Master's thesis

The effects of dietary fumonisin B_1 on growth and physiology of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Traditionally, fish meal and fish oil have been used as main sources of proteins and energy in the commercial fish feeds. Nowadays these ingredients have been partially substituted with materials of plant-origin (e.g. maize, wheat and soy) due to their more inexpensive price and easier availability. If contaminated plant material is used in the making of fish feed, the finished product can contain harmful substances called mycotoxins. Mycotoxins are the secondary metabolite products of moulds that are produced mainly for competition against other moulds. Feed mycotoxins have been observed to suppress growth and cause diseases on several domestic animals, but their effects on fish have been studied scarcely. One of the most common mycotoxins is fumonisin B_1 (FB_1) produced by fungus *Fusarium moniliforme*. FB_1 contaminates mainly maize and maize products. In this 8-week study, the effects of dietary FB_1 on the growth and physiology of juvenile rainbow trout (*Oncorhynchus mykiss*) were examined. The fish were divided randomly into treatment groups that were fed with graded concentrations of FB_1: 0, 1, 5, 10 or 20 mg/kg. The growth of the fish and the feed consumption was monitored during the trial. After the trial, the blood samples of the fish were analysed for haematocrit, glucose and plasma chloride, and hepatosomatic index and liver water content were measured. Samples were taken from livers to examine possible histopathological abnormalities. The results indicate that FB_1 does not affect growth, haematology or livers of rainbow trout at tested concentrations. Rainbow trout is known to be sensitive towards mycotoxins deoxynivalenol and aflatoxin, but appears more resistant towards FB_1. This is maybe due to different metabolic pathways at cellular level and differences in biotransformation between different toxins. However, a longer experiment and/or increased amounts of FB_1, may be needed to track possible adverse effects of FB_1 on rainbow trout.

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TIIVISTELMÄ

Kalanrehuissa on perinteisesti luotettu kalajauhoon ja -öljyyn rehujen pääasiallisina proteiinin- ja energianlähteinä. Näitä raaka-aineita on nykyään alettu korvata kasviperäisillä ainesosilla niiden edullisemman hinnan ja helpomman saatavuuden vuoksi, esimerkiksi maissilla, vehnällä ja soijalla. Pilaantuneen kasviperäisen raaka-aineen mukana rehuun pääsee haitallisia mykotoksiineja. Mykotoksiinit ovat homesienten sekundäärisiä metaboliatuotteita, joita ne pääasiassa tuottavat kilpailuun muita homeita vastaan, ja joiden on havaittu aiheuttavan kasvun heikentymistä ja sairauksia monilla eri kotieläimillä, mutta niiden vaikutuksista kaloihin tiedetään varsin vähän. Yksi yleisimmistä mykotoksiineista on pääasiassa maissilla esiintyvän *Fusarium moniliforme* -homeen tuottama fumonisiini B_1 (FB_1). Tässä tutkimuksessa tutkittiin rehun sisältämän FB_1:n vaikutusta kirjolohen (*Oncorhynchus mykiss*) kasvuun ja fysiologiaan. 1+ -ikäiset kirjolohet jaettiin satunnaisesti koeryhmiin, joita ruokittiin kasvatuskokeessa kahdeksan viikon ajan rehulla, johon oli lisätty viisi eri FB_1 -pitoisuutta: 0, 1, 5, 10 ja 20 mg/kg. Kalojen kasvua ja rehunkulutusta seurattiin kokeen aikana. Kokeen jälkeen kaloista otettiin verinäytteet, joista analysoitiin hematokriitti, glukoosipitoisuus ja plasman kloridipitoisuus. Kalojen maksoista tutkittiin painojen ohella niiden vesipitoisuus. Osasta maksoista valmistettiin leikkeet mahdollisten histopatologisten poikkeamien havaitsemiseksi. Tulokset osoittivat, ettei FB_1 vaikuttanut kalojen kasvuun, niiden veriarvoihin tai maksoihin annetuilla pitoisuuksilla. Kirjolohi on herkkä kahdelle muulle mykotoksiinille, deoksinivalenolille ja aflatoksiinille, mutta laji vaikuttaa kestävän FB_1:tä varsin hyvin. Tämä voi johtua erilaisista solutason aineenvaihdunnan reiteistä ja biotransformaation eroista mykotoksiinien välillä. Kuitenkin lisätutkimuksia pitemmillä altistusajoilla ja/tai suuremmilla FB_1 -pitoisuuksilla tarvitaan havaitsemaan mahdolliset vaikutukset kirjoloheen.

Contents

1. INTRODUCTION

Fish meal and fish oil, usually of marine origin, are traditionally used as main sources of protein and energy in commercial fish feeds. However, due to decline of marine fish resources and supply, the prices of fish-based raw materials have increased in recent years. This, along with the increasing production of worldwide aquaculture, has led to increasing interest of replacing the fish-based raw material with the ingredients of plant origin which are generally cheaper and more readily available than fish raw material (New & Wijkström 2002). For example, wheat (*Triticum aestivum*), soy (*Glycine max*), maize (*Zea mays*), peanut (*Arachis hypogaea*), cottonseed (*Gossypium* spp.), rice (*Oryza* spp.) and canola (*Brassica rapa* subsp. *oleifera*) have been used in manufacturing fish feeds (Wilson & Poe 1985, Silvenius et al. 2012).

The switch from using plant proteins instead of animal proteins in fish feeds comes with some arising issues: concerns about decreasing growth rate and alterations in the fish flesh quality, especially the composition of healthy fish oils (New & Wijkström 2002). Also, using plant-based raw materials raises a question about mycotoxins (New & Wijkström 2002), especially if the plant material used in manufacturing the commercial feed is low of quality.

Mycotoxins are secondary metabolites produced by several different genera of fungal moulds: the most common belonging to genera *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. These moulds contaminate different plant material, especially raw grain material and grain products, and produce different toxic substances (some fungi can produce more than one toxin) for competition against other fungi (Manning 2001). Hundreds of different mycotoxins with vast chemical diversity have been found and described (Jestoi 2008), however *Aspergillus* toxins, aflatoxins and ochratoxin A, and *Fusarium* toxins fumonisins, deoxynivalenol, moniliformin and zearalenone being amongst the most common ones (Manning 2001).

The adverse effects of mycotoxins on domestic animals are widely researched and well-documented (D'Mello et al. 1999). Mycotoxins can suppress growth, reduce feed consumption and affect animals' productivity e.g. decrease milk production and quality in dairy cattle (Applebaum et al. 1982, Charmley et al. 1993), and reduce egg count and quality in hens (Wyatt et al. 1975). They can also be carcinogenic, neurotoxic and hepatotoxic and can cause severe diseases on animals, depending on mycotoxin (D'Mello et al. 1999).

Although the effects of FB_1 towards several domestic animals are well-known, there are surprisingly few studies about the effects of FB_1 on fish. In this study juvenile rainbow trout were fed with feed containing graded concentrations of mycotoxin fumonisin B_1 in an eight-week trial. The aim was to observe potential adverse effects of the FB_1 on the growth and physiology of rainbow trout. It was hypothesized that the feed FB_1 suppresses feed intake and growth of the fish and FB_1 also impacts on the physiology of the fish. It was also hypothesized that the higher the concentration of FB_1 given to fish, the more negative impact it cause.

2. BACKGROUND

2.1. Fumonisin B_1, the structure and the pathways of action

Fumonisin B_1 (FB_1) mycotoxin is a secondary metabolite produced by mould *Fusarium moniliforme* (syn. = *F. verticillicoides*) (Gelderblom et al. 1988). *F. moniliforme* infects mainly maize, causing common plant disease called "maize ear rot" with distinctive white mycelia growth. The mould can also infect the kernels of the plant ("kernel rot") if the surface of the kernels are damaged by insects or birds (Richard et al. 2003). *F. moniliforme* can produce three types of fumonisins, labelled as B_1, B_2 and B_3, of which B_1 is usually the most prevalent (Placinta et al. 1999).

Fumonisin B_1 is very polar and thus, water soluble compound. It has long carbon chain backbone (Figure 1) that separates the compound from other mycotoxins (Griessler & Encarnação 2009). In spite of the hydrophilic nature of FB_1, it can apparently pass the blood-brain barrier (BBB) of the organisms and can cause neurotoxic symptoms in animals. However, the actual mechanism of the passage is not known (Kovačić et al. 2009).

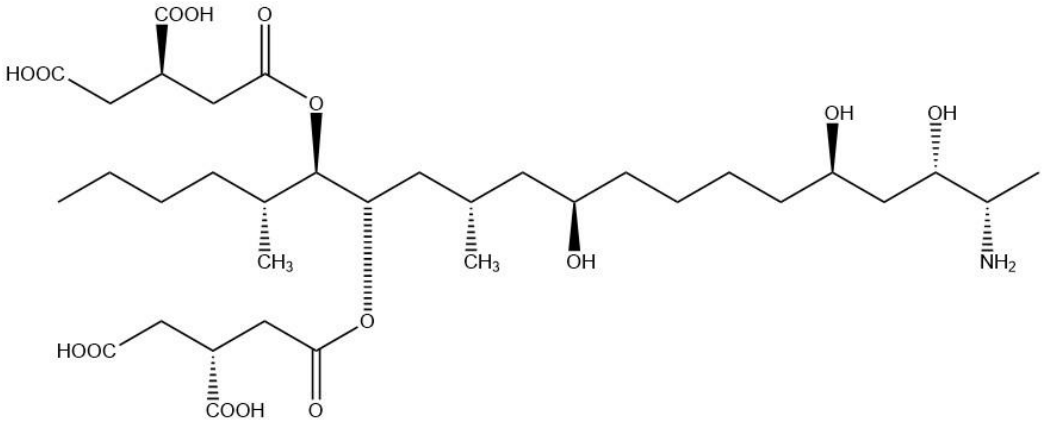


Figure 1. The chemical structure of FB_1. Drawn after ApSimon (2001) with ChemDraw Professional 15.1, PerkinElmer, Inc.

The long carbon backbone of FB_1 is structurally analogous with the base backbone of sphingosine (Wang et al. 1991, Plattner & Shackelford 1992, ApSimon 2001). Sphingosine is a structural component of several sphingolipids like ceramides, sphingomyelins, glycolipids and gangliosides. These compounds are part of cellular structures, especially membranes, and are mostly located in the tissues of nervous system (Wang et al. 1991, Plattner & Shackelford 1992, Merrill et al. 2001). There sphingolipids function in cell regulation as secondary messengers for various different substances e.g. growth hormones and cytokines and thus participates in cell to cell and cell to substratum interactions (Wang et al. 1991, Merrill et al. 2001).

The toxicity of FB_1 is based on this structural similarity of sphingosine. FB_1 interferes with sphingolipid metabolism and inhibits the ceramide synthesis, more specifically inhibiting sphingosine N-acyltransferase (Wang et al. 1991, D'Mello et al. 1999, Merrill et al. 2001, Shephard et al. 2007). This inhibition generally causes an elevation in free sphinganine (Sa) amounts and an elevation in free sphinganine to free sphingosine ratio (Sa:So) in dose-dependent manner (Merrill et al. 2001, Shephard et al. 2007). These elevated ratios of free sphingoid bases are useful tools (biomarkers) in animal

studies, especially when high doses are presented. These ratios can be observed in the blood serum and urine of animals (Shephard et al. 2007).

Tuan et al. (2003) observed significantly elevated Sa:So ratios in the livers of Nile tilapia (*Oreochromis niloticus*) fed with 150 mg/kg of FB_1 compared to control fish. The analogous elevation of Sa:So ratios was observed previously by Goel et al. (1994) in channel catfish (*Ictalurus punctatus*) fed with diet containing FB_1 in concentrations ranging between 0.3 to 240 mg/kg. The observed Sa:So ratios were considerable higher in feeding groups receiving 10, 20, 40 and 80 mg/kg of FB_1 compared to the other feeding groups. The elevated Sa:So ratios were observed in kidney, blood serum, liver and muscle of the catfish.

2.2. Interactive effects with other mycotoxins

Mycotoxins rarely appear solitarily in animal feedstuff i.e. domestic animals are frequently exposed to feedstuffs containing diverse, complex blend of *Fusarium* and *Aspergillus* mycotoxins. The effects can be either additive or synergistic (D'Mello et al. 1999, Richard et al. 2003). Fumonisin B_1 is usually accompanied with fumonisin B_2: in maize samples from Southeast Asia fumonisins B_1 and B_2 were found in 50 % of the samples. It is also noticeable that 48 % of screened samples contained both fumonisins and aflatoxins, two mycotoxin groups that are considered carcinogenic (Yamashita et al. 1995).

Both Javed et al. (1993) and Kubena et al. (1995) noticed additive effects of mixtures of different *Fusarium* mycotoxins (FB_1, FB_2, T-2 and moniliformin) on chicken and turkey hatchlings: The clinical symptoms appeared more quickly to birds served with combined mixture of mycotoxins than to birds fed with single mycotoxin at the time. Also, the intensity of the symptoms and physiological abnormalities were greater with the mixture feeds.

Yildirim et al. (2000) observed reduced growth (weight gain), decreased feed intake and lower haematocrit values in juvenile channel catfish fed with two different, combined mixtures of FB_1 and moniliformin, another *Fusarium* mycotoxin, (20:40 and 40:40 mg/kg, respectively) than the control groups (0 mg/kg). In another study done with rainbow trout, Carlson et al. (2001) noticed that FB_1 promotes the carcinogenic properties of aflatoxin B_1, thus causing possible hepatocarcinogenesis in rainbow trout. In conclusion, the mixtures of different mycotoxins in fish feeds can have adverse effects on the performance and health of the reared fish.

2.3. Effects of fumonisin B_1 on humans

Humans (as well as other animals) are exposed to mycotoxins usually via digestive tract by ingesting contaminated food, but it is considered that mycotoxins can possibly have other routes of exposure such as via inhalation and direct physical contact. There are few studies about human mycotoxicoses (diseases caused by dietary, respiratory or dermal exposure to toxic fungal metabolites, Bennett & Klich 2003) caused by FB_1 but there have been rising interests in research towards this subject (Richard et al. 2003).

The mouldy maize containing both FB_1 and FB_2 has been linked with high prevalence of human esophageal cancer in some regions in South Africa (Marasas et al. 1981, Sydenham et al. 1990) and China (Chu & Li 1994, Yoshizawa et al. 1994), but, however, direct causal connection has not been confirmed yet (Richard et al. 2003). Williams et al. (2010) noticed positive correlative connection between human immunodeficiency virus (HIV) transmission rates and maize consumption (thus, possible FB_1 exposure) in sub-Saharan Africa. They stated that consumption of FB_1 contaminated

maize might be promoting HIV transmission, but obviously more studies are needed to confirm this connection.

Also, possible residues of different mycotoxins in food products for human consumption may produce potential health risk for humans. These residues may be present in different commercial cereal products, such as flours, snacks (Patel et al. 1997, Silva et al. 2007) and breakfast cereals (Patel et al. 1997), and in animal products, such as milk, eggs, meat and entrails (D'Mello et al. 1999).

2.4. Effects of fumonisin B_1 on domestic animals

Juvenile animals tend to be more susceptible towards mould toxins than adults of same species, but the severity of toxicity towards different animal species varies between toxins (Manning 2001). Toxicity of fumonisins towards different domestic animals is well-documented. Feed containing fumonisins causes suppressed growth and variety of physiological damage on morphological, cellular and biochemical levels to domestic animals: lesions in brain, liver, kidneys, lungs and gastrointestinal track have been found in several domestic animal species. Also, as mentioned above, mycotoxins may alter the productivity of domestic animals: animals fed with diet containing mycotoxins produce less with inferior quality (D'Mello et al. 1999)

Equine species (e.g. horses, ponies and donkeys, belonging to genus *Equus*) are very vulnerable towards fumonisin B_1: concentrations as small as 5 mg/kg of FB_1 in feed can cause a lethal condition called equine leukoencephalomalacia (ELEM) (Manning 2001). Clinical signs of ELEM include several behavioural and neurological symptoms, e.g. apathy, refusal of eating/drinking, paralysis in mouth and difficulties in movement. Symptoms are caused by liquefactive lesions/oedema in brains of the horses (Marasas et al. 1988, Kellerman et al. 1990).

In domestic pigs (*Sus scrofa domesticus*) mycotoxicosis caused by FB_1 is identified with pulmonary oedema, often called porcine pulmonary oedema (PPE) (D'Mello et al. 1999). Along with PPE, FB_1 has been observed to cause abnormalities in livers, pancreases, hydrothoraxes, lungs (Harrison et al. 1990, Haschek et al. 1992, Osweiler et al. 1992) and in cardiovascular systems of the pigs (Smith et al. 1996). Also, FB_1 apparently can in some cases be lethal to swine and cause abortions in pregnant sows (Harrison et al. 1990, Osweiler et al. 1992)

In broiler chicken, especially in 10-16 days old chicks, FB_1 can cause acute mortality in flock, often described as "spiking mortality syndrome" (D'Mello et al. 1999). Javed et al. (1993) observed similar kind of high, acute mortality in 1-day old male chicks when fed with purified FB_1 material in 125 and 274 ppm concentration. They also noticed dose-dependent clinical signs (e.g. movement difficulties, apathy, refusal of feed and drink) and lower weight gain. In study by Kubena et al. (1997), they observed that FB_1 (fed with 300 mg/kg FB_1 to broiler hatchlings) caused enlargement of kidneys and liver and cause increase in blood serum biochemical values and enzyme activity values. In other common poultry species, turkey (*Meleagris gallopavo*) FB_1 causes poor performance (reduced growth and feed efficiency) and enlargement of the organs, especially liver, kidneys and gizzard (Kubena et al. 1995).

Mathur et al. (2001) observed alterations in liver function and injuries in liver, bile ducts and kidneys in milk-fed calves (*Bos taurus*) administered with 1 mg/kg of FB_1 intravenously. These injuries indicate that FB_1 is hepatotoxic and nephrotoxic to young cattle.

2.5. Worldwide occurrence of fumonisins

Globally the plant-based raw materials, feed ingredients and manufactured feeds may contain high amount of mycotoxins, and the contamination rate of the goods is still increasing due to the rising trend of the usage of plant materials in animal feeds (Santos et al. 2010).

In global survey conducted by BIOMIN in year 2009, the overall results showed that great number of samples contained mycotoxins and the maize was found to be the most contaminated commodity (Rodrigues 2009). Fumonisin B_1 was detected in 11 % to 60 % of tested samples, depending on the region: the most contaminated samples were found in North Asia. In a review by Santos et al. (2010) FB_1 was most prevalent in South America, especially in Brazil, where 87 % of tested samples were found to be positive. For the other regions, the results varied between 0 and 81 % of tested samples being contaminated.

Survey of similar kind was conducted in year 2012 and analogous results appeared this time as well: the FB_1 positive results ranged from 12 to 100 % of tested samples. The FB_1 was most prevalent in Africa. Overall, the observed FB_1 levels were slightly elevated since previous surveys (Rodrigues & Naehrer, 2013a). Also, similar as in previous surveys, FB_1 was most prevalent in maize (86 % FB_1 positive samples). However, the maize itself and processed maize products were the most analysed goods and main interests in these surveys (Rodrigues & Naehrer, 2013b).

In recent survey done in year 2015 showed that in maize samples from Central and Southern Europe, the FB_1 was the most prevalent mycotoxin in samples from Southern Europe (95 % of FB_1 positive samples) and in samples from Central Europe half (50 %) of the samples were contaminated. It was stated that the occurrence of FB_1 has been increased since the year 2014 possibly due to warmer summer experienced in year 2015 that could be favoured by FB_1 (Schwab, 2016).

In summary, FB_1 is very common contaminant in raw grain material and commercial grain products, especially in maize products, and is universally spread throughout the globe though the mycotoxin is more prominent in regions with humid and warm climate.

However, FB_1 contaminations do not limit only to be found in raw grain material or finished grain products. In a survey conducted by Nutriad in year 2016, 14 different commercial cat and dog foods of premium/super premium brands were sampled and analyzed for the occurrence of seven mycotoxins. The most prevalent mycotoxin was FB_1 (93 % of samples being contaminated), followed by FB_2 with 85 % of positive samples. It is common to supplement pet foods with grain products such as maize, soya, wheat and rice, even in feedstuff targeted to carnivores, and therefore they are being the most crucial source of mycotoxin contamination in pet foods (Borutova 2016).

2.6. Regulations and guidance values of mycotoxins

According to van Egmond et al. (2007), by the end of year 2003 at least 100 countries (covering nearly 85 % of the world population) had taken action in regulating or having detailed guidance values for mycotoxins in food and animal feed. Regulative actions are covering at least 19 different mycotoxins of various origins: aflatoxins (B_1, B_2, G_1, G_2 and M_2), trichothecenes (deoxynivalenol, diacetoxyscirpenol, T-2 and HT-2 toxins), fumonisins (B_1, B_2 and B_3), agaric acid, ergot alkaloids, ochratoxin A, patulin, phomopsins, sterigmatocystin and zearalenone. All regulating countries had guidance values for aflatoxin B_1 and combination of other aflatoxins, but for other mycotoxins

presented above specific guidelines may vary between countries (van Egmond & Jonker, 2004).

FDA (The Food and Drug Administration of the United States) has given guidance values for fumonisins $B_{1,}$ B_2 and B_3 in animal feed varying between 5 mg/kg and 100 mg/kg, depending on animal. For catfish the upper limit is 20 mg/kg of total fumonisins in grain products (Bhatnagar et al. 2004, Anonymous 2011). As well, European Commission has given own limits to member countries of European Union about FB_1 and FB_2: for maize and maize products (raw material) the limit is 60 mg/kg and in complete animal feedstuff the limits are varying between 5 to 50 mg/kg. For the farmed fish the upper limit is set at 10 mg/kg of fumonisins in feedstuff (Anonymous 2006).

2.7. Prevention of mycotoxins

The handling of mycotoxins in raw materials and manufactured feedstuff can be separated into two strategies: the prevention of contamination and if contamination has occurred, the actions to reduce concentrations of toxins in finished products (Manning 2001). These preventive actions should cover all steps from field to harvest into storage and eventually, into the finished product (Griessler & Encarnação 2009).

The primary production is the key issue for producing mould free (or at least, less contaminated) raw material as high quality as possible. The management actions that aim at maximising crop yield and reducing plant stress apparently decrease mould production in the field. These actions include suitable fertilisation and pest control, optimal crop density and rotation with careful selection of the cultivated seeds. The properly timed harvest (Griessler & Encarnação 2009) and quick drying of the harvested goods before storage (Marín et al. 1999) are also important. Careful use of different fungicides or selection of pathogen resistant plants can also be sustainable options for mycotoxin prevention (Placinta et al. 1999).

After harvesting the proper storage facilities with good, suitable conditions are essential in preventing mould growth or further contamination in raw material. This same principal is valid with finished products as well (Manning 2001). The optimal growth conditions for *Fusarium* spp. moulds (thus, production of fumonisins) were observed to be in between 15 – 30 °C and in relative high air humidity (Marín et al. 1999). The storage conditions should be below these favourable conditions. These can be achieved by controlling the air humidity and temperature, measuring the moisture content of the grains (below 12 % is recommended) and keeping grains in clean, proper containers. Applying mould inhibitors such as propionic acid onto surface of the goods can also decrease the risk of contamination. The pest control is also important: the pests (mainly rodents and insects) can spoil the grains with their excrement and may damage the hard outer layers of grains and thus promote fungal growth by giving potential sites for invasion and growth (Manning 2001). However, these preventive actions described above do not eliminate, but only limit the risk of contamination (Griessler & Encarnação 2009).

The reductive procedures for contaminated feedstuff can be further divided into physical, chemical (Placinta et al. 1999, Bhatnagar 2004) and biological treatments (Griessler & Encarnação 2009). The degree of spoilage and the distribution of fungal growth in the feedstuff affect the efficiency of physical treatments (Griessler & Encarnação 2009). Physical treatments include actions like milling of the grain (Placinta et al. 1999, Bullerman & Bianchini 2007), sorting and cleaning away the spoiled ingredients (Bhatnagar 2004, Bullerman & Bianchini 2007), dilution of the spoiled feedstuff with fungi free ingredients (Placinta et al. 1999) and treat contaminated grains with high

temperature and/or pressure (Bhatnagar 2004, Bullerman & Bianchini 2007). These physical processes tend to be rather expensive and may decrease the nutritional values of the feed (Kolossova et al. 2009)

The chemical treatments intend to detoxify mycotoxins entirely or to hinder the absorption of the mycotoxins in the gastrointestinal track. That is achieved by adding different chemical substances, called adsorbents, into feedstuff. The adsorbents function by binding mycotoxins and/or rendering them into indigestible form and thus reduce the bioavailability of digested toxins (Manning 2001, Griessler & Encarnação 2009). Several different compounds have been used, such as ammonia (NH_3), calcium hydroxide-monomethylamine ($Ca(OH)_2$-MMA), sodium bisulphite ($NaHSO_3$) and different deoxidising compounds (Placinta et al. 1999, Manning 2001, Bhatnagar 2004). However, chemical compounds often can alter the palatability of the feedstuff, form harmful by-products or decrease the nutritional values (Griessler & Encarnação 2009, Kolossova et al. 2009). Use of different clays (e.g. bentonites, zeolites, silicas and aluminium silicates (Griessler & Encarnação 2009)) is probably the most common way to try preventing toxin absorption. Using adsorbents have been fairly effective with aflatoxins, but their ability to bind fumonisins are restricted or more often without success (Ledoux & Rottinghaus 1999, Griessler & Encarnação 2009, Kolossova et al. 2009)

The biological treatments aim to enhance the natural biotransformation taking place in the system of organisms i.e. transforming or breaking up these organic toxins in naturally occurring metabolic processes. Comparing with the treatments presented above, the biotransformation is usually toxin-specific and irreversible. However, these different microbial and enzymatic pathways are still under research and development (Griessler & Encarnação 2009).

3. MATERIALS AND METHODS

3.1. Experimental fish and setup

The experiment was conducted at the research facilities of the Department of Biological and Environmental Science, University of Jyväskylä, Finland, between 2 April and 25 May 2013. The experimental fish were rainbow trout yearlings (age 1+). The fish were obtained from a commercial fish farm Hanka-Taimen Ltd., Hankasalmi, Finland, and the 400 fish (mean weight 23 ± 0.92 g) were divided into two stainless steel tanks for acclimation.

Fish were acclimated for 5 days at 15 °C. During acclimation period fish were hand-fed to apparent satiation twice a day with a commercial fish feed (Vital Plus, Rehuraisio Ltd.) that had previously given to fish at the fish farm. Later during the acclimation, the experimental control feed (Sparos Ltd.) was added in commercial feed in 50:50 mixture to accustom the fish to a larger pellet size of the experiment feed.

After acclimation, fish were separated randomly into 15 stainless steel tanks so that each tank contained 26 fish. Each flow-through tank (90x80x60 cm, volume 432 l) was covered with net lids to prevent fish from jumping out of the tanks. The water, originating from a well, was temperature-regulated through automated heating regulator (Ouman, model EH-201/V) and temperature was adjusted to 15.5 °C. Water was constantly aerated by conducting pressurised air through air stones (1-2 stones per tank) into tanks. For additional aeration air pumps (Mouse, M106) were used if necessary i.e. if oxygen level declined under 7.0 mg/l in tank. Water flow into tanks was set to 900 ml/min and daily

photoperiod was set at 12 hours dark: 12 hours light (12D:12L) using fluorescent lights. Temperature and oxygen levels were measured daily using handheld oxygen meter (YSI ProODO).

During this 8 week experiment period, fish were treated with five different mycotoxin fumonisin B_1 (FB_1) levels: 0 (control), 1, 5, 10 and 20 mg/kg of feed. Each level of FB_1 had three replicate tanks. Experiment feed was produced by Sparos Ltd. (Portugal). Feed was made by extruding and was 2 mm of diameter. Feed was stored in a freezer (-18 °C) during the experiment.

Fish were hand-fed twice a day to apparent satiation six days a week. Uneaten pellets were collected from tank bottom by siphoning after the first daily meal. Pellets were dried in the oven (Memmert, m500) at 65 °C overnight and weighted (0.01 g accuracy).

Fish and tanks were checked daily. Dead or fish in poor health condition were removed (and euthanized with a sharp hit to the head) from tank when observed. The experiment had the license for animal experiment granted by Regional State Administrative Agency of Southern Finland (ESAVI/5561/04.10.03/2011).

3.2. Measurements and calculations

In the beginning of the experiment, before dividing into tanks, fish were weighed individually (to 0.1 g). Length was measured from first 10 individuals per tank (to 0.1 cm). During the experiment, the fish were weighed in groups (to 1 g) in every 2 weeks totalling 3 weighings. In the end of trial, fish were weighed and measured in the same way than in the beginning of the experiment. In all measurements, fish were starved the day before the weighing i.e. fish were weighed with empty stomachs.

Fish were anesthetised using clove oil during individual weighings. Ethanol and clove oil mixture (9:1) was added to water in relation to 2 ml clove oil solution to 5 litres of water (clove oil concentration 40 mg/l). The anaesthetic solution was constantly aerated with air pumps. After measurements, fish were returned to their original tanks. Fish recovered from anaesthesia within approximately 4 to 5 minutes.

Condition factor (K) for fish was calculated as:

$$K = W / L^3 * 100$$

where, W= fish weight (g) and L= fish length (cm). Fish growth was defined as specific growth rate (SGR):

$$SGR\ (\%\ /\ day) = 100 * (\ln W_2 - \ln W_1) / t,$$

where W_1= fish weight (g) in the beginning of the experiment, W_2= fish weight (g) in the end of the experiment and t= time as feeding days. Total feed consumption (total amount of pellets eaten) was calculated as:

Feed consumption (g) = dry weight of offered pellets (g) – dry weight of uneaten pellets (g)

Feed conversion ratio (FCR) was calculated as:

FCR = total feed consumption (g) / fish weight gain (g)

Relative feed intake (RFI) as percentage of fish body weight per feeding day was calculated as:

RFI (% body weight / day) = (100 * feed consumed (g) / fish mean weight (g)) / t,

where t= number of feeding days.

Total amounts of fumonisin B_1 (mg) consumed during this trial were calculated as:

Total amount of FB_1 = total amount of feed eaten (kg) * FB_1 level of feed (mg/kg).

3.3. Blood, liver and excrement samplings

After the feeding trial, all the fish were weighted and measured as described above. For blood and liver samples, six fish from each replicate group (tank) were euthanised by a sharp hit to the head, behind the eyes.

Blood were collected from caudal vein using Lithium heparinised disposable needles (23Gx1¼") and syringes (1 ml). Collected blood was transferred to Eppendorf tubes and centrifuged (8000 rpm x 5 min, Heraeus Biofuge A). Plasmas were transferred to new Eppendorf tubes and put into ice and later frozen at -18°C. Before centrifuging, haematocrit samples were taken and the haematocrit tubes were centrifuged (12,000 rpm x 5 min, Heraeus Biofuge Haemo).

Replicate (2-4) plasma samples (20 µl) were analysed for chloride (Cl^-) using chloride analyser (Sherwood 926S, UK). 2-4 replicates (20 µl per replicate) were sampled from each plasma sample. Maximum allowed difference between replicates was ± 2 mmol/l.

For liver removal, fish were opened from anus to gills using a scalpel. Livers were removed from body cavity using tweezers. Livers were transferred to tared Eppendorf tubes and weighed (to 0.0001 grams). After weighing, the livers were dried in the oven at 75 °C for 3 days and weighed again (to 0.0001 g). The liver water content (%) was calculated as:

Liver water content (%) = (100- (liver dry weight (g) / liver wet weight (g)) * 100),

and hepatosomatic index (HSI) was calculated as:

HSI = (liver wet weight (g) * 100)/ fish weight (g))

3.4. Liver histology

For liver histology, livers were removed as intact as possible and were preserved in buffered formalin (37 %) solution. For fixation, a 10 % phosphate buffered dilution was made from preservation solution (3.7 % formalin concentration, pH 7.4). The samples were fixed overnight. The fixed samples were taken to pathology laboratory of the Central hospital of Central Finland where the samples were processed and embedded into paraffin. From paraffin blocks, 4-5 µm thin sections were cut and inserted into microscope slides. The slides were deparaffinised and stained with haematoxylin and eosin solutions. Finished microscope slides were covered with glass slips.

Only liver samples from the control groups and highest treatment groups (20 mg/kg) were examined using fluorescence microscope (Leica Leitz DMRBE) with UV-filter. Each sample was examined for any abnormalities (for example tissue lesions, signs of inflammation or lipid accumulations) by an expert pathologist, Professor Markku Kallajoki. Sections were photographed using camera and software (Olympus Soft Imaging Solutions, analySIS 5.0) integrated within microscope.

3.5. Statistical analyses

The data were entered to Microsoft Excel 2010 (version 14). Statistical analyses were performed using IBM SPSS Statistics software (version 20). To avoid pseudo-replication, average values of each tank were used as observations in statistical analyses.

To test for possible differences in growth and feed efficiency and physiology between different FB_1 levels, one-way ANOVA (1-ANOVA) was used if conditions (the dependent variables are normally distributed and variances are homogenous) were met. If not, non-parametric Kruskal-Wallis test was used instead. Levene's test was used to check homogeneity of variances and Shapiro-Wilk's to check normality of the data. When needed, Tukey's honestly significant difference (HSD) *post hoc* – test was used for comparing means between the treatment levels. Statistical significance level was set up for p value < 0.05.

4. RESULTS

4.1. Growth and feed efficiency

In total 9 fish died or were euthanised due to poor health during the trial. There were fish from all treatment groups among the dead ones: control 1 fish, 1 mg/kg 3 fish, 5 mg/kg 2 fish, 10 mg/kg 2 fish and 20 mg/kg 1 fish.

There were no statistically significant differences between different FB_1 feeding groups in initial weight (1-ANOVA: $F_{4,10}$= 0.400, p= 0.804), initial length (1-ANOVA: $F_{4,10}$= 1.373, p= 0.311) and initial condition factor, K (1-ANOVA: $F_{4,10}$= 0.446, p= 0.773) nor final weight (1-ANOVA: $F_{4,10}$=1.301, p= 0.334), final length (Kruskal-Wallis: χ^2= 8.233, df= 4, p= 0.083) and final condition factor, K (1-ANOVA: $F_{4,10}$=2.284, p=0.132) (Table 1).

No statistically significant differences were found between different treatment groups in total feed consumption (1-ANOVA: $F_{4,10}$= 0.863, p= 0.518), total weight gain (1-ANOVA: $F_{4,10}$= 1.835, p= 0.199) nor in feed conversion ratio (1-ANOVA: $F_{4,10}$= 0.532, p= 0.716) as measured for whole experiment period (Table 1).

However, different feed FB_1 levels had statistically significant difference in specific growth rate (SGR) measured for whole experiment period (1-ANOVA: $F_{4,10}$= 3.629, p= 0.045). Tukey's *post hoc* – comparison between groups revealed that control group differed significantly from the FB_1 10 mg/kg group (Tukey HSD: p=0.042) (Table 1).

Table 1. The measured parameters of rainbow trout yearlings fed with feeds containing different concentrations (mg/kg) of mycotoxin fumonisin B_1. SGR and FCR were calculated for whole experiment period. The presented values are treatment group averages ± S.D., n= 3 in each group. The statistical significance is marked with uppercase letters.

	Fumonisin FB_1 concentration (mg/kg)				
	0	1	5	10	20
Initial weight (g)	21.5± 0.54	22.4± 0.24	22.5± 1.50	22.0± 0.69	22.1± 1.61
Initial length (cm)	12.6± 0.23	13.0± 0.22	12.9± 0.31	13.0± 0.24	12.7± 0.33
Initial condition factor	1.03± 0.05	1.01± 0.01	1.00± 0.02	1.01± 0.03	1.00± 0.03
Final weight (g)	111.34± 2.82	107.80± 3.91	110.03± 5.12	103.29± 5.74	104.55± 7.40
Final length (cm)	20.57± 0.20	20.32± 0.44	21.18± 0.16	20.12± 0.05	20.12± 0.71
Final condition factor	1.34± 0.03	1.32± 0.05	1.30± 0.01	1.27± 0.03	1.33± 0.02
Weight gain (g)	89.84± 3.32	85.43± 3.87	87.51± 3.63	81.27± 5.34	82.42± 5.86
SGR (%/day)	3.83^{a}± 0.12	3.66± 0.09	3.69± 0.05	3.59^{b}± 0.10	3.61± 0.05
FCR	0.73± 0.01	0.75± 0.03	0.74± 0.01	0.74± 0.04	0.75± 0.04
Total feed intake (g)	1676±75.56	1590±79.30	1574±52.20	1521±190.32	1507±163.73
Total fumonisin FB_1 intake (mg/kg)	0	1.59± 0.08	7.87± 0.26	15.21± 1.90	30.15± 3.27

4.2. Haematological analyses

The different feed FB_1 levels had no effect on blood haematocrit (1-ANOVA: $F_{4,10}$= 2.206, p= 0.142) or glucose levels (1-ANOVA: $F_{4,10}$= 1.291, p= 0.337) of the fish (Table 2). Similarly, no statistically significant differences were found in blood chloride levels (1-ANOVA: $F_{4,10}$=0.461, p=0.763) (Table 2).

Table 2. The results of sampled livers and blood from rainbow trout yearlings fed with feeds containing mycotoxin fumonisin B_1 in different concentrations (mg/kg). The presented values are treatment group averages ± S.D., n= 3 in each group.

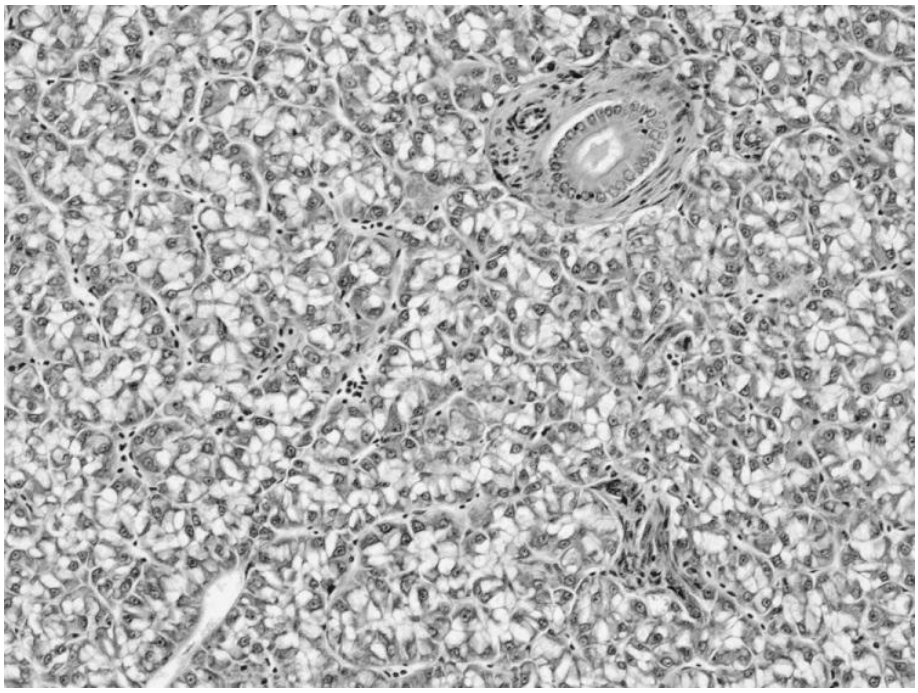
	Fumonisin FB_1 concentration (mg/kg)				
	0	1	5	10	20
Liver wet weight (g)	1.19 ± 0.09	1.16 ± 0.14	1.26 ± 0.14	1.04 ± 0.17	1.14 ± 0.10
Liver dry weight (g)	0.32 ± 0.03	0.31 ± 0.04	0.33 ± 0.04	0.28 ± 0.04	0.31 ± 0.03
HSI	1.03 ± 0.02	1.01 ± 0.06	1.02 ± 0.02	1.01 ± 0.06	1.00 ± 0.05
Liver water content (%)	73.27 ± 0.34	73.52 ± 0.32	73.52 ± 0.87	73.16 ± 0.14	73.12 ± 0.57
Haematocrit (%)	33.67 ± 0.58	34.00 ± 2.65	31.67 ± 4.16	30.00 ± 1.73	29.33 ± 1.53
Glucose (mmol/l)	4.67 ± 0.70	4.30 ± 0.30	3.83 ± 0.12	4.03 ± 0.61	4.50 ± 0.60
Chloride (mmol/l)	123.94 ± 2.91	123.90 ± 0.86	124.32 ± 0.25	125.48 ± 1.13	123.30 ± 3.29

4.3. Liver

There were no statistically significant differences between different feeding groups in liver wet weight (1-ANOVA: $F_{4,10}$= 1.050, p= 0.429), liver dry weight (1-ANOVA: $F_{4,10}$= 0.894, p= 0.502), hepatosomatic index (HSI) (1-ANOVA: $F_{4,10}$= 0.233, p= 0.914) nor in liver water content (1-ANOVA: $F_{4,10}$= 0.415, p= 0.794) (Table 2).

Liver histology sections from control and treatment groups treated with FB_1 20 mg/kg feed had no signs of lesions, signs of inflammation or lipid accumulations within liver tissue (Figure 2).

a)



b)

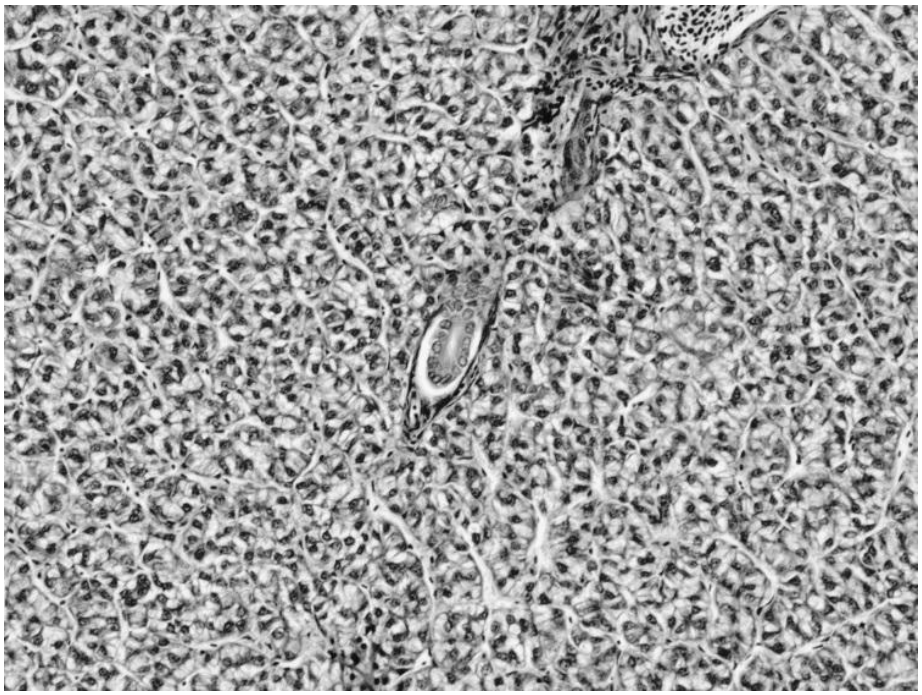


Figure 2. The liver sections of rainbow trout photographed in 10x magnification a) Fumonisin B_1 level 0 mg/kg (control) b) Fumonisin B_1 level 20 mg/kg.

5. DISCUSSION

The present study was conducted to examine possible effects on growth performance and physiology of rainbow trout yearlings fed on five different concentrations (mg/kg) of mycotoxin fumonisin B_1. The maximum concentration in this study was 20 mg FB_1/kg feed as it being FB_1 level that possibly can occur in infected feed stuff (Juhani Pirhonen, personal communication). This mycotoxin is a secondary metabolite produced by mould *Fusarium moniliforme* and occurs mainly in *F. moniliforme*-infected maize (Gelderblom et al. 1988). However, the present study did not support hypotheses presented above as none of the concentrations had adverse effects on performance or physiology of juvenile rainbow trout.

5.1. Mortality and growth performance

It is unclear what caused the mortality of the fish during the experiment. The fish behaved and swam normally during the experiment and no signs of continuous feed refusal were observed. There were no signs of bacterial infections on fish. Also the fact that all treatment groups were present among the removed fish suggests that the FB_1 concentrations have not affected survival of the fish.

In this study the fish had an average specific growth rate of 3.68 %/day. In their model, Austreng et al. (1987) estimate growth rate of 3.5 %/day to rainbow trout weighing 40 – 100 g reared at 16°C. Comparing to the model, the experimental fish grew well. However, it is possible that experimental fish in this study were a little underfed occasionally as there were multiple days when some tanks consumed all the feed added during the first meal of the day. If this happened, it was tried to compensate by increasing the amount of the feed given in the second meal, but it is possible that it was not enough to keep the fish satiated state.

In this study there were no significant differences in measured parameters: final weight and length, feed conversion ratio (FCR), final condition factor (K) and total weight gain. Only specific growth rate (SGR) measured for whole experiment period had significant difference between control and 10 mg/kg dose group. It is unclear what caused this difference. But, since the other measured fish performance parameters did not show statistically significant differences between feeding groups and the measured final weights were nearly identical, the observed statistical difference on SGR could be regarded as unconvincing.

Carrera García (2013) studied the effects of FB_1 on Baltic salmon (*Salmo salar* L.) in an analogous experiment, however the duration was two weeks longer and haematological analyses were not performed on Baltic salmon. Unfortunately, the salmon juveniles rejected continuously the feed offered thus having very low growth rates (SGR on average 0.5 %/day) throughout experiment. However, the results did not differ from the results obtained from this experiment: there were no significant differences between feeding groups in feed intake, SGR, feed conversion ratio, final length and weight of the fish. Only the condition factor (K) in fish fed with 20 mg/kg FB_1 was found to be lower from other feeding groups with statistical significance, but the author stated that as the other growth parameters did not statistically differ from one another, the observed significance should be considered inconclusive.

Several studies conducted on non-salmonid fish have shown that fish fed with feeds containing fumonisin B_1 had reduced growth rate and weight gain or fish had lost weight during the experiment (Li et al. 1994, Lumlertdacha et al. 1995, Yildirim et al. 2000, Pepeljnjak et al. 2002, Tuan et al. 2003, Kovačić et al. 2009, Gbore et al. 2010). Also, in

two studies (Li et al. 1994, Lumlertdacha et al. 1995) the channel catfish fed with highest amounts of FB_1 (240 and 720 mg/kg, respectively) continuously rejected offered feed. In the light of these studies, dietary FB_1 concentrations varying between 5 to 720 mg/kg has adverse effects on fish growth performance.

Feed conversion ratio (FCR) did not differ between treatment groups in recent study. In study with African catfish (*Clarias gariepinus*) fed with graded concentrations of FB_1 (0, 5, 10 and 15 mg/kg) measured FCR values did not differ significantly between treatment groups (Gbore et al. 2010). However, Yildirim et al. (2000) noticed in study done with young channel catfish fed with 0, 20 or 40 mg/kg concentrations of FB_1 that measured FCR value increased with an increase in concentrations of dietary FB_1. Respectively, in study done with Nile tilapia by Tuan et al. (2003), fish fed with maximum concentration of FB_1 (150 mg/kg) had 78 % higher FCR than control fish.

It is possible that there might be differences between different fish species in the tolerance for toxic effects of FB_1. These species described above might be more sensitive for dietary introduced FB_1 than rainbow trout or other salmonids. However, the some of the doses presented in previous studies were also noticeable higher than in the present study. Also, Lumlertdacha et al. (1995) found out that 2-year old channel catfish (initial weight 31 g) needed higher dose of dietary FB_1 to cause reduced weight gain than catfish yearlings (initial weight 1.3 g). This finding suggests that there may be dose/size relationship between reduced weight gain and digested fumonisin B_1 concentration i.e. larger fish needed higher doses of dietary FB_1 to cause lower growth comparing to smaller fish. There might be differences in doze/size relationship between different studies, i.e. smaller fish fed with higher amounts of FB_1. Also, in this study, the eight-week duration of the trial might not have been long enough to cause notable changes between treatment groups.

5.2. Haematology

According to Wedemeyer (1996), monitoring the haematological values of fish under culture system is useful tool for observing the health and physiological condition of fish. Significant differences from normal haematological values can be a sign of problems in fish health and farming conditions. Also the observed values can imply effects of different contaminants and disease pathogens. Elevated blood glucose, haematocrit values and decreased plasma chloride levels can be used as indicators of acute stress response in fish (Barton & Iwama 1991, Wedemeyer 1996, Barton 2002). The observed blood values in this study, however, fall within the values of clinically healthy rainbow trout under farming conditions (Wedemeyer 1996).

The observed haematocrit values, varying between 29 – 34 %, are well within the normal range (24 – 43 %, Wedemeyer 1996). The observed haematocrit values in this study did not differ between different treatment groups. Pepeljnjak et al. (2002) received no significant differences in haematocrit values in their studies done with carp (*Cyprinus carpio* L.) yearlings (weighing 120 – 140 g) fed with feed containing 0.5 and 5.0 mg kg^{-1} fumonisin B_1 for 42 days. Same kind of result was reported by Brown et al. (1994) with adult channel catfish fed with five different doses (from 0 to 313 mg/kg) of FB_1. On the other hand, few studies had pointed out that haematocrit values decreased when increasing the concentration of dietary FB_1 (Li et al. 1994, Lumlertdacha et al. 1995, Yildirim et al. 2000, Tuan et al. 2003, Gbore et al. 2010). However, these studies were conducted with non-salmonid fishes.

Observed blood glucose levels varied between 3.8 – 4.6 mmol/l and are well within normal range when comparing with normal values (2.8 – 8.4 mmol/l, Wedemeyer 1996). Measured blood glucose levels did not significantly differ between treatment groups in this study. Gbore et al. (2010) showed with African catfish that feed-introduced FB_1 lowered significantly the serum glucose levels of fish fed with highest concentration (15.0 mg/kg) of FB_1. However, in their paper the authors did not state any possible reasons for this decrease. Stoev et al. (2012) noticed a decrease in the serum glucose level together with kidney damage in a trial conducted on pigs fed with feed containing both FB_1 and ochratoxin A, another mycotoxin with levels 10 and 0.5 mg/kg respectively. Also, in a study done with calves, Mathur et al. (2001) observed decrease in serum glucose level in both control and treated animals (1 mg/kg of FB_1), but the decrease in glucose levels with FB_1 fed calves was faster. Stoev et al. (2012) suggest that observed decrease in serum glucose might be due to impaired kidney function and/or possible disturbance in their reabsorption caused by dietary mycotoxins.

Observed plasma chloride levels varied between 123 – 125 mmol/l and are well within normal range when comparing with normal values (84 – 132 mmol/l, Wedemeyer 1996). Measured plasma chloride levels did not significantly differ between treatment groups in this study. Haschek et al. (1992) observed no abnormalities in blood chloride values in swine given FB_1 intravenously (0, 4.6 and 7.9 mg/kg). They also fed different group of swine with maize contaminated with FB_1 (116 mg/kg) and FB_2 (48 mg/kg) and no abnormalities were found either in chloride values. Tardieu et al. (2004) observed decreased plasma chloride values during their 12-day trial done with domesticated mallard ducks (*Anas platyrhynchos*) force-fed with maize containing 0, 10 or 20 mg/kg of FB_1. However, they did not specify reasons for this decrease but stated that the force-feeding (handling stress) might have an effect on plasma biochemistry.

5.3. Liver damage

The liver water content is a simple way observing the health of fish: the more water is present in the liver, the less glycogen is deposited, and vice versa (Wedemeyer 1996). Observed water content values can imply about acute stress or nutritional imbalances in fish diet: in acute stress the liver glycogen is depleted due to elevated glucose metabolism and when dietary deficiencies are presented, glycogen is deposited in fish liver, impairing normal hepatic functions (Wedemeyer 1996).

Gelderblom et al. (1991) stated that the rat liver is the main target organ to be affected by dietary FB_1. Bailey et al. (1996) noted that regardless of the exposure route, trout liver is the most common organ to respond to carcinogens. Respectively, Lumlertdacha et al. (1995) noted that livers of channel catfish were apparently the target organs for toxicity of dietary FB_1. In their study, they fed two age groups of channel catfish with graded concentrations of FB_1: 0.3 (acted as control), 20, 80, 320 and 720 mg/kg. They found lesions from livers of fish from both age groups fed with 20 mg/kg or higher FB_1 concentrations. They also noted that abnormality prevalence and severity increased with concentrations of dietary FB_1. In same kind of study done with channel catfish, Li et al. (1994) found mild abnormalities from livers of the fish. Also, fish fed with FB_1 concentrations of 40 mg/kg or more (up to 240 mg/kg) had elevated levels of glycogen in livers (glycogen accumulations).

In the light of these findings, it was little surprising that no abnormalities (mild nor severe) were found from the livers in this study. Similar results were observed in experiment done previously by Carrera García (2013). Carlson et al. (2001) found no significant lesions from the livers of the rainbow trout fry fed with graded concentrations

(from 0 up to 104 mg/kg) of fumonisin B_1 either. Also, in studies done with Nile tilapia fingerlings (Tuan et al. 2003) and young and adult channel catfish (Brown et al. 1994, Yildirim et al. 2000) fed with different FB_1 concentrations respectively, histological abnormalities were not found from livers of the fish.

Bailey et al. (1996) stated that rainbow trout has a very low (0.1 %) spontaneous (not toxic-related) tumour incidence in liver thus making rainbow trout a good fish model for environmental carcinogenic research. Carlson et al. (2001) suggested that FB_1 do not act as complete carcinogen in livers, but promotes lesions or tumours already existing or spontaneously initiated within the tissue thus making it in that way a "complete" carcinogen. Since rainbow trout is a species with very low spontaneous tumour incidence, the possible lack of tumours for promoting can be the reason for the deficiency of the "complete carcinogenesis" of FB_1.

In studies where liver abnormalities were found the experiment durations were 12 weeks (Li et al. 1994) and 10 and 14 weeks, respectively (Lumlertdacha et al. 1995). The other studies without observable liver damages had durations from 5 weeks (Brown et al. 1994) up to 10 weeks (Yildirim et al. 2000, Tuan et al. 2003, Carrera García 2013). Interestingly, Carlson et al. (2001) examined rainbow trout fry feeding different concentrations of FB_1 (from 0 up to 104 mg/kg) for total duration of 34 weeks without liver lesions, suggesting that there might be several causes behind rainbow trout's sustainability to liver damages. As described before, there might be differences between species on sensitivity towards the toxicity of FB_1 and the FB_1 dose/fish size relationships may have been greater in other studies.

5.4. Effects of other mycotoxins on rainbow trout

Poston et al. (1982) fed 1-g rainbow trout with feed complemented with graded levels (0, 1, 2.5, 5, 10 and 15 mg/kg) of T-2 mycotoxin, another toxic secondary metabolite of *Fusarium* species. After their 16-week study, fish fed with 5 mg/kg or higher concentrations had significantly lower weight gain, feed acceptance and haematology values than control fish or fish fed with 1 and 2.5 mg/kg dietary levels. It is possible that rainbow trout is more sensitive to this particular toxin than FB_1.

Hooft et al. (2011) found that rainbow trout is very sensitive towards another common *Fusarium* mycotoxin, deoxynivalenol (DON). Fish (with initial weight of 24 g) had decreased growth rates and weight gains when fed with increasing levels of DON (0.3, 0.8, 1.4, 2.0 and 2.6 mg/kg). Fish fed with highest level of DON (2.6 mg/kg) had also abnormalities on their livers. Ryerse et al. (2016) fed rainbow trout fingerlings with diet containing three different concentrations of DON (0.5, 4 and 6 mg/kg) and after 4 weeks, infected fish intraperitoneally with *Flavobacterium psychrophilum*, a pathogen causing bacterial coldwater disease. During the 4-week feeding period, the fish receiving the two highest concentrations of DON had notably reduced feed intake than the control fish (fed with 0.5 mg/kg DON). However, fish fed with two highest concentrations of DON had significantly reduced post-infection mortality than control fish. These findings suggest that DON exposure together with reduced or restricted feed intake can alter the sensitivity of rainbow trout to coldwater disease as providing a defensive effect against *F. psychrophilum* bacterium. Manning et al. (2014) had received similar results earlier in experiment done with channel catfish fed with feed contaminated with graded concentrations (0, 2.5, 5.0 and 7.5 mg/kg) of DON and infected with pathogenic bacterium *Edwardsiella ictaluri*.

The extreme sensitivity of rainbow trout, especially certain strains, towards mycotoxin aflatoxin B_1 is well documented. Aflatoxin B_1, produced by fungus *Aspergillus flavus*, has toxic and carcinogenic effects on the fish (Sinnhuber & Wales, 1978). Lee et al. (1971) fed young rainbow trout with feed containing 0.02 mg/kg purified aflatoxin B_1 for 1, 5, 10, 20 and 30 days. Aflatoxin-induced hepatomas (liver tumours) were observed 3, 12, 10, 40 and 36 % of treated fish respectively, after 12 months. In their study done with young rainbow trout given known doses of aflatoxin intraperitoneally for 10-day period, Bauer et al. (1969) determined that LD_{50} value for aflatoxin B_1 was 0.81 mg/kg and the livers of fish had abnormal colour and showed extreme necrosis. In comparison, in study done with rats, Butler (1964) determined the LD_{50} for aflatoxin B_1 given intraperitoneally was 6.0 mg/kg and when given orally the LD_{50} value was 7.2 mg/kg.

Rainbow trout seems to be also sensitive towards ochratoxin A, a secondary metabolite product produced by *Aspergillus ochraceus*. In their 10-day experiment Doster et al. (1971) administered ochratoxin A and B daily to juvenile rainbow trout intraperitoneally with graded dose levels: ochratoxin A 2, 3, 4, 5, 6 and 8 mg/kg and ochratoxin B 16.7, 33.3 and 66.7 mg/kg. They used 0.1 N-sodium bicarbonate solution as control. During their experiment, only ochratoxin A proved to be lethal towards rainbow trout: at graded doses 2, 3, 4, 5, 6 and 8 mg/kg mortalities were 0, 0, 30, 60, 80 and 100 % respectively. The LD_{50} value for intraperitoneally dosed ochratoxin A was 4.67 mg/kg. Fish dosed with ochratoxin A showed also physiological abnormalities: oedema and several haemorrhages in the visceral fat along with discoloured livers and kidneys. There were no mortality or physical abnormalities amongst fish receiving ochratoxin B doses.

These findings suggest that there is some variation within the species on sensitivity towards different *Fusarium* and *Aspergillus* mycotoxins. These differences might be due to different pathways of action at cellular level.

5.5. Conclusions

Although rainbow trout is sensitive towards other *Fusarium* and *Aspergillus* mycotoxins, rainbow trout seems to be quite tolerant against dietary introduced fumonisin B_1. There are only few studies done with mycotoxins and their effects on rainbow trout and other salmonids, especially Atlantic salmon, despite their relative importance to aquaculture globally: in year 2011, totally 1,721,254 tons of Atlantic salmon and 770,385 tons of rainbow trout were produced worldwide (Anonymous, 2013). In year 2013, the amount of Atlantic salmon and rainbow trout reared in aquaculture had increased to over 2 million tons and 800,000 tons, respectively with economic value together near 16.4 billion US dollars (Anonymous, 2016).

In Finland, rainbow trout is the most reared fish in Finnish fish farms, over 90 percent of total farmed fish production for human consumption. In year 2014, totally 12,400 tons of rainbow trout were produced with economical value of 47.1 million euros (Savolainen, 2015). Comparing to global numbers, the Finnish aquaculture is very limited but on regional scale, especially in small communities, the fish farms and its' depending industry are valuable. The mycotoxin-contaminated feed may cause economic impact in fish farms due to straight loss of fish (mortality) or loss of productivity (inferior quality and growth). It is estimated that in the US annual economic losses in agriculture caused by mycotoxins (mainly aflatoxins, fumonisins and DON) rises over 900 million US dollars (Richard et al. 2003).

Salmonid fish feeds produced in Finland contains, on average, soy (18 %), wheat (13 %), canola oil (9 %) and maize (5 %) as their main plant-based ingredients, according to

the survey done by Silvenius et al. (2012). Also, broad bean (*Vicia faba*) is sometimes used in feeds as a protein source (Silvenius et al. 2012). As fumonisin B_1 mainly infects maize (Gelderblom et al. 1988) and feeds contain relatively small amounts of maize, it is quite unlikely that broad FB_1 outbreaks might happen in Finnish aquaculture scheme, at least when using Finnish feeds.

As the fish feed industry is prone to replace more costly fish protein with cheaper plant-origin proteins in their manufactured feeds, it is crucial to understand the possible adverse effects this might have on farmed fish. Selection of the best, mould-free raw materials, modern and hygienic manufacturing and packaging lines and proper storage facilities are the first step in preventing the occurrence of mycotoxins. Unfortunately, this is not always achievable, especially in humid climates. Researching pathways of action, developing practices to identify mouldy feedstuff efficiently and developing feed additives are ways to improve our knowledge about these substances. But there is still much to learn about these toxins. To know whether salmonids are sensitive towards fumonisins, more studies, with a longer duration and/or increased amounts of FB_1, may be needed to track possible adverse effects of FB_1 on salmonid fish.

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Appendix. The experimental setup and tank layout for 8-week feeding trial on rainbow trout (*Oncorhynchus mykiss*).