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Using self-selection to evaluate the acceptance of a new diet formulation by farmed fish

Hanna Carlberg[a], Ken Cheng[b], Torbjörn Lundh[c], Eva Brännäs[a,*]

[a] Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Science, 901 83 Umeå, Sweden
[b] Department of Food Science, Swedish University of Agricultural Science, Uppsala BioCenter, PO Box 7051, 750 07 Uppsala, Sweden
[c] Department of Department of Animal Nutrition and Management, Swedish University of Agricultural Science, PO Box 7024, 750 07 Uppsala, Sweden

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ABSTRACT

The evaluation of new diet composition is commonly achieved by performing time-consuming growth trials, which may negatively impact the welfare of a large number of fish if the feed is not accepted. Instead, the fish's behavioural responses to a new diet composition can be used as a first step in the evaluation of new diet composition. The taste acceptance of a new diet by Arctic charr (*Salvelinus alpinus*) was evaluated over 16 days based on the self-selection of a test diet and a control diet. The test diet contained ingredients from the nutrient-enriched Baltic Sea, and it is hoped that this diet can contribute nutrients to the nutrient-poor waters in northern Sweden in which Arctic charr are farmed. The main ingredients in the test feed were blue mussel meat (*Mytilus edulis*), meat from sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), baker's yeast (*Saccharomyces cerevisiae*), fish oil and regionally produced rapeseed oil. Individual fish ($n = 15$) were allowed to choose between abundances of both the test diet and a fishmeal based control diet that mimicked a standard feed for Arctic charr. After only a few days, the fish demonstrated a significant preference for the Baltic Sea test feed. These results may depend on betaine, which was found at concentrations that were four times higher in the test feed than in the control feed. Betaine is a known attractant for many fish species and is abundant in marine animals, such as mussels. Thus, the test diet can be evaluated further without additional taste stimulants before the new feed formula can be used commercially to ensure fish welfare, the product quality and the economic feasibility of the new formula.

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1. Introduction

Gustation and olfaction are the most important senses for feeding and thus for fish survival (Hara, 2007). Indeed, taste buds are more numerous in many fish species than in any other animals (Kasumyan and Doving, 2003). Preferences for tastes in fish are highly species-specific and even vary between individuals of the same species (Kasumyan and Doving, 2003). The diets of farmed fish must not only meet species-specific nutritional requirements but also be appetizing (Raubenheimer et al., 2012). A reduced acceptance of the feed results in poor fish welfare and increased feed waste that result in negative environmental effects and increased production costs (Jobling, 2001). The fishmeal and fish oil that are used to feed carnivorous farmed fish are limited resources; thus, the use of plant ingredients as substitutes is increasing (Naylor et al., 2000; FAO, 2014). However, there are drawbacks to the use of plant materials in fish diets because they commonly contain anti-nutritional factors that adversely affect fish, particularly carnivorous species like salmonids (Cain and Garling, 1995; Li and Robinson, 1997; Hughes and Soares, 1998). Additionally, many plant materials, such as soybeans, which are commonly used in feed for farmed fish, are important protein sources for the growing human population (Brown, 2012).

A future goal is therefore to develop fish feed that is created from ingredients that are either unsuitable or unattractive for human consumption and are not limited resources or key species in ecosystems but nevertheless produce healthy and attractive products for the table market (Kiessling, 2009). However, new feed compositions, especially those composed of ingredients that are not found in the natural diets of carnivorous fish, must be thoroughly evaluated before use.

Feeds need to be designed to attract the specific fish species, and the incorporation of feeding stimulants may be necessary to encourage feeding (Jobling, 2001). Amino acid attractant mixtures and betaine are examples of substances that improve feed

* Corresponding author. Tel.: +46 907868592.
E-mail addresses: hanna.carlberg@slu.se (H. Carlberg), ken.cheng@slu.se (K. Cheng), torbjorn.lundh@slu.se (T. Lundh), eva.brannas@slu.se (E. Brännäs).

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acceptability and growth on plant-based diets (Tandler et al., 1982; Papatryphon and Soares, 2000). Attractants are of particular importance when designing diet compositions for marine fish larvae (de la Higuera, 2001). The evaluations of new diet compositions are most commonly performed via time-consuming growth trials that may negatively impact the welfare of large numbers of fish if the feed is not accepted.

The fish's behavioural approach to a new diet composition could instead be used as a first step in the evaluation of a new diet formula. The fish's acceptance can be evaluated in short-term studies based on the self-selection of a test diet and a reference diet by the fish. A fish's preference for a feed may be determined by several methods of self-selection. Self-feeders that are activated when the fish actuate a rod are commonly used to measure feeding behaviour related to several aspects, such as diel or annual variations in appetite and diet preferences (Jobling et al., 2001). Geurden et al. (2005) used self-feeders to demonstrate that rainbow trout (*Oncorhynchus mykiss*) can discriminate between diets that contain fish oil and those that contain plant oils. Sea bass (*Dicentrarchus labrax*) are able to discriminate and prefer diets that contain fishmeal over those that contain soybeans and the enzyme phytase, which improves the nutritional efficiency of soy (Sugiura et al., 2001). Sea bass also exhibit preferences for fishmeal diets over those that contain soybean meal as assessed with the same method (Fortes-Silva et al., 2011). One problem with the use of self-feeders with groups of fish involves the social interactions between the individuals. When passive integrated transponders (PIT-tags) were used to study individual variations in trigger activation in groups of 15 Arctic charr, all individuals activated the trigger in the first days after the trial started, but thereafter, only one individual continued to exploit the activations (Brännäs and Alanärä, 1993). Self-feeding by trigger actuation has been tested with individual Arctic charr, but these tests were unsuccessful (Brännäs unpublished results). Thus, another method of assaying diet preference was tested on individual fish; this method involved measurements of the given and rejected feed in aquaria that were divided by one or several walls that separates two or more diets but allowed the fish to swim between the compartments (Pettersson et al., 2009). In such setups, rainbow trout exhibit a self-selection preference for feeds with 100% fish oil compared with feeds containing 25 to 75% rapeseed oil replacements and fishmeal as the protein fraction (Pettersson et al., 2009).

A conceptual model has been created for the small scale Swedish farming of Arctic charr (Eriksson et al., 2010). This model focuses on Arctic charr that are farmed in northern Sweden in net pens within extremely oligotrophic water bodies that are affected by hydropower regulations. The fish feed used in this system contains regionally produced ingredients that are unsuitable or unattractive for direct human consumption and are mainly sourced from the eutrophicated southern Baltic Sea (Ronnberg and Bonsdorff, 2004). Sourcing the feed ingredients from the Baltic Sea transports nutrients to the oligotrophic waters in which the Arctic charr are farmed and is thus beneficial for both ecosystems (Eriksson et al., 2010).

The protein fraction of the feed that was used in this study (i.e., the test feed) was sourced primarily from the Baltic Sea and was composed of one-third of each of the following: Baltic Sea blue mussels (*Mytilus edulis*), fishmeal from two fatty fish (i.e., sprat (*Sprattus sprattus*) and herring (*Clupea harengus*)), and baker's yeast (*Saccharomyces cerevisiae*). Blue mussels are excellent nutrient assimilators, but due to the low salinity of the Baltic Sea, they never grow large enough to be attractive for the table market (Schütz, 1964). Blue mussels have a favourable amino acid profile (Langeland et al., 2014) and a fat composition that is suitable for salmonid diets (Berge and Austreng, 1989), but very few studies have been published regarding the use of blue mussels as a protein source in fish diets. The two fatty fish, i.e., the sprat and herring, are very abundant in the Baltic Sea presumably due to the intensive cod (*Cadus morhua*) fisheries that have resulted in ecosystem imbalances and stocks that need to be decreased by selective fishing (Persson et al., 2014). Because the protein content of baker's yeast varies between 40 and 65% (Halasz and Lasztity, 1991; Nasseri et al., 2011), it replicates rapidly and can grow on multiple substrates why there is substantial interest in the use of yeast as an alternative protein source for fishmeal (Rumsey et al., 1990; Omar et al., 2012; Overland et al., 2013; Vidakovic et al., 2015).

This study represents the first step of a thorough evaluation of the test feed that contains these three different protein fractions and began with assessments of the fishes' behavioural approaches using the same methods employed by Pettersson et al. (2009). Mussel meat and its extract have been found to be efficient attractants in the diets of, for example, common sole (Mackie, 1982). However, very few trials of mussel meal in fish diets have been performed recently. Marine invertebrates, such as mussels, contain high quantities of betaine (Meyers, 1987), which is a known attractive substance to some fish species (Mackie et al., 1980; Tusche et al., 2013). However, bioassay studies have not identified betaine as a stimulant for two salmonid species, i.e., the rainbow trout (Jones, 1989) and the Chinook salmon (*Oncorhynchus tsawytscha*) (Hughes, 1993), and its effects on Arctic charr are uncertain. Feeds in which fishmeal composes the protein fraction are preferred to plant-based substitutes by sea bass (Fortes-Silva et al., 2011) and are also attractive to Arctic charr (Pettersson et al., 2009). To our knowledge, there are no reported data regarding the taste responses of fish to yeast. Yeast is often deficient in certain essential amino acids (Overland et al., 2013), and yeast was therefore used in the present case as a partial replacement for the fishmeal. The potential adverse or indifferent taste responses to the yeast are likely to be masked by the other ingredients.

2. Material and methods

2.1. Experimental feeds

The fish were given a choice between two feeds with the same amounts of protein and energy, i.e., the test feed and a fishmeal-based control feed with a composition that mirrored that of a commercial Arctic charr feed. Cooked and de-shelled mussels were used (Royal Frysk Muscheln GmbH Emmelsbüll-Hornsbül, Germany). The fishmeal from the Baltic Sea was produced by TripleNine (Esbjerg, Denmark), and the fishmeal in the control diet originated from the Atlantic Ocean. The Baker's yeast was cultured on molasses, ammonia, phosphorus, magnesium and vitamins and then dried on a fluidized bed (Jästbolaget®, Stockholm, Sweden). The oil fractions of the two feeds both contained regular commercial fish oil and regionally produced rapeseed oil.

The Finnish Game and Fisheries Research Institute manufactured the experimental feeds at the Laukaa Aquaculture station in Finland. Pellets were extruded using a twin-screw extruder (3 mm die, BC-45 model, Clextral, Creusot Loire, France), and lipids were then added with a vacuum coater (Pegasus PG-10VC, Dinnissen, Sevenum, Netherlands). The pellets were 3 mm long (See Tables 1 and 2 for further details about the feeds). The amounts of betaine (glycine-betaine) in the feeds were analysed because this compound is a feeding stimulant for salmonids and may be contained in marine invertebrates, such as mussels (Yamashita et al., 2006). The higher betaine content of the test feed was four times greater than that of the control feed (Table 2)

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