

Aus dem Institut für Tierzucht und Tierhaltung
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

OPTIMIZED USE OF POTATO PROTEIN CONCENTRATES
IN ORGANIC AQUACULTURE DIETS FOR RAINBOW
TROUT (*ONCORHYNCHUS MYKISS*)

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„Wo nur ein leerer Platz zu finden ist, soll die Kartoffel angebaut werden, da diese Frucht nicht allein sehr nützlich zu gebrauchen, sondern auch dergestalt ergiebig ist, daß die darauf verwendete Mühe sehr gut belohnt wird.“

Friedrich der Große - Kartoffelbefehl 14.03.1756

Table of Contents

General introduction		1
Chapter 1:	Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (<i>Oncorhynchus mykiss</i>)	7
Chapter 2:	Evaluation of feed attractants in potato protein concentrate based diets for rainbow trout (<i>Oncorhynchus mykiss</i>)	32
Chapter 3:	Wheat gluten and potato protein concentrate – Promising protein sources for organic farming of rainbow trout (<i>Oncorhynchus mykiss</i>)	51
Chapter 4:	High performance of rainbow trout (<i>Oncorhynchus mykiss</i>) fed diets with potato protein concentrates supplemented with feed attractants	68
General Discussion		83
General Summary		98
Zusammenfassung		101

List of Tables

Table 1.1	Nutrient and amino acid composition (g kg^{-1} dry matter (DM)) and concentrations of antinutritional factors of the used main protein sources in the experimental diets.	11
Table 1.2	Ingredients and proximate composition (g kg^{-1} DM) of the experimental diets.	12
Table 1.3	Growth performance (mean \pm SD, n=3) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg^{-1} DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg^{-1} DM). Values with the same superscript are not significantly different ($p < 0.05$).	15
Table 1.4	Body composition (g kg^{-1} wet weight; MJ kg^{-1} DM) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg^{-1} DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg^{-1} DM). Initial body composition was expressed on a wet weight basis as dry matter 266 g kg^{-1} ; ash 33 g kg^{-1} ; crude protein 163 g kg^{-1} ; crude lipid 70 g kg^{-1} ; energy 23.8 MJ kg^{-1} . Values (mean \pm SD, n=3) with the same superscript are not significantly different ($p < 0.05$).	16
Table 1.5	Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml^{-1}) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg^{-1} DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg^{-1} DM). Values (mean \pm SD, n=3) with the same superscript are not significantly different ($p < 0.05$).	17
Table 1.6	Occurrence (%) of liver types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg^{-1} DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg^{-1} DM); (n=9).	19
Table 1.7	Occurrence (%) of the stomach types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg^{-1} DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg^{-1} DM); (n=9).	21

Table 1.8	Occurrence (%) of the anterior intestine types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg ⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg ⁻¹ DM); (n=9).	23
Table 2.1	Nutrient composition, amino acid concentration, antinutritional phytic acid and glycoalkaloid concentration of the investigated protein sources and attractants utilized in the experimental diets in g kg ⁻¹ dry matter (Tab. 2.2). FM - fish meal; PPC - potato protein concentrate with a low glycoalkaloid content.	36
Table 2.2	Ingredients, nutrients, amino acid concentration and composition the experimental diets in g kg ⁻¹ dry matter. FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg ⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 0.5% or 1% free amino acid as attractant.	38
Table 2.3	Growth performance (mean ± SD, n=3) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg ⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Values with the same superscript are not significantly different (p<0.05).	41
Table 2.4	Body composition (g kg ⁻¹ wet weight; MJ kg ⁻¹ DM) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg ⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Initial body composition was expressed on a wet weight basis as dry matter (253 g kg ⁻¹ ; ash 26 g kg ⁻¹ ; crude protein 139 g kg ⁻¹ ; crude lipid 92 g kg ⁻¹ ; energy 26.7 MJ kg ⁻¹). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).	42
Table 2.5	Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml ⁻¹) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg ⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).	43

Table 3.1	Nutrient composition, amino acid concentration, antinutritional phytic acid and glycoalkaloid concentration of the investigated protein sources utilized in the experimental diets in g kg ⁻¹ dry matter (Tab. 3.2).	56
Table 3.2	Ingredients, amino acid concentration and antinutritional glycoalkaloid concentration of the protein sources utilized in the experimental diets in g kg ⁻¹ dry matter.	57
Table 3.3	Growth performance (mean ± SD, n=3) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Values with the same superscript are not significantly different (p<0.05).	60
Table 3.4	Body composition (g kg ⁻¹ wet weight; MJ kg ⁻¹ DM) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Initial body composition was expressed on a wet weight basis as dry matter 264 g kg ⁻¹ ; ash 24 g kg ⁻¹ ; crude protein 163 g kg ⁻¹ ; crude lipid 86 g kg ⁻¹ ; energy 27.1 MJ kg ⁻¹ . Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).	61
Table 3.5	Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml ⁻¹) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).	61
Table 4.1	Nutrient composition, amino acid profile and concentration of antinutritives (phytic acid, solanin, chaconin) in the ingredients (FM – fish meal, PPC – potato protein concentrate, WG – wheat gluten, SBM – soy bean meal) used as protein sources in the experimental diets in g kg ⁻¹ dry matter (Tab. 4.2).	72
Table 4.2	Ingredients, nutrient composition, amino acid profile and concentration of antinutritives (solanin, chaconin) in the experimental diets in g kg ⁻¹ dry matter.	73
Table 4.3	Growth performance (mean ± SD, n=3) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed diets at partial substitutions of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Values with the same superscript are not significantly different (p<0.05).	76

- Table 4.4 Body composition (g kg^{-1} wet weight) and gross energy (MJ kg^{-1} DM) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed diets at partial substitutions of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Initial body composition was expressed on a wet weight basis as: dry matter 264 g kg^{-1} ; ash 24 g kg^{-1} ; crude protein 163 g kg^{-1} ; crude lipid 86 g kg^{-1} ; energy 27.1 MJ kg^{-1} . Values (mean \pm SD, $n=3$) with the same superscript are not significantly different ($p<0.05$). 77
- Table 4.5 Blood parameters (hematocrit in %; protein, triglyceride and glucose in mg ml^{-1}) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed a diet at a partial substitution of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Values (mean \pm SD, $n=3$) with the same superscript are not significantly different ($p<0.05$). 77

List of Figures

- Figure 1.1 Histological sections (HE-stained, 200x) of the liver of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM), representative for severe hypertrophy (A, in LG100), mild hypertrophy (B, in the control), normal state (C, in the control) and hypotrophy (D, in HG25 diets). BD, bile duct; BV, blood vessel; LV, lymphatic vessel; Cp, ceroid pigment; N, nucleus; Si, sinusoid; V, vacuole. 19
- Figure 1.2 Histological sections (HE-stained, 100x) of the stomach of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM), representative for normal state (A, control) and impeded development (B, HG100). E, Epithelium; LP, Lamina propria; MU, Tunica muscularis; SC, Stratum compactum. 20
- Figure 1.3 Histological sections (HE-stained, 100x) of the anterior intestine of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM), representative for normal state (A, control), slight alterations (B, LG75), strong alterations (C, LG100) and under developed (D, HG75). LEM, lamina epithelialis mucosae; LP, Lamina propria; MU, Tunica muscularis; SC, Stratum compactum; V, vacuole. 21

General introduction

The production of feeds for aquaculture demanded huge amounts of fish meal (3.7 million tonnes in the year 2006) (Tacon and Metian, 2008). Due to the depletion of wild stocks of small pelagic fish species e.g. *Engraulis spp.* (converted into meal and oil) and restricted capture fishery quotas, the availability of these raw materials rich in highly digestible amino acids or fatty acids stagnated and the price increased dramatically in the last decades (Adelizi et al., 1998). Additionally, the increasing aquaculture market as well as further animal production systems (e.g. pigs and poultry) demanding increasing amounts of feeds revealing a high product quality (Tacon and Metian, 2008). Regarding the supply with certified fish feed ingredients for organic aquaculture, it became obvious that the claimed animal derived protein and fat sources were not available in sufficient amounts at the moment for fish species with enhanced demand for these feed ingredients (Bergleiter, 2009; Mente et al., 2011). To ensure a high quality and a continuously supply with feeds as well as to diminish the pressure on the feed availability by raw material scarcity, alternative feed ingredients were investigated and used in aquaculture diets (Moyano et al., 1992). Various plant meals, isolates and concentrates had shown a potential for the use as protein sources instead of fish meal. Soy bean, rape seed and lupine seed products were widely included in conventional aquaculture diets (Gatlin et al., 2007). The nutritive characteristics of these raw materials (e.g. reduced protein amounts, insufficient amino acid compositions, antinutritives and reduced digestibility) have been enhanced by several processing techniques: The generation of protein concentrates as known from hexane extraction in soy, methanol treatments in rape or hydrochloric acid extraction in lupine seed resulted in increased protein amounts in the raw materials (Zhang and Liu, 2005; Xu and Diosady, 1994; Sironi et al., 2005; Slawski et al., 2011). This is a general requirement for the nutrition of species with an enhanced protein demand. If the essential amino acid composition exhibited a deficiency, a supplementation with free amino acids (AA) provided a balanced AA supply via the feeds. Considering the presence of plant burden substances which acted normally as storage molecules (phytic acid) and repellents against insects (saponins, tannins), in protein concentrate production an accumulation of these substances took place. Similarly to their function in the plants in the natural environment, the mentioned molecules acted on nutritive processes in fish fed on the plant burden raw materials – mostly with negative impacts (Francis et al., 2001). The presence of higher amounts of phytic acid resulted in decreased phosphorous availability via the diets. A lot of repellents provoked a bitter and annoying taste resulted in decreased

feeding rates (Glencross et al., 2006). Some of the mentioned processing methods could be used to counteract some of the described ingredient effects (e.g. removing or inactivation via hexane or temperature treatments) and enhanced thereby the nutritive quality of the raw materials.

But regarding the specific and strict regulations for processing of feed and feed ingredients in organic aquaculture, it became obvious that only a small amount of alternative raw materials are available at the moment (Bergleiter, 2009; Mente et al., 2011). Especially the extraction and concentration of proteins using chemical extraction methods is prohibited and excluded rape, soy and lupine from the use in this highly regulated organic market (Naturland, 2010). Furthermore, any supplementation with synthetics (e.g. free AA or enzymes supporting digestion processes) is not allowed and obviates raw materials with insufficient AA composition and/or higher amounts of indigestible nutrients (e.g. carbohydrates) (European Union, 2009).

The claim for alternative protein sources - especially in organic aquaculture - was spotted to a new and promising feed ingredient: potato protein concentrate (PPC). This protein source is a by-product of the potato starch industry and exhibited extremely high crude protein amounts in the raw material with a nutritional balanced AA composition (Refstie and Tiekstra, 2003). Furthermore, the preparation process of PPC via heat steam extraction, coagulation and drying can be positively certified for organic aquaculture (IFOAM, 2010). First examinations with PPC in aquaculture diets revealed a slight negative result for use and showed that antinutritives (solanine and chaconine) influenced the quality of the diets (Tacon and Jackson, 1985; Xie and Jokumsen, 1997a, b; 1998). A further processing resulted in reduced amounts of antinutritives and thereby in enhanced quality of the raw material (Lokra et al., 2008). Such a processed PPC was positively used and evaluated in diets for Atlantic salmon (*Salmo salar*) (Refstie and Tiekstra, 2003). Regarding the global potato production of 329 million tonnes in the year 2009 (FAO, 2011), it should be stated that the potato starch by-product PPC has the potential to act as alternative fish meal protein source. Thereby a special emphasis should focus on the restricted organic feed market and production systems.

The *first chapter* of this thesis was set to investigate PPCs with varying processing qualities (low and high amounts of glycoalkaloids in the raw material) on the potential to replace fish meal in diets for rainbow trout (*Oncorhynchus mykiss*). Next to the data of growth performance (e.g. feed intake, feed conversion and growth), further parameter of animal

health (blood parameter, body indices and histological examinations) were analysed to evaluate the PPC efficiency.

As a consequence was defined that only a highly purified PPC could be used as fish meal supplement, if the problems concerning the palatability of diets rich in this raw material have been solved. To increase the attractiveness of the feed particles three different feed attractants were included in some of the experimental diets in *chapter two*. Blood meal (Johnstone and Mackie, 1990; Kikuchi, 1999; Millamena, 2002), mussel meal (Berge and Austreng, 1989; Kikuchi et al., 2002) or a mixture of free AA (Mackie et al., 1980; Papatryphon and Soares, 2000; Yamashita et al., 2006) were successfully used as feeding stimulants in former investigations and the findings were adapted to the own experimental setup. Furthermore, parameters of growth performance, physiology and health status were documented and analysed to evaluate the physiological response of PPC and attractant administrations on a nutrient supply level by the raw materials.

Next to the high quality of the raw material, PPC exhibited also a high price at the market. If it is used as fish meal supplement, the price of the PPC containing diet increases clearly in contrast to a fishmeal based diet. To soften this effect, an alternative to PPC was evaluated in *chapter three*. Wheat gluten is available in huge amounts at the market and reveals a lower price than PPC. Regarding the nutritional characteristics, wheat gluten exhibited only a minor quality compared with other plant protein sources (NRC, 1993). Nevertheless, it was successfully used in aquaculture diets (Storebakken et al., 2000) and met the claim of organic legislation and certification organisations (Mente et al., 2011). These preconditions resulted in a comparative examination of varying levels of wheat gluten and potato protein concentrate combinations at a constant fish meal replacement in diets for rainbow trout in *chapter three*. The main focus laid on the search for the optimum supplementation rate of these both alternative feed ingredients.

The aim of a maximized fish meal replacement by PPC was combined with the positive findings from all previous trials in the final experiment presented in *chapter four*. In addition to the PPC inclusion, a basal plant protein mixture (wheat gluten and soy bean meal) was used to decrease to overall fish meal demand in the diets. For the evaluation of a total fish meal replacement the data of growth performance and health parameter were analysed.

References

- Adelizi, P., Rosati, R., Warner, K., Wu, Y., Muench, T., White, M., Brown, P., 1998. Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*. *Aquacult. Nutr.* 4 (4), 255–262.
- Berge, G., Austreng, E., 1989. Blue mussel in feed for rainbow trout. *Aquaculture* 81 (1), 79–90.
- Bergleiter, S., 2009. Organic aquaculture 2009: Production and markets. Naturland, Gräfelfing, 120 pp.
- European Union, 2009. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union* (52), 15–34.
- FAO (2011): <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Gatlin, D., Barrows, F., Brown, P., Dabrowski, K., Gaylord, T., Hardy, R., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.* 38 (6), 551–579.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- IFOAM, 2010. Organic Aquaculture. EU Regulations (EC) 834/2007, (EC) 889/2008, (EC) 710/2009. BACKGROUND, ASSESSMENT, INTERPRETATION. International Federation of organic Agricultural movements.
http://www.ifoam.org/about_ifoam/around_world/eu_groupnew/positions/publications/aquaculture/IFOAMEU_IAMB_organic_aquaculture_dossier.pdf. Accessed 17 March 2011.
- Johnstone, A., Mackie, A., 1990. Laboratory investigations of bait acceptance by the cod, *Gadus morhua* L.: identification of feeding stimulants. *Fish. Res.* 9, 219–230.
- Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179 (1-4), 3–11.

- Kikuchi, K., Ueda, A., Sugita, H., Takeda, S., 2002. Effect of dietary inclusion of blue mussel extract on growth and body composition of Japanese flounder *Paralichthys olivaceus*. *J. World Aquacult. Soc.* 33 (1), 41–47.
- Lokra, S., Helland, M., Claussen, I., Straetkvern, K., Egelanddal, B., 2008. Chemical characterization and functional properties of a potato protein concentrate prepared by large-scale expanded bed adsorption chromatography. *LWT Food Sci. Technol.* 41 (6), 1089–1099.
- Mackie, A., Adron, J., Grant, P., 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). *J. Fish Biol.* 16.
- Mente, E., Karalazos, V., Karapanagiotidis, I., Pita, C., 2011. Nutrition in organic aquaculture: an inquiry and a discourse. *Aquaculture Nutrition*, no.
- Millamena, O., 2002. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. *Aquaculture* 204 (1-2), 75–84.
- Moyano, F., Cardenete, G., La Higuera, M. de, 1992. Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquat. Liv. Res.* 5 (1), 23–29.
- Naturland, 2010. Naturland Standards for Organic Aquaculture. Naturland - Association for Organic Agriculture, Registered Association.
http://www.naturland.de/fileadmin/MDB/documents/Richtlinien_englisch/Naturland-Standards_Aquaculture.pdf. Accessed 17 March 2011.
- NRC (National Research Council), 1993. Nutrient Requirements of Fish. *National Academy Press, Washington, DC, USA.*, 1–117.
- Papatryphon, E., Soares, J., 2000. The effect of dietary feeding stimulants on growth performance of striped bass, *Morone saxatilis*, fed-a-plant feedstuff-based diet. *Aquaculture* 185 (3-4), 329–338.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Sironi, E., Sessa, F., Duranti, M., 2005. A simple procedure of lupin seed protein fractionation for selective food applications. *Eur. Food Res. Technol.* 221 (1-2), 145–150.
- Slawski, H., Adem, H., Tressel, R.-P., Wysujack, K., Koops, U., Kotzamanis, Y., Wuertz, S., Schulz, C., 2011. Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquacult Int.*
- Storebakken, T., Shearer, K., Beaverfjord, G., Nielsen, B., Asgard, T., Scott, T., De Laport, A., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements

- and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. *Aquaculture* 184 (1-2), 115–132.
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London ;, Orlando, pp. 119–145.
- Tacon, A., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285 (1-4), 146–158.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.
- Xu, L., Diosady, L., 1994. The production of Chinese rapeseed protein isolates by membrane processing. *J. Am. Oil Chem. Soc.* 71 (9), 935–939.
- Yamashita, S., Yamada, T., Hara, T.J., 2006. Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *J Fish Biology* 68 (3), 783–800.
- Zhang, W., Liu, D., 2005. A new process for preparation of soybean protein concentrate with hexane-aqueous ethanol mixed solvents. *J. AOAC Int.* 88 (4), 1217–1222.

Chapter 1:

Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A feeding trial was conducted to evaluate the effect of potato protein concentrate (PPC) replacing fishmeal in nutrition of rainbow trout (*Oncorhynchus mykiss*) according to organic aquaculture guidelines. Eight diets were formulated by partial replacement of fishmeal (25%, 50%, 75% and 100%) with PPC of two different qualities (LG-PPC with low glycoalkaloid content of 7.41 mg kg⁻¹ dry weight, HG-PPC with high glycoalkaloid content of 2150 mg kg⁻¹ dry weight) and one experimental diet without PPC served as control. Experimental diets were fed over a period of 84 days to triplicate experimental groups until apparent satiation.

Best growth performance was observed in the control group. The inclusion of LG-PPC resulted in significantly reduced feed intake, feed utilization and growth with increasing replacement of 25 % and higher. Increasing amount of dietary LG-PPC inclusion resulted in alterations in liver histology and in anterior intestine indicating an impact of glycoalkaloids on the different gastrointestinal tissues. The HG replacement revealed most prominently a decreased feed intake and growth parameters indicating massive undernourishment. Congruently, hypotrophy of the liver with ceroid pigment accumulation, and villi degeneration in the intestine were observed in all HG groups. Blood parameters were equally affected by an inclusion of PPC (LG and HG) in the diets involving reduced plasma TG (HG75), glucose (HG100) as well as protein (HG100). As a conclusion PPC as alternative protein in fish nutrition is only feasible with LG contents as HG diets resulted in severe undernourishment of fish, lowest growth performance and irreversible intestinal damages.

Introduction

Organic aquaculture is ultimately committed to sustainability, including the sustainable use of resources for feeding. About 3724 thousand tonnes of fishmeal from capture based fisheries (16.6 million tonnes of small pelagic forage fish) were used for aquaculture feed production in the year 2006 mainly originating from decreasing wild stocks (Tacon and Metian, 2008). A sustainable growth and production of aquaculture is aiming for a progressive reduction of fishmeal from wild fisheries used in aqua feeds (Francis et al., 2001). As a consequence, alternative protein sources need to be explored, preferentially plant proteins with huge market supply. Restricted plant protein utilization in fish nutrition is commonly related to the presence of antinutritional ingredients, lower protein quality and palatability (Tacon and Jackson, 1985). But in recent years purification of plant proteins has been applied successfully to eliminate antinutrients and produce high quality feed sources for fish nutrition (Adelizi et al., 1998; Francis et al., 2001; Gatlin et al., 2007; Slawski et al., 2011).

In accordance to the European guideline EC 710/2009 for organic aquaculture fish feed ingredients shall be sourced from (a) certified organic aquaculture; (b) certified trimmings (e.g. MSC-certified); (c) sustainable trimmings for human fish consumption or (d) plant derived and other animal materials (listed in ANNEX V, European Union, 2009). In addition utilization of plant derived products should not exceed 60 % in the nutrition of carnivorous fish. Background for that is the claim of the legislation and organic movement that a carnivorous fish should be fed species-appropriate (European Union, 2009; IFOAM, 2010). But: A sufficient supply of certifiable animal protein sources as feed is currently not available with regard to an increasing demand for organic fish products (Bergleiter, 2009; IFOAM, 2010). Thus, an increased demand of alternative protein and fat sources compliant to the ANNEX V of the Commission Regulation (EC) 710/2009 is beneficial for the development of organic aquaculture production (European Union, 2009).

The use of protein concentrates from plant origin – with crude protein contents often achieving 70-90 % DM - are restricted to concentrates produced without the application of solvent extractors or other chemical agents (IFOAM, 2005). Consequently, the use of protein concentrates and isolates from e.g. soybean and rape seed meals (both hexane or methanol extracted for oil separation) and lupine seed (hydrochloric acid extraction for protein recovery) is thereby excluded from organic aquaculture (Zhang and Liu, 2005; Xu and

Diosady, 1994; Sironi et al., 2005). In addition, the use of synthetic AA used to compensate for AA deficient plant protein profiles in organic feeds is prohibited (IFOAM, 2005).

Potato protein concentrates (PPC) are produced by thermal coagulation as a by-product of the industrial production of potato starch (Strolle et al., 1973; Refstie and Tiekstra, 2003; Friedman, 2006). This process has been approved by the European Union and organic agricultural associations for organic feed including aqua feed production (IFOAM, 2005; Naturland, 2010; European Union, 2008). Furthermore, PPCs are well balanced in the AA profile and of higher protein quality than commonly used soy and rape seed products which do not require any supplementation of free (synthetic) AA. Still, methionine has been reported as a potentially limiting AA in PPC-containing diets in trout nutrition (Xie and Jokumsen, 1997a; 1998; Refstie and Tiekstra, 2003).

Nevertheless, remaining antinutritional substances predominantly glycoalkaloids may impede both growth performance and the well-being of farmed fish (Tacon and Jackson, 1985; Friedman, 2006; Glencross et al., 2006). In addition to the impact on growth performance, glycoalkaloids and their metabolites may adversely affect gastrointestinal tract and liver. It has been reported that the inclusion of freeze-dried potato berries in mice diets (glycoalkaloid contents of 50-1600 mg kg⁻¹) resulted in histopathological abnormalities in the stomach and liver tissue (Friedman, 2006). To our knowledge, no data on health impact of potato proteins (including glycoalkaloids) are available for fish.

The aim of the present study was to explore the replacement of fishmeal by PPC with varying glycoalkaloid contents on growth performance, feed intake, homeostasis of plasma parameters and intestinal histopathology of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Experimental diets

Eight isonitrogenous and isoenergetic experimental diets were formulated by partial replacement of fishmeal (FM) at approximately 25%, 50%, 75%, 100%, with potato protein concentrate (PPC) of two qualities, LG with low glycoalkaloid content (7.41 mg kg⁻¹ dry weight; K5, Emsland Group, Emlichheim, Germany) and HG with high glycoalkaloid content (2150 mg kg⁻¹ dry weight; IG, Emsland Group, Emlichheim, Germany) as documented in Tab. 1.1. and 1.2. One diet with low temperature (LT) fish meal (VFCUX, Cuxhaven, Germany) as main protein source served as control diet. In order to meet the council

regulation (EC) No. 710/2009 for organic aquaculture animal production no free AA were added to the diets.

All ingredients were pelletized with a fodder press L 14-175 (AMANDUS KAHL, Reinbek, Germany) to pellets of 4 mm diameter. Due to the high protein contents of PPC, magnesium silicate was used as inert filler in the diets to realise equal nutrient contents in the diets (ANNEX V, European Union, 2009).

Tab. 1.1: Nutrient and amino acid composition (g kg^{-1} dry matter (DM)) and concentrations of antinutritional factors of the used main protein sources in the experimental diets.

	diet ingredients		
	fish meal	LG-PPC	HG-PPC
<i>Nutrient composition (g kg^{-1})</i>			
Dry matter	918	955	919
Crude protein	689	859	841
Crude lipid	69	32	29
Crude fibre	5	0.5	6
NfE ¹	31	24	34
Crude ash	206	85	90
Phosphorus	25	22	21
<i>Essential amino acids (g kg^{-1})</i>			
Arginine	37.0	43.2	40.1
Histidine	12.7	19.1	17.3
Isoleucine	23.0	47.8	44.2
Leucine	40.9	90.5	82.2
Lysin	41.5	71.2	64.0
Methionine + Cysteine	20.1	32.9	30.2
Phenylalanine	22.3	57.8	52.6
Threonine	24.7	52.6	47.1
Valine	28.2	57.0	52.2
<i>Glycoalkaloids² (mg kg^{-1})</i>			
Solanin		4.40	1060
Chaconin		3.01	1090
<i>Phytic acid (g kg^{-1})</i>		<0.1	<0.1

¹ NfE, Nitrogen-free extract = $1000 - (\text{Crude protein} + \text{Crude lipid} + \text{Ash} + \text{Crude fibre})$

² analyzed at LUFA-ITL, Kiel, Germany, according to LC-MS

Tab. 1.2: Ingredients and proximate composition (g kg⁻¹ DM) of the experimental diets.

	Experimental diets								
	control	LG 25	LG 50	LG 75	LG 100	HG 25	HG 50	HG 75	HG 100
<i>Ingredients (g kg⁻¹)</i>									
fish meal ¹	686	514	343	171	-	514	343	171	-
LG-PPC ²	-	132	265	397	529	-	-	-	-
HG-PPC ²	-	-	-	-	-	140	281	421	561
fish oil ¹	100	107	114	121	129	107	115	122	130
wheat starch ³	145	137	129	120	112	135	125	116	106
wheat gluten ³	20	20	20	20	20	20	20	20	20
vitamin ⁴ + mineral mixture ⁵	20	20	20	20	20	20	20	20	20
cellulose ⁶	20	20	20	20	20	20	20	20	20
magnesium silicat ⁷	-	40	80	120	160	33	66	99	133
<i>Nutrient composition (g kg⁻¹)</i>									
Dry matter	929	936	944	952	959	930	932	935	938
Crude protein	488	490	486	483	477	492	491	488	485
Crude lipid	145	158	158	156	145	156	153	158	154
Crude ash	183	179	186	192	199	178	177	179	181
Phosphorus	14.4	13.7	13.0	12.3	11.6	13.7	13.1	12.4	11.8
NfE + crude fibre	166	141	116	100	97	152	116	110	104
Gross energy (MJ kg ⁻¹ DM)	20.4	20.3	20.6	20.4	20.4	20.3	20.8	21.1	21.1
<i>Essential amino acids (g kg⁻¹)</i>									
Arginine	28.2	27.3	26.3	25.4	24.5	27.4	26.6	25.8	25.1
Histidine	9.8	10.1	10.4	10.6	10.9	10.1	10.4	10.6	10.9
Isoleucine	17.7	20.1	22.4	24.7	27.0	20.2	22.7	25.1	27.6
Leucine	31.7	36.6	41.5	46.4	51.3	36.6	41.5	46.4	51.3
Lysin	31.3	33.4	35.5	37.6	39.7	33.3	35.3	37.3	39.4
Methionine + Cysteine	15.6	16.4	17.2	18.0	18.8	16.5	17.3	18.2	19.0
Phenylalanine	17.5	21.3	25.2	29.0	32.8	21.4	25.2	29.1	33.0
Threonine	18.9	21.5	24.2	26.9	29.5	21.4	24.0	26.6	29.2
Valine	21.7	24.3	27.0	29.6	32.2	24.4	27.1	29.8	32.5
<i>Glycoalkaloids (mg kg⁻¹)</i>									
Solanine	-	0.4	0.8	1.2	1.6	166	306	459	612
Chaconine	-	0.6	1.2	1.7	2.3	149	298	446	595

¹VFCUX, Cuxhaven, Germany.

²Emsland-Stärke GmbH, Emlichheim, Germany.

³Cargill Deutschland GmbH; Krefeld, Germany.

⁴Vitamin-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: vitamin A, 1,000,000 IU kg⁻¹; vitamin D3, 200,000 IU kg⁻¹; vitamin E (as α -tocopherol-acetate), 40,000; vitamin K3 (as menadione), 4,000; vitamin B1, 4,000; vitamin B2, 8,000; vitamin B6, 4,000; vitamin B12, 8; vitamin C (as monophosphate), 60,000; pantothenic acid, 8,000; nicotinic acid, 40,000; folic acid, 1,600; biotin, 100; inositol 40,000.

⁵Mineral-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: cobalt (as cobaltsulphate), 400; manganese (as manganese sulphate), 2,500; iodine (as calciumiodine), 500; copper (as coppersulphate), 2,500; selen, 25; zinc (as zincsulfate), 28,000.

⁶Pellan, Mikro-Technik, Bürgstadt, Germany.

⁷Magnesia 4371, Magnesia GmbH, Lüneburg, Germany.

Tryptophane was not analysed.

Experimental setup

The feeding trial was conducted at the experimental facilities of the Gesellschaft für Marine Aquakultur (GMA, Büsum, Germany). Prior to the experiment, 600 rainbow trout (Forellenzucht Trostadt GbR, Trostadt, Germany) were randomly stocked to 27 tanks (175 l each), resulting in 9 feeding groups in triplicates of 20 fish (27.8 ± 0.3 g) each, which were acclimatised for 14 d in the experimental system. The tanks were integrated in a closed recirculation system (9 m³ total water volume, water turnover rate 4 h⁻¹, daily water exchange 1.700 l kg⁻¹ feed), involving a mechanical sponge filter and a moving bed biofilter. Water parameters were determined daily ($14.3 \pm 0.4^{\circ}\text{C}$, 9.7 ± 0.4 mg l⁻¹ O₂, 0.15 ± 0.3 mg l⁻¹ NH₄-N and 0.7 ± 0.4 mg l⁻¹ NO₂-N). The photoperiod was adjusted to 12h light : 12h dark. During acclimatisation fish were fed a commercial trout feed (Performa 45/20, 2mm; AllerAqua A/S, Christiansfeld, Denmark). Over the entire experimental period of 12 weeks, fish were fed to apparent satiation twice per day.

Sampling

Prior to the experimental start, 10 fish were sampled to assess whole body composition and stored at -20°C until analysed. At the end of the experiment, final biomass of each tank was recorded, 3 fish (bulk sample) of each tank were utilised for determination of whole body composition and stored at -20°C. Three additional individuals per tank were utilized to take blood samples from the caudal vein for the determination of blood parameters. In addition fish weight and length as well as liver weight were recorded and tissue samples of liver, stomach and proximal and distal intestine were dissected for histological analyses. For each treatment, mortalities and growth parameters were calculated as average of the triplicates, including specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), final condition factor (FCF) and protein productive value based on the formulas given in Tab. 1.3.

Whole body composition

For the analysis of the whole body composition 3 fish per tank were freeze-dried for 120 h, homogenized and stored at -20°C, experimental diets were homogenized and stored at -20°C. Analysis for dry matter (DM), ash, crude protein, crude fat and crude fiber was performed according to EU guideline (EC) 152/2009. Briefly, crude protein content (N x 6.25) was determined by the Kjeldahl method (InKjel 1225 M, WD 30; Behr, Düsseldorf, Germany), crude fat content after hydrolysis with hydrochloric acid succeeded by petroleum ether

extraction with a Soxhlet extraction system (R 106 S; Behr), DM after drying at 103°C until constant weight was obtained and ash content after 4 h incineration at 550°C with a combustion oven (P300; Nabertherm, Lilienthal, Germany). Gross energy was measured in a bomb calorimeter (C 200; IKA, Staufen, Germany).

Blood parameters

Upon blood sampling with a heparinised syringe, the hematocrit value was determined in micro-hematocrit-tubes (Type 563, NA-heparinized, 75 mm/135 µm; Assistant, Sondheim, Germany) upon centrifugation (10,000 g, 5 min) with a hematocrit 210 centrifuge (Hettich, Tuttlingen, Germany) using a hematocrit tube card reader. Remaining blood was centrifuged (1,000 g, 5 min) and plasma was separated and stored at -80°C. Glucose and triglycerides were determined by the GPO-PAP method using an enzymatic colorimetric kit (Greiner, Frickenhausen Germany). Plasma protein was quantified according to Bradford with a Roti-Quant kit and a BSA standard dilution series. All colorimetric assays were measured in duplicate with a microplate reader Infinite 200 (Tecan, Männedorf, Switzerland) and calculated from a standard dilution series to compensate for run-to-run variation. Recoveries were determined by spike experiments.

Histological analysis

Sections of liver, stomach, proximal and distal intestine were taken from three fish of each tank as described. After fixation in 10% phosphate-buffered formalin, the samples were dehydrated in a graded series of ethanol and embedded in paraffin (Wuertz, 2005), cut at 5 µm and stained with haematoxylin-eosin (HE). Analysis was carried out with an Olympus CKX 41 microscope equipped with a Canon EOS 500D digital camera. Sections were typified according to the most frequently observed characteristics and compared between treatments. Three cross sections per individual were used to observe and classify a histological type of the different sections. Nine individuals per treatment were recorded and used to calculate the occurrence of different histological types.

Statistical Analyses

The statistical analysis was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, U.S.). Data are presented as mean ± standard deviation (SD) for each treatment and were compared between treatments. Data were analysed for normal distribution by Kolmogorov-Smirnov and equal variance by Kruskal-Wallis One Way Analysis of Variance (confirmed if

p< 0.05). Post-hoc multiple comparison was carried out by parametric Tukey-HSD (p<0.05) or non-parametric Dunnett-T3 test.

Results

Growth

At the end of the experiment, the control group revealed the highest final body weight (127.9 ± 12.5 g) and the highest SGR 1.84 ± 0.1 % BW d⁻¹. The growth performance of all other feeding groups was significantly lower than the control (Table 1.3). Within all LG-groups no significant differences in final body weight were detected and the fish had doubled their weight during the trial. In all HG-groups negative growth was observed as experimental fish lost weight. Consequently, FCR, PER and PPV were not calculated in these groups and thus marked as not determined (n.d.) in Tab. 1.3.

Tab. 1.3: Growth performance (mean \pm SD, n=3) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM). Values with the same superscript are not significantly different (p<0.05).

Variable	Experimental diets									
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100	HG100
IBW ¹	27.8 \pm 0.4	27.5 \pm 0.2	27.6 \pm 0.1	27.8 \pm 0.3	27.8 \pm 0.3	27.8 \pm 0.2	27.7 \pm 0.4	28.0 \pm 0.3	28.1 \pm 0.1	
FBW ²	127.9 ^a \pm 12.5	52.9 ^b \pm 6.4	46.5 ^b \pm 2.0	43.3 ^b \pm 4.3	50.7 ^b \pm 1.0	27.6 ^c \pm 1.4	22.9 ^c \pm 0.4	20.9 ^c \pm 1.7	20.7 ^c \pm 1.5	
DFI ³	2.07 ^a \pm 0.1	1.46 ^b \pm 0.1	1.47 ^b \pm 0.0	1.45 ^b \pm 0.1	1.32 ^b \pm 0.1	0.78 ^c \pm 0.2	0.48 ^d \pm 0.2	0.29 ^{de} \pm 0.0	0.03 ^e \pm 0.0	
SGR ⁴	1.84 ^a \pm 0.1	0.78 ^b \pm 0.2	0.63 ^b \pm 0.0	0.53 ^b \pm 0.1	0.72 ^b \pm 0.0	-0.01 ^c \pm 0.1	-0.26 ^d \pm 0.0	-0.35 ^{de} \pm 0.1	-0.37 ^e \pm 0.1	
FCR ⁵	1.13 ^a \pm 0.0	2.00 ^b \pm 0.2	2.51 ^{bc} \pm 0.1	3.05 ^c \pm 0.3	1.91 ^b \pm 0.1	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	
PER ⁶	1.95 ^a \pm 0.1	1.10 ^b \pm 0.2	0.87 ^{bc} \pm 0.1	0.72 ^c \pm 0.2	1.14 ^b \pm 0.1	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	
PPV ⁷	32.9 ^a \pm 0.8	18.0 ^{bc} \pm 2.6	16.4 ^{bc} \pm 1.6	13.4 ^c \pm 2.7	19.7 ^b \pm 1.3	n.d. ^d	n.d. ^d	n.d. ^d	n.d. ^d	
HSI ⁸	1.12 ^{ab} \pm 0.1	1.13 ^a \pm 0.2	1.16 ^a \pm 0.2	1.21 ^a \pm 0.2	1.26 ^a \pm 0.2	0.81 ^b \pm 0.1	0.85 ^{ab} \pm 0.3	0.83 ^b \pm 0.1	0.82 ^b \pm 0.1	
FCF ⁹	1.20 ^a \pm 0.1	1.06 ^a \pm 0.2	1.09 ^a \pm 0.1	1.06 ^a \pm 0.2	1.06 ^a \pm 0.1	0.87 ^b \pm 0.1	0.86 ^b \pm 0.1	0.81 ^b \pm 0.0	0.80 ^b \pm 0.1	

¹initial body weight (g); ²final body weight (g); ³daily feed intake (% BW day⁻¹); ⁴specific growth rate (% BW day⁻¹) = [ln (FBW) – ln (IBW)] / feeding days x 100; ⁵feed conversion ratio = feed intake (g) / weight gain (g); ⁶protein efficiency ratio = weight gain (g) / protein intake (g); ⁷protein productive value = 100 x [(crude protein final fish x biomass final fish) – (crude protein initial fish x biomass initial fish)] / (crude protein diet x total feed intake); ⁸hepatic somatic index = (liver weight / final body weight) x 100; ⁹Fulton condition factor = 100 x final body weight x final body length⁻³; n.d. = not determined.

Daily feed intake (DFI) of fish fed the control diet was highest (2.07 % BW d⁻¹). A significantly reduced daily feed intake of 1.32 (LG100) - and 1,47 % BW d⁻¹ (LG50) was observed in the LG groups. In the HG groups, DFI drastically decreased with increasing supplementation, being significant in all HG groups compared to the control as well as the LG groups. No feed intake was observed in the group HG100.

The control group exhibited the lowest FCR (1.13 ± 0.0). Supplementation with LG-PPC resulted in significantly higher FCR of 1.91 (LG 100) - 3.05 (LG75). Within the LG fed groups only LG75 differed significantly from the other. PER and PPV confirmed these observations, highest values were observed in the control group and LG75 differed significantly from the other LG fed groups. Hepatosomatic index and fulton condition factor (FCF) were not significant different between control group and LG groups, but HSI and FCF were significantly lower in all HG groups compared to the control and the LG groups.

No mortality was observed during the experiment.

Body composition

The inclusion of potato protein concentrate in the different diets had no effect on the ash content (Table 1.4). DM, crude lipid and gross energy were highest in the control (FM) and significantly different to all other groups. DM in the LG group ranged between 267-287 g kg⁻¹, but no consistent trend correlated to PPC inclusion was detected.

Tab. 1.4: Body composition (g kg⁻¹ wet weight; MJ kg⁻¹ DM) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM). Initial body composition was expressed on a wet weight basis as dry matter 266 g kg⁻¹; ash 33 g kg⁻¹; crude protein 163 g kg⁻¹; crude lipid 70 g kg⁻¹; energy 23.8 MJ kg⁻¹. Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).

Nutrient	Experimental diets								
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100
Dry matter	305 ^a ± 0.7	276 ^{bc} ± 0.6	287 ^b ± 0.5	267 ^c ± 1.0	282 ^{bc} ± 0.2	224 ^d ± 0.4	228 ^d ± 0.4	227 ^d ± 0.3	229 ^d ± 0.3
Crude protein	167 ^{ab} ± 0.5	163 ^{abc} ± 0.4	173 ^a ± 0.3	170 ^{ab} ± 0.9	167 ^{abc} ± 0.2	155 ^c ± 0.4	160 ^{bc} ± 0.3	155 ^c ± 0.2	160 ^{bc} ± 0.3
Crude lipid	111 ^a ± 0.3	82 ^c ± 0.3	86 ^{bc} ± 0.2	67 ^d ± 0.3	91 ^b ± 0.0	39 ^e ± 0.1	36 ^e ± 0.1	41 ^e ± 0.1	39 ^e ± 0.1
Crude ash	34 ^{ab} ± 0.1	35 ^{ab} ± 0.3	37 ^b ± 0.1	37 ^b ± 0.2	32 ^a ± 0.1	34 ^{ab} ± 0.1	36 ^{ab} ± 0.1	36 ^{ab} ± 0.1	36 ^{ab} ± 0.1
Gross energy	27.1 ^a ± 0.1	26.0 ^b ± 0.1	25.9 ^b ± 0.3	24.9 ^c ± 0.0	26.9 ^a ± 0.2	23.0 ^d ± 0.0	21.9 ^e ± 0.1	22.6 ^d ± 0.1	22.4 ^{de} ± 0.1

In the HG groups DM was significantly lower compared to the control and the LG groups, but again no PPC concentration effect within the HG group was recorded. Crude lipid and gross energy concentrations in the whole body composition revealed the same trend with major differences between control, LG and HG but not within the respective LG and HG groups. In the LG groups, no significant effect on body crude protein content was detected compared to the control ($167 \pm 0.5 \text{ g kg}^{-1} \text{ DM}$). In contrast, protein content was reduced in the HG groups though not significant in HG50 and HG100.

Blood plasma composition

Blood parameters revealed moderate differences which are shown in table 1.5. The hematocrit value was highest in the control group (33.8%). Based on the high variance of the hematocrit values, no significant differences between the control and all the other groups were detected. Some significant differences in the other blood parameter were detected between groups. A high individual variation was observed. Highest plasma protein concentrations (41.9 mg ml^{-1}) were detected in the control, which decreased with increasing amounts of PPC in the diets. In the HG100 group, plasma protein concentration was 15.0 mg ml^{-1} , thus only round 1/3 of the control. Triglycerides in the plasma revealed the same tendency and fall from 3.54 mg ml^{-1} in the control group to 2.40 mg ml^{-1} in LG 75 to 0.76 mg ml^{-1} in HG 75. Congruently, glucose decreased from 1.43 mg ml^{-1} in the control to 0.99 in the LG100. Lowest glucose concentration was observed at the highest LG-PPC concentration in the diet (HG100) at 0.90.

Tab. 1.5: Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml^{-1}) of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of $7.41 \text{ mg kg}^{-1} \text{ DM}$) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of $2150 \text{ mg kg}^{-1} \text{ DM}$). Values (mean \pm SD, n=3) with the same superscript are not significantly different ($p < 0.05$)

Blood parameter	Experimental diets									
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100	
Hematocrit	33.8 ^{ab} \pm 6.7	28.4 ^a \pm 6.1	25.2 ^a \pm 5.0	29.8 ^a \pm 6.0	30.3 ^a \pm 5.8	28.2 ^b \pm 5.0	29.3 ^a \pm 5.4	24.8 ^{ab} \pm 4.0	25.7 ^b \pm 6.9	
Protein	41.9 ^a \pm 2.8	27.0 ^{bc} \pm 11.1	30.3 ^b \pm 6.4	27.4 ^{bc} \pm 5.2	21.2 ^{bcd} \pm 7.0	23.8 ^{bcd} \pm 4.8	19.4 ^{cd} \pm 4.8	19.8 ^{cd} \pm 2.7	15.0 ^d \pm 5.7	
Triglyceride	3.54 ^a \pm 1.2	3.48 ^a \pm 1.0	2.23 ^{abc} \pm 0.9	2.40 ^{ab} \pm 0.6	0.93 ^{bc} \pm 0.7	0.84 ^{bc} \pm 0.2	1.02 ^{bc} \pm 0.6	0.76 ^{bc} \pm 0.5	1.67 ^c \pm 2.1	
Glucose	1.43 ^a \pm 0.2	1.20 ^{ab} \pm 0.2	1.21 ^{ab} \pm 0.4	1.09 ^b \pm 0.2	0.99 ^b \pm 0.2	1.04 ^b \pm 0.2	0.99 ^b \pm 0.1	0.96 ^b \pm 0.1	0.90 ^b \pm 0.2	

Histological examination

The histopathology of the fish fed on a fishmeal-based control diet was used as a reference to identify abnormalities caused by increasing glycoalkaloid content of potato supplemented diets (LG25-LG100, HG25-HG100). Characteristics considered are subsequently described:

Liver tissue of the control group (Fig. 1.1C) was characterized by evenly distributed polygonal hepatocytes with prominent nuclei in a peripheral position. Medium sized slightly eosinophilic (nearly transparent) vacuoles indicated a balanced vacuolization, considered as normal state here. Sinusoids subdivide the parenchyma, lined by endothelial cells. These vascular spaces were normally widened and regularly distributed. No ceroid pigment clusters (melano-macrophage centres) were observed in the visible area. This type of liver was subsequently identified as type 1 (Table 1.6).

In type 2, some abnormal alterations classified as moderate were observed. Here an increasing vacuolization and slightly enlarged hepatocytes became noticeable. The ratio of vacuoles to hepatocytes increased from approximately 1:4 to 1:2. The sinusoidal space decreased slightly due to the enlargement of the hepatocytes. Ceroid pigment cluster were occasionally found. This state characterises a mild hypertrophy of liver tissue. Type 2 was observed in approximately 33 % of the control samples (Fig.1.1B).

Increasing substitution of fishmeal by PPC (LG25-LG100) intensified hypertrophy of the liver (type 3) as denoted by increasing vascularisation and reduced sinusoidal space to a degree, where the decompressed space is hardly visible. Here, hypertrophic hepatocytes predominantly constituted the parenchyma (Fig. 1.1A). In some cases, no cytoplasm was noticeable and the nucleus was pressed against the cell wall. The ratio of vacuoles and normal hepatocytes range from 1:2 to nearly 1:1. In addition, the liver increased substantially as indicated by increased HSI. Ceroid pigment cluster were moderately present in all sections.

Finally, all HG exhibited a severe hypotrophy (Fig. 1.1D; type 4). Here, vacuoles were nearly absent (ratio of vacuoles to normal hepatocytes 0:1). In all sections of the liver ceroid pigment cluster were encountered near the blood vessels. The occurrence of these histopathological liver types in each feeding group is summarized in table 1.6.

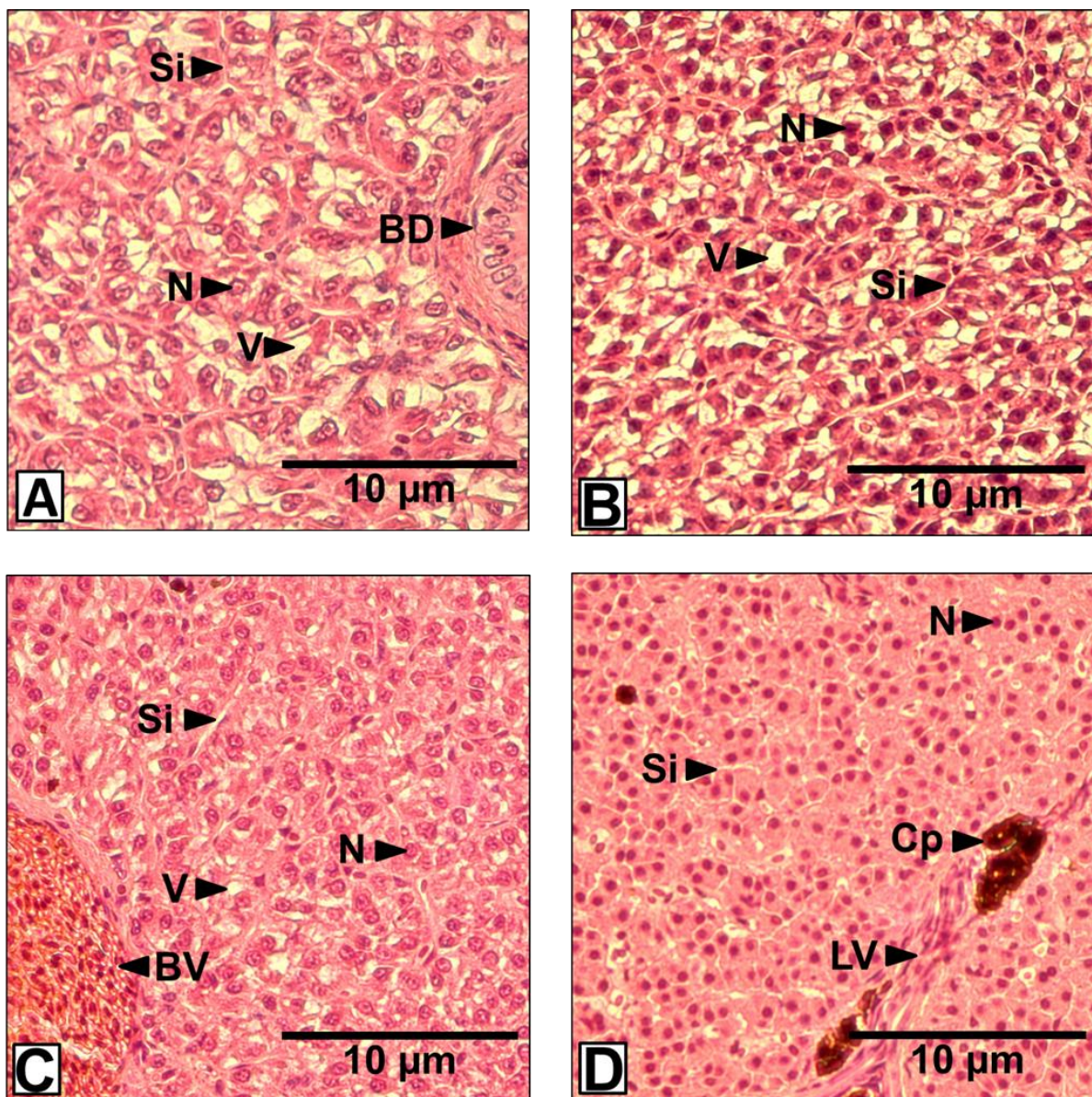


Fig. 1.1: Histological sections (HE-stained, 200x) of the liver of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM), representative for severe hypertrophy (A, in LG100), mild hypertrophy (B, in the control), normal state (C, in the control) and hypotrophy (D, in HG25 diets). BD, bile duct; BV, blood vessel; LV, lymphatic vessel; Cp, ceroid pigment; N, nucleus; Si, sinusoid; V, vacuole.

Tab. 1.6: Occurrence (%) of liver types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM); (n=9).

liver type	Experimental diets								
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100
(1)	67	56	23	22	22	-	-	-	-
(2)	33	33	33	22	22	-	-	-	-
(3)	-	11	33	34	56	-	-	-	-
(4)	-	-	11	22	-	100	100	100	100

The stomach tissue of the control group (Fig. 1.2A, type 1) revealed the typical four layered structure consisting of a folded mucosa, the submucosa, the muscularis and the serosa. The inner surface was composed of a single-layered columnar epithelium, followed by the lamina propria, a loose connective tissue with blood vessels, gastric glands, muscle fibers and nerves. These layers and the stratum compactum jointly define the tunica mucosa. Tela submucosa surrounding the stratum compactum was encircled by tunica muscularis. The tunica muscularis consisted of two different layers of smooth (tunica muscularis circularis) and skeletal (tunica muscularis longitudinalis) muscles. The muscle layers of the stomach were strongly developed in contrast to the other parts of the intestinal tract (see below). The enclosing outer layer (not shown in the figures) was a peritoneal epithelium (tunica serosa). Tunica mucosa and tela submucosa were clearly folded, increasing the overall inner surface area. Between the folds deep crypts were observed.

In the LG groups no changes in the morphology of the stomach were observed. All areas were clearly differentiated, normally developed and without abnormal findings.

In the HG groups (no differences observed between HG-groups) severe abnormalities were recorded (Fig. 1.2B, type 2). The tunica mucosa and tela submucosa were prevailing but less well developed. The lamina propria was only developed as a small layer which rarely reached the columnar epithelium. Also all muscle layers were reduced. It became apparent that the folding of the tunica mucosa and tela submucosa was rudimentary. The occurrence of the histopathological types in each feeding group is summarized in table 1.7.

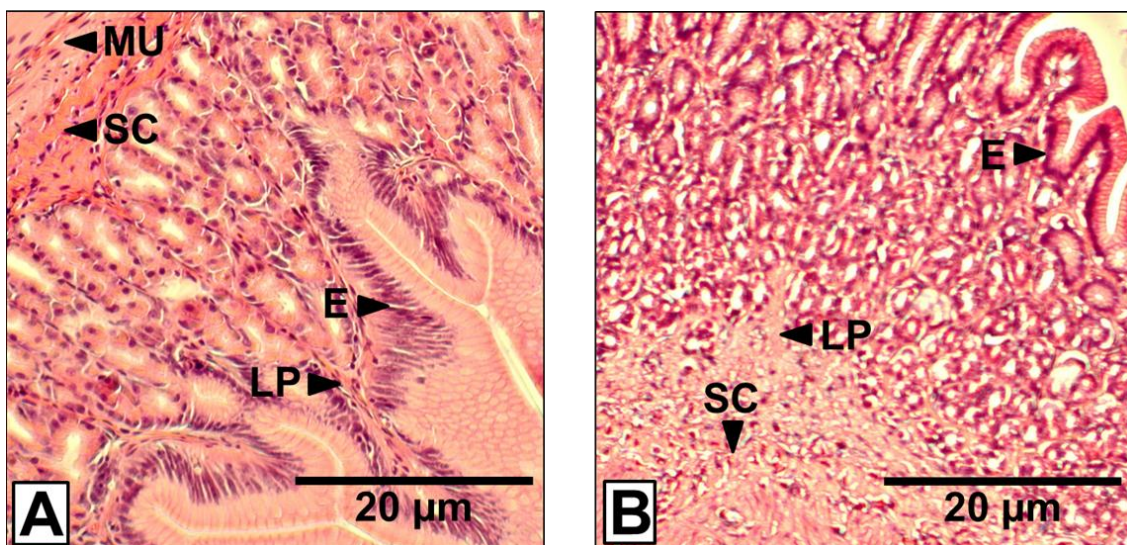


Fig. 1.2: Histological sections (HE-stained, 100x) of the stomach of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of $7.41 \text{ mg kg}^{-1} \text{ DM}$) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of $2150 \text{ mg kg}^{-1} \text{ DM}$), representative for normal state (A, control) and impeded development (B, HG100). E, Epithelium; LP, Lamina propria; MU, Tunica muscularis; SC, Stratum compactum.

Tab. 1.7: Occurrence (%) of the stomach types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM); (n=9).

stomach type	Experimental diets								
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100
(1)	100	100	100	100	100	22	-	-	-
(2)	-	-	-	-	-	78	100	100	100

The anterior intestine of fish in the control had the same basic structure as the entire four-layered gastrointestinal tract (Fig. 1.3A, type 1). The surface of the tunica mucosa comprised a single-layered, high prismatic epithelium (lamina epithelialis mucosae), consisting of intestinal absorptive cells (enterocytes), which were terminated to the inner lumen by a brush border. Within this layer a lot of goblet cells characterized by large vacuoles were regularly distributed. The tunica mucosa was strongly folded increasing the surface area. The lamina propria was similar in the stomach and the intestine, comprising connective tissue ensuring supply and disposal of the enterocytes. The stratum compactum was developed as a regularly curled band. In contrast to the stomach, the small intestine did not comprise a tela submucosa and the tunica muscularis though noticeable was relatively thin.

In the LG groups, with increasing glycoalkaloid content, the regularly shaped brush border became irregular due to vacuolization (Fig. 1.3B; type 2). Enterocytes were not affected by this manifestation. Furthermore, the size of the lamina propria as well as the overall shape of the tunica mucosa was reduced. In addition, the tunica mucosa appeared rather scattered and irregular. The mucosal folding was less pronounced as noticeable in the heights of the villi. The stratum compactum turned from a straight arrangement in the control to a rather curled one.

In the LG100 group, most severe alterations of anterior intestine were recognized (Fig. 1.3C; type 3). The vacuolization of the brush border was most prominent in this group. Parts of the tunica mucosa were extremely irregularly shaped or even ruptured. The lamina propria was reduced to a thin layer between the folded lamina epithelialis mucosae and phased out to unidentifiable cellular debris. No inflammations were observed and the stratum compactum and tunica muscularis revealed normal characteristics.

In the HG groups (Fig. 1.3D; type 4), most strikingly a drastic reduction in size of the anterior intestine was recorded, although all characteristic structures described were still developed. Furthermore, increased vacuolization was detected compared to the control. No

inflammation was noticed. The occurrence of these histopathological types in each feeding group is summarized in table 1.8.

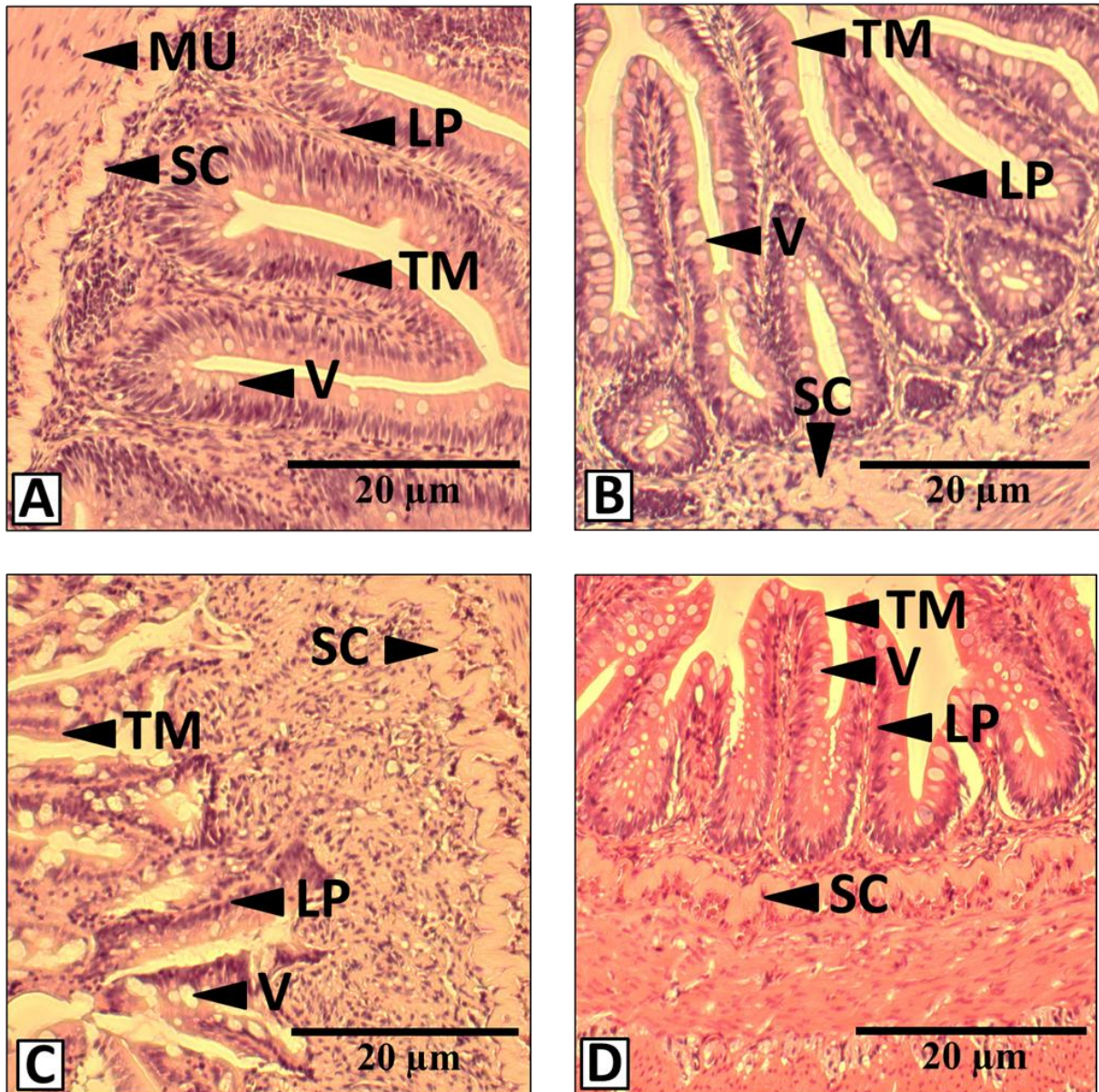


Fig. 1.3: Histological sections (HE-stained, 100x) of the anterior intestine of rainbow trout in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of $7.41 \text{ mg kg}^{-1} \text{ DM}$) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of $2150 \text{ mg kg}^{-1} \text{ DM}$), representative for normal state (A, control), slight alterations (B, LG75), strong alterations (C, LG100) and under developed (D, HG75). LEM, lamina epithelialis mucosae; LP, Lamina propria; MU, Tunica muscularis; SC, Stratum compactum; V, vacuole.

Tab. 1.8: Occurrence (%) of the anterior intestine types described based on histopathological characteristics (details see text) in the fishmeal (FM) control, the LG groups (supplementation of FM with PPC with a glycoalkaloid content of 7.41 mg kg⁻¹ DM) and the HG groups (supplementation of FM with PPC with a glycoalkaloid content of 2150 mg kg⁻¹ DM); (n=9).

anterior intestine type	Experimental diets								
	FM	LG25	LG50	LG75	LG100	HG25	HG50	HG75	HG100
(1)	89	78	56	22	22	-	-	-	-
(2)	11	22	44	56	22	-	-	-	-
(3)	-	-	-	22	56	-	-	-	-
(4)	-	-	-	-	-	100	100	100	100

In the posterior intestine no alterations between groups were observed.

Discussion

The growth parameters revealed that any inclusion of PPC in the diets of rainbow trout resulted in a significantly lower growth performance compared to the control. Fish fed on diets with low glycoalkaloid concentration in the diet (LG25 – LG100) doubled their weight during the experiment (control group tripled weight). DFI and SGR were significantly lower than in control while FCR increased. Also PER and PPV showed significantly decreased values. Although amino acid composition and amount of glycoalkaloids in LG-PPC diets revealed good nutritional composition, decreased PER and PPV values were indicative for insufficient amino acid supply and/or utilisation. Amino acid utilization could be negatively influenced by additional antinutritional substances (Tacon and Jackson, 1985; Francis et al., 2001). Further, with decreased amount of supplied nutrients by decreased feed intake, fish used proteins for maintenance energy metabolism.

Moyano et al. (1992) documented appropriate PER (1.67-1.74) for rainbow trout fed on diets with replacement of fishmeal by 50 and 70% plant protein mixture of lupine seed meal, corn gluten meal and potato protein concentrate. Same observations were made for PPV (35.7 and 39.3%). Feed ingestion was not affected. These findings were confirmed by Espe et al. (2006) in diets of Atlantic salmon with replacement of fishmeal by 100% plant protein mixture of wheat gluten meal and corn gluten meal. In contrast, an inclusion of HG-PPC in presented diets resulted in negative growth and drastically decreased DFI (up to refused feed intake).

Xie and Jokumsen (1997a) reported that an inclusion of PPC of even 94 g kg⁻¹ (glycoalkaloid concentration 1000 mg kg⁻¹, 18% replacement of fishmeal by PPC) in the diet (crude protein 460 g kg⁻¹) caused a significant decrease in growth performance of rainbow trout. With increasing amounts of PPC up to 510 g kg⁻¹ (100% replacement of fishmeal by

PPC) the feed intake was significantly decreased and growth became negative. A second trial of Xie and Jokumsen (1997b) with reduced amounts of PPC (glycoalkaloid concentration of 1000 mg kg⁻¹ PPC) in the diets (crude protein 430 g kg⁻¹) resulted in comparable trends to their previous study. A PPC incorporation of more than 22 g kg⁻¹ decreased growth and feed utilization in rainbow trout significantly. The results of the HG-groups of presented trial were in order to these findings. Any inclusion of LG-PPC and HG-PPC in the diets reduced feed intake and growth significantly to control up to refused feed intake and negative growth. But in contrast to the results of Xie and Jokumsen (1997a, b), an inclusion of 529 g kg⁻¹ LG-PPC in the diet (100% fish meal replacement) resulted in a higher SGR (0.72 % BW d⁻¹). This could be indicative for an action of glycoalkaloids and other antinutritive substances in PPC-diets on palatability and thereby on nutritional status and growth of fish fed on them. Tacon and Jackson (1985) reported that a replacement of fishmeal by a low glycoalkaloid PPC (glycoalkaloid concentration of 137 mg kg⁻¹) up to an inclusion level of 30% revealed no significant differences in growth parameters compared to a control diet for rainbow trout. Similar observations were recorded in Atlantic salmon by Refstie and Tiekstra (2003). They evaluated four different diets (crude protein 480 g kg⁻¹) with 0%, 7%, 14% and 21% FM replacement by PPC and resulting 0, 7, 14, 21 mg kg⁻¹ glycoalkaloid comparable to glycoalkaloid levels of diets LG25, 50, 75 and 100 used in present study. In contrast to the presented study, DFI, SGR, FCR and PPV and thus overall growth performance of Atlantic salmon were not affected by various PPC inclusion levels.

Regarding the whole body composition of fish, presented results are in line with previous studies. Xie and Jokumsen (1997a, b; 1998) reported significantly reduced dry matter, lipid and energy content when compared to the FM control, whereas ash and protein contents were not different between treatments.

Poor palatability of diets with PPC incorporation result in a reduction of the DFI as observed here and previously reported (Xie and Jokumsen, 1997a, b; 1998). Nevertheless, the DFI within the LG groups was not significant different, but revealed significant difference between the HG groups. Thus it can be assumed, that glycoalkaloid concentration (LG: 1.0-3.9 mg kg⁻¹, HG 315-1207 mg kg⁻¹) rather than PPC replacement itself influences the palatability of the diet, since a concentration-dependent effect of glycoalkaloids on DFI is observed, congruent with the data presented by Xie and Jokumsen (1997a). Furthermore, in the HG100 group, deterioration of the diet was observed as fish actually swam away from the pellets. Water solubility of glycoalkaloids reported by Jensen et al. (2009) additionally support the hypothesis the glycoalkaloids leach out of the pellet and act as taste-suppressor in

a concentration-dependent gradient around the feed particle. In humans, Friedman (2006) reported at a threshold of 140 mg kg⁻¹ (glycoalkaloid concentration in fresh potato products) a bitter taste recognized by a taste panel on fresh potato products. At 220 mg kg⁻¹ some test persons described irritation of mucosa in the mouth and pharynx. In presented study no observation on post-feeding rejection was observed.

Beside the potential effect on the palatability, a further minor antimicrobial effect of glycoalkaloids on digestibility and feed conversion needs to be considered. Jin et al. (2008) reported that glycoalkaloids from PPC had an antimicrobial influence on the digestion in weanling piglets, although actual concentrations were not determined. Friedman (2006) documented from antibiotic effects in mice and underlines the antimicrobial influence of glycoalkaloids. The increased FCR in the LG-groups may be indicative for this scenario. Such an effect could furthermore be anticipated by other plant derived antinutritional substances like protease inhibitors (Tacon and Jackson, 1985).

Due to the high variance of the hematocrit values in the control, no significant differences between groups could be found. Nevertheless, there was a decrease upon PPC inclusion. Still, no consistent difference between LG and HG was observed. Consequently, a concentration-dependent impact of PPC and glycoalkaloids on the hematocrit value was not observed. Wells and Weber (1991) defined an optimal hematocrit in rainbow trout at 30% in total blood sample. Hematocrit of the control was 33.8% indicative of a normal status. In all PPC groups a decrease in hematocrit was detected ranging from 24.8% to 30.3%, indicating some mild impact of dietary PPC inclusion. Friedman (2006) reported that in rabbits fed on high-glycoalkaloid diets (49-53 mg kg⁻¹ body weight day⁻¹) the red blood cell count and hemoglobin levels were reduced compared to the control, suggested as indicator of hemolytic anemia. Here, the reduced hematocrit as well as lower protein, triglyceride and glucose concentrations in all PPC groups were considered indicators of undernourishment and starvation. For good conditioned fish, Congleton and Wagner (2006) reported plasma protein of 35.6 mg ml⁻¹, triglycerides of 3.05 mg ml⁻¹ and glucose of 0.85 mg ml⁻¹, congruent with 41.9 mg ml⁻¹ protein, 3.54 mg ml⁻¹ triglycerides and 1.43 mg ml⁻¹ glucose in the plasma of control fish in the present study. In contrast, nutritional status indicated by 27.0-15.0 mg ml⁻¹ protein, 3.48-0.76 mg ml⁻¹ triglycerides and 1.20-0.90 mg ml⁻¹ glucose decreased in all PPC-groups, most extreme in the HG-groups. Again, this confirmed observations of Congleton and Wagner (2006) in fish starved over 3 weeks. Although direct adverse effects of glycoalkaloids as well as antinutritive effects of PPC (e.g. essential fatty acids and amino acids, mineral content etc.) incorporation cannot be ruled out, it has to be considered that symptoms on

plasma protein, triglycerides and glucose reflect severe starvation and substantial loss of appetite and reduced feeding were observed here.

This rather unspecific mode of action as exemplified by extreme undernourishment in the HG100 group is further supported by the histological findings. No observations such as inflammation or necrosis - indicative of toxic effects - were observed in fish fed PPC supplemented diets. Again, abnormalities observed in the intestine were previously described for malnutrition and undernourishment. In the liver, HG groups revealed hypotrophy characteristic for starvation as described by Glencross (2006). The hypertrophy observed with increasing LG incorporation furthermore suggests undernourishment. The vacuolization observed is most likely related to fat deposition as described by Yamamoto et al. (2010). LG groups revealed increasing occurrence of hypertrophy with increasing PPC incorporation, accompanied by increasing HSI values. Friedman (2006) observed that mice fed with up to 20% freeze-dried potato berries in the diets (glycoalkaloid content up to 76 mg kg⁻¹ diet) resulted in increased liver weights and decreased body-weight gain and resumed that the increasing of liver size (in some cases described as hepatomegaly) was an adaptation to glycoalkaloid load, since fat vacuoles may accumulate the lipophilic glycoalkaloids. Between 40% and 80% of the individuals in the LG-groups (table 1.6) exhibited hypertrophy of the liver (type 2, 3).

Yamamoto et al. (2010) reported mild abnormalities of the liver of rainbow trout in a feeding trial with fermented and unfermented soybean meal as fishmeal substitute. Most strikingly, an inclusion of soybean (fermented and unfermented) resulted in hypotrophic liver tissue, particularly in fish fed unfermented soybean meal additionally characterized by reduced feed intake (2.28 % BW day⁻¹) and lowest feed efficiency ratio (0.86). In presented trial, no growth or even negative growth was observed in the HG groups. As a consequence of undernourishment individuals used stored energy reservoirs. These are mainly based on glycogen or fat deposits, e.g. in the vacuoles of the liver (Yamamoto et al., 2010). Thus, with progressing starvation, HSI decreases and vascularization vanishes as observed in the HG, which turned highly hypotrophic. It is therefore most probable that hypotrophy is mainly a result of starvation, indirectly caused by the diets in the HG groups. Glencross et al. (2006) demonstrated that the inclusion of the lupine alkaloid gamine (>100 mg kg⁻¹) in diets resulted in increased occurrence of ceroid clusters in liver and kidney. These clusters were respectively responsible for trapping and removing of cellular debris and toxicants in the surrounding tissue area. But there was no indication for toxic action of gamine on fish health. In that way, it was concluded that the extreme decreased feed intake with increased gamine

levels in the diets resulted in starvation and thus increased the occurrence of ceroid clusters. Their incidence was used as indicator for starvation processes.

Histopathological analysis of the stomach revealed abnormalities in the HG groups, but not in the LG group. At increasing PPC incorporation, the typical four layered structure consisting of a folded mucosa, the submucosa, the muscularis were substantially reduced in all HG groups. Again, this is most probably attributed to malnutrition and starvation as reported by Glencross et al. (2006). No specific histopathological findings such as necrosis or inflammation suggested a direct toxicity of glycoalkaloids on the stomach.

In contrast, the anterior intestine was already affected in the LG-groups. Especially in LG75 and LG100 indicated functional impairment as indicated by a shortening of heights of the mucosal folding, an abnormal vacuolization of absorptive cells and an infiltration of inflammatory cells (lymphocytes, macrophages and leucocytes) in lamina propria. At an inclusion of soybean meal (40% of protein in the diet, crude protein content not specified) Beaverfjord and Krogdahl (1996) reported similar alterations in Atlantic salmon and Burrells et al. (1999) confirmed these in a feeding trial at soy protein inclusion of more than 50% in rainbow trout, concluding that antinutritional factors (glycinin and β -conglucine) were responsible for inflammatory effects and structural changes in anterior intestine tissues. The subsequent structural alterations decreased nutrient digestion and absorption (Van den Ingh et al., 1991; Burrells et al., 1999; Refstie et al., 2000). Although no inflammation became apparent in the presented study, the extreme irregular shape of mucosa and submucosa must have influenced nutrient absorption. Referring to the HG-groups again severe reduction of all layers was most likely related to malnutrition and starvation. As a conclusion of this comprehensive histopathological analysis, direct toxicity of glycoalkaloids has not been observed in any of the tissues studied, including the liver, stomach, the anterior and posterior intestine, but abnormalities showed characteristic impacts of undernourishment and malnutrition in the examined tissues.

The knowledge about the influence of PPC and glycoalkaloids on the taste, palatability and digestibility of PPC-diets in trout nutrition has to be explored in future studies, clarifying synergistic effects. Nevertheless, it was assumed, that a reduced feed intake resulted in an unbalanced nutritional status. Due to same genetic origin of fish and same environmental influences it is suggested that fish, reared in the present study, have same requirements for maintenance energy metabolism. However, decreased feed intake of LG-groups and HG-groups resulted in decreased energy supply required for growth. The use of LG-PPC as fish

meal supplement in organic diets could be possible if the acceptance and feed intake will be enhanced.

Conclusion

The use of PPC as FM replacement in organic rainbow trout nutrition is highly dependent on the quality, in particular the quantity of glycoalkaloids and other antinutritional substances as well as the substitution applied. Palatability, digestion and adsorption effect are affected at higher PPC incorporation. It should be concluded that a low glycoalkaloid PPC can provide a high quality in dietary AA composition. The acceptance of the diet is a major obstacle to be overcome by reduced glycoalkaloid contents and/or an addition of appetizers. Thereby, potato protein concentrates have a potential to fill in the gap regarding alternative protein sources substituting FM in diets according to organic guidelines and to provide a balanced amino acid profile without impact on animal welfare. Further research has to be done to ensure high plant protein utilization in organic aquaculture.

References

- Adelizi, P., Rosati, R., Warner, K., Wu, Y., Muench, T., White, M., Brown, P., 1998. Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*. *Aquacult. Nutr.* 4 (4), 255–262.
- Baeverfjord, G., Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *J. Fish Dis.* 19 (5), 375–387.
- Bergleiter, S., 2009. Organic aquaculture 2009: Production and markets. Naturland, Gräfelfing, 120 pp.
- Burrells, C., 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. *Vet. Immunol. Immunopathol.* 72 (3-4), 277–288.
- Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.* 69 (2), 473–490.
- Espe, M., Lemme, A., Petri, A., Elmowafi, A., 2006. Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? *Aquaculture* 255 (1-4), 255–262.

- European Union, 2008. Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Off. J. Europ. Union* 51, 1–84.
- European Union, 2009. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union* 52, 15–34.
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Friedman, M., 2006. Potato Glycoalkaloids and Metabolites: Roles in the Plant and in the Diet. *J. Agric. Food Chem* 54 (23), 8655–8681.
- Gatlin, D., Barrows, F., Brown, P., Dabrowski, K., Gaylord, T., Hardy, R., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.* 38 (6), 551–579.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- IFOAM, 2005. Basic standards for organic production and processing. International Federation of organic Agricultural movements.
http://www.ifoam.org/about_ifoam/standards/norms/norm_documents_library/IBS_V3_20070817.pdf. Accessed 17 March 2011.
- IFOAM, 2010. Organic Aquaculture. EU Regulations (EC) 834/2007, (EC) 889/2008, (EC) 710/2009. BACKGROUND, ASSESSMENT, INTERPRETATION. International Federation of organic Agricultural movements.
http://www.ifoam.org/about_ifoam/around_world/eu_group/new/positions/publications/aquaculture/IFOAMEU_IAMB_organic_aquaculture_dossier.pdf. Accessed 17 March 2011.
- Jensen, P.H., Jacobsen, O.S., Henriksen, T., Strobel, B.W., Hansen, H.C.B., 2009. Degradation of the Potato Glycoalkaloids – α -Solanine and α -Chaconine in Groundwater. *Bull. Environ. Contam. Toxicol.* 82 (6), 668–672.

- Jin, Z., Yang, Y.X., Choi, J.Y., Shinde, P.L., Yoon, S.Y., Hahn, T.-W., Lim, H.T., Park, Y., Hahm, K.-S., Joo, J.W., Chae, B.J., 2008. Potato (*Solanum tuberosum* L. cv. Gogu valley) protein as a novel antimicrobial agent in weanling pigs. *J. Anim. Sci.* 86 (7), 1562–1572.
- Moyano, F., Cardenete, G., La Higuera, M. de, 1992. Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquat. Liv. Res.* 5 (1), 23–29.
- Naturland, 2010. Naturland Standards for Organic Aquaculture. Naturland - Association for Organic Agriculture, Registered Association.
http://www.naturland.de/fileadmin/MDB/documents/Richtlinien_englisch/Naturland-Standards_Aquaculture.pdf. Accessed 17 March 2011.
- Refstie, S., Korsoen, O., Storebakken, T., Baeverfjord, G., Lein, I., Roem, A., 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture* 190 (1-2), 49–63.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Sironi, E., Sessa, F., Duranti, M., 2005. A simple procedure of lupin seed protein fractionation for selective food applications. *Eur. Food Res. Technol.* 221 (1-2), 145–150.
- Slawski, H., Adem, H., Tressel, R., Wysujack, K., Koops, U., Wuertz, S., Schulz, C., 2011. Replacement of fish meal with rapeseed protein concentrate in diets fed to wels catfish (*Silurus glanis* L.). *Aquacult. Nutr.*, DOI: 10.1111/j.1365-2095.2011.00857.x.
- Strolle, E., Cording, J., Aceto, N., 1973. Recovering potato proteins coagulated by steam injection heating. *J. Agric. Food Chem* 21 (6), 974–977.
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London ;, Orlando, pp. 119–145.
- Tacon, A., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285 (1-4), 146–158.
- Van den Ingh, T., Krogdahl, A., Olli, J., Hendriks, H., Koninkx, J., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture* 94 (4), 297–305.
- Wells, R.M.G., Weber, R.E., 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *J. Fish Biol.* 38 (1), 53–65.

- Wuertz, S., 2005. The paracrine IGF-I system and its role in the regulation of gonad maturation of sterlet, *Acipenser ruthenus* Linnaeus, 1758.: PhD Thesis, ISBN 3-86624-119-4. dissertation.de, Berlin.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.
- Xu, L., Diosady, L., 1994. The production of Chinese rapeseed protein isolates by membrane processing. *J. Am. Oil Chem. Soc.* 71 (9), 935–939.
- Yamamoto, T., Iwashita, Y., Matsunari, H., Sugita, T., Furuita, H., Akimoto, A., Okamatsu, K., Suzuki, N., 2010. Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 309 (1-4), 173–180.
- Zhang, W., Liu, D., 2005. A new process for preparation of soybean protein concentrate with hexane-aqueous ethanol mixed solvents. *J. AOAC Int.* 88 (4), 1217–1222.

Chapter 2:

Evaluation of feed attractants in potato protein concentrate based diets for rainbow trout (*Oncorhynchus mykiss*)

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Abstract

A feeding trial was carried out with rainbow trout to evaluate different feed attractants in isonitrogenous ($472 \pm 8 \text{ g CP kg}^{-1}$) and isoenergetic ($21.7 \pm 0.3 \text{ MJ kg}^{-1}$) diets upon fish meal (FM) replacement by potato protein concentrates (PPC) at 50% and low glycoalkaloid content (7 mg kg^{-1}). After one week of acclimatisation, two fish meal diets - one with 7 mg kg^{-1} synthetic glycoalkaloids (GFM) and one control (FM), a PPC control without additional attractants (PP), and six PPC diets with blood meal (BM 4 or 8 %), mussel meal (MM 4 or 8 %) and a free amino acid mixture (AM 0.5 or 1.0 % alanine, glycine and betaine) as attractant were fed in triplicate until apparent satiation over a period of 56 days to the experimental groups (7 fish per tank, average initial weight $52.8 \pm 0.3\text{g}$).

All PPC groups revealed reduced growth when compared to the fish meal group (FM). Furthermore, all PPC groups exhibited lower growth when compared to the GFM group fed at comparable glycoalkaloid concentrations suggesting antinutritional effects apart from glycoalkaloids. Nevertheless, a PER of more than 2 in all groups indicated acceptable protein utilisation in PPC groups, additionally supported by FCR ranging between 0.92-1.15. Highest daily feed intake (DFI) was observed in the FM and GFM group, but reduced in all PPC groups. Among the attractants used, BM and AM resulted in the highest DFI. A good nutritional status did not reveal severe impact of PPC in general.

Introduction

Potato protein concentrates (PPC) are by-products of the potato starch industry and are gained out of potato juice by thermal coagulation and drying (Strolle et al., 1973; Friedman, 2006). This gentle way of processing enables the use of PPC in organic aquaculture diets according to European organic aquaculture guideline (European Union, 2009b). The amino acid amount and profile is of a high quality and especially the high amounts of lysine, methionine and cysteine assign an extraordinary nutritional value for aqua feeds (Jeroch et al., 1999). Beside the high protein contents, these pure PPCs incorporate different anti-nutritional substances. Most prominent are the glycoalkaloids e.g. solanine and chaconine (secondary plant metabolites and repellents against pathogens such as insects) (Jeroch et al., 1999; Refstie and Tiekstra, 2003). These alkaloids are extremely heat stable and evoke bitter flavor/taste in PPC products at minimal concentrations (Friedman, 2006). As a result reduced palatability of pellets could be observed in trout fed on PPC diets with varying amounts of glycoalkaloids (Tusche et al., 2011). In rainbow trout reduced feed intake and growth has been described after incorporation of PPC (Xie and Jokumsen, 1997a, b; 1998). Minimal PPC inclusion of 56 g kg⁻¹ diet (glycoalkaloid concentration 1,110 mg kg⁻¹ PPC) resulted in significantly reduced feed intake and growth (Xie and Jokumsen, 1997a; Tusche et al., 2011), compromising the use of potato proteins for aqua feeds. More recently, organic solvents (e.g. ethanol) have been used to eliminate glycoalkaloids in the PPC from 2,000 mg kg⁻¹ to < 50 mg kg⁻¹ (Strolle et al., 1973). Such a purification seems to be essential before commercial application in aqua feeds. Nevertheless, it has been reported that even the inclusion of purified PPC is limited to 210 g kg⁻¹ in Atlantic salmon diets (Tacon and Jackson, 1985; Refstie and Tiekstra, 2003). Similarly, alkaloid gramine in lupine meals has been reported for anti-palatability in rainbow trout nutrition thereby constraining its utilization although characterized by a good nutritional quality as indicated by high protein content and a balanced amino acid profile (Glencross et al., 2006). On the other hand to compensate reduced palatability and increase dietary attractiveness several stimulants can be used.

Blood meal has successfully been used as attractant, e.g. in angling baits (Johnstone and Mackie, 1990) and in diets rich in plant based raw materials (Kikuchi, 1999; Millamena, 2002). Furthermore, this animal protein is highly digestible in rainbow trout (Bureau et al., 1999), rich in the amino acids valine, leucine and histidine (Tacon and Jackson, 1985) and was successfully used as alternative protein source in various fish species. In addition, mussels and processing products such as mussel meal (MM) were successfully incorporated

in aqua feeds to increase palatability (Mackie et al., 1980) or as alternative protein source replacing fish meal (Kikuchi, 1999). In the wild rainbow trout feed on molluscs, e.g. snails and mussels (McCarter, 1986) and in that way it can be assumed that such a feed ingredient acts as natural attractant in the diet. Reporting a good AA composition of blue mussel (*Mytilus edulis*) for rainbow trout nutrition (Berge and Austreng, 1989), the inclusion of MM in fish diets increased feed intake and enhanced growth performance (Kikuchi, 1999; 2002; Kikuchi and Furuta, 2009a, b). In addition, mussel meat is rich in the amino acids glycine, alanine and the non-amino acid betaine (trimethylglycine) which had been identified as main feed attractants (Mackie et al., 1980). Although betaine as single stimulant did not result in an increase of feed intake in Atlantic cod (*Gadus morhua*), it enhances L-amino acids as feed attractant (Johnstone and Mackie, 1990). Similar results were documented for different mixtures of betaine and amino acids such as alanine, glycine and other L-amino acids in the diets of rainbow trout (*Oncorhynchus mykiss*), Gibel carp (*Carassius auratus gibelio*) and Dover sole (*Solea solea*) (Mackie et al., 1980; Jones, 1989; Franco et al., 1991; Dias et al., 1997; Papatryphon and Soares, 2000; 2001; Xue and Cui, 2001; Yamashita et al., 2006).

Until now, there are only a few investigations on the utilization of PPC in aquaculture diets. The reduced DFI and the adverse effects reported in the study by Tusche et al. (2011), indicate severe impact of starvation and malnutrition rather than intoxication by secondary plant metabolites. As a consequence, the present study was conducted to explore the use of feed attractants to compensate repellent effects of PPC, e.g. glycoalkaloids in order to explore the feed potential for organic aquaculture. The aim was to evaluate the utilization of a mussel meal (MM), blood meal (BM) as well as a free AA mixture (alanine, glycine and non-AA betaine) as food attractant to compensate reduced feed acceptance of PPC based diets and thereby increase growth performance of PPC based diets in rainbow trout (*Oncorhynchus mykiss*). In addition to the parameters of growth and growth performance, blood samples were taken and analyzed in order to evaluate the physiological response of PPC and attractant administrations on a nutrient supply level by the raw materials.

Materials and Methods

Experimental diets

For evaluation of selected feed attractants, fish meal was replaced by PPC at a level of 50% of the protein (PPC with low glycoalkaloid content: 7.41 mg kg⁻¹ dry weight; K5, Emsland Group, Emlichheim, Germany) and nine isonitrogenous (472 ± 8 g CP kg⁻¹) and isoenergetic (21.7 ± 0.3 MJ kg⁻¹) diets were formulated (Tab. 2.1, 2.2):

Tab. 2.1: Nutrient composition, amino acid concentration, antinutritional phytic acid and glycoalkaloid concentration of the investigated protein sources and attractants utilized in the experimental diets in g kg⁻¹ dry matter (Tab. 2.2). FM - fish meal; PPC - potato protein concentrate with a low glycoalkaloid content.

	diet ingredients	
	FM	PPC
<i>Nutrient composition</i>		
Dry matter	918	955
Crude protein	689	859
Crude lipid	69	32
NfE ¹ + Crude fibre	36	25
Crude ash	206	85
Phosphorus	25	22
<i>Essential amino acids (g kg⁻¹)</i>		
Arginine	37.0	43.2
Histidine	12.7	19.1
Isoleucine	23.0	47.8
Leucine	40.9	90.5
Lysin	41.5	71.2
Methionine + Cysteine	20.1	32.9
Phenylalanine	22.3	57.8
Threonine	24.7	52.6
Valine	28.2	57.0
<i>Nonessential amino acids (g kg⁻¹)</i>		
Aspartic acid	52.5	107.7
Serine	25.7	48.0
Glutamic acid	79.0	96.2
Proline	29.7	43.9
Glycine	51.5	43.3
Alanine	40.6	43.8
Valine	28.2	57.0
Tyrosine	16.6	48.6
Tryptophan	5.3	10.2
<i>Glycoalkaloids² (mg kg⁻¹)</i>		
Solanin		4.40
Chaconin		3.01
<i>Phytic acid (g kg⁻¹)</i>		
		<0.1

¹NfE, Nitrogen-free extract = 1000 – (Crude protein + Crude lipid + Ash)

² analyzed at LUFA-ITL, Kiel, Germany, according to LC-MS

1) a diet with low temperature fish meal (VFCUX, Cuxhaven, Germany) as control (FM), 2) a fish meal diet added with pure glycoalkaloids at 7.4 mg kg⁻¹ (chaconine 3.01 mg kg⁻¹, 4.4 mg kg⁻¹ solanine) corresponding to the glycoalkaloid concentration in PPC diets, 3) a PPC diet without any attractant (PP), 4) a PPC diet with 4% blood meal (BM4), 5) a PPC diet with 8% BM (BM8), 6) a PPC diet with 4% green lip mussel meal (MM4), 7) a PPC diet with 8% green lip mussel meal (MM8), 8) a PPC diet with a 0.5% free amino acid mixture of betaine, alanine and glycine (AM05), and 9) a PPC diet with 1% AM (AM1). The nutritional formulation of several diets was tailored to suit the metabolic demand of the individuals. Especially the amount of essential AA and phosphorous were in line with the dietary requirement for salmonids in accordance to NRC (1993).

All ingredients were pelletized with a fodder press L 14-175 (AMANDUS KAHL, Reinbek, Germany) to pellets of 4 mm diameter. Due to the high protein contents of PPC, magnesium silicate was used as inert filler in the diets to realise equal nutrient contents in the diets (ANNEX V, European Union, 2009b).

Tab. 2.2: Ingredients, nutrients, amino acid concentration and composition the experimental diets in g kg⁻¹ dry matter. FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC or 4% and 8% mussel meal, AM - diet with 50% PPC and 0.5% or 1% free amino acid as attractant.

	Diets								
	FM	GFM	PP	BM4	BM8	MM4	MM8	AM05	AM1
<i>Ingredients (g kg⁻¹)</i>									
Fish meal ¹	595	595	262	205	147	231	200	262	262
LG-PPC ²	-	-	268	268	268	268	268	268	268
Blood meal ³	-	-	-	40	80	-	-	-	-
Mussel meal ⁴	-	-	-	-	-	40	80	-	-
Free AA-mix ⁵	-	-	-	-	-	-	-	5	10
Fish oil ¹	95	95	109	113	116	116	112	109	109
Wheat starch ⁶	241	241	223	225	226	206	189	216	209
Wheat gluten ⁶	60	60	60	60	60	60	60	60	60
Vitamin ⁷ + Mineral mixture ⁸	10	10	10	10	10	10	10	10	10
Magnesium silicat ⁹	-	-	68	80	93	75	81	70	72
Glycoalkaloids ¹⁰ (mg kg ⁻¹)	-	2.0	-	-	-	-	-	-	-
<i>Nutrient composition (g kg⁻¹)</i>									
Dry matter	917	880	927	927	935	932	936	934	936
Crude protein	484	479	469	470	471	460	466	469	483
Crude lipid	169	171	151	146	143	147	136	145	145
Crude ash	113	112	124	128	135	135	146	131	131
Phosphorus	13.9	13.9	12.5	12.0	11.5	12.6	12.6	12.5	12.5
NfE + crude fibre	234	238	256	256	251	258	252	255	241
Gross energy (MJ kg ⁻¹)	22.1	22.1	21.9	21.7	21.6	21.7	21.1	21.6	21.6
<i>Essential amino acids (g kg⁻¹)</i>									
Arginine	23.6	23.6	22.9	22.2	21.6	23.5	24.2	22.9	22.9
Histidine	8.5	8.5	9.4	11.6	13.8	9.7	9.9	9.4	9.4
Isoleucine	15.3	15.3	20.5	19.3	18.2	20.9	21.3	20.5	20.5
Leucine	27.5	27.5	38.2	40.6	43.0	38.8	39.4	38.2	38.2
Lysin	25.5	25.5	30.7	31.6	32.5	31.0	31.3	30.7	30.7
Methionine + Cysteine	13.6	13.6	15.7	15.1	14.5	15.8	15.9	15.7	15.7
Phenylalanine	15.6	15.6	23.7	24.9	26.2	24.3	24.9	23.7	23.7
Threonine	15.9	15.9	21.7	21.5	21.2	22.0	22.2	21.7	21.7
Valine	18.6	18.6	24.5	26.1	27.8	24.8	25.2	24.5	24.5
<i>Glycoalkaloids (mg kg⁻¹)¹¹</i>									
Solanine	-	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Chaconine	-	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

¹VFCUX, Cuxhaven, Germany.

²Emsland-Stärke GmbH, Emlichheim, Germany.

³Euroduna-Technologies GmbH, Barmstedt, Germany.

⁴Sera GmbH, Heinsberg, Germany.

⁵AA-Mix (g kg⁻¹); Carl Roth GmbH, Karlsruhe, Germany: Alanine, 333; Glycine, 333; Betaine, 333.

⁶Cargill Deutschland GmbH; Krefeld, Germany.

⁷Vitamin-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: vitamin A, 1,000,000 IU kg⁻¹; vitamin D3, 200,000 IU kg⁻¹; vitamin E (as α -tocopherol-acetate), 40,000; vitamin K3 (as menadione), 4,000; vitamin B1, 4,000; vitamin

B2, 8,000; vitamin B6, 4,000; vitamin B12, 8; vitamin C (as monophosphate), 60,000; pantothenic acid, 8,000; nicotinic acid, 40,000; folic acid, 1,600; biotin, 100; inositol 40,000.

⁸Mineral-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: cobalt (as cobaltsulphate), 400; manganese (as manganese sulphate), 2,500; iodine (as calciumiodine), 500; copper (as coppersulphate), 2,500; selen, 25; zinc (as zincsulfate), 28,000.

⁹Magnesia 4371, Magnesia GmbH, Lüneburg, Germany.

¹⁰Carl Roth GmbH, Karlsruhe, Germany.

¹¹Calculated by inclusion level of PPC in the diet.

Experimental setup

The feeding trial was conducted at the experimental facilities of the Gesellschaft für Marine Aquakultur (GMA, Büsum, Germany). Prior to the experiment, 189 rainbow trout (52.8 ± 0.3g; Forellenzucht Troststadt GbR, Troststadt, Germany) in 9 triplicate groups of 7 fish were randomly stocked in 27 tanks (50 l each) and acclimatised for 14 d in the experimental system. The tanks were arranged in a closed recirculation system (2 m³ total water volume, water turnover rate 6 h⁻¹, daily water exchange 1.700 l kg⁻¹ feed) and photoperiod was adjusted to 12 h light : 12 h dark. Water parameters were determined daily (13.3 ± 0.5°C, 9.7 ± 0.3 mg l⁻¹ O₂; Handy Polaris; OxyGuard International A/S, Birkerød, Denmark; 0.4 ± 0.2 mg l⁻¹ NH₄-N, 0.7 ± 0.3 mg l⁻¹ NO₂-N; Microquant test kit for NH₄ and NO₂; Merck KGaA, Darmstadt, Germany). During acclimatisation fish were fed a commercial trout feed (Performa 45/20, 2mm; AllerAqua A/S, Christiansfeld, Denmark). Over the entire experimental period of 8 weeks, fish were fed to apparent satiation twice per day and daily feed intake was recorded.

Sampling

Prior to the start of the experiment, 10 fish were sampled to assess whole body composition and stored at -20°C until analysed. At the end of the experiment, final biomass of each tank was recorded, 3 fish (bulk sample) of each tank were sampled for the determination of whole body composition and stored at -20°C. Three additional individuals per tank were utilized to draw blood samples from the caudal vein for the determination of blood parameters. In addition, fish weight and length as well as liver weight were documented. For each treatment, mortalities and growth parameters were calculated as average of the triplicates, as well as specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and fulton condition factor (FCF), according to the formulas in Tab. 2.3.

Whole body composition

For the analysis of whole body composition, 3 fish per tank were freeze-dried for 120 h, homogenized and stored at -20°C, experimental diets were homogenized and stored at -20°C. Analysis for dry matter (DM), ash, crude protein, crude fat and crude fiber was determined according to EU guideline (EC) 152/2009 (European Union, 2009a). Briefly, crude protein content ($N \times 6.25$) was determined by the Kjeldahl method (InKjel 1225 M, WD 30; Behr, Düsseldorf, Germany), crude fat content after hydrolysis with hydrochloric acid followed by a petroleum ether extraction with a Soxhlet extraction system (R 106 S; Behr), DM after drying at 103°C until constant weight remained stable and ash content after 4 h incineration at 550°C with a combustion oven (P300; Nabertherm, Lilienthal, Germany). Gross energy was measured in a bomb calorimeter (C 200; IKA, Staufen, Germany).

Blood parameters

Upon blood sampling with a heparinised syringe, the hematocrit value was determined in micro-hematocrit-tubes (Type 563, NA-heparinized, 75 mm/135 µm; Assistent, Sondheim, Germany) upon centrifugation (10,000 g, 5 min) with a hematocrit 210 centrifuge (Hettich, Tuttlingen, Germany) using a hematocrit tube card reader. Remaining blood was centrifuged (1,000 g, 5 min) and plasma was separated and stored at -80°C. Glucose and triglycerides were determined by the GPO-PAP method using an enzymatic colorimetric kit (Greiner, Frickenhausen, Germany). Plasma protein was quantified according to Bradford with a Roti-Quant kit and a BSA standard dilution series. All colorimetric assays were measured in duplicate with a microplate reader Infinite 200 (Tecan, Männedorf, Switzerland) and calculated from a standard dilution series to compensate for run-to-run variation. Recoveries were determined by spike experiments and were >90% for all plasma parameters analysed.

Statistical Analyses

The statistical analysis was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, U.S.). Data are presented as mean \pm standard deviation (SD) for each treatment and were compared between treatments. Data were analysed for normal distribution by Kolmogorov-Smirnov and equal variance by Kruskal-Wallis One Way Analysis of Variance (confirmed if $p < 0.05$). Post-hoc multiple comparison was carried out by parametric Tukey-HSD (if test for homogeneity was confirmed) ($p < 0.05$) or non-parametric Dunnett-T3 test (if test for homogeneity failed).

Results

Growth

No mortalities were observed and all groups more than doubled their weight during the experimental period.

At the end of the experiment the control group (FM) revealed the highest final body weight (FBW) (190.6 ± 4.3 g) and the highest SGR 2.42 ± 0.0 % BW d⁻¹. The growth rates of all PPC diets were slightly decreased and significantly different to the FM group, with the exception of a slightly higher value of 2.00 % BW d⁻¹ in the AM1 group. Among all PPC groups, no significant differences in FBW and SGR were detected, but FBW and SGR were not significantly different in the GFM and AM1 compared to the FM control (Table 2.3). The lowest final body weight and SGR were found in MM8 and AM05 and same trend was observed in the final condition factor.

Tab. 2.3: Growth performance (mean \pm SD, n=3) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC or 4% and 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Values with the same superscript are not significantly different ($p < 0.05$).

Variable	Experimental diets									
	FM	GFM	PP	BM4	BM8	MM4	MM8	AM05	AM1	
IBW ¹	53.1 \pm 0.7	52.8 \pm 0.3	53.0 \pm 0.6	52.5 \pm 0.4	53.1 \pm 0.9	53.2 \pm 0.6	53.0 \pm 1.1	52.3 \pm 0.1	52.3 \pm 0.6	
FBW ²	190.6 ^a \pm 4.3	175.8 ^{ab} \pm 16.7	144.4 ^{bc} \pm 20.6	140.1 ^{bc} \pm 8.4	143.5 ^{bc} \pm 11.6	133.9 ^c \pm 2.8	125.1 ^c \pm 10.2	126.6 ^c \pm 12.6	159.1 ^{abc} \pm 16.2	
DFI ³	2.42 ^a \pm 0.1	2.42 ^a \pm 0.1	1.69 ^{bc} \pm 0.3	2.04 ^{ab} \pm 0.1	1.94 ^{abc} \pm 0.1	1.75 ^{bc} \pm 0.2	1.64 ^{bc} \pm 0.1	1.48 ^c \pm 0.3	1.87 ^{bc} \pm 0.2	
SGR ⁴	2.31 ^a \pm 0.0	2.17 ^{abc} \pm 0.2	1.80 ^{bcd} \pm 0.3	1.77 ^{bcd} \pm 0.1	1.79 ^{bcd} \pm 0.2	1.67 ^{cd} \pm 0.0	1.54 ^d \pm 0.1	1.59 ^{cd} \pm 0.2	2.00 ^{abc} \pm 0.2	
FCR ⁵	1.05 ^{ab} \pm 0.1	1.12 ^{ab} \pm 0.1	0.94 ^b \pm 0.1	1.15 ^a \pm 0.1	1.09 ^{ab} \pm 0.0	1.05 ^{ab} \pm 0.1	1.06 ^{ab} \pm 0.0	0.92 ^b \pm 0.1	0.93 ^b \pm 0.0	
PER ⁶	2.16 ^{ab} \pm 0.1	2.13 ^{ab} \pm 0.2	2.47 ^b \pm 0.2	2.00 ^a \pm 0.2	2.09 ^{ab} \pm 0.1	2.23 ^{ab} \pm 0.2	2.16 ^{ab} \pm 0.1	2.47 ^b \pm 0.2	2.38 ^{ab} \pm 0.1	
HSI ⁷	1.41 \pm 0.1	1.56 \pm 0.4	1.39 \pm 0.2	1.55 \pm 0.1	1.49 \pm 0.2	1.61 \pm 0.1	1.76 \pm 0.1	1.49 \pm 0.1	1.59 \pm 0.1	
FCF ⁸	1.36 ^a \pm 0.0	1.33 ^{ab} \pm 0.0	1.26 ^{ab} \pm 0.1	1.27 ^{ab} \pm 0.1	1.30 ^{ab} \pm 0.0	1.28 ^{ab} \pm 0.0	1.27 ^{ab} \pm 0.1	1.22 ^b \pm 0.0	1.33 ^{ab} \pm 0.0	

¹ initial body weight (g); ² final body weight (g); ³ daily feed intake (% day⁻¹); ⁴ specific growth rate (% day⁻¹) = [ln (FBW) - ln (IBW)] / feeding days x 100; ⁵ feed conversion ratio = feed intake (g) / weight gain (g); ⁶ protein efficiency ratio = weight gain (g) / protein intake (g); ⁷ hepatosomatic index = (liver weight / final body weight) x 100; ⁸ Fulton condition factor = 100 x final body weight x final body length⁻³.

The FM group revealed the highest daily feed intake DFI (2.42 ± 0.1 % BW d⁻¹), though not significantly different to the blood meal groups (BM4 2.04 ± 0.1 % BW d⁻¹; BM8 1.94 ± 0.1 % BW d⁻¹). The DFI was significantly decreased in all other groups: MM4 1.75 ± 0.2 % BW d⁻¹, MM8 1.64 ± 0.1 % BW d⁻¹, AM05 1.48 % BW d⁻¹, AM1 1.87 ± 0.2 % BW d⁻¹ and PP group 1.69 ± 0.3 % BW d⁻¹. All attractants resulted in slightly increased DFI when compared to the PP group without significant differences to each other, only MM8 and the AM05

revealed even lower DFI as the PP group. The PP and AM AA diets exhibited the best FCR (0.92 – 0.94 ± 0.1) but without significant differences. All the other groups showed a slight increased FCR above 1 (1.05 – 1.15). PER of all groups were inverse correlated with calculated FCR. Highest PER was again observed in AM05 (2.47 ± 0.2) and AM1 (2.38 ± 0.1) and was slightly decreased in all other groups (2.23 – 2.00).

The hepatosomatic index (HSI) ranged between 1.39 – 1.76 without significant differences between all groups.

Body composition

DM of all groups was approximately at 294 g kg⁻¹ and highest in the BM4 group (308 ± 3.4 g kg⁻¹). The mussel meal groups MM4 (264 ± 4.2 g kg⁻¹) and MM8 (263 ± 1.8 g kg⁻¹) were significantly lowered in contrast to DM of all other groups. Same tendencies were observed in the crude lipid content. Strikingly, the mussel meal groups again exhibited the lowest crude lipid and gross energy contents (MM4 83 ± 1.7 g kg⁻¹ DM, 26.4 ± 0.0 MJ kg⁻¹ DM; MM8 81 ± 0.3 g kg⁻¹ DM, 25.8 ± 0.1 MJ kg⁻¹ DM), significantly different from all other groups. The highest crude lipid content was met in BM4 (128 ± 1.9 g kg⁻¹ DM) whereas the highest gross energy was found in AM1 (28.2 ± 0.2 MJ kg⁻¹ DM). The effect of the different diets on body crude protein content in all groups was moderate. Highest value was recorded in AM05 (159 ± 2.0 g kg⁻¹ DM). The use of potato protein concentrate and attractants in the different diets had no effect on the ash content (Table 2.4).

Tab. 2.4: Body composition (g kg⁻¹ wet weight; MJ kg⁻¹ DM) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Initial body composition was expressed on a wet weight basis as dry matter (253 g kg⁻¹; ash 26 g kg⁻¹; crude protein 139 g kg⁻¹; crude lipid 92 g kg⁻¹; energy 26.7 MJ kg⁻¹). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05)

Nutrient	Experimental diets									
	FM	GFM	PP	BM4	BM8	MM4	MM8	AM05	AM1	
Dry matter	303 ^{ab} ± 1.3	302 ^{ab} ± 1.1	301 ^{ab} ± 1.2	308 ^a ± 3.4	303 ^{ab} ± 7.0	264 ^c ± 4.2	263 ^c ± 1.8	304 ^{ab} ± 4.3	294 ^b ± 1.0	
Crude protein	153 ^a ± 1.4	153 ^{ab} ± 1.9	154 ^{ab} ± 1.9	158 ^{bc} ± 2.6	153 ^a ± 3.6	156 ^{abc} ± 2.1	155 ^{abc} ± 3.7	159 ^c ± 2.0	151 ^a ± 1.5	
Crude lipid	126 ^{ab} ± 0.4	123 ^b ± 2.5	123 ^b ± 1.7	128 ^a ± 1.9	125 ^{ab} ± 3.7	83 ^d ± 1.7	81 ^d ± 0.3	121 ^{bc} ± 1.4	119 ^c ± 0.6	
Crude ash	28 ± 1.1	30 ± 3.4	27 ± 3.5	27 ± 1.0	28 ± 3.1	26 ± 1.7	26 ± 1.3	25 ± 3.1	26 ± 3.2	
Gross energy	28.1 ^a ± 0.1	27.3 ^{bc} ± 0.2	27.2 ^c ± 0.1	27.6 ^b ± 0.1	27.6 ^b ± 0.1	26.4 ^d ± 0.0	25.8 ^e ± 0.1	28.1 ^a ± 0.1	28.2 ^a ± 0.2	

Blood plasma composition

Blood parameters revealed only minor differences between groups (table 2.5). The hematocrit was the highest in the FM group (33.8 ± 6.7 %). Due to the high individual variance of the hematocrit, no significant differences between this group and all the other groups were detected. Same effects were observed in the other physiological blood parameters, neither in plasma protein ($33.3 - 38.5$ mg ml⁻¹), triglycerides ($3.43 - 5.74$ mg ml⁻¹) nor glucose ($1.20 - 1.64$ mg ml⁻¹).

Tab. 2.5: Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml⁻¹) of rainbow trout fed the experimental diets (FM - fishmeal control, GFM - diet with fishmeal and 7.4 mg kg⁻¹ glycoalkaloids, PP - diet with 50% PPC, BM - diet with 50% PPC and 4% or 8% blood meal as attractant, MM - diet with 50% PPC and 4% or 8% mussel meal, AM - diet with 50% PPC and 4% or 8% free amino acid as attractant). Values (mean \pm SD, n=3) with the same superscript are not significantly different ($p < 0.05$)

Blood parameter	Experimental diets									
	FM	GFM	PP	BM4	BM8	MM4	MM8	AM05	AM1	
Hematocrit	33.8 ^{ab} \pm 6.7	28.4 ^a \pm 6.1	25.2 ^a \pm 5.0	29.8 ^a \pm 6.0	30.3 ^a \pm 5.8	28.2 ^b \pm 5.0	29.3 ^a \pm 5.4	24.8 ^{ab} \pm 4.0	25.7 ^b \pm 6.9	
Protein	37.3 ^a \pm 5.2	38.5 ^a \pm 2.8	36.5 ^a \pm 4.4	34.1 ^a \pm 4.0	37.3 ^a \pm 3.3	33.3 ^a \pm 2.4	37.0 ^a \pm 4.8	34.3 ^a \pm 5.8	36.0 ^a \pm 3.4	
Triglyceride	3.43 ^a \pm 0.7	5.47 ^a \pm 1.0	3.45 ^a \pm 1.2	4.52 ^a \pm 2.0	5.74 ^a \pm 2.1	5.69 ^a \pm 3.0	3.75 ^a \pm 1.6	3.97 ^a \pm 1.8	5.16 ^a \pm 1.9	
Glucose	1.38 ^a \pm 0.2	1.47 ^a \pm 0.3	1.48 ^a \pm 0.4	1.51 ^a \pm 0.4	1.64 ^a \pm 1.0	1.20 ^a \pm 0.2	1.31 ^a \pm 0.2	1.44 ^a \pm 0.3	1.22 ^a \pm 0.2	

Discussion

PPC has been used as fishmeal replacement source, but low feed acceptance and subsequent starvation caused a substantial reduction in growth performance as well as histopathological alterations (Tusche et al., 2011). These effects increased not just with increasing replacement of fishmeal, but even more significantly with regard to the glycoalkaloid residues in the PPC, when different qualities (low and high glycoalkaloid content) were used. In the present study, a highly purified PPC with low glycoalkaloid content (7.41 mg kg⁻¹ PPC, resulting in 2.0 mg kg⁻¹ diet) was consequently used as fishmeal substitute. Thus glycoalkaloids have successfully been reduced here with regard to previous studies exploring potato based diets in rainbow trout nutrition with glycoalkaloid contents of $24.2 - 566.1$ mg kg⁻¹ (Xie & Jokumsen, 1997a, b; 1998). Congruently, the reduced DFI and the adverse effects reported in the study by Tusche et al. (2011), revealed strong evidence for severe impact of starvation and malnutrition rather than intoxication by secondary plant

metabolites. It was suggested to compensate the repellent effect of glycoalkaloids by dietary supplementation with attractants.

The feed intake of the GFM was identical to the fish meal control diet (2.42 % BW d⁻¹). With regard to SGR of the GFM group (2.17 % BW d⁻¹) it could be suggested that the glycoalkaloids increased feed conversion and thereby reduced growth performance compared to the control (2.31 % BW d⁻¹).

Comparing the GFM group with the PPC based diets, DFI was reduced in all PPC diets although glycoalkaloid concentrations were set to a comparable amount by the ingredients. Although this reduction was not always significant it can be suggested that the reduced palatability of PPC is not exclusively a result of glycoalkaloid content as GFM did not reveal a reduced DFI. Nevertheless, fish meal itself may act as strong feed attractant compensating for the anti-palatability effect of glycoalkaloids in the GFM. In general, L-amino acids with short, unbranched and uncharged aliphatic chains are among the most effective stimuli of the gustatory system in salmonids (Hara et al., 1994). Furthermore, salmonids are among the species that only respond to a few AA, e.g proline, alanine and betaine (Marui et al., 1983; Hara, 2006; Yamashita et al., 2006). Among the salmonids rainbow trout exhibits a high number of taste buds when compared to other species such as Sockeye salmon (*Oncorhynchus nerka*), Lake whitefish (*Coregonus clupeaformis*) and others, indicating the importance of gustatory sensation (Hara et al., 1994). Furthermore, for fish meal can be mentioned that it contains high amounts of free alanine (50 mg kg⁻¹) and leucine (52 mg kg⁻¹) (Li et al., 2011). The lower limits of perception for these AA in rainbow trout are between 10⁻⁶ to 10⁻⁷ mol L⁻¹, which demonstrate that only smallest amounts of these free AA in the water and/or on the feed particle surface were able to innervate taste bud reactions (Hara et al., 1994).

In this study, highest DFI in PPC based diets was observed by inclusion of 4 % or 8 % blood meal (DFI of 2.04 % BW d⁻¹ and 1.95 % BW d⁻¹, respectively). Kikuchi (1999) reported similar appetizing effects in juvenile Japanese flounder (*Paralichthys olivaceus*) incorporating 10 % blood meal in soybean meal based diets. Upon supplementation, the DFI increased from 4.38 % BW d⁻¹ in the FM control to 4.44 % BW d⁻¹ in the BM diet. Li et al. (2011) reported that blood meal contained high concentrations of free AA e.g. alanine and proline at 78 and 114 mg kg⁻¹. Similar to the observations in presented investigation, Millamena (2002) reported highest DFI (3.1% BW d⁻¹ and 3.04 % BW d⁻¹) when blood meal was used (control 2.95% BW d⁻¹) at 6 % and 8 % in grouper nutrition. But in contrast to the

highest feed intake among the PPC groups, BM exhibited the worst feed conversion ratios (1.15 BM4; 1.09 BM8). Therefore it must be mentioned that blood meals and other animal protein ingredients in feeds underlay varying qualities resulting in e.g. varying apparent digestibilities. Bureau et al. (1999) reported apparent digestibility coefficients between 80-99 % for BM in different trials with rainbow trout. The industrial processing conditions and the amino acid profile were responsible for high variation. In this way it could be supposed, that the BM used in the present trial was of a reduced nutritional quality. The decreased PER (2.00 BM4 and 2.09 BM8) in both BM groups could be indicative for this fact. All the other feeding groups exhibited higher PER.

Nevertheless, it must be mentioned that BM as feed ingredient exhibited an excellent binding quality in the diets and had in this way an unexpected positive side effect also on the faeces consisting and water quality by reduced abrasions.

MM as feed attractant was ineffective as DFI could not be increased (1.75 % BW d⁻¹ and 1.64 % BW d⁻¹, respectively) compared to the PP group (1.69 % BW d⁻¹) in the present study. These results were rather surprising and in clear contrast to previous studies. Kikuchi (1999) reported that the inclusion of 5 % mussel meat in a soybean meal (300 g kg⁻¹) based diet resulted in a significantly increased feed intake (5.16 % BW d⁻¹) compared to control (4.38 % BW d⁻¹) in juvenile Japanese flounder. Similarly, Kikuchi and Furuta (2009b) reported an effect of MM as attractant in tiger puffer (*Takifugu rubripes*). In this study it was concluded, that a high availability of alanine and glycine increased DFI. These two AA and additionally the non-amino acid betaine were also identified by Mackie et al. (1980) as main feed attractants in the mussel meat which acted as stimulant in diets for Dover sole (*Solea solea*). The AA composition of mussel meal diets showed low amounts of related AA and a stimulating effect of L-AA via olfaction and/or gustation should be excluded. There was no advancement on DFI of mussel meal diets in contrast to PPC diets. Additionally the FCR (1.05 MM4; 1.06 MM8) was slightly increased and resulted in an overall reduced growth of the individuals in the mussel meal groups. This slight restricted feed utilization and thereby nutrient supply was confirmed by the reduced amount of fat deposits in the whole body composition of these experimental groups. A possible explanation for that could be the increased amount of ash in the MM diets. Concluding should be stated that the use of green lip mussel meal had no stimulating effect on feed intake and growth in PPC based rainbow trout feed. Unfortunately, the reasons for that were hardly understood. In further investigations should be proved if varying MM sources and qualities which acts as feed

attractants in plant protein based diets resulting in comparable or better data. Background for such an investigation is the lack of possible certified raw materials in organic aquaculture. May mussel meal can act as attractant, if the problems solved in the future.

The mixture of betaine, alanine and glycine (AM1) resulted in slight higher feed intake ($1.87 \% \text{ BW d}^{-1}$) than in the PP group. At this inclusion level a positive influence was recorded but without significant differences to the PP group. As mentioned before, the stimulating effect of free betaine and L-AA in fish nutrition was discussed by several authors. Johnstone and Mackie (1990) reported from investigations with bait preferences of Atlantic cod (*Gadus morhua*) and found that an inclusion of betaine as single stimulant in a diet had no increasing effect on feed intake. On the other hand, betaine enhanced the stimulating effect of L-amino acids. These findings were confirmed by Franco et al. (1991) in similar experiment with Atlantic cod. A synergistic action between betaine and neutral L-AA was recorded. Betaine, alanine and glycine had a positive influence on palatability in diets for rainbow trout (Jones, 1989; Yamashita et al., 2006), striped bass (*Morone saxatilis*) (Papatriphon and Soares, 2000; 2001) and European sea bass (*Dicentrarchus labrax*) (Dias et al., 1997). The results of the AM1 group in the own investigation were in consensus with earlier investigations. As mentioned in the blood meal groups, the data indicated that rainbow trout were able to detect the included L-AA when added with 1% in the AM diet and became stimulated in form of increased feed intake. In addition, it is possible that parts of the attractant mix were used in or for metabolic and energy producing processes (Papatriphon and Soares, 2001). This process could result in better FCR and PER as recorded in the free AA groups. But especially with regard to FCR and PER, it must be noted that the lowered feed intake in contrast to the fish meal group was compensated by better feed utilization in fish fed PP, AM05 and AM1 diets. The most potent attractant (1% free AA) which was identified in the present study should thus be explored in future studies.

With a view on the nutrient composition of the several diets it should be excluded that there was a nutrient dependent effect on feed intake in the present study e.g. deficient AA. The amounts of essential AA (Table 2.2) were in accordance or above required levels for rainbow trout (Rodehutschord et al., 1997). Nevertheless, there is no information about the negative influence of antinutritional factors present in PPC on amino acids digestibility as known from studies with other plant protein sources (Francis et al., 2001).

The hematological characteristics of the feeding groups in the present study were statistically similar to each other. Compared with the parameter for juvenile salmonids (Congleton and Wagner, 2006) it can be assumed that the evaluated individuals showed a good nutritional status and condition factor. The data display that all the provided feeds were sufficient to cover the nutritional demand of the fish. This was necessary to maintain growth and avoid malnutrition as found in former investigations (Tusche et al., 2011).

As conclusion should be stated, all PPC groups exhibited reduced feed intake and growth when compared with the FM group. Furthermore, all PPC groups exhibited lower growth when compared to the GFM group fed at comparable glycoalkaloid concentrations suggesting on the one hand antinutritional effects apart from glycoalkaloids or on the other hand, still present antinutritional effects by glycoalkaloids were compensated by the strong feed attractant action of fish meal. Nevertheless, PER was higher than 2 in all groups indicating acceptable protein utilization in PPC groups, further supported by FCR ranging between 0.92-1.15. Highest daily feed intake (DFI) was observed in the FM and GFM group, but reduced in all PPC groups. The effect of highest fish meal amounts on the palatability of a diet was more intensive than any used feed attractants. Among the attractants used, BM and AM resulted in the highest DFI. A good nutritional status did not reveal severe health impact of PPC in general.

In further studies combinations of feed additives and attractants (8% BM as binding agent plus 1% free AA as attractant agent) should be evaluated in order to achieve comparable performance data as generated with fish meal based diets. In addition general processing quality of PPC must be taken into account to improve its binding characteristic and palatability aspects.

References

- Berge, G., Austreng, E., 1989. Blue mussel in feed for rainbow trout. *Aquaculture* 81 (1), 79–90.
- Bureau, D., Harris, A., Cho, C., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 180 (3-4), 345–358.
- Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.* 69 (2), 473–490.

- Dias, J., Gomes, E., Kaushik, S., 1997. Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquat. Liv. Res.* 10 (6), 385–389.
- European Union, 2009a. Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Europ. Union*, 52, 1–130.
- European Union, 2009b. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union*, 52, 15–34.
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Franco, J., Johnstone, A., Mackie, A., 1991. Studies of bait preference in the cod, *Gadus morhua* L.: characterisation of feeding stimulants using an operant conditioning technique. *Fish. Res.* 10, 229–242.
- Friedman, M., 2006. Potato Glycoalkaloids and Metabolites: Roles in the Plant and in the Diet. *J. Agric. Food Chem* 54 (23), 8655–8681.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- Hara, T.J., 2006. Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *J Fish Biology* 68 (3), 810–825.
- Hara, T., Kitada, Y., Evans, R., 1994. Distribution patterns of palatal taste buds and their responses to amino acids in salmonids. *Journal of Fish Biology* 45 (3), 453–465.
- Jeroch, H., Drochner, W., Simon, O., 1999. Ernährung landwirtschaftlicher Nutztiere: Ernährungsphysiologie, Futtermittelkunde, Fütterung. Ulmer, Stuttgart, 544 S.
- Johnstone, A., Mackie, A., 1990. Laboratory investigations of bait acceptance by the cod, *Gadus morhua* L.: identification of feeding stimulants. *Fish. Res.* 9, 219–230.
- Jones, K.A., 1989. The palatability of amino acids and related compounds to rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biology* 34 (1), 149–160.
- Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179 (1-4), 3–11.

- Kikuchi, K., Furuta, T., 2009a. Use of Defatted Soybean Meal and Blue Mussel Meat as Substitute for Fish Meal in the Diet of Tiger Puffer, *Takifugu rubripes*. *Journal of the World Aquaculture Society* 40 (4), 472–482.
- Kikuchi, K., Furuta, T., 2009b. Inclusion of blue mussel extract in diets based on fish and soybean meals for tiger puffer *Takifugu rubripes*. *Fish Sci* 75 (1), 183–189.
- Kikuchi, K., Ueda, A., Sugita, H., Takeda, S., 2002. Effect of dietary inclusion of blue mussel extract on growth and body composition of Japanese flounder *Paralichthys olivaceus*. *J. World Aquacult. Soc.* 33 (1), 41–47.
- Li, X., Rezaei, R., Li, P., Wu, G., 2011. Composition of amino acids in feed ingredients for animal diets. *Amino Acids* 40 (4), 1159–1168.
- Mackie, A., Adron, J., Grant, P., 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). *J. Fish Biol.* 16.
- Marui, T., Evans, R.E., Zielinski, B., Hara, T.J., 1983. Gustatory responses of the rainbow trout (*Salmo gairdneri*) palate to amino acids and derivatives. *J. Comp. Physiol* 153 (4), 423–433.
- McCarter, N.H., 1986. Food and energy in the diet of brown and rainbow trout from Lake Benmore, New Zealand. *New Zealand J. of Marine & Freshwater Res* 20 (4), 551–559.
- Millamena, O., 2002. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. *Aquaculture* 204 (1-2), 75–84.
- NRC (National Research Council), 1993. Nutrient Requirements of Fish. *National Academy Press*, Washington, DC, USA, 117p.
- Papatryphon, E., Soares, J., 2001. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, *Morone saxatilis*. *Aquaculture* 202 (3-4), 279–288.
- Papatryphon, E., Soares, J., 2000. The effect of dietary feeding stimulants on growth performance of striped bass, *Morone saxatilis*, fed-a-plant feedstuff-based diet. *Aquaculture* 185 (3-4), 329–338.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Rodehutsord, M., Becker, A., Pack, M., Pfeffer, E., 1997. Response of Rainbow Trout (*Oncorhynchus mykiss*) to Supplements of Individual Essential Amino Acids in a Semipurified Diet, Including an Estimate of the Maintenance Requirement for Essential Amino Acids. *J. Nutr.* (126), 1166–1175.

- Strolle, E., Cording, J., Aceto, N., 1973. Recovering potato proteins coagulated by steam injection heating. *J. Agric. Food Chem* 21 (6), 974–977.
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London, Orlando, pp. 119–145.
- Tusche, K., Wuertz, S., Susenbeth, A., Schulz, C., 2011. Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 319 (1-4), 122-131.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.
- Xue, M., Cui, Y., 2001. Effect of several feeding stimulants on diet preference by juvenile gibel carp (*Carassius auratus gibelio*), fed diets with or without partial replacement of fish meal by meat and bone meal. *Aquaculture* 198 (3-4), 281–292.
- Yamashita, S., Yamada, T., Hara, T.J., 2006. Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *J Fish Biology* 68 (3), 783–800.

Chapter 3:

Wheat gluten and potato protein concentrate – Promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

In contrast to other plant proteins, wheat gluten does not require complex and costly purification and may thus be utilized in aquaculture diets according to aquaculture guideline EC 710/2009. Therefore, a feeding trial was conducted to evaluate the effect of various combinations of wheat gluten (WG) and potato protein concentrate (PPC) in nutrition of rainbow trout (*Oncorhynchus mykiss*). Seven isonitrogenous (459 ± 6 g CP kg⁻¹) and isoenergetic (21.4 ± 0.1 MJ kg⁻¹) diets were formulated to contain varying levels of wheat gluten and potato protein concentrate (6/27; 9/24; 11/21; 14/19; 17/16; 19/13 as proportion of WG/PPC in % of the diet) at constant fishmeal replacement level of 56% on protein level and one fish meal diet served as control. Experimental diets were fed over a period of 56 days to triplicate experimental groups until apparent satiation.

Growth and growth performance showed no significant differences between all feeding groups at the end of the experimental period. No influences on health and nutritional status were documented between the treatments in the body composition and blood parameter. Any inclusion level of wheat gluten and PPC as fish meal replacement in organic diets for rainbow trout revealed positive results for use.

Introduction

The bottleneck of organic aquaculture development of carnivorous fish species is a sufficient supply of certified, inexpensive raw materials for diet formulation (Bergleiter, 2009). Although feeding in organic aquaculture has to be species-appropriate as dictated by most certifying organization as well as the European regulation on organic aquaculture (European Union, 2009b) enforcing the use of certified fish meal and fish (IFOAM, 2010), supply with these protein and fat sources in fact are limited and organic aquaculture standards are impossible to realize nowadays (Mente et al., 2011). The possible back door for a continuous supply with high quality nutrients relies unequivocally on the use of vegetable raw materials. Nevertheless, fish meal and fish oil replacement is limited by antinutritional substances (e.g. glycoalkaloids) such as repellents (Tusche et al., 2011b) and/or inadequate amino acid (AA) compositions (Francis et al., 2001; Glencross et al., 2006; Xie and Jokumsen, 1997b). Still, processed plant proteins, partial replacement and/or supplementation with essential substances (e.g. free AA, phosphorous) resulted in comparable feed acceptance and utilization at a comparable growth performance (Refstie and Tiekstra, 2003; Slawski et al., 2011; Tusche et al., 2011a), but has to be established for each single species.

The variety of potential raw materials is limited by 3 obligatory requirements. 1) Legislation as well as certifying associations dictated the use of raw material that has been approved for use in organic diets (legislative level). 2) Raw material or combinations of various has to meet the species-specific nutritional requirements without supplementation of any synthetics (biology/physiology level). 3) The raw material obviously has to be available at an economically feasible price (economic level). Consequently, potential feed ingredients are evaluated regarding these three criteria before commercial use in organic aquaculture, turning feed formulation in organic farming less flexible than regular aqua feed production, where ingredients may be shifted substantially.

Potato protein concentrate (PPC) was evaluated as a potential plant protein for the use in aquaculture diets. In diets for Atlantic salmon it has been demonstrated that fishmeal can be replaced by PPC (38% of the fish meal protein) without impact on growth performance or animal health (Refstie and Tiekstra, 2003). Tusche et al. (2011b) documented appropriate feed intake and growth performance with PPC diets (inclusion level at 56% of the protein in the diet) in rainbow trout. The future fish meal supplementation with this protein source should be held at this consistently high level. The raw material PPC is produced and certified organic by thermal coagulation (ANNEX V, European Union, 2009b), revealing a high protein

content ($>800 \text{ g kg}^{-1}$) with a well-balanced AA profile (Tacon and Jackson, 1985), although methionine was identified as potentially limited (Xie and Jokumsen, 1998). Still, all solanaceous plants including potato produce glycoalkaloids (e.g. solanine and chaconine) that function as pest repellents and exhibit antinutritional effects in fish (Jeroch et al., 1999). Glycoalkaloids typically elicit a bitter taste and result in a reduced palatability and feed intake upon feeding (Xie and Jokumsen, 1997b; Tusche et al., 2011b). A technical purification of the PPC by downstream processing with membrane absorbers reduces the amount of glycoalkaloids from more than $2,000 \text{ mg kg}^{-1}$ to $<50 \text{ mg kg}^{-1}$ PPC, thereby enhancing the feed quality and acceptance, which is detrimental for increased supplementation in organic aquaculture diets (Refstie and Tiekstra, 2003; Tusche et al., 2011b). Particularly obstructive, in comparison to other plant protein sources, PPC purification is costly and nowadays produced in low quantities only.

A possible alternative protein source could be found in wheat gluten. This plant protein is characterized by high market availability and lower prices in contrast to PPC (price list, commercial wheat gluten producer). Furthermore, this raw material is accepted as feed material of plant origin in organic aquaculture diets (Mente et al., 2011; European Union, 2009b). On the other hand, the nutritional quality of this protein source displays some aspects for a limited potential for use in organic diets.

Although wheat gluten displayed digestibility coefficients of more than 99% in diets for Atlantic salmon and Atlantic cod (Storebakken et al., 2000; Tibbetts et al., 2006) the AA profile reveal low lysine and lysine deficiency may occur with regard to the actual demand of rainbow trout (NRC, 1993). Consequently, a supplementation with L-lysine enhances the nutritional quality of WG when incorporated as solely protein source in fish diets (Pfeffer et al., 1992; Davies et al., 1997), but excludes an application in organic farming which is prohibited by the legislation (European Union, 2009b; IFOAM, 2010).

The main focus of this investigation was to evaluate the effect of varying levels of wheat gluten and potato protein concentrate combinations at a constant fish meal replacement in diets for rainbow trout (*Oncorhynchus mykiss*) on feed consumption and growth performance. In accordance to the organic aquaculture guideline EC 710/2009 no additional free AA and/or other non-acceptable materials were added to tailor the diets for nutritional demand of the specie.

Materials and Methods

Experimental diets

Seven isonitrogenous and isoenergetic diets were formulated as documented in Tab. 3.1 and 3.2. One diet contained low temperature fish meal (VFCUX, Cuxhaven, Germany) as main protein source served as control diet. Six diets were formulated by a fixed replacement of fish meal at a level of 56% by a supplementing mixture of potato protein concentrate (PPC with low glycoalkaloid content: 23.2 mg kg⁻¹ dry weight; K5, Emsland Group, Emlichheim, Germany) and wheat gluten (Cargill Deutschland GmbH; Krefeld, Germany). The partial replacement of PPC by wheat gluten was approximately at 0%, 10%, 20%, 30%, 40% and 50%. The amount of wheat gluten in the diet was limited by the amount of essential AA in the raw material. In order to meet the council regulation (EC) No. 710/2009 no free AA were added to the diet. An initial wheat gluten amount of 6% was necessary to realise binding processes in the preparation of all experimental diets including the control diet. The plant protein based diets were named by the amount of included WG (W6 – W19) in the diet.

The nutrient formulation of the several diets was tailored to suit the metabolic demand of the individuals. Especially the amount of essential AA and phosphorous laid above the dietary requirement for salmonids.

All ingredients were pelletized with a fodder press L 14-175 (AMANDUS KAHL, Reinbek, Germany) to pellets of 4 mm diameter. Due to the high protein contents of PPC and wheat gluten, magnesia was used as inert filler in the diets to realise equal nutrient contents in the diets (ANNEX V, European Union, 2009b).

Tab. 3.1: Nutrient composition, amino acid concentration, antinutritional phytic acid and glycoalkaloid concentration of the investigated protein sources utilized in the experimental diets in g kg⁻¹ dry matter (Tab. 3.2).

	diet ingredients		
	fish meal	LG-PPC	Wheat gluten
<i>Nutrient composition (g kg⁻¹)</i>			
Dry matter	918	955	909
Crude protein	689	859	857
Crude lipid	69	32	63
Crude fibre	5	0.5	1.3
NfE ¹	31	24	5.9
Crude ash	206	85	73
Phosphorus	25	22	25
<i>Essential amino acids (g kg⁻¹)</i>			
Arginine	37.0	43.2	40.1
Histidine	12.7	19.1	17.3
Isoleucine	23.0	47.8	44.2
Leucine	40.9	90.5	82.2
Lysin	41.5	71.2	64.0
Methionine + Cysteine	20.1	32.9	30.2
Phenylalanine	22.3	57.8	52.6
Threonine	24.7	52.6	47.1
Valine	28.2	57.0	52.2
<i>Glycoalkaloids² (mg kg⁻¹)</i>			
Solanin		9.19	
Chaconin		14.00	
<i>Phytic acid (g kg⁻¹)</i>		<0.1	2.1

¹ NfE, Nitrogen-free extract = 1000 – (Crude protein + Crude lipid + Ash + Crude fibre)

² analyzed at LUFA-ITL, Kiel, Germany, according to LC-MS

Tab. 3.2: Ingredients, amino acid concentration, antinutritional phytic acid and glycoalkaloid concentration of the protein sources utilized in the experimental diets in g kg⁻¹ dry matter.

	Diets						
	FM	W6	W9	W11	W14	W17	W19
<i>Ingredients (g kg⁻¹)</i>							
fish meal ¹	606	265	265	265	265	265	265
LG-PPC ²	-	267	241	214	187	160	134
fish oil ¹	61	91	90	90	89	89	88
wheat starch ³	264	240	241	241	241	242	242
wheat gluten ³	60	60	87	114	141	167	194
vitamin ⁴ + mineral mixture ⁵	10	10	10	10	10	10	10
magnesia ⁶	-	66	66	66	67	67	67
<i>Nutrient composition (g kg⁻¹ DM)</i>							
Dry matter	923	932	932	932	931	932	927
Crude protein	457	461	464	462	460	462	445
Crude lipid	146	142	142	146	144	145	145
Crude ash	127	132	131	132	131	134	130
Phosphorus	14.1	12.6	12.6	12.7	12.7	12.8	12.8
NfE + crude fibre	270	265	263	260	265	260	280
Gross energy (MJ kg ⁻¹ DM)	21.5	21.3	21.4	21.4	21.4	21.4	21.3
<i>Essential amino acids (g kg⁻¹)</i>							
Arginine	24.0	22.9	22.5	22.1	21.6	21.2	20.7
Histidine	8.6	9.4	9.3	9.2	9.2	9.1	9.0
Isoleucine	15.5	20.5	19.9	19.4	18.8	18.3	17.7
Leucine	28.0	38.2	37.2	36.2	35.1	34.3	33.3
Lysin	25.9	30.8	29.3	27.7	26.1	24.6	23.0
Methionine + Cysteine	13.8	15.8	15.6	15.5	15.4	15.2	15.1
Phenylalanine	15.9	23.7	23.2	22.7	22.2	21.7	21.2
Threonine	16.1	21.8	20.9	20.0	19.1	18.2	17.4
Valine	18.9	24.5	23.8	23.1	22.4	21.7	20.9
<i>Glycoalkaloids (mg kg⁻¹)</i>							
Solanine	0.0	2.5	2.2	2.0	1.7	1.5	1.2
Chaconine	0.0	3.7	3.4	3.0	2.6	2.2	1.9

¹VFCUX, Cuxhaven, Germany.

²Emsland-Stärke GmbH, Emlichheim, Germany.

³Cargill Deutschland GmbH; Krefeld, Germany.

⁴Vitamin-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: vitamin A, 1,000,000 IU kg⁻¹; vitamin D3, 200,000 IU kg⁻¹; vitamin E (as α -tocopherol-acetate), 40,000; vitamin K3 (as menadione), 4,000; vitamin B1, 4,000; vitamin B2, 8,000; vitamin B6, 4,000; vitamin B12, 8; vitamin C (as monophosphate), 60,000; pantothenic acid, 8,000; nicotinic acid, 40,000; folic acid, 1,600; biotin, 100; inositol 40,000.

⁵Mineral-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: cobalt (as cobaltsulphate), 400; manganese (as manganese sulphate), 2,500; iodine (as calciumiodine), 500; copper (as coppersulphate), 2,500; selenium, 25; zinc (as zincsulfate), 28,000.

⁶Magnesia 4371, Magnesia GmbH, Lüneburg, Germany.

Tryptophane was not analysed.

Experimental setup

The feeding trial was conducted at the experimental facilities of the Gesellschaft für Marine Aquakultur (GMA, Büsum, Germany). Prior to the experiment, 420 rainbow trout (Forellenzucht Troststadt GbR, Troststadt, Germany) were randomly distributed to 21 tanks (175 l each), resulting in 7 triplicate feeding groups of 20 fish (52.2 ± 0.1 g) each, and acclimatised for 14 d in the experimental system. The tanks were arranged in a closed recirculation system (4.5 m³ total water volume, water turnover rate 6 h⁻¹, daily water exchange 1.700 l kg⁻¹ feed) and photoperiod was adjusted to 12 h light : 12 h dark. Water parameters were determined daily ($13.4 \pm 0.5^\circ\text{C}$, 9.6 ± 0.3 mg l⁻¹ O₂; Handy Polaris; OxyGuard International A/S, Birkerød, Denmark; 0.25 ± 0.2 mg l⁻¹ NH₄-N, 0.3 ± 0.2 mg l⁻¹ NO₂-N; Microquant test kit for NH₄ and NO₂; Merck KGaA, Darmstadt, Germany). During acclimatisation fish were fed a commercial trout feed (Performa 45/20, 2mm; AllerAqua A/S, Christiansfeld, Denmark). Over the entire experimental period of 8 weeks, fish were fed to apparent satiation twice per day at 9.00 a.m. and 5.00 p.m.

Sampling

Prior to the start of the experiment, 10 fish were sampled to assess whole body composition and stored at -20°C until analysed. At the end of the experiment, final biomass of each tank was recorded, 3 fish (bulk sample) of each tank were sampled for the determination of whole body composition and stored at -20°C. Three additional individuals per tank were utilized to draw blood samples from the caudal vein for the determination of blood parameters. In addition, fish weight and length as well as liver weight were recorded. For each treatment, mortalities and growth parameters were calculated as average of the triplicates, as well as specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and Fulton condition factor (FCF), according to the formulas in Tab. 3.3.

Nutritional composition of experimental diets and fish

For the analysis of whole body composition, 3 fish per tank were freeze-dried for 120 h, homogenized and stored at -20°C, experimental diets were homogenized and stored at -20°C. Analysis for dry matter (DM), ash, crude protein, crude fat and crude fiber was performed according to EU guideline (EC) 152/2009 (European Union, 2009a). Briefly, crude protein content (N x 6.25) was determined by the Kjeldahl method (InKjel 1225 M, WD 30; Behr, Düsseldorf, Germany), crude fat content after hydrolysis with hydrochloric acid followed by a

petroleum ether extraction with a Soxhlet extraction system (R 106 S; Behr), DM after drying at 103°C until constant weight remained stable and ash content after 4 h incineration at 550°C with a combustion oven (P300; Nabertherm, Lilienthal, Germany). Gross energy was measured in a bomb calorimeter (C 200; IKA, Staufen, Germany).

Blood parameters

Upon blood sampling with a heparinised syringe, the hematocrit value was determined in micro-hematocrit-tubes (Type 563, NA-heparinized, 75 mm/135 µm; Assistent, Sondheim, Germany) upon centrifugation (10,000 g, 5 min) with a hematocrit 210 centrifuge (Hettich, Tuttlingen, Germany) using a hematocrit tube card reader. Remaining blood was centrifuged (1,000 g, 5 min) and plasma was separated and stored at -80°C. Glucose and triglycerides were determined by the GPO-PAP method using an enzymatic colorimetric kit (Greiner, Frickenhausen, Germany). Plasma protein was quantified according to Bradford with a Roti-Quant kit and a BSA standard dilution series. All colorimetric assays were measured in duplicate with a microplate reader Infinite 200 (Tecan, Männedorf, Switzerland) and calculated from a standard dilution series to compensate for run-to-run variation. Recoveries were determined by spike experiments and were >90% for all plasma parameters analysed.

Statistical Analyses

The statistical analysis was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, U.S.). Data are presented as mean ± standard deviation (SD) for each treatment and were compared between treatments. Data were analysed for normal distribution by Kolmogorov-Smirnov and equal variance by Kruskal-Wallis One Way Analysis of Variance (confirmed if $p < 0.05$). Post-hoc multiple comparison was carried out by parametric Tukey-HSD (if test for homogeneity was confirmed) ($p < 0.05$) or non-parametric Dunnett-T3 test (if test for homogeneity failed).

Results

Growth

Data of growth and growth performance showed that all experimental groups were not significant different to each other at the end of the experiment (table 3.3). No mortalities were recorded and all groups more than doubled their weight during the experimental time span.

The W17 group (137.3 ± 6.9 g) exhibited the highest final body weight and thereby the highest SGR 1.73 ± 0.1 % BW d⁻¹. The growth performance of all other groups was slightly decreased to these results but without significant differences and/or a clear tendency. Although an increasing amount of wheat gluten primary resulted in slightly decreasing SGR (from 1.68 ± 0.1 % BW d⁻¹ in W6 to 1.53 ± 0.2 % BW d⁻¹ in W14), but at higher WG inclusion in W17 or W19 this tendency developed reversely. Same unspecific variations were documented in the daily feed intake (DFI).

The fish meal control revealed the lowest FCR (1.16 ± 0.1). In all plant protein-based diets was the FCR slightly enhanced, although the treatments differed not significantly from the control diet. The highest FCR could be observed in the groups W9 and W14 (1.32). The PER and PPV of all groups correlated with the calculated FCR. In addition, it was recognized that the control diet showed the highest PPV and PER value (31.89 ± 4.1 , 2.05 ± 2.0). The replacement of fish meal by plant protein mixture resulted in moderately elevated PPV and PER and correlated with the SGR. The hepatosomatic index (HSI) ranged between 1.40 and 1.60 without significant differences between all groups. Furthermore, the Fulton condition factor (FCF) of all groups ranged between 1.14 and 1.21 and was without indication for statistical significance.

Tab. 3.3: Growth performance (mean \pm SD, n=3) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Values with the same superscript are not significantly different ($p < 0.05$).

Variable	Experimental diets											
	FM	W6	W9	W11	W14	W17	W19					
IBW ¹	52.2 \pm 0.1	52.2 \pm 0.1	52.1 \pm 0.1	52.2 \pm 0.1	52.1 \pm 0.1	52.1 \pm 0.0	52.2 \pm 0.0					
FBW ²	135.6 \pm 9.1	134.2 \pm 5.9	124.9 \pm 13.1	125.7 \pm 10.4	123.3 \pm 10.1	137.3 \pm 6.9	131.8 \pm 9.8					
DFI ³	1.97 \pm 0.0	2.12 \pm 0.1	2.05 \pm 0.1	1.99 \pm 0.2	2.01 \pm 0.1	2.24 \pm 0.1	2.15 \pm 0.1					
SGR ⁴	1.70 \pm 0.1	1.68 \pm 0.1	1.56 \pm 0.2	1.57 \pm 0.1	1.53 \pm 0.2	1.73 \pm 0.1	1.66 \pm 0.1					
FCR ⁵	1.16 \pm 0.1	1.26 \pm 0.1	1.32 \pm 0.1	1.27 \pm 0.1	1.32 \pm 0.2	1.30 \pm 0.1	1.29 \pm 0.1					
PER ⁶	2.05 \pm 0.2	1.85 \pm 0.1	1.75 \pm 0.2	1.84 \pm 0.1	1.78 \pm 0.2	1.79 \pm 0.1	1.87 \pm 0.1					
PPV ⁷	31.89 \pm 4.1	29.41 \pm 1.6	29.27 \pm 2.1	27.33 \pm 1.9	27.18 \pm 3.3	28.28 \pm 2.7	29.99 \pm 0.6					
HSI ⁸	1.60 \pm 0.2	1.54 \pm 0.1	1.40 \pm 0.2	1.46 \pm 0.3	1.52 \pm 0.3	1.48 \pm 0.2	1.52 \pm 0.3					
FCF ⁹	1.20 \pm 0.1	1.18 \pm 0.1	1.14 \pm 0.1	1.21 \pm 0.1	1.14 \pm 0.0	1.25 \pm 0.1	1.17 \pm 0.1					

¹initial body weight (g); ²final body weight (g); ³daily feed intake (% BW day⁻¹); ⁴specific growth rate (% BW day) = [ln (FBW) - ln (IBW)] / feeding days x 100; ⁵feed conversion ratio = feed intake (g) / weight gain (g); ⁶protein efficiency ratio = weight gain (g) / protein intake (g); ⁷protein productive value = 100 x [(crude protein final fish x biomass final fish) - (crude protein initial fish x biomass initial fish)] / (crude protein diet x total feed intake); ⁸hepatosomatic index = (liver weight / final body weight) x 100, ⁹Fulton condition factor = 100 x final body weight x final body length⁻³.

Body composition

The inclusion of varying amounts of fish meal or plant proteins in the diet had no significant effect on the body composition of all groups (Table 3.4). The dry matter (DM) of all groups was approximately at 288 g kg⁻¹ and highest in the W6 group (294 ± 6.2 g kg⁻¹). No effect on body crude protein content was detected compared between all groups. The highest crude protein content was met in W9 (164 ± 1.4 g kg⁻¹) whereas the highest crude lipid contents were found in W6 (106 ± 9.0 g kg⁻¹) and W11 (106 ± 12.0 g kg⁻¹). Furthermore, the amount of crude ash was approximately 27.9 g kg⁻¹ without statistical indication between treatments as well. Comparable results were recorded in the amounts of gross energy of the different groups. This parameter correlates with the amount of crude fat in the whole body composition.

Tab. 3.4: Body composition (g kg⁻¹ wet weight; MJ kg⁻¹ DM) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Initial body composition was expressed on a wet weight basis as dry matter 264 g kg⁻¹; ash 24 g kg⁻¹; crude protein 163 g kg⁻¹; crude lipid 86 g kg⁻¹; energy 27.1 MJ kg⁻¹. Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05)

Nutrient	Experimental diets							
	FM	W6	W9	W11	W14	W17	W19	
Dry matter	287 ± 9.1	294 ± 6.2	287 ± 15.2	286 ± 13.6	283 ± 2.3	291 ± 1.4	291 ± 5.4	
Crude protein	157 ± 6.9	159 ± 1.7	164 ± 1.4	154 ± 9.3	155 ± 1.6	158 ± 5.2	160 ± 6.9	
Crude lipid	103 ± 5.5	106 ± 9.0	96 ± 15.4	106 ± 12.0	102 ± 4.2	103 ± 3.4	103 ± 3.2	
Crude ash	28 ± 4.6	29 ± 4.0	28 ± 2.3	26 ± 3.6	26 ± 3.3	30 ± 1.0	28 ± 1.4	
Gross energy	27.3 ± 0.7	27.3 ± 0.7	26.8 ± 0.9	27.6 ± 0.8	27.4 ± 0.5	27.1 ± 0.2	27.2 ± 0.1	

Blood plasma composition

The measured blood parameters of all groups revealed moderate differences, but due to the high individual variances within the groups no significant differences were detected (Table 3.5). The highest hematocrit value was measured in the W17 group (34.1 ± 4.4%). The other groups revealed only minor differences (30.2 – 34.1%). Same tendencies were observed in the other physiological blood parameters, either in plasma protein (33.14 – 37.24 mg ml⁻¹), triglycerides (2.36 – 4.20 mg ml⁻¹) or glucose (0.71 – 1.01 mg ml⁻¹).

Tab. 3.5: Blood parameter (hematocrit in %; protein, triglyceride and glucose in mg ml⁻¹) of rainbow trout fed the experimental diets (FM – fishmeal control, PPC groups – supplementation of potato protein concentrate with wheat gluten at fixed fish meal exchange of 56 % in the diet). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05)

Blood parameter	Experimental diets							
	FM	W6	W9	W11	W14	W17	W19	
Hematocrit	30.8 ± 2.6	33.1 ± 3.3	33.0 ± 4.6	30.2 ± 2.8	33.5 ± 5.5	34.1 ± 5.0	31.8 ± 4.4	
Protein	35.95 ± 4.3	33.14 ± 3.3	34.27 ± 2.3	34.47 ± 2.9	33.19 ± 3.5	37.24 ± 4.4	36.66 ± 2.6	
Triglyceride	2.36 ± 1.1	2.90 ± 1.2	3.21 ± 1.4	3.41 ± 0.9	4.20 ± 2.6	2.83 ± 1.0	3.19 ± 1.5	
Glucose	1.00 ± 0.5	0.84 ± 0.2	0.76 ± 0.1	0.71 ± 0.0	1.01 ± 0.3	0.73 ± 0.1	0.78 ± 0.1	

Discussion

Present study demonstrated that no significant performance differences occurred with various combinations of WG and PPC at a fixed fish meal substitution level of 56 % in rainbow trout nutrition. Especially when compared with the fish meal control no reduced growth performance was documented at the different plant protein inclusion levels. This is in contrast to former investigations, especially when PPC served as single plant protein source (Tusche et al., 2011a, b). Additionally, the fish of all groups more than doubled their weight during the experimental period and no mortality occurred in this time. This could be used as first indicators for an adequate supply with nutrients and living conditions for the test organisms.

Although the use of PPC or WG as fish meal replacement was successfully demonstrated in diets for rainbow trout (Rodehutsord et al., 2000; Storebakken et al., 2000; Refstie and Tiekstra, 2003; Tusche et al., 2011b), it was shown that both ingredients exhibited poor results when used as single raw material in high or total supplementation amounts (e.g. reduced palatability or insufficient nutrient supply) (Xie and Jokumsen, 1997b; Helland and Grisdale-Helland, 2006; Tusche et al., 2011a). Reasons for that were found in a reduced feed intake by reduced palatability of PPC diets caused by glycoalkaloids, their secondary metabolites and further antinutritional substances from solanaceous plants (Xie and Jokumsen, 1997a). A technical reduction of these plant metabolites (<50 mg kg⁻¹ raw material) resulted in enhanced feed intake (in comparison with feed intake of fish meal based control) and thereby growth performance, when PPC was used in amounts up to 210 g kg⁻¹ in diets for Atlantic salmon (Refstie and Tiekstra, 2003). Higher inclusion rates of purified PPC up to 268 g kg⁻¹ were possible without risks for fish health and nutritional status (Tusche et al., 2011b), but turned to decreasing growth performance when 400 g kg⁻¹ PPC in the diet was exceeded (Tusche et al., 2011a). The detailed mechanisms of these low glycoalkaloid concentrations and other PPC burden substances in the diets on fish olfaction and gustation are hardly understood, but it became clear that also purified raw materials reveals varying qualities with varying influences on growth performance. The results of the actual investigation confirmed that a purified PPC can replace 56 % (267 g kg⁻¹ PPC in the diet) of dietary fish meal protein without consequences for feed intake and feed conversion in rainbow trout. Thereby must be stated that the incorporation was clearly advanced (processing of the pellets; stability in the water; feed intake; feed conversion; water quality) in contrast to the own former trials (Tusche et al., 2011a, b). Thus PPC showed a good potential as partial

replacement of fish meal in organic aquaculture diets. Nevertheless, an excessive use could be prevented by the high market price of the raw material.

The action of antinutritional substances was not described when wheat gluten was used as fish meal replacement. In contrast to the observations in PPC, WG reveals a minder nutritional quality as total single replacement. Especially the AA composition shows deficiencies e.g. lysine when compared with the demand of rainbow trout and other species, (Helland and Grisdale-Helland, 2006). This reduced nutrient density resulted in the fact that WG was commonly used as feed ingredient to determine lysine requirements in rainbow trout and turbot (Pfeffer et al., 1992; Rodehutscord et al., 2000; Helland and Grisdale-Helland, 2006). In organic aquaculture diets this raw material could only be used up to amounts of non-limiting lysine supply because of the prohibited supplementation by free L-lysine. For rainbow trout the lysine requirement was determined at 27.7 g kg⁻¹ DM of the diet (Rodehutscord et al., 1997). This demand was met in all diets up to a WG inclusion level of 167 g kg⁻¹ (W17) and was slightly decreased in W19.

With view on feed intake and growth performance of the different diets in the present study it became obvious that there were no indication for negative influences of the two plant protein sources. In all groups the feed intake laid at the same height and in contrast to previous investigations (Tusche et al., 2011a, b) exhibited the fish meal control (1.97 % BW d⁻¹) no enhanced feed intake compared with all plant protein groups. Additionally were the specific growth rates (1.53 – 1.73 % BW d⁻¹) as well as the feed conversion (1.16 – 1.32) comparable between the treatments and without significant differences. Furthermore, the data of protein utilization revealed a good nutritional status of all groups. The PER (2.05 – 1.75) of all groups were above the values documented by Moyano et al. (1992), where it was claimed that diets with high plant protein contents (lupine seed meal, corn gluten meal, potato protein concentrate) exhibited appropriate PER between 1.64 – 1.74. Same observations were made with the PPV (27.18 – 31.89) between all treatments. These data confirm a good nutrient supply and utilization by the diets in contrast to former investigations especially with high PPC concentrations. Tusche et al. (2011a) documented in trials with highly purified PPC in rainbow trout diets extremely reduced PER (0.72 – 1.14) and PPV (13.4 – 19.7). Additionally was the feed intake (1.32 – 1.45 % BW d⁻¹) as well as the feed conversion (1.91 – 3.05) negatively affected. With a further look on processing qualities there is to mention, that a higher wheat gluten inclusion resulted in harder and more stable feed particle. This should be assumed as positive side effect on water quality by reduced leaching and higher faeces stability.

With regard to the whole body composition of the several treatments, there was no indication that the inclusion of plant proteins and thereby an increase of wheat gluten affected the contents of dry matter, crude protein, crude lipid, crude ash and gross energy of the experimental individuals. Whereas in former trials an increasing amount of plant proteins in the diets resulted in decreased feed intake and thereby malnutrition, starvation and unbalanced body composition (e.g. significantly reduced crude lipid and protein contents) (Xie and Jokumsen, 1997a, b; Glencross et al., 2006; Tusche et al., 2011a). All actual treatments revealed a well-balanced body composition comparable with fish fed on fish meal based diets (Tusche et al., 2011b).

The hematological characteristics of all treatments in the present study were in all parameters statistically similar to each other. Although high individual variances a homogenous subset between all groups could be observed. It could be assumed that the evaluated individuals exhibited a good nutritional status and condition factor. The optimal hematocrit of around 30% of the total blood sample (Wells and Weber, 1991) was measured in all treatments. Furthermore, the measured plasma protein ($33.14 - 37.24 \text{ mg ml}^{-1}$), triglycerides ($2.36 - 4.20 \text{ mg ml}^{-1}$) and glucose ($0.71 - 1.01 \text{ mg ml}^{-1}$) were in accordance to blood parameter of well-fed rainbow trout (Congleton and Wagner, 2006). Thus all the actual provided feeds were sufficient to cover the nutritional demand of the individuals in order to maintain growth and to avoid malnutrition as described in former trials (Tusche et al., 2011a).

As an overall result it should be stated, that all varying combinations of wheat gluten and potato protein concentrate were able to substitute 56% fish meal (on the protein level) in diets for rainbow trout. Thereby no negative influence on feed intake, feed utilization and growth were recorded. In addition, the unaffected body composition showed that the provided diets were able to meet the demand of the experimental fish. Both ingredients can be recommended as raw materials for organic aquaculture diets not only from a legal point of view but also from a nutritional. Up to a certain amount both ingredients revealed a good nutritional supply with all required nutrients (wheat gluten up to 190 g kg^{-1} in the diet without lysine deficiency) when combined in line with the nutritional demands. By the combination of both ingredients it becomes possible to utilize the cheaper wheat gluten with its deficiencies supplemented with a minimum amount of the high quality PPC to create a sustainable organic aqua feed of high nutritional and binding qualities.

References

- Bergleiter, S., 2009. Organic aquaculture 2009: Production and markets. Naturland, Gräfelfing, 120 pp.
- Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.* 69 (2), 473–490.
- Davies, S., Morris, P., Baker, R., 1997. Partial substitution of fish meal and full-fat soya bean meal with wheat gluten and influence of lysine supplementation in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Research* 28 (5), 317–328.
- European Union, 2009a. Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Europ. Union* (52), 1–130.
- European Union, 2009b. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union* (52), 15–34.
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- Helland, S., Grisdale-Helland, B., 2006. Replacement of fish meal with wheat gluten in diets for Atlantic halibut (*Hippoglossus hippoglossus*): Effect on whole-body amino acid concentrations. *Aquaculture* 261 (4), 1363–1370.
- IFOAM, 2010. Organic Aquaculture. EU Regulations (EC) 834/2007, (EC) 889/2008, (EC) 710/2009. BACKGROUND, ASSESSMENT, INTERPRETATION. International Federation of organic Agricultural movements.
http://www.ifoam.org/about_ifoam/around_world/eu_groupnew/positions/publications/aquaculture/IFOAMEU_IAMB_organic_aquaculture_dossier.pdf. Accessed 17 March 2011.
- Jeroch, H., Drochner, W., Simon, O., 1999. Ernährung landwirtschaftlicher Nutztiere: Ernährungsphysiologie, Futtermittelkunde, Fütterung. Ulmer, Stuttgart, 544 S.
- Mente, E., Karalazos, V., Karapanagiotidis, I., Pita, C., 2011. Nutrition in organic aquaculture: an inquiry and a discourse. *Aquaculture Nutrition*, no.

- Moyano, F., Cardenete, G., La Higuera, M. de, 1992. Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquat. Liv. Res.* 5 (1), 23–29.
- NRC (National Research Council), 1993. Nutrient Requirements of Fish. *National Academy Press, Washington, DC, USA.*, 1–117.
- Pfeffer, E., Al-Sabty, H., Haverkamp, R., 1992. Studies on lysine requirements of rainbow trout (*Oncorhynchus mykiss*) fed wheat gluten as only source of dietary protein. *Journal of Animal Physiology and Animal Nutrition* 67 (1-5), 74–82.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Rodehutsord, M., Becker, A., Pack, M., Pfeffer, E., 1997. Response of Rainbow Trout (*Oncorhynchus mykiss*) to Supplements of Individual Essential Amino Acids in a Semipurified Diet, Including an Estimate of the Maintenance Requirement for Essential Amino Acids. *J. Nutr.* 127 (6), 1166-1075.
- Rodehutsord, M., Borchert, F., Gregus, Z., Pack, M., Pfeffer, E., 2000. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. *Aquaculture* 187 (1-2), 163–176.
- Slawski, H., Adem, H., Tressel, R., Wysujack, K., Koops, U., Wuertz, S., Schulz, C., 2011. Replacement of fish meal with rapeseed protein concentrate in diets fed to wels catfish (*Silurus glanis* L.). *Aquacult. Nutr.*, DOI: 10.1111/j.1365-2095.2011.00857.x.
- Storebakken, T., Shearer, K., Beaverfjord, G., Nielsen, B., Asgard, T., Scott, T., De Laport, A., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. *Aquaculture* 184 (1-2), 115–132.
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London ;, Orlando, pp. 119–145.
- Tibbetts, S., Milley, J., Lall, S., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture* 261 (4), 1314–1327.
- Tusche, K., Wuertz, S., Susenbeth, A., Schulz, C., 2011a. Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 319 (1-4), 122–131.

- Tusche, K., Berends, K., Wuertz, S., Susenbeth, A., Schulz, C., 2011b. Evaluation of feed attractants in potato protein concentrate based diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 321 (1-4), 54-60.
- Wells, R.M.G., Weber, R.E., 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *J. Fish Biol.* 38 (1), 53–65.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.

Chapter 4:

High performance of rainbow trout (*Oncorhynchus mykiss*) fed diets with potato protein concentrates supplemented with feed attractants

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Abstract

Potential of potato protein concentrate (PPC) as fish meal replacer was evaluated in rainbow trout (*Oncorhynchus mykiss*) nutrition. Five diets were formulated by partial replacement of fish meal (20%, 40%, 60%, 80% and 100%) with PPC (LG-PPC with low glycoalkaloid content of 23.2 mg kg⁻¹ dry weight) and one experimental diet without PPC as control. Blood meal and a free amino acid mixture were utilized in constant amounts in order to increase palatability and increase particle stability. Experimental diets were fed twice per day over a period of 56 days to triplicate experimental groups until apparent satiation. Within all groups the feed intake was not negatively affected compared to control. Above a fish meal replacement of 60% feed conversion and growth performance became significantly reduced compared to the control. The good nutritional status (body composition, blood parameters) of all groups did not reveal severe health impact of PPC in general. The use of feed attractants suppressed the repellent effect of high amounts of PPC and other plant protein sources in the diets.

Introduction

The increasing demand for feeds in the production of aquatic animals is characteristic and one of the main bottlenecks for the fast growing aquaculture market. The price for fish meal reached a record level of more than 2,100 USD per ton in March 2010 (FAO/GLOBEFISH, 2010). Although the price per ton went down in the subsequent period, the upward trend it is foreseen to continue in the future due to restricted and/or decreased capture fishery quotas along with increasing production and processing cost for fish meal (Tacon and Metian, 2008; FAO, 2010). Currently, the costs for diets with fish meal as main protein source reveals the highest costs in the production process and contributes substantially to the ecological pressure on the wild stocks (Adelizi et al., 1998; Tacon and Metian, 2008). A reliable increase of aquaculture production is only feasible if fish meal in fish feeds will be replaced by raw materials of high quality and continuous supply. Such protein sources mainly originate from agricultural crops and their processing products (Adelizi et al., 1998; Davies et al., 1997).

Nevertheless, excessive use of plant materials for fish meal substitution is often limited by high amounts of antinutritional substances and unbalanced nutrient composition (Francis et al., 2001; Adelizi et al., 1998; Glencross et al., 2006). Potato protein concentrates (PPC) (Xie and Jokumsen, 1997a,b, 1998; Tusche et al., 2011a), wheat gluten (Pfeffer et al., 1992; Storebakken et al., 2000; Tibbetts et al., 2006) and soy proteins (Davies et al., 1997; Burrells, 1999) are widely used as fish meal replacement in diets for rainbow trout, Atlantic salmon (*Salmo salar*) and Atlantic cod (*Gadus morhua*). Still, high amounts of glycoalkaloids (from PPC) and saponines (from soy bean) resulted in reduced palatability and thereby reduced feed intake. Further negative aspects were documented in case of saponines (e.g. depression of mucosal enzyme activity in the distal intestine) (Francis et al., 2001). Technical processing and purification (downstream processing of PPC; water extraction for reduced saponine content and fermentation of soy products) can enhance the nutritional quality of the different raw materials, but result in higher processing costs. In particular purified PPC were used as alternative protein source in organic aquaculture diets (Tusche et al., 2011b, 2012). This processed raw material revealed high crude protein contents ($>800 \text{ g kg}^{-1}$) with a balanced AA composition for rainbow trout (Tacon and Jackson, 1985) without the need of additional supplementation of free AA (prohibited in organic aquaculture) (Refstie and Tiekstra, 2003). Nevertheless, high replacement of fish meal with purified PPC is limited by residual glycoalkaloids and their metabolites which influence, i.p. the taste of the diets (Tusche et al., 2011a) and by processing problems due to the extreme fine graining of the raw material (diet

particle stability). To address these problems, solution-oriented feeding trials with feed attractants, binders and additional protein sources were conducted in the past to enhance the nutritional quality, extend the use of PPC in aqua feeds and ultimately increase competitiveness of PPC in rainbow trout nutrition (Tusche et al., 2011b, 2012). As an outcome of these studies, up to 56% of the fish meal could consequently be replaced by PPC. Furthermore, wheat gluten, blood meal and appetizers can be used in conformity with the organic guideline EC 710/2009. Nevertheless, the use of higher amounts of wheat gluten normally requires supplementation with free AA (e.g. lysine) to meet the nutritional demand of rainbow trout (Rodehutschord et al., 2000). This limits an intensified utilization of plant proteins in organic aquaculture. For future expansion of fish meal supplementation rates (not exclusively in organic diets) a combination of varying plant protein sources need to be addressed, thereby reducing negative impacts of antinutritives observed in most plant proteins and providing better balanced proteins when compared with a single raw material. So far, adverse effects were not studied in mixtures of PPC, blood meal, wheat gluten and soy bean meal diets in rainbow trout.

Based on previous findings of PPC utilization in organic fish diets (Tusche et al., 2011 a, b, 2012), this study aimed at an optimization of PPC use in rainbow trout nutrition, in combination with wheat gluten and soy bean meal as basal protein sources as well as blood meal as binding agent. For the concluding evaluation, feed consumption, growth performance as well as the nutritional status (body and blood composition) of the fish was assessed for each experimental group.

Materials and Methods

Experimental diets

Five isonitrogenous and isoenergetic diets were formulated by partial replacement of fish meal (FM) at approximately 20%, 40%, 60%, 80%, 100% with potato protein concentrate (with low glycoalkaloid content: 23.2 mg kg⁻¹ dry weight; K5, Emsland Group, Emlichheim, Germany). Fixed levels of blood meal, soy bean meal and wheat gluten as supplementary plant proteins and binding agents were included according to Table 4.1 and 4.2. One diet served as control containing a low temperature herring fish meal (VFCUX, Cuxhaven, Germany) as main protein source. All diets containing PPC were named according to the fish meal replaced (in percent). All experimental diets met the nutritional requirements of rainbow

trout and the final AA composition was considered balanced according to the specifications of the dietary requirements of salmonids (NRC, 1993). Blood meal (8%) was added as additional protein source to support binding during pelletizing. Furthermore, 1% free AA was added, acting as feed stimulant in all diets as previously reported (Tusche et al., 2011b).

All ingredients were pelletized with a fodder press L 14-175 (AMANDUS KAHL, Reinbek, Germany) to pellets of 4 mm diameter. Due to the high protein contents of PPC, magnesia was used as inert filler in the diets to realise equal nutrient contents in the diets.

Tab. 4.1: Nutrient composition, amino acid profile and concentration of antinutritives (phytic acid, solanine, chaconine) in the ingredients (FM – fish meal, PPC – potato protein concentrate, WG – wheat gluten, SBM – soy bean meal) used as protein sources in the experimental diets in g kg^{-1} dry matter (Tab. 4.2).

	diet ingredients			
	FM	PPC	WG	SBM
<i>Nutrient composition (g kg^{-1})</i>				
Dry matter	918	955	909	910
Crude protein	689	859	857	571
Crude lipid	69	32	63	51
Crude fibre	5	0.5	1.3	0.5
NfE ¹	31	24	5.9	278.5
Crude ash	206	85	73	99
Phosphorus	25	22	25	29
<i>Essential amino acids (g kg^{-1})</i>				
Arginine	37.0	43.2	40.1	45.0
Histidine	12.7	19.1	17.3	16.6
Isoleucine	23.0	47.8	44.2	28.3
Leucine	40.9	90.5	82.2	48.0
Lysin	41.5	71.2	64.0	39.1
Methionine + Cysteine	20.1	32.9	30.2	17.8
Phenylalanine	22.3	57.8	52.6	32.4
Threonine	24.7	52.6	47.1	25.2
Valine	28.2	57.0	52.2	30.9
<i>Glycoalkaloids² (mg kg^{-1})</i>				
Solanin		9.19		
Chaconin		14.00		
<i>Phytic acid (g kg^{-1})</i>				
		<0.1	2.1	5.3

¹ NfE, Nitrogen-free extract = $1000 - (\text{Crude protein} + \text{Crude lipid} + \text{Ash} + \text{Crude fibre})$

² analyzed at LUFA-ITL, Kiel, Germany, according to LC-MS

Tab. 4.2: Ingredients, nutrient composition, amino acid profile and concentration of antinutritives (solanine, chaconine) in the experimental diets in g kg⁻¹ dry matter.

	Diets					
	FM	PP20	PP40	PP60	PP80	PP100
<i>Ingredients (g kg⁻¹)</i>						
fish meal ¹	339	270	202	134	66	-
PPC ²	-	53	107	160	214	267
blood meal ³	80	80	80	80	80	80
soy bean meal ⁴	177	177	177	177	177	177
free AA-Mix ⁵	10	10	10	10	10	10
fish oil ¹	84	90	96	102	109	115
wheat starch ⁶	200	195	191	186	182	177
wheat gluten ⁶	60	60	60	60	60	60
vitamin ⁷ + mineral mixture ⁸	10	10	10	10	10	10
magnesia ⁹	40	53	67	80	93	107
<i>Nutrient composition (g kg⁻¹ DM)</i>						
Dry matter	929	930	933	934	936	939
Crude protein	463	468	461	466	464	459
Crude lipid	143	143	150	150	149	147
Crude ash	136	139	139	138	138	143
Phosphorus	11.9	11.5	11.2	10.9	10.6	10.4
NfE + crude fibre	258	250	250	246	248	251
Gross energy (MJ kg ⁻¹ DM)	21.3	21.3	21.4	21.4	21.4	21.2
<i>Essential amino acids (g kg⁻¹)</i>						
Arginine	25.0	24.8	24.6	24.4	24.2	24.0
Histidine	14.1	14.2	14.4	14.5	14.7	14.8
Isoleucine	14.8	15.8	16.7	17.7	18.7	19.7
Leucine	35.1	37.2	39.2	41.3	43.3	45.4
Lysin	28.3	29.3	30.2	31.2	32.2	33.1
Methionine + Cysteine	12.7	13.1	13.5	13.9	14.3	14.6
Phenylalanine	20.7	22.3	23.9	25.4	27.0	28.6
Threonine	16.3	17.5	18.6	19.7	20.8	22.0
Valine	23.4	24.5	25.6	26.7	27.8	29.0
<i>Glycoalkaloids (mg kg⁻¹)</i>						
Solanine	0.0	0.5	1.0	1.5	2.0	2.5
Chaconine	0.0	0.8	1.5	2.2	3.0	3.7

¹VFCUX, Cuxhaven, Germany.

²Emsland-Stärke GmbH, Emlichheim, Germany.

³Euroduna-Technologies GmbH, Barmstedt, Germany.

⁴Hamlet Protein A/S, Horsens, Denmark.

⁵AA-Mix (g kg⁻¹); Carl Roth GmbH, Karlsruhe, Germany: Alanine, 333; Cystine, 333; Betaine, 333.

⁶Cargill Deutschland GmbH; Krefeld, Germany.

⁷Vitamin-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: vitamin A, 1,000,000 IU kg⁻¹; vitamin D3, 200,000 IU kg⁻¹; vitamin E (as α -tocopherol-acetate), 40,000; vitamin K3 (as menadione), 4,000; vitamin B1, 4,000; vitamin B2, 8,000; vitamin B6, 4,000; vitamin B12, 8; vitamin C (as monophosphate), 60,000; pantothenic acid, 8,000; nicotinic acid, 40,000; folic acid, 1,600; biotin, 100; inositol 40,000.

⁸Mineral-Premix (mg kg⁻¹); Vitfoss, Grasten, Denmark: cobalt (as cobaltsulphate), 400; manganese (as manganese sulphate), 2,500; iodine (as calciumiodine), 500; copper (as coppersulphate), 2,500; selen, 25; zinc (as zincsulfate), 28,000.

⁹Magnesia 4371, Magnesia GmbH, Lüneburg, Germany.

Tryptophane was not analysed.

Experimental setup

The feeding trial was carried out with 360 rainbow trout (Forellenzucht Trostadt GbR, Trostadt, Germany), which were randomly distributed to 18 tanks (175 l each) at the facilities of the Gesellschaft für Marine Aquakultur (GMA, Büsum, Germany), resulting in 6 feeding groups, assessed in triplicates of 20 fish ($52.2 \pm 0.1\text{g}$). The tanks were arranged in a closed recirculation system (4.5 m^3 total water volume, water turnover rate 6 h^{-1} , daily water exchange 1.700 l kg^{-1} feed) at a photoperiod of 12 h light : 12 h dark and a water temperature of $13.4 \pm 0.5^\circ\text{C}$. Water quality was determined daily ($9.6 \pm 0.3 \text{ mg l}^{-1} \text{ O}_2$; Handy Polaris; OxyGuard International A/S, Birkerød, Denmark; $0.25 \pm 0.2 \text{ mg l}^{-1} \text{ NH}_4\text{-N}$, $0.3 \pm 0.2 \text{ mg l}^{-1} \text{ NO}_2\text{-N}$; Microquant test kit for NH_4 and NO_2 ; Merck KGaA, Darmstadt, Germany). During acclimatisation of two weeks, fish were fed to apparent satiation twice per day (9.00 a.m. and 5.00 p.m) a commercial trout feed (Performa 45/20, 2mm; AllerAqua A/S, Christiansfeld, Denmark), subsequently change to the experimental diets upon acclimatisation.

Sampling

On day 0, 10 fish were randomly sampled to assess whole body composition and stored at -20°C . At the end of the experiment, final biomass of each tank was recorded, 3 fish (bulk sample) of each tank were sampled for the determination of whole body composition, revealing 3 bulk samples per treatment. Additionally, blood was drawn from the caudal vein of three individuals per tank for the determination of blood parameters and fish weight as well as liver weight was recorded. For each treatment, mortalities, growth parameters (specific growth rate, SGR; Fulton condition factor, FCF) and feed utilisation (feed conversion ratio, FCR; protein efficiency ratio, PER; protein productive value, PPV) were calculated as average of the triplicates according to the formulas in Tab. 4.3.

Nutritional composition of experimental diets and fish

Chemical analysis of the nutritional composition comprising dry matter (DM), ash, crude protein, crude fat and crude fiber was carried out according to EU guideline (EC) 152/2009 (European Union, 2009a). In brief, crude protein content ($\text{N} \times 6.25$) was determined by the Kjeldahl method (InKjel 1225 M, WD 30; Behr, Düsseldorf, Germany). Crude fat content was obtained after hydrolysis with hydrochloric acid and successive petroleum ether extraction with a Soxhlet extraction system (R 106 S; Behr). DM was measured after drying at 103°C until successive weighing revealed constant values. Ash content was quantified after 4 h of incineration at 550°C with a combustion oven (P300; Nabertherm, Lilienthal,

Germany). Gross energy was measured in a bomb calorimeter (C 200; IKA, Staufen, Germany).

Blood parameters

Blood sampling was taken from the caudal vein and the hematocrit value was determined in five replicates in micro-hematocrit-tubes (Type 563, NA-heparinized, 75 mm/135 µm; Assistant, Sondheim, Germany) upon centrifugation (10.000 g, 5 min) with a hematocrit 210 centrifuge (Hettich, Tuttlingen, Germany) and a hematocrit tube card reader. Remaining blood was centrifuged (1.000 g, 5 min) to separate the plasma and stored at -80°C. Glucose, triglycerides (TG) and protein were determined photospectrometrically in duplicate with a microplate reader (Infinite M200, Tecan) using commercial kits adapted to a microtiter scale. Glucose and TG were determined by the GPO-PAP method (Greiner, Frickenhausen, Germany) and plasma protein was quantified according to Bradford with a Roti-Quant kit and a BSA standard dilution series. Concentrations were calculated from a standard dilution series to compensate for run-to-run variation. Recoveries were determined by spike experiments and were >90% for all plasma parameters analysed.

Statistical Analyses

The statistical analysis was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, U.S.). Data are presented as mean ± standard deviation (SD) for each treatment. For comparison between treatments, data were analysed for normal distribution by Kolmogorov-Smirnov and equal variance by Kruskal-Wallis One Way Analysis of Variance (confirmed if $p < 0.05$) and compared by post-hoc multiple comparison, either parametric Tukey-HSD (if test for homogeneity was confirmed) ($p < 0.05$) or non-parametric Dunnett-T3 test (if test for homogeneity failed).

Results

Growth

At the end of the experiment, the control group revealed the highest final body weight (138.9 ± 8.5 g) and the highest specific growth rate (SGR, 1.75 ± 0.1 % BW d⁻¹). The group PP20 revealed comparable values. A fish meal replacement of 40% (PP40) and 60% (PP60) by PPC resulted in slightly decreased values, though statistical significance was not detected due to high individual variances of PP60. At the highest fish meal exchange levels, the SGR (PP80 1.40 ± 0.1 % BW d⁻¹; PP100 1.34 ± 0.0 % BW d⁻¹) and thereby the final body weight

(PP80 114.4 ± 3.4 g; PP100 110.5 ± 1.8 g) significantly decreased. In all groups, the daily feed intake did not differ significantly nor could tendencies be recorded ($1.96 - 2.18$ % BW d⁻¹).

Regarding the feed conversion, a consistent trend was observed with increasing supplementation of FM. The lowest FCR was observed in the control group (1.20 ± 0.0) and up to a fish meal supplementation level of 40% no significant differences were observed. At an exchange of 60% or higher, a significant increased FCR was detected ($1.34 - 1.46$). Same observations and trends were revealed for PER and PPV. The protein utilization decreased at increasing amounts of PPC in the diet and significant differences to the control diet were found at fish meal exchange levels of more than 40% for PER or more than 60% for PPV.

The hepatosomatic index (HSI) ranged between 1.40 and 1.60 without significant differences between groups. Furthermore, the Fulton condition factor (FCF) of all groups ranged between 1.16 and 1.25 and was without significant differences. No mortalities were recorded and all groups more than doubled their weight during the experimental time.

Tab. 4.3: Growth performance (mean \pm SD, n=3) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed diets at partial substitutions of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Values with the same superscript are not significantly different ($p < 0.05$).

Variable	Experimental diets											
	FM		PP20		PP40		PP60		PP80		PP100	
IBW ¹	52.1	± 0.0	52.2	± 0.1	52.2	± 0.0	52.2	± 0.0	52.1	± 0.1	52.2	± 0.0
FBW ²	138.9 ^a	± 8.5	138.8 ^a	± 4.7	129.4 ^{a,b}	± 0.4	129.6 ^{a,b}	± 15.8	114.3 ^b	± 3.4	110.5 ^b	± 1.8
DFI ³	2.10	± 0.1	2.18	± 0.1	2.11	± 0.0	2.15	± 0.2	2.03	± 0.1	1.96	± 0.0
SGR ⁴	1.75 ^a	± 0.1	1.75 ^a	± 0.1	1.62 ^{a,b}	± 0.0	1.61 ^{a,b}	± 0.2	1.40 ^b	± 0.1	1.34 ^b	± 0.0
FCR ⁵	1.20 ^a	± 0.0	1.25 ^{a,b}	± 0.0	1.30 ^{a,b}	± 0.0	1.34 ^{b,c}	± 0.1	1.45 ^c	± 0.1	1.46 ^c	± 0.0
PER ⁶	1.94 ^a	± 0.1	1.84 ^{a,b}	± 0.0	1.79 ^{a,b}	± 0.0	1.72 ^{b,c}	± 0.1	1.59 ^c	± 0.1	1.59 ^c	± 0.0
PPV ⁷	30.15 ^a	± 1.4	28.10 ^{a,b}	± 3.3	29.23 ^a	± 0.6	27.55 ^a	± 1.4	26.65 ^b	± 0.7	24.64 ^b	± 3.8
HSI ⁸	1.60	± 0.3	1.45	± 0.2	1.40	± 0.2	1.48	± 0.2	1.46	± 0.2	1.56	± 0.3
FCF ⁹	1.18	± 0.1	1.24	± 0.1	1.21	± 0.1	1.24	± 0.1	1.25	± 0.1	1.16	± 0.1

¹initial body weight (g); ²final body weight (g); ³daily feed intake (% BW day⁻¹); ⁴specific growth rate (% BW day) = $[\ln(\text{FBW}) - \ln(\text{IBW})] / \text{feeding days} \times 100$; ⁵feed conversion ratio = feed intake (g) / weight gain (g); ⁶protein efficiency ratio = weight gain (g) / protein intake (g); ⁷protein productive value = $100 \times [(\text{crude protein final fish} \times \text{biomass final fish}) - (\text{crude protein initial fish} \times \text{biomass initial fish})] / (\text{crude protein diet} \times \text{total feed intake})$; ⁸hepatosomatic index = (liver weight / final body weight) $\times 100$; ⁹Fulton condition factor = $100 \times \text{final body weight} \times \text{final body length}^{-3}$.

Body composition

The replacement of fish meal by PPC did not affect the body composition substantially (Table 4.4). The dry matter (DM) of all groups was approximately 296 g kg^{-1} . Furthermore, no differences in crude protein (approx. 159 g kg^{-1}), as well as in crude lipid content

(approx. 112 g kg⁻¹) and gross energy (approx. 27.6 MJ kg⁻¹) were detected in all groups. Solely the crude ash content revealed some differences. Up to a fish meal exchange of 80% comparable crude ash amounts were recorded, but significant decrease was detected upon total fish meal replacement in PP100.

Tab. 4.4: Body composition (g kg⁻¹ wet weight) and gross energy (MJ kg⁻¹ DM) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed diets at partial substitutions of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Initial body composition was expressed on a wet weight basis as: dry matter 264 g kg⁻¹; ash 24 g kg⁻¹; crude protein 163 g kg⁻¹; crude lipid 86 g kg⁻¹; energy 27.1 MJ kg⁻¹. Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).

Nutrient	Experimental diets					
	FM	PP20	PP40	PP60	PP80	PP100
Dry matter	289 ± 13.7	298 ± 12.3	300 ± 6.7	303 ± 5.2	297 ± 9.2	290 ± 17.8
Crude protein	157 ± 2.8	155 ± 11.4	161 ± 0.6	160 ± 2.4	164 ± 2.0	158 ± 13.4
Crude lipid	102 ± 12.2	117 ± 6.2	117 ± 5.5	116 ± 7.6	108 ± 11.1	110 ± 2.4
Crude ash	30 ^{a,b} ± 3.6	26 ^{a,b} ± 2.6	31 ^a ± 2.2	27 ^{a,b} ± 1.5	26 ^{a,b} ± 2.4	23 ^b ± 3.1
Gross energy	27.0 ± 0.5	28.0 ± 0.3	27.1 ± 0.1	27.9 ± 0.4	27.6 ± 0.6	28.0 ± 0.4

Blood plasma composition

Blood parameters did not reveal significant differences between groups (Table 4.5). The highest hematocrit value was measured in the PP100 group (38.0 ± 7.4 %) and the other groups revealed only minor differences (33.0 – 35.4 %). Plasma protein (33.74 – 36.77 mg ml⁻¹), triglycerides (2.53 – 4.02 mg ml⁻¹) and glucose (0.74 – 0.99 mg ml⁻¹) were comparable between treatments.

Tab. 4.5: Blood parameters (hematocrit in %; protein, triglyceride and glucose in mg ml⁻¹) of rainbow trout fed the fish meal control diet (FM) compared to the experimental groups fed a diet at a partial substitution of fish meal by potato protein concentrate (PPC with low glycoalkaloid content) at 20%, 40%, 60%, 80% and 100% (PP20-PP100). Values (mean ± SD, n=3) with the same superscript are not significantly different (p<0.05).

Blood parameter	Experimental diets					
	FM	PP20	PP40	PP60	PP80	PP100
Hematocrit	34.1 ± 4.4	34.2 ± 3.9	35.4 ± 2.3	33.0 ± 1.9	34.7 ± 4.2	38.0 ± 7.4
Protein	36.76 ± 3.9	36.18 ± 3.7	33.74 ± 3.2	35.54 ± 4.5	35.34 ± 1.6	36.77 ± 4.2
Triglyceride	2.53 ± 0.5	4.02 ± 1.3	2.98 ± 2.1	3.37 ± 1.6	2.73 ± 1.7	3.40 ± 1.0
Glucose	0.77 ± 0.1	0.78 ± 0.2	0.99 ± 0.6	0.75 ± 0.1	0.74 ± 0.1	0.85 ± 0.2

Discussion

The use of purified potato protein concentrates (PPC) has been evaluated as a suitable protein source to replace fish meal in organic aquaculture diets (Tusche et al., 2011b, 2012). Despite the high price of this raw material, which restricts emerging use yet, it was shown that

a total replacement of fish meal by PPC is difficult to realize. Mainly, this has been attributed to minor antinutritive effects of residual glycoalkaloids and their metabolites and, more importantly reduced palatability (Tusche et al., 2012). Additionally, the extreme fine graining of the raw material resulted in processing problems and a low pellet stability in water (Tusche et al., 2012). Still, in accordance with the EU organic guidelines, PPC could be included at 267 g kg⁻¹ without any impact on the growth performance, feed utilization and animal welfare. Furthermore, it seems recommendable to use a combination of alternative proteins to increase the PPC potential as fish meal replacer. Currently, this strategy is already considered in commercial aqua feeds for rainbow where the remaining percentage of fish meal is reduced to 15 – 20% (Tacon and Metian, 2008). In the present trial, mixed plant based diets using soy bean meal (constant level) and potato protein concentrate as fish meal replacement were evaluated. In addition, blood meal (80 g kg⁻¹ diet) was incorporated to increase palatability and improve processing, thereby decreasing the fish meal utilization to 339 g kg⁻¹ in the control diet (crude protein 463 g kg⁻¹). Previously, Tusche et al. (2011a) used 686 g fish meal kg⁻¹ in the control diet to provide 490 g kg⁻¹ crude protein content in the diet. Consequently, based on this initially control diet, a fish meal reduction of approximately 50% was achieved in addition to PPC based fish meal substitution. Refstie and Tiekstra (2003) reported a control diet for Atlantic salmon 589 g kg⁻¹ fish meal as main protein source (crude protein 480 g kg⁻¹) and a combination of blood meal (100 g kg⁻¹) and soy bean meal (250 g kg⁻¹) in experimental control diets was successfully used in Japanese flounder (*Paralichthys olivaceus*) without negative impacts on health and nutritional status of the experimental individuals (Kikuchi, 1999). Considering the present results, the control diet (177 g/kg soy meal, 80 g/kg blood meal, 339 g/kg fish meal) is considered a valid control to assure optimal growth, feed conversion and animal health.

Despite the high inclusion rate of alternative protein sources no influences on feed intake between all feeding groups were detected in this study. Again, DFI revealed no significant differences, although a slight decrease at 80% and 100% fish meal replacement was recorded. In former trials with higher inclusion of dietary vegetable proteins, the palatability and feed intake decreased dramatically up to a complete refusal of feed (Xie and Jokumsen, 1997a; Glencross et al., 2006; Slawski et al., 2011a, Tusche et al., 2011a). Especially the reduction of fish meal, with its own strong and distinctive taste, influenced the feed intake in a significant extent (Tusche et al., 2011b). In the past, a mixture of alanine, cysteine and betaine was successfully used as feed attractant in PPC-based diets in rainbow trout (Tusche et al., 2011b) and based on these result, a comparable mixture was added in the actual treatments. Thereby,

feed intake between all groups showed that the feed attractants had the potential to compensate the previously observed (Tusche et al., 2011b, 2012) repellent effects at high incorporations of plant proteins.

Considering growth parameters, minor decreases were observed at high fish meal replacement. Consequently, a fish meal replacement up to 60% by PPC is realized here without an impairment of growth rate. Still, feed conversion and protein efficiency ratio was affected above 40%. Clearly, decreased growth rates and feed conversions were recorded at 80% and 100% fish meal replacement, most probably related to glycoalkaloids in the PPC based diets. Furthermore, a slightly lower ash content in the diets (Tab. 4.2) and a slightly increase of phytic acid which was furthermore contributed to the higher PPC inclusion was detected. This may affected the dietary availability of phosphorus. Such an undersupply in phosphorus is characterized by reduced growth, decreased feed conversion and reduced energy supply (Skonberg, 1997). A supplementation with additional phosphorus (e.g. dicalcium phosphate) in the diet may thus circumvent such metabolic problems (Kaushik et al., 2004) and should be addressed in the future.

Regarding the whole body composition in the present trial, the amount of crude ash ($23 \pm 3.1 \text{ g kg}^{-1}$) in PP100 was significantly reduced compared to the control group. This result may indicate insufficient phosphorus supply as suggested by Slawski et al. (2011b). Still, other impact on whole body composition (dry matter, crude protein, crude fat and gross energy) was not observed here. In contrast to former trials (Xie and Jokumsen, 1997a, b; Glencross et al., 2006; Tusche et al., 2011a), an increasing amount of dietary plant proteins revealed decreased feed intake and thereby malnutrition, starvation and unbalanced body composition (e.g. significantly reduced crude lipid and protein contents). With the exception of the ash content of PP100, all actual treatments revealed a balanced and well-nourished body composition comparable with fish fed on fish meal based diets (Tusche et al., 2011b).

The parameters of the hematological investigation were comparable between treatments and the control values in previous studies (Tusche et al, 2011a, b, 2012), indicating a good nutritional status and condition of the individuals (Wells and Weber, 1991; Congleton and Wagner, 2006;). Same observations and conclusions were made for the amounts of protein, triglycerides and glucose in the blood plasma. Thus, all plant based diets did not affect nutritional homeostasis of the fishes and thus have to be regarded ideal to satisfy the nutritional demand, assure optimal growth and exclude malnutrition of rainbow trout, in sharp contrast to previous trials on PPC replacement and the reported adverse effects reported (Tusche et al., 2011a).

In conclusion, a PPC inclusion of up to 160 g kg⁻¹ (60% fish meal replacement) is feasible in rainbow trout diets without any negative impact, neither on feed intake, feed conversion, growth and nutritional status nor animal welfare. This is an improvement on fishmeal replacement by PPC compared to previous results (Tusche et al., 2011a, b, 2012) where PPC was included at 268 g kg⁻¹ (only representing 56% fish meal replacement on the protein level) but revealed comparable performance. Still, the inclusion of AA mix restricts a use in organic trout farming. In retrospective of the first studies (Tusche et al., 2011a) utilizing purified PPC, it is possible to replace more than 80% of the initial fish meal amount in presented diets. This is a substantial progress for the utilization of potato protein concentrates as alternative protein source in aquaculture diets. Still, it has to be mentioned that the legislation for organic aquaculture diets (European Union, 2009b) currently claims a maximum amount of dietary plant products of carnivorous species of 60%. Therefore, with regard to the necessity to replace fish meal in the context of global depletion of fish stocks an amendment of the current EU guidelines is needed to support a sustainable development of aquaculture.

References

- Adelizi, P., Rosati, R., Warner, K., Wu, Y., Muench, T., White, M., Brown, P., 1998. Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*. *Aquacult. Nutr.* 4 (4), 255–262.
- Burrells, C., 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. *Vet. Immunol. Immunopathol.* 72 (3-4), 277–288.
- Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.* 69 (2), 473–490.
- Davies, S., Morris, P., Baker, R., 1997. Partial substitution of fish meal and full-fat soya bean meal with wheat gluten and influence of lysine supplementation in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Research* 28 (5), 317–328.
- European Union, 2009a. Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Europ. Union*, 52, 1–130.
- European Union, 2009b. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of

- Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union* (52), 15–34.
- FAO. The State of World Fisheries and Aquaculture 2010. Rome, FAO. 2010. 197p.
- FAO/GLOBEFISH, 2010. Highlighths: A quarterly update based on the GLOBEFISH databank (4), 1–46.
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- Kaushik, S., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 230 (1-4), 391–404.
- Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179 (1-4), 3–11.
- Pfeffer, E., Al-Sabty, H., Haverkamp, R., 1992. Studies on lysine requirements of rainbow trout (*Oncorhynchus mykiss*) fed wheat gluten as only source of dietary protein. *Journal of Animal Physiology and Animal Nutrition* 67 (1-5), 74–82.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Rodehutschord, M., Borchert, F., Gregus, Z., Pack, M., Pfeffer, E., 2000. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. *Aquaculture* 187 (1-2), 163–176.
- Skonberg, D., 1997. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 157 (1-2), 11–24.
- Slawski, H., Adem, H., Tressel, R., Wysujack, K., Koops, U., Wuertz, S., Schulz, C., 2011a. Replacement of fish meal with rapeseed protein concentrate in diets fed to wels catfish (*Siluris glanis* L.). *Aquacult. Nutr.*, DOI: 10.1111/j.1365-2095.2011.00857.x.
- Slawski, H., Adem, H., Tressel, R.-P., Wysujack, K., Koops, U., Kotzamanis, Y., Wuertz, S., Schulz, C., 2011b. Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquacult Int.*

- Storebakken, T., Shearer, K., Beaverfjord, G., Nielsen, B., Asgard, T., Scott, T., De Laport, A., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. *Aquaculture* 184 (1-2), 115–132.
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London ;, Orlando, pp. 119–145.
- Tacon, A., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285 (1-4), 146–158.
- Tibbetts, S., Milley, J., Lall, S., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture* 261 (4), 1314–1327.
- Tusche, K., Wuertz, S., Susenbeth, A., Schulz, C., 2011a. Feeding fish according to organic aquaculture guidelines EC 710/2009: Influence of potato protein concentrates containing various glycoalkaloid levels on health status and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 319 (1-4), 122–131.
- Tusche, K., Berends, K., Wuertz, S., Susenbeth, A., Schulz, C., 2011b. Evaluation of feed attractants in potato protein concentrate based diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 321 (1-4), 54-60.
- Tusche, K., Arning, S., Wuertz, S., Susenbeth, A., Schulz, C., 2012. Wheat gluten and potato protein concentrate - Promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 344-349, 120-125,.
- Wells, R.M.G., Weber, R.E., 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *J. Fish Biol.* 38 (1), 53–65.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.

General discussion

The aim of the present thesis was to examine the potential of potato protein concentrates (PPC) as fish meal replacement in diets for rainbow trout (*Oncorhynchus mykiss*). Next to the evaluation of feeding and growth performance, there was an expanded focus on the nutritional status and health parameter of the experimental individuals to explain the influence of feed ingredients and diet formulations. The following paragraphs illustrate the experiences and advantages with PPC of varying quality as feed ingredient in rainbow trout nutrition. Thereby a special emphasis was laid on the legislation/requirements of organic aquaculture and on the feeding purposes of carnivorous fish in particular.

Feed intake

When PPC was used in aquaculture diets, it became obvious that untreated raw materials were rich in glycoalkaloids ($>1,000 \text{ mg kg}^{-1}$ PPC) and could only be used in low amounts (56 g kg^{-1}) without negative impacts in diets for rainbow trout (Xie and Jokumsen, 1997a, b; 1998). The results of the HG groups (glycoalkaloid concentration in the diets $315 - 1,207 \text{ mg kg}^{-1}$ diet) *chapter 1* confirmed these findings. Especially in these groups was shown that the palatability of the feed particles was affected extremely negative, up to a refuse of the diets with glycoalkaloid concentrations of more than $1,000 \text{ mg kg}^{-1}$ diet. Solanine, chaconine (main glycoalkaloids in potato) and other secondary metabolites of solanaceous plants act normally as repellents against insects and other animals which feed on green plant compartments (Friedman, 2006; Jeroch et al., 1999). This mode of action is still present in PPC rich in glycoalkaloids and was normally exhibited as a bitter taste of the feed particles (Xie and Jokumsen, 1997a, b). The quality (mostly reduction of secondary plant metabolites) of the raw material can be enhanced by different processing techniques (Lokra et al., 2008) and can be further improved in the future. Such a process often results in increasing production costs for the raw material and thereby increasing prices for the aquaculture diets.

The use of purified PPC (glycoalkaloid content $<100 \text{ g kg}^{-1}$) exhibited improved results in the three past decades (Tacon and Jackson, 1985; Refstie and Tiekstra, 2003). Most prominent was an increase of feed acceptance (enhanced palatability) by the fish, thus increased feed intake. The data of the first own trial exhibited good and enhanced results. The differences between the LG groups and HG groups as well as the fish meal control in the *first chapter* were highly indicative for the repellent action of glycoalkaloids in the diets. Although the feed intake of all LG groups was significantly increased compared to the HG groups, it was

only ordinary when the fish meal control acts as comparison. A possible explanation was set in the hypothesis that the glycoalkaloids were highly water soluble (Jensen et al., 2009). Thereby the glycoalkaloids may acted in a concentration-dependent layer/gradient around the feed particle. Additionally, this mode of action had an influence on the whole system induced by the water flow. The system wide (not clearly detectable) effect was successfully turned off by using an active coal filtration unit in following examinations.

In general, the most impressive finding in the *first chapter* was the heavily influenced and decreased feed intake. For this case it must be concluded that an unfed fish was not and will never be able to utilize enough energy and nutrients for growth (Glencross et al., 2006). The major obstacle for diets rich in PPCs was set to improve the feed particle palatability and feed intake. In this way the individuals should be enabled to swallow in the amount of feed which is demanded for metabolic purposes. In the trial described in the *second chapter*, varying feed attractants were used to reach the aim of enhanced PPC utilization in diets for rainbow trout. With the inclusion of blood meal, mussel meal or free amino acids (AA) an increased feed intake should be generated. Additionally it was proofed, if glycoalkaloids in a fish meal based diet result in similarly decreased feed intake as observed in normal PPC diets. The last mentioned experimental approach revealed absolutely comparable data to the control group. This could lead to a rejection of the hypothesis that low amounts of glycoalkaloids affected the palatability of a feed particle. Nevertheless, it has to be mentioned, that fish meal has the potential to act as a good feeding stimulant by itself. The strong smell and taste of the fish meal compensated the anti-palatability effect of the added solanine and chaconine in the diet. High amounts of free alanine and other AA in the fish meal (Li et al., 2011) are known to stimulate the gustatory system in salmonids. Thereby the short, unbranched and uncharged L-AA were most effective in rainbow trout (Hara et al., 1994). Smallest amounts of such free AA in the water and on the feed particle surfaces resulted in a taste bud innervation and induced a locomotion reaction of the fish (Hara, 2006; Yamashita et al., 2006). The PP group without additional feed attractants and the mussel meal groups (*second chapter*) exhibited only a low amount of alanine in the diets as well as a significantly reduced feed intake. In particular, a stimulating effect of mussel meal in diets for rainbow trout (corresponding AA: alanine, glycine, betaine) was not documented in the presented trial as described by Mackie et al. (1980). Thus excluded green lip mussel meal as feed additive in PPC based diets.

The effect of blood meal or a mixture of free AA as feed attractants in PPC based diets was in contrast to the formerly mentioned. Regarding the blood meal (4 and 8% inclusion level) the highest appetizing effects within all PPC groups were documented. This confirmed the

results from former investigations with this feed ingredient (Kikuchi, 1999; Millamena, 2002). Additionally, it was important to note that blood meal was identified to be rich in the free AA alanine (Li et al., 2011) and in this way it could influenced the palatability of the particles, locomotion and feed intake of the experimental individuals. The same effect should be reached with the mixture of free AA as feed attractants. As previously mentioned, the neutral AA alanine was of importance in palatability of rainbow trout and other salmonids. Additionally, other AA with comparable functions as well as enhancing effects were documented. The combination of betaine, glycine and alanine had a positive outcome as feed attractants in diets for rainbow trout (Jones, 1989; Yamashita et al., 2006), European sea bass (*Dicentrarchus labrax*) (Dias et al., 1997) and striped bass (*Morone saxatilis*) (Papatriphon and Soares, 2000, 2001). The stimulus was also present in the actual investigation. But a certain threshold concentration have to be exceeded until a reaction (in form of increased feed intake) can be documented. This threshold concentration was reached at free AA amounts at 1% in the diet. Especially in this group (AM1) the amount of free alanine was highest in the described feeding trial.

The findings of the palatability and the feed intake in *chapter three* and *four* will be combined in this paragraph. The main reason was that both trials took place in the same experimental facilities and time span and no implications of findings from the *third trial* could be used in a subsequent experiment (until now). Nevertheless, both experimental setups showed good results for feed intake of PPC based diets around 2 % BW d⁻¹. These values were in order to the different control groups and were used as indication that no negative influences of the use plant protein sources took place anymore. In the case of PPC replacement by wheat gluten (*chapter 3*) it could be speculated, that a higher amount of wheat gluten resulted in a better binding quality of the feed particles, thus the water solubility of the glycoalkaloids was decreased. With decreased amount of glycoalkaloids around the pellet the palatability of the diets increased inversely proportional. Furthermore, the used fish meal acted as strong feed attractant which increased the attractiveness of the used diets. The feed intake of the diets in the *fourth chapter* also revealed no influences between all groups although fish meal replacement took place up to 100%. This was achieved by the use of the positive evaluated mixture of free AA from the *second trial*. Especially at highest exchange rates, a significantly decreased palatability and feet intake was documented in former trials with total fish meal replacement (Xie and Jokumsen, 1997a; Glencross et al., 2006; Slawski et al., 2011).

Generally should be concluded that the feed intake in PPC based diets was stabilized at 2% BW d⁻¹ by the improvement of particle stability (and water stability), reduction of glycoalkaloids in the raw material and the use of appetizers. This amount reflects a positive result for further investigations and use of PPC in aquaculture diets for salmonids.

Metabolism and growth

Regarding the metabolic processes and thereby increase of body substance of the individuals using PPC in varying concentrations and combinations as protein source in the diets, it became obvious that the parameter of growth performance as well as other parameters of feed utilization were strongly correlated with the feed intake – as discussed in the preceding paragraphs.

The data of the initial experiment (*chapter one*) showed significantly decreased growth rates in any case of PPC inclusion compared to the fish meal control group. Due to the varying qualities of the used PPC the results must be evaluated separately according to the PPC origin. When fish meal was replaced by the HG-PPC, only minimal growth in HG25 and negative growth in all other HG groups was documented. The individuals were exposed to an undersupply with nutrients and used endogenous nutritive substances for the maintenance energy metabolism. It was impossible to realize positive growth under these conditions which were also documented by Xie and Jokumsen (1997a). Furthermore, a calculation of protein utilization and feed conversion was misleading when negative growth occurred and was not determined in the HG groups. The data of the LG groups showed another result. It became clear that all supplementation rates with this raw material revealed positive growth, but depending on the reduced feed intake (1.32–1.47 % BW d⁻¹) of the LG diets only worse feed conversion and protein utilisation occurred. A possible explanation should be found in the decreased amount of supplied nutrients and the experimental individuals thereby used the low amounts of applied protein as source for maintenance energy metabolism. Thus decreased automatically the FCR, PPV and PER. Normally, in rainbow trout fed on diets with high amounts of plant protein (lupine seed meal, corn gluten meal and PPC) appropriate PER was documented at 1.67-1.74 and for PPV at 35.7–39.3% and the nutritional status of the individuals was without negative indication (Moyano et al., 1992). These values were never achieved at any LG group. Nevertheless, the highly purified PPC used in the *first chapter* showed some positive aspects for further use in aquaculture diets.

As mentioned above, the daily feed intake of all PPC groups in the *second* and the *following chapters* was increased and laid above the values from all PPC groups of the *first*

trial. In general, this simple but fundamental increase resulted in better feed conversion, significantly increased protein utilization and finally better growth.

Next to the action of feed attractants on palatability in PPC diets there is to mention that these additives could also had a side effect on nutrient utilization and acted as well as nutrient source. The use of blood meal resulted in an increased feed conversion and decreased PER. A possible explanation for that was given by Bureau et al. (1999). In trials with rendered animal protein sources in rainbow trout nutrition was found that blood meals underlayed fluctuations in the nutritional quality. It could be supposed that the processing quality of the actual used blood meal was of a minder quality which resulted in a decreased digestibility and conversion into endogenous energy and body substance. In the case of mussel meal as feed attractant only minor influences on feed conversion were detected. Due to slight increased ash contents in the mussel meal diets, the digestion and adsorption processes in the gastrointestinal tract were affected. The AM groups as presented in the *second chapter* revealed excellent data for FCR and PER and it was concluded, that parts of the attractant mix were used for or in metabolic and energy producing processes (Papatriphon and Soares, 2001). For the *second trial* is to add, that all experimental groups with a reduced feed intake (especially the PP group) revealed best data for feed conversion which correlates mostly with a longer retention time in the gastrointestinal tract and this enhanced the digestion and adsorption processes of nutrients. Though, the feed intake reduction in these groups was less impressive compared to the LG groups from *chapter one* and the observed effects resulting from it.

The growth performance of the individuals in the *third trial* was in consensus with the unaffected feed intake of all experimental groups. The offered ingredients in the diets were normally used for energy metabolism (crude lipids and carbohydrates) and growth (crude proteins). The values for PER and PPV were in order to the mentioned above (Moyano et al., 1992) and the insignificance between the fish meal control and the plant protein groups was indicative for an adequate supply. The combination of PPC and wheat gluten as fish meal replacement showed a good potential regarding feed utilization and growth performance when 56% of fish meal (on the protein level of the diet) were replaced. With regard to the feed conversion, a very slight increase (without statistical indication) was observed when plant proteins were included in the diets. An initial conclusion was based on an insufficient amino acid supply (e.g. lysine) (Rodehutsord et al., 2000), which could not be confirmed in the AA composition of the diets and fish bodies. A second and highly speculative explanation could be found in small amounts of phytic acid with increased amount of plant proteins in the diets, especially wheat gluten. This antinutritional substance was determined in the raw material,

but not in the diets due to marginal amounts. Nevertheless, a possible but only low influence on feed conversion and nutrient supply of the organism could be supposed. A decreased growth performance was indicative for phytic acid activity from plant protein sources in aquaculture diets (Francis et al., 2001). The use of artificial phosphorous could work against this potential negative impact (Kaushik et al., 2004).

In the *fourth chapter* no influences on feed conversion and protein recovery were detected up to a fish meal replacement of 40% and no influence on growth in the experimental individuals up to a replacement of 60%. Above 60% fish meal replacement, all parameters of growth performance were negatively affected. Furthermore, the high amounts of plant burden raw materials which decreased the crude ash content with a simultaneously slight increased amount of phytic acid. In this case the phosphorous availability for the fish was also decreased. This resulted in increased feed conversion, reduced energy supply and finally reduced growth (Skonberg, 1997). A supplementation with alternative phosphorous sources (e.g. dicalcium monophosphate) should avoid such problems.

In general is to say, that the initial trial with PPC (*first trial*) as feed ingredient resulted in poor growth performance regardless which origin and quality the raw material had. Due to residues of glycoalkaloids and other plant burden substances in the diets, the palatability of the feed particles was negatively affected. The advancement of the feed intake in the form of feed attractant application, an advancement of binding quality of the pellets (reduced leaching of taste suppressors) as well as a combination of alternative protein sources, offered the possibility to use high amounts of PPC and other plant proteins as fish meal replacement without negative effects on the growth performance of rainbow trout.

Nutritional status and health parameter

In general should be stated, that the characteristics of whole body composition and further parameters of the control groups can be used as a standardized health model. This is due to the application of nutrients and raw materials, which were produced as appropriate to the demand of the specie. As a summary of the data of all presented experimental control groups the total body composition in the original matter laid at approximately: Dry matter 296 g kg⁻¹, crude protein 159 g kg⁻¹, crude lipid 111 g kg⁻¹, crude ash 30 g kg⁻¹ and a gross energy content of 27.4 MJ kg⁻¹ in the dry matter. The values were in consensus with data from literature for healthy and well-nourished rainbow trout (Davies et al., 1997; Xie and Jokumsen, 1998; Rodehutsord et al., 2000; Slawski et al., 2011). Normally, an increase of dry matter, crude protein and gross energy should occur during experimental feed application. When data of the

groups feed on diets rich in plant protein materials (especially PPC) were used in comparison to the standard control a generally expected development became visible: All PPC groups in the *first trial* were affected by the PPC inclusion. The LG groups showed slight decreased amounts of dry matter, crude protein and crude lipid as well as a slight decreased amount of gross energy in the whole body composition. The HG groups were characterized by a stronger decrease of these composition parameters. The reduction of dry matter and crude lipid has to be emphasized. With regard to the initial body composition of the experimental individuals a reduction of the amounts of body nutrients were detected. It was concluded that the supply or rather undersupply with feeds (decreased feed intake) and nutrients provoked an unbalanced body composition with decreased nutrient amounts. Individuals of the LG group were not able to increase the amount of dry matter and other nutrients. The offered feeds were only sufficient to meet the maintenance needs of the individuals. A slight growth could be realised, but no sustainable and substantial increase of body energy and nutrient buffers. This was necessary for high quality aquaculture products as well as for animal welfare aspects. The HG groups showed an extremely negative change from the ideal and good constitution. All body nutrients decreased, with the exception of crude ash. This was highly indicative for long-term starvation processes and the use of body reserves for the maintenance requirements of the individuals (Glencross et al., 2006). The hepatosomatic index and the Fultons condition factor represented the same trend. The inclusion of PPC in the diet decreased feed intake and affected the nutrient availability which was necessary for increase and a good constitution of whole body composition. Metabolic processes compensated deficiencies by the use of body reserves (decrease of body substance). The Fultons condition factor (FCF) as proportion of body weight against body length (gives information about the amount of incorporated nutrients in the body substance) decreased with PPC inclusion in the *first trial*. In the control group this FCF laid at 1.2, the LG groups revealed values slightly above 1.0 and the HG groups exhibited values around 0.85. The decrease of the FCF was in line with the overall decreased feed intake and utilization and suggested that especially the individuals of the HG groups had an extreme lack of body substance in contrast to their length. A fish within such a malnourished condition had problems to withstand against environmental problems (e.g. pathogens) and was minder or not marketable.

Congruently with increased palatability and feed intake of the diets in the *continuing examinations* the overall body constitution of the most treatments revealed nutritional values comparable with the well-nourished status described above. It was stated that the varying

combinations of fish meal, plant protein sources and feed attractants were able to provide the demanded nutrients and energy for metabolism and growth.

Two exceptions must be reminded: The mussel meal groups in the *second trial* revealed decreased dry matter, crude lipid and gross energy contents in the whole body composition. The slightly restricted feed utilization and thereby nutrient supply was confirmed by the reduced amount of fat deposits in the whole body composition of these experimental groups. The reasons for that were hardly understood. A possible explanation for that could be the increased amount of ash in the MM diets. The second observation was made in the PP100 group in the *fourth* experiment. The ash content of this group with total fish meal replacement and high plant protein proportions was significantly reduced. As described in the paragraphs before, a slightly decreased amount of phosphorous and simultaneously a slightly increased amount of phytic acid provoked an insufficient supply with phosphorous. This nutrient was demanded for various tissues structures and metabolic processes. A decreased supply with phosphorous resulted in both reduced growth and ash contents (Skonberg, 1997; Francis et al., 2001).

The replacement of fish meal by PPC, other plant protein sources, feed attractants and binding agents could be realized without negative impacts on the whole body compositions. Even through a total replacement could be arranged, first negative impacts occurred below a residual fish meal amount of 130 g kg⁻¹. It should be concluded, that the whole body composition was highly influenced by the amount of eaten diet particles in the *first, second* and the *third experiment*. In the *last trial* a slight nutrient dependent effect was documented at the highest level of fish meal replacement (PP100).

For the sake of completeness the blood plasma composition should be mentioned. The scientific approach of these parameters was set on an illustration of digestion and nutritional transport in the blood. The literature works with optimal values for hematocrit at approximately 30% of the whole blood (Wells and Weber, 1991). This factor describes oxygen transport capacity of the blood. A decreased amount could be indicative for undersupply and/or hemolytic processes due to toxic substances (Wells and Weber, 1991; Friedman, 2006). The level of blood plasma nutrients were determined and used to indicate level of nutrients supply. Congleton and Weber (2006) determined levels for plasma protein (35.9 mg ml⁻¹), plasma triglycerides (3.05 mg ml⁻¹) and plasma glucose (0.85 mg ml⁻¹) in well-nourished rainbow trout in starvation and non-starvation experiments. The fish from the presented *experimental setups 2 – 4* and the values of the control group from *trial 1* ranged in all cases around these values which were defined as optimum. This documentation displays

the trend generally observed in all trials and indicates good nutritional supply via feeds (Slawski et al., 2011). Though, two facts seem problematic concerning the explanatory power of these parameters. Firstly, the nutritional values showed only a cutting of the day of examination and these values were highly dependable on incalculably factors (e.g. time to the last feeding, environmental stressors, day time variations). Secondly, a very high variance of the measured data within the groups mostly resulted in an insignificance of the data (some trends only suspected, but not calculable). These findings lead to the conclusion that blood plasma parameter only could act as confirmation of specific facts but not for a sole explanation of conditional status and nutritional processes. One exception to this showed the data of the PPC groups in the *first trial*. The malnutrition and strong starvation processes in the most individuals was clearly seen and significantly decreased in all blood plasma parameter. For these cases must be recognized that the duration of decreased feed intake was long enough that clear detectable parameters were developed.

As closing parameter for the health status the overall histological aspects should be discussed and concluded. It should only be discussed briefly in contrast to the extensive description and discussion in the *first chapter*. In general is to say that the histopathological observations of the control group act again as a standardized model for healthy and well-nourished rainbow trout. The findings were confirmed by descriptions from literature (Glencross et al., 2006; Yamamoto et al., 2010). The inclusion of LG-PPC in the diets resulted in slight alterations in the liver and the anterior intestine. These alterations were indicative for malnutritive processes as well as for some interactions of antinutritional substances which were hardly understood. Especially the increased amount of vacuolization of the liver tissues and anterior intestines (additional: irregular shape of tissue structures) were indicative for that (Baeverfjord and Krogdahl, 1996; Burrells, 1999). Congruently, a direct toxicity of the glycoalkaloids has to be decline due to missing of inflammatory tissue areas and destructed cell compartments. But it was obvious that structural changes directly influenced nutrient digestion and absorption (Van den Ingh et al., 1991; Burrells, 1999; Refstie and Tiekstra, 2003). Furthermore, the structural reduction of tissue layers in the gastrointestinal tract and the strong hypotrophy of the liver tissues in the HG groups were highly correlated with starvation processes. Comparable observations were made in rainbow trout fed on diets with high alkaloid contents (gramine) resulted in rejection of the diet particles and starvation processes (Glencross et al., 2006). The increased feed intake and increased nutritional supply in the *following examinations* led to the expectation that

histological structures were not affected anymore. In the *following examinations*, further histopathological observations were declined.

Processing / Particle quality

The general product description defined potato protein concentrates as fine grained powder. This physical property influenced the pressing quality and stability of the feed particles by reduced binding ability. The diet particles from the *first trial* showed increased instability against water contact with an increased amount of PPC in the diet. Leaching processes of the particles increased as well. Carboxymethylcellulose (CMC) (20 g kg⁻¹) and wheat gluten (20 g kg⁻¹) were used as binding agents but without sufficient effects especially at the highest PPC inclusion level. As a consequence the use of CMC was dismissed and the initial amount of wheat gluten was increased to 60 g kg⁻¹ in the *second trial*. All diets showed good results in this and the following trials and the handling during feeding time was enhanced for the fish and also the person who applied the diets in the tanks. Best particle quality was shown in the blood meal groups. The animal derived raw material with its high protein content, exhibited excellent binding facilities in diets. Also an increasing amount of wheat gluten (*chapter three*) revealed positive results for use. The ability to curing under the influence of pressure and heat (functional principle of the present used fodder press) was highest in WG19. In the last trial (*chapter four*) the properties of blood meal (80 g kg⁻¹) and wheat gluten (60 g kg⁻¹) were effectively used to produce both hard and water stable feed particles. This was necessary to ensure that all applied nutrients were swallowed in by the individuals and no leaching of nutrients would take place. Additionally, the leaching was responsible for a further disposal of glycoalkaloids from the particles into the surrounding area with the known and discussed effects.

The best processing results were reached between pressing temperatures of 55 and 70°C in the fodder press. Below 55°C no binding processes took place within the raw materials. Above 70°C the raw materials dried out and pressing became impossible to realise. In further investigations should be proofed if extruding of the diets enhances the processing and overall quality of PPC based feeds.

Potential of PPC, WG and varying protein combinations as organic feed ingredients

The lack of adequate raw materials for organic aquaculture diets for carnivorous fishes with enhanced protein demand for growth is the bottleneck for a sustainable increase of production (Mente et al., 2011). The organic legislation of the European Union claims the use

of organic certified fish meal as protein source which should be mainly sourced from fish from certified aquaculture or trimmings (European Union, 2009). At the moment there are barely enough raw materials available from these origins to meet those organic aqua feed production demands. Furthermore, the production of certified fish for the use as fish meal or fish oil in certified aquaculture diets is unsustainable and should be rejected as potential source (IFOAM, 2010). As a solution for the problematic deficiency and sustainability alternative protein sources (plants and terrestrial animals) should be focused (Mente et al., 2011). The positive evaluated feed ingredients and processing methods of feed ingredients for organic aquaculture diets were listed in the ANNEX V of the Commission Regulation (EC) 710/2009 (European Union, 2009). Potato protein concentrates met the processing requirements (steam extraction and precipitation) claimed from organic associations and legislation. They provided an AA composition which was appropriate to the metabolic demand of rainbow trout (Tacon and Jackson, 1985). Therefore a supplementation with free AA can be avoided as it is forbidden in organic aquaculture. The nutrient composition of this raw material seems ideally suited for the use in aquaculture diets.

Congruently, the first results (*chapter one*) were disenchanting about the overall potential of PPC as feed ingredient (regardless the organic market). Although a highly purified PPC was used, in four experimental diets a decreased feed intake, slow growth and unacceptable feed conversion were documented. The reasons for that were discussed in the paragraphs above. A PPC application (268 g kg^{-1}) was realized at a stable replacement level of 56% of the fish meal protein in the *second trial*. This became possible by the use of a higher amount of organic certified wheat gluten. This wheat gluten was of minor nutritional quality (AA composition). At an inclusion level of 60 g kg^{-1} though, the AA composition of the diets was nearly unaffected. The used feed attractant blood meal showed improved feed intake but is prohibited from an organic point of view (not listed in the ANNEX V) (European Union, 2009). Before recommending the product as feed additive in organic diets, blood meal as raw material has to be certified by legislation and organic associations. Nevertheless, this protein source revealed qualities as feed attractant as well as binding agent in the feed particles. Opposed to that, wheat gluten had also the potential to be used in increased amounts in the organic diets for rainbow trout (*chapter three*). This plant protein source is characterized by a lower price in contrast to the PPC and a higher market availability. The results of the *third trial* showed that varying combinations of wheat gluten and PPC were able to substitute 60% of fish meal. The residual amount of fish meal in the diets laid at 265 g kg^{-1} . In combination with the used fish oil (90 g kg^{-1}) in the diets the overall remaining amount of animal products

in the feed ration comprised 40%. This amount is claimed by the European legislation for organic aquaculture of carnivorous fish species (European Union, 2009).

References

- Baeverfjord, G., Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *J. Fish Dis.* 19 (5), 375–387.
- Bureau, D., Harris, A., Cho, C., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 180 (3-4), 345–358.
- Burrells, C., 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. *Vet. Immunol. Immunopathol.* 72 (3-4), 277–288.
- Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. *J. Fish Biol.* 69 (2), 473–490.
- Davies, S., Morris, P., Baker, R., 1997. Partial substitution of fish meal and full-fat soya bean meal with wheat gluten and influence of lysine supplementation in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Research* 28 (5), 317–328.
- Dias, J., Gomes, E., Kaushik, S., 1997. Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquat. Liv. Res.* 10 (6), 385–389.
- European Union, 2009. Commission Regulation (EC) No 710/2009 of 5 August amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. *Off. J. Europ. Union* (52), 15–34.
- Francis, G., Makkar, H., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199 (3-4), 197–227.
- Friedman, M., 2006. Potato Glycoalkaloids and Metabolites: Roles in the Plant and in the Diet. *J. Agric. Food Chem* 54 (23), 8655–8681.
- Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingharn, M., Sipsas, S., 2006. The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture* 253 (1-4), 512–522.
- Hara, T., Kitada, Y., Evans, R., 1994. Distribution patterns of palatal taste buds and their responses to amino acids in salmonids. *Journal of Fish Biology* 45 (3), 453–465.

- Hara, T.J., 2006. Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *J Fish Biology* 68 (3), 810–825.
- IFOAM, 2010. Organic Aquaculture. EU Regulations (EC) 834/2007, (EC) 889/2008, (EC) 710/2009. BACKGROUND, ASSESSMENT, INTERPRETATION. International Federation of organic Agricultural movements.
http://www.ifoam.org/about_ifoam/around_world/eu_groupnew/positions/publications/aquaculture/IFOAMEU_IAMB_organic_aquaculture_dossier.pdf. Accessed 17 March 2011.
- Jensen, P.H., Jacobsen, O.S., Henriksen, T., Strobel, B.W., Hansen, H.C.B., 2009. Degradation of the Potato Glycoalkaloids – α -Solanine and α -Chaconine in Groundwater. *Bull. Environ. Contam. Toxicol.* 82 (6), 668–672.
- Jeroch, H., Drochner, W., Simon, O., 1999. Ernährung landwirtschaftlicher Nutztiere: Ernährungsphysiologie, Futtermittelkunde, Fütterung. Ulmer, Stuttgart, 544 S.
- Jones, K.A., 1989. The palatability of amino acids and related compounds to rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biology* 34 (1), 149–160.
- Kaushik, S., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 230 (1-4), 391–404.
- Kikuchi, K., 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179 (1-4), 3–11.
- Li, X., Rezaei, R., Li, P., Wu, G., 2011. Composition of amino acids in feed ingredients for animal diets. *Amino Acids* 40 (4), 1159–1168.
- Lokra, S., Helland, M., Claussen, I., Straetkvern, K., Egelanddal, B., 2008. Chemical characterization and functional properties of a potato protein concentrate prepared by large-scale expanded bed adsorption chromatography. *LWT Food Sci. Technol.* 41 (6), 1089–1099.
- Mackie, A., Adron, J., Grant, P., 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). *J. Fish Biol.* 16.
- Mente, E., Karalazos, V., Karapanagiotidis, I., Pita, C., 2011. Nutrition in organic aquaculture: an inquiry and a discourse. *Aquaculture Nutrition*, no.
- Millamena, O., 2002. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. *Aquaculture* 204 (1-2), 75–84.
- Moyano, F., Cardenete, G., La Higuera, M. de, 1992. Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquat. Liv. Res.* 5 (1), 23–29.

- Papatryphon, E., Soares, J., 2000. The effect of dietary feeding stimulants on growth performance of striped bass, *Morone saxatilis*, fed-a-plant feedstuff-based diet. *Aquaculture* 185 (3-4), 329–338.
- Papatryphon, E., Soares, J., 2001. Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, *Morone saxatilis*. *Aquaculture* 202 (3-4), 279–288.
- Refstie, S., Tiekstra, H., 2003. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 216 (1-4), 283–298.
- Rodehutsord, M., Borchert, F., Gregus, Z., Pack, M., Pfeffer, E., 2000. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. *Aquaculture* 187 (1-2), 163–176.
- Skonberg, D., 1997. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 157 (1-2), 11–24.
- Slawski, H., Adem, H., Tressel, R.-P., Wysujack, K., Koops, U., Kotzamanis, Y., Wuertz, S., Schulz, C., 2011. Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquacult Int.*
- Tacon, A., Jackson, A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds, in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), *Nutrition and feeding in fish*. Academic Press, London ;, Orlando, pp. 119–145.
- Van den Ingh, T., Krogdahl, A., Olli, J., Hendriks, H., Koninkx, J., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture* 94 (4), 297–305.
- Wells, R.M.G., Weber, R.E., 1991. Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. *J. Fish Biol.* 38 (1), 53–65.
- Xie, S., Jokumsen, A., 1997a. Replacement of fish meal by potato protein concentrate in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum): growth, feed utilization and body composition. *Aquacult. Nutr.* 3 (1), 65–69.
- Xie, S., Jokumsen, A., 1997b. Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquacult. Nutr.* 3 (4), 223–226.
- Xie, S., Jokumsen, A., 1998. Effects of dietary incorporation of potato protein concentrate and supplementation of methionine on growth and feed utilization of rainbow trout. *Aquacult. Nutr.* 4 (3), 183–186.

- Yamamoto, T., Iwashita, Y., Matsunari, H., Sugita, T., Furuita, H., Akimoto, A., Okamatsu, K., Suzuki, N., 2010. Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 309 (1-4), 173–180.
- Yamashita, S., Yamada, T., Hara, T.J., 2006. Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *J Fish Biology* 68 (3), 783–800.

General Summary

The replacement of fish meal (FM) is one of the major challenges in aquaculture. Whereas in conventional aquaculture the main impulse for a reduction of FM is most frequently the high price of the raw material due to depletion of wild fish stocks (decreased raw material supply). The main impulse to replace fish meal in organic fish production is furthermore situated in a lack of adequate amounts of this raw material. But in contrast to the conventional production only a few alternative raw materials were certified for use. Especially plant burden meals rich in protein are lack or of a weak quality for carnivorous fish nutrition. Nevertheless, these ingredients are necessary to create a sustainable increase of organic production. In this scientific work two plant burden protein sources were used to investigate their potential for use in organic aquaculture diets.

In *chapter 1*, potato protein concentrates (PPC) of varying qualities as FM replacement in organic rainbow trout (*Oncorhynchus mykiss*) nutrition were evaluated. Palatability, digestion and adsorption effect were affected at higher PPC incorporation. It became obvious that a PPC inclusion was highly dependent on the quality. Thereby, the quantity of glycoalkaloids and other antinutritional substances as well as the substitution applied was strongly influencing the feed intake processes. It should be stated that only a highly purified PPC (low in glycoalkaloid content) was able to provide a high quality in dietary AA composition. The palatability of the feed particles is a major obstacle that has to be overcome by a further reduction of glycoalkaloid contents or an addition of appetizers. The highly purified PPC exhibited the potential to fill the gap regarding alternative protein sources substituting FM in diets according to organic guidelines. To ensure increased and stable high plant protein utilization in organic aquaculture diets additional research has to be done.

Therefore, the feeding trial in *chapter two* was carried out with rainbow trout. An evaluation of different feed attractants in aquaculture diets upon FM replacement by PPC at 50% and low glycoalkaloid content (7 mg kg^{-1}) took place. Two fish meal based diets - one with 7 mg kg^{-1} synthetic glycoalkaloids (GFM) and one control without any glycoalkaloids (FM), a PPC control without additional attractants (PP), and six PPC diets with blood meal (BM 4 or 8 %), mussel meal (MM 4 or 8 %) and a free amino acid mixture (AM 0.5 or 1.0 % alanine, glycine and betaine) as feeding stimulant were used. Concluding should be stated, that any PPC inclusion in the experimental diets resulted in reduced feed intake and growth in comparison to the FM control. Additionally, all PPC groups showed decreased growth when compared to the GFM group fed at comparable glycoalkaloid concentrations. Therefore, it

was suggested that the antinutritional effect of the glycoalkaloids on the palatability of the diets were compensated by the strong feed attractant FM. Furthermore, it should be supposed that next to the antinutritional effect of the glycoalkaloids further antinutritional substances and their metabolites were still present in the PPC diets. Nevertheless, the protein utilization within all PPC groups was at an acceptable high level. The highest daily feed intake (DFI) was observed in the FM and GFM group, but reduced in all PPC groups. Among the attractants used in the trial, BM and AM exhibited highest DFI. The good nutritional status of all treatments was used as indicator for adequate supply with nutrients and did not reveal severe health impact in general.

In *chapter 3*, wheat gluten (WG) was examined as a plant burden raw material which does not require complex and costly purification as described for potato protein concentrate processing. Additionally, wheat gluten as protein source can be certified and thus may be utilized in aqua feeds according to aquaculture guideline EC 710/2009. Therefore, the *third* feeding trial was conducted to evaluate the effect of various combinations of WG and PPC in nutrition of rainbow trout (*Oncorhynchus mykiss*). Seven diets were formulated to contain varying levels of WG and PPC (constant FM replacement level of 56% on the protein level) and one fish meal diet served as control. Concluding was stated, that a substitution of FM (56%) by varying combinations of WG and PPC was positively realized in diets for rainbow trout. All parameters of feed intake, feed utilization and growth were unaffected within all treatments. Regarding the metabolic and energetic demand of the individuals, it became obvious that the provided diets were able to offer the demanded nutrients and energy. This became visible in the unaffected body composition of the individuals. Finally, both ingredients should be recommended as good alternative proteins sources for organic aquaculture diets not only from a legislation point of view but also from a nutritional. Additionally, the substitution of fish meal assures sustainable use of aquatic resources in the context of depleting global fish stocks. Furthermore, substituting PPC with cheap wheat gluten had a mayor economic benefit.

In *chapter four*, the potential of PPC as total FM substitute was evaluated in rainbow trout (*Oncorhynchus mykiss*) nutrition. Five diets were formulated by partial replacement of fish meal with PPC and one experimental diet without PPC acted as control group. Soy bean meal and wheat gluten were used as basal protein sources in the feed mixtures. For the increase of palatability and particle stability of the diets, a free amino acid mixture and blood meal were utilized in constant amounts (as determined in *chapter 2*). The feed intake within all groups was not negatively affected compared to control. Above a fish meal replacement of 60% feed

conversion and growth performance became significantly reduced compared to the control. The good nutritional status (body composition, blood parameters) of all groups did not reveal severe health impact of PPC in general. The use of feed attractants suppressed the repellent effect of high amounts of PPC and other plant protein sources in the diets. A combination of PPC and further plant protein sources should be recommended for use up to a residual fish meal amount of 134 g kg⁻¹ in organic aquaculture diets for carnivorous fish.

The results of all presented experiments showed that the use of certified raw materials in organic aquaculture diets was highly dependent from varying quality parameters of these ingredients. The initial problems with PPC were reduced by the use of several ingredients to increase the attractiveness, stability and digestibility of the diets. Furthermore, by the use of higher amounts of wheat gluten in PPC diets the price became more economic, which is necessary for a wider use in the aqua feed industry (organic or conventional). Both protein sources exhibited a great potential to be used as fish meal replacement in organic aquaculture diets for rainbow trout.

Zusammenfassung

Der Austausch von Fischmehl (FM) in Fischfuttermitteln ist eine der größten Herausforderungen für die Aquakultur. In der konventionellen Produktion liegt der stärkste Anreiz für eine Reduzierung des FM-Anteils im steigenden Preis dieses Rohstoffs. Durch Quotierung und Rückgang der Fangmengen und steigende Produktionskosten nimmt der Preis ständig zu. Um tragbare Preisniveaus zu halten, werden (günstigere) alternative Rohstoffe in der Futtermittelherstellung eingesetzt. In der biologisch-organischen Aquakultur übt der Mangel an zertifiziertem FM für die Futtermittelherstellung den stärksten Druck auf die Suche nach neuen Futterproteinquellen aus. Im Gegensatz zur konventionellen Produktion stehen hier sehr wenige zertifizierte bzw. zertifizierungsfähige Rohstoffe zur Verfügung. Zusätzlich weisen diese oft noch reduzierte Qualitäten für die Ernährung von karnivoren Arten auf. Dennoch sind diese Rohstoffe erforderlich, um eine Steigerung in der ökologischen Aquakultur zu realisieren. Um die Lücke im Rohstoffbedarf zu reduzieren, wurden in dieser Arbeit zwei pflanzliche Proteinquellen verwendet, um ihr Potential für den Einsatz in Ökofischfuttermitteln zu ermitteln.

Im *Kapitel 1* wurden konzentrierte Kartoffelproteinkonzentrate (PPC) unterschiedlicher Qualität als Fischmehlersatz in biologisch-organischen Futtermitteln für Regenbogenforellen (*Oncorhynchus mykiss*) untersucht und ausgewertet. Der Geschmack, die Verdaulichkeit und Resorption der Nährstoffe wurden bei höheren PPC Gehalten stark beeinflusst. Weiterhin wurde aufgezeigt, dass die PPC Verwendbarkeit von der Rohstoffqualität abhängig ist. Vor allem der Gehalt an Glykoalkaloiden sowie anderen antinutritiven Inhaltsstoffen und das Substitutionslevel selbst hatten den stärksten Einfluss auf die aufgenommene Futtermenge. Es wurde festgehalten, dass lediglich ein hochgereinigtes PPC (geringer Glykoalkaloidgehalt) die nutritiven Anforderungen erfüllte. Der Geschmack der Futtermittel und damit reduzierte Futterraufnahme war das größte Hindernis im Versuchsvorhaben. Durch eine weitere Reduktion der Glykoalkaloidgehalte im PPC oder eine Verwendung von geschmacksverstärkenden Inhaltsstoffen in den Futterpartikeln könnte dieses Problem gelöst werden. Generell zeigte das hochgereinigte Kartoffelproteinkonzentrat das Potential, die Lücke im Bereich der alternativen Proteinquellen als Fischmehlersatz zu schließen. Um einen hohen und stabilen Austausch mit diesem pflanzlichen Protein zu gewährleisten, sollten weitere Untersuchungen erfolgen.

Diesbezüglich wurde der Fütterungsversuch im *Kapitel 2* angesetzt. Es wurde eine Bewertung von verschiedenen geschmacksverstärkenden Inhaltsstoffen in Futtermitteln

vorgenommen. Bei diesen Futtermitteln wurde der Anteil an FM zu 50% durch PPC (geringer Glykoalkaloidgehalt von 7 mg kg^{-1}) ersetzt. Zusätzlich kamen 2 Fischmehl-basierte Futter zum Einsatz (ein Futter bei dem 7 mg kg^{-1} synthetische Glykoalkaloide hinzugesetzt wurden (GFM)) und ein Fischmehlkontrollfuttermittel (FM) ohne weitere Zusatzstoffe. Weiterhin wurde ein PPC Futter verwendet, bei dem keine weiteren Inhaltsstoffe (PP) und sechs PPC Futtermittel denen Blutmehl (BM 4 oder 8%), Muschelmehl (MM 4 oder 8 %) oder ein Mix aus freien Aminosäuren (AM 0,5 oder 1 %) als Futterstimulanzien hinzugefügt wurden. Es konnte festgehalten werden, dass alle PPC Futtermittel eine geringere Futteraufnahme und Wachstum im Vergleich zu den FM Kontrollgruppen aufwiesen. Auch die Fischmehlgruppe mit zusätzlichen Glykoalkaloidgehalten (GFM) zeigte bessere Wachstumsleistungen. Es wurde geschlussfolgert, dass der antinutritive Effekt der Glykoalkaloide auf den Geschmack des Futters durch den starken und intensiven Eigengeschmack des FM überlagert wurde. Darüber hinaus kann vermutet werden, dass in den PPC neben den Glykoalkaloiden weitere (nicht erfasste) antinutritive Inhaltsstoffe und deren Metabolite vorhanden sind und den Fisch wirken. Dennoch war auch in diesen PPC Futtermitteln die Proteinverwertung auf einem hohen Niveau. Die höchste tägliche Futteraufnahme (DFI) wurde in den Fischmehlgruppen aufgezeichnet und war in allen PPC Gruppen reduziert. Innerhalb der PPC Gruppen zeigten die Verwendung von Blutmehl und freien Aminosäuren die höchsten Futteraufnahmemengen. Generell zeigten alle Fütterungsgruppen einen guten Ernährungszustand, was als Indikator für eine gute und ausreichende Versorgung mit Nährstoffen gewertet werden kann und zeigt, dass die PPC keinen Einfluss auf die Tiergesundheit ausübten.

In Kapitel 3 wurde Weizenkleberprotein (WG) als weiterer pflanzlicher Rohstoff in der Forellenernährung untersucht. Im Gegensatz zu PPC ist der Herstellungs- und Verarbeitungsprozess weniger komplex und der Rohstoff damit preislich günstiger. Zusätzlich kann WG als Proteinquelle im Rahmen der EU Ökoverordnung EC 710/2009 zertifiziert werden und damit in Ökofuttermitteln eingesetzt werden. Der *dritte Versuch* wurde durchgeführt, um den Einfluss von verschiedenen Kombinationen an WG und PPC in der Ernährung von Regenbogenforellen zu untersuchen. Sieben Futtermittel mit verschiedenen Mischungsverhältnissen von WG zu PPC (FM-Austausch zu 56% auf der Proteinebene) und ein FM-Kontrollfuttermittel wurden hergestellt. Über den Versuchszeitraum wurde erfasst, dass eine Substitution von FM (56%) durch die verschiedenen Kombinationen von WG und PPC in der Forellenernährung mit positiven Ergebnissen realisiert werden konnte. Alle Parameter der Futteraufnahme, Futtermittelverwertung und Wachstum waren innerhalb aller Gruppen unbeeinflusst. Bei der Betrachtung des metabolischen und energetischen Bedarfs der

Versuchstiere konnte aufgezeigt werden, dass die Futtermittel die benötigten Nährstoffe und Energie liefern konnten. Auch die ausgewogene und artspezifische Ganzkörperzusammensetzung deutete auf eine bedarfsgerechte Ernährung hin. Beide verwendeten pflanzlichen Rohstoffe erhielten abschließend, eine Empfehlung für die Verwendung in ökologisch-organischen Fischfuttermitteln aus Sicht der gesetzlichen Rahmenbedingungen und auch nach ernährungsphysiologischen Gesichtspunkten. Darüber hinaus kann festgehalten werden, dass ein Austausch von Fischmehl in den Futtermitteln sicherstellt, dass die aquatischen Ressourcen im Kontext der zunehmenden Überfischung der Meere nachhaltiger genutzt werden können. Darüber hinaus hat der Rückaustausch von PPC durch den preiswerteren WG einen wirtschaftlichen Nutzen.

In einem abschließenden Versuchsvorhaben (*Kapitel 4*) wurde wiederholt das Potential von PPC als Totalfischmehlsubstitut in der Forellenernährung untersucht. Es wurden 5 Futtermittel unter sukzessivem Austausch von FM durch PPC hergestellt und ein Futtermittel ohne PPC diente als Kontrollgruppe. Sojabohnenmehl und WG wurden als basale Proteinquellen in den Futtermischungen zugesetzt. Zur Steigerung des Geschmacks und der Partikelstabilität in den einzelnen Diäten wurden freie Aminosäuren und Blutmehl hinzugefügt (wie in *Kapitel 2* ermittelt). Die Futteraufnahme war im Vergleich zur Kontrollgruppe in keiner weiteren Versuchsgruppe negativ beeinflusst. Oberhalb einer FM-Substitution von 60% wurden die Futteraufnahme und Wachstumsleistungen signifikant eingeschränkt. Dennoch zeigten alle Gruppen einen guten und tiergesundheitslich guten Gesamtzustand. Aus den Ergebnissen konnte abgeleitet werden, dass eine Kombination von PPC und weiterer Pflanzenproteine eine gute Alternative zu Fischmehl in organisch-biologischen Futtermitteln für karnivore Arten darstellt. Dabei sollte ein Restanteil an Fischmehl von 134 g kg^{-1} Futtermittel enthalten bleiben.

Die Ergebnisse der hier präsentierten Experimente zeigten, dass die Verwendung von zertifizierten bzw. zertifizierfähigen Rohstoffen in biologisch-organischen Aquakulturfuttermitteln im hohen Maße von variierenden Qualitätsmerkmalen dieser Inhaltstoffe abhängig war. Die anfänglichen Probleme mit PPC in den Futtermitteln wurden durch die Verwendung von verschiedenen Zusätzen zur Steigerung der Attraktivität, Stabilität und Verwertbarkeit der Futterpartikel reduziert. Weiterhin konnte durch die Verwendung von höheren Anteilen an WG in PPC Futtermitteln ein ökonomisch günstigerer Herstellungspreis eingestellt werden, was für eine Nutzung in der Futtermittelindustrie (biologisch-organisch oder konventionell) notwendig ist. Beide Proteinquellen zeigten ein gutes Potential als Alternative zum FM in biologisch-organischen Futtermitteln für Regenbogenforellen.

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