D3.4_1 Report on meat proteomics of slow growing poultry breed

Deliverable 3.4_1

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1. Introduction

The POULTRYNSECT Work Package 3 *"Laboratory and Sensorial Analyses"* aims to evaluate the impact of Black Soldier Fly (BSF) live larvae inclusion as a feed ingredient in the chicken diet on chicken health and meat quality. Animal welfare and health affect many metabolic processes, which may impact meat quality after slaughter (Petracci, Bianchi, & Cavani, 2010). Differences in feed composition may also be translated to differences in the chemical composition of meat and, thus, changes in sensory attributes. This Deliverable reports the changes in muscle protein composition and degradation, as affected by the feed type, studied using the Label-free quantification proteomic method.

2. Material and Methods

A total of 36 samples, six samples per six experimental groups, consisting of three diet groups (control, live larvae and sustainable) and two slaughter time, were included in the study (Table 1). Subsamples were selected by choosing one of the biological replicates randomly per box.

Table 1. List of the samples and number of identified proteins after LC-MS/MS analysis

Sample nr.	TREATMENT	BOX	Slaughter time	Nr. of identified proteins
25	CONTROL	1C	1	484
29	CONTROL	2C	1	398
33	CONTROL	3C	1	486
37	CONTROL	4C	1	487
41	CONTROL	5C	1	445
45	CONTROL	6C	1	456
73	CONTROL	1C	2	442
53	CONTROL	2C	2	471
57	CONTROL	3C	2	451
61	CONTROL	4C	2	469
65	CONTROL	5C	2	404
93	CONTROL	6C	2	436
28	LIVE LARVAE	1PV	1	474
32	LIVE LARVAE	2PV	1	308
36	LIVE LARVAE	3PV	1	471
16	LIVE LARVAE	4PV	1	485
20	LIVE LARVAE	5PV	1	418
24	LIVE LARVAE	6PV	1	468
52	LIVE LARVAE	1PV	2	468
56	LIVE LARVAE	2PV	2	465
84	LIVE LARVAE	3PV	2	450
88	LIVE LARVAE	4PV	2	459
92	LIVE LARVAE	5PV	2	470

72	LIVE LARVAE	6PV	2	415
26	SUSTAINABLE DIET	1P	1	445
30	SUSTAINABLE DIET	2P	1	479
10	SUSTAINABLE DIET	3P	1	456
14	SUSTAINABLE DIET	4P	1	474
18	SUSTAINABLE DIET	5P	1	462
46	SUSTAINABLE DIET	6P	1	505
50	SUSTAINABLE DIET	1P	2	414
54	SUSTAINABLE DIET	2P	2	468
58	SUSTAINABLE DIET	3P	2	483
62	SUSTAINABLE DIET	4P	2	416
66	SUSTAINABLE DIET	5P	2	465
94	SUSTAINABLE DIET	6P	2	408

Total proteins were extracted by SDT-lysis buffer (4% SDS, 100mM Tris-HCl, pH 7.6, 0.1M DTT), and protein concentration was measured using a Bio-Rad protein assay kit. Sixty µg proteins were digested by trypsin/Lys-C at 37 °C overnight, and one µg tryptic peptide was analyzed by a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer. The mass spectral data were processed by MaxQuant (version 2.1.4.0) (Cox and Mann 2008) for protein identification and quantification. Further data analyses including statistical analyses were carried out by Perseus (version 2.0.6.0) (Tyanova et al. 2016).

3. Preliminary results and discussion

On average, 451 proteins were identified from the samples (Table 1). This was a slightly lower number compared to the first experiment which identified in average 495 proteins. PCA analysis was performed (Figure 1) and showed no clear separation between diets or slaughter time. Student's t-test were performed comparing between control and live larvae and between control and sustainable diet. No significant difference was found in protein expression according to feed types. Student's t-test were also performed to compare samples slaughter in two different time points. However, the results did not show significant difference. The results indicated that neither diet nor slaughter time had significant effect on protein expression.
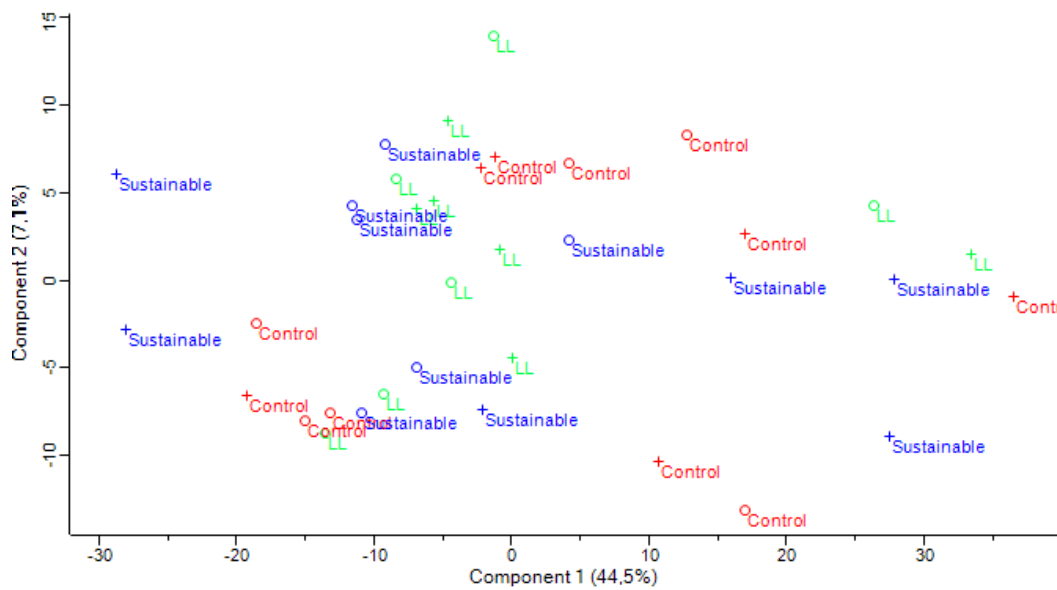
Figure 1 PCA analysis of 36 samples. Red color represents control. Green color represents samples fed with live larvae (LL). Blue color represents samples fed with sustainable diet (Prima). Cross and circle symbols represent slaughter times, one and two, respectively.

4. References

Cox, J. and Mann, M. 2008. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nature Biotechnology 26:1367-1372.

Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M. and Cox, J. 2016. The Perseus computational platform for comprehensive analysis of (prote)omics data. Nature Methods 13:731-740.

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