Spirulina (*Arthrospira platensis*) als alternative Proteinquelle für Fische

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Auswirkungen eines vollständigen Austausches von Fischmehl auf Wachstum, Mikrobiom, Produktqualität und Konsumentenakzeptanz

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Widmung

Diese Arbeit widme ich meinem noch ungeborenen Kind.

Vorwort

Die vorgelegte Dissertation ist aus einem geförderten Projekt des Ministeriums für Wirtschaft und Kultur mit dem Arbeitstitel „Sustainable Trout Aquaculture Intensification“ (SusTAIn) entstanden. Das Projekt hatte das Ziel eine genetische Anpassung an sich wandelnde Futtermittelressourcen zu erforschen. Durch die Intensivierung der Aquakultur und dem Bestreben nach einer nachhaltigeren Produktion wurde das Potenzial von Mikroalgen (*Arthrospira platensis*) und Insekten (*Hermetia illucens*) als alternative Proteinquelle zu Fischmehl untersucht. Unterschiedliche Regenbogenforellenstämme (*Oncorhynchus mykiss*) wurden dabei auf ihre genetische Variabilität für deren Adaptationsfähigkeit an diese alternativen Proteinquellen untersucht. Die Forschungsergebnisse sollen für eine nachhaltigere, tier- und umweltgerechtere Intensivierung der Aquakultur genutzt werden.

Alle Teilprojekte dieser Arbeit wurden unterstützt durch Ressourcen des SusTAIn-Projekts und durch die Arbeitsgruppen Functional Breeding, Produktqualität tierischer Erzeugnisse und Aquakultur des Departements für Nutztierwissenschaften der Universität Göttingen.

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Liste der Publikationen

Folgende Publikationen sind in dieser Dissertation enthalten:

Paper I:

Rosenau S., Oertel E., Mott A. C., Tetens J. (2021). The Effect of a Total Fishmeal Replacement by *Arthrospira platensis* on the Microbiome of African Catfish (*Clarias gariepinus*). Life, 11:1-15. DOI: 10.3390/life11060558

Paper II:

Rosenau S., Oertel E., Dietz C, Wessels S., Tetens J., Mörlein D., Ciulu M. (2021). Total Replacement of Fishmeal by Spirulina (*Arthrospira platensis*) and Its Effect on Growth Performance and Product Quality of African Catfish (*Clarias gariepinus*). Sustainability, 13:1-19 DOI: 10.3390/su13168726

Paper III:

Rosenau S., Ciulu M., Reimer C., Mott A. C., Tetens J., Mörlein, D. (2022). Feeding green: Spirulina (*Arthrospira platensis*) Induced Changes in Production Performance and Quality of Salmonid Species. Aquaculture Research, 00:1-12. https://doi.org/10.1111/are.15925.

Paper IV:

Rosenau S., Wolgast T., Altmann B., Risius A. (2022). Consumer Preferences for Altered Fillet Color in Rainbow Trout (*Oncorhynchus mykiss*) Induced by Spirulina (*Arthrospira platensis*). Eingereicht am 11.07.2022 in Aquaculture (Elsevier)

Abkürzungsverzeichnis

ASC	alternativ-spezifische Konstante
ANF	antinutritive Faktoren
ANOVA	Varianzanalyse
BHA	Butylhydroxyanisol
CAT	Katalase
cDNA	komplementäre Desoxyribonukleinsäure
CMC	Carboxymethylcellulose
COO	Herkunftsland
DCE	Discrete Choice Experiment
DHA	Docosahexaensäure
DMSO	Dimethylsulfoxid
DNA	Desoxyribonukleinsäure
EPA	Eicosapentaensäure
FAME/FAMEs	Fettsäuremethylester
FCR	Futterumsetzungsrate
FID	Flammenionisationsdetektor
FIFO	„fish in/fish out“
FM100	experimentelle Diät mit Fischmehlanteil
GC-FID	Gaschromatographie-Flammenionisationsdetektor
LEDs	Licht emittierende Dioden
MUFA	einfach ungesättigte Fettsäuren
n	Größe der Stichprobe
N-free extracts	Stickstoff-freie Extraktstoffe
NCBI	National Center for Biotechnology Information
NGS	Next-Generation Sequenzing
NRC	National Research Council
NSP	Nicht-Stärke-Polysaccharide
OTU	operationale taxonomische Einheit
PCA	Hauptkomponentenanalyse

PCA	Polymerase Kettenreaktion
PCoA	Hauptkoordinatenanalyse
PIT	passive integrierte Sender
PUFA	mehrfach ungesättigte Fettsäuren
Q-Q-plot	Quantile-Quantile-Plot
RAS	recirculating aquaculture system
RLP	random parameter logit model
SD	Standardabweichung
SGR	spezifische Wachstumsrate
SFA	gesättigte Fettsäuren
SOD	Superoxid-Dismutase
SP	Spirulina
SP100	experimentelle Diät mit vollständiger Fischmehlsubstitution durch Spirulina
WTP	Zahlungsbereitschaft
ω-3	Omega-3
ω-6	Omega-6
16S rRNA	16S ribosomal RNA

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1 Einleitung

In der gegenwärtigen globalen Situation, die geprägt ist von Klimawandel, Zerstörung von Ökosystemen sowie dem damit einhergehenden Artensterben und der übermäßigen Nutzung natürlicher Ressourcen, rückt das Thema Nachhaltigkeit immer weiter in den Fokus der öffentlichen Diskussion. Im Kontext der Notwendigkeit eine ausreichende Versorgung mit Nahrungsmitteln für die stetig wachsende Weltbevölkerung sicherzustellen, werden besonders im Bereich der Agrar- und Tierproduktion Ansätze für eine nachhaltigere Erzeugung diskutiert (Alexandratos und Bruinsma 2012). Wobei gerade der Aquakultursektor seit geraumer Zeit einer der am stärksten wachsenden Märkte ist (FAO 2020). In der Aquakultur steht die Fütterung der Fische in der Kritik, da ein Teil der Diäten aus Fischmehl- und öl besteht. Vor dem Hintergrund stagnierender Erträge der Fangfischerei (FAO 2020) und ökologischer Bedenken bzgl. nicht-selektiver und teilweise gewässerstruktur-schädigender Fangmethoden sowie dem hohen Bedarf an Energie und Wasser für die Fischmehlproduktion, erscheint die Suche nach geeigneten Proteinquellen für die Fischernährung unumgänglich (Hall 2010; García 2003; Little und Edwards 2003). Deshalb wird seit den 1950iger Jahren nach einem geeigneten Ersatz für Fischmehl geforscht (Becker 2007).

Bereits jetzt gibt es verschiedene pflanzliche und tierische Alternativen für Fischmehl auf dem Futtermittelmarkt. Viele dieser Ressourcen sind jedoch mit Nachteilen in Bezug auf die Verdaulichkeit, Wachstum und Fischgesundheit aufgrund unerwünschter Inhaltsstoffe verbunden, wodurch die Einsatzmenge eingeschränkt wird. Dazu kommt ein hoher Bedarf an direkter und indirekter Energie, um pflanzliche oder tierische Erzeugnisse zu produzieren (Troell et al. 2010). Statt diese Ressourcen direkt in die Humanernährung einfließen zu lassen, erfolgt eine weitere Konversion mit zusätzlichen Umwandlungsverlusten.

Eine nachhaltige Alternative zu herkömmlichen Futterproteinen können Mikroalgen darstellen. Diese erreichen auf kleiner Fläche hohe Produktionskapazitäten und, je nach Anbausystem, mit sehr geringem Energieaufwand und Wasserverbrauch produziert werden (Singh und Sharma 2012). Bisher zeigt sich, dass Mikroalgen das Potenzial haben nachhaltiger als z.B. Fischmehl und andere konventionelle Proteinquellen zu sein (Draganovic et al. 2013).

Aus der Vielzahl von Mikroalgenarten hat sich vor allem Spirulina als vielversprechend für den Ersatz von Fischmehl herausgestellt. Spirulina ist ein mehrzelliges, fadenförmiges Cyanobakterium, welches sich nicht nur durch einen hohen Proteingehalt auszeichnet, sondern zudem ein ausbalanciertes Fettsäure- und Aminosäuremuster aufweist (Becker 2007). Außerdem ist Spi-

rulina als Mikroalge Teil der natürlichen Nahrungskette und somit für eine Vielzahl an Jungfischen eine Nahrungsgrundlage. Dementsprechend liegt es nahe Spirulina als alternative Proteinquelle in der Aquakultur zu nutzen, denn die Mikroalge besitzt nutritive Eigenschaften, welche sich sowohl auf das Wachstum, die Gesundheit der Fische, als auch auf die Produktqualität positiv auswirken können (Rosas et al. 2019; Alagawany et al. 2021). Wie viel Fischmehl durch Spirulina ersetzt werden kann, hängt stark von der genutzten Fischart, dem Alter der Fische und weiteren Faktoren ab (Rosas et al. 2019; Alagawany et al. 2021). Ein vollständiger Ersatz von Fischmehl durch Spirulina ist bisher meist bei cypriniden Arten untersucht worden (Nandeesha et al. 2001). Dementsprechend bleiben noch viele Fragen hinsichtlich der Einsatzmenge, der Nutzbarkeit für unterschiedliche Fischarten und der Auswirkung auf die Produktqualität ungeklärt.

Ziel der vorliegenden Dissertation ist die Untersuchung eines vollständigen Austauschs von Fischmehl gegen Spirulina für in Deutschland kommerziell genutzte Warm- und Kaltwasserfische. Für die Studien wurden Afrikanische Welse (*Clarias gariepinus*) und Tilapia (*Oreochromis niloticus*) als wichtige Warmwasserfische für Kreislaufanlagen in Deutschland ausgewählt (Statistisches Bundesamt 2022). Als Vertreter der Forellenteichwirtschaft wurden die nach Brämick (2019) am häufigsten produzierten salmoniden Arten, Regenbogenforelle (*Oncorhynchus mykiss*), Bachforelle (*Salmo trutta fario*) und Bachsaibling (*Salvelinus fontinalis*), genutzt. In mehreren Versuchen wird die Nutzbarkeit von Spirulina als vollständiges Fischmehlsubstitut und dessen Auswirkung auf die Wachstumsleistung und Umsetzungsrate der unterschiedlichen Fischarten untersucht und mit einer Kontrolldiät verglichen. Zusätzlich sollen Mikrobiomanalysen Aufschluss über mögliche Effekte von Spirulina auf die Zusammensetzung der Darmbakterien und damit einen Erklärungsbeitrag zu Unterschieden in der Verdaulichkeit leisten. Darüber hinaus werden auch ökonomische Kriterien wie die Produktqualität sowie die Verbraucherakzeptanz ermittelt.

Die Ergebnisse der vorliegenden Arbeit sollen einen Beitrag zur nachhaltigeren Gestaltung der Fischernährung leisten. Dabei werden sowohl die Chancen als auch Herausforderungen einer vollständigen Substitution von Fischmehl durch Spirulina diskutiert.

2 Literaturübersicht

2.1 Globale Aquakultur

Als Aquakultur wird die Aufzucht von Wasserorganismen wie Fischen, Weichtieren, Krebstieren und Wasserpflanzen bezeichnet, bei der wenigstens eine Phase des Wachstums bzw. ein Lebensabschnitt durch den Menschen kontrolliert wird (FAO 2021b). Die Produktion von aquatischen Organismen hat eine lange Tradition, ist jedoch erst im letzten Jahrhundert stark gestiegen (Abbildung 1). Seit 1970 wächst die Aquakulturproduktion um 7,5 % pro Jahr an und es wird prognostiziert, dass bis 2030 rund 109 Millionen Tonnen aquatische Organismen in der Aquakultur produziert werden (FAO 2020). 2018 waren es rund 82,1 Millionen Tonnen. Währenddessen stagniert der Anteil der Fangfischerei weitestgehend, da die Ozeane ihre Kapazitätsgrenze erreicht haben. Seit 1986 stagnierten dementsprechend die Fangerträge zwischen 86,9 – 96,4 Millionen Tonnen (FAO 2020).

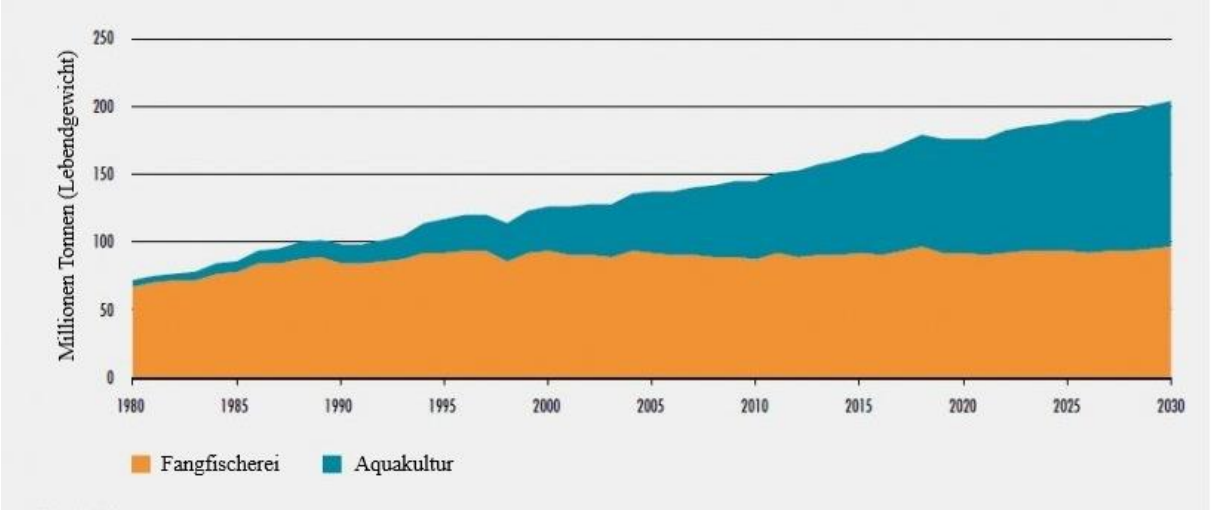

Abbildung 1. Weltweite Erträge der Fangfischerei und Aquakultur (1980 – 2030). Modifiziert nach FAO (2020).

Dennoch ist die weltweite Nachfrage nach Fisch weiter gestiegen und ein immer größer werdender Anteil der Bevölkerung in den Industrieländern kann sich die verhältnismäßig teuren Produkte leisten – ein Trend, der sich in den Entwicklungsländern fortsetzt (Delgado et al. 2003). Durch die hohe Nachfrage, insbesondere in Asien, zeigt sich hier nicht nur die größte Wachstumsrate der Aquakultur, sondern auch die weltweit höchste Produktionsmenge (FAO 2020). Dabei macht die Aquakulturproduktion in Asien insgesamt 89 % der weltweiten Produktion aus (FAO 2020). Monetär gesehen sind Norwegen und Vietnam nach China die größten

Exporteure von Fischerzeugnissen (FAO 2021a). Währenddessen stagniert der Aquakultursektor in Deutschland aufgrund von rechtlichen Vorgaben und dem Wettbewerb durch günstigere Produkte aus dem Ausland (BLE 2017).

Zu den weltweit am häufigsten produzierten Fischarten gehören hauptsächlich herbivore und omnivore Fischarten wie Graskarpfen, Silberkarpfen und Tilapia, doch monetär gesehen, machen karnivore Arten wie Lachse, Forellen und Welse ebenfalls einen großen Anteil aus (Cai et al. 2019). Außerdem steigt die Nachfrage nach karnivoren Fischarten weiter an (Bostock et al. 2010).

Begründet durch den starken Anstieg der Aquakulturproduktion ist auch die Nachfrage nach Fischmehl und -öl stark gestiegen. Diese Futtermittelkomponenten sind Hauptbestandteil des Fischfutters und bilden auch die Referenz für physiologische Nährstoff- und Ernährungsstandards. Rund 68,2 % des Fischmehls und 88,5 % des Fischöls werden in der Aquakultur verbraucht (Tacon und Metian 2008). Bis 2025 wird in der Aquakultur ein Futtermittelverbrauch von rund 73,2 Millionen Tonnen prognostiziert (Tacon und Metian 2008). Die benötigten Ressourcen stammen größtenteils aus dem Fang von Wildfisch und nur zu einem kleinen Anteil auch aus Schlachtabfällen der Fischverarbeitung (FAO 2020). Allerdings steht die Produktion von Fischmehl und -öl aufgrund verschiedener Nachhaltigkeitsbedenken stark in der Kritik. Dazu zählen die zunehmende Überfischung der Weltmeere sowie nicht nachhaltige und wenig selektive Fangmethoden, die ggf. die Gewässerböden schädigen, ein hohes Maß an Energie verbrauchen und zur Umweltverschmutzung beitragen (FAO 2020; Hall 2010; Lebreton et al. 2018; Little und Edwards 2003).

Die Preise für Fischmehl- und öl sind in den vergangenen Jahren stark gestiegen und werden voraussichtlich auch in näherer Zukunft weiter steigen (World Bank 2013). Die Gründe dafür sind vielfältig und liegen hauptsächlich in den stagnierenden Erträgen aus der Fangfischerei, steigenden Marktpreisen für pelagische Futterfische, Energie, Verarbeitung, Versand und Transport sowie den stagnierenden oder teilweise abnehmenden Export von Fischmehl- und öl (Tacon und Metian 2008). Zusätzlich übt die Zivilgesellschaft zunehmend Druck auf den Einzelhandel aus und fordert eine nachhaltigere Nutzung von aquatischen Ressourcen.

Insgesamt macht das Futter in der Produktion von aquatischen Organismen mit 50 – 70 % den größten Anteil der Betriebskosten aus (Rana et al. 2009). Aus diesem Grund wird vermehrt nach (kostengünstigeren) Alternativen für Fischmehl und -öl gesucht. Im Zeitraum von 1990 bis 2013 konnte am Beispiel von konventionellen Lachsfuttermitteln der Anteil von Fischmehl von über 65 % auf rund 18 % und Fischöl von 24 % auf rund 11 % reduziert werden, ohne

Einbußen in der Produktion zu verursachen (Ytrestøyl et al. 2015). Dieser Trend setzt sich auch bei einer Vielzahl anderer Fischarten weltweit fort (Tacon und Metian 2008). Substituiert werden die Futtermittelkomponenten hauptsächlich durch pflanzliche Proteine und Öle, aber auch Tiermehle von Landtieren können für die Ernährung von aquatischen Organismen eingesetzt werden. Allerdings zeichnet sich immer wieder ab, dass je nach Substitut nur ein gewisser Prozentsatz von Fischmehl- und öl austauschbar ist, da es sonst zu negativen Auswirkungen in Bezug auf das Wachstum und die Gesundheit der Fische kommen kann.

2.2 Alternative Proteinquellen in der Aquakultur

Fischmehl gilt aufgrund von ernährungsphysiologischen Eigenschaften als hochwertigste Proteinquelle für die Ernährung von aquatischen Organismen und zeichnet sich zudem durch eine hohe Schmackhaftigkeit, Verdaulichkeit und ein ideales Nährstoffprofil aus (Jannathulla et al. 2019). Trotz dieser Vorteile wird angesichts der zuvor genannten ökologischen und ökonomischen Problematik seit den 1950er Jahren vermehrt nach geeigneten alternativen Proteinquellen für die Ernährung von Fischen gesucht (Becker 2007). Dabei ist das Ziel die Menge an Fischmehl, die verwendet wird, um ein Gewichtsäquivalent an Wildfisch in ein Gewichtsäquivalent an Zuchtfisch umzuwandeln, also das sogenannte „fish in/fish out“ (FIFO), zu reduzieren.

Proteine tierischer Herkunft sind in den heutigen Diäten regelmäßig zu finden. Doch auch verschiedene pflanzliche Proteine haben sich bereits aufgrund ernährungsphysiologischer und wirtschaftlicher Aspekte in der Diät von aquatischen Organismen etabliert. Es ist anzumerken, dass nicht alle Proteinquellen für jede Fischart gleichermaßen verdaulich sind und der Proteinbedarf je nach Fischart zwischen 20 – 55 % (Ayadi et al. 2012) variiert. Bei Larven und Brut, insbesondere von karnivoren Arten, ist der Proteinbedarf erhöht (National Research Council 2011). Alternative Proteine müssen nicht nur den Nährstoffbedarf decken, sie sollten idealerweise auch bezahlbar und nachhaltig sein. Weitere wichtige Aspekte sind die Verfügbarkeit der Ressourcen sowie die anfallenden Transportkosten und die Lagerfähigkeit.

In den folgenden Unterkapiteln werden die wichtigsten alternativen Proteinquellen und deren Eignung für die Produktion von Fischen in Aquakultur beschrieben.

2.2.1 Tierische Proteinquellen

In der Aquakultur werden tierische Proteinquellen aufgrund ihrer hohen physiologischen Wertigkeit häufig eingesetzt. Nichtdestotrotz unterliegen tierische Proteine einer sehr hohen Konversion, da sie aus Nahrungsmitteln stammen, die direkt in die Humanernährung einfließen

könnten, stattdessen jedoch zunächst in der Tierernährung Anwendung finden (Salami et al. 2019). Allerdings entsteht der Großteil der Proteine aus Nebenerzeugnissen der Schlachtung sowie der Verarbeitung von Landtieren und sind daher häufig nicht mehr für den menschlichen Verzehr vorgesehen. Aus dem Grund sind diese Ressourcen in der Regel auch deutlich preiswerter als Fischmehl.

Zu den Landtierproteinquellen, die sich für die Substitution von Fischmehl eignen, gehören z.B. Mehle aus der Geflügelverarbeitung (Bureau et al. 1999; Rawles et al. 2006). Geflügelmehle zeichnen sich nicht nur durch eine hohe Verdaulichkeit aus (Yang et al. 2006), sie sind zudem auch noch günstiger als Fischmehl (Shapawi et al. 2007). Bei Doraden, Forellen, Streifen-, und Wolfsbarschen haben Untersuchungen gezeigt, dass Geflügelmehle bis zu 100 % des Fischmehls ersetzen können (Nengas et al. 1999; Takagi et al. 2000; Gaylord und Rawles 2005; Sealey et al. 2011; Dawson et al. 2018).

Andere Nebenprodukte der tierischen Verarbeitung sind Fleisch- und Knochenmehle. Diese zeichnen sich ebenfalls durch hohe Proteingehalte sowie ein ausgeglichenes Aminosäuremuster aus und sind zudem eine gute Quelle für Mineralien wie Phosphor und Calcium (Suloma et al. 2013). Nachteilig wirkt sich hingegen der hohe Aschegehalt im Mehl aus, welcher die Verwertung des Futters einschränkt (Bureau et al. 1999). Deshalb können Fleisch- und Knochenmehle in der Regel nur zu Anteilen von 20 – 45 % das Fischmehl ersetzen (Lee et al. 2012; Ai et al. 2006; Bureau et al. 2000; Esmaeili et al. 2017).

Blutmehl ist ebenfalls ein Nebenprodukt, welches bei der Schlachtung von Landtieren entsteht und zeichnet sich durch eine hohe Proteinqualität aus. Zusätzlich stellt es eine reichhaltige Quelle für Lysin dar (El-Haroun und Bureau 2007). Dennoch ist meist nur ein geringer Austausch von Fischmehl ohne Wachstumseinbußen möglich (Aliu und Dako 2018; Kirimi et al. 2016).

Krillmehl wird bereits in vielen kommerziellen Diäten für Fische angewendet und zeichnet sich durch einen hohen Proteingehalt von 33 -55 % und moderaten Fettgehalt von 15 – 20 % (Hardy und Brezas 2022) mit hohem Anteil an essentiellen Fettsäuren aus (Storebakken 1988). Besonders interessant ist diese Ressource für salmonide Arten, denn Krillmehl kann bis zu 60 % des Fischmehls ersetzen ohne negative Auswirkungen auf die Wachstumsleistung und Futterumsetzung zu haben (Rungruangsak-Torrissen 2007) und wird zusätzlich aufgrund des hohen Karotinoidgehaltes (Storebakken 1988; Wei et al. 2019; Roncarati et al. 2011) als sog. „finishing feed“[1] eingesetzt, um die typische rote Färbung des Filets von Salmoniden zu erhalten (Mørkøre

[1] finishing feed bezeichnet die Fütterung einiger Monate/Wochen vor der Schlachtung.

et al. 2020). Für weitere Fischarten konnte eine gleichbleibende, oder sogar verbesserte Wachstumsleistung, durch den Austausch von Fisch durch Krillmehl im Futter nachgewiesen werden (Saleh et al. 2018; Yoshitomi et al. 2007; Shi et al. 2021; Choi et al. 2020). Zwar lässt sich mit Krillmehl ein größerer Anteil von Fischmehl ohne Einbußen reduzieren, wirklich nachhaltiger ist die Ressource durch den hohen Energiebedarf und der möglichen Umweltauswirkungen jedoch nicht (Draganovic et al. 2013).

Neuere tierische Proteinquellen, wie Insektenproteine, sind durch die Anpassung der Verordnung (EG) 999/2001 seit Mitte Juni 2017 in der europäischen Aquakultur zugelassen. Insektenmehle repräsentieren eine besonders nachhaltige Alternative zu Fischmehl und anderen Landtierproteinen, da die Produktion von Insekten deutliche ökologische Vorteile bietet (Smetana et al. 2016). Zu den wichtigsten weltweit eingesetzten Arten gehören schwarze Soldatenfliegen (*Hermetia illucens*), Mehlkäfer (*Tenebrio molitor*), Stubenfliegen (*Musca domestica*), Seidenspinner (*Bombyx mori*) und weitere Insektengruppen wie Heuschrecken, Grashüpfer, Termiten, Grillen und Käfer (Mousavi et al. 2020). Insektenmehle können je nach Art einen Proteingehalt von über 70 % aufweisen (Al-Qazzaz und Ismail 2016) und auch die meisten wichtigen Aminosäuren sind vorhanden (Henry et al. 2015). Des Weiteren sind Insekten reich an Mineralstoffen wie Phosphor, Calcium, Eisen, Magnesium, Zink und Selen (DeFoliart 1992; Finke 2002; Banjo et al. 2006; Schabel 2010; Rumpold und Schlüter 2013). Zahlreiche Studien hinsichtlich der Fütterung von Insektenprotein und deren Auswirkung auf die Wachstumsleistung von verschiedenen Fischarten wurden bereits durchgeführt. Dabei zeigte sich insbesondere, dass bei karnivoren Arten negative Effekte auf das Wachstum und die Futterumsetzungsrate (FCR) eintreten können (Gasco et al. 2016; Dumas et al. 2018; Tilami et al. 2020; Weththasinghe et al. 2021; Jeong et al. 2021; Ng et al. 2001; Katya et al. 2017; Wang et al. 2017). Andere Arten wie Tilapia (Tippayadara et al. 2021) und Zebrabärblinge (Fronte et al. 2021) zeigen hingegen auch bei einer vollständigen Substitution kein reduziertes Wachstum.

2.2.2 Pflanzliche Proteinquellen

In den meisten Formulierungen von Futtermitteln für aquatische Organismen werden bereits unterschiedlich hohe Gehalte an proteinreichen pflanzlichen Bestandteilen verwendet. Dies ist unter anderem abhängig von der Verfügbarkeit der einzelnen Ressourcen und ihrem Preis. Einschränkungen ergeben sich jedoch, wie bei anderen Nutztieren auch, durch das Aminosäureprofil und die enthaltene Menge an antinutritiven Faktoren (ANF). ANF sind Stoffe, welche die Verwertung von Nährstoffen aus der Nahrung einschränken. Zu den häufigsten ANF gehören z.B. Proteaseinhibitoren, Saponine, Tannine, Lektine, Oligosaccharide, Phytoöstrogene, Nicht-

Stärke-Polysaccharide, Phytate und antigene Verbindungen (Francis et al. 2001). Die biologische Wertigkeit der Pflanzenproteine ist deshalb abhängig von der Art der Verarbeitung, der Wahl des Extraktionsverfahrens sowie dem Zusammenspiel von verschiedenen enthaltenen ANF (Kaushik und Hemre 2008).

Seit diesem Jahrhundert wird Soja vermehrt in Fischdiäten, insbesondere für karnivore Arten, eingesetzt (Ytrestøyl et al. 2015), da die Ressource weltweit in großen Mengen verfügbar ist und zuverlässig als Fischmehlalternative genutzt werden kann. Sojamehl hat einen Proteingehalt von 44 %, während Sojaproteinkonzentrat einen Gehalt von 65 % aufweist (National Research Council 2011). ANF kommen hauptsächlich in Form von Trypsin-Inhibitoren, Saponinen, Lektinen und Oligosacchariden im Sojaprotein vor, haben einen negativen Einfluss auf die Verdaulichkeit und somit auf das Wachstum der Fische (Friedman und Brandon 2001). Insbesondere karnivore Fischarten wie Lachse können Darmentzündungen entwickeln, welche hauptsächlich durch Saponine, β-Conglycinin und Glycinin hervorgerufen werden (Buttle et al. 2001; Kumar et al. 2020; Zhang et al. 2013). Die meisten Untersuchungen zeigen, dass Soja zwar effizient einsetzbar ist, aber sich hohe Gehalte an Sojamehl oder Sojaproteinkonzentrat negativ auf die Wachstumsleistung von Fischarten auswirken (Chen et al. 2019; Kalhoro et al. 2018; Wu et al. 2015; Xu et al. 2012; Zhu et al. 2020; Deng et al. 2006). Generell lässt sich in den Studien feststellen, dass Sojamehle von Fischen ineffizienter verstoffwechselt werden können als Sojaproteinkonzentrate.

Für Deutschland können Rapserzeugnisse als regionale Alternative für Fischmehl, aber auch Sojaprotein eingesetzt werden. Bei der Rapsölgewinnung entsteht als Nebenprodukt Rapsölkuchen, welcher sich unbearbeitet, oder bearbeitet, einsetzen lässt. Rapsmehl zeichnet sich durch einen Proteingehalt zwischen 36 und 39 % aus (Enami 2010). Hingegen können Rapsproteinkonzentrate deutlich höhere Gehalte bis zu 71 % aufweisen (Slawski et al. 2011b). Insbesondere das Konzentrat weist sowohl hohe Gehalte an Lysin und Methionin (Enami 2010; Slawski et al. 2011b) als auch an essentiellen Fettsäuren auf (Adem et al. 2014). Auch hier deutet die Studienlage darauf hin, dass Rapsproteine als Substitut für Fischmehl geeignet sind, es aber bei einer hohen Substitution zu einer geringeren Wachstumsleistung der Fische kommt (Burr et al. 2013; Slawski et al. 2012; Slawski et al. 2011b; Slawski et al. 2011a; Drew et al. 2007; Zhang et al. 2020b). Als nachteilig erweist sich beim Einsatz von Raps eine reduzierte Schmackhaftigkeit und dadurch geringere Futteraufnahme der Fische (Adem et al. 2014). Zusätzlich enthält auch Raps mehrere ANF, welche sich negativ auf die Verwertung der Inhaltsstoffe auswirken. Darunter fallen Glucosinolate, Phytate und weitere unverdauliche Kohlenhydrate (Francis et al. 2001; Mawson et al. 1994). Diese sind jedoch im Rapsproteinkonzentrat in geringerem Maß

vorhanden als im Rapsmehl, weshalb sich ein höherer Gehalt an Fischmehl durch Rapsproteinkonzentrat austauschen lässt (Adem et al. 2014; Drew 2004).

Weizengluten ist eine weitere pflanzliche Proteinquelle, welche häufig im kommerziellen Fischfutter enthalten ist. Es ist ein Nebenprodukt der Stärkegewinnung und verbindet ernährungsphysiologische und technische Eigenschaften. Aus ernährungsphysiologischer Sicht besitzt Weizengluten mit 75 % einen der höchsten Proteingehalte im Vergleich zu anderen pflanzlichen Futtermitteln (Day et al. 2006). Zusätzlich ist Weizengluten eine Quelle für essentielle Aminosäuren, jedoch mit Defiziten für Lysin, Methionin und Threonin (Apper-Bossard et al. 2013; Day et al. 2006; Allan et al. 2000). Trotz geringer Phosphorgehalte im Weizengluten ist das enthaltene Phosphor durch die Abwesenheit von Phytat gut verfügbar (Apper-Bossard et al. 2013). Generell ist die Ressource gut einsetzbar, doch haben hohe Substitutionsraten auch hier nachteilige Effekte für die Gesundheit und die Wachstumsleistung der Fische (Johny et al. 2020; Saleh et al. 2021; Helland und Grisdale-Helland 2006). Nichtsdestotrotz besitzt Weizengluten durch seine Bindeeigenschaften technologische Vorteile, wodurch es bei der Extrusion von Futtermitteln und der Stabilität von Pellets (als Bindemittel) zum Einsatz kommt (Apper-Bossard et al. 2013; Draganovic et al. 2011).

2.2.3 Mikroalgen als Proteinquelle

Seit Inkrafttreten der Verordnung (EU) 68/2013 sind Mikroalgen als Einzelfuttermittel für Fische in der EU zugelassen. Als Lebendnahrung werden Mikroalgen bereits seit einigen Jahren in der Aquakulturproduktion von Muscheln, Krebstieren und einigen Fischarten (hauptsächlich in der Brutaufzucht) genutzt (Guedes und Malcata 2012). Zu den wichtigsten Arten gehören *Spirulina sp., Arthrospira sp., Chlorella sp.* und *Scenedesmus obliquus* (Becker 2007). Zurzeit werden Algenmehle überwiegend in kommerziellen Diäten von Zierfischen genutzt, da sie die Hautpigmentierung und damit die Farbgebung des Schuppenkleids verstärken können (Bakshi et al. 2018; Sudirman et al. 2020). Aufgrund der gegenwärtig hohen Produktionskosten werden Algen kaum in kommerziellen Diäten für Nutzfische eingesetzt, dabei bieten Mikroalgen eine hochwertige Proteinquelle.

Die meisten Mikroalgen zeichnen sich durch einen hohen Proteingehalt aus, der jedoch innerhalb und zwischen den Arten stark variieren kann (Tabelle 1). Je nach Algenart kann der Proteingehalt zwischen 6 und 71 % liegen. Ein großer Vorteil von Algen ist, dass sie in der Lage sind alle Aminosäuren selbst zu synthetisieren. Das Aminosäuremuster fast aller Algen bietet

vergleichbar hohe Gehalte an essentiellen Aminosäuren, wie die Referenzaminosäuren von Eiern, Soja oder Fischmehl, jedoch treten Mängel bei schwefelhaltigen Aminosäuren wie Methionin und Cystein auf (Clément et al. 1967; Volkmann et al. 2008; Becker 2007; Hall 2010).

Tabelle 1. Zusammensetzung verschiedener Mikroalgenarten (in % Trockenmasse). Modifiziert nach Becker (2007).

Art	Familie	Protein	Kohlenhydrate	Fette
Anabaena cylindrica	Nostocaceae	43 – 56	25 – 30	4 – 7
Aphanizomenon flos-aquae	Nostocaceae	62	23	3
Chlamydomonas rheinhardii	Chlamydomonadaceae	48	17	21
Chlorella pyrenoidosa	Chlorellaceae	57	26	2
Chlorella vulgaris	Chlorellaceae	54 – 58	12 – 17	14 – 22
Dunaliella salina	Dunaliellaceae	57	32	6
Euglena gracilis	Euglenaceae	39 – 61	14 – 18	14 – 20
Porphyridium cruentum	Porphyridiaceae	28 – 39	40 – 57	9 – 14
Scenedesmus obliquus	Scenedesmaceae	50 – 56	10 – 17	12 – 14
Spirogyra sp.	Zygnemataceae	6 – 20	33 – 64	11 – 21
Arthrospira maxima	Microcoleaceae	60 – 71	13 – 16	6 – 7
Spirulina platensis	Microcoleaceae	46 – 63	8 – 14	4 – 9
Synechococcus sp.	Synechococcaceae	63	15	11

Der Gehalt an Antioxidantien, Vitaminen, Karotinoiden, Mikro- und Makronährstoffen ist stark Abhängig von der Mikroalgenspezies (Muchlisin 2012; Sandgruber et al. 2021). Grundsätzlich beinhalten Mikroalgen aber alle essentiellen Vitamine, darunter A, B_1, B_2, B_6, B_{12}, C, E, Nicotinsäure, Biotin, Folsäure und Pantothensäure (Becker 2008), sowie wichtige Mineralien wie Calcium, Eisen, Magnesium und Zink (Sandgruber et al. 2021).

Mikroalgen enthalten außerdem natürlicherweise Karotinoide, die auch in Landpflanzen vorkommen, wie Zeaxanthin, Lutein und Antheraxanthin, aber auch spezifische Karotinoide, welche nur in Algen, Bakterien und Hefen vorkommen, darunter auch das in der Aquakultur häufig eingesetzte Astaxanthin (Jin et al. 2003; Ambati et al. 2014). *Haematococcus pluvialis* enthält

beispielsweise hohe Gehalte an Astaxanthin, das für eine rote Farbe des Fischfilets verantwortlich ist (Chen et al. 2017). Andere Mikroalgen führen zu einer Gelbfärbung des Filets wie *Schizochytrium limacinum* oder *Spirulina platensis* (Katerina et al. 2020; Teimouri et al. 2013; Roohani et al. 2019).

Die Studienlage zeigt, dass ein geringer Einsatz an Mikroalgenmehl sowohl förderlich für das Wachstum als auch die Gesundheit und die Produktqualität von unterschiedlichen Fischarten sein kann (Roy und Pal 2015; Nagappan et al. 2021). Es ist jedoch auch bekannt, dass sich eine zu hohe Substitution negativ auf das Wachstum auswirken kann (El-Sayed 1994; Dallaire et al. 2007). Insgesamt weist die Literatur darauf hin, dass Mikroalgen als Fischmehlersatz viele Vorteile gegenüber anderen alternativen Proteinen haben. Zum einen ist die Nährstoffzusammensetzung hinreichend balanciert und kann zum anderen durch Variationen im Anbauprozess positiv beeinflusst werden (Kapitel 2.3.5). Verschiedene Fütterungsexperimente haben darüber hinaus gezeigt, dass Mikroalgen das Wachstum und die Gesundheit der Fische fördern können. Im Vergleich der verschiedenen Mikroalgenarten sticht Spirulina besonders hervor. Diese Art ist eine der am häufigsten untersuchten und genutzten Mikroalgenarten im Bereich Aquakultur. Im Folgenden wird die Gattung Spirulina näher beschrieben sowie deren Anbau und Einsatzmöglichkeiten in der Aquakultur.

2.3 Spirulina

Obwohl Spirulina in manchen Ländern wie z.B. Mexiko und Afrika seit Jahrhunderten als Nahrungsmittel verwendet wird (Ciferri 1983), ist sie erst seit ca. 50 Jahren kommerziell verfügbar (Borowitzka 1999). Aufgrund verschiedener als vorteilhaft diskutierter Eigenschaften für die Humanernährung wird Spirulina auch oft als sog. „Superfood“ bezeichnet. Gegenwärtig werden etwa 70 % der Mikroalge für den menschlichen Verzehr produziert (Koru 2012), ein weiterer Teil findet Anwendung in der Ernährung von Nutztieren wie Geflügel (Selim et al. 2018; Ross und Dominy 1990; Bonos et al. 2016), Schweinen (Grinstead et al. 2000; Saeid et al. 2013) und Fischen (Zhang et al. 2020a; Alagawany et al. 2021; Habib et al. 2008). Außerdem kann Spirulina auch für die Biokraftstoffherstellung genutzt werden (Mostafa und El-Gendy 2017; Pradana et al. 2020; Rahman et al. 2017). Um das Potenzial von Spirulina genauer einzuordnen, werden in diesem Kapitel die wichtigsten Eigenschaften, ihre Verbreitung, Kultivierung und Einsatzmöglichkeiten in der Aquakultur erläutert.

2.3.1 Taxonomie und Morphologie

Lange Zeit wurden die Arten *Arthrospira* und *Spirulina* getrennt voneinander betrachtet, bis 1932 beide Arten aufgrund ihrer spiralförmigen Morphologie unter dem Namen Spirulina zusammengefasst wurden (Habib et al. 2008). Im Folgenden dieser Arbeit werden daher auch die beiden Arten unter dem Begriff Spirulina zusammengefasst. Spirulina ist ein mehrzelliges, photosynthetisches, alkaliphiles und fadenförmiges Cyanobakterium (Abbildung 2), welches zur Familie der *Microcoleaceae* gehört. Cyanobakterien werden häufig als Blaualgen oder Blau-Grün-Algen bezeichnet (Whitton und Potts 2000), da sie durch ihre fadenförmige Struktur ein ähnliches Erscheinungsbild wie Algen besitzen (Strasburger et al. 2008). Spirulina gehört zu den Prokaryonten, wodurch Zellorganelle wie Zellkern, Lysosomen, Mitochondrien, membranbegrenzende Chloroplasten und das endoplasmatische Retikulum fehlen (Becker 1984). Auffällig ist die typische Anordnung der zylindrischen Trichome mit einem Zelldurchmesser von 6 – 12 µm (Belay 2013), welche sich spiralförmig und unverzweigt in einer offenen Linkshelix mit einem Helixdurchmesser von 30 – 70 µm anordnen (Sili et al. 2012). Trichotome sind durch mehrfache interkalare Zellteilung entlang des gesamten Filaments dazu in der Lage ihre Struktur zu verlängern, oder einen Bruch durch die Zerstörung einer Interkalarzelle des Trichoms herbeizuführen und sich zu teilen (Vonshak und Tomaselli 2000).

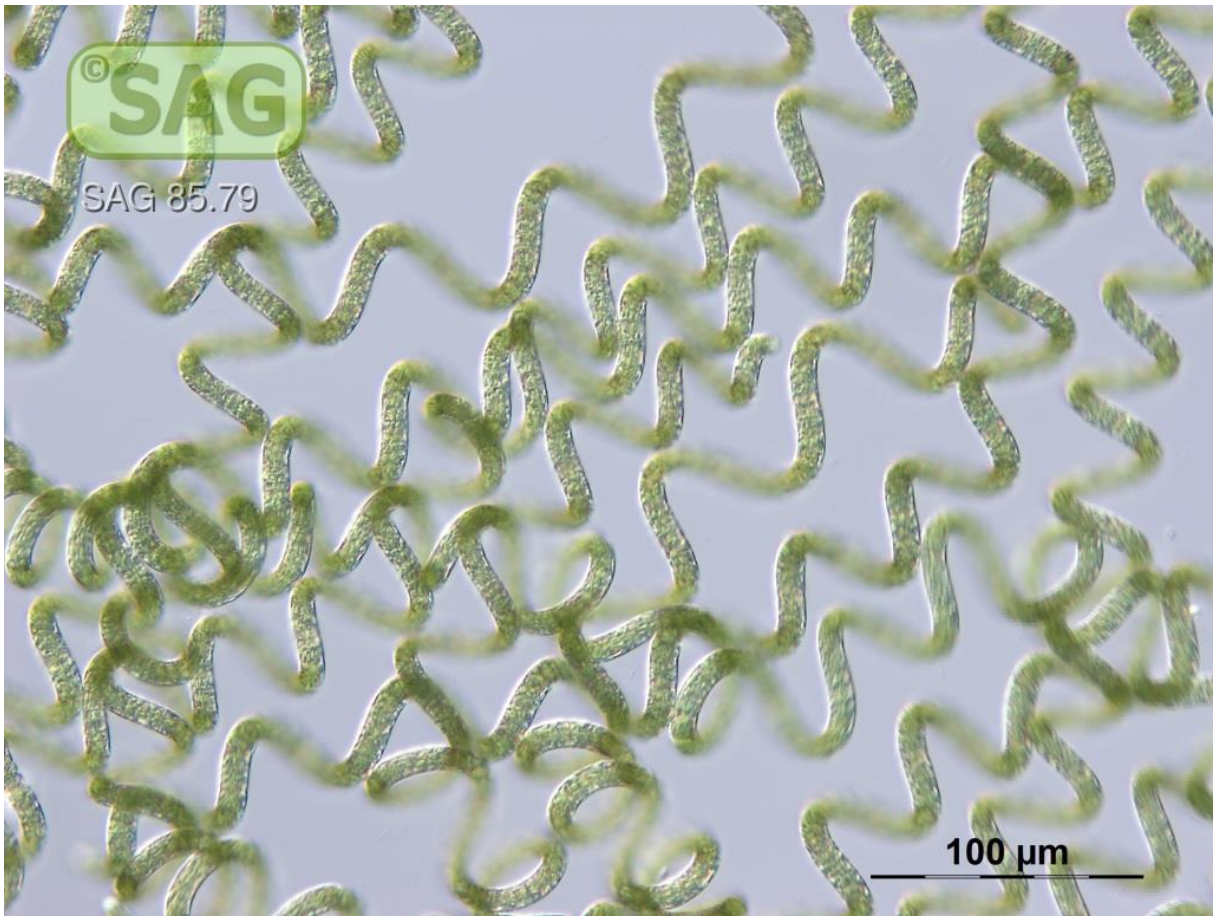

Abbildung 2. Trichome des Cyanobakteriums *Arthrospira platensis* (Darienko 2017).

2.3.2 Verbreitung und Lebensweise

Vorrangig kommt Spirulina in tropischen sowie subtropischen, alkalischen Salzseen und teilweise auch im Süßwasser vor. Spirulina ist photoautotroph, weshalb die Mikroalge auf Gewässer mit hoher Sonneneinstrahlung angewiesen ist (Udayan et al. 2017; Komárek et al. 2008). Deshalb ist sie hauptsächlich in Afrika, aber auch in Asien und Südamerika zu finden (Vonshak und Tomaselli 2000). Die größten Spirulina-Seen befinden sich in Zentralafrika, Tschad, Niger und Ostafrika (Habib et al. 2008). Aufgrund des limitierten Nährstoffangebots in den natürlichen Seen bilden sich in der Regel Wachstumszyklen der Cyanobakterien aus (Habib et al. 2008). Je nach Höhe des Nährstoffangebots kann sich die Population rasant vermehren und stirbt ab, sobald die Nährstoffe aufgezehrt werden. Die abgestorbenen Algen setzen dann wiederum Nährstoffe in das Gewässer frei und ein neuer Zyklus beginnt.

Wie Pflanzen sind Cyanobakterien in der Lage Lichtenergie in chemische Energie umzuwandeln und deshalb auf eine bestimmte Lichtintensität und Einstrahlungsdauer angewiesen (Vonshak 2010). Zusätzlich werden primäre Nährstoffe wie Kohlenstoff, Sticksoff und Phosphor für das Wachstum benötigt (Belay 2013). Für ein optimales Wachstum benötigt Spirulina einen pH

von 8,5 – 11,0 und eine Salinität von über 30 g pro L (Habib et al. 2008). Dabei liegt die optimale Temperatur für einen hohen Biomassezuwachs unter Laborbedingungen bei 35 °C. Dennoch gibt es auch genetische Linien, deren optimales Wachstum bei 24 °C oder sogar bei 40 °C liegt (Sili et al. 2012). Aufgrund der hohen Variabilität der Art kann auch ein Wachstum bei Extremtemperaturen von 10 oder 50 °C noch stattfinden (Venkataraman 1997).

Andere Mikroorganismen wachsen bei diesen extremen Umweltbedingungen kaum oder gar nicht, sodass in der Regel reine Spirulina-Kulturen produziert werden, ohne das Risiko einer Kontamination von toxinbildenden Mikroorganismen (Ciferri 1983; Habib et al. 2008). Dies stellt einen wichtigen Vorteil der Kultivierung von Spirulina dar und sorgt für eine hohe Biosicherheit. Die verschiedenen Kultivierungsmethoden werden im folgenden Kapitel näher erläutert.

2.3.3 Kultivierung

Aufgrund der Vielfalt moderner Kultivierungsmethoden kann Spirulina mittlerweile weltweit angebaut werden. Im Jahr 2019 wurden rund 56.208 t Spirulina weltweit produziert (Cai et al. 2021). Global zeigt sich allerdings ein starker Fokus auf den asiatisch-pazifischen Raum (Hu 2004; Cai et al. 2021). Durch die Optimierung der Nährmedien sind die Produktionskosten mittlerweile deutlich gesunken (Soni et al. 2017). Durch die modernen Anbausysteme können auch in landwirtschaftlich schwachen Regionen Erträge durch die Produktion von Algen generiert werden (Foley et al. 2011). Generell wird zwischen offenen und geschlossenen Kultivierungssystemen unterschieden, welche im Folgenden näher charakterisiert werden.

2.3.3.1 Offene Systeme

Bei offenen Systemen handelt es sich z.B. um Teiche oder natürliche Gewässer wie Seen und Lagunen. Es können aber auch künstliche Behältnisse für die Produktion von Spirulina genutzt werden. Am häufigsten werden große, flache Teiche genutzt oder sog. „circular ponds“ und „raceway ponds“ (Soni et al. 2017; Shen et al. 2009; Borowitzka 2018). Alle Systeme weisen eine geringe Wassertiefe auf und werden teilweise mit einem Rührwerk oder einem Schaufelrad durchmischt (Abbildung 3).

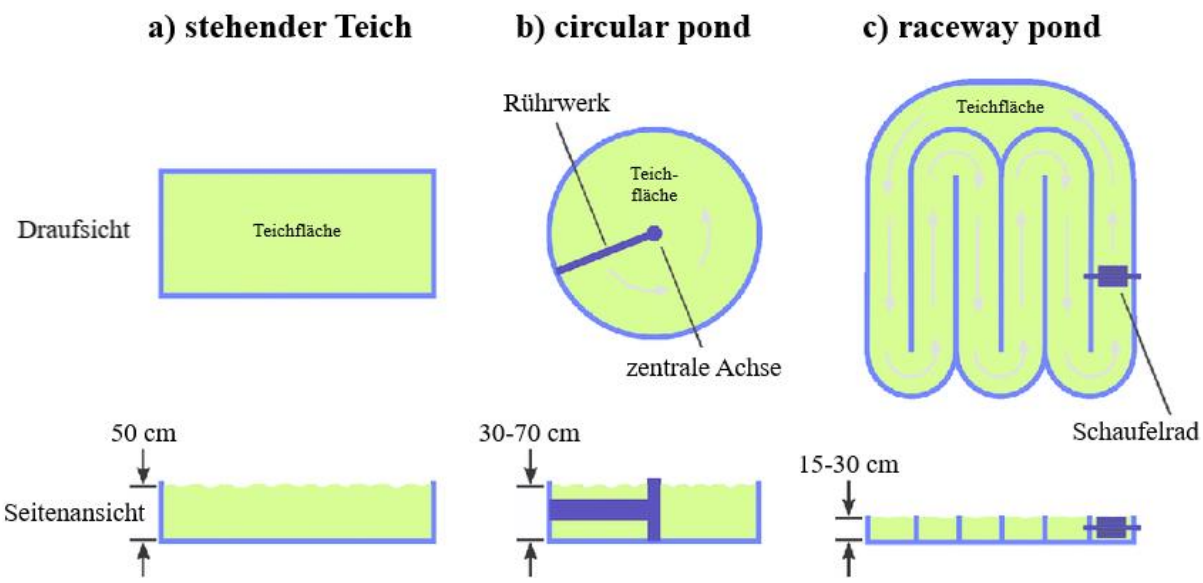

Abbildung 3. Schematische Darstellung von offenen Systemen für die Algenkultivierung. a) stehender Teich, b) circular pond, c) raceway pond. Modifiziert nach Hallmann (2016).

Stehende Teiche sind die ältesten und technisch einfachsten Systeme, welche ohne großen technischen Aufwand betrieben werden können. Sie sind meist bis zu 50 cm tief. Circular ponds haben eine Tiefe von 30 – 70 cm und werden mit einem Rührwerk durchmischt. Raceway ponds besitzen einer Wassertiefe von 15 – 30 cm und bestehen meist aus Beton oder in den Boden eingelassener Folie (Hallmann 2016; Singh und Sharma 2012). Die Kanäle von raceway ponds sind mit einfachen oder mehrfachen Schlaufen angelegt (Chaumont 1993), wobei die Algenmasse mit einem Schaufelrad ständig in Bewegung gehalten wird.

Bei diesen Systemen handelt es sich um die wirtschaftlichsten und technisch am wenigsten aufwendigen Kulturmethoden (Shen et al. 2009). Jedoch sind die offenen Systeme aufgrund einer erhöhten Evaporation auch am ineffektivsten in Bezug auf die Biomasseproduktion und Wassernutzung (Hallmann 2016). Zudem sind sie stärker von den vorherrschenden Umweltbedingungen wie Temperatur, Lichteinstrahlung und Verdunstung abhängig, wodurch nicht nur die Wachstumsrate, sondern auch der Proteingehalt und die Pigmentsynthese beeinflusst werden (Saeid und Chojnacka 2015). Dementsprechend variiert die Qualität durch jahreszeitlich bedingte Änderungen der Umweltbedingungen. Außerdem ist das Risiko für Kontaminationen von außerhalb mit invasiven Algen und Bakterien in den offenen Systemen höher (Chaumont 1993; Hallmann 2016). Aus diesem Grund ist eine ständige Qualitätskontrolle unumgänglich.

2.3.3.2 Geschlossene Systeme

Durch die Intensivierung in der Mikroalgenproduktion wurden Photobioreaktoren entwickelt, welche auf die biologischen und physiologischen Eigenschaften der Algen abgestimmt sind und

nahezu standortunabhängig produzieren können. Meist werden hierfür Platten-, Säulen-, Ring- und Röhrenreaktoren verwendet (Abbildung 4). Bedingt durch die hohen Anschaffungs- und Betriebskosten sind diese Systeme im Vergleich zu den zuvor beschriebenen offenen Systemen deutlich kostenintensiver.

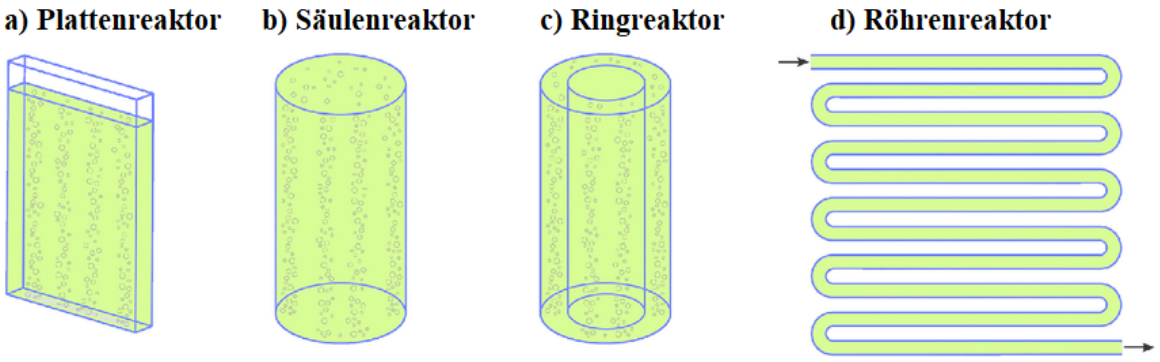

Abbildung 4. Schematische Darstellung von geschlossenen Systemen für die Algenkultivierung. a) Plattenreaktor, b) Säulenreaktor, c) Ringreaktor, d) Röhrenreaktor. Modifiziert nach Hallmann (2016).

Meist werden die Reaktoren mit künstlichem Licht versorgt, welches durch Licht emittierende Dioden (LEDs) oder Leuchtstoffröhren erzeugt wird (Da Prates et al. 2018), jedoch kann das System je nach Standort auch mit natürlichem Sonnenlicht betrieben werden (Borowitzka 1999). Zudem bieten die geschlossenen Systeme den Vorteil, dass die Umweltbedingungen vollständig kontrollierbar sind und dadurch ein höherer Biomasseoutput als auf offener Teichfläche produziert werden kann (Singh und Sharma 2012). Beispielsweise besteht die Möglichkeit, die Temperatur konstant im optimalen Produktionsbereich zu halten, die Nährstoffzufuhr ist einfach steuerbar und die Lichtnutzung optimiert. Durch die Kontrolle der Umweltparameter kann auch der Nährstoffgehalt im Endprodukt gezielt beeinflusst werden (Kapitel 2.3.5). Darüber hinaus ist die Wahrscheinlichkeit, dass andere Organismen in das System eingetragen werden und dieses kontaminieren, sehr gering (Mata et al. 2010). Dies garantiert ein unbedenkliches und sicheres Produkt. Die geschlossenen Systeme ermöglichen es zudem den Verlust von Wasser durch Verdunstung sowie den Verlust von CO_2 zu minimieren und tragen dadurch zur Einsparung von Ressourcen bei (Singh und Sharma 2012).

2.3.4 Ernte und Verarbeitung

Die Ernte von Mikroalgen ist aufgrund der hohen Wassermenge in Bezug auf die relativ niedrige Algenkonzentration sehr aufwendig (Shen et al. 2009). Üblich verwendete Methoden sind die Zentrifugation, Koagulation und Flockung, Filtration, Flotation sowie Kombinationen aus mehreren Verfahren (Salim et al. 2011).

Eine kostengünstigere Methode ist die Filtration. Hier werden die Algen von der Flüssigkeit getrennt, indem das Gemisch mit Hilfe von Druck oder Vakuum durch ein permeables Medium geleitet wird, das die Feststoffe zurückhält (Shen et al. 2009). Bei hohem Wasservolumen kommt das System durch die begrenzte Filtrationsgeschwindigkeit jedoch an seine Grenzen.

Mit einem Wirkungsgrad von bis zu 100 % ist die Zentrifugation die effektivste Methode für die Mikroalgenernte (Najjar und Abu-Shamleh 2020). Aufgrund des hohen Energieverbrauchs ist die Methode aber auch die kostenintensivste Erntemethode (Najjar und Abu-Shamleh 2020; Shen et al. 2009). Für die Zentrifugation können unterschiedliche Geräte wie Scheibenstapel-, Vollmantel-, Röhren-, Mehrkammerzentrifuge und Hydrozyklon genutzt werden (Najjar und Abu-Shamleh 2020).

Bei der Flockung werden meist chemische Flockungsmittel oder Mikroorganismen in das Kulturmedium gegeben, sodass die Algenzellen aggregieren und sich die Partikelgröße erhöht. Das Ausflocken kann auch auf natürlichem Wege durch den Abbau von CO_2 während der Photosynthese und dem damit einhergehenden Anstieg des pH-Wertes auf über neun entstehen (Vandamme et al. 2013). Die Flockung wird häufig in großem Maßstab für die Algenernte praktiziert, da die Mittel verhältnismäßig kostengünstig sind. Die Methode wirkt allerdings nur sehr spezifisch für bestimmte Algenstämme, da es hier einen genetischen Einfluss auf die Wirksamkeit gibt und die Wiedergewinnung des Flockungsmittels darüber hinaus sehr schwierig ist (Shen et al. 2009). Zudem werden häufig Metallverbindungen eingesetzt, die im Nachhinein wieder aufwändig entfernt werden müssen (Hallmann 2016). Alternativ können z.B. polymere Flockungsmittel (Vandamme et al. 2013), Elektroflockung (Lee et al. 2013), Elektroflockung in Kombination mit Fe_3O_4 Nanopartikeln (Gerulová et al. 2022) oder Ultraschall (Bosma et al. 2003) genutzt werden.

Die weiteren Verarbeitungsschritte werden im Folgenden nach Habib et al. (2008) beschrieben: Nach der Gewinnung der Algenbiomasse muss diese zunächst gewaschen werden, um den Salzgehalt zu reduzieren. Es folgt ein weiterer Konzentrationsschritt, mit dem Ziel möglichst viel interstitielles Wasser aus den Algen zu entfernen. Im nächsten Schritt muss der pH-Wert der Biomasse durch Zugabe einer sauren Lösung neutralisiert werden. Daraufhin können die Trichome mit Hilfe eines Mahlwerks zerkleinert und anschließend z.B. durch Sprühtrocknung getrocknet werden. Zum Schluss werden die getrockneten Algen luftdicht verpackt und können gelagert werden.

2.3.5 Nährstoffgehalt

Spirulina ist eine besonders interessante Nährstoffquelle für die Ernährung von aquatischen Organismen und Landtieren sowie die menschliche Ernährung. In Abhängigkeit von den jeweiligen Produktionsfaktoren unterliegt der Nährstoffgehalt im Endprodukt starken Schwankungen (Chung et al. 1978). Einflussfaktoren sind z.B. die Temperatur, Lichtintensität und der pH-Wert (Rafiqul et al. 2004). Aber auch die Verarbeitung und die thermische Behandlung der Mikroalge können einen starken Einfluss auf den nutritiven Wert des Endprodukts haben (Ho und Redan 2020; Mishra 2015; Becker 2008)

Spirulina zeichnet sich vor allem durch einen hohen Proteingehalt von teilweise mehr als 70 % (Tabelle 2) und einer hohen biologischen Wertigkeit aus (Gutiérrez-Salmeán et al. 2015), da alle für den Menschen essentiellen Aminosäuren vorhanden sind (Becker 2007). Jedoch muss beachtet werden, dass bei der Betrachtung des Rohproteingehalts auf die Hydrolyse von Algen oder auf die Schätzung des Gesamtstickstoffs zurückgegriffen wird. Dadurch wird der tatsächliche Proteingehalt durch den Anteil an nicht-Protein-Stickstoff von Spirulina mit ca. 11,5 % überschätzt (Becker 2007).

Tabelle 2. Nährwerte von Spirulina (*Arthrospira*) in % Trockensubstanz.

Nährstoff	Gehalt [%] in Trockensubstanz	Quelle
Protein	57,8 – 76,7	Borowitzka (2009), Belay (2013), Becker (2007), Ennaji et al. (2021), Seghiri et al. (2019), Sandgruber et al. (2021)
Fett	2,5 – 7,0	Becker (2007), Ennaji et al. (2021), Seghiri et al. (2019), Sandgruber et al. (2021)
Kohlenhydrate	6,5 – 36,9	Becker (2007), Ennaji et al. (2021), Seghiri et al. (2019), Sandgruber et al. (2021)
Asche	5,8 – 14,6	Ennaji et al. (2021), Seghiri et al. (2019), Sandgruber et al. (2021)

Der Fettgehalt der Mikroalge liegt zwischen 2,5 – 7,0 %. Darüber hinaus ist Spirulina eine geeignete Quelle für einfach ungesättigte Fettsäuren (MUFA) und mehrfach ungesättigte Fettsäuren (PUFA) (Ötleş und Pire 2001). Die enthaltenen PUFA lassen sich in Omega-3 (ω-3) und

Omega-6-Fettsäuren (ω-6) klassifizieren. Spirulina hat einen besonders hohen Anteil an γ-Linolsäure sowie an essentiellen PUFA wie z.B. Eicosapentaensäure (EPA) und Docosahexaensäure (DHA) (Dibeklioglu et al. 2009). Studien haben gezeigt, dass EPA und DHA vor einer Vielzahl an chronisch-degenerativen Krankheiten schützen und antientzündliche und kardioprotektive Effekte aufweisen (Abdelhamid et al. 2018; Kones et al. 2017; Yang et al. 2019; Marventano et al. 2015).

Generell kommen in der Mikroalge Kohlenhydrate in Form von Stärke, Cellulose, Zuckern und anderen Polysacchariden vor (Roy und Pal 2015). Spirulina besteht zu 6,5 – 36,9 % aus Kohlenhydraten. Diese besitzen keine gesonderte ernährungsphysiologische Bedeutung, können aber Anwendung in der Bioraffinerie finden (Liu et al. 2019).

Spirulina bietet eine reichhaltige Quelle für Kalium, Kalzium, Kupfer, Eisen, Magnesium, Mangan, Phosphor, Selen, Natrium und Zink (Habib et al. 2008). Zusätzlich enthält Spirulina eine große Menge an wichtigen Vitaminen wie Vitamin A, K, B1, B2, B3, B6 und B12 (Habib et al. 2008). Insbesondere der sehr hohe Gehalt an Vitamin B12 wurde in der Vergangenheit ernährungsphysiologisch positiv hervorgehoben, allerdings setzt sich dieses Vitamin nach neueren Studien zu 83 % aus dem Pseudovitamin B12 zusammen, welches das Potenzial hat den Vitamin-B12-Metabolismus zu blockieren (Watanabe et al. 1999). Dementsprechend wird Spirulina nicht als zuverlässige Vitamin B12-Quelle angesehen. Darüber hinaus sind im Spirulina verschiedene Pigmente wie Chlorophyll a, β-Carotin, Xanthophyll, Echinenon, Myxoxanthophyll, Zeaxanthin, Canthaxanthin, Diatoxanthin, 3-Hydroxyechinenon, Beta-Cryptoxanthin, Oszillaxanthin, Phycobiliproteine, c-Phycocyanin und Allophycocyanin enthalten (Habib et al. 2008).

Trotz des höheren Kohlenhydratgehalts ist die Verdaulichkeit von Mikroalgen sehr hoch (Becker 2004). Zusätzlich beinhalten Algen zwischen 2 und 22 % Fett, mit einem hohen Anteil ungesättigter Fettsäuren, darunter auch langkettige, mehrfach ungesättigte Fettsäuren (Mishra 2015). Durch die Steuerung von Produktionsfaktoren, wie z.B. dem Herbeiführen eines Stickstoffmangels, der Erhöhung der Salinität oder Änderung im Lichtmanagement, können Änderungen im Fettstoffwechsel der Algen herbeigeführt werden, um eine erhöhte Akkumulation von EPA und DHA zu provozieren (Yokochi et al. 1998).

2.3.6 Einsatz von Spirulina für die Fischernährung

Der erfolgreiche Austausch von Fischmehl gegen alternative Proteinquellen ist von einer Vielzahl unterschiedlicher Faktoren abhängig. Dazu zählt die Höhe der Substitution, welche je nach

Fischart und deren Ernährungsphysiologie unterschiedlich stark variiert werden kann (Kapitel 2.2). Die günstige Nährstoffzusammensetzung von Spirulina macht den Einsatz bei Fischen besonders interessant und trägt zur Reduktion des FIFO bei. Auf der einen Seite kann Spirulina als Fischmehlersatz dienen und auf der anderen Seite aufgrund biochemischer Eigenschaften Produktionsparameter wie Wachstum, Gesundheit und Qualität der Erzeugnisse verbessern. Deshalb werden im Folgenden die Vorteile und Limitationen des Einsatzes von Spirulina in der Fischernährung diskutiert.

2.3.6.1 Wachstum und Fischgesundheit

Die Einsatzmöglichkeiten von Spirulina wurden bereits für verschiedene Fischarten evaluiert. Darunter sind Zierfische wie Guppy (*Poecilia reticulata*) (Dernekbasi et al. 2010), Goldfisch (*Carassius auratus*) (Vasudhevan und James 2011) und kommerziell produzierte Speisefische, wie cyprinide Fische z.B. Karpfen (*Cyprinus carpio*) (Nandeesha et al. 1998) und Giebel (*Carassis auratus gibelio*) (Cao et al. 2018), aber auch karnivore und omnivore Arten wie Regenbogenforellen (*Oncorhynchus mykiss*) (Yeganeh et al. 2015; Teimouri et al. 2013; Sirakov et al. 2012; Ahmadzade-Nia et al. 2011), Afrikanische Welse (*Clarias gariepinus*) (Raji et al. 2018; Raji et al. 2020) und Tilapia (O*reochromis sp.*) (Lu et al. 2003; Velasquez et al. 2016; Olvera-Novoa et al. 1998; Plaza et al. 2019). Grundsätzlich deutet die Studienlage darauf hin, dass die Auswirkungen der Futtermittelsubstitution mit Spirulina auf die Wachstumsleistung artspezifisch und von der Höhe der Spirulina-Substitution abhängig sind. Bei einem zu hohen Spirulina-Anteil im Futtermittel wird die Wachstumsleistung reduziert. Eine vollständige Substitution ist nur bei herbivoren Arten wie z.B. Karpfen, Giebel und teilweise für Afrikanische Welse möglich (Cao et al. 2018; Nandeesha et al. 1998; Raji et al. 2020). Bei karnivoren Arten wie Forellen ist ein zehnprozentiger Austausch von Fischmehl gegen Spirulina ohne Wachstumseinbußen realisierbar (Teimouri et al. 2013; Sirakov et al. 2012). Im Rahmen von Versuchen mit Tilapia konnte bei einer Substitution von Fischmehl durch Spirulina von 30 bis 45 % eine erhöhte Gewichtszunahme beobachtet werden, jedoch kam es ab einer Substitutionsrate von 75 % zu einer deutlichen Abnahme und somit zu einer entgegengesetzten Entwicklung der Wachstumsleistung (Velasquez et al. 2016). Ähnliche Ergebnisse sind für Tilapia-Brut berichtet worden. Hier ist eine Substitution des Fischmehls durch bis zu 40 % Spirulina möglich, ohne eine verminderte Wachstumsleistung hervorzurufen (Olvera-Novoa et al. 1998).

Ein negativer Einfluss auf die Überlebensrate wurde in keiner der zuvor genannten Studien beobachtet. Ganz im Gegenteil, die Fütterung von Spirulina scheint mehrere Vorteile für die

Fischgesundheit zu haben. El-Sheekh et al. (2014) beobachteten sogar signifikant höhere Überlebensraten bei Tilapiahybride, die mit einer Spirulina-Diät gefüttert wurden.

Weiterhin besitzt Spirulina immunstimulierende Effekte, welche sich positiv auf die Immunantwort auswirken (Watanuki et al. 2006; Ragap et al. 2012; Yeganeh et al. 2015). Ein Indiz dafür sind immunrelevante Gene, welche durch die Fütterung mit Spirulina hochreguliert werden (Sheikhzadeh et al. 2019) und der Antikörper-Titer steigt (Duncan und Klesius 1996). Für mehrere Fischarten konnte im Rahmen von *in vivo*- und *in vitro*-Versuchen eine höhere Resistenz von Spirulina-gefütterten Fischen gegenüber häufig auftretenden bakteriellen Erregern nachgewiesen werden (Ibrahem et al. 2013; Cao et al. 2018; Adel et al. 2016; Sheikhzadeh et al. 2019; Yu et al. 2018). Zusätzlich gilt die Mikroalge als Antioxidant, welches im Fisch durch die verstärkte Expression von Superoxid-Dismutase (SOD) und Katalase (CAT) zur Reduktion von oxidativem Stress führt (Teimouri et al. 2019).

Diese antioxidativen Effekte konnten sowohl im Serum (Sayed et al. 2017) als auch in Darm, Haut und Kiemen der Fische nachgewiesen werden (Sheikhzadeh et al. 2019). In mehreren Studien zeigten sich zudem ein verbesserter Schutz gegenüber toxischen Verbindungen wie Insektiziden (Abdel-Daim et al. 2020b; Abdelkhalek et al. 2015), Aflatoxin B1 (Abdel-Daim et al. 2020a) oder Schwermetallen (Bangeppagari 2014; Mohanty und Samanta 2018; Soliman et al. 2021). Zusammenfassend weist die Substitution von Spirulina für die untersuchten Fischarten gleich mehrere Vorteile für die Gesundheit auf und kann dadurch zu einer effizienteren Aquakulturproduktion beitragen.

2.3.6.2 Mikrobiom

Das Mikrobiom beschreibt die Gesamtheit aller Mikroorganismen (Bakterien, Archaeen, Eukaryoten und Viren) in einem Habitat (Yukgehnaish et al. 2020; Marchesi und Ravel 2015). Veränderungen der Futtermittelzusammensetzung gehen in der Regel mit Veränderungen der Zusammensetzung des Mikrobioms von Fischen einher (Ringø et al. 2016). Dabei ist gerade dieses Organ besonders wichtig für die Immunantwort und Fischgesundheit (Romero et al. 2014; Llewellyn et al. 2014). Auf Basis der zuvor beschriebenen Vorteile für die Fischgesundheit, liegt der Rückschluss nahe, dass Veränderungen der Zusammensetzung des intestinalen Darmmikrobioms eine zentrale Rolle spielen. In diesem Kontext wären Analysen des Mikrobioms hilfreich, um die Kausalitäten zu überprüfen und zu erklären.

Bislang sind nur wenige Studien bekannt, welche sich mit der Auswirkung von Spirulina auf das Darmmikrobiom von Fischen auseinandersetzen. Bei Zebrabärblingen konnte durch die

Zugabe von 15 % Spirulina in der experimentellen Diät ein signifikant höherer Anteil an *Cetobacterium* im Darm gefunden werden (Ma et al. 2022). Indessen konnten Plaza et al. (2019) bei der Fütterung von Tilapia mit einer Beimischung von 3 % Spirulina keine Veränderung der bakteriellen Diversität des Mikrobioms beobachten. Die Variabilität zwischen den einzelnen Versuchstieren war insgesamt hoch. Größtenteils wurde das Mikrobiom dominiert von *Proteobakterien* und *Fusobakterien*, andere Individuen wiesen wiederum verstärkt *Actinobakterien* auf.

Weitere Studien mit höheren Gehalten an Spirulina im Futtermittel könnten zu stärkeren Änderungen im Darm-Mikrobiom führen und ggf. Aufschluss darüber geben, welche Bakterien in einem höheren Maße durch die Spirulina-Substitution gefördert werden.

2.3.6.3 Reproduktion

Spirulina weist einen hohen Gehalt an Pigmenten auf, darunter Karotinoide wie z.B. Astaxanthin und Zeaxanthin (Kapitel 2.3.5). In der Regel steigern Karotinoide die Reproduktionsleistung von Fischen (Craik 1985; Hue et al. 2021; Vassallo-Agius et al. 2001). Folglich liegt es nahe, dass sich die Fütterung mit Spirulina positiv auf den Reproduktionserfolg auswirken kann. Darüber hinaus wirken sich die in Spirulina enthaltene Ascorbinsäure und PUFA potentiell begünstigend auf die Eierzeugung und deren Qualität aus (Scabini et al. 2011).

Im Rahmen von Versuchen mit Zierfischen wie Gelbflossenmaulbrütern (*Pseudotropheus acei*) oder Gourami (*Trichopodus trichopterus*) konnte durch die Substitution von Spirulina eine erhöhte Reproduktionsrate erzielt werden (Güroy et al. 2012; Khanzadeh et al. 2016). Der Trend kehrte sich in den Studien jedoch bei höherer Substitution von Fischmehl durch Spirulina um. Geffroy und Simon (2013) beobachteten bei Zebrabärblingen (*Danio rerio*) nicht nur eine höhere Überlebensrate, sondern auch eine schnellere Entwicklungsrate der Larven durch die Fütterung von Spirulina. Nicht signifikant unterschiedlich waren in dieser Untersuchung hingegen die Größe und das Gewicht der Eier sowie der Larven.

Bislang gibt es nur vereinzelt Studien zum Einfluss von Spirulina als Futtermittel auf die Reproduktionsrate von Nutzfischen. Eine Untersuchung mit Welsen (*Clarias macrocephalus*) konnte bei einer Zumischung von 5 und 10 % Spirulina keine Änderung der Fortpflanzungsleistung und Eiqualität gegenüber der Kontrolldiät feststellen (Chainapong und Traichaiyaporn 2013). Laut Wahbi und Sangak (2017) zeigten Tilapia (*Oreochromis niloticus*) eine erhöhte Schlupfrate bei 10 und 20 g pro kg Spirulina-Beimischung im Futter. Zusätzlich wiesen die Larven eine höhere Körperlänge, sowie ein höheres Gewicht als die Fische auf, die mit der

Kontrolldiät gefüttert wurden. Zudem konnte die Mortalität sowie die Häufigkeit von Deformationen durch den Einsatz von Spirulina verringert werden. Lu und Takeuchi (2004) untersuchten über drei Generationen Tilapia, die ausschließlich mit rohem Spirulina gefüttert wurden und verglichen diese mit Tieren, die mit einer kommerziellen Diät gefüttert wurden. Es resultierten keine signifikanten Unterschiede in der Fruchtbarkeit, den Laichintervallen und der Eigröße, jedoch enthielten die Eier der Spirulina-Gruppen einen höheren Gesamtgehalt an ω-6-Fettsäuren. Darunter waren insbesondere Linolensäure und γ-Linolensäure, welche Vorstufen der Arachidonsäure sind und eine wesentliche Rolle bei der Bildung von Prostaglandinen spielen, wodurch die Reifung der Eizellen und der Eisprung beeinflussen werden (Patiño und Sullivan 2002).

2.3.6.4 Produktqualität

Die Qualität von Fischerzeugnissen ist anhand einer Vielzahl an organoleptischen Eigenschaften zu bewerten. Dazu zählen in erster Linie die Ansprüche der Verbraucher an Geschmack und Aussehen des Produkts. Außerdem rückt mehr und mehr der Wert von gesunden Lebensmitteln (Rana und Paul 2017), sprich den nutritiven Eigenschaften, in den Fokus der Humanernährung. Ein weiterer wichtiger Aspekt ist die Haltbarkeit des Produkts.

Durch den Einsatz von neuen Futtermittelkomponenten besteht die Möglichkeit, dass es zu einer Änderung des Geschmacks im Endprodukt kommt. Für Karpfen konnte bisher kein signifikanter Effekt auf organoleptische Eigenschaften von rohen und gekochten Fisch hinsichtlich der Spirulina-Fütterung festgestellt werden (Nandeesha et al. 1998; Sobczak et al. 2021). Auch für Tilapia, die ausschließlich mit Spirulina gefüttert wurden, war kein Unterschied zur Kontrollgruppe festzustellen (Lu und Takeuchi 2002). Bei anderen häufig konsumierten Arten liegen bisher keine Forschungsergebnisse vor.

Die in Spirulina enthaltenen Pigmente und Karotinoide können die Haut und Filetfarbe der Fische beeinflussen. Generell werden Karotinoide ohne weiteren Umbau in den Fischmuskel integriert (Hata und Hata 1974). In mehreren Studien konnte durch die Fütterung von Spirulina eine Gelbfärbung des Fischfilets beobachtet werden (Teimouri et al. 2013; Roohani et al. 2019; Plaza et al. 2019). Die Untersuchungen von Teimouri et al. (2013) zeigten, dass die Spirulina-induzierte Färbung bis zu drei Monate bei -20 °C stabil bleibt. Die Gelbfärbung der Filets wird dabei in der Literatur als positiver Effekt kommuniziert. Dennoch wird gleichzeitig berichtet, dass eine Gelbfärbung von Regenbogenforellen-Filets, die durch das saisonale Auftreten von Algen in italienischen Aquakulturen hervorgerufen wird, zu Umsatzeinbußen führt (Welker et

al. 2001). Die Studien stützen sich auf deskriptive und keine quantitativen Ergebnisse oder empirische Studien. Deshalb können zum jetzigen Zeitpunkt keine eindeutigen Aussagen über die Akzeptanz der Verbraucher für die geänderte Filetfarbe getroffen werden.

Der Fischmuskel setzt sich aus etwa 70 – 84 % Wasser, 15 – 24 % Protein und 0,1 – 22 % Fett zusammen (Abraha et al. 2018). Für die Bewertung des Schlachtkörpers wird die Zusammensetzung der zuvor genannten Komponenten als gängiger Qualitätsparameter herangezogen. Die beiden wichtigsten Parameter sind dabei der Protein- und Fettgehalt (Alagawany et al. 2021). Beim Einsatz von Spirulina als Fischmehlsubstitut wurde bei Karpfen und Regenbogenforellen kein Unterschied im Proteingehalt festgestellt (Nandeesha et al. 1998; Teimouri et al. 2016), jedoch konnte bei Doraden (*Rhabdosargus sarba*) ein verringerter Proteingehalt nachgewiesen werden (El-Sayed 1994). Ebenfalls kann es zu einer Verringerung des Fettgehalts im Muskel von verschiedenen Fischarten durch die Fütterung von Spirulina kommen (Teimouri et al. 2016; Nandeesha et al. 1998; Mustafa et al. 1994). Untersuchungen mit Catlabarbe (*Catla catla*) und Rohu (*Labeo rohita*) ergeben konträre Ergebnisse. Beide Fischarten zeigen einen erhöhten oder gleichbleibenden Muskelfettgehalt infolge des vollständigen Austausches von Fischmehl durch Spirulina (Nandeesha et al. 2001). Zusammenfassend lässt sich festhalten, dass nach gegenwärtigem Stand der Literatur der Einfluss von Spirulina auf den Fett- und Proteingehalt stark abhängig von der Fischart ist.

Bei der Betrachtung des Fettsäuremusters ist insbesondere der Gehalt an PUFA im Filet relevant für die Bewertung der Produktqualität. Potenziell dient Spirulina durch den hohen Gehalt an PUFA als wichtiger Lieferant für essentielle Fettsäuren. Teimouri et al. (2016) zufolge führt die Erhöhung des Anteils von Spirulina im Futtermittel von 50 auf 100 g pro kg zu einem Anstieg an DHA und weiteren PUFA im Muskel von Regenbogenforellen. Ähnliche Ergebnisse wurden von Jafari et al. (2014) erzielt. In ihren Untersuchungen zeigte sich, dass geringe Mengen Spirulina in der Diät den Anteil der PUFA im Muskel von Regenbogenforellen erhöht. Im Gegensatz dazu stellen Twibell et al. (2020) einen Abfall von ω-3-Fettsäuren wie EPA und DHA und eine Zunahme der Gehalte an ω-6-Fettsäuren im Filet bei der Fütterung von jungen Regenbogenforellen mit Spirulina fest. Bei Stören (*Acipenser baeri*) reduzierte sich ebenfalls der Anteil an EPA und DHA bei Erhöhung des Spirulina-Anteils im Futter (Palmegiano et al. 2005). Insgesamt reduzierte sich in der Studie der Anteil an MUFA, während der Anteil an SFA (gesättigte Fettsäuren), und hier besonders der Anteil an Palmitinsäure, zunahm. Ähnliche Resultate zeigten sich für Welse (*Clarias macrocephalus*) in einer Studie von Chainapong et al. (2018). Der Einsatz von 5 und 10 % Spirulina in der Ration der Fische führte ebenfalls zu einer

Reduktion von DHA und EPA. Zugleich stieg der Gehalt an Palmitinsäure und der gesamte Anteil an SFA stark an.

Die Literaturlage deutet also darauf hin, dass aus dem Austausch eines hohen Anteils von Fischmehl gegen Spirulina ein ungünstigeres Fettsäuremuster im Endprodukt resultieren kann. Dieser Effekt ist in der Aquakultur bereits bekannt, denn im Vergleich zum Wildfisch ist der Anteil an ω-3-Fettsäuren in Zuchtfischen durch die Fütterung konventioneller Futtermittel verringert (Little et al. 2018).

Durch neue Futtermittelkomponenten ist auch ein Effekt auf die Haltbarkeit von Fischprodukten möglich (Ruff et al. 2002). Plaza et al. (2019) zufolge setzt bei Tilapia beispielsweise der *rigor mortis* schneller ein, wodurch die Autoren von einer geringeren Haltbarkeit des Endprodukts ausgehen. Diese Ergebnisse stehen jedoch im Widerspruch mit den Ergebnissen von Güroy et al. (2019). Die Autoren untersuchten die Haltbarkeit von Regenbogenforellenfilets, welche auf Grundlage einer Fischmehldiät, einer Diät mit hohem Anteil an pflanzlichem Protein oder einer Kombination aus Fischmehldiät und 4 % Spirulina, bzw. einer Diät mit hohem pflanzlichen Proteinanteil und 4 % Spirulina gefüttert wurden. Die Diät mit der geringsten Anzahl an coliformen Keimen im Filet war die mit hohem Anteil an pflanzlichem Protein und 4 % Spirulina, gefolgt von der Diät mit hohem Anteil an pflanzlichem Protein ohne Spirulina. Die Autoren schlussfolgern, dass der Spirulina-Anteil im Futtermittel die Haltbarkeit von Fischerzeugnissen verbessert. Allerdings muss erwähnt werden, dass die aus der mit Fischmehl und 4 % Spirulina gefütterten Diät stammenden Filets, die höchste Anzahl an coliformen Keimen aufwiesen. Dementsprechend kann zwar ein begünstigender Effekt auf die Haltbarkeit angenommen werden, dieser ist auf Basis der gegenwärtigen Studienlage jedoch nicht gesichert.

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3 Veröffentlichungen

3.1 The Effect of a Total Fishmeal Replacement by *Arthrospira platensis* on the Microbiome of African Catfish (*Clarias gariepinus*)

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Abstract: An increasing number of fishmeal supplements are becoming the focus of aquaculture research, with a special emphasis on microalgae / cyanobacteria such as spirulina being considered as sustainable alternatives. New feed ingredients can have a far-reaching impact on the intestinal microbiome and therefore play an important role in the development and the health of fish. However, the influence of these alternatives on the microbiome is largely unknown. We undertook a 10 weeks feeding experiment on 120 African catfish with an initial body weight of 50.1 ± 2.95 g. To understand the effect of the spirulina supplementation, two isoenergetic experimental diets were formulated, containing either fishmeal or spirulina as a protein source. The 16S rRNA sequencing was used to analyze the intestinal bacteria microbiota. Results show that the observed richness indicated no significant statistical difference, but Chao1, ACE, Shannon, and Simpson indices indicate a possible increase in bacterial richness for the spirulina diet. The most abundant bacteria in both experimental groups were *Fusobacteriia* with the only taxa from the genus *Cetobacterium*. The bacterium from genus *Romboutsia* was more likely to be found in the microbiome of fish fed the fishmeal diet. In spirulina fed fish, the genera *Plesiomonas* and *Bacteroides* were the most dominant microbes observed. Even though some genera were more abundant in the spirulina group, the overall microbial community structure was not affected by diets.

Keywords: microbiome; microalgae; cyanobacteria; *Arthrospira platensis*; *Clarias gariepinus*; 16S rRNA; bacteria

1 Introduction

The production of fish for human consumption through aquaculture is increasing steadily (FAO 2016), and an increasing demand for fishmeal is predicted to continue (World Bank 2013). As a result, the global marine fish stocks became strongly overfished (FAO 2016) with rising concerns for the ecosystem and the future of fish populations (García 2003). Since the 1950s, the search for alternative protein sources is a major focus of aquafeed research (Becker 2007). However, due to the expected rise in pricing and availability, this is now a priority, as the operational costs for aquafeed are between 50 – 70 % (Rana et al. 2009). Current trends in aquafeed production are now more focused on the exploitation of plant ingredients, simultaneously decreasing fishmeal and oil (Ytrestøyl et al. 2015). New feed ingredients such as microalgae and cyanobacteria (including multiple species such as Arthrospira, Schizochytrium, Tetraselmis, etc.) are suggested as potentially cost-effective and sustainable substitute (Ragaza et al. 2020) and increasingly gaining importance as feed-stuff (Yarnold et al. 2019), but their impact on the fish gut is yet to be investigated.

The cyanobacteria Arthrospira platensis and A. maxima, known collectively as spirulina, are photosynthetic, filamentous, and spiral-shaped cyanobacteria of 0.5 mm in length (Becker 2007). It contains high proportions of protein, between 59 – 65 % (Dernekbasi et al. 2010), and it is known that low level inclusions can boost growth performance and feed conversion ratio in African catfish (Promya and Chitmanat 2011; Raji et al. 2018). Spirulina supplementation also contribute to increase the carotenoid content in the fish muscle and has an immune stimulating effect (Raji et al. 2018). In other species, a positive effect on the fatty acid composition was observed, resulting in enhanced polyunsaturated fatty acids (Jafari et al. 2014; Roohani et al. 2019; Teimouri et al. 2016).

It is not only the performance of the fish that is affected by the diet but also the whole gastrointestinal tract and its microbiome (Desai et al. 2012; Estruch et al. 2015; Ingerslev et al. 2014; Ringø et al. 2006; Schmidt et al. 2016; Xia et al. 2014; Zarkasi et al. 2016). Souza et al. (2020) studied the microbiome of Nile tilapia fed with low concentrations of unicellular microalgae, indicating an influence of microalgae on the microbial community due to an alteration in the bacterial abundance. In support of this, Cerezuela et al. (2012) showed that diets containing unicellular alga altered the intestinal microbiota and decreased the bacterial diversity in gilthead seabream (*Sparus aurata*). In contrast, Lyons et al. (2017) found a greater microbial diversity in rainbow trout (*Oncorhynchus mykiss*) fed with 5 % microalgae meal. However, there are a number of other factors, such as environment (Sullam et al. 2012; Roeselers et al. 2011; Schmidt

et al. 2015; Zhang et al. 2016), season (Ray; Zarkasi et al. 2014), geographic location (Smith et al. 2015; Ye et al. 2014), and host genetics (Schmidt et al. 2015), influencing the microbiome. The health of the gastrointestinal tract is of particular importance for host health, because it is considered to be one of the main routes of infection in fish (Ringø et al. 2016). Nevertheless, the microbiome plays an important role in host immune system and fish nutrition (Llewellyn et al. 2014; Romero et al. 2014), which are two of the most important factors for a successful aquaculture production. For this reason, research focuses on microbiome modulating effects of feed ingredients and additives (Romero et al. 2014).

Since limited information is available about the effect of microalgae on the microbiome, the objective of this study was to investigate the effect spirulina (obtained from the cyanobacterium *Arthrospira platensis*) has on the microbiome. In a controlled feeding trial, we used African catfish (*Clarias gariepinus*), which are one of the most efficient warm water species and became increasingly important in global aquaculture production (Bovendeur et al. 1987; Palm et al. 2018). To avoid an interaction between supplements and nutrients on the microbiome, two isoenergetic experimental diets with different protein sources were utilized. The control diet was based on fishmeal (FM100), and, in the experimental diet, the fishmeal was completely exchanged to spirulina (SP100). We hypothesized that we would find a shift in the microbial community structure. Therefore, we investigated the impact of the algal component in the diet of African catfish on the microbiome, utilizing 16S rRNA amplicon sequencing and based on a previous study on product quality and fatty acid composition (Rosenau et al. submitted) (data not yet published).

2 Materials and Methods

2.1 Fish and Rearing Conditions

A total of 120 African catfish (Clarias gariepinus) full siblings with an average size of 50.1 ± 2.95 g were fed for ten weeks on two different experimental diets (Table 1 and Table 2) (Dietz et al. 2020). Both groups were fed pelleted 3 mm feed, with the control group (FM100) consisting of 20 % fish meal. In the treatment group (SP100), the fish meal was completely replaced by an *Arthrospira platensis* meal. Each treatment was run in triplicate and consisted of 20 fish, which were kept in a recirculated aquaculture system comprising 200 L aquariums with 10 h of dim light and 14 h of darkness. The daily amount of feed consisted of 2 % of the fish biomass and was applied in two portions. In order to maintain a constant stocking density, dead fish were removed and replaced with another full sibling of the same weight (± 5 g) and marked with a PIT tag. Replaced fish were excluded from further microbiome analysis. The body weight of the fish was regularly measured, and feed rations were adjusted accordingly.

The temperature and the oxygen saturation/content were recorded daily for the recirculation system by a Pond Master sensor (OxyGuard, Farum, Denmark). Over the period of the study, the mean water temperature was 27.0 ± 0.43 °C, while mean oxygen content was 9.9 ± 0.41 mg/L. The pH value was measured weekly by a color scale for indicator solution UNISOL 410 (MACHEREY-NAGEL, Düren, Germany). A NANOCOLOR 300 D (MACHEREY-NAGEL, Düren, Germany) was used to measure ammonium and nitrate contents photometrically. The mean pH of the water was 6.9 ± 0.23, with 0.05–0.12 mg/L ammonium and 0.12–0.26 mg/L nitrate. Both groups were fed pelleted 3 mm feed, with the control group (FM100) consisting of 20 % fish meal. In the treatment group (SP100), the fish meal was completely replaced by an Arthrospira platensis meal. Each treatment was run in triplicate and consisted of 20 fish, which were kept in a recirculated aquaculture system comprising 200 L aquariums with 10 h of dim light and 14 h of darkness. The daily amount of feed consisted of 2 % of the fish biomass and was applied in two portions. In order to maintain a constant stocking density, dead fish were removed and replaced with another full sibling of the same weight (± 5 g) and marked with a PIT tag. Replaced fish were excluded from further microbiome analysis. The body weight of the fish was regularly measured, and feed rations were adjusted accordingly. The temperature and the oxygen saturation/content were recorded daily for the recirculation system by a Pond Master sensor (OxyGuard, City, Denmark). Over the period of the study, the mean water temperature was 27.0 ± 0.43 °C, while mean oxygen content was 9.9 ± 0.41 mg/L. The pH value was measured weekly by a color scale for indicator solution UNISOL 410 (MACHEREY-NAGEL, Düren, Germany). A NANOCOLOR 300 D (MACHEREY-NAGEL, Düren, Germany) was used to measure ammonium and nitrate contents photometrically. The mean pH of the water was 6.9 ± 0.23, with 0.05–0.12 mg/L ammonium and 0.12–0.26 mg/L nitrate.

Table 1. Feed ingredients of FM100 and SP100 diets.

Ingredient (% Dry Matter)	FM100	SP100
Fishmeal[1]	20.00	0.00
Spirulina[2]	0.00	20.00
Fish oil	10.70	10.70
Wheat meal	14.00	12.50
Wheat gluten	20.00	21.50
Soy protein concentrate[3]	20.00	20.00
Rapeseed oil	10.70	10.70
Vit./Min. Premix	1.00	1.00
$CaHPO_4$	1.00	1.00
CMC (Binder)	1.29	1.08
TiO_2 (Marker)	0.50	0.50
Fe_3O_4–black (Dye)	0.07	0.07
L-Lysin (HCL-Lys, 78 % Lys)	0.70	0.90
D,L-Methionine	0.01	0.04
L-Tryptophan	0.03	0.01

[1]Crude protein: 62 % as is, [2]Crude protein: 63 % as is, [3]Crude Protein: 67 % as is.

Table 2. Approximate composition (% fresh matter) of FM100 and SP100 diets.

Approximate Composition (%)	FM100	SP100
Dry matter	94.6	94.0
Crude protein (Nx6.25)	45.4	45.7
Crude lipids	24.6	23.9
N-free extracts	17.5	19.0
Crude ash	7.1	5.4
Gross energy [MJ/kg]	23.4	23.5
Digestible energy [MJ/kg]	20.0	20.0
Essential amino acids[1]	26.6	26.8

[1]% in feed dry matter

2.2 Microbiome

2.2.1 Sampling

The microbiome sampling took place at day 70 of feeding. To ensure that the gastrointestinal tract was equally filled, feed was applied to each tank 4 h (staggered every 30 min) before the sampling. Three fish per tank (9 fish/treatment) were killed by a sharp blow to the head and processed for microbiome analysis. The external surfaces of the fish were cleaned with 99.8 % pure ethanol and dissected with sterile syringes and forceps. Thereafter, the lower third of the intestine was removed and squeezed out on a sterile petri dish. In total, 220 mg of feces were put into a 2 ml bead beating tube (Sarstedt AG & Co. KG, Nümbrecht, Germany) and placed on dry ice pellets until further processing the same day.

2.2.2 DNA Extraction

DNA was extracted with the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Venlo, Netherland) following the manufacturers protocol with following modifications. After the application of InhibitEX buffer, samples were disrupted by a Bead Ruptor Elite (OMNI International, Kennesaw, USA) with 300 µg 0.1–0.2 mm, 100 µg 0.4–0.6 mm, and three 1.4–1.6 mm ceramic beads (Biolabproducts, Bebensee, Germany) on two cycles for 45 s with 6 m/s and 5 min of rest on ice in between. A total of 45 µl of Proteinase K was used, and 50 µl ATE buffer was placed on the QIAmp spin column membrane, incubated for 5 min and centrifuged for 1 min. The filtrate was then applied again on the same QIAmp membrane (to maximize the microbial DNA output) and repeated. The DNA yield was quantified by an Infinite® 200 Pro (TECAN Group Ltd., Männedorf, Switzerland).

2.2.3 16S rRNA Gene Amplification and Sequencing

In order to amplify 16S rRNA sequences, we used 16S rRNA with the bacterial gene primer pairs S-D-Bact-0341-b-S-17: 5'-CCTACGGGNGGCWGCAG-3' and S-D-Bact-0785-a-A-21: 5'-GACTACHVGGGTATCTAATCC-3' targeting the V3–V4 region (Klindworth et al. 2013) by using the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Schwerte, Germany). The reaction (50 µl) contained 10 µl of 5x Phusion GC buffer, 0.2 µl 50 mM $MgCl_2$ solution, 2.5 µl DMSO, 200 µM of each of the four deoxynucleoside triphosphates, and 1 U of Phusion DNA Polymerase. We used 20–30 ng of DNA and 1 µl cDNA per reaction. The PCR reaction was started by an initial denaturation at 98 °C for 1 min, followed by 25 cycles of denaturation at 98 °C for 45 s, annealing at 60 °C for 45 s, and elongation at 72 °C for 30 s. The final elongation was done at 72 °C for 5 min. Each PCR was performed in triplicate so as to reduce any PCR bias. The PCR products were visualized on a 1 % agarose gel at 100 V for 1 h

to check for bacterial DNA amplicons. The presence of these was then verified with the identification of a PCR product at ~550 bp. These amplicons were then purified using the MagSi-NGS PREP Plus magnetic beads (AMS Biotechnology, Abingdon, United Kingdom) with 30 µl bead solution on 25 µl amplicon solution and an elution volume of 30 µl EB buffer. Purified amplicons were sequenced with an Illumina MiSeq and Nextera XT DNA Library Prep Kit chemistry (Illumina, San Diego, USA), resulting in paired-end reads of 2x 300 bp length by using the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Schwerte, Germany). The reaction (50 µl) contained 10 µl of 5x Phusion GC buffer, 0.2 µl 50 mM $MgCl_2$ solution, 2.5 µl DMSO, 200 µM of each of the four deoxynucleoside triphosphates, and 1 U of Phusion DNA Polymerase. We used 20–30 ng of DNA and 1 µl cDNA per reaction. The PCR reaction was started by an initial denaturation at 98 °C for 1 min, followed by 25 cycles of denaturation at 98 °C for 45 s, annealing at 60 °C for 45 s, and elongation at 72 °C for 30 s. The final elongation was done at 72 °C for 5 min. Each PCR was performed in triplicate so as to reduce any PCR bias. The PCR products were visualized on a 1 % agarose gel at 100 V for 1 h to check for bacterial DNA amplicons. The presence of these was then verified with the identification of a PCR product at ~550 bp. These amplicons were then purified using the MagSi-NGS PREP Plus magnetic beads (AMS Biotechnology, Abingdon, United Kingdom) with 30 µl bead solution on 25 µl amplicon solution and an elution volume of 30 µl EB buffer. Purified amplicons were sequenced with an Illumina MiSeq and Nextera XT DNA Library Prep Kit chemistry (Illumina, San Diego, USA), resulting in paired-end reads of 2x 300 bp length.

2.2.4 Sequence Processing and Analyses

Amplicon sequencing was performed by the Göttingen Genomics Laboratory using the CASAVA software (Illumina, San Diego, USA) for demultiplexing and clipping of adapter sequences from the raw amplicon sequences. Quality filtering was performed using fastp (v0.20.0) (Chen et al. 2018), and sequences with a phred score of ≥ 20 and a length of ≥ 50 bp were excluded. Soft clipping of low quality base pairs took place with phred score of 20 and a sliding window size of four bases as well as a read correction by overlap and adapter of Illumina Nextera primers. These quality-filtered reads were then merged with the paired end read merger (PEAR v.0.9.11) with default settings (Zhang et al. 2014). The program cutadapt (v2.5) was used with default settings to remove forward and reverse primer sequences (Martin 2011). Subsequently, sequences were then size filtered (≤ 300 bp were removed) and dereplicated by vsearch (version 2.14.1) (Rognes et al. 2016). The vsearch module UNOISE3 (default settings) was used for denoising reads with a minimum size of eight reads, and the UCHIME module further excluded chimeric sequences (including de novo chimera and reference-based chimera)

against the SILVA SSU 138 NR database (Quast et al. 2013; Bolyen et al. 2019). The resulting amplicon sequence variants were clustered at 97 % by vsearch. OTUs were taxonomically assigned with BLASTn (version 2.9.0) against the SILVA SSU 138 NR database with an identity threshold of 90 %. Uncertain blast hits were marked by using identity and query coverage. Additionally, taxonomic assignments for blast hits with (pident + qcovs)/2 ≤ 93 % were removed (recommended by SILVA ribosomal RNA database project).

2.3 Data Processing

Analysis was carried out with R (version 3.6.3) (R Core Team 2020). Microbiome data were normalized per sample and processed with R package "phyloseq", and a PcoA plot was computed on phylum level (McMurdie und Holmes 2013). Alpha diversity was calculated for common metrics (Chao1, ACE, Shannon, Simpson, and Fischer) and a Wilcoxon rank test was performed to compare the observed diversity in FM100 and SP100. Bar plots were produced with "ggplot2" (Wickham 2016).

2.4 Sequence Data Deposition

The 16 S rRNA gene amplicon sequences were submitted to the NCBI Sequence Read Archive4 (SRA) under the NCBI BioProject accession number PRJNA723703.

3 Results

Bacterial DNA was isolated from 17 of 18 samples. One sample from the FM100 group indicated poor PCR amplification and was excluded from the study. Overall, the microbial community could be classified to 8 phyla, 10 classes, 25 orders, 40 families, 49 genera, and 69 species.

Alpha diversity metrics are shown in Figure 1. Between 32–51 bacterial species were observed in the microbiome. Observed richness showed no statistically significant difference (P = 0.499). Chao1 and ACE showed two samples of the SP100 group with a notably higher diversity in the FM100 group, but also one sample within this group had the lowest diversity in regard to the FM100 group. Similar findings were represented by the Shannon and the Simpson indices with the lowest diversity in a sample in FM100 and the highest in a sample from SP100. However, Fisher's alpha parameter seemed to be only slightly different.

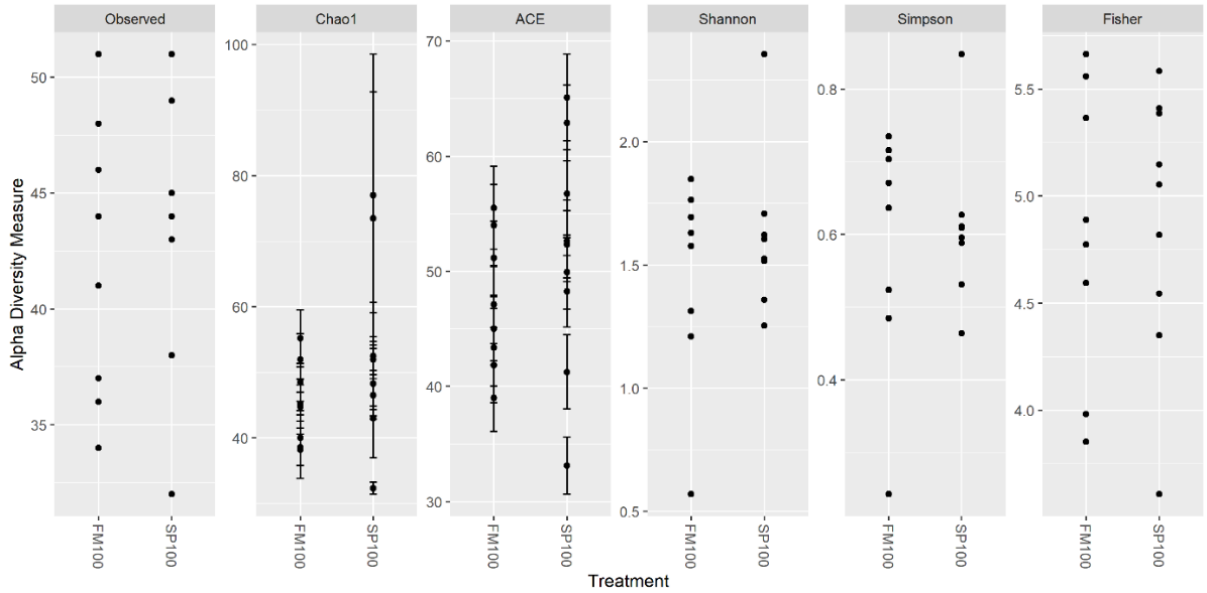

Figure 1. Alpha diversity metrics of microbiome community of African catfish fed with FM100 (n = 8) and SP100 (n = 9) diet. Each point represents one microbiome sample.

Sequencing resulted in 652,898 counts, 248,486 for FM100 and 404,412 for SP100 group. The numbers of counts varied between the samples between 14,419–72,937. We observed the counts per class in the most frequent observations (Figure 2) and the most abundant bacteria on genus level (Table 3). The highest number of counts were dominated by *Fusobacteriia* in FM100 and SP100 with the only taxon from the genus *Cetobacterium*. The second highest counts in FM100 were *Clostridia*, followed by *Bacteroidia*, *Bacilli*, *Gammaproteobacteria,* and *Desulfovibrionia*. In SP100, the second highest counts were observed for *Bacteroidia,* followed by *Clostridia*, *Gammaproteobacteria,* and *Desulfovibrionia*. The class of *Clostridia* was highly abundant in FM100 group, dominated by the genus *Romboutsia*. The family of *Peptostreptococcaceae*, *Bacteroidaceae,* and *Barnesiellaceae* were predominant in *Bacteroidia* and mainly found in spirulina fed fish. Additionally, *Gammaproteobacteria* was mostly abundant in SP100 group and contained primarily the genus *Plesiomonas*.

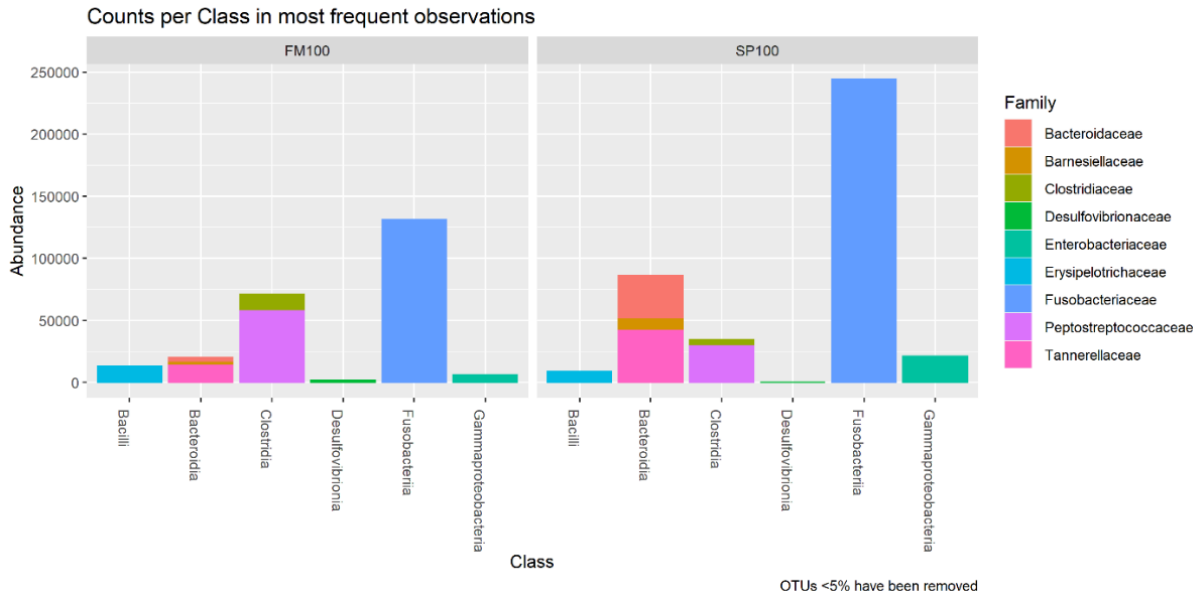

Figure 2. Bacterial counts per class of the most frequent observations (OTUs < 5 % were removed) of FM100 (n = 8) and SP100 (n = 9).

Table 3. Mean values ± SD of percentual abundant of bacteria on genus level in FM100 and SP100.

Genus	FM100	SP100
Cetobacterium	54.98 ± 20.43	58.90 ± 13.34
Romboutsia	20.30 ± 14.88	8.14 ± 3.26
Macellibacteroides	4.15 ± 4.10	5.05 ± 2.50
Plesiomonas	2.95 ± 2.47	5.66 ± 2.95
Bacteroides	1.50 ± 0.61	8.82 ± 5.72
[Anaerorhabdus] furcosa group	3.44 ± 2.96	1.88 ± 1.92
[Barnesiellaceae] uncultured	1.30 ± 1.15	2.17 ± 1.72
Clostridium sensu stricto 1	2.39 ± 1.94	0.72 ± 0.38
Turicibacter	2.25 ± 2.30	0.21 ± 0.19

Relative abundances for each sample on family level are shown in Figure 3. OTUs (operational taxonomic units) with < 5 % were declared as “rare OTUs” (represented in Figure 4). Similar to Figure 2, high percentages of *Fusobacteriia* represented the most abundant bacteria in most of the probes. There were, however, two exceptions to this with 3_FM100 indicating a high proportion of *Peptostreptococcaceae* and 6_SP100 with a high abundancy of *Bacteroidaceae*. As such, overall, we could observe a high variation between samples.

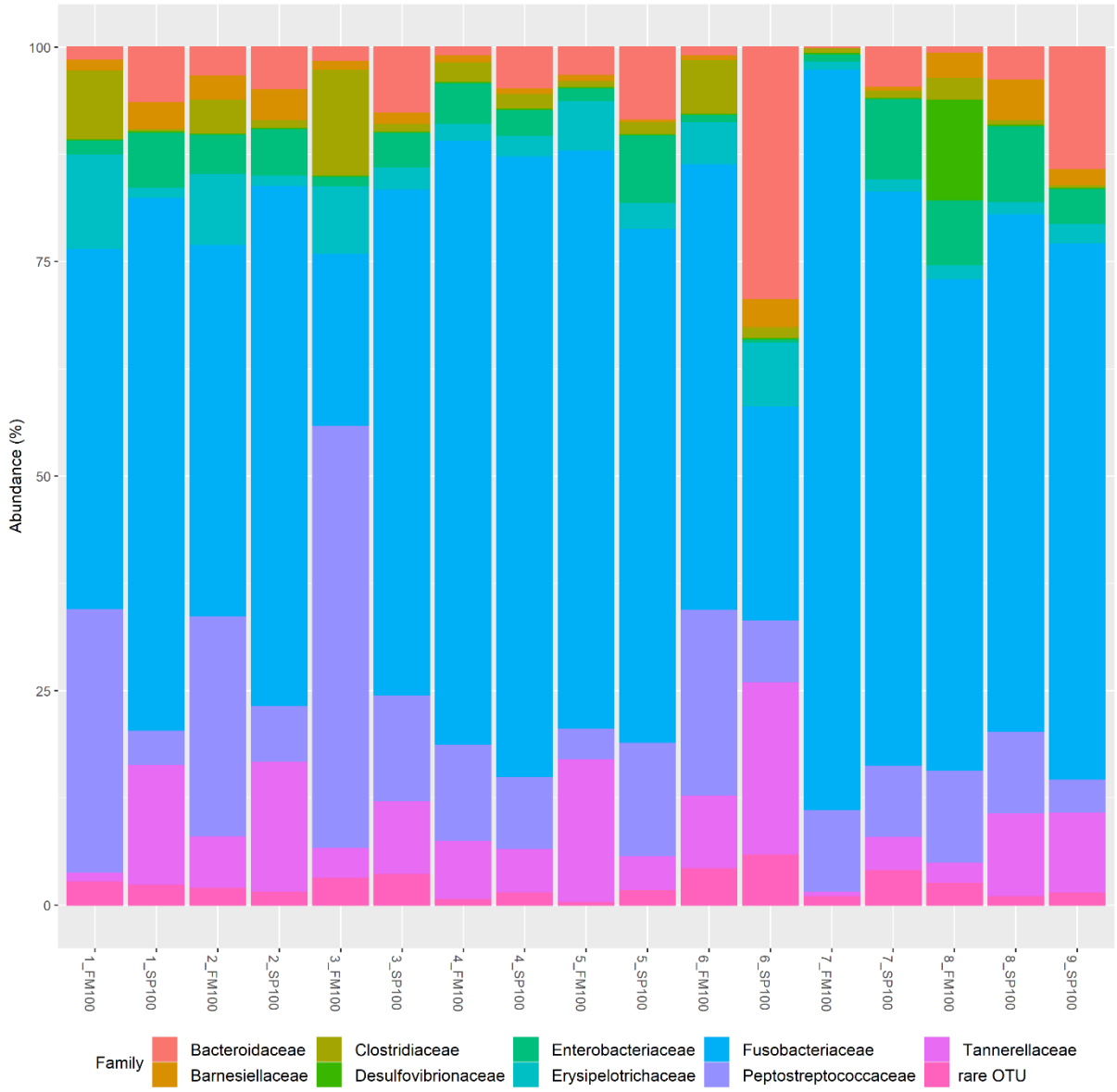

Figure 3. Relative abundance (%) at family level for samples in FM100 (n = 8) and SP100 (n = 9).

Between 40–53 rare OTUs in FM100 and between 48–58 rare OTUs in SP100 were found to be present in the microbial probes (Figure 4). Rare OTUs were observed in both groups, whereby 6_SP100 showed the highest percentage of rare bacteria in the microbiome. As with the other OTUs, the rare OTUs also showed a high variation between samples.

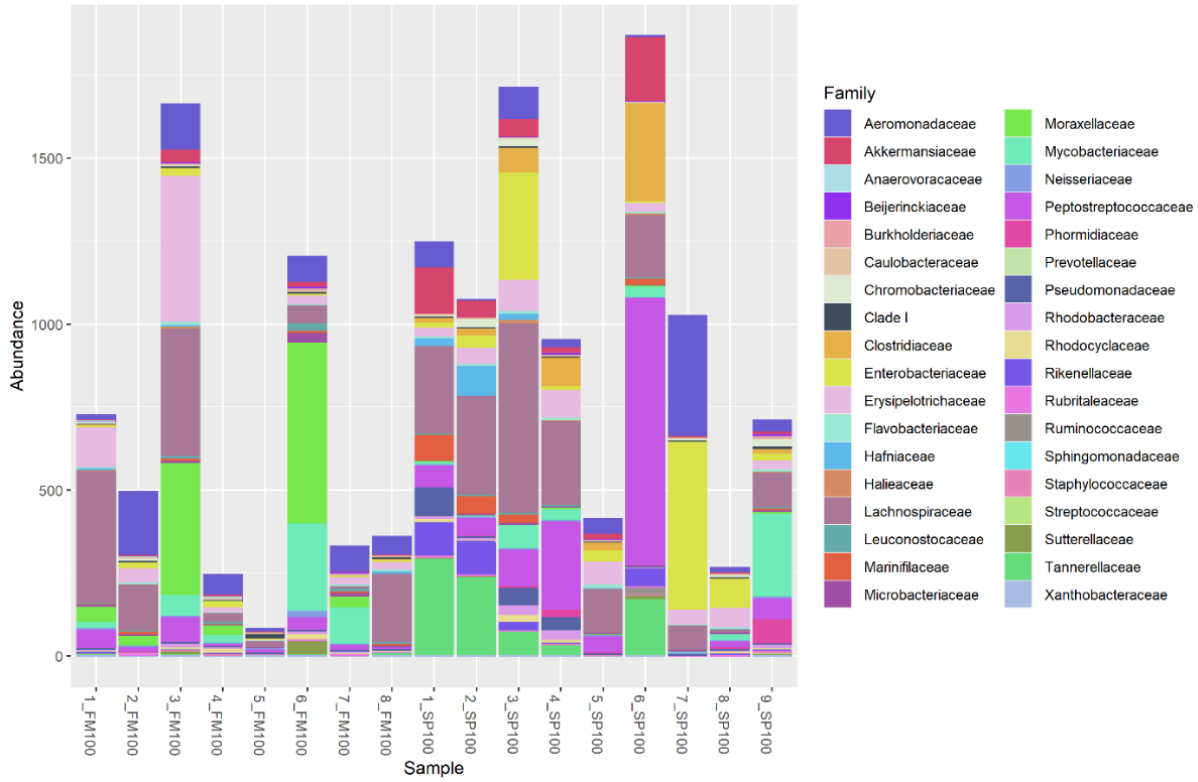

Figure 4. Relative abundance (%) of rare OTUs (< 5 %) of FM100 (n = 8) and SP100 (n = 9) samples.

We used a principal coordinate analysis (PCoA) to confirm previous statistical analysis. The distance matrix was used to detect similarities and differences in microbial community structures (Figure 5). However, neither samples nor taxa were visually distinguishable due to the close clustering. The PCoA showed only low level of variation, as all values were clustered near the origin; therefore, the samples showed only small or no differences. In this case, we were not able to distinguish between dietary groups, and the overall microbiome structure of the gastrointestinal tract was not seen to be affected by the treatment.

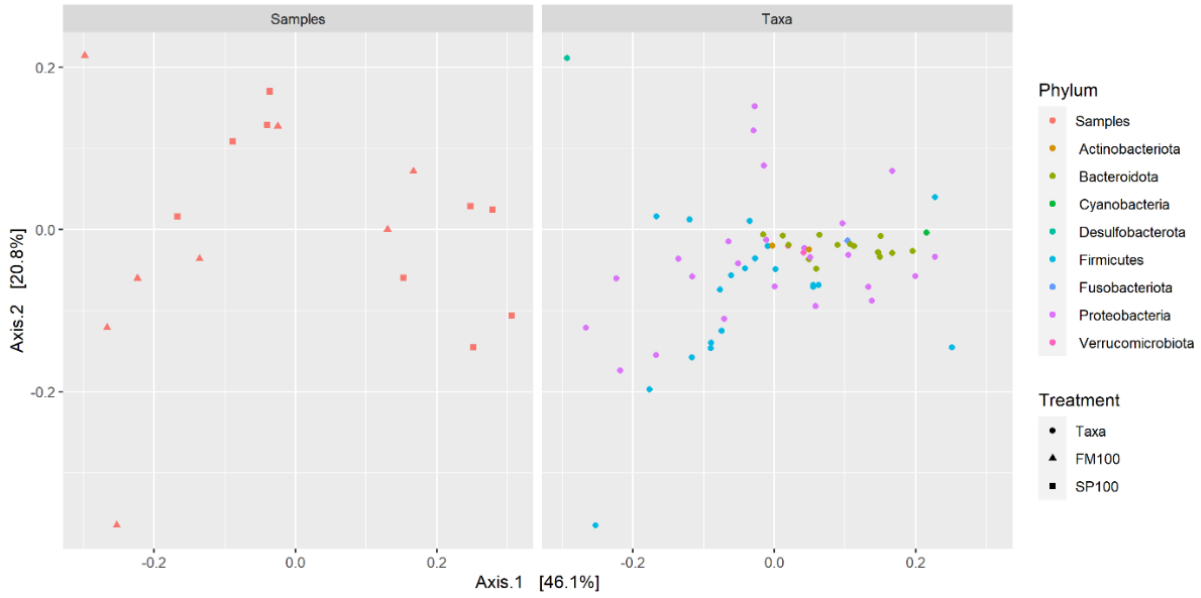

Figure 5. Principal coordinate analysis (PCoA) of FM100 (n = 8) and SP100 (n = 9) samples at phylum level. Each item represents an individual sample.

4 Discussion

Microalgae/cyanobacteria such as spirulina are promising supplements for fishmeal, but information about the impact on the intestinal fish microbiome is highly limited. Only a few studies concentrated on algae supplementation and its effect on the microbiome, but the supplementation levels were rather low. Our study is the first one evaluating a complete microalgae/cyanobacteria supplementation, hoping to find the exclusive effect of spirulina on the microbiome.

In general, the most frequent phyla in fish gut microbiota are *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Firmicutes*, and *Proteobacteria*, which account for over 80 % of the total gut bacteria (Yukgehnaish et al. 2020), which is also in accordance with our study. We were able to demonstrate that the most abundant microbial family in African catfish was *Fusobacteriia* with the only taxon from the genus *Cetobacterium* in FM100 and SP100 groups. This bacterium is also highly abundant in other fish species such as carp (Eichmiller et al. 2016), rainbow trout (Lyons et al. 2017), and tilapia (Souza et al. 2020; Fan et al. 2017; Zhang et al. 2016). Both (Minich et al. 2018) and (Bledsoe et al. 2016) observed *Cetobacterium somerae* as the most abundant microbial bacteria for African catfish and channel catfish (*Ictalurus punctatus*), respectively. *Cetobacterium somerae* plays an important role in the intestinal microbiome due to its physiological benefits of synthesizing vitamin B-12 and antimicrobial metabolites (Tsuchiya et al. 2008). The second most abundant genus in our study was *Romboutsia*, which was found to be lower in spirulina-fed fish. This genus is able to utilize carbohydrates and have the capability to ferment numerous amino acids, anaerobic respiration, and metabolic end products in

the human intestine (Gerritsen et al. 2019). In farmed tilapia (*Oreochromis niloticus*), the abundance of *Romboutsia* is reduced in fish fed with low protein levels (Zhu et al. 2020). However, both of our experimental diets contained approximately the same percentages of crude protein and essential amino acids. Therefore, we suggest that *Romboutsia* may be unable to degrade the cell wall of microalgae component and, as such, led to the reduced bioavailability of the spirulina protein. Another change on genus level can be seen in the abundance of *Gammaproteobacteria*. The supplementation of microalgae increased the abundance of this genus in fish fed the spirulina-diet. The same effect could be observed with microalgae (Souza et al. 2020). In humans, high levels of *Gammaproteobacteria* can be observed in patients with nonalcoholic fatty liver disease (Michail et al. 2015). The class of *Gammaproteobacteria* are able to produce endogenous alcohol (Ren et al. 2007), which may affect liver health. In this case, higher feed intensity and long-term effects of microalgae application may have a negative effect on liver health, but this would require further examination. Within the class of *Gammaproteobacteria*, the genus Plesiomonas was more abundant in SP100 than in FM100 samples. *Plesiomonas shigelloides*, which is known to be an aerogenic gram-negative bacteria (Ahmad et al. 1998), is often found in tropical climates (Brenden et al. 1988) and in various fish species (Bledsoe et al. 2016; Suphoronski et al. 2019; Sugita et al. 1996). As *P. shigelloides* was proven to have an antimicrobial effect, its presence in gut microbiome may have a positive effect on the overall health of the fish (Sugita et al. 1996).

Our study indicates that a full supplementation of fishmeal with spirulina has the potential to alter the diversity of microbiome samples, leading to a higher bacterial diversity in the gut. In this context, we were able to find a possible increase of diversity in different metrics but were not able to find a significant statistical difference in the observed richness. Due to a small number of samples and a high variation between all samples, the overall microbial community structure was not affected. Similar findings were presented by Lyons et al. (2017), who also found higher OTU counts and a higher diversity (for Chao index) in rainbow trout (*Oncorhynchus mykiss*) fed with a 5 % whole-cell microalgae ingredient (*Schizochytrium limacinum*) for 15 weeks, but they were also unable to observe changes in the microbial community structure. The authors explained this as an effect by polysaccharides contained in the microalga that led to an adaptation of the gut microbiota but did not affect the bacterial community structure. Aside from this, they were also able to observe a strong variation between microbiome samples. Souza et al. (2020) determined also an increasing diversity (for Chao index) with a 1.2 *% Schizochytrium sp.* supplementation for Nile tilapia (*Oreochromis niloticus*).

It is known that, in mammals, the microbial gut diversity increases from carnivorous to omnivorous to herbivorous (Ley et al. 2008), and the same occurs in fish (Li et al. 2016). In addition, the gut length also increases from carnivorous to omnivorous to herbivores species (Karachle und Stergiou 2010). Normally, a long gut favors anaerobic bacteria, which are more common in herbivorous gut microbiome (Escalas et al. 2021). A slow passage of feed ingredients through the longer digestive tract may increase the time for microbial fermentation, leading to an increase in the microbial diversity (Lyons et al. 2017) and thus associated with an improved dietary digestion (Bäckhed et al. 2005). In herbivorous fish, the abundance of cellulolytic bacteria is found to be higher in the gut (Li et al. 2009; Li et al. 2016; Wu et al. 2012). We were able to see this effect due to an increase of some microbial phyla such as *Firmicutes* and *Bacteroidia* in the microbiome of those spirulina-fed. These bacterial members are able to improve cellulose digestion in herbivorous fish (Wu et al. 2012; Nayak 2010). As the African catfish is an omnivorous fish with a relatively short gut length, the rate of passage for microalgae components may be too short in the gastrointestinal tract for the fermentation process to occur and to influence the microbiome structure. Furthermore, dietary factors also have the ability to alter the intestinal morphology (Santigosa et al. 2008). Souza et al. (2020) did not find an effect on the morphological structure or integrity of the intestinal villi due to a microalgae supplementation for Nile tilapia.

As described earlier, environmental factors can affect the microbiome of fish. To minimize the environmental effect, we comprised all experimental fish with the same water from a recirculation aquaculture system. The water temperature was within the optimum range of 25–28 °C (Ogunji und Awoke 2017), and dissolved oxygen was in an appropriate range (Peteri et al. 1992). Nitrate and ammonium concentrations were below the upper limit throughout the whole test period (Peteri et al. 1992; Schram et al. 2010; Schram et al. 2014). It cannot be ruled out that the effects of the environmental factors have a stronger shaping effect on the gastrointestinal tract and its microbiome than those of the diet.

5 Conclusions

Spirulina is a promising microalgae/cyanobacteria for fish nutrition and can be considered as a suitable alternative for fishmeal. Previous studies on the impact of microalgae on the microbiome were performed using low levels of algae inclusion. Our study is the first one focusing on a total replacement of fishmeal with spirulina and its effect on the microbiome of African catfish. While the data were not statistically significant, there was some indication that the diversity levels could be altered by supplementation; this will need to be further investigated in more

depth. A further adaptation of the intestinal microbiome to the supplemented microalgae was seen at the bacterial genus level. We were unable to see an effect on the overall microbiome structure, but further investigation into the effect on alternative fish species could prove more insightful. As manipulation of the microbiome was shown to improve health as well as nutrient utilization in fish, it could therefore lead to further improvements in aquaculture production as a whole.

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3.2 Total Replacement of Fishmeal by Spirulina (*Arthrospira platensis*) and Its Effect on Growth Performance and Product Quality of African Catfish (*Clarias gariepinus*)

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Abstract: Microalgae are increasingly being studied to replace fishmeal in aquafeed production. Low level Spirulina supplementation to various fish species has been widely investigated, demonstrating enhanced growth and better product quality. In order to evaluate the effects of a full fishmeal replacement with Spirulina (Arthrospira platensis) on growth and product quality in African catfish (Clarias gariepinus), two isoenergetic diets were formulated and fed for ten weeks to 120 fish with an average initial weight of 50 ± 3 g. Full supplementation of Spirulina resulted in reduced growth ($p < 0.001$) whereas feed conversion ratio was on par ($p > 0.05$). Furthermore, Spirulina-fed fish showed a more intense yellow coloration in skin, and raw and cooked fillet ($p < 0.001$). The analysis of fatty acids revealed higher proportions of C16:0 ($p < 0.001$) and C18:2n6 ($p < 0.05$) in fish fed the Spirulina-diet while C24:0 ($p < 0.01$) and C20:5n3 ($p < 0.001$) were found to be higher in the control group. Even though no statistically significant differences in the overall SFA, MUFA and PUFA were detected, a slight increase of the n6/n3 ratio was observed in the Spirulina-fed fish. Without further optimization of the feed ration, a complete fishmeal replacement with Spirulina can lead to economic losses. It remains to be studied whether the observed changes in product quality affect consumer acceptance.

Keywords: aquaculture; plant protein; cyanobacteria; aquafeed; fatty acids; carotenoid; fillet color; omnivorous fish

1 Introduction

Nowadays, the worldwide fish populations are endangered by various anthropogenic factors. Climate change threatens the overall biodiversity (Lovejoy et al. 2019; Sala et al. 2000; Thomas et al. 2004; Mooney et al. 2009) and can have an effect on the worldwide fish populations (Rijnsdorp et al. 2009; Cheung et al. 2009; Booth et al. 2017). Further, water pollution can be identified as another crucial factor, which has been largely investigated (Lawrence et al. 2007; Austin 1998; Heath 2018; Khoshnood 2017). In addition, the intensive use of fishmeal represents a threat to the safeguarding of marine ecosystems (Little und Edwards 2003). Seawater consumption, the high amount of energy required, and the emission of effluents with a high organic content are some of the reasons why the processing of raw fish into produce fishmeal is raising environmental concerns. In addition, fisheries lead to far-reaching changes in the ecosystem and endanger the world's fish populations (García 2003). These observations underlie the urgent need to identify feasible alternatives to common fishmeal for the aquaculture sector.

Micro- and macroalgae constitute the first trophic level in the food chain, representing an interesting source of proteins for aquafeed production. They are also considered to be more sustainable in terms of resource use than other aquafeed ingredients (Taelman et al. 2013). The protein content of algae is between 6 and 71 % of dry matter, highly depending on the species (Becker 2007).

Spirulina (SP) seems to be suitable for aquafeed production, containing a high proportion of proteins, usually between 59 and 65 % of dry matter (Dernekbasi et al. 2010). In fact, various studies showed that low levels of SP addition can be beneficial to the growth performance of different fish species (Velasquez et al. 2016; Teimouri et al. 2013; Sirakov et al. 2012; Nandeesha et al. 2001; Raji et al. 2018; Promya und Chitmanat 2011; Meng-Umphan 2009; Roy und Pal 2015).

Besides the protein, SP is also a source of natural carotenoids, containing β-carotene and xanthophylls (zeaxanthin, echinenone and cryptoxanthin) (Miki et al. 1986; Mathew et al. 1995). These carotenoids are integrated into the fish muscle without further modification (Hata und Hata 1974), producing a more intense red and yellow coloration of the fillet, along with blood carotenoids (Kop und Durmaz 2008). In previous studies, the luminosity (L*), redness (a*) and yellowness (b*) was found to be higher in SP-fed trout fillets (Teimouri et al. 2013; Roohani et al. 2019). Although the presence of carotenoids in the fillet could represent a sort of value adding in terms of their nutraceutical properties (Vílchez et al. 2011), the trade-off might be a decreased consumer acceptance due to an unfamiliar coloring effect (Welker et al. 2001).

The fatty acid profile plays a major role in product quality of animal products, and polyunsaturated fatty acids (PUFA) are especially crucial for human health (Tapiero et al. 2002). Since algae are considered to have a positive effect on the lipid metabolism in fish (Nakagawa 1997), several studies were already conducted in this direction and reported an enhanced level of PUFA in the fish muscle (Roohani et al. 2019; Jafari et al. 2014; Teimouri et al. 2016).

In our study, we aimed at evaluating the effects of a full supplementation of SP on the growth and product quality of fish. African catfish (*Clarias gariepinus*) was selected for the experiment, being an omnivorous fish species and given its increasing importance in global aquaculture production (FAO 2020). A ten-week feeding trial was undertaken with two isoenergetic diets containing either fishmeal or SP (*Arthrospira platensis*) meal as a protein source. In order to estimate the general effects of a fishmeal-free fully SP-substituted diet on weight, feed conversion ratios (FCR), color of skin and fillet, cooking loss and fatty acids were determined.

2 Materials and Methods

2.1 Ethical Clearance

All animal work followed relevant national guidelines. Good veterinary practice was applied in all procedures whenever animals were handled. The study was in accordance with the German legal and ethical requirements of appropriate animal procedures. The procedures in this study were approved by the Institutional Animal Welfare Body (no. T2-2019, 27 June 2019).

2.2 Rearing of Experimental Fish

Reproduction, rearing and the feeding trial of fish took place in a warm water recirculation aquaculture system (RAS) of the Georg August University of Göttingen. Catfish larvae (full siblings) were reared with trout starter feed (Inicio Plus, Biomar, Aarhus Denmark) until they reached the required weight. Then a total number of 120 African catfish (Clarias gariepinus) with an average body weight of 50 ± 3 g were fed for ten weeks on two isoenergetic experimental diets (Tables 1 and 2), developed and analyzed by Dietz et al. (2020). Nutritional value was in accordance with the recommendations of the National Research Council (NRC) for catfish (National Research Council 2011). The control group (FM100) was fed with a pelleted feed consisting of 20 % fishmeal, whereas in the experimental group (SP100), the fishmeal was completely replaced by SP meal. Each treatment was run in randomized triplicates and consisted of 20 fish per aquarium. All experimental fish were kept in 200 L aquariums connected to a RAS and exposed to a light regime of 10 h dim light and 14 h darkness. Fish were hand fed twice a day according to body weight and water temperature, thus daily rations amounted to 2 % of the fish biomass. One fish died over the course of the trial and was replaced with a full

sibling comparable in body weight (±5 g) of the dead fish's weight in order to keep a constant stocking density. The body weight of fish was regularly measured (after two, six and ten weeks) in order to adjust rations applied. Water temperatures and oxygen contents were recorded daily using a Pond Master sensor (OxyGuard, Farum, Denmark). Average water temperature was kept at 27.0 °C throughout the study period, and oxygen saturation in the RAS showed an average value at 127.0 %, resembling an average oxygen content of 9.9 mg/L. The pH was measured weekly applying a color scale for indicator solution UNISOL 410 (Macherey-Nagel, Düren, Germany). Ammonium and nitrate concentrations were measured photometrically by a NANOCOLOR 300 D (Macherey-Nagel, Düren Germany). The average pH of the water was 6.9, while the ammonium and nitrate concentrations ranged in the intervals 0.05–0.12 mg/L and 0.12–0.26 mg/L, respectively.

Table 1. Feed ingredients of FM100 and SP100 diets.

Ingredient (% Dry Matter)	FM100	SP100
Fishmeal [1]	20.00	0.00
Spirulina [2]	0.00	20.00
Fish oil	10.70	10.70
Wheat meal	14.00	12.50
Wheat gluten	20.00	21.50
Soy protein concentrate [3]	20.00	20.00
Rapeseed oil	10.70	10.70
Vit./min. premix	1.00	1.00
CaHPO4	1.00	1.00
CMC (Binder)	1.29	1.08
TiO_2 (Marker)	0.50	0.50
Fe_3O_4—black (dye)	0.07	0.07
L-Lysin (HCL-Lys, 78 % Lys)	0.70	0.90
D,L-Methionine	0.01	0.04
L-Tryptophan	0.03	0.01

[1] Crude protein: 62 % as is, [2] Crude protein: 63 % as is, [3] Crude protein: 67 % as is.

Table 2. Approximate composition (% fresh matter) of FM100 and SP100 diets.

Approximate Composition (%)	FM100	SP100
Dry matter	94.6	94.0
Crude protein (Nx6.25)	45.4	45.7
Crude lipids	24.6	23.9
N-free extracts	17.5	19.0
Crude ash	7.1	5.4
Gross energy [MJ/kg]	23.4	23.5
Digestible energy [MJ/kg]	20.0	20.0

2.3 Sampling of Experimental Fish

After 10 weeks of feeding, 120 fish were anesthetized with a sharp blow to the head and killed by immediate withdrawal of blood. Body weight, length and carcass weight were recorded for all experimental fish. Approximate FCR was calculated as followed:

$$\text{approximate FCR} = \frac{\text{mean amount of feed per fish [g]}}{\text{weight gain [g]}}$$

Daily weight gain was calculated as follows:

$$\text{daily weight gain} = \frac{\text{weight gain [g]}}{\text{days of feeding}}$$

Eighteen fish (9 per treatment) were used for microbiome analysis (data presented elsewhere) and were not used for further product quality measurements. After the direct samplings, fish were filleted and each fillet was cut in two parts (anterior and posterior muscle) and frozen at –20 °C until further analyses.

2.4 Color Measurements of Fish

A total of 102 fish (51 fish per treatment) were used to determine skin and fillet color (Figure 1). Color values were displayed as lightness (L*), red/green (a*) and blue/yellow (b*). Chroma (C*) describes the color saturation and was calculated as follows:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

h° describes the color appearance and was calculated as follows:

$$h° = \arctan\left(\frac{b^*}{a^*}\right)$$

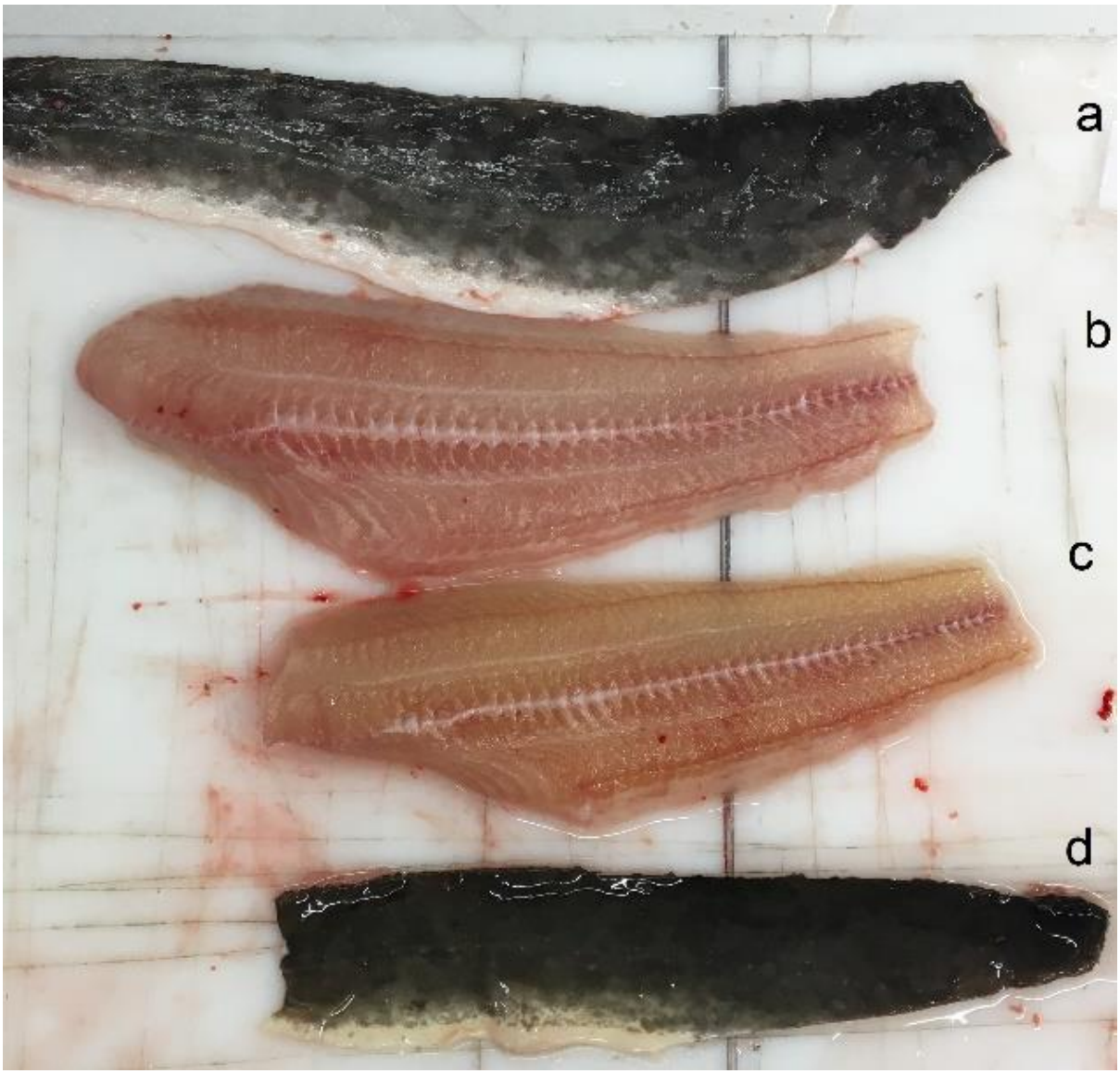

Figure 1. Skin and fillet of FM100 and SP100-fed fish after 10 weeks of experimental feeding: (**a**, **b**) skin and fillet of a fish fed the FM100 diet; (**c**, **d**) fillet and skin of a fish fed SP100 diet.

Immediately after slaughtering, skin color was measured with a CM-600d spectrophotometer (Konica Minolta, Chiyoda, Japan) with the following settings: Illumina D65 and two degrees observer. Both sides of the fish, left and right, were sampled on the dorsal skin at three points: between the head and dorsal fin, below the beginning of the dorsal fin and above the middle of the dorsal fin. In addition, three measurements were taken along the ventral skin at the abdomen: between the pectoral fins, between the pectoral and pelvic fins, and between the pelvic fins.

For the fillet color evaluation, both sides of the skinless fillets were cleaned with tap water and measured with a CR-400 spectrophotometer (KONICA MINOLTA, Japan) on the internal surface of the skinless fillet, as described by Iwona et al. (2016) for African catfish. Subsequently,

the right fillet was frozen at –72 °C for fatty acid analysis and the left fillet was frozen at -20 °C for the determination of color stability upon cooking and cooking loss.

2.5 Sampling of Experimental Fish

Further measurements were taken once the 102 (51 fish per treatment) fillets reached room temperature after thawing. The core temperature of raw and also of cooked fillets was measured with a thermometer (735-2, testo, Titisee-Neustadt, Germany). First, the right dorsal part of the fillets was thawed for 24 h at 4 °C, then the fillet color was measured (CM-600d, Konica Minolta, Chiyoda, Japan) once again in three measurements along the dorsal back muscle. The color change was calculated as follows:

$$\Delta\text{color} = \text{fillet color cooked} - \text{fillet color raw}$$

2.6 Fatty Acids

The method for the fatty acid analysis was adapted from (Du et al. 2000) with modifications. Both of the experimental diets and the front dorsal muscle of 20 fish per treatment were used for analysis. Fish fillets and feed samples were freeze-dried overnight and minced (EGK 200 spice and coffee grinder, Rommelsbacher, Dinkelsbühl, Germany). The samples were stored at 4 °C in hermetically sealed boxes until further processing.

2.6.1 Chemical and Reagents

Butylated hydroxyanisol (BHA) and 3 M methanolic HCl were provided by Sigma-Aldrich (Munich, BY, Germany). Methanol, chloroform and n-hexane were purchased from Carlo Roth (Karlsruhe, BW, Germany). Sodium chloride (NaCl) was provided by VWR Chemicals (Darmastadt, Germany).

2.6.2 Fish Samples Treatment

Two grams of freeze-dried fish were accurately weighed and recorded to two decimal spaces. A 20 ml chloroform/methanol solution (2/1, *v*/*v*, Folch I solution) and 32 µl of 10 % (*w*/*v*) BHA ethanolic solution were added. The mix was homogenized with an Ultra Turrax T25 (IKA, Germany) for 45 s. Next, the homogenate was filtered through paper and 5 ml of 0.88 % NaCl (aq) was added. After 15 min the lower phase was withdrawn and evaporated with a Multivapor P-12 (BÜCHI, Switzerland) at 50 °C. Afterwards, 1 ml of 3 M methanolic HCl was added and incubated in a 60 °C water bath for 40 min. After cooling for 5 min at room temperature, 2 ml hexane was added. The upper phase was transferred into a 1.5 ml vial for the GC-FID analysis.

2.6.3 Feed Samples Treatment

Of freeze-dried feed, 0.5 g was accurately weighed. Three milliliters of 3Mmethanolic HCl was added and incubated at 60 °C for 2 h. The mix was brought to room temperature and centrifuged for 5 min at 10 °C and 4000 x *g*. One milliliter of the supernatant was mixed with 1 ml of n-hexane and 200 µL of the resulting upper phase was put into a GC-vial for the GC-FID analysis.

2.6.4 GC-FID Analysis of FAMEs

Fatty acid methyl esters (FAMEs) were analyzed by means of a TRACE^{TM} 1310 gas chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA) and an AS 1310 autosampler (Thermo Fisher Scientific Inc., Waltham, USA). Extractions were performed in duplicate for the fish fillets and for the experimental diets in quadruplicate, respectively. The gas chromatograph was equipped with Chromeleon software 7.2 SR 9 software. Separation of methyl esters was performed on a Supelcowax^{TM-10} column (30 m, 0.32 mm, 0.25 µm) provided by Thermo Fisher, USA. A flame ionization detector (FID) was used with a heater temperature of 260 °C, an air flow of 350 ml/min, H_2 flow of 35 ml/min and auxiliary gas flow of 40 ml/min. The SSL injector temperature was set at 250 °C with a split ratio of 1:50 and a purge flow of 2.4 ml/min. The oven temperature was set at 160 °C for 1 min, then increased by 10 °C/min until 220 °C and then held for 3 min. The final temperature reached 250 °C (increased also by 10 °C/min) and held for 3 min. The total run time amounted to 16 min. Hydrogen was used as a carrier gas with a flow rate of 1.2 ml/min. Identification of fatty acids was performed by comparison of retention times with the Supelco 37 Component FAME Mix standard (Sigma-Aldrich, Munich, Germany). The relative amount of each fatty acid was expressed as percentage of total area.

2.7 Statistical Analysis

The statistical analysis was conducted with R (R Core Team 2020). Even though a normal distribution is initially assumed for a fish population, we visually checked normality by Q-Q-plot and confirmed it with a Shapiro–Wilk test. Welch two-sample t-tests for unequal variances were carried out to compare the means of all quantitative variables from FM100 and SP100. ANOVA was calculated to obtain differences between the initial experimental units. Pearson's correlation was used for linear association between fish body weight and cooking loss. A principal component analysis (PCA) was then performed on standardization (i.e., to mean 0 and standard deviation 1) such that the PCA was merely based on correlations of the studied variables. Multivariate analysis was applied to growth, color parameters, cooking loss as well as fatty acids. For visualization, the R package's ggplot2 (Wickham 2016) and factoextra (Kassambara und

Mundt 2020) were used. Additionally, R code snippets from Allen et al. (2019) were adjusted to create raincloud plots.

3 Results

3.1 Growth Parameters

Mortality was generally low during the ten-week trial, since only one fish died for unknown reasons in the SP100 group. The initial body weight did not show any statistical differences between the experimental units ($p > 0.05$). The final body weight differed significantly among experimental groups, which showed average values of 280.84 ± 110.80 g for FM100 and 219 ± 82 g for SP100, respectively (Figure 2 and Table A1). The final weight was found to be significantly reduced for SP100 ($p < 0.001$). No statistically significant difference was observed in FCR between both groups ($p > 0.05$), but within the last weeks of the feeding experiment, we observed an increase in FCR for both groups (Table A 2).

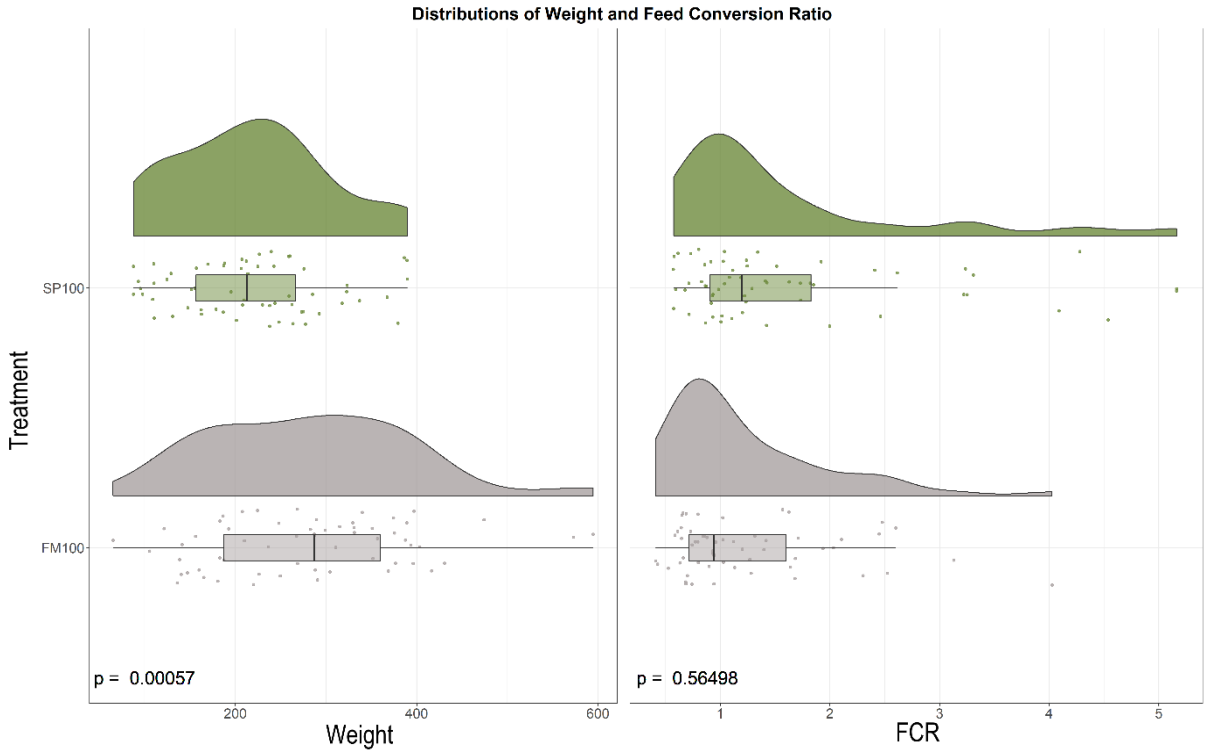

Figure 2. Raincloud plot of body weight and approximate food conversion ratio (FCR) for experimental groups FM100 (gray, $n = 60$) and SP100 (green, $n = 60$) after 10 weeks. Upper histograms show the distribution of data and boxplot with each value for every individual measurement.

3.2 Color Parameters and Cooking Loss

Dorsal skin and ventral skin showed significant differences between FM100 and SP100 in redness (a*) ($p < 0.01$), yellowness (b*) and C* ($p < 0.001$), but not in h°-values ($p > 0.05$). In

SP100 samples, redness (a*) was lower in the skin and higher in the fillets ($p < 0.001$), when compared to FM100 group (Table 3). In skin and fillet samples of SP100, significantly higher values for the color yellow (b*) were observed ($p < 0.001$). No relevant differences for brightness (L*) were observed in both tissues ($p > 0.05$). In addition, SP100 values were elevated in treatment groups in skin and fillet; however, h°-values showed only slight increase in skin samples and decreased values in fillets.

The effect of cooking on the color stability of the fillet is expressed in the delta values between raw and cooked fillet samples. Color parameters changed after cooking for both treatments. Brightness, redness and yellowness increased due to the cooking process. The color changes were comparable for L* and b*, showing no statistically significant difference ($p > 0.05$). However, significant differences in Δa* were observed between the two groups ($p < 0.01$).

SP replacement affected cooking loss ($p < 0.05$). Specifically, cooking loss average values of 11.24 ± 3 % and 9.82 ± 3 % were recorded for FM100 and SP100 samples, respectively. No correlation was found between body weight and cooking juice loss ($p > 0.05$).

Table 3. Color parameters of skin, and raw and cooked fillet in FM100 (n = 51) and SP100 (n = 51).

Skin (Dorsal Back Muscle)					
Diet	**L***	**a***	**b***	**C***	**h°**
FM100	30.42 ± 4.92	1.17 ± 0.46	1.97 ± 1.42	2.40 ± 1.29	67.29 ± 63.92
SP100	29.81 ± 4.63	0.77 ± 0.46	4.18 ± 2.04	4.30 ± 2.00	78.14 ± 17.43
p-Value	0.455	<0.001	<0.001	<0.001	0.091
Skin (Abdomen)					
Diet	**L***	**a***	**b***	**C***	**h°**
FM100	71.19 ± 8.28	1.56 ± 1.74	2.49 ± 2.53	3.53 ± 2.37	107.89 ± 100.11
SP100	68.60 ± 7.45	0.61 ± 2.35	11.25 ± 3.95	11.46 ± 4.09	87.34 ± 10.46
p-Value	0.058	0.002	<0.001	<0.001	0.056
Raw Fillet					
Diet	**L***	**a***	**b***	**C***	**h°**
FM100	48.66 ± 5.05	0.35 ± 1.69	9.15 ± 2.25	9.32 ± 2.23	89.90 ± 13.58
SP100	47.98 ± 5.52	2.32 ± 2.17	13.17 ± 3.55	13.52 ± 3.66	82.00 ± 11.83
p-Value	0.247	<0.001	<0.001	<0.001	<0.001
Cooked Fillet					
Diet	**ΔL***	**Δa***	**Δb***	**ΔC***	**Δh°**
FM100	24.36 ± 3.68	2.22 ± 1.28	8.34 ± 2.61	7.83 ± 2.41	16.58 ± 8.65
SP100	24.19 ± 3.37	1.80 ± 1.09	8.83 ± 2.96	8.81 ± 2.96	7.19 ± 4.17
p-Value	0.690	0.003	0.142	0.037	<0.001

Values are means ± SD. *p*-values were calculated by Welch *t*-test. L* (+ = lighter, − = darker), a* (+ = redder, − = greener), b* (+ = yellower, − = bluer), C* (+ = brighter, − = duller), h° (hue angle).

The effect of cooking on the color stability of the fillet is expressed in the delta values between raw and cooked fillet samples. Color parameters changed after cooking for both treatments. Brightness, redness and yellowness increased due to the cooking process. The color changes were comparable for L* and b*, showing no statistically significant difference ($p > 0.05$). However, significant differences in Δa* were observed between the two groups ($p < 0.01$).

SP replacement affected cooking loss ($p < 0.05$). Specifically, cooking loss average values of 11.24 ± 3 % and 9.82 ± 3 % were recorded for FM100 and SP100 samples, respectively. No correlation was found between body weight and cooking juice loss ($p > 0.05$).

3.3 Fatty Acids

The five most abundant fatty acids in our investigation included oleic (C18:1n9), palmitic (C16:0), linoleic (C18:2n6c), lignoceric (C24:0) and eicosapentaenoic acids (C20:5n3) (Figure 3 and Table A2). Higher proportions of C16:0 ($p < 0.001$) were observed in fish fed the SP100 diet with 19.37 ± 1.25 % compared to 17.89 ± 1.22 % in the FM100 group. C24:0

($p < 0.01$) and C20:5n3 ($p < 0.001$) were higher in the FM100 group (8.01 ± 1.47 % and 6.00 ± 0.53 %) than in the SP100 group (6.58 ± 1.33 % and 4.99 ± 0.59 %). No significant difference ($p > 0.05$) was observed for C18:1n9, which was the most abundant fatty acid in both FM100 (36.36 ± 2.42 %) and SP100 (37.07 ± 2.20 %) groups. No significant difference was found for C18:2n6c, with 11.48 ± 0.72 % in FM100 and 11.33 ± 0.64 % in the SP100 group. Overall, no differences were observed in sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) between the two diets. Total n-6 fatty acids amounted to 12.79 ± 0.70 % in FM100 and were higher ($p < 0.001$) in the SP100 group with 14.27 ± 0.73 %, whereas n-3 fatty acids were lower in content ($p < 0.001$) in SP100 with 8.53 ± 0.68 % towards 9.87 ± 0.56 %. This led to a significantly lower ($p < 0.001$) n-6/n-3 ratio in FM100 (1.30 ± 0.09) than in SP100 (1.68 ± 0.12).

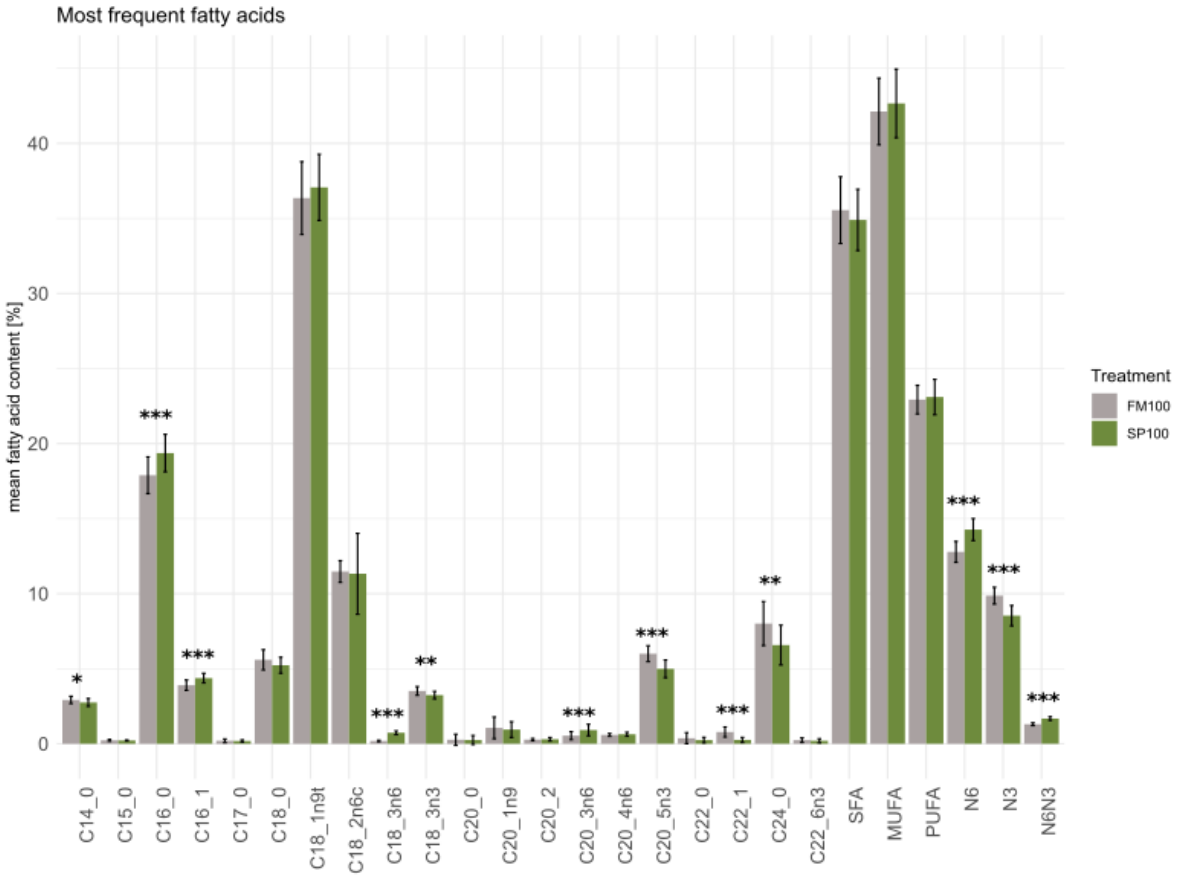

Figure 3. Most frequent fatty acids (%) in fish muscle of FM100 ($n = 20$) and SP100 ($n = 20$). SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, N6: sum of omega-6 fatty acids, N3: sum of omega-3 fatty acids. N6N3: ratio N6 : N3. The values are means ± SD. *p*-values were calculated by Welch *t*-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.01$).

The fatty acid profile in the feed was characterized by higher values of SFA in SP100 (32.05 ± 0.28 %) than in FM100 (25.37 ± 0.54 %) and in MUFA for SP100 (52.16 ± 0.39 %)

than in SP100 (43.93 ± 1.97 %) Table A3. Overall, PUFA levels were lower in SP100 (15.73 ± 0.17 %) than in the FM100 (30.60 ± 1.48 %) diet. It is particularly noticeable that the concentration of C20:5n3 in SP100 was much lower, at 0.62 ± 0.05 %, than in FM100, with 8.58 ± 0.63 %.

3.4 Principal Component Analysis (PCA)

The PCA biplot illustrates that fillet color was influenced the most by the complete replacement of FM with SP (Figure 4). In addition, skin color parameters (dorsal back muscle and belly) b* and C*, and to a lesser extent a*, were also affected by SP-based feeding. As for the other parameters, PCA confirms the results of the *t*-test, with fatty acids being one of the distinguishing factors among the two groups; FCR, weight, length, carcass weight, cooking loss and delta color parameter had only a subordinate influence.

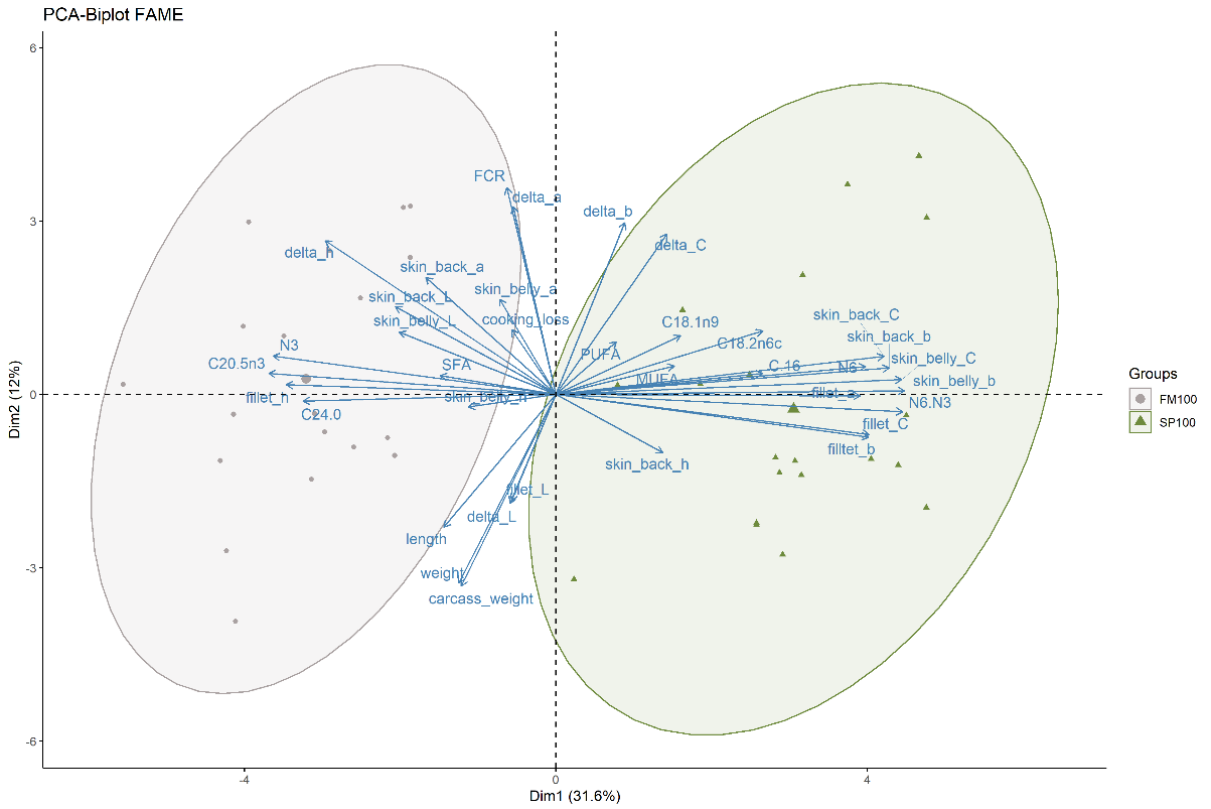

Figure 4. PCA biplot of weight, length, carcass weight, FCR, skin and filet color parameters, color stability (delta values), cooking loss and fatty acids for FM100 (*n* = 20) and SP100 (*n* = 20). Individual values are shown as gray points for FM100 and green triangles for SP100 group.

4 Discussion

The results of this study indicate that a full supplementation of SP has a negative effect on the growth parameters in African catfish. Furthermore, our study implies that SP might affect consumer acceptance, as color parameters of skin and fillet change according to the SP supplementation. Additionally, effects on cooking loss and fatty acid composition were observed.

Growth performance is one of the most relevant factors for aquaculture economics, considering that the operational costs for aquafeed are between 50 and 70 % (FAO 2018). In this trial, fish fed SP100 showed a significantly lower growth performance than the FM100 group. This confirms previous observations that a full replacement of fishmeal with SP resulted in reduced growth performance in other fish species (Olvera-Novoa et al. 1998; El-Sayed 1994). More specifically, supplementation rates of up to 50 % produced comparable growth to that of the control group, but beyond that level, a lower growth rate and – with a 100 % SP replacement – a lower feed utilization was observed. On the other hand, a low (5 %) to intermediate (75 %) inclusion level of SP in the diet of African catfish has been shown to boost growth performance (El-Sayed 1994; Promya und Chitmanat 2011). In our study, a full supplementation of SP had no effect on FCR. Over the course of the trial, a declining feed acceptance was observed within the last weeks of the trial and this resulted in uneaten food particles, which may have influenced the FCR and explain the tendency to increasing FCR values. Compared to other studies with a lower SP supplementations for African catfish, a full supplementation showed comparable FCR values (Promya und Chitmanat 2011; Chavez und Bolviar 2018; Raji et al. 2019). However, the feed ingredients, housing conditions and the age of the catfish were different between experiments and especially SP composition can vary in protein, carbohydrate and lipid content (Becker 2007).

Previous studies showed that the carotenoid pigments in the SP algae change the flesh color of rainbow trout to brown-yellow (Choubert 1979). Our study delivered similar results, as skin and fillet color were highly influenced by the SP replacement. More specifically, yellow intensified in skin and fillet; red decreased in skin, but increased in the fillet; brightness was not affected. The different coloring effect on skin and fillet might be due to a diverse accumulation tendency by the various pigments in different tissues. Red and yellow values in skin and fillet of SP-fed trout increased, while brightness/darkness only showed remarkable differences in fillet color (Teimouri et al. 2013; Roohani et al. 2019). Both studies indicate SP as a good source of carotenoids to improve skin and fillet pigmentation. However, it must be taken into account that alterations in flesh color are known to have a potentially negative impact on consumer

acceptance (Peterson et al. 1966). Traditionally, the fillet color of African catfish is classified as white (Young et al. 1996; Jäger 1991). On the one hand, a higher yellow coloration of catfish fillet might be perceived as a discoloration leading to lower consumer acceptance – for example, in countries like USA (Li et al. 2013). On the other hand, the characteristic coloration might also be used for sustainability marketing purposes, but more studies should be made on this topic.

The cooking process increased L*, a* and b* values for both groups. This observation could be justified by the already documented thermal stability of yellow pigments, such as lutein (Iwona et al. 2016).

Dietary nutrients have the potential to improve the flesh quality of fish (Larsson et al. 2014). To ensure a high product quality, a minimal weight loss after cooking is required to fulfill consumer expectations (Morkore und Einen 2003). The fish consists of high proportions of water and fat. Its moisture content mostly depends on feeding and usually ranges from 69 to 74 % (Solomon 2018; Chukwu und Shaba 2009; Akpambang 2015) and the fat content is about 5.7 % (Rosa et al. 2007). Due to the cooking process, liquid components leach out of the fillet (Offer et al. 1989). In our study, cooking loss was reduced in the SP100 group. A study performed on African catfish suggested that the fattening and – as a consequence – the weight of the fish could have an influence on the cooking loss (Wedekind 1991). More specifically, the author observed higher cooking losses in stronger fattened African catfish. However, in this case we found no statistically significant correlation between body weight and cooking loss, so that the difference might be due to a fattening effect, the diets or could be attributable to other aspects not considered in this study.

Our research showed that fatty acids profile was affected by the presence of SP in the diet. C18:1n-9 was one of the most prevalent fatty acids but did not differ significantly in the FM100 group. SFA, MUFA and PUFA also did not show any significant differences. In contradiction to our study, MUFA was reduced, while PUFA levels were elevated in Siberian sturgeon (*Acipenser baeri*) fed with high SP inclusion of up to 60 % (Palmegiano et al. 2005). However, this led to increased proportions of C16:0, which is in accordance with our study. Teimouri et al. (2016b) reported similar findings for MUFA and PUFA in rainbow trout (*Oncorhynchus mykiss*) fed up to 100 g kg^{-1} SP. Additionally, SFA reduced with high inclusions of SP. However, Jafari et al. (2014) found this effect in rainbow trout only with low SP inclusion of 5 %, but not at 7.5 %. Increasing amounts of SFA and PUFA could be found in Roohani et al. (2019) at 52.8 g kg^{-1} SP inclusion in the feed of Caspian brown trout (*Salmo trutta* caspius). In walking

catfish (*Clarias macrocephalus*) fed with a 10 % SP inclusion, C16:0 increased too, but other highly abundant fatty acids such as C18:0, C18:1n-9, C18:2n-6 and C22:6n3 showed opposite results. Furthermore, SFA and MUFA increased and PUFA was reduced (Chainapong et al. 2018). Differences in the fatty acid composition between the experimental groups can largely be explained by the fatty acid composition of the diets. In this respect, it is noted that the C20:5n3 was largely reduced in the SP100 diet, but in the fillet the strong difference was not reflected in the fish muscle. This might be because of the biosynthesis of long-chain PUFA towards C20 and C22 PUFA and the expression of the elongase of the very long chain fatty acid gene (Oboh et al. 2016). Nevertheless, the fatty acid composition is species-specific (Passi et al. 2002) and feed composition of the experimental diets differed strongly, which means that comparisons between species can only provide limited information. Differences in the microbial community of the gastrointestinal tract could be a reason for this effect. The slightly increased n6/n3 ratio observed in SP100 fillets reflects the fatty acids composition of diets. In fact, results showed how the replacement of FM with SP led to an increase of dietary n6 fatty acids and a contemporary reduction of the n3. Although from a mere nutritional point of view these data can be interpreted as a (slight) worsening of the lipid profile of the fillet, the observed n6/n3 ratio was still well below the limit of 5 recommended for the fatty acid balance in the human diet (Elvevoll und James 2000).

5 Conclusion

This is the first study regarding total replacement of fishmeal with SP for African catfish. Even though a full SP supplementation could produce several benefits in terms of sustainability, the full replacement of fishmeal with SP could return economic losses due to a lower growth performance. It remains to be shown whether catfish with elevated yellow skin and fillet coloration is acceptable to consumers. Moreover, investigations on the chemical nature of pigments accumulated in the fish muscle are necessary in order to evaluate possible health benefits deriving from the consumption of SP-fed African catfish. Even though the replacement of fishmeal reduced the PUFA content in feed drastically, this difference was comparably low in flesh and the resulting n6/n3 ratio is still considered acceptable. Further optimizations in feed composition and feed application time should be investigated as well as the performance of other fish species.

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Appendix

Table A1. Final growth parameters, carcass weight and approximate FCR and daily weight gain of FM100 (n = 60) and SP100 (n = 60).

Diet	Weight [g]	Length [mm]	Carcass weight [g]	Approximate Feed Conversion Rate (FCR)	Daily Weight Gain [g]
FM100	280.84 ± 110.80	315.85 ± 38.84	256.67 ± 99.91	0.97 ± 1.89	3.31 ± 1.59
SP100	218.66 ± 82.24	291.33 ± 33.31	199.61 ± 72.87	1.16 ± 1.16	2.41 ± 1.18
df	108.74	115.32	107.93	97.921	108.74
t-Statistics	3.550	3.711	3.617	−0.577	3.550
p-Value	<0.001	<0.001	<0.001	0.565	<0.001

Values are means ± SD. p-values were calculated by Welch t-test.

Table A2. Growth parameters over the course of the ten-week trial for FM100 and SP100.

Weeks		0–2			2–6			6–10	
Diet	Weight [g]	FCR	Daily Weight Gain [g]	Weight [g]	FCR	Daily Weight Gain [g]	Weight [g]	FCR	Daily Weight Gain [g]
FM100	74.47 ± 0.64	0.67 ± 0.03	1.714 ± 0.08	169.33 ± 8.81	0.66 ± 0.05	3.388 ± 0.25	280.84 ± 13.19	1.33 ± 0.19	3.982 ± 0.60
SP100	71.07 ± 1.06	0.75 ± 0.03	1.526 ± 0.07	147.95 ± 5.87	0.78 ± 0.04	2.756 ± 0.16	218.66 ± 41.57	1.72 ± 0.28	2.526 ± 0.44
df	2.606	4.000	3.753	3.588	3.986	3.439	2.399	3.561	3.671
t-Statistics	3.396	−3.084	3.053	3.506	−3.245	3.738	2.469	−1.991	3.412
p-Value	0.053	0.037	0.041	0.030	0.032	0.0264	0.111	0.126	0.031

Values are means ± SD of triplicate goups (n = 20 per replicate). p-values were calculated by Welch t-test.

Table A3. Fatty acid content (%) of FM100 (n = 20) and SP100 (n = 20) in the fillet.

Fatty Acid	FM100	SP100	*p*-Value
C6:0	0.00 ± 0.00	0.00 ± 0.00	NA
C8:0	0.00 ± 0.00	0.00 ± 0.00	0.586
C10:0	0.00 ± 0.00	0.00 ± 0.00	0.690
C11:0	0.00 ± 0.00	0.00 ± 0.00	0.622
C12:0	0.07 ± 0.02	0.07 ± 0.01	0.957
C13:0	0.01 ± 0.01	0.01 ± 0.01	0.700
C14:0	2.92 ± 0.24	2.75 ± 0.26	0.026
C14:1	0.02 ± 0.01	0.02 ± 0.01	0.247
C15:0	0.23 ± 0.06	0.22 ± 0.03	0.535
C15:1	0.00 ± 0.00	0.00 ± 0.00	0.834
C16:0	17.89 ± 1.22	19.37 ± 1.25	<0.001
C16:1	3.90 ± 0.34	4.37 ± 0.32	<0.001
C17:0	0.20 ± 0.12	0.18 ± 0.07	0.639
C17:1	0.00 ± 0.00	0.00 ± 0.00	0.155
C18:0	5.59 ± 0.67	5.23 ± 0.53	0.061
C18:1n9	36.36 ± 2.42	37.07 ± 2.20	0.312
C18:2n6c	11.48 ± 0.72	11.33 ± 0.64	0.811
C18:3n6	0.18 ± 0.05	0.73 ± 0.13	<0.001
C18:3n3	3.52 ± 0.29	3.24 ± 0.25	0.001
C20:0	0.27 ± 0.37	0.25 ± 0.30	0.806
C20:1n9	1.06 ± 0.72	0.95 ± 0.53	0.580
C20:2	0.27 ± 0.07	0.30 ± 0.11	0.389
C20:3n6	0.54 ± 0.27	0.91 ± 0.39	<0.001
C21:0	0.00 ± 0.01	0.00 ± 0.00	0.223
C20:4n6	0.59 ± 0.10	0.63 ± 0.13	0.195
C20:3n3	0.11 ± 0.02	0.10 ± 0.03	0.264
C20:5n3	6.00 ± 0.53	4.99 ± 0.59	<0.001
C22:0	0.37 ± 0.36	0.24 ± 0.18	0.099
C22:1	0.78 ± 0.34	0.26 ± 0.17	<0.001
C22:2	0.00 ± 0.00	0.00 ± 0.00	NA
C23:0	0.00 ± 0.00	0.00 ± 0.00	NA
C24:0	8.01 ± 1.47	6.58 ± 1.33	0.001
C24:1n9	0.00 ± 0.00	0.00 ± 0.01	0.324
C22:6n3	0.24 ± 0.15	0.20 ± 0.13	0.277
SFA	35.55 ± 2.22	34.90 ± 2.04	0.299
MUFA	42.12 ± 2.22	42.66 ± 2.29	0.428
PUFA	22.93 ± 0.95	23.10 ± 1.18	0.604
Total n-6	12.79 ± 0.70	14.27 ± 0.73	<0.001
Total n-3	9.87 ± 0.56	8.53 ± 0.68	<0.001
n-6/n-3	1.30 ± 0.09	1.68 ± 0.12	<0.001

Values are means ± SD. *p*-values were calculated by *t*-test

Table A4. Fatty acid content (%) in FM100 and SP100 diet.

Fatty Acid	FM100	SP100
C6:0	0.00 ± 0.00	0.15 ± 0.00
C8:0	0.02 ± 0.02	0.10 ± 0.02
C10:0	0.00 ± 0.00	0.00 ± 0.00
C11:0	0.00 ± 0.00	0.28 ± 0.00
C12:0	0.06 ± 0.02	0.11 ± 0.02
C13:0	0.01 ± 0.00	0.05 ± 0.00
C14:0	3.65 ± 0.33	4.59 ± 0.33
C14:1	NA	NA
C15:0	0.24 ± 0.02	0.54 ± 0.02
C15:1	NA	NA
C16:0	12.60 ± 0.35	19.03 ± 0.35
C16:1	3.93 ± 0.26	4.85 ± 0.26
C17:0	0.20 ± 0.39	0.45 ± 0.39
C17:1	0.09 ± 0.00	0.09 ± 0.00
C18:0	2.61 ± 0.18	3.73 ± 0.18
C18:1n9	36.80 ± 2.25	44.75 ± 2.25
C18:2n6c	15.77 ± 0.53	11.81 ± 0.16
C18:3n6	0.12 ± 0.02	1.19 ± 0.05
C18:3n3	4.83 ± 0.36	1.34 ± 0.04
C20:0	0.58 ± 0.04	1.80 ± 0.03
C20:1n9	1.87 ± 0.13	1.96 ± 0.05
C20:2	0.10 ± 0.01	0.06 ± 0.01
C20:3n6	0.07 ± 0.02	0.06 ± 0.00
C21:0	0.00 ± 0.00	0.00 ± 0.00
C20:4n6	0.56 ± 0.06	0.12 ± 0.01
C20:3n3	0.00 ± 0.00	0.00 ± 0.00
C20:5n3	8.58 ± 0.63	0.62 ± 0.05
C22:0	0.22 ± 0.06	0.59 ± 0.28
C22:1	1.24 ± 0.15	0.50 ± 0.28
C22:2	NA	NA
C23:0	NA	NA
C24:0	5.18 ± 0.24	0.61 ± 0.03
C24:1n9	0.00 ± 0.00	0.00 ± 0.01
C22:6n3	0.67 ± 0.16	0.58 ± 0.12
SFA	25.37 ± 0.54	32.05 ± 0.28
MUFA	43.93 ± 1.97	52.16 ± 0.39
PUFA	30.60 ± 1.48	15.73 ± 0.17
Total n-6	16.51 ± 0.60	13.19 ± 0.20
Total n-3	14.09 ± 0.88	2.54 ± 0.12
n-6/n-3	1.17 ± 0.30	5.21 ± 0.03

Values are means ± SD.

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3.3 Feeding Green: Spirulina (*Arthrospira platensis*) Induced Changes in Production Performance and Quality of Salmonid Species

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Abstract: Spirulina is an interesting candidate for fish nutrition. This study aims to investigate the effect of the complete replacement of fishmeal with spirulina (*Arthrospira platensis*) in the diets of brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*), in relation to growth and product quality. Two isoenergetic and isonitrogenous experimental diets containing either fishmeal or spirulina as a main source of protein were used for a 10-week feeding trial. Differences in the spirulina acceptance and conversion between species were observed. The experimental diets were well accepted except for brown trout. A species–diet interaction was observed, which led to a reduction in final body weight due to the spirulina supplementation for brook and rainbow trout ($p < 0.05$). Parallel, the feed conversion ratio increased to the same extent in the spirulina-fed fish ($p < 0.05$), fostering the assumption, that both species did not differ in converting the spirulina diet. Spirulina led to a significant increase ($p < 0.05$) in yellow and red coloration in both raw and cooked fillets. The diet had a significant effect on the fatty acid profile, resulting in an increase in SFA and MUFA, while PUFA levels decreased significantly in spirulina-fed fish ($p < 0.05$). Overall, total replacement of fishmeal with Spirulina goes along with a reduced production performance and effects on major product quality traits such as fillet colour and fatty acid pattern. In particular, consumer acceptance of the yellow fillet colour should be further investigated.

Keywords: cyanobacteria, fishmeal replacement, growth performance, microalgae, pigmentation.

1 Introduction

The current trend in aquaculture is moving towards the more intensive production of carnivorous fish (Bostock et al. 2010), whose main protein source is fishmeal (Teles et al. 2019). Carnivorous fish have a dietary protein demand between 40 % and 55 %, higher than that of the herbivorous or omnivorous fish species with 25 %–35 %, (National Research Council 2011). Considering this trend, and the predicted further increase in global aquaculture production (World Bank 2013), an even higher demand on fishmeal for aquafeed can be expected. Despite the strict reduction of fishmeal in aquafeed over the past years, the change to full supplementation with alternative protein sources has shown to lead to reduced growth performance and health issues in fish (Teles et al. 2019).

Algae recently gained attention as potential supplements for fishmeal (Rosas et al. 2019). Microalgae/cyanobacteria such as *Arthrospira platensis* and *A. maxima*, which are known collectively as *Spirulina*, have both a high protein content and represent an important source of vitamin B12 (Estrada 2001). In nature, algae also contribute to the food chain as a primary source of polyunsaturated fatty acids (PUFA) (Legeżyńska et al. 2014). In Europe, about half of the algae produced is exclusively spirulina (Araújo et al. 2021). These algae species are of interest as a substitution for fishmeal, as they contain high amounts of essential amino acids and essential fatty acids (Becker 2007; Becker 2013).

Dietary ingredients can have an impact on the product quality of fish (Larsson et al. 2014; Webster et al. 2004), and in salmonid fish, spirulina can improve nutritional value by increasing the content of PUFA (Roohani et al. 2019; Teimouri et al. 2016). Consumer demands and their requirements on fish product quality vary geographically (Rasmussen 2001). In Europe, for example, the flesh colour of trout is described as white or red/pink when carotenoids are added to the diet. It has been known for some time that organisms like plants and protists are able to synthesize carotenoids and thus are the source for the pigmentation of fish flesh (Isler et al. 1971; Steven 1948). These carotenoids are incorporated directly into the fish muscle without further conversion (Hata und Hata 1974). This pigmentation of the fish fillets can affect consumer acceptance: For example, in salmon, this leads to a higher willingness to pay for fillets with increased redness (Alfnes et al. 2006). However, algae-like spirulina were shown to cause a yellow coloration of the fillet colour in trout (Roohani et al. 2019; Teimouri et al. 2013). In Italian trout farms, an unusual yellow flesh coloration occurs during spring and summer times, induced by algae (*Cladophora glomerata*) resulting in high economic losses due to the reduced acceptability to consumers (Welker et al. 2001). This study utilized three different salmonid

species which were fed for 10 weeks with two experimental isoenergetic and isonitrogenous diets, containing either fishmeal or spirulina (*Arthrospira platensis*) as the main protein source. Therefore, rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta fario*), which are the three most important salmonid species for German aquaculture production (Brämick 2019), were used. We hypothesized that the conversion of spirulina differs between species, regarding growth performance and product quality.

2 Material and Methods

2.1 Rearing of Experimental Fish

Fish eggs of rainbow, brook and brown trout were collected and fertilized in the experimental farm of the University of Goettingen in Relliehausen (Dassel, Germany) and transferred in plastic bags in a Styrofoam box to the Department of Animal Science in Göttingen (distance 44 km). Hatching took place in vertical incubators comprised with 10 °C water. Larvae (full siblings) were reared in a recirculated aquaculture system and fed with commercial trout starter and fattening feed (BIOMAR, Denmark) until they reached the required body weight for the experimental trial. All three species were transferred into experimental tanks (250 L) one week before the experiment started to acclimate. Afterwards, the fish were divided into two experimental groups with $n = 60$ fish per treatment in three tanks (replicates) with $n = 20$ fish. All fish were fed, for 10 weeks, with two isoenergetic and isonitrogenous diets, based on the recommendation for rainbow trout from the National Research Council (2011), consisting of 20 % fishmeal (FM100), whereas in the experimental group, the fishmeal was completely replaced by spirulina (SP100) (Table 1). Diet quantity was adjusted to 1 % of the fish biomass (based on a previous study by Dietz et al. (2020) and fed once a day per hand (about 50 % of the feed ration), and the rest was applied by an automatic feeder (FIAP GmbH, Germany). The rainbow trout were stocked first, followed two days later by the brook trout, and the brown trout seven weeks later, due to slower growth. The initial average body weight ± standard deviation of brook trout was 111.80 ± 8.92 g, rainbow trout was 100.8 ± 5.96 g and for brown trout 98.25 ± 9.76 g. All experimental fish were exposed to 14 h of light and 10 h of darkness. In order to keep the stocking density constant, dead fish were replaced with full siblings of a comparable body weight (±10 g) and tagged with a passive integrated transponder. These fish were still used for growth parameters, but excluded from other analyses, since they were fed with a commercial trout fattening diet (BIOMAR, Denmark) in advance. Every two weeks, fish weight was recorded and rations were adjusted on the basis of biomass and feed conversion ratio (FCR). Water temperature and oxygen contents were recorded daily in the tanks with a

handheld sensor (OxyGuard, Denmark). A colour scale for indicator solution UNISOL 410 (MACHEREY-NAGEL, Germany) was used weekly to measure the pH value. Ammonium and nitrite concentrations were measured photometrically by a NANOCOLOR 300 D (MACHEREY-NAGEL, Germany). The mean water temperature was 16.82 ± 0.69 °C with a mean oxygen saturation of 92.21 ± 0.03 % and a mean oxygen content of 8.75 ± 0.30 mg/L. The mean pH value was 7.42 ± 0.36 as well as low amounts of ammonium and nitrate with 0.06 ± 0.03 and 0.09 ± 0.08 respectively.

Table 1. Feed ingredients and approximate composition of FM100 and SP100 diets.

Ingredient (% dry matter)	**FM100**	**SP100**
Fishmeal[a]	20.00	0.00
Spirulina[b]	0.00	20.00
Fish oil	10.70	10.70
Wheat meal	14.00	12.50
Wheat gluten	20.00	21.50
Soy protein concentrate[c]	20.00	20.00
Rapeseed oil	10.70	10.70
Vit./Min. Premix	1.00	1.00
$CaHPO_4$	1.00	1.00
Carboxymethyl Cellulose (Binder)	1.29	1.08
TiO_2 (Marker)	0.50	0.50
Fe_3O_4 – black (Dye)	0.07	0.07
L-Lysin (HCl-Lys, 78% Lys)	0.70	0.90
D,L-Methionine	0.01	0.04
L-Tryptophan	0.03	0.01
Approximate composition (%)		
Dry matter	94.6	94.0
Crude protein (Nx6.25)	45.4	45.7
Crude lipids	24.6	23.9
N-free Extracts	17.5	19.0
Crude ash	7.1	5.4
Gross Energy [MJ/kg]	23.4	23.5
Digestible Energy [MJ/kg]	20.0	20.0

[a]Crude protein: 62% as is, [b]Crude protein: 63% as is, [c]Crude Protein: 67% as is

2.2 Sampling of Experimental Fish

120 fish per species were anaesthetized with a sharp blow to the head and killed by exsanguination at the end of the 10-week feeding experiment. Body weight and length (fork length) were recorded for all experimental fish. Due to the large number of fish, sampling took place on two consecutive days. For product quality measurements, all fish were filleted and the right side of the fillet was frozen at −20 °C and the left side at −80 °C until further processing.

2.3 Colour Measurements

In order to maintain consistency and remove any storage effects the colour was measured immediately after slaughtering the fish, skin and fillet colour parameters were measured on a white filleting board with a CM-600d spectrophotometer (KONICA MINOLTA, Japan) with following settings: illuminant D65, two degrees observer and a measuring unit of 10 mm in diameter. Skin colour was measured on three points on each side of the fish: between the head and dorsal fin, below the dorsal fin and below the adipose fin (Figure 1). For the fillet colour, skinless fillets were cleaned with tap water and measured on three points on the internal surface of the dorsal fish muscle: between the head and dorsal fin, below the dorsal fin and below the adipose fin (Figure 2). Colour values based on the CIELAB system (CIE 1977) and displayed as lightness (L^*), red/green (a^*) and blue/yellow (b^*). Chroma (C^*) describes the colour saturation and was calculated as follows:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$h°$ describes the colour appearance and was calculated as follows:

$$h° = \arctan\left(\frac{b^*}{a^*}\right)$$

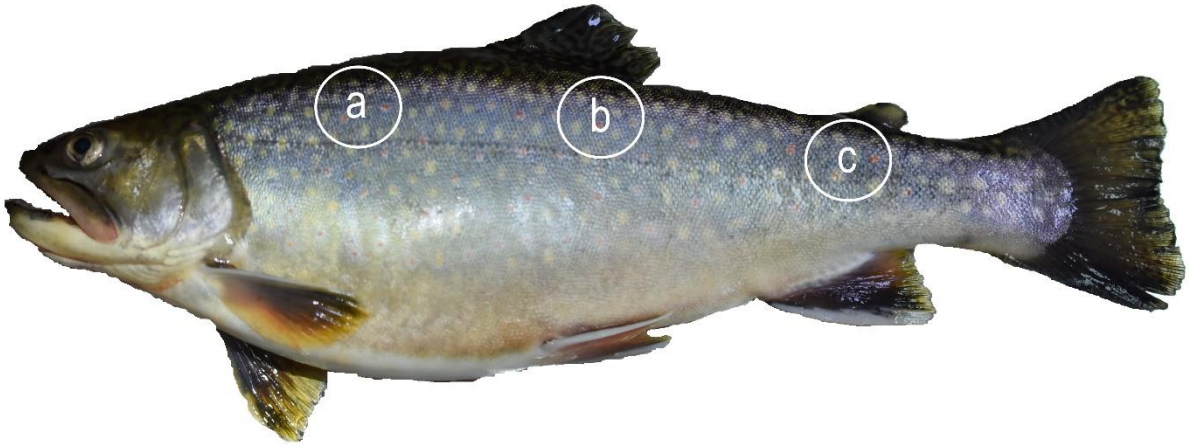

Figure 1. Fish skin color measurement locations. (a) between the head and dorsal fin, (b) below the dorsal fin and (c) below the adipose fin.

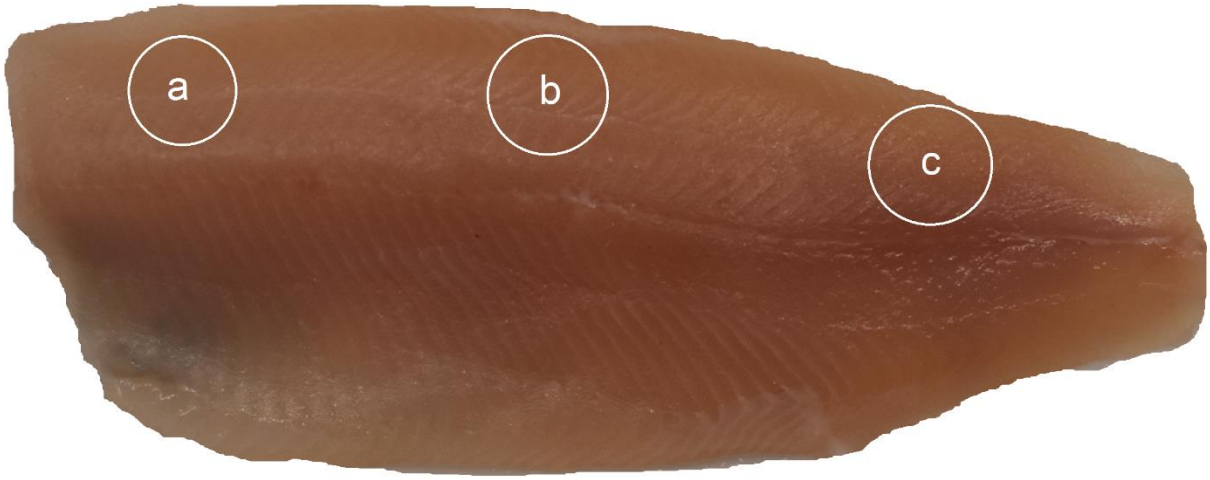

Figure 2. Fillet color measurement locations. (a) between the head and dorsal fin, (b) below the dorsal fin and (c) below the adipose fin.

2.4 Colour stability and cooking loss

120 fillets (right side) were thawed for 24 h at 4 °C, then brought to room temperature. Before the cooking process, fillets were weighed and put in plastic bags and then boiled in a water bath at 78°C until the core temperature reached 70 °C. Released fluids were removed using paper tissues and the fillet weight was once again determined when the cooked fillets had reached room temperature. Cooking loss was calculated as follows: Afterwards, fillet colour was measured again as described previously for skinless fillet.

$$Cooking\ loss\ [\%] = \frac{(initial\ weight\ [g]\ - cooked\ weight\ [g])}{initial\ weight\ [g]} * 100$$

Afterwards, fillet colour was measured again as described previously for skinless fillet.

2.5 Shear force

Cooked fillets were cut with a template into rectangular probes matching the blade holder frame, and the weight of each probe was determined. The myomeres of the sample were placed parallel to the shear blades. A 5-blade Allo-Kramer shear cell (Stable Micro Systems Ltd., Surrey, UK), which was mounted to a TA.XT Plus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 50-kg load cell, was used with following settings: pre-test speed of 5 mm/s, test speed of 2 mm/s, post-test speed of 10 mm/s (Aussanasuwannakul et al. 2010) and triggering force of 1000 g.

2.6 Fatty acids

40 fish per species (20 per treatment) were used for fatty acid analyses. Fish fillets and feed samples were freeze-dried overnight. Fillets were weighed before and after dry-freezing to calculate the water content. Then, freeze-dried fillets and feed were minced with an EGK 200 spice and coffee grinder. The samples were stored in hermetically sealed boxes in the refrigerator until further processing the next day. Gas chromatography Flame Ionization Detector (GC-FID) analysis of fatty acid methyl esters (FAMEs) for fish and feed samples was conducted as described by Rosenau et al. (2021).

2.7 Statistical analysis

R was used for statistical analysis (R Core Team 2020). The visualization of the data was performed with ggplot2 (Wickham 2016) and factoextra (Kassambara und Mundt 2020) package. A principal component analysis (PCA) was computed on standardized data (i.e., to mean = 0 and standard deviation = 1) such as the PCA is merely based on correlations of the studied variables. Normality was checked visually with quantile–quantile plot (Aldor-Noiman et al. 2013). Fixed effects of species, diet and their interaction on performance and product quality traits were analysed by two-way ANOVA with subsequent Tukey's honestly significant difference test for mean differences when appropriate (Barros 2013). A multiple linear regression model was calculated (Stats und R 2021) to observe interactions between final weight, species, diet and initial fish weight with the following equation:

$$y_{ikl} = \mu + S_i + S_i(T_k) + S_i(W_l) + e_{ikl};$$

with y_{ikl} being the final body weight in gram, S_i the fixed effect of species (rainbow trout and brook trout), T_k fixed effect of the type of diet (FM100 and SP100), and W_l as the regression

of the initial tank weight mean, both nested within species and e_{ikl} the residual error per individual.

3 Results

3.1 Performance

The brown trout had accepted the fishmeal diet but not the spirulina diet, causing a weight loss in the spirulina groups after two weeks. We aborted the trial for this species due to animal welfare concerns. However, the experimental diets were well received by the brook and rainbow trout. Results are presented in Figure 3 and Table S1. The initial body weight of brook and rainbow trout groups differed significantly between species ($p < 0.05$). A higher final body weight was observed in FM100 brook trout group (298.03 ± 3.61 g) than in SP100 brook trout group (250.12 ± 4.73 g) and rainbow trout FM100 (226.47 ± 2.29 g) and SP100 (201.70 ± 0.44 g) groups respectively. Final weight and weight gain showed a significant species–diet interaction ($p < 0.05$). Length, specific growth rate (SGR) and FCR showed a statistically significant difference between species and diets respectively ($p < 0.05$). The overall FCR was low (=efficient) across all groups. We were able to find lower FCR values for FM100 than for SP100 groups ($p < 0.05$), but no significant interaction of species and diet was observed ($p > 0.05$).

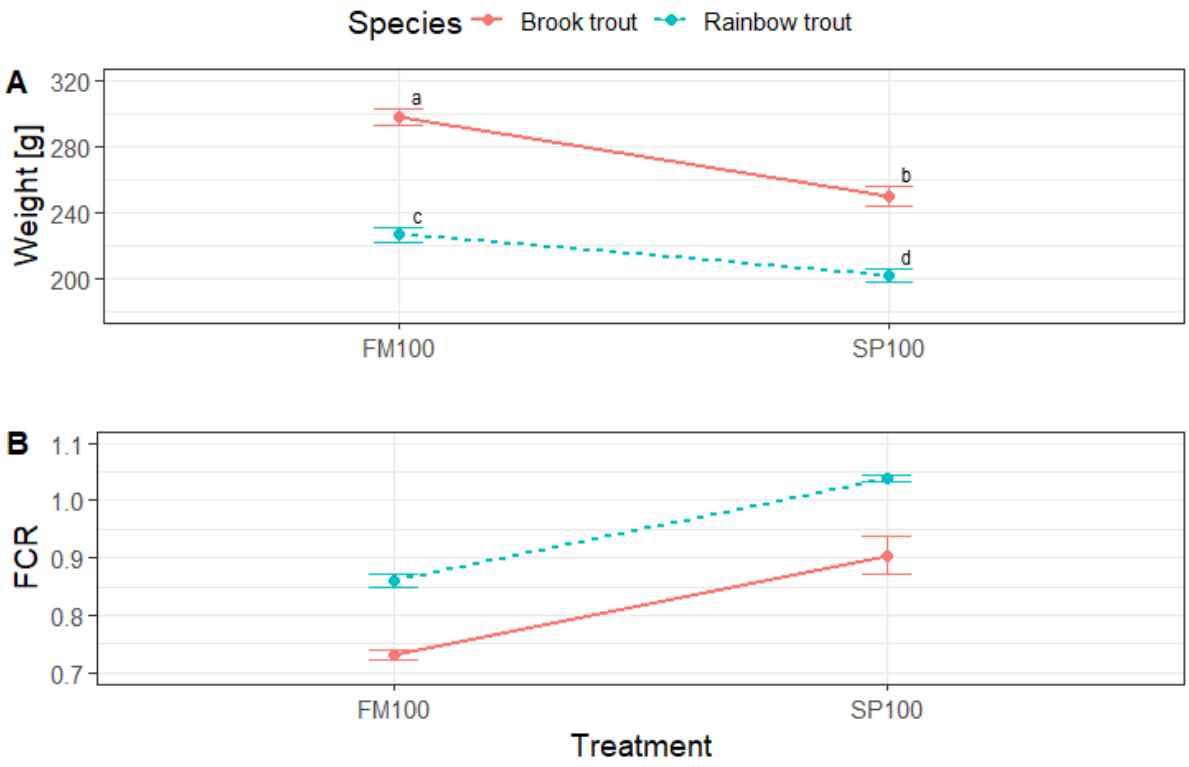

Figure 3. Line chart of estimated mean with standard error of performance parameters for brook trout and rainbow trout groups fed ten weeks FM100 and SP100 diets. Body weight (A) is calculated from every fish (n = 120 per species) and feed conversion ratio (B) are means of triplicate groups. Different letters in the same plot are significant different at a level of $p < 0.05$ (Tukey's test).

A multiple linear regression was calculated to predict the final body weight based on species, diet and initial fish weight (Figure S1). A significant regression equation was observed ($F[5, 234] = 42.99$, $p < 0.05$) with an R^2 of 0.479. Predicted final weight for brook trout and rainbow trout is equal to 412.59–148.31 (species rainbow trout) – 45.15 (brook trout SP100) – 24.36 (rainbow trout SP100) – 1.04 (brook trout tank weight) – 0.38 (rainbow trout tank weight), where final weight is measured in gram. The dietary treatment for brook trout and rainbow trout were significant predictors of final weight ($p < 0.05$).

3.2 Colour Parameters

Species–diet interactions were observed for a^*, b^* and C^* values in all treatment groups ($p < 0.05$). A more intense yellow/orange fillet colour was observed visually in spirulina-fed fish (Figure 4). The photometric data (Table 2) show that redness and yellowness increased the skin of brook trout for SP100 diet ($p < 0.05$), whereas the diet had no significant influence on all skin colour parameters in rainbow trout ($p > 0.05$). The fillet redness, yellowness and chroma

increased due to the spirulina supplementation in both species ($p < 0.05$). Yellow and red coloration was higher in brook trout fed with SP100 than in rainbow trout fed with SP100 diet ($p < 0.05$). Yellow coloration increased after the cooking process of the fillet for all groups, whereas redness slightly decreased in SP100 groups. However, most of the significant differences between the two diets in brook and rainbow trout are also displayed in the cooked fillets. In cooked fillets, no significant difference was observed between species fed with FM100 diet ($p > 0.05$).

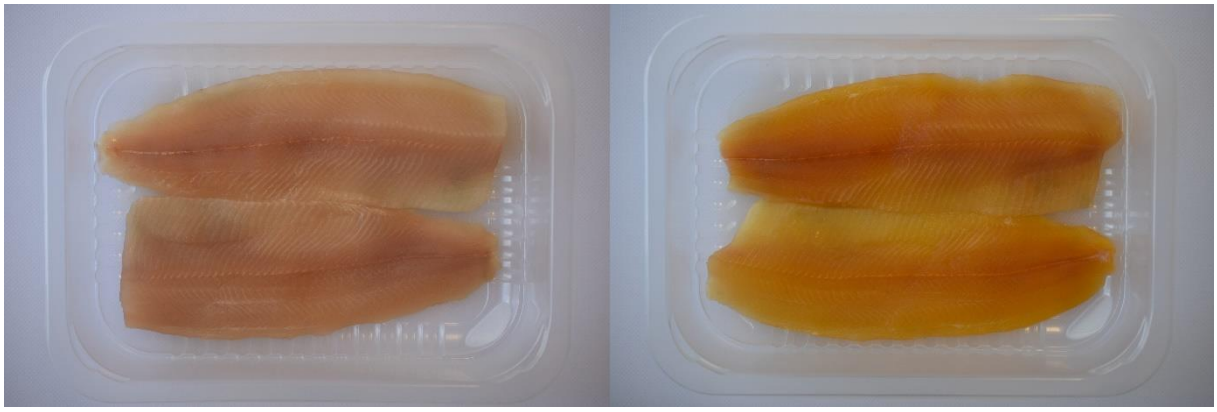

Figure 4. Rainbow trout fillets fed for ten weeks with FM100 (left) and SP100 (right).

Table 4. Color parameter of skin, fillet and cooked fillet of FM100 and SP100 diet in brook trout (n = 116) and rainbow trout (n = 120).

		Brook trout		Rainbow trout		*p* - values		
		FM100	**SP100**	**FM100**	**SP100**	**Species**	**Diet**	**Species x Diet**
Skin Color	L*	60.23 ± 4.43	58.81± 4.18	58.96 ± 5.89	58.6 ± 5.37	ns	ns	ns
	a*	0.88 ± 0.63^{c}	1.88 ± 0.57^{a}	1.48 ± 0.94^{b}	1.63 ± 0.97^{ab}	ns	***	***
	b*	0.07 ± 1.53^{c}	2.64 ± 1.62^{b}	4.08 ± 1.27^{a}	4.34 ± 1.74^{a}	***	***	***
	C*	2.93 ± 0.72^{c}	4.18 ± 1.08^{b}	5.07 ± 0.90^{a}	5.43 ± 1.35^{a}	***	***	***
	h°	192.87 ± 57.04^{a}	114.22 ± 51.49^{b}	87.21 ± 31.65^{c}	80.5 ± 21.01^{c}	***	***	***
Raw Fillet	L*	48.87 ± 1.76	46.8 ± 1.84	47.86 ± 1.48	46.5 ± 2.27	*	***	ns
	a*	0.77 ± 1.18^{c}	7.19 ± 1.87^{a}	0.33 ± 0.68^{c}	4.29 ± 1.46^{b}	***	***	***
	b*	11.78 ± 1.25^{c}	23.6 ± 3.39^{a}	9.45 ± 1.01^{d}	17.3 ± 2.73^{b}	***	***	***
	C*	11.90 ± 1.34^{c}	24.7 ± 3.72^{a}	9.60 ± 1.00^{d}	17.9 ± 2.92^{b}	***	***	***
	h°	87.12 ± 5.08	73.5 ± 2.74	89.26 ± 3.96	77.4 ± 3.66	***	***	ns
Cooked Fillet	L*	82.03 ± 1.58	80.8 ± 2.83	81.43 ± 1.45	80.8 ± 1.25	ns	***	ns
	a*	1.16 ± 0.92^{c}	4.63 ± 1.79^{a}	0.68 ± 0.62^{c}	2.17 ± 1.00^{b}	***	***	***
	b*	17.11 ± 1.15^{c}	29.6 ± 5.55^{a}	16.12 ± 1.10^{c}	23.0 ± 2.87^{b}	***	***	***
	C*	17.18 ± 1.21^{c}	30.0 ± 5.64^{a}	16.16 ± 1.11^{c}	23.2 ± 2.93^{b}	***	***	***
	h°	86.30 ± 2.76	83.0 ± 12.55	87.76 ± 2.13	85.0 ± 2.04	*	***	ns

L* (lightness), a* (red/green), b* (yellow/blue), C* (Chroma) and h° (hue angle). Values are means ± SD, followed by different letters in the same row are significant different at a level of $p < 0.05$ (Tukey's test).

3.3 Water Content and Cooking Loss

The water content was similar between species and diets (Table 3) and no significant effects were observed ($p > 0.05$). Mean cooking loss was higher in brook trout than in rainbow trout ($p < 0.05$). Statistical differences were observed between species and diet, resulting in higher cooking loss for SP100 fed fish ($p < 0.05$).

Table 3. Water content (n = 80) and cooking loss (n = 236) of FM100 and SP100 diet in brook trout and rainbow trout.

Parameter	Brook trout		Rainbow trout		*p* - values		
	FM100	SP100	FM100	SP100	Species	Diet	Species x Diet
Water content [%]	74.05 ± 0.64	75.16 ± 1.52	73.90 ± 1.35	74.13 ± 2.06	ns	ns	ns
Cooking loss [%]	11.67 ± 1.66	13.78 ± 1.92	8.89 ± 1.48	10.24 ± 2.51	***	***	ns

Values are means ± SD. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not significant.

3.4 Shear Force

Results show that the fillet of brook trout ($n = 116$) is more tender than rainbow trout fillet ($n = 120$) ($p < 0.05$). Also the species–diet interaction had an impact on the tenderness of the fillet ($p < 0.05$). In brook trout, the spirulina-fed fillets were less tender with 0.79 ± 0.11 kg*g^{-1} than control group with 0.71 ± 0.17 kg*g^{-1} ($p < 0.05$). The opposite effect was observed in rainbow trout with more tender fillets in the SP100 group (0.96 ± 0.14 kg*g^{-1}) than in FM100 group (1.04 ± 0.21) ($p < 0.05$).

3.5 Fatty Acids

The most abundant fatty acids in the fish muscle were docosahexaenoic (C22:6n3; DHA), oleic (C18:1), palmitic (C16:0), eicosapentaenoic (C20:5n3; EPA) and linoleic acid (C18:2n6c) (Figure 5 and Table S2). Species showed significant differences for C16:0, DHA and EPA ($p < 0.05$), while diet, that is replacement of fishmeal with spirulina affected all of the most abundant fatty acids ($p < 0.05$). DHA and C16:0 content decreased significantly due to the spirulina feeding ($p < 0.05$). In general, SP100 diet lowers the EPA content in the fish muscle for brook and rainbow trout ($p < 0.05$). C18:2n6c was higher in SP100 group than in FM100 ($p < 0.05$), and C18:1 did not show any statistical differences between species ($p > 0.05$), but between experimental diets ($p > 0.05$). Saturated fatty acid (SFA) were significantly influenced by species and diet ($p > 0.05$), while monounsaturated fatty acids (MUFA) and PUFA were

affected only by diet. SFA and MUFA content showed a significant increase and PUFA levels were reduced in SP100 group ($p > 0.05$). Both species responded similarly to the diets in terms of the omega-6 fatty acid (n6), omega-3 fatty acid (n3) content and n6/n3 ratio, leading to statistical differences between experimental diets ($p < 0.05$).

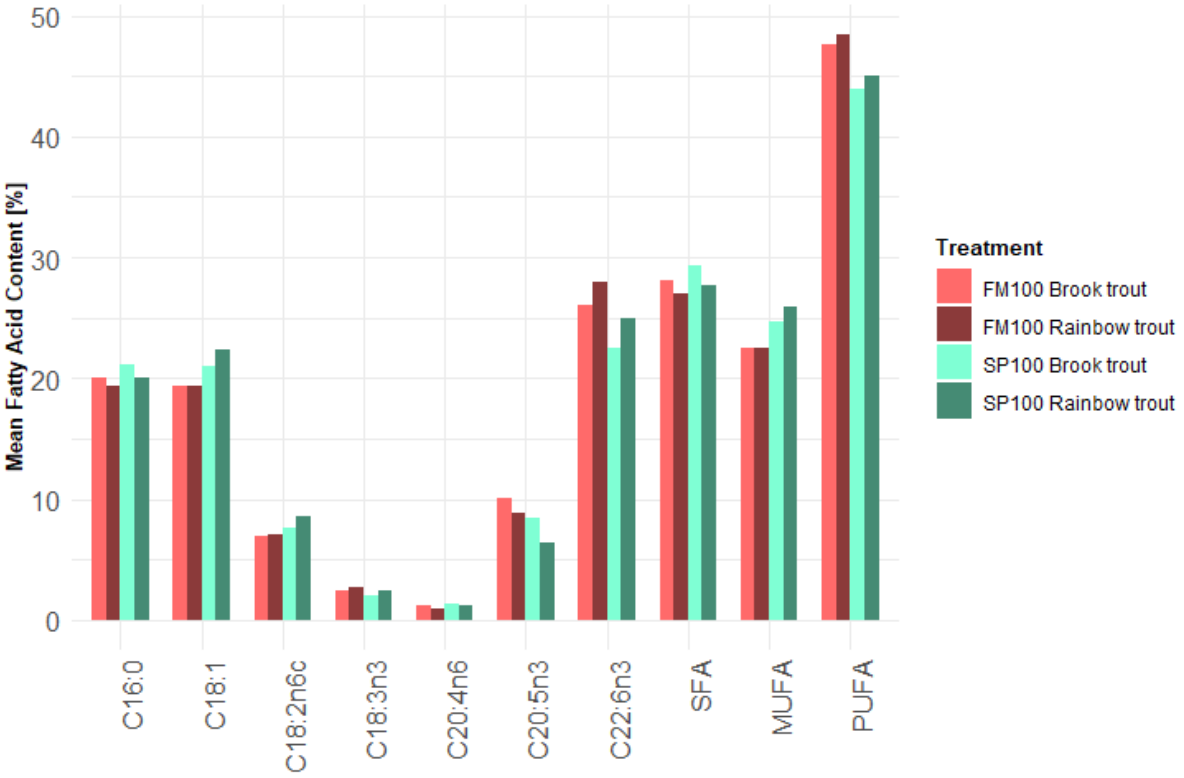

Figure 5. Most abundant fatty acids (%) in fish muscle of brook trout (n = 40) and rainbow trout (n = 40) fed with FM100 and SP100 diet; SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n6: omega-6 fatty acids, n3: omega-3 fatty acids.

The fatty acid content in the experimental diets was characterized by higher values of SFA in SP100 with 29.47 ± 0.44 % than in FM100 with 22.86 ± 0.01 % (Table 4). In SP100, MUFA accounted for half (50.69 ± 0.38 %) of the fatty acids, while in FM100, the amount of MUFA was lower (43.79 ± 0.26 %). Overall, the proportion of PUFA was higher in the FM100 diet (33.23 ± 0.27 %) than in SP100 (19.77 ± 0.06 %). Also noticeable was a reduced DHA and EPA content in SP100 diet.

Table 4. Fatty acid content (%) in FM100 and SP100 diet.

Fatty acid	FM100	SP100
C6:0	0.00 ± 0.00	0.29 ± 0.07
C8:0	0.02 ± 0.00	0.07 ± 0.01
C10:0	0.01 ± 0.00	0.01 ± 0.00
C11:0	0.00 ± 0.00	0.02 ± 0.00
C12:0	0.08 ± 0.01	0.09 ± 0.01
C13:0	0.02 ± 0.00	0.02 ± 0.00
C14:0	4.15 ± 0.14	5.25 ± 0.27
C14:1	0.02 ± 0.00	0.01 ± 0.00
C15:0	0.25 ± 0.01	0.29 ± 0.01
C15:1	0.00 ± 0.00	0.00 ± 0.00
C16:0	13.56 ± 0.05	18.78 ± 0.23
C16:1	4.30 ± 0.09	5.42 ± 0.16
C17:0	0.19 ± 0.00	0.25 ± 0.02
C17:1	0.78 ± 0.04	0.38 ± 0.01
C18:0	2.88 ± 0.07	3.41 ± 0.10
C18:1	36.96 ± 0.34	43.00 ± 0.48
C18:2n6c	15.47 ± 0.11	13.02 ± 0.14
C18:3n6	0.14 ± 0.02	1.41 ± 0.08
C18:3n3	4.74 ± 0.05	2.11 ± 0.04
C20:0	0.34 ± 0.01	0.42 ± 0.02
C20:1n9	1.67 ± 0.00	1.76 ± 0.04
C20:2	0.11 ± 0.01	0.07 ± 0.00
C20:3n6	0.06 ± 0.00	0.09 ± 0.01
C20:4n6	0.50 ± 0.01	0.19 ± 0.02
C20:3n3	0.05 ± 0.00	0.02 ± 0.00
C20:5n3	7.79 ± 0.11	1.80 ± 0.14
C22:0	0.20 ± 0.01	0.18 ± 0.01
C22:1	0.07 ± 0.00	0.12 ± 0.01
C22:2	0.00 ± 0.00	0.03 ± 0.01
C23:0	0.31 ± 0.00	0.10 ± 0.01
C24:0	0.85 ± 0.02	0.27 ± 0.01
C22:6n3	4.48 ± 0.07	1.10 ± 0.06
C24:1n9	0.00 ± 0.00	0.00 ± 0.00
SFA	22.86 ± 0.01	29.47 ± 0.44
MUFA	43.79 ± 0.26	50.69 ± 0.38
PUFA	33.23 ± 0.27	19.77 ± 0.06
n6	16.16 ± 0.12	14.71 ± 0.20
n3	17.07 ± 0.16	5.03 ± 0.25
n6/n3	0.95 ± 0.00	2.92 ± 0.18

Values are means of three replicates ± SD. SFA: saturated fatty acids, MUFA: monounsaturated acids, PUFA: polyunsaturated fatty acids, n6/n3: omega-6, omega-3 fatty acid ratio.

3.6 Principal Component Analysis

PCA plot (Figure 6) implies that colour parameters of the raw and cooked fillet had a stronger influence on the descriptive characteristics than the skin colour. Also, fatty acid profile contributes to the distinction between the diets. Performance parameters indicated only a low explanatory contribution. Product quality traits, especially colour and fatty acids, are main factors distinguishing between FM100 and SP100 groups. The SP100 groups are associated with increased fillet colour values for red (*a**) and yellow (*b**), whereas the FM100 groups are associated with higher PUFA and n3 levels. It is also notable that the variability in the SP100 groups is higher than in FM100 groups as to be seen from the size of the confidence ellipses.

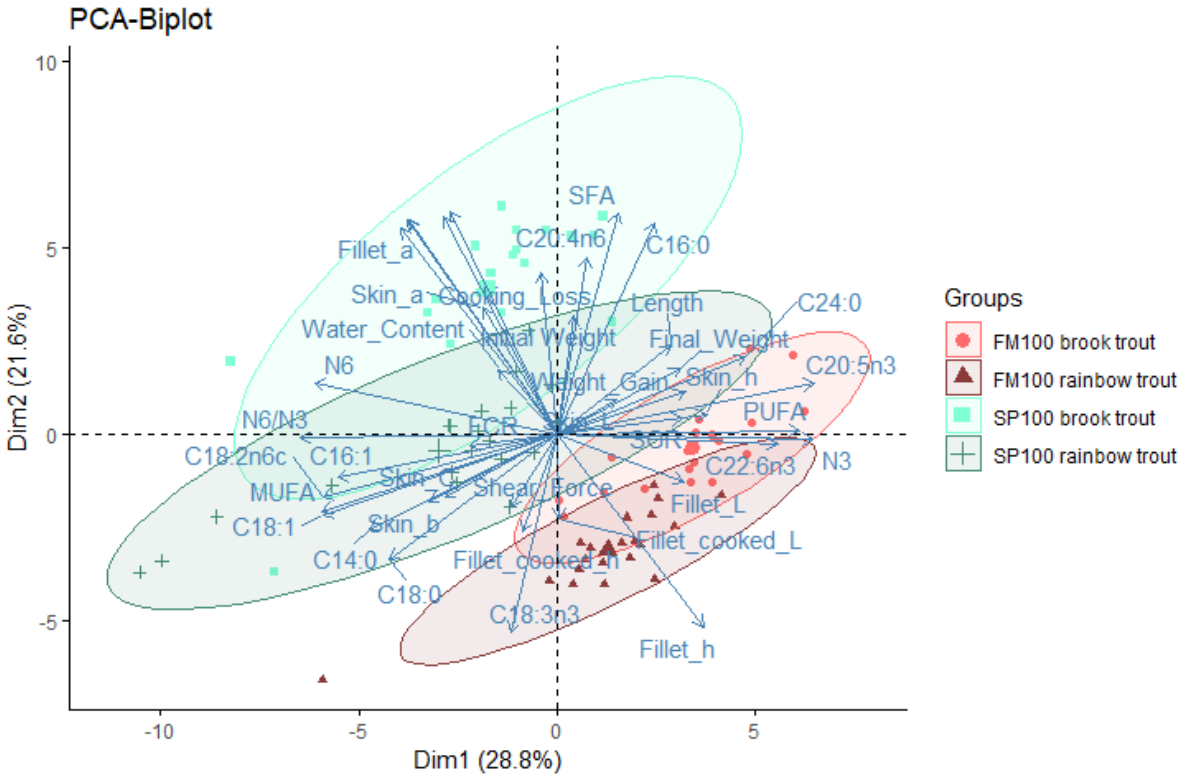

Figure 6. PCA biplot of performance parameters, colour parameters, cooking loss, water content, shear force and most frequent fatty acids for rainbow trout and brook trout fed with FM100 and SP100 diet.

4 Discussion

While brook and rainbow trout accepted the experimental spirulina diet, it led to uneaten food particles and a decline in mean body weight of the brown trout. Since the FM100 diet had been eaten by the brown trout, we considered that brown trout may have an aversion to the flavour of the diet. The trial was aborted for brown trout due to animal welfare concerns. Brook and

rainbow trout showed overall reduced growth performance in response to the spirulina supplementation. In a multiple linear regression model, we observed that the significant predictor for final weight was the dietary treatment and the slight differences in the initial body weight did not affect the final weight. In that way, we were able to show that brook trout had a higher growth rate and were stronger influenced by the spirulina diet. The feed conversion was efficient in all groups in accordance with previously observed data from studies examined by Teles et al. (2019) for brook and rainbow trout. Brook and rainbow trout fed with FM100 diet showed to the same extent a significantly lower FCR than the SP100 group. This observation could be explained by the fact that part of the chemical composition of microalgae is indigestible by non-ruminants. It is estimated that 10 % of the microalgae consist of cell walls, which are not degradable by the fish gastrointestinal tract (Becker 2007; Coelho et al. 2020). Current technological approaches to destroy cell walls in order to increase the digestibility of the material have so far only been used for human consumption (Makkar et al. 2016). Further studies would be necessary to see whether such technologies could also be used in the aquaculture sector.

Moreover, it was noticed that especially in spirulina-fed brook trout, the variance was high in final growth, indicating that some fish are either quite effective in converting the spirulina proteins into biomass or some fish had a higher acceptance for the diet, resulting in a significantly higher feed intake. Even though we worked with full siblings, genetic effects of the implementation of the spirulina cannot be excluded for both species. Selective breeding programs could be the key for an improved implementation of alternative protein sources (Le Boucher et al. 2012; Verdal et al. 2018), since some fish seem to use the spirulina protein more effectively.

As expected, colour parameters changed to more yellow and red pigments, induced by the Spirulina carotenoids. About 10 % of the total carotenoids of young trout are stored in the skin (No und Storebakken 1991). We were able to observe a shift towards more red and yellow coloration in the skin of brook trout, but we were not able to find any statistical differences in rainbow trouts' skin. Other parameter like luminosity were also unaffected by species or diet. Differences in chroma und hue were only observed in brook trout. This is contrary to previous reports on trout (Roohani et al. 2019; Teimouri et al. 2013), where a low-level spirulina supplementation led to a significant increase in red and yellow skin colour. Since the pigments are mainly found along the lateral line of trout (Storebakken und No 1992), it is possible that our measurement of the skin was not performed at the location with the most carotenoids. Another reason for this effect could be due to a different carotenoid metabolism or differences in the amount of lipids in the skin, since carotenoids tend to be linked to a higher lipid content in salmonid

fish (Bjerkeng et al. 1997; Einen und Roem 1997; Jensen et al. 1998). The pigment deposition, due to carotenoid in the feed, depends also on genetic factors (Blanc und Choubert 1985; Torrissen und Naevdal 1984). With all variables considered, we are not able to make an accurate statement about the different colouring effect of yellow and red pigments in the skin of the experimental fish. In order to further elucidate the colouring effect, further investigations of the carotenoid content in the skin would be necessary.

The fillet colour is an important product quality criterion for salmonid fish (Christiansen et al. 1995). In European countries, trout fillet is preferred as white or red/pink. In the present study, however, the spirulina-fed fish increased significantly in yellow fillet coloration. Our investigation confirms previous results for trout (Roohani et al. 2019; Teimouri et al. 2013). Fish fed the fishmeal diet were not distinguishable by coloration of the cooked fillet, but the yellow coloration was also displayed in both species. During the cooking process, yellow colour values increased due to the cooking process. It is suspected that carotenoids from spirulina are heat stable up to 70 °C as previously described for catfish (Rosenau et al. 2021). To what extent consumers may reject the product due to the yellow fillet coloration is largely unknown. A recent study from Altmann et al. (2022) on chicken breasts, produced using spirulina as feed, showed that mainstream consumers rejected the product, due to the altered colour. However, they were able to reduce this effect by providing information. Therefore, it should be noted that this unusual colour could also be used as a sustainability quality mark. Still, it remains questionable, whether consumers have enough knowledge about fish nutrition to understand the complexities of sustainable aquaculture. In this context, a consumer-specific marketing would be necessary.

Cooking loss and firmness are important characteristics of product quality (Brinker und Reiter 2011). Fifty-two to 82 % of the fish muscle consist of water (Petricorena 2015). During the cooking process, liquid components leak out of the fillet. Low levels of fluid loss through cooking can lead to a higher solubilization of intramuscular collagen-based tissue, and consequently to a more tender fillet (Pathare und Roskilly 2016). Herein, cooking loss in rainbow trout was in accordance with the literature (Secci et al. 2019), while there are no data currently available for brook trout. We were able to find a dietary effect on the cooking loss in both species, resulting in significantly higher water loss in spirulina-fed fish in both species. The investigation of Dallaire et al. (2007) demonstrates that algae-fed trout fry have an increased water content in the carcass. The authors explain this observation with a lower protein and lipid content in the

diet, leading to reduced lipid content in the muscle. Lower lipid content was observed for several other species fed with spirulina diets (Mustafa et al. 1994; Nandeesha et al. 1998; Teimouri et al. 2016). Accordingly, this lower lipid content is associated with a higher water content in the fish muscle (Guillaume und Watson 2001).

Shear force is an important tool to estimate the tenderness of a product. Brook trouts were generally more tender than rainbow trouts. However, we found an opposite effect of spirulina supplementation on the tenderness of fillets. While brook trout fed with SP100 were more tender, rainbow trout fed with SP100 were less tender in comparison with the FM100 diet. One influence on this aspect could be the previously described water content of the fish muscle, which tends to increase in spirulina-fed fish, but values were neither significantly different in brook trout nor in rainbow trout.

Fish is the major source for essential fatty acids in human nutrition and provide high amounts of EPA and DHA (Taşbozan und Gökçe 2017). Omega-3 fatty acids are known to reduce myocardial infarction and coronary heart disease and therefore they are of great importance for human health (Zheng et al. 2012). However, the fatty acid profile of fish is highly species specific (Passi et al. 2002) and that is why we expected changes in the fatty acid profile between species, and it also varies due to diet. Results show slight differences between species and strong differences between diets. Due to the spirulina supplementation, important fatty acids like EPA decreased in both species, but DHA decreased only in brook trout. Overall, SFA was increased and PUFA decreased significantly in SP100 fed brook trout, while MUFA was not affected at all. Even though the fatty acid composition tended to react similarly to the spirulina diet than the brook trout; we were unable to find statistically significant differences in SFA and MUFA for rainbow trout, but we observed a significant reduction in PUFA. This might be an indicator that because of the lower final body weight of the rainbow trout, the differences were not represented clearly enough in the fatty acid pattern.

The fatty acid pattern of the flesh reflected the fatty acid pattern of the diet. Even though DHA and EPA levels are very low in the SP100 feed, this difference was not observed in the muscle. Also, PUFA levels were quiet low in the feed, but still high after 10 weeks of feeding. Previous studies showed that rainbow trout are able to synthesize long-chain PUFAs up to a certain amount by regulating hepatic expression fatty acyl desaturase (Gregory et al. 2016; Hixson et al. 2014). Furthermore, n6/n3 ratio was still below the recommended level of five in the fish muscle (Elvevoll und James 2000). In previous studies with trout, the amount of beneficial PUFA with low-level spirulina supplementation increased (Roohani et al. 2019; Teimouri et al.

2016). With increasing exchange rates of fishmeal with spirulina, this effect was reversed (Jafari et al. 2014), indicating that spirulina supplementation could be limited to a certain extent to avoid unwanted reduction of desirable PUFA. It remains unknown, whether long time feeding with high exchange rates might lead to an even stronger reduction of PUFA.

Overall, the main performance and product quality characteristics were influenced by the diet. Especially colour parameters and fatty acid content differed significantly between treatments. It must be emphasized that the variability in these variables was higher in the spirulina-fed fish than in the control fish. It is possible that the experimental fish were genetically adapted to the fishmeal diet due to selection in the aquaculture facility, as the conventional diets contain high proportions of fishmeal. Both tested species reacted in a similar way to the spirulina supplementation. Finally, sole use of spirulina as the main protein source still leads to acceptable growth performance, but product quality traits were strongly affected too. In the end, possible ways to compensate the losses in production could be achieved by a marketing scheme that addresses sustainability of fish production, but it remains unclear, if the consumer would accept the alteration in fillet colour.

5 Conclusion

For a sustainability transition of aquaculture production, replacing traditional protein sources like fishmeal is paramount. Our study shows that the conversion of spirulina differs in some growth and production traits between species. A total replacement of fish meal with spirulina was accepted by brook trout and rainbow trout, but not by brown trout; we hypothesize that the brown trout had an aversion to the flavour of the spirulina diet. A complete replacement of fishmeal with spirulina comes along with reduced growth performance and feed efficiency. Yet, growth performance observed in brook trout and rainbow trout is considered acceptable. A species–diet interaction was found for final weight and weight gain, but not for the feed conversion. Total replacement of fishmeal reduced PUFA and n-3 fatty acid levels, which is regarded a disadvantage from a human health perspective. The spirulina-induced change in fillet coloration towards a strong yellow may have an impact on the consumer acceptance, but might also be a chance for marketing. Based on the presented investigation, we plan to conduct a consumer study, to get more insights on the consumer preferences.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Simon Rosenau: Conceptualization; Methodology; Visualization; Formal Analysis; Investigation; Writing – Original Draft. **Marco Ciulu:** Methodology; Writing – Review & Editing. **Christian Reimer:** Formal Analysis; Validation, Writing – Review & Editing. **Alexander Charles Mott:** Writing – Review & Editing. **Jens Tetens:** Resources; Writing – Review & Editing; Project administration. **Daniel Mörlein:** Resources; Writing – Review & Editing; Supervision; Project administration.

Ethical Clearance

All animal work followed relevant national guidelines. Good veterinary practice was applied to all procedures. The study is in accordance with the German legal and ethical requirements of appropriate animal procedures. The experiment was approved by the Institutional Animal Welfare Body (no. T2-2019, 27.06.2019).

Appendix

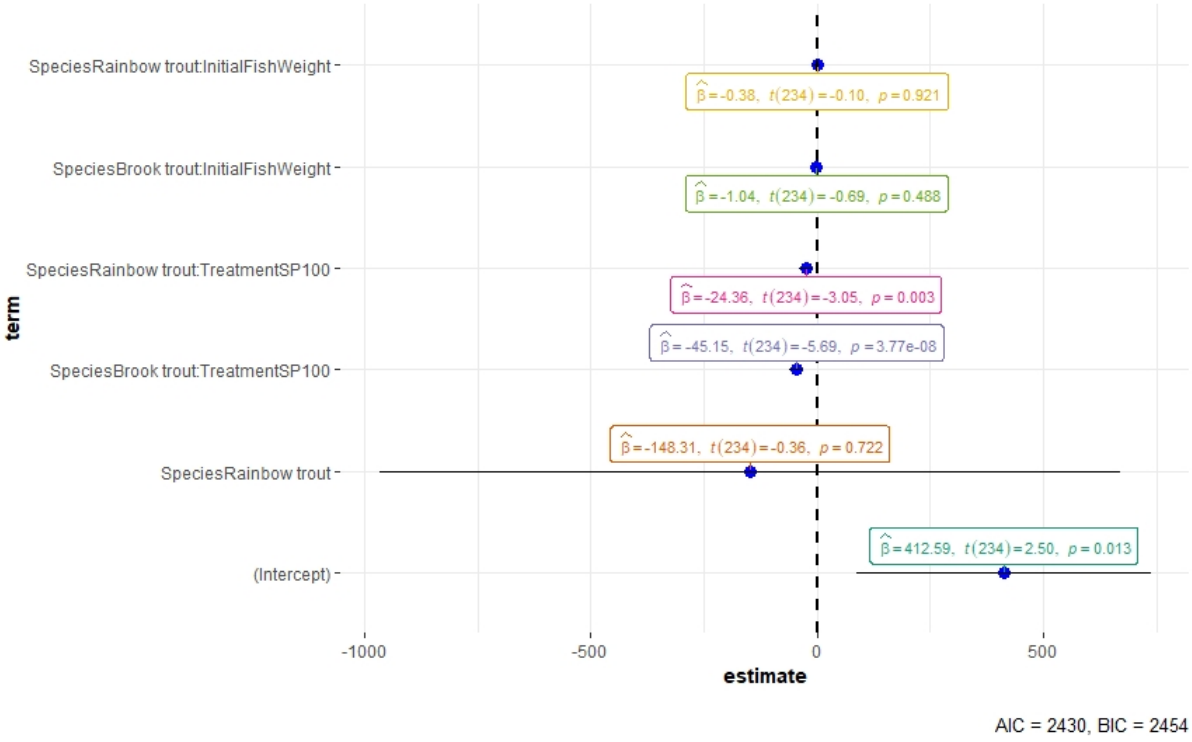

Figure S1. Multiple linear regression model coefficients of brook trout and rainbow trout fed FM100 and SP100 diet.

Table S2. Intramuscular fatty acid composition (% of detected fatty acids) of brook trout (n = 40) and rainbow trout (n = 40) fed with FM100 and SP100.

Parameters	Brook trout		Rainbow trout		*p* - values		
	FM100	SP100	FM100	SP100	Species	Diet	Species x Diet
C6:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	ns	ns	ns
C8:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	ns	ns	ns
C10:0	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	ns	**	ns
C11:0	0.01 ± 0.00	0.21 ± 0.05	0.02 ± 0.00	0.29 ± 0.07	ns	***	ns
C12:0	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	ns	ns	ns
C13:0	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	*	ns	ns
C14:0	1.83 ± 0.06	2.01 ± 0.05	2.12 ± 0.05	2.21 ± 0.07	**	ns	ns
C14:1	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	***	ns	ns
C15:0	0.16 ± 0.00	0.19 ± 0.00	0.16 ± 0.01	0.18 ± 0.00	ns	ns	ns
C15:1	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	***	ns	ns
C16:0	20.06 ± 0.33	21.19 ± 0.34	19.41 ±0.31	20.08 ± 0.37	***	***	ns
C16:1	1.88 ± 0.16	2.38 ± 0.09	1.93 ± 0.09	2.33 ± 0.07	ns	**	ns
C17:0	0.13 ± 0.01	0.20 ± 0.01	0.11 ± 0.01	0.15 ± 0.01	*	***	ns
C17:1	0.13 ± 0.01	0.13 ± 0.01	0.20 ± 0.03	0.21 ± 0.02	***	ns	ns
C18:0	2.43 ± 0.10	2.55 ± 0.13	2.86 ± 0.07	3.01 ± 0.09	***	*	ns
C18:1	19.39 ± 1.08	21.00 ± 1.11	19.39 ± 0.58	22.35 ± 0.5	ns	*	ns
C18:2n6c	6.99 ± 0.35	7.67 ± 0.24	7.09 ± 0.28	8.53 ± 0.18	ns	*	ns
C18:3n6	0.10 ± 0.01	0.38 ± 0.02	0.13 ± 0.09	0.30 ± 0.04	ns	***	ns
C18:3n3	2.43 ± 0.09	2.05 ± 0.06	2.64 ± 0.09	2.43 ± 0.06	***	***	ns
C20:0	0.07 ± 0.02	0.12 ± 0.04	0.09 ± 0.01	0.10 ± 0.01	ns	*	ns
C20:1n9	0.83 ± 0.10	0.92 ± 0.08	0.77 ± 0.04	0.82 ± 0.04	ns	ns	ns

C20:2	0.66 ± 0.09[b]	0.93 ± 0.19[a]	0.35 ± 0.05[c]	0.33 ± 0.05[c]	***	ns	*
C20:3n6	0.78 ± 0.12	1.44 ± 0.13	0.33 ± 0.01	0.81 ± 0.03	***	***	ns
C20:4n6	1.17 ± 0.05	1.33 ± 0.06	0.98 ± 0.03	1.26 ± 0.05	**	***	ns
C20:3n3	0.15 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.09 ± 0.00	**	*	ns
C20:5n3	10.04 ± 0.27	8.45 ± 0.25	8.87 ± 0.20	6.35 ± 0.19	***	***	ns
C22:0	0.09 ± 0.03	0.09 ± 0.03	0.04 ± 0.01	0.05 ± 0.01	***	ns	ns
C22:1	0.26 ± 0.03	0.20 ± 0.04	0.18 ± 0.04	0.15 ± 0.06	*	ns	ns
C23:0	0.46 ± 0.08	0.39 ± 0.13	0.22 ± 0.06	0.15 ± 0.03	***	ns	ns
C24:0	2.87 ± 0.05	2.38 ± 0.05	2.02 ± 0.04	1.38 ± 0.05	***	***	ns
C24:1n9	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	*	ns	ns
C22:6n3	25.99 ± 0.83	22.45 ± 0.65	27.98 ± 1.03	24.92 ± 1.28	*	***	ns
SFA	28.13 ± 0.28	29.37 ± 0.34	27.07 ± 0.37	27.64 ± 0.34	***	***	ns
MUFA	22.52 ± 1.31	24.68 ± 1.26	22.48 ± 0.67	25.88 ± 0.63	ns	*	ns
PUFA	47.66 ± 0.81	43.91 ± 0.80	48.49 ± 0.94	45.04 ± 1.32	ns	***	ns
n6	9.04 ± 0.33	10.83 ± 0.30	8.53 ± 0.33	10.91 ± 0.15	ns	***	ns
n3	38.61 ± 1.02	33.08 ± 0.81	39.61 ± 1.15	33.80 ± 1.43	ns	***	ns
n6/n3	0.24 ± 0.01	0.34 ± 0.02	0.22 ± 0.02	0.35 ± 0.02	ns	***	ns

Values are means ± SD, followed by different letters in the same row are significant different at a level of $p < 0.05$ (Tukey's test). SFA: saturated fatty acids, MUFA: monounsaturated acids, PUFA: polyunsaturated fatty acids, n6: omega-6 fatty acids, n3: omega-3 fatty acids, n6/n3: omega-6/omega-3 ratio, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not significant.

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3.4 Consumer Preference for Altered Color of Rainbow Trout (*Oncorhynchus mykiss*) Fillet Induced by Spirulina (*Arthrospira platensis*)

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Abstract: Growing global demand for fish in combination with stagnating capture fishery production challenges aquaculture. Aquaculture is dependent on fishmeal which is criticized for its overall environmental impact. Sustainable growth of aquaculture requires the integration of alternative protein sources. Spirulina has been found to be a viable fishmeal substitute in various fish species and it is known to elevate yellow pigmentation which results in an unusual yellow color for trout fillets. For this reason, the influence of fillet color and other attributes like, country of origin, price and feed claims have on consumer preferences for trout fillet was tested. A discrete choice experiment was conducted to assess German consumer preferences and willingness to pay for yellow fillet color compared to conventional white fillet. Results indicate that spirulina-fed yellow fillet color had positive influence on preference and consumers are willing to pay more compared to a white fillet. These results show that spirulina leads to an improved product quality and value for trout fillets as perceived by consumers. Furthermore, respondents revealed the strongest preferences for fillets of domestic origin (Germany) before Denmark and Turkey. In contrast, feed claims showed no significant effect, except for the claim "fed with sustainable resources", which had marginal impact. Marketing activities to successfully integrate sustainable feeds like spirulina for rainbow trout into the German fish market should foremost focus on consumers' interest in domestic or nearby production. Nevertheless, it still seems valuable/important to raise consumers' awareness of sustainable value chains and production methods in aquaculture through effective transfer of information.

Keywords: fishmeal replacement, microalgae, product quality, pigmentation, sustainability, choice.

Highlights

- Spirulina improves product quality and value for rainbow trout fillets
- Spirulina-induced yellow fillet color is preferred to a more typical white fillet
- Consumers strongly prefer domestic rainbow trout fillets
- Claims related to feed sourcing had minor to no effect on consumer choice

1 Introduction

The global fish consumption increased at an annual rate of 3.1 % on average from 1961 to 2017, exceeding all other animal protein foods, e.g. meat, dairy (FAO, 2020). This increase in consumption is largely due to the rapid growth of aquaculture. Over the last decades, the aquaculture sector has been one of the fastest-growing food-producing industries worldwide (Olsen and Hasan, 2012); therefore requiring increasing amounts of fishmeal. Aquaculture has become the main user of fishmeal (IFFO, 2022). Amidst stagnating yields of capture fisheries (FAO, 2020) and ecological concerns regarding fishing techniques, high demand for energy and water for fishmeal production, the search for alternative protein sources for fish nutrition seems inevitable (García, 2003; Hall, 2010).

There are already several plant and animal protein alternatives to fishmeal on the feed market. However, many of these resources are associated with disadvantages in terms of digestibility, growth and health, due to undesirable compounds like anti-nutritive substances (Francis et al., 2001). Furthermore, other alternatives such as, soybean meal are in direct competition with the production of other livestock and are associated with land use changes (Masuda and Goldsmith, 2009).

Recently, cyanobacteria, often referred to as microalgae, have been discussed as a natural and sustainable feed source (Becker, 2007), since they are the first trophic level in aquatic food chain. Especially, *Arthrospira platensis* and *Spirulina platensis* (both species are summarized under the term spirulina) have been successfully investigated as a feed source for various fish species (Alagawany et al., 2021). Spirulina can have a positive influence on growth and health (Rosas et al., 2019); yet new feed components can also lead to changes in product quality (Webster et al., 2004). Spirulina is known to consist of substantial amounts of carotenoids like β-carotene and xanthophylls (Deng and Chow, 2010; Habib et al., 2008). In a previous study, the feeding of spirulina led to a higher pigmentation in rainbow trout, resulting in an increased

yellow coloration of the fillet (Rosenau et al., 2022). Several other studies have reported this effect for different fish species (Plaza et al., 2019; Roohani et al., 2019; Rosenau et al., 2021; Teimouri et al., 2013). While Teimouri et al. (2013) described the coloration as a positive effect for product quality, Welker et al. (2001) assume that yellow pigmentation of rainbow trout fillet could be perceived as unusual and result in economic losses. Although the study from Teimouri et al. (2013) describes that a more yellow pigmentation in rainbow trout could have positive effects on consumers preference, to date there has been no study providing evidence and quantifying the effects of coloration on consumer perceptions and preferences.

The visual appearance of food is evaluated by its color and thereby has a strong impact on consumers perception of food quality (León et al., 2006; Nisha et al., 2011). It is of great importance to measure the consumer acceptance for fish fillets produced with spirulina as a protein source to ascertain their marketability. Consumers are also progressively sensitive to production processes in the food sector (Olynk et al., 2010) and therefore tend to consider different aspects like ethics of food products, such as animal welfare and sustainability of the production system (Garnier et al., 2003); claims and labels are an important information source for consumers to evaluate sustainable qualities of aquaculture products (Feucht and Zander, 2015; Risius et al., 2019; Stoll and Johnson, 2015). Sustainability claims and country of origin (COO) can have a positive impact on consumers towards trout products (Risius et al., 2017).

Spirulina has the potential to replace fishmeal in aquaculture, but the success of spirulina-based feed depends largely upon consumers' preferences and their willingness to pay for these products. Since consumer choice depends not only appearance, but also claims and labels, by conducting a discrete choice experiment (DCE) we tested the consumer acceptance for yellow compared with white rainbow trout fillet, while including credence attributes. Rainbow trout was chosen, as it is a well-established aquaculture product in Germany (Brämick, 2019; Feucht and Zander, 2015; Risius et al., 2017). We hypothesized that consumer preference is influenced by fillet color as well as other attributes like, COO, price and claims related to feed production characteristics (feed-claim).

2 Materials and Methods

2.1 Ethical Clearance

All animal work followed relevant national guidelines. Good veterinary practice has been applied to all procedures whenever animals were handled. The study is in accordance with the German legal and ethical requirements of appropriate animal procedures. The procedures in

this study were approved by the Institutional Animal Welfare Body (no. T2-2019, 27.06.2019). All respondents gave informed consent and were able to terminate their participation at any time while filling out the survey.

2.2 Fish & Image Preparation

The experimental fish originate from a previous experiment, which focused on growth performance and product quality (Rosenau et al., 2022). Rainbow trout with a mean weight of 100.80 ± 5.96 g were reared in a recirculation system and fed for ten weeks two isoenergetic and isonitrogenous diets, based on the recommendations for rainbow trout (National Research Council, 2011). The control diet contained 20 % of fishmeal and in the experimental diet, the fishmeal was completely replaced by spirulina (Arthrospira platensis). After ten weeks, all fish were stunned with a sharp blow to the head and killed by exsanguination. Fillets were cut, vacuumed-packed in plastic bags and frozen at -80 °C until further process.

Fillets were thawed over 24 hours in a fridge at 7 °C. Thereafter, fillets were rinsed with water; overlapping skin and residues of blood were removed. Fillets were photographed in pairs using a reflex camera (NIKON D7200, Tokyo, Japan) mounted on a photographic bench and set up with the lens aligned 44 cm from the fillet surface. Each photo contained either two white fillets obtained from the control group, or two yellow fillets obtained from the spirulina group. All photos were taken without flash against a white neutral background. Fillets were placed in the same standardized plastic tray without plastic film seal to avoid undesired light reflections on the foil. Sales tag and feed-claims were digitally added to photos using “GNU Image Manipulation Program” (GIMP, version 2.10.30). The sales tag contained the name of the product, country of origin, weight in kilogram, price per 100 g, total price and was placed in the lower left corner. A feed-claim was added in the lower right corner (Figure 1). The final edited photos were intended to mimic retail-ready fresh trout fillets packed in plastic trays, as consumers may encounter them in retailers.

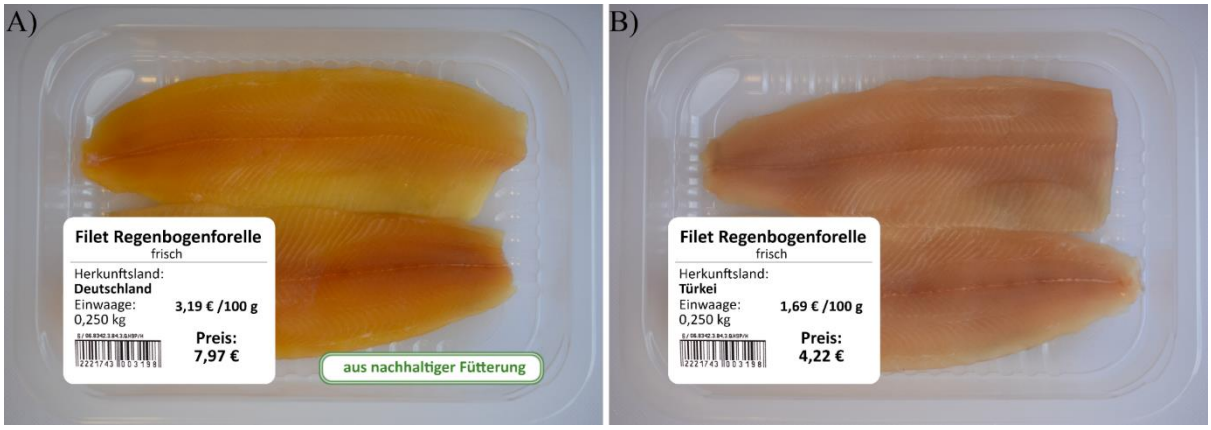

Figure 1. Example retail-ready trout fillets as shown to respondents. A) 0.25 kg of yellow Rainbow trout fillet from Germany with a price per 100 g of 3.19 €, an overall price of 7.97 € and a feed-claim for sustainable feeding. B) 0.25 kg of white Rainbow trout fillet from Turkey with a price per 100 g of 1.69 €, an overall price of 4.22 € and no claim stated.

2.3 Survey & Discrete Choice Experiment

The cross-sectional consumer survey was programmed using EyeQuestion survey data col-lection software (Elst, Netherlands). A commercial field research provider (Respondi AG, Cologne, Germany) distributed the survey to an online panel. Participants were invited based on previously set age and gender quotas representative for German census data (Statistisches Bundesamt, 2022). Participants had to meet two main recruitment criteria: they had to be at least 18 years old and they had to buy fish fillet at least occasionally.

The questionnaire was divided into three parts: purchasing behavior, DCE, demographics. In the first section, participants were asked about their fish fillet purchasing frequencies, pre-ferred processing form, most frequent place of purchase and familiarity with spirulina. Af-terwards, participants had to rate the importance of different product attributes i.e., price, production method, labeling, organic production, color, freshness, and geographical origin. In the second part of the survey, participants received the following written information on spirulina in aquaculture diets:

- The microalgae spirulina can be used as a fishmeal alternative in trout farming.
- The use of spirulina in the feed of aquaculture fish can reduce fishing pressure on natural fish stocks (e.g., less fishing for fishmeal production, less bycatch).
- Fishing for fishmeal production deprives marine ecosystems of their primary food base.
- Spirulina is a natural part of the aquatic food chain and it can be produced in aquaculture.

- The pigments (carotenoids) contained in spirulina give trout fillets an intense yellow/ orange color.

Subsequently a DCE was used to evaluate consumer preference and WTP for the attributes fillet color, COO and feed-claim for rainbow trout fillets. In the third section of the questionnaire sociodemographic data, including education, household size, household net income, diet (e.g. pescatarian), and geographical residence was collected. The DCE imitated a purchase situation, in which three different packages of fillets varied in four different attributes: color, feed-claim, country of origin and price (Table 1). The participants were instructed to imagine being in a supermarket, trying to decide for the product they would most likely buy. Subsequently, participants completed nine choice-sets. Consumers could also decide for a "no buy" alternative in each set to incite a more realistic choice scenario and to avoid bias as a consequence of forced choice (Dhar and Simonson, 2003). The selection of attributes was based on literature (Ankamah-Yeboah et al., 2019; Risius et al., 2017) and market inventory research.

Table 1. Design of the choice experiment with tested attributes and levels.

Attributes	Levels
Fillet-color	White
	Yellow
Country of origin	Germany
	Denmark
	Turkey
Price (per 100g trout fillet)	1.69 €
	2.19 €
	2.69 €
	3.19 €
	3.69 €
Feed-claim	no claim
	fed with sustainable resources
	fed without fishmeal
	fed on plant-based proteins
	fed with spirulina

The fillets of the control group had a typical white to pinkish color and were referred to as "white". The fillets of the spirulina-fed fish had an intense yellow/orange color and were referred to as "yellow", since the yellow color values increased stronger than the red color values (Rosenau et al., 2022).

The geographical origins were tested in the DCE based on their appearance on the German trout market. Denmark and Turkey were included as attributes, representing an European Union neighing country and a non-European Union country and both are the main import countries of rainbow trout products in Germany (Risius et al., 2019; Risius et al., 2017). A label with "no origin" was not applicable, since legislative regulations prescribe that the origin of fish must be declared on product packages (Regulation (EU) No 1379/2013).

Five different price levels were included in the experiment. The offered price range was based on market inventory in research prior to the consumer study at local discounters, supermarkets, fish markets and farm shops in Lower Saxony and Hesse. A total of 42 prices was collected, ranging from 1.30 € to 3.85 € per 100 g of rainbow trout fillet from conventional as well as organic aquaculture. It was adjusted using the prices for rainbow trout fillet of large German fish markets in Hamburg, Nuremberg, and Munich (Fischer und Teichwirt, 2021, 2020). Based on a calculated average price of 2.72 € per 100 g rainbow trout fillet were offered at five price levels ranging around 2.69 € per 100 g.

Four feed-claims with increasing levels of specificity informed consumers about the feed (and indirectly sustainability) used to produce the fish. The four feed-claims were: "fed with sustainable resources", "fed without fishmeal", "fed on plant-based proteins", and "fed with spirulina". In the design a restriction was integrated where the "fed with spirulina" claim could not be combined with "white" fillet color, as it would represent an unrealistic product. We intentionally included vague claims, such as "fed with sustainable resources" to compare the effects of different levels of claim specificity on consumer preferences. A no claim level was included, where products did not receive a feed-claim. Combining attributes and levels resulted in 150 possible sets. The underlying experimental design is a reduced d-efficient design, created with the NGene software (ChoiceMetics, 2012) which resulted in nine choice sets, relying on priors based on studies of Risius et al. (2017) and (2019), also using trout fillet. To avoid confounding effects, the order of the choice sets and the position of choices (top, middle, bottom) varied randomly per respondent. Choice tasks were introduced with an information treatment regarding fishmeal in aquafeed and spirulina as a potential substitute.

To ensure purpose and objectivity, the questionnaire and the choice experiment were pre-tested using a 70-person sample group. The participants had the opportunity to give open ended feedback on the tasks and questions. Participants who chose the "no buy" alternative in every choice situation were asked for their motivations in an open-ended question. The answers were used to ensure a high-quality data cleaning.

2.4 Econometric Model

A random parameter logit model (RPL) was applied to measure consumer preferences and WTP for the above-mentioned attributes. RPL models refer to utility theory which assumes that the utility of goods are determined by their attributes (Lancaster, 1966; Thurstone, 1927). The model was developed based on McFadden's (1973) utility theory and the conditional logit modelling, which assumes that consumers have homogenous preferences. The RPL model (also known as mixed logit model) overcomes this limitation of homogeneous preferences by assuming that preferences vary among consumers (Revelt and Train, 1998). This means that the utility U consists of an observable component X_{ijs} (based on attributes and individual characteristics) and an unobservable (random) component which is departed in an error term ε_{ijs}.and a random term zq + ηq, integrating distribution over individuals which depends on underlying characteristics and thus is bound to individual preferences ('taste heterogeneity'). ε_{ijs} is independent and identically distributed over individuals i, alternatives j and scenarios s. β_i is an attribute parameter vector, which is specific to the individual preference for this attribute. The utility received by the individual i from alternative j (in this manuscript a package of rainbow trout fillets) given choice scenario s is explained by:

(1) $U_{ijs} = \beta_i X_{ijs} + \varepsilon_{ijs}$

The observable component X_{ijs} is assumed to have a linear relationship with observed attribute levels x of each alternative j and their weights β with a positive scale factor σ_n:

(2) $U_{ijs} = \sigma_n \sum_{k=1}^{K} \beta_{ik}\, x_{isjk} + \varepsilon_{ijs}$

The marginal utility associated with attribute k for respondent i in choice situation s is represented by β_{ik} (Hensher et al., 2015). Population parameter weights vary randomly around a mean are estimated, where $\bar{\beta}_k$ represents the mean of the distribution of marginal utilities derived by the sampled population; η_k is the deviation of preferences among the respondents and z_{ik} stands for the random draws taken from a pre-specified distribution for each individual i and attribute k:

(3) $\beta_{ik} = \bar{\beta}_k + \eta_k z_{ik}$

Based on the assumption that individuals try to maximize their utility the probability of individual i to select alternative j in choice situation s is:

(4) $P_{ijs} = P(U_{ijs} > U_{iks} \forall\, k \neq j)$

A respondent's utility is specified for choosing one of the three rainbow trout fillet packages or the "no buy" option with a known price and product attributes. Fillet color, feed-claims, COO were coded as dummy. In our model, price is in put as a continuous fixed variable in order to align with microeconomic theory as well as ensure interpretable WTP estimates. All other attributes were considered as random variables. The "no buy" option was modelled as the base alternative (ASC). YELLOW corresponds with yellow fillet color. SUSTAIN represents the "fed with sustainable resources" claim. NOFISH represents the "fed without fishmeal" claim. PLANT represents the "fed on plant-based proteins" claim. SPIRUL represents the "fed with spirulina" claim. GER corresponds with the COO Germany. DEN corresponds with COO Denmark. An exemplary choice set is shown in Table S1. The specification on our model is describes as followed:

(5) $U_i = \beta_i PRICE + \beta_{0i} + \beta_i YELLOW + \beta_i SUSTAIN + \beta_i NOFISH + \beta_i PLANT + \beta_i SPIRUL + \beta_i GER + \beta_i DEN$

(6) $U_{ascnB} = \beta_i NoBuy$

WTP was estimated in preference space for the fillet color, COO and claims according to Hensher et al. (2015). The negative ratio between the attribute and the price coefficient yields the mean WTP for the respective attribute relative to the reference category.

(7) $WTP = -\frac{\beta_{attribute}}{\beta_{price}}$

Currently, rainbow trout production depends on fishmeal-based feeds, which lead to a "white" fillet color. Reference level Turkey is one of the major importers of rainbow trout to Germany and based on the findings from Risius et al. (2017), we assume it to be the least preferred COO in our experiment because it is geographically distant (Risius et al., 2017). Therefore, to determine marginal utility and WTP, attribute reference levels used were "white", "no claim", and "Turkey".

2.5 Statistical Analysis

Choice data were prepared using R version 4.1.1 (R Core Team, 2021) with the "fastDummies" package (Kaplan, 2020). Choice data were modelled using the panel-data mixed logit choice model command using the package "cmxtmixlogit" from STATA (StataCorp., 2021), while specifying the Halton sequence for integration using 3500 points and normal distribution for random coefficients. The total sample was modelled along with split samples based on familiarity with spirulina. Respondents were considered familiar with spirulina when they responded positively to knowing about one or more spirulina product (first part of the questionnaire); all other respondents were considered to be unfa-miliar with spirulina.

3 Results

3.1 Sample Characteristics

A total of 741 people participated in the survey, of which 75.17 % (n = 557) of responses were complete and considered for analysis. Data was quality checked based on attention checks embedded in the questionnaire and respondents who did not pass the quality checks were not considered for analysis. As a consequence, 84 (11.34 %) responses were excluded from the analysis. The other 98 (13.22 %) responses were incomplete and discarded. It turned out that 42 participants chose "no buy" in every presented set. Reasons for choosing the "no buy" option were "too expansive" or "I do not buy packed fish" and "I only buy frozen fish". Two of the 42 responses (0.27 %) were excluded, as they did not seem to understand the procedure of the questionnaire. The respondents reasoned their decisions by "instructions are incomprehensible" and "it is how it is".

This study aimed to reflect market conditions as realistic as possible; therefore, we kept the remainder of responses that opted out ("no buy") even from respondents who opted out in all choice sets. The fact that respondents decided against each of the products offered does not exclude them as a potential market reflecting determinant. Respondents who made a reasonable decision to opt out may also reflect realistic market conditions.

Respondents were asked if they had ever heard of or knew about spirulina products. The majority of the sample was not familiar with spirulina (66.25 %). Most of the respondents that were familiar with spirulina stated that they knew spirulina tablets and flakes.

3.2 Sociodemographic Characteristics

The sample consisted of 45.8 % female and 54.2 % male respondents aged 18 to 88 years old. For analysis, the sample was divided into three age groups: 18 to 35 years, 36 to 56 years, 57 years and older. Most respondents belong to the age group from 57 years and above with 42.6 % (Table 2), which is a little over-represented compared to the current demographics within Germany (approx. 30 %) (Statistiches Bundesamt 2020). This over-representation likely arose due to the nature of the questionnaire, i.e. only persons who conduct grocery shopping were questioned. The other age groups closely mirrored the general population. The sample covered the main education and employment groups monitored in Germany; however, highly educated and employed are over-represented within the sample. The largest share of the household net income was between 1,300 and 2,600 € and the proportion of high-income households with more than 5,000 € was over-represented. Importantly, 83.3 % of the participants stated to be the responsible person for shopping of the everyday commodities in their household.

Table 2. Sociodemographic characteristics of the sample (n = 557).

Demographic attributes	Level	Total sample [%]	Census 2020 [%] (Statistisches Bundesamt, 2022)
Age (in years)	18 - 35	24.6	25.5
	36 - 56	32.7	34.0
	≥ 57	42.6	40.5
Gender	Female	45.8	50.7
	Male	54.2	49.3
Education (highest level of formal education)	No qualification	0.2	0.2
	Intermediate qualification	30.0	23.5
	University qualification	21.9	33.5
	Professional school degree	19.8	46.6
	University degree	28.2	18.5
Income (net household income per month)	< 1300	12.6	13.3
	1,300 to < 2,600 €	33.9	29.7
	2,600 to < 3,600 €	24.6	17.8
	3,600 to < 5,000 €	20.3	16.9
	5,000 € and more	8.6	22.2
Recent activity	Employee	52.1	NA
	Student	5.8	NA
	Self-employed	5.0	NA
	Trainee	0.2	NA
	Other activity	37.0	NA
Purchase of everyday commodities	Responsible	83.3	NA
	Sometimes responsible	14.9	NA
	Not responsible	1.8	NA

3.3 Purchase Behavior

Generally, respondents bought fish fillet weekly (23.88 %), twice per month (25.13 %), or once per month (18.13 %) (Table 3). A cumulative share of 30.96 % of the participants purchased trout at least once per month, but most of the respondents bought trout products less than once per month (46.50 %). Nearly a quarter (23.31 %) of the respondents stated that they never buy trout products.

Table 3. Relative shares of fish fillet and trout purchasing frequencies (n=557). Participants were asked how often they buy fish fillet and how often they buy rainbow trout.

Parameters	Frequency of fish fillet purchase [%]	Frequency of trout purchase [%]
Daily	0.72	0.36
Multiple times per week	5.92	0.72
Once per week	23.88	4.49
Twice per month	25.13	13.11
Once per month	18.13	11.49
Less	26.21	46.50
Never	NA	23.34

Respondents stated purchasing their fish fillet in frozen (51.61 %) or fresh (28.01 %) forms at the supermarket (54.4 %), followed by discounter supermarkets (25.31 %). The majority of respondents (85.46 %) stated that the main reason for fish consumption is the taste (Table 4), closely followed by respondents who characterize fish as a healthy food (74.69 %). Around 45.06 % of respondents considered fish fillet as easy to prepare and 36.09 % perceived it as a natural product. Approximately 12 % of the respondents consider fish fillets sustainable and affordable. Only 5.57 % were of the opinion that fillets are easy to find on the market. Respondents categorized as familiar or unfamiliar with spirulina did exhibit minor differences in the perception of affordability of trout fillets; 14.09 % familiar respondents, yet only 7.98 % of unfamiliar respondents stated that fish fillets are affordable.

Table 4. Relative frequency of most important fish fillet characteristics (n=557). Relative frequency of most important fish fillet characteristics (n=557). Participants were able to select the three most important items. Questionnaire item: "I buy fish fillet, because: a) it is healthy, b) it is tasty, c) it is affordable, d) it is easy to prepare, e) it is easy to find, f) it is sustainable and g) it is natural."

Parameter	Total Sample [%] n=577	Spirulina familiar [%] n=369	Spirulina unfamiliar [%] n=188
Taste	85.46	87.26	81.91
Healthy	74.69	72.63	78.72
Easy to prepare	45.06	46.34	42.55
Natural	36.09	34.15	39.89
Sustainable	12.39	10.57	15.96
Affordable	12.03	14.09	7.98
Easy to find	5.57	5.42	5.85

The importance to respondents of different fillet attributes was investigated (Table 5). Freshness was considered as the most important attribute followed by the fillet color. Price, production system, and label were considered to be of equal importance, closely followed by the label. In comparison, COO, nutritional value, organic label and the brand were rated as less important, nearing "neither important nor unimportant".

Table 5. Fish fillet attributes and consumer importance in a purchase situation (n=557).

Attribute	Mean Score[1]
Freshness	1.54
Fillet color	2.02
Price	2.14
Cultivation system	2.14
Label	2.16
COO	2.33
Nutritional value	2.41
Organic label	2.55
Brand	2.79

[1] 1 = very important; 2 = rather important; 3 = neither; 4 = rather unimportant; 5 = very unimportant.

3.4 Consumer Preferences

RPL model results (Table 6) were statistically significant ($p < 0.001$). Standard deviations of the random attributes were also significant ($p < 0.01$) which underlines preference heterogeneity within our sample(s) (Hensher et al., 2015).

Table 6. RPL model estimates of coefficient means and standard deviation (SD) for the total consumer sample and according to previous exposure to spirulina

Variable	Total sample n=557			Spirulina familiar n=188			Spirulina unfamiliar n=369		
	coefficient	**SE**	***p*-value**	**coefficient**	**SE**	***p*-value**	**coefficient**	**SE**	***p*-value**
PRICE	-1.08	0.07	***	-1.20	0.09	***	-0.88	0.11	***
YELLOW	0.39	0.10	***	0.39	0.13	**	0.38	0.15	*
SUSTAIN	0.48	0.18	**	0.50	0.23	*	0.33	0.29	n.s.
NOFISH	-0.12	0.18	n.s.	-0.10	0.23	n.s.	-0.22	0.30	n.s.
PLANT	-0.11	0.16	n.s.	-0.28	0.21	n.s.	0.06	0.26	n.s.
SPIRUL	-0.22	0.19	n.s.	-0.37	0.25	n.s.	-0.02	0.31	n.s.
GER	2.65	0.18	***	2.58	0.23	***	2.85	0.30	***
DAN	1.67	0.15	***	1.64	0.19	***	1.71	0.24	***
SD									
YELLOW	1.78	0.10	***	1.92	0.13	***	1.53	0.15	***
SUSTAIN	1.48	0.12	***	1.68	0.17	***	1.16	0.19	***
NOFISH	0.90	0.13	***	0.94	0.18	***	0.68	0.25	**
PLANT	1.51	0.11	***	1.68	0.15	***	1.26	0.17	***
SPIRUL	1.07	0.24	***	0.98	0.33	***	1.06	0.34	***
GER	2.60	0.15	***	2.70	0.19	***	2.24	0.23	***
DAN	1.72	0.12	***	1.80	0.15	***	1.49	0.18	***
Constant alt1	1.25	0.18	***	1.19	0.22	***	1.49	0.28	***
Constant alt2	1.09	0.17	***	1.13	0.22	***	1.20	0.28	***
Constant alt3	1.35	0.18	***	1.36	0.22	***	1.49	0.29	***
Log likelihood	-5183.71			-3399.20			-1744.01		

Significances levels: *** p < 0.001, ** p < 0.01, * p < 0.05, n.s. not significant. SD: Standard deviation, YELLOW: “yellow fillet color”, SUSTAIN: “fed with sustainable resources”; NOFISH: “fed without fishmeal”, PLANT: “fed on plant-based proteins”, SPIRUL: “fed with spirulina”, GER: “Germany”, DEN: “Denmark”.

As is consistent with utility theory, price had a linear negative and significant effect on the participants choice ($p < 0.001$). Furthermore, respondents preferred to participate and choose a fillet compared to refusing to buy one of the products. This is evident through the positive and significant ($p < 0.001$) constant coefficients for the choices 1 through 3 (per choice set), and where the base alternative was the "no buy" option.

Amongst the attributes under investigation, COO influences preferences to the largest degree, based on the absolute size of the coefficients. Germany was highly preferred to Turkey (the reference variable) by the respondents ($p < 0.001$), followed by Denmark ($p < 0.001$). Significant SD values of the coefficients for Germany ($p < 0.001$) and Denmark ($p < 0.001$) indicate that heterogeneity amongst respondents and their choices. Therefore, there is consistent pattern of preference, but preferences for COO are respondent specific.

Given that the coefficient for yellow fillet color was positive and significant ($p < 0.001$), it can be assumed that spirulina has a perceptible effect on appearance. The spirulina-induced altered color of trout fillets was preferred by respondents. However, the large and significant SD reveals heterogeneity amongst respondents. Nevertheless, slight effects were observed between respondents familiar with spirulina and those who were not. So, it cannot be assumed that all respondents prefer the yellow color compared to the reference.

The feed-claims did not influence respondent choices to a great extent. The vage claim "fed with sustainable resources" was preferred over no claim ($p < 0.01$). In contrast to the participants who knew spirulina beforehand, this claim was only significant in the group who did not know spirulina ($p < 0.05$). The other feed claims "fed without fishmeal", "fed on plant-based proteins" and "fed with spirulina" were neither significant in the total sample nor in the groups ($p > 0.05$) and therefore did not effect respondent choice.

3.5 Willingness to Pay

Table 7 summarizes the marginal willingness to pay in preference space for each attribute level. Unsurprisingly based on the coefficient sizes, COO is the most lucrative attribute. On average, respondents would be willing to pay 2.44 € more per 100 g of rainbow trout produced in Germany compared to fillets that are produced in Turkey. Fillets produced in Denmark would also earn an average mark-up of 1.54 € per 100 g from Turkish fillets. Overall, yellow fillets are also preferred, and respondents would be willing to pay 0.36 € more per 100 g, on average. Respondents would be willing to pay 0.44 € more per 100 g for the "fed with sustainable resources" claim, on average. As "fed without fishmeal", "fed on plant-based proteins" and "fed

with spirulina" claims had no significant effect in the model, they would neither result in a possible price premium nor mark-down. In general, the specific feed-claims do not appear to have a positive effect on consumer preference. Overall, the analysis shows only slight differences between participants who are familiar with spirulina and those who are not. Interestingly, respondents familiar with spirulina have a higher willingness to pay for German and Danish trout. The WTP calculated in these results is only for the global average; individual consumers would have their own willingness-to-pay thresholds, which likely vary greatly based on the preference heterogeneity found across all attributes (statistically significant coefficient standard deviations) for all attributes.

Table 7. Willingness to pay in preference space for production attributes of rainbow trout fillets.

Variable	Total sample n=557			Spirulina unfamiliar n=369			Spirulina familiar n=188		
	WTP	**ll**	**ul**	**WTP**	**ll**	**ul**	**WTP**	**ll**	**ul**
YELLOW	0.36 ***	0.18	0.55	0.33 ***	0.11	0.54	0.43 ***	0.07	0.78
SUSTAIN	0.44 **	0.14	0.74	0.42 *	0.06	0.77	0.38 n.s.	-0.23	0.98
NOFISH	-0.11 n.s.	-0.43	0.22	-0.08 n.s.	-0.46	0.29	-0.25 n.s.	-0.93	0.43
PLANT	-0.10 n.s.	-0.39	0.19	-0.24 n.s.	-0.57	0.10	0.06 n.s.	-0.51	0.64
SPIRUL	-0.20 n.s.	-0.56	0.15	-0.32 n.s.	-0.73	0.10	-0.03 n.s.	-0.73	0.67
GER	2.44 ***	1.98	2.90	2.15 ***	1.66	2.65	3.23 ***	2.17	4.28
DAN	1.54 ***	1.23	1.86	1.37 ***	1.01	1.72	1.93 ***	1.26	2.60

Significances levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. not significant.

WTP: willingness to pay, ul: upper bounds, ll: lower bounds, YELLOW: "yellow fillet color", SUSTAIN: "fed with sustainable resources"; NOFISH: "fed without fishmeal", PLANT: "fed on plant-based proteins", SPIRUL: "fed with spirulina", GER: "Germany", DEN: "Denmark".

4 Discussion

As the first empirical study looking at consumer preferences for the color of fish fillet, COO and utilitarian claims regarding production sustainability this research adds to the limited literature on consumer preferences for sustainably produced fishproducts (Asche and Bronnmann, 2017; Banovic et al., 2019; Bronnmann and Asche, 2017; Bronnmann and Hoffmann, 2018). The heterogeneity in consumer preferences observed in our study corresponds with other studies providing information regarding production attributes in aquaculture (Bronnmann and Asche, 2017; Bronnmann and Hoffmann, 2018). Despite heterogeneity, consumers still display clear preferences towards fillet color and COO; however, the feed-based claims used in this study had little effect on influencing consumer preferences, if at all.

Our study confirmed that consumers display preferences regarding the color of fish fillet produced with spirulina as a feed. Our findings support those of Altmann et al. (2022) who observe distinct consumer preferences for chicken breast produced with spirulina as a feed and add to the discussion on the boundaries of consumer acceptance and willingness-to-pay for animal productcolor (Altmann et al., 2022; Grebitus et al., 2013; Lusk et al., 2018). Specifically, the discrete choice experiment carried out in this study conveys that German consumer prefer the yellow fillet color corresponding to feeding spirulina in comparison to the status quo white color derived from fishmeal-based feed. This aligns with the assumptions of Teimouri et al. (2013) that consumers may prefer the color of fish fillet produced with spirulina as a feed and rejects the uninvestigated hypothesis of Welker et al. (2001) that consumers reject will the product based on its unfamiliar color.

Altered color resulting from spirulina feed can also be a way of transmitting credence attributes, such as aspects of sustainability, into searchable attributes. This may be one of the reasons why the yellow color was so highly preferred. Since the respondents were provided information that spirulina can replace fishmeal, the altered color may have been perceived to signal sustainability and therefore confounded the feed-claim effects. Color may have even been considered more trustworthy, and therefore more strongly preferred, than the sustainability claims themselves. The phenomenon of using search attributes to make assumptions regarding credence attributes already exists in the realm of health and wellness; consumers are already trained to receive information regarding health attributes through intrinsic search attributes, such as marbling (Ardeshiri and Rose, 2018).

Unlike as predicted by Welker et al. (2001) that an unfamiliar color could lead to economic losses, our research shows that consumers are willing to pay a price premium (0.36 € / 100g) for the yellow fish fillet. In other words, the feeding of spirulina could result in economic gains for producers, not losses; this being dependent on spirulina prices in comparison to fishmeal. Furthermore, in aquaculture, pigments (astaxanthin) are often supplemented in feeds to intensify filletcolor (Lim et al., 2018; Storebakken and No, 1992). Astaxanthin is usually only added to the finishing feed, because of the increased costs associated with its incorporation. Therefore, incorporating spirulina into fish feed has an additional economic advantage; spirulina acts as a pigment and replaces fishmeal as a protein source, both of which tend to be expensive components to fish feed. A price premium and cutting costs by doing away with other expensive feed ingredients will be necessary in order to make spirulina economically advantageous, since the

price of spirulina still exceeds that of fishmeal (Benemann et al., 2018). Nevertheless, spirulina remains a potential alternative presenting multiple advantages as an aquafeed ingredient.

Olsen (2004) state that freshness is a major determinant for the anticipated quality of sea-food. This is confirmed by freshness being stated the number one criterion when purchasing fresh fish in our study. Moreover, the color of food is often also taken into account when determining freshness (Lee et al., 2013). Consequently, color is one of the most decisive attributes for the consumer (Pathare et al., 2013) and likely the reason that color was stated as the second most important criterion. Although, fillet color was stated as the second most important criteria (behind freshness) while purchasing fish, COO played a much larger role in determining decisions than fillet color. Color persisted to be an important criterion in the choice experiment. This points to the strengths of conducting DCEs to elicit consumer preferences and behaviors compared to direct questioning.

Even though consumers initially classified COO behind freshness and fillet color, the highest preference and willingness to pay was shown for COO in the DCE. Consumers significantly prefer German rainbow trout fillets, followed by products from Denmark compared to trout that originated from Turkey. These results contribute to the findings of Risius et al. (2017) and Ankamah-Yeboah et al. (2018) who found that German consumers attach great importance to domestic trout products. In our study, German consumers were, on average, willing to pay 2.41 € per 100 g more for rainbow trout fillets that originated in Germany compared to those originating from Turkey. These results suggest that German products have a competitive advantage over products imported from Denmark and Turkey (Ankamah-Yeboah et al., 2018). Nonetheless, Denmark remains the largest import country of fresh rainbow trout fillet by market value since 2016, while Turkey has emerged to be the world's second largest producer of rainbow trout from aquaculture behind Iran (FAO, 2021). Especially frozen rainbow trout products from Turkey are common on the German market. In addition, imports from Turkey will likely become increasingly important in the future, due to the collapse of the rainbow trout market in Ukraine. The continued participation of Denmark and Turkey within the German market for rainbow trout ensures that local producers can continue to benefit from local consumer preferences. Therefore, domestic production should be visually highlighted on packaging to make it easy to find for consumers.

Finally, in our experiment respondents did not display strong preferences for the feed related claims. As mentioned above, this may be due to the confounding information of color with spirulina-fed fillets. The sustainable feed claim was the only significant claim across the total

sample ($p < 0.01$). The claim remained significant ($p < 0.05$) for consumers familiar with spirulina; however, "fed with sustainable resources" did not significantly influence consumers unfamiliar with spirulina. Although assumed to concisely communicate credence attributes and therefore be easily understood (Grunert et al., 2014), all three ("fed without fishmeal", "fed on plant-based proteins" and "fed with spirulina") of the singular-specific claims applied in our experiment elicited no consumer preferences. This does not conform to the assumption that specific claims are better at communicating utilitarian aspects of a product and are therefore more accepted by consumers to be used as marketing tools (Davis, 1993). Succinct claims are not always found to be the most credible and indicator of the highest product quality; they may need to be elaborated to increase consumer acceptance (Ertz et al., 2017). Likely, the "fed with spirulina" claim was weakly rejected to limited knowledge and familiarity of spirulina and/or aquaculture production (Zander et al., 2018) and indicates limited consumer sensitivity toward this succinct information (Simoes et al., 2015). Alternatively, confusion around claims, e.g. difference between claims, information behind the claim, can result in negative associations (Moon et al., 2017). The vaguest claim "fed with sustainable resources" was the only claim to positively influence consumer preferences. This could partially be due to the fact that sustainability, as a more general concept, is a familiar for consumers (Hanss and Böhm, 2012). As in the experiment of van Loo et al. (2014), our study depicts that consumers exhibit preference hierarchies towards utilitarian claims on animal products, where the least specific is preferred and the most specific was negatively perceived.

5. Conclusion

The development of alternative protein sources for fishmeal is essential for sustainable aquaculture production. Spirulina presents itself as a suitable alternative for fishmeal, yet affects fillet pigmentation towards yellow coloration. The altered fillet appearance should not be taken as a drawback, rather an opportunity to increase consumer perceived product quality. The results of this study show that a yellow fillet color, as produced with spirulina as a feed, increases consumer preference for rainbow trout fillet. For this reason, spirulina is not only suitable as a sustainable source of protein for fish, but at the same time increases the value of trout fillets. Fillet color is stated as the second most important criterion, behind freshness, while searching for fish fillet; yet discrete choice analysis shows that country of origin (COO) is the most important attribute influencing consumer preference.

When provided information on spirulina as a feed, on average a domestically produced product with a yellow fillet with our German consumer sample. Feed-based utilitarian claims had limited to no effect on consumer preferences, yet. Marketing measures and information about sustainable aquafeed could help to boost consumer orientation and acceptance, leading to a sustainable transformation of the aquaculture sector.

6. Limitations of the Study

Although the yellow coloration of the fillets was perceived positively compared to white fillets, it remains unknown how more traditional red fillets, as produced by feeding astaxanthin, would be perceived in comparison to the yellow/orange fillets produced with spirulina. In addition, other pigments in other species of algae, e.g. Lutein and Zeaxanthin in *Cladophora glomerata* are responsible for fillet coloration and can lead to differing colors of end production. Finally, the confounding effects of information on spirulina as fish feed, altered color and feed-claims is something that cannot be disentangled from our design. We provided respondents with information on spirulina as a fish feed, because we sided with Welker et al. (2001): that an unexplained unfamiliar color is likely not marketable. Spirulina-fed trout would likely be marketed with providing information regarding color and benefits of the novel feed.

Therefore, although the results of this study could be extrapolated to other pink fish fed with spirulina (and marketed as such), further research should confirm or deny our results and focus on the acceptance of traditional white fish and fish fed other microalgae. It should be noted that the effect of fillet color could also vary between countries; processing and storage forms of yellow fillets, e.g. smoked rainbow trout, are very common in Germany (Zander et al., 2018) and exposure and familiarity with yellow fillets may influence the positive consumer preference observed in this study. Overall, studies are needed for countries outside Germany/Europe to evaluate the preference for spirulina-fed fish. Finally, a split-sample study including blinded and informed consumer groups is also needed to ascertain whether the spirulina fillets were preferred in our study due to color alone or other associated credence attributes, i.e. sustainability.

Author Contribution

Simon Rosenau: Conceptualization, Methodology, Resources, Writing – Original Draft, Funding acquisition, Supervision; **Thiemo Wolgast:** Methodology**,** Formal Analysis, Investigation, Data Curation, Visualization, Writing – Original Draft; **Brianne Altmann:** Methodology, Writing – Review & Editing, Formal Analysis, Investigation, Supervision; **Antje Risius:** Conceptualization, Methodology, Formal Analysis, Writing – Review & Editing, Supervision, Project administration

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Description

Table S1. Exemplary choice set of rainbow trout filets.

Entscheiden Sie sich bitte zwischen den folgenden Produkten. Wenn Sie sich für keines der Produkte entscheiden können, wählen Sie bitte "Kein Kauf". Hinweis: Wenn Sie ein Smartphone benutzen, drehen Sie es bitte ins Querformat.



Kein Kauf

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4 Diskussion

Im Rahmen der vorliegenden Dissertation wurden grundlegende Aspekte der Nutzbarkeit von Spirulina als Fischmehlsubstitut in der Ernährung von kommerziell genutzten Fischarten untersucht. Dabei wurde mit einer vollständigen Substitution von Fischmehl durch Spirulina gearbeitet, um Effekte auf Ebene des Wachstums, der Futterverwertung, des Mikrobioms, der Produktqualität und der Konsumentenakzeptanz zu untersuchen.

In Bezug auf das Wachstum und die damit assoziierten Parameter, wie Körpergewicht, Länge und Futterumsetzungsrate, sind in erster Linie ökonomische Zielgrößen relevant. Deshalb wurde bei Afrikanischen Welsen, Regenbogen- und Bachforellen sowie Saiblingen erforscht, ob die experimentellen Diäten (Kontroll- und Spirulina-Diät) angenommen werden und welchen Einfluss sie auf die Wachstumsleistung und Umsetzung des Futters ausüben (Kapitel 3.2 und 3.3). In diesem Kontext wurde auch die Auswirkung auf die Bakterienzusammensetzung im Darmtrakt untersucht, um mögliche Zusammenhänge zwischen Mikrobiom und Futterverwertung herzustellen (Kapitel 3.1).

Im Zuge der Literaturrecherche für die geplanten Versuchsreihen wurde deutlich, dass Spirulina als neue Diätkomponente einen potenziellen Einfluss auf die Produktqualität besitzt. Aus diesem Grund wurden umfangreiche Untersuchungen zu Qualitätsparametern wie der Haut- und Filetfarbe, aber auch dem Kochsaftverlust und der Textur des Filets vorgenommen (Kapitel 3.2 und 3.3). Darüber hinaus liegt der Fokus auf der Zusammensetzung des Fettsäuremusters im Filet, die einen wichtigen Hinweis auf die ernährungsphysiologische Qualität des Produkts gibt. Auf Grundlage der Ergebnisse wurde in einer weiterführenden Studie die Konsumentenakzeptanz von Regenbogenforellen-Filets mit der durch die Spirulina-Fütterung verursachten Gelbfärbung gegenüber den üblichen weißen Filets verglichen (Kapitel 3.4). Die Ergebnisse dieser Studien wurden zusammen mit weiteren Projektergebnissen zum Einsatz von Spirulina in der Tierernährung als Kommunikationsartikel publiziert (Kapitel 9).

Die Ergebnisse der in dieser Dissertation enthaltenen Publikationen werden im Folgenden diskutiert. Ergänzend werden weitere Ergebnisse, welche nicht in die aufgeführten Publikationen eingeflossen sind, behandelt. Dazu gehört ein Fütterungsversuch mit Tilapia (Kapitel 4.1) und die Mikrobiomanalyse von Saiblingen und Regenbogenforellen (Kapitel 0).

4.1 Wachstum

Eine der wichtigsten Voraussetzungen für den Einsatz von Spirulina als Fischmehlsubstitut ist die Akzeptanz der Fische gegenüber der neuen Proteinquelle. In dieser Dissertation wurden insgesamt fünf Fischarten mit zwei experimentellen Diäten gefüttert. Die Kontrolldiät enthielt Fischmehl, während in der Spirulina-Diät das Fischmehl vollständig durch Spirulina ersetzt wurde. Die entsprechenden Forschungsergebnisse für Afrikanische Welse sind in Kapitel 3.2 und für Salmonide in Kapitel 3.3 aufgeführt. Da die Spirulina-Diät lediglich von Regenbogenforellen, Saiblingen und Afrikanischen Welsen akzeptiert und gefressen wurde, sind für diese Arten Veröffentlichungen entstanden. Für Tilapia und Bachforellen konnten aufgrund der Ablehnung des Futters keine Wachstumsdaten erfasst und publiziert werden. Die Gründe dafür werden im Folgenden diskutiert.

Der Fütterungsversuch mit Tilapia musste nach zwei Wochen vorzeitig abgebrochen werden. Ursächlich war vermutlich die geringe Besatzdichte der Aquarien sowie das zu geringe Ausgangsgewicht von 50 g, das dazu führte, dass die Fische nicht gesext[2] werden konnten. Folglich kam es dann vorrangig unter den Männchen zu verstärkten Revierkämpfen und einer stark reduzierten Futteraufnahme. Dadurch war eine Weiterführung des Versuchs im Sinne des Tierschutzgesetzes nicht möglich. Bei Bachforellen wurde hingegen ersichtlich, dass die Fische das Futtermittel komplett ablehnten. Vereinzelt wurde beobachtet, wie die Bachforellen die Spirulina-Futterpellets aufnahmen und danach wieder ausspuckten. Dieses Verhalten konnte für die Kontrolldiät nicht beobachtet werden. Als Resultat zeigte sich für die Spirulina-Gruppen nach zwei Wochen eine Gewichtsabnahme. Der Versuch wurde daher ebenfalls aufgrund von Tierwohlbedenken abgebrochen.

Möglicherweise könnte der Zusatz von Lockstoffen und Aromen helfen, die Futteraufnahme von Bachforellen zu verbessern. Verschiedene Untersuchungen an pflanzenbasierten Futtermitteln weisen darauf hin, dass Aromen die Futteraufnahme steigern und dadurch die Wachstumsleistung verbessern sowie die Fütterungsdauer reduzieren, was in einer geringeren Futtermittelverschwendung resultiert (Oliveira und Cyrino 2004; Dias et al. 1997; Kader et al. 2012; Nagel et al. 2014). Allerdings muss erwähnt werden, dass die Futterzusätze meist aquatische Organismen wie z.B. Muscheln oder Tintenfische enthalten, was sich nachteilig auf marine Ökosysteme auswirken kann (Caccavo et al. 2021). Dennoch kann es eine Möglichkeit darstellen die Substitutionsrate von Spirulina für Arten mit geringer Akzeptanz zu erhöhen.

[2] Determinierung des Geschlechts (z.B. durch äußere Geschlechtsmerkmale)

Insgesamt ist eine vollständige Substitution von Fischmehl durch Spirulina für Afrikanische Welse, Regenbogenforellen und Saiblinge möglich, geht aber mit einer reduzierten Wachstumsleistung und teilweise mit einer erhöhten FCR einher. Verantwortlich für die verringerte Verdaulichkeit könnte die Zusammensetzung der Mikroalge sein, denn Spirulina besteht zu 10 % aus – für Nichtwiederkäuer weitestgehend unverdaulichen – Zellwänden (Becker 2007; Coelho et al. 2020). Durch den langen Darmtrakt herbivorer Arten und der damit verbundenen längeren Passagezeit sind Bakterien in der Lage Cellulase durch die Sekretion von Cellulase zu verdauen (Li et al. 2008). Bei Graskarpfen (*Ctenopharyngodon idellus*) und Tilapia (*Oreochromis mossambica*) konnten *Bacillus circulans* und *Bacillus megaterium* als celluloseabbauende Bakterien identifiziert werden (Saha et al. 2006). Aus diesem Grund ist die vollständige Substitution nur für herbivore Fische wie Karpfen möglich, ohne die Wachstumsleistung maßgeblich zu reduzieren. Daher wurde nach den Fütterungsexperimenten das Mikrobiom der Fische untersucht, um mögliche Änderungen der Bakterienzusammensetzung zu erfassen (Kapitel 0).

Trotz der reduzierten Wachstumsleistung durch den vollständigen Austausch von Fischmehl konnte in den Fütterungsexperimenten kein Effekt auf die Vitalität und Überlebensrate festgestellt werden. Somit zeigt sich, dass Spirulina auch bei einer vollständigen Substitution die Gesundheit der Fische nicht zu beeinflussen scheint. Diese Ergebnisse stimmen mit den vorangehenden Versuchen, die bereits in Kapitel 2.3.6.1 ausführlich beschrieben wurden, überein.

Letztendlich müssen für die Feststellung des Potenzials einer kommerziell genutzten, vollständigen Substitution von Fischmehl durch Spirulina weitere Forschungsbemühungen unternommen werden. Dabei wären beispielsweise Untersuchungen zur Aufschließung der Spirulina-Zellwände durch technische (Makkar et al. 2016) oder enzymatische Verfahren (Córdova et al. 2019) relevant. Weiterhin ist die Verdaulichkeit ein wichtiges Kriterium für die Nachhaltigkeit des Futtermittels, da unverdaute Futtermittelbestandteile ins Wasser gelangen und dessen Qualität beeinflussen (Kong et al. 2020) (Kapitel 4.6). Darüber hinaus können ökonomische Verluste aufgrund reduzierter Wachstumsleistung nur durch einen niedrigeren Marktpreis von Spirulina im Vergleich zu Fischmehl oder durch einen höheren Produktpreis der Fischerzeugnisse ausgeglichen werden (Kapitel 4.5).

4.2 Mikrobiom

Seit Anfang des 21. Jahrhundert hat sich ein Trend zur Analyse des humanen Mikrobioms etabliert und spiegelt sich in der steigenden Anzahl an Publikationen sowie der Fördermittel wieder (Jones 2013). Dieser Trend setzt sich nun auch in der Tierproduktion fort. Durch den Einsatz neuer Sequenzierungsmethoden, wie beispielsweise der Next-Generation Sequencing (NGS)

Technologie, wurde es möglich, das Mikrobiom ohne den Einsatz von Kulturmedien zu analysieren. Eine der am häufigsten angewandten Methoden zur Identifizierung von Mikroben ist die 16S rRNA Sequenzierung (Woese und Fox 1977). Dennoch ist die Anzahl von Veröffentlichungen über das Mikrobiom von Fischen im Vergleich zu Untersuchungen des humanen Mikrobioms noch gering. Die Untersuchungen am Mikrobiom von Fischen fokussieren sich hauptsächlich auf Untersuchungen zum Einfluss von Futtermittelkomponenten und der Modulation der Darmmikrobiota (Egerton et al. 2018), denn das Mikrobiom spielt eine Schlüsselfunktion für die Ernährung und das Immunsystem von Fischen (Romero et al. 2014; Llewellyn et al. 2014) und wird unter anderem von Umwelt (Sullam et al. 2012), Jahreszeit (Zarkasi et al. 2014), geografischen Lage (Smith et al. 2015) und Genetik (Schmidt et al. 2016) beeinflusst.

Trotz der vielfältigen Einflüsse sind bei Mikrobiomuntersuchungen vor allem Variationen des sehr spezifischen und individuellen Mikrobioms Ursache für unregelmäßige Ergebnisse (Wu et al. 2010; Wagner Mackenzie et al. 2015). In den Forschungsergebnissen des Kapitels 3.1 wird diese Variabilität des Mikrobioms ersichtlich. Die festgestellte Variabilität innerhalb des Mikrobioms einzelner Individuen deckt sich mit Ergebnissen anderer Autoren (Givens et al. 2015; Wu et al. 2013). Die Untersuchungen von Lyons et al. (2017) und Plaza et al. (2019) zeigen ebenfalls, dass beim Einsatz von Mikroalgen als Futtermittel eine hohe Variabilität im Mikrobiom zwischen einzelnen Individuen zu beobachten ist.

Infolge der hohen Variation zwischen den Individuen und der verhältnismäßig kleinen Stichprobe konnte im Rahmen der beschriebenen Versuche keine Änderung der Mikrobiomstruktur festgestellt werden. Im Mittel repräsentiert die gefundene bakterielle Zusammensetzung aber den in der Literatur beschriebenen physiologischen Aufbau des Darmmikrobioms von Fischen (Yukgehnaish et al. 2020). Zu ähnlichen Ergebnissen kamen auch Plaza et al. (2019) bei der Untersuchung von Tilapia, allerdings beinhaltete in dieser Publikation die Diät nur 3 % Spirulina. Die in den Untersuchungen dieser Dissertation beobachtete leichte Erhöhung des Anteils an *Cetobacterium* im Mikrobiom der Spirulina-Gruppen findet sich auch in den Ergebnissen von Ma et al. (2022). Hier zeigten sich höhere Anteile an *Cetobacterium* im Mikrobiom von Zebrabärblingen, die mit einer Spirulina-Diät gefüttert wurden.

Gleichwohl muss beim Vergleich der wenigen verfügbaren Studien berücksichtigt werden, dass allesamt Unterschiede in der Methodik aufweisen. Es ist bekannt, dass die Probensammlung, die Behandlung der Proben und die DNA-Extraktion wesentlichen Einfluss auf die Ergebnisse der Mikrobiomanalyse von Fischen besitzen (Hart et al. 2015). Bisher fehlt es an einheitlichen

Protokollen für die Analyse des Darmmikrobioms, wodurch die Studien nur bedingt vergleichbar sind.

In der beschriebenen Veröffentlichung erschien es zur Sicherstellung der einheitlichen Behandlung der Proben aufgrund der schwankenden Zeitintervalle zwischen erster und letzter Probenahme zuträglicher, diese zunächst auf Trockeneis zu gefrieren und dann am selben Tag im Labor zu verarbeiten. Diese Methode wird für Mikrobiomuntersuchungen zwar häufig angewendet (Fouhy et al. 2015), jedoch muss berücksichtigt werden, dass das Gefrieren von Proben und auch die Lagertemperatur zur Verschiebung der bakteriellen Zusammensetzung, führen kann (Carda-Diéguez et al. 2014; Larsen et al. 2015). Neuere Forschungsergebnisse lassen darauf schließen, dass geeignetere Methoden für die Probenlagerung existieren. So kann beispielsweise 96-prozentiger Ethanol mit einer Darmmukusprobe vermischt werden, wodurch die DNA-Quantität und -Qualität gegenüber dem reinen Gefrieren der Probe erhöht wird (Hildonen et al. 2019).

Ebenfalls ist bekannt, dass unterschiedliche Extraktionskits (Tarnecki et al. 2017) oder DNA-Extraktionsprotokolle (Larsen et al. 2015) einen erheblichen Einfluss auf die Ergebnisse der Mikrobiomstudien haben können. Dazu kommt, dass das 16S rRNA-Gen etwa 1600 Basenpaare und neun hypervariable Regionen umfasst, welche als V1 – V9 bezeichnet werden (Stackebrandt und Goebel 1994; Tringe und Hugenholtz 2008; Wang und Qian 2009). Die genutzten Primer können immer nur bestimmte Regionen der bakteriellen DNA abdecken, was einen Einfluss auf die erfasste Diversität hat (Tarnecki et al. 2017; Bukin et al. 2019). In den eigenen Untersuchungen wurde ein Primerpaar genutzt, welches Klindworth et al. (2013) zufolge im Vergleich zwischen 175 Primern und 512 Primerpaaren die höchste Gesamtabdeckung von Bakterien und Archaeen bietet. Dadurch konnte das Risiko für Verzerrungen von PCR-basierten mikrobiellen Diversitätsstudien in den durchgeführten Versuchen minimiert werden. Dieser Primer zielt auf die V3 – V4 Region ab und wurde bereits erfolgreich in vielen anderen Mikrobiomstudien bei Fischen genutzt (Cerezo et al. 2021; Dehler et al. 2017; Rimoldi et al. 2018).

Der Primer für die bakterielle DNA von Afrikanischen Welsen zeigte bei nahezu allen Fischen sichtbare Banden nach der Gelelektrophorese bei 550 Basenpaaren (Kapitel 3.1). Bei Regenbogenforellen und insbesondere Saiblingen (Daten nicht veröffentlicht) waren die PCR-Produkte nur bei der Kontrollgruppe sichtbar, aber nicht für die Spirulina-Gruppe. Dieses Ergebnis könnte auf mögliche, aus den Spirulinazellen stammende Inhibitoren hinweisen. Diese Inhibitoren, zu denen z.B. Huminsäure, Gallensalze und Polysaccharide zählen (Schrader et al. 2012),

könnten die PCR der extrahierten DNA der Spirulina-Gruppe beeinflusst haben. Weitere Anpassungen der Methodik, wie z.B. Aufreinigungsschritte, sollten erprobt werden.

Die Heterogenität der Methoden von Mikrobiomstudien macht einen direkten Vergleich zwischen einzelnen Untersuchungen nur teilweise möglich. Hinzu kommt, dass einzelne Studien in ihrem Kontext zutreffend, ihre Schlussfolgerungen jedoch wegen unterschiedlicher Methoden nicht immer auf andere Studien übertragbar sind (Pollock et al. 2018). Dennoch schreitet die Forschung auch hier weiter voran und deshalb muss zwingend für zukünftige Experimente die neueste Literatur stets herangezogen werden, um eine gute Vergleichbarkeit zu schaffen.

4.3 Produktqualität

Der Verbraucher legt zunehmend Wert auf gesunde Produkte und deren Qualität (Vanhonacker et al. 2013). Dabei haben die eingesetzten Futtermittel einen maßgeblichen Effekt auf die Produktqualität und können Geruch, Geschmack, Farbe, Textur, Festigkeit und Nährstoffgehalt beeinflussen (Webster et al. 2004). Die Auswirkungen der Substitution von Fischmehl durch Spirulina auf die Produktqualität sind in Kapitel 3.2 und 3.3 zu finden und werden im Folgenden diskutiert.

Die Fütterung von Spirulina führte in den beschriebenen Versuchen zu einer sichtbaren Änderung der Filetfarbe von weiß/pink zu gelb/orange. Die Messung der Farbe des Fischmuskels mittels Spektralphotometer erfasste erhöhte Gelb- und Rotwerte und konnte somit die optische Wahrnehmung verifizieren. Dieser Effekt wurde bereits von mehreren Autoren beschrieben und wird durch die in Spirulina enthaltenen Karotinoide hervorgerufen (Kapitel 2.3.6.4). Darüber hinaus konnten die im Rahmen dieser Arbeit durchgeführten Untersuchungen zeigen, dass Spirulina auch zu einer stärkeren Hautpigmentierung führt. Da insbesondere die Änderung der Filetfarbe einen potenziellen Einfluss auf die Kaufentscheidung von Konsumenten hat (Steine et al. 2005; Alfnes et al. 2006), wurde getestet, ob der Kochprozess die Farbpigmente zerstört und das Filet weniger gelb erscheinen lässt. Allerdings wurde die Gelbfärbung durch den Kochprozess weiter erhöht, jedoch im gleichen Maße wie für die Filets der Kontrollgruppe. Daraus konnte geschlussfolgert werden, dass die Karotinoide im Muskel bis mindestens 70 °C hitzestabil sind. Da die Gelbfärbung des Filets durch den Kochprozess nicht verringert werden konnte, wurde in einem Folgeversuch die Auswirkung der veränderten Filetfarbe auf die Akzeptanz des Konsumenten untersucht (Kapitel 4.4).

Die Untersuchung weiterer Qualitätsparameter wie die Höhe des Kochsaftverlusts, zeigten zwischen Regenbogenforellen, Saiblingen und Afrikanischen Welsen gegensätzliche Ergebnisse.

Während sich bei Regenbogenforellen und Saiblingen ein höherer Kochsaftverlust in der Spirulina-Gruppe ergab, zeigte sich bei Afrikanischen Welsen ein geringerer Kochsaftverlust in den mit Spirulina gefütterten Gruppen. Auch die Scherkraftmessungen von Regenbogenforellen und Saiblingen zeigten zwischen den Versuchsgruppen und Arten gegensätzliche Ergebnisse. Verantwortlich könnten hier die tendenziell steigenden Wassergehalte im Fischmuskel sein, die durch die Spirulina-Fütterung hervorgerufen werden. Auch Änderungen im Protein- und Fettgehalt könnten einen Einfluss auf den Wassergehalt im Muskel haben (Kapitel 2.3.6.4).

Ein weiteres Kriterium der Produktqualität ist das Fettsäuremuster von Fischprodukten, da es einen hohen Stellenwert für die menschliche Gesundheit hat (Calder und Yaqoob 2009). Dabei ist insbesondere ein hoher Anteil an PUFA aus ernährungsphysiologischer Sicht positiv zu bewerten (Zárate et al. 2017). Gerade Spirulina hat durch einen hohen Gehalt an PUFA das Potenzial wichtige essenzielle Fettsäuren zu liefern (Remize et al. 2021). Bei einem vollständigen Austausch von Spirulina zeigte sich jedoch im Rahmen der in Kapitel 3.2 beschriebenen Studie an Afrikanischen Welsen, dass wichtige PUFA wie EPA reduziert wurden. Dennoch konnten für die Gesamtmenge an SFA, MUFA und PUFA im Fischmuskel keine signifikanten Unterschiede zwischen Kontroll- und Spirulina-Diät nachgewiesen werden. Infolge der Spirulina-Fütterung kam es ebenfalls bei Regenbogenforellen und Saiblingen zu einer Reduktion von EPA und DHA. Gleichzeitig erhöhte sich der Gehalt an SFA und MUFA. Mit einer Ausnahme (Eicosadiensäure) konnte für das Fettsäuremuster keine Interaktion zwischen Spezies und Diät gefunden werden. Das bedeutet, dass die im Spirulina enthaltenen Fettsäuren von beiden Arten gleichermaßen in den Fischmuskel integriert werden – somit ist keine der beiden Arten im Hinblick auf die Akkumulation von wichtigen Fettsäuren effizienter.

Wie bereits in Kapitel 2.3.6.4 beschrieben, können – abhängig von der untersuchten Fischart – mit geringeren Substitutionsraten erhöhte Gehalte an PUFA im Muskel festgestellt werden. Zusammenfassend führt die vollständige Substitution von Fischmehl durch Spirulina zu Veränderungen im Fettsäuremuster des Produkts. Da sich das Verhältnis von ω-6- zu ω-3-Fettsäuren jedoch weiterhin im für die Humanernährung empfohlenen Bereich von unter fünf bewegt (Elvevoll und James 2000), kann davon ausgegangen werden, dass die ernährungsphysiologische Qualität des Fettsäuremusters durch die Spirulina-Fütterung nicht vermindert wird. Es bedarf weiterer Untersuchungen und Optimierungen der Diäten, um einen möglichst großen Teil der essenziellen Fettsäuren im Produkt zu akkumulieren.

4.4 Akzeptanz des Konsumenten

Auch während der COVID-19-Pandemie ist die Nachfrage nach Aquakulturerzeugnissen in Deutschland weiter gestiegen (Schäfer et al. 2021). Bei der Lebensmittelauswahl von Konsumenten wirkt ein komplexes Zusammenspiel aus einer Vielzahl an Faktoren (Köster 2009). Vor allem das Abweichen von der Norm kann eine starke Ablehnung von Lebensmitteln bei Menschen hervorrufen, was auch als Neophobie bezeichnet wird (Pliner und Hobden 1992). Insbesondere wird, aufgrund der durch die Spirulina-Fütterung veränderten Filetfarbe, ein Effekt auf die Konsumentenakzeptanz vermutet, da die Farbe zu den ersten Produktmerkmalen gehört, die vom Konsumenten wahrgenommen werden. Die Filetfarbe stellt daher eine der wichtigsten Eigenschaft für den Konsumenten dar und kann darüber entscheiden, ob ein Produkt vom Verbraucher akzeptiert oder abgelehnt wird (Pathare et al. 2013). Fischprodukte werden in der Regel verpackt verkauft, sodass die olfaktorische und haptische Wahrnehmung als Faktor für das Kaufverhalten des Konsumenten nicht ausschlaggebend ist.

Aufbauend auf den vorangehenden Untersuchungsergebnissen wurde im Rahmen einer für Deutschland repräsentativen Online-Studie mit 557 Teilnehmern die Präferenz und Zahlungsbereitschaft für die veränderte Filetfarbe ermittelt (Kapitel 3.4). Die Regenbogenforelle wurde als am häufigsten konsumierte Süßwasserart in Deutschland für die Studie ausgewählt (Statista 2022a). Dabei stellte sich heraus, dass die Mehrheit (ca. 66 %) der Befragten Spirulina nicht kannte. Zu ähnlichen Ergebnissen kamen Kamenidou und Priporas (2012). Die Autoren berichten, dass knapp 56 % der Befragten die Mikroalge nicht bekannt war. Generell scheint das Wissen von Konsumenten über Spirulina und deren Eigenschaften limitiert zu sein (Mellor et al. 2022).

Das Discrete Choice Experiment zeigte einen Unterschied in der Präferenz von weißen (Kontrolle) und gelben (Spirulina) Filets. Die Konsumenten präferierten nicht nur die gelbe Filetfarbe, sie waren auch bereit 3,40 € pro kg mehr dafür zu bezahlen. Dennoch war das Merkmal der heimischen Produktion das wichtigste Kriterium für die Kaufentscheidung. Auch Risius et al. (2017) beobachteten in ihren Untersuchungen die stärkste Präferenz für heimisch produzierte Fischprodukte. Die Präferenz für heimische Produkte wurde nicht nur für Fisch- und Aquakulturprodukte beobachtet, sondern auch im weiteren Lebensmittelsektor (Waterlander et al. 2019; Steenhuis et al. 2011). Die verwendete Proteinquelle hatte hingegen kaum Einfluss auf die Auswahl und die Kaufbereitschaft der Konsumenten. Lediglich der allgemeiner formu-

lierte Claim „aus nachhaltiger Fütterung“ ließ möglicherweise genug Spielraum zur Interpretation, sodass Konsumenten Produkte mit diesem Merkmal präferierten und auch die Zahlungsbereitschaft gesteigert wurde.

Verantwortlich für die Ergebnisse könnte ein hohes Vertrauen in die Qualität deutscher oder dänischer Aquakulturerzeugnisse sein, oder auch ein zu geringes Verständnis der Konsumenten für die Aquakultur und deren Produktion (Zander et al. 2018). Die Resultate bedeuten für die Aquakultur, dass der Einsatz von Spirulina als Fischmehlsubstitut zu einer Wertsteigerung von Fischfilets führen kann. Diese Forschungsergebnisse können als Grundlage für die weitere Etablierung von Spirulina auf dem Futtermittelmarkt dienen und somit zu einer nachhaltigeren Gestaltung der Aquakultur beitragen. In diesem Fall sollte das Marketing in Richtung heimischer oder sogar regionaler Produktion ausgeweitet werden. Zukünftig könnte die Bereitstellung von Informationen, ähnlich wie bereits bei anderen alternativen Proteinquellen wie Insekten nachgewiesen, zu einer weiter steigenden Akzeptanz unter den Konsumenten führen (Baldi et al. 2021). Konsumenten müssen verstärkt über die Aquakultur, die Fischproduktion und die Einsatzmöglichkeiten von Spirulina informiert werden, um eine differenzierte Kaufentscheidung zu ermöglichen.

4.5 Ökonomischer Nutzen

In Anbetracht der aus der vorliegenden Arbeit resultierenden Forschungsergebnisse einer vollständigen Substitution von Fischmehl durch Spirulina, muss auch die wirtschaftliche Tragfähigkeit der Futtermittelalternative betrachtet werden. Um eine möglicherweise verringerte Wachstumsleistung (Kapitel 3.2 und 3.3) aus ökonomischer Sicht auszugleichen, ist eine wichtige Voraussetzung, dass Spirulina im Einkauf auf einem ähnlichen, bzw. niedrigeren Preisniveau als Fischmehl liegen muss.

Der Marktpreis für Spirulina liegt bei einem Massenabsatz zwischen 10,00 und 30,00 US$ pro kg (9,51 – 28,54 € pro kg; durchschnittslicher Umrechnungskurs Dollar in Euro aus 2018 (European Central Bank 2022)) (Benemann et al. 2018). Dabei sind die Herstellungskosten stark abhängig vom Anbausystem und variieren zwischen 3,86 und 9,59 € pro kg für open pond Systeme und 18,71 und 75,29 € pro kg für Photobioreaktoren (Delrue et al. 2017). Dementgegen steht jedoch ein verhältnismäßig geringerer Fischmehlpreis von ca. 1,45 € pro kg für das Jahr 2021 (Statista 2022b). Der hohe Preisunterschied macht den Einsatz von Spirulina somit unrentabel. Dennoch ist anzunehmen, dass durch die Optimierung von Produktionsprozessen die Produktionskosten in Zukunft sinken werden. Verbesserungen der Produktionsprozesse sind

beispielsweise im Rahmen der Steigerung der Produktivität von Anbausystemen sowie der Optimierung der Energienutzung und der Nährmedien möglich (Delrue et al. 2017; Tredici et al. 2016; Soni et al. 2017).

Ergänzend muss die Akzeptanz und Zahlungsbereitschaft in die ökonomische Analyse mit einbezogen werden. In der zugehörigen Untersuchung zeigte sich, dass die Verbraucher die Filetpigmentierung durch den Einsatz von Spirulina präferieren und zudem bereit sind einen höheren Produktpreis zu zahlen (Kapitel 3.4). In der Fischproduktion muss für die Pigmentierung des Filets dem Futter natürliches oder synthetisches Astaxanthin beigemischt werden, welches den Preis des Futtermittels erhöht (Lim et al. 2018; Storebakken 1988).

Zusammenfassend wird Spirulina zum jetzigen Zeitpunkt im Vergleich zu Fischmehl nicht rentabel einsetzbar sein, doch weist der aktuelle Forschungsstand darauf hin, dass durch den optimierten Anbau Produktionskosten gesenkt werden können und sich die Preisspanne zu Fischmehl verringern wird (Taelman et al. 2013). Darüber hinaus spricht sich ein potenziell besseres Wachstum bei geringer Substitution in Kombination mit einer gesteigerten Konsumentenpräferenz und Zahlungsbereitschaft für die Pigmentierung des Filets für den Einsatz von Spirulina in der Diät von Fischen aus. Gegenwärtig scheint die vollständige Substitution von Fischmehl durch Spirulina noch nicht rentabel gestaltbar zu sein, weshalb sie in der Praxis noch keine Anwendung findet.

4.6 Nachhaltigkeitsaspekte

Mikroalgen werden in der Literatur häufig als nachhaltige Alternative für Fischmehl beschrieben (Nagappan et al. 2021; Ahmad et al. 2022; Gamble et al. 2021). Ein Vorteil von Mikroalgen, wie z.B. Spirulina, gegenüber den pflanzlichen und tierischen Proteinquellen ist, dass die Produktion umweltfreundlich und ressourcenschonend ist. Aufgrund der hohen flächenbezogenen Biomasseproduktivität mit geringem Wasser- sowie Ackerlandverbrauch bieten Mikroalgen ein sehr hohes wirtschaftliches Potential (Nagappan et al. 2021). Durch den geringen Wasser- und Platzbedarf finden die Anbausysteme Anwendung in Gebieten, in denen traditionelle Landwirtschaft kaum oder nur begrenzt möglich ist (Ciani et al. 2021). Aus diesem Grund steht die Mikroalgenproduktion in der Regel nicht in Konkurrenz zu anderen Feldfrüchten.

Spirulina ist in Bezug auf die Nutzung von Lichtenergie effizienter als andere Nutzpflanzen und kann im Wasser gelöstes CO_2 aufzunehmen (Radmer und Kok 1977). Anbausysteme wie offene und geschlossene raceway ponds können durch die Produktion von Algenbiomasse zu einer Reduzierung des Treibhausgaspotenzials führen (Duran Quintero et al. 2021). Dennoch

zeigen Smetana et al. (2017), dass Mikroalgen, abhängig vom Anbausystem und Standort, einen deutlich höheren Energieverbrauch als andere traditionelle Proteinkonzentrate wie Soja oder Erbsen haben können. Ein Großteil der für die Produktion von Spirulina aufgewendeten Energie wird laut Taelman et al. (2013) für die Kultivierung und die Trocknung der Mikroalgen benötigt. Gerade in diesem Produktionsabschnitt erkennen die Autoren ein großes Einsparungspotenzial, das durch die Optimierung der Anbausysteme und Prozessabläufe erreicht werden kann. Insbesondere die Intensivierung der Produktion zeigte in der Studie ein deutliches Potenzial zur Reduktion der CO_2-Emissionen. Zusammenfassend kamen die Autoren zu dem Schluss, dass durch die Hochskalierung der Produktion ein geringerer ökologischer Fußabdruck von Mikroalgen im Vergleich zu herkömmlichen Fischfuttermitteln erreicht wird.

In einer Untersuchung zu Umweltauswirkungen von Fischmehlsubstituten haben Draganovic et al. (2013) mit Hilfe von Energie- und Exergieanalysen[3] sieben hypothetische Futtermittelformulierungen für Salmoniden, darunter eine auf Basis von Mikroalgen, evaluiert. Die Analyse umfasste alle Produktionsschritte vom Anbau bis hin zum fertigen Produkt als Fischfutter. Die Produktion einer auf Fischmehl basierenden Diät verbrauchte rund 15 bis 31 % mehr Energie als eine pflanzenbasierte Diät und 9 % mehr als eine Mikroalgen-Diät. In Bezug auf die Exergie benötigt Fischmehl sogar 24 bis 30 % mehr als eine pflanzliche und viermal so viel wie eine Algendiät. Die Autoren schlussfolgern, dass insbesondere Mikroalgen als nachhaltige Proteinalternative für Fischmehl die Umweltkosten in Zukunft drastisch senken könnten.

Auch die Produktion von Spirulina in der Kreislaufwirtschaft erscheint aussichtsreich. Im Bereich der Aquakultur werden solche Konzepte als integrierte Aquakultursysteme bezeichnet. In solchen Systemen werden Organismen unterschiedlicher Trophiestufe eingesetzt, um Abfallstoffe nutzbar zu machen und Ressourcen effektiver einzusetzen. Dabei können wertvolle Nebenprodukte entstehen, die andernfalls in der Produktion ungenutzt bleiben und verloren gehen. Han et al. (2019) zufolge ist es beispielsweise möglich Mikroalgen zusammen mit Fischen in Teichen oder Becken zu kultivieren. Dabei können die Fische Mikroalgen als direkte Nahrungsquelle aufnehmen und zugleich nutzen die Mikroalgen die von den Fischen abgegebenen Stoffwechselprodukte, um diese in Biomasse umzuwandeln. Infolgedessen können auf umweltschonende Weise Futterkosten reduziert und die Nährstofflast im Abwasser gesenkt werden. Zudem

[3] Die Exergie berücksichtigt auch den zweiten Hauptsatz der Thermodynamik, da zwischen Energie, die Arbeit verrichten kann, und Energie, die als Wärme an die Umgebung abgegeben wird, unterschieden wird. Energie bleibt stets erhalten (erster Hauptsatz der Thermodynamik), während sie bei der Verrichtung von Arbeit (oft als Exergie genannt) unweigerlich verloren geht (zweiter Hauptsatz der Thermodynamik). Exergie ist definiert als die Menge an Arbeit, die ein betrachtetes System verrichten kann, wenn es mit der Umgebung ins Gleichgewicht gebracht wird.

führen die Autoren an, dass durch die Mikroalgen der Sauerstoffgehalt des Haltungswassers erhöht wird und der Energieverbrauch für technische Applikationen von Luftsauerstoff oder technischem Sauerstoff reduziert werden kann. Aufgrund der verbesserten Wasserqualität kann wiederum Wasser eingespart werden. Eine weitere Möglichkeit sehen Zhang et al. (2020) in der Behandlung von Abwässern (einschließlich der Abwässer von Fischzuchtbetrieben). So können beispielsweise in integrierten Systemen die Nährstoffe aus Abwässern für die Produktion von Mikroalgen genutzt werden. Dadurch könnte die Nährstofflast im Abwasser reduziert und Futtermittel in Form von Spirulina produziert werden.

Insgesamt hat Spirulina ein hohes Potenzial, um als nachhaltige Alternative für Fischmehl in der Aquakultur Anwendung zu finden. Eine anteilige Substitution kann von Vorteil sein, da diese meist nicht im Konflikt mit der Wachstumsleistung und Futterverwertung steht. Zudem resultierten aus einer hohen oder vollständigen Substitution bei karnivoren und omnivoren Fischarten eine verringerte Wachstumsleistung und Verdaulichkeit. Dadurch könnten unverdauliche Bestandteile von Spirulina wieder ausgeschieden werden, welche dann die Wasserqualität beeinträchtigen und die Umwelteffizienz reduzieren. Das hat zur Folge, dass die Wasserqualität von nachgelagerten, natürlichen Gewässern beeinflusst wird, denn die Hauptquelle für Nährstoffbelastungen wie Stickstoff und Phosphor stammen aus den Futtermitteln (Jegatheesan et al. 2011).

Verdaulichkeitsstudien zeigen, dass Spirulina zwar die Lipidverdaulichkeit verbessern, es aber auch zu einer reduzierten Verdaulichkeit von Aminosäuren kommen kann (Jiang et al. 2022; Yang 2011; Sarker et al. 2016; Sevgili et al. 2019). Sevgili et al. (2019) vermuten, dass sich eine zu hohe Substitution negativ auf das Aminosäuregleichgewicht auswirkt und es dadurch zu einer schlechteren Verwertung einzelner Aminosäuren kommt. Allerdings wurden die Aminosäuremuster der Versuchsdiäten im Rahmen der vorliegenden Dissertation ausbalanciert, weshalb der zuvor beschriebene Effekt für diese Untersuchungen vernachlässigt werden. Das beobachtete verringerte Wachstum ist vermutlich darauf zurückzuführen, dass nicht alle Bestandteile von Spirulina für den Fisch verdaulich sind.

Der Einsatz von Spirulina als Fischmehlsubstitut mag zwar das FIFO-Verhältnis verbessern, der Anteil an Fischöl ist in den vorgestellten Untersuchungen aber nicht reduziert worden. Vor allem für Salmonide müsste der Anteil an Fischöl mittels adäquater Alternativen ersetzt werden, um einer Reduktion von wertvollen ω-3-Fettsäuren im späteren Produkt vorzubeugen (Twibell et al. 2020).

Auf der einen Seite hat Spirulina als Proteinquelle das Potenzial durch Verbesserung des Anbaus die Aquakultur mit nachhaltigem und hochwertigem Futterprotein zu versorgen, auf der anderen Seite müssen die Diäten auch an die Ansprüche der Fischarten angepasst sein. Insbesondere für herbivore Arten scheinen deutlich höhere Substitutionsraten ohne Nachteile für Wachstum und Futterverwertung möglich zu sein. Es sollte besonders darauf geachtet werden, dass durch den Einsatz von Spirulina die Verdaulichkeit des Futtermittels nicht herabgesenkt wird, da dies zu erhöhten Emissionen an Nährstoffen wie Stickstoff und Phosphor führen könnte.

5 Limitationen

Die Ergebnisse der vorliegenden Dissertation beschränken sich auf die hier untersuchten Fischarten. Da aber die Verwertung und Akzeptanz von Spirulina, wie bereits mehrfach beschrieben, artspezifisch ist, sind die Untersuchungen nur begrenzt auf andere Fischarten übertragbar. Des Weiteren muss erwähnt werden, dass die in dieser Dissertation genutzten Fischarten über viele Generationen unter konventionellen Aquakulturbedingungen gehalten und reproduziert wurden. Alle Fische sind somit an konventionelle Futtermittel, welche Fischmehl als Hauptproteinquelle enthalten, adaptiert. Ein interessanter Ansatz wäre die gezielte Selektion und Untersuchung von an Spirulina adaptierten Fische, über eine oder mehrere Generationen. Laut Le Boucher et al. (2012) konnte bei Regenbogenforellen, die mit einer vollständig pflanzlichen Diät gefüttert wurden, bereits nach einer Generation die Mortalität gesenkt und das Wachstum gesteigert werden. Sollte sich Spirulina in Zukunft als Proteinquelle durchsetzen, sind Konzepte für eine zielgerichtete Selektion auf spirulinaadaptierte Zuchtlinien essenziell für eine effiziente und ökonomisch verträgliche Aquakulturproduktion.

Weiterhin muss beachtet werden, dass nur ein vollständiger Austausch von Fischmehl gegen Spirulina erforscht wurde. Dies war in Belangen der Mikrobiomanalyse zwar hilfreich, um einen möglichst starken Kontrast zur Kontrolldiät zu erlangen, doch für den Vergleich der Wachstumsleistung und Verdaulichkeit zwischen unterschiedlichen Spezies hätte sich eine Abstufung der Substitutionsraten durchaus angeboten. In diesem Kontext hätten dann Aussagen über die maximale Austauschrate von Fischmehl getroffen werden können, bei dem keine Reduzierung der Wachstumsleistung beobachtet werden kann. Damit verbunden wäre auch eine ökonomische Betrachtung für die groß skalierte Aquakulturproduktion sinnvoll. Um das Futtermittel noch nachhaltiger zu gestalten, sollte in einem darauffolgenden Schritt auch der Anteil des Fischöls durch vegetarische Alternativen herabgestuft werden.

Eine weitere Limitation stellt der auf zehn Wochen begrenzte Zeitraum der experimentellen Fütterung dar. Langzeitstudien über den Produktionsablauf vom juvenilen bis zum adulten Fisch sollten infolgedessen auch Aufschluss über den optimalen Einsatz von Spirulina in allen Lebensstadien bringen und sind besonders für die spätere Praxis in der Aquakultur von großer Bedeutung.

Eine wesentliche Limitation der Konsumentenbefragung ist die Tatsache, dass die Ergebnisse sich nur auf deutsche Konsumenten beziehen. Die Präferenz für die Filetfarbe kann zwischen Ländern und Verarbeitungs- und Lagerform variieren. Die Ergebnisse dieser Studie zeigen

zwar, dass die gelbe gegenüber der weißen Filetfarbe bevorzugt wurde, doch auch die Rotfärbung, vergleichbar mit der Färbung von Lachs oder Lachsforelle, wird vom Kunden präferiert. Daher sollte im Rahmen der weiteren Untersuchungen auch die Konsumentenpräferenz von weißen, gelben und roten Forellenfilets verglichen werden. Vor allem für die nachhaltige Gestaltung der Lachsindustrie, die in Europa einen sehr hohen Stellenwert hat, wären die Ergebnisse relevant. Sollte sich dabei eine geringere Akzeptanz für die aus der Spirulina-Fütterung resultierenden veränderten Filetfarbe von Lachsen ergeben, wären weitere Untersuchungen notwendig, um zu erforschen, wie die Gelbfärbung verhindert oder ggf. durch andere Pigmente ausgeglichen werden kann. Dabei könnte dann ein finishing feed mit Astaxanthin zum Einsatz kommen, oder erprobt werden, inwieweit Astaxanthin auch in eine Spirulina-Diät eingemischt werden kann, um dadurch die rote Pigmentierung zu erhöhen. In diesem Kontext muss erforscht werden, ob sich die gelbe Filetfarbe im Produktionsprozess ausgleichen lässt, wie lange dieser Prozess dauert und welche Ressourcen sowie Änderungen im Prozessablauf dafür benötigt werden.

6 Fazit

Spirulina stellt insgesamt eine geeignete Proteinquelle für eine Vielzahl von Fischarten dar und kann Fischmehl vollständig in der Diät von Afrikanischen Welsen, Saiblingen und Regenbogenforellen ersetzen. Dennoch gehen mit einer vollständigen Substitution eine verringerte Wachstumsleistung und zum Teil reduzierte Futterverwertung durch die genannten Fischarten einher. Hinzu kommt, dass die Akzeptanz gegenüber der Spirulina-Diät artspezifisch ist und ggf. zur Verringerung oder Einstellung der Futteraufnahme führen kann. Eine geringe Akzeptanz konnte beispielsweise für Bachforellen beobachtet werden. Hier könnten Lockstoffe und Aromen zum Einsatz kommen, um die sensorische Wahrnehmung des Futters zu verbessern. Dementsprechend müssen artspezifische Limits für die Substitution von Fischmehl durch Spirulina weiter erforscht werden, um die Ressource rentabel und ohne Nährstoffverluste in der Fischernährung einzusetzen.

Eine vollständige Substitution kann Auswirkungen auf die Produktqualität haben. Die Spirulina-Fütterung führte bei den Fischen zu einer stärkeren Haut- und Filetpigmentierung. Insbesondere die damit verbundene Gelbfärbung der Filets wurde vom Konsumenten gegenüber einer weißen Filetfarbe präferiert und steigerte die Zahlungsbereitschaft. Die Untersuchung des Fettsäuremusters im Endprodukt zeigten eine Reduktion von PUFA durch die Spirulina-Substitution. Dennoch blieb das Verhältnis von ω-6- zu ω-3-Fettsäuren in einem für die Humanernährung empfohlenen Bereich.

Der kommerzielle Einsatz von Spirulina im Fischfutter ist demnach von vielen Faktoren abhängig. Besonders der Marktpreis von Spirulina im Vergleich zu anderen Futtermittelkomponenten wie Fischmehl wird einen entscheidenden Einfluss auf die ökonomische Bewertung haben. Momentan wird eine vollständige Substitution infolge einer reduzierten Wachstumsleistung nicht rentabel eingesetzt werden können. Zunächst müsste sich der Marktpreis für Spirulina dem von Fischmehl angleichen. Gleichzeitig sollte auch die mögliche Wertsteigerung des Produkts durch eine stärkere Filetpigmentierung berücksichtigt werden. Darüber hinaus muss Spirulina zu jeder Zeit in großen Mengen für die industrielle Futtermittelherstellung verfügbar sein. Sollte sich der Trend der steigenden Nachfrage weiter fortsetzen, wird dies voraussichtlich zu einer Produktionssteigerung und einem damit verbundenen Absinken des Marktpreises von Spirulina führen.

Die Möglichkeit eines wirtschaftlichen Einsatzes von Spirulina wäre wünschenswert, da Spirulina eine nachhaltige Alternative zu Fischmehl darstellt. Spirulina ist zum einen effektiv in der Biomasseproduktion, zum anderen steht der Anbau meist nicht in Konkurrenz mit anderen

Nutzpflanzen und ist je nach Produktionssystem energiesparender. Zusätzlich ermöglicht die Fischmehlsubstitution eine Entlastung der Meere und deren Fischpopulationen. Auch integrierte Aquakultursysteme mit Spirulina könnten zielführend für eine nachhaltigere Aquakulturproduktion sein.

Zusammenfassend bietet der Einsatz von Spirulina in der Fischernährung ein hohes Potential für eine nachhalitgere Aquakultur. Gleichwohl wird Spirulina aufgrund der beschriebenen Herausforderungen noch nicht im großen Maßstab für die Produktion von Fischen eingesetzt. Es müssen zunächst weitere Optimierungen, vom Anbau und der damit verbundenen Senkung der Produktionskosten, bis zur Ermittlung der optimalen Einsatzmenge für die artspezifische Ernährung, vorgenommen werden, damit sich der Einsatz nicht nur ökologisch, sondern auch ökonomisch rentiert.

7 Zusammenfassung

Eine nachhaltigere Produktion von Nutztieren steht immer stärker im Fokus der Konsumenten. Durch die voranschreitende Überfischung der Weltmeere wird auch die Aquakultur als Hauptabnehmer für Fischmehl kritisiert, denn die Produktion dieser Ressource kann nicht nur das Ökosystem und Fischpopulationen nachhaltig schädigen, sondern verbraucht zudem viel Energie. Als Resultat steigen die Preise für Fischmehl seit Jahren an und die Suche nach einer geeigneten nachhaltigeren Alternative scheint unumgänglich.

Die Mikroalge Spirulina (*Arthrospira platensis*) hat sich bereits in mehreren Studien als geeignete Proteinquelle für Fische herausgestellt. Ein anteiliger Austausch von Fischmehl gegen Spirulina kann förderlich für das Wachstum und die Gesundheit der Fische sein. Im Gegensatz zu Fischmehl können Mikroalgen in Abhängigkeit von Produktionssystem und Standort deutliche Vorteile im Bereich der Nachhaltigkeit aufweisen. Ziel dieses Promotionsvorhabens war es daher, die Eignung von Spirulina als vollständiges Fischmehlsubstitut zu erproben. In diesem Kontext wurden vier Studien durchgeführt und die Auswirkungen der Substitution auf das Wachstum, das intestinale Mikrobiom, die Produktqualität sowie die Konsumentenakzeptanz untersucht.

In der ersten Studie wurde der Effekt eines vollständigen Austauschs von Fischmehl gegen Spirulina auf das Darmmikrobiom von Afrikanischen Welsen (*Clarias gariepinus*) mittels 16s rRNA-Sequenzierung untersucht. Aus den Sequenzierungsdaten konnten keine statistischen Unterschiede in der bakteriellen Vielfalt gefunden werden. Insgesamt dominierten Fusobakterien als einzige Taxa aus der Gattung *Cetobacterium*. Im Mikrobiom der Fische der Kontrolldiät war hingegen vorwiegend das Bakterium der Gattung *Romboutsia* zu finden. In den Spirulina-Gruppen konnten vermehrt Bakterien der Gattung *Plesiomonas* und *Bacteroides* gefunden werden. Dennoch wurde die Gesamtstruktur der mikrobiellen Gemeinschaft des Darms durch die experimentellen Diäten nicht beeinflusst.

Die zweite Untersuchung beschäftigte sich mit den Auswirkungen einer vollständigen Substitution von Fischmehl durch Spirulina auf das Wachstum und die Produktqualität von Afrikanischen Welsen (*Clarias gariepinus*). Die Welse wurden über zehn Wochen mit einer Diät auf Fischmehlbasis (Kontrolldiät) und einer Diät, in der das Fischmehl vollständig durch Spirulina ersetzt wurde, gefüttert. Dabei zeigte sich in den Spirulina-Gruppen eine abnehmende Wachstumsleistung, während sich die Futterverwertung der beiden Diäten nicht unterschied. Außerdem zeigten mit Spirulina gefütterte Fische eine intensivere Gelbfärbung der Haut sowie des rohen und gekochten Filets. Die Analyse der Fettsäuren ergab höhere Anteile von Palmitinsäure

und Linolsäure, aber abnehmende Gehalte an Lignocerinsäure und Eicosapentaensäure bei Welsen, die mit Spirulina gefüttert wurden. Insgesamt wiesen die Gruppen die gleiche Gesamtmenge an gesättigten, einfach ungesättigten und mehrfach ungesättigten Fettsäuren auf. Die Ergebnisse führten zu dem Schluss, dass eine Optimierung der Spirulina-Diät notwendig ist, um wirtschaftliche Verluste in der Produktion von Welsen zu vermeiden.

Die dritte Studie erforschte den Effekt einer vollständigen Substitution von Fischmehl durch Spirulina auf das Wachstum und die Produktqualität von Bachsaibling (*Salvelinus fontinalis*), Regenbogenforelle (*Oncorhynchus mykiss*) und Bachforelle (*Salmo trutta fario*). In einem zehnwöchigen Fütterungsversuch wurden zwei Versuchsdiäten verwendet, die entweder Fischmehl (Kontrolldiät) oder Spirulina als Hauptproteinquelle enthielten. Es wurden Unterschiede zwischen den Arten in der Akzeptanz und Verwertung von Spirulina festgestellt. Mit Ausnahme der Bachforellen nahmen alle Fischarten die Spirulina-Diät auf. Da die Bachforellen die Versuchsdiät ablehnten, wurde der Fütterungsversuch für diese Art aufgrund von Tierwohlbedenken abgebrochen. Bei Saiblingen und Regenbogenforellen resultierte die Fütterung von Spirulina in einer Abnahme der Wachstumsleistung sowie einer reduzierten Futterverwertung. Außerdem führte die Spirulina-Diät zu einer Zunahme von gelben und roten Pigmenten im rohen und gekochten Filet und wirkte sich zudem auf das Fettsäuremuster der beiden Arten aus. Insbesondere der Anteil an gesättigten und einfach ungesättigten Fettsäuren stieg durch die Spirulina-Fütterung an, gleichzeitig reduzierte sich der Gehalt an mehrfach ungesättigten Fettsäuren. Insgesamt geht der vollständige Ersatz von Fischmehl durch Spirulina mit einer verringerten Produktionsleistung und Auswirkungen auf wichtige Produktqualitätsmerkmale wie die Filetfarbe und das Fettsäuremuster einher.

Auf Grundlage der Ergebnisse der dritten Studie wurde die gelbe Filetfarbe von Regenbogenforellen, die aus der vollständigen Substitution von Fischmehl durch Spirulina resultierte, hinsichtlich ihrer Akzeptanz durch den Konsumenten untersucht. Die zugrundeliegende Hypothese lautete, dass die Filetfarbe neben anderen Attributen wie Herkunftsland, Preis und Futtermittelangaben die Akzeptanz der Verbraucher beeinflussen würde. In einem Discrete Choice Experiment wurde die Präferenz und die Zahlungsbereitschaft deutscher Verbraucher für die geänderte Filetfarbe untersucht. Die empirische Analyse basierte auf einer repräsentativen Onlinebefragung mit 557 Teilnehmenden. Die Ergebnisse zeigen, dass die gelbe Filetfarbe einen positiven Einfluss auf die Präferenz der Verbraucher hatte und zu einer höheren Zahlungsbereitschaft führte. Auf Grundlage dieser Ergebnisse kann angenommen werden, dass Spirulina sowohl die Produktqualität verbessert als auch zu einer Wertsteigerung von Forellenfilets führt. Zudem konnte eine stärkere Präferenz der Verbraucher für Filets aus heimischer Herkunft

(Deutschland) gegenüber Filets aus Dänemark und der Türkei beobachtet werden. Produktinformationen wie "aus nachhaltiger Fütterung" einen positiven Einfluss auf die Kaufentscheidung. Im Gegensatz dazu hatten spezifische Futtermittelangaben wie "ohne Fischmehl gefüttert", "mit Spirulina gefüttert" und "mit pflanzlichen Proteinen gefüttert" keinen Einfluss auf die Kaufentscheidung. Marketingaktivitäten zur erfolgreichen Vermarktung von Regenbogenforellen, die mit Spirulina gefüttert wurden, sollten sich auf die hohe Nachfrage der Verbraucher für inländisch produzierte Produkte konzentrieren. Darüber hinaus ist es notwendig, das Bewusstsein und die Akzeptanz der Verbraucher für nachhaltige Produktionsmethoden in der Aquakultur mittels Informationsvermittlung zu erhöhen.

Zusammenfassend zeigen die Forschungsergebnisse dieser Dissertation, dass Spirulina eine geeignete alternative Proteinquelle für den die vollständige Substitution von Fischmehl darstellt. Sollte ein hoher Anteil an Fischmehl gegen Spirulina ausgetauscht werden, muss eine artspezifische Optimierung der Diät vorgenommen werden, um reduzierten Wachstumsleistungen vorzubeugen. Ebenso muss beachtet werden, dass die ernährungsphysiologische Relevanz der veränderten Fettsäuremuster noch weiterer Forschung bedarf. Dennoch ist Spirulina geeignet, um Produktqualitätsmerkmale wie die Pigmentierung der Filets zu verbessern, wodurch es zu einer höheren Kauf- und Zahlungsbereitschaft der Konsumenten kommen kann. Zudem bietet der Einsatz von Spirulina in der Fischernährung vielversprechendes Potenzial für eine nachhaltigere Aquakulturproduktion, denn die Mikroalge bietet gleich auf mehreren Ebenen ökologische Vorteile. Allerdings müssen in Zukunft weitere Optimierungen der Anbauprozesse vorgenommen werden, damit der Marktpreis für Spirulina sinkt und die Ressource in konstant hohen Mengen verfügbar ist, sodass sich der Einsatz sowohl aus ökologischer als auch aus ökonomischer Sicht rentiert.

8 Summary

The sustainability of livestock production is becoming increasingly important for consumers. Due to increasing levels of overfishing of the world's oceans, aquaculture has also been criticized for its role as the main consumer of fishmeal. Production of this resource not only causes damage to the ecosystem and fish populations, but also requires high energy consumption. As a result, the price of fishmeal has been rising over the last decade and the search for a suitable and more sustainable alternative is inevitable.

The microalgae spirulina (*Arthrospira platensis*) has already been identified in several studies as a suitable source of protein for fish. A proportional substitution of fishmeal with spirulina can be beneficial for growth and health of fish. In contrast to fishmeal, microalgae can have advantages in sustainability depending on the production system and location. The aim of this dissertation project was therefore to test the suitability of spirulina as a complete fishmeal substitute. In this context, four studies were conducted to investigate the effects of substitution on growth, the intestinal microbiome, product quality and consumer acceptance.

In the first study, the effect of a complete replacement of fishmeal by spirulina on the intestinal microbiome of African catfish (*Clarias gariepinus*) was investigated using 16s rRNA sequencing. Analysis of the sequencing data showed no statistical differences for bacterial diversity. Overall, in the Spirulina group Fusobacteria dominated as the only taxa from the genus *Cetobacterium*. In contrast, the microbiome of the control group was dominated by bacteria of the genus *Romboutsia*. In the spirulina group, bacteria of the genus *Plesiomonas* and *Bacteroides* were found more frequent. Nevertheless, the overall gut microbiome structure was not affected by the experimental diets.

The second study dealt with the effects of a complete substitution of fishmeal with spirulina on the growth and product quality of African catfish (*Clarias gariepinus*). The catfish were fed either a fishmeal-based diet (control diet) or a diet in which the fishmeal was completely replaced by spirulina for ten weeks. There was a reduction in growth performance observed in the spirulina group, whilst no difference was observed in the feed conversion rates between the two diets. In addition, Spirulina-fed fish showed a more intense yellow coloration of the skin and of the raw and cooked fillet. Analysis of fatty acids revealed higher levels of palmitic and linoleic acid but decreasing levels of lignoceric and eicosapentaenoic acid from the catfish fed with spirulina. Overall, the groups had the same levels of total saturated, monounsaturated and polyunsaturated fatty acids. The results concluded that optimization of the spirulina diet is necessary to avoid economic losses in catfish production.

The third study investigated the effect of a complete substitution of fishmeal with spirulina on the growth and product quality of brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*). In a ten-week feeding trial, two experimental diets were used, containing either fishmeal (control diet) or spirulina as the main protein source. Differences were found between species in the acceptance and utilization of spirulina. Except for the brown trout, the spirulina diet was accepted by the fish. As the brown trout rejected the experimental diet, the feeding trial for this species was terminated due to animal welfare concerns. Spirulina feeding resulted in a decrease in growth performance and a reduction in feed conversion for both brook trout and rainbow trout. In addition, Spirulina diet increased yellow and red pigments in raw and cooked fillets, as well as also affecting the fatty acid pattern. In particular, the proportion of saturated and monounsaturated fatty acids increased with spirulina feeding, whilst concurrently the content of polyunsaturated fatty acids decreased. Overall, the complete replacement of fishmeal by Spirulina is associated with reduced production performance andd further effects on important product quality traits such as fillet color and fatty acid pattern were determined.

Based on the results of the third study, the yellow fillet colour of rainbow trout resulting from the complete substitution of fishmeal with spirulina was investigated with regard to its acceptance by consumers. The hypothesis was that the fillet color would influence consumer acceptance along with other attributes such as country of origin, price and feed information. In a discrete choice experiment, the preference and willingness of German consumers to pay for the changed fillet color was investigated. The empirical analysis was based on a representative online survey with 557 participants. The results show that the yellow fillet color had a positive influence on consumer preference and consumers were willing to pay more than for a white fillet. These results, show that spirulina improves product quality and increases the value of trout fillets. Moreover, a stronger consumer preference for fillets of domestic origin (Germany) was observed ahead of Denmark and Turkey. Product information such as "from sustainable resource" had a positive influence on the purchase decision. In contrast, specific feed claims such as "fed without fishmeal", "fed with spirulina" and "fed on plant-based proteins" had no influence on the purchase decision. Marketing activities to successfully market spirulina-fed rainbow trout should focus on the high consumer demand for domestic products. Furthermore, there is a necessity to increase consumer awareness and acceptance of sustainable production methods in aquaculture.

In summary, the research results of this dissertation show that Spirulina is suitable as an alternative for the complete substitution of fishmeal. If a high content of fishmeal is replaced by

spirulina, a species-specific optimization of the diet should be carried out to prevent reduced growth performance. It must also be noted that the nutritional relevance of the altered fatty acid patterns still requires further research. Nevertheless, Spirulina is suitable to incease product quality characteristics such as the pigmentation of the fillets, which can lead to a higher purchase decision and willingness to and pay of consumers. In addition, the use of spirulina in fish nutrition offers promising potential for a more sustainable aquaculture production, because the microalgae has ecological advantages on several levels. However, further optimisation of the cultivation processes will be necessary in the future so that the market price for spirulina decreases and the resource is available in constantly high quantities, so that its use is profitable from both an ecological and economic perspective.

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9 Anhang

9.1 Spirulina as animal feed: opportunities and challenges

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Abstract: Increasing demand for protein, especially animal-based proteins and the large amounts of protein feed inputs required for production, has largely driven the research on spirulina as an animal feed. This short communication summarizes the results from two larger research projects investigating spirulina as an animal feed. Overall, spirulina appears to be a prospective protein source in poultry and pork production, as well as aquaculture. However, spirulina as a feed can have implications for system productivity and end product quality, depending on animal production system. Neither swine productivity nor product quality was negatively affected with spirulina as a feed, which is likely due to the low amounts of protein required in swine finishing diets. Spirulina as a feed does negatively affect poultry and fish productivity as well as alter product quality, primarily raw meat color. Therefore, future research focused on sustainability analysis and product processing and acceptance should investigate the trade-offs of incorporating spirulina into poultry and fish diets.

Keywords: microalgae; alternative protein source; animal nutrition; product quality; meat science; soybean meal replacement; fishmeal replacement

1. Introduction

Meat production requires large amounts of inputs and is therefore in many countries and cultures considered a high value food product. Yet, due to increasing wages and a global population, global demand for animal-based proteins continues to grow (OECD-FAO 2021). Spirulina (*Arthrospira platensis*) has moved into the spotlight as a sustainable source of protein for direct consumption (Wang et al. 2021) as well as animal feed (Martins et al. 2021). The attention on spirulina stems from its high crude protein content (> 60 % in dry matter content) and as a source of essential amino (Mišurcová et al. 2014) and fatty acids (Gutiérrez-Salmeán et al. 2015). Furthermore, as a cyanobacteria, spirulina presents an interesting opportunity to utilize resources otherwise not included in the food production system. Spirulina can be cultivated free of arable land, in bioreactors or open ponds (Chen et al. 2016). Utilizing alternative resources will be important in ensuring a sustainable development of food production systems to meet growing global protein demands (Röös et al. 2017). As a part of multiple larger research projects, the Department of Animal Sciences at the University of Goettingen has thoroughly investigated spirulina as an animal feed ingredient. The project "Sustainability Transitions in Food Production" investigated the opportunities and barriers of spirulina transitioning into European animal production. In the project "Sustainable Trout Aquaculture Intensification (SusTAIn)" alternative proteins such as spirulina were investigated for their possible effects on growth parameters and product quality traits in trout and other commercially important fish species. This communication summarizes the findings of these projects.

2. Spirulina in Poultry Feed

Through a series of feed trial, Neumann et al. (2018c) investigated the graded inclusion of spirulina into broiler diets. The authors were successfully able to fully substitute soybean meal for spirulina; experimental diets were supplemented and balanced according to animal amino acid requirements (Neumann et al. 2018c). Although a successful substation and incorporation of spirulina without adverse effects to animal growth was shown to be possible within numerous studies (Neumann et al. 2018c; Velten et al. 2018a; Neumann et al. 2018a; Velten et al. 2018b), lower live weights and decreased feed intake were observed when animal diets including spirulina were not balanced according to amino acid requirements (Velten et al. 2018a).

Thereafter, the effect of spirulina as a feed on resulting meat quality was investigated. First, Altmann et al. (2018; 2020) monitored meat quality using numerous physico-chemical parameters as well as sensory analysis. Perceivable differences in meat quality were identified in

broiler chickens fed spirulina. In accordance with previous research (Toyomizu et al. 2001; Venkataraman et al. 1994), Altmann et al. (2018; 2020) observed a more intense color of meat produced with spirulina as a feed; both breast and thigh meat exhibits higher red (a*) and yellow (b*) hues as recorded with the CIELAB color system. In other words, poultry meat turns an intense orange color when spirulina is included at high rates as a feed ingredient (Altmann et al. 2022). Based on this observation, research into implications regarding marketability was conducted. A study on consumer preferences for poultry meat appearance found that consumers will reject (not purchase) poultry meat produced with spirulina unless information explaining the unfamiliar color is provided (Altmann et al. 2022).

Other subtler changes in meat quality included: increased umami and therefore chicken flavor (Altmann et al. 2020) and decreased off-flavor (Altmann et al. 2018; Altmann et al. 2020) in breast meat produced with spirulina as a feed. Although spirulina is often cited as an antioxidant (Gutiérrez-Salmeán et al. 2015), meat samples produced with spirulina as a feed exhibited higher rates of lipid oxidation compared to other (soybean meal or insect meal) treatment groups, especially when meat samples were packaged in highly oxygenated modified atmosphere packaging (Altmann et al. 2020). Evaluation of meat quality also included monitoring fatty acid composition of intramuscular fat. Despite its reputation as a good source polyunsaturated fatty acids (PUFA), particularly gamma-linolenic acid (GLA) (Gutiérrez-Salmeán et al. 2015), spirulina as a feed did not result in higher GLA nor omega-3 levels in intramuscular fat, when comparing with meat samples raised on soybean meal (Altmann et al. 2020). The reason for stagnant levels of GLA remains unknown; even as GLA made up approx. 1% of fatty identifiable fatty acids in spirulina-based diets (Altmann et al. 2020). PUFA levels did not increase compared to soybean meal because soybeans are also a good source of PUFA and levels were similar across experimental diets (Altmann et al. 2020). Future research should focus on better understanding the physiological uptake of spirulina-derived fatty acids.

Gkarane et al. also conducted in-depth analysis on the effect of spirulina as a feed on meat aroma (Gkarane et al. 2020b) and flavor precursors (Gkarane et al. 2020a). Spirulina as a feed decreased levels of endogenous bioactive compounds (i.e., anserine, creatine and carnosine); whereas it increased amounts of flavor-related compounds (i.e., inosine and insosine-50-monophosphate) (Gkarane et al. 2020a). Furthermore, the aroma profile of spirulina fed chicken was found to be distinguishable from samples reared on other feeds; the profile was partially characterized by compounds associated with lipid oxidation (Gkarane et al. 2020b).

These above-mentioned studies illustrate that spirulina can be successfully incorporated into poultry diets. However, due to amino acid requirements of poultry, spirulina is not sufficient in itself as a protein source; amino acid supplementation is required. This has implications for use in organic or low-input rearing systems. Furthermore, spirulina as a feed affects meat quality, in ways that are subjectively positive (e.g., increased flavor compounds and perceptible flavor and intensified color). Yet its effect as an antioxidant and its effect on improving fatty acid composition remain contested according to the results of this study.

3. Spirulina in Swine Feed

Based on the research carried out in the "Sustainability Transitions" project, spirulina appears to be a good candidate as a protein source in swine diets. Two studies investigated spirulina: one from an animal nutrition perspective and the other from the product quality perspective.

In their feeding trials with piglets and barrows, Neumann et al. (2018) observed that soybean meal could be completely replaced by spirulina (with appropriate lysine supplementation) in swine diets without compromising overall protein quality. However, the authors also observed that supplementing histidine in high amounts when in combination with lysine, methionine and threonine improves protein quality of swine diets containing spirulina (Neumann et al. 2018). In additional trials, spirulina as a feed did result in slightly lower carcass weights, however, results remained insignificant compared to the soybean meal-fed group (Altmann et al. 2019).

Furthermore, Altmann et al. (2019) investigated the resulting product quality of barrows reared on diets containing spirulina as a protein source. In their study, finishing diets completely substituted soybean meal for spirulina; spirulina composed 9.5% of the ration (Altmann et al. 2019). Physico-chemical parameters, such as meat pH, water holding capacity, proximate composition, meat and fat color, lipid oxidation in meat and fatty acid composition of backfat were analyzed. Overall, spirulina at a rate of 9.5% of the finishing ration did not affect meat and fat quality compared to the control diet containing soybean meal (Altmann et al. 2019). One exception is that the backfat of spirulina fed barrows had a marginally lower proportion of monounsaturated fatty acids (MUFA) and increased PUFA compared to the soybean meal-fed control (Altmann et al. 2019). Unlike in the poultry experiment (Altmann et al. 2020), a higher proportion of GLA was also found in the spirulina-fed samples (Altmann et al. 2019). However, the authors caution that these results should not be taken out of context; soybean oil was added in greater amounts to the experimental diets containing spirulina, which also likely confounded the effects of spirulina as a feed ingredient (Altmann et al. 2019).

Sensory analysis was also conducted and showed that meat reared with a diet containing spirulina increased the overall odor of loin meat; spirulina as a feed was also associated with an increased astringent aftertaste in pork loin (Altmann et al. 2019). As results were marginal, the authors came to the conclusion that spirulina does not lead to drawbacks in product quality when included in swine feed (Altmann et al. 2019).

Although only two pieces of literature were published, both include multiple replicates and different trials, illustrating the robustness of results. Spirulina can be included in swine feed at high rates, with appropriate amino acid supplementation, without disadvantaging animal nutrition or product quality.

4. Spirulina in Fish Feed

The use of spirulina has already been tested for various fish species, but high substitution levels often lead to a reduction in growth performance and increase in feed conversion ratio in carnivorous fish (Ragaza et al. 2020). The objective of the SusTAIn project was to evaluate a whole fishmeal substitution with spirulina in terms of animal growth and product quality. Therefore, Dietz et al. (2020) developed two isoenergetic and amino acid balanced diets, based on the recommendation from the National Research Council (2011) for trout. The control diet contained fishmeal, but in the experimental diet fishmeal was completely exchanged by spirulina. The authors tested the diets on different rainbow trout (*Oncorhynchus mykiss*) genetic lines but did not find significant interaction in growth parameters and feed conversion between breed and diet.

In feeding trials with rainbow trout, brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta fario*) and African catfish (*Clarias gariepinus*), Rosenau et al. (2022; 2021b) investigated the acceptance and performance of experimental diet containing spirulina. Overall, acceptance for the diets was high across all species, with the exception of brown trout. The authors hypothesized that this species may have rejected the spirulina diet due to an aversion to unfamiliar flavor. Overall, the digestibility for both diets was high, but the feed conversion ratio was increased for spirulina-fed rainbow trout and brook trout and resulted in significantly lower growth rates in all species. A subsequent investigation of the intestinal microbiome in African catfish, using 16S rRNA sequencing, found only slight changes for some bacterial genus, but the overall microbial community structure was not affected by spirulina-diet (Rosenau et al. 2021a).

Rosenau et al. (2022; 2021b) also investigated the spirulina-induced changes in product quality. The authors observed higher red (a*) and yellow (b*) coloration for skin and also for raw fillet,

leading to a more yellow-orange coloration in spirulina treatment groups. This change in color could have a major effect on consumer acceptance. Preliminary results of an online consumer survey suggest that fish consumers are not put-off by the unfamiliar color. In fact, the orange-yellow coloration may be preferred in trout fillet. These results contrast with results pertaining to poultry consumers (Altmann et al. 2022) and are currently being prepared for publication.

Another important criterion for product quality is the fatty acid composition, which is strongly influenced by diet. While in African catfish, the saturated fatty acids (SFA), MUFA and PUFA showed no significant differences (Rosenau et al. 2021b), in salmonid fish, a significant reduction in PUFA was observed (Rosenau et al. 2022). Among the PUFA, important long-chain fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) were reduced in spirulina treatment groups (Rosenau et al. 2022).

These results show that advantages pertaining to sustainability and even consumer preferences can be linked to spirulina substitution of fishmeal. However, animal growth and product quality, especially food nutritional aspects, may come at a cost when fishmeal is completely replaced by spirulina. In addition, the viability of replacing fishmeal with spirulina in fish production and its implications are highly dependent on each species of fish and its trophic level. Spirulina as a fish feed impacts fish production to a lesser extent in omnivorous rather than carnivorous fish species.

5. Opportunities and Challenges

Using spirulina as an animal feed enables the nutritional quality of food to be improved so as to meet human nutritional requirements. Although spirulina can be processed and consumed directly (Grahl et al. 2018a; Grahl et al. 2018b), it is often rejected in Western cultures due to unfamiliarity and organoleptic characteristics, i.e., flavor (Grahl et al. 2018a; Grahl et al. 2020). In addition, spirulina does not meet all essential requirements for human development; although it contains small amounts of vitamin B12, primarily pseudovitamin B12 is found in spirulina (Watanabe et al. 1999). Including spirulina as a protein source in animal feed culminates in a nutritional end product; meat contains numerous essential fatty and amino acids, as well as vitamins, including vitamin B12 (Gille and Schmid 2015), important for human growth and development. Overall, both meat reared using spirulina as a feed and direct consumption of spirulina should be seen as part of a well-balanced diet.

However, before spirulina can become a mainstream animal feed ingredient, a few challenges need to be overcome. Firstly, spirulina remains very expensive compared to other protein feedstuffs, such as soybean meal (Chen et al. 2016; Ragaza et al. 2020). Although, due to the

exorbitant cost of fishmeal compared to other protein sources, with improvements in production efficiency and utilizing waste streams as production media, spirulina could quickly become competitive on the market for fish feed (Ragaza et al. 2020). Secondly, the cultivation of spirulina has remained, with few exceptions, small-scale and primarily for the nutritional supplement sector (Ragaza et al. 2020). Thirdly, although spirulina is often heralded as a sustainable source of protein, its environmental footprint is extremely variable based on the production system and regional climate and comparable protein source (Ragaza et al. 2020). For example, spirulina is not more sustainable to produce than soybean meal (Smetana et al. 2017); however microalgae may be more sustainable than fishmeal, especially incorporated into salmonid diets (Draganovic et al. 2013). Nonetheless, improvements are needed to make production more sustainable. To this end, research continues to improve sustainability by including biogas effluent (Hultberg et al. 2017) or wastewater (Olguín et al. 2003) as nutrient sources. As spirulina requires warm temperature (35 – 37 °C) for cultivation (Habib et al. 2008), integrating waste heat sources, such as is produced during biogas production, also has the potential to optimize cultivation facilities within Europe (Taelman et al. 2015). Upscaling and optimizing production will play a big part in overcoming the challenges presented here. Finally, although spirulina has a high proportion of crude protein, improvements to protein quality could be possible through breeding and nutrition/production research, as has been the case for other feedstuffs, such as soybean and faba bean (Stein et al. 2016).

There are two main aspects to consider when evaluating the advantages of spirulina as an animal feed: first, production efficiency (animal growth) and meat quality; secondly, is its ability to substitute less sustainable protein sources, i.e., fishmeal. As illustrated in this article, spirulina can be incorporated into poultry and swine diets without largely forfeiting animal productivity and product quality. The same holds mostly true for omnivorous fish species, such as the African catfish. Regarding its sustainability potential, once optimized, spirulina could be produced with high production capacities, small space requirement and low energy and water consumption (Singh und Sharma 2012). This would grant it a leg up on replacing other protein sources dependent upon arable land and fishmeal with its problems of bycatch and fossil fuel powered ships. However, although spirulina may have the greatest sustainability potential through its incorporation into carnivorous fish aquaculture, it also negatively impacts animal growth and end product nutritional quality. Research needs to investigate these trade-offs further. In addition to these system-wide implications, research on product color and consumer preferences highlights spirulina as a good possible pigment medium. The application of pigments may be pursued to influence consumer liking and acceptance, as well as customize an intrinsic product

attribute into a marker for extrinsic characteristics (Ardeshiri und Rose 2018), such as production system or feed type.

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Eidesstattliche Erklärung

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

   Göttingen, den 15.07.2022

   

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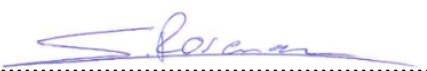
   (Unterschrift)

2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

   Göttingen, den 15.07.2022

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   (Unterschrift)