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The Nutrient Composition of Three Mosquito (Diptera: Culicidae) Species, *Aedes caspius*, *Anopheles hyrcanus*, and *Culex pipiens*, Harvested from Rice Fields for Their Potential Utilization as Poultry Feed Ingredients

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Abstract: Increasing pressure on the world's livestock production sector has stirred interest towards the exploration of insects as an alternative feed source. We examined the potential of wild-caught mosquitoes, harvested from rice-fields, to be utilised as poultry feed. Three mosquito species were identified in high abundance, namely *Aedes caspius*, *Anopheles hyrcanus*, and *Culex pipiens*, and their nutritional and microbiological profile was assessed at the species-level and as mixed samples collected from two different seasons (summer 2020 and 2021). Their nutritional potential was evaluated based on protein, fat, moisture and ash content, fatty acid and mineral profile, and antioxidant activity. The microbiological profile of each mosquito species was assessed by measuring the population of total viable count, *Enterobacteriaceae*, lactic acid bacteria, and coagulase-positive *Staphylococci*. Obtained values were compared to common edible insects and conventional livestock feedstuff. All mosquito samples presented an overall promising nutritional composition, stable between the two harvest seasons. Mosquitoes' protein and fat content ranged from 54 to 62% and 16 to 28%, respectively. The examined species presented statistically significant differences in certain cases; *Anopheles hyrcanus* had the highest protein content (61.8% dry weight basis; dwb) and the highest antioxidant potential (45.9% ability to scavenge the DPPH radical). All mosquito samples were rich in minerals, containing high concentrations of calcium, phosphorus, and magnesium, minerals essential for poultry development. The GC/MS fatty acid profile revealed a high unsaturated character (65.2–71.5%), a predominance of palmitic (23.8–30.4%), palmitoleic (28.5–37.0%), and oleic (18.3–29.1%) acids, as well as the presence of essential linolenic (1.4–5.2%) and eicosapentanoic (1.5–2.4%) acids. The presence of microorganisms was confirmed across all species, at levels comparable to fresh food harvested from soil and farmed edible insects. Mosquitoes harvested from rice-fields exhibited an overall highly nutritious, stable profile, comparable and even superior to common feedstuff and edible insects, showing potential to be utilised as poultry feed components.

Keywords: insects; alternative protein source; minerals; feed safety; animal feed

1. Introduction

The ever-growing global population, coupled with the increasing demand for livestock production, has amplified the need for livestock feed, inadvertently threatening food security and environmental welfare. Conventional animal feed contains substantial amounts of fishmeal and crops such as soybean, wheat, maize, and barley, directly competing with human consumption. Each year, over a third of the world's total cereal production [1], and approximately 85% of the world's soybeans are used for animal feed [2,3]. Additionally, the supply of staple grains is becoming volatile due to military conflict among Russia and

Ukraine, two of the top three exporters of wheat and maize [4]. Feed production also leads to harmful greenhouse gas emissions, further land occupation, and extensive use of water resources for irrigation [5]. Subsequently, the prospects of entomophagy have captured the interest of western societies, which are seeking sustainable alternatives to ensure food and feed security [6].

Insects are a natural food source for poultry and fish [7], and provide the necessary amounts of proteins, amino acids, fatty acids, and micronutrients to feed livestock [3,8]. As a result, insect farming systems have significantly advanced in the last decade, while the global regulatory landscape is being gradually adapted to encourage the development and trade of insect-based products; European regulations already allow the use of eight species (*Acheta domesticus*, *Gryllobates sigillatus*, *Gryllus assimilis*, *Bombyx mori*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Hermetia illucens*, and *Musca domestica*) as feed for aquaculture [9], poultry, and porcine animals [10], while *T. molitor* and *Locusta migratoria* have been authorised as novel food [11,12]. United States regulations have been established for the use of black soldier fly larvae (BSFL, *H. illucens*) in aquaculture, poultry, and swine feed [13], while insects reared with the intention of human consumption, following good manufacturing practices, are considered as food [14].

Studies on the nutritional value of insects have been performed on a variety of reared [15–18] and wild harvested species [19–22]. Some insect species have been researched extensively as a source for food and feed (BSFL [2,17,23]; mealworm [24–26]; house cricket [16,27,28]; African palm weevil [19,29,30]), while others have remained understudied.

Mosquitoes (Diptera: Culicidae) are widely distributed across the globe, except for Antarctica [31], and are important vectors of life-threatening pathogens (i.e., Malaria parasite; West Nile virus; Dengue virus [32]). They are inadvertently produced in massive numbers in intensive agricultural environments, such as rice farming regions [33–35], where they are a major nuisance for animals, including humans. Despite their ubiquitous nature and high populations, there is currently, to the best of our knowledge, no information on the nutritional value of adult, free-flying mosquitoes as animal feed. The aim of this study was to assess the nutritional characteristics, as well as the microbial profile, of the most abundant mosquito species produced in a rice-field environment for their potential exploitation as poultry feed ingredients.

2. Material and Methods

2.1. Mosquito Sampling and Identification

Mosquitoes were collected in the context of an arbovirus surveillance program in the rice-field region of West Thessaloniki near the Axios River Delta (N 40°34.600 E 22°45.230) from July to August 2020. The Centre for Disease Control (CDC) miniature light traps baited with dry ice were used for mosquito collections, selectively targeting host-seeking female mosquitoes. Traps were placed in the field, adjacent to rice-paddies for maximum collection efficiency. Collected mosquitoes were identified [36] and analysed to species level to account for interspecies differences in nutritional characteristics.

Collections were also performed during the follow-up summer (June–August 2021), following the same procedures, to allow for qualitative comparisons of the nutritional profile between mixed mosquito populations from the two harvest seasons. Mosquito samples (mixed species) were randomly analysed from the total collections of each given season.

Samples (single species; mixed species) were stored at -20°C until processing for nutritional and microbiological analysis.

2.2. Proximate Analysis

2.2.1. Crude Protein Content

The total nitrogen content was determined using the Dumas method following the manufacturer's protocol (DUMATHERM Analyzer, Gerhardt, Königswinter, Germany). Approximately 100 mg of freeze-dried mosquito powder was used per sample. Percent nitrogen content was determined based on a calibration curve constructed from standard

ethylene diamine tetraacetic acid (EDTA) samples. The universal protein conversion factor $N \times 6.25$ was used [24].

2.2.2. Crude Fat Content

Crude fat was determined with the Soxhlet extraction method. The process was as follows: Crude fat was extracted from freeze-dried mosquito samples (~2.0 g) using a Soxhlet apparatus for 24 h and petroleum ether as a solvent (400 mL, CAS nr. 64742-49-0). Upon completion of the extraction, petroleum ether was removed from the flasks containing the crude fat extracts via rotary evaporation (IKA RV8, Werke GmbH & Co., Staufen, Germany) at a pressure of approximately 350 mbar in a water bath at 40 °C for about 30 min [37]. The flasks with the crude fat extracts were then dried in an oven at 107 °C for 24 h to determine the final crude fat weight gravimetrically.

2.2.3. Moisture and Ash Content

Percent moisture and ash were calculated gravimetrically as the difference in weight between pre- and post-dried or ashed mosquito samples, respectively. Fresh samples were dried in an oven at 107 °C for 2 h (determined as sufficient time to reach constant weight). Percent ash was determined by incinerating freeze-dried samples in a furnace (F47900, Thermolyne, Thermo Fisher Scientific, Waltham, MA, USA) at 600 °C for 4 h (determined as sufficient time to reach constant weight). In both cases, samples were cooled down in a desiccator, to prevent reabsorption of moisture, before measuring the final weight.

2.3. Fatty Acid Profile

Fatty Acid Methyl Esters (FAMES) were prepared according to O'Fallon et al.'s [38] direct FAME synthesis with slight modifications. In brief, in 0.5 g of freeze-dried mosquito powder, 0.7 mL of 10 N aqueous KOH and 5.3 mL of MeOH were added. The mixture was incubated in a 55 °C water bath for 1.5 h under constant agitation (150 rpm). After cooling, in a cold-water bath, 0.58 mL of 24 N H₂SO₄ aqueous solution was added. Incubation at 55 °C was repeated followed by another cooling step and the addition of 3 mL of hexane. The mixture was vortexed for 2 min and centrifuged for 5 min at 3500 rpm. Analysis of the organic layer containing FAME was performed after 1/10 dilution with hexane according to Psathas et al.'s [39] GC/MS instrumentation and analytical protocol. Compounds were identified using a mass spectra library. Samples were run in randomised order and results were expressed as mean % area values.

2.4. Mineral Content

The mineral content of freeze-dried, powdered mosquito samples was analysed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) after wet digestion in a microwave oven [40] using certified machinery (Hellenic Accreditation System (ESYD), accreditation number 848-3). The process was as follows: (1) 0.5 g of sample was mixed with 5 mL of HNO₃ and 1 mL of H₂O₂ in XP-1500 Plus digestion vessels; (2) mixtures were allowed to sit for 15 min before closing the vessels to prevent pressure build-up from vigorous chemical reactions; (3) vessels were placed in a microwave system (MARS 5, CEM) to complete digestion at 200 psi, 600 W, with a ramp to 200 °C in 25 min for 35 min total; (4) after cooldown, final solutions were diluted to 25 mL using DI H₂O; (5) the minerals of interest (Ca, Mg, Na, K, Fe, Cu, Zn, Mn, and P) were detected using ICP-OES (Agilent Technologies 5110, Santa Clara, CA, USA) at radial viewing mode with limit of detection (LOD) < 0.5 mg/kg.

2.5. Antioxidant Activity

The antioxidant activity of mosquitoes was determined according to a 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging in vitro assay according to Nenadis et al. [41], with some modifications. Extracts were prepared by adding 1.0 g of material (freeze-dried powdered mosquitoes or food-grade *T. molitor* powder (Kreca Ento-Food BV, Ermelo, The

Netherlands)) in 25 mL of methanol and the mixture was shaken in the dark at 150 rpm for 24 h. The supernatant was separated from the mixture and used for the antioxidant activity assessment. An aliquot (0.3 mL) of the methanolic extracts was mixed with 2.9 mL of methanolic DPPH solution (0.1 mM) and the mixture was allowed to react for 30 min in the dark at ambient temperature (25 °C). Absorbance was then measured at 517 nm (spectrophotometer UV-1800, Shimadzu Canby, OR, USA). For the control, a respective methanol aliquot was mixed with the DPPH solution. Antioxidant activity was expressed as % Radical Scavenging Activity (%RSA) according to Formula (1):

$$\%RSA = [1 - (\text{Absorbance sample} / \text{Absorbance control})] \times 100 \quad (1)$$

Gallic acid was used as a reference compound and its ability to scavenge the DPPH radical at various concentrations (4.5–30 µg/mL) was determined as before, to create a standard curve. The antioxidant activity of mosquitoes and *T. molitor* methanol extracts was expressed as gallic acid equivalents (GAE; µg of gallic acid scavenging 0.29 µmol DPPH radical), using the regression equation of the obtained gallic acid standard curve. A regression equation from the %RSA values of harvest 2020 and harvest 2021 extracts (4–32 mg of dry material weight scavenging 0.29 µmol DPPH radical) was used to determine the IC₅₀ value (mosquito concentration at which 50% of DPPH radical solution is scavenged).

2.6. Microbiological Analyses

For the microbial analysis, approximately 2.0 g of fresh mosquitoes were mixed with Ringer's solution (1:10 dilution) and homogenised using a stomacher homogenizer (BL Style, Astori Tecnica, Poncarale, Italy) at maximum speed (260 rpm) for 2 min. The homogenates were further diluted with Ringer's solution in 10-fold serial dilutions and aliquots of dilutions 10⁻¹ to 10⁻⁵ were spread on different types of agar plates depending on the target microbial groups: total viable aerobic bacteria on plate count agar (PCA) incubated at 30 °C for 48 h; *Enterobacteriaceae* on pour-plates of violet red bile glucose agar (VRBGA) with an overlay of the same medium, incubated at 37 °C for 24 h; lactic acid bacteria (LAB) on pour-plates of de Man, Rogosa, and Sharpe (MRS) agar with an overlay of the same medium, incubated at 30 °C for 48 h; coagulase-positive *Staphylococci* (CPS) on Baird-Parker (BP) agar with 5% egg yolk tellurite, incubated at 37 °C for 48 h. Microbial counts were recorded as colony forming units (CFU) per gram.

2.7. Statistical Analysis

The data obtained for proximate composition (protein, fat, moisture, and ash content), the antioxidant activity, FAME, and the microbiological analysis were the outcome of at least three independent replicates and were analysed with one-way ANOVA using the IBM SPSS statistic program (IBM Schweiz AG, Zurich, Switzerland). Tukey's test was used to compare groups. Statistical difference was considered significant if $p < 0.05$.

3. Results and Discussion

3.1. Mosquito Collections

A total of 1.5 and 7.5 kg of mosquito biomass were collected during the 2020 and 2021 season, respectively, corresponding to ~500,000 and 2.5 million mosquitoes. Three mosquito species were identified in high abundance across both seasons, namely *Aedes caspius*, *Culex pipiens*, and *Anopheles hyrcanus*, and were analysed separately. All three species commonly breed in rice-field environments and are competent vectors of a variety of pathogens of medical and veterinary importance.

3.2. Proximate Composition

The three examined mosquito species appear most beneficial for different nutrients, though differences were not always statistically significant (Table 1). Among these species, *An. hyrcanus* had a significantly higher percentage of crude protein content (61.8%), while *Cx. pipiens* had the lowest percentage of crude protein (55.6%) and moisture (37.3%). Both

fat and ash content were similar among the three species (21.0–27.6% and 6.6–8.5%, respectively). Given that mosquito development and collection for all three species occurred at the same seasonal period and in the same region, environmental parameters are unlikely to have affected the interspecies variation observed. Species-specific structural, physiological, and behavioural traits are likely responsible for these differences. As expected, the proximate compositional characteristics of mixed mosquito populations were similar to those of the three species analysed separately, as these populations are mixtures of the individual species. Interestingly, there was no significant difference in the proximate composition of mixed populations harvested in 2020 and 2021, indicating that the nutritional quality of mosquitoes is stable across different seasons and unaffected by fluctuations in species populations.

Table 1. Proximate composition of the mosquito samples examined (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, mixtures of harvest 2020 and harvest 2021, \pm SD) compared to standard livestock feed.

	<i>An. hyrcanus</i>	<i>Ae. caspius</i>	<i>Cx. pipiens</i>	Harvest 2020	Harvest 2021	Fishmeal ¹	SBM ¹
Moisture (%)	55.5 (\pm 1.0) ^a	57.2 (\pm 1.7) ^a	37.3 (\pm 1.5) ^c	48.9 (\pm 2.7) ^b	53.0 (\pm 0.5) ^{ab}	7.7–7.9	12.0–12.3
Crude protein (% dwb)	61.8 (\pm 0.3) ^a	56.8 (\pm 0.7) ^b	55.6 (\pm 0.3) ^{bc}	53.9 (\pm 0.7) ^c	53.8 (\pm 1.1) ^c	67.8–74.8	49.5–55.2
Crude fat (% dwb)	23.4 (\pm 6.2) ^a	21.0 (\pm 4.3) ^a	27.6 (\pm 6.0) ^a	15.9 (\pm 2.5) ^a	15.7 (\pm 1.0) ^a	9.8–10.3	1.7–1.9
Ash (% dwb)	7.7 (\pm 0.1) ^b	8.5 (\pm 0.3) ^a	6.6 (\pm 0.6) ^c	7.7 (\pm 0.3) ^b	7.3 (\pm 0.2) ^b	15.2–19.2	7.1–7.4

¹ INRAE-CIRAD-AFZ (2017–2021); SBM = soybean meal; dwb = dry weight basis; different letters across each row indicate significant difference ($p < 0.05$).

The proximate compositional characteristics of the mosquito samples were considered comparable and even superior to conventional animal feedstuff (Table 1). According to reported data [42], mosquitoes are similar to soybean meal (SBM) in terms of crude protein and ash content, while fishmeal has a slightly higher protein and ash content than mosquitoes. Though fishmeal is nutritionally beneficial, it is problematic due to its high prices, low availability, and damage to the ecosystem caused by overfishing [43]. Mosquito-based poultry feed could counteract these issues. The high protein content in mosquitoes is an important finding as proteins are expensive feed components and limiting ingredients. Insect-derived proteins are seen as a sustainable, environmentally friendly alternative that benefits food security [3,44]. The considerably higher fat content of mosquitoes compared to fishmeal and SBM is expected as both feedstuffs are being processed for oil extraction prior to animal consumption, resulting in low fat content [45,46]. The proximate compositional characteristics of mosquitoes are comparable to those of the three most common edible insects with the potential to be used as animal feed (*T. molitor*, *A. domesticus*, and *H. illucens*; Table S1 see in the Supplementary Materials). Mosquitoes have a similar protein (54–62% vs. common edible insects: 49–63%) and fat content (16–28% vs. common edible insects: 18–37%). Fat, although of significant nutritional importance, both for humans and animals, has received little attention in strategies to sustainably close the gap in fat supply [47]. Mosquitoes' fat content could be a useful source of fat, contributing to the food and feed system sustainability.

It has been reported that the diet of insects (wild-harvested or reared) has a significant effect on their nutritional profile [48]. Therefore, the values reported here may not apply to all mosquito species or mosquitoes reared/collected from different environments. Additional studies should target a wide variety of wild and reared mosquito species from different geographical origins.

3.3. Fatty Acid (FA) Profile

All mosquito samples presented a qualitatively similar FA profile (Table 2). Quantitative differences observed in the proportion of individual FAs, although statistically significant in certain cases, did not affect the overall quality of the FA profile. Importantly, the two examined harvests had a similar FA content, indicating that harvested mosquitoes

have a stable nutritional profile, which is a beneficial trait for commercial exploitation. Total amounts of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) ranged from 29.6 to 32.7%, 57.3 to 63.1%, and 5.1 to 12.7%, respectively (Figure 1). The higher total unsaturated fatty acid (UFA = MUFA + PUFA) content is considered beneficial in accordance with the nutritional suggestions for human diets for reduction in SFAs and replacement with UFAs [49,50]. The predominance of MUFAs in the UFA fraction is also considered favourable, as, apart from the health benefits related to the former [50], high PUFA concentrations may enhance lipid susceptibility and protein oxidation, leading to unfavourable changes in meat quality [51].

Table 2. Fatty acid composition (mean percentage of total fatty acids % \pm SD) of the mosquito samples examined (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, mixtures of harvest 2020 and harvest 2021).

Fatty Acids *	% of Total Fatty Acids				
	<i>An. hyrcanus</i>	<i>Ae. caspius</i>	<i>Cx. pipiens</i>	Harvest 2020	Harvest 2021
Lauric acid; C12:0	0.23 (\pm 0.06) ^{a,b}	0.15 (\pm 0.06) ^c	0.27 (\pm 0.01) ^a	0.19 (\pm 0.01) ^{b,c}	0.18 (\pm 0.01) ^{b,c}
Myristic acid; C14:0	0.94 (\pm 0.01) ^c	0.82 (\pm 0.01) ^d	1.75 (\pm 0.06) ^a	0.91 (\pm 0.01) ^c	1.00 (\pm 0.01) ^b
Tetradecenoic acid; C14:1	0.23 (\pm 0.01) ^b	0.32 (\pm 0.11) ^b	0.99 (\pm 0.01) ^a	0.23 (\pm 0.06) ^b	0.30 (\pm 0.01) ^b
Palmitic acid; C16:0	25.52 (\pm 0.15) ^b	23.78 (\pm 0.12) ^d	30.43 (\pm 0.10) ^a	25.25 (\pm 0.06) ^b	25.82 (\pm 0.17) ^b
Palmitoleic acid; C16:1n7	28.54 (\pm 0.06) ^d	31.04 (\pm 0.23) ^b	37.04 (\pm 0.06) ^a	30.59 (\pm 0.12) ^c	31.33 (\pm 0.25) ^b
Stearic acid; C18:0	2.73 (\pm 0.01) ^c	3.11 (\pm 0.10) ^a	2.12 (\pm 0.01) ^d	3.04 (\pm 0.12) ^{a,b}	2.90 (\pm 0.01) ^b
Oleic acid; C18:1n9	26.57 (\pm 0.10) ^c	29.10 (\pm 0.10) ^a	18.29 (\pm 0.06) ^d	28.18 (\pm 0.15) ^b	27.42 (\pm 0.26) ^c
Octadecenoic acid; C18:1	1.22 (\pm 0.06) ^d	1.84 (\pm 0.01) ^b	3.19 (\pm 0.01) ^a	1.19 (\pm 0.01) ^d	1.31 (\pm 0.01) ^c
Linoleic acid; C18:2n6	4.60 (\pm 0.10) ^a	2.25 (\pm 0.06) ^c	1.48 (\pm 0.01) ^d	3.01 (\pm 0.10) ^b	2.83 (\pm 0.12) ^b
Linolenic acid; C18:3n3 (ALA)	5.19 (\pm 0.06) ^a	2.60 (\pm 0.01) ^c	1.35 (\pm 0.06) ^d	3.24 (\pm 0.06) ^b	3.33 (\pm 0.15) ^b
Eicosanoic acid; C20:0	0.14 (\pm 0.06) ^c	0.21 (\pm 0.01) ^{b,c}	0.26 (\pm 0.01) ^a	0.17 (\pm 0.01) ^{b,c}	0.27 (\pm 0.06) ^b
Arachidonic acid; C20:4n6 (ARA)	1.10 (\pm 0.01) ^a	0.73 (\pm 0.06) ^c	0.73 (\pm 0.01) ^c	0.95 (\pm 0.06) ^b	0.79 (\pm 0.10) ^c
Eicosapentaenoic acid; C20:5n3 (EPA)	1.79 (\pm 0.06) ^c	2.36 (\pm 0.12) ^a	1.49 (\pm 0.01) ^d	2.04 (\pm 0.06) ^b	1.70 (\pm 0.10) ^c

* Other minor fatty acids (e.g., 15:0, 16:1, 17:1, 18:1, and 18:3n6) that are not included here contributing in amounts < 0.1% were also identified; different letters across each row indicate significant difference ($p < 0.05$).

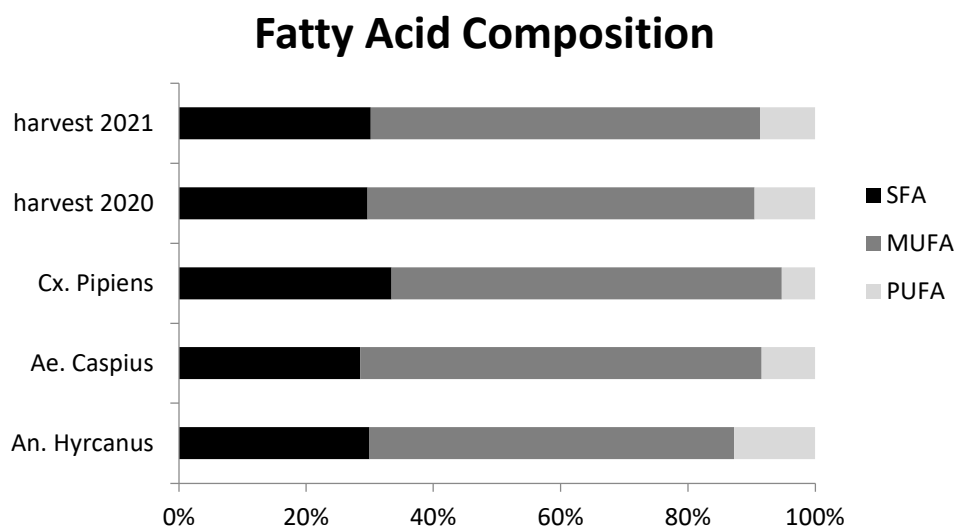


Figure 1. Total amount % of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids of the mosquito samples examined (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, mixtures of harvest 2020 and harvest 2021).

In all cases, the three dominant fatty acids accounting for ~80–85% of the total FA content were the palmitic (24–30%), palmitoleic (29–37%), and oleic (18–29%) acids. These findings resemble the mosquito lipid profile reported by Sushchik et al. [52] and Komínková et al. [53], where the same fatty acids predominate.

Mosquitoes in the current study contained the essentials for poultry consumption, omega-3 linolenic (ALA) and omega-6 linoleic acids. Omega-3 and omega-6 fatty acids in poultry diets are known to improve poultry growth and health, as well as the nutritional quality of the produced meat and eggs [51]. Specifically for the essential linoleic acid, its requirement for poultry consumption has been set as 1% of the diet [54].

Mosquitoes' UFA levels are close to those of *A. domesticus* and slightly lower than those of *T. molitor* [37,55]. Compared to *T. molitor*, mosquitoes have similar SFA, higher MUFA, and lower PUFA amounts [18,37,55–58]. Compared to *A. domesticus*, mosquitoes have higher oleic and linoleic acid contents [37,55]. Moreover, the *H. illucens* FA profile has higher linoleic acid and lower MUFA contents compared to mosquitoes [48,59,60].

As previously discussed, conventional livestock feedstuffs go through significant processing pre-consumption, resulting in a lower lipid content. In the small lipid fraction remaining in SBM post-processing, linoleic acid predominates (~50%) [42,46,61]. Fish meal contains considerable amounts of beneficial lipids, such as eicosapentaenoic acid [45]; however, off-flavours appear in the final product, adversely affecting consumers' acceptability [51].

Globally, poultry meat is a main source of proteins and lipids for humans and its FA composition may be regulated by dietary lipids to meet demands for human nutrition. In general, nutritionists recommend fat consumption reduction, a reduction in SFAs, and an increase in UFAs, a diet that includes ALA, EPA, and docosahexaenoic acid [49,50,62,63]. In this direction, the mosquito FA profile seems promising considering its low SFA, high UFA, and presence of ALA and EPA.

3.4. Mineral Profile

The examined wild-harvested mosquitoes appeared to be a rich source of minerals, and in most cases, superior to conventional feedstuff and commonly consumed insects (Table 3). Mosquitoes showed a very stable mineral profile, irrespectively of harvest season. Compared to SBM, the examined mosquito samples showed higher concentrations for all minerals (calcium (~3×), phosphorus (~2×), manganese (~4×), and zinc (~3×)) except potassium and iron. Fishmeal has higher calcium and phosphorus contents (~3.5× and ~1.5×, respectively), but mosquitoes showed higher manganese and zinc concentrations (~2× and ~1.5×, respectively).

Table 3. Mineral content of the mosquito samples (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, mixtures of harvest 2020 and harvest 2021) compared to standard livestock feed.

Mineral (mg/kg, dwb)	<i>An. hyrcanus</i>	<i>Ae. caspius</i>	<i>Cx. pipiens</i>	Harvest 2020	Harvest 2021	SBM ¹	Fishmeal ¹
Calcium (Ca)	9773	12,113	6626	11,371	11,122	3867	42,367
Potassium (K)	9884	10,305	9079	10,216	10,941	24,200	8733
Phosphorus (P)	18,600	18,800	13,700	17,500	17,300	7100 *	27,767
Magnesium (Mg)	3667	4604	2753	4136	4062	3200	2367
Sodium (Na)	2593	2556	2414	2625	2668	127	11,067
Iron (Fe)	131.8	146.8	146.4	111.5	118.5	249.7	367
Manganese (Mn)	261.9	218.6	86.9	185.2	176.7	41.7	17.3
Copper (Cu)	23.6	19.0	19.7	20.1	20.8	17.3	11.3
Zinc (Zn)	188.0	158.0	166.4	151.1	159.3	53.3	105.7

¹ INRAE-CIRAD-AFZ (2017–2021); SBM = soybean meal; dwb = dry weight basis. * 4267 mg/kg of this phosphorus (60%) is in the form of Phytate Phosphorus, which is not bioavailable.

Out of all the minerals examined, phosphorus was detected in the highest concentration across all mosquito samples. Among mosquito species, the concentrations of the

macrominerals (Ca, K, P, Mg, and Na) were lowest in *Cx. pipiens* and highest in *Ae. caspius*, except for sodium, which was slightly higher in *An. hyrcanus*. The concentrations of the microminerals (Fe, Mn, Cu, and Zn) were highest in *An. hyrcanus*, except for iron. For the remaining micronutrients, the lowest concentrations of copper and zinc were found in *Ae. caspius*, while the lowest concentration of manganese was found in *Cx. pipiens*.

The mineral content of animal feed is important for the proper growth and development of livestock. Calcium, phosphorus, and magnesium are the essential minerals for broiler chicken development [54]. Calcium and phosphorus are essential for intracellular communication and cellular structure, respectively, and they are both necessary for skeletal formation and maintenance. In laying fowl, calcium is also important in eggshell formation [2]. Magnesium is necessary for proper neuromuscular function, growth, and survival [54]. Trace elements such as zinc and manganese are essential co-factors to enzymes, and zinc is necessary for DNA structural motifs (zinc fingers) [54]. Calcium and phosphorus are usually deficient in conventional feed, while zinc and manganese show low bioavailability [54].

According to Finke [20], the limiting minerals for most insectivores are calcium and manganese. Unlike most wild-harvested and reared insects (with the exception of black soldier fly, *H. illucens*, that has a mineralised exoskeleton), mosquitoes appear to be rich in calcium (Table S2). Compared to *T. molitor* (adult and larvae), mosquitoes present a superior mineral content. On average, mosquitoes have $\sim 14\times$ more calcium, $2\times$ more phosphorus, and $\sim 18\times$ more manganese. Compared to *A. domesticus*, mosquitoes have on average $5\times$ more calcium and manganese, $3\times$ more phosphorus and iron, and up to $6\times$ more magnesium. *Hermetia illucens* larvae are the most comparable reared insect to wild-harvested mosquitoes, but their mineral profile varies and is highly dependent on their diets. In general, *H. illucens* has similar levels of magnesium, lower phosphorus, and higher iron than mosquitoes. Overall, mineral content findings for the studied mosquito samples concur to its promising profile for being used as an alternative to conventional and unsustainable animal feed.

3.5. Antioxidant Properties

The antioxidant potential of mosquitoes was assessed via the DPPH assay, which is the most frequently used method for the in vitro antioxidant potential assessment of edible insects [64]. DPPH is a stable free radical, whose solutions change colour (from violet to yellow) upon its scavenging from antioxidants. Free radicals induce oxidative damage to biomolecules, leading to an array of diseases and adverse effects, while antioxidant compounds protect biological systems by inhibiting such harmful effects [65]. The higher the antioxidant activity of a substance, the higher its ability to scavenge the DPPH radical (%RSA) and the smaller its IC₅₀ value [41].

The antioxidant activity of the examined mosquito extracts compared with food-grade *T. molitor* extracts is presented in Figure 2a. The three examined mosquito species, as well as the mixture samples from the two harvest seasons, exhibited promising antioxidant activity with %RSA values ranging from 32.6% to 50.5%. *Anopheles hyrcanus* showed a significantly higher antioxidant activity (45.9%), when the other mosquito species (*Ae. caspius* and *Cx. pipiens*) and *T. molitor* performed similarly (39.2%, 32.6%, and 36.0%, respectively). The scavenging capacity of the mosquito extracts increased linearly ($R^2 \sim 0.95\text{--}0.97\%$) as the amount of mosquito increased in the sample (Figure 2b). Inhibition of 50% of the DPPH radicals (IC₅₀ values) was achieved using 15.9 mg and 13.8 mg of the mosquito sample (dwb) from harvest 2020 and harvest 2021, respectively, showing a quite stable antioxidant potential of the harvested material.

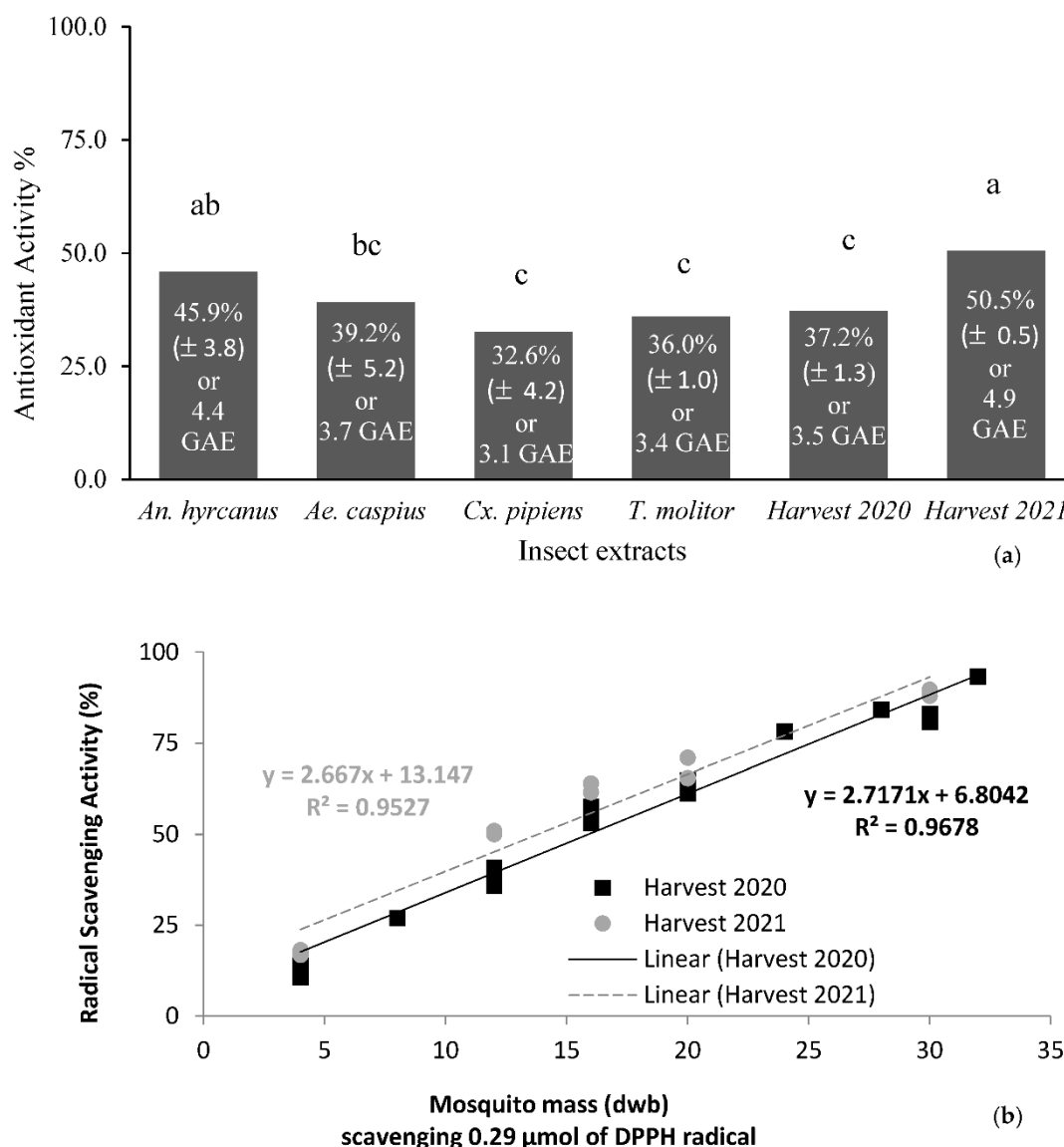


Figure 2. (a) Mean antioxidant activity (expressed as % scavenging activity of the DPPH radical \pm SD, and as gallic acid equivalents (GAE)) of methanolic extracts prepared from the mosquito samples examined (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, mixtures of harvest 2020 and harvest 2021). Different letters indicate a significant difference ($p < 0.05$). (b) Linear dose-dependent capacity of mosquito extracts (prepared from mixtures of harvest 2020 and harvest 2021) to scavenge the DPPH radical.

Direct comparisons with published data on the DPPH radical scavenging activity of other edible insects and feedstuffs are not easy, due to differences in the employed experimental approaches (e.g., reaction times, solvents, pH, standard compounds, and extract characteristics), which may considerably affect findings. Identical procedures must be followed when comparing antioxidant activities across different materials for reliable evaluations [41,65–67]. For this reason, in the current study, the scavenging ability of food-grade *T. molitor* was examined in parallel with that of mosquito and the results revealed a comparable antioxidant performance of the materials. Mosquitoes, as with other edible insects, could perform as an antioxidant source of low ecological impact able to modulate oxidative stress in animals [64]. Antioxidants preserve unsaturated fatty acids and vitamins from destruction due to oxidation. Vitamin deficiencies in chickens cause brain and skin lesions, skeletal and nervous disorders, haemorrhage, muscular

dystrophy, cardiac myopathy, and diarrhoea [54]. As a preventative measure, synthetic antioxidants are supplemented in animal feed. In this view, the antioxidant potential of mosquitoes is considered advantageous because natural antioxidants are considered safer than synthetic ones and have more potential on acceptability and palatability from livestock [68]. Nonetheless further assessments employing animal models and dietary intervention trials are needed to confirm their antioxidant efficacy in animals.

3.6. Microbiological Screening

The presence of food microbes was confirmed for all wild-harvested, fresh mosquitoes (Table 4). For each microbial group, the three mosquito species showed similar bacterial loads. The total viable aerobic count (TVC) for *An. hyrcanus*, *Ae. caspius*, and *Cx. pipiens* was 6.47, 6.61, and 6.63 log cfu/g respectively, averaging at 6.6 log cfu/g. The microbial loads of *Enterobacteriaceae* (5.46, 5.47, and 5.61 log cfu/g), LAB (5.48, 5.59, and 5.26 log cfu/g), and coagulase-positive *Staphylococci* (CPS) (5.41, 5.11, and 5.29 log cfu/g) were similar for all species, averaging at 5.5, 5.4, and 5.3 log cfu/g, respectively.

Table 4. Mean microbiological load (Log CFU/g \pm SD) of fresh mosquito samples (*Anopheles hyrcanus*, *Aedes caspius*, and *Culex pipiens*, and mixture of harvest 2020).

Mosquito Samples	Total Viable Bacteria	<i>Enterobacteriaceae</i>	Lactic Acid Bacteria	Coagulase-Positive <i>Staphylococci</i>
<i>An. hyrcanus</i>	6.47 (\pm 0.06) ^b	5.46 (\pm 0.06) ^a	5.48 (\pm 0.01) ^a	5.41 (\pm 0.3) ^a
<i>Ae. caspius</i>	6.61 (\pm 0.02) ^b	5.47 (\pm 0.05) ^a	5.59 (\pm 0.01) ^a	5.11 (\pm 0.1) ^a
<i>Cx. pipiens</i>	6.63 (\pm 0.02) ^b	5.61 (\pm 0.02) ^a	5.26 (\pm 0.02) ^a	5.29 (\pm 0.2) ^a
Harvest 2020	7.01 (\pm 0.02) ^a	5.91 (\pm 0.04) ^a	5.28 (\pm 0.04) ^a	4.34 (\pm 0.2) ^b

Different letters along each column indicate significant difference ($p < 0.05$).

The similarities in microbial loads between the three mosquito species are expected as mosquitoes were collected from the same environment and handled in an identical manner. When examined as a mixture (harvest 2020), TVC load was significantly higher than the loads of the individual species (7.0 vs. 6.6 log cfu/g), while the CPS load was significantly lower (4.3 vs. 5.3 log cfu/g). *Enterobacteriaceae* and LAB loads showed no statistical differences.

As fresh, whole insects, mosquitoes have initial bacterial loads (\sim 6.6 log cfu/g) comparable and slightly lower to bacterial loads reported in the literature for *A. domesticus* and *T. molitor* (7.5 log cfu/g and 7.4 log cfu/g, respectively; [25,28]). For *Enterobacteriaceae* (the only group of microorganisms with existing regulations for feedstuff, Regulation (EU) No 142/2011), the mosquito initial load (\sim 5.5 log cfu/g) is comparable to that of *A. domesticus* (5.5 log cfu/g) but lower than that of *T. molitor* (7.0 log cfu/g). Lactic acid bacteria can be probiotics, thus beneficial, and their presence (\sim 5.4 log cfu/g) is not concerning. Coagulase-positive *Staphylococci* (\sim 5.3 log cfu/g in mosquitoes) are common human pathogens but have also been found in ruminants and chickens. These are typical levels for fresh food harvested from soil or having been in contact with soil materials, but it is worth noting that wild-harvested mosquitoes display comparable, and slightly lower, microbial loads than insects reared in farms under controlled conditions [25,28].

Generally, most pathogens (viruses, bacteria, fungi, protists, and nematodes) that affect insects are associated with the insect larval stages and are invertebrate-specific, posing no threat to livestock [69]. Bringing insects to domestication, separating them from their natural habitat, and clustering them in confined spaces for mass production increase their susceptibility to infectious diseases [69]. Therefore, harvesting insects from their natural habitat may minimise the exposure to the aforementioned harmful parameters.

According to our data, if mosquitoes are to be utilised as animal feed, some form of processing will be required to reduce *Enterobacteriaceae* and CPS loads and eliminate

Salmonellae as per EU Regulations. A form of heat treatment is usually involved in reducing microbial load. Thermal processing (boiling, (sun)-drying, roasting, toasting, frying, and smoking) occurs in temperatures of 55–105 °C and exposure times of 5–20 min [70]. Studies have shown that heat treatment effectively reduces microbial levels in insects and ensures their safety as feed. Klunder et al. [25] decreased the total microbial load and *Enterobacteriaceae* of *T. molitor* and *A. domesticus* below the limit of detection (LOD < 1.0 log cfu/g) by boiling (~100 °C) for 10 and 5 min, respectively. Vandeweyer et al. [26], using a different heat treatment (blanching for 40 s and then microwave drying for 16 min), decreased the total microbial load of *T. molitor* to 1.3 log cfu/g and the *Enterobacteriaceae* population below the LOD. Future studies should focus on determining and optimising a processing method appropriate for wild-harvested mosquitoes to ensure that harmful pathogens are eliminated while maintaining the nutritional integrity of the insects.

4. Conclusions and Moving Forward

The need for alternative feed sources to ensure global food security and environmental sustainability has stirred people's interest in insect feed. Preliminary studies have shown that when conventional feed is partially or fully replaced with insect feed, there are no significant differences in the development of broiler chickens, indicating that insects are a viable option for feed. Utilising wild-harvested mosquitoes, which, until now, have only been considered a major pest of veterinary and public health importance, is a novel and promising approach. Due to the notion that mosquitoes are unable to provide any function other than harm, scientists have gone as far as to say that if mosquitoes were eliminated from the planet, it would be for the best [71]. Through this study, we suggest that there may be a value to mosquitoes not considered before. Wild-harvested mosquitoes show potential as feed components in poultry diets due to their high protein content, rich micromineral profile, and promising antioxidant potential. Our data justify additional studies to further assess the nutritional value and safety of mosquito-based diets and investigate their impact on poultry performance and meat quality.

While this research project is still in its infancy, it is important to try and envision the applicability of this new approach taking into consideration the already available technologies and infrastructures. Mosquito mass-rearing is already applied across the world to support Sterile Insect Technique (SIT) programs [72] and their production is expected to increase in the coming years. Therefore, mass-rearing activities could be modified and upscaled for feed production if needed. Furthermore, SIT strategies need only male mosquitoes for release into the environment, while the remaining 50% of the biomass that corresponds to the female population is eventually disposed. Utilizing mosquitoes as animal feed shall add value to the byproducts of mass mosquito rearing by transforming female mosquitoes into animal feed.

From another angle, mosquitoes could be harvested from intensive agricultural environments, such as rice-fields, where they are produced in massive numbers. There are mass-trapping technologies available for the purpose of controlling mosquito populations [73], but whether these systems could be modified and upscaled for the purpose of insect harvesting remains to be seen. Mosquitoes are seasonal pests and, realistically, harvesting their populations from the wild for animal feed is not a feasible stand-alone approach, perhaps only for small-scale production. Farmed insects could satisfy consistently high feed demands, but harvesting insects could be a complementary, high-value, low-investment strategy for taking advantage of mosquitoes—byproducts of intensive agricultural activity—produced massively in agricultural landscapes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su142113852/s1>, Table S1: Proximate composition of common edible insects reported in literature (% dwb); Table S2: Mineral composition of common edible insects reported in literature (mg/kg, dwb). References [2,23,24,27,74–77] are cited in the Supplementary Materials.

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