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Production and nutritional composition of white worms *Enchytraeus albidus* fed different low-cost feeds

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ABSTRACT

The marine oligochaete, white worm, *Enchytraeus albidus* (Henle, 1837), was evaluated as a potential live feed by exploring its production capacity and nutritional composition fed coffee grounds, spent brewing grains, stale bread, mixed produce, or sugar kelp over the course of 6-, 9-, or 12-week production cycles. Feed type and production cycle duration affected white worm biomass, reproductive potential, and proximate and fatty acid composition. In general, white worm cultures fed coffee grounds, stale bread, and spent brewing grains had higher production yields than cultures fed mixed produce or sugar kelp. Dependent on feeds and production cycle duration, white worms were high in protein (49–69%) and lipids (10–27%) and low in ash (5–8%), indicating that they would meet the dietary needs of species requiring a high protein, relatively high lipid, low ash diet. Compared to fatty acid profiles reported for standard live feeds like rotifers, *Artemia*, and copepods, white worms provided less n-3 long-chain polyunsaturated fatty acid content (DHA 0–0.5%, EPA 2–18%, total LC-PUFAs 4–25%), with the highest levels in worms fed mixed produce or sugar kelp. White worms exhibit many attractive characteristics as feeds, but commercialization will require improved culture techniques to produce greater worm biomass while reducing production costs. Depending on the target species, white worms may need enrichment to increase n-3 LC-PUFA levels.

1. Introduction

White worms (*Enchytraeus albidus,* Henle, 1837) show great promise as a feed (either live, frozen, or an ingredient in processed feeds) for a diverse set of cultured organisms during some period of their development, including freshwater and marine fishes (including ornamentals) as well as some crustaceans, amphibians, reptiles, and birds. White worms are robust, found worldwide (reviewed in Ghabbour, 1966), and grow in terrestrial systems to 2–4 cm in length (Ivleva, 1973). White worms are euryhaline and will survive in both fresh and full-strength seawater (Ivleva, 1973), wriggle and attract predators (Walsh, 2012), and do not noticeably impair water quality when added to aquaculture systems (Walsh, 2012), making them ideal live feeds for cultured aquatic species. In addition, they are retained longer in aquaculture systems since they are bottom-dwelling and are not easily flushed from rearing tanks like other traditional live feeds (e.g., *Artemia*; Walsh, 2012). White worms also are easy and inexpensive to rear on a small scale (Walsh, 2012); research organism suppliers grow the worms for biological and toxicological studies as worm tissue is sensitive to chemical composition (e.g., Amorim and Scott-Fordsmand, 2012), and aquarium hobbyists cultivate them as a live feed for ornamental fishes. Despite their many positive attributes and proven value as live feeds for ornamental fish, large-scale commercial culture of white worms has yet to develop and white worms have not received considerable attention among those involved in intensive aquaculture.

Scant information is available regarding optimal white worm rearing protocols, particularly for large-scale production. White worm cultivation was developed in the former Soviet Union in the 1940s in conjunction with expanding fish culture programs. A few English-language review papers describe culture rooms with wall-to-wall stacks of culture boxes and yields of 100–300 kg white worms per week (with a peak biomass 35,000 g worms/m^3) used to feed 2.5–3 million juvenile sturgeon (Ivleva, 1973; Vedrasco et al., 2002). Despite the apparent success of such operations, as far as is known, large-scale white worm production no longer exists - cessation appears to correspond with the breakup of the Soviet Union – but rudimentary white worm life history and production techniques are known. The life span of an individual white worm is 8–9 months, during which time it produces about 1000 viable eggs of which 93–95% develop successfully. The eggs are laid in cocoons that may contain as few as 2–3 eggs or as many as 20–35 eggs,

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depending on the age and condition of the worms (Ivleva, 1973). White worms grow best under controlled temperatures (~15–21 °C), in the dark, in small containers filled with soil as a culture medium. Russian culturists traditionally used wooden boxes, but plastic containers prevent the media from drying out too rapidly and are inexpensive, readily available, and easy-to-clean culture vessels (Walsh, 2012; Walsh et al., 2015). White worm survival is high throughout all growth and developmental stages (Ivleva, 1973).

White worms are notoriously unfastidious and indiscriminate when it comes to feeding protocols; white worms will survive on just about anything. Russian and Turkish researchers reported that growth and reproduction of white worms were greatest when milled grain products (i.e., oatmeal, wheat and rye flour, groats) were provided as compared to bran, potato, bread, fruit, or vegetable products (Ivleva, 1973; Memiş et al., 2004). Further study suggested that white worms exhibit a preference for brewery waste, with worms forming dense aggregations to feed on spent brewers' yeast (Ivleva, 1973). Dense feeding aggregations also formed when white worms were fed formulated aquaculture feeds, however, the authors advised against the use of feeds containing animal products (e.g., fish meal or other rendered animal proteins) as they tended to attract pests (i.e., flying insects, mites; Memiş et al., 2004). Pest concerns aside, protein- and lipid-rich aquaculture feeds contain costly ingredients, meaning they are likely too expensive to be cost-effective in white worm production. Worms should be harvested during the period of peak biomass growth (45–50 d from start of culture), however, peak biomass may vary with the type of feed provided, hence a range of production cycles warrants evaluation (Ivleva, 1973). Proximate analysis of worms fed baby rice cereal verified that the nutritional profile of current white worm stocks at the University of New Hampshire (UNH, Durham, New Hampshire, USA) is similar to that documented in the Russian literature (Ivleva, 1973; Walsh, 2012): 70–75% protein, 15% lipid, and 6% ash (dry matter basis). Fatty acid analysis of these same cultures revealed that white worms are a good source of n-3 long chain polyunsaturated fatty acids (LC-PUFAs; fatty acids with ≥ 20 carbons and ≥ 3 double bonds), though the docosahexaenoic acid (DHA, 22:6n-3) content may be limiting (Fig. 1; Walsh, 2012). Knowing that the nutritional composition of white worms is dependent on their diet and can be altered by adding supplements to their food (Ghabbour, 1966), the effects of different feed types and additional enrichment strategies should be investigated to develop white worms as a nutritionally customized feed for specific organisms.

Using low- or no-cost byproducts as feed inputs in aquaculture is attractive in terms of reducing feed costs and environmental footprints for aquaculture operations, as well as helping to address the challenges other industries face regarding byproduct disposal. The indiscriminate feeding behavior white worms exhibit is one of the main advantages of worm production. Many industries generate waste or byproducts that white worms will eat, however, we do not know which of these resource streams are optimal for worm growth, production, and nutritional profile. Our aim is to produce a live feed that can easily and cost-effectively be incorporated into the routine of aquaculturists. Thus, the following experiment was framed to examine intensive white worm culture in the context of "local recycling" and to assess culture productivity and worm composition as functions of different low- or no-cost feed resources and culture period durations.

2. Methods

2.1. Experimental parameters

A common garden experiment was designed to assess the productivity and nutrient composition of white worms fed various low- or no-cost feeds over the course of production cycles of different durations at UNH. Five feed treatments (spent coffee grounds [coffee], spent brewing grains [grain], stale bread [bread], mixed produce [produce], and sugar kelp [kelp]) were evaluated in white worm cultures held at ambient temperatures and harvested after production cycles lasting 6, 9, or 12 weeks. All feeds were wastes generated by local commercial entities and provided free of charge, except for sugar kelp which was a donated UNH product. Produce was the most variable of the feeds and mostly included leafy greens, but during some weeks also celery, carrots, broccoli, cabbage, green beans, and berries. The feeds represented an array of products known to promote worm growth and reproduction (Ivleva, 1973). The production cycles ranged from harvesting recommendations at 6–7 weeks to longer durations (9 and 12 weeks) since it was possible that some feeds could depress white worm growth and reproduction (Ivleva, 1973).

To start the experimental cultures, 45 plastic containers (5 feed treatments × 3 production cycle durations × 3 replicates per treatment combination) measuring 33 × 19 × 10 cm (6.4 L) were filled to a depth of 5 cm with sieved, seawater-dampened, organic, potting soil for culture media. Containers were stocked with white worms at an initial density of approximately 0.3 g worms/100 cm^3 soil (~210 worms/100 cm^3), based on recommended stocking densities for starter cultures (Ivleva, 1973). All containers were sealed with lids and kept in darkness by covering them with black plastic sheeting.

Feeds were sourced weekly and fed to worms shortly after acquisition. Except for coffee, all feeds were homogenized with a home-use grade food processor to form coarse particulates (bread and kelp), thick liquid (produce), or paste (grain) prior to feeding. Feed (initially 0.8 g feed/g worms/week) was applied to the bottom of containers and

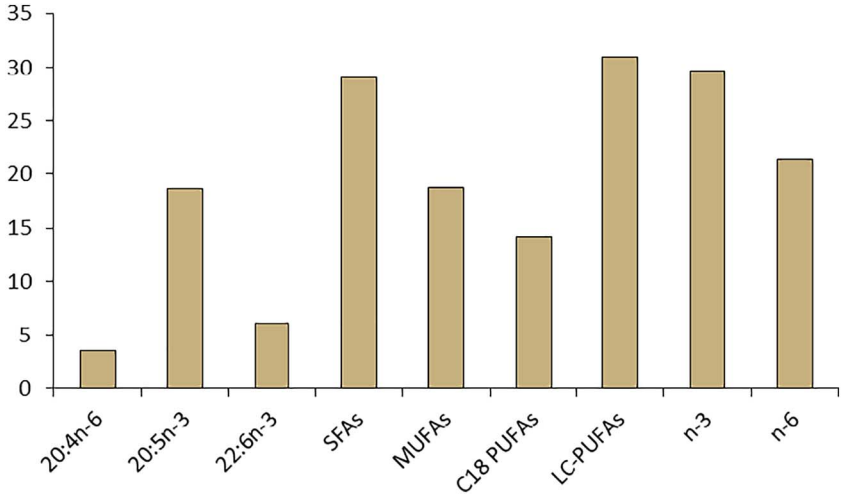

Fig. 1. Representative fatty acid profile of white worms, fed baby rice cereal, with respect to select individual fatty acids, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), 18-carbon polyunsaturated fatty acids (C18 PUFAs), long-chain polyunsaturated fatty acids (LC-PUFAs), n-3 fatty acids, and n-6 fatty acids. Columns represent least square-means of triplicate samples; standard deviations were ≤ 0.1% for all fatty acids and fatty acid groupings. Data reproduced from Walsh (2012).

covered with soil to minimize infestation by mites or small flying insects. Feed levels were monitored by viewing the underside of the clear container and replenished weekly. If feeds were consumed completely within one week, weekly amounts were increased to ensure food was not a limiting factor to worm production. Total processing time to prepare and administer these feeds was recorded for each container.

On a weekly basis, soil pH and temperature, and feed pH were measured. The target soil moisture level was 22–25%, but due to inaccuracies with the moisture meter, all containers were checked visually several times/week during the experiment. To maintain acceptable moisture levels, cultures were moistened with seawater or supplemented with additional dry soil, as needed.

At the end of each production cycle, the worm population and reproductive output were calculated from each replicate. We were not confident with our current harvesting techniques to guarantee a complete worm harvest from each container, thus production metrics were estimated from three subsamples per replicate culture container. Each subsample was composed of multiple cores, from various locations in the container, that collectively amounted to 40 cm^3 of material containing both soil and worms. Worms and cocoons were enumerated for each subsample and mean subsample densities were extrapolated to the entire culture volume to estimate worm density and reproductive potential, respectively, for each culture. No attempts were made to assess the number of eggs per cocoon.

2.2. Compositional analysis

To evaluate the effects of feed type and production cycle duration on worm composition, subsamples of worms from each replicate culture container were collected for proximate composition and fatty acid analysis. To gather sufficient sample volumes for analysis, worms were harvested by placing each container on a heating pad until the worms began to congregate on the top of the soil away from the heat source. Worm aggregations were collected, transferred to seawater to remove adhering soil, drained, placed in labeled, plastic 2-dram vials, and held on dry ice until samples could be transferred to −80 °C storage. Triplicate samples of feeds were collected weekly and stored similarly. After all worm and feed samples had been collected, they were packaged with dry ice and shipped overnight to Southern Illinois University Carbondale (SIUC, Carbondale, Illinois) for compositional analysis.

Feeds and white worms were analyzed to determine proximate and fatty acid composition. Samples were lyophilized (Freezone 6, Labconco Corporation, Kansas City, Missouri) and then pulverized. Protein (LECO FP-528, LECO Corporation, St Joseph, Michigan), ash (muffle furnace, 650 °C for 4 h, gravimetric determination), and lipid content (chloroform/methanol extraction [Folch et al., 1957], gravimetric determination) were determined for each pulverized sample. Proximate analyses were conducted in duplicate when sample mass was adequate. Lipid extracts were analyzed for fatty acid composition per standard SIUC protocols. Crude lipid samples were subjected to acid-catalyzed transmethylation performed overnight at 50 °C (Christie, 1982). The resultant fatty acid methyl esters (FAMEs) were separated using a gas chromatograph equipped with a flame-ionization detector fitted with a permanently bonded polyethylene glycol, fused silica capillary column (Omegawax 250, 30 m × 0.25 mm I.D., 0.25 μm film; Supelco, Bellefonte, Pennsylvania). The injection volume was 1.0 μL, helium was the carrier gas (30 cm/s, 205 °C), and the injector temperature was 250 °C. A split injection technique (100:1) was used, and the temperature program was as follows: 50 °C held for 2 min, increased to 220 °C at 4 °C/min, and held at 220 °C for 15 min. Individual FAMEs were identified by reference to external standards (Supelco 37 Component FAME Mix, PUFA-1, PUFA-3, 20:4n-6, and 22:6n-3, Supelco, Bellefonte, Pennsylvania).

2.3. Statistical analysis

White worm production data (worm and cocoon densities), and proximate and fatty acid composition data were subjected to two-way ANOVA (PROC GLIMMIX) to determine the significance of feed treatment and production cycle duration as main effects, as well as their interaction (SAS Version 9.4, SAS Institute, Cary, North Carolina). When omnibus ANOVA tests indicated significant main effects of feed type, mean worm production values were compared within production cycles using post-hoc procedures for partitioned analysis of means (also known as analysis of simple effects; SLICE statement); mean proximate and fatty acid composition values were compared across production cycles using Tukey's HSD pairwise comparison tests.

Weekly worm culture temperatures and pH datasets were unbalanced, as these data were not recorded for the 6-week and 9-week production cycle groups after the requisite period had elapsed and the cultures were harvested. To avoid missing data-related issues associated with comparing these datasets to each other or to the complete 12-week production cycle dataset, temperature and pH data were analyzed independently by production cycle using repeated measures one-way ANOVA (PROC GLIMMIX). When omnibus ANOVA tests indicated significant main effects of feed type, mean temperature and pH values were compared across sampling times using Tukey's HSD pairwise comparison tests.

Although many of the response variables reflected data collected or calculated from many pooled worms per sample or subsamples collected from an individual culture container, replicate culture containers were considered experimental units ($N = 3$) for all statistical analysis. All effects/differences were considered significant at $P < 0.05$.

3. Results

3.1. Worm production

The combination of feed treatment and production cycle significantly affected worm production, in terms of worm density and cocoon density (2-way ANOVA; df = 8; $P < 0.001$; Supplemental Table 1). Both worm and cocoon densities increased over time in all feed treatments, except for kelp which decreased from weeks 9 to 12 (Fig. 2, Supplemental Table 1). Overall, bread yielded the greatest worm production, but other feedstuffs were comparable, depending on production cycle duration. After 6 weeks, worm and cocoon densities were comparable among the various cultures, except for kelp-fed worms which had numerically lower worm density (134 vs. 221–327 worms/100 cm^3). By week 9, cultures fed coffee, bread, and grain had significantly greater worm densities than the other cultures (380–705 vs. 202–277 worms/100 cm^3), whereas cultures fed bread had significantly higher cocoon densities (40 vs. 25–29 cocoons/100 cm^3). By week 12, cultures fed bread yielded significantly more worms than the other cultures (1321 vs. 153–711 worms/100 cm^3), and both bread- and grain-fed cultures had higher cocoon densities than the others (53–58 vs. 17–42 cocoons/100 cm^3).

Worm culture temperatures ranged from 17.7 to 18.5 °C during the 12-week study (Nov.–Jan.) and generally decreased over time as expected (Fig. 3, Supplemental Table 2). Feed treatment had no effect on worm culture temperature, but the pH of the worm cultures was affected by both feed treatment and time (Fig. 3, Supplemental Table 2). Generally, pH decreased over time, regardless of feed treatment with the 12-week production cycle cultures becoming most acidic. Since feedstuffs were sourced opportunistically from industry partners, they varied from week to week (e.g., bread type, beer brew, produce mixture, coffee variety), resulting in variation in pH (Fig. 4, Supplemental Table 2) and nutritional composition (Fig. 5, Supplemental Table 3).

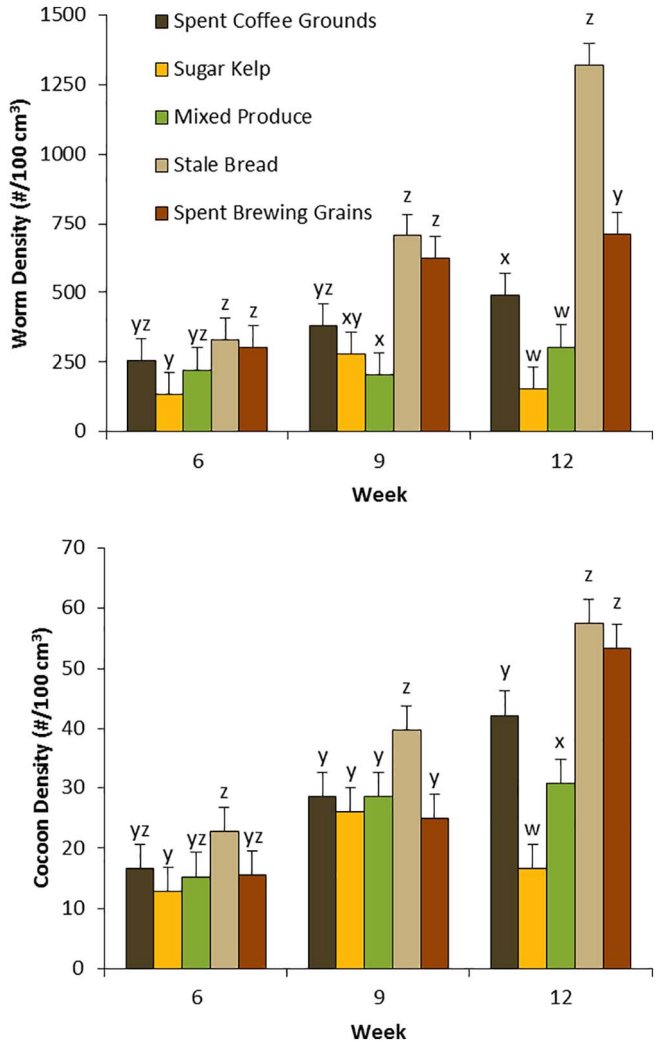

Fig. 2. Worm (upper figure) and cocoon densities (lower figure) of white worm cultures by feed and production cycle duration (weeks) treatments. Columns represent least-square means ± pooled SEs. Columns within a production cycle duration treatment with common letter labels are not significantly different ($P < 0.05$). Results of omnibus tests applied to proximate composition data are provided in Supplemental Table 1.

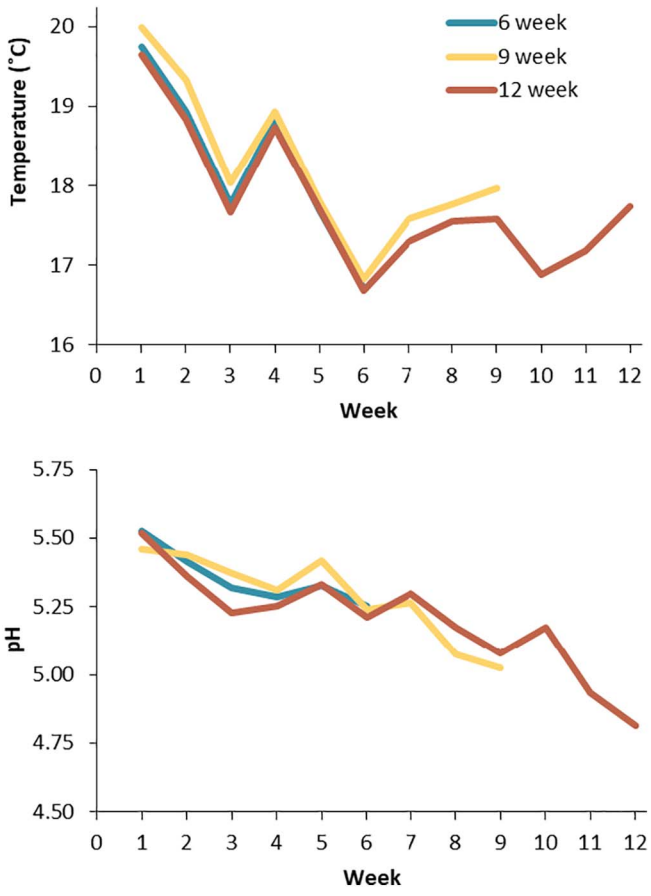

Fig. 3. Temperature (upper figure) and pH (lower figure) of white worm cultures throughout the experimental period according to production cycle duration (weeks) treatment. Experiments were conducted simultaneously and data were recorded weekly, but only for as long as the worms were being cultivated according to the production cycle duration treatment. Therefore, datasets for the 6-week and 9-week cycles are truncated to the first 6 or 9 weeks of the experimental period. Results of omnibus tests applied to worm culture temperature and pH data are provided in Supplemental Table 2.

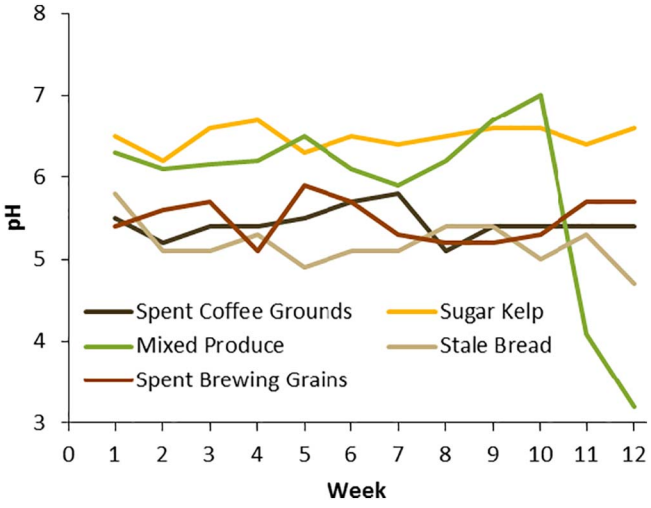

Fig. 4. pH of feedstuffs over time.

3.2. Worm composition

Proximate composition of white worms was significantly affected by feed treatment and production cycle duration (Fig. 6, Supplemental Table 1). Production cycle duration significantly affected carbohydrate, protein, and ash content, but these effects were not associated with clear temporal trends. With respect to feed treatment effects, coffee-fed worms had significantly lower protein (49–54%) and ash (5%) levels and significantly higher lipid content (24–27%) compared to worms fed the other feedstuff types. Although significant treatment differences were noted in some cases, kelp-, produce-, bread-, and grain-fed worms had more comparable compositional profiles that reflected higher protein (57–69%) and ash (6–8%) and lower lipid (10–16%) levels.

Worm fatty acid composition was significantly affected by feed treatment and production cycle duration; however, as with proximate composition, fatty acid profile did not vary predictably with length of the production cycle (Fig. 7, Supplemental Table 3). In many cases, particular fatty acids were notably higher or lower in one treatment, with statistically significant differences existing between some, but not all of the other intermediate groups. Saturated fatty acid (SFAs; fatty acids with no double bonds) were highest among bread-fed worms (24% FAMEs) and lowest among produce-fed worms (18.4% FAMEs), in comparison with most of the other feed treatment groups (20.0–21.7% FAMEs). Monounsaturated fatty acids (MUFAs; fatty acids with one double bond) were lowest among grain-fed worms (10.1% FAMEs), and highest among bread-fed worms (30.7% FAMEs) in comparison with most of the other feed treatment groups (12.5–14.2% FAMEs). Long-

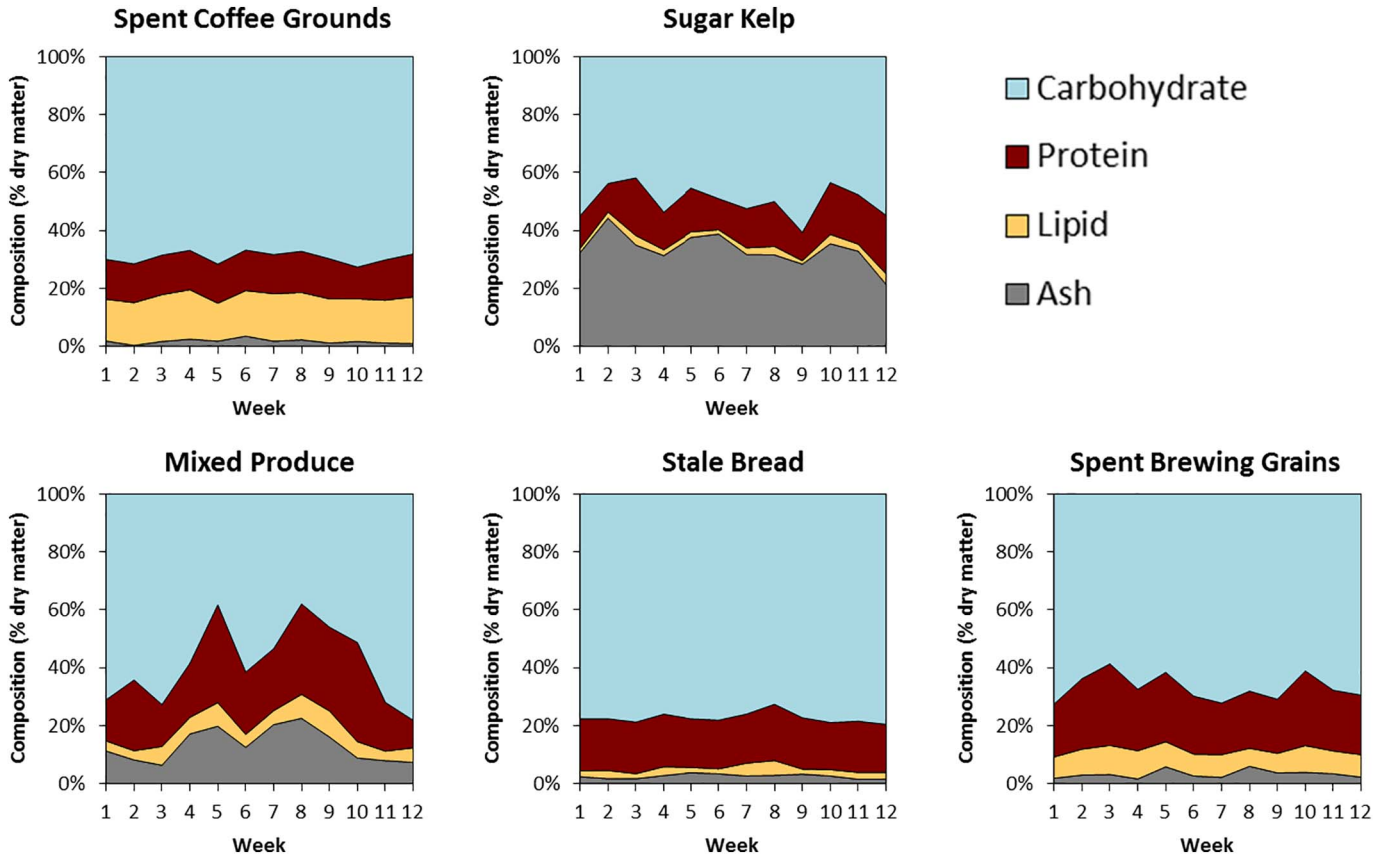

Fig. 5. Proximate composition (dry matter basis) of feedstuffs over time. Note that carbohydrate content was calculated by difference, not analytically verified.

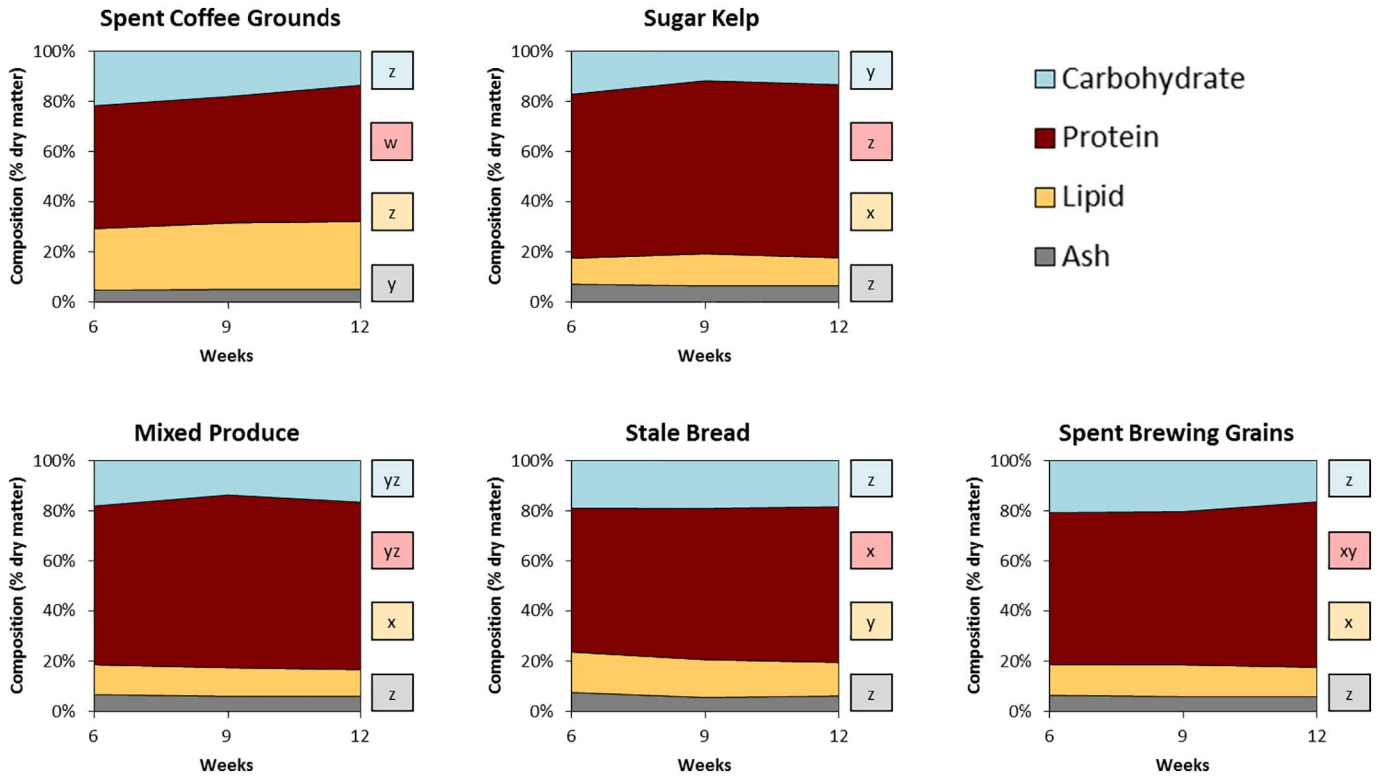

Fig. 6. Proximate composition (dry matter basis) of white worms by feed treatment and production cycle duration (weeks). Note that carbohydrate content was calculated by difference, not analytically verified. Letter labels indicate differences in carbohydrate, protein, lipid, or ash content based on significant main effects of feed treatment across all production cycle durations; feed treatments with common labels for the same compositional parameter are not significantly different. The absence of letter labels indicates the lack of a significant main effect of feed treatment. Results of omnibus tests applied to proximate composition data are provided in Supplemental Table 1.

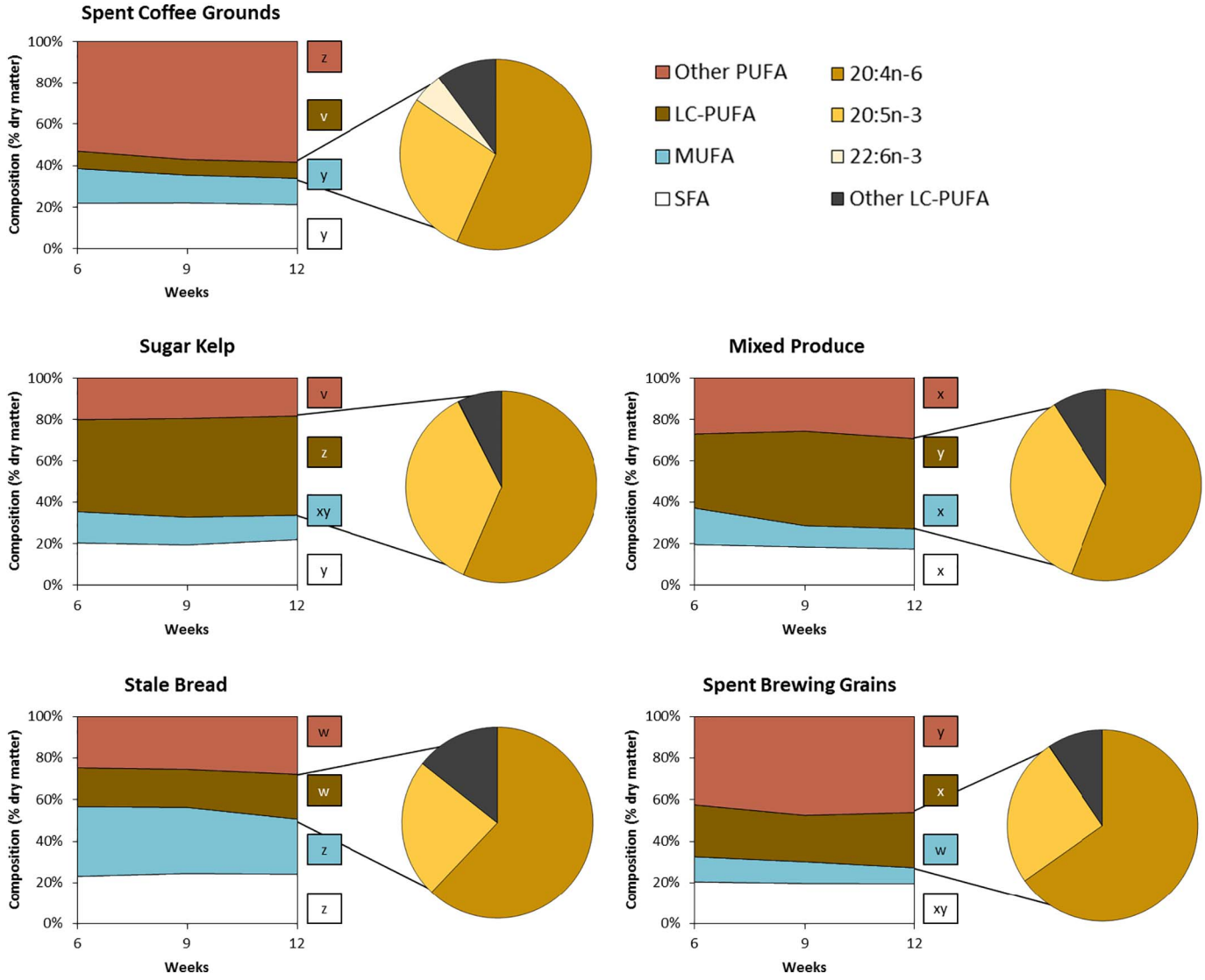

Fig. 7. Fatty acid composition of white worms over time by feed treatment and production cycle duration (weeks). Stacked area plots illustrate composition with respect to saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), long-chain polyunsaturated fatty acids (LC-PUFAs), or other polyunsaturated fatty acids (PUFAs); pie charts provide additional detail regarding the relative contributions of arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) to the LC-PUFA fraction. Letter labels indicate differences in levels of SFAs, MUFAs, LC-PUFAs, or other PUFAs based on significant main effects of feed treatment across all production cycle durations; treatments with common labels for the same grouping are not significantly different. For complete fatty acid profiles, including information regarding treatment differences for individual fatty acids, see Supplemental Table 3.

chain polyunsaturated fatty acids varied significantly among each of the feed treatment groups, ranging from 7.9 to 46.8% FAMEs as follows: coffee < bread < grain < produce < kelp. With respect to specific LC-PUFAs of interest, DHA (22:6n-3) was observed in trace amounts in kelp- and coffee-fed worms (0.1–0.4% FAMEs), but not in the other cultures. Eicosapentaenoic acid (EPA, 20:5n-3, range: 2.2–16.8% FAMEs) and arachidonic acid (ARA, 20:4n-6, range: 4.5–26.5% FAMEs) levels varied significantly among all of the treatment groups according to the pattern described for LC-PUFAs in general. A similar, but not identical, pattern was observed for total n-3 fatty acids (range: 4.5–21.9% FAMEs), whereas total n-6 fatty acids were highest among coffee- (52.5% FAMEs) and grain-fed worms (44.7% FAMEs) in comparison with the other feed treatment groups (28.5–30.8% FAMEs).

4. Discussion

The potential of white worms as a live feed, as well as the constraints that might limit their value or applicability for intensive aquaculture operations, is underscored by the production and compositional analyses in this study. In order for white worms to be a beneficial live feed, several criteria must be met.

4.1. First, white worm cultures must be productive and consistent.

In this study, white worm productivity (e.g., worm and cocoon densities, yield) was not as high as expected, and this may be related to how the cultures were established. Worm production from the start of the experiment (approx. 210 worms/100 cm^3) to week 6 was more gradual than expected (Fig. 2), and overall production was much lower compared to historic Russian worm cultures. Peak Russian worm culture density reached as high as 3.5 g worms/100 cm^3 (Ivleva, 1973; Vedrasco et al., 2002), which for UNH worm production weights, is the equivalent of roughly 2450 worms/100 cm^3. The maximum worm density achieved in this experiment was only 50% of the Russian capacity (1.9 g or 1321 worms/100 cm^3; bread-fed worms at 12 weeks; Supplemental Table 1). It is possible that the containers and media used in the Russian experiments (i.e., wooden boxes and humus- and carbonate-rich chernozem soil) are partly responsible for the increased

Table 1
Average fatty acid contents (mg/g, dry matter basis) of white worms from the present study and various live feeds as reported by Evjemo and Olsen (1997). Note the values from Evjemo and Olsen (1997) were adapted from a graphical presentation of results (i.e., a column graph) and are therefore estimates, not precise figures.

Source/treatment or attributes	20:5n-3	22:6n-3	n-3	n-6	SFA	MUFA	PUFA
White worms/spent coffee grounds	5	1	11	126	52	34	154
White worms/sugar kelp	19	0	23	34	23	15	73
White worms/mixed produce	15	0	23	31	19	13	72
White worms/stale bread	6	0	11	39	33	42	62
White worms/spent brewing grains	7	0	11	51	23	11	79
Artemia franciscana/newly hatched	4	0	49	9	31	49	206
A. franciscana/enriched	36	22	80	9	22	36	246
A. franciscana/enriched	18	40	80	9	22	49	213
Brachionus plicatilis/slow growth rate, enriched	13	18	62	4	9	27	119
B. plicatilis/fast growth rate, enriched	4	4	31	4	9	22	66
Calanus finmarchicus/early life stages	9	18	31	< 4	22	9	110
C. finmarchicus/early life stages	9	18	36	< 4	18	4	89
C. finmarchicus/late life stages	22	18	53	< 4	36	18	169
Temora longicornis/source #1	9	18	31	< 4	13	< 4	76
T. longicornis/source #2	9	18	31	< 4	13	< 4	76
Eurytemora sp./source #1	13	22	36	< 4	13	4	93
Eurytemora sp./source #2	9	18	27	< 4	9	4	76

productivity observed in these trials. However, we used potting soil and plastic culture containers because these outperformed coconut fiber and wooden boxes in preliminary white worm cultivation experiments conducted at UNH (Walsh, 2012; Walsh et al., 2015) and were inexpensive, readily-available supplies. Perhaps it is more likely that the reduced productivity observed in our study was related to the use of new, sterile media. According to Ivleva (1973), new worm cultures do best when used or 'conditioned' media and new media are mixed together. However, we did not reuse conditioned soil in the present work because it would have contained unaccounted worm cocoons, eggs, and small juveniles and we wanted all cultures to be started with a known quantity of worms. The slow growth we observed likely represented an acclimation period as the worms adjusted to the new soil in the containers. In hindsight, using irradiated or otherwise sterilized conditioned soil might have provided better culture conditions and dampened the effects of the acclimation period on overall white worm production. Considering the new soil impeded faster worm production, we compared worm density of some older, more established worm cultures at UNH as a more realistic proxy: mean worm density of three older (15- to 29-month-old) cultures maintained on a diet of baby rice cereal was 2.4 ± 0.5 g or 1702 ± 327 worms/100 cm^3. Although this level of productivity is greater than what we observed in the present study, it still falls short of that reported for Russian worm cultures. Despite the interest in using low- or no-cost industry byproducts, for these types of feeds to be of value in large-scale white worm production, they should yield equal if not greater worm biomass than using powdered rice cereal. Future production studies should exercise caution in how white worm cultures are started and consider reuse of conditioned media.

4.2. Second, white worms must have a compositional profile that satisfies the macronutrient demands of cultured organisms.

Although needed nutrient levels will vary depending on the target species, generally the younger the fish, the higher the protein demands (Cahu and Infante, 2001). For example, the protein requirement level is 53% for goldfish *Carassius auratus* larvae, but only 29% for adults (Sales and Janssens, 2003). Recommended protein levels for other freshwater ornamental fishes range from 30 to 50%, depending not only on life history stage but also species (Lubzens et al., 1989; Sales and Janssens, 2003). For marine fishes, optimal diets are typically formulated at protein levels of 40–70% for juveniles (Cahu and Infante, 2001) and at lipid levels of 13–16% (Lubzens et al., 1989). Whereas live feeds often are nutritionally tailored (i.e., enriched) to meet specific dietary requirements, the feed organisms must contain enough protein so that they can sustain growth in the cultured fish. At 49–69% protein content (Fig. 6, Supplemental Table 1), white worms appear likely to satisfy that protein demand of cultured fishes, even during early life history. In addition to protein, live feeds must provide energy, usually as lipid, to fuel bioenergetic process and support growth. Compared to other live feeds, the total lipid content of white worms (10–27%; Supplemental Table 1) is comparable to copepods (9–24%, depending on stage), enriched *Artemia* nauplii (21–23%), and enriched rotifers (7–11%; Evjemo and Olsen, 1997). Results of the present work indicate that white worms would meet the needs of species that demand high protein, high lipid, low ash food items, regardless of feed treatment or production cycle duration.

4.3. Third, the fatty acid composition of white worms must meet the needs of the target species.

Essential fatty acids in live feeds probably is the most important factor to rearing cultured fish. Many fish cannot synthesize LC-PUFAs and even for those that can produce LC-PUFAs de novo, biosynthetic capacity is limited during early life stages. As a result, direct dietary inclusion of these physiologically critical compounds is vital to ensuring maximum larval survival (Watanabe et al., 1983). Since fatty acid profiles can be modified in live prey, live feeds like rotifers, *Artemia*, and copepods usually are enriched. Compared to these other live feeds, regardless of their larval stage or whether they were enriched, the fatty acid profile of white worms typically contains lower percentages of LC-PUFAs, especially EPA and DHA (Fig. 7, Supplemental Table 3). However, it is more important to consider the fatty acid *content* of the live feeds, rather than their fatty acid *profile*. Factoring in the total lipid content of the live feed organism and the fatty acid composition of that lipid (assuming 0.93 g FAME/g lipid; Weihrauch et al., 1977), one can calculate the absolute amounts of fatty acids present in a sample, which is much more informative than relative percentages in determining the nutritional value of live feed organisms (Table 1). Expressed in this context, our results indicate that white worms provide comparable amounts of EPA (5–19 mg/g, dry matter basis) as *Artemia franciscana*, *Brachionus plicatilis* (rotifer), *Calanus finmarchicus, Temora longicornis,* and *Eurytemora* sp. (copepods) (4–36 mg/g), but substantially less DHA (0–1 mg/g for white worms vs. 4–40 mg/g for other organisms) (Table 1). Whereas composition is broadly consistent between white worms and the aforementioned live feed organisms for SFA (19–52 mg/g for white worms vs. 9–36 mg/g for other organisms), MUFA (11–42 mg/g for white worms vs. < 4–49 mg/g for other organisms), and PUFA (62–154 mg/g for white worms vs. 66–246 mg/g for other organisms) contents, white worms tended to provide less n-3 fatty acid

content (11–23 mg/g for white worms vs. 31–80 mg/g for other organisms) and more n-6 fatty acid content (31–126 mg/g for white worms vs. < 4–9 mg/g for other organisms)(Table 1). Depending on the target species, the composition of live white worms may need to be enhanced to increase n-3 fatty acid content in general and DHA content in particular. This may be possible via established enrichment techniques used for other live feeds (i.e., by feeding n-3 LC-PUFA-rich oil emulsions or other n-3 LC-PUFA-rich feedstuffs). Future research is recommended to determine how white worms can take up additional essential fatty acids, particularly n-3 LC-PUFAs, what kind of enrichments to use, and the duration of enrichment needed to affect the fatty acid profile of white worms.

4.4. Fourth, white worms must be an appropriate consumable size for the target species.

The most nutritionally optimal live diet for a specific cultured fish may exist, but if the fish is incapable of eating it, then the nutritional aspects are irrelevant. To be a suitable feed, live prey dimensions should be comparable to or smaller than the gape of the fish's mouth. Compared to other commonly used live feeds (*Brachionus rotundiformis* S-type rotifers: 0.10 to 0.21 mm, Fukusho, 1989; *Brachionus plicatilis* L-type rotifers: 0.13 to 0.34 mm, Fukusho, 1989; *Artemia* Instar I nauplii: 0.42–0.52 mm, Bengtson et al., 1991, Merchie, 1996; *Artemia* Instar II-V metanauplii: 0.5–0.8 mm, Bengtson et al., 1991; Calanoid copepods: on average 0.22 mm, 0.49 mm, and 0.79 mm for nauplii, copepodites, and adults, respectively, Delbare et al., 1996), white worms are longer (1.0–1.5 mm at hatch to 2.5–4.5 cm as adults, Ivleva, 1973). However, Walsh (2012) noted that white worms can be cut into smaller pieces and the individual segments continue to wiggle in water. We verified this by cutting adult worms into progressively smaller pieces and observing their movements after 15 min in freshwater. Movement was seen consistently in cut sections as small as 1.5 mm. Depending on the fish species, white worms may not be a size-appropriate early larval diet, but more useful as a transitional or conditioning diet during other ontogenetic stages. Live white worms should be tested with a variety of cultured organisms to identify which species and stages would benefit most.

4.5. Lastly, to be an effective feed, white worm production must be cost-effective.

For this study, all feeds tested were readily available, local, and resulted in promising white worm production and nutritional outcomes. Minimal processing time (< 15 min/feeding) was required to create a worm-ready feed at this small, experimental scale; coffee was the least labor-intensive to prepare, followed by grain and bread, then produce, and lastly kelp. Though likely not a hindrance to white worm production, mold grew in the bread-fed cultures, and occasionally required some additional time to remove it. It is not clear whether fungal contamination of white worms would affect target organisms, but given what is known regarding the negative effects of aflatoxins associated with mold in industrially compounded aquafeeds, it seems prudent to avoid feeds that encourage fungal growth in white worm cultures. For small-scale experiments, there was no cost for any of the feeds tested. However, sugar kelp is a high value ($15/kg wet weight; UNH price as of 8/2016), seasonal product, currently available in northern New England, USA only in spring-early summer. Though attractive as a potential source of LC-PUFAs, due to the long processing time of kelp (relative to the other feeds), low productivity of kelp-fed worms, and its high cost (if not available as a donated resource), sugar kelp would not be a cost-effective white worm feed for large-scale white worm production.

5. Conclusion

White worm production, both in terms of white worm biomass and reproductive potential, generally is higher in cultures fed coffee grounds, stale bread, and spent brewing grains than in those cultures fed mixed produce or sugar kelp. White worm proximate and fatty acid composition results suggest two distinct strategies: one for producing worms as a source of 'bulk nutrition' and energy (e.g., protein and lipid) and another for using worms as vehicles for specific nutrients, such as LC-PUFAs. If worms are to be used as a source of bulk nutrition, feeding coffee, grains, or bread appears to be the best approach, as this yields worms with low ash and mid-to-high protein and lipid content at minimal cost. However, the lipid composition of coffee- and bread-fed worms did not favor n-3 LC-PUFA accumulation specifically, and additionally, the bread-fed worm cultures experienced mold outbreaks which could compromise the biosecurity of using white worms as a live feed. If n-3 LC-PUFAs are desired, feeding kelp or produce appears to be a better strategy, though this strategy will yield white worms with lower lipid content and will increase the cost of worm production due to the high value of kelp and the reduced white worm productivity associated with these feeds. That said, none of the feed treatments we tested resulted in more than trace levels of DHA, so an alternative strategy will be needed to enhance levels of this particular n-3 LC-PUFA, if essential to the target organism. As with any live feed, white worm commercialization and use in intensive aquaculture will depend on standardizing culture techniques to consistently and cost-effectively produce high quality feed organisms in relevant volumes.

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