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BRIEF COMMUNICATIONS

A comparative study of phagocytic activity and lymphoproliferative response in five varieties of tilapia *Oreochromis* spp.

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The immunological status of three native species of tilapia *Oreochromis* spp. and two hybrids were compared, showing a greater potential of the hybrid strain *O. niloticus* Rocky Mountain for aquaculture and a better capacity to resist the stressful conditions of a fish farm.

Key words: hybrid crosses; lymphoproliferation; *Oreochromis* spp.; phagocytosis.

The stressful conditions of aquaculture are known to compromise the defence mechanisms of fishes. As a consequence, there is a major need to study their immune response to obtain a better understanding of the host resistance to stress and pathogens (Sarder *et al.*, 2001).

The fish immune system is divided into two responses: the innate and the adaptative. Within the innate immune response, phagocytosis is a fundamental and generally efficient mechanism that provides the host with a continuous surveillance against foreign invaders and is ultimately responsible for the destruction of the phagocytosed pathogens (Silva *et al.*, 2002). In the adaptative immune response, T and B lymphocytes play a pivotal role in directing both specific humoral and cellular responses (Dixon & Stet, 2001).

'Tilapia' *Oreochromis* spp. is the common name given to a group of cichlids endemic to Africa and this species is especially successful in warm-water aquaculture. Today five varieties of tilapia are economically important to the aquaculture industry worldwide: the three species *Oreochromis mossambicus* (Peters,

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1852), *Oreochromis aureus* (Steindachner, 1864), *Oreochromis niloticus* Egypcia (L., 1758) and the two hybrids *O. niloticus* Rocky Mountain (cross of *O. niloticus* and *O. aureus*) and *O. niloticus* Stirling (cross of *O. niloticus* and *O. mossambicus*). The characterization of the different tilapia varieties is based mainly on productive variables (Garduño-Lugo *et al.*, 2003) and, as far as is known, no report dealing with the comparison of their immune system has been put forward until now. In order to answer the question as to which of the five varieties under study has the greatest ability to respond immunologically to foreign antigens, phagocytic index (I_P), percentages of phagocytic cells (% PC), lymphoproliferation rates and relative spleen mass (M_{RS}) were examined.

Experimental tilapia were hatched, grown and maintained in the aquarium facilities of the University of Guadalajara, Jalisco, Mexico. Animals were kept individually in heated (28° C, range ± 2° C) and aerated 40 l glass aquariums for a 10 day acclimation period, before commencing the experiment. Ten male animals between 3 and 5 months-old with a body mass of *c.* 250 g were used from each variety. Animals were anaesthetized with 0·4 ml l^{-1} of 2-phenoxyethanol (Sigma-Aldrich Inc., St Louis, MO, U.S.A.) (Cecchini *et al.*, 2004).

Functional phagocytic assays, using the glass adherence method were performed with peripheral blood obtained with a syringe from the caudal vein of the fish. Blood cells were incubated in a wet chamber at 28° C, using *Candida albicans* opsonized with 20% autologous serum and incubated at room temperature for 30 min. The I_P was determined as the average number of yeast cells engulfed per cell, calculated by dividing the total number of yeast cells engulfed by the cell count (Ainsworth *et al.*, 1991). The % PC was quantified by counting a total of 100–200 phagocytic and non-phagocytic neutrophils, the result of which was considered as a percentage of the positive phagocytic cells (Watanuki *et al.*, 1999).

The M_{RS} was considered as the average spleen mass (M_S, g) and fish mass (M_F, g), according to the following equation: $M_{RS} = M_S M_F^{-1}$ (Garg *et al.*, 2004).

For the lymphoproliferation assays, a suspension of the spleen tissue was prepared, from which mononuclear cells were purified on a density gradient using Histopaque 1077 (Sigma-Aldrich Inc.) for 30 min at 352 *g*. Approximately 200 000 viable cells were cultivated in 96 well flat-bottom plates (Corning Inc., New York, NY, U.S.A.) according to the method described by Gogal *et al.* (1999) using RPMI-1640 (Sigma-Aldrich Inc.), supplemented with 5% foetal bovine serum (Gibco-Invitrogen Corporation, Grand Island, NY, U.S.A.), and stimulated with 50 μg ml^{-1} of phytohaemagglutinin (PHA, Sigma-Aldrich Inc.) as a polyclonal mitogen, while un-stimulated cells only received RPMI-1640 to be used as a control. Plates were incubated in a humidified atmosphere at 28° C in a mixture of 95% air and 5% CO_2. After 48 h, 1 μCi of ^{3}H-thymidine (specific activity 6·7 Ci μmol^{-1}; Perkin Elmer, Life Sciences Inc., Boston, MA, U.S.A.) was added and plates were incubated for an additional 24 h. Cells were harvested onto glass fibre filters (Nunc A/S, Roskilde, Denmark) and the incorporation of ^{3}H-thymidine to newly synthesized DNA was measured in a Beckman beta counter. Data expressed the mean of counts per minute (cpm) and the stimulation index (I_S) was calculated according to the following equation: I_S = cpm of PHA stimulated cells per cpm of un-stimulated cells

(Le Morvan-Rocher *et al.*, 1995). Data are expressed as mean ± S.D., and analysed with a one-way ANOVA using the Sigma Stat 2·03 software.

The species *O. mossambicus* showed the highest I_P (1·40 ± 0·08), followed by *O. niloticus* Rocky Mountain (1·28 ± 0·11) and *O. niloticus* Stirling presented the lowest one (1·1 ± 0·3). The I_P of *O. mossambicus* was significantly higher than *O. niloticus* Egypcia and *O. aureus* ($P < 0·001$), and also compared to the other two species ($P < 0·05$) (Table I). It was observed that *O. aureus* had the highest % PC (88·1 ± 3·2) while *O. mossambicus* showed the lowest one (74·3 ± 6·2). Statistically, *O. mossambicus* manisfested lower % PC than *O. aureus*, *O. niloticus* Egypcia and *O. niloticus* Rocky Mountain. No significant differences were observed between the % PC of *O. mossambicus* and *O. niloticus* Stirling. The highest values of M_{RS} were recorded with *O. niloticus* Egypcia (0·18 ± 0·02), while the lowest were obtained with *O. mossambicus* (0·05 ± 0·02).

The I_S of *O. niloticus* Rocky Mountain lymphocytes reached 3·6 ± 0·63, a value significantly higher than obtained with the other four varieties of tilapia, *i.e. O. mossambicus* (1·6 ± 0·9) ($P < 0·05$), *O. niloticus* Stirling (1·52 ± 0·54), *O. niloticus* Egypcia (1·44 ± 0·71) and *O. aureus* (1·25 ± 0·30) ($P < 0·001$). No significant differences were observed in the I_S between the remaining four varieties (Table I).

Overall the species *O. mossambicus* presented low immunological variables compared to the other four tilapia varieties, especially lower M_{RS} and % PC values. These results are comparable with the data put forward by Cnaani *et al.* (2004) who studied different innate immunity variables in response to stress with regard to four varieties of tilapia. In the present study, however, the I_S of spleenocytes from *O. mossambicus* did not significantly differ from the other varieties, except with *O. niloticus* Rocky Mountain. The latter variety manifests intermediate values for I_P, % PC and M_{RS} compared with the four other varieties.

Oreochromis niloticus Rocky Mountain presented the best response to PHA stimulation (Table I). This particular response could be the result of a combination of the parental traits, as this variety corresponds to a hybrid derived from a cross between the varieties *O. aureus* and *O. niloticus* Egypcia (Barriga-Sosa

TABLE I. Immunological variables from five varieties of tilapia (*Oreochromis* spp.)

	Species			Hybrid	
Immunological variables	*O. aureus*	*O. mossambicus*	*O. niloticus* Egypcia	*O. niloticus* Stirling	*O. niloticus* Rocky Mountain
Phagocytic index	$1·18 \pm 0·05^{b}$	$1·40 \pm 0·08^{d}$	$1·16 \pm 0·04^{cb}$	$1·10 \pm 0·26^{ab}$	$1·28 \pm 0·11^{a}$
Percentage of phagocytic cells	$88·1 \pm 3·2^{b}$	$74·3 \pm 6·2^{d}$	$86·4 \pm 3·8^{cb}$	$76·8 \pm 4·7^{ad}$	$80·7 \pm 3·5^{a}$
Relative spleen mass	$0·10 \pm 0·01^{ad}$	$0·05 \pm 0·02^{d}$	$0·18 \pm 0·02^{c}$	$0·09 \pm 0·01^{ad}$	$0·12 \pm 0·01^{a}$
Stimulation index	$1·25 \pm 0·30^{a}$	$1·60 \pm 0·90^{a}$	$1·44 \pm 0·71^{a}$	$1·52 \pm 0·54^{a}$	$3·60 \pm 0·63^{b}$

Values are mean ± S.D. ($n = 10$ fish); data with different superscript letters differ significantly ($P < 0·05$) across the row.

et al., 2004), and may be in connection with the fact that it is more resistant to stressful conditions and tolerant to disease (Cano *et al.*, 2001; Wang & Xia, 2002). Previous work comparing the reproduction traits of nine genetic groups of tilapia suggested that hybrids of tilapia, among which the *O. niloticus* Rocky Mountain, have the best commercial value, because of their final mass and colouration (Muñoz, 2000). This observation was also supported by Cohen (1995) who recommends the variety *O. niloticus* Rocky Mountain as an adequate species for aquaculture due to its high growth rate, uniform size, shape and colour and its tolerance to low temperature and high salt concentration.

The growth data collected from previous studies, as well as the data presented here indicate that the hybrid strain *O. niloticus* Rocky Mountain is a suitable variety to be developed in aquaculture due to its superior immune response which provides this hybrid with a better capacity to respond to stress.

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