DIETARY EFFECTS ON GROWTH AND ENERGY STATUS OF JUVENILE BALLAN WRASSE (*LABRUS BERGYLTA*) IN SALMON PENS

Thesis for the degree

Master of Science in Aquaculture Biology

Ida Lee Liseth Hansen

Department of Biological Sciences

University of Bergen

June 2022

Supervisor: Øystein Sæle, Institute of Marine Research

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Abstract

The use of cleaner fish as an ectoparasite countermeasure is well established in Norwegian salmon farming. In 2019, around 61 million cleaner fish were deployed in Norwegian salmon farms (Grefsrud et al. 2021). Farmed lumpfish and different species of wild caught wrasses dominated, followed by a growing amount of farmed Ballan wrasse. Unfortunately, poor welfare and high mortality has been reported for cleaner fish rapidly after they been transferred to the salmon pens (Imsland et al. 2020), Wilmann et al. (2020)). Poor nutrient is hypothesised to play a major role on poor welfare and consequently survival. This study aimed to investigate dietary effect on growth, energy status and survival of juvenile Ballan wrasse fed three different diets offered in both pellets and blocks with and without salmon in net pens. The first diet, Control, was based on previous studies, and consisted of cod filet proteins (30%) and shrimp meal (28%). The second diet, Smart, contained Black soldier fly larvae meal (20%), krill meal (24%) and some shrimp meal (14%) for palatability. The third diet, Soft, was a standard commercial feed used in many salmon farms as wrasse feed today. Fish were divided in nine net pens with salmon and each feed was offered to fish in three units. Energy status of muscle filet, liver and viscera were analysed using bomb calorimetry for the trial period. After two months in salmon net pens, fish fed the Soft showed a negative SGR of 0.12 % day^{-1}, whereas fish fed Control and Smart put on weight with a SGR of 0.08 and 0.11 accordingly. The growth performance reflected the energy storage and survival of fish over the winter, where fish fed Control and Smart achieved better results in terms of increased growth (i.e., weight gain, SGR and CF), energy status and survival compared to fish fed standard commercial diet. This study showed that there is potential of optimising growth, energy status and survival by offering the right feed composition.

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Abbreviation

BSF	Black soldier fly
CP	Crude Protein
DIFF	Difference
EAA	Essential amino acids
GE	Gross energy
IMR	Institute of marine research
LE	Liver energy
LSI	Liver somatic index
ME	Muscle energy
NFE	Nitrogen-free-extract
PL	Phospholipid
SD	Standard deviation
SGR	Specific growth rate
TAG	Triacylglycerid
VE	Viscera energy
VSI	Viscera somatic index
OWI	Operational welfare indication

1. INTRODUCTION

1.1 THE NORWEGIAN AQUACULTURE AND THE SEA LICE PROBLEM

Since the 1970s, the Norwegian salmon production has been a success story in terms of production volume and income. From a starting point of approximately 600 tons Atlantic salmon and rainbow trout (*Oncorhynchus mykiss)* in the 1970s , to reaching a production volume of 1 140 000 ton of salmon and 72 000 ton of trout in 2020, with a total export value of 7.4 billion € (Hersoug 2021, SSB 2021). Norway is the leading producer of salmon and trout and produce more than half of the world's production. The rapid growth of the industry has played a significant part for the Norwegian economy, making salmon farming the most important export industry next to oil and gas in terms of revenue (Hersoug 2021).

The rapid growth of the industry has not come without negative effects, and the industry are facing multiple challenges. To this date, sea lice has been reported to be one of the most harmful pathogenic marine parasites for Atlantic salmon (Costello 2006). Pre-adult and adult sea lice feed of the mucus, skin, and blood on the salmon, eventually wearing down the protective surface of the fish (Grimnes and Jakobsen 1996, Hamre et al. 2013b). A stress response, triggered by increased blood levels of cortisol and glucoses, can develop into chronic stress if infected with large numbers of lice over a long period (Grimnes and Jakobsen 1996, Mustafa et al. 2000). This can further lead to osmoregulatory failure, lesions, anaemia, and increased vulnerability of secondary bacterial infections and eventually death (Mustafa et al. 2000, Grant et al. 2016, Overton et al. 2019). The genera *Caligus* and *Lepeophtheirus Salmonis* are the most common genera infecting salmonids, where *Lepeophtheirus* is responsible for most of the disease outbreaks and economic losses throughout the northern hemisphere (Mustafa et al. 2000). In 2015, the economic loss of sea lice on a global scale was estimated at 822 million €, or around 9% loss in farm revenues (Brooker et al. 2018).

1.1.1. Sea lice Treatments

Some solutions fighting sea lice include thermal, mechanical, and chemical treatment such as warm- or freshwater bath, laser technology and different chemicals. Challenges with these methods are their effect on salmonids welfare in terms of high stress levels and/or mechanical injures, that result in increased mortality (Olaussen 2018, Overton et al. 2019). Use of chemical treatments such as hydrogen peroxide, organophosphates, and emamectin benzoate has showed resistance and reduced sensitivity for the sea lice (Jones et al. 2006, Lees et al. 2008, Helgesen et al. 2015). Other troubles with chemical treatments are leakages to the surrounding environment and animals, with negative impacts on shrimps and other crustaceans (Olaussen 2018). This has

resulted in a blooming interest of using cleaner fish as an alternative biological treatment for lice control. Cleaner fish has been presented as a more environmentally sustainable alternative, less expensive and reduced stress for salmon (Skiftesvik et al. 2013, Imsland et al. 2014). Perhaps the most sought-after cleaner fish species is Ballan wrasse (*Labrus bergylta)*.

1.2 Ballan Wrasse (*Labrus Bergylta*)

Ballan wrasse is a saltwater sequential hermaphrodite fish in the family Labridae, the wrasses. There are six wrasse species in Norway where Ballan wrasse is the largest. (Rimstad et al. 2017, Svaasand et al. 2017). Out of the six wrasses in Norway, Goldsinny (*Ctenolabrus rupestris*), Corkwing (*Symphodus melops)*, and Ballan wrasse are the most used cleaner fish in aquaculture. However, Ballan wrasse is the only one farmed wrasse (Svaasand et al. 2017).

1.2.1. Ballan wrasse as cleaner fish

The use of wrasses can be dated back to the 1980s, but the growing demand for wrasses (**Figure 1**) did not start until 30 years later (Geitung et al. 2020). In later years, lumpfish has been introduced as a cleaner fish. Since 2008 to 2019, the use of cleaner fish in Norway increased from 1.7 million to 61 million cleaner fish (Geitung et al. 2020, Grefsrud et al. 2021). Out of the 61 million cleaner fish that were deployed in salmon farms in 2019, 17.3 million were a mixture of wild wrasse species and 681 000 were farmed Ballan wrasse. The remaining 39 million were farmed lumpfish (Grefsrud et al. 2021).

The growing demand for wrasses has led to increased pressure on wild populations, resulting in a new challenge for the salmon industry. Intensive culture of Ballan wrasse needed to be developed quickly in order to cope with the increased fishing pressure. However, cultivation of Ballan wrasse has showed challenges regarding biological knowledge and its nutritional requirements along the production cycle. Although substantial progress has been made in the last decade, the Ballan wrasse production is still struggling with slow growth, poor feeding performance, high mortality and costly feeds (Geitung et al. 2020).

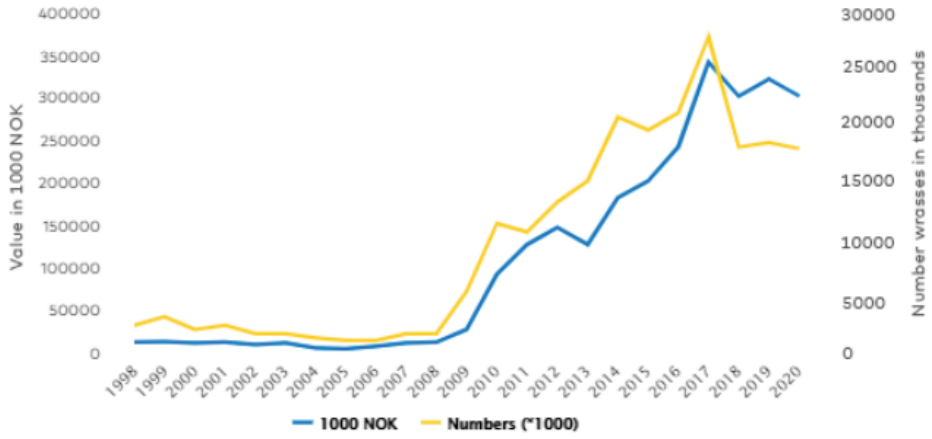

Figure 1: *Commercial catches of all species of wild wrasses in Norway. Showing both number wrasses and first-hand value for the fisher during 1998-2020. From (Tallaksen Halvorsen et al. 2021).*

1.2.2. The importance of intestinal function and health

Cultivation of lumpfish take around 4 months until it is ready to deploy in salmon pens, while Ballan wrasse take approximately 1.5 years to reach their deployment size of 40-50g (Brooker et al. 2018, Powell et al. 2018). The slow growth of Ballan wrasse can be an advantage as Ballan wrasse can follow the salmon throughout the production cycle. Nevertheless, a mortality rate of 40% has been reported during the production period of salmon (Wilmann et al. 2020). The largest causes of mortality is reported to be illness, followed by poor fish quality from the supplier, damage from handling and no clear cause (Wilmann et al. 2020). However, it has also been assumed that poor nutrition has a direct or indirect effect on health and mortality (Skiftesvik et al. 2013, Geitung et al. 2020). Good intestinal function and health are of crucial importance for any animal's production, health, and resistance to disease. The intestines contain complex immunogenic organ, but also other structures and mechanisms that can help neutralise substances or other organisms that can threaten the function or health of the fish. The immune system of the intestines are also actively communicating with other organs withbarrier functions such as the gills, skin, head kidney and spleen (Lein et al. 2021). Therefore, it can be assumed that the right feed composition and feeding strategy are important in terms of proper fish welfare. To optimize rearing, feed and feeding strategy for Ballan wrasse, better understanding of anatomical, physiological, and functional knowledge is needed.

1.2.3. General intestinal physiology

Ballan wrasse has a different digestive system than many other fishes. Unlike gastric fish, wrasses lack stomach (agastric) and pyloric caeca, have a short oesophagus, and a short intestine. For most fish, the intestines are longer than its own body length, but Ballan wrasse intestines has a length of only 2/3 of its body length and 1.5-2% of the total body weight (Hamre et al. 2013a). The unique digestive system of Ballan wrasse makes them more dependent on higher levels of digestible protein compared to those for gastric species (Lie et al. 2018).

1.3. FEEDING AND DIET APPROACHES

1.3.1. Protein and lipids

Important components of fish feed are proteins and lipids. They have a major role as sources of metabolic energy for growth, development, reproduction, movement and migration in fish (Tocher 2003).

Fish consumes proteins to access the amino acids. The level and the availability of essential amino acids (EAA) is an important factor in determining the protein quality for fish nutrition (Sargent et al. 2002). New amino acids are always needed when they are constantly used by the fish, either to build new proteins or replace existing ones. Inadequate protein in the diet can lead to reduced or cessation of growth and loss in weight. This is due to abandonment of protein from less essential tissues to preserve the functions of more essential tissues. In case of a surplus of dietary proteins, proteins with adequate amino acids be utilised for growth and the rest will be stored as fat (Brett and Groves 1979, Wilson 2002).

Lipids are another important source of energy and are the main form of storage of usable energy and are categorised as either polar or neutral, depending on their polarity. The lipids triacylglycerols (TAG) and phospholipids (PL) are the ones that have been given a lot of attention in lipid digestion compared to other groups of lipids. PL are polar lipids, an important source of energy, but even more so important for structural and functional components of cell membranes, eye tissues and brain, posttranscriptional regulation of proteins and as a messenger molecule (Sargent et al. 2002, Rønnestad et al. 2013). TAG's are neutral storage lipids that function as the key source of energy. TAG's have twice the energy as carbohydrates due to the relative amount of oxygen, hydrogen, and carbon in compounds. The mean energy of lipid, proteins and carbohydrates are 9.4, 5.6 and 4.1 kcal/g, respectively (Bureau et al. 2002). Therefore, high lipid and high protein feed has more energy compared to high-carbohydrate feed. Finding the optimal balance between lipid, proteins and carbohydrate is thus highly important in fish nutrition.

1.3.2. The optimal balance of feed

Several studies have analysed different feed compositions to find the optimal feed composition for Ballan wrasse's special digestive system. A study done by Hamre et al (2013) studied the balance between protein, lipid and carbohydrate in feed for small Ballan wrasse (1-5g) (Hamre et al. 2013a). The result showed that the highest growth occurred when the feed contained approximately 65% protein, 12% lipid and 16% carbohydrates (Hamre et al. 2013a).

The study also showed that the growth rate increased when the feed contained more than 45% polar lipid. The experiment used increased levels of soy lecithin in the feed to balance the phospholipid level (Hamre et al. 2013a). The optimal amount of lipids in the diet for Ballan wrasse can vary depending on the source (polar vs neutral, marine vs vegetable), but also other factors such as size of the fish and water temperature (Lein et al. 2021).

Another study done by Lein et al. (2021) built on the results from Hamre et al (2013) and reported that with different total lipids levels, phospholipid levels, and sources of phospholipid (marine vs vegetable), the highest growth were achieved with high lipid content and a high proportion of marine phospholipids. This also gave better intestinal health and increased fat in muscle tissue and liver. Increased vegetable phospholipids resulted in reduced growth, but also a tendency to a higher degree of inflammatory reactions in the intestine (Lein et al. 2021).

The source of marine ingredients has also proven to be an important factor in feed for Ballan wrasse as they have shown to be a picky eater.

1.3.3. A picky eater

Like many other marine fish such as Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*), Ballan wrasse have a less developed digestive system during initial feeding and depend on live feed for start feeding than Atlantic salmon. The transition from live feed to pellets however, has showed to be challenging regarding the palatability of the feed for Ballan wrasse (Kousoulaki et al. 2015). Ballan wrasses have showed to be very picky in terms of palatability and conventional fish meal has proved not to be a favourite. It has also been reported that feed containing fish meal resulted in higher mortality rates compared with feed without fish meal (Kousoulaki et al. 2015). Marine ingredients containing crustaceans, such as shrimp or krill has showed to be favourable for the species. This is consistent with the natural diet of wild Ballan wrasse where fish accounts for less than 0.1 %, whereas Gastro-poda, Decaopoda and Echinodermata accounts for 11%, 27% and 46% respectively, by weight (Figueiredo et al. 2005). To replace fish meal, cod muscle meal which has a more neutral taste, in combination with shrimp or krill has showed to be a good recipe for successful weaning diets of Ballan wrasse (Kousoulaki et al. 2015). This selection of high-quality marine ingredients on the

other hand also means high costs and with limited resources available from the sea, it is not a very sustainable choice in the long run (Kousoulaki et al. 2015, Kousoulaki et al. 2021). It is therefore in the industry's own interest to find a cheaper and more sustainable feed source.

1.3.4. Black Soldier Fly (BSF)

In later years, there has been an increased interest for insects as an alternative sustainable feed for variety of livestock species (Barroso et al. 2014, Henry et al. 2015). Insects have the benefits of high feed conversion rate and minimal water and land utilisation (Oonincx and Boer 2012, Henry et al. 2015).

One particular insect species that showed promising features is the black soldier fly (*Hermetia illucens*). Black soldier fly (BSF) is native to the Americas, but occurs worldwide in tropical and temperate regions (Sheppard et al. 1994). BSF larva (BSFL) are capable of efficiently converting a wide variety of organic materials, such as food waste, animal manure, plant residues and agriculture waste into insect biomass that can be implemented in animal feed (Sheppard et al. 1994). The larvae are rich in protein and fat, containing around 40-44% crude protein (CP) (Makkar et al. 2014). The amount of fat is highly variable depending on the type of diet: 21 % for larvae fed on cow manure (Makkar et al. 2014), 28% on swine manure (Newton et al. 2005), and 42-49% on oil-rich food waste (Barry 2004). It has also been showed that feeding BSFL with a diet containing fish offal, increased the levels of desirable omega-3 fatty acids and thus a way to enrich the final biomass (St-Hilaire et al. 2007).

The use of BSFL meal as a valuable feed ingredient has been reported for several fish species such as Atlantic salmon (Lock et al. 2016), rainbow trout (*Oncorhynchus mykiss*)(Sealey et al. 2011, Stadtlander et al. 2017), turbot (*Psetta maxima*) (Kroeckel et al. 2012) and grass carp (*Ctenopharyngodon idellus)* (Lu et al. 2020).

Whether the use of BSFL meal in Ballan wrasse feed can replace some of the expensive cod, shrimp, or krill meal in terms of palpability, has not yet been confirmed.

1.3.5. Feed technology and feed source

Feed source plays an important role for nutrition condition, health and survival for cleaner fish (Imsland et al. 2020). In present time, Ballan wrasse stocked in commercial salmon pens are being fed extruded pellets placed in fine mesh bags. Challenges with this method are that the feed has been found to disintegrate within hours in water leading to substantial wastage, compromising the validation and quantification of feed intake (Leclercq et al. 2015). A recent study also demonstrated that conventional extruded feeds negatively affect survival and

condition during the on growing face for Ballan wrasse (Kousoulaki et al. 2021). These authors suggest that Ballan wrasse feed for all stages should be processed using low temperatures, as is done for cold extruded or agglomeration technologies.

A solution to reduce pellets waste have been the use of feed blocks as feed source. The use of feed blocks have had positive effects for wrasse species stocked with salmon (Leclercq et al. 2015), but also for lumpfish fed with blocks had a better health and survival (Imsland et al. 2019, Imsland et al. 2020). In other words, both feed technology and feed source can have a major impact in fish nutrition.

1.4 Energy

1.4.1. Energy balance

Organisms in all life stages should balance their energy budget between maintenance, growth, reproduction and storage in a way that maximise their fitness. In order for fish to maintain their body mass, the absorbed dietary energy must equal energy loss for maintenance and activity. In cases when dietary energy exceeds the requirements for maintenance and activity, growth and energy storage can occur from the deposition of nutrients, that for fish is largely protein (Brett and Groves 1979). Factors such as water temperature, metabolism, and quality of feed regulate the ability of fish to convert energy from food into body mass (Yuen et al. 2019).

Metabolic rates for Ballan wrasse are influenced directly by the water temperature. At colder temperature the metabolic rates decrease, and fish has been reported to be less active bellow 10°C , and bellow 6°C Ballan wrasse can stop feeding and enter torpor (Imsland et al. 2014, Brooker et al. 2018, Powell et al. 2018, Yuen et al. 2019). If feed intake is reduced or even stopped for a prolonged period, energy saving becomes especially important for survival. The energy status of the fish is thus essential, as it can be an indicator of health and fitness of the fish.

1.4.2. Fish bioenergetics

As feed is the major production cost (>50%) in aquaculture (Iversen et al. 2020), formulating the right diet is important for minimising feed cost and enhance fish productivity, health and survival. Fish bioenergetics is the study of the balance between dietary energy intake, expenditure and gain (Bureau et al. 2002). Energy intake in fish happens trough feed and energy loss happen through feces, urine and gaseous losses. In general, energy is the most common currency used to convert the amount of food consumed by a fish into weight loss or gain.

Therefore, bioenergetics can allow nutritionists to formulate the ration per energy needed for the fish, which can further improve the evaluation of different feed.

1.5 Aim of Study

This master thesis is part of the Institute of Marine Research (IMR) project "Optimal feeding of Ballan wrasse in salmon cages (OPTIfeed)". The project is funded by FHF (The Fisheries and Aquaculture industry's research funding) project number: 901694. The present study aims to compare growth, energy status and survival of juvenile Ballan wrasse fed three different diets offered in both pellets and blocks with and without salmon in net pens. The diet Control were based on previous studies, and consisted of cod filet proteins (30%) and shrimp meal (28%). This is a diet that has been showed to be favourable in terms of palatability, but on the other side not particularly sustainable and is relatively expensive (Kousoulaki et al. 2015). The diet Smart were made as a more sustainable feed compared to Diet Control, containing Black soldier fly larvae meal (20%), krill meal (24%) to replace cod filet proteins and some shrimp meal (14%) for palatability. The third and last diet, was the Soft diet, a standard commercial feed used in many salmon farms as wrasse feed today. Antenna measurements was used to see if there was a preference between pellets and blocks.

Energy status of muscle filet, liver and intestine were analysed using bomb calorimetry for the trial period.

Results from this study can be used to finding a more suitable feed for Ballan wrasse when stock with salmon, and potentially improving the farming efforts of the aquaculture industry.

2. MATERIALS AND METHOD

2.1 EXPERIMENTAL OVERVIEW

The experimental period took place the months of August - November 2021 at the IMR Austevoll Aquaculture Research Station, Storebø, Norway. The analytical work took place at IMR Nordnes, Bergen, Norway, where, energy status of muscle sample, liver samples, and intestinal samples were analysed using a Bomb calorimetry.

2.2 FISH AND EXPERIMENTAL DESIGN

2.2.1. Ballan wrasse

All Ballan wrasses were farmed and provided by IMR Austevoll, born May 2020. Approximately 480 Ballan wrasse with initial mean body weight of 39.9g, were individually labelled by an intraperitoneal injection of PIT tags. The fish were randomly distributed into one of three 500 litre tanks (160 fish/tank) with 24 h light, and water temperature of 12°C. Twenty days were used as an acclimation period to the experimental conditions before starting the adaptation of the different diets. Following the acclimation period, another 20 days were used for adaptation to the different feed. The fish were fed continuously using belt feeders.

After being fed the experimental diets for 20 days, the fish were transferred outside to net pens (5 × 5m square, 5 m deep) with shelter and without salmon at Austevoll (**Figure 2)**. Fifteen days later the salmon were transferred to the net pens with the Ballan wrasse. In order to have the opportunity to take out fish during sampling, the percent admixture of Ballan wrasse was high from the start (10%).

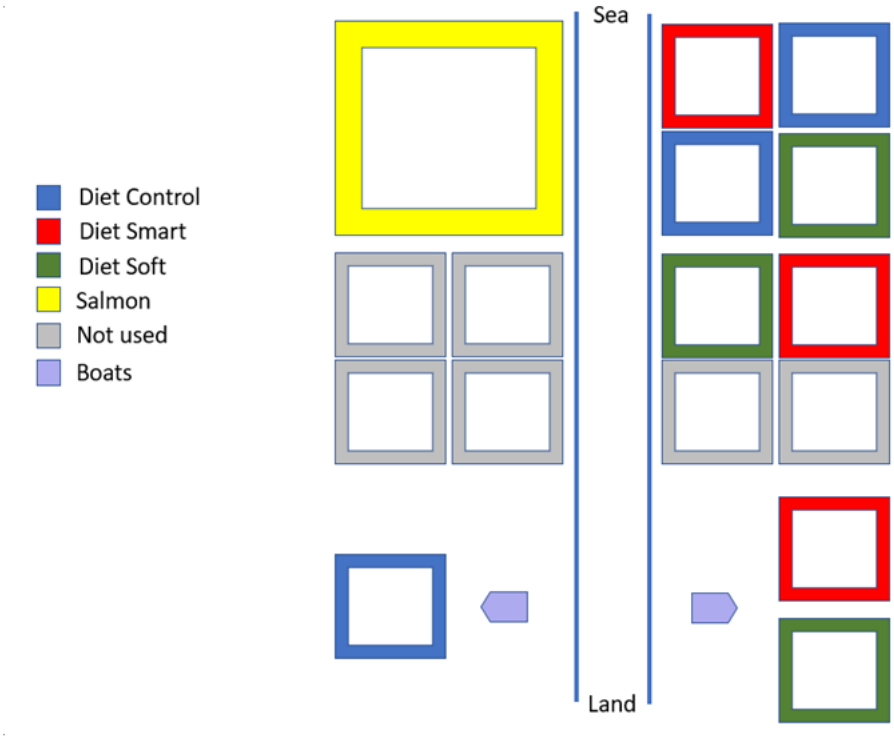

Figure 2: *Schematic of the sea cages and location of the different diets. Cages closes to land were dependent on a boat to board.*

In triplicate, each feed was offered in both bags of 50g pellets and 50g blocks. Six of the net pens were steel framed sea cages connected to land, while three of the net pens were float cages that were dependent on a boat as a bridge to board (**Figure 2)**. The feed was changed every day and placed inside an antenna, making it possible to track whenever the fish were eating. To avoid constant logging of fish hiding in the shelter, the feed and antenna were placed half a meter in front of the shelter (**Figure 3**).

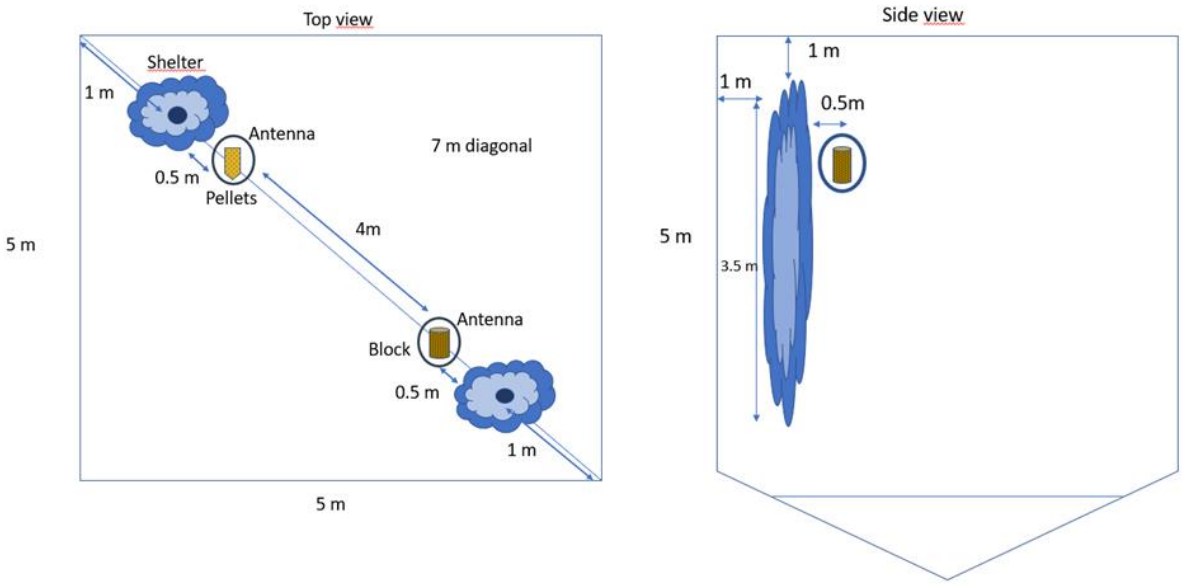

***Figure 3**: Overview of the setup inside the cages with shelter, antenna, and feed. All cages had the same setup.*

Fish from each tank fish were counted and weighed initially, before deployed at sea and at the end of the trial period . Samples for energy analysis were collected four times during the trial period. An initial group of six fish were sampled before the start of the feed experiment, and then six samples of six fish per net pens were taken after 20, 49 and 75 days from the groups fed the different diets. See **Figure 4** for timeline of the trial period. At the end of trail period in November, the fish were transferred to a larger net pen for storage over winter. Survival over the winter was reported for the different treatments over the winter.

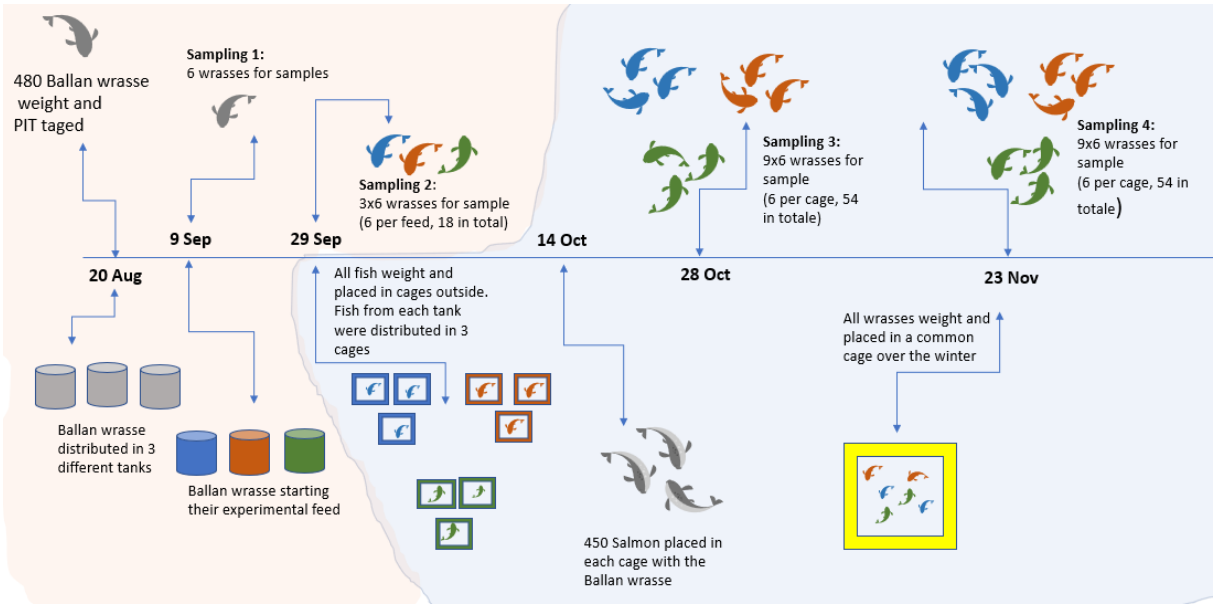

Figure 4: *Study timeline showing the different sampling points, trial phases and duration. Orange colour indicate the land phase while blue colour indicate the sea phase.*

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2.2.2. Atlantic salmon

The salmon was delivered by BIOFISH AS on 22 July 2021 and transferred directly to 12*12 m cage (**Figure 2**) until the start of the experiment. When relocated to the cages with the Ballan wrasse on 14 October, the mean weight for the salmon was 160 grams.

2.3 Feed

2.3.1. Pellets

Three different feeds were used in this experiment. Diet Control and Diet Smart were specifically made for this project by Katerina Kousoulaki at Nofima. In accordance with previous study by Kousoulaki et al. (2021), the feed was prepared using cold extrusion; i.e. feed production technologies using lower processing temperatures. The balance of the feed composition was based on the recommendation by Hamre et al. (2013) of 65% protein, 12% lipid and 16% carbohydrate. The chemical composition in the Control and Smart diets were 58.9% protein, 11% lipid and 11.1 % carbohydrate and 57.9% protein, 10.8% lipid and 12.9% carbohydrate respectively. Diet Soft was a commercial wrasse feed made by Skretting named CLEAN Soft. CLEAN Soft is designed to attract water and be water stable for a while so that wrasses can graze on a soft pellet in a bait bag. Its chemical composition values may vary within a certain range (min and max). This is to give the factory the opportunity to adjust the feed in case of challenges with the physical quality of the pellets. Protein range between 40-45%, lipid 15-16% and Nitrogen-free-extract (NFE) 16-22%. NFE indicates the proportion of carbohydrates, excluding fibre (which

is cellulose and threads and is what the fish cannot break down. This feed was prepared using extrusion.

% NFE = 100% - (% Ether Extract +% Crude Protein +% Ash +% Crude Fibre)

Table 1 presents the percentage of the different feed composition values.

Table 1: *Nutrient composition of the diets fed to juveniles Ballan wrasse*

Diet number		1	2	3
Diet name		**Control**	**Smart**	**Soft ****
Protein	**%**	58,9	57.9	40 - 45
Lipid	**%**	11.0	10.8	15 - 16
Carbohydrates	**%**	11.1	12.9	-
NFE*	**%**	-	-	16 - 22
Ash	**%**	11.9	10.6	7 - 10
Water	**%**	7.1	7.9	-
Fibre	**%**	**-**	-	0.6 – 4.5
Other	**%**	**-**	-	2.5-21.4
Sum		**100**	**100**	**100**
Pellets size	**mm**	3	3	12

The major difference between Control and Smart diet is the protein source (**Table 2**). In the Control diet, the main protein sources were cod muscle and shrimp meal. The main protein sources in Diet Smart were insect meal of black solider fly and some shrimp and krill meal for taste. The percentage of each ingredient in the commercial Soft diet is unknown, but the diet consisted of fishmeal, shrimp meal, fish oil, SPC, faba bean, Calanus finmarchicus, rapeseed meal, wheat and wheat gluten.

Table 2: *Formulation of the experimental diets used in the juvenile Ballan wrasse trials of the current study*

Diet number		1	2
Diet name		**Control**	**Smart**
Shrimp meal [a]	%	28	14
Cod muscle meal [a]	%	30	-
Krill meal [b]	%	-	14
Insect meal(Black soldier fly) [c]	%	-	20
Wheat gluten [d]	%	9.00	24.50
Krill oil [e]	%	4.20	-
Tapioca starch [f]	%	6.56	2.72
SPC [g]	%	8.50	8.00
Krill hydrolysate[h]	%	6.60	6.60
BIOMOSS[i]	%	0.50	0.50
Vitamin C[j]	%	0.23	0.23
Choline chloride[j]	%	0.50	0.50
Cholesterol[k]	%	0.20	0.20
vitamin mix[j]	%	0.50	0.50
Organic mineral[i]	%	0.68	0.68
Rapseed lecithin[l]	%	2.00	3.60
Yttrium oxide[m]	%	0.010	0.010
Lysin[j]	%	-	0.44
MSP[j]	%	2.50	2.90
Methionine[j]	%	-	0.30
Threonine[j]	%	-	0.30
Taurine[m]	%	0.020	0.020
Total	**%**	**100**	**100**

a Provided by Seagarden AS, Norway
b Krill meal made of graske?? Produced by Nofima provided from the raw material of Aker Biomarine Antarctic AS, Norway
c Provided by Innovafeed, France
d Provided by Roquette, France
e Provided by Aker Biomarine Antarctic AS, Norway
f Provided by Idun AS, Norway
g Provided by Selecta AS, Norway
h Provided by Rimforst As, Norway
i Provided by Alltech, Norway
j Supplied by Vilomix, Norway
k Provided by Carbogen AMCIS, Switzerland
l Provided by NOBA, Netherland
m Provided by VWR, Norway

2.3.2. Feed blocks

The feed blocks were made by Karl Sveinsvoll. The blocks were made by soaking pellets in water until soft, and then mix it with 1 kg wheat gluten for every 20 kg pellets. The mixture was then pressed together forming a block. The blocks were stored in a freezer due to the amount of water used to soak the pellets. The blocks were cut to 50 g portions before placed in a bag.

2.4 SAMPLING

Fish for analysis were placed in a bath with an overdose of 100 g/l Finquel for euthanizing. Weight (g), standard length (cm), and OWI-score (Operational welfare indication) for each individual were recorded. The OWI-score focus on physical condition of the fish together with record of fin, skin, and eye damage. The OVI-scored were given a score between 0-3 (**Table 3**)

Table 3: *Scores and definitions of welfare indicators.*

	Score	Definition
Fins	0	No erosion, splitting or rays exposed
	1	Minor erosion or split damage, not to be considered at high risk
	2	Erosion and spilt damaged are more widespread. Not to be consider at high risk.
	3	Clear evidence of erosion or split damaged on fins, recovery unlikely, health status compromised.
Eyes	0	No damage
	1	Some minor damage in one or both eyes
	2	Clear eyes damaged in one or both eyes.
	3	Server damaged in one or both eyes. Health status compromised
Shell loss	0	No shell loss
	1	Some shell loss
	2	Shell loss areas are well defined

	3	Server shell loss, recovery unlikely. Health status compromised.
Skin	0	No damage
	1	Some skin damage or previous wounds (evidence of scars)
	2	More widespread skin damaged. Not to be considered at high risk
	3	Clear evidence of skin damaged, recovery unlikely. Health status compromised.
Operculum	0	No damage
	1	Minor damage, not to be considered at high risk
	2	Damaged areas more prevalent.
	3	Clear evidence of damaged, recovery unlikely. Health status compromised.
Head necrosis	0	No damage
	1	Minor damage, not to be considered at high risk
	2	Damaged areas more prevalent.
	3	Clear evidence of damaged, recovery unlikely. Health status compromised.

After that, dissection of the abdominal part was performed to remove the digestive system. Liver was separated as one sample and the intestinal and the sounding viscera fat as another (referred as viscera from now). An example of appearance of liver and viscera for two different fish are presented in **Figure 5.** The separated organs and the whole fish were weighed, and then put into separate test tubes. The samples were transferred to IMR Nordnes on dry ice and preserved at -32°C for proximate composition analyses.

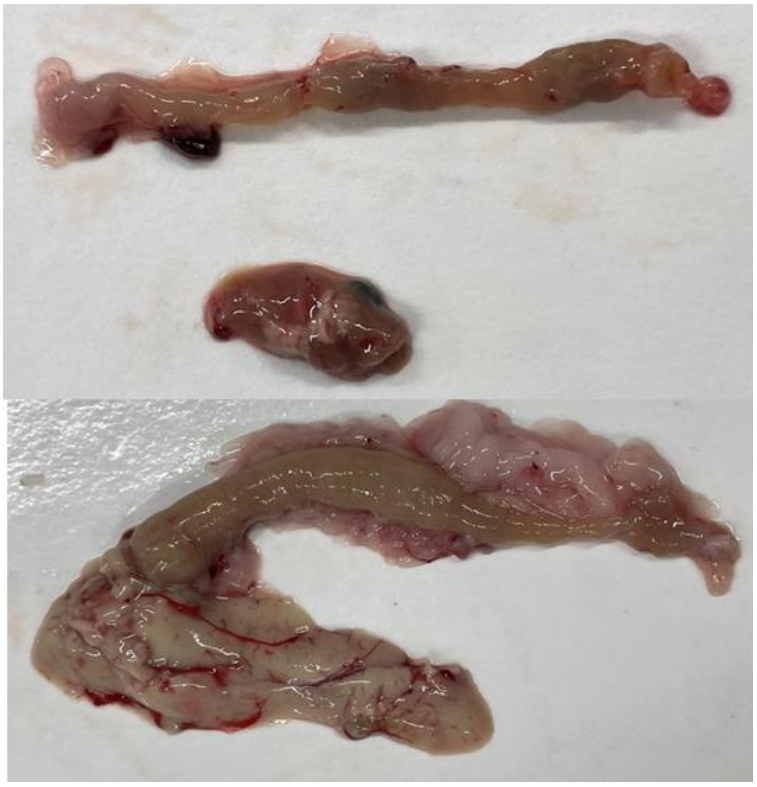


Figure 5*: Picture of liver and viscera of two different sampled fish. Photo: Ida Hansen*

The first sampling happened at 9th of September where an initial group of six fish were taken out (see **Figure 4** for timeline). At this time, the fish had not started their experimental diets.

The second sampling happed at 29th of September. The fish were still on land and been adapted to the experimental diets for 20 days. Prior to sampling, the fish had fasted for approximately 24 h. Eighteen fish were collected for energy analysis and the remaining Ballan wrasses were individually OWI-scored and weighted before transferred outside in net pens. Length was not taken and assumed that it had not changed since the August weighting.

The third sampling happened 29 days after the second one, on 28th of October. Six fish per cage (18 per diet) were randomly collected for further analysis to a total of 54 sampled fish. As previous samplings, OVI- score, weight, liver, and intestine were taken. This time the fish had not been fasted in advance, and intestinal content, if any, was checked by pressing out the feed with a scalpel.

The last sampling happened on 23rd and 24th of November. This time all fish from all the net pens were weighed, measured for length and OWI-scored. Six fish from each net pen were taken for analysis. Tissue samples from the intestine and the brain were also taken for RNA test. The remaining fish were transferred to a common cage (12*12m) for overwintering, given the feed that had the best result so far in the experiment.

There were in total 5 extra fish that were taken out for test sampling, two fish from the Control diet, two from the Soft diet and one from the Smart diet at 29 September. These samples were not used in the results of the sampled fish. 11 fish were also injured after PIT tagging and were removed.

2.5 Analytical methods

2.5.1. Freeze-drying

The gutted sampled fish were filleted free from skin and bones for muscle samples. The muscle samples, liver and intestine were further analysed for dry matter by freeze-drying, conducted by qualified personnel at IMR. Freeze drying is done by placing the frozen samples in a vacuum and then the frozen water is vaporized and extracted from the frozen samples.

2.5.2. Bomb calorimetry

Measurements of energy is expressed in calories (Cal) or Joule (J). The calorie used in nutrition is the energy needed to raise the temperature of 1 g water from 14.5 to 15.5 °C (Bureau et al. 2002). One 15°C calorie is equivalent to 4.184 J.

In nutrition, the enthalpy (ΔH) of combustion is normally referred as Gross energy (GE) (Bureau et al. 2002). GE is the amount of heat liberated when the content of a substance is burned in a bomb calorimeter. The substance is burned in an oxygen-filled cylinder, called a bomb, which is immersed in water. Under these conditions, the hydrogen and carbon are completely oxidized to water and carbon dioxide, as they are in vivo. The heat released during burning of the sample will raise the surrounding temperature of the water. The GE can then be calculated from the weight of the sample, the weight of the water and the rise in temperature.

Finished freeze dried muscle samples were blended to a homogenous mixture using Retch GM 200. Liver and intestine samples were homogenised to a fine mixture using a mortar.

The energy of the homogenised samples was determined using a bomb calorimetry. The GE was determined Parr Calorimeter 6400 Automatic isoperibol calorimeter, and standardised using pellets of benzoic acid at regular intervals with energy density: 26.4 kJ g^{-1} (6.3 Kcal g^{-1}). For muscle samples, between 0.25-0.30 g dry weight material were measured using a gross weight (Sartorius, Universal). Further, the material was pressed using a pellet press, then transferred in a crucibles and weighed on an analysis weight with 4 decimals, then run in the calorimeter.

Due to small amount of liver material, samples were pooled. Samples for the same day, feed and cage were combined, given six samples in each batch and a total of 22 samples. The samples were combined and homogenised using a mortar. 0.25 - 0.35 g of the mixed and pressed material were run in the bomb calorimeter.

The viscera were homogenised in a mortar together with an equal amount of cellulose. The viscera were individually used in bomb calorimetry. Between 0.15 and 0.35 g of the mixed samples were used to make pellets for the calorimetry. A minimum of 0.15 g was needed for the bomb calorimetry to measure the energy. Due to small amounts of material, some samples did not fire, and energy was not taken. From the Control diet, there was one sample from 29 September and two from 23 November that did not run. From diet Smart there were one from 28 October, and three from 23 November that did not run, two samples from same cage were also pooled due to small amount making so it possible to run. From diet soft, there was one from

both 29 September and 28 October, and three from 23 November, that did not run. Here, there were also two samples from the same cage that was pooled and run together from 28 October.

2.5.3. Energy in the feed

Samples of pellets and blocks were checked for energy using bomb calorimetry. Due to unknown amount of moister for some of the feed, energy was calculated assuming no moister and the energy in the feed can thus represent a higher energy than it actually is.

2.5.4. Statical analysis

Statical analysis and data treatment were analysed using Microsoft 365 Excel 2022 (version 2204), and R-studio 2021 (version 4.1.2).

Growth and energy data were analysed by a one-way or a two-way analysis of variance (ANOVA) using "Diet" and "Date" as factors, followed by Tukey's Hones Significant Difference test (Tukey's HSD) when relevant to find out which specific diets and date that were different compared with each other. Differences were considered statistically significant at $p < 0.05$.

Person's product-moment correlation test were used to see if there was a correlation between organs. For correlation tests, the *p-value* was based on the null hypothesis; that true correlation is equal to 0. All data were assumed to be normally distributed when constructing confidence intervals.

Specific growth rate (SGR), condition factor (K), Liver somatic index and Viscera somatic index were calculated as follow:

SGR (% day^{-1}) = 100 * (ln (final biomass, g) – ln (initial biomass, g))/ (time, days)

LSI (%) = (Liver weight/ body weight) *100

VSI (%) = (Viscera weight/ body weight) * 100

Fulton's condition factor (K) = (weight/ length^3) *100

3. RESULTS

3.1 GROWTH PERFORMANCE

3.1.1. Weight

Both diet and time had an effect on mean body weight (g fish^{-1}) (ANOVA $p < 0.001$). There was a significant difference in weight between treatments at each time point except between fish fed Smart and Soft in September (diff= 1.1 g, Tukey $p = 0.5$) (**Table 4**). Fish fed Control and Smart had the highest growth pre-deployment (20 Aug-29 Sep) and increased in weight by 22.8% (Tukey $p < 0.001$) and 15.0% (Tukey $p < 0.001$) respectively. After deployment to the sea and until the end of trial (29 Sep- 23 Nov), the weight gain was less for fish fed Control and Smart with 4.7% (Tukey $p = 0.14$) and 5.8% (Tukey $p = 0.057$) respectively. Overall, fish fed Control and Smart increased in weight with 28.6% (Tukey $p < 0.001$) and 21.7% (Tukey $p < 0.001$) respectively, for the whole trial period (**Table 4**).For fish fed Soft there was weight gain of 3.3% (Tukey $p = 0.23$) pre-deployment. After deployment and until the end of trial, fish fed Soft decreased in weight with 6.4% (Tukey $p = 0.011$). Overall, fish fed Soft decreased in weight with 3.3% (Tukey $p = 0.34$).

The six fish that were taken for sampled on 9 September had a mean body weight of 35.6 (SD: 7.09) g. While fish taken for sampled on 28 October had a mean body weight of 49. 0 (SD: 8.9, n=18), 42.2 (SD: 5.82, n=18), 38.7 (SD: 8.05, n=18) g for fish fed Control, smart and Soft respectively.

3.1.2. Condition factor

Both diet and time had an effect on condition factor (g cm^{-3}) (ANOVA $p < 0.001$). There was a significant difference in K-factor between treatments at each time point except between fish fed Smart and Soft in September (diff= 0.058 g cm^{-3}, Tukey $p = 0.18$) and between fish fed Control and Smart in November (diff= 0.035 g cm^{-3}, Tukey $p = 0.21$) (**Table 4**). Overall, fish fed Control had significant higher K-factor then fish fed Soft for each time point. Fish fed Control and Smart had a significant increased condition factor from 1.83 (SD: 0.15) to 2.25 (SD: 0.29) and 1.75 (SD: 0.17) to 2.00 (SD:0.27) g cm^{-3} ,respectively in the land phase. There was no significant difference in K-factor from 1.88 (SD: 0.17) to 1.94 (SD: 0.29) for fish fed Soft diet in the land phase. In the sea phase there was a significant decrease in K-factor for all treatments, varying from 1.80 (SD: 0.19), 1.76 (SD: 0.17) and 1.60 (SD:0.13) for fish fed Control, Smart and Soft, respectively.

Table 4:*Mean body weight (g) and condition factor (K, g cm-3) development off all fish for the different diets at three time point. Standard deviation in paratheses, Samples size(n). Samples were taken initially when the fish were on land and had not started their experimental diets (20 Aug), right before transferred to sea (29 Sep), and at end of the trial period (23 Nov). Weight and K-factor were analysed by a One-way ANOVA, with significant levels (*p<0.05*; *p<0.01**; *p<0.001***). Orange colour indicates land phase, while blue colour indicates the sea phase.*

	Control	**Smart**	**Soft**	***p*-value ANOVA**
20 August	39.8 (7.8) K = 1.83 (0.15) n= 162	37.3 (7.7) K = 1.75 (0.17) n=162	42.6 (7.1) K = 1.88 (0.17) n= 162	*p* <0.001***
29 September	48.9 (9.7) K = 2.25 (0.29) n= 156	42.9^{a} (9.3) K = 2.00^{b} (0.27) n= 158	44.0^{a} (8.7) K = 1.94^{b} (0.29) n=155	*p* <0.001***
23 November	51.2 (12.2) K = 1.80^{c} (0.19) n= 129	45.4 (11.3) K = 1.76^{c} (0.17) n= 127	41.2 (7.6) K = 1.60 (0.13) n=123	*p* <0.001***

Notes:
Different letters in superscripts indicate no significant differences (p > 0.05)

3.1.3. Specific Growth Rate (SGR)

There was a significant difference in SGR between all treatments for period 1 (20 Aug – 29 Sep), varying from 0.48 (SD: 0.29), 0.33 (SD: 0.36) and 0.04 (SD: 0.54) % day^{-1} for fish fed Control, Smart and Soft respectively (**Figure 6**). For period 2 (29 Sep – 23 Nov) there was no significant difference in SGR between fish fed Control and Smart with an SGR of 0.08 (SD: 0.23) and 0.11 (SD: 0.25) % day^{-1}, accordingly. Fish fed Soft had a significantly lower SGR than fish fed Control and Smart, with an SGR of -0.12 (SD: 0.41) % day^{-1}. Going from Period 1 to Period 2 the SGR decreased significantly for all diets.

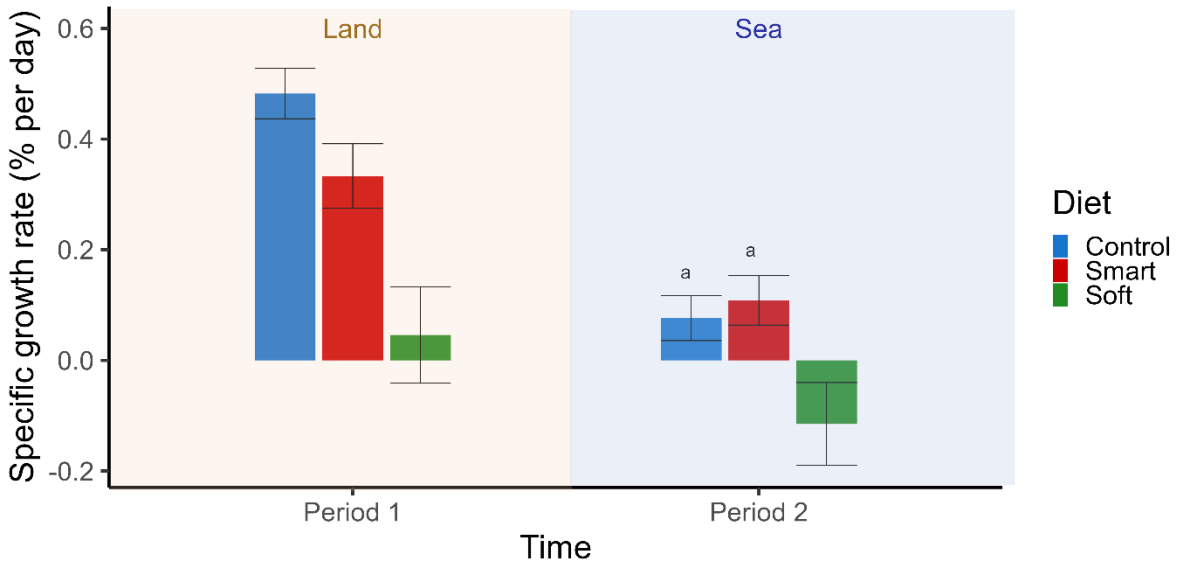


Figure 6: *Specific growth rate (SGR, % day^{-1}) with confidence intervals for all Ballan wrasse over two periods. In Period 1 the fish were on land, while in period 2 the fish were at sea. Period 1 lasted for 40 days while period 2 lasted for 55 days. For period 1 (Control n=151; Smart n=149; Soft n=150), for period 2 (Control n=129; Smart n=121; Soft n=118). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase.*

3.1.4. OWI-score

Ballan wrasses were most affected by pectoral fin erosion. All fish independent of treatment had rather similar percentage damage on pectoral fin erosion. Fish fed the Smart and Soft diet had higher percentage of damage of shell loss compared to fish fed Control diet pre-deployment (**Figure 7**). In general, independent of treatment, the OWI-score was improved in the sea compared to land (**Figure 8**). There was almost no registered shell loss at end of trial, compared to pre-deployment and there was less severe damage on pectoral fin.

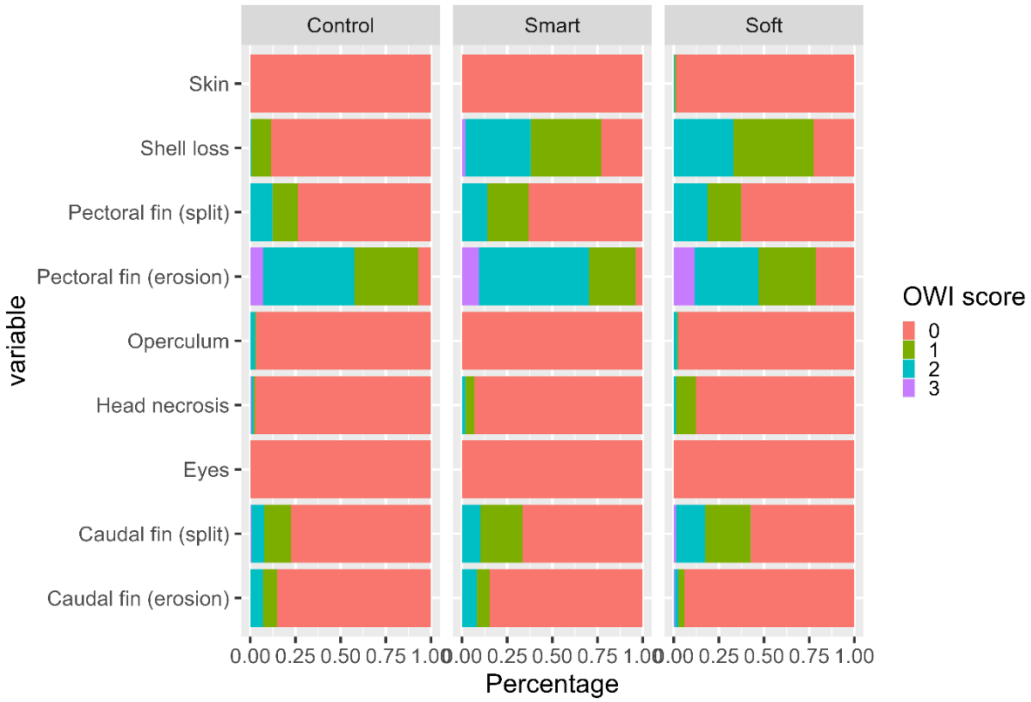


Figure 7: *Operational welfare indicators (OWIs) of all fish before deployment (29 Sep, n=469) shown are the percentage of fish scored on a 4-point scale (score 0-3). Each indicator, depending on the extent and severity of each condition*

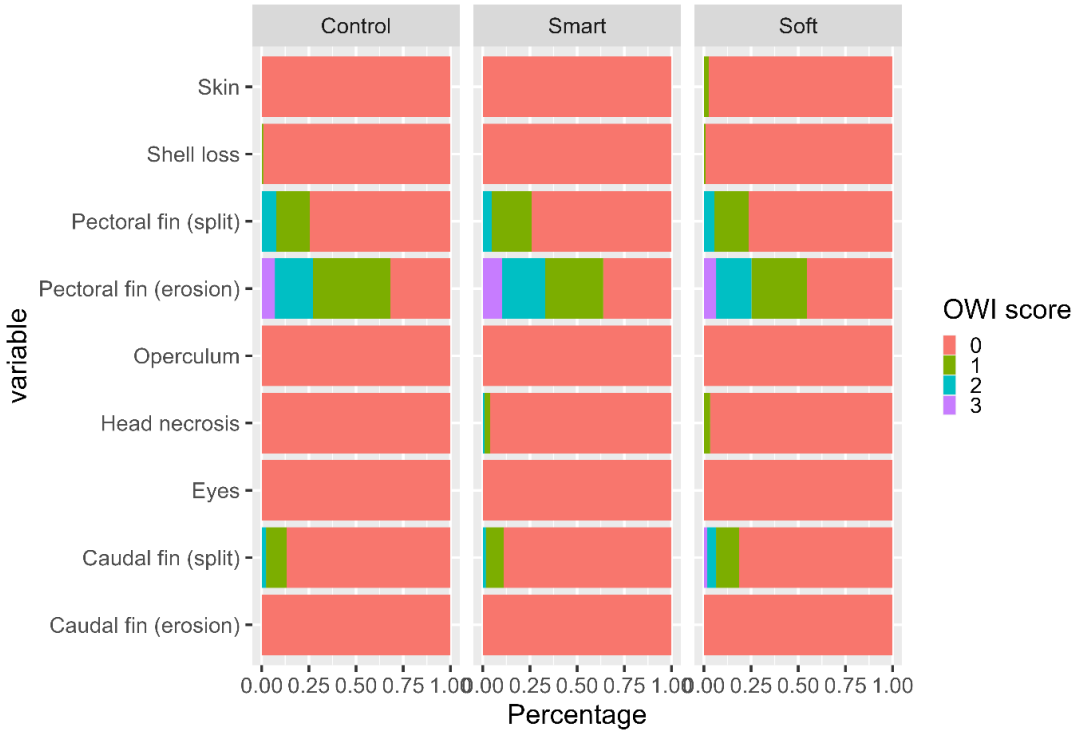


Figure 8: *Operational welfare indicators (OWIs) of all fish at end of trial (23 Nov, n=379) shown are the percentage of fish scored on a 4-point scale (score 0-3). Each indicator, depending on the extent and severity of each condition*

3.2 Organ Index

3.2.1. Viscera somatic index

There were no significant interactions between time and diet on Viscera somatic index (VSI, %) of total body weight (ANOVA *p*= 0.4). Both time and diets did have an effect on ISI *(*ANOVA *p*=0.001) and (ANOVA *p* <0.001) respectively. There were no significant differences in VSI between diet Control and Smart at any time point (**Figure 9**). Fish fed Control had an VSI of 3.04(SD: 1.08), 2.24 (SD: 0.68) and 2.62 (SD: 1.01)%, while fish fed Smart had an VSI of 2.51 (SD: 0.35) 1.86 (SD: 0.47) and 2.21 (SD: 0.86)% in September, October and November respectively. Fish fed Soft had a mean VSI of 2.26 (SD: 0.50), 1.57 (SD: 0.32) and 1.38 (SD: 0.29)% for Sep, Oct and Nov respectively. Diet Control and Soft were not different in Sep, but diet Soft was significantly lower than diet Control in Oct and November. Diet Smart and Soft were only significant different in November.

The fish that were given the Soft diet, had a significant lower VSI when in the sea than on land (29 Sep-23 Nov) (diff = 0.88%, Tukey *p* = 0.0014). The Control and Smart diet had no significant change in VSI between dates.

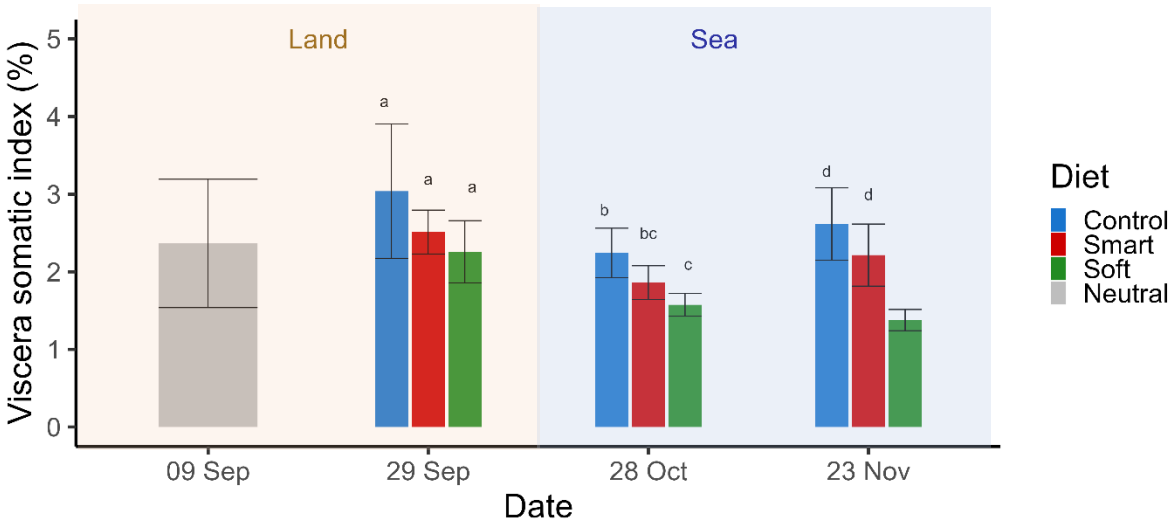


Figure 9: *Viscera somatic index (ISI, %) with confidence intervals of total body weight for Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land and had not started their experimental diets (Netural). 29 September (Control n=6; Smart n=6; Soft n=6), the fish been on land, but transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Significant codes (*p<0.05*; p<0.01**; p<0.001). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase.*

3.2.2. Liver Somatic Index

There was no significant interaction between diet and time on Liver somatic index (LSI, %) (ANOVA p=0.07). Both time and diet did have an effect on LSI (ANOVA p<0.001). On 9 September the Liver somatic index (LSI, %) (Neutral) was 1.22 (SD: 0.33) % (**Figure 10**). There were no significant differences in LSI between diet Control and diet Smart at any of the time points. The LSI for fish fed Control was 1.49 (SD: 0.51), 1.02 (SD: 0.53), and 2.04 (SD: 1.07) %, while the LSI for fish fed Smart was 1.20 (SD: 0.17) 1.07 (SD: 0.58) and 1.94, for September, October and November, accordantly. The LSI for fish fed Soft was 0.79 (SD: 0.28), 0.69 (SD: 0.14) and 0.86 (SD: 0.33) % for September, October and November, respectively. There was no significant difference in LSI between fish offered diet Control and Soft in October (diff =0.33 *%,* Tukey p = 0.091). In September and November, the fish offered diet Soft had a significantly lower LSI of with 0.70 and 1.18%, respectively, than fish fed Control. There was no significant difference in LSI between diet Smart and Soft in September (diff = 0.41%, Tukey *p = 0.14)*. In October and November, fish fed Smart had a significant higher LSI with 0.39 and 1.08% respectively, than fish fed Soft.

There was no significant change in LSI for fed Control and Smart between 9 September and 28 October, but both diets had a significant increase in LSI in the second half of the sea phase (28 October- 23 November) with 1.02 (Tukey p = 0.0014) and 0.87% (Tukey p < 0.0089) accordantly. Fish offered diet Soft had a significant decrease in LSI with 0.37% (Tukey p = 0.028) from 9 September until the end of trial.

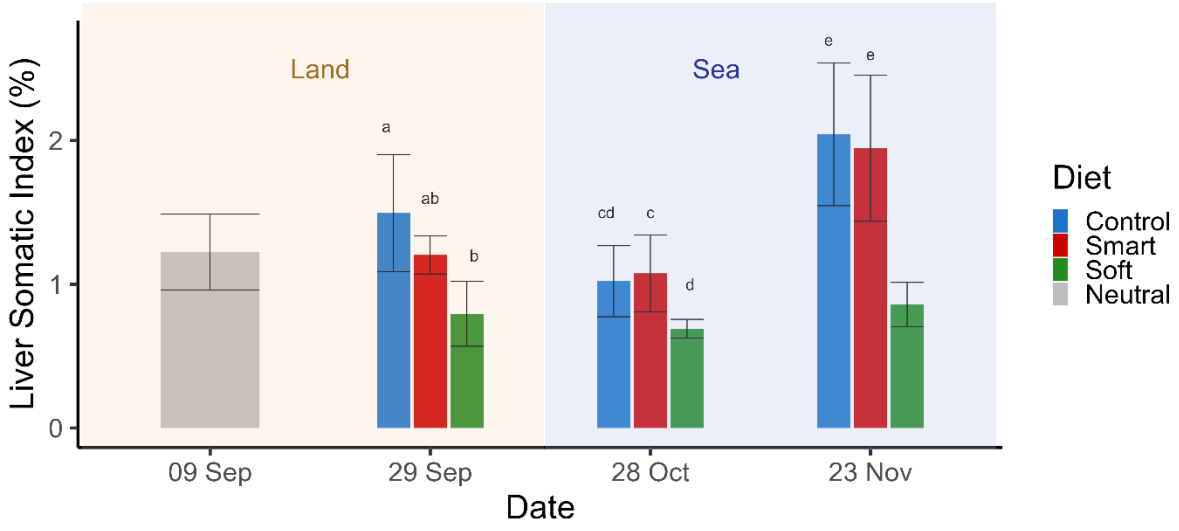


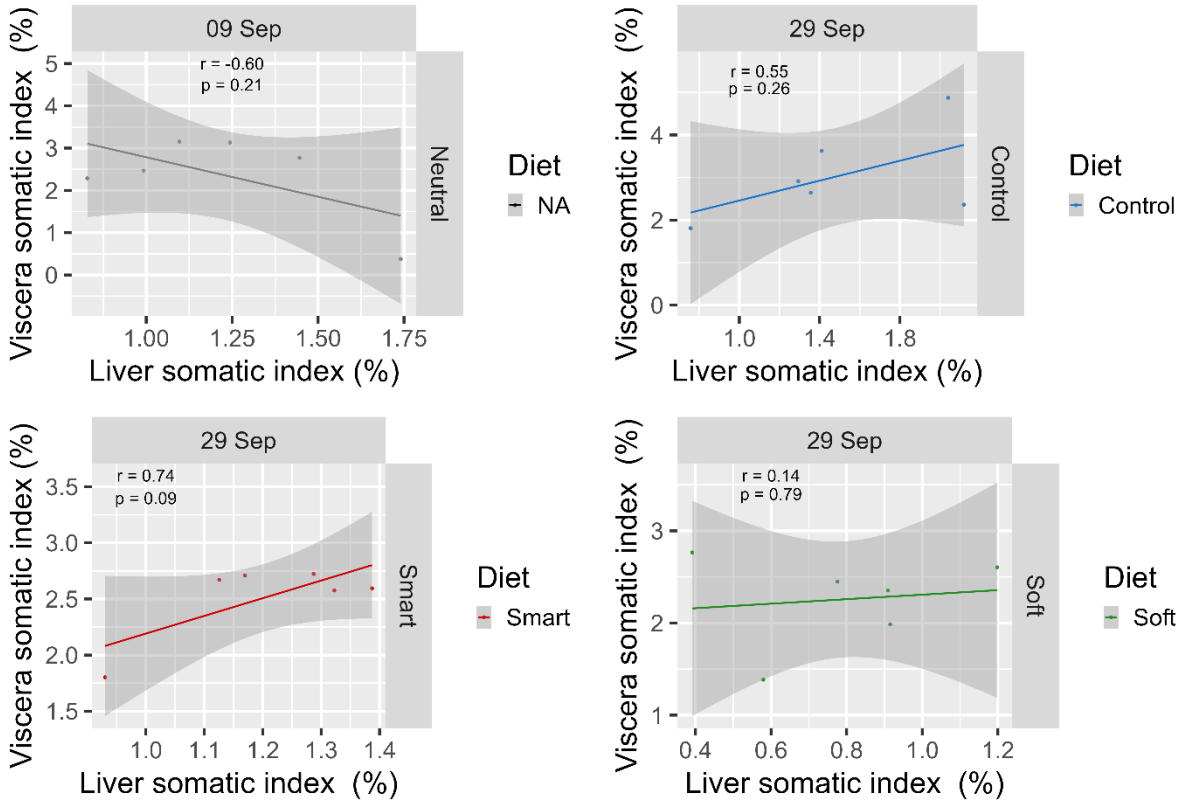
Figure 10: *Liver somatic index (LSI, %) with confidence intervals of total body weight for Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land and had not started their experimental diets (Neutral). 29 September (Control n=6; Smart n=6; Soft n=6), the fish been on land, but transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Arrow indicated line between land and sea. Significant codes (*p<0.05*; p<0.01**; p<0.001). *Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase.*

3.2.3. Correlation Index

There was no significant correlation between LSI and VSI from 9 Sep (r=-0.60, p=0.21) (**Figure 11**). There was also no significant correlation between LSI and VSI for any of the different diets on 29 Sep; Control (r=0.55, p=0.26); Smart (r=0.74. p=0.094); Soft (r=0.14, p=0.79).

From 28 October there were a significant correlation between LSI and VSI for Control (r=0.66, p=0.0027) and Smart (r=0.72, p=0.00084). For the Soft diet on the other hand there were no significant correlation between LSI and VSI index (r=0.38, p=0.12).

Diet Control (r=0.90, p=<0.0001) and Smart (r=0.85, p= <0.0001) did have a significant correlation between LSI and VSI on 23 November. There was no significant correlation between LSI and VSI for diet Soft at any time point.



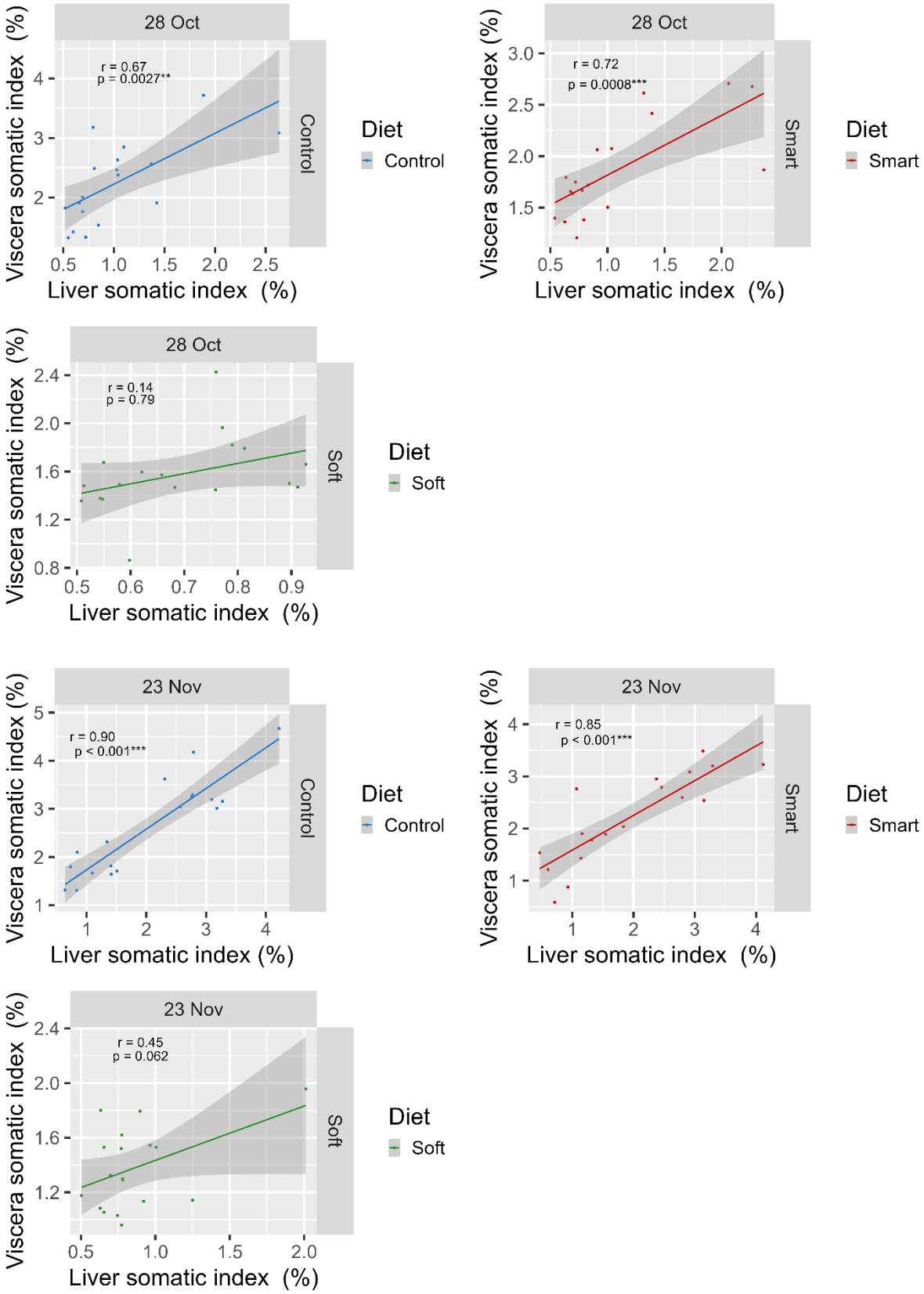


Figure 11: *Pearson correlation coefficient, r, and p-values for each diet between liver somatic index (LSI) and viscera somatic index (ISI) at each time point.*

3.3 ENERGY

3.3.1. Feed

The energy in pellets was 463, 481 and 480 (kcal/100g) for Control, Smart and Soft respectively. While the energy in Blocks were 367, 369 and 378 (kcal/100g) for Control, Smart and Soft respectively.

3.3.2. Muscle Energy

The mean start energy (Kcal/100g) from the muscle samples are presented in From 9 September the energy was 122 (SD: 5) **(Figure 12).** For 29 September, October and November fish given the Control diet had a mean energy of 121 (SD: 4), 115 (SD: 4), and 113 (SD: 7) respectively. While diet Smart had mean energy of 118 (SD: 4), 111 (SD: 4) and 111 (SD: 6), and diet Soft had a mean energy of 120 (SD: 5), 109 (SD: 3) and 106 (SD: 3) from.

The interaction between diet and date did not have a significant effect on energy (ANOVA, p=0, 20). However, both time and diet had a significant effect on energy in muscle (ANOVA, $p < 0.001$). Fish offered the Control diet decreased with 7.4% (Tukey p = 0.0046) at the end of trial compared 9 September, while fish offered the Smart and Soft diets decreased with 9.0% (Tukey, $p < 0.001$) and 13.1% (Tukey, $p < 0.001$) respectively. There was no significant difference in energy between the various diets in September (ANOVA p = 0.79). For October, fish fed Control had a significant higher energy compared to both fish fed Smart (diff = 4.5 Kcal/100g, Tukey *p = 0.0012)* and fish fed Soft (diff = 5.9 Kcal/100g, Tukey *p < 0.001).* For November, fish fed Control had a significant higher energy than fish fed Soft (diff = 6.9 Kcal/100g, Tukey *p < 0.001)*, but not fish fed Smart (diff = 2.4 Kcal/100g, Tukey *p = 0.45)*. There was no significant difference in energy between fish offered the Smart and Soft at any time (ANOVA p = 0.055).

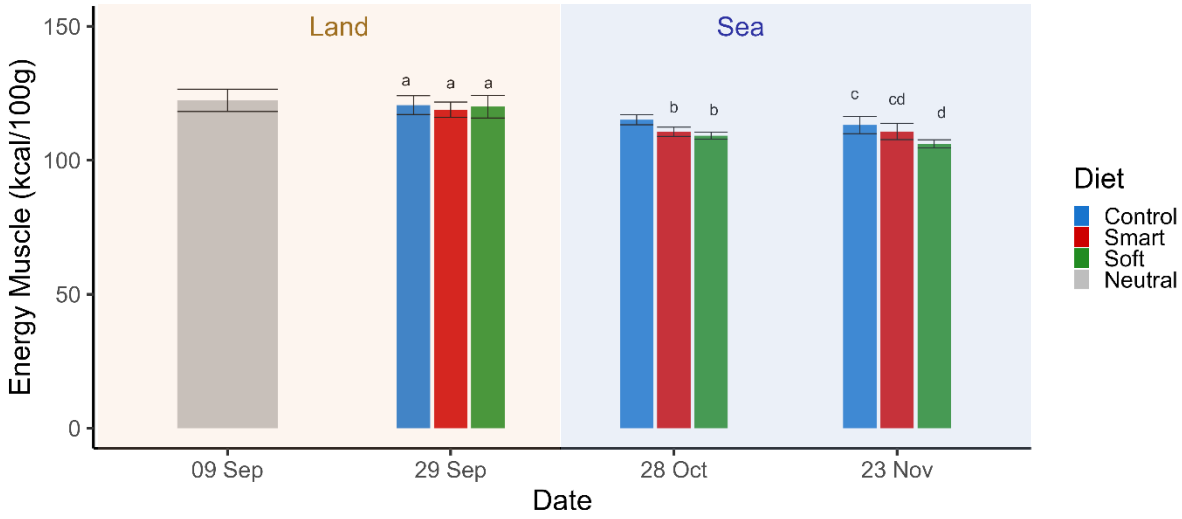


***Figure 12**: Mean Energy (Kcal/100g) with confidence intervals from muscle samples from Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land and had not started their experimental diets (Neutral). 29 September (Control n=6; Smart n=6; Soft n=6), the fish were transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Significant codes (*p*<0.05*; *p*<0.01**; *p*<0.001). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase*

3.3.3. Viscera Energy

Before starting the experimental diets, the mean energy (Kcal/100g) in the viscera was 349 (SSD: 70) **(Figure 13**). In September, October and November, fish offer the Control diet had an energy of 375(SD: 147), 275 (SD: 101) and 235 (SD: 78) respectively. For fish fed Smart the mean energy was 263 (SD: 101), 179 (SD: 55), and 227 (SD: 79), while the mean energy for fish fed Soft was 345 (SD: 139), 173 (SD: 78) and 151 (SD: 66). The interaction between time and diet did not have a significant effect on energy (ANOVA $p = 0.058$). both time and diet had an effect on energy with (ANOVA $p < 0.001$) for both. Fish fed Control decreased with 32.7% (Tukey, p = 0.081) at the end of trial compared 9 September, while fish offered the Smart and Soft diets decreased with 35.0 % (Tukey *p = 0.0072)* and 56.7 % (Tukey p < 0.001) respectively. There was no significant difference in energy between fish fed Control and Smart (diff = 111 Kcal/100g, Tukey *p = 0.33),* and between Control and Soft (diff = 29 Kcal/100g, Tukey *p = 0.92)* in September. From October the energy of Control fish was significantly higher than for fish fed Smart (diff = 96 Kcal/100g, Tukey *p = 0.0031)* and Soft (diff = 102 Kcal/100g, Tukey *p = 0.0023)*. There were no significant differences in energy between fish offered the Smart and Soft (diff = 6 Kcal/100g, Tukey *p = 0.98)* diets in October. From November fish fed Control and Smart had significantly higher energy than fish fed Soft.

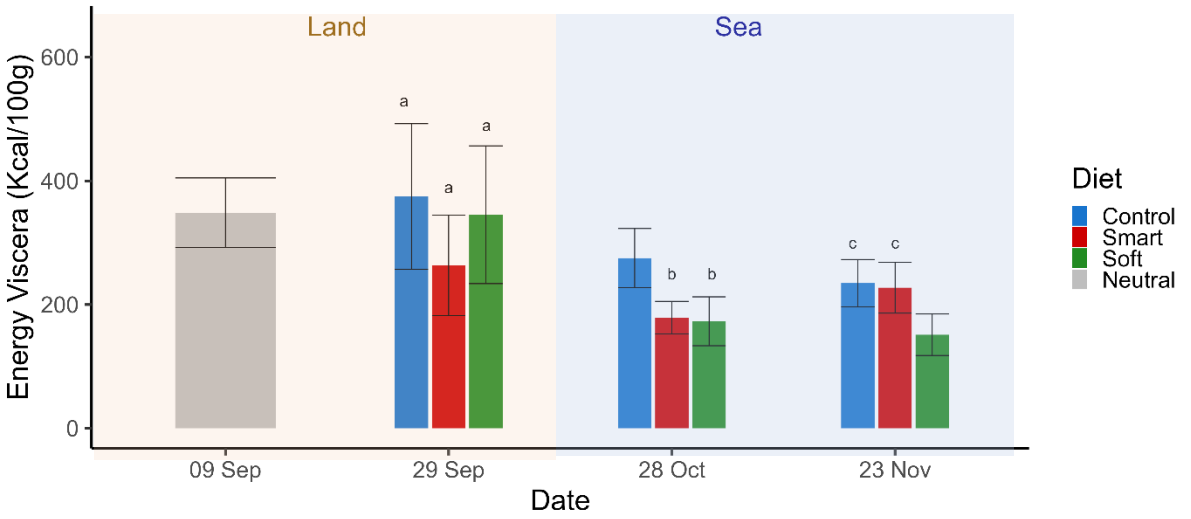


Figure 13: *Mean Energy (Kcal/100g) with confidence intervals from Viscera samples for Ballan wrasse given the different diets at all four samplings date. On 9 September (n=6), the fish were on land and had not started their experimental diets (Neutral). On 29 September (Control n=6; Smart n=6; Soft n=6), the fish were transferred to sea. 28 October (Control n=17; Smart n=16; Soft n=15) and 23 November (Control n=16; Smart n=14; Soft n=15), the fish were at sea. Significant codes (*p<0.05*; p<0.01**; p<0.001). *Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase*

3.3.4. Liver Energy

The pooled mean energy (Kcal/100g) from the liver samples from 9 September (Neutral) was 272 (**Feil! Fant ikke referansekilden.**) From September, October and November the pooled energy for fish offered the Control diet was 247, 250 (SD: 33) and 241 (SD: 9). While fish offered the Smart diet had a mean energy of 214, 215 (SD: 61) and 249 (SD: 15), and for fish offered the Soft diet the energy was 249, 164 (SD: 47) and 231 (SD: 72). Fish offered the Control diet decreased with 11.4% at the end of trial compared 9 September, while fish offered the Smart and Soft diets decreased with 8.5% and 15.1% respectively.

Due to small sample size, no statistic was run.

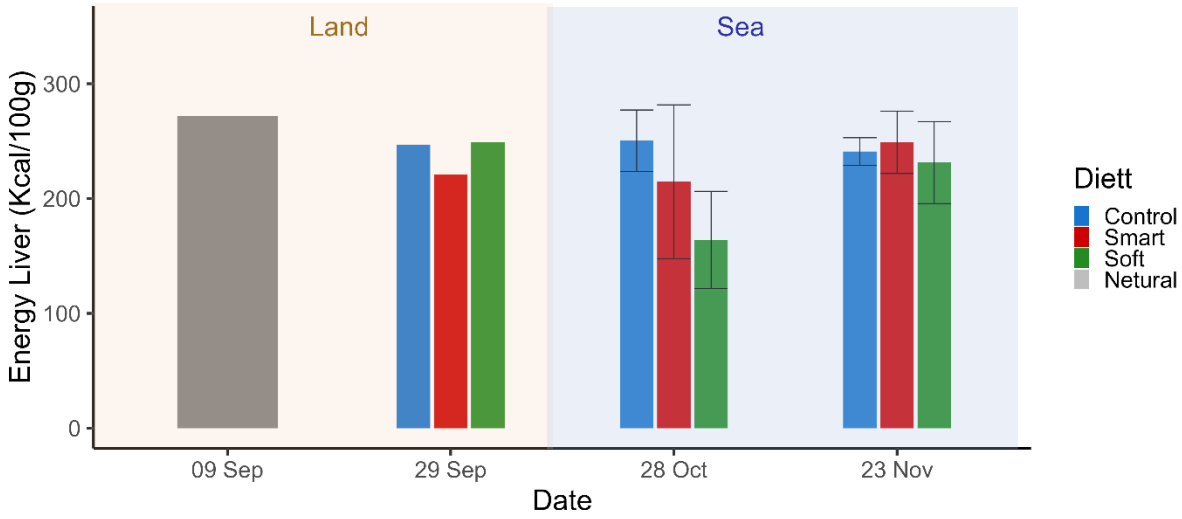
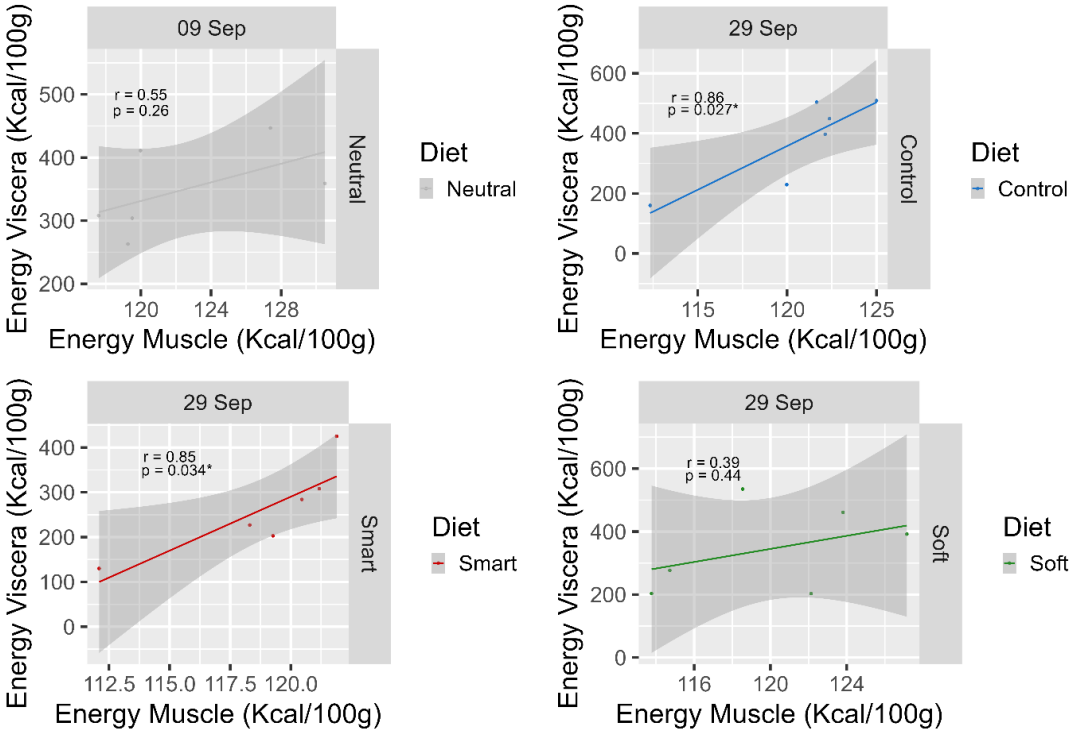


Figure 14: *Mean pooled energy (Kcal/100g) with confidence intervals from liver samples for Ballan wrasse given the different diets at all four samplings date. On 9 September (n=1), the fish were on land and had not started their experimental diets (Neutral). On 29 September (Control n=1; Smart n=1; Soft n=1), the fish were transferred to sea. 28 October (Control n=3; Smart n=3; Soft n=3) and 23 November (Control n=3; Smart n=3; Soft n=3), the fish were at sea. Orange colour indicates the land phase, while blue colour indicates the sea phase*

3.3.5. Correlation Viscera-Muscle Energy

There was no significant correlation between viscera (VE) and muscle energy (ME) in the start of the trial (r=0.55, *p*=0.26) on 9 September (**Figure 15)**. Fish fed Control had a significant correlation between VE and ME for all the sampling dates. Fish fed Smart had a significant correlation between VE and ME in Sep (r= 0.85, p = 0.034) and Nov (r= 0.90, p < 0.001) but not in October (r= 0.46, p= 0.062). Fish fed Soft had only a significant correlation in November (r = 0.76, p = 0.0026).



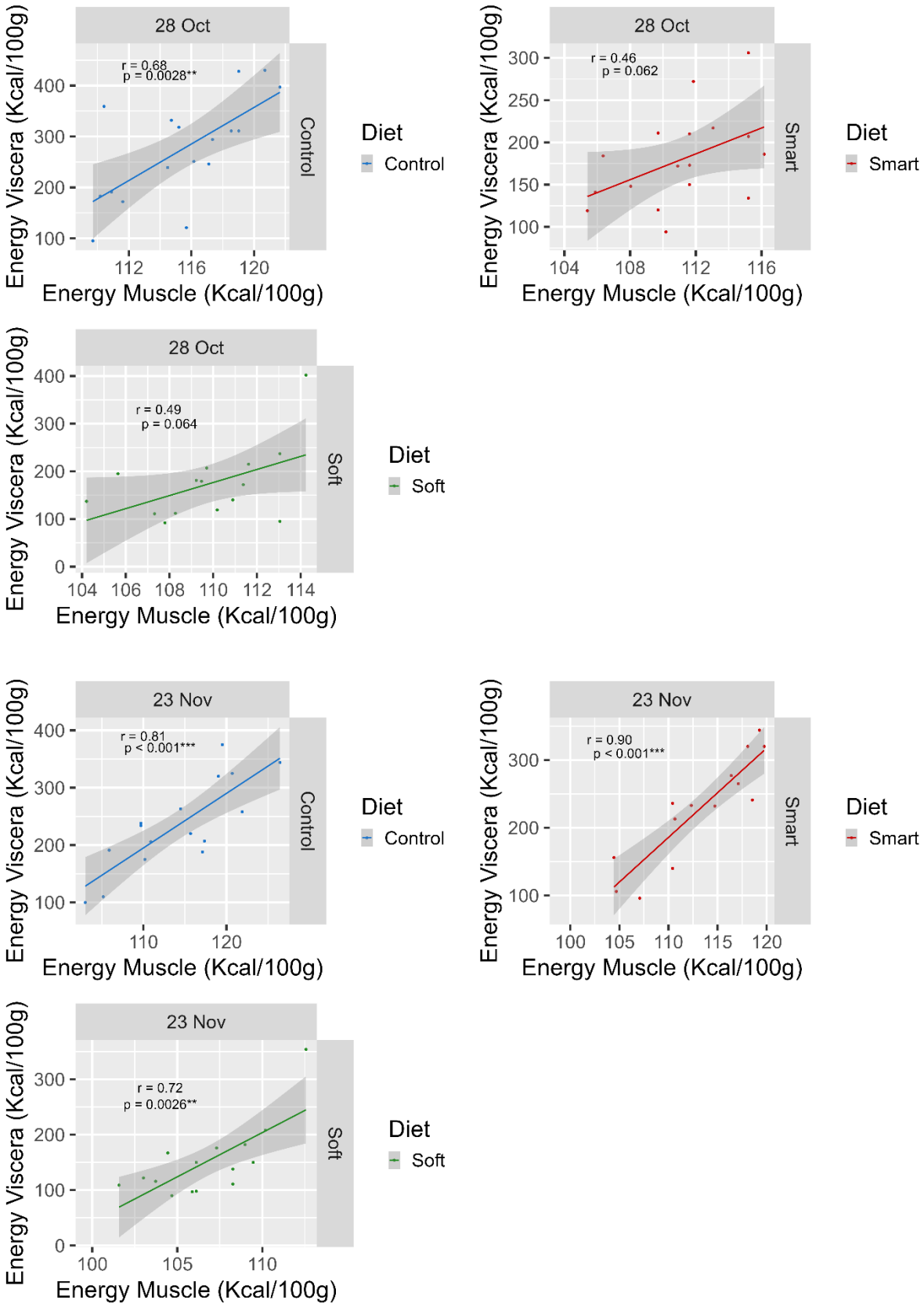
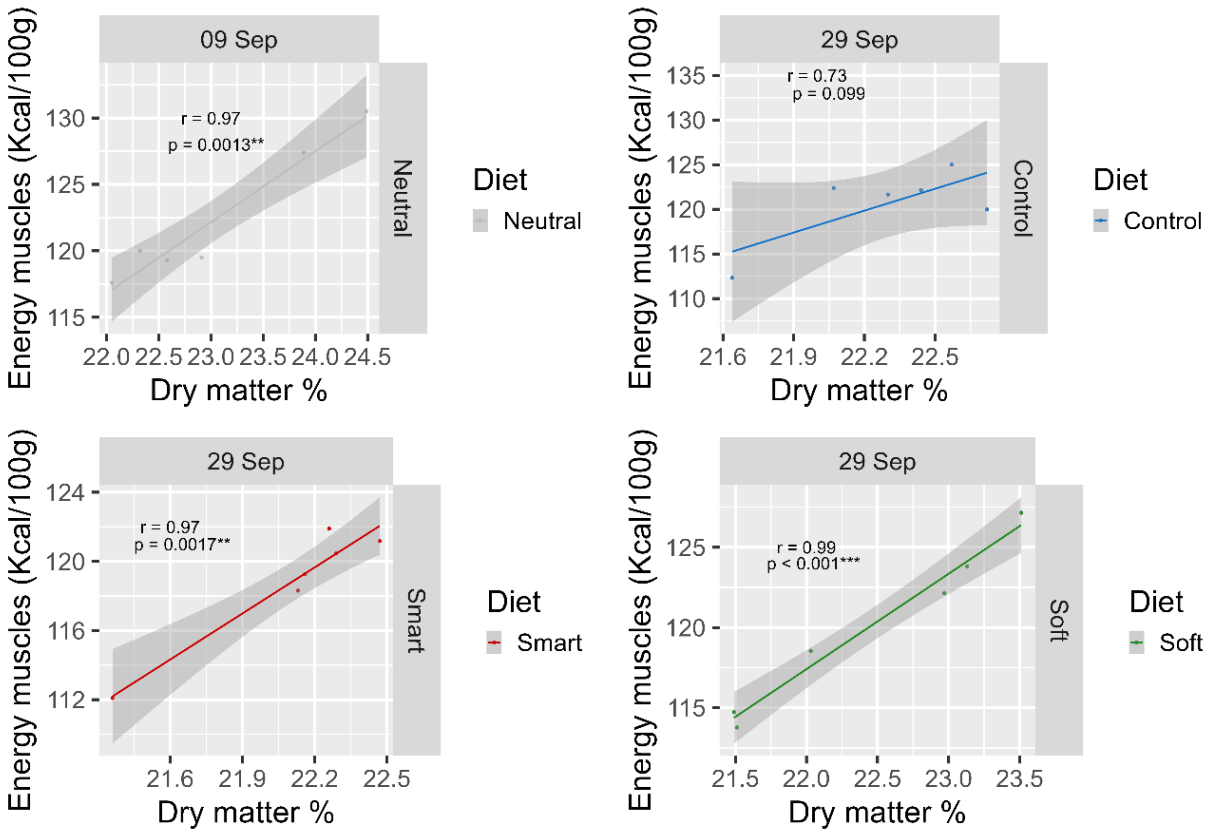


Figure 15: *Pearson correlation coefficient, r, and p-values for each diet between viscera energy (VE, kcal/100g) and Muscle energy (Kcal/100g) from different time points.*

3.4 Dry matter Correlation

3.4.1. Muscle Dry matter

There was a significantly correlation (r > 0.97) between the dry matter percentage and energy in the muscles for all the samples, except for fish offered the Control diet on 29 September (r = 0.73, p = 0.099) (**Figure 16**).



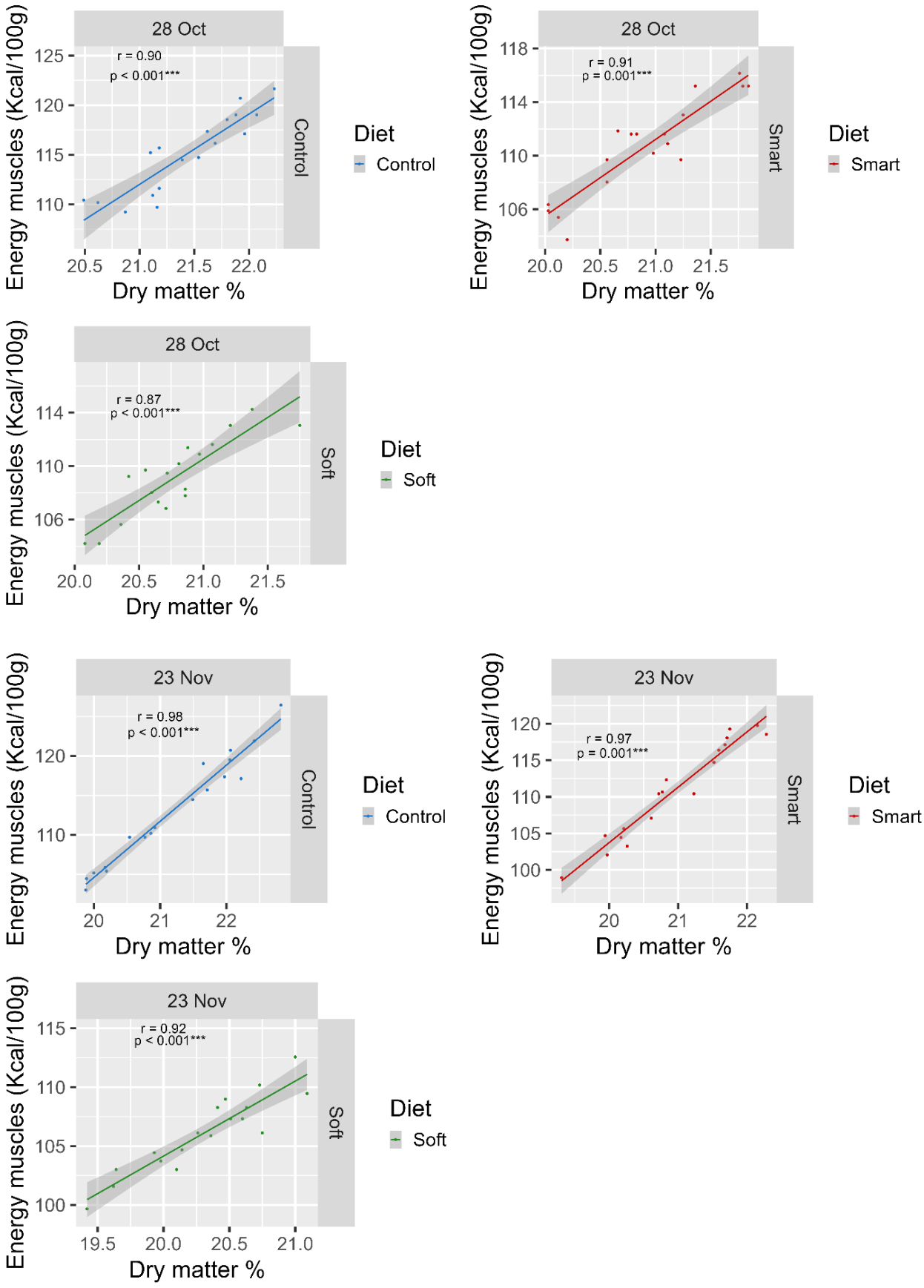
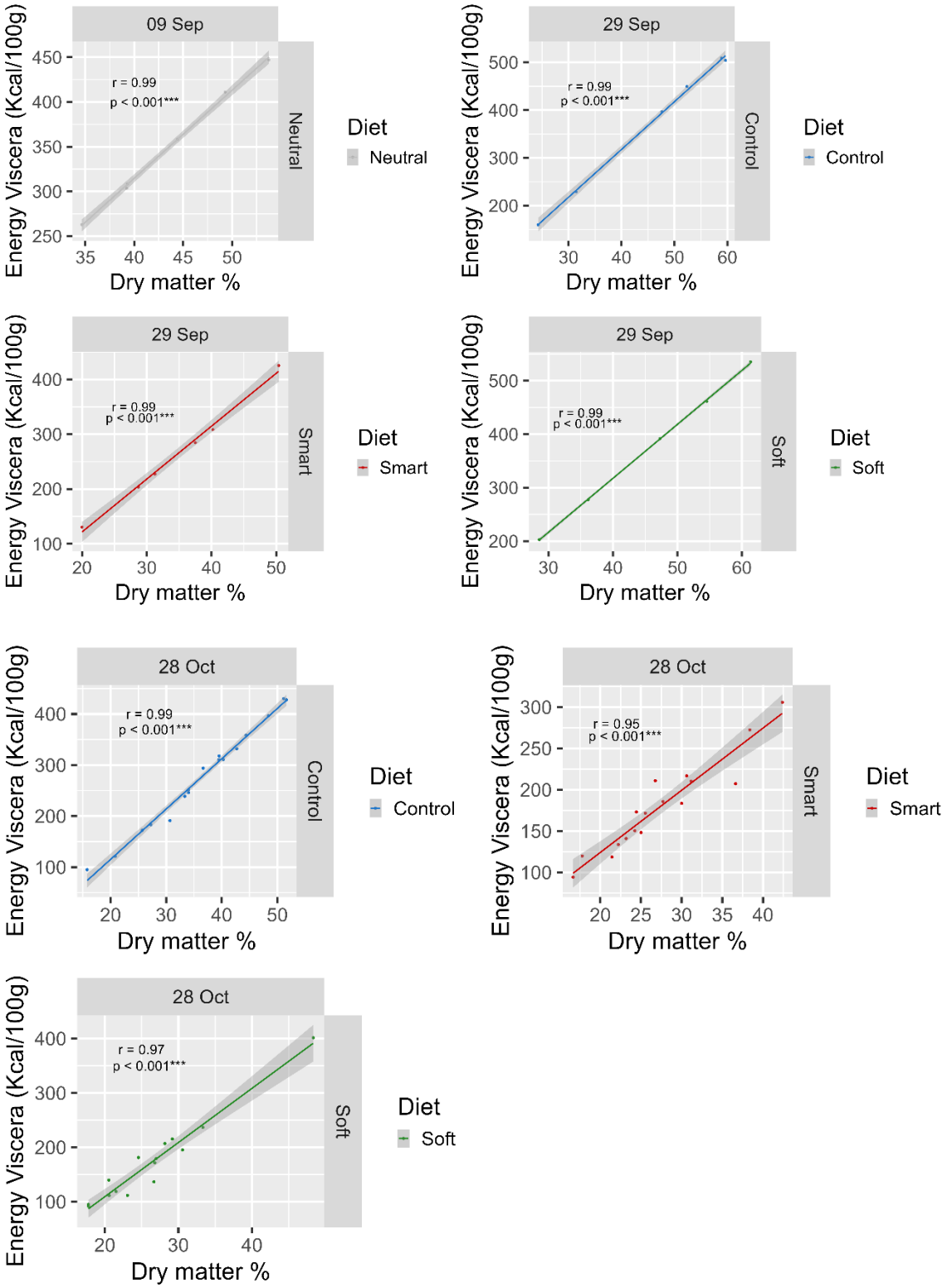


Figure 16: *Correlation between dry matter (%) and energy in muscles (kcal/100g) at different time point.*

3.4.2. Viscera Dry matter

There was a significant correlation between all samples for all time points (**Figure 17**)



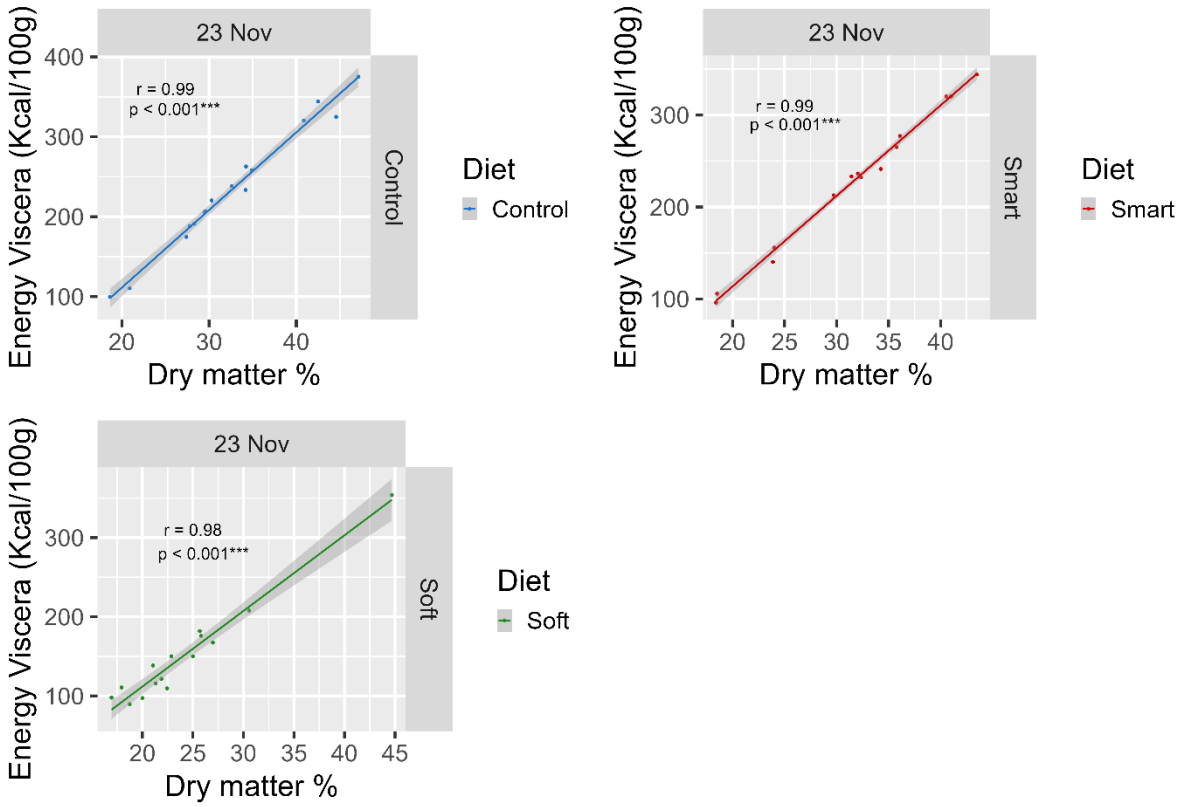


***Figure 17**: Correlation between dry matter (%) and energy in the intestinal (kcal/100g) at different time point.*

3.4.3. Liver Dry matter

There was a significant correlation between dry matter and energy for fish offered the Smart diet for both dates, while fish offered the Soft diet had a significant correlation in October but not in November **Figure 18**. There was no significant correlation between dry matter and energy for any of the dates for fish offered the Control diet.

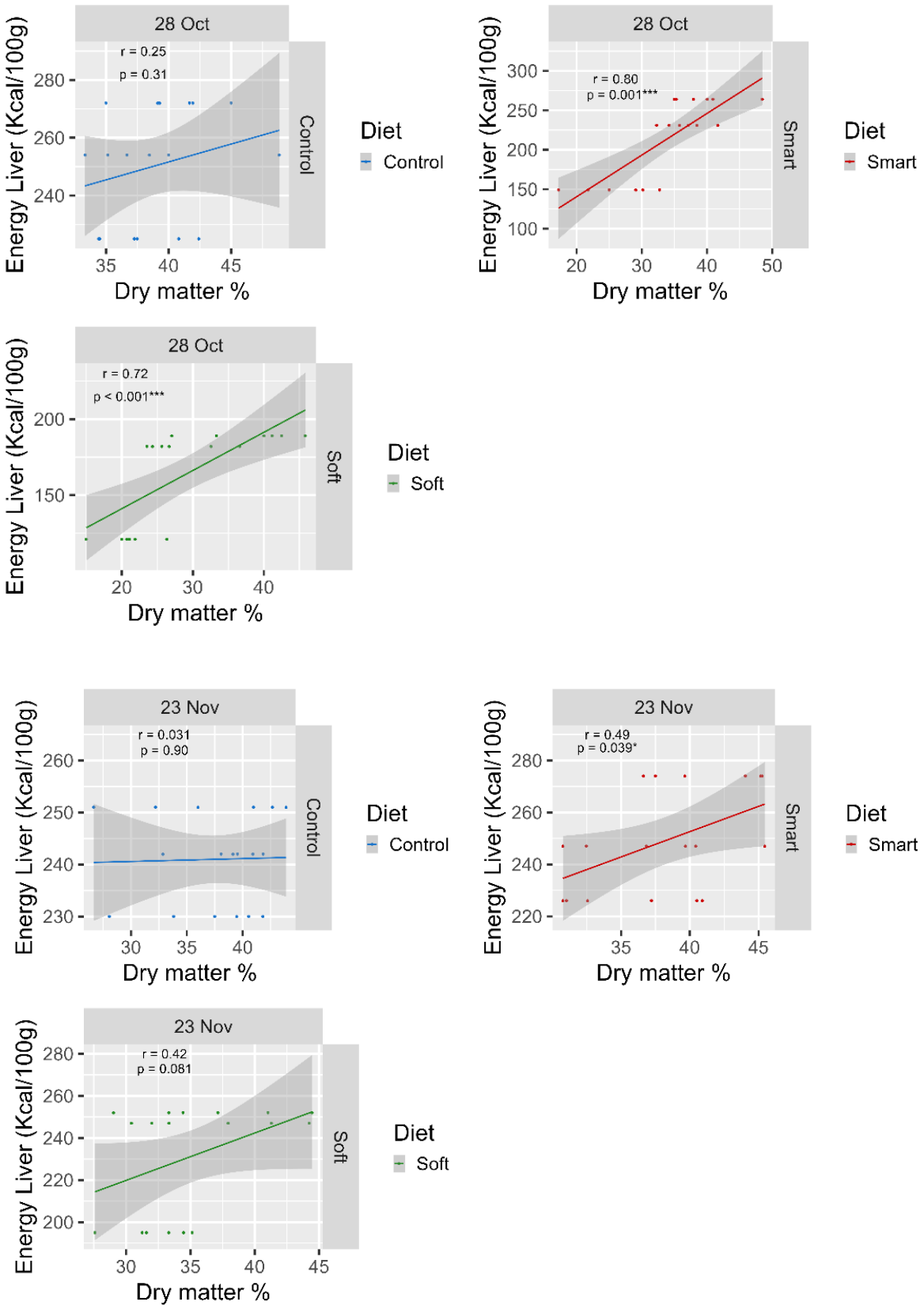


***Figure 18**: Correlation between dry matter (%) and energy in the liver (kcal/100g) at different time point.*

3.5 SURVIVAL

There was no registered death during the land phase. During the sea phase mortality rate was not significantly different indicated by overlapping confidence intervals between treatments (**Table 5**), varying between 0.8, 4.5 and 4.7% for fish fed Control, Smart and Soft, respectively.

Mortality rate over winter in common cage had a higher mortality rate than during the trial period, varying between 22.5, 19.3 and 27.6% for fish fed Control, Smart and Soft (**Table 6**). There was no significantly difference in mortality rate between treatments indicated by overlapping confidence intervals. There was also registered fish that were missing, if including missing fish the mortality rate increased to 55.0, 53.2 and 69.5% for fish fed Control, Smart and Soft respectively.

Table 5: *Mortality rates for fish during the sea phase (29 Sep – 23). ns = no significant difference ($p < 0.05$) indicated by overlapping confidence intervals (CI).*

	Control	Smart	Soft	Significance
Start fish number (29 Sep)	148	151	147	-
Fish number for sample (28 Oct)	18	18	18	-
End fish number (23 Nov)	129	127	123	-
Final mortality (%)	0.8 (CI: 0 – 2.3)	4.5 (CI: 1.0 – 8.0)	4.7 (CI: 1.0 – 8.3)	ns

Table 6: Mortality rates for fish over winter (Nov 2021 – Feb 2022). ns = no significant difference p < 0.05) indicated by overlapping confidence intervals (CI).

	Control	Smart	Soft	Significance
Fish number transferred to common cage for overwintering	111	109	105	-
Fish number in February 2022	50	51	32	-
Number dead fish	25	21	29	-
Number missing fish	36	37	44	-
Mortality rate (%) Not included missing	22.5 (CI: 14.8 – 30.3)	19.3 (CI: 11.9 – 26.7)	27.6 (CI: 19.1 – 36.2)	ns
Mortality rate (%) Included missing	55.0% (CI: 45.2-64.4)	53.2% (CI: 43.8 – 62.6)	69.5% (CI: 60.7 – 78.3)	ns

3.6 REGISTRATION

3.6.1. Temperature

Temperature (°C) was registered during the trial period at 0.5-meter and 5-meter depth (**Figure 19**). Temperature was measured every day.

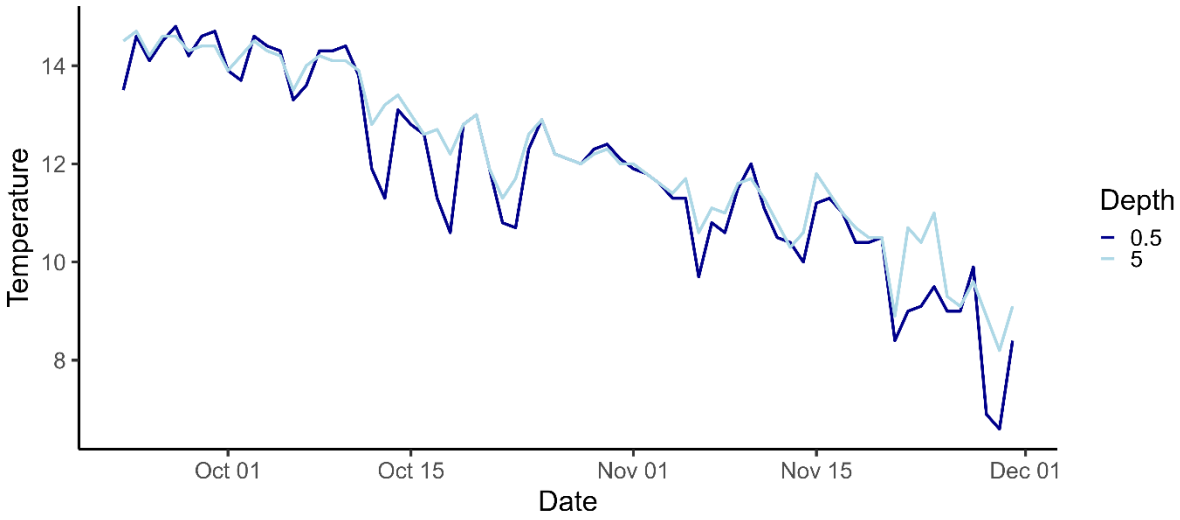


Figure 19: Temperature (°C) at sea during the trial period at 0.5 m and 5 m depth

3.6.2. Antenna registration

Fish fed Control had most registrations from antenna data on block in October but change to pellets in November. However, there was no large differences in registration between pellets and Block for fish fed Control in November (**Figure 20**) While fish fed the other diets had most registration on pellets. There was an increase in number of registration in November compared to October for all the treatments. The antenna data also showed a clear daily feeding pattern, where the wrasse was feeding between 08:00 and 15:00, following the daylight in the winter region

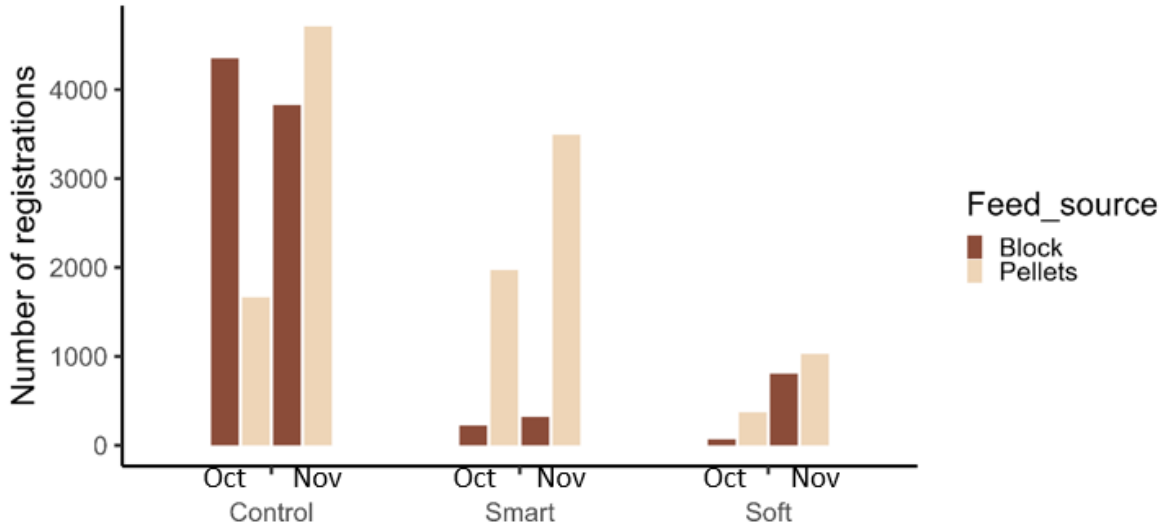


Figure 20: *Number of antenna registrations in October and November for fish offered the different diets and their preference feed source.*

3.6.3. Intestinal content

Most of the sampled fish from October and November did not have any feed (NA) in the intestinal **Figure 21**. However, some fish fed Control and Smart, had some fish feed in the intestinal.

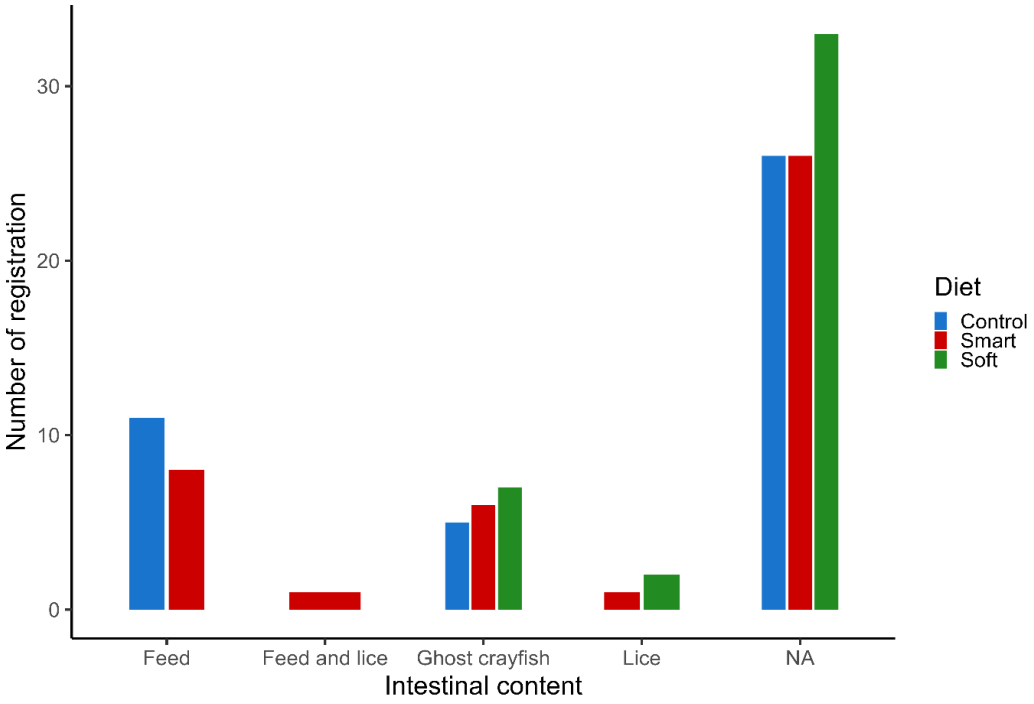


Figure 21: *Feed content from sampled fish from October and November. NA indicates no feed content.*

4. Discussion

4.1. Main findings

The salmon industry would like to increase the number farmed Ballan wrasse, deployed to fight sea lice and to reduce pressure on wild stocks. However, challenges regarding slow growth, poor welfare and high mortality rate in all life stages, have hindered the dominating use of farmed Ballan wrasse. This study has focused on Ballan wrasse nutrition in the salmon net pens, theorising that the right nutrition and feed composition could address these challenges.

Results from the present study demonstrated that fish offered the Control diet achieved better results in terms of increased growth (i.e., weight gain and CF), energy status at the end of trial, closely followed by fish offered the Smart diet. Fish offered the Control diet increased their body weight with 28.6% over the three-month trial period while fish fed the Smart diet increased their body weight with 21.7%. For fish fed the commercial Soft diet the weight decreases by 3.3%. This group also had lower energy storage and survival over the winter compared to fish offered the other experimental diets.

4.2 Dietary effect on Growth

The present trial supports previous studies that inclusion of high-quality protein sources such as, shrimp, krill and cod muscle in Ballan wrasse diets had a positive effect on growth and survival of the fish compared to diets containing fishmeal (Kousoulaki et al. 2015, Bogevik et al. 2016, Kousoulaki et al. 2021). This study also shows a similar positive effect on growth for diet with insect meal (Smart), as for diet with cod muscle meal (Control).

Before deployment, there was a clear effect on SGR between treatments. Fish offered the Control diet had an SGR of 0.48 % day^{-1}, which is in accordance with previous studies by Cavrois-Rogacki et al (2019) at similar rearing temperature (SGR of 0.5 % day^{-1}). The fish used in the study by Cavrois-Rogacki et al (2019) were about half the size of the fish used in the present study. Other studies have demonstrated that at the same temperature, SGR is at its highest during the early stages of the fish and declines thereafter (Brett and Groves 1979, Jobling 1996). Considering that the SGR of fish offered the Smart diet (0.33% day^{-1}) were not as high as Control diet or the study by Cavrois-Rogacki, it could still be a reasonable SGR in relation to the fish size. This does not explain why fish offered the Soft diet had an SGR of 0.04% day^{-1}, but can rather be explained by the diets.

As previously described, Hamre et al (2013a) found that the highest growth for juvenile Ballan wrasse was obtained with 65% protein. In the present study fish offered the commercial Soft diet

had a protein level between 40-45%. Previous studies have shown that increasing the protein level in the diets from 45%to 55% for olive flounder (*Paralichthys olivaceus)* and European sea bass (*Dicentrarchus labrax)* resulted in increased weight gain of 16% in flounder and 23% in sea bass (Ballestrazzi et al. 1994, Kim et al. 2002). The low protein levels can be an explanatory factor why fish fed Soft did not increase as much in weight, compared to the fish fed Control and Smart that had a protein level of almost 60%.

Another reason for the slow growth for fish fed Soft, can be due to the content of free amino acids and small peptides. Krill and other crustaceans has higher level of free amino acids and small peptides than those found in fishmeal (Kousoulaki et al. 2013). Kousoulaki et al (2015) suggest that the content of these free amino acids and small peptides can have positive effects on stimulating increased feed consumption, and that juvenile Ballan wrasse might need higher levels of available protein than what is found in fishmeal, and in this case the commercial Soft diet. Bogevik et al (2016) also suggested that ethoxyquin present in fish meal or/and the higher lipid oxidation level of fish oil may act as a feeding repellent or mask the attractants received from the crustacean feed components for Ballan wrasse larvae. This might be the case for juvenile Ballan wrasse as well. That the slow growth in the present study for fish offered the Soft diet, may be due to the negative effect of dietary fishmeal on feed intake.

The slow growth of fish offered the Soft diet can also be related to feed technology. All diets were extruded, but it is uncertain if the Soft diet was extruded with high or low temperature. Considering high temperature is the most common, this could be the case here as well. Kousoulaki et al (2021) presented that slow growth was related with high temperature extrusion due to poorer digestibility of some proteins and minerals. However, a study by Cavrois-Rogacki et al (2022) contradicts the finding of Kousoulaki et al (2021), where there was no large effect of high temperature extrusion on growth, condition and survival.

In general, the fish performance on land reflected the fish performance in the net pens. There was a significant decrease in SGR for all the diets in the salmon net pens. However, while fish offered the Soft diet had a negative SGR (0.12% day^{-1}) and lost weight, fish fed Control and Smart had a positive SGR and continued to gain weight. The SGR of fish fed Smart was (0.11 % day^{-1}) higher than fish fed Control (0.08% day^{-1}), but they were not significantly different. The weight gain for fish offered the Control and Smart diet is in contrast to a previous study where Ballan wrasse lost weight within six weeks of deployment (Skiftesvik et al. 2013). Despite that, fish fed Control and Smart did better in terms of growth. They also had the highest standard deviation implying that there were large differences within the group. If comparing the number of visits at

the feeding stations (antenna data) and intestinal content during the sea phase, there was fewer registrations for fish offered the Soft diet and no feed was registered in the intestine compared to fish offered the other diets. This can indicate that fish given the Soft diet had a lower feed intake, thus supporting Ballan wrasse pickiness towards feed that contains fish meal.

Irrespective of the diet offered, all the fish performed better in terms of weight gain, K-factor and SGR when they were on land compared to the sea. The decrease in K-factor and SGR after deployment can be due to increased stress due to handling and changing habitat. Additionally, fish can exhibit a reduced swimming activity in tanks compared to their natural environment at sea, hence resulting in a higher condition factor and SGR on land (Skiftesvik et al. 2013). However, wild fish has often been used as a reference of the assumption that their nutrient requirements are met by their natural diet. In this study, the K-factor for all fish at sea (1.6-1.8) were rather similar to what has been found for wild Ballan wrasse (Hamre et al. 2013a, Cavrois-Rogacki et al. 2021).

4.3 Dietary effect on Energy

Ballan wrasse store energy in both liver, muscle and viscera, where the viscera had the highest energy, followed by the liver. There was a significant difference in energy between treatments. At the end of trial, fish offered the Soft diet had a 4.1%, 6.2% and 35.7% lower energy in the liver, muscle and viscera respectively, compared to fish offered the Control diet. Compared to fish fed Smart, fish fed Soft had 7.2%, 4.5% and 33.5% lower energy in the liver, muscle, and viscera respectively. Fish fed Smart and Control had small differences in energy in all the organs. Fish fed Control had also a strong correlation between energy in the viscera and muscle for all the time points ($r > 68$). This correlation can indicate that when energy is absorbed, it is stored both in the muscles and viscera. Considering all diets contain some percentage of shrimp meal, this can indicate that changing cod muscle meal with fishmeal compared to insect - and krill meal, results in lower energy storage.

4.3.1. Growth vs Energy storage

Ballan wrasse natural habitat is from northeastern Atlantic Ocean outside Norway to Morocco and thus able to tolerate large differences in temperature (Yuen et al. 2019). As temperatures changes so does the energy requirement. Several studies have showed that many wrasses exhibit reduced feeding and activity behaviour in the winter (Sayer et al. 1993, Deady et al. 1995, Yuen et al. 2019). If feed intake is reduced over the winter, energy storage becomes important for

survival. For juvenile fish, growth and energy storage, stored primarily as lipids, are the most important factors to overcome the two major causes of mortality: predation and starvation (Sogard 1997, Huss et al. 2008). The fish must therefore balance the need to out-grow predators by maximising their growth, while accumulate a sufficient energy storage that can help them sustain through the winter. When resources are limited, juvenile fish usually face a trade-off of balancing demands for energy between growth or storage. Vulnerability to size-dependent predators decreases with size, and selection for fast growth rather than store energy is normally preferred for juvenile fish (Sogard 1997). Increasing growth can also increase size of prey ingested and thus increase their probability of survival during the winter when temperature decreases. This might explain why fish in this present study fish gained weight at the end of trial compared to the neutral start on 9 September, despite their energy storage decreased. The increased growth usually leads to an higher level of water and lower levels of lipids in the fish (Shearer 1994). The larges decrease in energy for all treatments happened in the viscera. Fish offered the Control diet decreased with 32.7% at the end of trial compared 9 September, while fish offered the Smart and Soft diets decreased with 35.0 % and 56.7 % respectively. This demonstrates that the primary organ for energy storage and use is the viscera. Villegas-Rios et al (2014) also suggested that the major energy allocated to reproduction derived from the viscera for Ballan wrasse.

4.3.2. Organ Index

By comparing the organ index with energy there was a decrease in LSI and VSI for all diets after deployment. This was probably due to utilization of stored fat which is also showed by a decrease in energy storage. However, fish offered the Control and Smart diets approximately doubled the LSI towards the end of trial compared a month after deployment. If comparing this to the temperature there was a decrease in temperature from October towards November, going from around 12-13 to around 9-10 degrees. The increase in LSI is in line with previous findings by Cavrois-Rogacki (2019) were fish had increased LSI reared in 10°C compared to 13°C and 16°C. The authors suggested that at a lower temperature, Ballan wrasse juveniles use their liver to stock energy from lipids, probably as glycogen. This is in a similar way to that of cod (Jobling 1988) and common carp (*Cyprinus carpio*) (Shikata et al. 1995). In addition, Cavrois-Rogacki (2019) observed that the lipid content in the liver decreased as the temperature decreased and hypothesised that their liver glycogen reserves was used as energy sources at low temperatures. If comparing the LSI with the energy, there was not that much change in energy compared to the

increase in LSI during this period. As glycogen may bind between 2.7 and 4 time its own weight in water (Fenn 1939), the increase in LSI may represent a high water content.

There was large difference in correlation between LSI and ISI index for the different treatment but also the different date. Reasons for this is unclear but could be impacted by both diet and temperature. Analysis of lipid, protein and carbohydrate could thus be interesting to shed further light on the effect from the different diets.

4.4 Dry matter and energy

There was a strong correlation between dry matter and energy in the intestine for all diets ($r \geq 0.97$). This is in accordance with previous work for a variety of species where high correlation between energy and dry matter ($r > 0.97$) has been reported (Johnson et al. 2017). There was a similar strong correlation between energy and dry matter in the muscles ($r = 0.87$-0.99). However, for the Control diet on 29 September, the correlation between dry matter and muscle energy is associated with higher uncertainty ($r = 0.73$, p-value= 0.099). Reasons for this is unclear, but could be due to an incorrectly executed method, such as poorly homogenised samples resulting in a misleading energy. For the liver samples the correlation between dry matter and energy were associated with great uncertainty, as several correlations between dry matter and energy were not statistically significant. Two samples had a significant correlation, smart and soft from 29 October. Again, the reason for this is unclear, but an explanation could be that samples were pooled. For some of the samples there were larger differences in liver size, dry matter, and thus probably large difference in energy within groups. If the mixtures were not homogenised properly this could result in a misleading result, either too high or too low energy, and therefore not correlating with the dry matter. Another reason could be that during freeze-drying some of the sample “popped” thus contaminating other samples and loosing energy themselves. However, there were only two samples that popped, and one of the contaminated one from the same group, while the other contaminated several. But this did not seem to have a big impact on the results when comparing it to the other groups.

4.5 Feed source

Based on previous study, feed blocks showed a positive effect on growth and survival for both lumpfish (Imsland et al. 2020) and wrasses (Leclercq et al. 2015). For fish fed Control there was a similar preference of block and pellets from the antenna registrations, while there were more registrations on pellets for fish fed Smart and Soft. It can therefore be theorised that preference in feed source depends on feed composition.

4.6 Dietary effect on OWI- score and Survival

Results from the OWI-score showed the most reported damage was on the pectoral fin erosion, followed by shell loss when the fish were on land. The severity of pectoral fin erosion was rather similar between treatments, but fish fed Control had less shell loss compared to fish fed Smart and Soft. On the positive side, after having been transferred to net pens, all fish improved their OWI-score. This can indicate that fish recover over time when transferred to the net pens compared to land. The recovery of fish have been reported for farmed escaped seabream (*Sparus aurata*) and European seabass, were they improved their fin and splitting erosion in the wild, compared to the farming conditions in open-sea cage (Arechavala-Lopez et al. 2013).

There was no significant difference in mortality rate between treatments during the trial period with a rather low mortality rate of 0.8, 4.5% and 4.7% for fish fed Control, Smart and Soft respectively. After the fish were transferred to a common cage for overwintering, they were all fed the Smart diet. During the winter the mortality rate increased for all treatments. The performance during the trial period, affect the mortality rate over the winter. In general, fish fed Soft during the trial period, had a higher mortality rate over the winter with 27.6%, compared to fish fed Control and Smart with 22.5 and 19.3% accordantly. These numbers are lower than what have been reported in the National inspection campaign (Wilmann et al. 2020), where 40% of Ballan wrasse die during the production period of salmon. However, there was a large number of missing Ballan wrasse during the winter. The reason for this is unclear, but there was a small population of cormorants living around the cages that might have affected the end result. Due to the large number of missing fish, the mortality rate increased to 55.0, 53.2 and 69.5% for fish fed Control, Smart and Soft respectively. This is higher than what have been reported by the National inspection campaign (Wilmann et al. 2020). Independent of missing fish, fish fed the commercial Soft diet had the highest mortality, reflecting their general performance during the trial period and can it be suggest feeding the right feed composition can enhance survival during winter, but mortality rate is still high.

4.8 Conclusion

In conclusion, there was a clear nutritional effect on growth and energy status. Fish fed Control performed better in terms of growth and energy status, closely followed by fish feed Smart. This shows that there is a potential of replacing the expansive cod muscle meal, with a more sustainable feed such as insect meal. Fish offered feed containing fishmeal had a negative effect

on growth, energy status and higher mortality rate. In general, offering the right diet for juvenile Ballan wrasse before deployment and during deployment can improve their growth, energy storage and survival during the winter.

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APENDIX

Table A.1: Nutrient composition of the diets fed to juveniles Ballan wrasse

Diet number		1	2	3
Diet name		**Control**	**Smart**	**Soft ****
Protein	**%**	58,9	57.9	40 - 45
Lipid	**%**	11.0	10.8	15 - 16
Carbohydrates	**%**	11.1	12.9	-
NFE*	**%**	-	-	16 - 22
Ash	**%**	11.9	10.6	7 - 10
Water	**%**	7.1	7.9	-
Fibre	**%**	-	-	0.6 – 4.5
Other	**%**	-	-	2.5-21.4
Sum		**100**	**100**	**100**
Pellets size	**mm**	3	3	12

Table A.2: Formulation of the experimental diets used in the juvenile Ballan wrasse trials of the current study

Diet number		1	2
Diet name		**Control**	**Smart**
Shrimp meal [a]	%	28	14
Cod muscle meal [a]	%	30	-
Krill meal [b]	%	-	14
Insect meal(Black soldier fly) [c]	%	-	20
Wheat gluten [d]	%	9.00	24.50
Krill oil [e]	%	4.20	-
Tapioca starch [f]	%	6.56	2.72
SPC [g]	%	8.50	8.00
Krill hydrolysate[h]	%	6.60	6.60

BIOMOSS[i]	%	0.50	0.50
Vitamin C[j]	%	0.23	0.23
Choline chloride[j]	%	0.50	0.50
Cholesterol[k]	%	0.20	0.20
vitamin mix[j]	%	0.50	0.50
Organic mineral[i]	%	0.68	0.68
Rapseed lecithin[l]	%	2.00	3.60
Yttrium oxide[m]	%	0.010	0.010
Lysin[j]	%	-	0.44
MSP[j]	%	2.50	2.90
Methionine[j]	%	-	0.30
Threonine[j]	%	-	0.30
Taurine[m]	%	0.020	0.020
Total	**%**	**100**	**100**

a Provided by Seagarden AS, Norway

b Krill meal made of graske?? Produced by Nofima provided from the raw material of Aker Biomarine Antarctic AS, Norway

c Provided by Innovafeed, France

d Provided by Roquette, France

e Provided by Aker Biomarine Antarctic AS, Norway

f Provided by Idun AS, Norway

g Provided by Selecta AS, Norway

h Provided by Rimforst As, Norway

i Provided by Alltech, Norway

j Supplied by Vilomix, Norway

k Provided by Carbogen AMCIS, Switzerland

l Provided by NOBA, Netherland

m Provided by VWR, Norway

Table A.4: Mean body weight (g) and condition factor (K, g cm-3) development off all fish for the different diets at three time point. Standard deviation in paratheses, Samples size(n). Samples were taken initially when the fish were on land and had not started their experimental diets (20 Aug), right before transferred to sea (29 Sep), and at end of the trial period (23 Nov). Weight and K-factor were analysed by a One-way ANOVA, with significant levels (p<0.05*; p<0.01**; p<0.001***). Orange colour indicates land phase, while blue colour indicates the sea phase.

	Control	Smart	Soft	*p*-value ANOVA
20 August	39.8 (7.8) K = 1.83 (0.15) n= 162	37.3 (7.7) K = 1.75 (0.17) n=162	42.6 (7.1) K = 1.88 (0.17) n= 162	*p* <0.001***
29 September	48.9 (9.7) K = 2.25 (0.29) n= 156	42.9[a] (9.3) K = 2.00[b] (0.27) n= 158	44.0[a] (8.7) K = 1.94[b] (0.29) n=155	*p* <0.001***
23 November	51.2 (12.2) K = 1.80[c] (0.19) n= 129	45.4 (11.3) K = 1.76[c] (0.17) n= 127	41.2 (7.6) K = 1.60 (0.13) n=123	*p* <0.001***

Notes:
Different letters in superscripts indicate no significant differences (p > 0.05)

Table A.7: Mortality rates for fish during the sea phase (29 Sep – 23). ns = no significant difference (p < 0.05) indicated by overlapping confidence intervals (CI).

	Control	Smart	Soft	Significance
Start fish number (29 Sep)	148	151	147	-
Fish number for sample (28 Oct)	18	18	18	-
End fish number (23 Nov)	129	127	123	-
Final mortality (%)	0.8 (CI: 0 – 2.3)	4.5 (CI: 1.0 – 8.0)	4.7 (CI: 1.0 – 8.3)	ns

Table A.8: Mortality rates for fish over winter (Nov 2021 – Feb 2022). ns = no significant difference p < 0.05) indicated by overlapping confidence intervals (CI).

	Control	Smart	Soft	Significance
Fish number transferred to common cage for overwintering	111	109	105	-
Fish number in	50	51	32	-

February 2022				
Number dead fish	25	21	29	-
Number missing fish	36	37	44	-
Mortality rate (%) **Not included missing**	22.5 (CI: 14.8 – 30.3)	19.3 (CI: 11.9 – 26.7)	27.6 (CI: 19.1 – 36.2)	ns

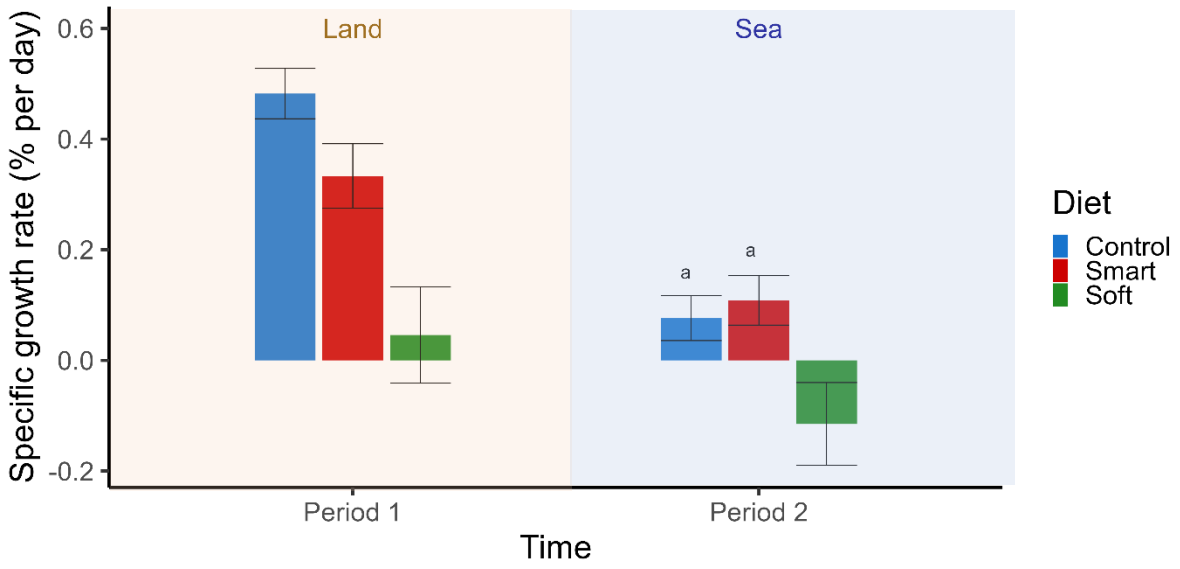


Figure A.6: Specific growth rate (SGR, % day^{-1}) with confidence intervals for all Ballan wrasse over two periods. In Period 1 the fish were on land, while in period 2 the fish were at sea. Period 1 lasted for 40 days while period 2 lasted for 55 days. For period 1 (Control n=151; Smart n=149; Soft n=150), for period 2 (Control n=129; Smart n=121; Soft n=118). Different letters in superscripts indicate no significant differences ($p > 0.05$). Orange colour indicates the land phase, while blue colour indicates the sea phase.

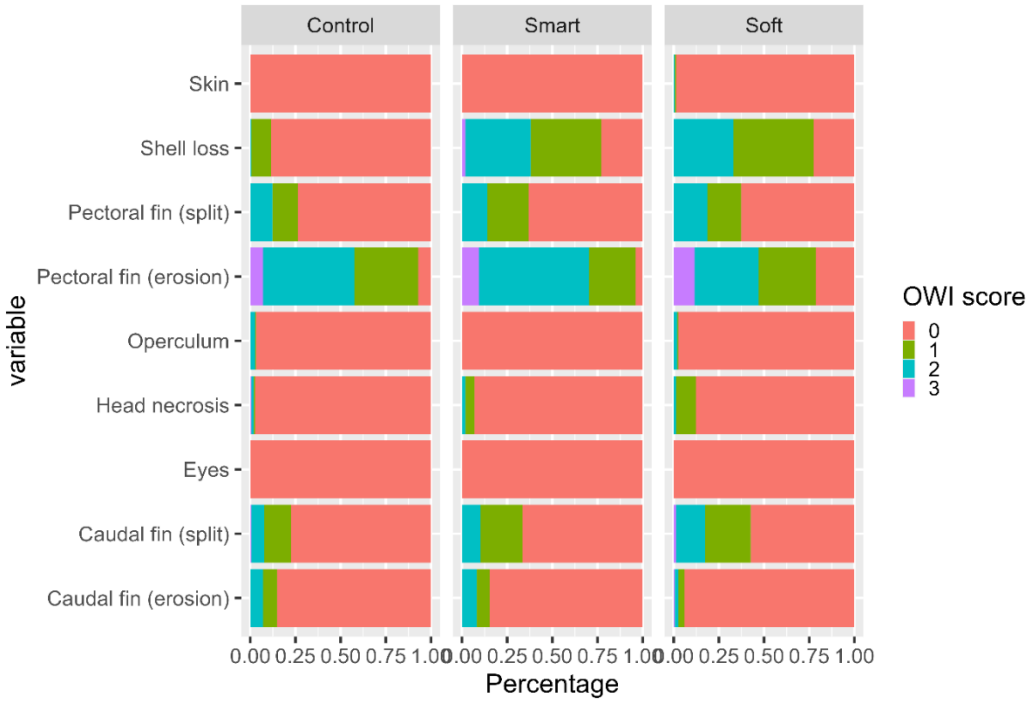


Figure A.7: Operational welfare indicators (OWIs) of all fish before deployment (29 Sep, n=469) shown are the percentage of fish scored on a 4-point scale (score 0-3). Each indicator, depending on the extent and severity of each condition

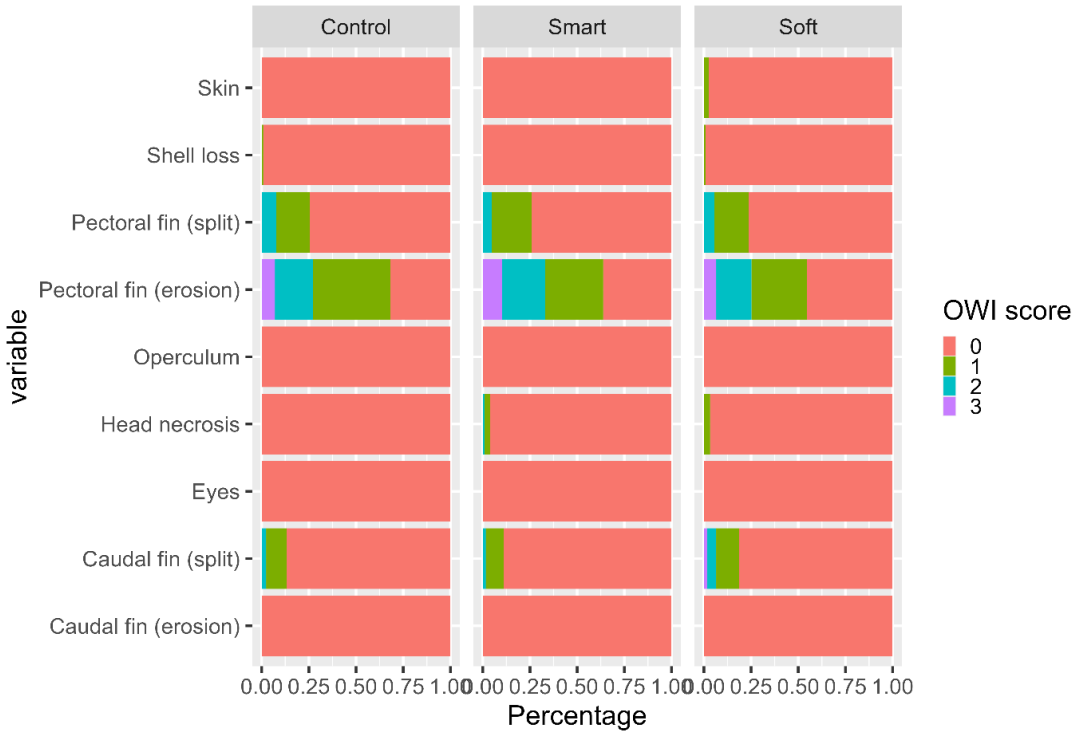


Figure A.8: Operational welfare indicators (OWIs) of all fish at end of trial (23 Nov, n=379) shown are the percentage of fish scored on a 4-point scale (score 0-3). Each indicator, depending on the extent and severity of each condition

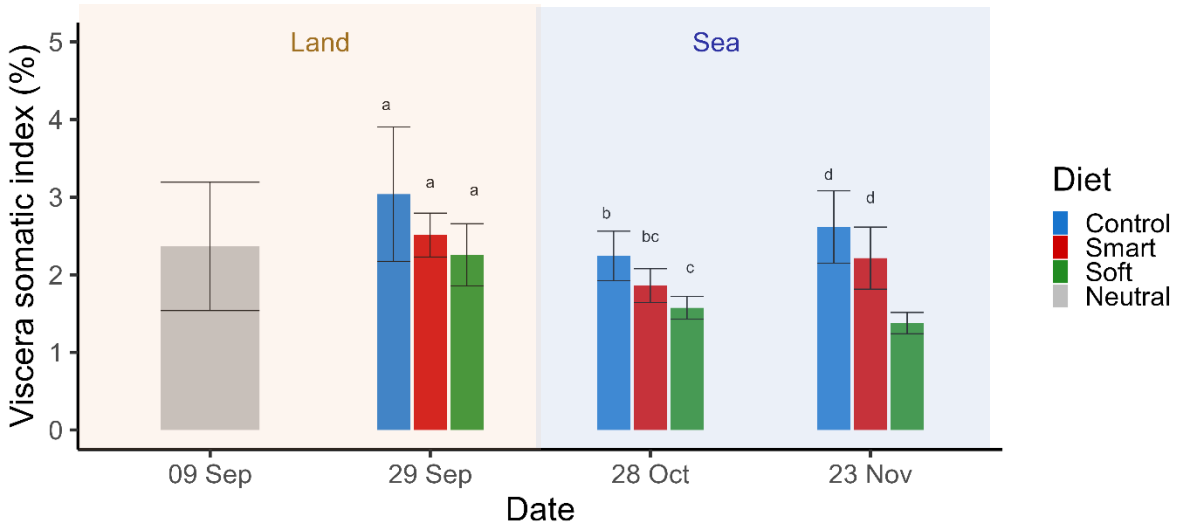


Figure A.9: Viscera somatic index (ISI, %) with confidence intervals of total body weight for Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land and had not started their experimental diets (Netural). 29 September (Control n=6; Smart n=6; Soft n=6), the fish been on land, but transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Significant codes ($p<0.05$*; $p<0.01$**; $p<0.001$). Different letters in superscripts indicate no significant differences ($p > 0.05$). Orange colour indicates the land phase, while blue colour indicates the sea phase.

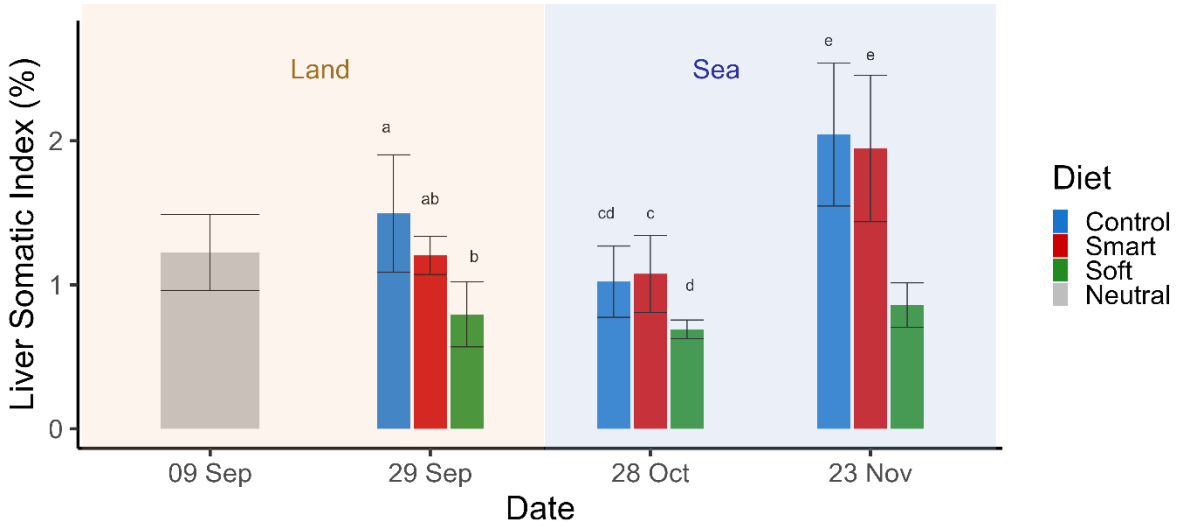
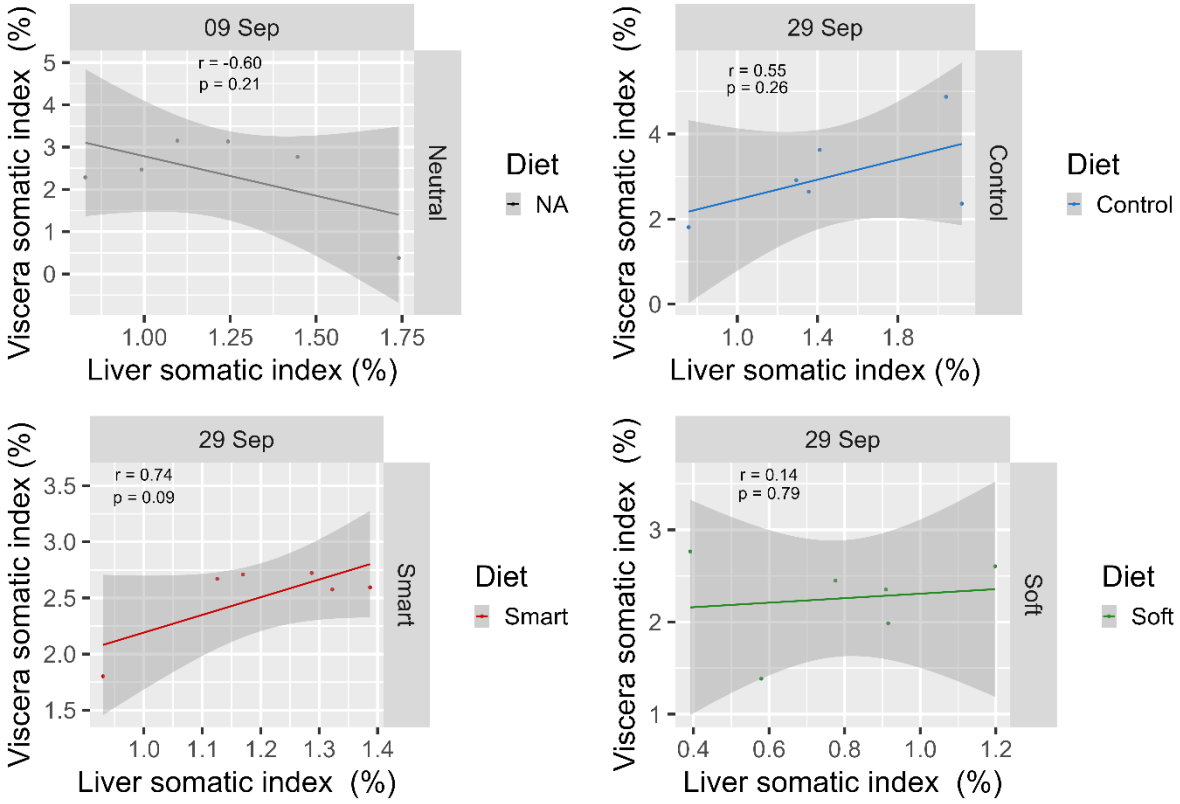


Figure A.10: Liver somatic index (LSI, %) with confidence intervals of total body weight for Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land

and had not started their experimental diets (Neutral). 29 September (Control n=6; Smart n=6; Soft n=6), the fish been on land, but transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Arrow indicated line between land and sea. Significant codes (p<0.05*; p<0.01**; p<0.001). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase.



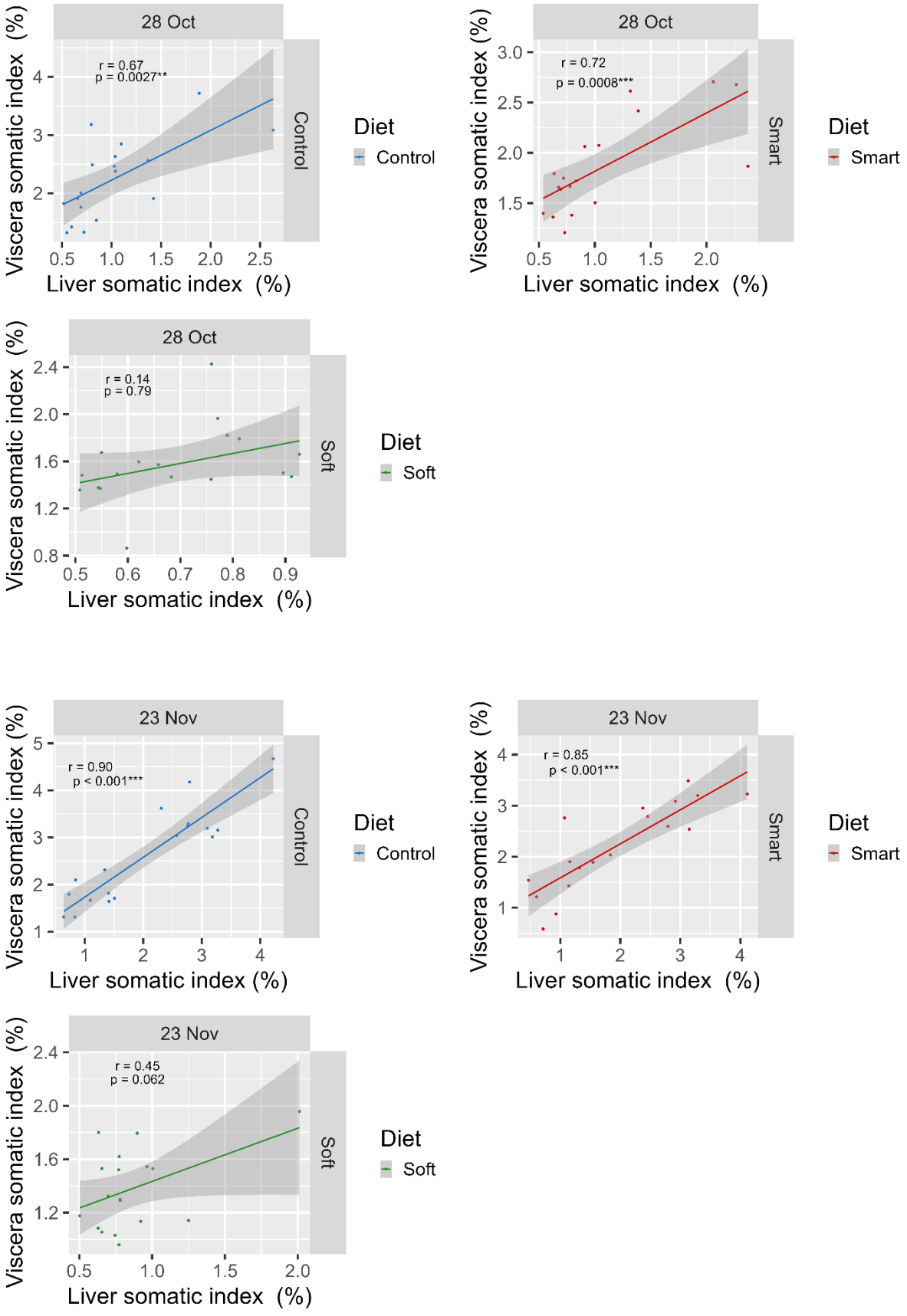


Figure A.11: Pearson correlation coefficient, r, and p-values for each diet between liver somatic index (LSI) and viscera somatic index (ISI) at each time point

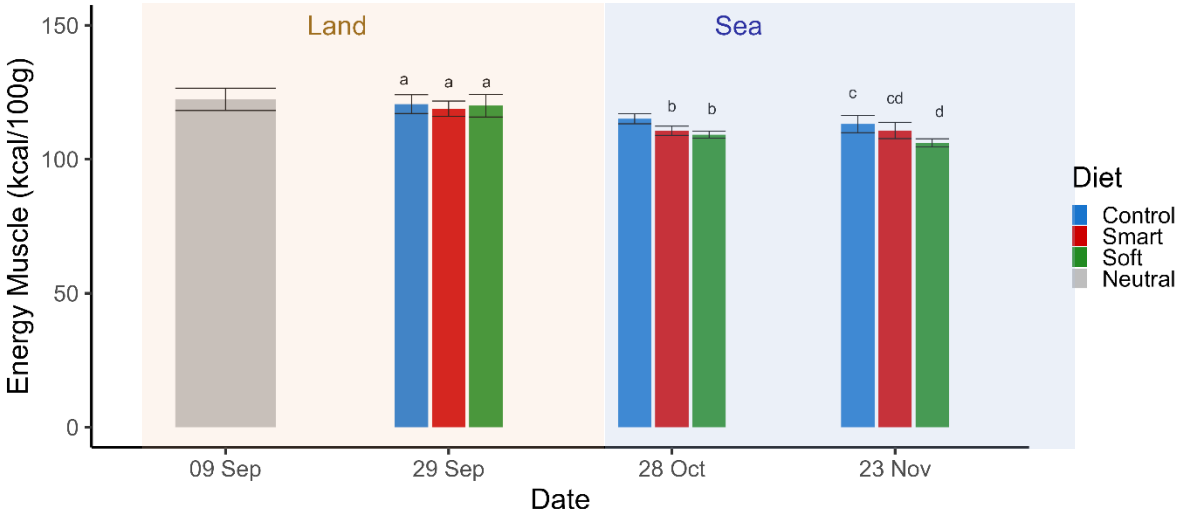


Figure A.12: Mean Energy (Kcal/100g) with confidence intervals from muscle samples from Ballan wrasse given the different diets at all four samplings date. 9 September (n=6), the fish were on land and had not started their experimental diets (Neutral). 29 September (Control n=6; Smart n=6; Soft n=6), the fish were transferred to sea. 28 October (Control n=18; Smart n=18; Soft n=18) and 23 November (Control n=18; Smart n=18; Soft n=18), the fish were at sea. Significant codes (p<0.05*; p<0.01**; p<0.001). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase

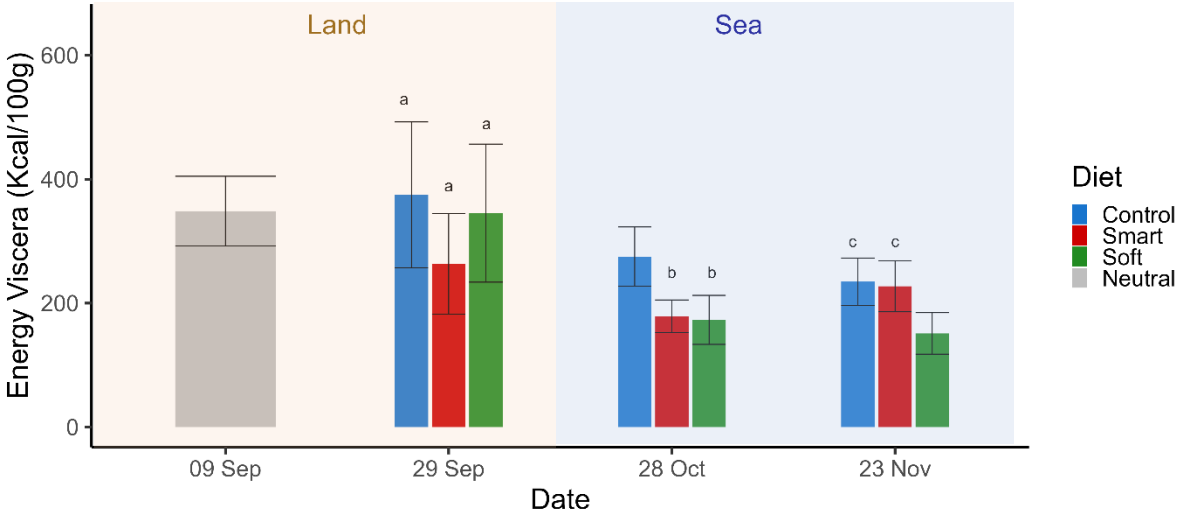


Figure A.13: Mean Energy (Kcal/100g) with confidence intervals from Viscera samples for Ballan wrasse given the different diets at all four samplings date. On 9 September (n=6), the fish were on land and had not started their experimental diets (Neutral). On 29 September (Control n=6; Smart n=6; Soft n=6), the fish were transferred to sea. 28 October (Control n=17; Smart n=16; Soft n=15) and 23 November (Control n=16; Smart n=14; Soft n=15), the fish were at sea. Significant codes (p<0.05*; p<0.01**; p<0.001). Different letters in superscripts indicate no significant differences (p > 0.05). Orange colour indicates the land phase, while blue colour indicates the sea phase

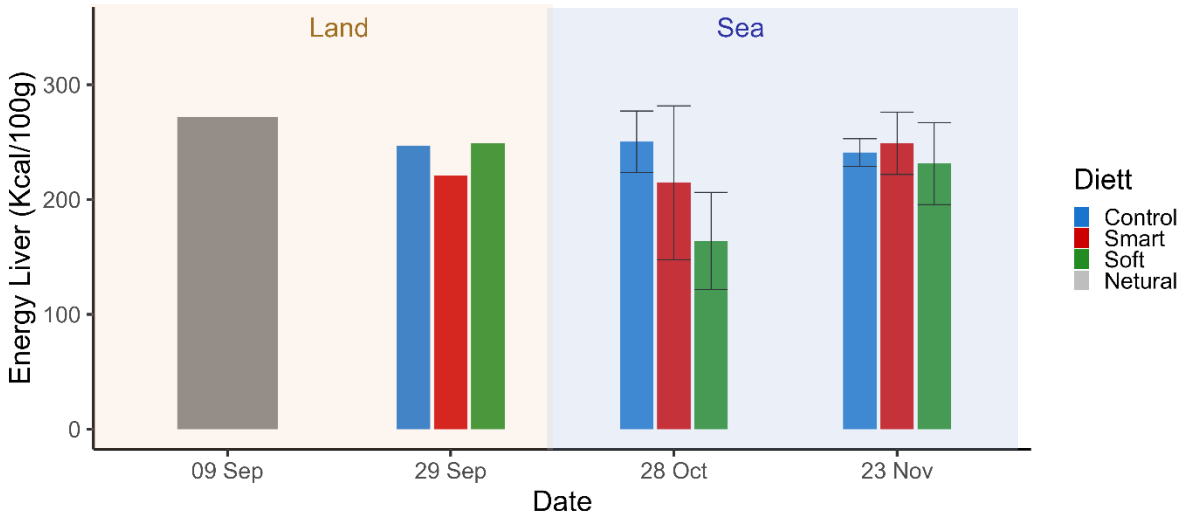
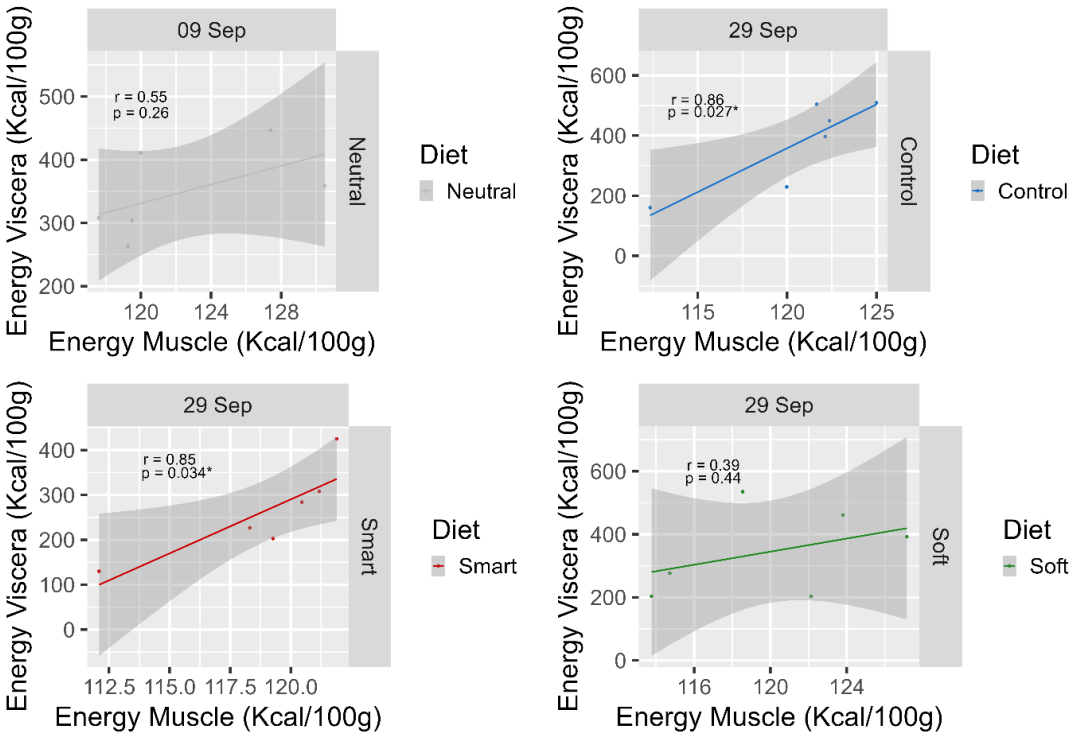


Figure A.14: Mean pooled energy (Kcal/100g) with confidence intervals from liver samples for Ballan wrasse given the different diets at all four samplings date. On 9 September (n=1), the fish were on land and had not started their experimental diets (Neutral). On 29 September (Control n=1; Smart n=1; Soft n=1), the fish were transferred to sea. 28 October (Control n=3; Smart n=3; Soft n=3) and 23 November (Control n=3; Smart n=3; Soft n=3), the fish were at sea. Orange colour indicates the land se, while blue colour indicates the sea phase



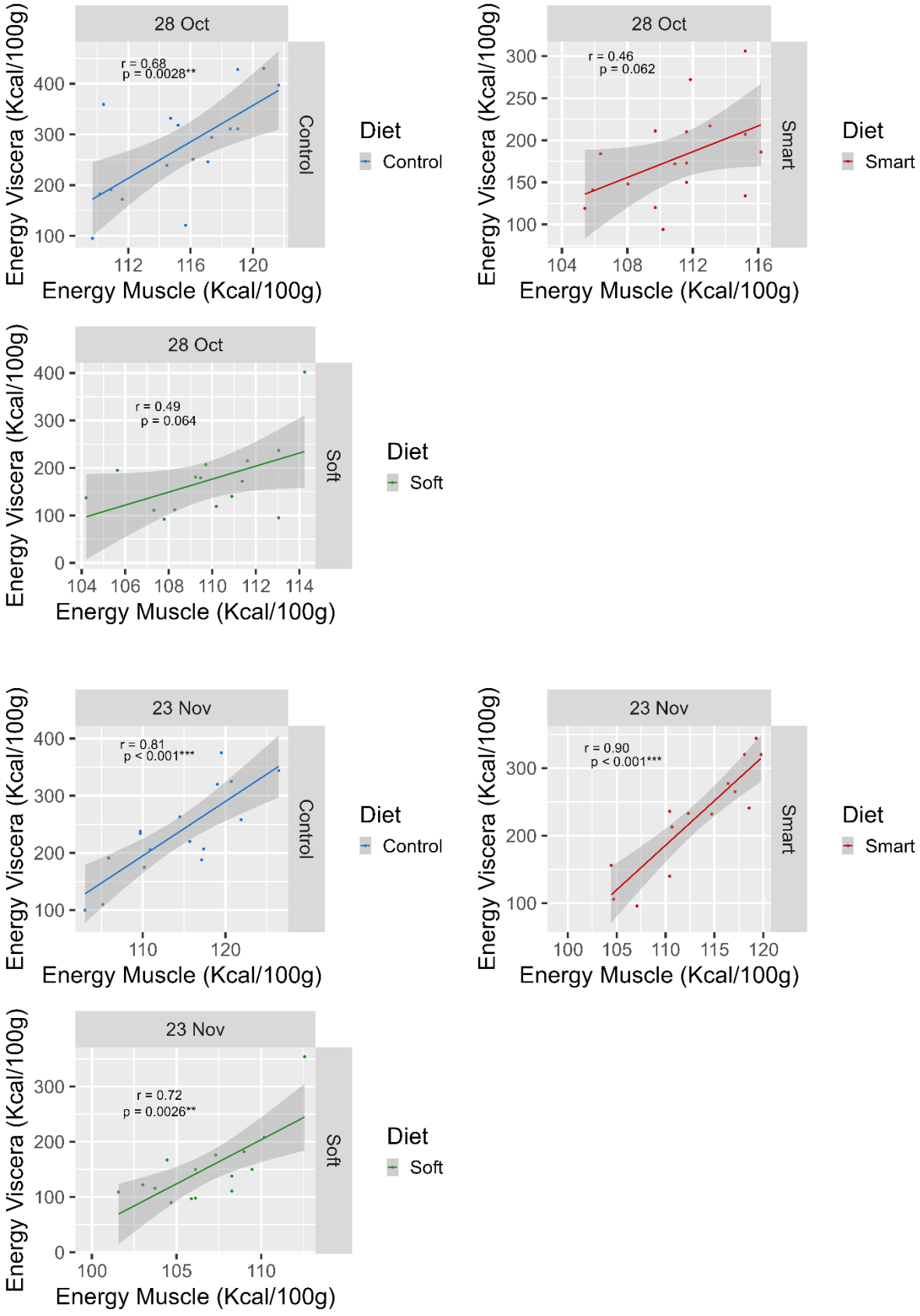
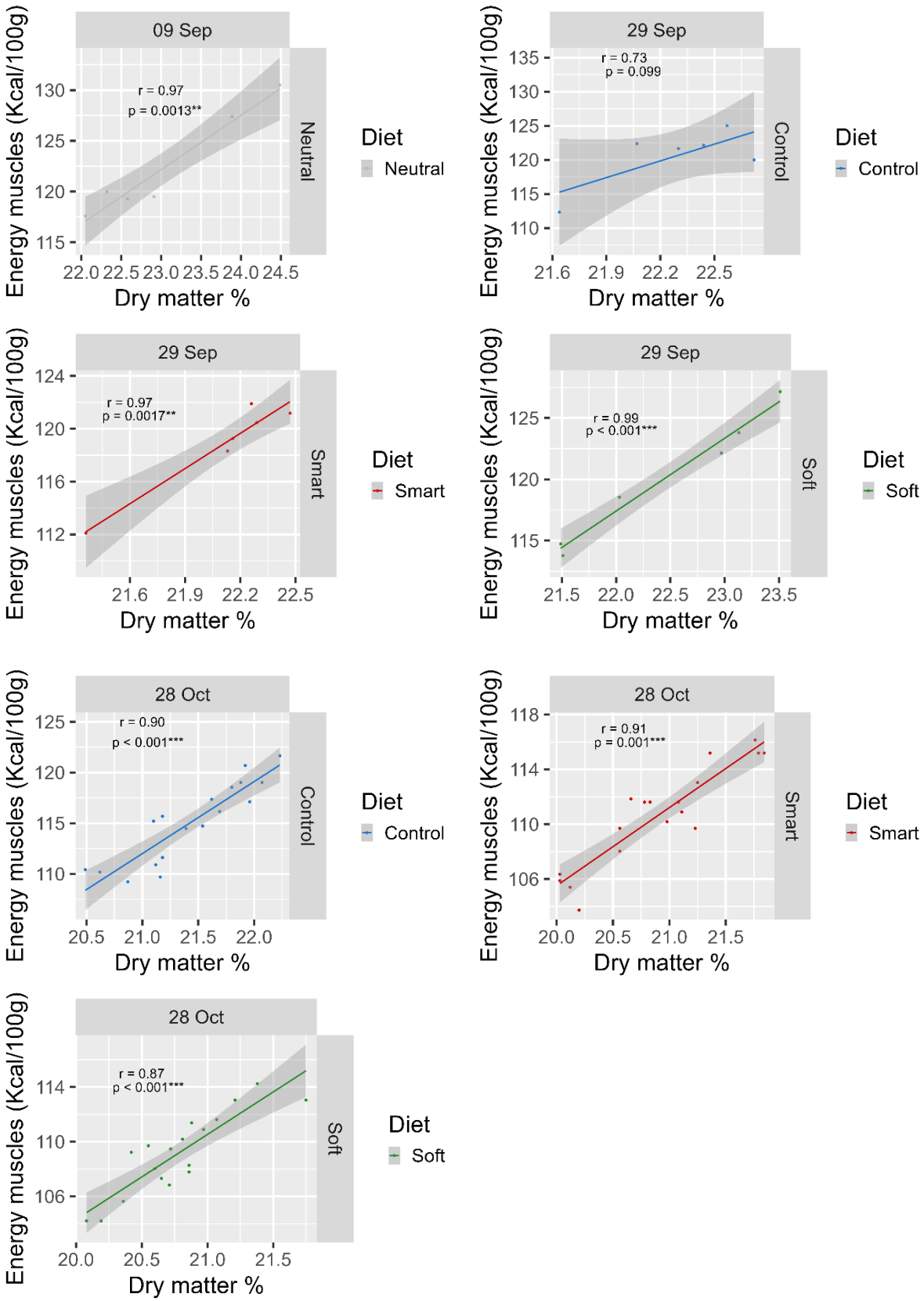


Figure A.15: Pearson correlation coefficient, r, and p-values for each diet between viscera energy (VE, kcal/100g) and Muscle energy (Kcal/100g) from different time points.


09 Sep
r = 0.97
p = 0.0013**
Neutral
Energy muscles (Kcal/100g)
Dry matter %
Diet
Neutral
29 Sep
r = 0.73
p = 0.099
Control
Diet
Control
29 Sep
r = 0.97
p = 0.0017**
Smart
Diet
Smart
29 Sep
r = 0.99
p < 0.001***
Soft
Diet
Soft
28 Oct
r = 0.90
p < 0.001***
Control
Diet
Control
28 Oct
r = 0.91
p = 0.001***
Smart
Diet
Smart
28 Oct
r = 0.87
p < 0.001***
Soft
Diet
Soft

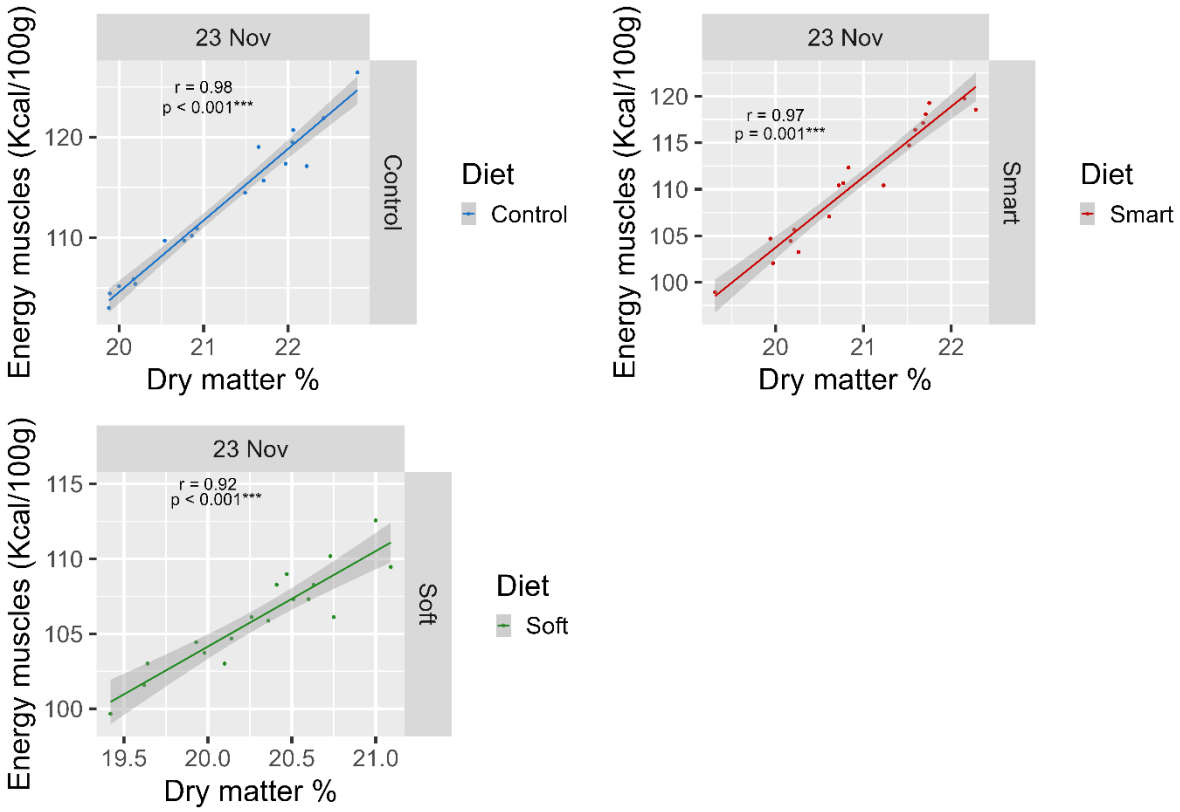
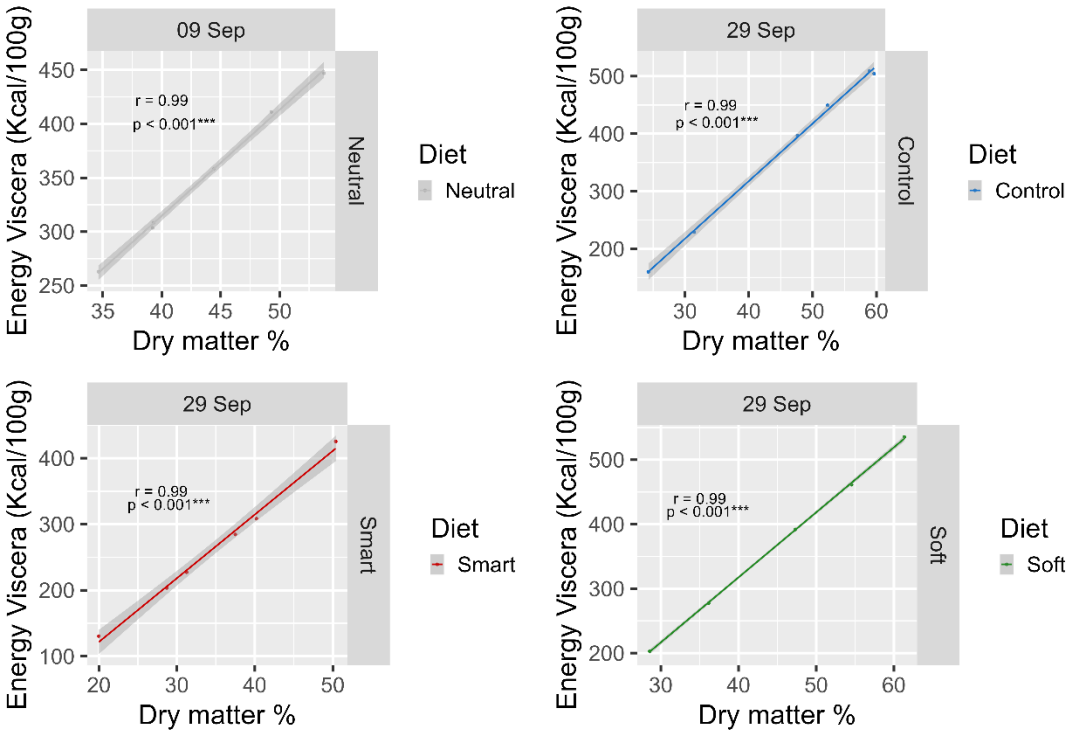


Figure A.16: Correlation between dry matter (%) and energy in muscles (kcal/100g) at different time point.



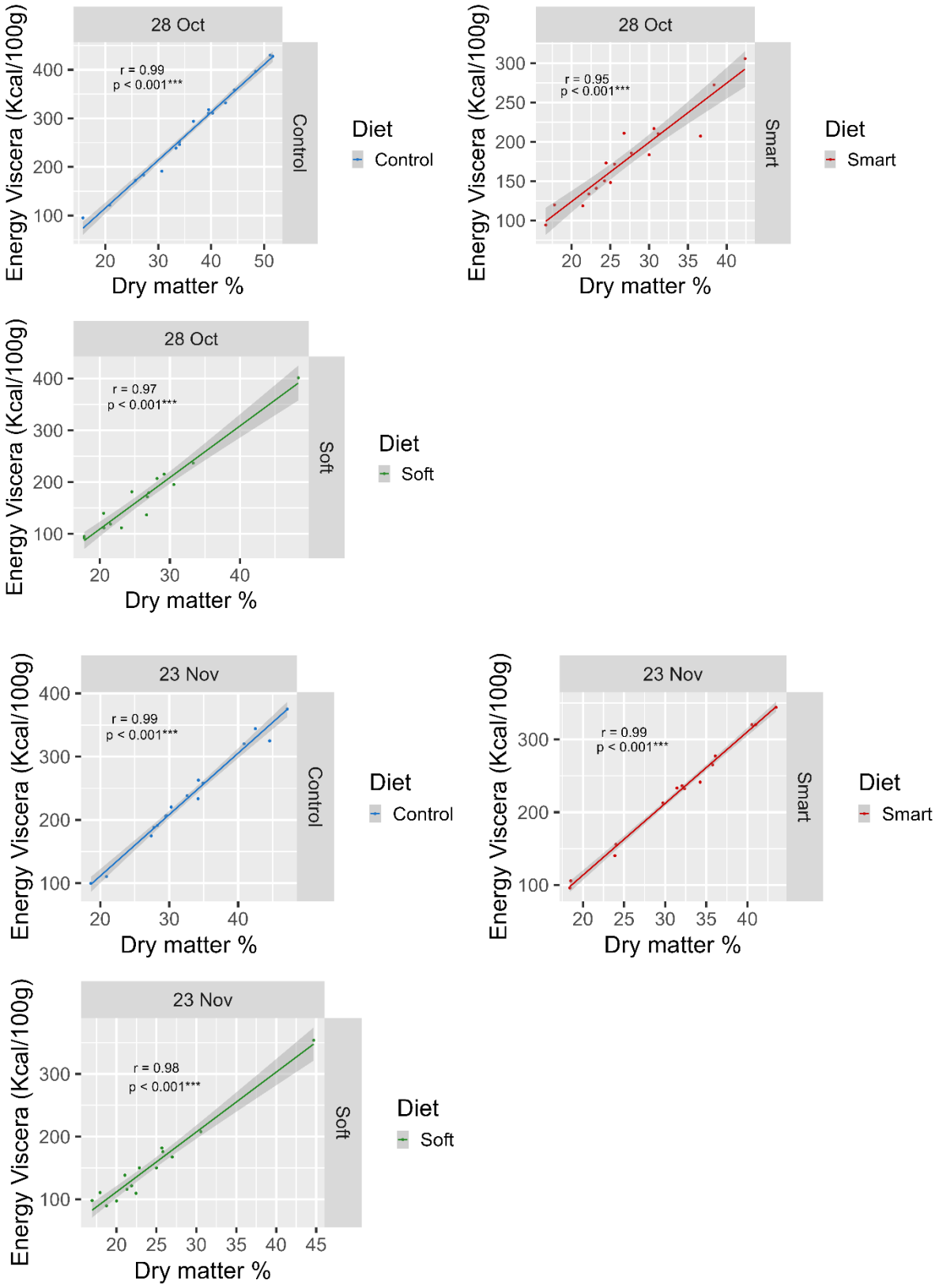


Figure A.17: Correlation between dry matter (%) and energy in the intestinal (kcal/100g) at different time point.

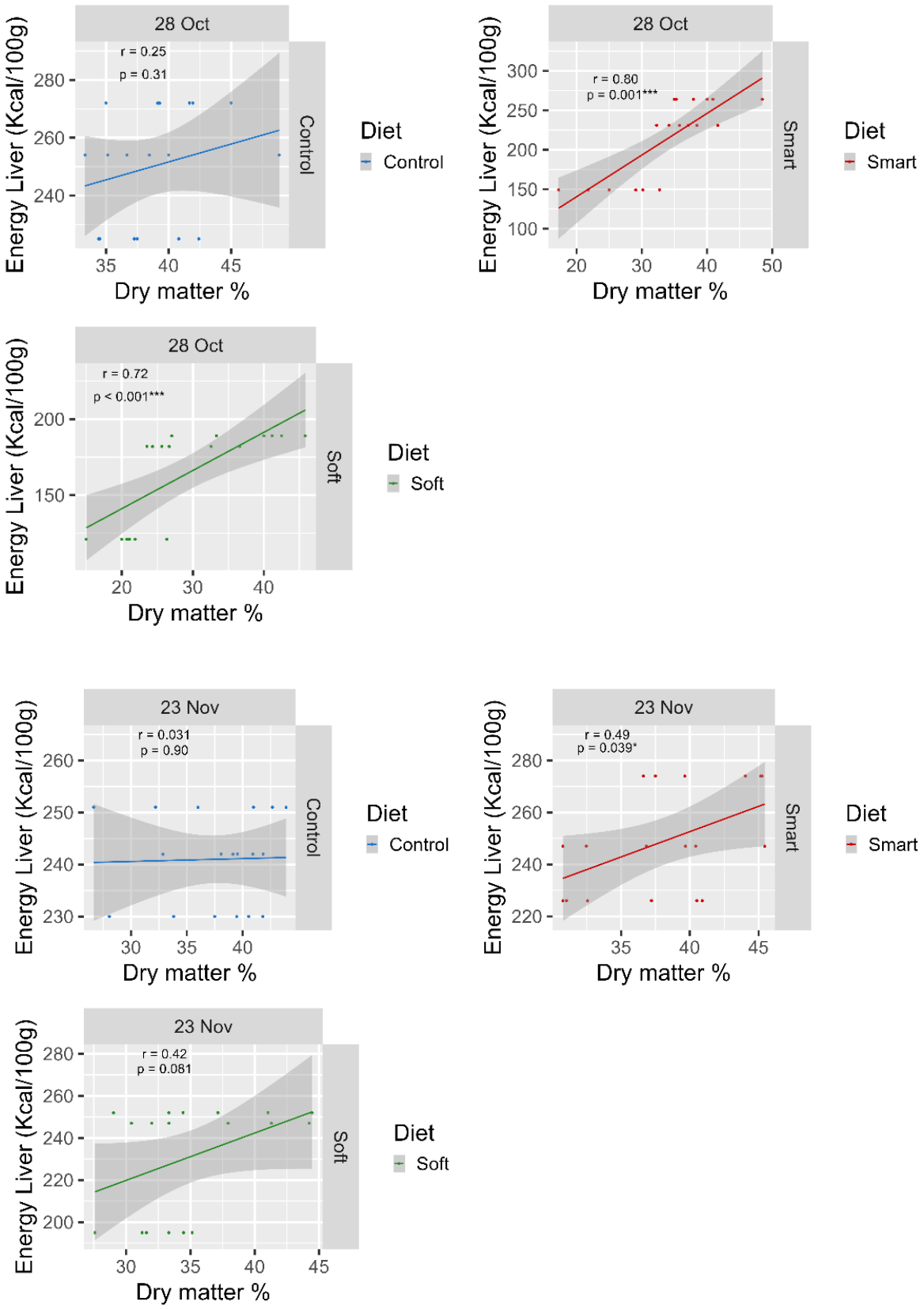


Figure A.18: Correlation between dry matter (%) and energy in the liver (kcal/100g) at different time point.

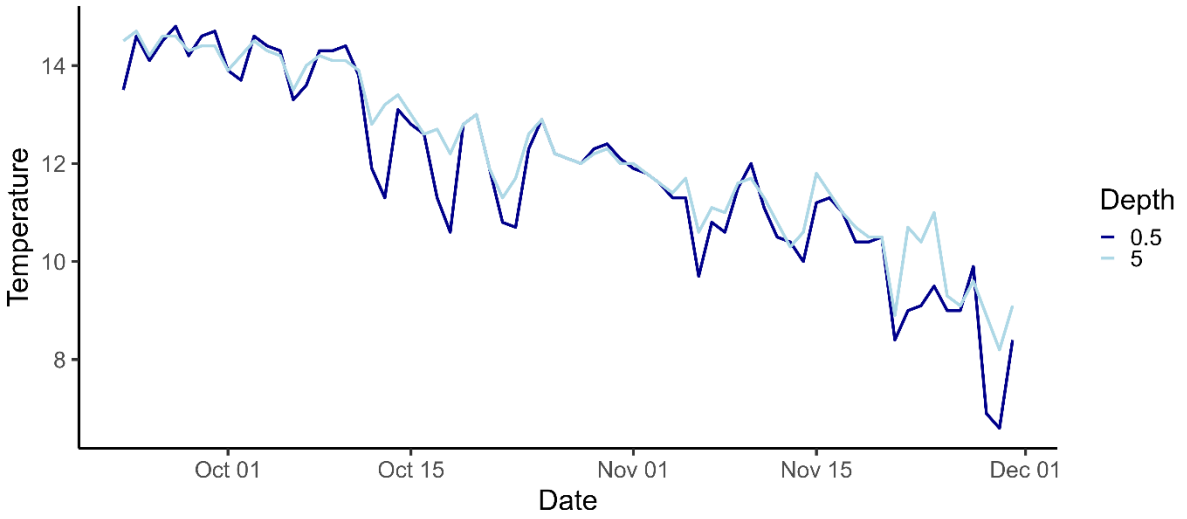


Figure A.19: Temperature (°C) at sea during the trial period at 0.5 m and 5 m depth

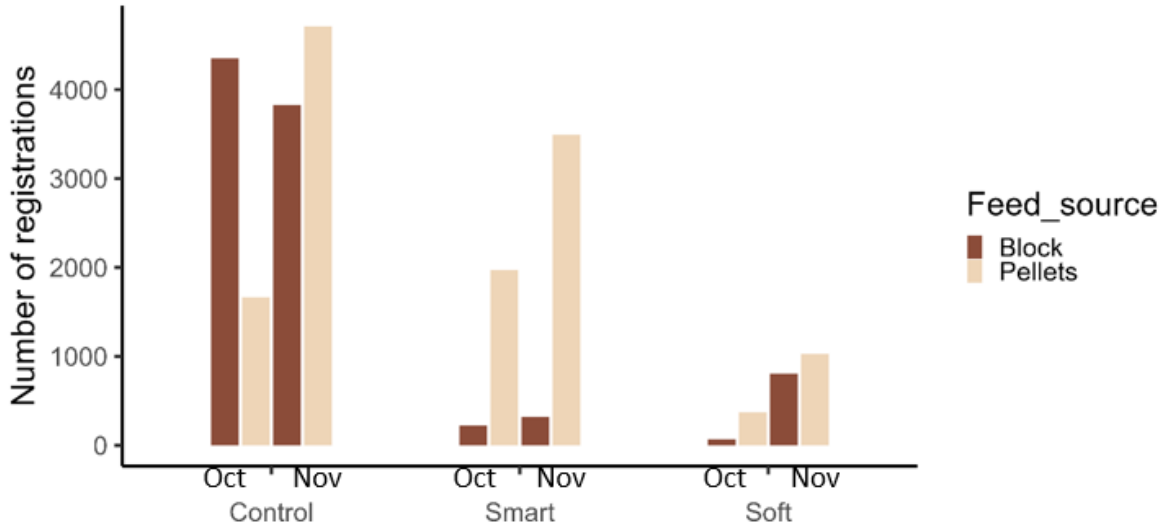


Figure A.20: Number of antenna registrations in October and November for fish offered the different diets and their preference feed source.

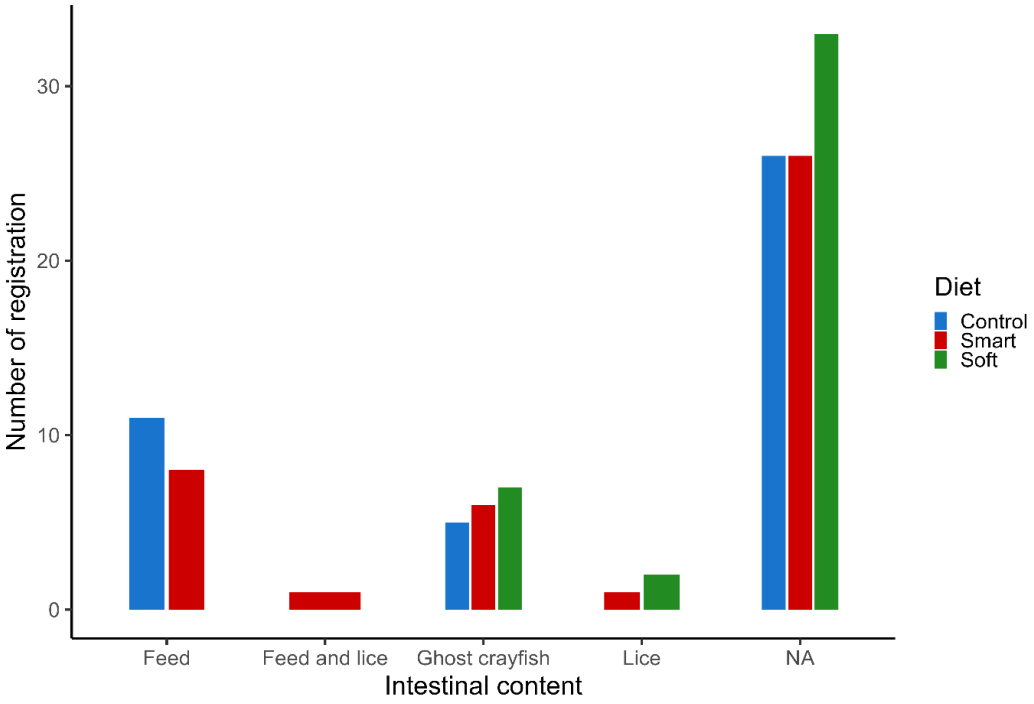


Figure A.2122: Feed content from sampled fish from October and November. NA indicates no feed content.