

# The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (Homarus gammarus, L.)

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**Abstract**

Releasing hatchery-reared juveniles in the wild can mitigate the general decline in the natural stocks of European lobster, Homarus gammarus, L. However, growth and survival rates in lobster culture are low, presumably due to suboptimal nutrition and feeding. With the aim of determining appropriate nutrient levels, we tested different formulated extruded feeds for the culture of juvenile European lobster. Baseline metabolism (standard metabolic rate, SMR), in combination with the metabolic cost of feeding (specific dynamic action, SDA), and nitrogen retention during digestion and assimilation was investigated for six experimental diets. Diets were formulated to contain two different levels of protein (400 and 500 g kg-1), with three lipid to carbohydrate (L:CHO) ratios (low, medium, and high). These experimental diets were tested over a 32-day period, against a conventional control diet (Antarctic krill, Euphausia superba). During this period, the growth performance of the juveniles was assessed as molting frequency, increments in carapace length and whole body wet weight. At the end of the growth performance trial, oxygen consumption (MO2) and nitrogen excretion rates of individual lobsters were determined prior to and following the ingestion of a single meal. Molting occurred more frequently in juveniles fed with krill and krill resulted in a significantly higher specific growth rate than experimental dry feeds except for the 500-low diet. However, lobsters fed any of the three 500 and the 400-low diets had carapace length increments, SMR, SDA, and nitrogen retention similar to those fed the krill diet. Results suggest that protein is an important macronutrient for juveniles of this species and must be included above 40 %. Also, lobsters have a dietary requirement for carbohydrates ranging from 24% to 35% probably related to the need for glycogen in chitin synthesis. The lower the protein content, the higher the requirement in carbohydrates.



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Highlights:

- Lobster juveniles perform better with 50 than 40% protein formulated extruded feeds.
- Best performance of formulated diets ranging from 24 to 35% in carbohydrate content.
- Dietary carbohydrate has a protein-sparing effect.

# The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (*Homarus gammarus*, L.)

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Releasing hatchery-reared juveniles in the wild can mitigate the general decline in the natural stocks of European lobster, *Homarus gammarus*, L. However, growth and survival rates in lobster culture are low, presumably due to suboptimal nutrition and feeding. With the aim of determining appropriate nutrient levels, we tested different formulated extruded feeds for the culture of juvenile European lobster. Baseline metabolism (standard metabolic rate, SMR), in combination with the metabolic cost of feeding (specific dynamic action, SDA), and nitrogen retention during digestion and assimilation was investigated for six experimental diets. Diets were formulated to contain two different levels of protein (400 and 500 g $kg^{-1}$), with three lipid to carbohydrate (L:CHO) ratios (low, medium, and high). These experimental diets were tested over a 32-day period, against a conventional control diet (Antarctic krill, *Euphausia superba*). During this period, the growth performance of the juveniles was assessed as molting frequency, increments in carapace length and whole body wet weight. At the end of the growth performance trial, oxygen consumption ($MO_2$) and nitrogen excretion rates of individual lobsters were determined prior to and following the ingestion of a single meal. Molting occurred more frequently in juveniles fed with krill and krill resulted in a significantly higher specific growth rate than experimental dry feeds except for the 500-low diet. However, lobsters fed any of the three 500 and the 400-low diets had carapace length increments, SMR, SDA, and nitrogen retention similar to those fed the krill diet. Results suggest that protein is an important macronutrient for juveniles of this species and must be included above 40 %. Also, lobsters have a dietary requirement for carbohydrates ranging from 24% to 35% probably related to the need for glycogen in chitin synthesis. The lower the protein content, the higher the requirement in carbohydrates.

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## 1. Introduction

The European lobster (*Homarus gammarus*) is an economically important decapod crustacean distributed from Northern Norway to Morocco and Eastern Mediterranean (Triantafyllidis et al., 2005). Commercial landings of this species are declining and efforts to enhance natural populations have been made by restocking with hatchery-reared juveniles (Agnalt et al., 2007). Hatchery production of European lobster aims to enhance growth and survival rates by securing optimal water quality, reducing predation, and by improving access to nutritional rich diets (Powell, 2016). Research efforts have been devoted mainly towards improving water quality conditions and the development of novel rearing systems (Daniels et al., 2013, 2015; Drengstig and Bergheim, 2013; Halswell et al., 2016; Middlemiss et al., 2015), but less so towards the development of a species-specific formulated diet. The transition from live or frozen feeds to the use of dry formulated diets may be one way to support a simpler and more sustainable production through the ease of application, reduced cost, and a more consistent nutritional quality (Powell et al., 2017).

The development of a nutritionally balanced formulated feed requires species-specific information on nutritional requirements. A considerable research effort on formulated feed development for spiny lobsters has been made in the last 30 years, the results of which indicate a dietary demand for high protein (> 45%), low lipid (<10%), and moderate carbohydrate (~20%) (Williams, 2007). However, the performance of spiny lobsters fed on formulated feeds remains poor, partially as a consequence of a lack of understanding of how they digest and assimilate this type of feeds (Perera and Simon, 2015). There is less literature available for homarid species than for spiny lobsters, and while some nutritional studies have been conducted on American and European lobster, appropriate nutritional levels are still to be defined. Based on the idea that diets should match the dry matter biochemical composition of an organism (Dall et al., 1991), the proximate composition content of *Homarus americanus* post-larvae suggests that an appropriate diet for this species should contain 53% protein, 4% lipid, and 12% carbohydrates (Haché et al., 2015). The observation that European lobster possesses a variety of carbohydrases (Glass and Stark, 1995), is indicative of a digestive capacity of different carbohydrate sources. Furthermore, Powell et al. (2017) identified glycogen deficiencies in the biochemical composition of *H. gammarus* larvae reared on formulated dry feed. Taken together, these findings suggest that the development of a formulated dry feed for European lobster should consider carbohydrates as a potential non-protein energy source.

The standard metabolic rate (SMR) represents the minimum energy expenditure of an ectotherm animal (Rosewarne et al., 2016). Although individuals with higher SMR have higher maintenance metabolism, previous studies also suggest this is indicative of an increased growth potential (Álvarez and Nicieza, 2005; Auer et al., 2015; Reid et al., 2012; Van Leeuwen et al., 2012). The magnitude of the postprandial metabolism, commonly referred to as the specific dynamic action (SDA), depends largely on the size and nutritional composition of a meal. It provides information on the cost and duration of the nutritional processes, including the energy expended towards food handling, absorption and storage of nutrients, deamination of amino acids, protein and lipids synthesis for growth, and synthesis of excretory products (Jobling, 1993). In crustaceans, the mechanical costs of digestion are calculated to be 5% to 8% while protein synthesis accounts for 20% to 37% of SDA (Whiteley et al., 2002). Therefore, SDA determination is a useful performance parameter in nutritional studies. In principle, the higher the fraction of the meal energy allocated to SDA, the less energy will be retained to fuel locomotion, growth, and reproduction (Stieglitz et al., 2018). The present information on the metabolism in European lobster is sparse. Whiteley et al,. (1990) compared aquatic and aerial rates of $MO_2$ (mass-specific oxygen consumption) of this species at a temperature range 10-20˚C. Later, Drengstig (2017) reported data of standard metabolism in *H. gammarus* at 20˚C. However, to our knowledge, no studies on the effects of dietary composition on SMR or the SDA of European lobster have been performed.

Lobsters excrete the majority of the end product of protein metabolism across the gill epithelium in the form of ammonia (Burger, 1957), while a smaller part of nitrogenous waste is converted into urea in the antennal and maxillary glands (Binns and Peterson, 1969). Wickins (1985) investigated the effect of feeding on ammonia excretion by *Homarus gammarus* and observed that lobsters exhibited a significant increase in ammonia excretion after a meal. The efficiency with which dietary amino acids are deposited as new tissue can be estimated by the quantification of nitrogen excretion during digestion (Ming, 1985). Amino acids that are deaminated for *de novo* lipogenesis and glycogenesis, or oxidized for fuel, turn into nitrogenous waste (Skov et al., 2017). Therefore, high nitrogen excretion rates are indicative of reduced protein retention.

This study aimed to investigate the role of protein inclusion levels combined with different non-protein energy sources (lipids and carbohydrates) in the metabolism and growth of European lobster. For that purpose, the present work compared the respiration and nitrogen excretion rates of European lobster juveniles (< 1 g) reared on Antarctic

krill or experimental extruded dry feeds with different inclusion levels of protein, lipids, and carbohydrates. SMR, SDA response, nitrogen retention, and growth performance were determined and discussed.

## 2 - Materials and methods

### *2.1. Experimental animals*

Experiments were conducted at the aquaculture facilities at the Technical University of Denmark, Section for Aquaculture, Hirtshals. All experimental animals were hatched from eggs obtained from wild European lobster females caught along the Skagerrak coast of North Jutland, Denmark. Experimental lobster juveniles were reared individually in cassette systems consisting of 200 mL compartments. Cassettes were placed in raceways supplied by a flow-through semi-closed seawater system at a constant flow rate of 330 L $h^{-1}$ (18±0.5˚C temperature, 34±1 PSU salinity, > 90% dissolved oxygen, <0.1mg $L^{-1}$ ammonia-N), subjected to a photoperiod cycle of 8h light: 16h dark. Lobsters were fed once daily with thawed Antarctic krill, *Euphausia superba* (Akudim A/S, Denmark).

### *2.2. Growth trial*

Animals were held under the above-described conditions for four months from settling, after which they were randomly divided into seven treatment groups (N=10, per diet) while ensuring animals were of similar size across all treatments (0.86 ± 0.06 g wet weight, mean ± SEM). An experimental period of 32 days before respirometry and nitrogen excretion trials was used for evaluation of growth performance. During this period, each juvenile was individually fed its respective diet in excess each morning, and allowed to feed for 4h before uneaten food was removed. Additionally, juveniles were allowed to feed on their molted exoskeletons. Molt occurrences were recorded daily. At the beginning and end of the experimental period, lobsters were gently blotted dry with a paper towel and body wet weight was recorded to the nearest 0.01g. Carapace length was recorded with a vernier caliper from the base of the eye socket to the posterior edge of the cephalothorax. The following formulas were used:

$$Cumulative\ molting\ (CM, \%) = \sum_{i=0}^{n} (Mi \times Lob^{-1}) \times 100$$

where: i = the day; Mi = number of molts on the day i; Lob = number of lobsters in each treatment.

$$Specific\ growth\ rate\ (SGR, \%\ day^{-1}) = [ln\ (BW_f) - ln\ (BW_i)] \times days^{-1} \times 100$$

where, $BW_f$ = final wet body weight, $BW_i$ = initial wet body weight.

$$Carapace\ length\ increment\ (iCL, \%) = (CL_f - CL_i) \times CL_i^{-1} \times 100$$

where: $CL_f$ = final carapace lentgh; $CL_i$ = initial carapace length.

*2.3. Experimental diets*

Six formulated dry diets were evaluated using Antarctic krill as a reference diet. The six experimental dry diets were formulated to have two fixed protein levels (400 or 500 g $kg^{-1}$), and for each protein level, three L:CHO ratio levels (low: 0.3, medium: 0.5, and high: 0.8-1.0). Different protein, lipid, and carbohydrate contents were achieved by altering squid meal, wheat gluten, wheat starch, and fish oil inclusion levels. Experimental diets were extruded as 4 mm pellets and were manufactured by SPAROS Lda (Olhão, Portugal). Proximal analysis of krill and experimental diets were performed in duplicate. Briefly, the diets were finely ground using a Krups Speedy Pro homogenizer and analyzed for crude protein, (i.e. Kjeldahl N × 6.25, ISO 5983-2 (2005), crude fat (Bligh and Dyer, 1959), dry matter and ash (NMKL 23, 1991). Formulation and proximate composition are presented in Table 1.

*2.4. Respirometry trial*

Measurements were performed on 10 intermolt lobsters per dietary treatment. All animals used were fasted for 48h prior to respirometry measurements to ensure a post-absorptive state. Experiments were performed in 75 mL respirometers supplied with temperature-controlled aerated seawater, using 8 chambers at a time. The bottom of each chamber was equipped with a perforated base plate, under which a magnetic stirrer ensured water mixing in the chamber. Oxygen content was registered every 15 sec using a sensor connected to an optical oxygen meter (FireSting O2, Pyro Science GmbH, Aachen, Germany) installed in each chamber. $MO_2$ measurements were performed by computerized intermittent flow, in loops of 60 min (consisting of a 5 min flushing period, followed by a 55 min closed period). The oxygen consumption rate was determined by linear regression of the decline in oxygen content during the closed period. The mass-specific oxygen consumption ($MO_2$) was calculated based on the slope of the regression according to Steffensen (1989) as:

$$MO_2 = \alpha \times V_{resp} \times \beta \times BW^{-1}$$

where: α = slope ($\Delta pO2 \times \Delta t^{-1}$), $V_{resp}$ = volume of the chamber minus the volume of the lobster (using a lobster density of 1), β = oxygen solubility at the experimental temperature, and BW = wet body weight of the lobster.

Standard metabolic rate (SMR) was estimated from the first 48h $MO_2$ measurements, which was calculated as described by Skov et al. (2011). Briefly, $MO_2$ measurements were grouped in frequency classes, and SMR was calculated from the most frequently occurring bins and their relative contribution. Following SMR measurements, chambers were opened and a pre-weighed piece of thawed krill or a feed pellet was offered to each lobster. During

feeding, chambers were kept unsealed, the flushing pump was stopped, and external aeration was provided. After a 2h feeding period, the remaining krill or pellet was carefully removed and $MO_2$ postprandial measurements resumed for 48h for estimation of SDA response. The uneaten feed fraction was collected, filtered, and dried for voluntary feed intake (VFI) estimation employing the following formula (Nguyen et al., 2014):

$$VFI = dF - uF - L$$

where: dF = distributed feed, uF = unconsumed feed, L = leaching after 2h. Leaching was estimated by placing a pre-weighed quantity of each diet in the chambers under the same conditions as in the feeding period but in this case, without animals.

For calculation of SDA variables digestion was determined to be completed when $MO_2$ postprandial measurements plotted over time fell within 15% of the SMR previously recorded for that chamber (Jordan and Steffensen, 2007). According to Secor (2009), the following SDA variables were calculated to describe the postprandial $MO_2$: $SDA_{dur}$ (h) is the time from feeding until $MO_2$ converged with the SMR + 15%. The $SDA_{cost}$ (µg $O_2$ $g^{-1}$) is the post-feeding integrated excess $MO_2$ above SMR. The $SDA_{peak}$ (µg $O_2$ $g^{-1}$ $h^{-1}$) is the maximum value of $MO_2$ above SMR during the SDA course and $SDA_{ttp}$ (h) is the time from feeding to $SDA_{peak}$. $SDA_{coef}$ (%) is the $SDA_{cost}$ converted to energy using an oxycalorific coefficient of 14.06 J $mg^{-1}$ $O_2$ (Dejours, 1981) and divided by the energy content of the meal which was calculated from the estimated feed intake. The scope is the $SDA_{peak}$ divided by the SMR.

*2.5. Nitrogen excretion trial*

Following oxygen consumption measurements, each lobster was transferred to a 130 mL seawater container supplied with aeration. Water samples of 15mL were collected manually from individual chambers at time 0h and 48h for baseline screening of total ammonia and nitrogen excretion rates. After this period, lobsters were offered a pre-weighed pellet or krill piece for 2h. After the meal, lobsters were transferred into containers with fresh seawater. Water samples were manually collected at time 0h and 48h for the determination of total postprandial ammonia and nitrogen excretion rates. Collected water samples were filtered (0.2µm, Filtropur Sarstedt, Numbrecht, Germany) and stored at 0°C until analysis. Total nitrogen and ammonia nitrogen of collected water samples were determined in duplicate according to ISO 11905-1 (1997) and DS (1975), respectively. The voluntary feed intake was calculated from the

uneaten fraction that was collected, filtered, and dried. N intake was calculated as 16% of protein intake (Chibnall et al., 1943). Total postprandial nitrogen excretion was calculated using the following formulas:

$$N_{pre-feeding} = [(N_{48h} \times V_{48h}) - (N_{0h} \times V_{0h})] \times BW^{-1}$$

$$N_{post-feeding} = [(N_{48h} \times V_{48h}) - (N_{0h} \times V_{0h})] \times BW^{-1}$$

$$N_{excreted} = N_{post-feeding} - N_{pre-feeding}$$

where: $N_{excreted}$ = total postprandial nitrogen excreted (mg total nitrogen per mg wet weight), $N_{pre\text{-}feeding}$ = pre-feeding nitrogen excretion (mg total nitrogen per mg wet weight), $N_{post\text{-}feeding}$ = post-feeding nitrogen excretion (mg total nitrogen per mg wet weight), $N_{48h}$ = total nitrogen concentration at time 48h (mg total nitrogen per mL); $N_{0h}$ = total nitrogen concentration at time 0h (mg total nitrogen per mL), $V_{48h}$ = volume of the chamber at time 48h (mL), $V_{0h}$ = volume of the chamber at time 0h (mL), BW = wet body weight of the lobster. Nitrogen retention was expressed as percentage of total N intake.

### *2.6. Data analysis and statistics*

Data are expressed as means ± SEM unless otherwise specified. All dietary treatments were subjected to a one-way ANOVA to test the experimental formulated dry feeds against the control diet (krill). Whenever significant differences were identified, comparisons against the krill diet were conducted using the Dunnett t-test. Data from experimental formulated dry feed treatments were subsequently subjected to a two-way ANOVA, considering protein level and L:CHO ratio as variables. Following a two-way ANOVA and whenever significant differences were identified, means were compared by the Holm-Sidak post hoc test. Data were checked for normal distribution and homogeneity of variances and, when necessary, log-transformed. Data expressed as a percentage were arcsin transformed. Carapace length increment was log(x+2) transformed due to a high frequency of null observations. Statistical significance was set at $p \leq 0.05$. All statistical tests were performed using the IBM SPSS Statistics 25.0 and graphics were generated by GraphPad Prism version 5.0 software package. The linear regression of the decline in oxygen content was computed using R version 3.5.1 software (R Core Team, 2018).

## 3. Results

### *3.1. Growth performance*

Observation during feeding showed that *H. gammarus* juveniles were attracted to all experimental diets and actively manipulated the offered feed. Minimum cumulative molting during the 32-day growth trial was recorded as 10% for the group of animals fed the 400-high diet. Maximum cumulative molting (90%) was observed for lobsters fed the krill diet (Figure 1). SGR was significantly higher ($F_{6,69} = 4.90$, $p < 0.001$) in krill-fed lobsters in comparison to lobsters fed experimental dry diets, with the exception of the 500-low diet (Figure 2A). Among experimental diets, SGR was unaffected by protein level ($F_{1,59} = 2.63$, $p = 0.11$), L:CHO ratio ($F_{2,59} = 2.62$, $p = 0.08$), or the interaction of the two variables ($F_{2,59} = 1.05$, $p = 0.36$). Nevertheless, protein content caused a significant positive effect on the carapace length increment (iCL) increment (Figure 2B). The iCL increment in lobsters fed the 500-high diet was significantly higher compared to animals fed the 400-high diet ($F_{1,59} = 6.12$, $p = 0.02$). The animals fed the 400-medium and 400-high experimental diets presented a significantly lower iCL in opposition to the krill-fed animals ($F_{1,69} = 2.89$, $p = 0.02$). Voluntary feed intake expressed as dry weight was higher ($F_{6,71} = 3.19$, $p = 0.01$) for the 400-low and 500-medium diets compared to the krill. No significant effect on feed intake for protein or L:CHO ratio was detected on experimental diets. However, the interaction of both was statistically meaningful ($F_{2,62} = 3.33$, $p = 0.04$). The L:CHO ratio did not affect the 500 group of diets but in the 400 group, the 400-medium diet had a significantly lower VFI compared to the 400-low (Figure 3).

### *3.2. Metabolic rates*

The pre- and postprandial metabolic data for lobsters fed control and experimental diets are presented in Table 2. Individuals that did not ingest sufficient feed to induce a clear postprandial metabolic response were omitted from the analyses. Comparing the results of all treatments, animals fed the 400-medium and 400-high diets had significantly lower SMR compared to the krill-fed animals (Table 2, Figure 5). Within experimental diets, SMR was significantly affected by protein level and L:CHO ratio, but unaffected by the interaction of protein × L:CHO. With the exception of the 400-low, animals fed diets with 40% protein were observed to have significantly lower SMR values compared to the 50% protein diets (Table 2, Figure 5). The amount of ingested energy was significantly higher for lobsters fed on the 400-low and 500-medium diets compared to the krill-fed group (Table 2). No significant differences in meal energy were observed between experimental diets. $SDA_{dur}$ was significantly longer in lobsters fed 400-low when compared to krill-fed lobsters. Within experimental diets, duration of SDA was affected by protein

level and the interaction of protein × L:CHO, but was unaffected by L:CHO ratio (Table 2, Figure 4). Results showed lobsters fed the 500-low diet had a shorter SDA response compared to the animals fed on the 400-low diet.

### *3.3. Nitrogen retention*

The effects of dietary treatment on nitrogen budgets are shown in Table 3. As for the respirometry trials, animals that did not feed sufficiently were not included in the analysis. Within the experimental dietary groups, protein level, L:CHO ratio, and the interaction of the two, all significantly affected nitrogen intake. N intake was generally higher for the 50% protein diets with no effect of L:CHO. Among the 40% protein diets, N intake was highest for the 400-low diet. Animals fed the 400-medium and 400-high diets presented a lower N intake in comparison to krill. No significant differences were observed for the total nitrogen excreted. Comparing all the dietary treatments, nitrogen retention was significantly lower in animals fed the 400-high diet against lobsters fed krill. Within the experimental diets the % total N retention was significantly affected by protein level, L:CHO ratio, and the interaction of protein × L:CHO ratio. Nitrogen retention was higher for the 50% protein diets with no effect of L:CHO. The lowest N retention was observed in the 40% protein, with a significant decrease with increasing L:CHO ratios (Table 3). The contribution of ammonia to the total nitrogen excreted varied between 64% and 88% among dietary treatments (data not presented) but no statistical differences were found ($F_{6,33} = 1.37$, $p = 0.27$).

## Discussion

The present study demonstrates a limited successful growth of European lobster juveniles fed on formulated dry feeds. Results indicate that SGR of European lobster juveniles fed the 500-low diet (50% protein, 9% lipid, 26% carbohydrate) was not significantly lower than the control group fed on krill. However, the cumulative molting rate was lower in all treatment groups fed formulated experimental diets. Results suggest that the poor growth performance of *H. gammarus* fed on formulated feeds remains one of the principal obstacles in the development of sustainable aquaculture of this species. Further optimization of formulated diets in terms of mechanical and chemical digestion is imperative. Improving pellet size, format, and texture, and supplementing feeds with additives (digestible binders, pH buffers, and exogenous enzymes) need to be addressed in future research.

Results from this study establish that the dietary regime affects the SMR in European lobster juveniles. Animals fed the 400-medium (40% protein, 15% lipid, and 31% carbohydrate) and 400-high diets (40% protein, 23% lipid, and 23% carbohydrate) showed the lowest SMR. According to Biro and Stamps (2010) a higher SMR is associated with a larger metabolic capacity. In the same study, the authors suggested that individuals with high metabolism were able to process larger meals. Therefore, under this hypothesis, SMR is expected to produce a positive impact on performance (Burton et al., 2011). Our results showed that the juveniles fed the 400-medium and 400-high experimental diets presented the poorest performance in terms of cumulative molting, SGR, and CL increment, confirming Biro and Stamps (2010) hypothesis. The voluntary feed intake for the group of animals fed these two diets was also the lowest, which most likely contributed to the poorest growth performance. Protein synthesis, a crucial process in growth, is strongly affected by the feed intake. Previous studies in several crustacean species have demonstrated that protein synthesis rates generally decrease in starved or less frequently fed animals (Carter and Mente, 2014). The reason why the feed intake was lower for the 400-medium and 400-high diets remains unclear but it might be related to lower palatability of these two diets, or that high lipid levels cause faster satiation. The growth compensation for animals fed the 400-low diet, i.e., low lipid (11%) and high carbohydrate (35%) is in agreement with the effect observed between SMR and L:CHO ratio. This result supports our initial hypothesis that carbohydrate represent an important macronutrient for *H. gammarus* especially in diets with reduced protein content. In fact, carbohydrates are important for crustacean species as glycogen is an essential precursor of chitin synthesis, serving a critical role during the molt cycle (Wang et al., 2016).

Studies on the SDA in lobster species are scarce, and, to our knowledge, there is no information for European lobsters. In this work, we observed that feeding caused a rise in oxygen consumption 2 to 3 times above SMR levels in European lobster juveniles fed the different diets. In a previous study, in 3.2 g *Homarus americanus* fed on formulated diets, Koshio et al. (1992) reported an SDA scope of 1.5. The smaller size of the lobsters tested in this study can explain the difference in the SDA scope, as the animal size is known to influence the SDA variables (McCue, 2006). In the present study, the time to achieve the SDA peak ranged between 8h to 11h, with elevated oxygen consumption rates lasting for 17 to 24h. The SDA duration was significantly longer for the 400-low experimental diet compared to krill, which is likely related to the higher meal energy or protein intake (Secor, 2009). SDA duration in southern rock lobster (*Jasus edwardsii*) fed squid was longer than what we observed in European lobster juveniles. Crear and Forteath (2000) reported that 750 g *J. edwardsii* took 42h to return to the pre-feeding oxygen consumption level, while Radford et al. (2004) observed that SDA response in 16 g animals of the same species lasted 30h. In this study, SDA coefficient results showed that juveniles fed on the tested diets spend between 3.4% to 7.0% of the meal energy on digestive processes. These results are in agreement with the findings by Crear and Forteath (2000) who reported an SDA coefficient of 6.6% in *J. edwardsii*. Nevertheless, the amount of meal energy allocated to SDA reported for crustacean species is highly variable even within the same species. For example, Houlihan et al. (1990) observed an $SDA_{coef}$ of 13.3% for 37 g *Carcinus maenas* while Wallace (1973) reported an $SDA_{coef}$ of 3.4% in 10 g individuals of the same species. Collectively, these findings suggest that the duration of the SDA response and the SDA coefficient increases with increasing body sizes.

In this study, we observed that juveniles excreted the majority of the nitrogenous waste in the form of ammonia (64-88%). Results reported here suggest that the mechanism for nitrogen excretion in *H. gammarus* is similar to other aquatic crustacean species, namely, the *H. americanus* (Burger, 1957) and *J. edwardsii* (Binns and Peterson, 1969). Total nitrogen budget results showed that the nitrogen retention of juveniles fed the 400-high diet (40% protein, 23% carbohydrate, and 23% lipid content) was significantly lower than in juveniles fed the other experimental or control diets. The severely reduced N intake in this group of animals induced a negative nitrogen balance, i.e., nitrogen excretion exceeded the nitrogen intake. The incapacity of this group of animals for nitrogen retention suggested that, rather than protein deposition, animals were periodically undergoing tissue protein catabolism (Guo et al., 2012).

Results from this study suggest that European lobster juveniles with low SMR and low nitrogen retention have a reduced growth capacity. Nevertheless, the estimated SMR and nitrogen retention were highly affected by the feed intake of the different tested diets. Therefore, the results presented in this study should be interpreted with caution. Moreover, the growth performance indices were calculated over a 32-day period, which could be considered relatively short, particularly in the case of crustacean species. In these animals, wet weight changes follow a typical pattern through the molt cycle. The highest increase occurs in the brief period of rapid water uptake at ecdysis. Further moderate gains are related to carapace mineralization and tissue growth. Finally, during the intermolt period, there is a relative stabilization of fresh weight until the onset of the successive ecdysis (Nguyen et al., 2014). As follows, nutritional studies targeting evaluation of growth performance should allow at least one complete molt cycle per individual.

SMR and nitrogen retention results from this study corroborate the hypothesis that juvenile *H. gammarus* perform better fed on 500 against 400 g $kg^{-1}$ protein content in their diet. This level agrees with the protein content (52% DM) in European lobster larvae reported by Powell et al. (2017) supporting the idea that diets should meet the organism's biochemical composition. The results of this study show that protein is a fundamental nutrient in formulated dry feeds. However, its inclusion can potentially be reduced when compensated with appropriate carbohydrate levels. Carbohydrates are the least expensive energy source for aquatic animals (Wang et al., 2016) and therefore, this is an important opportunity for the production of sustainable and economically viable formulated feeds for this species.

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**Table 1.** Formulation and chemical composition of experimental diets.

| Protein level | 400 g kg$^{-1}$ | | | 500 g kg$^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| L:CHO ratio | Low | Medium | High | Low | Medium | High | Krill |
| *Ingredients (g kg$^{-1}$)* | | | | | | | |
| Antarctic krill | | | | | | | 1000.0 |
| Fish meal [a] | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | |
| Squid meal [b] | 125.0 | 125.0 | 125.0 | 255.0 | 255.0 | 255.0 | |
| Krill meal [c] | 250.0 | 250.0 | 250.0 | 200.0 | 200.0 | 200.0 | |
| Wheat gluten [d] | 20.0 | 20.0 | 20.0 | 50.0 | 50.0 | 50.0 | |
| Wheat meal [e] | 172.5 | 172.5 | 172.5 | 172.5 | 172.5 | 171.5 | |
| Wheat starch [f] | 229.0 | 171.0 | 89.0 | 141.0 | 93.0 | 30.0 | |
| Fish oil [g] | 22.0 | 80.0 | 160.0 | 0.0 | 48.0 | 112.0 | |
| Soy lecithin [h] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | |
| Vitamin & minerals premix [i] | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | |
| Astaxanthin [j] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | |
| *Proximal composition (g kg$^{-1}$ as fed)* | | | | | | | |
| Moisture | 78.0 | 81.0 | 82.0 | 86.0 | 81.0 | 71.0 | 916.1 |
| Ash | 68.1 | 68.0 | 66.2 | 68.70 | 68.2 | 66.3 | 11.6 |
| Protein | 400.0 | 397.0 | 385.0 | 497.0 | 495.0 | 481.0 | 58.2 |
| Lipids | 107.0 | 147.0 | 233.0 | 85.8 | 119.0 | 172.0 | 9.6 |
| Carbohydrates [x] | 346.9 | 307.0 | 233.8 | 262.5 | 236.8 | 209.7 | 4.5 |
| L:CHO ratio | 0.3 | 0.5 | 1.0 | 0.3 | 0.5 | 0.8 | 2.1 |
| Gross energy (KJ. g$^{-1}$) [y] | 19.0 | 19.8 | 21.6 | 18.7 | 19.5 | 20.8 | 1.8 |
| Protein/Energy (g MJ$^{-1}$) | 21.0 | 20.1 | 17.8 | 26.5 | 25.4 | 23.1 | 32.6 |

[a] Micronorse: 70.9% CP, 8.7% CF, Tromsø Fiskeindustri AS, Norway.

[b] Squid meal: 83% CP, 4% CF, Sopropêche, France.

[c] Krill meal: 61.1% CP, 17.4% CF, Aker Biomarine, Norway.

[d] VITAL: 80.4% CP, 5.6% CF, Roquette, France.

[e] Wheat meal: 11.7% CP, 1.6% CF, Molisur, Spain.

[f] Meritena 200: 0.4% CP, 0.1% CF, 90% starch, Tereos, France.

[g] Fish oil: 98.1% CF, 16% EPA, 12% DHA, Sopropêche, France.

[h] P700IPM, Lecico GmbH, Germany.

[i] Vitamins (IU or mg kg$^{-1}$ diet): DL-alpha tocopherol acetate, 200 mg; sodium menadione bisulphate, 50 mg; retinyl acetate, 40000 IU; DL-cholecalciferol, 4000 IU; thiamine, 60 mg; riboflavin, 60 mg; pyridoxine, 40 mg;

cyanocobalamin, 0.2 mg; nicotinic acid, 400 mg; folic acid, 30 mg; ascorbic acid, 1000 mg; inositol, 1000 mg; biotin, 6 mg; calcium pantothenate, 200 mg; choline chloride, 2000 mg, betaine, 1000 mg. Minerals (g or mg $kg^{-1}$ diet): copper sulphate, 18 mg; ferric sulphate, 12 mg; potassium iodide, 1 mg; manganese oxide, 20 mg; sodium selenite, 0.02 mg; zinc sulphate, 15 mg; sodium chloride, 800 mg; excipient wheat gluten, Premix Lda., Portugal.

[j] Carophyll Pink 10% CWS, 10% astaxanthin, DSM Nutritional Products, Switzerland.

[x] Carbohydrate (%) = 100 – (Crude protein % + crude lipid % + moisture % + ash %)

[y] Gross energy (MJ $kg^{-1}$) = Protein content × 21.3 kJ $g^{-1}$ + Lipid content × 39.5 kJ $g^{-1}$ + Carbohydrate content × 17.6 kJ $g^{-1}$) / 1000 kJ $MJ^{-1}$ (Cuzon and Guillaume, 1997)

**Table 2.** Overview of pre and postprandial metabolism responses for European lobsters fed six different experimental and one control diet.

| Protein ($g.kg^{-1}$) | L:CHO | SMR ($\mu g\ O_2\ g^{-1}\ h^{-1}$) | $SDA_{peak}$ ($\mu g\ O_2\ g^{-1}\ h^{-1}$) | Scope | $SDA_{ttp}$ (h) | $SDA_{cost}$ ($\mu g\ O_2\ g^{-1}$) | $SDA_{dur}$ (h) | $SDA_{coef}$ (%) | Meal energy (J) | N |
|---|---|---|---|---|---|---|---|---|---|---|
| 400 | Low | $75.6 \pm 10.4^{ab}$ | $249.4 \pm 44.6$ | $3.3 \pm 0.4^{*,a}$ | $9.0 \pm 1.5$ | $2079.6 \pm 554.4$ | $23.6 \pm 0.5^{*,a}$ | $3.9 \pm 1.5$ | $580.0 \pm 130.2^{*}$ | 5 |
| | Medium | $51.2 \pm 6.1^{*,bc}$ | $137.6 \pm 16.6$ | $2.7 \pm 0.2^{ab}$ | $10.3 \pm 0.9$ | $704.2 \pm 291.3$ | $19.0 \pm 1.7^{ab}$ | $6.1 \pm 2.5$ | $245.1 \pm 55.3$ | 6 |
| | High | $42.7 \pm 5.0^{*,c}$ | $135.1 \pm 27.2$ | $3.2 \pm 0.5^{*,a}$ | $10.8 \pm 1.3$ | $581.9 \pm 229.6$ | $20.0 \pm 2.2^{ab}$ | $4.8 \pm 3.6$ | $278.9 \pm 99.1$ | 5 |
| 500 | Low | $97.9 \pm 11.9^{a}$ | $214.0 \pm 33.1$ | $2.2 \pm 0.2^{b}$ | $9.8 \pm 0.7$ | $1099.3 \pm 247.0$ | $18.0 \pm 0.4^{b}$ | $3.5 \pm 0.6$ | $445.1 \pm 160.9$ | 6 |
| | Medium | $80.7 \pm 4.0^{a}$ | $224.2 \pm 45.5$ | $2.7 \pm 0.5^{b}$ | $11.3 \pm 1.2$ | $1683.8 \pm 750.5$ | $20.7 \pm 0.8^{ab}$ | $3.4 \pm 1.2$ | $473.0 \pm 42.7^{*}$ | 6 |
| | High | $73.1 \pm 6.5^{a}$ | $149.5 \pm 22.5$ | $2.0 \pm 0.2^{b}$ | $9.6 \pm 0.7$ | $621.0 \pm 233.7$ | $17.2 \pm 1.3^{ab}$ | $2.5 \pm 1.0$ | $438.7 \pm 110.1$ | 5 |
| Control (Krill) | | $107.3 \pm 19.3$ | $186.3 \pm 17.6$ | $1.9 \pm 0.2$ | $7.6 \pm 0.2$ | $779.7 \pm 229.5$ | $17.2 \pm 1.4^{ab}$ | $7.0 \pm 1.7$ | $103.6 \pm 5.6$ | 5 |
| [1] One-Way ANOVA | | $F_{6,37}=5.27^{**}$ | $F_{6,37}=1.99$ | $F_{6,37}=2.83^{*}$ | $F_{6,37}=1.50$ | $F_{6,37}=1.45$ | $F_{6,37}=2.90^{*}$ | $F_{6,37}=0.58$ | $F_{6,37}=2.76^{*}$ | |
| [2] Two-Way ANOVA | | | | | | | | | | |
| P | | $F_{1,32}=17.70^{***}$ | $F_{1,32}=0.63$ | $F_{1,32}=7.69^{**}$ | $F_{1,32}=0.39$ | $F_{1,32}=0.39$ | $F_{1,32}=4.50^{*}$ | $F_{1,32}=0.51$ | $F_{1,32}=1.19$ | |
| L:CHO | | $F_{2,32}=6.79^{**}$ | $F_{2,32}=3.39^{*}$ | $F_{2,32}=0.08$ | $F_{2,32}=2.82$ | $F_{2,32}=2.82$ | $F_{2,32}=1.38$ | $F_{2,32}=0.21$ | $F_{2,32}=0.50$ | |
| P x L:CHO | | $F_{2,32}=0.15$ | $F_{2,32}=1.73$ | $F_{2,32}=1.95$ | $F_{2,32}=1.36$ | $F_{2,32}=1.36$ | $F_{2,32}=4.21^{*}$ | $F_{2,32}=0.13$ | $F_{2,32}=2.57$ | |

Values are mean ± standard error.

[1] Superscript * indicate dietary groups significantly different from control (Krill).

[2] Means in the same column with a different superscript letter are significantly different.

$^{*}p < 0.05$; $^{**}p<0.01$; $^{***}p<0.001$

**Table 3.** Dietary effects on nitrogen budgets in juvenile European lobster.

| Protein ($g.kg^{-1}$) | L:CHO | $N_{intake}$ ($\mu g.mg\ WW^{-1}$) | $N_{excreted}$ ($\mu g.mg\ WW^{-1}$) | $N_{retention}$ (%) | N |
|---|---|---|---|---|---|
| 400 | Low | 806.44 ± 269.46 [a] | 65.80 ± 43.32 | 86.02 ± 9.54 [ab] | 5 |
| | Medium | 65.32 ± 23.35 [*,b] | 30.41 ± 9.63 | 41.58 ± 15.47 [b] | 6 |
| | High | 42.73 ± 8.50 [*,b] | 55.17 ± 25.91 | -22.08 ± 38.85 [*,c] | 4 |
| 500 | Low | 839.33 ± 153.47 [a] | 71.02 ± 13.42 | 90.85 ± 1.36 [a] | 6 |
| | Medium | 1166.83 ± 387.53 [a] | 72.78 ± 11.34 | 91.98 ± 2.17 [a] | 4 |
| | High | 873.94 ± 298.23 [a] | 127.34 ± 71.63 | 87.12 ± 4.49 [a] | 5 |
| Control (Krill) | | 657.18 ± 74.53 | 190.85 ± 87.83 | 73.30 ± 12.11 | 4 |
| [1] One-Way ANOVA | | $F_{6,33}=17.14^{***}$ | $F_{6,33}=1.00$ | $F_{6,33}=6.96^{***}$ | |
| [2] Two-Way ANOVA | | | | | |
| P | | $F_{1,29}=52.21^{***}$ | $F_{1,29}=2.71$ | $F_{1,29}=19.03^{***}$ | |
| L:CHO | | $F_{2,29}=9.49^{**}$ | $F_{2,29}=0.07$ | $F_{2,29}=6.68^{**}$ | |
| P x L:CHO | | $F_{2,29}=9.64^{**}$ | $F_{2,29}=0.38$ | $F_{2,29}=5.79^{**}$ | |

Values are mean ± standard error.

[1] Superscript * indicate dietary groups significantly different from control (krill).

[2] Means in the same column with different superscript letter are significantly different.

* $p < 0.05$; ** $p<0.01$; *** $p<0.001$

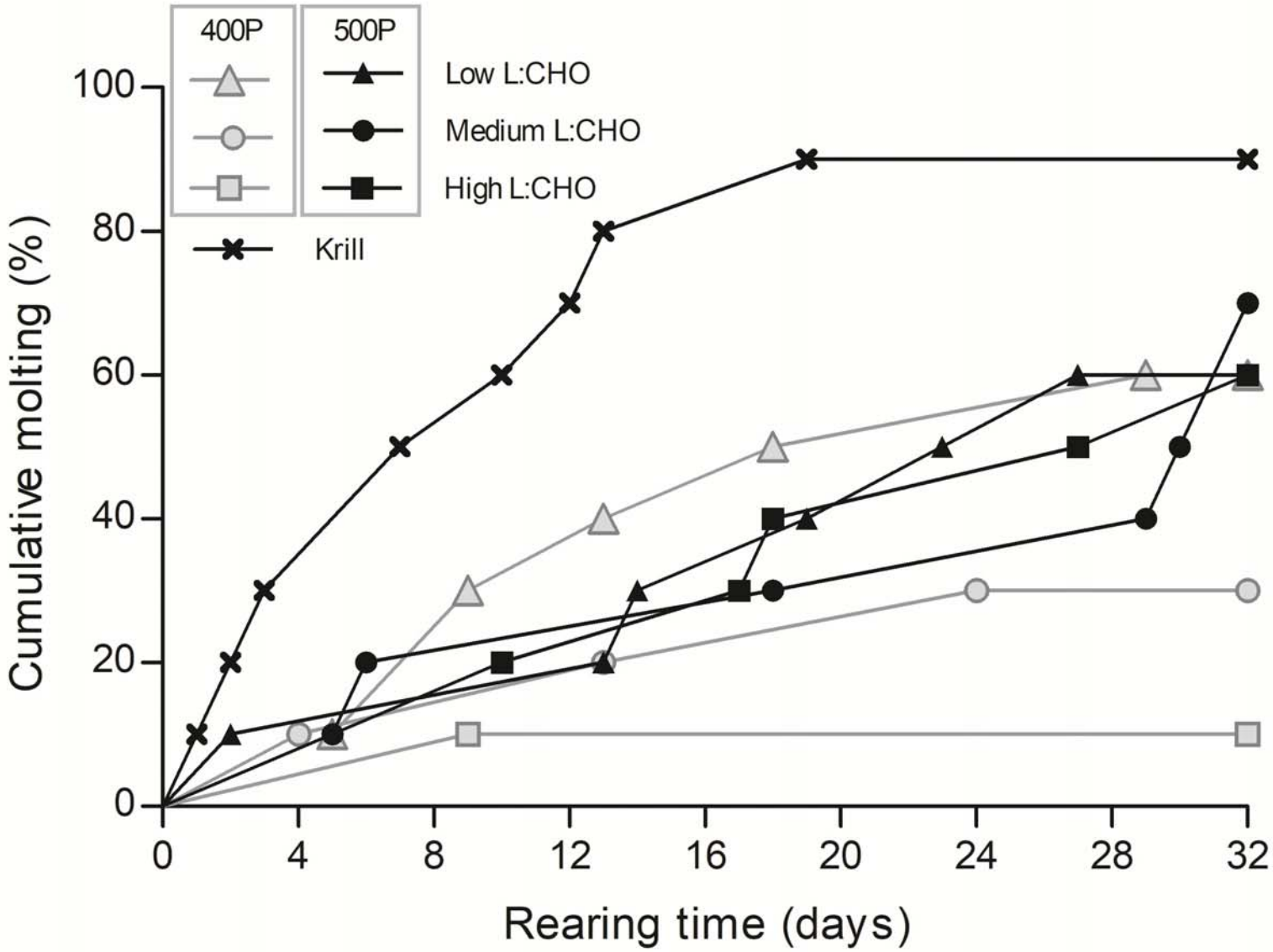
**Fig. 1.** Cumulative molting of European lobster juveniles (% of initial numbers) fed the different diets.

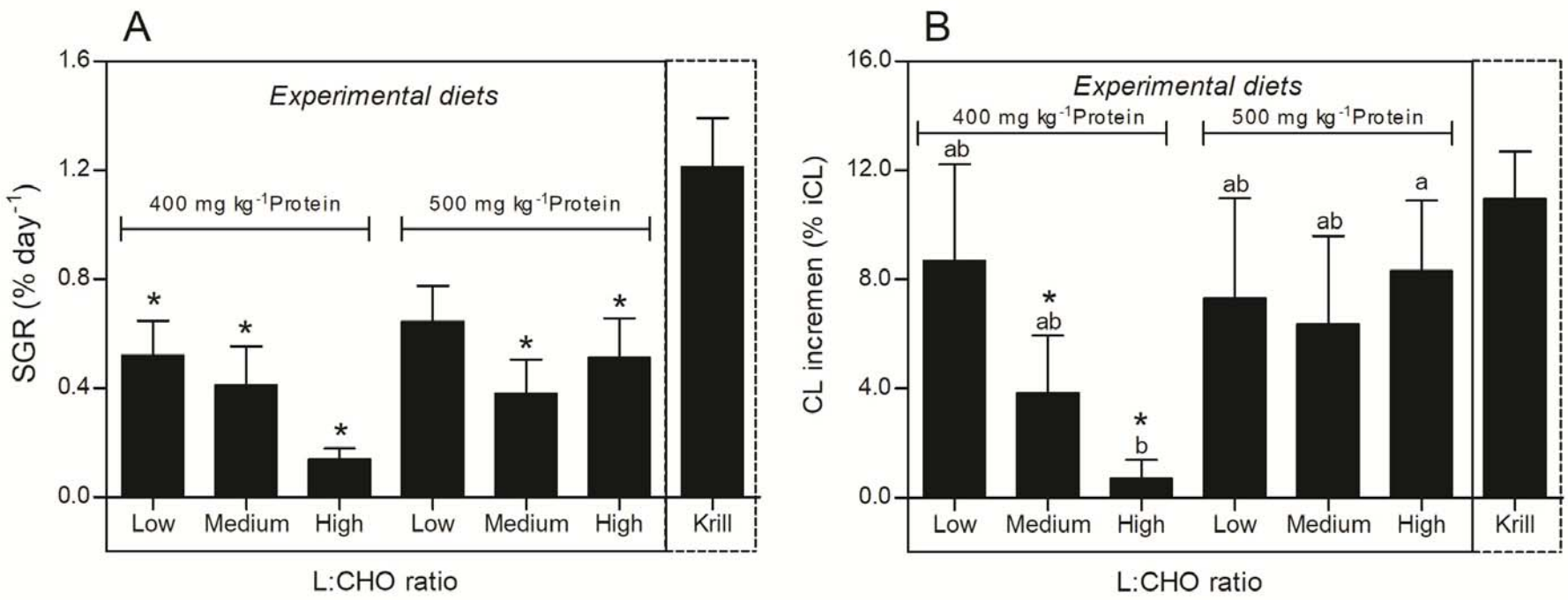
**Fig. 2.** Specific growth rate (A) and carapace length increment (B) after a 4-week period for European lobster juveniles fed on different diets. Data represents the mean ± SEM of 10 animals per treatment. Dietary treatments that were significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences between experimental formulated diets.

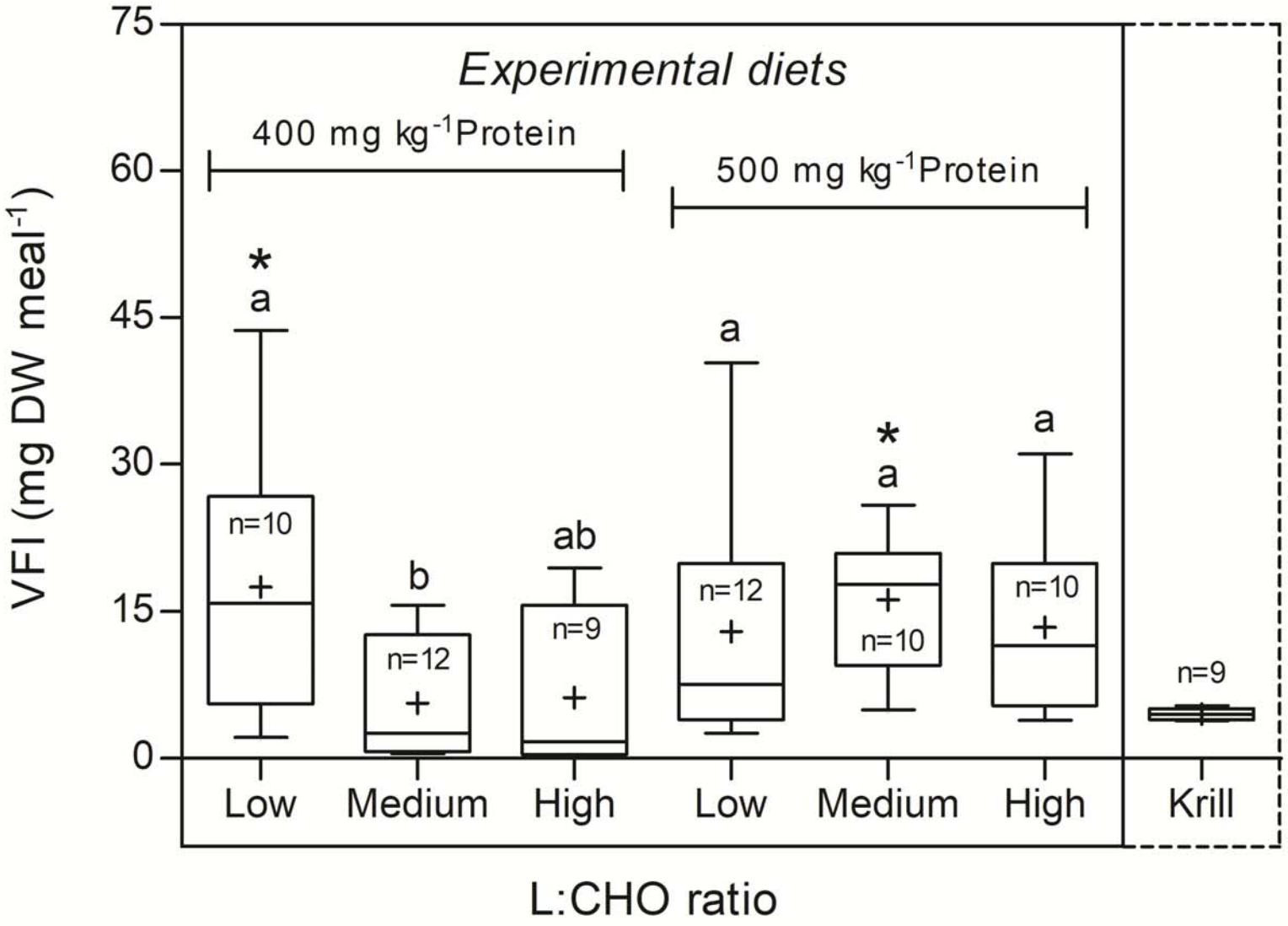
**Fig. 3.** The effect of dietary treatment on voluntary feed intake. Estimated individual feed intake values from both respirometry and nitrogen excretion trials were pooled. The box includes observations from the $25^{th}$ to the $75^{th}$ percentile and the whiskers above and below the box indicate the $10^{th}$ and $90^{th}$ percentiles. The horizontal line within the box represents the median value and the symbol (+) indicates the mean. Dietary treatments significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences among experimental formulated diets.

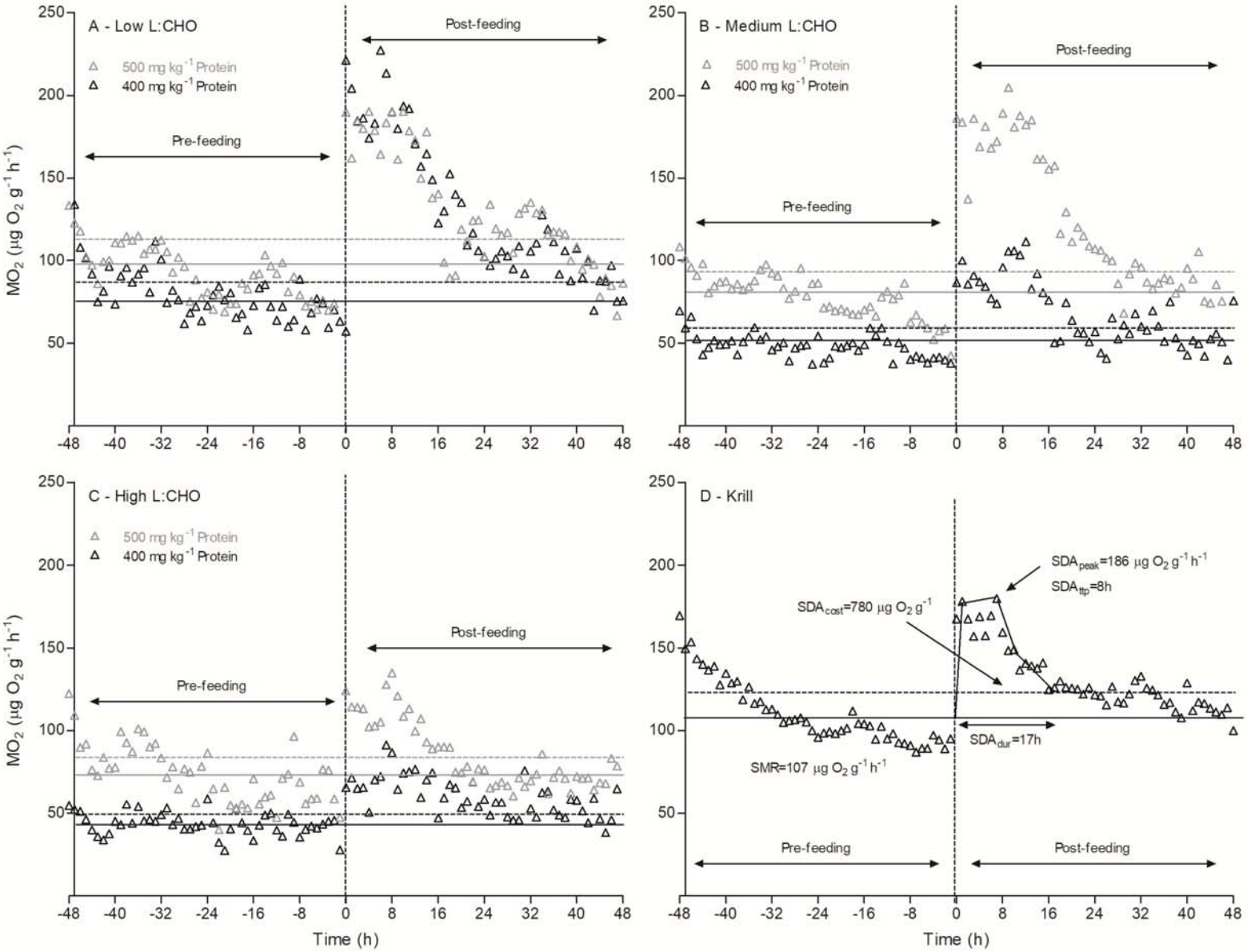
**Fig. 4.** Representative plots of pre and post-feeding metabolic rates (μg $O_2$ $g^{-1}$ $h^{-1}$) over time in lobsters fed experimental (A, B, and C) and control (D) diets. The solid line represents SMR and the dashed line represents SMR + 15%. The lobsters were fed at 0h (vertical dashed line). The SDA variables accounting for MO2 postprandial metabolism are visually explained in panel D.

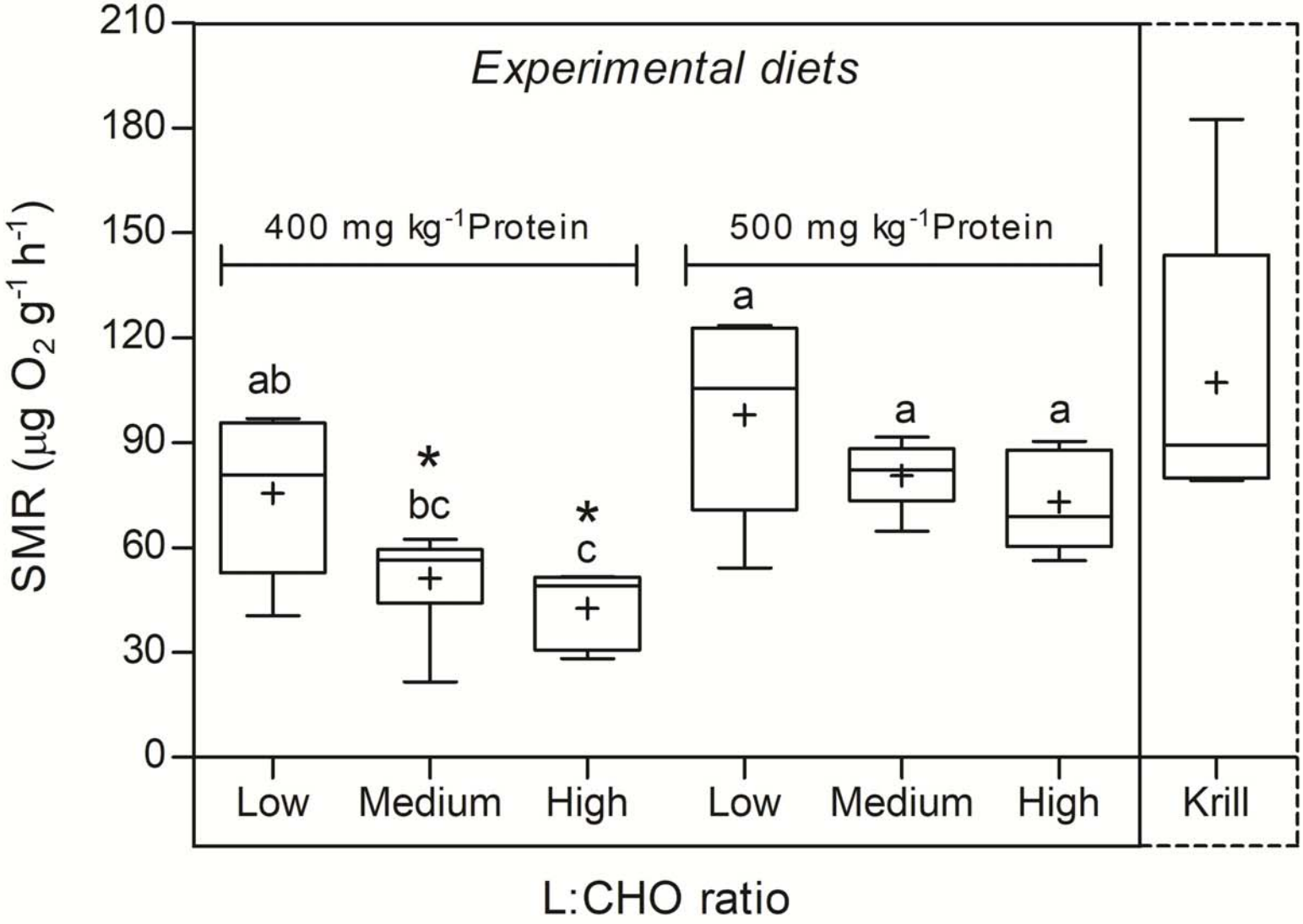
**Fig. 5.** The effect of dietary treatment on the standard metabolic rate of European lobster juveniles. The box includes observations from the $25^{th}$ to the $75^{th}$ percentile and the whiskers above and below the box indicate the $10^{th}$ and $90^{th}$ percentiles. The horizontal line within the box represents the median value and the symbol (+) indicates the mean. Dietary treatments significantly different from control (krill) are marked with an asterisk. Different letters indicate significant differences among experimental formulated diets.


400P
500P
Low L:CHO
Medium L:CHO
High L:CHO
Krill
Cumulative molting (%)
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
Rearing time (days)


A
Experimental diets
400 mg kg-1Protein
500 mg kg-1Protein
SGR (% day-1)
1.6
1.2
0.8
0.4
0.0
*
*
*
*
*
Low
Medium
High
Low
Medium
High
Krill
L:CHO ratio
B
Experimental diets
400 mg kg-1Protein
500 mg kg-1Protein
CL incremen (% iCL)
16.0
12.0
8.0
4.0
0.0
ab
*
ab
*
b
ab
ab
a
Low
Medium
High
Low
Medium
High
Krill
L:CHO ratio


Experimental diets
400 mg kg-1Protein
500 mg kg-1Protein
VFI (mg DW meal-1)
75
60
45
30
15
0
*
a
n=10
b
n=12
ab
n=9
a
n=12
*
a
n=10
a
n=10
n=9
Low
Medium
High
Low
Medium
High
Krill
L:CHO ratio


A - Low L:CHO
B - Medium L:CHO
C - High L:CHO
D - Krill
500 mg kg-1 Protein
400 mg kg-1 Protein
Pre-feeding
Post-feeding
MO2 (µg O2 g-1 h-1)
Time (h)
SDApeak=186 µg O2 g-1 h-1
SDAttp=8h
SDAcost=780 µg O2 g-1
SDAdur=17h
SMR=107 µg O2 g-1 h-1


Experimental diets
400 mg kg-1Protein
500 mg kg-1Protein
SMR (µg O2 g-1 h-1)
210
180
150
120
90
60
30
0
ab
bc
c
a
a
a
*
*
Low
Medium
High
Low
Medium
High
Krill
L:CHO ratio

**Declaration of interests**

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

| Credit / Author | Renata Goncalves | Ivar Lund | Manuel Gesto | Peter Vilhelm Skov |
|---|---|---|---|---|
| Conceptualization | x | x | x | x |
| Methodology | x | x | x | x |
| Validation | x | | | x |
| Formal analysis | x | | | x |
| Investigation | x | | | |
| Resources | | x | x | x |
| Data Curation | x | | | |
| Writing – Original draft | x | | | |
| Writing – Review and Editing | | x | x | x |
| Visualization | x | | | |
| Supervision | | x | x | x |
| Project administration | | x | x | |
| Funding acquisition | | x | | |