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Telah melaksanakan penelitian pada tahun 2015 dengan judul sebagai berikut :

Perendaman Ekstrak *Spirulina plantesis* Terhadap Ig-M, Jaringan Limpa dan Diferensial Leukosit Ikan Mas Setelah Diinfeksi *Aeromonas hydrophila*

Adapun penelitian ini sudah mengacu pada prosedur pertimbangan etik dari *American Fisheries Society* (AFS, 2014) yang berjudul *Guidelines for the Use of Fishes in Research* dan *Canadian Council on Animal Care* (CCAC, 2005) yang berjudul *Guidelines on the Care and Use of Fish in Research, teaching and Testing*. Sehingga penelitian tersebut tidak perlu dilakukan ***Uji Ethical Clearence*** karena menggunakan ikan yang minimal dan menghasilkan *out put* yang sangat baik untuk ikan Mas dan telah mengacu kepada (CCAC, 2005;: Guideline-97, hal 57).

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Lektor Kepala** atas nama **Dr. Woro Hastuti Satyantini, Ir. M.Si.**

Surabaya, 19 Juni 2023
Dekan

UNIVERSITAS AIRLANGGA
FAKULTAS PERIKANAN DAN KELAUTAN

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AUN-QA ASIIN BSI BAN-PT

Guidelines for the Use of Fishes in Research

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4. Animal Welfare Considerations

4.1 General Considerations

Research involving living animals, including fishes, must be based on experimental designs and animal care practices that can lead to scientifically valid results. Fishes are acutely sensitive to stress (e.g., Barton and Iwama 1991), and responses may include changes in behavior (e.g., Martins et al. 2012), reduced growth, changes in osmotic status, suppressed immune systems (with consequent disease onset), and altered reproductive capacity (Iwama et al. 2006; Schreck et al. 2001; Schreck 2010). Accordingly, unless the experimental objectives require actions or conditions designed to test responses to stress, fishes should be maintained, handled, and tested under conditions that will not create such responses. The Guidelines addresses the conduct of scientific research and focuses on established facts and the processes through which knowledge is developed. Research plans submitted to IACUCs should address animal care considerations, in addition to the details of research goals, objectives, and procedures. The extent to which IACUCs incorporate personal values concerning animal welfare into their institutional guidelines is determined within each institution.

4.2 Stress

The study of stress has focused on how animals have evolved physiological and behavioral mechanisms to address the challenges of changing environmental conditions and then to permit them to maintain homeostasis, or self-sustaining balance. The set of environmental variables (conditions) best suited for the well-being of each species typically encompasses a specific range for each factor and species (see section 5.7 Facilities for Temporary Holding and Maintenance), as stress responses are species-specific (Schreck 2010). Accordingly, when fishes are maintained within these ranges, a state of homeostatic balance is expected. Deviations from homeostasis characterize a stress response. While many definitions for stress have been proposed, we employ the definition of Schreck (2000) and Schreck et al. (2001): "a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult." When stressed, fish generally attempt to reestablish homeostasis via a process known as "allostasis regulation in which they adjust their physiological function to re-establish a dynamic balance" (Sterling and Eyer 1988). While allostasis is generally adaptive because it helps keep animals alive in the face of a short-term stressor(s), it can be maladaptive over the long term and have negative consequences on growth, reproduction, and immunological health (Schreck 2010). Accordingly, investigators need to understand those factors that might cause stress in their experimental animal(s), the potential consequences, and how stress might be avoided by optimizing experimental conditions.

Each investigator and the IACUC should understand the conditions that minimize stress for the species in question. Extrapolation between taxa, however, must be avoided because differences exist among species (Schreck 2010). The factors and range of conditions appropriate for fishes typically will deviate substantially from those used for mammals. Assumptions and perceptions based on experiences with mammals, especially primates, must not be extrapolated to fishes; however, investigators should be aware of APHIS policy (i.e., Policy 11, USDA 2011, http://www.aphis.usda.gov/animal_welfare/policy.php?policy=11).

4.2.1 Stages of Stress

Stress responses are elicited after a fish detects a threat. Recognizing and understanding the three stages of stress is important. Each warrants consideration in the design of animal care protocols:

Stage 1. Primary stress responses vary among species but are characterized by immediate neuroendocrine responses including catecholamine and corticosteroid release and can be quantified by measuring blood hormones. Sometimes behavioral changes accompany these endocrine responses that help the animal cope with the stressor and, in and of themselves, have few consequences to health.

Stage 2. The secondary stage of a stress response is characterized by changes in blood and tissue function evoked by the primary response. Secondary stress typically occurs within minutes of the primary response and is characterized by increased blood glucose and heart rate, diuresis, alteration of leukocyte count, altered osmolyte balance, and behavioral changes (see section 5.6 Handling and Transport). Although these responses can have short-term positive effects, many also are negative, so they should be avoided when possible. They can be evaluated through the study of extracted blood (see section 5.9 Collection of Blood and Other Tissues).

Stage 3. Tertiary stress responses are associated with long-term exposure and negatively affect the well-being of the organism. Effects associated with tertiary stress include decreased growth, propensity to contract disease, and decreased reproductive function (Selye 1976; Schreck et al. 2001; Iwama et al. 2006; see sections 5.8 Field Acclimation and 7.3 Acclimation to Laboratory Conditions). The best way to avoid a tertiary stress response is to care for animals so as to minimize stress responses.

4.2.2 Measuring and Avoiding Stress

While the nature of stress is insidious, it also tends to be polymorphic, changing with time and taking different forms in different species at different stages in their lives. It is rarely feasible to measure changes in blood hormones to assess primary or secondary stress; therefore, investigators are advised to design experiments that avoid stress unless the purposes of the research require measurements of stress indicators. Important indicators of a lack of stress are persistence of normal behavioral activity and propensity to feed and grow. Careful experimental design and planning can ensure study results that are not confounded by unrecognized or unmeasured stress. Unless the aim of the research is to establish optimal conditions for holding particular species of fish in captivity, such as captive propagation of endangered species, it is generally advisable for investigators to select species for experiments whose optimal holding conditions are known and can be recreated in the laboratory. Specific factors to consider include (1) choice of species, (2) history of the animals under study, (3) water chemistry, (4) water flow, (5) water temperature, (6) light conditions and cycles, (7) bottom substrate, (8) noise and other physical stimuli, (9) shelter, (10) stocking density, and (11) size of tank relative to body size and activity rate. Other variables, such as fish density or the presence or absence of tank covers, may be important. Species that are known as reliable laboratory models (e.g., Zebrafish or Japanese Medaka) or that are commonly used in fish culture (e.g., Channel Catfish *Ictalurus punctatus* or Rainbow Trout *Oncorhynchus mykiss*) might be selected whenever such a choice is compatible with research objectives.

In addition to the aforementioned factors that are associated with long-term maintenance, additional considerations apply when fishes are handled or subjected to various experimental manipulations.

- <u>Handling should be minimized.</u> Merely catching fish in nets can induce release of stress hormones, such as cortisol, within one minute. Fishes should be given time to recover from handling prior to use in experiments. The amount of recovery time needed may vary with species and conditions; therefore, preliminary tests would help to establish the appropriate recovery period.
- <u>Effects of stressors can be reduced through the use of sedatives or by adding environmental salts to the holding water to reduce osmotic and related stress.</u> (Note that marine fishes, due to their osmoregulatory requirements, can be an exception.) The specific salts and concentrations will vary depending on each fish species and environmental conditions. Sedatives themselves, however, can evoke physiological stress responses (Trushenski et al. 2012a), so they should be employed cautiously and in accordance with established guidelines.
- <u>Environmental conditions from which fish originated, or are held, should not be changed rapidly.</u> This is especially true for temperature conditions. An instantaneous change of 2°C in water temperature generally is not lethal, but it can cause detectable stress responses. Tolerable changes depend on the species, the life history stage, previous thermal history, and the initial holding conditions. Effects due to previous thermal history have been detected for as long as a month posttreatment. Rapid, substantial changes in water quality also should be avoided (see section 7.7 Water Quality).
- <u>Fish densities should be appropriate.</u> Fish which live in shoals should be kept as groups but not in such large groups that they are crowded and compete for food and space or degrade water quality.

Canadian Council on Animal Care

guidelines on:

the care and use of fish in research, teaching and testing

This document, the CCAC *guidelines on: the care and use of fish in research, teaching and testing*, has been developed by the *ad hoc* subcommittee on fish of the Canadian Council on Animal Care (CCAC) Guidelines Committee.

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particularly following resuscitation, to ensure that the administered agent is retained. However, many fish have a J- or U-shaped stomach or pyloric flexure that prevents the introduced substance from being regurgitated, providing the tube is inserted into the stomach past this flexure. In general, no more than 1% body weight should be administered orally in a single dose, although many species have highly distensible stomachs and can tolerate larger percentages.

Electronic transmitters may be inserted via the oral route into the stomach using a hollow plastic tube with a central blunt trocar to push the transmitter into the stomach.

4.3 Injection

Guideline 97:

Care should be taken during injection to introduce the needle in spaces between the scales. Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, taking care to avoid the lateral line and ventral blood vessels. Intraperitoneal (IP) injections should avoid penetrating abdominal viscera as substances that cause inflammation may lead to adhesion formation.

The most useful routes for injection in fish are intravascular, intraperitoneal and intramuscular. Details of injection techniques, suggested needle sizes, and injection volumes are available, e.g., Summerfelt & Smith (1990), Stoskopf (1993) and Black (2000b).

Chemicals to be injected should be dissolved directly in sterile physiological saline. However, hydrophobic chemicals should be dissolved in very small quantities of co-solvent (e.g., ethanol, methanol, or dimethyl sulfoxide [DMSO]) prior to dilution in saline. For chemicals that are not soluble or stable at neutral pH, the pH of the injection solution may be adjusted with an acid or base (Perry & Reid, 1994).

Final injection volumes should be as small as possible to minimize physiological disturbances to the fish. In addition, control fish (vehicle and/or sham injected) should be part of the experimental protocol to correct for any effects of the injection procedure or the vehicle.

4.4 Implants, windows and bioreactors

Guideline 98:

Implanted materials should be biocompatible and aseptic, and should be implanted using sterile techniques.

Bioabsorbable pellet implants of bioactive compounds in absorbable and nonabsorbable matrix vehicles are available from commercial sources or can be custom fabricated. These can be surgically implanted in the peritoneal cavity or implanted with a trocar introducer into muscle masses. Osmotic minipumps can be implanted in a similar fashion as can transmitters and telemetry units. Windows to visualize visceral changes, such as splenic size change during blood loss, have been successfully used (Yamamoto *et al.*, 1985).

5. Tagging and Marking

Tagging and marking techniques are used in both field and laboratory studies. For field studies, general principles are outlined in the CCAC *guidelines on: the care and use of wildlife* (CCAC, 2003a). As well, *Concerted Action for Tagging of Fishes* (www.hafro.is/catag/) provides detailed information on current best practices for tagging and telemetry in field research.

When choosing a marking method, primary consideration should be given to methodologies that are not invasive, do not require recapture for identification, and will remain visible for the duration of the study. Where possible, investigators are encouraged to use natural features as marks, rather than removing or damaging tissues or attaching auxiliary markers.

Guideline 99:

Investigators must aim to minimize any adverse effects of marking and tagging procedures on the behaviour, physiology or survival of individual study animals. Where such effects are unknown, a pilot study should be implemented.

The following criteria should be applied as far as possible:

- marking should be quick and easy to apply;