POULTRYNSECT

D3.4 Report on meat composition of medium growing poultry breed

Deliverable 3.4

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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

Content

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 (3.4) reports the meat composition preliminary results obtained from the second in vivo trials performed on Bianca di Saluzzo chickens, by the UNITO project partner. For this trial, males of Bianca di Saluzzo (BS) breed (slow-growing genotype) were reared for 150 and 180 days. The choice of focusing only on males was made due to the predisposition of BS males to have higher slaughter yields than females, as stated by Bongiorno et al., (2022).

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.

2. Second poultry trial

2.1 Material and Methods

A total of 96 birds were distributed in 2 experimental groups according to treatment:

basal organic feed (Control)
basal organic feed with live BSF larvae supplementation (Live larvae)
The birds were fed with the experimental diets from day 39 to day 150 (first slaughtering age, 12 birds/ treatment) or day 180 (second slaughter time, 12 birds/treatment) as described in D2.4.
After slaughter, pH, color and drip loss of the right chicken breast filets were recorded on the fresh fillet by the CNR partner. The left filets were weighed, vacuum packed and frozen at -20°C before frozen transport (-20 °C) in Norway to the NOFIMA laboratories.

Thaw loss, NIR-scanner and subsample selection at Nofima

At Nofima all 48 fillets were thawed and dried with paper towels 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 sent through an NIR scanner to screen for muscle abnormalities (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 fillets. Therefore, a subset of samples from each treatment group were selected for these analyses. To do so the number of fillets per treatment group was reduced from 12 to 6 by randomly choosing one of the two fillets from birds reared in the same box.

In vitro protein digestibility and content of oxidation products

The *in vitro* protein digestibility and TBARS of chicken breast fillets were determined on a subset of samples (selection described in 1.1). A small section (from the thin end of each fillet) was cut off and saved for analysis of raw chicken breast before the remainders were vacuum packed and heat treated in a water bath at 70°C for 30 min as described in 1.3 for the Warner Bratzler method. The heat treated chicken fillets were used to measure instrumental tenderness (Warner Bratzler method see 1.3) and the remaining of cooked fillets from each treatment group were pooled and minced together. For protein digestibility measurements, 350 mg of minced chicken (corresponding to 100 mg protein) were weighed in triplicates into 50 mL centrifuge tubes for in vitro digestion. For TBARS measurements triplicates of 1g raw minced chicken or 1 g cooked minced chicken were weighed per treatment group. The moisture and protein content of the minced chicken fillets (both raw and cooked) 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 separately 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 digestibility as DSEC (Rieder et al., 2021). As a measure of lipid oxidation and oxidative stress the content of TBARS was determined in raw chicken breast filet, heat treated chicken breast filet as previously described (Steppeler, Haugen, Rødbotten, &

Kirkhus, 2016).

Instrumental tenderness (Warner-Bratzler)

The thawed chicken filets were individually vacuum packed in plastic bags and immersed into a water bath 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 cm3 along the muscle fiber direction. Three samples from each filet were sheared across the fiber direction with a Warner-Bratzler device in an Instron Materials Testing Machine (model 5944) as described (Hildrum et al., 2009).

2.2 1st and 2nd slaughtering age preliminary results

Fillet weight, thaw loss and protein content with NIR

In Table 5 the weight of all the left fillets after thawing, the thaw loss and the estimated protein content are summarized per treatment. Compared to the 1st feeding trial the fillets weight of the 2nd trial was much lower (115 vs 195 g in average for male birds), but no significant effects of feed or slaughter time on fillet weight were found. The thaw loss was on average around 2.9 %, which was considerably lower than in the first feeding trial were thaw loss amounted to about 8.4% in average. However, also with thaw loss no significant differences according to feed or slaughter time could be observed. The average estimated protein content with NIR was 23.4%, which was slightly higher than the 22.3% from the 1st trial.

Table 5: 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, $p<0.05$).

	Time of slaughter	fillet weight	thaw loss	estimated protein content with NIR
		g	%	%
CONTROL	1	115 +/- 6^{A}	3.4 +/- 1.4^{A}	23.4 +/- 0.3^{A}
LIVE LARVAE	1	115 +/- 10^{A}	3.6 +/- 1.3^{A}	23.2 +/- 0.3^{A}
CONTROL	2	113 +/- 13^{A}	3.1 +/- 1.4^{A}	23.6 +/- 0.7^{A}
LIVE LARVAE	2	115 +/- 14^{A}	2.6 +/- 0.6^{A}	23.6 +/- 0.9^{A}

TBARS

The raw chicken fillets contained only very low amounts of lipid oxidation products measured as malondialdehyde (MDA) equivalents (Table 6). No effect of treatment on lipid oxidation products was seen in the raw samples. The content of MDA increased with heat treatment (Table 2). This was the same in the 1st feeding trial, but the extent of increase with heat treatment was much more pronounce in the 2nd trial. While the 1st trial did not reveal any significant effects of treatment on the amount of MDA equivalents in heated chicken fillets, significant differences were observed in the 2nd feeding trial. At the first slaughter time, control diet resulted in higher lipid oxidation compared to the treatment with live larvae. There was a decrease in MDA equivalents at slaughter time 2 compared to 1, with the control diet at

slaughter time 2 gave the lowest values.

Table 6: 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, $p<0.05$).

	Time of slaughter	raw fillet	heat treated fillet
		µmol MDA/kg	µmol MDA/kg
CONTROL	1	1.8 +/- 0.2^{A}	51.5 +/- 2.5^{A}
LIVE LARVAE	1	1.8 +/- 0.3^{A}	37.3 +/- 2.6^{B}
CONTROL	2	1.6 +/- 0.2^{A}	23.6 +/- 4.1^{C}
LIVE LARVAE	2	1.8 +/- 0.4^{A}	24.8 +/- 3.4^{C}

In-vitro protein digestibility

During *in vitro* digestion almost all the protein in the chicken fillet samples was solubilized and no significant differences between slaughter times and diets were found. As expected, all the chicken fillets were very well digested and the peptide size distributions showed a content of small, bioavailable peptides (<1kDa) above 93% for all groups. Consequently, no significant differences were found between *in vitro* protein digestibility of chicken fillets from birds fed different diets or slaughtered at different time points. Compared to the first feeding trial, the measured protein digestibility was slightly higher in the second trial (88.6 – 90.6 vs 91.2 – 91.7).

Table 7: *in vitro* protein digestibility of heat treated chicken breast fillets obtained from 3 parallels of a pooled sample prepared from 6 cooked fillets. Protein solubilization and size of peptides generated during in vitro digestion were measured and used to calculated protein digestibility (D_{SEC}).

	Time of slaughter	Soluble protein	Small peptides	Protein digestibility (D_{SEC})
		%	%	%
CONTROL	1	98.2 +/- 0.5	93.2 +/- 0.1	91.6 +/- 0.5
LIVE LARVAE	1	98.2 +/- 0.2	93.3 +/- 0.1	91.6 +/- 0.2
CONTROL	2	97.7 +/- 1.7	93.3 +/- 0.1	91.2 +/- 1.7
LIVE LARVAE	2	98.2 +/- 0.1	93.4 +/- 0.1	91.7 +/- 0.1

Warner-Bratzler shear force (instrumental tenderness)

A subset of 6 fillets were selected from each of the six experimental groups. The recorded shear force values are shown in Table 8. There were significant differences between the individual fillets, but no significant difference was observed between the experimental groups. The range of WB shear force was slightly higher in this 2nd feeding trial compared to the first (average shear force 19.2 vs 16.6 N/cm^2)

Table 8: Average Warner-Bratzler shear force values (N/cm^2) obtained from 4 parallels of each cooked chicken breast fillet. Numbers in the same column sharing the same letter are not significantly different (Tukey test, $p<0.05$).

	Time of slaughter	n	WB shear force (N/cm^2)
CONTROL	1	6	18.5 +/-6.0^A
LIVE LARVAE	1	6	16.6 +/- 3.5^A
CONTROL	2	6	22.8 +/- 6.0^A
LIVE LARVAE	2	6	19.5 +/- 6.8^A

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