* and *Channa striatus*. The decrease in growth and conversion efficiency may be due to the diversion of more energy for the stress put up against the toxic effect of the pesticide, as suggested by Arunachalam *et al* (1980).

4.3. *Respiration*

Respiratory rate decreases up to 16·7% in endosulfan treated fishes. This result is in conformation with the earlier reports by Baskaran (1980) in *C. striatus* using DDT and methyl parathion and by Carolyn *et al* (1976) using Carbaryl and Dieldrin on Rainbow trout. Blood smear studies made in the present experiments have revealed the severe damage caused to the red blood corpuscles in the endosulfan treated fishes. A reduction in the RBC count in *C. striatus* following exposure to Metasystox (Demeton) has been reported by Natarajan (1981). Therefore, it is presumed that due to the injury caused to the red blood corpuscles by the pesticides, the efficiency of the fish to trap the dissolved oxygen is considerably reduced resulting in the reduced rate of respiration.

4.4. *Nutritive value of the fish*

Finally the data obtained in the present experiments have shown that the nutritive value of the fish treated with pesticide is significantly reduced (16 to 35% drop in protein, 17 to 34% in lipid and 9 to 20% drop in sugar level). A similar phenomenon has been demonstrated by Ramana Rao and Ramamurthi (1980) and Kabeer Ahmed *et al* (1978) in Sumithion treated *Pila globosa* and Malathion treated *Lamellidens marginalis*. Although the above references are from invertebrates, they prompt one to think that such a phenomenon (reduced level of organic constituents) is common to all animals irrespective of species.

5. Conclusion

From this investigation it is obvious that the toxic nature of pesticides produces lethal effect in fishes at higher doses. Even in sublethal concentrations, it results in degraded metabolic changes, affecting the nutritive value of the animal. Therefore it may be suggested that necessary care may be taken to avoid contamination of fresh water bodies while spraying pesticides.

Acknowledgement

Facilities provided for this work by the Management, Principal and the Professors are gratefully acknowledged.

References

Amminikutty C K and Rege M S 1977 Effects of acute and chronic exposure to pesticides, Thiodan BC 35 and Agallol '3' on the liver of Widowtetra Cymnocorymbus ternetzi ; *Indian J. Expl. Biol.* 15 197–200

Anderson J M 1971 Sublethal effects and changes in ecosystems. Assessment of the pollutants on physiology and behaviour ; *Proc. R.Soc. London* 177 307–320

APHA 1971 American Public Health Association New York 1971 *In Standard methods for the examination of water and sewage* 13th edition.

Arunachalam S, Jeyalakshmi K and Aboobucker S 1980 Toxic and sublethal effects of Carbaryl on a fresh water catfish *Mystus vittatus* ; *Arch. Environ. Contam. Toxicol.* 9 307–316

Baskaran R 1980 Biological studies on a chosen thermo conformer (*Channa striatus*) ; Ph.D. thesis submitted to Madurai Kamaraj University, Madurai

Bragdon J H 1951 Colorimetric determination of blood lipids ; *J. Biol. Chem.* 190 513

Carolyn R Lunn, Daniel P Toews and David J Pree 1976 Effects of three pesticides on respiration, coughing and heart rates of Rainbow trout ; *Can. J. Zool.* 54 214–219

Carter F L 1971 *In vitro* studies of brain cholinesterase inhibition by organo phosphate and carbamate insecticides in fish ; Ph.D. thesis, University of Louisiana, Baton rouge, Louisiana (Diss. Abstr. Int.) 13 2772–73

Desi Ilees, Lili Genczi Gyorgy Simon, Ildiko Farkas and Zsuzsa Kneffel 1974 Neurotoxicological studies of two carbomate pesticides in sub-acute animal experiments ; *Toxicol. Appl. Pharmacol.* 27 465

*Johnson H E 1967 The effects of Endrin in the reproduction of a freshwater fish, *Cryzias latipes* ; Ph.D. thesis, Univ. Washington, Seattle, Wash.

Kabeer Ahamed I, Begum Md R, Sivaiah S and Ramana Rao K V 1978 Effect of Malathion on free amino acids, total proteins, glycogen and some enzymes of Pelecypod *Lamellidens marginalis* ; *Proc. Indian Acad. Sci.* (*Anim. Sci.*) 87 377–380

Kingsley Kay 1973 Toxicity of pesticides ; Recent advances, *Environ. Res.* 6 202

Korschgen L J and Murphy D A 1967 Missouri federal aid project progress report

Kosi Onodera 1962 Some data on Eel culture in Japan ; *Indo-Pac. Fish. Counc., Occasional Pap.* 6216 4

Lowry O H, Rosebrough N J, Farr A L and Randal R J 1951 Protein measurement with Folin phenol reagent ; *J. Biol. Chem.* 193 265–275

Maynard A L and Loosli K J 1962 *Animal nutrition* 5th ed. (New York : McGraw-Hill)

Mukhopadhyay R K and Dehadrai P V 1980 Studies on air breathing catfish *Clarias batrachus* under Malathion sublethal exposure ; *Indian J. Exp. Biol.* 18 348–352

Munawar Ahmed Anees 1975 Acute toxicity of our organophosphorous insecticides to a fresh water teleost *Channa punctatus* ; *Pakistan J. Zool.* 7 135

Natarajan G M 1981 Changes in the bimodel gas exchange and some blood parameters in the air breathing fish, *Channa striatus* following lethal ($LC_{50/48}$ hrs) exposure to Metasystox (Demeton) ; *Current Sci.* 50 40–41

Petrusewicz and MacFadyen A 1970 *Productivity of terrestrial animals* ; *IBP handbook No.* 13 (Oxford : Blackwell)

Ramanarao M V and Ramamurthi R 1980 Effect of sublethal concentration of sumithion on some biochemical constituents of the freshwater snail *Pila globosa Geobios* 7 247–250

Seifter S and Dayton S 1950 The estimation of glycogen with anthrone reagent ; *Arch. Biochem. Biophys.* **25** 191–200

Sprague J B 1973 *The ABC's of pollutant Bioassay using Fish* ; American Society for Testing and Materials 6–30

Winkler 1948 In Welch P S *Limnological methods* (New York : McGraw-Hill)

Yaganobano, Seikh Amjad Ali and Tariq Hameed 1981 Effects of sublethal concentration of DDT on muscle constituents of an air breathing catfish, *Clarias batrachus* ; *Proc. Indian Acad. Sci. (Anim. Sci.)*, **90** 33–37

* Originals not referred.