



# ALTERNATIVE FORMS OF DIETARY PROTEIN FOR RECOVERY: TRADITIONS, CHALLENGES AND OPPORTUNITIES

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## KEY POINTS

- Dietary protein ingestion to support skeletal muscle recovery, acute reconditioning and longer-term adaptations to exercise training represents a cornerstone within modern sports nutrition. However, most of our recommendations tacitly assume protein needs can be met by the (increased) consumption of animal-derived protein sources.
- There is interest for an evidence base for increasing alternative dietary protein choices within sports nutrition. While protein 'source' has traditionally been less studied within sports nutrition, data are now accumulating such that we can begin to incorporate this issue within modern sports nutrition recommendations.
- A clear practical challenge for the athlete wishing to become less reliant on animal-derived foods is obtaining sufficient dietary protein. However, it is clear the accelerating availability of alternative protein rich foods is making this achievable.
- It has traditionally been assumed that animal-derived proteins are the most anabolic (i.e. the most robust at stimulating muscle protein synthesis rates), attributable to a high bioavailability and leucine content. Our evolving understanding and expanded dataset now suggests anabolic parity between animal and carefully selected alternative proteins can be achieved.
- Promising alternative dietary protein sources that have been investigated include a variety of plant- (including blends), fungal-, algal- and insect-derived proteins. All these proteins provide differing challenges and opportunities, with each existing at various stages of maturity concerning the data supporting their application, and broader applied and commercial viability.
- Acute studies of muscle protein synthesis and translational studies of muscle adaptive responses to training now show equivalent magnitude responses can be achieved using traditional omnivorous diets and/or animal-derived protein supplementation compared with (more) alternative protein-based approaches, assuming sufficient protein is consumed.
- However, many limitations in our knowledge base still exist; including how alternative diets interact with different training regimens, populations and various adaptive responses.

## INTRODUCTION

Dietary protein ingestion to support skeletal muscle recovery, acute reconditioning and longer-term adaptations to exercise training represents a cornerstone within modern sports nutrition. Refined dietary protein guidelines, far more precise and individualized than broad-brush society-wide daily recommended dietary allowances/intakes (RDA/Is), have been widely agreed upon within the academic sports nutrition community. This increased resolution offers many (by no means complete) protein recommendations concerning optimal daily (e.g.  $\geq 1.2$ - $1.6$  g protein/kg body mass [bm] per day), single meal doses (e.g.  $\geq 20$  g), timings (e.g. proximity to exercise, prior to sleep, etc.) and distribution over the day to optimize recovery and adaptation, all within the context of differing sports, level and population (reviewed in Morgan et al., 2022). However, until the last 5-10 years, protein source has been somewhat neglected, with our evidence base overly reliant on experiments using animal-derived proteins, most notably, whey protein; often regarded as the 'gold standard' within sports nutrition. This forms an uncomfortable nexus, with society at large now wishing to reduce reliance on animal agriculture due to increased awareness of ethical and environmental concerns associated with increasing animal-derived protein production.

Recent data show the often touted inferiority of non-animal proteins within sports nutrition may have been premature. Human in vivo studies have begun to refine the concept of 'protein quality' regarding sports nutrition. Carefully selected plant, fungal, insect or algal proteins, often in tandem with particular production techniques (e.g. protein isolation, blending, fortification, etc.), are all viable and novel strategies to optimize the utility of dietary protein within sports nutrition. It is the purpose of this Sports Science Exchange (SSE) article to discuss the impact of these studies through the narrative of the emergence of the optimal application of alternative proteins within evidence-based sports nutrition. Ultimately, the aim is to succinctly provide the scientific state-of-play along with practical and applied recommendations.

## RESEARCH REVIEW

### Alternative Proteins: Quantity

Many elite athletes and high-profile media sources now push the anecdotal benefits of vegan diets for health, recovery and performance (reviewed in West et al., 2023a). Increasing proportions of the sporting and wider public aim to move to vegan diets, or at least reduce their reliance on animal-derived foods, coined 'flexitarian' or 'reducetarian'

diets. The precise prevalence of vegan or flexitarian diets within athlete populations of differing competitive standards is difficult to ascertain. However, by assessing non-athlete large cohort studies, it can be surmised that spontaneously adopted vegan diets typically reduce absolute and relative (to total energy intake or bm) dietary protein intakes by ~30% (West et al., 2023a). When considering the general scientific consensus (Morton et al. 2018) that optimal protein intakes for athletes of all type is almost certainly above the RDI of 0.75-0.8 g/kg bm (likely more than double this at ~1.6 g/kg bm/day), this poses a concern that vegan or flexitarian athletes may be more predisposed to below optimal protein intakes. This is largely driven by the typically lower protein density of plant- compared with animal-derived foods.

Athletes, often more cognizant of food choices than non-athletes (especially with the support of coaches and nutritionists), can compensate for this by consciously selecting from a growing number of alternative protein isolates, supplements and novel protein-dense meat alternative foods. In support, various studies show high(er) protein intakes, in line with proposed optimal intakes is eminently achievable in vegan athletes, although possibly more practically challenging (Hevia-Larrain et al., 2021; Monteyne et al., 2023a). Therefore, in practice, vegan sports nutrition likely requires a more mindful and diligent approach compared with the more traditional omnivorous diet.

### Alternative Proteins: Quality

Beyond total daily protein intakes, perhaps the key consideration for the increasing consumption of alternative protein sources within sports nutrition is their quality. Non-animal-derived protein sources generally rank lower on accepted indices of dietary protein quality, such as the protein digestibility-corrected amino acids score (PDCAAS) or the digestible indispensable amino acid score (DIAAS) (van der Heijden et al., 2023a). Such observations underpin the traditional view of animal-derived proteins being ideal choices within sports nutrition, maximizing opportunity for both quantity and quality of intake. This leads to the supposition that, even with a sufficient quantity of protein, it may be necessary for the athlete wishing to reduce animal-derived protein intake to compensate with greater intakes. However, unequivocally ascribing the quality of a whole diet containing mixed (protein-rich) meals from metrics of individual proteins, particularly within the sporting context and from a muscle-centric perspective, is questionable. Many workers have examined this concept more precisely by examining the effect of individual alternative proteins on muscle protein metabolism both on a per-meal and broader diet perspective.

Dietary protein ingestion supports recovery, reconditioning and longer-term muscle adaptive responses by providing the signal and substrate for increasing muscle protein synthesis (MPS) rates. In the postabsorptive state, the continuous parallel processes of MPS and muscle protein breakdown (MPB) are tipped in the balance of greater MPB rates, meaning a net loss of muscle protein. The ingestion of protein at a meal stimulates MPS thereby allowing muscle protein accretion and replacing net losses experienced in the postabsorptive state. Prior exercise amplifies the effect of each protein containing meal for at least 24-48 h (Wall et al., 2016). Therefore, to some degree, acute

muscle reconditioning in response to a single bout of exercise and, over time, the muscle adaptive response to prolonged exercise training regimens, is governed by the magnitude of this MPS response to each protein containing meal. It is therefore possible to more precisely define the quality of alternative protein sources by assessing their ability to stimulate post-exercise MPS rates in comparison with more traditionally consumed and studied animal-derived proteins.

The properties of any given protein source thought most relevant when predicting its anabolic potential per gram of total protein are its amino acid digestibility (both speed and total postprandial peripheral availability) (Pennings et al., 2011), essential amino acid content (Tipton et al., 1999) and in particular, the presence of the particular amino acid leucine (Rieu et al., 2016). While a complete protein source appears necessary to prevent any substrate level limitations for optimal duration MPS responses (Fuchs et al., 2019), the presence of the essential amino acids, and leucine, have been assumed to be the limiting factors in terms of initiating the intracellular signalling processes that govern MPS. It has therefore followed that, even when comparing per gram of protein (i.e. eliminating concerns over protein density of whole foods), the typically lower digestibility and essential to non-essential amino acid ratio, as well as the presence of various anti-nutritional factors, of plant- compared with animal- derived proteins, not only dictates their lower PDCAAS and DIAAS scores, but is also assumed to result in inferior post-exercise MPS responses. Early stable isotope experiments directed at these questions largely confirmed animal-derived proteins to be more anabolic to muscle compared with those from plants; wheat (even when pre-hydrolysed) or soy protein isolate shown to result in lower resting or post-exercise MPS rates compared with isonitrogenous animal-derived comparators (isolated milk, casein and/or whey protein) (Gorissen et al., 2016; Tang et al., 2009; Wilkinson et al., 2007; Yang et al., 2012).

These data implied that, beyond concerns only over the quantity of protein they consume, the challenge for the athlete wishing to follow a (more) plant-based diet may be the quality of the protein within the more limited food choices available to them. However, with the appreciation of a broader range of possible plant proteins, together with the availability of non-animal derived protein isolates and meat-alternative products rapidly proliferating, research in this space has begun to accelerate. This is now providing an evolving understanding of the mechanistic regulation of postprandial MPS as well as a wider dataset from which to draw more modern sports nutrition recommendations and encompassing consumer drive towards reducing reliance on animal-derived foods.

### Leucine as an Anabolic Signal to Contracted Muscle

Leucine as the key anabolic amino acid has been a robust framework with predictive potential in studies ranging from in vitro cell-line approaches and translating to in vivo rodent work and human studies where participants have ingested crystalline amino acid mixtures or animal-derived protein isolates (Monteyne et al., 2024). This has provided an ostensible challenge to those wishing to increase their intake of non-animal derived proteins as they are typically lower in leucine per gram

of total protein. However, the increase in recent research assessing the (post-exercise) MPS response to diverse protein sources, with respect to their origin (i.e. animal vs plant) and form (e.g. isolated proteins vs protein-rich whole foods, macro/micro-nutrient co-ingestion, mixed meals and/or the impact of industrial protein processing techniques) has “muddied the water” of leucine’s exclusive role.

By incorporating these modern research considerations, two recent systematic reviews (Wilkinson et al., 2023; Zaromskyte et al., 2021) broadly concluded that, while an association between the leucine load of a protein meal (though not a specific parameter of the subsequent leucinaemia it elicited post-ingestion) and the magnitude of the (post-exercise) MPS response was found, it was far less predictive than assumed. The latter was even more true within younger compared with older adults. In fact, both reviews demonstrated that, although leucine was clearly of major relevance (perhaps still the major nutrient signal), the relatively minor proportion of the anabolic response it predicted left room for a multitude of other regulatory nutrients. This appeared especially true as the complexity of single meals, and the diets they ultimately comprise, increases. Although the lack of other obvious candidate mechanisms is perplexing, this interpretation of the data does demonstrate the opportunity to view alternative proteins through a more promising lens regarding their potential anabolic capacity, and not have ambitions hamstrung by assessing leucine content only.

### **Beyond the ‘Plant vs Animal Proteins’ Narrative**

The seminal work establishing plant protein ingestion as inferior for the stimulation of MPS focussed heavily on soy protein (Tang et al., 2009; Wilkinson et al., 2007; Yang et al., 2012), a source exhibiting relatively poor bioavailability, low essential amino acid and leucine contents and a deficiency (according to the World Health Organization (WHO)/Food and Agriculture Organization (FAO)/United Nations University (UNU) statements) in methionine. It is therefore possible that insufficient (single) postprandial amino acid availability was limiting the initiation of MPS (e.g. essential amino acids), or one or more specific amino acids were substrate limiting for the continuation of MPS. In the intervening years, other plant-derived protein isolates such as potato (Pinckaers et al., 2022), wheat (Gorissen et al., 2016; Pinckaers et al., 2021), pea (Pinckaers et al., 2024a; West et al., 2023b) and corn (Pinckaers et al., 2024b), all possessing different isolation purities and amino acid profiles, have been compared with animal proteins for their anabolic potential within muscle tissue. Although the studies are not entirely consistent, a growing theme of this work has been, provided sufficient (i.e. 25- ≥ 30 g) plant-derived protein is ingested, the post-exercise postprandial MPS response will be as robust as that following animal protein. In this way, it has been suggested that consuming more of plant-derived proteins (at least in isolated form) can compensate for any lack of bioavailability and/or single/multiple amino acid deficiencies.

Although the traditional view within (particularly applied) sports nutrition has been to view protein source as a dichotomy of animal vs plants, this unnecessarily oversimplifies the issue. Recent work has begun to identify other potential food groups that may possess higher density of protein, a more favourable essential amino acid content and/or a more

balanced amino acid profile that avoids amino acid deficiencies. For example, proteins derived from fungi (mycoproteins) (Monteyne et al., 2020), algae (van der Heijden et al. 2023b; 2024a) or insect (Hermans et al., 2021) sources and single amino acid enriched plant proteins (Kouw et al., 2021) or plant/animal or plant exclusive protein blend isolates (Lim et al., 2024; Pinckaers et al., 2023; Reidy et al., 2013; van der Heijden et al., 2024b) have all been investigated. Moreover, these (as well as animal proteins) have increasingly been investigated as more complex foods (i.e. within their natural whole-food matrix or as part of mixed meals) (van Vliet et al., 2017; West et al., 2023c) and as components of the wider diet (Hevia-Larrain et al., 2021; Monteyne et al., 2023), with the ensuing effects on longer-term measures of MPS and/or muscle adaptive responses determined. This work has shed light on the many opportunities that are available from the perspectives of basic research, industry and commercial avenues for novel product development, and expanding the choices for athletes and practitioners.

### **Fungi**

Mycoproteins benefit from having favorable sustainability credentials by virtue of being produced in a closed system continuous fermentation process, and a protein density and amino acid composition closer to that of animal proteins (albeit with a relatively lower leucine content). Following ingestion, the amino acids appear slowly in the circulation and this is attributable to the protein being encapsulated within a fibrous cell wall with a hyphal cell structure which remains mainly intact during the early phases of digestion and relying on protease permeation of the cell membrane during intestinal digestion and absorption (West et al., 2024). Despite the slower digestion and absorption kinetics, the total postprandial peripheral amino acid availability following mycoprotein ingestion is comparable to milk protein (Monteyne et al., 2020). This observation is atypical of the traditional animal vs plant protein comparisons where plant-based proteins are reliably less bioavailable (van der Heijden et al., 2023a), often even when ingested in an isolated form to remove the majority of potentially anti-nutritional factors (van der Heijden et al., 2024b).

Despite the slower and lower postprandial leucine response, work reported that ingestion of mycoprotein (containing 31.5 g protein) stimulated resting and post-exercise MPS rates to a greater degree than a leucine-matched bolus of milk protein isolate (Monteyne et al., 2020). Whether this was attributable to the slightly greater protein content (milk protein isolate contained 26.2 g protein), energetic load or the whole-food nature of the mycoprotein was not clear but implied that leucine-independent mechanisms are responsible. Though similar work has previously suggested the whole food nature of eggs may be responsible for their anabolic potency being higher (also despite lower plasma leucine responses) than that of egg whites (van Vliet et al., 2017), this hypothesis was not confirmed with respect to mycoprotein (West et al., 2023c).

These findings concerning mycoprotein illustrate the opportunities available if we diversify the search for alternative proteins and do not assume traditional metrics of protein quality and/or leucine content of

a protein source will be entirely predictive when moving into human metabolic experimentation.

### **Insects**

Although not strictly speaking 'non-animal', an intriguing alternative dietary protein source is that derived from insects. At least partly due to the drive for more sustainable protein sources, insect farming for food has increased globally, and this has resulted in the increased availability of insect protein as a powder, food ingredient or as the protein base of novel food products and supplements. Farmed edible insects tend to be of a high protein content, compliant to food processing techniques (e.g. removal of cytoskeletal or non-edible parts, protein isolation, etc.) and possess essential amino acid rich and balanced profiles more analogous with traditional meats and, resultantly, a relatively high PDCAAS score (0.89). Human experiments corroborate these indicators of protein quality. Most notably, the creation and application of intrinsically stable isotopically labelled mealworms allowed the simultaneous demonstration that its ingestion (30 g) led to equivalent postprandial amino acid bioavailability and rises in resting and post-exercise MPS rates as compared with similarly labelled milk protein in young men (Hermans et al., 2021).

### **Algae**

Recent work has expanded our definition of potential alternative dietary proteins to the Protista and Monera kingdoms; eukaryotic (algae) and prokaryotic (cyanobacterium) photosynthetic organisms, which can be the substance of tightly controlled closed system agriculture to rapidly produce biomass with minimal environmental cost. Such species are naturally rich in protein of which the essential amino acid content is higher and more balanced than most plant-based proteins, though PDCAAS and DIAAS ratings are not available. In vitro work has suggested that amino acid bioavailability from algae (chlorella) or cyanobacteria (spirulina) ingestion may be lower than animal- or plant-derived proteins, attributed to the rigid structures of the cellulosic and bacterial walls, respectively (Becker, 2007). Human work has confirmed this lower amino acid bioavailability post chlorella ingestion but has shown that spirulina appears highly digestible allowing for equivalent postprandial amino acid availability as animal-derived comparators (van der Heijden et al., 2024a). Work has also shown that bolus ingestion of chlorella (despite poor bioavailability) or spirulina (containing 25 g protein) stimulates post-exercise MPS rates to a comparable degree as mycoprotein (van der Heijden et al., 2023b). Therefore, algal type foodstuffs appear to offer a novel modern opportunity to exploit for diversification of alternative proteins, though its current palatability and consumer acceptability still represent commercially relevant challenges to be overcome.

### **Barriers for Refining Sports Nutrition Recommendations**

The encouraging datasets discussed above have largely been the result of researchers establishing proof-of-concept data as to the viability and quality of novel proteins. However, this work is in its infancy, and the study designs have stark limitations when translating to real-world recommendations for sports nutrition.

For all data described generated from plant-, fungal-, algal- or insect-derived proteins, a reasonable interpretation could be that these sources have only been shown to be robustly anabolic when sufficient quantities are ingested (i.e. > 20 g). This is in excess of what has previously been suggested to be required for an optimal acute (i.e. ~4 h) MPS response to whey (Witard et al., 2014) or egg (Moore et al., 2009) proteins and implies that an optimal response may only occur if more total protein is ingested to compensate if the protein is, in fact, of poorer quality. In the virtual absence of studies of alternative proteins taking a dose-response approach, applying smaller less optimal boluses or comparing to a 'gold standard' within sports nutrition (i.e. whey; Devries & Phillips, 2005), it is hard to rebut this theory at present. However, a recent study (van der Heijden et al., 2024b) took a plant protein isolate blend (pea, rice, cannola) approach to theoretically optimize amino acid composition and thus maximize anabolic signalling while avoiding amino acid deficiencies. The work showed an equivalent post-exercise MPS response following ingestion of the plant-blend as compared with an isonitrogenous bolus of whey. This is in keeping with similar plant-blend approaches that have shown parity with whey (Lim et al., 2024), milk protein (Pinckaers et al., 2021, 2023) or chicken (Kouw et al., 2021) in resting and/or exercised muscle, with mycoprotein post-exercise (West et al. 2023) or when used as part of a plant-animal blend (Reidy et al., 2013). Data are therefore accumulating to support the concept that optimal anabolic responses may be achievable with carefully selected alternative protein sources, though sub-optimal doses are yet to be studied. It is also worth remarking that only a single study of those cited has assessed the interesting additional variable of potentially greater systemic amino acid demands of more ecologically valid whole-body exercise bouts (West et al., 2023a).

Much of our protein guidelines in sports nutrition are reliant on studies assessing acute postprandial MPS rates post-exercise. It is important to note that such capturable metabolic responses do not necessarily predict a given endpoint concerning muscle adaptation or performance (Mitchell et al., 2015). The number of additional variables present when examining longer-term studies and responses (e.g. mixed and repeated meals, the diet as a whole, study adherence, training variables and fluctuations, sleep, stress, variability of responses, etc.) make the determination of MPS predictive of broad-brush muscle reconditioning per se, rather than any given adaptive parameter (e.g. muscle mass, strength,  $VO_2$ max, etc.). It also illuminates the logistical issues that have led to fewer well controlled longer-term diet and exercise intervention studies in the scientific literature. One intervention study (Monteyne et al., 2023) has taken the acute data described for mycoprotein to use as the foundation of high protein (~2 g/kg bm) vegan diets consumed by young adults engaging in a high-volume resistance training regimen over 10 weeks. The vegan diet supported equivalent longer-term MPS rates (i.e. captured over the course of days encompassing multiple meals, exercise, sleep cycles), muscle hypertrophy and increases in strength compared with an optimal omnivorous diet. Hevia-Larrain et al. (2021) compared habitual vegans and omnivores, increasing their protein intakes to 1.6 g/kg bm/day using soy and whey, respectively, and also showed comparable increases in muscle size and strength during



prolonged resistance training between groups. While these studies essentially examined wholesale dietary habits, agreement can be found from studies applying protein supplementation on top of habitual or prescribed diets; pea (Babault et al., 2015), rice (Joy et al., 2013)) and soy (Brown et al., 2004) protein isolates all having been compared with whey supplementation. Conspicuously, for both wholesale dietary and supplemental protein intervention studies, unusual examples can be found (e.g. Hartman et al., 2007) where animal proteins appear superior, and these appear to be when participants have consumed what is likely sub-optimal protein intakes (i.e. < 1.0 g/kg bm/day).

Thus, data available to date imply that acute data reported for alternative protein sources can be translated to longer-term, wholesale dietary change; that is, evidence-based alternative proteins can be used to construct (more) vegan diets without compromising training adaptations and/or performance, with the potential caveat that sufficient total protein is consumed. However, we should be mindful that there is still a paucity of work investigating the potential influence of other variables (e.g. training status, sex, types of exercise/sport, varying adaptive responses, etc.) with the efficacy of alternative protein consumption during training.

## PRACTICAL APPLICATIONS

- Consumption of sufficient daily (~1.6 grams per kg bm) and per meal (~20 g) dietary protein represent fundamental recommendations within modern evidence-based sports nutrition to support optimal muscle recovery and adaptation to training.
- Athletes wishing to reduce their protein intake from animal-derived sources should be mindful of achieving sufficient total daily and per meal protein, with alternative protein isolates and novel foods offering protein-rich alternatives to help achieve this.
- Various plant- (e.g. pea protein), plant-blend, fungal- (mycoprotein), algal- or insect- derived proteins are evidence-based high-quality protein choices, and can be consumed as supplements, food ingredients or within novel products and can make up significant proportions of daily intakes.
- Alternative proteins exist at various places on a spectrum in terms of feasibility; for example, palatability, acceptability, commercial availability, product diversification, etc. They therefore may require more effort or create additional challenges to source and/or consume compared with traditional protein choices. These are important practical considerations for the athlete and practitioner when designing diets.
- Absent more data, it may still be prudent to consume modestly more (~10-20%) of alternative dietary proteins per meal and per day to account for any potential inferiority if selected in place of high-quality animal-derived proteins (e.g. whey).

## SUMMARY

A rich framework of human muscle protein metabolism research has built dietary protein guidelines for sports nutrition to a far higher resolution than those available for society at large. However, contemporary issues are forcing our hand; it should be acknowledged that until recently we have neglected protein sources in research settings and, thus, do not have well developed guidelines for where our protein should come from. While this is a multidisciplinary issue, recent work concerning the mechanisms regulating postprandial muscle protein metabolism, diverse protein sources and training responses, are uncovering new insights and highlighting new opportunities. The data here are emergent, but offer a new frontier for the scientific, applied and commercial sports nutrition communities to exploit novel, socially responsible and sustainable options to support optimal and evidence-based sports nutrition (policy).

The views expressed are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

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