POULTRYNSECT

D3.3_1 Report on meat proteomic of intermediate growing poultry breed

Deliverable 3.3_1

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Content

Introduction

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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.

1. Material and Methods

A total of 24 Label Naked Neck chicken bird samples, six samples per four experimental groups according to gender and treatment, were included in the study (Table 1). Subsamples were selected systematically based on the results from the chemical composition (Deliverable 3.3) to ensure that they represented the variation in each experimental group.

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

Sample Name	Sample ID	Nr of identified proteins
CF1	20	493
CF2	33	472
CF3	151	257
CF4	165	500
CF5	167	500
CF6	213	206
CM1	6	505
CM2	66	485
CM3	82	517
CM4	159	463
CM5	189	461
CM6	205	514
LF1	71	529
LF2	99	495
LF3	107	503
LF4	148	553
LF5	162	507
LF6	221	496
LM1	8	507
LM2	53	499
LM3	81	484
LM4	123	449
LM5	232	492

LM6	235	474

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. The statistical analyses were carried out by Perseus (version 2.0.6.0) (Tyanova et al. 2016) and Matlab. The protein quantities of each sample were normalized by SNV using the mean and standard deviation of all the detected proteins in that sample. Each protein was then tested using Welch's T-test, with all samples in which the protein was detected except samples 3 and 6 due to their low detection rate. The covariances of the proteins were extracted using PLS. Both analyses were done on both the normalized and raw data. Only the result for the normalized data is included here since they have a larger significance. Autoscaling the variables was also tested, but the results are similar and, therefore, not included here.

2. Preliminary results and discussion

On average, 495 proteins were identified from the samples except two of six samples from one of the groups (females fed basal organic feed +10% BSF supplementation). PCA analysis showed that these two samples (CF3 and CF6) were separated from the others (Fig. 1). The digestion of proteins by trypsin/Lys-C was inadequate as peptide concentration was lower than others after tryptic digestion in one of the samples. The reason for poor identification in another sample is unknown. Because of the poor identification of proteins in these two samples, the data for these two samples were omitted from further data analyses.

PCA analysis was carried out again without the two samples (CF3 and CF6) (Fig. 2). No clear separation was observed according to their diet or sex. Welch's t-test showed a few differentially expressed proteins between the diets (control vs. larvae), but those proteins
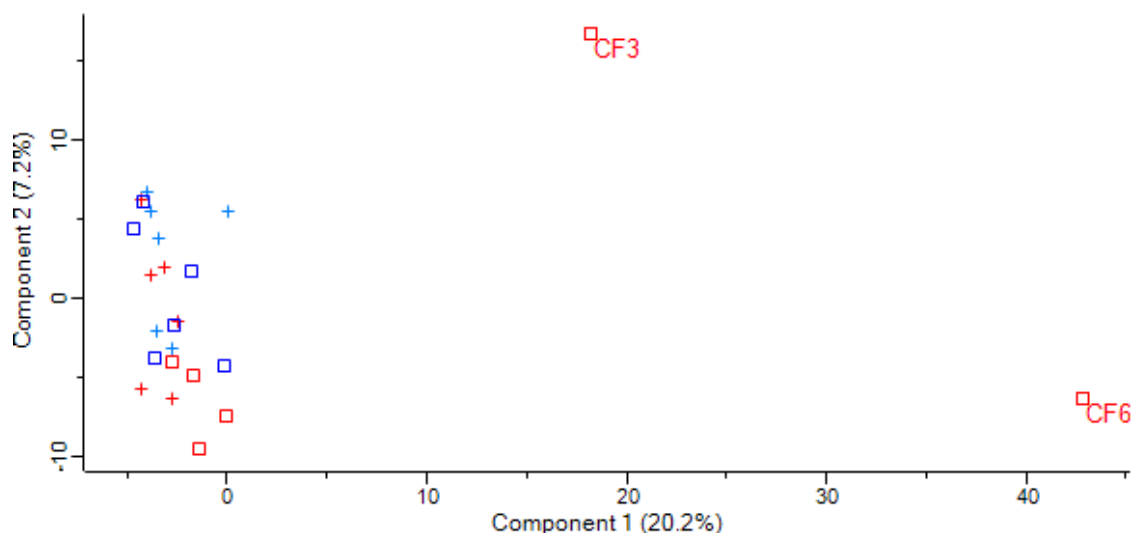

Figure 1 PCA analysis of 24 samples. Red and blue colors represent female and male, respectively. The square symbols represent control feed, while the cross symbols represent feed with larvae.

were within limits expected by the false discovery rate. In contrast, 45 proteins were differentially expressed between females and males at False Discovery Rate (FDR)=0.05. Among 45 proteins, 27 were highly expressed in male chickens, while 18 were highly expressed in female chickens (Fig. 3).
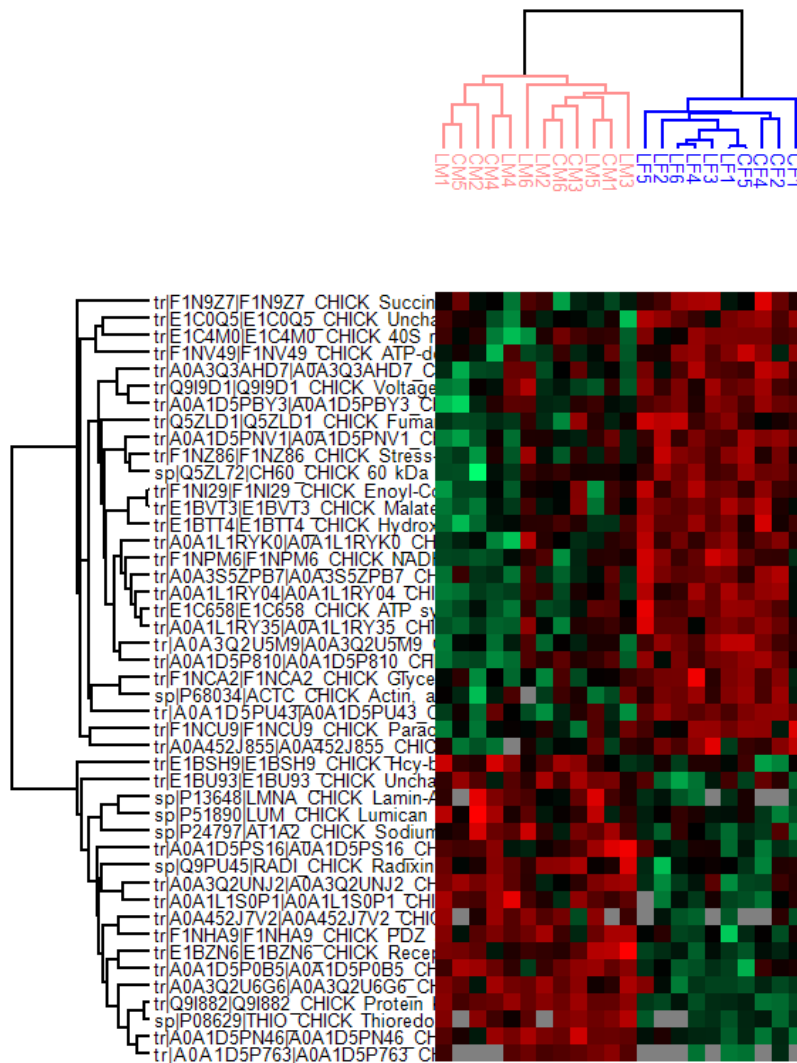

Figure 2 Hierarchical clustering of samples according to the proteins significantly differentially expressed between the female and male chicken. A row cluster is a list of significantly differentially expressed proteins. A column cluster is a list of samples. Green color represents lower intensities while red color represents high intensities.
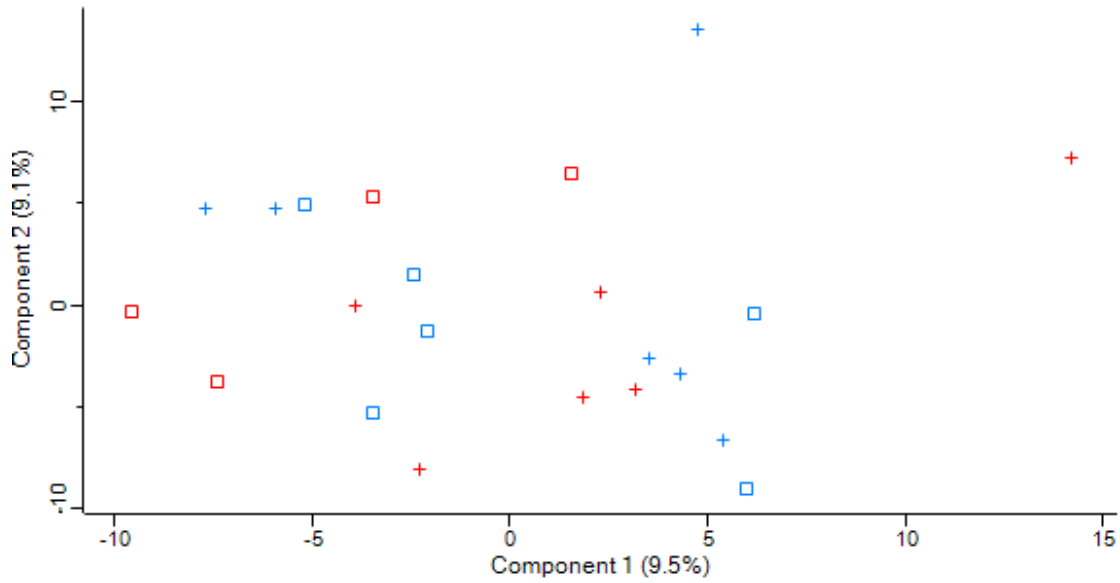
Figure 3 PCA analysis of 22 samples. Red and blue colors represent female and male, respectively. The square symbols represent control feed, while the cross symbols represent feed with larvae.

Partial least squares (PLS) were conducted to find the proteins involved in separating samples between the diets (control vs. larvae) (Fig. 4, Table 2). The diets were separated, but the changes in the individual proteins responsible for this separation were minute and within the distribution already shown by the control group. Considering this, combined with the low significance level found by Welch's T-test, this study reveals no evidence that using larvae as feed would be detrimental.

Another grouping appeared in the PLS scores. This grouping showed up in both feed groups, interfering with the separation of the feed groups. The two proteins with the largest impact in the PLS model were responsible for separating this other group (Table 2), without which separating the feed group is not possible. If the smaller group is excluded, the separation between the feed groups becomes more apparent, and the samples start grouping by gender.
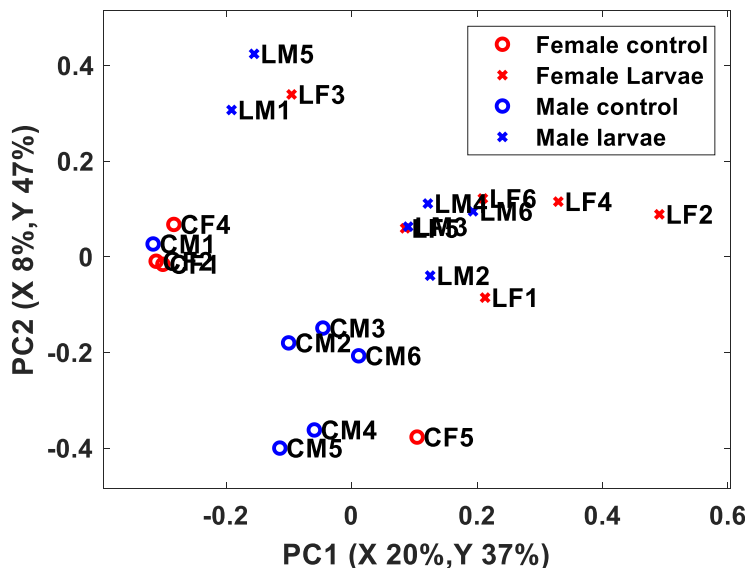

Figure 4 Score plot of partial least squares analysis of chicken samples fed by control and larvae. Red and blue colors represent female and male, respectively. The circle symbols represent control feed, while the cross symbols represent feed with larvae.

Table 2 List of proteins that has a significant impact on separating PC1 and PC2

Uniprot identifier	Gene name	Name	Inpact on PC 1	Inpact on PC2
			(Y-loadings * X-scores)	
A0A1D5P525	MYH1C	Myosin, heavy chain 1C, skeletal muscle	-5.27	2.97
R4GIG1	MYH1B	Myosin heavy chain 1B, skeletal muscle	-3.81	3.36
F1NII7	FBN1	Fibrillin 1	2.07	1.15

The results indicated that feed types have no practical impact on the expression of proteins. On the other hand, 45 proteins were differentially expressed between female and male chicken. However, those proteins were not studied further as this is not the scope of the study, but it is possible if interested.

References

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