

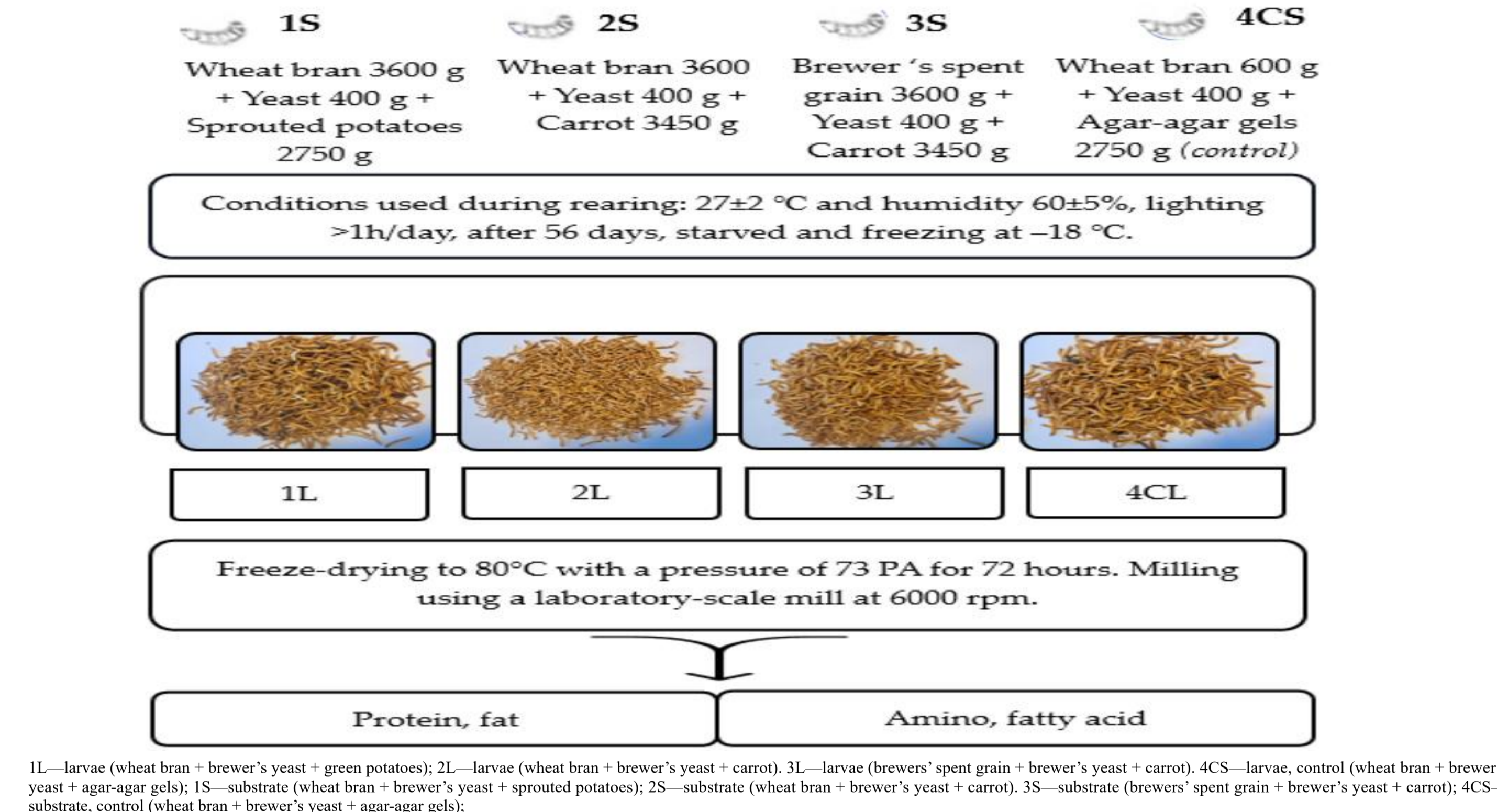
## Introduction

- It is projected that the world's population will reach nearly 9.8 billion people By 2050.
- Consequently, environmental sustainability concerns underscore the importance of developing alternative protein strategies, including new sources of high-quality proteins and fats. When rearing mealworms, it should be taken into account that they are grown quickly with the lowest costs and the resulting biomass is of high quality.
- The rearing of *Tenebrio molitor* larvae (Linnaeus, 1758), a member of the darkling beetle family (*Tenebrionidae*), using plant-based by-products offers multiple benefits.
- Together with a shift towards an economical, safe, and sustainable outcome, there is an increasing trend of attempting to rear mealworms using by-products from production and agriculture.
- The impact of these by-products on the mealworms themselves can vary, depending on the chosen raw materials and their proportions.
- Analyzing the nutritional value of mealworms, besides the quantity of proteins and fats, it's crucial to consider the specific AAs and FAs present.

This study **aims** to explore the potential of using local by-products or food residues as substrates in the mass production of *Tenebrio molitor* larvae (mealworms), focusing on achieving an optimal amino and fatty acid profile.

The **object** of this study encompasses the use of various plant-based by-products such as brewers' spent grain, wheat bran, and notably, sprouted potatoes, which are considered waste from farms and are no longer suitable for human consumption.

## Methodology



1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

Figure 1. Chart illustrating the complete research procedure.

## Results

### Proteins and amino acids

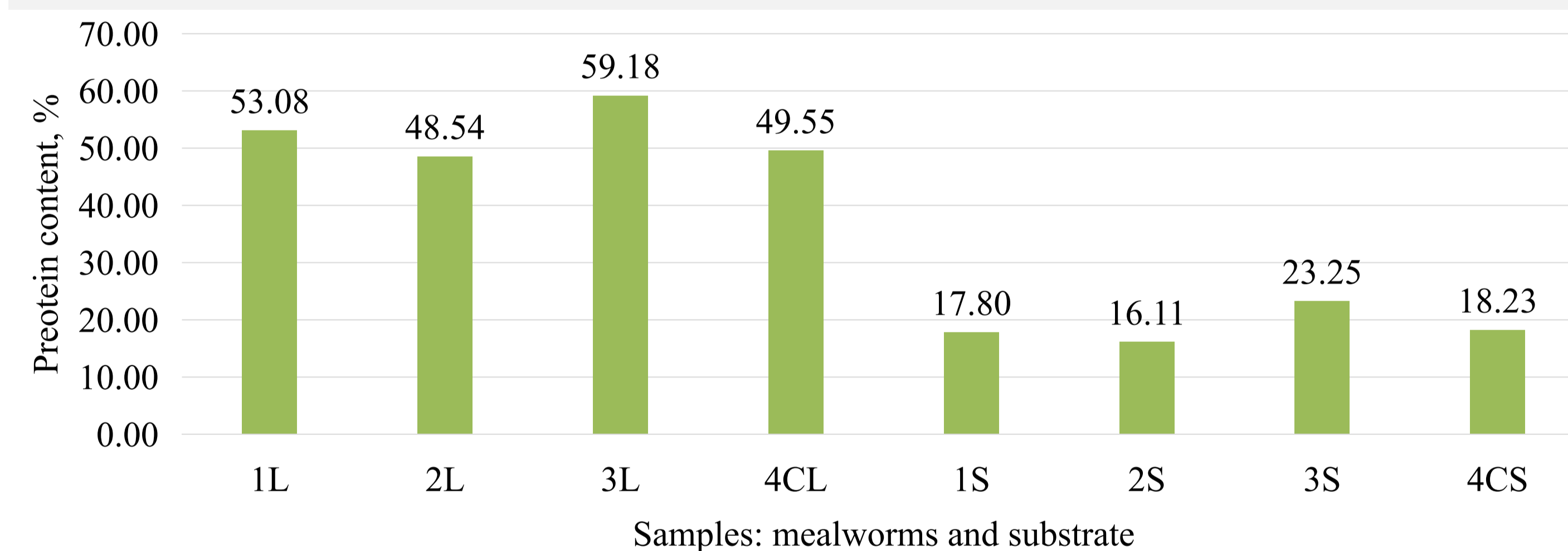


Figure 2. Protein content in substrate and mealworms

Table 1. Amino acid composition in lyophilized larvae and substrate, g/100g of dry matter, average ± standard error, n=3.

Samples	Larvae				Substrate			
	1L	2L	3L	4CL (control)	1S	2S	3S	4CS (control)
Valine	2.88±0.134 a	2.25±0.013 b	3.14±0.130 a	2.93±0.174 a	0.84±0.089 a	0.74±0.061 a	1.19±0.025 b	0.83±0.038 a
Leucine	3.54±0.130 a	3.02±0.002 b	3.79±0.183 a	3.45±0.262 ab	1.09±0.060 a	1.00±0.071 a	1.53±0.009 b	1.10±0.041 a
Isoleucine	2.01±0.104 a	1.61±0.011 b	2.14±0.096 a	2.02±0.111 a	0.63±0.085 ac	0.47±0.059 b	0.79±0.007 c	0.52±0.042 ab
Threonine	1.39±0.082	1.27±0.045	1.45±0.002	1.34±0.111	0.45±0.004 a	0.39±0.019 b	0.52±0.013 c	0.41±0.017 b
Methionine	0.60±0.027 a	0.46±0.044 b	0.62±0.023 a	0.57±0.026 ab	0.18±0.011 a	0.24±0.001 b	0.30±0.017 c	0.26±0.012 b
Phenylalanine	1.63±0.065 ab	1.63±0.039 ab	1.83±0.097 a	1.61±0.089 b	0.72±0.034 a	0.66±0.045 a	1.11±0.010 b	0.69±0.034 a
Lysine	2.73±0.060 a	2.46±0.016 b	2.92±0.152 a	2.80±0.075 a	0.80±0.048 a	0.87±0.044 ab	1.07±0.001 c	0.95±0.042 b
Histidine	1.60±0.116 abc	1.49±0.032 b	1.81±0.116 cd	1.89±0.009 d	0.83±0.005 a	0.61±0.021 b	0.71±0.008 c	0.67±0.002 d
Aspartic acid	3.92±0.132 a	3.21±0.035 b	4.30±0.257 a	3.78±0.306 ab	1.22±0.056 a	1.10±0.051 a	1.47±0.048 b	1.19±0.060 a
Glutamic acid	5.88±0.238 a	3.73±0.121 b	6.49±0.903 a	6.17±0.046 a	3.86±0.254 a	2.80±0.199 b	3.67±0.171 a	3.00±0.081 b
Glycine	2.41±0.062 a	2.87±0.067 b	2.66±0.091 b	2.43±0.090 a	0.83±0.054	0.74±0.061	0.81±0.003	0.76±0.03
Serine	1.55±0.116 ab	1.39±0.041 a	1.73±0.002 b	1.68±0.111 b	0.75±0.003 a	0.66±0.033 b	0.74±0.013 a	0.67±0.004 b
Alanine	3.35±0.109 a	3.38±0.094 ab	3.74±0.166 b	3.40±0.148 ab	0.80±0.05 a	0.72±0.046 a	0.99±0.029 b	0.79±0.045 a
Proline	2.94±0.106 ab	2.67±0.020 a	2.98±0.143 ab	3.09±0.174 b	1.12±0.043 a	1.06±0.063 a	1.88±0.029 b	1.13±0.025 a
Tyrosine	3.25±0.037 a	3.47±0.144 a	3.88±0.160 b	3.54±0.070 a	0.50±0.013 a	0.64±0.034 b	0.85±0.026 c	0.40±0.020 d
Cystine	0.38±0.005 a	0.20±0.005 b	0.37±0.013 a	0.38±0.007 a	0.34±0.014 a	0.50±0.001 b	0.50±0.001 b	0.48±0.013 b

a,b,c,d - means marked with different letters in the row (in the groups Larvae and Substrate separately) differed significantly (p<0.05, Fisher's LSD criterion); 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

## Results

### Fats and fatty acids

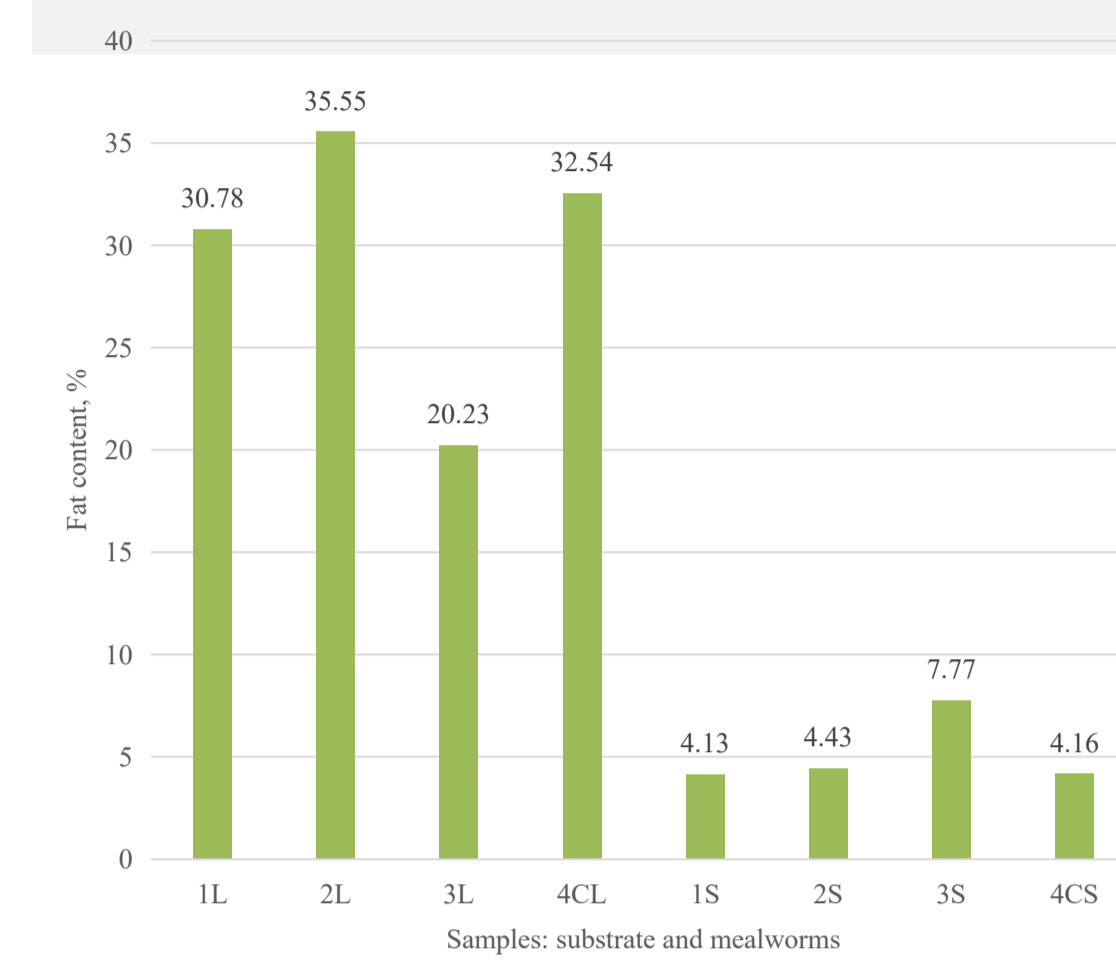


Figure 3. Fat content in substrate and mealworms

Table 3. Comparison of fatty acid in lyophilized larvae and substrate, % of total FAs content, average ± standard error, n=3.

Fatty acids Samples	C12:0	C16:1	C17:0	C18:0	C18:1 cis	C18:2 trans	c18:3 gama	C20:1	C20:3 w6	C22:1 w9	C20:3w3	C22:4 w6	C20:5 w3
	Larvae	2.46 ±1.411***	1.90 ±1.373***	3.39 ±3.821*	4.48 ±3.960*	35.72 ±2.472***	0.46 ±0.613***	0.62 ±0.504**	1.41 ±0.431***	1.58 ±1.341**	1.32 ±1.294**	4.14 ±5.674*	2.03 ±2.027**
Substrate	0.11 ±0.201	0.07 ±0.132	0 ±0	1.75 ±0.51	18.12 ±1.273	0 ±0	0.14 ±0.233	7.16 ±2.084	0 ±0	0 ±0	0.53 ±0.411	0.03 ±0.062	0.08 ±0.135

In the groups larvae and substrate separately differed significantly (\* - p<0.05; \*\* - p<0.01; \*\*\* - p<0.001, Fisher's LSD criterion); 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

Table 4. Fatty acid ratio in lyophilized larvae and substrate, % of total FAs content, n=3.17

Samples	Larvae				Substrate			
	1L	2L	3L	4CL	1s	2s	3s	4cs
Total SFA	26.42 a	24.15 b	52.64 c	17.33 d	20.94 a	20.79 b	28.59 c	20.25 d
Total MUFA	45.12 a	38.02 b	35.89 c	43.37 d	24.64 a	28.47 b	24.13 c	24.18 d
Total PUFA	28.47 a	36.24 b	10.18 c	35.11 d	54.42a	50.74 b	44.63 c	54.05 d
Omega 6 FA	27.12 a	18.65 b	7.82 c	29.22 d	54.00 a	49.54 b	42.92 c	53.91 d
Omega 3 FA	0.32 a	4.39 b	1.69 c	6.99 d	0.03 a	0.29 b	2.65 c	1.53 d
Omega 6/3 FA	85.05 a	4.25 b	4.632 c	4.18 d	1838.83 a	170.51 b	16.20 c	35.34 d

a,b,c,d - means marked with different letters in the row (in the groups larvae and substrate separately) differed significantly (p<0.05, Fisher's LSD criterion); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids;

## Main conclusions

- The results showed that the highest protein content (59.18 ± 0.07 %) was observed in samples grown on brewers' spent grain, while wheat bran yielded the highest fat content (34.22 ± 0.491 %).
- The amount of FAs in the larvae was influenced by the substrate used, with variations in monounsaturated, omega-3, and oleic acids.
- Notably, wheat bran showed the highest content of total polyunsaturated FAs (36.23 %). In the analysis of 16 distinct amino acids, a sample with brewers' spent grain, consistently demonstrated the highest content in 11 instances.
- This adaptation renders the larvae suitable for diverse purposes, including animal or human nutrition and health enhancement.
- In conclusion, our study underscores the significance of substrate optimization in harnessing the full potential of *Tenebrio molitor* larvae for targeted applications.