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The Usage of Antibiotics and their Alternatives in Scottish Salmon Farms
Az Antibiotikumok és Alternaívááik Használata Skót Lazactenyészetekben

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Abstract:

The potential role of fish as a means to providing a solution for diminishing land availability as well as forming a well-balanced diet is well documented. Despite this, the public perception of aquaculture can be coloured easily by media as well as personal experiences with aquaculture.

The tradition of capture fisheries in Scotland has lent itself to a more negative impression of salmon farming, when compared to countries such as Germany, where the perception of aquaculture in media is more positive. Some major concerns about aquaculture are the interaction of wild fish stocks as well as the environment with these potential issues having a knock-on effect to people's health. Now, antibiotics administered to salmon are becoming a point of contention due to concerns with Antimicrobial Resistance (AMR) emerging in modern society and there is a significant increase in mortality (due to previously treatable causes) projected. There is a global movement to address this concern, with the UK collecting annual data related to the use of antibiotics, with salmon farms showing a year-on-year increase in their overall use with individual fluctuations in the type of antibiotic used. Alternatives to antibiotics are trying to minimise the need for antibiotics through a variety of methods, including the development of new probiotics that effectively prevent bacterial adhesion in the gut, but they also minimise the effect on the environment.

Vaccinations are already part of effective herd health strategies but now there are studies looking at making new vaccination administration routes that are less stressful on salmon as well as an adoption of autologous vaccinations which are endeavouring to create a more farm focused health plan.

Good welfare has also been found to have a two-fold benefit to Scottish salmon farming in that it provides an added market value, and it improves the finished product through better carcase conformation. Biosecurity is also capable of decreasing a reliance on antibiotics, through ensuring compliance across the production chain, implementation of a biosecurity plan and peracetic acid has been found to be an effective cleaning tool in low doses as it doesn't cause damage to salmon.

Összefoglalás

Közismert a halak potenciális szerepe abban, hogy megoldást nyújthatnak a Föld élelmiszer-forrásainak csökkenésére, valamint a népességnél egy kiegyensúlyozottabb étrend kialakítására. Ennek ellenére az akvakultúra megítélését a média, valamint az akvakultúrával kapcsolatos személyes tapasztalatok is könnyen színezhetik.

A skóciai halászat hagyománya negatívabb benyomást keltett a lazactenyésztésről, mint például Németországban, ahol az akvakultúra megítélése a médiában pozitívabb. Az akvakultúrával kapcsolatos néhány fő aggodalomra ad okot a vadon élő halállományok, valamint a környezet kölcsönhatása, és ezekkel a potenciális problémákkal, amelyek az emberek egészségére kihatnak. A lazacoknak adott antibiotikumok most vita tárgyává válnak a modern társadalomban megjelenő antibiotikum-rezisztenciával kapcsolatos aggodalmak miatt. Az antibiotikum használat csökkentése miatt a mortalitás jelentős növekedése várható.Világszerte mozgalom zajlik ennek a problémának a megoldására: az Egyesült Királyságban évente gyűjtenek adatokat az antibiotikumok használatával kapcsolatban, a lazacfarmokon pedig évről évre nőtt általános használatuk, az alkalmazott antibiotikumok típusának egyéni ingadozása mellett. Az antibiotikumok alternatívái különféle módszerekkel próbálják minimalizálni az antibiotikumok szükségességét, többek között új probiotikumok kifejlesztésével, amelyek hatékonyan akadályozzák meg a baktériumok megtapadását a bélben, ugyanakkor minimalizálják a környezetre gyakorolt hatást is.

Az oltások már a hatékony állomány-egészségügyi stratégiák részét képezik, de mostanában vannak olyan tanulmányok, amelyek új, lazacok esetében kevésbé megterhelő vakcinázási módok kidolgozását, valamint autológ oltások bevezetését vizsgálják, amelyek egy gazdaság-központúbb egészségügyi terv létrehozására törekednek.

Az állatok jólléte kétszeres előnyt jelent a skót lazactenyésztés számára, mivel hozzáadott piaci értéket biztosít, és javítja a készterméket a jobb hasított testfelépítés révén. A biológiai biztonság az antibiotikumoktól való függést is képes csökkenteni azáltal, hogy biztosítja a megfelelőséget a termelési láncban, egy biológiai biztonsági terv végrehajtását, és a perecetsav hatékony szernek bizonyult alacsony dózisban, mivel nem okoz kárt a lazacban.

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1. Introduction:

Historically, antibiotics formed an important part in helping maximise the yield of the finished product in many branches of livestock and aquaculture. Nowadays though, we are beginning to see increasing levels of antibiotic resistance in human medicine, with more illnesses (which were once treatable with antibiotics) becoming resistant to the conventional antimicrobials used. Upon investigation, it was found, antimicrobials that were used as growth promotors in livestock farming (including aquaculture) were able to persist in the meat that was then consumed. As a result of this, it become imperative to eradicate the use of antimicrobials as a growth promotor. With this gap emerging, farms had to develop other methods to ensure that their product reached a maximum potential with a reasonable economic cost.

A multi-faceted approach was taken in order to help bridge the gap opened up by the banning of growth promoting anti-microbials. Now there is an even more concentrated effort to minimise antibiotics as a treatment option. This has included taking a deeper look into nutraceuticals and how the general role of nutrition could help reduce the need for antimicrobials by promoting a healthier animal. There has also been an increase in the utilisation of vaccinations as a means of disease prevention. This further drives the need to innovate and create new ways by which producers can still ensure a good quality, safe product whilst still remaining economically viable.

Therefore, this review aims to look at what methods salmon farms are utilising in the wake of this movement away from antimicrobials. Scotland offers a unique opportunity to examine how it has adapted to the decrease in the use of antimicrobials as it continues to form one of the world's highest Atlantic salmon producers (just behind Norway and Chile). Scotland has also had to contend with the impact that Brexit has had on their market but have shown that they managed to maintain their position on the global salmon market despite these issues. This review will look at the place Scotland has in the global market, as well as the alternatives that are being used and to what extent these measures are being adopted in Scotland.

2. Objectives/Questions:

This literature review will endeavour to look at a number of questions:

What effect does the public have on the production methods in aquaculture? I want to establish how the public perceive aquaculture and what concerns (if any) that they may have. As an extension of these concerns, it can influence the economic worth of fish (through sales) which can drive development in the sector (through possible policy development).

Is Scotland successfully reducing reliance on antibiotics in salmonid farms? This is key in helping to establish whether or not it is possible to mitigate the effects of antimicrobial resistance (AMR) in human medicine. It would be feasible to imagine that there is a decreasing reliance on antibiotics, however, it would stand to reason that if there is an intensification of farming, that the use of antibiotics may also increase.

Does nutrition have a positive impact on salmonid species, and does it help avoid disease? Do vaccines have an impact on disease prevention? How? What are the future trends in vaccine production? Does biosecurity play a role in disease prevention, and can this help reduce the reliance on antibiotics? Can adequate welfare standards help prevent disease and decrease reliance on antibiotics? These questions will all help ascertain if these are viable long-term strategies in reducing reliance on antibiotics. I want to establish how these various factors can (if at all), affect fish.

3. Literature Review:

At first, this literature review will provide an overview of the global fish market in terms of the reliance on fish. It will examine the significance of fish internationally, before moving onto examining the European fish market and finally it will examine the fish market of Scotland the United Kingdom (UK). It is useful to provide this picture of fish production as it provides the reader with an idea of just how important it is to properly manage the use of antibiotics in fish and to find adequate alternatives to antibiotics. The literature review will continue by establishing the value of fish as a food source and the role of aquaculture in helping to form a secure source of food and the nutritional value of fish. This is important as it provides a reason as to why the issue of antibiotics in fishes used for human consumption is so significant both now and into the future.

The next chapter will continue by examining the issues that plague aquaculture with a focus on public perception of aquaculture. It is important to understand the role that the public has on formulating the direction and future of aquaculture. In an economic sense, consumers are showing an increasing desire to purchase from welfare friendly and ethical sources. Therefore, it is important to outline the concerns that the public have regarding various components of aquaculture.

An examination of the antibiotics used in aquaculture will follow. It will examine the current trends in place (both in Europe and the UK) in the usage of antibiotics. It will examine what benefits these antibiotics once had and what necessitated a change in the previous trends.

Finally, the literature review will examine the alternatives that are used for fishes and how they affect the fish. It will look at the role that vaccinations, nutrition and welfare play on the ultimate outcome of fish and look at how they affect the fish.

At this juncture, it is important to clarify the difference between aquaculture and capture fisheries. Capture fisheries refers to the harvesting of naturally occurring fish populations (through activities such as trawling and fishing) but aquaculture refers to the deliberate cultivation and subsequent harvesting of both marine plants, molluscs, crustaceans and fish (including activities such as farm fisheries) [1].

3.1: Global Picture of Fish Production

Given the expanding global population, food security is becoming an increasing issue of importance [2–5]. In tackling this issue, the role of fisheries and aquaculture is often overlooked in favour of looking at the role of agriculture and livestock farming. However, the contribution of aquaculture and capture fisheries to the economy of developing countries, exceeded that of meat, rice and tobacco in 2018 [6] which shows just how much aquaculture can contribute to the economy. Fish forms a staple diet for many populations around the world [6]. In the European Union (EU), fish is an important food source for its citizens, especially in countries such as Spain and demand for marine food sources is increasing [6] Therefore, it is evident that aquaculture has an important role in helping meet the demand for increased food in the face of an increasing population. It can also offer a potential solution to the future issues related to a lack of land availability for the rearing of terrestrial livestock by utilising water (both at sea and in lakes) to help produce food.

Fish is considered a valuable source of nutrients for people, due to this, some countries have based their recommended daily intake based on the levels of Omega-3 (expressed as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) [6]. In Europe, the recommendation for fish intake varies widely based on the recommendation laid out by the various national health agencies within the EU [6]. In the UK, the Scientific Advisory Committee on Nutrition (SACN) recommend that people consume at least two portions per week (with one portion equalling 140g) [7]. Adequate fish consumption has been shown to help people (especially in developing countries) to meet the recommended daily amount (RDA) of micronutrients [6] and there has been a positive correlation between fish-based diets and helping reduce diseases associated with micronutrient deficiencies [6]. Furthermore, a low to moderate weekly intake of fish has been shown to reduce the likelihood of contracting coronary disease or a stroke [6].

In the UK, the purchase and consumption of fish products fall in line with the health of the economy, with declines in purchases falling during periods of economic hardship [7]. This is due to the fact that fish proteins are amongst some of the most expensive proteins available and households would no doubt move to purchase cheaper alternatives during periods of increased financial pressure [7]. Interestingly, during the Covid-19 pandemic, there was an increase in the purchase of ambient and frozen seafood products (prompted by the uncertainty created by the situation at the time) [7].

In more recent times, sales of salmon in the UK amounted to £1.2 billion in 2022 [8]. Even more so, the shortage of turkeys in the 2022 Christmas period (caused by the outbreak of Avian Influenza), opened up a market gap which was filled by Scottish salmon (eventually, salmon accounted for 29.6% of total fish sales in 2022) [8]. In 2022, Scottish salmon formed one of the UK's largest food exports (worth £578 million) (which was still down 6% compared to 2021, due in part to the increased domestic consumption) [8]. Some of the major markets for this product is the EU market (making up 64% of the market share) but there is a major increase in demand being seen amongst the USA and China [8]. It is clear that Atlantic salmon (*Salmo salar*) farmed in Scotland occupies a premium place on the market and is viewed as a high value product and people are willing to purchase Scottish salmon both domestically and on an international level. Given the situation with Brexit, Scottish salmon producers are looking to further their international reach (through the removal of trading barriers) [8]. This would be considered a wise move given that there is a general downward trend in the purchase of salmon (in line with the increasing financial pressures on homes in the UK [7]) and the avian influenza outbreak is not a stable, long-term strategy for the promotion of salmon farming. There is an opportunity for growth of Scottish salmon in the future given the "salmon tax" proposed by the Norwegian government creating turmoil within the Norwegian sector [8]. The ensuing speculation about the potential effect of this new tax may somewhat limit the Norwegian producers and allow the Scottish sector to fill in the new space created on the market [8].

3.2: The Role of Aquacultures and Fisheries in Food Security

There is a broad consensus that "food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" [9]. There are 5 key considerations to determine if a foodstuff can attain a sufficient level of food security [5]. These include:

1. Quantity of food produced
2. Production sustainability

3. Economic viability (can it sustain jobs?)
4. The nutritional content of food
5. Is the food safe?

These are the parameters that must be met by aquaculture and capture fisheries in order to meet increasing demand. The quantity of food should be maximised as this is the crux of sustaining an increasing global population and the nutritional value of fish has been outlined multiple times [6]. The production sustainability depends on the resources available in order to produce sufficient amounts of fish. The safety of the food produced considers whether or not the food is safe for human consumption. Finally, the economic viability is necessary as it will establish whether or not this method of food production is worthwhile for people to continue pursuing. The profitability of production can depend on producing as much safe food as sustainably possible whilst also minimising costs required to meet such criteria. Good quality food can fetch a higher price, and this frees up more money after costs have been paid off (such as wages).

Aquaculture and fisheries are receiving an increasing amount of attention by various governments as it holds a great deal of promise in helping achieve food security and it is undergoing a rapid growth rate (especially when it is compared to other food production methods) [2, 6]. Countries like Iceland and the Faroe Islands already have a heavy reliance on fish and it can contribute up to 40% in their economy [6]. In fact, it has been projected that consumption of fish will continue to increase as it can produce a rapidly available protein with a fraction of the greenhouse gases compared to that of traditional terrestrial livestock farming [3, 10]. Aquaculture, as a whole, is taking a larger role in terms of production and it has bypassed capture fisheries in 2014 and ended up providing up to 50.4% of global seafood for direct human consumption [10]. Within this, however, fish (finfish and molluscs) only provide 18.25% of fish for human consumption [10]. It is clear from these figures that it is possible to develop the contribution of aquaculture to global fish supplies. It could be argued that in increasing the amount, of fish obtained from fish farming can alleviate the pressure on wild fish stocks that are typically targeted by capture fisheries. This could help to minimise the levels of fish being removed from the world's oceans and help to stabilise wild populations in allowing aquaculture to act as a type of "buffer" system.

3.3: Concerns Regarding Aquaculture and Fisheries

It is important to consider the complex interplay of socio-economic factors that come into play when trying to establish the role of finfish aquaculture within the framework of a stable food supply network [3, 4, 6]. The parties that are typically concerned with the role of finfish aquaculture are Non-Governmental Organisations (NGO's), Government (as they are involved in the formation of policies), local fishing communities and consumers in the general public [11].

3.3.1: *Factors Influencing Public Perception of Aquaculture*

As with any production system involving living organisms, it is important to remember that there is going to be a level of scrutiny applied to the chain of production. The role of the internet and social media now allows for a greater level of scrutiny and observation to be applied to finfish aquaculture on a level not seen before. Public perception is one of the major hurdles that policy makers concern themselves with when trying to establish the sustainability of fisheries [3]. There is an increasing interest in aquaculture as people become more concerned with how food is produced and what is involved in the production of their food [12]. It is important to consider that public perception, and subsequent demand can have a chain reaction for finfish aquaculture. The possibility for expansion of finfish aquaculture is driven by possibilities created by new legislation and grant aid availability from government policies [12, 13]. These government policies and legislation can be derived and formulated based on public demand and their desire for fish [10, 12, 13] so therefore, it is logical to consider the role and influence of the public.

Major public concerns cite potential ecological issues or health risks for consumers as the major issues with aquaculture [3, 12, 13]. Studies in Germany examined the public perception of aquaculture as presented to the public through the lens of major German media. The media plays a crucial role in presenting information to the public and has a proven influence on fish sales [12]. Given this level of influence, managing public image becomes more important for aquaculture-based enterprises. In the research conducted by Feucht and Zander (2017), they adopted the stance that the media "tell people what to think about," when forming the basis for evaluation. Throughout the 5-year period involved in their study, the majority of articles appeared in "*Süddeutsche Zeitung*" which has head offices in Southern Germany, where there is a high number of finfish aquaculture enterprises [12]. There was a noted increase in articles related to aquaculture and there was an increase in negative press coverage when there were disease outbreaks which called into question farming practises (especially as the issue at hand involved an outbreak of Infectious Salmon Anaemia) [12]. The main issues that German papers were concerned with were the economic possibilities presented by aquaculture, animal welfare and the ecological effect of finfish aquaculture [12]. However, food safety and availability of fish is not something that the German public appear to be concerned with [14] and there was less concern with issues surrounding the ocean compared to other issues surrounding the environment [13].

In Greece, the majority of the public (62.7%) were found to possess a moderate amount of knowledge regarding the state of the marine environment after being presented with a series of statements to which they had to respond true, false or don't know to [13]. The sample population were representative of the populations of the three major port cities of Athens, Thessaloniki and Volos due to the fact that they presented the highest level of maritime activities [13]. The study found that there was a largely positive attitude towards aquaculture and that people felt that aquaculture provided a valid alternative to fishing with 65.92% of respondents stating this. On top of this, 53% of people believe that fishing in the traditional sense poses a significant threat to marine life [13] which helps strengthen the perception of aquaculture as a viable option for secure, sustainable food production.

In the United Kingdom (UK), public perception is mainly focused on the conservation of marine environments [13] and there is a concern with pollution and climate change [14]. As with Greece, there was a correlation between the level of interaction individuals had with marine environments (through living near, working on or near marine environments) and the level of understanding they exhibited towards issues affecting the marine environment [13]. Out of the countries that make up the UK, Scotland is a major contender for salmon production [3] with Scottish Parliament aiming to increase production of salmon to 300,000-400,000 tonnes by 2030 as part of their economic development plan [11]. However, the potential viability of aquaculture is at odds with public perception of these farms and their presentation in the media has largely negative connotations [11] with salmon farming garnering a less favourable coverage when compared to a generally positive perception of aquaculture in the media. In the UK, more global studies have found that there is a general trend towards increased media coverage in terms of aquaculture [15]. When the sentiment of various headlines is further investigated, Scotland was found to have a higher level of negative media coverage when compared to the rest of the UK [15]. The reason for this could be Scotland is one of the highest producers of salmon in the world (after Norway and Chile, respectively) [3], with capture fisheries dominating salmon production there. Therefore, there is a natural resistance to the increasing number of large corporations seeking to open salmon farms. This reticence would typically be most prevalent in fishing families, and they would no doubt perceive this production model as a threat to their own livelihoods and it would trickle into various facets of society, including media coverage.

This attitude would no doubt impinge on the "Social License to Operate" (SLO) linked to aquaculture (an SLO refers to "an ongoing negotiation between a host community and an organisation (industry, NGO, business) which has environmental and social implications associated with its activities, where the organisation is held to certain standards set by the local community in exchange for the trust and support of the community," [11]). In the study carried out by Billing (2018), public perception of aquaculture was examined based on comments from the public about planning permission applications lodged by a number of companies seeking to establish fish farms on lochs. The major reasons the public supported the establishment of fish farms were the potential economic benefit through the establishment of stable jobs and attraction of younger workers into rural communities [11]. The objections to the establishment of fish farms were more complex in that there was a type of ripple effect noted . Objections were often raised on more emotive grounds and at the behest of objectors that had a large number of resources with which to pursue objections via legal challenges to the planning permission [11]. These objections simplified complex matters relating to the biophysical system at play (for example, certain objectors established an environmental NGO which published fliers that pushed the idea that fish farms would "pollute the loch" or create "holes at the bottom of the sea") [11]. The major concern with aquaculture tended to have a more general, overarching theme of "environmental concern" (which can encompass the shooting of predators, farm fish debris left on shores, animal welfare etc) which may mean that there are objections by people that have had less exposure to traditional fishing [11]. The specific concern of wild fish interactions (relating to the spread of sea lice) falls much lower on the list of concerns but indicates a certain level of expertise and experience working with fish (since these were very specific points raised and traditional fishermen would have experienced this during their life) [11].

From these studies, the public perception of aquaculture varies from country to country. Germany, having a coastline of 3,624 km and an exclusive fishing zone of 37,438 km^2 [16], would most likely mean that there is a limited invested interest on the issue of aquaculture based on the fact that the vast majority of the population would have limited exposure to this issue. Greece and Scotland however have a more interesting disparity in views as both possess a large coastline (15,147 km and 19,717 km, respectively) with a large exclusive fishing zone (114,914 km^2 and 753,752 km^2 respectively) [17, 17]. It seems that some countries are willing to accept that aquaculture can play a role in managing overfishing of the world's oceans (such as in the case of Greece). However, it can also be an emotive subject, especially when the establishment of these farms is occurring on a more local level (such as in people's own vicinity) and can lead to embittered legal battles (such as in the case of Scotland) if the objecting individual has enough resources to maintain a prolonged campaign against these farms. The disparity between Greece and Scotland reveals that there is a complex interplay of social factors and the level of invested interest in the industry plays a large role in the public perception (and acceptance). Therefore, it becomes even more important that farms are able to work with and address the concerns raised by local communities if these farms are to last into the future.

3.3.2: *Public Concerns About Antibiotics in Aquaculture*

The food chain is becoming more scrutinised by the general public and people are becoming more concerned with how the diets fed to animals and fish could affect their own health [12]. In France, a largely negative picture of aquaculture was painted in the country after the airing of an investigative documentary, "*Envoyé Spécial, Fish: Farming in Murky Water*" in 2013 [18]. The documentary targeted the alleged secrecy surrounding Norwegian salmon production (although Scotland escaped scrutiny), including the use of antibiotics in production which prompted a large uptick in negative coverage of salmon in France [18]. The natural response to this was the distribution of a statement on behalf of the Norwegian Seafood Council defending their use, outlining the rules for use of antibiotics in salmon (the use of withdrawal periods, EU regulation) and emphasised the increasing use of vaccinations to prevent epidemics [19].

The use of antibiotics in feed has become one of the more concerning issues for the public [12]. Efforts have been made in the media to highlight the fact that antibiotic usage in food producing animals has been greatly reduced despite also highlighting its potential to have a negative effect on human health [12]. However, France, once again saw an increase in negative media coverage surrounding the use of antibiotics in Chilean salmon in 2017 but the increased use of vaccinations by Norway meant that there was a decreased coverage of Norway when it came to this particular matter [18]. In the UK, there are only five antibiotics allowed for use in aquaculture [20]. The concern about the use of antibiotics voiced in the media is echoed by scientists as they too, believe that antibiotic usage and subsequent risk of contaminating the food chain is of concern to the public [1]. A more comprehensive discussion surrounding the issues of antibiotic usage will be discussed in the next chapter.

Furthermore, the European Union (EU) has been seeking to further tighten up the use of antibiotics across the union (although admittedly, it remains to be seen how the UK will handle this issue post-Brexit) [21]. In 2006, the EU issued a total ban on the use of antibiotics as a growth promotor in livestock [21]. In 2018, a new ruling was passed by the EU to ban the use of prophylactic antibiotics, and this is due to enter into effect in 2022 [21]. As a consequence of Brexit, the UK will still have to adhere to these standards if they wish to remain trading with the rest of Europe [22]. This now means that livestock practises and technologies must improve to compensate for the newly enacted law.

3.3.3: *Public Trust and its Effect on Aquaculture*

As previously iterated, the importance of public perception cannot be underestimated as it can determine the ease with which aquaculture can be established in a particular region [11]. Stakeholders in aquaculture (enterprises, NGO's, Government etc.) have an interest in building and maintaining trust with the public in order to push their own particular agenda. In Greece, there seems to be a high-level of trust placed in the so-called experts of their field. When asked who they (the Greek public) would trust to manage the marine environment, research centres and scientists were the two most trusted stakeholders, followed by the

European Union and environmental organisations [13]. On the flip side, there was a marked lack of trust in their own central government, local authorities, businesses and the private sector [13]. One could stipulate that this shows a willingness to trust the people that are perceived as experts and those that would typically be considered to have an investment in the preservation of the environment.

In the UK, there also seems to be an inherent distrust of government bodies with trust placed in scientific journals and academic publications [11, 13]. However, it has also been brought to light that this trust in academics could potentially be undermined by "grassroots" movements that are pushing highly personal agendas [11]. With some planning request objections, there was a clear distrust of perceived authorities like the Scottish Environmental Protection Agency (SEPA) and there was a belief that they allowed pollution of lochs to continue without consequence [11]. There was a strong belief that authorities, including the government were getting bribes and environmental groups (like SEPA) were accused of being selective in the information that they present in their studies [11]. The cases examined in Scotland revealed just how much personal opinions can drive or hinder aquaculture development. As a result of this distrust, it would be feasible to imagine that finfish aquaculture is under greater scrutiny in the areas they operate in. Therefore, it would be especially important that they operate to the highest possible standards in order to avoid fractious confrontations within their locality. This can be achieved by addressing the concerns raised by locals in their objections and working to alleviate these risks (such as managing the interaction between their own farmed stock and wild fish to minimise the spread of disease etc.).

3.4: The Issue of Antibiotics: A General Overview

Antimicrobials are a class of drugs that encompass antibacterial, antifungal and antiviral agents. Antibiotics are specifically used to treat bacterial infections by either inhibiting bacterial growth or outright destroying the bacteria cell [1] and the European Medicines Agency (EMA) defines an antibiotic as "any substance with a direct action on bacteria that is used for treatment or prevention of infections or infectious disease," [1]. Antibiotics encompasses various drug classes (e.g., aminoglycosides, fluoroquinolones, penicillin, macrolides, tetracyclines etc.) and each class of drug has different pharmacodynamic properties [1]. The main division of these antibiotics is based on whether they have a bactericidal effect (such as in the case of aminoglycosides, cephalosporins, fluoroquinolones, metronidazole, penicillin, polypeptides and potentiated sulphonamides) or a bacteriostatic effect (such as lincosamides, macrolides, pleuromutilins, phenicols and tetracyclines).

Antibiotics have been frequently used in order to treat various illnesses and found popularity in food production enterprises, not just as a curative agent, but also in low doses as a growth promotor [23].

3.4.1: *WHO said that? The Emerging Issues with Antibiotics*

Despite the benefits of antibiotics in terms of increasing the productivity of finfish systems, the WHO have raised serious concerns regarding the increasing levels of antimicrobial resistance occurring worldwide [24]. There is a concern that this resistance will lead to the decreased efficacy of antibiotics in human medicine since previously susceptible bacteria will now be resistant to these antibiotics [24]. In fact, this issue is already becoming more prevalent with increasing numbers of deaths caused by antibiotic resistant strains across the world [1, 24]. In Europe, there was an increase in mortality due to antibiotic resistance in 2015 (33,000) with projected increases by 2030 [1]. The European Commission have raised concerns that there may be more far-reaching effects with increased bacterial resistance, medical procedures such as organ transplants, hip replacement and chemotherapy may pose a greater threat to human life due to a greater risk of infection and combined immunosuppression of the patient [21]. In terms of veterinary medicine, this concern can translate to the increased suffering of animals if they contract these particular resistant strains of bacteria [21].

The WHO has gone on to classify the most important antibiotics for human medicine as "Critically Important Antibiotics (CIA)." These antibiotics are the ones that will be limited for use in veterinary medicine in order to preserve their efficacy in the field of human medicine [25]. Furthermore, there is a further classification of Highest Priority Critically Important Antibiotics (HP-CIA) [21, 25] for the antibiotics which the WHO feels must be absolutely restricted in veterinary medicine. The most important antibiotics in this field are;

colistin, fluoroquinolones, macrolides and 3rd/4th generation cephalosporins [21]. The EMA follows the WHO's classification for HP-CIA but they use the classification of "Restricted" to describe these antibiotics (however, macrolides are not included in this category) [21].

These concerns have been echoed by and picked up by the EU. As such, there has been an effort to minimise the reliance of production systems on antibiotics [21] and an attempt has been made by the European Commission to limit the amount of antibiotics being introduced into the food-chain, by the implementation of Maximum Residue Levels (MRL's) (by tracking levels of marker residues) in EU Reg. No. 37/2010 [26]. In 2006, the use of antibiotics as a growth promotor was banned for both European farms and on animal-derived products being imported into the EU (as outlined in article 107.2 of "Regulation 2019/6 of 11 December 2018 on Veterinary Medicinal Products" [21]. Following this, in 2018, the EU voted to ban the use of antibiotics for use as a prophylactic agent and also restrict their use as a metaphylactic treatment (where this kind of treatment can only be implemented when there is no other option available) [21]. This regulation came into effect in January 2022, however there was also meant to be a list of antibiotics reserved for human use released at this time but there is still no list compiled and it is expected to be released in the latter half of 2022 [21]. In the future, veterinarians will be expected to record on which species they are using antibiotics on, so as to form a more comprehensive image on the usage of antibiotics. This will be a phased procedure with data usage for cattle, pigs and poultry expected to begin in 2024, from 2027 usage information for other food-producing animals (including fish) is to begin and in 2030 reports for companion animals and fur farmed animals are to be kept [21].

In order to track these antibiotics and their use across member countries, the EMA, European Food Safety Authority (EFSA) and the European Centre for Disease Control (ECDC) established a set of harmonised indicators [21, 24, 25]. The aim of this was to produce a map that can track the emergence and prevalence of Antimicrobial Resistance (AMR) across the EU (including Norway and Iceland) [24]. The indicators that are linked to animal production are divided into primary and secondary factors, primary indicators provided an overarching image of antibiotic use (overall sales of antibiotics (mg (active substance)/kg (estimated weight at treatment of livestock and slaughtered animals) indicated in mg/PCU) and secondary indicators provided more detailed information about sales of 3rd/4th generation cephalosporins, polymyxins and quinolones (measured in mg/PCU) [25].

In the UK, there is a question as to how Brexit will affect the use of antibiotic use given that the UK is no longer subject to legislation enacted by the EU. This naturally brought into question whether the UK would continue to reduce the amount of antibiotics (in line with Europe) [21]. There seems to be a paucity of information regarding any tangible timeline regarding future safeguarding antibiotics in the future, similar to what has been laid out by the EU. However, the Department for the Environment, Food and Rural Affairs (DEFRA) agreed that it would continue to push to limit the use of antibiotics in food producing animals [22]. Even more specifically, another report in the 2017 to 2019 period, there was a more concentrated examination of the use of antibiotics in the veterinary field and there has been a continued downward trend in the sale of antibiotics [27]. Between 2015 and 2019, there was a 45% reduction in the sales of CIA and a 5% reduction when compared with 2018 [27]. The UK has indicated a multi-modal approach is needed for their reduction in antibiotic usage [22, 25] and they also used the same harmonised indicators as the EU (with quinolone oxolinic acid used for the fish sector) [25]. These reports were carried out in 2019 whilst the UK were still considered a member state of the EU [27] but more recent debates in parliament have shown a continued concern with reducing the level of antibiotics in all areas of farm production [22].

3.4.2: *Antibiotic Usage in Aquaculture*

As with terrestrial animal production, low-dose antibiotics were used in aquaculture as a growth promotor [1, 23]. The major use for antibiotics across the globe is as a treatment for a range of bacterial infections that can devastate fish stocks and pose a risk to profits and food safety [23]. Aquaculture benefited hugely from the use of antibiotics as they were used as growth promotors, prophylactic agents and in a therapeutic manner [1, 23, 28, 29]. Studies carried out by the European Food Safety Authority (EFSA) and European Centre for Disease Control (ECDC) points out the more direct link between human resistant strains and food producing animals, when compared to human antibiotic usage [21]. The use of antibiotics in feeds and their subsequent entry into the food chain has been an issue the public have been concerned about (as detailed in "Section 3.3.2: *Public Perception of Antibiotics in Aquaculture*").

As mentioned in the previous section, the EU has outlawed this use of antibiotics in aquaculture as have the United States of America (USA) and China, but there is a concern that developing countries still rely on antibiotics as a growth promotor (in some countries where it is banned there is still a reliance on them in an illegal capacity) [1, 2, 23]. This has a worrying implication for food safety for countries where this is allowed to happen and countries that import these affected fish products

In the UK, "Salmon Scotland" is able to provide 100% coverage of the salmon populations and therefore help provide a more complete picture of the usage of antibiotics in Scotland [27, 30]. The information is collected by means of a national survey of all salmon farms in conjunction with the Scottish Government [27, 30]. The drugs that are used for salmon farming, are florfenicol, oxytetracycline, oxalinic acid and amoxicillin [27, 30]. Of the antibiotics used, oxytetracycline continues to be the antibiotic of choice in salmon farming, followed by florfenicol [30]. For the first time since records began (in 2017) oxalinic acid was not used in 2021 (since it has become classed as a CIA) [30]. Despite 2017 being a benchmark year, there was an initial decrease in antibiotic use, but, since 2018, there has been a consistent overall use of antibiotics with 43.1 mg/kg of active ingredient used in 2021 (up from 29.3 mg/kg in 2020) [27, 30]. This increase in antibiotic usage has been heavily criticised in the media [31–33]. Despite the heavy criticism, the representative body for salmon production, Salmon Scotland, reiterated the continued drive to decrease the use of antibiotics in salmon farms [30]. They stressed that treatment within the salmon farming sector is considered infrequent with a total of 13.4% of salmon farms (8.5% freshwater; 4.9% marine farms) requiring antibiotics in 2021 and that there is a continued preventative approach applied with a strong focus on alternatives used [30].

3.4.3: Antibiotic Resistance and Aquaculture

The methods that can increase the prevalence of antibiotic resistance include the formation of biofilms, climate change, anthropogenic activities (antibiotic administration in hospitals, antibiotic usage in farms), mixing of farmed and wild fish stocks [1, 34]. Sub-optimal doses, either through mixing of wild and farmed stocks or else, through human error (when it comes to administering the correct dose) can also contribute to AMR [35–37].

Antimicrobial resistance in aquaculture is a concern given the nature of finfish aquaculture and its proximity to wild fish stocks, and such concerns have been consistently raised by those that resist the establishment of fish farms with a large concern surrounding the level of fish escapes from farms [3, 38]. The proximity of the farmed stocks to wild stock can bring about interaction between the two groups, through escaped farm stock, as well as through the increased sediment, parasites and other problematic agents [11]. This proximity also increases the risk of resistant bacteria transferring between the two groups and the impact that these infections can have on wild stocks is poorly understood (as sick fish in the wild will typically die before they are detected) [38]. As a result of this, there is an increased risk of resistant bacteria being able to replicate, unchecked and undetected, in the environment [37]. This also poses a risk of wild fish introducing resistant bacteria into the food chain if captured and processed. In Scotland, the interaction between wild and farmed stocks has shown to be a common and persistent source of concern amongst people [11]. However, despite these concerns, there is a scarcity of information relating to disease transmission between farmed and wild stocks, with evidence of disease transmission being very limited [38, 39]. Although a study has found that Atlantic salmon are considered poor colonisers if they escape into the wild [40]. Other studies commissioned have found a potential indirect indicator of the effect that proximity between farmed and wild stocks has on wild stocks [41]. Drastic population reductions have been found in wild fish and there were increased levels of wild fish in the proximity of fish farms with lice above the critical limit, especially in the period between 2003 and 2013 [41]. The Scottish Government introduced their current marine plan in 2015 which called for a more cautious approach to the control of factors that could potentially cause damage to wild fish stocks, where these factors lacked scientific evidence [42]. This approach appears to have had a somewhat beneficial effect on wild stocks as a study in 2017 found there was a reduction in the level of lice found on wild stocks surrounding farmed fish [43]. Whilst these studies show there is a transfer of lice between wild and farmed stocks, there is a poorer level of understanding about the transfer of bacterial infections between farmed and wild stocks [38]. Given that lice can cause fish to become moribund [41], it would stand to reason that infested fish become more prone to infection and it can lead to increased mortality through this level of infection. There is limited information regarding the spread of bacterial disease between farmed and wild fish stocks which may be because there is in fact a limited spread or due to the fact that wild fish (infected by infections stemming from farmed fish) have died and were not included in studies [44]. In more recent investigations, there have been decreased levels of sea lice found on wild populations of fish around Scotland, which can be indicative of efforts made to diminish the risk posed by farmed salmon [43, 44]. Given the outlined risks of stock mixing, it would be wise for Scottish producers to adopt a more cautious approach to ensure that AMR is not given a chance to establish in the wild.

The high stocking density of fish [26] coupled with the antimicrobial administration systems utilised (either in feed or in the water [45]) provide a favourable environment for the development of AMR. Of these samples, *Escherichia coli, Enterococcus faecalis* and *Pseudomonas* spp. were cultured in salmon (but the presence of *E. coli* and *Enterococcus faecalis* can be due to insufficient observance of food hygiene procedures during processing and not necessarily originate at the site of salmon farms) [35]. Given the lack of individual treatment options in farmed fisheries, the administration of antibiotics becomes more complex, since antibiotic administration is typically done through water or feed, and this increases the risk that some of the treated animals receive sub-therapeutic doses [45].

In terms of the resistance that can emerge, numerous studies have found that the gut microbiome of fish are capable of facilitating this change [36]. The gut provides a favourable environment for the proliferation of various bacteria [23]. In 2016, various samples of fresh and fileted salmon (originating from Norway, Scotland and Denmark) were taken, various bacterial strains were cultured and tested for AMR [35]. Accumulation of antibiotics in the gut of fish may also promote the establishment of resistant bacterial strains through selective propagation [23]. Sub-therapeutic doses can inhibit the growth of some, but not all bacteria [36]. These bacteria that are able to survive the unfavourable conditions created by the antibiotics can develop coping strategies to help them resist the pharmacodynamics of various antibiotics (through altering surface receptors, increased efflux pump action etc) [46]. This information is subsequently stored on the plasmid of the bacteria which then allows for the increased exchange of this information amongst other bacteria and facilitates the emergence of resistant strains of bacteria [36, 45]. Studies carried out on zebrafish in 2021 found that antibiotics significantly altered gut biochemistry (florfenicol decreased total cholesterol, triglyceride, pyruvate levels and oxytetracycline increased ACP) and the mucin production in the gut decreased significantly after 21 days of treatment with oxytetracycline and florfenicol [36]. There is a paucity of studies carried out on salmon farms and the emergence of AMR within the microbiome of salmon in European and UK waters, with the majority of studies focused on Chile, which has a documented issue with the consistent use of high levels of antibiotics in the production of salmon.

There is a documented concern regarding the effect of sub-therapeutic doses of antibiotics on the microbiota of other fish species [36]. The alteration of the gut microbiota can have a subsequent negative effect on the fish that are treated [36]. The typical gut microbiota has a beneficial effect on fish and has been known to play a role in promoting nutrient metabolism, gut lining proliferation and the innate immune response [46]. Antibiotics can affect the gut microbiota through its indiscriminate action (affecting both beneficial and harmful bacteria) and can result in dysbiosis [36, 46]. The gut microbiota in zebrafish has been found to be negatively affected by treatment with oxytetracycline and florfenicol with decreased relative abundance found in all cases [36]. This dysbiosis could lead to issues in terms of nutrient absorption etc. which may lead to subsequent medicated feed passing through the fish unaltered and therefore, the medicated feed remains in the environment where it can be consumed by the surrounding marine life and the treated fish stocks receive sub-therapeutic antibiotic levels [46].

Interestingly, the control of sea lice may pose a risk for the formation of AMR through the formation of biofilms [47]. A biofilm is a type of capsule that forms typically in pipes, tanks or other areas that are exposed to water [48]. The issue with these biofilms is, that they typically contain high numbers of microorganisms that are protected by layers of non-cellular materials such as polysaccharides, lipids and nucleic acids which are able to withstand the action of cleaning agents and disinfectants [48]. As a result, microbes within the biofilm are protected and genetic material is able to be swapped between microbes [47]. The treatment of the subsequent infection may be further complicated by the presence of AMR strains of bacteria that were formed in the biofilm [47]. Studies found that treating a variety of infections at sub-minimum inhibitory concentrations (MIC) led to an increase in the formation of biofilms [47–49] and it has been posited as a more general, acute defence on behalf of the microbes which affords them more time to formulate a more specific defence against the antimicrobial [48].

Studies were previously done in bacteria that affect swine (*Streptococcus suis*), where it was found that amoxicillin, lincomycin and oxytetracycline, at sub-MIC, increased biofilm formation [49]. In 2020, a study was carried out in the UK to establish the effect that sub-MIC of antimicrobials has on biofilm formation in finfish aquaculture with a specific focus on atypical strains of *Aeromonas salmonicida* [47]. Atypical *Aeromonas salmonicida* is of indirect importance to salmon farms in Scotland, as the atypical strain does not infect salmonid fish, but rather the ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) [47]. The aforementioned fish are used as "cleaner fish" and they act in the capacity as a biological control for sea lice (especially *Lepeophtheirus salmonis*) which would otherwise pose a threat to Atlantic salmon that are farmed [47]. In the event of an outbreak of atypical *Aeromonas salmonicida*, antibiotics become the only feasible option for the treatment of the affected fish, therefore, it becomes important to understand just how antimicrobials used may affect the formation of biofilms [47]. Sequencing, culturing and phenotypic tests were carried out in order to identify the atypical strains of *Aeromonas salmonicida* (of which there were 28) before culturing was carried out on well-plates under a variety of conditions (control = uninoculated wells, inoculated wells, inoculated wells plus florfenicol (8 mg/L) and inoculated wells plus oxytetracycline (2, 8 or 32 mg/L)) [47]. Sub-MIC typically had one of two majorly observed effects, either formation of a new biofilm (when there was none formed in inoculated wells) or an increased biofilm formation (where there had been growth seen in inoculated wells) [47]. The quantity of biofilm formation increased for at least one sub-MIC of only oxytetracycline (68% of strains (19/28); highest number of isolates showed an increased biofilm quantity at ¼ MIC), at least one sub-MIC of florfenicol (61% (17/28); highest number of isolates showed increased biofilm quantity at ½ of MIC) and 13/28 (46%) of isolates showed increases in biofilm production in the presence of both antibiotics [47]. This study was carried out *in vitro*, and the formation of biofilms in a farmed situation also depends on other factors (such as the nature of the surface to which adhesion occurs, temperature, cleaning agents used etc.) [47] but the fact that biofilm formation occurred and showed an ability to increase the quantity of biofilm should not be ignored.

There is also a concern that climate change is driving the emergence of AMR in aquaculture as well as in agriculture [34]. After an extensive study comparing the mortality rates of significant bacterial pathogens (*Aeromonas* spp., *Edwardsiella* spp., *Flavobacterium* spp., *Streptococcus* spp., *Lactococcus* spp., *Vibrio* spp. and *Yersinia* spp.) between temperate and warm water, the increased temperatures were found to have increased mortality rates [34]. Given the increasing temperature of the world's oceans, this is no doubt a worrying result as it may indicate increasing levels of serious infections which may necessitate more antibiotic usage and increase the risk of AMR emerging [34]. An increase of 1^{o}C in the ocean's temperature could lead to an increased mortality of 2.82%-4.12% (temperate waters) or 3.87-6.00% (warm waters) [34]. The fact that increasingly unstable weather patterns due to climate change can facilitate increased numbers of escape stocks [3]. As stated above, these escapees can place more pressure on the wild populations [3] and lead to increased levels of AMR. This pressure is especially concerning when we consider the fact that 90% (85% of which are considered critical, vulnerable or endangered) of the wild population of Atlantic salmon is located in the oceans around Norway, Ireland, Scotland and Iceland [3]. These results paint a sobering picture, especially for the preservation of wild fish stocks.

3.5: Alternatives to Antibiotics

Given the serious nature that AMR poses to both human and animal health, it is increasingly important to establish viable alternatives to decrease the use of antibiotics and mitigate the risk of AMR emergence where possible [24]. In attempting to determine what alternatives can be used, it is important to understand the nature of the production system as well as the socio-economic factors that are at play and this can be done by sitting down with stakeholders and establishing the critical points during production [50]. Identifying these key areas could allow for a more targeted control and risk mitigation strategy to be applied. It is also important for workers to understand the importance of the role that they play in terms of maintaining the healthy environment for fish to thrive and meet their production potential [50]. The key strategies to minimise the use of antibiotics on salmonid fish farms are focused on the prevention of disease and promoting fish health [51]. These strategies include the increased focus on animal welfare, increased biosecurity to help best achieve the environment that is most conducive to healthy fish production, which are discussed later in the chapter. Health strategies can also be implemented on farms to minimise the effect diseases may have on fish, these strategies typically include the use of vaccinations [52]. It is also possible to utilise adequate nutrition in order to best establish an internal environment that helps to prevent the establishment of pathogens [53]. The key benefits of achieving adequate production include the ability to market an enhanced quality product (especially given consumers seeking more naturally produced products).

3.5.1: *Probiotics and Prebiotics*

Probiotics are micro-organisms that have a beneficial effect on the host's gut microbiome when they are provided in a sufficient concentrations [53–56]. There has been an increasing interest in the use of probiotics as an alternative to antibiotics given the rise of resistant pathogens that pose a risk to human health (such as *Aeromonas* spp., *Escherichia tarda, E. coli, Vibrio vulnificus, V. parahaemolyticus, V. cholerae* etc.) [53]. Probiotics were utilised in aquaculture as early as the 80's after the beneficial effects on humans and poultry were noted [53]. To further enhance the action of probiotics, prebiotics are also administered (substances used to help enhance the establishment of probiotics) [51]. The major probiotics that are used include bacteria from the genus *Lactobacillus, Bacillus, Bifidobacterium, Enterococcus,* bacteriophages which can inhibit certain bacteria and yeast such as *Saccharomyces cerevisiae* [53, 56].

The benefits of probiotics are typically seen as improvements in growth performance, immunity and the fish's own physiology [53, 55]. There is some disagreement in literature regarding the effect of probiotics on the quality of water in which they are used, with some literature arguing there is a beneficial effect [53, 57], and other papers citing potential undesired effects of live probiotics on the environment through interactions with already existing probiotics in the environment [55]. The probiotics that are used have various documented benefits, such as reducing pathogen loads, improving feed efficiency, enhanced immunity, stress tolerance and blood quality [53]. Preventing the adhesion of pathogenic bacteria on the gut mucosa is another benefit yielded by probiotics [54]. This adhesion prevention leads to a diminished capacity for bacteria to establish infection in fish [53]. The use of bacteriophages in Atlantic salmon in case of infection with *Vibrio harveyi* yielded unfavourable results with 100% mortalities in Atlantic salmon during the treatment period with damage seen in the liver, kidneys and muscle [56]. This indicates a serious risk with the persistent use of bacteriophages in salmon farming as there is an exchange of virulence factors which can pose a threat to the vitality of farmed salmon and therefore, not a viable long-term probiotic for salmon [56]. In the desire to minimise the use of antibiotics, understanding the effect of probiotics on the immune system helps to establish other potential candidates for probiotics [53]. There has been a documented improvement in the survival rate of Atlantic salmon when there was supplementation of probiotics (such as *Lactobacillus, Photobacterium, Vibrio etc*) in the diet [46]. This can be due to the increased resistance to infection by enhancing the ability to withstand stress as well as the role probiotics play in helping to inhibit the establishment of pathogenic bacteria (through adhesions) [46]. Other probiotics act by stimulating an immune response in fish [46, 51, 58]. The main immune responses of fish include lysozyme (in the mucous membranes of fish skin), alternative complement pathway, phagocytosis, respiratory burst, superoxide dismutase and the production of mucous [51]. The combined use of prebiotics (substances used to help enhance the establishment of probiotics) with probiotics further enhanced the immune system activation [51]. In Atlantic salmon, the alterations in the gut microbiome depended heavily on the environment in which they are reared [59]. Smolts reared in saltwater showed significant suppression of genes related to gut immune function but an increase in the diversity of the gut microbiome (when compared to smolts reared in freshwater) [59]. Lactic Acid Bacteria (LAB) were found to have a positive effect on promotion of immune action in salmon [59]. As an added bonus, probiotics were also shown to have a beneficial effect on preventing disease caused by viruses and improving survivability of fish (fed probiotics) that contract viral infections [60].

More recently there has been a drive to tailor probiotics more specifically to fish since earlier probiotics didn't account for the physiological differences between fish and other animal species or the differences in an aquatic versus a terrestrial environment [53]. When a potential candidate for a probiotic is proposed, there is an extensive selection criterion applied, involving both *in vitro* and *in vivo* stages [53]. Potential probiotics should meet the following criteria: (i) does not cause harm to the host, (ii) non-invasive and non-carcinogenic, (iii) be effective when it reaches the host's target site, (iv) should not have resistance genes for virulence or antibiotics encoded in their plasmid, (v) it should be able to stabilise and colonise within the host for a time period and (vi) it should work *in vivo* as well as *in vitro* [53, 61]. The initial stage involves screening so as to rule out any organism that would be detrimental to the end goal of aquaculture [53]. Potential probiotics can be isolated from the gastrointestinal tract of healthy fish and then the 16S rRNA gene was amplified with the use of RT-PCR before cross checking with a genetic database in order to identify the strain isolated [54]. After the identification of potential strains, the ability for these strains to act as an inhibiting agent is assessed through a variety of methods, initially *in vitro,* through culturing and streaking the sample onto a plate to detect inhibitory or antagonistic action [53, 54]. Parallel and streak plate methods use plates which contain the pathogen that is being considered and the potential probiotic is inoculated onto the plate, incubated before the inhibitory zone is considered [54]. The stability of probiotics in the changing pH of the digestive tract (through spectroscopic methods that track the level of proliferation at varying pH) and the added digestive benefits of probiotics (through culturing in a media mixed with a certain substrate and looking at the alterations in the quality of the culture media) [53, 54] are also examined. Once these initial tests have been completed *in vitro*, there is a movement to *in vivo* tests [53]. The adhesive ability is examined through the experimental inoculation of live fish, keeping them in controlled conditions for a set period of time before inoculating the gut sample to plates for culturing and evaluation of the population of the potential probiotic strain [54]. The antibiotic resistance of probiotic strains is important to examine as we want to minimise the risk of AMR becoming established (due to a potential risk of genetic exchange occurring, especially with pathogenic bacteria). The resistance is tested through agar gel diffusion (where we look for areas of alteration in the agar, indicating interaction between antibiotics and the probiotic) [53, 54].

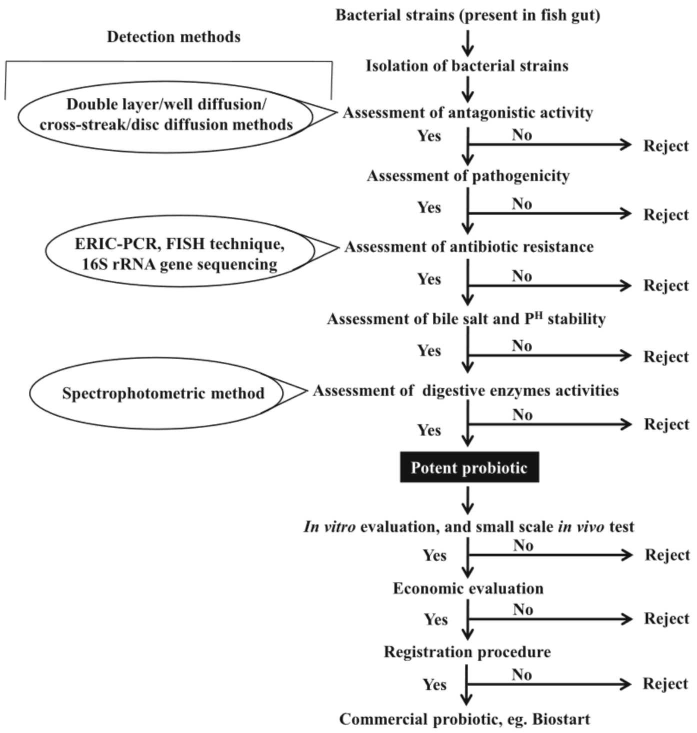

Figure 1: Flow chart indicating the selection criteria for adoption of new probiotics [taken from Hasan and Banerjee, 2020]

Given the concern about the effect of probiotics on the environment, attempts have been made to make probiotics more sustainable. Heat treatment of probiotics can be used to inactivate the bacteria without having a detrimental effect on the overall efficacy of the probiotic [55]. Aside from potentially mitigating the damage to the environment into which probiotics are added, heat treated probiotics also don't have the risk of antimicrobial resistance emerging (due to the damage done to the plasmid during heat treatment) [55]. When studies were carried out to establish the potential benefits of heat treated probiotics, it was found that there is a similar ability to adhere to the gut mucosa of fish [61] but this is limited as these bacteria are inactive and the adhesion time is shorter, which necessitates more frequent application, and therefore the feed cost (due to increased utilisation) could be higher [55]. The inactivation of bacteria also prevents the production of extracellular enzymes which are utilised by fish to improve digestibility and utilisation of feed [55]. This decrease in feed utilisation may also influence the amount of feed that is absorbed in the fish's gut and also increase the cost of feed used. The effect of heat-treated probiotics on the immunomodulatory response of fish is variable with some strains of bacteria showing a diminished ability to activate the immune response (when compared to untreated strains) [55]. This variability in response could be linked to the alteration in structural integrity that occurs when the probiotic undergoes heat treatment and subsequently, there is a change in the type of immune pathways that become activated [55].

When looking for information regarding the prevalence of usage of probiotics in Scottish salmon farming, there is a distinct lack of information regarding the prevalence with which they are used.

Probiotics have already proven their worth in aquaculture given their ability to increase the growth rate and overall production value of salmon in which these probiotics are used, without causing the level of dysbiosis seen with antibiotics [53]. This is achieved through an ability to compete successfully against pathogenic bacteria and incite an immune response in host fish [53, 54, 56]. This beneficial effect can be amplified through a multi-modal approach (like combining vaccines and probiotics) [56]. The major concerns regarding the future of probiotics, includes establishing an environmentally friendly probiotic that has a longevity to match (or exceed) currently used probiotics [55]. Heat treatment is showing promise through its ability to minimise its effect on the environment with a similar level of gut adhesion [55]. However, a lack of effective active time in fish could be a stumbling block for heat treated probiotics [55].

3.5.2: Vaccines in Salmon

Given the therapeutic role that vaccinations play in aquaculture, it forms a valid branch in the strategy to decrease the use of antibiotics [20, 46]. The key role of vaccinations is the promotion of an immune response in a controlled manner (typically through the use of non-pathogenic strains of the pathogen or the use of particles of the pathogen that induce an immune response) [52]. Vaccines act in a preventative capacity and help decrease the severity of clinical signs of disease in fish [62]. There are a number of vaccines that are already on the market for salmon aquaculture [52]. Vaccine strategies in place either target the fish directly (protecting them directly from severe infection of a number of pathogens) or indirectly, through the vaccination of cleaner fish (which act to prevent the accumulation of sea lice on salmon, with an accumulation of sea-lice increasing the fish's susceptibility to disease) [63–65]. Vaccinations in aquaculture typically act on the antigen presenting cells of the lamina propria of the gut [66]. Viral diseases are normally the target of vaccination protocols, but they are also vaccinations for a number of bacterial infections that affect salmonids (such as *Flavobacterium psychrophilum, Yersinia ruckeri, Vibrio* spp. *Aeromonas* etc.) [52, 67].

Some vaccinations are administered through incorporation of the vaccine into feed before being fed to fish [62]. The issue with this administration method lies in the fact that uptake and duration of action of the vaccination can be inhibited [62]. This inhibition is typically due to the lack of antigen (in the vaccine) reaching the immune cells that can induce an immune response [62, 63]. However, the benefits of oral administration lie in the fact that it is a less stressful administration method for fish and less labour intensive for workers (compared to injectable vaccines) [63]. Injectable vaccinations can induce a better immune response but the drawback with injectable vaccinations lies in the cost and complexity of administration. Injectable vaccination is typically considered for species which have a high economic value (such as Atlantic salmon) [20].

Studies have been made to try and develop new vehicles for the delivery of *per os* vaccines that will provide adequate protection to fish [63]. The ideal delivery matrix for fish should minimise adverse effects on the fish gut [63]. Adverse effects on the gut may have a combined effect of diminished vaccine efficacy and increased feed conversion efficiency (resulting in increased feed wastage due to decreased gut absorption, increased feed costs and decreased finishing weight) [63]. Polyethylene glycol and alginate were studied as potential vehicles for oral vaccine delivery given their already established use in the pharmaceutical industry [63]. Alginate has the added benefit of being used in the stabilisation of pellets used for fish feed [63], which can minimise the loss of the vaccine through leaching. Whilst the study was carried out on rainbow trout, the results provide a basis for the potential of these for use in salmon production. Pellets were combined with either alginate, polyethylene glycol, both, and the control contained neither agent [63]. After the trial period, the degree of inflammation in the entire gut was examined through the use of histology in order to ascertain the potential effect the matrices would have on the uptake of the vaccine, with RNA tests run to detect the presence of inflammatory markers [63]. Polyethylene glycol showed a decreased growth rate with significant, undesired changes in the morphology of the gut (especially in the posterior intestine which is most significant in the initiation of an immune response) [63]. There was a lack of inflammatory indicators such as cytokines, indicating that the inflammation was not truly severe but the alteration in morphology would be enough to impair the vaccine delivery [63]. The inclusion of alginate, however, showed some ability to mitigate the negative effect of polyethylene glycol [63]. Alginate by itself showed similar trends to polyethylene glycol in the alterations of the pyloric caecum but significantly wider villi in the mid-intestine [63]. Given that the pyloric caecum is the major region for the recruitment of IgM and IgT B-cells, any alterations in this region are undesired, as it impacts the ability of the fish to formulate an effective immune response with exposure to oral vaccinations [63].

The use of adjuvants in vaccinations enhance the immune reaction of the treated fish for a longer period of time (in cases where the pathogen itself would not produce a sufficiently strong immune reaction) [67] but the use of these adjuvants have, in themselves, side-effects which may not be favourable for the treated fish (including local reactions at the site of injection which leads to subsequent downgrading of the fish carcass at harvest, which has a knock on effect on the amount of money the farm receives) [67].

Due to this, there is a consistent drive to improve the adjuvants used to ensure the vaccine's efficacy and minimise the loss of quality in fish. Development of potential adjuvants requires *in vivo* testing and experimental infection of control groups of fish to evaluate the IgM level in serum, histology (to detect signs of inflammation or adverse reactions) and level of immune response that occurs in fish (through the presence of CD-4, IL-8, IFN-γ etc.) [67]. Hoare et al. developed three potential adjuvants for use in combination with vaccination (in the form of formalin-killed cells) against *Flavobacterium psychrophilum* in Atlantic salmon and trout. The adjuvants considered were Montanide (an oil/water mixture) or a squalene/alum adjuvant (squalene/glycerol/Tween 80/Aluminium hydroxide) with a phosphate buffer solution used as the control and a vaccine without any adjuvant [67]. The groups were subsequently infected with *F. psychrophilum* and mortality curves were derived [67] as this indicated which adjuvant promoted an effective immune response. Other tests were run in order to establish if the adjuvant enabled the vaccine to formulate a specific immune response and histopathology was carried out to determine if there was any sign of side-effects on the fish (which might result in downgrading of the fish at harvest) [67]. Montanide showed a good survivability and a depot-like effect (with high levels of IgM still found six weeks post-vaccination) but there were high levels of inflammation found in various organs during histological examination (with some fish even exhibiting signs of adhesions around the organs) [67]. In comparison, the squalene/alum adjuvant showed a lower survivability (75.5%) when compared to Montanide (95.2%) and the unadjuvanted vaccine (85.7%) but there was also mild to minimal signs of inflammation found (vs. Montanide) [67]. Even more, when a novel strain of *F. psychrophilum* was introduced into the vaccinated fish, there was a marked immune response in fish treated with adjuvanted vaccines, which indicates a potential for cross immunisation [67]. The use of adjuvants presents an important consideration for fish farmers when selecting viable strategies for alternatives to antibiotics. Adjuvants can play an important role in helping maximise the survivability and the strength of the immune response induced but also bearing in mind the impact side-effects that adjuvants may have on the quality of their stock [67]. Montanide, whilst effective, may have less favourable effects on the value of the stock when being harvested, whereas squalene/alum adjuvants imbue a level of protection to fish with less severe inflammatory reactions at the site of injection whilst also offering the potential for cross protection [67].

A more modern proposal for the utilisation of vaccinations as an alternative to antibiotics is to promote the use of autogenous vaccines [67]. Autogenous vaccines are generated with samples of the pathogen taken from the farm in question and a vaccine is produced targeting the particular strain found on the farm, thus providing a more tailored treatment plan [64] and could potentially provide the next step for Scotland in terms of reducing dependence on antibiotics. In terrestrial farming, the use of autogenous vaccines was found to decrease the use of antibiotics [64] and have been found to be effective in the control of atypical *Aeromonas salmonocida* [65], which is important when the risk of biofilm formation increases in the presence of two commonly used antibiotics in Scottish salmon farms [30, 47]. *Yersinia ruckeri* has also been targeted as a potential candidate for autogenous vaccines, especially given the increasing incidence in Atlantic salmon [68]. In studies carried out on Atlantic salmon in Scotland over a period of 14 years; through O-serotyping, it was found that there were over 109 isolates recovered [68] and this was partly due to O-antigen modification (which is common in gram negative bacteria) [68]. This modification was proposed to have emerged from the selective pressure applied when vaccination is used and promotes the proliferation of strains that are not affected by the currently applied vaccines [68]. The range of *Y. ruckeri* strains in Atlantic salmon varied widely when compared to rainbow trout in Scotland due to the geographical differences in their production sites and the limited interaction between the two species [68]. As a consequence of this, the use of a "one size fits all" approach to a vaccination protocol is not feasible. Therefore, Scotland adopted the use of autogenous vaccines to help formulate a more effective and precise disease management plan [68].

The main benefits conferred by vaccinations lie in their ability to decrease the severe effects of pathogens on fish [62]. Increased survivability and an ability to maintain growth rates despite infection are two benefits that have been understood of good vaccination protocol. Although with the increasing desire to reduce the use of antibiotics, their ability to protect fish stock from serious disease outbreaks means that the improvement of vaccination technology will be more seriously considered [52]. This is already being evidenced by the studies carried out into more fully understanding the effect of vaccines on fish with a view to improving the modalities of vaccine delivery [63, 67, 68]. The benefits of effective vaccination strategies through the reduction of antibiotics is seen both in Norway and Scotland [19].

3.5.3: Welfare

The environment in which salmon are reared are just as important as vaccinations and the types of feed that are utilised in reducing the reliance of antibiotics. Adherence and improvement of welfare allows salmon that are farmed to best realise their genetic potential and achieve their maximal growth rate [69]. This is achieved through minimising stress on the fish by providing them an optimal environment that respects the five freedoms indicated by the UK Farm Animal Welfare Council in 1992: (i) Freedom from pain, injury and disease, (ii) Freedom from hunger and thirst, (iii) Freedom from physical and thermal discomfort, (iv) Freedom from fear and distress and (v) Freedom to express natural behaviours. Animal welfare, including fish, has become an important issue amongst governmental agencies and animal advocacy groups [70]. This concern with fish welfare has led to the establishment of standard operating procedures, that are focused on welfare, by official bodies [71]. By achieving high welfare standards, the susceptibility to disease is decreased and, by extension, the need for antibiotics is not required. There is a further benefit to ensuring that the welfare needs of salmon are met as consumers are becoming increasingly conscious of purchasing ethically produced salmon [3].

The use of medication is linked to improving welfare and reduction of antibiotic usage in farmed salmon in Scotland but it should not take the place of prevention [71]. Therefore, it becomes important to ensure that the husbandry of fish stocks is sufficient to ensure that fish don't easily become sick of moribund and the use of medication does not become the norm [71]. The role of the veterinarian in determining the course of treatment is highlighted with a focus on the appropriate management of any medications used and kept on the farm [71]. There are a number of programmes that aim to hold farms to higher welfare standards, such as the RSPCA's "Welfare Standards for Farmed Atlantic Salmon" [71].

When assessing the quality of fish welfare, it is important to not only observe the behaviour of fish (through their physical appearance and swimming behaviour) but also the quality of the water in which they are placed (such as oxygen saturation, pH, salinity, temperature) and the general environment (such as lighting, noise, feeding and handling) [69, 70]. It is important to remember that some of the welfare indicators that are discussed require specialised equipment in order for it to be properly assessed [70], therefore, when in a practical situation, some of these parameters cannot be readily assessed by workers. In this case, welfare indicators which can be readily assessed on site (such as water temperature) are classed as operational welfare indicators and more complicated indicators are classed as lab-based welfare indicators [70]. In a practical sense, it is important that the operational welfare indicators are tracked by trained personal regularly in order to detect and mitigate any deviations outside of the optimal range. A deviation from the optimal could result in placing fish under undue stress which can increase the risk of fish contracting infections requiring the use of antibiotics.

Given the expansion of aquaculture into open waters (such as out at sea), the impact the environment has on the welfare of Atlantic salmon is important in determining its robustness. Swimming behaviour can be used to assess the welfare of fish and it can provide information about the strength of the current in which they are placed [72]. In weaker currents, salmon tend to carry out circling behaviour and form schools (so as to avoid colliding with one another), but as currents become stronger, salmon have to expend more energy on remaining stationary before being pressed against the net in excessively strong currents [72]. The issue with stronger currents means that fish have to expend more energy trying to simply remain stationary (becoming exhausted in prolonged scenarios) and in cases where the current is too strong, fish are crushed against a net and are subject to an increased risk of injury [72]. Therefore, it stands to reason that in allowing fish to become weakened or injured, there is an increased risk of pathogens entering these fish and causing disease. However, it is also postulated that a moderate degree of current (to the point fish are stationary) allows for fish to express natural behaviours and provides a level of enrichment to fish kept in nets, so a certain level of current is considered acceptable [72]. As an added benefit, the increased challenge when swimming in moderate currents may produce a leaner product due to smolts putting energy into muscle production (as opposed to sexual maturation through development in slower currents, which diminishes the carcass quality) [72]. The benefits of a controlled, moderate current can help promote the health and carcass quality of Atlantic salmon [72], but this would not necessarily be an easy parameter to control given the exposed nature of the sea to the north-west of Scotland.

3.5.4: Biosecurity

Biosecurity encompasses the environment in which salmon are reared and it accounts for the sanitary measures including the water management as well as the worker hygiene [50, 73, 74]. Biosecurity is also important in ensuring food chain safety, as it aims to prevent the introduction of disease to the farm (or spread of disease between wild and farmed salmon) [38]. To ensure that disease introduction is minimised, it becomes imperative that the various parties involved in salmon production work together to ensure that sanitation measures are properly observed along the production chain [38, 75]. Biosecurity has been mentioned as of benefit in helping reduce the level of antibiotics used in salmon farming in Scotland [30, 38]. In an attempt to promote biosecurity, Scotland has made it necessary for salmon farms to draft a biosecurity plan (and have it approved) in order to obtain a license to operate [75]. One significant measure implemented to protect the consumer and the food chain in the UK, is the ban on feeding meat and bone meal of mammalian origin to fish (after the outbreak of Bovine Spongiform Encephalopathy) [74]. Through avoiding disease establishment, there is an added benefit to the farm through the reduced costs associated with the treatment costs and losses associated with dead and moribund fish and it has been proven that good adherence to biosecurity measures helps decrease the occurrence of disease [76].

The focus of implementing biosecurity measures includes appropriate training of staff and this point has been raised repeatedly when trying to promote an effective biosecurity plan [50, 75]. Worker compliance is especially important as these people will be the ones effectively on the ground and implementing the biosecurity plan [75]. Ensuring compliance can be achieved through properly educating workers about the importance of why certain rules are in place. Adequate training is also necessary as it allows for a rapid detection of any issues arising during day-to-day operations (for example, training workers to recognise signs of sick or moribund fish) and a clear plan of action in the case of detected deviations [75]. In the UK, there is a heavy focus on ensuring cross compliance with other sections of the production chain (by ensuring that they too, have a biosecurity plan in place), ensuring there is a clear chain of command (a biosecurity manager is appointed and that the veterinarian's details are clearly and easily available), workers receive regular training and visitors are recorded when entering the premises [75].

Management of the environment is an important consideration for the biosecurity of salmon. The failure to adequately manage the organic load (such as waste from salmon etc.) in farms that use re-circulated water can lead to decreased growth rates in salmon as well as increased risk of infection [73]. Therefore, peracetic acid is used to help disinfect waters containing salmon due to the fact it acts on a wide range of microbes, rapidly and stably (even at low temperatures and pH) whilst also having a low ecological impact (with harmless decomposition products) [73]. Studies on the impact of peracetic acid have shown that the stress effect on salmon varies widely based on the life stage of the salmon (with significant differences in the harmful dose seen between salmon smolt (4.8 mg/L can lead to undesired health effects) and salmon parr (3.2 ml/L causing undesired health effects) [73]. However, low-dose administration of peracetic acid to disinfect water has been found to have a minimal effect on salmon and it is still appropriate for the disinfection of water to avoid the build-up of undesired organic matter [77].

4. Method:

The literature review was carried out using the web browser Mozilla Firefox and the search engine Google Scholar. In order to ensure the information was as up to date as possible, I applied a filter for the year and selected papers from 2017 and later.

When searching for various papers related to each chapter, I would specify the UK or Scotland in my searches. If papers failed to acknowledge Scotland or the UK, I would broaden my search to geographically similar regions (such as Ireland) or regions with a similar economic output (such as Norway). Results focusing on Chile were generally discounted due to the difference in the geographic region and economic situation (in that it is generally a poorer country when compared to Scotland, so the production chain would be at risk of being poorer when compared to Scotland). Other keywords searched included "Public perception," "Food Security," "Diet," "Vaccination," "Aquaculture," "Salmon" "Salmonid," "Vaccination," "Biosecurity," "Welfare," "Resistance," "Antimicrobials," "Probiotics," "Gut Health."

Papers selected came from databases such as "Plos One," "Wiley Online Library," "Elsevier" and "PubMed," as well as governmental sites such as "gov.co.uk," and "gov.scot." Scientific papers had to be peer reviewed in order to be selected for this. Reports selected had to come from recognised government organisations (such as the Scottish Government), recognised global bodies (such as the WHO and FAO) or recognised NGO's (such as Salmon Scotland). Papers that explicitly focused on Scotland and salmon farming in Scotland were prioritised. Papers that mentioned Scotland and salmon farming were then examined.

5. Results:

There was a total of 77 papers used in the literature review. Of the 77 papers used, 8 out of 77 (10.4%) were published before 2017 but they contributed significantly to the topic in question. The remaining papers (69 out of 77, 89.6%) fell within the desired range (2017 to present).

When looking at the papers that were selected. 23 out of the 77 papers had specific mentions of both Scotland and salmon (29.8%). 33 out of 77 papers (42.9%) specifically mentioned salmon only and 14 out of 77 papers (18.18%) only mentioned Scotland. 14 out of 77 (18.18%) papers used did not mention either Scotland or salmon, however these papers contributed to the topic through the research that they were carrying out.

6. Discussion/Conclusion

By and large, I was satisfied that the papers selected fell within the desired timeframe but there were certain areas that I would have like to have seen papers that were more up to date (such as in the case of autogenous vaccinations). Media articles were selected bearing in mind the potential for bias and swaying the public. Therefore, I tried to select from news sources. The information about the use of antibiotics is very well documented within Scotland, with the publication of annual reports, however, I would like to see more in-depth studies regarding the prevalence of vaccinations and nutraceuticals used. It should also be noted that the data about the use of antibiotics in salmon farms in Scotland are derived from surveys and therefore there is a chance of human error when filling out the surveys that are submitted to the Scottish government. Overall, there is satisfactory information regarding the effect of antibiotics on the gut of fish and the potential for AMR to emerge. There is a lot of documentation about the range of factors that contribute to the emergence of AMR. I also feel that there is a lot of information regarding the potential alternatives to antibiotics, but I also feel that there is room for more updated studies to examine the newer alternatives that are potentially arriving on the market.

7. Summary:

The potential role of fish both in terms of supplying a solution to food security and dealing with the issue of land availability is undeniable. Scotland has a strong foothold in the market with evidence that there is room for market expansion (seen by the increase in domestic sales during the avian influenza outbreak) and potential for expansion in foreign markets (by muscling on Norwegian markets that may be impacted by future taxation schemes).

The public perception will play a big role in the future of salmon farming within Scotland. Documentaries that examine the practices of aquaculture will end up calling for a greater accountability amongst these farms. Public perception in Scotland is highly driven by the fact these communities primarily made their living in capture fisheries and the emergence of new aquaculture farms poses a threat to these traditional jobs. It's important salmon farms foster good relationships with these local communities by promoting local employment and investing in these communities. This could help smooth out potential tensions.

Given the increasing overall use of antibiotics in salmon farming in Scotland despite Salmon Scotland claiming that the percentage of salmon farms using antibiotics has decrease, it could be indicative of an increasing number of salmon being kept on various farms. This increase may be indicative of farms needing a higher level of antibiotic medication administered in order to ensure the therapeutic dose is achieved.

There is continuing advancement in alternatives to antibiotics. The developments are starting to take the impact on the environment into account (through the potential offered by heat-treated probiotics). Future developments are also looking to improve salmon health through improving welfare and adjuvants that cause less irritation to fish. Through improving welfare, and joining recognised welfare schemes, there is a second benefit as it provides another marketing tool to salmon producers. However, public trust in these welfare schemes must be maintained for the marketing to be effective. Biosecurity is also important as it aims to prevent disease introduction and prevent disease becoming established in the first place. There is no quick fix for the issue of AMR, and Scotland is already adopting some of these measures. However, the co-operation of all stakeholders is essential in ensuring a successful decrease in the use of antibiotics.

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Thesis progress report for veterinary students

Name of student: Margaret Cronin

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Name and title of the supervisor: Professor Ferenc Baska

Department: Department of Exotic Animals and Wildlife Medicine

Thesis title: Comparing the use of conventional antibioties and the alternatives to antibiotics in fish farms in Scotland

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	10	11	Planning Consultation	[signature]
2.	2022	11	14	Progress report	[signature]
3.	2023	02	10	Review of lit. Review introduction	[signature]
4.	2023	03	15	Progress Report	[signature]
5.	2023	04	17	Planning consultation for the upcoming summer/semester	[signature]

Grade achieved at the end of the first semester: 5

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	07	11	Progress Report	[signature]
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3.	2023	09	01	Feedback and planning	[signature]
4.	2023	10	02	Submission of second draft	[signature]
5.	2023	11	02	Plagiarism check	[signature]

Grade achieved at the end of the second semester: ......5 (jeles)......

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

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Signature of the student: ......Margaret Cronin......

Signature of the secretary of the department: ....

Date of handing the thesis in..2023. 11. 14...........

I hereby confirm that I am familiar with the content of the thesis entitled

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Date: Budapest, 03 day 11 month 2023 year

Dr. BASKA FERENC

Supervisor name and signature

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