POULTRYNSECT

D3.3 Report on meat composition of medium growing poultry breed

Deliverable 3.3

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Abbreviations	
BSF	Black Soldier Fly
D_{SEC}	In vitro protein digestibility based on SEC analysis of generated peptides
MDA	Malondialdehyde
NIR	Near infrared spectroscopy
PCA	Principal component analysis
SEC	Size exclusion chromatography
TBARS	Thiobarbituric acid reactive substances

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Introduction

Introduction

The POULTRYNSECT Work Package 3 ***"Laboratory and Sensorial Analyses"*** aims to evaluate the impact of Black Soldier Fly (BSF) live larvae inclusion as feed ingredient in 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 chemical composition of meat and thus changes in sensory attributes. The assessment of bird`s health (D3.6 and 3.7) and the sensory analyses of chicken breast filets (D3.5) will be compiled in separate reports. This Deliverable (3.3) reports the meat composition **preliminary results** obtained from the first *in vivo* feeding trial performed with Label Naked Neck chicken. Besides chemical composition such as protein content and pH, physical parameters including drip loss, thaw loss and instrumental tenderness are reported and complemented by analysis of in vitro protein digestibility and content of oxidation products to evaluate potential health implications for human consumption.

1. Material and Methods

A total of 240 Label naked neck birds were distributed in four experimental groups according to gender and treatment (10 chickens/pen, 60 birds/treatment):

1. males fed basal organic feed;
2. males fed basal organic feed +10% BSF supplementation;
3. females fed basal organic feed;
4. females fed basal organic feed +10% BSF supplementation.

The birds were fed with the experimental diets from day 20 to day 82 (time of slaughter) as described in D2.2.
After slaughter, pH, color and drip loss of the right chicken breast filets were recorded aftre 24h on the fresh fillet by the CNR partner. The left filets were weighed, vacuum packed and frozen at -20 °C before frozen transport to Nofima.

1.1 Ultimate pH and colour parameters

At CNR laboratories on 60 fillets (15 sample/group) ultimate pH and colour parameters were recorded. The ultimate pH (pHu_{24}), color and drip loss were assessed after 24 h of storage at +4°C on the *Pectoralis major* muscle on the right side of the breast muscle. In particular, the pHu_{24} of the *Pectoralis major* muscle was measured in duplicate by means of a pHmeter (Crison, Crison Instruments, SA, Alella, Spain) equipped with a specific electrode suitable for meat penetration. The lightness (L*), redness (a*), and yellowness (b*) color indexes (Commission International de l'Eclairage, 1976) were measured in the same muscles (taken at multiple locations and averaged) using a portable Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan).

1.2 Thaw loss, NIR-scanner and subsample selection at Nofima

At Nofima all 240 fillets were thawed and dried with paper towles before weighing to determine the thaw loss. The thaw loss was calculated as:
Thaw loss % = ((weight before freezing – weight after thawing)/ weight before freezing) *100.
Each fillet was send through an NIR scanner to screen for muscle abnormalties (wooden breast) and estimate the protein content based on a calibration for chemical composition (Wold, Veiseth-Kent, Høst, & Løvland, 2017). Due to capacity limitations it was not possible to perform in vitro digestion and Warner-Bratzler measurements of all 240 fillets. Therefore a subset of samples from each treatment group were selected for these analyses. Fillet weight, thaw loss and protein content from NIR were used to select the subsamples from each treatment group. PCA was used for this selection to ensure that the subsamples represented the variation in each group, but not the extremes (Figure 1).

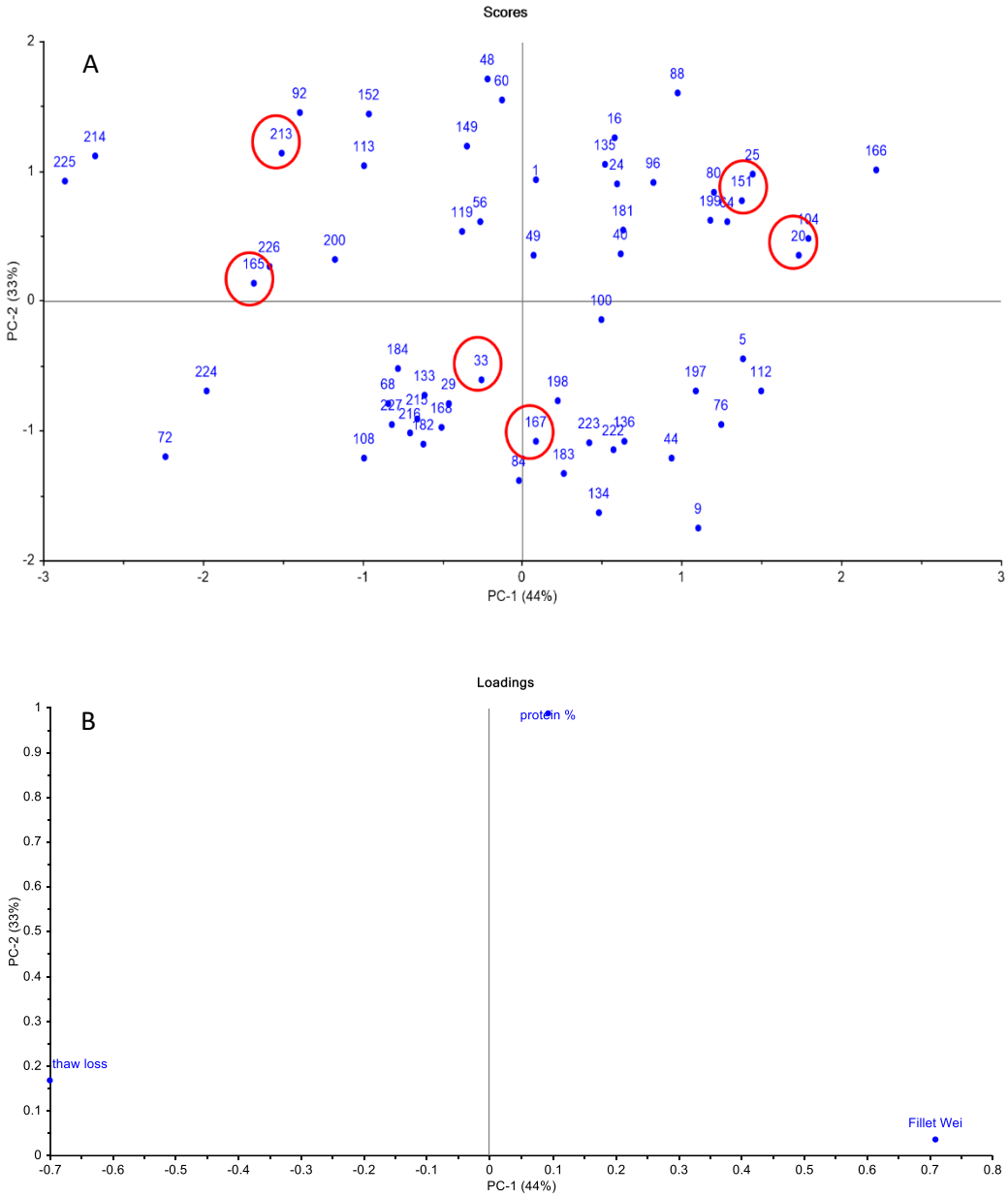

Figure 1: Example of PCA based selection of subsamples. A: Score plot for all samples in the group female control. The red circles indicate the samples selected for further analyses.B: Loadings plot of the PCA on protein%, thaw loss and fillet weight.

1.3 *In vitro* protein digestiblity and content of oxidation products

The *in vitro* protein digestibility of chicken breast fillets was determined on a subset of samples (selection described in 1.2). From each treament group 6 samples were chosen and diveded in two. One part of the fillet was minced and digested raw, the other part was vacuum packed and heat treated in a water bath at 70°C for 30 min as described in 1.4 for the Warner Bratzler method. The heat treated chicken fillets were also minced and a total of 1g samples was weighed into individual tubes for *in vitro* digestion. The moisture and protein content of the minced chicken fillets were determined by freeze-drying (weight loss) and combustion analysis

using a Nitrogen to protein conversion factor of 6.25. The *in vitro* digestion was performed according to the international consensus INFOGEST model (Brodkorb et al., 2019; Minekus et al., 2014). After 120 min of simulated small intestinal digestion, digestive enzymes were inactivated by heat (water batch at 90 °C for 10 min) and centrifuged. The protein content in supernatant and pellet were determined separtely as previously described (Rieder et al., 2021). The percentage of small peptides (less than 1kDa in MW) in the peptides size distribution of the supernatant (determined by SEC) and the amount of dissolved protein were used to calculate protein digestiblity as D_{SEC} (Rieder et al., 2021). As a measure of lipid oxidation and oxidative stress during digestion the content of TBARS was determined in raw chicken breast filet, heat treated chicken breast filet and heat treated chicken breast fillet after *in vitro* digestion as previously described (Steppeler, Haugen, Rødbotten, & Kirkhus, 2016).

1.4 Instrumental tenderness (Warner-Bratzler)

WB method

The thawed chicken filets were individually vacuum packed in plastic bags and imersed into a waterbath at 70.5°C for 30 minutes, which ensured a core temperature of 70°C in the muscle samples. After chilling in ice-water for 45 minutes, and further acclimatization to room temperature, the chicken filets were sliced into samples of size 3x1x1 cm^3 along the muscle fibre direction. Three samples from each filet were sheared across the fibre direction with a Warner-Bratzler device in an Instron Materials Testing Machine (model 5944) as descibed (Hildrum et al., 2009)

2. Preliminary results and discussion

2.1 Ultimate pH and colour parameters

In table 1 the pH_{u24}, colour parameters and drip loss of the breast muscles are summarized per treatment. No significant differences between larvae and control fed birds were observed.

Table 1: *Average values and standard error of the mean (SEM) of pHu_{24} and colour (L*, a*, b*) parameters of right chicken filets after 24h of slaughtering.*

	pHu_{24}	L*	a*	b*	Drip loss %
control male	5.62	49.7	0.52	14.1	1.45
larvae male	5.63	47.0	0.78	12.8	1.63
control female	5.59	47.8	0.44	15.9	1.56
larvae female	5.58	48.0	0.79	16.6	1.57
SEM	0.03	0.93	0.25	0.49	0.01

2.2 Fillet weight, thaw loss and protein content with NIR

In table 2 the weight of all the left fillets after thawing, the thaw loss and the estimated protein content are summarized per treatment. While we observed an expected difference in filet weight between male and female birds and a tendency towards slightly lower protein content in fillets from female vs male birds, no significant differences between larvae and control fed birds were observed.

Table 2: *Average values +/- standard deviation of weight, thaw loss and estimated protein content for all left chicken filets after thawing. Numbers in the same column sharing the same letter are not significantly different (Tukey test, p<0.05).*

	fillet weight	thaw loss	estimated protein content with NIR
	g	%	%
control male	196 +/- 23 ^{A}	8.2 +/- 2 ^{A}	22.5 +/- 0.5 ^{A}
larvae male	194 +/- 22 ^{A}	8.1 +/- 2.1 ^{A}	22.4 +/- 0.4 ^{A}
control female	151 +/- 17 ^{B}	8.7 +/- 2.2 ^{A}	22.1 +/- 0.7 ^{B}
larvae female	149 +/- 17 ^{B}	8.9 +/- 1.8 ^{A}	22.3 +/-0.7 ^{AB}

2.3 TBARS

The raw chicken fillets contained only very low amounts of lipid oxidation products measured as malondialdehyde (MDA) equivalents (Table 3). The content of MDA increased with heat treatment and during digestion (Table 3). Similar effects have been reported previously for

different types of meat (Steppeler et al., 2016). There was a higher variation between individual fillets of the same treatment, then there was between treatments (Figure 1) and no significant effects of bird sex or feed type on TBARS were found.

Table 3: *TBARS average values +/- standard deviations for the 6 individual filets of each treatment for raw fillets, heat treated fillets and digested fillets. Numbers in the same column sharing the same letter are not significantly different (Tukey test, p<0.05).*

	raw fillet	heat treated fillet	digested fillet
	µmol MDA/kg	µmol MDA/kg	µmol MDA/kg
control male	0.2 +/-0.7 ^{A}	5.9 +/- 1.7 ^{A}	24.8 +/-15.1 ^{A}
larvae male	0.02 +/- 0.2 ^{A}	5.2 +/- 2.8 ^{A}	20.7 +/- 15.7 ^{A}
control female	0.14 +/- 0.2 ^{A}	6.8 +/- 3.1 ^{A}	22.5 +/-12.8 ^{A}
larvae female	0.24 +/- 0.3 ^{A}	7.7 +/- 4.9 ^{A}	26.4 +/-19.0 ^{A}

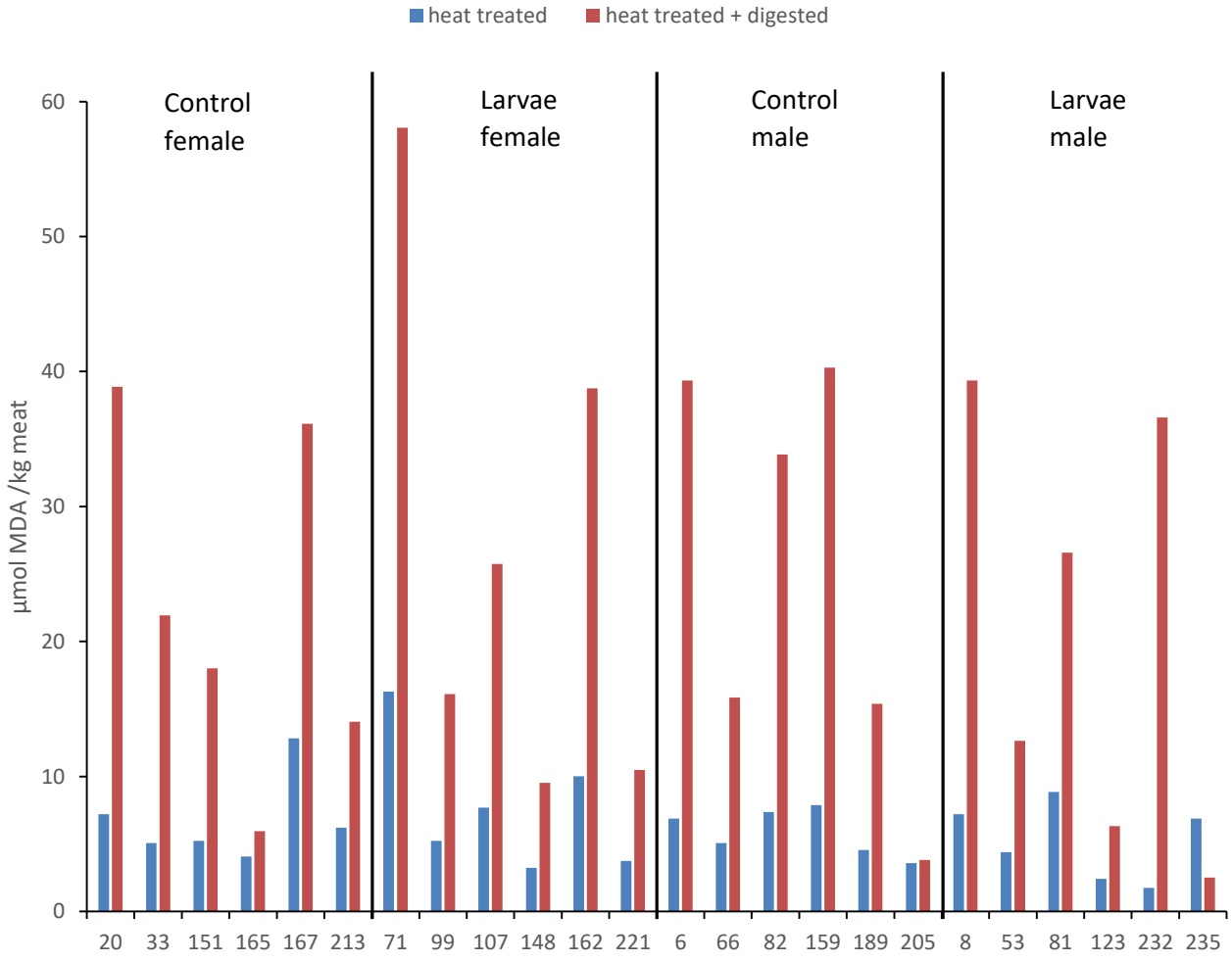

Figure 1: Content of MDA in individual selected chicken filets (bird number) after heat treatment and after heat treatment + digestion.

2.4 *in vitro* protein digestibility

Heat treatment of the chicken fillets before in vitro digestion resulted in a significant increase in protein content of the mince (measured by combustion), which was due to a decrease in moisture (Figure 2). However no significant effect of SEX or feed on moisture loss during heat treatment could be observed.

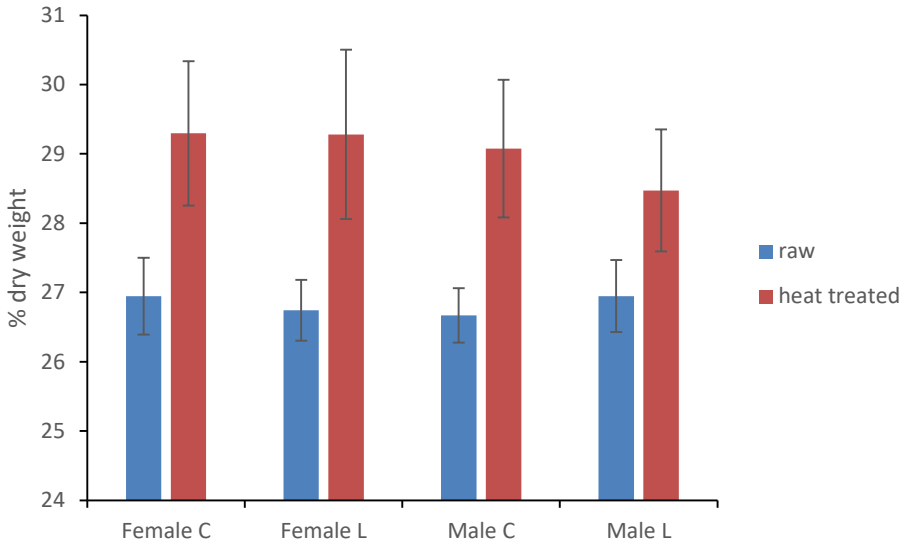

***Figure 2**: Average +/- standard deviation of dry weight content of selected chicken fillets before and after heat treatment.*

During *in vitro* digestion almost all the protein in the chicken fillet samples was solublized and no signficant differences between raw or heated samples, male or female chicken or normal or larvae feed were found. As expected, all the chicken fillets were very well digested and the peptide size distrubtions showed a content of small, bioavailable peptides (<1kDa) above 92% for all groups. However, the heat treated chicken fillets were slightly better degraded during simulated digestion with an average of 94.7% small peptides vs 92.1% for raw. This resulted in slightly higher values of protein digestiblity for heat treated vs raw chicken fillets (Figure 3). However, no significant effects of sex or larvae feed on protein digestibility of chicken fillets were found.

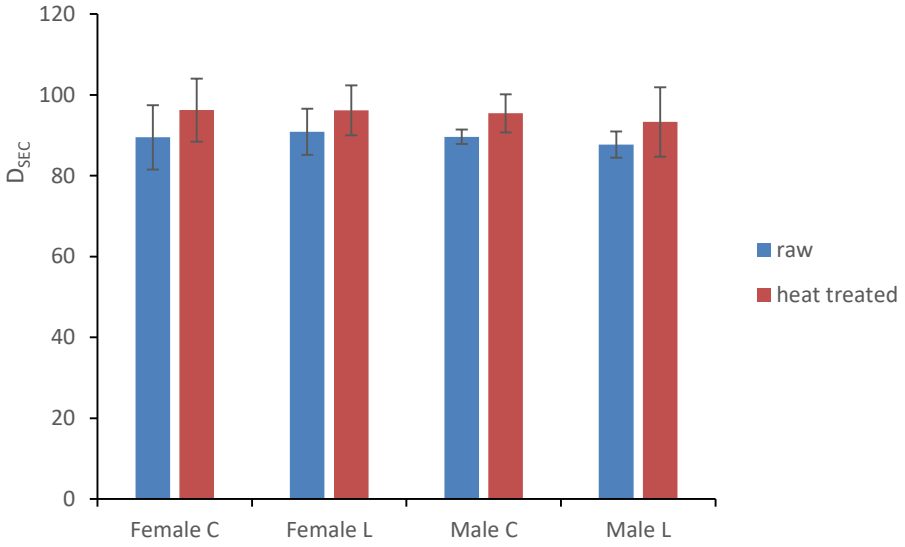

Figure 3: *Average +/- standard deviations of in vitro protein digestiblity expressed as* D_{SEC} *for the different groups of chicken fillets.*

2.5 Warner-Bratzler shear force (instrumental tenderness)

A subset of 15 fillets were selected from each of the four experimental groups. The recorded shear force values are shown in Table 4. There were significant differences between the individual fillets, but no significant difference was observed between the experimental groups. However, there was a tendency (p=0.10) for lower WB shear force values from the female compared to the male samples. This may indicate that female chicken could be perceived more tender than male chicken breast. No differences were found between birds given larvae or control feed.

Table 4: *Average Warner-Bratzler shear force values (N/cm²) obtained from 3 parallels of each cooked chicken breast fillet. Numbers in the same column sharing the same letter are not significantly different (Tukey test, p<0.05).*

	n	WB shear force (N/cm²)
control male	15	15.7 +/- 6.9 ^{A}
larvae male	15	15.9 +/- 4.6 ^{A}
control female	15	14.1 +/- 5.3 ^{A}
larvae female	15	13.8 +/- 4.5 ^{A}

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