POSTPRANDIAL AMINOACIDEMIA AND APPETITE RESPONSES TO A NOVEL SALMON-DERIVED PROTEIN PEPTIDE IN YOUNG HEALTHY ADULTS

By

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Abstract

Muscle plays a vital role in our body's health and relies on ingestion of amino acids in order to stimulate muscle protein synthesis (MPS). Research is endeavoring to search for novel, high-quality, sustainable protein sources, capable of supporting muscle mass globally. Marine protein hydrolysates offer a potential sustainable source of high-quality protein, although little research to-date has explored their postprandial aminoacidemic responses. This study investigated the postprandial aminoacidemic and appetite responses following ingestion of a novel Salmon Protein Peptide (SPP) supplement, compared with a dose-matched Milk Protein Isolate (MPI). 11 young healthy individuals (6 Male and 5 Female) took part in this randomized single-blind crossover study. After an overnight fast, participants consumed either SPP or MPI at a protein-matched dose of 0.3g/kg BM. Blood samples were taken before protein intake and periodically for 240minutes post-ingestion. Visual analog scales were utilized throughout to determine changes in perceived appetite, alongside a food diary and ad-libitum meal following the trial period to determine changes to dietary composition. Peak plasma values for total amino acid (SPP: 5004 ±296 vs. MPI: 3719 ±302μmol·L^{-1}, p<0.001), non-essential amino acid (SPP: 3376 ±197 vs. MPI: 2287 ±173μmol·L^{-1}, p<0.001), glycine (SPP: 456 ±24 vs. MPI: 284 ±33μmol·L^{-1}, p<0.001), hydroxyproline (SPP: 74.9 ±6.6 vs. MPI: 28.3 ±2.6 μmol·L^{-1}, p<0.001) and proline levels (SPP: 469 ±29 vs. MPI: 335 ±25 μmol·L^{-1}, p=0.029) were significantly greater following SPP consumption and all demonstrated significantly greater iAUC values (all p<0.05). No differences were observed in plasma EAA levels between proteins (peak EAA levels = SPP: 1361 ±196 vs. MPI: 1340 ±166μmol·L^{-1}, p=0.658; iAUC, p=0.551). Peak leucine levels did not significantly differ between proteins (SPP: 250 ±32 vs. MPI: 246 ±31μmol·L^{-1}; p=0.657); however, leucine levels were significantly higher overall following MPI consumption (iAUC, p=0.011). Fat intake was significantly reduced compared to habitual diet following both SPP and MPI (1.0±0.6g/kg/day, p=0.0156; 1.0±0.4g/kg/day, p=0.0236, respectively). No differences in plasma insulin, glucose or appetite were observed between supplements. To conclude, SPP demonstrated great promise

as a high-quality protein source when compared to MPI, although further investigation is needed to determine if SPP's postprandial aminoacidemia can robustly stimulate MPS.

List of Tables

Table 1. Participant Characteristics 23

Table 2: Supplement Composition 28

Table 3: Meal Nutritional Information 31

Table 4: Average Nutritional Intake Values 48

List of Figures

Figure 1. Illustration of muscle mass regulation – adapted from Breen and Philips. 2011 10

Figure 2. Illustration of VAS questions posed during trials. 26

Figure 3. Postprandial study design schematic. 27

Figure 4. Postprandial plasma glucose and insulin concentrations. 36

Figure 5. Postprandial plasma TAA, NEAA, EAA and leucine levels following SPP and MPI ingestion. 39

Figure 6. Postprandial plasma glycine, hydroxyproline and proline levels following SPP and MPI ingestion. 42

Figure 7. Appetite response following salmon and milk protein ingestion. 45

Figure 8. Daily dietary intake and during trial ad-libitum breakfast. 47

Abbreviations

AA	Amino Acid
iAUC	Incremental area under the curve
BCAA	Branch chain amino acids
CHO	Carbohydrates
EAA	Essential amino acids
EI	Energy intake
SPP	Salmon protein peptide
HAB	Habitual
MPI	Milk protein isolate
MPS	Muscle protein synthesis
NEAA	Non-essential amino acids
PI	Protein intake
PRO	Protein
TAA	Total amino acids
TDEI	Total daily energy intake
VAS	Visual analog scale

Table of Contents

List of Tables 5
List of Figures 6
Abbreviations 7
1. Introduction 10
1.1. Background 10
1.3.1. Protein Dose 12
1.4.1. Protein Quality 13
1.4.2. Nitrogen Balance 13
1.4.3. Amino Acid Composition 14
1.4.4. Digestibility 15
1.4.5. Influence on Appetite 16
1.4.6. Plant Proteins 18
1.5. Protein and Planetary Health 19
1.6. Marine Protein 20
1.7. Aims and Objectives 22
1.7.1. Determine and compare the postprandial aminoacidemic responses of milk protein isolate (MPI) to hydrolyzed Salmon protein peptide (SPP) in healthy young males and females. 22
1.7.2. Determine the effects of milk protein isolate (MPI) and hydrolyzed Salmon protein (SPP) on appetite regulation in healthy young males and females. 22
2. Methods 23
2.1. Participants 23
2.2. Ethical Approval 24
2.3. Protein Supplements 24
2.3.1. Novel Salmon Protein Peptide (SPP) 24
2.4. Study Design 25
2.5. Pre-Experimental Trial Protocol 29
2.6. Experimental Trial Protocol 29
2.7. Blood processing and analysis 32
2.8. Statistical Analysis 33
3. Results: 35
3.1. Postprandial plasma glucose and insulin levels. 35
3.2. Postprandial plasma TAA, NEAA, EAA and leucine levels. 37
3.3. Postprandial plasma glycine, hydroxyproline and proline levels 40

3.4. Protein ingestion demonstrated no impact on perceived appetite between groups during trials. 43
3.5. Protein ingestion showed no impact on dietary composition. 46
4. Discussion: 49
4.1. Overview. 49
4.1. Insulin and Glucose 49
4.2. TAAs 50
4.3. NEAAs 51
4.5. EAAs 52
4.6. Leucine 53
4.4. Glycine, Hydroxyproline and Proline 54
4.8. Appetite 55
Conclusion 56
References: 57

1. Introduction

1.1. Background

Skeletal muscle (SM) alone can make up to 40% of healthy body mass (Janssen et al. 2000). This abundant tissue plays vital roles in our body's health; from heat production (McCuller, Jessu and Callahan. 2016), metabolic regulation (Stump et al. 2016), nutrient storage (McCuller et al. 2016), immune responses (Afzali et al. 2018), to brain neuroplasticity (Walsh et al. 2015) and much more. Amino acids (AA) are the building blocks of muscle, linking via peptide bonds to form various combinations in polypeptides, which are utilized when building and remodeling muscle structures. There are two types of AA, essential amino acids (EAA): which the body cannot synthesize and are provided only through dietary sources; alongside non-essential amino acids (NEAA): which the body can synthesize but are heavily relied upon for physiological functions, such as building metabolic structures and maintaining nitrogen balance (Wu, 2010). Ingestion of AAs via dietary protein is vital to maintain muscle health by promoting growth, repair and reducing muscle atrophy.

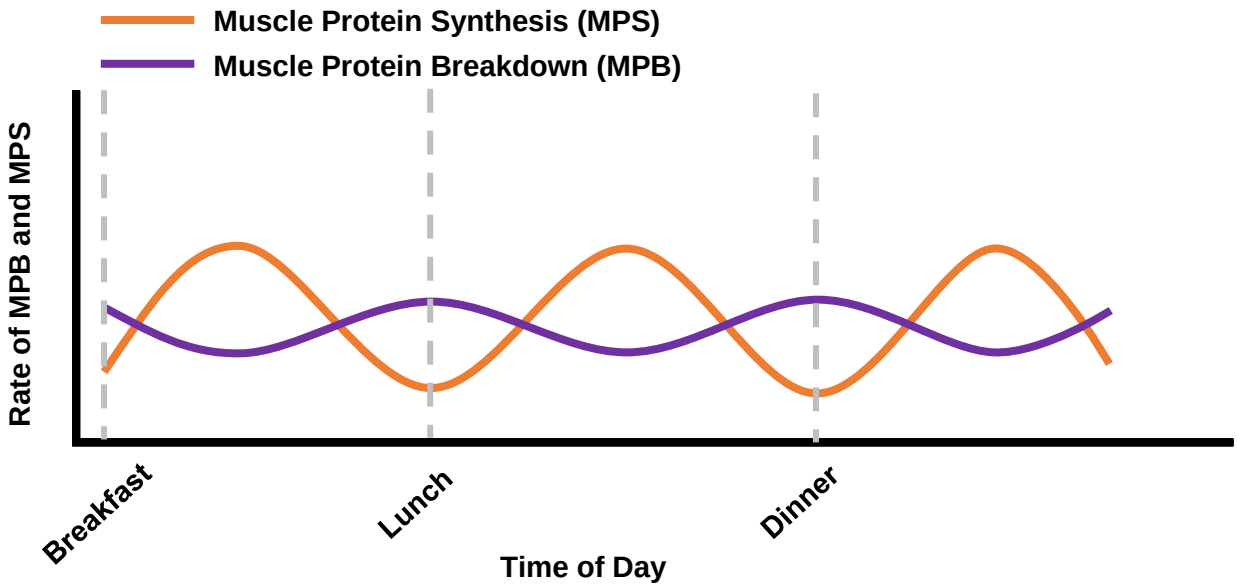


Figure 1. Illustration of muscle mass regulation – adapted from Breen and Philips. 2011

The relationship between Muscle Protein Synthesis (MPS) and Muscle Protein Breakdown (MPB) is demonstrated in **Fig.1**. MPS is the process by which new muscle mass is constructed. At the start of the day, the body wakes up in a fasted state; following digestion of a protein-containing meal, its constituent AAs are released into the blood stream, which trigger increases in MPS, therefore, meals high in protein robustly stimulate MPS. MPB is required for muscle remodeling to occur, allowing for increases in muscle mass, and training adaptations to take place (Tipton, Hamilton, and Gallagher. 2018). MPS and MPB both become elevated following resistance exercise and the availability of free plasma AAs appears to determine the balance between the two opposing mechanisms (Tipton and Wolfe. 1998), highlighting the importance of protein consumption following resistance training. MPB has been found to reduce following a meal (Breen and Philips. 2011); although research surrounding the postprandial control of MPB is sparce, hyperinsulinemia has demonstrated inhibitory effects on MPB (Chow et al. 2006), suggesting increased insulin levels following a meal contribute to ensure net muscle mass is balanced overall (MPS=MPB). Consequently, the synthesis–breakdown relationship can be manipulated through exercise and nutritional strategies. A low protein diet can lead to reductions in MPS, resulting in a negative net balance (MPS<MPB), leading to muscle atrophy. However, a high-protein diet, can create a positive net balance (MPS>MPB), which, is escalated when accompanied by exercise (in particular resistance exercise). Postprandial AA and insulin levels have demonstrated considerable influence in muscle remodeling responses by upregulating the mTOR pathway (Wang and Proud, 2006), highlighting the importance of investigations into nutritional variables that may impact MPS and MPB levels.

1.3.1. Protein Dose

Several factors can influence the protein dose required to maximally stimulate MPS. Younger individuals have high 'anabolic sensitivity', therefore, require ~0.24g/kg in one meal to maximally stimulate MPS. However, older people require almost double that of a younger person (0.40g/kg) to initiate the same level of MPS (Moore et al. 2015). A recent study found a protein dose of 0.49g/kg was sufficient to maximally stimulate MPS following endurance exercise in young males (Churchward-Venne et al. 2020). In addition, recent review, demonstrated a protein dose of 0.31g/kg was required to generate maximal MPS following resistance exercise in young males (Moore, 2019). The above studies suggest activity level should be considered in order to maximally stimulate MPS to gain the greatest benefits from protein consumption per meal, and in turn allow for maximal muscular adaptations during recovery. However, it should be noted that further research is required to determine if post-exercise protein dosage is impacted further by age and sex. Despite the above evidence supporting high levels of dietary protein intake to optimize muscle remodeling, the current recommended dietary allowance advises adults consume 0.8g/kg/day of protein (distributed throughout normal daily meals e.g., 0.16g/kg/meal when eating 5 times a day), which according to the current research discussed above, would not be sufficient to maximally stimulate MPS. MPS generated by protein intake is transient even following prolonged hyper aminoacidemia, this is known as the 'muscle full effect' (Atherton et al. 2010). Therefore, it is important dietary protein is well distributed throughout the day, rather than consumed all at once. A recent meta-analysis found, a plateau in the response of muscle hypertrophy with1.6g/kg/day of protein intake (Morton et al. 2018). Considering per meal protein recommendations of approx. 0.3g/kg/mass to maximally stimulate MPS (Areta et al. 2013 and Mallinson et al. 2023), the value suggested by Morton et al. would equate to 5 feeding events across the day equating to 0.25g/kg/meal), results in greater muscular adaptations alongside resistance exercise (Morton et al. 2018). This finding accentuates the need to promote the importance of sufficient protein intake during meals, and the emphasizes the need to conduct

further research into various protein sources available to the population, to enable greater maintenance of muscle mass for generations to come. Previous investigations have found distributing protein intake (PI) every 3hrs during the day maximizes MPS responses than a more frequent distribution of 1.5hrs, or longer intervals of 6hrs (Areta et al. 2013). This suggests that total daily protein should be distributed evenly across breakfast, lunch and dinner to augment muscular adaptations. In addition, immediately following exercise, it has commonly been thought that protein ingestion is vital to maximize muscle hypertrophy (Moore et al. 2009). Although, more recent research has demonstrated delayed PI post-exercise (up-to 24hrs) produces similar benefits to immediate ingestion (Burd et al. 2011). In addition, a recent meta-analysis demonstrated that the timing of post-exercise PI is of little importance, rather adequate and well distributed PI alongside resistance training is central to muscular adaptations (Schoenfeld, Aragon, and Krieger, 2013).

1.4.1. Protein Quality

1.4.2. Nitrogen Balance

Proteins are the main sources of nitrogen in the human diet. Protein quality is determined by a sources ability to achieve whole-body nitrogen balance. Research has often used the Protein Digestibility Corrected Amino Acid Score (PDCAAS) to quantify the quality of a protein. PDCAAS is calculated using whole protein content, AA profile, and digestibility of a source. However, limitations have since been identified with the PDCAAS; Firstly, its 0-1 scoring system does not account for proteins which score higher than 1, and secondly, the score uses rat fecal digestibility - the rat digestive system is physiologically dissimilar to humans and contains proteins produced by microbes in the gut, which potentially overestimates the quality of some sources (Bailey and Stein, 2019). Research has now shifted to the Digestible Indispensable Amino Acid Score (DIAAS). DIAAS uses a 3 patterned scoring system, allowing for more accurate representation for all proteins and more transferable insight into how several proteins in a mixed meal can

complement one another to generate a higher DIAAS score (Bailey and Stein, 2019); DIAAS takes individual AA digestibility into account rather than whole protein content alone; DIAAS also uses pig ileal tract samples to determine protein absorption - pigs have a physiologically similar digestive system to humans and ileal tract samples provide a more accurate representation of AA absorption as it occurs prior to the large intestine where microbes may impact the presence of AA in feces (Deglaire and Moughan, 2012).

1.4.3. Amino Acid Composition

Although protein scores are a useful nutritional guide, other factors should be considered when determining protein quality. A protein's ability to maintain muscle mass is determined by several factors, studies have found only EAA's are required to stimulate MPS following exercise (Tipton et al. 1999), emphasizing their anabolic importance; 'Complete' proteins are defined as containing all EAAs, and therefore have higher anabolic potential and subsequent quality compared to 'incomplete' proteins. However, protein can still be 'complete' yet have lower anabolic potential due to its EAAs ratio, for example, milk protein demonstrated increased MPS stimulation when compared to dose-matched Soy protein (Wilkinson et al. 2007), this was likely due to its higher leucine content. Leucine is an a key EAA driver of the mTORC1 pathway; mTORC1 is an essential regulator of cell metabolism and growth, playing a key role in initiating protein synthesis and suppressing autophagy (Takahara et al. 2020). Therefore, leucine is thought to play a key role in muscle growth and maintenance, with the ability to independently stimulate MPS (Pasiakos. 2012).

NEAAs are also important to consider when determining the quality of a protein. More research is emerging demonstrating the need for NEAAs in the diet, suggesting the term 'non-essential' is outdated (Hou and Wu, 2017). Studies have demonstrated deficiencies in NEAAs can lead to metabolic issues: specifically, proline deficiency has demonstrated resultant deregulation of mTORC1-4EBP1 and endoplasmic reticulum stress (Sahu et al. 2016); glutamine deficiency

appeared to lead to increased intestinal permeability, which was resolved following glutamine supplementation (Wang et al. 2015). NEAAs such as glycine, glutamine and serine make up large quantities of fetal plasma, and so are vital to fetal growth and development (Bazer et al. 2014). Hydroxyproline is thought to play an important role in regulating cellular redox states, and alongside glycine and proline is a key building block of collagen, a protein vital for maintaining the structural integrity of connective tissues (Wu et al. 2011, Li, and Wu, 2018.). However, many of the current commercially available 'high quality' proteins are deficient in glycine, hydroxyproline, and proline, therefore, have little stimulatory impact on connective tissue synthesis (Dideriksen et al. 2016). In light of this expanding area of research into NEAAs, it is important a proteins NEAA profile is investigated in future studies to determine additional potential nutritional benefits aside from MPS regulation.

1.4.4. Digestibility

Digestibility can impact on bioavailability of AAs and therefore protein quality; the faster AAs reach the blood stream, the quicker they can be absorbed, and used to stimulate MPS and muscle remodeling (Gaudichon, and Calvez, 2021). Previous studies have found higher bioavailability leads to greater muscle gains: when comparing milk to casein protein in , milks' MPS response was significantly higher. Crucially, not only did Milk possess higher leucine content, but it was also more rapidly digested (Hartman et al. 2007), therefore its constituents appeared in the blood stream at a faster rate and at higher concentrations, so were more readily available for MPS stimulation. Despite this, research has also found slower digestion of proteins may be more beneficial for overnight recovery, due to their gradual release into the blood stream supporting positive whole-body protein balance (Res et al. 2012).

The way in which a protein supplement is produced also impacts on its digestibility. Concentrate supplements tend to have the lowest purity at 35-80% protein content and have the slowest digestion rate typically due to: increased fat and lactose contents, alongside increased fibrous

structures in plants, all of which prolong food breakdown, and therefore delay postprandial rise in AAs (Gaudichon, and Calvez, 2021). Isolate supplements contain up to 90% protein and contain partly digested forms of protein so are broken down at a faster rate. Hydrolysates on the other hand are the purest supplement, containing over 90% protein and comprised of single amino acids and small peptides so are rapidly digested (Koopman et al. 2009).

1.4.5. Influence on Appetite

In addition to AA profiles and MPS responses, studies have investigated the potential of dietary protein as a tool to suppress appetite in order to combat the growing obesity pandemic (Chooi, Ding and Magkos. 2019). Appetite is controlled by many complex interactions between the digestive system, peripheral energy stores and the hypothalamus, most of which involve the use of chemical messengers.

The hormone Leptin, released from adipose tissue, acts to decrease appetite (Friedman and Halaas. 1998). Research has previously investigated the potential role of insulin in the regulation of leptin; it is thought glucose metabolism plays a paramount role in long-term transcription of leptin mRNA (Tsai, et al. 2012); insulin also has a known involvement in increasing mTOR pathway regulation (Wang and Proud, 2006), and increased postprandial insulin levels have been associated with elevated rates of leptin secretion (Carlson et al. 2009) - potentially from insulin's influence on mTOR increasing rate of translation and translocation. However, research into acute impacts of insulin on leptin regulation remains conflicted, with several studies showing no acute impact of insulin on leptin levels (Dagogo-Jack et al. 1996 and Pratley et al. 1996). It is well known AA consumption leads to elevations in insulin secretion (Floyd et al. 1966); as previously discussed, insulins involvement in leptin regulation, suggests protein could play an important part in tackling weight-loss, through indirectly increasing leptin-induced appetite reduction.

In addition to leptin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are released from endocrine cells in the bowel following meals, inducing satiety (Karra, Chandarana and Batterham.

2009, Turton et al. 1996). PYY and GLP-1 have both been found to increase significantly following a high protein meal (Batterham et al. 2006 and Chen et al. 2020); further indicating protein's potential for use in weight loss strategies.

The hormone ghrelin, produced mainly in the stomach, exhibits the opposite effect. Alongside stimulating growth hormone release, ghrelin has been shown to increase feeding (Nakazato et al. 2001) and premeal hunger (Cummings et al. 2001). Protein consumption has demonstrated superior ability to reduce postprandial ghrelin levels compared to carbohydrates (CHO) (Blom et al. 2006), leading to greater reductions in appetite for longer periods of time.

As well as the release of anorexigenic hormones, previous research has investigated the impact of serum AAs alone on appetite. A study comparing AA infusion to protein feeding found similar reductions in appetite in both groups: as serum AAs increased, hunger reduced, followed by a subsequent increase in hunger as AA concentrations fell (Mellinkoff et al. 1956). This research suggests, acute bouts of satiety following protein consumption are not reliant on stomach mechanoreceptor signaling, instead, that serum AAs themselves may act as a chemical signal to the brain triggering a reduction in appetite. More recent studies have supported this theory, demonstrating high protein diets increased vagal sensitivity to anorectic signals in comparison to high fat diets (Nefti et al. 2007). Additionally, the aminostatic control of appetite may be a safety mechanism incorporated by the body, to prevent serum AA levels not yest metabolized in MPS rising to toxic concentrations (Munro, 1978). Furthermore, investigations have shown certain AAs appear to have a greater anorexic impact than others. For instance, leucine alone demonstrated the same reduction in food intake compared to an AA mixture (Morrison et al. 2007); whereas, other AAs such as histidine or tyrosine did not demonstrate any effects, despite being key precursors to histamine and dopamine -key initiators of satiety (Bassil, Hwalla, and Obeid. 2007), suggesting AA composition of a protein source may affect its impact on appetite. However, current research is mixed with regard to protein source's impact on appetite; this may be due to previous

studies not taking into account timing of post protein meal, and in many cases not utilizing proteins in their single form, making it difficult to fully compare the anorexic properties of various protein sources (Veldhorst et al. 2008). Therefore, further studies using protein concentrates or hydrolysate forms are warranted to better test protein sources acute impact on appetite.

Overall, despite the various connections between protein and molecular regulators of appetite, there is still large disparity in the literature concerning protein's impacts on molecular appetite regulation (Leidy et al. 2010, Ben-Harchache et al. 2021), in addition to, conflicting relationships between perceived appetite and coinciding molecular regulation following PI (Smeets et al. 2008).

More robust investigation is required to better understand the interaction between protein and appetite regulation and explore how variations in AA profiles alter a source's impact on satiety. It is especially important for future studies into novel proteins to consider impacts on appetite regulation, in order to establish a wider comparative picture between sources.

1.4.6. Plant Proteins

Previous research has investigated various PP sources in an attempt to provide sustainable protein for the future. PP production requires far less energy (Pimentel, and Pimentel, 2003), water (Leitzmann, 2003) and land (Stoll-Kleemann and Schmidt, 2017) compared to animal-derived sources, giving it a lower carbon footprint overall. However, quality of individual PP sources are yet to match AP. Many PPs are considered 'incomplete', have lower %EAA or reduced leucine levels (van Vliet, Burd, and van Loon, 2015), which in many previous studies have demonstrated reduced ability to stimulate MPS (Wilkinson et al. 2007). Many PP also have slower digestibility compared to AP, likely due to their secondary protein structure (Carbonaro, Maselli, and Nucara, 2012); however, aforementioned, some studies suggest although slower digestibility delays anabolic signaling responses, it may benefit overnight recovery and lead to a greater protein balance overall (Res et al. 2012).

In an attempt to improve PP quality, studies have utilized various methods to improve its anabolic properties , and thus postprandial aminoacidemic responses, including: selective breeding (Bruce et al. 2019 and Prasanna et al. 2001); AA fortification (Fomon et al. 1973); blending PPs with contrasting amino acid profiles (Gorissen et al. 2018) and promising evidence now suggests, increasing quantity of PP intake, can successfully attain protein dietary requirements to the same level as AP (De Gavelle et al. 2017). Although PP development displays potential, further research is required to refine their anabolic properties and maximize capacity of PP sources for the future (van Vliet, Burd, and van Loon, 2015).

1.5. Protein and Planetary Health

Despite AP demonstrating superiority over more sustainable sources such as PP, there are many concerns regarding sustainability of AP's production. The UK government found that in 2022, agriculture accounted for around 44.8% of greenhouse gas (GHG) emissions in the UK alone. In addition, previous reports found livestock contribute 80% towards global agriculture emissions (McMichael et al. 2007), totaling higher than global transport emissions. Furthermore, around 44% of the most pollutive livestock – beef, is inedible, and therefore cannot contribute to global nutritional demands (USDA, 2011).

Therefore, more modern proteins such as mycoprotein, produced using fermentation processes are considered high in quality due to their relatively low carbon footprint (Finnigan et al. 2019). In addition, alternative animal protein sources such as marine hydrolysates which are produced largely from biproducts of the fishing industry, have recently captured the interest of the protein industry, due to its more sustainable production methods which are of a larger and more viable production scale, capable of providing greater world-wide food security than current commercial AP sources (Kristinsson and Rasco, 2000).As the global population is expected to rise above 8.5 billion by 2030 (United Nations), and climate change continues to accelerate at an alarming rate, sustainable sources of protein are now needed more than ever before. It is vital research

continues to discover novel, high-quality protein sources, which can sustainably meet nutritional demands; with the ultimate aim of maintaining muscle mass, and lengthening health spans for future generations.

1.6. Marine Protein

Furthering the search for high quality sustainable proteins, research has begun to explore the possibilities within Marine Protein Hydrolysates (MPH). MPHs comprise of marine protein broken down into its constituent peptides and amino acids (Venugopal. 2016).

Fish is one of the largest global proteins consumed by humans, with over 184.6 million metric tons consumed in 2022 alone (Shahbandeh, 2023). However, over 60% of products produced from fish farms are discarded as waste, such as skin, guts, and bones (Chalamaiah, Hemalatha and Jyothirmayi. 2012). MPH are able to make use of these protein-rich waste products, making its supply lucrative and sustainable. By harnessing the potential of MPHs, fish farms can more than double their nutritional export, thus reducing the reliance on other more pollutive livestock supplies of protein such as cattle, aiding in lowering livestock contribution to GHGs. In addition to indirectly reducing GHGs, MPHs have the potential to boost economies of countries such as Norway for example, where the fish industry is the third largest contributor to its economy behind oil and gas (The Heritage Foundation, 2023). As well as drive economies of developing countries such as Vietnam which rely heavily on the fishing industry (Nguyen, 2023). Although, more work needs to be done to establish the required infrastructure to produce MPHs in such countries, as the hydrolysis process can be a costly investment (Siddik et al. 2012).

Due to their great nutritional and economic potential, research is developing a growing interest in MPH as a potential high-quality sustainable protein source for the future. Current research has established promise in Blue-whitening fish species, demonstrating high aminoacidemic responses with plasma leucine levels in particular, which although significantly lower than dose-matched whey protein, produced an equivalent anabolic response in vitro (Lees et al. 2021).

Further research has enlightened promise in Tilapia fish species, which produced similar aminoacidemic responses to whey protein following a bout of resistance exercise (Cordeiro et al. 2020); therefore, can be considered a promising alternative to currently marketed protein sources. In addition to MPHs high anabolic potential, studies have noted additional health benefits such as improved intestinal health following consumption of pacific whiting MPH (Marchbank et al. 2008) and potential therapeutic effects of cuttlefish MPH on hypertension have also been demonstrated in animal models (Balti et al. 2015).

Further to rapid digestibility and potential muscle anabolic effects, by-products from the fishing industry typically comprise largely of connective tissues, therefore, contain a significant level of collagen (Pal, and Suresh, 2016; Rajabimashhadi et al. 2023). Aforementioned, collagen is rich in glycine, proline and hydroxyproline, thus their consumption has proved beneficial for connective tissue remodeling (Holwerda, and van Loon, 2022; Li and Wu, 2018.). However, at present, the processing of fish by-products into novel protein sources varies greatly between companies, which can largely impact on their AA profiles and digestibility. Consequently, it is important future studies investigate postprandial aminoacidemic responses of novel MPHs, in order to determine their potential to support connective tissue and muscle remodeling. This novel protein-origin has paved an exciting path for protein research, with many marine protein sources yet to be explored. Notably, BIOMEGA (Skogvag, Norway) has recently developed a promising salmon-derived MPH, with a relatively high EAA and NEAA profile compared to current high-quality sources such as milk protein; in particular the novel salmon MPH possesses high glycine, proline and hydroxyproline content (see **table 2**), offering a sustainable protein source with high digestibility (see section **2.3** for production process), with promising potential to robustly stimulate muscle and connective tissue remodeling. However, it has yet to be determined if this new Salmon Protein Peptide (SPP) source can match up to current AP supplements postprandially. Therefore, this study aims to investigate the following...

1.7. Aims and Objectives

The aims and objectives of this study were as follows:

1.7.1. Determine and compare the postprandial aminoacidemic responses of milk protein isolate (MPI) to hydrolyzed Salmon protein peptide (SPP) in healthy young males and females.

In order to achieve this aim, the current study will compare the plasma aminoacidemic responses of SPP and a dose matched MPI (e.g., high-quality control).

We hypothesized that SPP would generate higher NEAA responses, due to its greater glycine proline and hydroxyproline content. Despite SPP's lower EAA and leucine content, we hypothesized comparable postprandial levels would be observed, due to SPP's hydrolyzed form allowing for faster digestion and absorption of AAs.

1.7.2. Determine the effects of milk protein isolate (MPI) and hydrolyzed Salmon protein (SPP) on appetite regulation in healthy young males and females.

To compare perceived appetite responses to SPP and MPI, utilizing visual analog scales across a 4hr period followed by an ad-libitum meal.

We hypothesized an initial transient alteration in perceived appetite immediately post protein ingestion, which is generally observed following protein consumption. Alongside a secondary hypothesis, that no significant differences in perceived appetite, insulin or glucose levels would be apparent between the two supplements, due to the relatively small differences in overall protein content.

2. Methods

2.1. Participants

11 young, healthy people participated in this randomized single-blind crossover study, 6 male and 5 female (see Table 1 below for characteristics). Both sexes were recruited amongst the cohort during this investigation in (roughly) even numbers, in order to better represent the wider population. During a preliminary visit, participants received detailed explanations of study purpose, investigation procedures and potential risk factors, after which, informed consent was obtained in writing. Following consent, a general health questionnaire was conducted to determine participant eligibility for the study. Exclusion criterion included: Persons with a BMI of <18.5 or > 29.9 kg/m^2, any history of chronic disease or illness, individuals with any known allergies or intolerances to the protein supplements and ingredients to be digested as part of this study (including shellfish), individuals that habitually smoke and/ or regularly skip breakfast, finally, persons who regularly consumed any supplementary aids or medication known to affect muscle protein metabolism. Following a successful GHQ, participants were taken to the laboratory for height, weight, and body composition analysis (using Bioelectrical Impedance Analysis), to confirm healthy status for participation, and to determine if body composition significantly changed throughout the study.

Table 1. Participant Characteristics

Age (y)	21.9 ± 3.3
Height (cm)	175.4 ± 10.5
Weight (kg)	74.2 ± 17.2
BMI (kg/cm^2)	23.9 ± 3.6
Fat Mass (kg)	15.2 ± 6.0
Body Fat (%)	20.5 ± 6.6
Fat free mass (kg)	58.8 ± 14.3
Skeletal Muscle Mass (kg)	56.3 ± 14.1

Values represent mean ± standard deviation.

2.2. Ethical Approval

Ethical approval was provided by the School of Sport Exercise and Rehabilitation Science's Ethical Review Board at the University of Birmingham (Study Number: ERN_15-0522). The investigation was carried out in accordance with Ethical Review Board standards within the School of Sport Exercise and Rehabilitation Science's at the University of Birmingham - aligning with the standards of human experimentation and in accordance with the Declaration of Helsinki - 1975, as revised in October 2013.

2.3. Protein Supplements

The SPP was obtained from BIOMEGA (Skogvag, Norway) and MPI from MyProtein (Northwich, UK), previously used in a study by Smeuninx et al in 2017, (supplement characteristics can be found in Table 2). A mean dose of 21.7±4.6g of protein was administered to each participant during trials. Overall protein content of SPP was 93.5%, compared to 78% in MPI. Therefore, protein powder was adjusted accordingly, in order to dose-match protein during trials; on average participants received: 23.3±4.9g/kg of SPP or 27.9±5.9g/kg MPI during trials (more MPI powder was administered to account for the higher protein content in SPP).

2.3.1. Novel Salmon Protein Peptide (SPP)

SalMe Peptides was produced from food grade salmon raw materials, using commercial food grade, non-GMO proteases in a patented process at Biomega Norway AS (Skageneset, Norway). Following the enzymatic hydrolysis, all fractions were heated to >85°C before water-soluble content was separated from fat and the non-soluble fractions via centrifugation. Following this, water-soluble fraction is ultra- and nano filtered. The retentate from the nanofiltration was further concentrated using an evaporator, before being spray dried. The final product consisted of a mixture of peptides and free amino acids, alongside other water-soluble nutrients.

2.4. Study Design

The study was a randomized single-blind crossover design, to prevent knowledge of supplement consumed impacting on perceived appetite or ad-libitum energy intake. The primary outcome measure was postprandial plasma aminoacidemia following ingestion of SPP and MPI. Secondary outcome measures included postprandial levels of plasma Insulin, glucose, alongside perceived appetite. Participants attended two laboratory trials, at least one week apart. On both trials, individuals consumed either SPP or MPI at a protein-matched dose of 0.3g/kg BM (Dosage Mean ±SD: Salmon 23.3g ± 4.9. Milk 27.9g ± 5.9) to ensure PI was relative to body mass; this was mixed with 300ml water, which was later swilled with 200ml of water and ingested to ensure all content in the bottle was consumed. Participants were assigned supplement order by a computer randomizer; each participant was their own control, as all participants trialed both supplements in a blinded, randomized order on two separate occasions. Protein shakes were served in opaque bottles, mixed with dose-matched vanilla flavoring (MyProtein) and nose clips were worn during consumption to further strengthen blinding of trial conditions. Food diaries alongside periodical visual analog scales (VAS) were utilized to assess changes in appetite and dietary composition between conditions. During each trial, participants were asked to mark along a 100mm line, where they thought appropriately represented their appetite; the distance along the line was then measured to provide an appetite score for each question (see **Fig. 2** below for VAS questions presented to participants). Blood samples were taken prior to consumption and routinely for the following 4 hours. Following sampling, participants were provided with an ad-lib meal in order to assess appetite further (see **Fig. 3** below for a visual representation of the trial timeline). All trials were scheduled at the same time of day to ensure diurnal and intrasubject variation was minimized.

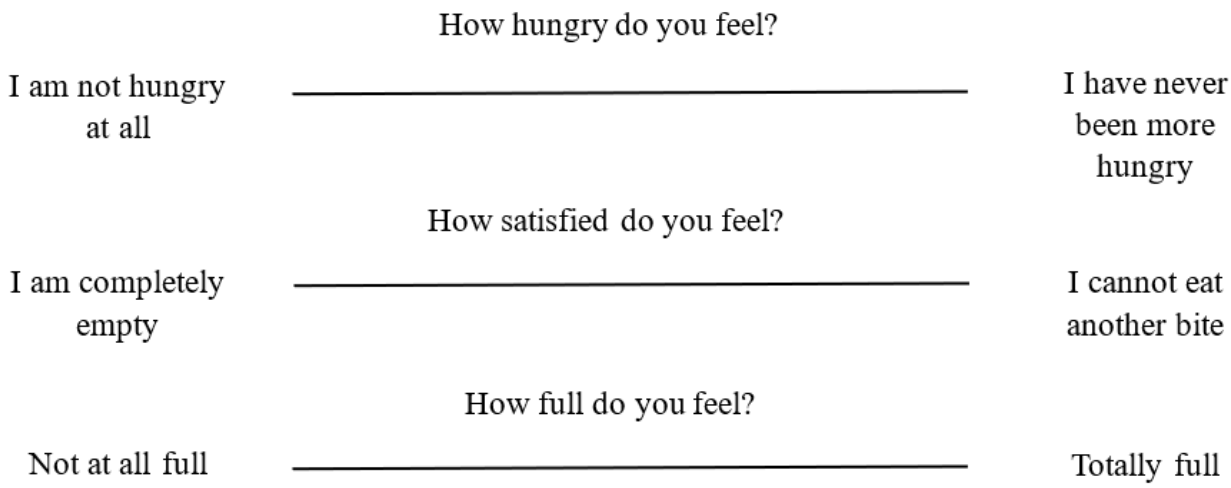


Figure 2. Illustration of VAS questions posed during trials.

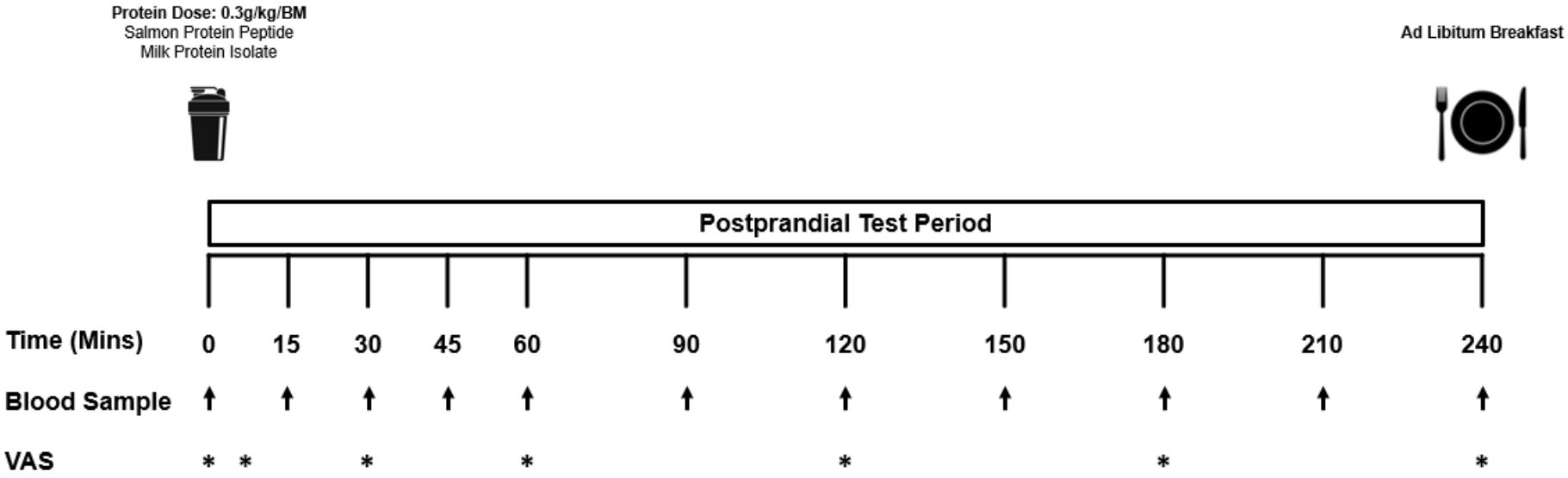


Figure 3. Postprandial study design schematic.

Table 2: Supplement Composition

Amino Acid Composition	Salmon Protein Peptide (%)	Milk Protein Isolate (%)
Aspartic Acid	9.4	7.4
Glutamic Acid	14.5	9.1
OH-Proline	4	
Serine	4.6	5.3
Glycine	15	1.7
Histidine	2	2.3
Arginine	7	3.7
Threonine	3.9	3.8
Alanine	7.8	3
Proline	7.3	9.9
Tyrosine	1.7	4.6
Valine	3.5	6
Methionine	2.6	2.6
Isoleucine	2.7	5.6
Leucine	4.8	9.8
Phenylalanine	2.6	4.7
Lysine	7.4	8.7
Tryptophan	0.81	1.7
Total cysteine/cystine	0.49	0.9
Total EAA	**30.31**	**43.8**
Total BCAA	**11**	**21.4**
Macro and Micronutrient Composition	**Salmon Protein Peptide (g)**	**Milk Protein Isolate (g)**
Fat	<0.5	1.4
Of which saturates	<0.5	1.2
Carbohydrates	n.d.	5.4
Of Which Sugars	<0.04	4.4
Protein	93.5	81
Salt	0.6	0.2

EAA = Essential Amino Acids. BCAA = Branch Chain Amino Acids

2.5. Pre-Experimental Trial Protocol

To gain baseline measures of appetite and habitual dietary composition, during the 3-5 days following preliminary visits, participants were asked to complete a food diary of their habitual diet. Individuals were provided with a standardized meal to consume by 2200 the night before trial days, which was personalized to each participant (each meal consisted of ~ 413 kcal, 56% CHO, 22% protein and 22% fat). Participants remained fasted (apart from water intake) before attending the laboratory the following morning; this was to ensure recent dietary intake had minimal impact on samples from either trial (see Table 3 for nutritional information of standardized meals). In addition, food diaries were completed the day before so control measures could be checked following study completion. Participants were also given a physical activity diary and pedometer to record daily activity the day before and the day of trials. Individuals were asked to abstain from strenuous physical activity 24 hours prior to and on the day of trials, and repeat the activity logged on their second visit.

2.6. Experimental Trial Protocol

Upon arrival at 0700, participants were seated on a bed and their arm placed under a heated blanket to arterialize venous blood for sampling. Following arterialization, an intravenous canular was inserted into the antecubital vein to allow repeated blood sampling (10ml every timepoint). Following a fasted baseline blood sample and VAS, participants consumed either a SPP or MPI shake. Participants could consume ad-libitum water throughout the 4-hour period, and water intake was repeated on the second trial. Blood Samples were taken at 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240minutes post-protein ingestion, in order to identify changes in postprandial plasma aminoacidemia and changes in insulin. VAS scales were completed at 5, 30, 60, 120, 180 and 240minutes following protein consumption. Following the final blood sample and VAS, the intravenous canular was removed and participants were provided with an ad-libitum meal consisting of: 500ml water, 75g Cornflakes, 500ml semi-skimmed milk, six slices of white bread,

10g margarine, 45g raspberry jam, 2 strawberry yogurt pots, three medium bananas, three royal gala apples, four breakfast bars, six scotch pancakes, four baked bean pots, four mini mature cheddar cheeses and two porridge pots (**Table 3**). Participants could eat as much or as little as they liked and were not aware meal consumption was monitored to assess appetite levels and dietary composition choices, to avoid knowledge of monitorization impacting on the volume of food eaten. Finally, for the rest of the day, participants were asked to complete a food diary and fill out 3 more VASs (Pre-lunch, Pre-dinner and Pre-sleep). Food diary and ad-libitum meal data was later assessed using Nutritics.

Table 3: Meal Nutritional Information

Item	Brand	Amount	Weight (g)	Energy (Kcal)	CHO (%Kcal)	FAT (%Kcal)	PRO (%Kcal)
Cottage Pie,	Sainsbury's	1	400	344	44	31	25
Lentil Cottage Pie	Sainsbury's	1	400	297	75	11	14
Spaghetti Bolognese	Sainsbury's	1	400	427	48	29.7	22.3
Mixed Frozen Vegetables	Sainsbury's	2	80	40	60	13	27
Cornflakes	Kellogg’s	1	75	282.5	90	2	8
SS-Milk	Sainsbury's	1	500	235	39	31	30
White Bread Slice	Warburtons Toasty	6	47.3	116	78	7	15
Margarine	Clover	1	10	59	1	98	1
Raspberry Jam	Sainsbury's	1	45	107.6	100	-	-
Scotch Pancakes	Sainsbury's	6	50	74	78	12	10
Strawberry Yogurt	Activia	2	115	104	54	28	18
Bananas	Sainsbury's	3	118	89	93	3	4
British Apples	Sainsbury's	3	100	54	95	-	5
Cereal Bar	Eat Natural	4	45	215	45	51	4
Porridge Pot	Oat So Simple Original	2	45	164	65	15	20
Bean Snap Pots	Heinz	4	200	158	71	3	26
Mature Cheddar	Cathedral	4	20	83.0	0.0	76	24
Water	Tap	1	500	0.0	0.0	0.0	0.0

Values with - contain <0.5%

2.7. Blood processing and analysis

Each 10ml blood sample was collected via syringe and divided evenly (x2 5ml) into two vacutainers (Becton Dickinson, UK); one pre-treated with K_2EDTA anticoagulant for plasma collection (catalog number: 367525), and the other pre-treated with Silica (Clot Activator)/Gel for serum collection (catalog number: 367958). Serum samples were rested at room temperature for 40 minutes to enable clotting. During this time, plasma samples were rested on ice to prevent clotting. All samples underwent centrifugation at 4000RCF for 10minutes at 4°C. Plasma and serum were aliquoted from samples and stored at –80°C prior to analysis.

Plasma glucose levels were determined using the glucose hexokinase method via a Randox rX Daytona chemistry analyzer (Randox Laboratories LTD, Crumlin, UK). Samples were analyzed in duplicate, following manufacturer protocols. All reagents utilized during analysis were obtained from Randox Laboratories LTD (Ref: GL8319).

Plasma insulin levels were examined in duplicate via an ultrasensitive insulin ELYSA kit (catalogue number: 10-1132-01; Mercodia AB, Sylveniusgatan, Sweden) following manufactures instructions. In brief, a solid two-site enzyme immunoassay was used, employing the direct sandwich technique. After administering 25µL of each sample, calibrators, and controls into their relevant wells, 100µL enzyme conjugate solution was added to each well and the plate was incubated for 1hr at room temperature. Plates were washed 6 times with an automatic plate washer to remove unbound enzyme labelled antibody. 200µL Substrate TMB was added to each well. Following 30minute incubation at room temperature, 50µL Stop Solution was added to each well to produce a colorimetric endpoint which was read spectrophotometrically.

Analysis of plasma AA levels was conducted using EZ: faast procedure according to the manufacturer's instructions. In brief, 50µL of plasma was combined with 50µL of EZ: faast internal AA standard and 50µL of DDH_2O in a sample preparation vial. Samples were transferred slowly (1 min) through a sorbent tip attached to a 1.5mL syringe. 200µL of wash solution (1-propanol

and H_2O) was added to sample preparation vials and transferred slowly through the sorbent tips into the syringe barrel. Liquid accumulated in the syringe barrel was then discarded. 200µL of freshly prepared elution medium (a NaOH-based solution) was added to the sample preparation vial. Using a 0.6mL syringe, the eluting medium was transferred slowly through sorbent particles to the filter plug in the sorbent tip. After, the liquid and particles were ejected from the syringe into the sample preparation vial; this step repeated a further two times. 50mL of derivatization solution (a mixture of CHCl3, 2, 2, 4 trimethylpentane and propylchloroformate) was added to the sample preparation vial and vortexed for 10 sec to emulsify. To separate the emulsion into 2 layers, 100ul of Acid solution (HCl-based) was added. The upper layer (containing derivatized AA's) was transferred to an auto sampler vial and analyzed via Gas Chromatography (Agilent 6890W) fitted with a Flame Ionization Detector. NEAA levels during the current study represent the sum of alanine, arginine, asparagine, citrulline, cysteine, glutamine, glutamic acid, glycine, ornithine, proline, taurine, and tyrosine.

2.8. Statistical Analysis

Changes in appetite throughout the trial day were assessed using a 2-way ANOVA with condition and time factors alongside iAUC to determine group effects. iAUC values were calculated with the trapezoid method using the Time Series Response Analyser (Narang et al., 2020). Changes in dietary composition were assessed using 2-way ANOVA with composition and condition as factors. Dietary composition during trial days were compared to habitual dietary intake values (HAB) gained from food diaries prior to experimental visits. A $p<0.05$ was considered statistically significant. Changes in plasma glucose levels were calculated using a mixed effects analysis, to a significance level of $p<0.05$. Changes in plasma insulin levels were calculated using a 2-way AVONA, A $p<0.05$ was considered statistically significant. iAUC was also used to assess group differences in glucose and insulin (Narang et al., 2020). Changes in plasma AA levels were calculated using a mixed effects analysis, to a significance level of $p<0.05$; iAUC was also utilized

to determine group effects on plasma AA levels (Narang et al., 2020). Data was presented as mean ± SEM. All statistical analyses were completed on Prism 9.3.1 Graph Pad.

3. Results:

3.1. Postprandial plasma glucose and insulin levels.

Daytona analysis of plasma Glucose Hexokinase demonstrated an overall decline in glucose levels following protein consumption (time effect: p=0.0123; **Fig. 4A**); in SPP glucose levels were significantly higher at 15minutes compared to fasted state (Mean Percentage Increase = 4.1±20.8, p= 0.0038), following this, glucose decreased steadily until 60minutes post-ingestion, then a secondary rise occurred until 120minutes, followed by a final decrease to 240minutes. Glucose levels following MPI did not significantly increase above fasted levels, although followed a similar pattern to SPP. No group differences were observed between SPP and MPI (p=0.9918). Likewise, no group x interaction effect was observed between the two trials (all p=0.9183). In addition, no significant difference was observed between glucose iAUC values (p=0.9247; **Fig. 4B**). ELISA of plasma insulin demonstrated an increase in plasma insulin following protein intake (time effect: p<0.0001; **Fig. 4C**); in SPP insulin levels were significantly higher at 30 (Mean Percentage Increase = 408.4±9.5% p<0.0001) and 60 minutes compared to fasted state (Mean Percentage Increase = 119.9±32.2% p=0.0125). SPP insulin levels remained elevated above fasted state until 90minutes post-protein intake. Similarly, insulin levels following MPI were significantly greater at 30 (Mean Percentage Increase = 323.2±13.3% p<0.0001) and 60 minutes compared to fasted levels (Mean Percentage Increase = 127.5±33.8% p=0.0190). MPI insulin and remained elevated above fasted state until 120minutes post-protein intake. No group differences were observed between the supplements (p=0.6070). Similarly, no group x interaction effect was observed between the two proteins (p=0.0664). Finally, iAUC comparison demonstrated no significant differences between supplements (p=0.7542; **Fig.4D**).

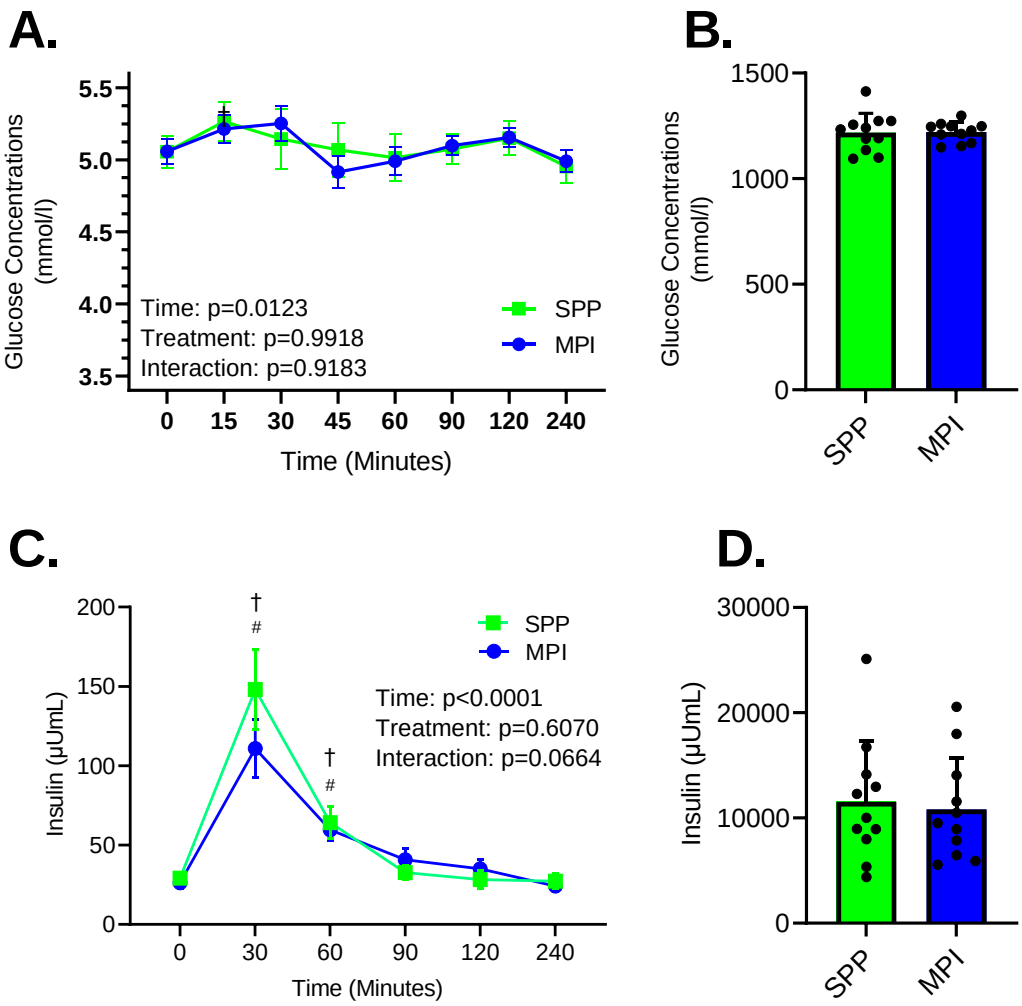


Figure 4. Postprandial plasma glucose and insulin concentrations.
Figures demonstrating mean ±SEM in plasma glucose and insulin levels throughout Salmon Protein Peptide (SPP) and Milk Protein Isolate (MPI) trials. (A) Post prandial plasma glucose concentrations found in blood samples taken across a 240-minute period. (B) Glucose area under the curve values (iAUC). (C) Post prandial plasma insulin concentrations found in blood samples taken across a 240-minute period. (D) Insulin iAUC. † SPP = p ≤ 0.05 compared to timepoint 0minutes, # MPI = ≤ 0.05 compared to timepoint 0minutes.

3.2. Postprandial plasma TAA, NEAA, EAA and leucine levels.

Gas chromatography analysis of TAA concentrations demonstrated an increase following protein consumption (time x interaction effect: P<0.001; **Fig. 5A**), Plasma TAA concentrations significantly increased above baseline values following 15 to 90 min following ingestion of SPP and MPI (all = p<0.05). Plasma TAA concentrations were significantly higher following SPP compared to MPI at 30, 45 and 60 min (all = p<0.001). SPP demonstrated significantly greater peak TAA levels compared to MPI (5004 ±296 vs. 3719 ±302μmol·L^{-1}, respectively; p<0.001). Time-to-peak TAA levels did not differ between proteins (SPP: 50 ±14 vs. MPI: 48 ±16min; p=0.552). Additionally, SPP demonstrated significantly greater plasma TAA levels following iAUC analysis compared to MPI (p=0.002; **Fig. 5B**).

Gas chromatography analysis of plasma NEAA levels (sum of alanine, arginine, asparagine, citrulline, cysteine, glutamine, glutamic acid, glycine, ornithine, proline, taurine, and tyrosine) demonstrated an increase following protein intake (time x interaction effect: p<0.001), NEAA levels following SPP intake were significantly higher than baseline at 30-90min (all= p<0.001). However, plasma NEAA only increased above fasted state at 45 and 60min post consumption of MPI (both = p<0.05). Plasma NEAA concentrations were significantly higher following SPP intake compared to MPI at 30, 45, 60 and 90min post intake (all = p<0.01). SPP demonstrated a significantly greater peak NEAA concentration compared to MPI (3376 ±197 vs. 2287 ±173μmol·L^{-1}, respectively; p<0.001). Although, time-to-peak TAA concentration did not differ between supplements (SPP: 49 ±18 vs. MPI: 49 ±17min; p=0.303) (**Fig. 5C**). In addition, SPP demonstrated significantly greater plasma NEAA levels following iAUC analysis compared to MPI, (p<0.001; **Fig. 5D**).

Gas chromatography analysis of plasma EAA concentrations demonstrated a significant increase following protein intake (time effect: p<0.001, **Fig. 5C**), although, no significant difference between proteins was identified (group effect: P=0.722; interaction effect: P=0.836). Plasma EAA

concentrations were increased above fasted state from 30-90min following intake of both SPP and MPI ($P<0.05$ for all). Peak EAA concentration did not significantly differ between SPP and MPI (1361 ±196 vs. 1340 ±166μmol·L^{-1}, respectively; $p=0.658$). In addition, time-to-peak EAA did not demonstrate a significant difference between SPP and MPI (52 ±21 vs. 47 ±18min, respectively; $p=0.196$). Finally, no differences in iAUC for EAA were observed between proteins ($p=0.551$, **Fig. 5D**).

Gas chromatography analysis of plasma leucine demonstrated an increase following protein ingestion (time x interaction effect: $p=0.0139$; **Fig. 5G**). Leucine concentrations increased above fasted state from 15-150min following ingestion of MPI (all $p<0.05$), whereas plasma leucine increased from 15-60min following SPP intake (all $p<0.05$). Leucine concentrations were significantly higher following MPI compared to SPP at 90 and 120min post intake (both $p<0.01$). Peak leucine levels did not significantly differ between proteins (SPP: 250 ±32 vs. MPI: 246 ±31μmol·L^{-1}; $p=0.657$). SPP reached peak leucine levels at a significantly faster rate compared to MPI (31 ±14 vs. 46 ±17min, respectively; $p=0.034$). MPI also demonstrated significantly greater plasma leucine levels following iAUC analysis compared to SPP ($p=0.011$; **Fig. 5H**).

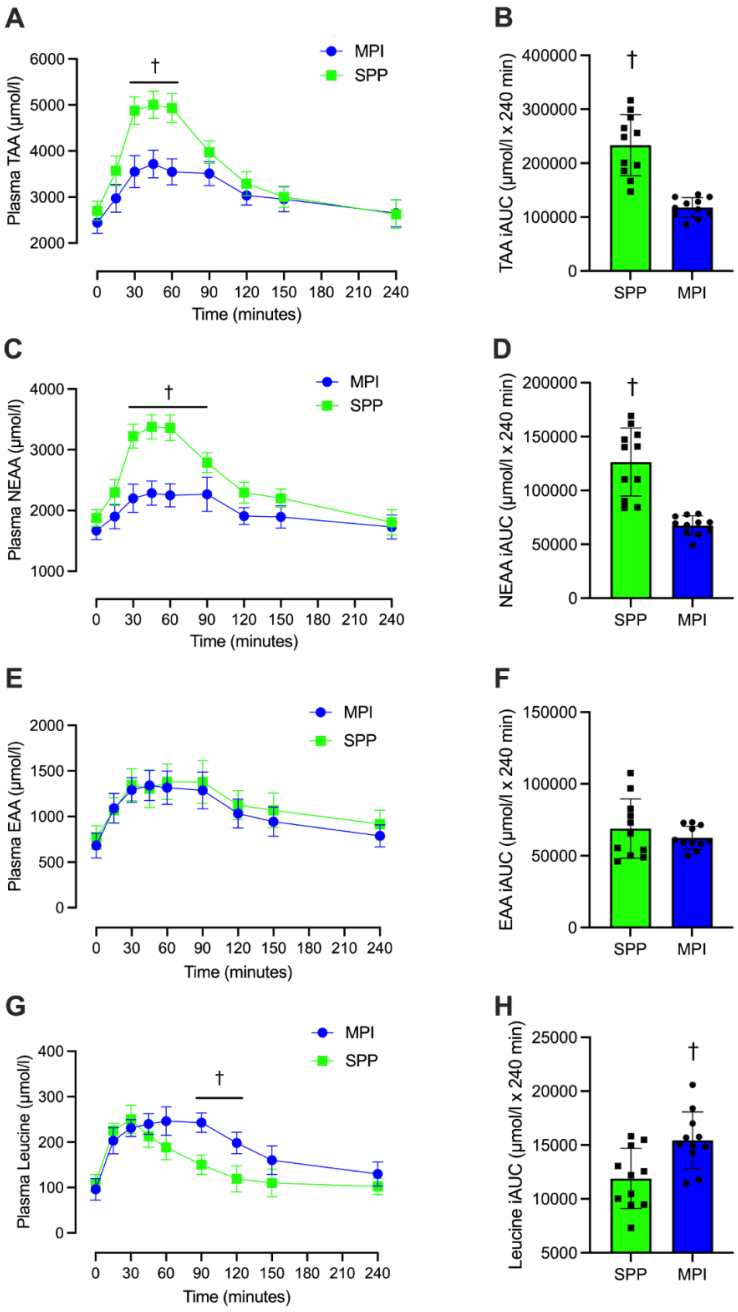


Figure 5. Postprandial plasma TAA, NEAA, EAA and leucine levels following SPP and MPI ingestion.

Figures demonstrating mean ±SEM of postprandial aminoacidemic responses following Salmon Protein Peptide (SPP) and Milk Protein Isolate (MPI) ingestion. (A) TAA levels in blood samples taken across a 240-minute period. (B) TAA area under the curve (iAUC). (C) NEAA levels in blood samples taken across a 240-minute period. (D) NEAA iAUC. NEAA level represents the sum of alanine, arginine, asparagine, citrulline, cysteine, glutamine, glutamic acid, glycine, ornithine, proline, taurine, and tyrosine. (E) EAA levels in blood samples taken across a 240-minute period. (F) EAA iAUC. (G) Leucine levels in blood samples taken across a 240-minute period. (H) Leucine iAUC. TAA = total amino acids. NEAA = non-essential amino acids. EAA = essential amino acids. † = $p \leq 0.05$.

3.3. Postprandial plasma glycine, hydroxyproline and proline levels .

Gas chromatography analysis of plasma glycine demonstrated a significant increase following protein intake (time x interaction effect: $p<0.001$; **Fig. 6A**)). Plasma glycine concentrations increased above fasted levels between 30-150min post SPP ingestion (all $p<0.05$). However, no changes in plasma glycine levels were observed post MPI ingestion. Plasma glycine concentrations were significantly higher following SPP compared with MPI between 30-120min post-intake (all $p<0.01$). Peak glycine level was significantly greater following SPP compared to MPI (456 ±24 vs. 284 ±33μmol·L^{-1}, respectively; $p<0.001$). Time-to-peak glycine concentration did not significantly differ between SPP and MPI (44 ±19 vs. 51 ±16 min, respectively; $p=0.127$). SPP also demonstrated significantly greater plasma glycine levels following iAUC analysis compared to MPI ($p<0.001$; **Fig. 6B**).

Gas chromatography analysis of plasma hydroxyproline (OH-Pro) demonstrated a significant increase following protein intake (time x interaction effect: $p<0.001$; **Fig 6C**). OH-Pro levels increased above fasted state from 30-240min following SPP intake (all $p<0.001$), whereas OH-Pro levels only increased at 60min post MPI ingestion ($p=0.044$). OH-Pro concentrations were significantly higher following SPP compared to MPI intake at timepoints 30-240min post-ingestion (all $p<0.01$). Time-to-peak OH-Pro levels did not differ between proteins (SPP: 51 ±21 vs. MPI: 46 ±13 min; $p=0.201$). Likewise, SPP demonstrated significantly greater iAUC values for OH-Pro compared to MPI ($P<0.001$; **Fig. 6D**)

Gas chromatography analysis of plasma proline concentrations demonstrated a significant increase following protein intake (time effect x interaction effect: $p<0.030$; **Fig. 6E**). Proline levels increased above fasted state at timepoints 30-90min following ingestion of SPP and MPI (all $p<0.001$). Proline concentrations were significantly higher following SPP intake compared to MPI at 30min post-ingestion ($P=0.037$). Time-to-peak plasma proline did not significantly differ

between supplements (SPP: 44 ±18 vs. MPI: 45 ±19 min; p=0.343). In addition, SPP demonstrated significantly greater proline levels following iAUC analysis (p=0.029; **Fig. 6F**).

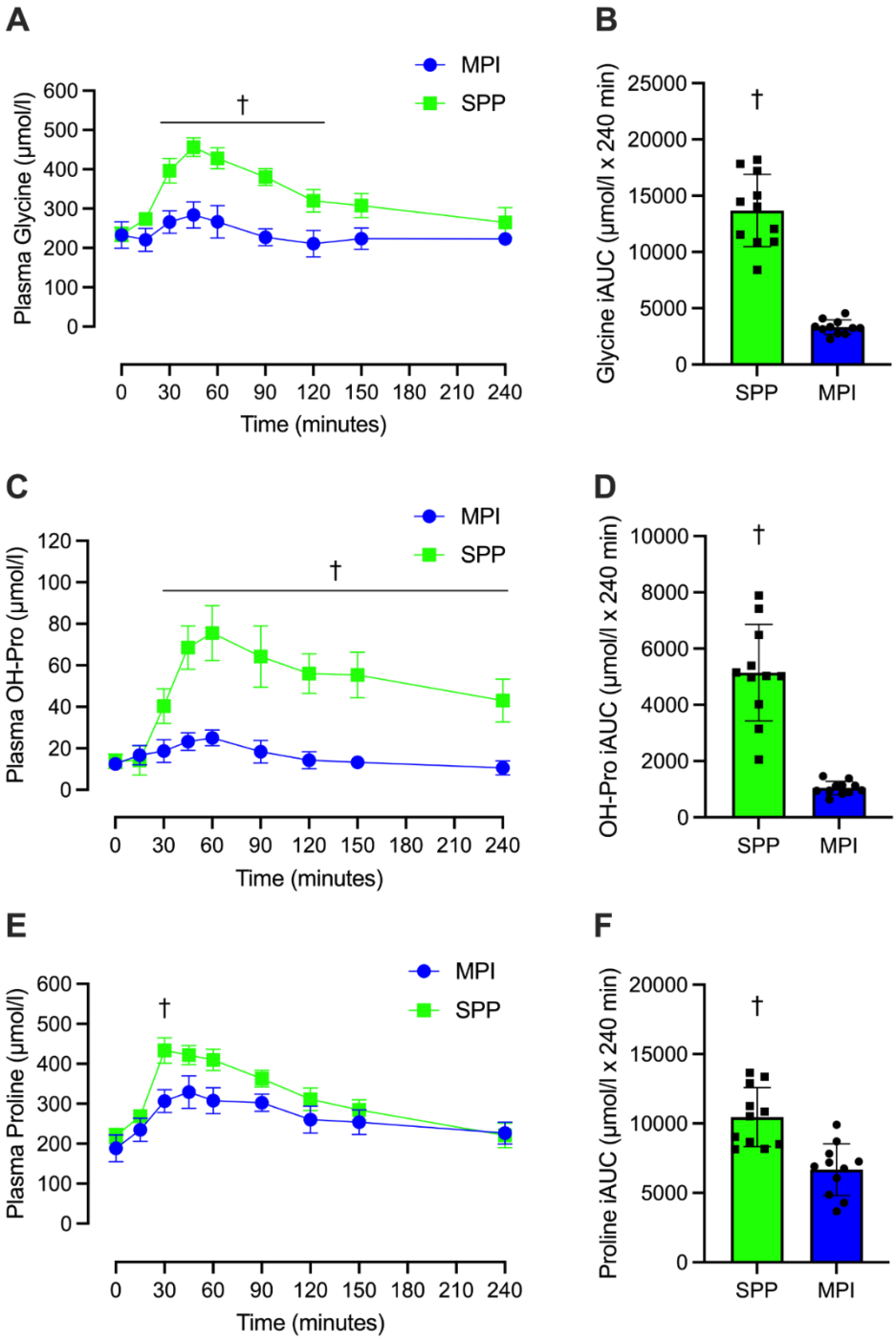


Figure 6. Postprandial plasma glycine, hydroxyproline and proline levels following SPP and MPI ingestion.

Figures demonstrating mean ±SEM of postprandial plasma amino acid levels following Salmon Protein Peptide (SPP) and Milk Protein Isolate (MPI) ingestion. (A) Glycine levels in blood samples taken across a 240-minute period. (B) Glycine area under the curve (iAUC). (C) Hydroxyproline levels (OH-Pro) in blood samples taken across a 240-minute period. (D) OH-Pro iAUC. (E) Proline levels in blood samples taken across a 240-minute period. (F) Proline iAUC. † = $p \leq 0.05$.

3.4. Protein ingestion demonstrated no impact on perceived appetite between groups during trials.

VAS scales for hunger (**Fig. 7A**), fullness (**Fig. 7C**) and satisfaction (**Fig. 7E**) demonstrated a time effect ($p<0.001$). Following SPP, hunger levels demonstrated a small decrease at 15minutes followed by a steady rise, but no significant changes compared to fasted state until 240minutes post-ingestion (Mean Percentage Increase = 58.6±27.2% $p=0.0189$; **Fig. 7A**). Fullness levels following SPP demonstrated a significant increase at 15minutes post-ingestion compared to fasted state (Mean Percentage Increase = 75.9±19.2% $p= 0.0019$; **Fig. 7C**); after which fullness levels decreased back to fasted levels, this decrease continued steadily throughout the trial. Despite a slight increase at 15minutes post-ingestion, followed by a steady decline back to fasted values, no significant changes in satisfaction were observed following SPP (**Fig. 7E**). Hunger levels following MPI demonstrated a small decrease at 15minutes followed by a steady rise, but no significant changes were observed compared to fasted state until 180minutes (Mean Percentage Increase = 55.7±30.9% $p=0.0387$; **Fig. 7A**) and 240minutes post-ingestion (Mean Percentage Increase = 70.2±19.9% $p=0.0025$; **Fig. 7A**). Following MPI Fullness demonstrated a significant increase at 15minutes post-ingestion compared to fasted state (Mean Percentage Increase = 93.3±16.7% $p= 0.0006$; **Fig. 7C**); after which fullness levels returned to fasted levels, this decline continued steadily throughout the trial. MPI demonstrated a significant increase at 15minutes post-ingestion (Mean Percentage Increase = 29±29.4% $p= 0.0300$ **Fig. 7C**), followed by a steady decline throughout the rest of the trial. Despite a slight increase at 15minutes post-ingestion, followed by a steady decline back to fasted values, no significant changes in satisfaction were observed following MPI throughout the trial (**Fig. 7E**). No group differences in perceived appetite were observed between SPP and MPI (hunger, $p=0.7328$., fullness, $p=0.8313$., and satisfaction, $p=0.8679$., **Fig. 7A-E**). Likewise, no group x interaction effect was observed

on any VAS (all p>0.7**, Fig. 7A-E**). Finally, no differences in iAUC were observed between groups for hunger (SPP,13363 ±1295 vs MPI, 14093 ±1527, p=0.6287, **Fig. 7B**), fullness (SPP, 6714 ±1469 vs MPI, 7035 ±1344, p=0.8955, **Fig. 7D**), satisfaction (SPP, 7707 ± 1518 vs MPI, 8172 ± 1598, p=0.7852, **Fig. 7F**).

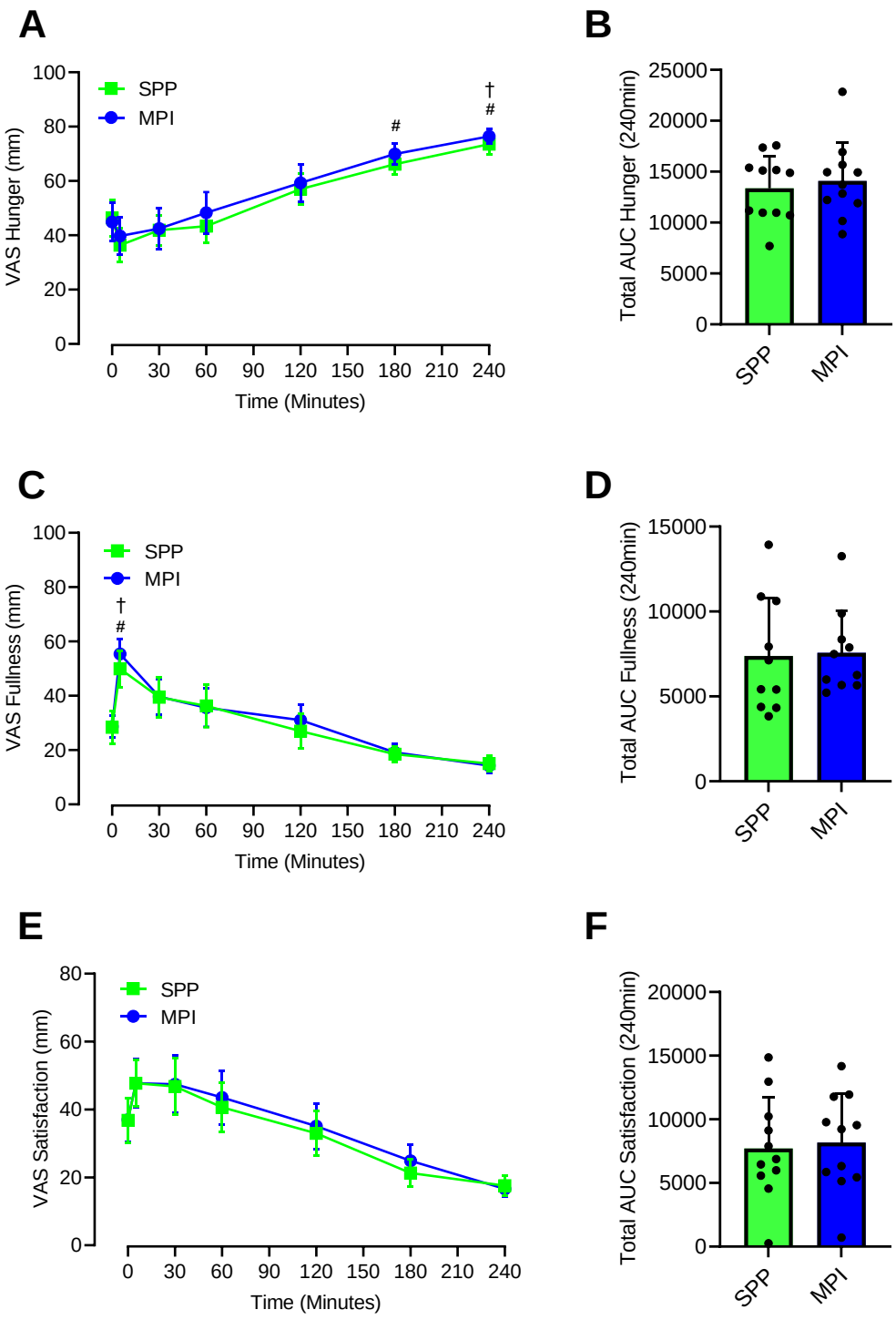


Figure 7. Appetite response following salmon and milk protein ingestion.
Figures demonstrating mean ±SEM in perceived appetite measures throughout Salmon Protein Peptide (SPP) and Milk Protein Isolate (MPI) trials. (A) Visual analog scores (VAS) of Hunger. (B) Hunger area under the curve (iAUC). (C) VAS scores of Fullness. (D) Fullness iAUC. (E) VAS scores of Satisfaction (F) Satisfaction iAUC. † SPP = $p \leq 0.05$ compared to timepoint 0 minutes. # MPI = $p \leq 0.05$ compared to timepoint 0 minutes. ns = No significant difference.

3.5. Protein ingestion showed no impact on dietary composition.

No significant differences in daily EI were observed following protein consumption compared to HAB (p=0.1303; **Fig. 8A**). No differences in dietary composition were identified between HAB, SPP and MPI for CHO (p=0.7922), PRO (p=0.1109) or Water (p=0.2892), **Fig. 8B, C, E**. However, daily FAT intake was significantly lower than HAB following SPP and MPI consumption (p=0.0156, p=0.0236, respectively), although no differences were observed between proteins (p=0.9805) (**Fig. 8D**). No significant differences in ad-libitum breakfast EI were found between proteins (p=0.9099; **Fig. 8F**), and no differences were identified between dietary composition of ad-libitum breakfast between trials (**Fig. 8G**) (see **Table 4** for average nutritional values).

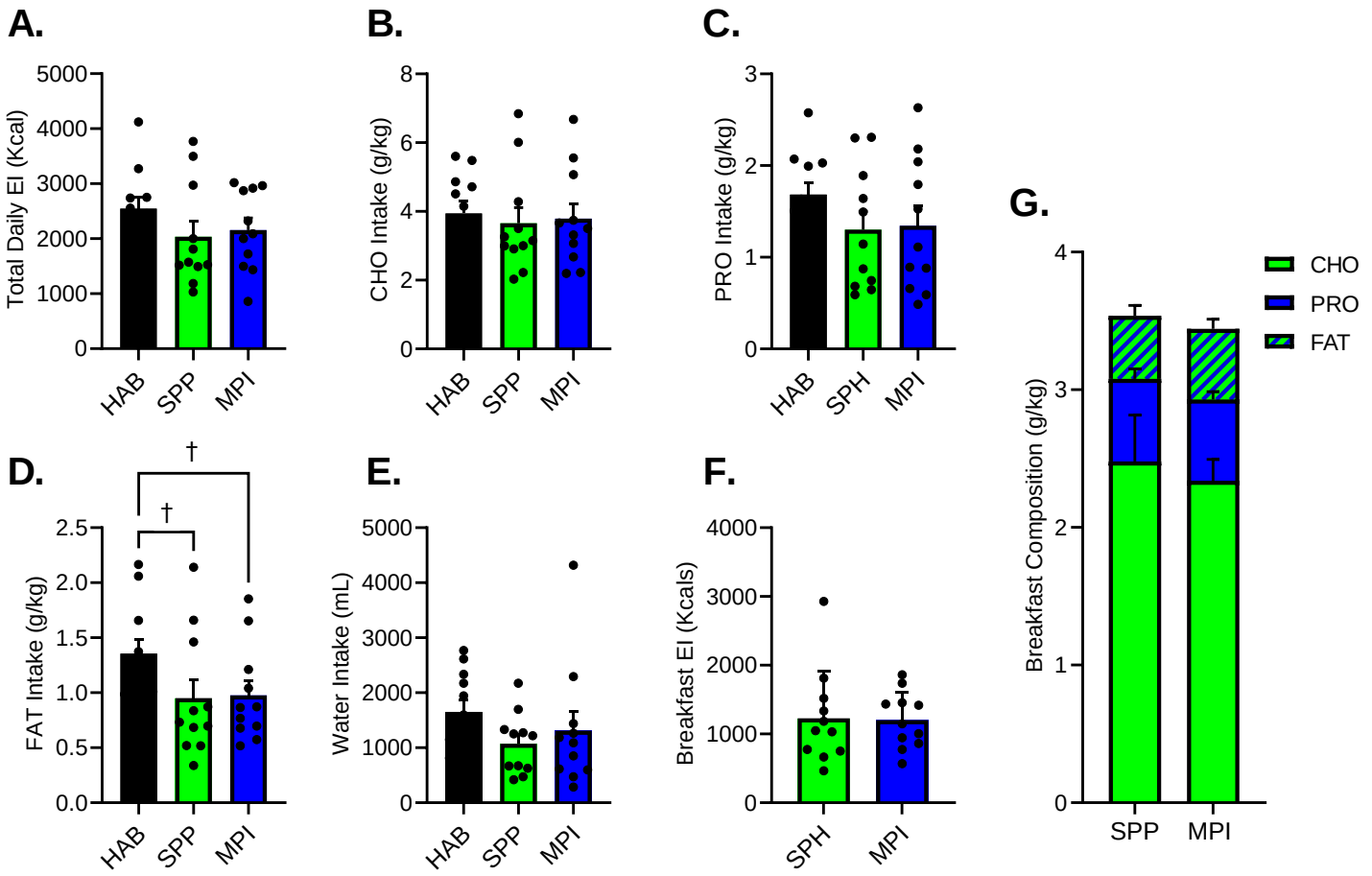


Figure 8. Daily dietary intake and during trial ad-libitum breakfast.
Figures demonstrating mean ±SEM of dietary composition: Habitually (HAB), alongside following Salmon Protein Peptide (SPP) and Milk Protein Isolate (MPI) trials. (A) Total daily energy intake (EI). (B) Daily carbohydrate (CHO) intake. (C) Daily protein (PRO) intake. (D) Daily fat intake. (E) Daily water intake. (F) Ad-libitum breakfast energy intake following trials. (G) CHO, PRO and FAT consumed during ad-libitum breakfast. † = $p < 0.05$.

Table 4: Average Nutritional Intake Values			
Dietary Component EI/Day	**HAB**	**SPP**	**MPI**
CHO (g/kg)	3.9±1.2	3.7±1.5	3.8±1.4
Protein (g/kg)	1.7.8±0.4	1.3±0.7	1.3±0.7
Fat (g/kg)	1.4±0.4	1.0±0.6	1.0±0.4
Total Water (ml)	1652.4±709.6	1073.9±528.1	1311.1±1088.9
Total EI (Kcals)	2549±699	2035±940	2155±732
Ad-lib Meal EI	**HAB**	**SPP**	**MPI**
CHO (g/kg)	0.9±0.3	2.5±1.1	2.3±0.5
Protein (g/kg)	0.3±0.2	0.6±0.2	0.6±0.2
Fat (g/kg)	0.2±0.1	0.5±0.2	0.5±0.2
Total EI (Kcals)	520±232	1229±688	1206±409

EI= Energy Intake HAB = Habitual. SPP = Salmon Protein Peptide. MPI = Milk Protein Isolate.

4. Discussion:

4.1. Overview.

This study investigated changes in postprandial plasma glucose, insulin, and aminoacidemia; as well as changes in perceived appetite, ad-libitum energy intake (EI) and dietary composition, following acute ingestion of protein-matched beverages relative to body mass, containing either SPP or MPI in young healthy individuals (**Table 1**). Overall, plasma TAA and NEAA were significantly greater following SPP ingestion, although, no differences were observed in EAAs (**Fig. 4A-F**) . Postprandial plasma leucine levels were significantly greater following MPI (**Fig. 4G-H**), although plasma glycine, hydroxyproline and proline were significantly greater following SPP (**Fig. 5**). No differences in postprandial plasma insulin or glucose levels were observed between proteins (**Fig. 3**). In addition, no differences in perceived appetite measures were evident between supplements (**Fig. 6**) and aside from a significant reduction in daily fat consumption, protein intake appeared to have little impact on dietary composition (**Fig. 7**).

4.1. Insulin and Glucose

No significant differences in postprandial plasma insulinemia were identified between supplements (**Fig. 3D**). Both Proteins followed a similar pattern, with significantly elevated insulin concentrations at 30 and 60minutes compared to fasted levels (**Fig. 3C**). The observed postprandial glucose response coincided with the lack of differences observed in insulinemia between proteins. Glucose demonstrated no significant differences between supplements for any timepoint, although, SPP did demonstrate significantly higher glucose levels compared to fasted state at 15 minutes post-ingestion (**Fig. 3A**). Research to-date appears mixed on the effects MPHs have on hyperglycemic control. One review by Sharkey et al, in 2020, found overall MPH appears to positively impact blood glucose and body weight. However, due to wide variation in study methodologies, it is yet to be determined by what mechanism MPHs might act on hyperglycemic control; several studies invitro and animal models coincide with the current investigation, demonstrating increased insulinemia following MPH consumption (Harnedy et al.

2018a, Parthsarathy et al. 2019). Although, in contrast to the current study, previous human research has shown insignificant impact on insulin levels (Sharkey et al, 2020). The smaller sample size, and short duration of the current study's trials, may have played a part in the lack of differences observed between glucose levels; SPP did demonstrate a significant rise in glucose at 15minutes post-ingestion compared to fasted levels; whereas MPI did not observe any significant changes compared to fasted state. Therefore, a larger sample size may aid in elucidating the impacts of SPP on glucose levels. Furthermore, studies have found MPHs appear to increase glucose uptake (Harnedy et al. 2018b), which may explain the sharp decrease in glucose levels following 15minutes into the trial. Many of the studies that observed positive impacts on glycemic control in the above review were longer-term interventions, with larger sample sizes of older populations; this may have allowed more scope to capture changes in glycemic control.

4.2. TAAs

At present, research comparing postprandial aminoacidemia from MPHs to commercially available high-quality protein sources is sparce. Previous investigations from Lees et al (2021) and Cordeiro et al (2020), demonstrated similar postprandial TAA levels following MPHs compared to dose-matched whey protein (WP) throughout trials. These findings differ from the current study; although time-to-peak TAA did not differ between the two supplements, SPP displayed a larger TAA response compared to MPI at time points 30-60min (**Fig 4A**), as well as a significantly higher iAUC value for SPP (**Fig 4B**). As both supplements were dose-matched for protein throughout the study (**Table 2**), the variance in TAA levels is likely due to a superior digestion rate by SPP. MPHs have been shown to possess faster digestion rates, due to their higher free AA content resulting from hydrolysis processing, allowing hydrolysates to cross the gastrointestinal membrane much quicker (Koopman et al. 2009). TAA levels in the two previous studies (Lees et al. 2021 and Cordeiro et al. 2020), also peaked at the same timepoint, supporting the theory that the larger TAA concentrations observed following SPP in the current study, are

due to rapid digestibility. Previous studies have demonstrated WP isolates and hydrolysates are digested at a significantly faster rate compared to milk protein (MP) (Mitchell et al. 2015) and so may have lessened the difference in time-to-peak generated by digestion in Lees and Cordeiro's studies. Despite previous studies mentioned comparing MPH to WP, this should not influence TAA comparison between studies, for all investigations provided protein-matched beverages relative to body mass, and previous studies have demonstrated little difference in postprandial TAA between whey and milk supplements (Mitchell et al. 2015), further supporting the theory that SPP possesses superior digestibility.

4.3. NEAAs

The larger NEAA response demonstrated by SPP (**Fig. 4C, D**) is potentially resultant from the greater glycine, hydroxyproline and proline content within SPP. (**Fig 5**). Although Cordeiro's study did not measure postprandial NEAAs, Lees's investigation demonstrated no difference in NEAA levels between proteins; the blue-whiting hydrolysate utilized in Lees's study had much lower glycine and proline levels compared to the SPP used in the current study, further supporting the theory that the higher NEAA concentrations observed following SPP resulted from its rich glycine, proline and potentially hydroxyproline content. However, both MPI and SPPs reached peak NEAA at the same timepoint, so SPP's rapid digestibility may have also played a part behind the large difference in NEAAs witnessed in the current study.

4.5. EAAs

The current study demonstrated similar postprandial EAA levels between SPP and MPI throughout the trial (**Fig. 4E**). Previous research investigating postprandial levels of EAA following MPHs is mixed; Lees et al. (2021) demonstrated lower EAA concentrations following MPH consumption compared to WP; however, Cordeiro et al (2020), coincided with the current study, finding similar concentrations of EAA between its MPH and WP. The variation between studies may have been due to the different hydrolysis processes or fish species utilized when producing the MPHs, although future studies are required in order to determine this. Notably, the past studies demonstrated time-to-peak EAA was faster in MPH compared to WP (Lees et al. 2021), whereas the current study found little difference in time-to-peak between SPP and MPI. Despite this dissimilarity, SPP and MPI from the current study reached peak EAA levels at the same timepoint as the MPH used in previous investigations (30min, **Fig. 4E**), suggesting the WP used in the past studies may have had lower digestibility. Although, digestibility was not directly measured in the current or previous studies and due to the smaller sample sizes used in the previous investigations, it is difficult to determine if the contrasts observed are due to lack of statistical power or differing digestibility.

The results in the current study demonstrate SPP produces the same level of EAA concentrations as the commercially available MPI, despite possessing lower EAA content (SPP: 30.31%, MPI: 43.80%. **Table 2**). SPP also reached peak EAA levels at the same time as MPI and at the same witnessed by WP in previous investigations (Lees et al. 2021 and Cordeiro et al. 2020). The similarity in EAA concentrations witnessed may have been due to a faster digestibility rate allowed by the hydrolyzed form of the novel SPP (Koopman et al. 2009). enabling the same level of bioavailability as MPI. Milk protein has been shown in previous studies to generate maximal MPS responses following these levels of plasma EAA acidemia (Mitchell et al. 2015). As such, the matched EAA response of SPP indicates potential to elicit a maximal MPS response, although further investigation is required to determine the stimulatory impacts of SPP on MPS.

4.6. Leucine

Previous research comparing postprandial leucine levels following MPH and current gold standard protein supplements is mixed (Lees et al. 2021 and Cordeiro et al. 2020). This investigation coincided with Lees et al (2021) and found MPI produced overall greater postprandial leucine levels compared with SPP (**Fig 4H**), likely due to MPI's substantially higher leucine content (**Table 2**). However, this potential inferiority, may not lead to anabolic differences between the two proteins; a previous study by Mitchell et al. 2015 showed, protein supplements with significantly different postprandial leucine levels, can still generate similar MPS activation, potentially because both proteins produced sufficient plasma leucine concentrations. The SPP utilized in this study, produced similar postprandial leucine levels to the milk protein used in the study by Mitchel et al (200µmol/L), which matched the anabolic stimulation of WP. Interestingly, a recent systematic review investigating the impact of dietary leucine on postexercise MPS rates by Wilkinson et al (2023), discovered no direct relationship between postprandial leucine availability and MPS responses; suggesting leucine's impact on MPS rates may be less potent than the current narrative has suggested. Furthermore, a study comparing skimmed milk and beef by Burd et al in 2015, demonstrated that despite significantly higher leucine availability following beef, skimmed milk produced significantly higher MPS rates. These recent findings suggest EAA availability may be more important than previously thought in MPS activation, therefore, the matched EAA levels by SPP and MPI (**Fig. 4E**), alongside similar leucine concentrations to supplements previously shown to maximally stimulate MPS (Leese et al. 2021), further suggests SPP may possess high anabolic potential. Although, MPS was not assessed in this study, as such, further investigation is required to determine how SPP's postprandial aminoacidemic responses translate into MPS activation in comparison with current high-quality protein sources.

4.4. Glycine, Hydroxyproline and Proline

The current study demonstrated SPP produced significantly higher glycine, hydroxyproline and proline levels compared to MPI (**Fig. 5**). There is currently little research exploring the postprandial plasma concentrations of glycine, hydroxyproline and proline. However, a review by Ghaly et al (2013), demonstrates that the amino acid composition possessed by current MPH, follows suit with the SPP investigated in current study - with the majority high in concentrations of glycine and proline (hydroxyproline concentrations were not presented in this review), suggesting similar aminoacidemia responses may be observed by other MPHs, although further research is required to determine this. Aforementioned, MPHs are produced from waste gathered from fish farming, resulting in their contents being produced largely from fish bones, skin, and guts, all rich in collagen. Therefore, SPP's high plasma glycine, hydroxyproline and proline contents are likely as a result of collagen breakdown during the hydrolysis process. Glycine, hydroxyproline and proline are major components of collagen, one of the key building blocks within bones, connective tissues, and skin. Studies have previously demonstrated EAAs do not promote collagen synthesis (Babraj et al. 2005), however, ingestion of glycine and proline appear to stimulate collagen synthesis (Vieira et al 2015 and Paxton, Grover, and Baar, 2010). In addition, previous studies in animals have found hydroxyproline supplementation can lead to increased growth, as well as muscle and collagen synthesis (Cao et al. 2022). Hydroxyproline has also been thought to be an important component in regulation of oxidative stress (Phang et al. 2010). Although no previous studies have directly examined postprandial hydroxyproline levels, the highly elevated levels demonstrated in the current study (**Fig. 5C, D**) could explain why previous investigations in vitro, such as a study by Da Rocha et al (2018), identified reductions in inflammatory markers following MPH consumption; increased hydroxyproline availability, could potentially enable greater regulation of cellular stress. Therefore, the high concentrations of glycine, hydroxyproline and proline produced by the novel SPP should be investigated further, to determine its potential

impacts on connective tissue remodeling and adaptation alongside exercise; as well as its potential therapeutic impact on metabolic stress.

4.8. Appetite

Coinciding with previous studies (Dale et al. 2018 and Zaïr et al. 2014), little differences in appetite or dietary composition were observed between supplements in any measures (**Fig 6 - 7**). This data is also supported by the lack of difference between proteins in insulin levels discussed above; studies have shown previously, insulin is associated with short-term appetite regulation and satiety signals in healthy individuals (Flint et al. 2007). Little research has reported effects on daily fat intake, however, both supplements in the current study coincided with significant reductions in daily fat intake (**Fig. 7D**), suggesting protein consumption may aid in dietary strategies amongst patients with obesity; although, it is highly likely the reduction in fat consumption is rather due to the offerings at the ad-libitum breakfast containing relatively low-fat concentrations (**Table 3**). The breakfast contributed largely to total EI and participants may not normally purchase such food options.

Both proteins demonstrated a significant short-term effect on fullness and satisfaction levels (**Fig. 6C and E**); this was likely due to the fast consumption of the drinks at the start of the trial, as studies have found stomach fullness is the largest influencer of appetite (Mook and Votaw, 1992). Despite effects on fullness, similarly to a study by Zaïr et al in 2014, hunger and satisfaction levels did not demonstrate any significant changes throughout the trial for either protein. Although previous studies demonstrated high serum AA levels reduced acute appetite levels (Mellinkoff et al. 1956) this did not coincide with either protein trial in the current study. A longer intervention may allow greater scope to identify changes in habitual dietary composition and appetite generated by SPP consumption, as appetite levels in the current study may have been impacted by the lengthy overnight fast and long trial duration. Despite the lack of impact on appetite by SPP suggesting an inability to aid in tackling the growing obesity pandemic, it would still provide a

beneficial source of protein for the ageing population, in which loss of, or dysregulated appetite can commonly cause health challenges (Donini, Savina, and Cannella, 2003). However, it should be noted, that the trials in the current study only spanned over a brief period, and it is uncertain what effects might arise on appetite following long-term consumption of SPP. Overall, the current study found that the acute impact on hunger, fullness and satisfaction did not differ between protein sources an neither source significantly impacted on perceived satisfaction or hunger levels.

Conclusion

Overall, SPP demonstrated a superior postprandial TAA and NEAA response, alongside comparable EAA response to MPI. SPP's postprandial leucine concentration was lower than MPI, likely as a consequence of MPI's higher leucine content. Further investigation is needed to determine if SPP's postprandial plasma leucine and EAA response can robustly stimulate MPS comparable to MPI and other high-quality animal-based proteins. In addition, SPP's high postprandial glycine, hydroxyproline and proline levels demonstrates potential beneficial properties for collagenous tissues, prompting additional investigation into SPP's impact on collagen synthesis and populations with compromised connective tissues such as the elderly. Accompanied by a lack of satiating differences between proteins, SPP has shown great promise for further investigation as a future sustainable high-quality protein source.

References:

Afzali, A.M., Müntefering, T., Wiendl, H., Meuth, S.G. and Ruck, T. (2018) 'Skeletal muscle cells actively shape (auto) immune responses', *Autoimmunity Reviews*, 17(5), pp.518-529. doi: https://doi.org/10.1016/j.autrev.2017.12.005

Areta, J.L., Burke, L.M., Ross, M.L., Camera, D.M., West, D.W., Broad, E.M., Jeacocke, N.A., Moore, D.R., Stellingwerff, T., Phillips, S.M. and Hawley, J.A. (2013) 'Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis', *The Journal of physiology*, 591(9), pp.2319-2331. doi: https://doi.org/10.1113/jphysiol.2012.244897

Atherton, P.J., Etheridge, T., Watt, P.W., Wilkinson, D., Selby, A., Rankin, D., Smith, K. and Rennie, M.J. (2010) 'Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling', *The American journal of clinical nutrition*, 92(5), pp.1080-1088. doi: https://doi.org/10.1096/fasebj.24.1_supplement.331.1

Babraj, J.A., Cuthbertson, D.J., Smith, K., Langberg, H., Miller, B., Krogsgaard, M.R., Kjaer, M. and Rennie, M.J. (2005) 'Collagen synthesis in human musculoskeletal tissues and skin', *American Journal of Physiology-Endocrinology and Metabolism*, 289(5), pp.E864-E869. doi: https://doi.org/10.1152/ajpendo.00243.2005

Bailey, H.M. and Stein, H.H. (2019) 'Can the digestible indispensable amino acid score methodology decrease protein malnutrition', *Animal Frontiers*, 9(4), pp.18-23. doi: https://doi.org/10.1093/af/vfz038

Balti, R., Bougatef, A., Sila, A., Guillochon, D., Dhulster, P. and Nedjar-Arroume, N. (2015) 'Nine novel angiotensin I-converting enzyme (ACE) inhibitory peptides from cuttlefish (Sepia officinalis) muscle protein hydrolysates and antihypertensive effect of the potent active peptide in spontaneously hypertensive rats', *Food chemistry*, 170, pp.519-525. doi: https://doi.org/10.1016/j.foodchem.2013.03.091

Bandegan, A., Courtney-Martin, G., Rafii, M., Pencharz, P.B. and Lemon, P.W. (2017) 'Indicator amino acid–derived estimate of dietary protein requirement for male bodybuilders on a nontraining day is several-fold greater than the current recommended dietary allowance', *The Journal of nutrition*, 147(5), pp.850-857. doi: 10.3945/jn.116.236331

Bazer, F.W., Wu, G., Johnson, G.A. and Wang, X. (2014) 'Environmental factors affecting pregnancy: endocrine disrupters, nutrients and metabolic pathways', *Molecular and cellular endocrinology*, 398(1-2), pp.53-68. doi: https://doi.org/10.1016/j.mce.2014.09.007

Ben-Harchache, S., Roche, H.M., Corish, C.A. and Horner, K.M. (2021) 'The impact of protein supplementation on appetite and energy intake in healthy older adults: a systematic review with meta-analysis', *Advances in Nutrition*, 12(2), pp.490-502. doi: 10.1093/advances/nmaa115

Bassil, M.S., Hwalla, N. and Obeid, O.A. (2007) 'Meal pattern of male rats maintained on histidine-, leucine-, or tyrosine-supplemented diet', *Obesity*, 15(3), pp.616-623. doi: https://doi.org/10.1038/oby.2007.565Batterham, R.L., Heffron, H., Kapoor, S., Chivers, J.E., Chandarana, K., Herzog, H., Le Roux, C.W., Thomas, E.L., Bell, J.D. and Withers, D.J. (2006) 'Critical role for peptide YY in protein-mediated satiation and body-weight regulation', *Cell metabolism*, 4(3), pp.223-233. doi: 10.1016/j.cmet.2006.08.001

Blom, W.A., Lluch, A., Stafleu, A., Vinoy, S., Holst, J.J., Schaafsma, G. and Hendriks, H.F. (2006) 'Effect of a high-protein breakfast on the postprandial ghrelin response', *The American journal of clinical nutrition*, 83(2), pp.211-220. doi: 10.1093/ajcn/83.2.211

Breen, L. and Phillips, S.M. (2011) ‘Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing’, *Nutrition & metabolism*, *8*(1), pp.1-11. doi: https://doi.org/10.1186/1743-7075-8-68

Bruce, R.W., Grainger, C.M., Ficht, A., Eskandari, M. and Rajcan, I. (2019) ‘Trends in soybean trait improvement over generations of selective breeding’, *Crop Science*, 59(5), pp.1870-1879. doi: https://doi.org/10.2135/cropsci2018.11.0664

Burd, N.A., Gorissen, S.H., Van Vliet, S., Snijders, T. and Van Loon, L.J. (2015) ‘Differences in postprandial protein handling after beef compared with milk ingestion during postexercise recovery: a randomized controlled trial’, *The American journal of clinical nutrition*, 102(4), pp.828-836. doi: https://doi.org/10.3945/ajcn.114.103184

Burd, N.A., West, D.W., Moore, D.R., Atherton, P.J., Staples, A.W., Prior, T., Tang, J.E., Rennie, M.J., Baker, S.K. and Phillips, S.M. (2011) ‘Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men’, *The Journal of nutrition*, 141(4), pp.568-573. doi: https://doi.org/10.3945/jn.110.135038

Cao, S., Xiao, Y., Huang, R., Zhao, D., Xu, W., Li, S., Tang, J., Qu, F., Jin, J., Xie, S. and Liu, Z. (2022) ‘Dietary supplementation with hydroxyproline enhances growth performance, collagen synthesis and muscle quality of Carassius auratus triploid’, *Frontiers in Physiology*, 13, pp.913800. doi: https://doi.org/10.3389/fphys.2022.913800

Carlson, J.J., Turpin, A.A., Wiebke, G., Hunt, S.C. and Adams, T.D., (2009) ‘Pre-and post-prandial appetite hormone levels in normal weight and severely obese women’, *Nutrition & metabolism*, 6, pp.1-8. doi: https://doi.org/10.1186/1743-7075-6-32

Chalamaiah, M., Hemalatha, R. and Jyothirmayi, T. (2012) ‘Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review’, *Food chemistry*, 135(4), pp.3020-3038. doi: https://doi.org/10.1016/j.foodchem.2012.06.100

Chen, Y.C., Smith, H.A., Hengist, A., Chrzanowski-Smith, O.J., Mikkelsen, U.R., Carroll, H.A., Betts, J.A., Thompson, D., Saunders, J. and Gonzalez, J.T. (2020) 'Co-ingestion of whey protein hydrolysate with milk minerals rich in calcium potently stimulates glucagon-like peptide-1 secretion: an RCT in healthy adults', *European journal of nutrition*, 59, pp.2449-2462. Doi: 10.1007/s00394-019-02092-4

Chooi, Y.C., Ding, C. and Magkos, F. (2019) 'The epidemiology of obesity', *Metabolism*, 92, pp.6-10. Doi: https://doi.org/10.1016/j.metabol.2018.09.005

Chow, L.S., Albright, R.C., Bigelow, M.L., Toffolo, G., Cobelli, C. and Nair, K.S. (2006) 'Mechanism of insulin's anabolic effect on muscle: measurements of muscle protein synthesis and breakdown using aminoacyl-tRNA and other surrogate measures', *American Journal of Physiology-Endocrinology and Metabolism*, 291(4), pp.E729-E736. Doi: https://doi.org/10.1152/ajpendo.00003.2006

Churchward-Venne, T.A., Pinckaers, P.J., Smeets, J.S., Betz, M.W., Senden, J.M., Goessens, J.P., Gijsen, A.P., Rollo, I., Verdijk, L.B. and van Loon, L.J. (2020) 'Dose-response effects of dietary protein on muscle protein synthesis during recovery from endurance exercise in young men: a double-blind randomized trial', *The American journal of clinical nutrition*, 112(2), pp.303-317. Doi: 10.1093/ajcn/nqaa073.

Cordeiro, E.M., de Oliveira, G.V., Volino-Souza, M., Velozo, O.D.C. and Alvares, T.S. (2020) 'Effects of fish protein hydrolysate ingestion on postexercise aminoacidemia compared with whey protein hydrolysate in young individuals', *Journal of food science*, 85(1), pp.21-27. Doi: https://doi.org/10.1111/1750-3841.14970

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. and Weigle, D.S. (2001) 'A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans', Diabetes, 50(8), pp.1714-1719. Doi: https://doi.org/10.2337/diabetes.50.8.1714

Dagogo-Jack, S., Fanelli, C., Paramore, D., Brothers, J. and Landt, M. (1996) 'Plasma leptin and insulin relationships in obese and nonobese humans', *Diabetes*, 45(5), pp.695-698. Doi: https://doi.org/10.2337/diab.45.5.695

Dale, H.F., Jensen, C., Hausken, T., Lied, E., Hatlebakk, J.G., Brønstad, I., Hoff, D.A.L. and Lied, G.A. (2018) 'Effect of a cod protein hydrolysate on postprandial glucose metabolism in healthy subjects: A double-blind cross-over trial', *Journal of nutritional science*, 7, pp.e33. doi: https://doi.org/10.1017/jns.2018.23

Da Rocha, M., Alemán, A., Baccan, G.C., López-Caballero, M.E., Gómez-Guillén, C., Montero, P. and Prentice, C. (2018) 'Anti-inflammatory, antioxidant, and antimicrobial effects of underutilized fish protein hydrolysate', *Journal of Aquatic Food Product Technology*, 27(5), pp.592-608. Doi: https://doi.org/10.1080/10498850.2018.1461160

De Gavelle, E., Huneau, J.F., Bianchi, C.M., Verger, E.O. and Mariotti, F. (2017) 'Protein adequacy is primarily a matter of protein quantity, not quality: Modeling an increase in plant: Animal protein ratio in French adults', *Nutrients*, 9(12), pp.1333. doi: https://doi.org/10.3390/nu9121333

Deglaire, A. and Moughan, P.J. (2012) 'Animal models for determining amino acid digestibility in humans–a review', *British Journal of Nutrition*, 108(S2), pp.S273-S281. Doi: https://doi.org/10.1017/S0007114512002346

Department for Environment Food & Rural Affairs. (2022) *Agri-climate report 2022*. Available at: https://www.gov.uk/government/statistics/agri-climate-report-2022/agri-climate-report-2022 (Accessed: 15th July 2023).

Dideriksen, K., Reitelseder, S., Malmgaard-Clausen, N.M., Bechshoeft, R., Petersen, R.K., Mikkelsen, U.R. and Holm, L. (2016) 'No effect of anti-inflammatory medication on postprandial and postexercise muscle protein synthesis in elderly men with slightly elevated systemic

inflammation', *Experimental gerontology*, 83, pp.120-129. doi: https://doi.org/10.1016/j.exger.2016.07.016

Donini, L.M., Savina, C. and Cannella, C. (2003) 'Eating habits and appetite control in the elderly: the anorexia of aging', *International psychogeriatrics*, 15(1), pp.73-87. doi: https://doi.org/10.1017/S1041610203008779

Finnigan, T.J., Wall, B.T., Wilde, P.J., Stephens, F.B., Taylor, S.L. and Freedman, M.R. (2019) 'Mycoprotein: the future of nutritious nonmeat protein, a symposium review', *Current developments in nutrition*, 3(6), p.nzz021. doi: https://doi.org/10.1093/cdn/nzz021

Flint, A., Gregersen, N.T., Gluud, L.L., Møller, B.K., Raben, A., Tetens, I., Verdich, C. and Astrup, A. (2007) 'Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies', *British Journal of Nutrition*, 98(1), pp.17-25. doi: https://doi.org/10.1017/S000711450768297X

Floyd, J.C., Fajans, S.S., Conn, J.W., Knopf, R.F. and Rull, J. (1966) 'Stimulation of insulin secretion by amino acids', *The Journal of clinical investigation*, 45(9), pp.1487-1502. doi: 10.1172/JCI105456

Fomon, S.J., Thomas, L.N., Filer, L.J., Anderson, T.A. and Bergmann, K.E. (1973) 'Requirements for protein and essential amino acids in early infancy 1 Studies with a Soy-Isolate Formula', *Acta Pædiatrica*, 62(1), pp.33-45. doi: https://doi.org/10.1111/j.1651-2227.1973.tb08062.x

Friedman, J.M. and Halaas, J.L. (1998) 'Leptin and the regulation of body weight in mammals', *Nature*, 395(6704), pp.763-770. doi: https://doi.org/10.1038/27376

Gaudichon, C. and Calvez, J. (2021) ‘Determinants of amino acid bioavailability from ingested protein in relation to gut health’, *Current opinion in clinical nutrition and metabolic care*, 24(1), pp.55. doi: 10.1097/MCO.0000000000000708

Ghaly, A.E., Ramakrishnan, V.V., Brooks, M.S., Budge, S.M. and Dave, D. (2013) ‘Fish processing wastes as a potential source of proteins. Amino acids and oils: A critical review’, *Microbial & Biochemical Technology*, 5(4), pp.107-129. doi: 10.4172/1948-5948.1000110

Gorissen, S.H., Crombag, J.J., Senden, J.M., Waterval, W.H., Bierau, J., Verdijk, L.B. and van Loon, L.J. (2018) ‘Protein content and amino acid composition of commercially available plant-based protein isolates’, *Amino acids*, 50, pp.1685-1695. doi: 10.1007/s00726-018-2640-5

Harnedy, P.A., Parthsarathy, V., McLaughlin, C.M., O'Keeffe, M.B., Allsopp, P.J., McSorley, E.M., O'Harte, F.P. and FitzGerald, R.J. (2018a) ‘Atlantic salmon (Salmo salar) co-product-derived protein hydrolysates: A source of antidiabetic peptides’, *Food Research International*, 106, pp.598-606. doi: https://doi.org/10.1016/j.foodres.2018.01.025

Harnedy, P.A., Parthsarathy, V., McLaughlin, C.M., O'Keeffe, M.B., Allsopp, P.J., McSorley, E.M., O'Harte, F.P. and FitzGerald, R.J., (2018b) ‘Blue whiting (Micromesistius poutassou) muscle protein hydrolysate with in vitro and in vivo antidiabetic properties’, *Journal of Functional Foods*, 40, pp.137-145. doi: https://doi.org/10.1016/j.jff.2017.10.045

Hartman, J.W., Tang, J.E., Wilkinson, S.B., Tarnopolsky, M.A., Lawrence, R.L., Fullerton, A.V. and Phillips, S.M. (2007) ‘Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters’, *The American journal of clinical nutrition*, 86(2), pp.373-381. doi: https://doi.org/10.1093/ajcn/86.2.373

Holwerda, A.M. and van Loon, L.J. (2022) ‘The impact of collagen protein ingestion on musculoskeletal connective tissue remodeling: a narrative review’, *Nutrition reviews*, 80(6), pp.1497-1514. doi: https://doi.org/10.1093/nutrit/nuab083

Hou, Y. and Wu, G. (2017) ‘Nutritionally nonessential amino acids: a misnomer in nutritional sciences’, *Advances in nutrition*, 8(1), pp.137-139. doi: 10.3945/an.116.012971

Janssen, I., Heymsfield, S.B., Wang, Z. and Ross, R. (2000) ‘Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr’, *Journal of applied physiology*, 89(1) pp.81-88. doi: https://doi.org/10.1152/jappl.2000.89.1.81

Karra, E., Chandarana, K. and Batterham, R.L. (2009) ‘The role of peptide YY in appetite regulation and obesity’, *The Journal of Physiology*, 587(1) pp.19-25. doi: https://doi.org/10.1113/jphysiol.2008.164269

Koopman, R., Crombach, N., Gijsen, A.P., Walrand, S., Fauquant, J., Kies, A.K., Lemosquet, S., Saris, W.H., Boirie, Y. and van Loon, L.J. (2009) ‘Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein’, *The American journal of clinical nutrition*, 90(1), pp.106-115. doi: 10.3945/ajcn.2009.27474

Kristinsson, H.G. and Rasco, B.A. (2000), ‘Fish protein hydrolysates: production, biochemical, and functional properties’, *Critical reviews in food science and nutrition*, 40(1), pp.43-81. doi: http://dx.doi.org/10.1080/10408690091189266

Lees, M.J., Nolan, D., Egan, B. and Carson, B.P. (2021) ‘A blue-whiting protein hydrolysate induces aminoacidemia in older adults and skeletal muscle anabolism in vitro’*, Proceedings of the Nutrition Society*, 80(OCE3), E109. doi:10.1017/S0029665121002329

Leidy, H.J., Armstrong, C.L., Tang, M., Mattes, R.D. and Campbell, W.W. (2010) 'The influence of higher PI and greater eating frequency on appetite control in overweight and obese men', *Obesity*, 18(9), pp.1725-1732. doi: https://doi.org/10.1038/oby.2010.45

Leitzmann, C., (2003) 'Nutrition ecology: the contribution of vegetarian diets', *The American journal of clinical nutrition*, 78(3), pp.657S-659S. doi: https://doi.org/10.1093/ajcn/78.3.657S

Li, P. and Wu, G. (2018) 'Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth', *Amino acids*, 50, pp.29-38. doi: https://doi.org/10.1007/s00726-017-2490-6

Mallinson, J.E., Wardle, S.L., O'Leary, T.J., Greeves, J.P., Cegielski, J.E.S.S.I.C.A., Bass, J.O.S.E.P.H., Brook, M.S., Wilkinson, D.J., Smith, K.E.N.N.E.T.H., Atherton, P.J. and Greenhaff, P.L. (2023) 'Protein dose requirements to maximize skeletal muscle protein synthesis after repeated bouts of resistance exercise in young trained women', *Scandinavian Journal of Medicine & Science in Sports*, 33(12), pp.2470-2481. doi: https://doi.org/10.1111/sms.14506

Marchbank, T., Limdi, J.K., Mahmood, A., Elia, G. and Playford, R.J. (2008) 'Clinical trial: protective effect of a commercial fish protein hydrolysate against indomethacin (NSAID)-induced small intestinal injury', *Alimentary pharmacology & therapeutics*, 28(6), pp.799-804. doi: https://doi.org/10.1111/j.1365-2036.2008.03783.x

Marti, D.L., Johnson, R.J., Mathews, K.H., United States Department of Agriculture (2011) *Where's the (Not) Meat? Byproducts From Beef and Pork Production*. Available at: https://www.ers.usda.gov/webdocs/outlooks/37427/8801_ldpm20901.pdf (Accessed: 15th July 2023).

McLeod, M., Breen, L., Hamilton, D.L. and Philp, A. (2016) 'Live strong and prosper: the importance of skeletal muscle strength for healthy ageing', *Biogerontology*, 17, pp.497-510. doi: 10.1007/s10522-015-9631-7

McCuller C, Jessu R, Callahan AL. (2022) *Physiology, Skeletal Muscle*, In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537139/

McMichael, A.J., Powles, J.W., Butler, C.D. and Uauy, R. (2007). 'Food, livestock production, energy, climate change, and health', *The lancet*, 370(9594), pp.1253-1263. doi: https://doi.org/10.1016/S0140-6736(07)61256-2

Mellinkoff, S.M., Frankland, M., Boyle, D. and Greipel, M., (1956). 'Relationship between serum amino acid concentration and fluctuations in appetite', *Journal of applied physiology*, 8(5), pp.535-538. doi: https://doi.org/10.1152/jappl.1956.8.5.535Mook, D.G. and Votaw, M.C. (1992) 'How important is hedonism? Reasons given by college students for ending a meal', *Appetite*, 18(1), pp.69-75. doi: https://doi.org/10.1016/0195-6663(92)90211-N

Moore, D.R. (2019) 'Maximizing post-exercise anabolism: the case for relative protein intakes', *Frontiers in nutrition*, 6, pp.147. doi: https://doi.org/10.3389/fnut.2019.00147

Moore, D.R., Churchward-Venne, T.A., Witard, O., Breen, L., Burd, N.A., Tipton, K.D. and Phillips, S.M. (2015) 'Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men', *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 70(1), pp.57-62. doi: https://doi.org/10.1093/gerona/glu103

Moore, D.R., Tang, J.E., Burd, N.A., Rerecich, T., Tarnopolsky, M.A. and Phillips, S.M. (2009) 'Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise', *The Journal of physiology*, 587(4), pp.897-904. doi: https://doi.org/10.1113/jphysiol.2008.164087

Morrison, C.D., Xi, X., White, C.L., Ye, J. and Martin, R.J. (2007) 'Amino acids inhibit Agrp gene expression via an mTOR-dependent mechanism', *American Journal of Physiology-Endocrinology and Metabolism*, 293(1), pp.E165-E171. doi: https://doi.org/10.1152/ajpendo.00675.2006

Morton, R.W., Murphy, K.T., McKellar, S.R., Schoenfeld, B.J., Henselmans, M., Helms, E., Aragon, A.A., Devries, M.C., Banfield, L., Krieger, J.W. and Phillips, S.M. (2018) 'A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults', *British journal of sports medicine*, 52(6), pp.376-384. doi: 10.1136/bjsports-2017-097608

Munro, H.N. (1978) 'Nutritional consequences of excess amino acid intake', *In Nutritional Improvement of Food and Feed Proteins*, pp. 119-129. Boston, MA: Springer US. doi: https://doi.org/10.1007/978-1-4684-3366-1_8

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K. and Matsukura, S. (2001) 'A role for ghrelin in the central regulation of feeding', *Nature*, 409(6817), pp.194-198. doi: https://doi.org/10.1038/35051587

Nefti, W., Darcel, N., Fromentin, G. and Tomé, D. (2007) 'Long-term exposure to high-protein diet or high-fat diet have opposite effects on vagal afferent sensitivity to luminal macronutrients, ip cholecystokinin and serotonin', *Appetite*, 49(1), p.316. doi: https://doi.org/10.1016/j.appet.2007.03.146

Nguyen, M. (2023) *Fishery and aquaculture in Vietnam - statistics & facts*. Available at: https://www.statista.com/topics/9875/fishery-and-aquaculture-in-vietnam/#topicOverview (Accessed: 16th July 2023).

Narang, B.J., Atkinson, G., Gonzalez, J.T. and Betts, J.A. (2020) 'A tool to explore discrete-time data: the time series response analyser', *International Journal of Sport Nutrition and Exercise Metabolism*, 30(5), pp.374-381. doi: https://doi.org/10.1123/ijsnem.2020-0150

Pal, G.K. and Suresh, P.V. (2016) 'Sustainable valorisation of seafood by-products: Recovery of collagen and development of collagen-based novel functional food ingredients', *Innovative food science & emerging technologies*, 37, pp.201-215. doi: https://doi.org/10.1016/j.ifset.2016.03.015Parthsarathy, V., McLaughlin, C.M., Harnedy, P.A., Allsopp, P.J., Crowe, W., McSorley, E.M., FitzGerald, R.J. and O'Harte, F.P. (2019) 'Boarfish (Capros aper) protein hydrolysate has potent insulinotropic and GLP-1 secretory activity in vitro and acute glucose lowering effects in mice', *International journal of food science & technology*, 54(1), pp.271-281. doi: https://doi.org/10.1111/ijfs.13975

Pasiakos, S.M. (2012) 'Exercise and amino acid anabolic cell signaling and the regulation of skeletal muscle mass', *Nutrients*, 4(7), pp.740-758. doi: https://doi.org/10.3390/nu4070740

Paxton, J.Z., Grover, L.M. and Baar, K. (2010) 'Engineering an in vitro model of a functional ligament from bone to bone', *Tissue engineering part A*, 16(11), pp.3515-3525. doi: https://doi.org/10.1089/ten.tea.2010.0039

Pimentel, D. and Pimentel, M. (2003) 'Sustainability of meat-based and plant-based diets and the environment', *The American journal of clinical nutrition*, 78(3), pp.660S-663S. doi: 10.1093/ajcn/78.3.660S

Prasanna, B.M., Vasal, S.K., Kassahun, B. and Singh, N.N. (2001) 'Quality protein maize', *Current science*, pp.1308-1319. doi: https://www.jstor.org/stable/24105845

Pratley, R.E., Nicolson, M.A.R.G.E.R.Y., Bogardus, C.L.I.F.T.O.N. and Ravussin, E. (1996) 'Effects of acute hyperinsulinemia on plasma leptin concentrations in insulin-sensitive and insulin-resistant Pima Indians', *The Journal of Clinical Endocrinology & Metabolism*, 81(12), pp.4418-4421. doi: https://doi.org/10.1210/jcem.81.12.8954052

Phang, J.M., Liu, W.E.I. and Zabirnyk, O. (2010) 'Proline metabolism and microenvironmental stress', *Annual review of nutrition*, 30, pp.441-463. doi: https://doi.org/10.1146/annurev.nutr.012809.104638

Rajabimashhadi, Z., Gallo, N., Salvatore, L. and Lionetto, F. (2023) 'Collagen derived from fish industry waste: progresses and challenges', *Polymers*, 15(3), p.544. doi: https://doi.org/10.3390/polym15030544

Res, P.T., Groen, B., Pennings, B., Beelen, M., Wallis, G.A., Gijsen, A.P., Senden, J.M. and Van Loon, L.J. (2012) 'Protein ingestion before sleep improves postexercise overnight recovery', *Med Sci Sports Exerc*, 44(8), pp.1560-9. doi: 10.1249/MSS.0b013e31824cc363

Sahu, N., Cruz, D.D., Gao, M., Sandoval, W., Haverty, P.M., Liu, J., Stephan, J.P., Haley, B., Classon, M., Hatzivassiliou, G. and Settleman, J. (2016) 'Proline starvation induces unresolved ER stress and hinders mTORC1-dependent tumorigenesis', *Cell metabolism*, 24(5), pp.753-761. doi: 10.1016/j.cmet.2016.08.008

Schoenfeld, B.J., Aragon, A.A. and Krieger, J.W. (2013) 'The effect of protein timing on muscle strength and hypertrophy: a meta-analysis', *Journal of the International Society of Sports Nutrition*, 10(1), pp.53. doi: https://doi.org/10.1186/1550-2783-10-53

Sharkey, S.J., Harnedy-Rothwell, P.A., Allsopp, P.J., Hollywood, L.E., FitzGerald, R.J. and O'Harte, F.P. (2020) 'A narrative review of the anti-hyperglycemic and satiating effects of fish protein hydrolysates and their bioactive peptides', *Molecular nutrition & food research*, 64(21), pp.2000403. doi: https://doi.org/10.1002/mnfr.202000403

Shahbandeh, M. (2023) *Fish production worldwide 2002-2022*. Available at: https://www.statista.com/statistics/264577/total-world-fish-production-since-2002/#:~:text=The%20volume%20of%20global%20fish,becoming%20more%20popular%20over%20time (Accessed: 16th July 2023).

Siddik, M.A., Howieson, J., Fotedar, R. and Partridge, G.J. (2021) 'Enzymatic fish protein hydrolysates in finfish aquaculture: a review', *Reviews in Aquaculture*, 13(1), pp.406-430. doi: https://doi.org/10.1111/raq.12481

Smeuninx, B., Mckendry, J., Wilson, D., Martin, U. and Breen, L. (2017) 'Age-related anabolic resistance of myofibrillar protein synthesis is exacerbated in obese inactive individuals', *The Journal of Clinical Endocrinology & Metabolism*, 102(9), pp.3535-3545. doi: https://doi.org/10.1210/jc.2017-00869

Smeets, A.J., Soenen, S., Luscombe-Marsh, N.D., Ueland, Ø. and Westerterp-Plantenga, M.S. (2008) 'Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch', *The Journal of nutrition*, 138(4), pp.698-702. doi: https://doi.org/10.1093/jn/138.4.698

Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J. and Mittendorfer, B. (2011) 'Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial', *The American journal of clinical nutrition*, 93(2), pp.402-412. doi: https://doi.org/10.3945/ajcn.110.005611

Stoll-Kleemann, S. and Schmidt, U.J. (2017) 'Reducing meat consumption in developed and transition countries to counter climate change and biodiversity loss: a review of influence factors', *Regional Environmental Change*, 17, pp.1261-1277. doi: https://doi.org/10.1007/s10113-016-1057-5

Stump, C.S., Henriksen, E.J., Wei, Y. and Sowers, J.R. (2006) 'The metabolic syndrome: role of skeletal muscle metabolism', *Annals of medicine*, 38(6), pp.389-402. doi: https://doi.org/10.1080/07853890600888413

Takahara, T., Amemiya, Y., Sugiyama, R., Maki, M. and Shibata, H. (2020) 'Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes', *Journal of Biomedical Science*, 27, pp.1-16. doi: https://doi.org/10.1186/s12929-020-00679-2

The Heritage Foundation (2023) *2023 Index of Economic Freedom – Norway*. Available at: https://www.heritage.org/index/country/norway#:~:text=Norway%20is%20one%20of%20the,world's%20largest%20sovereign%20wealth%20fund (Accessed 16th July 2023).

Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle Jr, D. and Wolfe, R.R. (1999) 'Postexercise net protein synthesis in human muscle from orally administered amino acids', *American Journal of Physiology-Endocrinology And Metabolism*, 276(4), pp.E628-E634. doi: https://doi.org/10.1152/ajpendo.1999.276.4.E628

Tipton, K.D., Hamilton, D.L. and Gallagher, I.J. (2018) 'Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans', *Sports Medicine*, 48, pp.53-64. doi: https://doi.org/10.1007/s40279-017-0845-5

Tipton, K.D. and Wolfe, R.R. (1998) 'Exercise-induced changes in protein metabolism', *Acta Physiologica Scandinavica*, 162(3), pp.377-387. doi: https://doi.org/10.1046/j.1365-201X.1998.00306.x

Tsai, M., Asakawa, A., Amitani, H. and Inui, A. (2012) 'Stimulation of leptin secretion by insulin', *Indian Journal of Endocrinology and Metabolism*, 16(Suppl 3), pp.S543. doi: 10.4103/2230-8210.105570

Turton, M.D., O'shea, D., Gunn, I., Beak, S.A., Edwards, C.M.B., Meeran, K., Choi, S.J., Taylor, G.M., Heath, M.M., Lambert, P.D. and Wilding, J.P.H. (1996) 'A role for glucagon-like peptide-1 in the central regulation of feeding', *Nature*, 379(6560), pp.69-72. doi: https://doi.org/10.1038/379069a0

United nations (no date) *Global issues, population*. Available at: https://www.un.org/en/global-issues/population#:~:text=The%20world%20population%20is%20projected,surrounding%20these%20latest%20population%20projections. (Accessed 16th July 2023).

van Vliet, S., Burd, N.A. and van Loon, L.J. (2015) 'The skeletal muscle anabolic response to plant-versus animal-based protein consumption', *The Journal of nutrition*, 145(9), pp.1981-1991. doi: https://doi.org/10.3945/jn.114.204305

Veldhorst, M., Smeets, A.J.P.G., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N. and Westerterp-Plantenga, M. (2008), 'Protein-induced satiety: effects and mechanisms of different proteins', *Physiology & behavior*, 94(2), pp.300-307. doi: https://doi.org/10.1016/j.physbeh.2008.01.003

Venugopal, V., (2016) 'Enzymes from seafood processing waste and their applications in seafood processing', *Advances in food and nutrition research*, 78, pp.47-69. doi: https://doi.org/10.1016/bs.afnr.2016.06.004

Vieira, C.P., Oliveira, L.P.D., Guerra, F.D.R., Almeida, M.D.S.D., Marcondes, M.C.C.G. and Pimentel, E.R. (2015) 'Glycine Improves Biochemical and Biomechanical Properties Following Inflammation of the A chilles Tendon', *The Anatomical Record*, 298(3), pp.538-545. doi: https://doi.org/10.1002/ar.23041

Walsh, J.J., Edgett, B.A., Tschakovsky, M.E. and Gurd, B.J. (2015) 'Fasting and exercise differentially regulate BDNF mRNA expression in human skeletal muscle', *Applied Physiology, Nutrition, and Metabolism*, 40(1), pp.96-98. doi: https://doi.org/10.1139/apnm-2014-029

Walston, J. (2004) 'Frailty--the search for underlying causes', *Science of Aging Knowledge Environment*, 2004(4), pp.pe4-pe4. doi: 10.1126/sageke.2004.4.pe4

Wang, X. and Proud, C.G. (2006) 'The mTOR pathway in the control of protein synthesis', *Physiology*, 21(5), pp.362-369. doi: https://doi.org/10.1152/physiol.00024.2006

Wang, H., Zhang, C., Wu, G., Sun, Y., Wang, B., He, B., Dai, Z. and Wu, Z. (2015) 'Glutamine enhances tight junction protein expression and modulates corticotropin-releasing factor signaling in the jejunum of weanling piglets', *The Journal of nutrition*, 145(1), pp.25-31. doi: https://doi.org/10.3945/jn.114.202515

Wilkinson, K., Koscien, C.P., Monteyne, A.J., Wall, B.T. and Stephens, F.B. (2023) 'Association of postprandial postexercise muscle protein synthesis rates with dietary leucine: A systematic review', *Physiological Reports*, 11(15), pp.e15775. doi: https://doi.org/10.14814/phy2.15775

Wilkinson, S.B., Tarnopolsky, M.A., MacDonald, M.J., MacDonald, J.R., Armstrong, D. and Phillips, S.M. (2007) 'Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage', *The American journal of clinical nutrition*, 85(4), pp.1031-1040. doi: https://doi.org/10.1093/ajcn/85.4.1031

Wolfe, R.R., Miller, S.L. and Miller, K.B. (2008) 'Optimal protein intake in the elderly', *Clinical nutrition*, 27(5), pp.675-684. doi: https://doi.org/10.1016/j.clnu.2008.06.008

Wu, G. (2010) 'Functional amino acids in growth, reproduction, and health', *Advances in nutrition*, 1(1), pp.31-37. doi: https://doi.org/10.3945/an.110.1008

Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D.A., Li, P., Li, X., McKnight, J.R., Satterfield, M.C. and Spencer, T.E. (2011) 'Proline and hydroxyproline metabolism: implications for animal and human nutrition', *Amino acids*, 40, pp.1053-1063. doi: https://doi.org/10.1007/s00726-010-0715-z

Zaïr, Y., Duclos, E., Housez, B., Vergara, C., Cazaubiel, M. and Soisson, F. (2014) ‘Evaluation of the satiating properties of a fish protein hydrolysate among overweight women: a pilot study’, *Nutrition & Food Science*, 44(5), pp.389-399. doi: https://doi.org/10.1108/NFS-06-2013-0075